315.871

VOLUME 11 · NUMBER 1—2 · 1977

HAEMATOLOGIA

11
1977

International Quarterly of Haematology

EDITOR-IN-CHIEF:
S. R. HOLLÁN

EDITOR:
I. BERNÁT

Editorial Board:
G. A. ALEXEIEFF (Moscow)
A. ANDRÉ (Liège)
A. ANTONINI (Rome)
V. APATEANU (Bucharest)
R. ARNAUD (Tours)
G. ASTALDI (Tortona)
I. BARTA (Pécs)
G. BAST (Rostock)
H. BEGEMANN (Munich)
E. BENEDEK (Budapest)
J. BERNARD (Paris)
E. BEUTLER (Duarte, California)
H. BRAUNSTEINER (Innsbruck)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Odense)
O. K. GAVRILOV (Moscow)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
T. J. GREENWALT (Washington, D.C.)
A. HÄSSIG (Bern)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
E. KELEMEN (Budapest)
P. J. LAH (Ljubljana)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Freiburg/Br.)
P. L. MOLLISON (London)
A. E. MOURANT (London)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Debrecen)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM †(Liège)
W. RUDOWSKI (Warsaw)
G. RUHENSTROTH-BAUER (Munich)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Pavia)
A. VIDEBAEK (Copenhagen)
A. S. WIENER †(Brooklyn, N. Y.)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

HAEMATOLOGIA

is an international quarterly publishing original papers on haematology. It also provides the reader with complex and up-to-date information on both research and clinical practice. A General Survey, an Open Forum, Book Reviews, Abstracts of more important papers from other periodicals and a Documentation of the well-known and the less accessible journals are to serve this purpose.

Haematologia is published at quarterly intervals, the four issues per year make up a volume of about 500 pages.

Subscription price: 36 per volume (per year). Orders may be placed with

KULTURA

Foreign Trading Company

Budapest 62. P.O.B. 149

or with its representatives abroad, listed on the verso of the cover.

VOLUME 11 · NUMBER 1—2

HAEMATOLOGIA

INTERNATIONAL QUARTERLY OF HAEMATOLOGY

EDITOR-IN-CHIEF:
S. R. HOLLÁN

EDITOR:
I. BERNÁT

MEMBERS OF THE EDITORIAL BOARD:

G. A. ALEXEIEFF (Moscow) · A. ANDRÉ (Liège) · A. ANTONINI (Rome) · V. APATEANU (Bucharest) · R. ARNAUD (Tours) · G. ASTALDI (Tortona) · I. BARTA (Pécs) · G. BAST (Rostock) · H. BEGEMANN (Munich) · E. BENEDEK (Budapest) · J. BERNARD (Paris) · E. BEUTLER (Duarte, California) · H. BRAUNSTEINER (Innsbruck) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle, Washington) · L. GARBY (Odense) · O. K. GAVRILOV (Moscow) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest). F. GRÁF (Budapest) · A. GRAFFI (Berlin) · T. J. GREENWALT (Washington, D. C.) · A. HÄSSIG (Bern) · L. P. HOLLÄNDER (Basel) · J. HOŘEJSÍ (Prague) · M. HRUBIŠKO (Bratislava) · E. KELEMEN (Budapest) · P. J. LAH (Ljubljana) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Freiburg/Br.) · P. L. MOLLISON (London) · A. E. MOURANT (London) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Debrecen) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM† (Liège) · W. RUDOWSKI (Warsaw) · G. RUHENSTROTH-BAUER (Munich) · V. SERAFIMOV-DIMITROV (Sofia) · I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIC (Belgrade) · E. STORTI (Pavia) · A. VIDEBAEK (Copenhagen) · A. S. WIENER † (Brooklyn, N. Y.)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES
BUDAPEST 1977

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója
Műszaki szerkesztő: Zacsik Annamária
A kézirat nyomdába érkezett: 1977. VIII. 24. — Terjedelem: 21,70 (A/5) ív, 67 ábra, 1 melléklet
78.4855 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

Contents

Rosenfield, R. E.: In memoriam Alexander S. Wiener 5
Astaldi, G., Bagnara, G. P., Brunelli, M. A., Karanovic, D., Karanovic, J., Scorza, R., Topuz, Ü.: Experimental and clinical investigations on stem cell take and colony formation 11
Mód, A., Harsányi, V., Jakschitz, M., Hollán, S. R.: The treatment of infections in granulocytopenic patients with granulocyte transfusions 31
Šulc, K., Neuwirt, J., Trávníček, T., Kobylka, P., Radikovská, E.: Bone marrow cell separation on Ficoll gradient 41
Fleischmann, T., Krizsa, F.: Chromosomes in malignant lymphomas 47
Климова, Н. Ф.: Десятилетний опыт лечения миелобромолом больных миелофиброзом 57
Amir, J., Forman, W. B., Rassiga, A., Bernier, G. M.: Nonsynthetizing multiple myeloma 73
Tichý, M., Hrnčíř, Z., Šimša, J.: Serum levels of IgA, IgM, IgD and IgE in IgG paraproteinaemias 79
Láng, I., Fekete, B., Gergely, P., Petrányi, Gy.: A simple allogeneic test system for the study of antibody-dependent cellular cytotoxicity (ADCC) in man 85
Alföldy, P., Dombi, É., Tóth, T., Gyódi, É., Hollán, S. R.: Cell-mediated immunity in subjects immunized with HLA antigens 93
Jókay, I., Karczag, E.: Antagonistic effect of heparin and a water-soluble glucocorticoid on the peripheral lymphocyte count in mice 103
Merkiel, K., Prokopowicz, J., Bielawiec, M., Kiersnowska, B.: Serum lysozyme activity in some myeloproliferative diseases 111
Хомченовский, Е. И.: Итоги экспериментального и клинического изучения нового противолейкозного препарата гексафосфамида 115
Rudowski, W., Daszyński, J., Schier, J., Kowalczyk, V., Gaczkowski, A., Góralski, S.: Infusion of physiological saline supplemented with adenine, pyruvate and phosphate 127
Fagiolo, E.: Immune haemolytic anaemia after exchange transfusion 133
Prodanov, P.: H-excess A_1 and A_{int} variants among the Bulgarian population 139
Krizsa, F., Cserháti, I.: Effect of F-leurosine (formyl-leurosíne) on platelet production in mice 149
Kosztolányi, G., Jobst, K., Kádas, I.: Effect of lanthanum ion on platelet electrophoretic mobility 155
Blaskó, Gy., Bédi, J., Machovich, R., Pálos, L. Á.: Effect of collagen on thrombin inactivation by antithrombin-III and heparin 163
Goldschmidt, B., Siegler, J., Schmidt, M.: Fibrinogen metabolism in cyanotic congenital heart disease 169
Szmitkowski, M., Prokopowicz, J., Szmitkowska, K.: Antihaemophilic factor (factor VIII) from human granulocytes 177
Book Reviews 189
Abstracts 195
From the International Literature of Haematology 203
News Items 247

In Memoriam

ALEXANDER S. WIENER, M. D.



1907 – 1976

Dr. Alexander S. Wiener, world famous for his work on Rh and other human blood types, and for his expertise in forensic medicine, died at the age of 69 of leukemia in New York City on 6 November 1976. In his distinguished professional career, Dr. Wiener was responsible for over 700 publications including 12 books. In addition to his work on Rh, he described blocking antibodies [1], the first test for IgG anti-Rh, and he showed the role of plasma proteins in support of Rh agglutination by IgG anti-Rh, adding that these antibodies, rather than saline agglutinins, crossed the placenta [2]. He independently rediscovered Kell (Si) [3], he discovered U in the MN system [4] and M^e as a cross-reaction between M and Henshaw [5]. He correctly estimated IgG half-life [6], he discovered Rh in non-human primates [7], and he defined both anti-I cold autoagglutinins and variably weak I blood types in adults [8]. He vigorously pursued Simian allotypes [9] for the last 13 years of his life.

Of all of his numerous discoveries, Dr. Wiener was best known for, and indeed proudest of, describing human Rh polymorphism and coining the term Rh, both in collaboration with the late Dr. Karl Landsteiner [10]. Following this enormously important contribution, Dr. Wiener went on to describe other Rh alloantigens: rh′ [11], rh″ [12], $\mathfrak{R}h_0$ [13], Rh^A [14], Rh^B [15], Rh^C [16], and Rh^D [17]. Astonishingly, never in his career – until his studies of Simian allotypes – did he ever encounter a new allelic antigen. As a consequence, Dr. Wiener was always an advocate of multiple complex alleles.

It is not possible to understand or appreciate Dr. Wiener's remarkable career in serology without knowing how it began and where it became complicated. After graduating from Cornell University with election to the Phi Beta Kappa honorary fraternity, Dr. Wiener studied medicine at Long Island College Hospital, later to become Downstate Division of State University of New York. In the third year of his four year curriculum, he published two reports, both with Lederer and Polayes (the former known for Lederer's anemia [18] and the latter to be known for ABO disease [19]). One of these reports [20] was a magnificent study of 500 mother–baby pairs showing that anti-A and anti-B activity in cord blood serum was dependent both on the mother having such antibody activity and on the baby being ABO compatible with its mother. Had Dr. Wiener then understood distribution theory as he was to know it later in his career, he would have noted from the active cord sera how type 0 mothers differed from non-0 mothers. The other report [21] mistakenly attributed blood typing to the ancient Chinese. This blunder annoyed Dr. Karl Landsteiner who had his assistant, Dr. Philip Levine, invite Dr. Wiener to Rockefeller Institute to discuss the matter. That initial meeting led not only to another report [22] – a formal retraction – but to the long extramural association between Dr. Wiener and Dr. Landsteiner which lasted until the latter's death in 1943.

Possibly because his father was a practicing attorney, Dr. Wiener became intensely interested in using human blood typing for forensic medicine, and he became Serologist for New York City's Chief Medical Examiner from 1938 until his death. Wiener's early forensic interests generated serious concerns about the reliability of MN typing. In 1935, he sought Dr. Landsteiner's advice on MN and was told to prepare rabbit anti-*rhesus* for anti-M as one possible route toward a solution. This he did, and in 1937 he noted that, if anti-M were removed from such immune sera, a new and hitherto undescribed specificity remained. But Dr. Landsteiner discouraged publication of these findings. Indeed, the 1937 report of Landsteiner and Wiener [23] not only fails to mention the new human polymorphism but it lacks a report of one crucial experiment: What happens when rabbit anti-*rhesus* is adsorbed with human type M red cells? Wiener always claimed, and stated [24], that he knew of Rh polymorphism in 1937. But Landsteiner may have known of it even earlier through the work of one of his Ph. D. students at Columbia University, Leon Buchbinder [25]. Buchbinder during his life repeatedly stated that he knew about Rh polymorphism but that Landsteiner refused to permit further studies or publication [26].

In 1940, however, H. Raymond Peters of Johns Hopkins University sent Dr. Wiener blood specimens on three cases of severe transfusion reaction, two of which were fatal. In each case, patient and donor were of the same ABO type but, with rabbit anti-*rhesus*, all three patients were Rh-negative and all incriminated donors were Rh-positive. Astonishingly two of the patients displayed weak, somewhat cold-reacting, posttransfusion serum hemagglutinins having the same specificity as rabbit anti-*rhesus*. In consequence, Landsteiner now agreed to publish the results of human blood typing with rabbit anti-*rhesus* [10], and this

report was followed almost immediately by publication of transfusion reactions due to allogeneic anti-Rh [27]. It is to be noted that the Landsteiner and Wiener report [10] lacked data on adsorption and elution. If Landsteiner remembered that this had been done by Buchbinder, he forgot that it had not been published [25].

Although the reports of Landsteiner and Wiener [10] and Wiener and Peters [27] had a great impact on the world of biomedical science, they were published at least a year too late. In 1939, Levine with Stetson [28] published a case report of a woman who, following delivery of a stillborn child, suffered a violent transfusion reaction when given her husband's AB0 compatible blood. Her posttransfusion serum contained hemagglutinins for a hitherto undefined alloantigen present in 80% of unselected Caucasian red blood cell samples, including the blood of her husband. This alloantigen was not named, but the idea that it might have been the cause of the stillbirth was ample reason for Levine to study other cases of erythroblastosis. In the course of doing this, Levine and Wiener in 1940 compared the agglutinating specificity of the serum of a patient of Dr. Peter Vogel, M. F. [29], with that of rabbit anti-*rhesus* [10]. The two seemed to be identical, and human Rh was soon perceived to be the cause of 90% of cases of erythroblastosis [29].

Priority questions were promptly raised both about the discovery of Rh polymorphism and the description of Rh as a cause of transfusion reactions. Did the Levine and Stetson unnamed 1939 polymorphism take priority over the 1940 rabbit anti-*rhesus* data of Landsteiner and Wiener, and did the Levine and Stetson transfusion reaction take priority over the 1940 report of Wiener and Peters? Perhaps Landsteiner should have published with Wiener in 1937, when Levine was only beginning to work on the Stetson case. But no one wanted to blame Landsteiner for anything, and an acrimonious situation arose, never to subside. Indeed, it only worsened with the introduction of CDE terminology by Race and Fisher [30]. This was a threat both to Wiener's Rh terminology and to his favored concept of multiple complex alleles [10, 11, 12, 13]. It was too late for rational dialogue and, although repeatedly challenged, Dr. Wiener was never to abandon his views.

The priority problem continued to warp the relationship between Wiener and Levine. And yet both men were to be jointly honored on seven separate occasions, more than any other two independent scientists. They received the Lasker Award (1946), the Ward Burdick Award (1946), the Passano Award (1951), the Karl Landsteiner Memorial Award (1956), the Kennedy Award (1966), Fellowships in the Royal College of Physicians (Levine 1973, Wiener 1974), and the William Allan Award (1975). Only the English were fearful of a direct confrontation, and gave their Fellowships on separate years.

Despite his many publications, Dr. Wiener always insisted that none of his reports was an easy chore, and that he worked most diligently on each one. Certainly, he was a perfectionist in his bench work where he personally interpreted almost every single test performed in his laboratories.

As a person Dr. Wiener was perceptive and intelligent. As a scientist he was generous in helping others with gifts of valuable reagents. But, although he considered himself as forthright, he was often extremely dogmatic, and the polemical nature of some of his publications was offensive to targets of his attacks. Nevertheless, when both contradictory facts and their underlying assumptions were made completely clear to him, he did accept criticism.

Alexander S. Wiener will always be remembered as a skillful and enterprising investigator. He also deserves respect for vigorous efforts to systematize observations. Using a currently popular American emphemism, to explore serological possibilities and organize the data was Wiener's "bottom line", the goal of his life's work.

Richard E. Rosenfield, M. D.
Department of Pathology,
The Mount Sinai School of Medicine,
City University of New York, USA

References

1. Wiener, A. S.: A new test (blocking test) for Rh sensitization. *Proc. Soc. exp. Biol. Med. 56*, 173 (1944).
2. Wiener, A. S.: Conglutination test for Rh sensitization. *J. Lab. clin. Med. 30*, 662 (1945).
3. Wiener, A. S., Soun-Gordon, E. B.: Réaction transfusionnelle hémolytique intragroupe due à une hémagglutinogène jusqu'ici non décrit. *Rev. d'Hématol. 2*, 3 (1947).
4. Wiener, A. S., Unger, L. J., Cohen, L.: Distribution and heredity of blood factor U. *Science 119*, 734 (1954).
5. Wiener, A. S., Rosenfield, R. E.: M^e, a blood factor common to the antigenic properties M and He. *J. Immunol. 87*, 376 (1961).
6. Wiener, A. S.: The half-life of passively acquired antibody globulin molecules in infants. *J. exp. Med. 94*, 213 (1951).
7. Wiener, A. S.: Blood group factors in anthropoid apes and monkeys. I. Studies on a chimpanzee, "Pan." *Amer. J. phys. Anthrop. 10*, 375 (1952).
8. Wiener, A. S., Unger, L. J., Cohen, L., Feldman, J.: Type-specific cold autoantibodies as a cause of acquired hemolytic anemia and transfusion reactions; biologic test with bovine red cells. *Ann. Intern. Med. 44*, 221 (1956).
9. Moor-Jankowski, J., Wiener, A. S., Rogers, C.: Blood groups of chimpanzees demonstrated with isoimmune serums. *Science 145*, 1441 (1964).
10. Landsteiner, K., Wiener, A. S.: An agglutinable factor in human blood recognizable by immune sera for rhesus blood. *Proc. Soc. exp. Biol. (N. Y.) 43*, 223 (1940).
11. Wiener, A. S.: Hemolytic reactions following transfusions of blood of the homologous group. II. Further observations on the role of property Rh, particularly in cases without demonstrable isoantibodies. *Arch. Path. 32*, 227 (1941).
12. Wiener, A. S.: Distribution and heredity of variants of the Rh type. *Science 98*, 182 (1943).
13. Wiener, A. S.: The Rh series of allelic genes. *Science 100*, 595 (1944).
14. Wiener, A. S., Geiger, J., Gordon, E. B.: Mosaic nature of the $\mathbf{Rh_0}$ factor of human blood. *Exp. Med. Surg. 15*, 82 (1957).
15. Unger, L. J., Wiener, A. S., Weiner, L.: New antibody (anti-Rh^B) resulting from blood transfusion in an Rh-positive patient. *J. Amer. med. Assoc. 170*, 1380 (1959).

16. Unger, L. J., Wiener, A. S.: A "new" antibody, anti-**Rh**^{C}, resulting from isosensitization by pregnancy with special reference to the heredity of a new Rh-Hr agglutinogen $\mathfrak{Rh}_2^S$. *J. Lab. clin. Med. 54*, 835 (1959).
17. Sacks, M. S., Wiener, A. S., Jahn, E. F., Spurling, C. L., Unger, L. J.: Isosensitization to a new blood factor, **Rh**^{D}, with special reference to its clinical importance. *Ann. Intern. Med. 51*, 740 (1959).
18. Lederer, M.: A form of acute hemolytic anemia probably of infectious origin. *Amer. J. med. Sci. 170*, 500 (1925).
19. Polayes, S. H.: Erythroblastosis fetalis in mothers with Rh-positive blood. *Amer. J. dis. Child. 69*, 99 (1945).
20. Polayes, S. H., Lederer, M., Wiener, A. S.: Studies on isohemagglutination. II. The Landsteiner blood groups in mothers and infants. *J. Immunol. 17*, 545 (1929).
21. Wiener, A. S., Lederer, M., Polayes, S. H.: Studies in isohemagglutination. I. Theoretical considerations. *J. Immunol. 16*, 469 (1929).
22. Wiener, A. S., Lederer, M., Polayes, S. H.: A note on the paper, "Studies in isoagglutination." *J. Immunol. 17*, 357 (1929).
23. Landsteiner, K., Wiener, A. S.: On the presence of M agglutinogens in the blood of monkeys. *J. Immunol. 33*, 19 (1937).
24. Wiener, A. S.: Rh-Hr Blood Types. Grune & Stratton, New York 1954. See opening of Preface.
25. Buchbinder, L.: The blood grouping of *Macacus rhesus* including comparative studies of the antigeneic structure of the erythrocytes of man and *Macacus rhesus*. *J. Immunol. 25*, 33 (1933).
26. Personal communications at the New York City Department of Health on numerous occasions between 1951 and 1965 when Buchbinder died.
27. Wiener, A. S., Peters, H. R.: Hemolytic reactions following transfusions of blood of the homologous group with three cases in which same agglutinogen was responsible. *Ann. Intern. Med. 13*, 2306 (1940).
28. Levine, P., Stetson, R. E.: An unusual case of intra-group agglutination. *J. Amer. med. Assoc. 113*, 126 (1939).
29. Levine, P., Burnham, L., Katzin, E. M., Vogel, P.: The role of isoimmunization in the pathogenesis of erythroblastosis fetalis. *Amer. J. Obstet. Gynec. 42*, 925 (1941).
30. Fisher, R. A., cited by Race, R. R.: An "incomplete" antibody in human serum. *Nature 153*, 771 (1944).

Haematologia 11 (1—2), pp. 11—30 (1977)

Experimental and Clinical Investigations on Stem Cell Take and Colony Formation[1]

G. ASTALDI, G. P. BAGNARA,* M. A. BRUNELLI,*
D. KARANOVIC,** J. KARANOVIC,**
R. SCORZA,*** Ü. TOPUZ****

The Blood Research Foundation Centre,[2] Hospital of Tortona, Italy

(Received August 29, 1976)

First, lymphocyte transplantation into total body-irradiated rats is discussed. The effect on spleen colony formation caused by the transplantation of untreated lymphocytes, as well as of lymphocytes previously incubated with PHA, or with PHA plus L-asparaginase, or with lymphokines, was studied. Then the effect of the urinary colony-stimulating factor *in vitro*, and the *in vitro* feeder-layer activity of leucocytes on colony formation of human and mice bone marrow cells in haematological diseases is dealt with.

The injection of rat lymphocytes previously incubated for 24 hours with PHA resulted in a higher number and a larger size of colonies in the spleen of the recipient rats. Lymphocytes preincubated with lymphokines gave rise to the formation of spleen colonies which were larger than those developing after the injection of untreated lymphocytes. When the lymphocytes were previously incubated with PHA plus L-asparaginase, PHA failed to stimulate colony formation in the spleen. The phenomenon is explained by assuming that PHA, as an aspecific stimulator of cell division, initiated the division of CFUs, thus the CFUs content of the preincubated samples increased, resulting in an increase in the number of colonies formed after the transplantation of lymphocytes pretreated with PHA. Another possible explanation is that CFUs division, or their spleen take is enhanced by the immunocompetent lymphocytes activated by PHA, either directly or via soluble mediators produced or released by immunocompetent lymphocytes such as lymphokines.

The study of colony-forming cells and colony-stimulating activity in primary myelofibrosis (PM) showed an increase in the number of circulating CFU_c in this condition, and an abnormal density of these cells reaching a peak below 1.062. The lowering of CSA in the first two peripheral blood gradient fractions agrees with the observation in the same fractions of a high percentage of CFUc at the expense of the CSC population. Thus, double cell population seems to exist in PM. One is greatly abnormal with a low specific density and high plating efficiency, whereas the other population is almost normal, showing a higher specific density and a lower plating efficiency.

[1] Paper delivered by G. Astaldi at the 1976 Congress of the Hungarian Society of Haematology on the occasion of his nomination for Honorary Membership.

[2] Supported by "The Blood Research Foundation", Washington, D. C., USA.

* Institute of Histology, University Medical School, Bologna, Italy.
** Institute of Nuclear Sciences, Beograd, Yugoslavia.
*** Fourth Department of Medicine, University Medical School, Milano, Italy.
**** University Medical School, Ankara, Turkey. (Present address: The Blood Research Foundation Centre, Tortona, Italy.)

Introduction

Let me first express my sincere thanks to every single member of the Hungarian Society of Haematology, to the Council of this Society, to our President Professor Susan Hollán for whom I have warm friendship and deep respect, as well as to the other numerous friends that I have among the Hungarian colleagues for the honour conveyed on me by you, in appointing me as Member of this Society. I will always do my best in order to be a member fully aware of this position.

I shall now discuss some of the results of studies carried out on stem cell take and colony formation under different experimental conditions in animals, and in some clinical disorders. I want to give credit to my colleagues G. P. Bagnara, M. A. Brunelli, C. Carnevali, D. and J. Karanovic, P. Paleani Vettori, O. Piletic, C. Rizzoli, R. Scorza, Ü. Topuz and G. Torlontano who have carried out most of the work.

A. *As to our animal experiments*, I shall discuss the effect on colony formation of the undifferentiated, pluripotent, self-maintaining stem cells (CFUs) in the spleen of irradiated rats injected intravenously with

– a pool of peripheral blood lymphocytes preincubated *in vitro* with PHA;

– a similar lymphocyte pool, preincubated with PHA plus L-asparaginase: this enzyme inhibits lymphocyte transformation caused either by PHA [1, 2] or PWM [3], as well as in MLC [4, 5], but does not depress the growth of the stem cells [6]:

– a similar lymphocyte pool preincubated *in vitro* with lymphokines.

B. *Concerning clinical disorders*, we shall deal with the

– morphological features and colony-forming capacity in agar culture of stem cells (CFUc) from primary myelofibrosis;

– humoral control of agar culture granulopoiesis developed by colony-stimulating factor(s) (CSF) from primary myelofibrosis and from chronic granulocytic leukaemia.

Materials and Methods

A. *In the animal experiments*, Wistar rats 2½ months old of either sex were used as donors of lymphocytes for transplantation. Lymphocytes were obtained from peripheral blood drawn by heart puncture under ether anaesthesia. The blood was immediately defibrinated by glass beads and then sedimented for one hour with dextran. The lymphocytes remained in the supernatant. They were pooled and used for transplantation either without any preliminary treatment, or after incubation with PHA alone, PHA plus L-asparaginase, or lymphokines.

Before transplantation, lymphocytes were incubated at 37 °C for 24 hours, in Medium 199 supplemented with 20% of isogenic serum, 1% glutamine, 100

I.U./ml of penicillin and 100 μg/ml of streptomycin. Either PHA alone, or PHA plus L-asparaginase, as well as lymphokines were added at the onset of incubation. Their doses were: 0.03 ml PHA-M (Difco) per ml of medium, the same dose for lymphokines, and 50 I.U. asparaginase per ml medium. The final lymphocyte concentration was 2000 cells per ml medium.

Rats of the same strain, but 25-day old, of either sex, were used as recipients. Before irradiation, their spleen still showed signs of active haemopoiesis. Then they were exposed to total body X-ray irradiation of 600 r ($LD_{80}/30$ days). A Roentgen Siemens apparatus at 200 kV, 16 mA, with 0.5 mm Cu filter was used, and the dose rate was 92 r/min. Ten days after irradiation, the spleen of

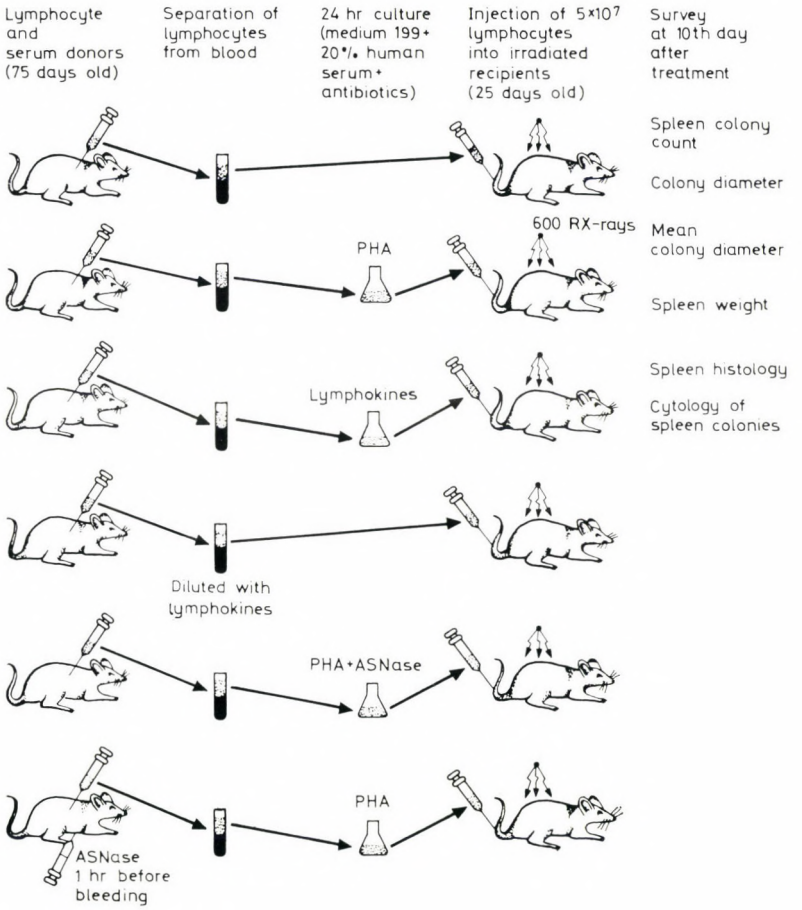


Fig. 1. Scheme of the different treatments applied to peripheral blood lymphocytes before their transplantation into total body-irradiated recipients and the parameters evaluated 10 days later

the above-mentioned control rats showed marked hypocellularity in both red and white pulp, no follicles, and no cell colonies.

Lymphocyte administration into irradiated rat recipients was made via their tail vein, and 5×10^7 lymphocytes per rat were administered. The recipients were divided in 8 lots. The first one did not receive any irradiation, and was kept as blank control. All the other rat lots, from the second one on, were irradiated. This second lot was just irradiated, and kept as irradiated control, whereas the third lot was injected with 5×10^7 untreated peripheral blood lymphocytes. The fourth lot was injected with 5×10^7 peripheral blood lymphocytes previously incubated with PHA-M for 24 hours, whereas the fifth lot was injected with the same amount of lymphocytes previously incubated with PHA plus L-asparaginase. The sixth lot was injected with a same amount of PHA-preincubated lymphocytes, but in this case the lymphocytes were drawn from donors who had previously been treated with L-asparaginase *in vivo* (Fig. 1). The seventh lot was injected with a pool of 5×10^7 peripheral blood lymphocytes preincubated with human lymphokines. Finally, the eighth lot received the same number of untreated lymphocytes together with 0.3 ml/5×10^7 cell/rat of lymphokines.

Lymphokines were prepared from human lymphocytes whose donors were 6 healthy volunteers of either sex, aged between 22 and 30 years. The procedure of lymphokine preparation was detailed elsewhere [7, 8].

All the animals were sacrificed 10 days after beginning the experiment, i.e. 10 days after the irradiation and transplantation procedures. After killing the rats, the spleen was immediately excised and weighed, the number of macroscopic surface colonies was counted and the size of each colony measured. Then, the spleens were either used to make smear and imprint preparations for cytologic examination, or were submitted to the usual procedures for histological observation, fixed in Bouin, cut into 5 μ sections and stained with haematoxylin and eosin. The smears were stained with May-Grünwald-Giemsa.

B. *In the clinical study*, first the colony-forming activity (CFA) and density distribution of the stem cell population (CFUc) in primary myelofibrosis were investigated; either unfractionated buffy-coat white cells, or cell fractions separated by discontinuous density gradient from human peripheral blood were used as overlayers in the agar culture model. Technical details have been described elsewhere [9]; in short, for the cultures the Pike and Robinson technique [10] was used and cell separation was obtained by the Dicke method [11].

A second series of experiments concerned the effect of the urine colony-stimulating factor (CSFu) obtained from two different myeloproliferative disorders, tested on the colony formation in agar cultures set up with stem cells (CFUc) obtained either from humans, or from mice. For details, see reference [9]; CSF was obtained from urines of patients with primary myelofibrosis or chronic granulocytic leukaemia and partially purified according to Stanley and Metcalf [12]. The CSFu concentration was 0.1 ml/dish, the optimum concentration shown by a dose-response curve.

Colony-stimulating activity (CSA) was tested by using the two-layer system

of Pike and Robinson [11]. The feeder-layer was prepared with blood cells, whereas the over-layer either by C57BL mouse bone marrow cells, or normal human ones. They were plated at a concentration of 2×10^5 cells/dish. CSA was evaluated by counting the number of colonies produced on the 8th day of incubation.

Results

A. *CFU take in experimental animals.* Data concerning spleen weight, the frequency of colonies and colony diameters are seen in Table 1.

Spleen weight. Total body irradiation with 600 r caused a 70% decrease when compared to the blank control rats.

Table 1

Spleen weight and frequency and size of colonies observed under different experimental conditions (Means±S.D.)

		Blank controls	Irradiated controls (600 R)	Lots of irradiated rats injected with lymphocytes cultured with:					
				Untreated	PHA	Lympho-kines	Untreated and diluted with lympho-kines	PHA and ASNase	PHA-treated cells from ASNase injected donors
No. of rats		20	20	22	26	11	13	9	9
Spleen weight (mg)(mean ± S.D.)		300.9 ±17.8	89.0 ±34.2	116.3 ±25.0	140.7 ±25.0	142.7 ±81.7	116.5 ±36.3	108.2 ±15.5	108.2 ±15.5
Colonies per spleen (mean ± S.D.)				5.2 ±2.3	11.2 ±2.5	2.5 ±1.4	3.5 ±2.1	4.3 ±1.9	5.2 ±1.4
Colony size distribution (~mm)	0.5	-	-	18(15%)	8(3%)		7(15%)	12(31%)	-
	1	-	-	26(22%)	41(14%)	5(18%)	17(36%)	19(50%)	30(64%)
	2	-	-	64(54%)	156(54%)	5(18%)	10(21%)	6(16%)	17(36%)
	3	-	-	6(5%)	47(16%)	4(14%)	5(11%)	1(3%)	-
	4	-	-	4(4%)	39(13%)	14(50%)	8(17%)	-	-
Colony diameter average (mm)				1.67	2.25	2.96	1.86	10.5	1.36

The loss in spleen weight was partially protected by the lymphocyte injection into the irradiated animals; spleen weight was about 20% higher in the rats irradiated and injected with untreated lymphocytes than in the irradiated controls. The protection was still more marked when lymphocytes were incubated with PHA prior to their administration into the irradiated recipients. Indeed, the spleen of the rats injected with PHA-stimulated lymphocytes was about 58% heavier

than that of the irradiated controls, and about 21% heavier than the spleens from rats injected with unstimulated lymphocytes.

The spleen weight of the fifth and sixth lots were similar to the spleen weight of the lot injected with unstimulated lymphocytes. Thus, spleen weight was not increased more by PHA lymphocyte culture when the lymphocytes were treated also with L-asparaginase *in vitro* (fifth lot) or *in vivo* (sixth lot).

Concerning the lymphokine effect, the spleen of irradiated rats injected with a pool of lymphocytes stimulated with the soluble mediators (seventh lot) was 60% heavier than the spleen of the irradiated controls, and 23% heavier than in the rats irradiated and injected with untreated lymphocytes.

Spleen colony frequency. No evidence of the presence of typical macroscopic surface colonies was noticed in either blank or irradiated controls (Table 1). In contrast, spleen colonies were present in all the rats irradiated and injected with the different pools of peripheral blood lymphocytes. Nevertheless, marked differences were noticed in colony frequency, according to the different experimental conditions, depending on whether the irradiated recipients were injected with untreated lymphocytes, or with PHA-stimulated lymphocytes, or with lymphocytes treated with PHA and L-asparaginase, or with lymphokines.

In detail, the irradiated recipients injected with untreated lymphocytes showed an average colony frequency of 5.2 per spleen, whereas the corresponding figure was 11.2 for the recipients of PHA-stimulated lymphocytes. On the other hand, L-asparaginase prevented the potentiating effect of PHA stimulation. Mean spleen colony frequency was 4.3 when PHA was supplemented with L-asparaginase in the lymphocyte cultures and 5.2 when the PHA cultures were set up with lymphocytes drawn from donors one hour previously injected with L-asparaginase. Concerning the rat lots injected with lymphocytes pretreated with lymphokines, the spleen colony frequency was 2.5 when the irradiated rats were injected with lymphocytes preincubated with lymphokines and 3.5 when lymphocytes diluted with lymphokines were administered. This particular behaviour will be discussed later on.

Spleen colony size. These data are recorded in Table 1. The spleen colonies of the irradiated rats injected with untreated lymphocytes were small or medium sized; 37% were under 1 mm, and 87% under 2 mm in diameter. In contrast, the colony size was higher in the spleens of the irradiated rats injected with PHA-stimulated lymphocytes. Mean colony diameter was 1.7 mm in the recipients of untreated lymphocytes, and 2.3 in the recipients of PHA-activated lymphocytes.

L-asparaginase prevented the effect of PHA-stimulation. In fact, no increase in the size of colonies was observed either when the enzyme was added directly to the PHA culture medium, or when it was injected into donors whose lymphocytes were cultivated in the presence of PHA; mean size was 1.05 in the former experiment, and 1.36 in the latter one.

Finally, mean colony diameter was 2.9 mm in the spleen of recipients of lymphokine-stimulated lymphocytes, the highest figure in the different experi-



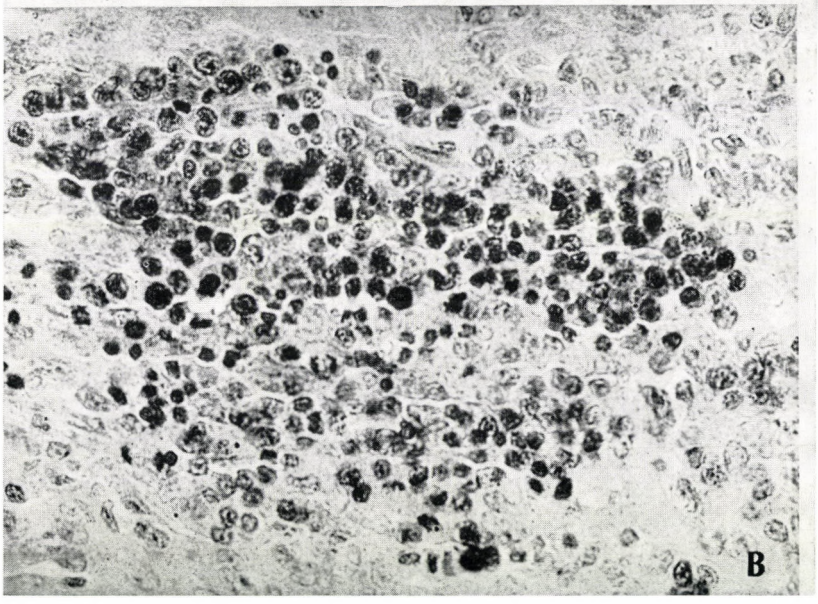


Fig. 2A. Spleen section from an irradiated control showing marked hypocellularity and no colonies

Fig. 2B. Spleen section from a rat injected with untreated lymphocytes: mixed colony made by either undifferentiated or erythroid cells

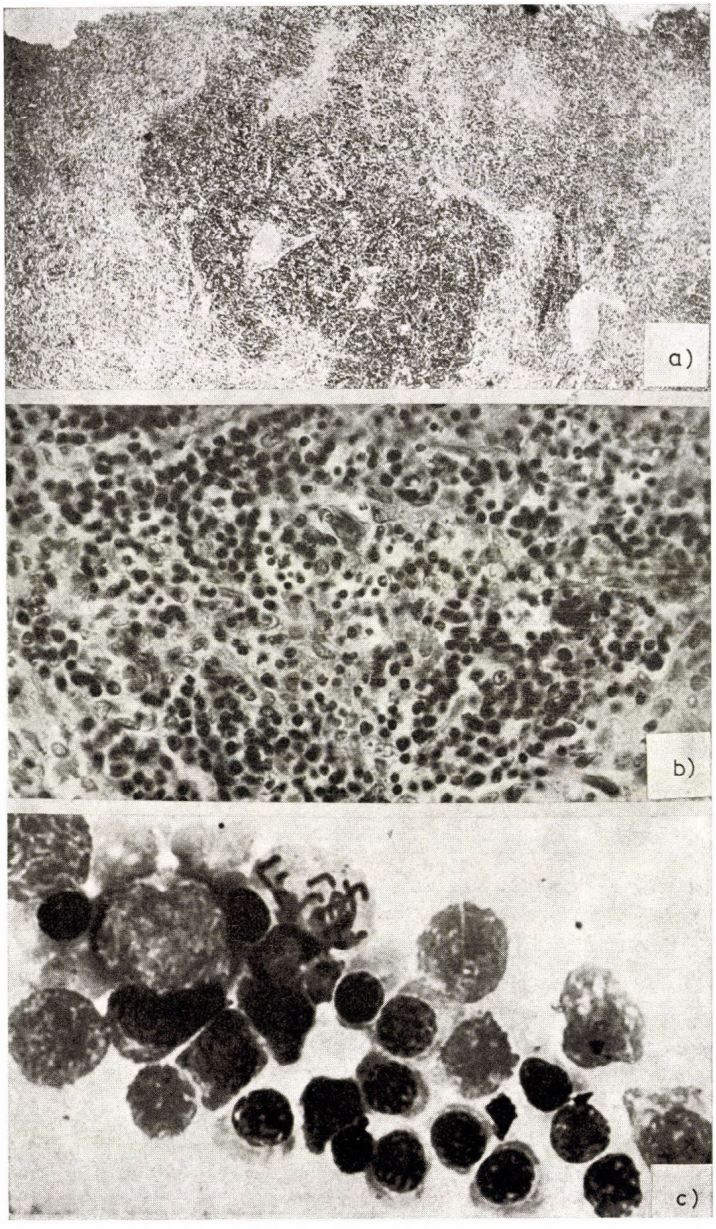


Fig. 3. Spleen section and imprint preparation from a rat injected with PHA-preincubated lymphocytes. Large erythroid colony with erythroblasts in different stages of maturation, one in mitosis

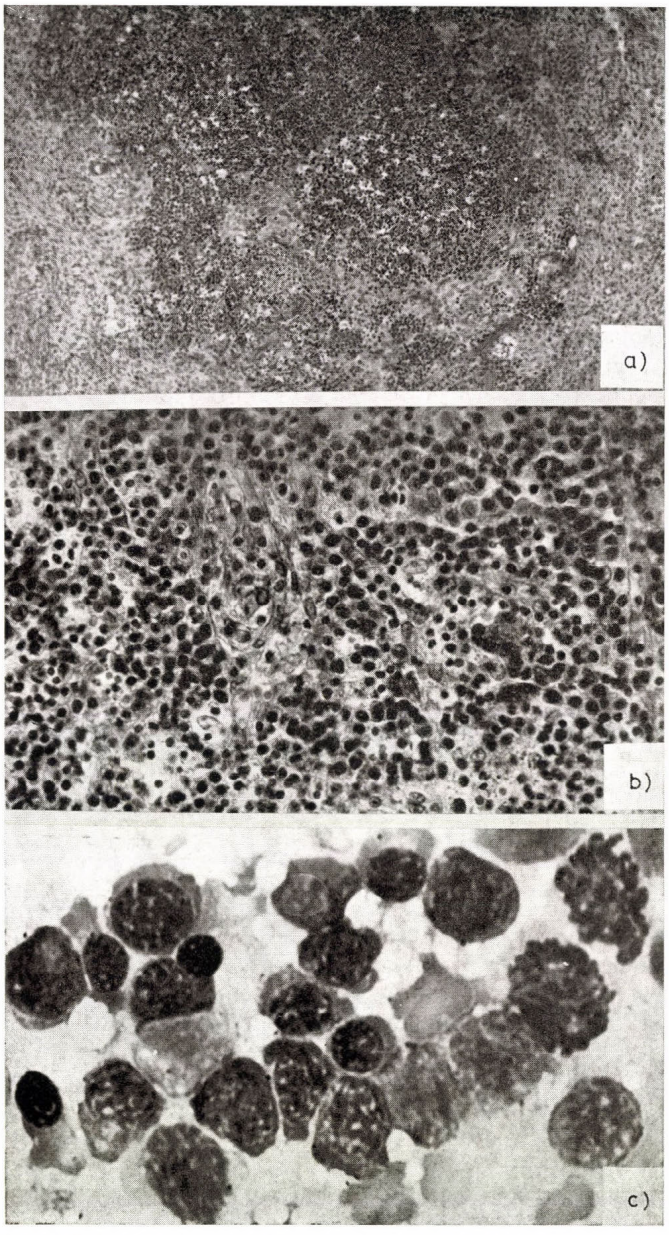


Fig. 4. Spleen sections and imprint preparation from a rat injected with lymphokine-pretreated lymphocytes; giant erythroid colony

ments. These large colonies originated from the coalescence of two or more colonies; this might explain the lower colony frequency observed in this particular experiment.

Spleen colony cytology. This study showed that the above-mentioned protection of the spleen weight loss caused by the administration of peripheral lymphocytes into the irradiated recipients, was accompanied by spleen repopulation, and either undifferentiated cells or erythroblasts were present. In fact, the spleens of the irradiated controls showed marked hypocellularity with poor spontaneous cell regeneration (Fig. 2A), whereas the spleens from the recipients of untreated lymphocytes showed small or medium size colonies, either undifferentiated, or mixed colonies (made by both undifferentiated and erythroid cells) (Fig. 2B), whereas the erythroid colonies were much fewer. Randomly dispersed small foci of erythroid cells could also be seen. In contrast, no granulocytic or megakaryocytic colonies were detected.

Within the spleen sections of the irradiated rats injected either with PHA-treated (Fig. 3), or with lymphokine-treated lymphocytes (Fig. 4), the colonies were more numerous and larger than in the previous lot, sometimes showing a tendency to merge with each other. Most of these colonies were mixed cell type or erythroid (Figs 3b, 4b), with densely concentrated erythroblasts in different maturation stage and sometimes also in mitosis, which was quite clearly seen in the imprint preparations (Figs 3c, 4c). Again, numerous dispersed erythroid

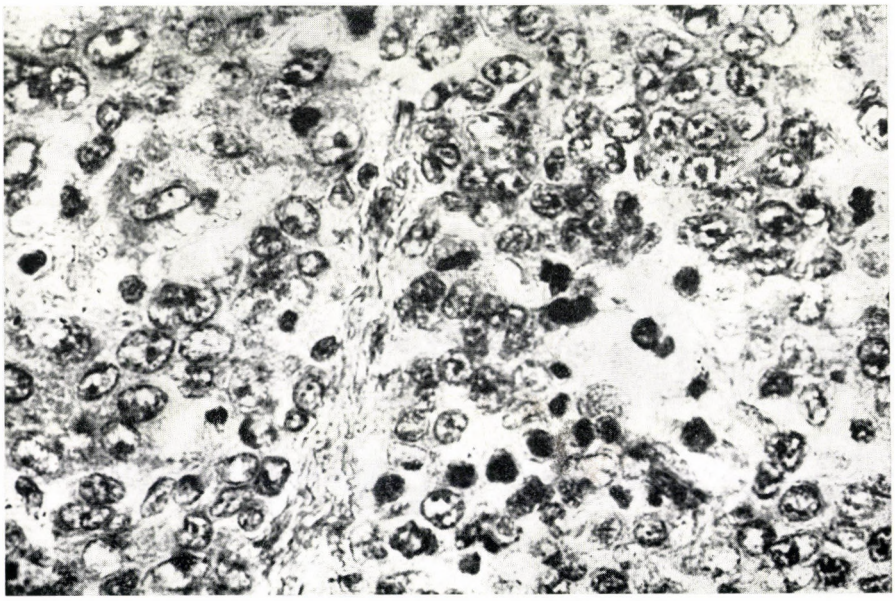


Fig. 5. Undifferentiated cell colony showing many mitotic figures in the spleen of a rat injected with PHA-preincubated lymphocytes

clusters as well as diffuse erythroid regeneration combined with repopulation of undifferentiated cells could be observed. Besides erythroid and mixed cell colonies, also undifferentiated ones were present (Fig. 5), though less in number than the erythroid ones. These undifferentiated cell colonies were larger in size than those in the lot of rats injected with untreated lymphocytes. Like the erythroblasts, the undifferentiated cells showed several mitotic figures. Granulocytic or megakaryocytic colonies were not seen in this experiment, either.

Apparently, L-asparaginase inhibited not only the enhancement of spleen colony frequency and size, but also the enhancement of cell repopulation (both

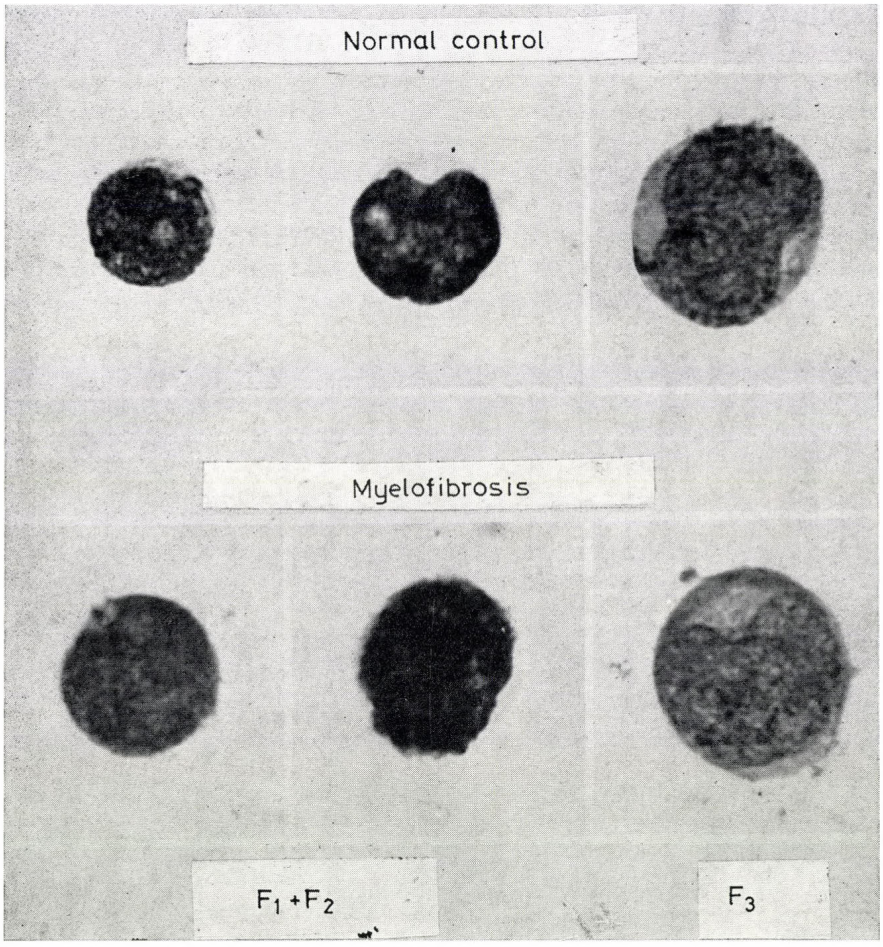


Fig. 6. Myelofibrosis blasts in fractions 1 + 2 show morphological features different from those shown by the control blasts. In contrast, control and the myelofibrosis blasts were similar in morphological features in fraction 3

undifferentiated and erythroid cells) caused by stimulation by PHA of the transplanted lymphocytes.

B. *CFUc colonies in myeloproliferative disorders.* In primary myelofibrosis (PM), peripheral blood white cell gradient fractionation showed blasts in fractions 1 to 3, with the highest frequency in fraction 3, whereas in the control free blasts were detected and only in fraction 3. Again it appeared that in fraction 1 + 2, the agranulated blasts from PM had different morphological features in comparison to the agranulated control blasts from normal bone marrow (Fig. 6). In contrast, in fraction 3 no morphological differences could be detected between the granulated blasts from PM and those from the control.

The above-mentioned different features were accompanied by biological differences (Figs 7, 8). Three out of 4 PM patients showed a much higher growth of colonies in fraction 1 + 2, and a progressive decrease in colony and cluster frequency in the following cell fractions, despite that in these patients too the highest blast frequency was in fraction 3. In contrast, the control (and the PM patient No. 1) showed the highest colony-forming activity in fraction 3.

The high colony growth of the first two fractions could be a consequence of the elevated proportion of "colony stimulating cells" in the same fractions. The percentual proportion of monocytes and PMN, together with the colony stimulating activity (CSA) in the different cell fractions has shown (Fig. 9) that:

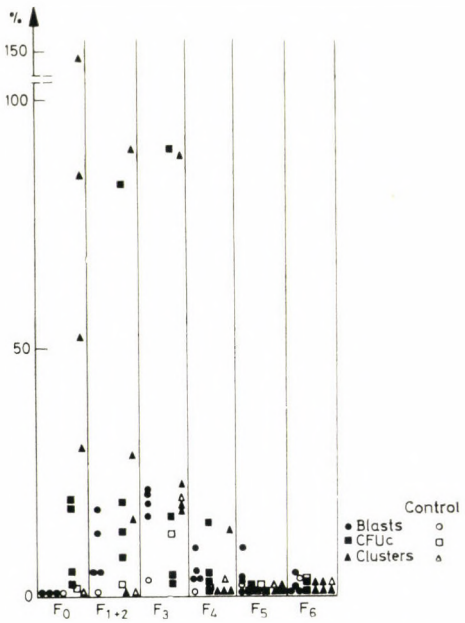


Fig. 7. Cluster and colony (CFUc) frequency caused by the different cell fractions from 4 patients with myelofibrosis and a control subject. The blast frequencies observed in the above cell fractions are also shown. Open circles: control. Black circles: patients with myelofibrosis

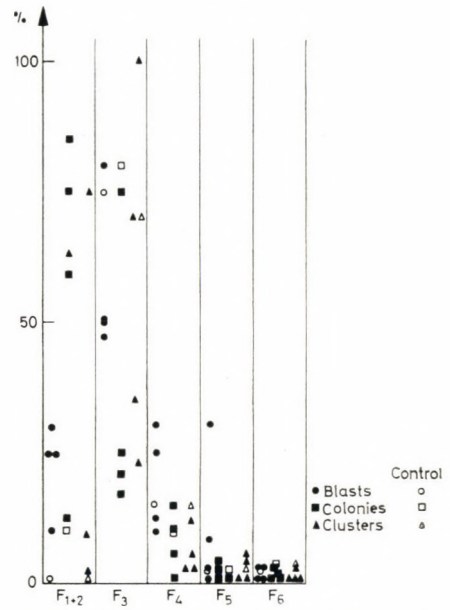


Fig. 8. Frequency of blasts and of clusters and colonies in the different cell fractions from the 4 patients with myelofibrosis and from the control subject. Open circles: control. Black circles: patients with myelofibrosis

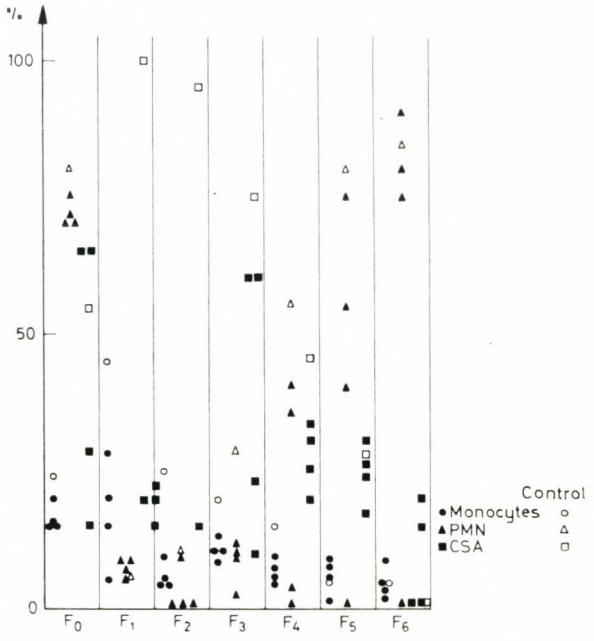


Fig. 9. Proportion of polynuclears and monocytes in the different cell fractions from the 4 patients with myelofibrosis and the control subject. Open circles: control. Black circles: patients with myelofibrosis

(a) in the 1st fraction, the monocyte proportion was higher in the control and in 2 out of 4 PM patients (in particular Nos 2 and 4) than in PM patients Nos 1 and 3;

(b) in the fractions from 4 to 6, the polynuclear proportion was progressively predominant over the control and the 4 PM patients;

(c) the colony stimulating activity of the first two fractions appears to be low compared to normal control values.

The number of colonies grown in both human and mouse bone marrow cultures supplemented with urine CSF (CSFu) obtained from chronic granulocytic leukaemia (CGL) and PM, are seen in Table 2.

It appears that urine CSA was much higher when tested in mouse marrow cultures than in human ones. This was true for both CGL and PM as well as for the control normal subjects, but this behaviour was the most marked in CGL. Indeed, the CSFu from 2 out of 3 CGL patients produced very few colonies in the human marrow cultures as compared with the corresponding controls or with those in MP. Nevertheless, as already mentioned, a reduced effect of the CSFu when passing from mouse to human marrow cultures was also evident in PM, though to a lesser extent. The CSFu from PM patient No. 4 stimulated formation of more human colonies than mouse colonies. It has to be noted that (a) this particular MP patient then progressed to acute granulocytic leukaemia, and (b) a similar behaviour was observed in 3 patients with blastic crisis of CGL; indeed, the CSFu from these latter 3 patients has almost no effect on mouse bone marrow cultures, whereas it produced a fairly high number of colonies in human marrow cultures.

Besides CSFu, the colony-stimulating activity of white blood cells (WBC-CSA) from all the above patients was tested on colony marrow culture growth. The results are summarized in Fig. 10. In PM, the WBC-CSA had a weaker effect than the CSA shown by WBC from normal subjects when tested either in mouse or human marrow cultures. (Due to the low number of patients tested, the mean values are not to be considered significant, only orientative.)

As it appears from Fig. 10, the WBC-CSA from CGL was tested only on mouse marrow cells, but as feeder-layers WBC either from peripheral blood, or from spleen were used. In general, the results show that spleen WBC stimulate formation of less colonies than do the peripheral blood WBC, though of more than the colony number given by the spleen WBC from the control donors. (In this particular experiment, as controls we used spleens removed for staging of patients with Hodgkin disease, who appeared to be in stage 1.) On the other hand, when comparing the spleen WBC from CGL to the WBC from the control spleens, it appeared that the former caused a higher colony frequency in mouse marrow cultures than did the latter.

The above-mentioned behaviour is in agreement with a previous observation of ours [13]. Indeed, in untreated CGL patients splenectomized at the onset of the disease, the number of colonies produced by the spleen cells was higher than that produced by the marrow cells, and roughly similar to the colony number

Table 2

Effect of urine-CSF (0.1 ml/dish) from chronic granulocytic leukaemia or from primary myelofibrosis on both human and mouse marrow colony frequency

Source of urine-CSF	Human bone marrow (2×10^5 cells/dish)	C57BL bone marrow (2×10^5 cells/dish)
Chronic granulocytic leukaemia	Number of colonies	
Patient No.		
1	—	94
2	—	160
3	5	125
4	8	244
5	32	260
6	—	310
7	—	180
Myelofibrosis		
Patient No.		
8	19	65
9	25	31
10	14	36
11	24	4
12	15	89
Normal subjects (Controls) Nos 13, 14, 15, 16, 17, 18	Mean±SD 37±3	144±11

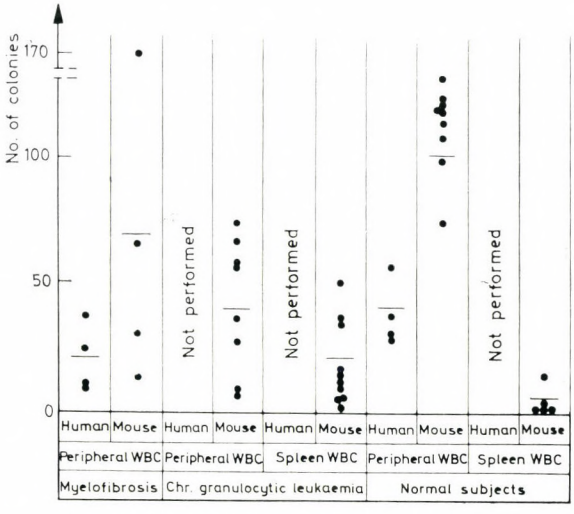


Fig. 10. WBC colony-stimulating activity (over-layer 2×10^5 cells; feeder-layer 1×10^5 cells)

produced by the blood cells. Though producing a large number of colonies, spleen cells in 3 out of 5 cases showed a growth curve definitely similar to that given either by bone marrow or peripheral blood white cells in the preleukaemic syndrome; in other words, a cluster recruitment, as well as a large number of blastic recruited clusters. Indeed, this behaviour may be dependent on the above-mentioned lower stimulating activity of the spleen cells as compared to the corresponding normal or CGL peripheral blood cells.

Discussion and Conclusions

Our observations on the take of CFUs showed that the administration of 5×10^7 peripheral blood lymphocytes into 25-day old Wistar rats previously subjected to total body irradiation with 600 r, causes the development of an average of 5.2 subcapsular macrocolonies with an average diameter of 1.67 mm/colony. Most of these colonies are made up by undifferentiated cells, few of them by mixed (undifferentiated and erythroid) cells and still fewer by erythroid cells only. In contrast, no granulocytic or megakaryocytic colonies are detectable. Spleen histology showed that there are also small erythroid foci (microcolonies or clusters), or erythroid cells intermingled with undifferentiated cells randomly dispersed within the spleen.

Intravenous administration into similarly irradiated rats of a same amount of peripheral lymphocytes stimulated with PHA for 24 hours in cell culture, markedly increased the number and size of subcapsular macrocolonies, their mean frequency being 11.2 per spleen, with mean diameter of 2.25 mm per colony. Besides, the cell composition of those spleen colonies grown in the rats injected with lymphocytes preincubated with PHA was more erythroid, the erythroid and mixed cell type colonies being more frequent than the undifferentiated cell type colonies. The dispersed erythroid microcolonies or cell clusters were also more numerous and diffuse cell regeneration was more intense.

Several interpretations could be discussed to explain the PHA stimulation of erythroid colony formation, but basically all of them may be referred to two main possibilities, namely: PHA acts directly on the processes of spleen colony formation and growth, or the mitogen acts indirectly via an activation of the immunocompetent lymphocytes.

The first explanation of the phenomenon is that PHA, as a non-specific stimulator of cell division, initiates the division of CFUs, thus the CFUs content of the preincubated samples increased resulting in an increase in the number of colonies formed and in the weight of the spleen after transplantation of lymphocytes pretreated with PHA. Indeed, among the injected lymphocytes a small number of stem cells may occur (in mice: 5 CFUs/10^6 leucocytes) on which the number of the formed colonies depend.

In the literature there is some evidence that PHA may either stimulate spleen haemopoiesis in normal mice [14, 15], or accelerate haemopoietic regener-

ation in irradiated mice [16, 17], as well as increase mitotic activity in the bone marrow erythroid cells [18]. Again, in transplantation experiments an increased frequency of spleen colonies was shown when the animal recipients of thymic cells were treated with PHA [19], or when donors of lymph node cells were subjected to PHA pretreatment [20]. It was also observed that normal human peripheral white blood cells plated in agar culture generate colonies of blastoid cells when stimulated *in vitro* by PHA [21, 22, 23]. It was even observed that PHA may enhance the growth of tumour cells in mice [24].

Another possible explanation is that CFUs division, or their spleen take is enhanced by the immunocompetent lymphocytes activated by PHA. Indeed, it is known from the literature that a T-subpopulation not exactly identified promotes the cell take of transplanted stem cells, although the lymphocytes themselves do not form spleen colonies. Again, from our experiment it appeared that L-asparaginase, that inhibits the PHA activation of the immunocompetent lymphocytes, definitely counterbalanced the PHA enhancement of the spleen colony formation and cell repopulation [25, 26]. The activated immunocompetent lymphocytes could possibly act via their production or release of soluble mediators such as lymphokines, and thus display a helper function for a better CFUs take and/or growth, as well as for a more intense stem cell differentiation of the erythroid unipotent progenitor. There are data which seem to support the interpretation that immunological cells or factors may regulate the proliferation and spleen take of stem cells injected into lethally irradiated recipients [27, 28]. The possibility that PHA itself or PHA products may act as stimulating factor(s) on colony growth in agar culture was also discussed in papers on the control and regulation of lympho- and granulopoiesis [29, 30]. The importance of immunological processes on CFUs proliferation and differentiation is also shown by the facts that (a) thymectomy causes depletion of CFUs growth [31], and (b) the extent of spleen colonies growing in sublethally irradiated mice after injection of marrow or spleen cells damaged by radiation is enhanced when thymus cells are injected simultaneously or one to two days after the injection of the above-mentioned damaged haemopoietic stem cells [32].

Our study has shown that the weight of the spleen is noticeably higher in rats irradiated and injected with 5×10^7 peripheral blood lymphocytes preincubated with lymphokines for 24 hours than in rats irradiated and injected with the same amount of untreated lymphocytes. Then, the spleen colony size is definitely higher in the former than in the latter rats (2.96 versus 1.67 mm). Again, the colonies grown in the spleen of rats injected with lymphocytes preincubated with lymphokines are mostly of the erythroid, or at least of the mixed cell type (erythroid and undifferentiated cells). On the contrary, the spleen colonies grown in rats injected just with untreated lymphocytes are mostly of the undifferentiated type.

The above-mentioned data, taken as a whole, substantiate the interpretation that besides a possible direct stimulating effect of the PHA itself, humoral mediators produced by the PHA activation of immunocompetent lymphocytes may

stimulate either stem cell growth, or erythroid differentiation and maturation in the spleen colonies. This in turn supports the concept that immunocompetent lymphocytes activated by PHA, and/or the factors produced via this activation, may help in the still complicated and unknown intimate processes relating to stem cell take and haemic differentiation. Again, as we tested human lymphokines on rat cells, it seems to suggest that such humoral mediators are able to act across the species barriers.

The present results seem to allow the conclusion that an increase in the number of circulating CFc does exist in PM, in agreement with previous data [33]. In most patients, the CFc behave abnormally as far as specific density is concerned, peaking in a density range below 1.062, as reported by others [34]. Finally, lowering of the colony-stimulating activity (CSA) in the first two peripheral blood gradient fractions agrees with the observation in the same fractions of a high percentage of CFUc at the expense of the CSC population.

Summing up, in PM there seems to be a double cell population. One shows low specific density and high plating efficiency, whereas the other one is more normal, showing higher specific density and lower plating efficiency. It is nevertheless questionable whether the blood CFUc may be regarded as really representative of the whole CFUc population, whether a complete exchange exists among the several stem cell pools of the organism. In any case, the above-mentioned interpretation is supported by the observation that in CGL the highest amounts of blood or marrow CFUc are present in the first (low) density fractions, just as in PM.

References

1. Astaldi, G., Burgio, G. R., Krč, I., Genova, R., Astaldi, A. Jr.: L-asparaginase and blastogenesis. *Lancet 1*, 423 (1969).
2. Astaldi, A. Jr., Burgio, G. R., Krč, I., Genova, R., Astaldi, G.: Inhibition of the PHA response by L-asparaginase. *Haematologia 3*, 395 (1969).
3. Krč, I., Micu, D., Astaldi, A. Jr., Krčova, V., Biscatti, G.: Effetto inibente della L-asparaginasi sulla blastogenesi da "Phytolacca Americana". *Boll. Ist. sieroter. milan. 58, 48* (1969).
4. Astaldi, G., Micu, D., Astaldi, A. Jr., Burgio, G. R.: Inibizione da L-asparaginasi della reazione linfocitaria in coltura mista. *Tumori 56*, 47 (1970).
5. Astaldi, G., Micu, D., Astaldi, A. Jr., Burgio, G. R., Krč, I.: Further investigation on L-asparaginase and immune reactions. In: Symposium International sur la L-asparaginase. Coll. int. Centre nat. Rech. sci. N° 197. Eds: J. Bernard, M. Boiron, Cl. Jacquillat, M. Weil, D. Levy. Paris 1971, p. 189.
6. Alexander, P., Delorme, E. J., Currie, G. A., Sime, G. C.: Inhibition of immunoblast response *in vivo* and *in vitro* by asparaginase from E. coli and agauti serum. In: Symposium International sur la L-asparaginase. Coll. int. Centre nat. Rech. sci., N° 197. Eds: J. Bernard, M. Boiron, Cl. Jacquillat, M. Weil, D. Levy. Paris 1971, p. 189.
7. Scorza, R., Topuz, Ü., Karanovic, D., Karanovic, J., Astaldi, G.: Frequenza di colonie spleniche nel ratto panirradiato e trasfuso con linfociti precoltivati *in vitro* in presenza di linfochine. In: Atti del 2° Incontro Nazionale di Ematologia Sperimentale. Eds: M. A. Brunelli, G. P. Bagnara, C. Castaldini. 1976, p. 229.
8. Astaldi, G., Karanovic, D., Scorza, R., Karanovic, J., Topuz, Ü.: Administration of

peripheral blood rat lymphocytes stimulated *in vitro* with human lymphokines and spleen colonies in the total body irradiated rat. *Boll. Ist. sieroter. milan. 55*, 386 (1976).

9. Bagnara, G. P., Baccarani, M., Brunelli, M. A., Castaldini, C., Marani, M., Valvassori, L., Santucci, M. A., Zaccaria, A., Tura, S., Rizzoli, C.: Crescita in agar delle cellule ematiche, midollari e spleniche della leucemia mieloide cronica (LMC) separate in gradiente di densità discontinuo. Lo studio *in vitro* delle cellule staminali. In: Atti del 2° Incontro Nazionale di Ematologia Sperimentale. Eds: M. A. Brunelli, G. P. Bagnara, C. Castaldini. 1976, p. 33.
10. Pike, B. L., Robinson, W. A.: Human bone marrow colony growth in agar gel. *J. Cell Physiol. 76*, 77 (1970).
11. Dicke, K. A.: Bone marrow transplant after separation by discontinuous albumin density gradient centrifugation. Thesis, Leiden 1971.
12. Stanley, E. R., Metcalf, D.: Purification and properties of human urine colony stimulating factor (CSF). In: Cell Differentiation. Eds: R. Harris, D. Viza. Munksgaard, Copenhagen 1972, p. 149.
13. Bagnara, G. P., Brunelli, M. A., Castaldini, C., Valvassori, L., Folicaldi, S., Zaccaria, A., Tura, S., Marini, M., Santucci, M. A.: La crescita in agar della leucemia mieloide cronica (LMC). In: Atti del 1° Incontro Nazionale di Ematologia Sperimentale. Eds: M. A. Brunelli, G. P. Bagnara, C. Castaldini. 1975, p. 41.
14. Lozzio, B. B., Machado, E. A., Cherhoff, A. I.: Functional and cellular alterations produced by phytohaemagglutinin. III. Haematologic finding in mice receiving phytohaemagglutinin. *Acta haemat. (Basel) 41*, 349 (1969).
15. Antal, S., Fehér, J., Gallyas, A.: Effect of phytohaemagglutinin in subtoxic doses on haematopoiesis in mice. I. Effect on normal mice. *Haematologia 7*, 319 (1973).
16. Antal, S., Fehér, J.: The effect of PHA on the hematological regeneration in mice irradiated with 350 R. Abstr. 8th Ann. Meeting Europ. Soc. Radiat. Res. Basko Polje 1971, p. 5.
17. Fehér, J., Antal, S.: Effect of phytohemagglutinin on CFU regeneration in mice. Abstr. 8th Ann. Meeting Europ. Soc. Radiat. Res. Basko Polje 1971, p. 40.
18. Morris, W. T.: *In vivo* effect of phytohaemagglutinin on mouse haemopoietic tissue. *Acta haemat. (Basel) 49*, 57 (1973).
19. Cooper, E. H., Barkman, P., Hale, A. J.: Observations on the proliferation of human leukocytes cultured with phytohaemagglutinin. *Brit. J. Haemat. 9*, 101 (1963).
20. Lozzio, B. B., Lopez, A.: Capacity of lymph node cells to form spleen colonies after *in vivo* stimulation with phytohaemagglutinin (PHA). Abstr. I. Meeting Europ. Div. Intern. Soc. Hematol., Milano 1971, p. 189.
21. Louwagie, A. C.: Increase in bone marrow CFU-s and CFU-c in CH mice after *in vivo* PHA administration. Abstr. Fourth Annual Conference Internat. Soc. Exper. Hematol., Trogir, Yugoslavia 1975, p. 4.
22. Rozenszajn, L. A., Shoham, D., Kalechman, I.: Clonal proliferation of PHA-stimulated human lymphocytes in soft agar culture. *Immunology 29*, 1041 (1975).
23. Riou, N., Boizard, G., Alcalay, D., Goube de Laforest, P., Tanzer, J.: *In vitro* growth of colonies from human peripheral blood lymphocytes stimulated by phytohaemagglutinin. *Ann. Immunol. 127*, 83 (1976).
24. Pappas, A., Schwarze, F., Wolff, C., Scheurlen, P. G.: Enhanced tumour proliferation in mice treated with phytohaemagglutinin. *Z. Immun.-Forsch. 145*, 449 (1973).
25. Astaldi, G., Karanovic, J., Karanovic, D.: L-asparaginase (Asn-ase) in ana hücre (stem cell) yapimi ve immunolojik keaksiyonlar uzerine etkisi. Cocuk Sagligi ve Hastaliklari dergisi *17*, 25 (1974).
26. Astaldi, G., Karanovic, D., Karanovic, J., Paleani Vettori, P., Piletic, O., Torlontano, G.: Immune competent lymphocytes and colony formation. *Wadley med. Bull. 5*, 88 (1975).

27. Vitale, B., Jurin, M., Plavljanic, D., Burek, V.: Immunological factors regulating the proliferation of bone marrow injected into lethally irradiated recipients. Exper. Hematol., *1*, 286 (1973).
28. Cerny, J.: Stimulation of bone marrow haemopoietic stem cells by a factor from activated T cells. *Nature (Lond.) 249*, 63 (1974).
29. Van Bekkum, D. W.: Pathways and control of lymphocytopoiesis. In: Proc. Internat. Conference: Leukemia and Aplastic Anemia. Naples, 1974. Eds: D. Metcalf, M., Condorelli, C. Peschle. Il Pensiero Scientifico, Roma 1976, p. 31.
30. Metcalf, D.: Some specifications on the regulation of normal and leukemic granulopoietic cells by colony stimulating factor. In: Proc. Internat. Conference: Leukemia and Aplastic Anemia. Naples, 1974. Eds: D. Metcalf, M. Condorelli, C. Peschle. Il Pensiero Scientifico, Roma 1976, p. 141.
31. Frindel, E., Croizat, H.: The possible role of the thymus in CFU proliferation and differentiation. *Biomedicine 19*, 392 (1973).
32. Lord, B., Schofield, R.: The influence of thymus cells in hemopoiesis: Stimulation of hemopoietic stem cells in a syngeneic, *in vivo*, situation. *Blood 42*, 395 (1973).
33. Chervenich, P. A.: Increase in circulating stem cells in patients with myelofibrosis. *Blood 41*, 67 (1973).
34. Moore, M. A. S.: *In vitro* studies in the myeloid leukaemia. In: Advances in Acute Leukaemias. North-Holland Publishing Company, Amsterdam 1974, p. 161.

Correspondence: Prof. G. Astaldi, The Blood Research Foundation Center, Hospital of Tortona, 15057 Tortona, Italy.

Haematologia 11 (1—2), pp. 31—40 (1977)

The Treatment of Infections in Granulocytopenic Patients with Granulocyte Transfusions*

Anna MÓD, Vera HARSÁNYI, Mária JAKSCHITZ,
Susan R. HOLLÁN

National Institute of Haematology and Blood Transfusion, Budapest, Hungary

(Received April 12, 1977)

Twenty-three severely neutropenic patients were given 31 courses of AB0 and HL-A compatible granulocyte-rich plasma transfusions between 1974—76 at the National Institute of Haematology and Blood Transfusion. The criteria for the effecr tiveness of therapy were disappearance of fever and survival for over 3 weeks aftegranulocyte transfusion. Of the 28 evaluated cases granulocyte transfusions were effective in 16 and ineffective in 12 cases. The effectiveness of granulocyte transfusion was favourably influenced by early introduction of therapy, and continuation for several days.

Since the introduction of intensive chemotherapy and radiotherapy the prognosis of malignant tumours and haematological diseases has changed fundamentally. Partial remissions and in many cases recovery may be attained. However, the majority of chemotherapeutics used today is myelotoxic and often bone marrow aplasia develops, which makes further cytostatic treatment of the underlying disease extremely difficult. Anaemia and haemorrhage due to thrombocytopenia can be adequately controlled with red blood cell and thrombocyte replacement. At the same time, however, the prevention and treatment of infections remain unsolved. At present, the primary cause of mortality in malignant haematological diseases treated with combinations of cytostatic drugs is infection [19, 22, 25]. The cause of defencelessness in the organism is due to bone marrow aplasia induced by the underlying illness, chemotherapy and immunosuppression [2, 4, 10, 19, 31].

The introduction of protective sterile or semisterile environments, the prophylactic administration of oral, non-absorbable antibiotics all serve the prevention of infection [6, 24, 37]. These approaches reduce the number of severe infections but remain ineffective if applied after the onset of the infectious episode. The mortality rate of patients with granulocytopenia due to infection has been somewhat reduced by using combinations of broad-spectrum antibiotics which affect gram-negative pathogenic bacteria [3, 5, 13, 28]. However, with the exception of carbenicilline the effect of these antibiotics is poor in patients with severe

* Dedicated to Prof. Dr. H. Begemann in honour of his 60th birthday.

granulocytopenia [8, 28]. Since granulocytopenia represents the major cause of susceptibility to infections, the introduction of granulocyte transfusions may mean a new outlook in the therapy of these malignant diseases.

The main problem related to granulocyte transfusions is the preparation of granulocyte concentrates containing a sufficient quantity of cells with intact function. In 1961 Freireich transfused white blood cells (WBC) obtained from chronic myeloid leukaemic (CML) patients into granulocytopenic children with infections. In 49% the transfusions were followed by a decline in fever [30]. The advantage of CML cell transfusion is that a very high number of WBC can be obtained from one single donor. The disadvantages are the frequently arising serious side-effects; several authors reported the development of graft-versus-host (GVH) reactions [16, 22, 26]. In 1964 Yankee prepared a granulocyte concentrate from the blood of 15–25 donors, the therapeutic effectivity of which was similar to that of the CML cell transfusion applied by Freireich et al. [36]. In 1965 Freireich, Yudson and Levine were the first to report the preparation of a granulocyte concentrate in a continuous flow cell separator (CFC) [14]. The leucocyte content of this concentrate was $2-6\times10^9$. The CFC enables isolated leukapheresis, which means that only the leukocytes are removed from the donor's blood, all other components are continuously reinfused to the donor. An injection of corticosteroid (50 mg prednisolone or 4 mg/m^2 dexamethasone) and etiocholanolone (4 mg/m^2) given to the donor before cell separation results in a threefold increase of the granulocyte yield [17, 21, 23, 27]. Addition of hydroxyethyl starch (HES) produces a twofold increase of the efficiency of continuous cell separation [27, 29]. With HES treatment and simultaneous steroid stimulation a tenfold increase is achieved of the granulocyte yield by CFC [27]. In 1970 Djerassi introduced filtration leukapheresis. When used together with CFC, it results in a further increase of the leucocyte count up to $20-50\times10^9$ [11, 12, 20]. However, granulocytes separated with this method are easily damaged and they do not raise the WBC count of the recipient to such an extent as non-filtered granulocyte concentrates, and, according to some authors, their function is not intact [20, 32]. Shivering and febrile episodes have been more often observed following the transfusion of granulocytes obtained by filtration leukapheresis [18]. Further investigations are necessary to determine whether the *in vivo* effectivity of granulocytes collected by CFC and filtration leukapheresis is higher compared with granulocyte concentrates prepared by other methods.

Granulocyte concentrates always contain red blood cells, therefore only AB0-matched donors should be used for these preparations. Furthermore, the determination of HL-A compatibility is also advisable, as the rise of the WBC of the recipient is proportional to HL-A compatibility. The higher WBC count, however, is not always proportional to the clinical effectiveness [18, 22, 23, 27]. Leucocyte antibodies play a significant role from the point of view of clinical efficacy. In the presence of these antibodies the WBC count of the recipient will not increase in the expected degree and very severe transfusion reactions may arise [15, 35].

In Hungary granulocyte concentrations prepared with dextran sedimentation technique have been used for transfusion at the National Institute of Haematology and Blood Transfusion since 1974.

The effect of granulocyte transfusions has been studied in relation to the underlying disease, the leukocyte count before the onset of therapy, the indications of granulocyte transfusions and the onset and length of treatment.

Patients and Methods

Twenty-three patients received a total of 300 bottles of granulocyte-rich plasma in 31 courses. In each case the granulocyte-rich plasma was prepared from AB0 and HL-A-matched blood. In 4 cases the donor was related to the recipient. The granulocyte plasma was prepared with the dextran sedimentation technique, the WBC count was between 0.96×10^9 and 2.45×10^9. The average age of the patients was 34.2 years. The sex distribution was 10 females and 13 males. The distribution of the patients according to their haematological disease is presented in Table 1. The table also indicates that some patients received repeated granulocyte transfusions in the course of their illness. These were considered as separate cases when the effectiveness of the 31 granulocyte transfusions was evaluated. In 8 cases granulocyte transfusions were given because of fever without any detectable infection. In 3 cases prophylactic granulocyte transfusions were given. In 20 cases the indication was a clinically or microbiologically confirmed infection (cultures of sputum, urine or excretion and/or haemocultures) unresponsive to antibiotics. In several cases various pathogenic bacteria were cultured from different parts of the body, thus 24 infections and 34 pathogenic bacteria were identified. Table 2 demonstrates the localization of infections and the types of infecting agents. The effectiveness of therapy was evaluated on the basis of re-

Table 1

Distribution of patients according to diagnosis

Diagnosis	Number of patients	Number of granulocyte transfusions
AML	14	17
ALL	3	6
CLL	1	1
CML blastic crisis	1	2
Hodgkin's disease	2	3
Reticulosarcoma	2	2
Total	23	31

Table 2

Localization of infections and cultured bacteria

Localization	Number of infections	Pathogenic agent	Number of cases
Upper respiratory tract	7	E. coli	7
Lung	6	Candida albicans	7
Urogenital	2	Proteus	6
Skin	3	Klebsiella	6
Anorectal	3	Ps. aeruginosa	2
Other	3	B. anitratum	2
		Citrobacter	2
		St. aureus	1
		Str. faecalis	1
Total	24	Total	34

duction in temperature and survival time. Granulocyte transfusions were considered effective if the temperature dropped and the patient lived longer than 3 weeks following this form of therapy.

Results

Twenty-eight granulocyte transfusion courses were evaluated. As the changes of temperature were considered, prophylactic transfusions were not included in this evaluation. The granulocyte transfusion was effective in 16 cases (56%) and ineffective in 12 cases (44%). A drop in temperature occurred only in 4 patients following the first transfusion. In 12 patients the therapy was effective after a few days of treatment. In cases of successful treatment fever was absent by the 5th day after the onset of therapy. With the exception of one case the patients remained

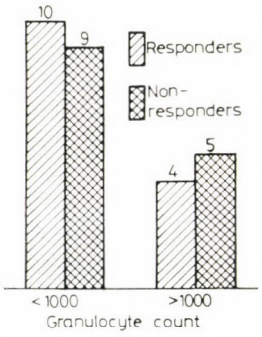


Fig. 1. Correlation between the response to granulocyte transfusion and the recipient's granulocyte count

afebrile over 7 days. The efficacy of the granulocyte therapy in relation to the granulocyte count of the recipient is displayed in Fig. 1. No difference was found in effectiveness in patients with different initial granulocyte counts. The efficacy of therapy in the various underlying diseases is demonstrated in Table 3.

Table 3

Effectiveness of granulocyte transfusions in relation to the underlying disease

Diagnosis	Number of granulocyte transfusions	Effective	Ineffective
ALL	5	4	1
AML	15	9	6
CLL	1	—	1
CML blastic crisis	2	1	1
Hodgkin's disease	3	2	1
Reticulosarcoma	2	—	2

(Prophylactic granulocyte transfusions are not included).

As a higher number of patients was found only in the group of acute leukaemia, only the cases of acute lymphoid leukaemia (ALL) and acute myeloid leukaemia (AML) were compared. In patients with ALL the granulocyte transfusions were effective in more cases than in patients with AML. The effectiveness of granulocyte concentrates in the function of time of their introduction is illustrated in Fig. 2. Granulocyte replacement was found to be much more effective when it was started within 4–5 days after the onset of fever. The mortality rate of acute leukaemic patients treated with granulocyte concentrations was compared to that of the untreated group (Table 4). There was no significant difference between the two groups. The cause of death was also compared in these two

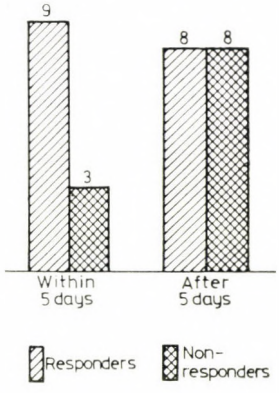


Fig. 2. Response to therapy related to the timing of granulocyte transfusion

Table 4

Mortality rate due to infectious and febrile episodes in the transfused and non-treated (control) groups

	Transfused	Control
Lethal infection	7/20	17/58
Lethal fever	2/8	10/16
Lethal infection + fever	9/28	27/74

Table 5

Causes of death in the transfused and non-treated (control) groups

Cause of death	Transfused	Control
Infection	9/16	17/39
Fever	1/16	10/39
Haemorrhage	3/16	4/39
Hepatitis	2/16	1/39
Pulmonary embolism	1/16	—
Side-effect of chemotherapy	—	3/39
Other	—	4/39

groups (Table 5) and no significant difference was found. Transfusion reaction occurred in 4 cases, in one case a fatal cerebral haemorrhage developed in the course of a severe pyrogenic reaction.

Discussion

The therapeutic effect of granulocyte transfusions is very difficult to evaluate due to the heterogeneity of the underlying disease, infection and fever of "unknown" origin (which is probably caused by undetectable infection).

The effect of granulocyte transfusions can be assessed on the basis of clinical parameters: the reduction of temperature, clearing of infection and survival. These factors, however, are also influenced by antibiotics and cytostatic drugs.

In 48 to 82% of immunosuppressed and severely neutropenic patients infections were cleared by combinations of antibiotics with a broad spectrum of action [3, 5, 6]. According to several authors antibiotic therapy supplemented with granulocyte transfusions results in clinical improvement in 46 to 86% of the cases [12, 13, 21, 23, 27, 33]. Although these reported preparations contained very different numbers of granulocytes (2×10^9 to 80×10^9) no significant difference

was found in their clinical efficacy, evaluated on the basis of clearing of infection and survival.

Our granulocyte preparations contained $0.67-1.7\times10^9$ granulocytes. The use of antibiotics with a broad spectrum of action and repeated granulocyte transfusions resulted in the disappearance of fever and clearing of infection in 58% of our patients. This result is very similar to that of Graw and McCredie who transfused $2-5.5\times10^9$ granulocytes and attained improvement in 46 to 49% [18, 27]. The effectiveness of granulocyte transfusion has not yet been unequivocally proved in an adequately high number of patients and carefully matched controls. Alavi and Fortuny comparing the course of infection in patients treated with granulocytes and in untreated patients found no significant difference between the two groups [1, 13]. We have also compared the difference in fever, infectious episodes and lethality in acute leukaemic patients treated with granulocytes and untreated. No significant differences were found between the two groups in the course of infection, death rate and causes of death.

Our results indicate that the clinical effectiveness of granulocyte transfusion depends largely on the time of the beginning of this therapy. At first, the indication of granulocyte transfusion was exclusively infection unresponsive to antibiotics. This meant that granulocyte replacement could only be started 4–5 days subsequent to the onset of the infectious episode. In agreement with other authors we also found that the earlier the granulocyte therapy is started, the more effective it will be [21, 26].

The early introduction of granulocyte transfusions is further substantiated by the finding that the effectiveness of broad-spectrum antibiotics is reduced in patients with severe granulocytopenia [8, 28]. Similarly to other authors we have also observed an increased effectiveness of granulocyte replacement when transfusions were administered repeatedly for several days [18, 26].

Since granulocyte concentrations are most effective in the first, critical stage of the infectious episode, this raises the question whether to give prophylactic transfusions. To our opinion there is no critical granulocyte level at which prophylactic granulocyte transfusion is necessary. In our patients there was no significant difference regarding the effectiveness of therapy in patients with various initial granulocyte counts. The explanation of this finding is that susceptibility and elimination of the infection is not solely dependent on the absolute number of granulocytes but depends also on the length of time in which their number decreases and on how prolonged the granulocytopenia is. Furthermore, it depends on the total granulocyte pool of the organism and the cellular and humoral immunoreactivity of the patient. When contemplating prophylactic granulocyte therapy the side-effects and dangers of granulocyte transfusions must also be considered. Literary data report 11 to 50% of toxic reactions [13, 21, 23]. We also observed side-effects in 4 (12%) of our own patients, one of which was a fatal pyrogenic reaction. Isoimmunization is one of the most dangerous side-effects reducing, on the one hand, the effectiveness of subsequent thrombocyte and granulocyte replacement and possibly inducing, on the other hand, severe trans-

fusion reactions. This danger can be substantially reduced by using AB0 and HL-A-matched granulocytes, which we regard as indispensable. Prophylactic granulocyte concentrates were only given when a patient at high risk had a sudden fall in the granulocyte count.

When analyzing the factors that influence the clinical efficacy of granulocyte transfusions, several authors have suggested that the underlying disease is of importance [13, 26, 33]. Due to the low number of our patients we only compared the cases of acute leukaemia. Similarly to Lowenthal et al. [26] we have also found that granulocyte transfusions were more often effective in cases of ALL as compared to cases of AML. Present methods of preparing granulocyte concentrates do not enable the preparation of such granulocyte concentrates which raise the granulocyte count to the normal level, these only raise the number of the circulating granulocytes for a short time [9]. In case of severe infection in patients with ALL, granulocyte transfusions may help the patient over the critical period. However, patients with AML are regularly on the border of bone marrow insufficiency and this appears to explain the lower therapeutic efficacy of granulocyte replacement.

Further comparison of the various granulocyte concentrates are necessary in a larger and more homogeneous patient series, for fuller understanding of the factors influencing their therapeutic efficacy, and for evaluating the correlations between the *in vitro* granulocyte count, function, and the *in vivo* therapeutic effectivity.

References

1. Alavi, J. B., Root, R. K., Remischowsky, J., Djerassi, I., Evans, A. E., Schreiber, A. D., Guerry, D., Cooper, R. A.: Leukocyte transfusions in acute leukaemia. *Proc. Amer. Soc. Hemat. 17*, 150 (1974).
2. Armstrong, D., Lowell, S., Young, R., Meyer, D., Blevius, A. H.: Infectious complications of neoplastic disease. *Med. Clin. N. Amer. 55*, 729 (1971).
3. Bloomfield, C. D., Kennedy, B. J.: Cephalotin, Carbenicillin and Gentamycin combination therapy for febrile patients with acute nonlymphocytic leukemia. *Cancer 34*, 431 (1974).
4. Bodey, G. P., Buckley, M., Sathe, J. S., Freireich, E. J.: Quantitative relationships between circulating leukocytes and infection in patients with acute leukaemia. *Ann. intern. Med. 64*, 328 (1966).
5. Bodey, G. P., Rodriquez, V., Luce, J. K.: Carbenicillin therapy of gram-negative bacilli infections. *Amer. J. med. Sci. 257*, 408 (1969).
6. Bodey, G. P., Gehan, F. A., Freireich, E. J., Frei, J.: Protected environment and prophylactic antibiotic program in the chemotherapy of acute leukaemia. *Amer. J. med. Sci. 262*, 138 (1971).
7. Bodey, G. P., Whitecar, J. P. Jr., Middleman, E., Rodriquez, V.: Carbenicillin therapy for Pseudomonas infections. *J. Amer. med. Ass. 218*, 62 (1971).
8. Bodey, G. P., Middleman, E., Umsawadi, T., Rodriquez, V.: Infections in cancer patients. Results with gentamycin sulfate therapy. *Cancer 29*, 1697 (1972).
9. Boggs, D. R.: Transfusion of neutrophils as prevention or treatment of infection in neutropenic patients. *New Engl. J. Med. 290*, 1055 (1974).

10. Deinard, A. S., Fortuny, I. E., Theologides, A., Anderson, G. L., Boen, J., Kennedy, B. J.: Studies on the neutropenia in cancer chemotherapy. *Cancer 33*, 1210 (1974).
11. Djerassi, I., Kim, J. S., Mitrakul, C., Suvansri, U., Ciesileka, W.: Filtration leukapheresis for separation and concentration of transfusable amounts of normal human granulocytes. *J. Med. (Basel) 1*, 358 (1970).
12. Djerassi, I., Kim, J. S., Suvansri, U., Mitrakul, C., Ciesileka, W.: Continuous flow filtration leukapheresis. *Transfusion 12*, 75 (1972).
13. Fortuny, I. E., Bloomfield, C. D., Hadlock, D. C., Goldman, A., Kennedy, B. J., McCullough, J. J.: Granulocyte transfusion: A controlled study in patients with acute nonlymphocytic leukemia. *Transfusion 15*, 548 (1975).
14. Freireich, E. J., Judson, G., Levin, R. H.: Separation and collection of leukocytes. *Cancer Res. 25*, 1516 (1965).
15. Goldstein, I. M., Eyre, H. J., Terasaki, P. I., Henderson, E. S., Graw, R. G.: Leukocyte transfusions: Role of leukocyte alloantibodies in determining transfusion response. *Transfusion 11*, 19 (1971).
16. Graw, R. G., Buckner, C. D., Whang-Peng, J., Leventhal, B. G., Krüger, G., Bevard, C., Henderson, E. S.: Complication of bone marrow transplantation. GVH disease resulting from chronic myelogenous leukaemic leucocyte transfusions. *Lancet 2*, 338 (1970).
17. Graw, R. G., Herzig, G. P., Eisel, R. J., Perry, S.: Leukocyte and platelet collection from normal donors with the continuous flow blood cell separator. *Transfusion 11*, 94 (1971).
18. Graw, R. G., Herzig, G. P., Perry, S., Henderson, E. S.: Normal granulocyte transfusion therapy: Treatment of septicaemia due to gram-negative bacteria. *New Engl. J. Med. 287*, 367 (1972).
19. Hersh, E. M., Bodey, G. P., Freireich, E. J.: Causes of death in acute leukemia. *J. Amer. med. Ass. 193*, 105 (1965).
20. Herzig, G. P., Root, R. K., Graw, R. G.: Granulocyte collection by continuous-flow filtration leukapheresis. *Blood 39*, 554 (1972).
21. Highby, D. J., Henderson, E. S., Holland, J. F.: Granulocyte transfusion therapy — clinical and laboratory studies. *Proc. Amer. Soc. clin. Oncol. 15*, 176 (1974).
22. Highby, D. J., Henderson, E. S.: Granulocyte transfusion therapy. *Ann. Rev. Med. 26*, 7151 (1975).
23. Kozs, I. J., Holland, F., Cohen, E.: Histocompatible leukocyte transfusion during granulocytopenia. *Neoplasma 18*, 185 (1971).
24. Levine, A. S., Siegel, S. E., Schreiber, A. D., Hauser, J., Preisler, H., Goldstein, I. M., Seidler, F., Simon, R., Perry, S., Bennet, J. E., Henderson, E. S.: Protected environments and prophylactic antibiotics. A prospective controlled study of their utility in the therapy of acute leukemia. *New Engl. J. Med. 288*, 477 (1973).
25. Levine, A. S., Schimpff, S. C., Graw, R. G. Jr., Young, R. C.: Hematologic malignancies and other marrow failure states. Progress in the management of complicating infections. *Semin. Hematol. 11*, 141 (1974).
26. Lowenthal, R. M., Goldman, J. M., Buskard, N. A., Murphy, B. C., Grossman, L., Storring, R. A., Park, D. S., Spiers, A. S. D., Galton, D. A. G.: Granulocyte transfusions in treatment of infections in patients with acute leukaemia and aplastic anaemia. *Lancet 1*, 7903 (1975).
27. McCredie, K. B., Freireich, E. J., Hester, J. P., Vallejos, C.: Leukocyte transfusion therapy for patients with host-defense failure. *Transplant. Proc. 5*, 1285 (1973).
28. Middleman, E. L., Watanabe, A., Kaizer, H., Bodey, G. P.: Antibiotic combinations for infections in neutropenic patients. *Cancer 30*, 573 (1972).
29. Mishler, J. M., Highby, D. J., Rhonberg, W., Holland, J. F., Nicora, R. W.: Hydroxyethyl starch and dexamethasone as an adjunct to leukocyte separation with the IBM blood cell separator. *Transfusion 14*, 352 (1974)

30. Morse, E. E., Bronson, W., Carbone, P., Freireich, E. J.: Effectiveness of granulocyte transfusion from donors with chronic leukaemia to patients with leukopenia. *Clin. Res. 9*, 332 (1961).
31. O'Loughlin, J. M.: Infections in the immunosuppressed patients. *Med. Clin. N. Amer. 59*, 495 (1975).
32. Roy, A. J., Yankee, R. A., Brivkalins, A., Fitch, M.: Viability of granulocytes obtained by filtration leukapheresis. *Transfusion 15*, 539 (1975).
33. Schwarzenberg, L., Mathé, G., Amiel, J. L., Cattan, A., Schneider, M., Schlumberger, J. R.: Study of factors determining the usefulness and complications of leukocyte transfusions. *Amer. J. Med. 43*, 204 (1976).
34. Tattersall, M. N. H., Spiers, A. S. D., Darrell, J. H.: Initial therapy with combination of five antibiotics in febrile patients with leukaemia and neutropenia. *Lancet 1*, 162 (1972).
35. Ward, N. H.: Pulmonary infiltrates associated with leukoagglutinin transfusion reactions. *Ann. intern. Med. 73*, 689 (1970).
36. Yankee, R. A., Freireich, E. J., Carbone, P. P., Frei, E.: Replacement therapy using normal and chronic myelogenous leukemia leukocytes. *Blood 24*, 844 (1964).
37. Yates, J. W., Holland, J. F.: A controlled study of isolation and endogenous microbial suppression in acute myelocytic leukemia patients. *Cancer 32*, 1490 (1973).

Correspondence: Dr. A. Mód, National Institute of Haematology and Blood Transfusion, Department of Haematology, Daróczi út 24, H-1113 Budapest, Hungary

Haematologia 11 (1—2), pp. 41—46 (1977)

Bone Marrow Cell Separation on Ficoll Gradient

K. Šulc, J. Neuwirt, T. Trávníček, P. Kobylka, Eva Radikovská

Institute of Pathological Physiology, Faculty of General Medicine, Charles University, Prague, Czechoslovakia

(Received April 12, 1977)

Cell fraction from rat and murine bone marrow were obtained by sedimentation at unit gravity. A linear gradient of polymerized sucrose (Ficoll) was used and some properties of individual cell fractions were studied. The 3rd cell fraction containing the lymphoblasts and lymphocytes contained also stem cells.

Bone marrow cell separation and extraction of comparatively pure cell fractions have been the subject of considerable research. Several methods have been described for obtaining and identifying cell fractions.

Some authors used centrifugation for separating cells. Thus, e.g. van Bekkum et al. [1, 2] were able to identify the haemopoietic stem cell in rodents and primates by means of cell suspension centrifugation in albumin gradient. The technique was further improved and employed not only in order to obtain haemopoietic stem cells from bone marrow but also to extract CFC from murine foetal liver [5], thrombocytes from peripheral blood [11], megakaryocytes from murine bone marrow [7], etc.

Another technique of separation of cell fractions in a relatively pure state involves cell suspension sedimentation at unit gravity. The method allows the identification of each particular cell fraction. In 1967, Peterson and Evans [9] described a technique of bone marrow cell separation in the linear gradient of saccharose. The technique was further developed by Miller and Phillips [6] and used by other research workers with a view to separating each of the bone marrow cell fractions [3, 10, 4, etc.]. Albumin, saccharose, and Ficoll were used as carriers. Sedimentation times ranging from 3 hours [6] up to 20 hours [9] were recorded.

In the present study bone marrow cell separation at unit gravity has also been attempted.

Methods

1. Cell suspension

Rats or mice were killed by decapitation, their femoral or tibial bones were removed and the bone marrow was taken out by a special needle into a preweighed paraffinized dish. The bone marrow was "homogenized" by being slowly

drawn up and ejected by needles of gradually decreasing lumina and, step by step, suspended in sterile Parker's solution [8] pH 7.4. The cell suspension was eluted three times using sterile Parker's solution at 700 *G* for a period of 5 minutes at a temperature of 4 °C. The number of nuclear elements was estimated.

2. Bone marrow cell separation

The separation column is cylindrical in shape with cones 20 mm in height attached to the upper and lower ends. The bottom cone bears a solid hemisphere to prevent turbulence while the column is being filled. The cylindrical part of the column is 90 mm long and 90 mm high. The column is filled from the bottom at a rate of 11 ml/5 min with the following layers:

(1) 40 ml Parker's solution
(2) 20 ml cell suspension in 0.2% Ficoll solution in Parker's medium
(3) 60 ml 0.33% Ficoll solution in Parker's medium
(4) 400 ml linear concentration gradient of Ficoll obtained by mixing 200 ml 1% and 200 ml 5% Ficoll solution in Parker's medium

Parker's solution used for the separation of cells contains a constant quantity of bovine serum albumin (0.1% BSA in Parker's medium).

Cell sedimentation at a temperature of 4 °C takes 16 hours. After sedimentation, the cell layers are eluted through a UV analyzer (type "Uvicord") by 254 nm into the respective test tubes at a rate of 11 ml/5 min (Fig. 1).

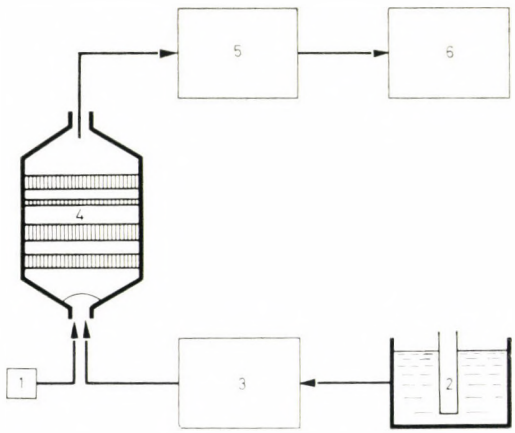


Fig. 1. Scheme of the apparatus: 1 = bone marrow cell suspension; 2 = linear Ficoll gradient; 3 = micropump; 4 = column for bone marrow cell separation; 5 = UV monitor; 6 = fraction collector

3. Cell viability

Cell viability was ascertained by means of trypan blue. The bone marrow cells were stained with 0.5% trypan blue in saline at 25 °C for 5 minutes; the number of living and dead cells was estimated in a Buerker chamber.

4. Morphology of cell fractions

Smears from each cell fraction were estimated following routine panoptic staining without counting of the cell types.

5. Spleen colony method

The recipient animals were irradiated by a lethal dose (850 r). The cell fractions were transplanted 2 hours after irradiation (250,000 cells per mouse). The animals were sacrificed 9 days after irradiation and transplantation and the number of macroscopic colonies was estimated.

Results and Discussion

Bone marrow cell suspensions were separated in four fractions. Figure 2 shows the UV analyzer recording of the process of separation of mouse and rat bone marrow cells. Table 1 shows the relative proportion of cells in each of the fractions, the quantity of cells obtained from the column, and the sedimentation rates for each particular fraction. Table 2 demonstrates cell viability. Table 3 shows the morphology of each of the cell fractions. Table 4 gives the number of exogenous colonies that had developed in irradiated murine recipients following the administration of cell fractions and cells of unseparated bone marrow. Figure 3

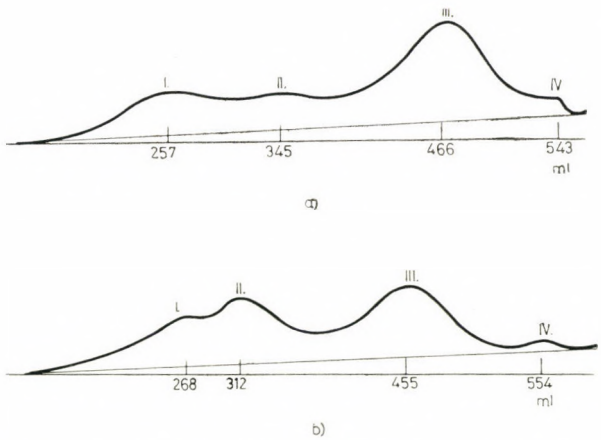


Fig. 2. Optical density of eluted fractions: a = bone marrow cells of mice; b = bone marrow cells of rats; thick line = optical density; thin line = Ficoll gradient

Table 1

Relative amount of cells in individual fractions, velocity of cell sedimentation

Fraction	Sedimentation time (mm/hr)	Relative amount of cells (per cent)	Amount of cells in column
1.	2.3	18.4 ± 1.5	Before separation,
2.	3.3	26.5 ± 2.1	$50.10^6 - 200.10^6$
3.	4.4	35.9 ± 1.9	After separation, $43.10^6 - 171.10^6$
4.	5.2	19.1 ± 2.1	$(85.7 \pm 5.2\%)$

Table 2

Living cells in the fractions

Fraction	Relative amount of living cells (per cent)
1.	87.0 ± 2.1
2.	81.0 ± 2.8
3.	85.2 ± 2.0
4.	82.3 ± 1.9

The cells were stained with 0.5% trypan blue in saline at 25 °C for 5 minutes

Table 3

Morphology of cell fractions (qualitative aspect)

Fraction	Cell types
1.	Polychromatic normoblasts (intermediate normoblasts); oxyphil normoblast (late normoblasts); reticulocytes; erythrocytes
2.	Erythrocytes; megakaryocytes; myeloblasts; neutrophil, eosinophil, basophil myelocytes; young and band forms of metamyelocytes; neutrophil, eosinophil, basophil polymorphonuclears; lymphoblasts; lymphocytes
3.	Lymphoblasts; lymphocytes; neutrophil, eosinophil, basophil polymorphonuclears; plasmoblasts
4.	Plasmoblasts; plasma cells; neutrophil, eosinophil, basophil polymorphonuclears; thrombocytes

Table 4

Spleen colonies in irradiated mice after transplantation of bone marrow cells

Groups	Number of animals	Number of colonies per animal
Controls (irradiated)	74	1.2±0.2
Administration of unseparated bone marrow cells	62	12.6±1.3
Administration of 1st fraction	66	5.2±1.1
Administration of 2nd fraction	64	8.7±2.1
Administration of 3rd fraction	88	45.2±2.8
Administration of 4th fraction	90	4.3±1.2

Number of transplanted cells: 250,000/1 mouse.

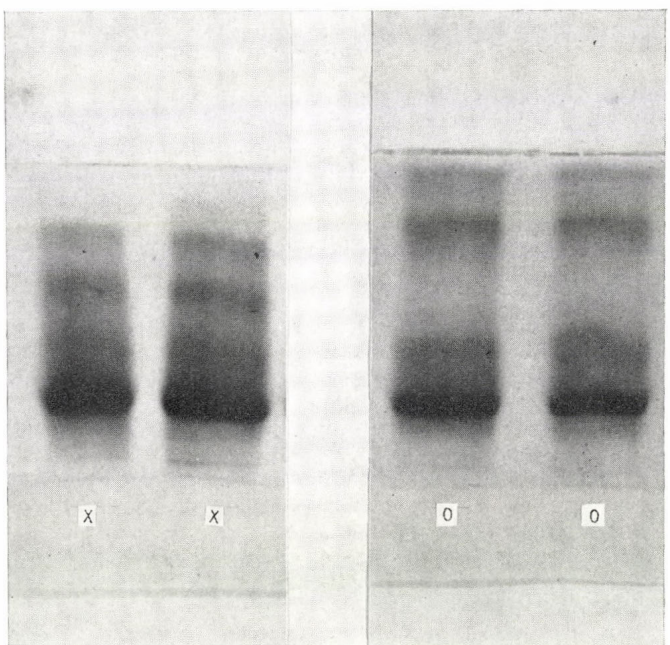


Fig. 3. Haemoglobin pattern in recipient rats 21 days after transplantation of the 3rd cell fraction. 0 = control recipients; X = chimera; Recipients = 3 main Hb types; Donors = 4 main Hb types; Number of transplanted cells = 1×10^6/rat; The recipients were irradiated by a dose of 850 r

shows the development of radiation chimeras in rats after the administration of cell fractions. Chimeras were obtained solely by administering cells of the 3rd fraction.

As the results show the cells always separated into four fractions, in agreement with the findings of other authors [3, 4]. Our experiments showed that after a period of 16 hours outside the body and following a passage through the column, most of the cells were fully viable. The separation column yielded about 80% of the cells placed in the column. Out of these, about 80 to 90% were alive according to trypan blue incubation, which means that 65 to 76% of the cells survived 16 hours in the column.

Observations of the development of exogenous colonies in mice and the development of radiation chimeras in rats show that the 3rd cell fraction contains, among other elements, also concentrated stem cells; this is obvious from Table 4.

The number of stem cells in the 3rd cell fraction was four times greater than in the cells of the intact, unfractionated bone marrow (compared with the same number of transplanted cells), while there are probably only few stem cells in the rest of the cell fractions. The survival rate of recipients irradiated after transplantation of unseparated bone marrow cells and each of the cell fractions all confirmed the finding.

References

1. van Bekkum, D. W., van Noord, M. J., Maat, B., Dicke, K. A.: Attempts at identification of hemopoietic stem cell in mouse. *Blood 38*, 547 (1971).
2. van Bekkum, D. W., Dicke, K. A., van Noord, M. J.: Attempts at morphological identification of the hemopoietic stem cell in rodents and primates. *Exp. Hemat. 1*, 36 (1973).
3. McCool, D., Miller, R. J., Painter, R. H., Bruce, W. R.: Erythropoietin sensitivity of rat bone marrow cells separated by velocity sedimentation. *Cell Tissue Kinet. 3*, 55 (1970).
4. Denton, M. J., Arnstein, H. R. V.: Characterization of developing erythroid cells. *Brit. J. Haemat. 24*, 7 (1973).
5. Löwenberg, B., Dicke, K. A., van Bekkum, D. W.: Density distribution of *in vivo* and *in vitro* CFC in mouse fetal liver. Ann. Report of REP-TNO (Netherlands) 153 (1972).
6. Miller, R. G., Phillips, R. A.: Separation of cells by velocity sedimentation. *J. Cell Physiol. 22*, 128 (1969).
7. Nakeff, A., Maat, B.: Separation of megakaryocytes from mouse bone marrow by velocity sedimentation. *Blood 43*, 591 (1974).
8. Parker, R. C.: Methods of Tissue Culture. 3rd ed. Hoeber, New York 1966, p. 58.
9. Peterson, E. A., Evans, W. H.: Separation of bone marrow cells by sedimentation at unit gravity. *Nature (Lond.) 214*, 824 (1967).
10. Stephenson, J. R., Axelrad, A. A.: Separation of erythropoietin-sensitive cells from hemopoietic spleen colony-forming stem cells of mouse fetal liver by unit gravity sedimentation. *Blood 37*, 417 (1969).
11. Walsh, P. N.: Albumin density gradient separation and washing of platelets and the study of platelet coagulant activity. *Brit. J. Haemat. 22*, 205 (1972).

Correspondence: Dr. K. Šulc, Institute of Pathological Physiology, Faculty of General Medicine, Charles University, U nemocnice 5, 128 53 Praha 2, ČSSR

Haematologia 11 (1–2), pp. 47–55 (1977)

Chromosomes in Malignant Lymphomas (Study on Short-Term Lymph Node Cultures)

T. FLEISCHMANN, F. KRIZSA

First and Second Departments of Medicine, University Medical School, Szeged, Hungary

(Received February 17, 1975)

In 15 cases, chromosome studies were performed in short-term lymph node cultures, using the banding technique. Clonal aberrations were found in two cases of Hodgkin's disease and in two of lymphosarcoma. In agreement with earlier observations, the cases of lymphosarcoma with abnormal karyotypes proved hyperdiploid. No chromosomal aberrations were found in three cases of reactive lymphoreticular hyperplasia.

Chromosome studies in malignant lymphoma have received considerable impetus in recent years, primarily as a result of the introduction of short-term lymph node cultures. The self-evident target of cytogenetic studies in this group of disease is the directly involved lymph node itself. Direct studies of lymph nodes, apart from being too elaborate, hardly ever yield adequate chromosome preparations. The results of long-term cultures are also questionable in the majority of cases. Short-term lymph node culture has thus become an essential tool in the cytogenetic study of malignant lymphomas. The technique yields a sufficient number of dividing cells and chromosome preparations of better quality, thus allowing cytogenetic studies to be carried out on a larger scale. The value of short-term cultures is enhanced by the fact that they give preparations suitable for the banding technique which requires chromosome preparations of good quality.

In the present study we have examined the chromosomes of 15 patients.

Material and Method

From surgically excised lymph nodes suspension cultures were prepared in Earle-Eagle medium supplemented with 20% foetal calf serum. The life-span of the cultures was 24 to 96 hours. The chromosome preparations were made according to Moorhead et al. [18]. The fluorescent procedure was a simplified modification described by us earlier [9]. Giemsa-banding was done with the ASG and BSG techniques [28, 29].

Patients

The essential clinical features of the patients have been summed up in Table 1. "Duration" refers to the time between the first manifestation of the disease and the cytogenetic studies, "Treatment" to the specific therapy of the primary disease. The diagnosis was based on histologic evidence in all cases.

Table 1

Clinical data

Case No.	Diagnosis	Age (years)	Sex	Duration (months)	Treatment
1	Hodgkin's disease	20	M	48	Radiotherapy
2	Hodgkin's disease	49	M	96	Radiotherapy, cyclophosphamide
3	Hodgkin's disease	32	F	5	Radiotherapy
4	Hodgkin's disease	29	F	12	—
5	Hodgkin's disease	48	F	12	—
6	Hodgkin's disease	77	M	7	—
7	Lymphosarcoma	62	M	1	—
8	Lymphosarcoma	70	M	10	Cyclophosphamide
9	Reticulosarcoma	49	F	6	—
10	Chronic lymphocytic leukaemia	67	F	6	—
11	Chronic lymphocytic leukaemia	60	M	3	—
12	Chronic lymphocytic leukaemia	80	F	12	—
13	Lymphoreticular hyperplasia	38	F	5	—
14	Lymphoreticular hyperplasia	20	M	2	—
15	Lymphoreticular hyperplasia	40	F	6	—

Results

Results of the cytogenetic studies have been summed up in Table 2. All analyzable metaphases were evaluated and banding was applied if it was compatible with the quality of the preparation.

The cases with clonal aberrations are reported below.

Case 3. Hodgkin's disease, lymph node culture, life-span 48 hr. Orcein staining. Mitoses of moderate quality and fairly large number. Chromosome count: $\frac{44}{1}$ $\frac{45}{2}$ $\frac{46}{17}$. Ten metaphases were fully analyzed. In all of them there was a long subtelocentric marker being closest in length to group B. In addition, in all analyzed metaphases two or three chromosomes of group B, one or two

Table 2

General cytogenetic features

Case No.	Method		Number of analyzed cells	Number of chromosomes (stem line)	Clonal aberrations, series tested
	Culture	Staining			
1	Lymph node	Fluorescent	11	46	–
2	Lymph node	Orcein	5	46	–
3	Lymph node	Orcein	20	46	M1, 2–3B–, 2–3C+, 1–2F–
4	Lymph node	ASG	10	46	–
5	Lymph node	ASG, BSG	15	52	M1, M2, M3, M4 1+, 9–, 15–
6	Lymph node	ASG	5	46	–
7	Lymph node	Orcein	15	48	2F+
8	Lymph node	ASG	10	48	2C+
9	Lymph node	ASG	5	46	–
10	Lymph node	Orcein	10	46	–
11	Lymph node	Orcein	8	46	–
12	Lymph node	ASG	10	46	–
13	Lymph node	ASG	10	46	–
14	Lymph node	ASG, BSG	15	46	–
15	Lymph node	ASG, BSG, fluorescent	16	46	–

chromosomes of group F were missing and there were two or three additional chromosomes of group C. In half of the analyzed cells trisomy or tetrasomy 2 were present.

Case 5. Hodgkin's disease. Lymph node cultures of 24, 48 and 72 hr. ASG, BSG fluorescent stainings, a fair number of metaphases of adequate quality. Chromosome count: $\frac{52}{12}$ $\frac{53}{2}$ $\frac{102}{1}$. There were 4 consistent markers and further 2 to 5 marker chromosomes varying between the cells. Moreover, trisomy 1 and monosomy 9 and 15 were found in the majority of the analyzed cells (Figs 1, 2).

The consistent markers were: M1 long submedian, resembling pair-2 in size but of entirely different banding pattern. M2 long, median, similar to pair-3 in length but of distinctive banding pattern. M3 related to group D in size and readily identifiable on grounds of its typical subtelocentric banding. M4 is a true "minute" marker, identifiable by the BSG procedure as the centromere of one of the chromosomes 9. Of the above markers M2 and M3 could not be identified by orcein staining since, on the grounds of length and centromere position, the first would be classified to group 3 and the second to group 15.

Case 7. Lymphosarcoma, 48 hr lymph node culture, orcein staining. Fair number and quality of dividing cells. Chromosome count: $\frac{46}{1}$ $\frac{47}{4}$ $\frac{48}{10}$. All metaphases used for counting were analyzed and in each one or two additional chromosomes E were found.

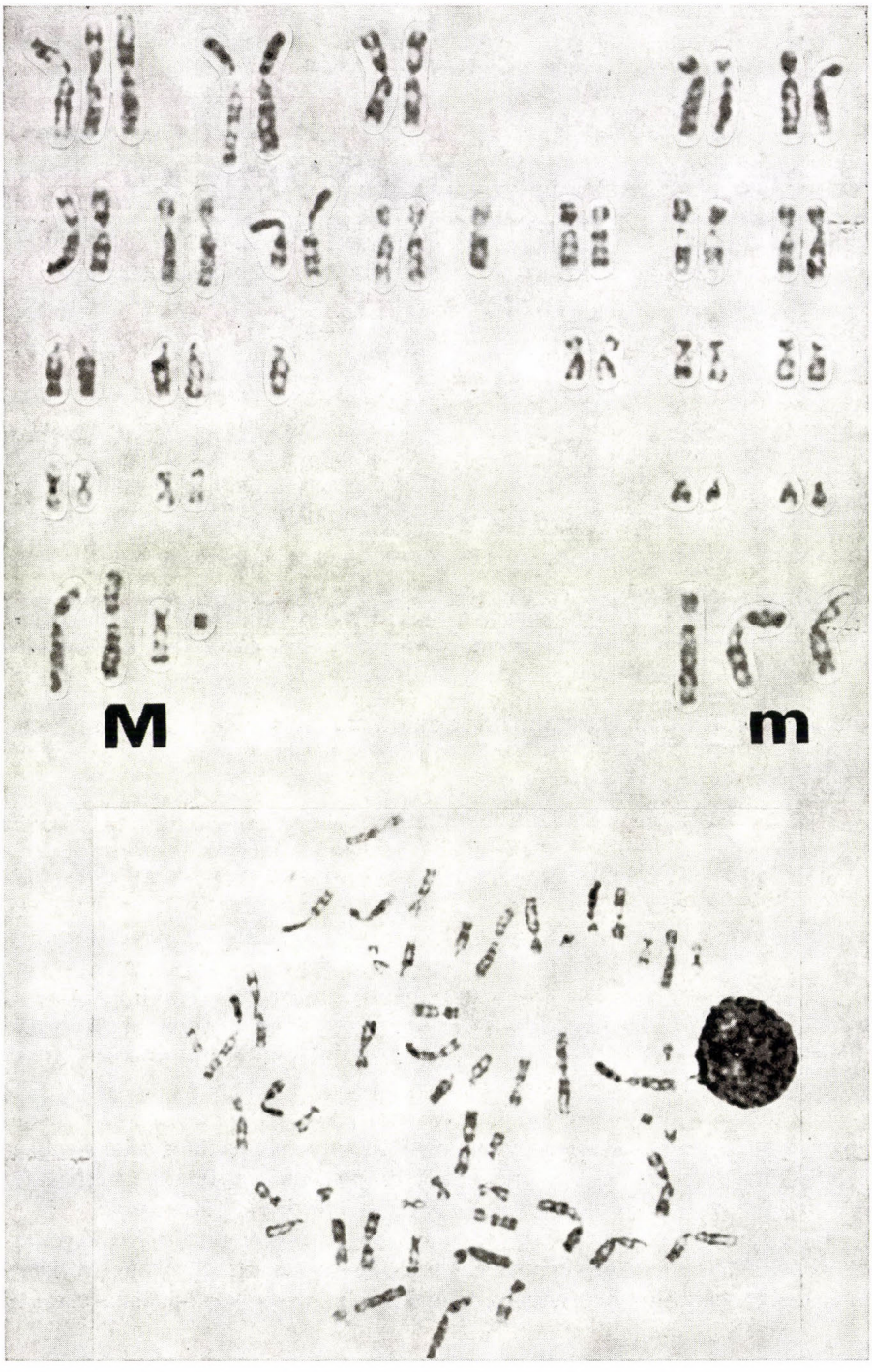


Fig. 1. Karyotype of Case 5. Short-term lymph node culture ASG method. Note trisomy 1, monosomy 9 and 15. Bottom, left side: four consistent markers (M). Bottom, right side: non-consistent markers (m) varying between the individual cells

Case 8. Lymphosarcoma, 48 hr lymph node culture, ASG staining, sparse dividing cells of poor quality. Chromosome count: $\frac{48}{4}$ $\frac{50}{1}$. All cells revealed two additional chromosomes C as a typical aberration. Owing to the poor quality of the preparation identification was not possible with absolute certainty, it may, however, be assumed that the chromosomes 8 and 11 were involved.

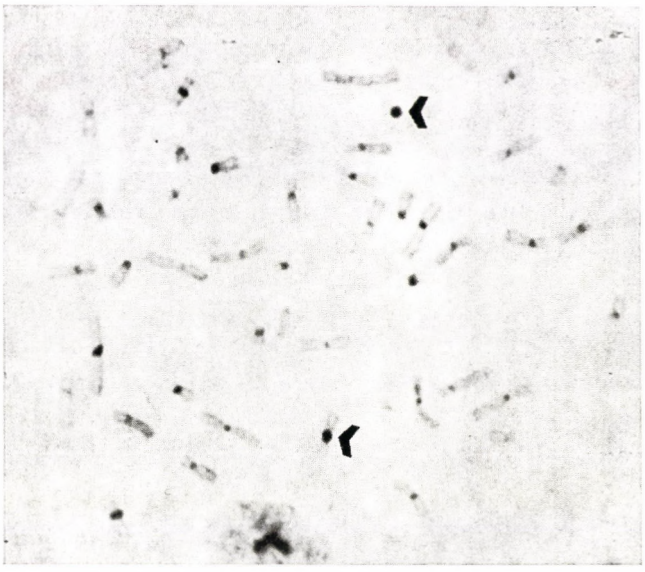


Fig. 2. Metaphase from Case 5. Short-term lymph node, BSG method. The arrows point to chromosome 9 and to the "minute" marker which latter is identifiable as the centromere of the other chromosome 9

Case 15 showed that even by the banding technique we may find it difficult to differentiate normal from abnormal findings. Clinical diagnosis was also far from simple. The patient had been admitted for progressive lymph node enlargement which, on admission, seemed indicative of malignancy, first of all of malignant lymphoma. What puzzled us, however, was the normality of all other findings. Biopsy carried out twice yielded the same finding of lymphoreticular hyperplasia of marked reactivity. During the follow-up period of six months, the patient has remained in a perfect general condition without showing any further lymph node enlargement. Cytogenetic study: 48-hr lymph node culture, ASG, BSG and fluorescent staining. Dividing cells of fair number and quality. Chromosome count: $\frac{46}{16}$. All counted metaphases were subjected to close analysis. With the exception of a single detail the karyotype was entirely normal. What seemed uncommon was the finding that one of chromosome 9 was longer than its pair, the difference being due to the prolonged and very marked secondary constriction. This was clearly demonstrated by all three banding procedures (Fig. 3). The finding represents in all likelihood a normal variety.

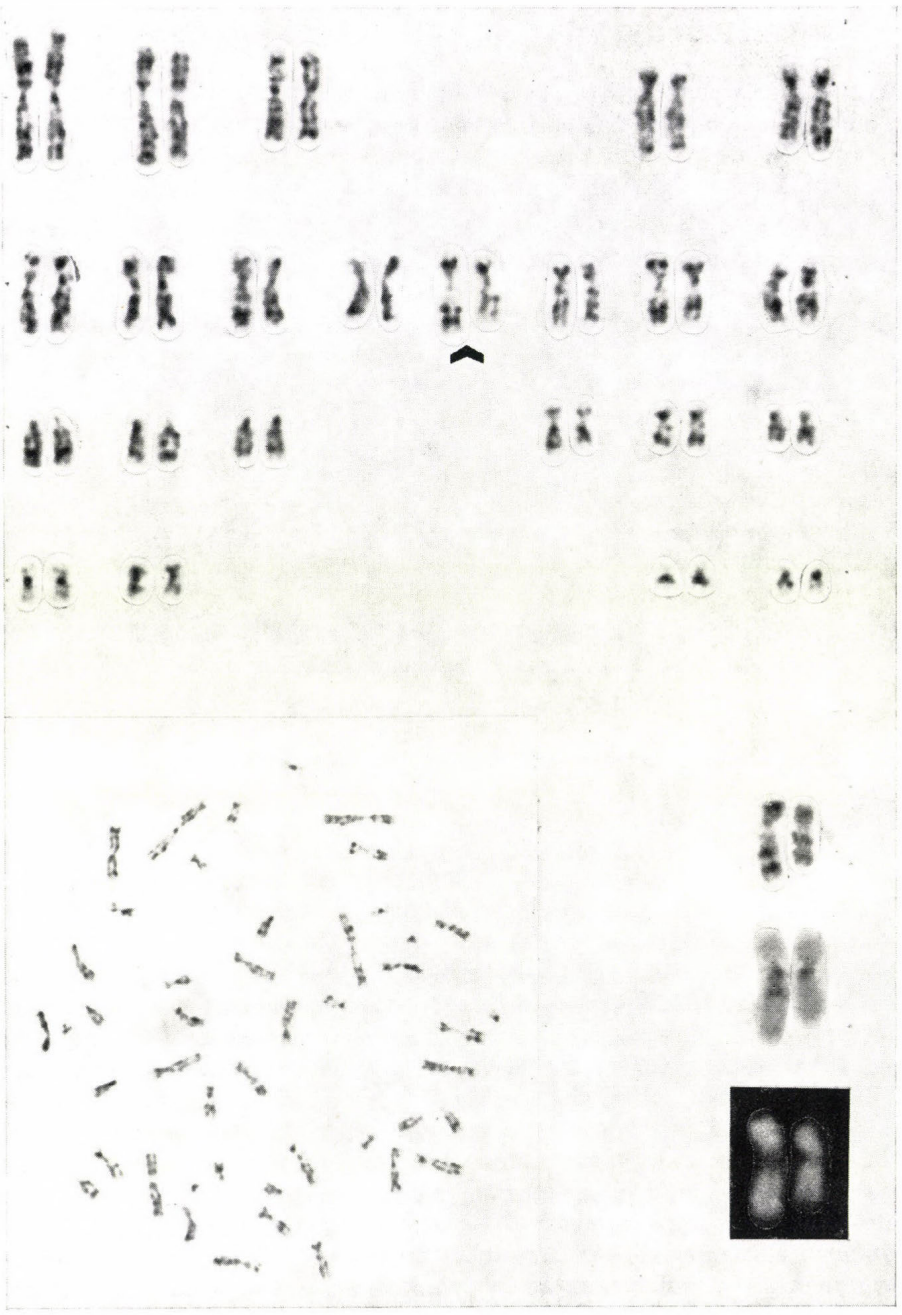


Fig. 3. Karyotype of Case 15. Short-term lymph node culture, ASG method. Arrow: chromosome-pair 9. Bottom, right side: chromosome-pair 9 stained by the use of three different (ASG, BSG, fluorescent) techniques

Discussion

The failure of cytogenetic studies to provide information in the early stage of malignant lymphomas is attributable to the use of inappropriate methods. Studies of blood cultures or of bone marrow are generally uninformative in the early stage. Early recognition of this problem prompted direct lymph node studies. Ricci et al. [19] and Spriggs and Boddington [26] were the first to undertake direct lymph node analysis in malignant lymphoma. This enabled them to detect chromosomal abnormalities. Later this extremely time-consuming procedure was replaced by short-term cultures of the malignant lymph node which permitted studies in a larger number of patients. Chronic lymphocytic leukaemia displayed in general a normal karyotype. It was confirmed later that the Christchurch-chromosome (deletion of the short arm of one of the chromosomes 22) is not typical of the disease. It seems to be of familial occurrence and may be regarded as a predisposing factor [12, 13].

Cytogenetic studies proved more informative in lymphosarcoma, reticulosarcoma and Hodgkin's disease. In lymphosarcoma the chromosomal number was generally diploid, in reticulosarcoma it was often normal but varied between 50 and 98 in individual cases. A subtetraploid set of chromosomes was regarded earlier by certain authors as typical of Hodgkin's disease, later studies on a larger scale failed, however, to confirm this finding [4, 5, 15, 23, 24]. Recently, Soviet authors have found chromosomal counts of 3 – 5 n in 10 to 30% of the metaphases in Hodgkin's disease [7].

The marker chromosomes, no less than numerical chromosomal anomalies, represent essential clues in the identification of these diseases. Spiers and Baikie [23, 24] describe the deletion of the short arms of one chromosome of pairs 17 – 18 and regard the marker thus produced as specific. Although the existence of this marker has not been confirmed by other workers, there is fairly general agreement concerning the prevalence of aberrations of the chromosome groups 17 – 18 in malignant lymphoma. Spiers and Baikie themselves expressed the same view on grounds of their later observations [25].

Various types of marker have been described in the conditions under discussion, their analysis has, however, failed to provide conclusive results. In the majority of cases large or medium-sized markers were found which were generally subtelocentric or submedian. Of the smaller markers the isomarkers were found to be the most common [1, 2, 3, 4, 5, 16, 19, 20, 21, 22, 23, 24, 30].

We have reported earlier on our chromosome studies in lymph node cultures in 20 cases of malignant lymphoma [8, 10, 11]. In all cases of lymphosarcoma with an abnormal karyotype the chromosomal number was hyperdiploid, and one or more isomarkers in the stemline of the abnormal cases were fairly common.

The present results are to some extent conflicting. Our further two cases of lymphosarcoma were also hyperdiploid and, although in the two series we had no more than 6 patients of this type, we have to regard this observation as consistent. On the other hand, we found no isomarkers in any of 4 further cases

with clonal aberrations. The fairly high prevalence noted earlier may well have been accidental and the true incidence is in fact lower. It is also conceivable that the prevalence of isomarkers is confined to particular forms of malignant lymphoma, therefore, further studies are needed to correlate the chromosomal pattern of the tumour to its microscopic features identified on the basis of histological examination. It is for instance possible that the various histologic types of Hodgkin's disease carry different chromosomal aberrations. We are, however, unable to test the possibilities outlined above, owing to the small number of our cases, even if both series are considered. It is to be noted that isomarkers have been demonstrated in other malignant processes as well [6, 17, 27], and Lobb et al. [14] even regard the presence of isomarkers in malignant tissue as common.

Our findings in lymphoreticular hyperplasia deserve interest by serving as control. Suspicion of malignant lymphoma which had made lymph node excision necessary was not confirmed by histologic study. Two lymph node cultures revealed definitely normal karyotypes and the atypical chromosome of group 9 had finally to be regarded as a normal variety. This third case exemplifies a situation in which the banding technique fails to provide an immediate and clear answer to the morphological problem. It also holds further perspectives for diagnostic chromosome studies in the absence of microscopic signs of malignancy. Though the diagnosis of malignant lymphoma obviously rests on histologic evidence, there are borderline cases in which, at least in the early stage, no differentiation between malignancy and a reactive tissue is possible on histologic grounds. In this situation an abnormal chromosomal pattern may offer clues to the identification of malignancy.

*

We are indebted to Miss Mária Heténvi for excellent technical assistance.

References

1. Atkin, N. B., Baker, M. C.: Chromosome abnormalities as primary events in human malignant disease: Evidence from marker chromosomes. *J. nat. Cancer Inst. 36*, 539 (1966).
2. Baker, M. C., Atkin, N. B.: Chromosomes in short-term cultures of lymphoid tissue from patients with reticulosis. *Brit. med. J. 1*, 770 (1965).
3. Castoldi, G. L., Ricci, N., Punturieri, E., Bosi, L.: Chromosomal imbalance in plasmocytoma. *Lancet 1*, 829 (1963).
4. Coutinho, V., Bottura, C., Falcao, R. P.: Cytogenetic studies in malignant lymphomas: A study of 28 cases. *Brit. J. Cancer 25*, 689 (1971).
5. Демин, А. А., Раджабли, С. И., Лосева, М. И., Метелкина, Н. В.: Цитогенетические исследование при лимфогранулематозе и некоторых других ретикулозах. Генетика *8*, 126 (1972).
6. Engel, E., McKee, L. C., Bunting, K. W.: Chromosome 17—18 in leukaemias. *Lancet 2*, 42 (1967).
7. Fleischmann, E. V., Prigogina, E. L., Platonova, G. M., Chudina, A. P., Kruglova, G. V., Kaverzneva, M. M.: Comparative chromosome characteristics of reticulosarcomas and Hodgkin's disease. *Neoplasma 21*, 51 (1974).

8. Fleischmann, T., Håkansson, C. H., Levan, A.: Fluorescent marker chromosomes in malignant lymphomas. *Hereditas 69*, 311 (1971).
9. Fleischmann, T., Gustaffson, T., Håkansson, C. H., Levan, A.: The fluorescent pattern of normal chromosomes in biopsies of malignant lymphomas, and its computer display. *Hereditas 70*, 75 (1972).
10. Fleischmann, T., Håkansson, C. H., Levan, A., Möller, T.: Multiple chromosome aberrations in a lymphosarcomatous tumor. *Hereditas 70*, 243 (1972).
11. Fleischmann, T., Håkansson, C. H., Levan, A.: Chromosomes of malignant lymphomas. Studies in short-term cultures from lymph nodes of twenty cases. *Hereditas 83*, 47 (1976).
12. Fitzgerald, P. H., Hamer, W. J.: Third case of chronic lymphocytic leukaemia in a carrier of the inherited Ch^1 chromosome. *Brit. med. J. 3*, 752 (1969).
13. Gunz, F. W., Fitzgerald, P. H., Adams, A.: An abnormal chromosome in chronic lymphocytic leukaemia. *Brit. med. J. 2*, 1097 (1962).
14. Lobb, D. S., Reeves, B. R., Lawler, S. D.: Identification of isochromosome 17 in myeloid leukaemia. *Lancet 1*, 849 (1972).
15. Miles, G. P.: Chromosomal aberrations in cancer. *Med. Clin. N. Amer. 50*, 875 (1966).
16. Millard, R. E.: Chromosome abnormalities in the malignant lymphomas. *Europ. J. Cancer 4*, 97 (1968).
17. Mitelman, F., Brandt, L., Levan, G.: Identification of isochromosome 17 in acute myeloid leukaemia. *Lancet 2*, 972 (1973).
18. Moorhead, P. S., Mowell, P. C., Mellman, W. J., Batipps, D. M., Hungerford, D.: Chromosome preparations of leukocytes from human peripheral blood. *Exp. Cell Res. 20*, 613 (1960).
19. Ricci, N., Punturieri, E., Bosi, L., Castoldi, G. L.: Chromosomes of Sternberg-Reed cells. *Lancet 2*, 564 (1962).
20. Sandberg, A. A., Ishihara, T., Kikuchi, Y., Crosswhite, L. H.: Chromosomes of lymphosarcoma and cancer cells in bone marrow. *Cancer 17*, 738 (1964).
21. Sasaki, M. S., Sofuni, T., Makino, S.: Cytologic studies of tumors. XLII. Chromosome abnormalities in malignant lymphomas in man. *Cancer 18*, 1007 (1965).
22. Seif, G. F. S., Spriggs, A. F.: Chromosome changes in Hodgkin's disease. *J. nat. Cancer Inst. 39*, 557 (1967).
23. Spiers, A. S. D., Baikie, A. G.: Cytogenetic studies in the malignant lymphomas. *Lancet 1*, 506 (1966).
24. Spiers, A. S. D., Baikie, A. G.: Cytogenetic studies in the malignant lymphomas and related neoplasms. *Cancer, 22*, 193 (1968).
25. Spiers, A. S. D., Baikie, A. G.: A special role of the group 17, 18 chromosomes in reticuloendothelial neoplasia. *Brit. J. Cancer 24*, 77 (1970).
26. Spriggs, A. I., Boddington, M. M.: Chromosomes in Sternberg-Reed cells. *Lancet 2*, 153 (1962).
27. Stich, W., Back, F., Dormer, P., Tsirimban, A.: Doppel-Philadelphia-Chromosom und Isochromosom 17 in der terminalen Phase der chronischen myeloischen Leukämie. *Klin. Wschr. 44*, 334 (1966).
28. Sumner, A. T., Evans, H. J., Buckland, R. A.: A new technique for distinguishing between human chromosomes. *Nature* (Lond.) *232*, 31 (1971).
29. Sumner, A. T.: A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res. 75*, 304 (1972).
30. Tjio, J. H., Marsh, J. C., Whang, J., Frei, E.: Abnormal karyotype findings in bone marrow and lymph node aspirates of a patient with malignant lymphoma. *Blood 22*, 178 (1963).

Correspondence: Dr. T. Fleischmann, National Institute of Oncology, Ráth György u. 7, H-1525 Budapest, Hungary

Haematologia 11 (1—2), pp. 57—72 (1977)

Десятилетний опыт лечения миелобромолом больных миелофиброзом

Н. Ф. КЛИМОВА

Научно-поликлиническое отделение (заведующая-проф. Г. В. Осеченская) Центрального ордена Ленина института гематологии и переливания крови (директор—член-корреспондент АМН СССР проф. О. К. Гаврилов) Министерства здравоохранения СССР (Москва)

(Сообщение поступило 6 мая 1976 года)

Идиопатический миелофиброз в настоящее время рассматривается как миелопролиферативное заболевание, характеризующееся фиброзной мезенхимной пролиферацией костного мозга.

Спленомегалия у больных миелофиброзом обусловлена не только компенсаторным механизмом, развивающимся в результате фиброретикулярной пролиферации костного мозга, но является выражением такого же процесса и в селезенке.

У больных миелофиброзом, помимо фиброретикулярной гиперплазии, наблюдается повышенное образование лейкоцитов, тромбоцитов и эритроцитов.

Заболевание протекает с прогрессированием сплено- и гепатомегалии, которые часто определяют тяжесть состояния, создавая абдоминальный дискомфорт.

С целью сокращения размеров селезенки и печени, в 1964 г. мы впервые применили препарат МИЕЛОБРОМОЛ-дибром-1,6-дидезокси-Д-манит (ДБМ), венгерское средство, изготовленное заводом „ХИНОИН".

В 1967 г. мы сделали первое сообщение о положительном влиянии МИЕЛОБРОМОЛА при миелофиброзе.

В настоящей работе обобщены результаты лечения ДБМ больных миелофиброзом за период с 1967 по 1974 гг.

Лечение проведено 65 больным, из них 35 женщин и 30 мужчин, в возрасте от 31 года до 71. Давность заболевания: от нескольких месяцев до 27 лет (от момента установления диагноза).

Надо отметить, что диагноз идиопатического миелофиброза у этих больных, хотя клинически и не вызывал сомнений, был подтвержден еще и цитохимическим, и цитогенетическим методами.

Методом трепанобиопсии у всех больных выявлен фиброз различной степени. Степень фиброза варьировала от вовлечения небольших участков костного мозга до диффузного, полностью вытесняющего кроветворную ткань. У 35 человек, наряду с фиброзом костного мозга, отмечались участки новообразования кости.

МИЕЛОБРОМОЛ назначался при выраженной сплено- и гепатомегалии, вызывавших дискомфорт в брюшной полости, мучительных оссалгиях, полиглобулии, гипертромбоцитозе.

В зависимости от гематологических показателей больные были разделены на три группы:

1-я. группа — 49 человек с сублейкемическим лейкоцитозом, нерезко выраженной анемией или нормальными показателями красной крови, нормальным количеством тромбоцитов или умеренным тромбоцитозом;

2-я. группы — 6 больных с явлениями геморрагической тромбоцитемии, развившейся после спленэктомии;

3-я. группа — 9 больных с полиглобулией.

Первая группа

7 больных из 49 прежде получали цитостатическую терапию; миелосан, допан и миелосан, миелосан и рентгенотерапию на область селезенки без положительного эффекта и циклофосфан с положительным, но кратковременным эффектом.

Основной жалобой всех больных была общая слабость, дискомфорт в брюшной полости из-за увеличения селезенки и печени, у двух больных селезенка достигала гигантских размеров, фактически лишившая их активного движения, большую часть времени они были вынуждены проводить в постели.

В периферической крови число лейкоцитов, хотя и было увеличено у всех больных, но, как правило, не превышало 65000 б1 мм^3, содержание незрелых гранулоцитов составляло 3—15—30%. Периодически встречались бластные формы от 0,5 до 2,5%, и только у трех больных достигали 6,5—8 и 11,5%.

Ни у одного больного не отмечено эозинофильно-базофильной ассоциации, столь характерной для хронического миелолейкоза.

Число тромбоцитов у 46 больных было в пределах нормы, у трех — умеренный тромбоцитоз в пределах 554—635 000 в 1 мм^3, и у трех — умеренная тромбоцитопения — 102—125—175 000 в 1 мм^3.

Нормохромная анемия отмечалась у 18 больных, выраженная (7,4—8,8г%) у четырех и умеренная (9—11,4%) — у 14.

В периферической крови неоднократно наблюдались ядерные формы красных клеток; в большинстве случаев они не превышали 5-3%, у отдельных больных они увеличивались до 8—10%.

В миелограмме — (22 больных), у 10 больных количество миелокариоцитов было нормальным (51 400—130 000) и у 12 – резко-сниженным (3500—29 000), бластные формы (до 4,4—7%) выявлялись у отдельных больных (5), незрелые гранулоциты, снижены либо в пределах нормы и только у двух —

повышено (до 42,8—44,2%); зрелые гранулоциты составляли 31—66,8%. У большинства больных (14 человек) наблюдалось снижение эритро- и нормобластов (0,8—12,5%), у остальных (восьми оно было) в пределах нормы. Обращало на себя внимание наличие в костном мозге большого количества свободно лежащих тромбоцитов.

Препарат мы назначали по 250 мг в день в течение 2—4 недель, затем доза уменьшалась до 125 мг., при количестве лейкоцитов 18—25 000 и числе тромбоцитов 180—200 000. При лейкоцитозе ниже 18 000 и количестве тромбоцитов 120 000 миелобромол назначался в сочетании с преднизолоном. Если число лейкоцитов не превышало 15 000 и тромбоциты составляли 100—110 000, то за одну-две недели до приема миелобромола назначали преднизолон (15—20 мг pro die). Обычно на 4—5-й день приема препарата наблюдался подъем лейкоцитов, а затем число их медленно снижалось.

Препарат отменяли в зависимости от темпа снижения лейкоцитов до 6—7—8000 и снижения тромбоцитов до 100—110 000.

При тенденции к интенсивному уменьшению количества лейкоцитов или тромбоцитов, при еще выраженной спленомегалии, назначали стероидные и анаболические гормоны.

У всех больных уже на 6—9—12-й день приема препарата значительно сокращались в размерах селезенка и печень, хотя нормализации размеров селезенки не было получено ни у одного больного. Надо отметить, что у трех больных селезенка и у семи печень после проведенного лечения стали пальпироваться у реберной дуги. Максимально размеры селезенки сокращались от 32—40—28 см до 18—32—18 см, минимально от 28—39—27 см до 20—32—22 см, печень с 10 до 1 см и с 7 до 5 см.

У всех больных исчезло чувство абдоминального дискомфорта, двое больных с гигантской селезенкой после проведенного лечения также вернулись к привычному образу жизни.

После отмены миелобромола наблюдалось последующее снижение числа лейкоцитов в течение 6—12 дней; причем у подавляющего большинства не ниже 3700—5000 и у 8 — до 1050—2100, и лишь у одной больной после приема 9250 мг наблюдалась глубокая лейкопения: 900 в 1 мм^3. Терапия стероидными и анаболическими гормонами способствовала нормализации числа лейкоцитов. У остальных 8 больных нормализация лейкопоэза наступила без применения каких-либо стимулирующих средств. У всех больных, за исключением трех, отмечено улучшение состава лейкоцитарной формулы, наблюдалось уменьшение незрелых гранулоцитов с 20—30% до 5—19% и с 20—15% до 2%—0, исчезли бластные формы с 6,5—8—11,5% до 0.

Угнетения тромбоцитопоэза у 34 больных не отмечено. Падение числа тромбоцитов ниже 100 000 (37—82 000) выявлено у 12 больных на 12—18-й день после отмены препарата, и у трех лечение миелобромолом было прекращено из-за снижения числа тромбоцитов (до 59—93 000); надо отметить, что миелобромол эти больные начали принимать при относительно низких показателях (102—125 и 175 000).

Уровень гемоглобина и число эритроцитов за период лечения миелобромолом оставались на исходном уровне. После окончания курса лечения снижение показателей красной крови наблюдалось у 10 больных; у 6 умеренное и у 4 выраженное (с 15 г% до 9,2, с 11,6—12 до 9,2—8,8 г%), у 29 больных гемоглобин и эритроциты держались на исходном уровне. У 5 больных с анемией сочетание лечения ДБМ с анаболическими и кортикостероидными гормонами способствовало повышению уровня гемоглобина и количеству эритроцитов (с 8—8,4 до 10,2—11,6 г% и с 9—10,6 до 11,8—12 г%).

Панцитопения, после применения лечебных доз миелобромола (4750—7000), развилась у 8 из 49 больных. Миелобромол у них отменен при нормальных или умеренно сниженных показателях красной крови, тромбоцитов и лейкоцитов (табл. 1).

Таблица 1

Развитие панцитопении после лечения миелобромолом

Инициалы больных	Возраст	Пол	Давность заболевания	Курс лечения	Суммарная доза ДБМ (мг)	ДБМ отменен при			Развитие панцитопении после отмены ДБМ (в днях)
						Гемоглобин (в г%)	Лейкоциты (в тыс.)	Тромбоциты (в тыс.)	
Ч. Н.	45	ж	12	7	5500	11,7 / 7,7	6,2 / 0,9	239,0 / 58,2	25
П. Е.	51	ж	16	1	4750	10,8 / 7,0	6,0 / 1,7	352,0 / 48,0	30
Л. М.	69	ж	9	1	5500	16,4 / 7,8	4,9 / 1,6	204,0 / 15,0	28
М. И.	67	ж	12	1	5500	12,0 / 8,4	8,0 / 0,6	175,2 / 12,5	12
З. Е.	63	ж	3	1	5000	8,0 / 5,6	8,0 / 0,4	567,0 / 50,0	25
В. А.	63	м	33	4	5000	11,5 / 6,8	5,1 / 0,5	201,0 / 32,0	8
К. М.	68	ж	15	1	7000	10,0 / 4,2	4,2 / 2,9	169,1 / 28,1	18
К. М. И.	63	ж	6	3	7250	11,0 / 7,6	8,0 / 1,4	191,3 / 22,0	4

Угнетение кроветворения наступало в различные сроки: на 8—12—18—30-й день после отмены миелобромола (в основном, после первого курса — у 5 больных, третьего и четвертого курса — у 2 больных и после седьмого курса лечения — у одного больного). Вначале снижалось количество лейко-

цитов, затем тромбоцитов и в последнюю очередь наступало угнетение эритропоэза (см. табл. 1).

Глубокая лейкопения (ниже 1000) наблюдалась у 4 больных; 400—500—600—900 с наличием в формуле миелоидных элементов — 28—41—32—23%, соответственно. У других 4 больных лейкоциты уменьшались до 1000—2950 в 1 мм^3. У всех больных наступила глубокая тромбоцитопения (в пределах 12500—50 000 в 1 мм^3); при этом геморрагии в виде кровоизлияний на коже наблюдались у трех больных. Анемия (в пределах 8,4—4,2 г%) (табл. 1).

С целью купирования панцитопении назначали стероидные (40—60 мг) и анаболические гормоны (40 мг) в сочетании с гемотрансфузиями. Подобная терапия проведена больным в условиях стационара с положительным эффектом. Таким образом, все больные были выведены из состояния гипоплазии костного мозга.

При повторных курсах лечения панцитопения наступила у одной больной (М) после приема минимальной дозы миелобромола (3000 мг), что было обусловлено повышенной чувствительностью больной к препарату.

После окончания курса лечения миелобромолом (22 больных) проведено исследование костного мозга, показавшее значительное уменьшение общего количества миелокариоцитов у всех больных. При подсчете миелограмм у 6 больных особых изменений, по сравнению с миелограммами до лечения, не выявлено: у 7 наблюдалась нормализация недифференцированных клеток и увеличение зрелых гранулоцитов почти вдвое, у 5 больных выявлено значительное увеличение эритро- и нормобластов (с 17,8 до 38,4, с 0,8 до 9 и т. д.), а у двух — резкое снижение их (с 26,6 до 9,5% и с 20 до 3%).И лишь у одной больной отмечена выраженная редукция гранулопоэза: у нее в миелограмме 70% составляли лимфоциты при 3400 лейкоцитах в периферической крови.

Гистологическое исследование костного мозга после окончания курса лечения миелобромолом проведено 15 больным; при этом у 12 из них обращало на себя внимание уменьшение количества клеточных элементов миелоидного ряда и мегакариоцитов, в то время как эритропоэз оставался прежним, либо несколько увеличенным (у трех больных).

Обращало на себя внимание наличие очагов острого опустошения стромы костного мозга и кровоизлияний. Фиброретикулярная ткань исчезала полностью или становилась более нежной, и количество ее уменьшалось (рис. 1), однако следует отметить неравномерность изменений в костном мозге; если в одних участках костного мозга наблюдались вышеописанные изменения, то в других полостях костного мозга сохранялась миелоидная гиперплазия и выраженный фиброз костного мозга.

Курс лечения миелобромолом продолжался от 17 до 40 дней. Максимальная доза препарата — 10 000 мг, минимальная — 3000 мг, средняя курсовая доза составила 6200 мг. Длительность ремиссии: минимальная — 3 месяца, максимальная — 7 лет, средняя — 15 месяцев.

Таблица 2

Клинические и гематологические показатели больных миелофиброзом с явлениями гемор-

Инициалы	Возраст	Пол	Давность заболевания	Предшествующее лечение — спленэктомия	Клинические проявления
Ш. П.	43	ж	1968	68 г., миелосан	Десневые, желудочно-кишечные кровотечения, тромбозы мезентериальных сосудов
К. М.	63	ж	1962	67 год	Десневые кровотечения
А. В.	57	м	Не установлена	71 год	Десневые кровотечения
Л. А.	56	ж	1963	64 год	Тромбозы нижних конечностей, инфаркт миокарда
С. В.	48	ж	1969	69 год	Носовые и десневые кровотечения, асцит
И. П.	33	м	Не установлена	71 год	Желудочно-кишечные, носовые, почечные кровотечения, тромбозы мезент. сосудов

Таким образом, миелобромол сокращал размеры селезенки и печени, снимал оссалгии. Миелобромол воздействовал на фиброретикулярную ткань, гранулопоэз, тромбопоэз и мало на эритропоэз.

Во вторую группу включено 6 больных с идиопатическим миелофиброзом, протекающим с явлениями геморрагической тромбоцитемии, развившейся после спленэктомии. Основными клиническими проявлениями у больных этой группы были геморрагии, тромбозы, гепатомегалия. Печень выступала из-под реберной дуги от 1,5 до 11 см (табл. 2).

У одной больной (С) гепатомегалия сопровождалась нарушение и портального кровообращения с развитием венозной сети на коже брюшной стенки живота и асцита.

У четырех больных заболевание протекало с геморрагиями (десневые, носовые, кишечные, почечные), у трех — с тромбозами (у двух — мезентериальных сосудов и у одной больной — сосудов нижних конечностей). Основным гематологическим признаком у всех больных был гипертромбоцитоз, у пяти количество тромбоцитов достигало 3—3 610 000, а у одной больной не превышало 516 000 в 1 мм^3.

Количество лейкоцитов у пяти больных колебалось в пределах 15,5—84 000, у одного — 7120. В лейкоцитарной формуле сдвиг до бластных форм (1,5—4%) у трех, у всех обнаружены незрелые гранулоциты в пределах 0,5—4%, а у одной больной — 25%. Как правило, обнаруживались эритро- и нормобласты (1—12%); у одного больного они составили 81,5%.

рагической тромбоцитемии до и после лечения миелобромолом

Гемоглобин г. %	Эритроциты, млн	Лейкоциты, тыс	Тромбоциты, тыс	Бласты, %	Незрелые гранулоциты %	Эритро- и нормобласты %	Печень: ниже реберной дуги	Доза ДБМ, мг
7 / 9	2,65 / 3,52	23,4 / 8,0	3000 / 650		11,5 / 0,5	12 / 5	5	6500
3 / 8	1,5 / 3,1	48 / 6	1490 / 650		2 / 0	10 / 4	11 / 6	3000
4,3 / 5,0	2,0 / 2,7	7,12 / 4,6	2000 / 140	1,5 / 0	2,5 / 0	81,5 / 9	6 / 4	2500
12,6 / 13,8	4,0 / 4,1	84 / 50	516 / 200	4 / 2	35 / 8	4 / 0	11 / 5	5000
10 / 11,6	3,1 / 3,6	18,4 / 7,6	1202 / 600	2 / 0	4 / 0	1 / 0	11 / 6	3000
11,2 / 7,6	3,3 / 3,5	15,5 / 7,2	2580 / 361	0	5 / 2	3,5 / 0	1,5	8700

Глубокая анемия Нв. 3—4—7 г.% наблюдалась у трех, умеренная (11,2—10 г.%) у двух больных.

В миелограмме преобладали зрелые гранулоциты. У всех выявлено сужение красного ростка (до 8,6—2,6%), скопление свободно лежащих тромбоцитов.

С целью подавления гипертромбопоэза и миелоидной метаплазии в печени мы решили применить миелобромол.

Препарат назначали по 250 мг в сутки в сочетании с нероболом (30—40 мг) и для одного больного — с гемотрансфузиями и антикоагулянтами. Курсовая доза составила 3000—5000, а у одного больного 8600 мг.

Миелобромол отменяли, в зависимости от темпа снижения лейкоцитов, при 7,2—7,6—8000. У одного больного лечение было начато при 7120 и прекращено при 4650.

У всех больных получен положительный клинико-гематологический эффект — сократилась в размерах печень: у четырех — с 11—6 до 6—4 см, у двух — стала пальпироваться в подреберье, исчез асцит, прекратились геморрагии и болевой синдром, обусловленный тромбозами.

У всех больных снижалось количество тромбоцитов, у двух — до 140—200 000, у других — до 600 000 в 1 мм^3 с последующей нормализацией. Угнетения лейкопоэза не отмечалось ни у одного больного, у всех улучшился состав лейкоцитарной формулы. Следует подчеркнуть благополучное влияние на эритропоэз миелобромола в сочетании с нероболом у этой группы

Таблица 3

Клинические и гематологические показатели больных миелофиброзом с полиглобулией

Инициалы	Возраст и пол	Предшествующее лечение	Результаты	Гемоглобин, г. %	Эритроциты, млн
К. М.	57, ж	—	—	19 13,2	7,9 5,32
О. А.	52, ж	Миелосан	Без эффекта	20,4 13	7,51 4,36
Х. К.	48, ж	Миелосан	Без эффекта	17,4 16,4	7,64 6,0
К. Е.	49, ж	Миелосан	Без эффекта	16,8 15,8	6,8 6,0
К. А.	50, ж	—	—	16,8 10,8	6,4 4,0
В. А.	47, ж	ДБМ	+	18 12	6,9 4,5
Д. Ю.	62, ж	Миелосан Р-32	Без эффекта	18,8 17,4	9,0 6,4
К. М.	55, м	—	—	15,2 14	6,6 5,0
С. М.	37, ж	—	—	17 14	5,5 4,3

больных. У всех больных улучшился состав красной крови: повысился уровень гемоглобина, значительно уменьшилось количество эритро- и нормобластов (с 10—12 до 4—5%, с 81,5 до 9%) и у трех больных они исчезли совсем. Стернальная пункция больным после лечения не проводилась. Средняя продолжительность ремиссии составляла 5 месяцев (максимальная — 48 месяцев, минимальная — 2 недели).

Таким образом, МИЕЛОБРОМОЛ оказался эффективным средством и у больных миелофиброзом, протекающим с явлениями геморрагической тромбоцитемии и гепатомегалией. Препарат сокращал размеры печени. Миелобромол воздействовал на гиперплазированный тромбопоэз, способствовал его нормализации и прекращению геморрагий, не влиял на гранулопоэз, а сочетание миелобромола с нероболом положительно влияло на эритропоэз.

Третья группа больных (9 человек) идиопатическим миелофиброзом, характеризовалась эритремическими показателями.

до и после лечения ДБМ

Лейкоциты, тыс	Тромбоциты (в тыс.)	Бласты %	Незрелые гранулоциты в %	Эритронормобласты в %	ниже реберной дуги (см)		Курсовая доза ДБМ (в мг)
					селезенка	печень	
22,6 / 4,8	282,9 / 218	—	10 / 0	2 / 0	15 / 5	12 / 2	9250
18,6 / 5,2	459,0 / 225,5	—	—	2 / 0	8	3	7000
10,6 / 5,6	388,0 / 300,0	1 / 0	1,5 / 0,5	5,5 / 0	17 / 8	8 / 5	7750
20,6 / 5,2	532 / 107,0	—	0,5 / 0	1 / 0	8 / 4	9	8250
14,2 / 6,4	145,0 / 69,4	—	16 / 3	1 / 0	31 / 16	9 / 5	8000
17,8 / 10,6	195,4 / 117,8	0	3 / 0	1 / 0	6	—	6050
16 / 20	117 / 27,3	—	5,5 / 3	2 / 0	25 / 15	3	6725
18,4 / 5,4	515 / 192	—	0	3 / 0	13 / 7	4 / 1	9000
17,6 / 5,7	420 / 211	—	0 / 0	1 / 0	9 / 2	19 / 10	4500

Эритремические показатели определялись с самого начала заболевания у шести больных, у одной на втором и другой на девятом году заболевания. В начале заболевания трем больным была диагностирована эритремия и проведено лечение миелосаном, другом — миелосаном и P^{32} без положительного терапевтического эффекта (табл. 3.).

Основные жалобы больных: слабость, головная боль, головокружение, боли в костях, дискомфорт в брюшной полости из-за резко увеличенной селезенки, чувство онемения в ногах. У всех больных выявлена гипертония.

Размеры селезенки у двух больных достигали 25—39—25 см и 31—40—30 см, печень выступала из-под реберья на 4 и 8 см, у остальных больных селезенка пальпировалась из-под реберья от 6 до 19 см, а печень от 2—14—20 см. У всех больных число лейкоцитов в периферической крови было повышенным, однако не превышало 22 600 в 1 $мм^3$, со сдвигом до бластов (1%) у одного больного, у четырех — до миелоцитов (1,5—16%) и у трех

было увеличено число палочкоядерных (13—14%). Число тромбоцитов было в пределах нормы у 5 больных, у двух понижено до 145—117 000 в 1 мм^3 и у двух повышено до 513—530 000. Уровень гемоглобина был в пределах от 20,4 до 15,8 г.%, эритроциты 6070—7900 000, высокий гематокрит, вязкость крови 7—14.

При стернальной пункции аспирация костно-мозгового содержимого была затруднена, а у двух больных костный мозг не получен. Содержание миелокариоцитов в костном мозгу у четырех — в норме, у трех понижено. В миелограмме бласты не превышали 1—2,2%, незрелые гранулоциты 3—18%, зрелые гранулоциты составляли 33,8—69,4%. Эритро- ц нормобласты у 5 больных в пределах нормы (24,8—19,6), у 3 — снижены — 4,8—12% и у одной больной повышены до 38%.

Миелобромол, как и больным предыдущих групп, назначали по 250 мг в день, а в дальнейшем, в зависимости от степени снижения числа лейкоцитов и тромбоцитов, суточную дозу препарата уменьшали до 125 мг. Для достижения ремиссии был необходим курс лечения миелобромолом длительностью в 32—43 дня и только у одной больной в 18 дней.

Минимальная курсовая доза — 4500 мг, максимальная — 12 000 мг, средняя —7250 мг. Максимальная длительность ремиссии —48,6 месяцев, минимальная — 6 месяцев, средняя — 24,5.

В результате лечения миелобромолом состояние всех больных улучшилось, исчезла головная боль, нормализовался цвет кожи лица и видимых слизистых, артериальное давление также оказалось в пределах нормы.

Следует особо подчеркнуть свойство миелобромола оказывать положительное влияние на оссалгии, которые в результате приема препарата полностью исчезали. Предшествующее лечение миелосаном P^{32} обычно не способствовало уменьшению болей в костях.

У всех больных значительно уменьшились размеры селезенки: так, у четырех она стала пальпироваться в подреберье, до лечения ее были размеры 4—12—8, 8—20—24, 5—13—10, 6—17—14 см, по Курлову.

Под влиянием миелобромола число лейкоцитов медленно снижалось, и при достижении 5200—6000 лечение прекращали; причем у одного больного лечение было прекращено из-за снижения тромбоцитов до 113 000.

После отмены миелобромола наблюдалось последующее снижение содержания лейкоцитов в течение 6—12—18 дней, у двух — до 250—750 в 1 мм^3, у трех — до 1650—2200, у четырех — до 3500—6000 в 1 мм^3.

Следует отметить, что у больной 0 миелобромол был отменен при 5250 лейкоцитах, а снижение до 250 (с 8% миелоидных элементов) произошло на 18-й день после прекращения приема препарата с одновременным уменьшением числа тромбоцитов с 225 000 до 0. Под влиянием гемотрансфузий, однократной инфузии лейкоцитной массы, преднизолона уже на 5-й день число лейкоцитов и тромбоцитов начало постепенно увеличиваться и на 30-й день достигло 5400 и тромбоциты 295 720 в 1 мм^3. При проведении последующих курсов лечения миелобромолом в общей дозе 12—8—4500 мг, коли-

чество лейкоцитов снижалось до 1580—1650—1800 в 1 мм^3, а тромбоцитов до 74—94 000. Через три недели после отмены миелобромола без применения каких-либо гемостимулирующих средств число лейкоцитов и тромбоцитов нормализовалось. При последующих двух курсах лечения миелобромолом количество лейкоцитов не уменьшалось ниже 5200, а тромбоцитов ниже 100 000 в 1 мм^3.

Больная *К.* получала миелобромол ежедневно по 250 мг в течение 28 дней, при снижении числа лейкоцитов до 10 000 по 125 мг в течение 10 дней, миелобромол был отменен, когда число лейкоцитов достигло 5250. На 7-й день после отмены миелобромола число лейкоцитов снизилось до 750 в 1 мм^3 (с 24% миелоидных элементов), тромбоцитов до 64 000. Через неделю без применения каких-либо гемостимулирующих средств число лейкоцитов возросло до 4000 и 217 5000. При проведении повторных курсов лечения миелобромолом депрессии лейко- и тромбопоэза не наступало.

У больного *Д.* миелобромол был отменен при снижении содержания тромбоцитов до 27 360, на 6-й день после отмены препарата число тромбоцитов снизилось до нуля. Ввиду высоких показателей красной крови (Нв. 17,4%, эритроцитов до 6 420 000), учитывая свойства эритроцитов принимать участие в гемостазе, а также отсутствие каких-либо геморрагических проявлений, терапию, стимулирующую тромбопоэз, не проводили. Через 40 дней количество тромбоцитов возросло до 376 420. Повторный курс лечения не вызывал тромбоцитопении.

У другой больной (К. А.) содержание тромбоцитов снизилось до 69 410, но через две недели оно нормализовалось.

В результате лечения миелобромолом у всех больных было достигнуто снижение уровня гемоглобина и числа эритроцитов. У больного *Д.* после длительного повторного курса ДБМ в общей дозе 21 250 удалось добиться снижения уровня гемоглобина до 14 г%, эритроцитов до 4 200 000. Вязкость крови у всех больных снизилась до нормального уровня. Лейкоцитарная формула крови улучшилась у всех больных, исчезли или уменьшились незрелые гранулоциты. У всех больных повысилась РОЭ (с 0,5 до 5—10 мм в час).

Трем больным после проведенного лечения миелобромолом произведена стернальная пункция (у одной больной костно-мозгового содержимого получить не удалось). Отмечалось снижение содержания миелокариоцитов, некоторое уменьшение незрелых гранулоцитов, увеличение эритро- и нормобластов (с 11,6 до 25,2, с 21,4 до 30,8%, с 20,6 до 59,2%).

Таким образом, миелобромол оказался эффективным средством при лечении больных идиопатическим миелофиброзом, протекающим с полиглобулией и выраженной сплено- и гепатомегалией, оссалгией. Следует отметить положительное действие миелобромола у больных резистентных к другим цитостатическим средствам (миелосан P^{32}).

Миелобромол хорошо переносился больными и не вызывал никаких побочных явлений.

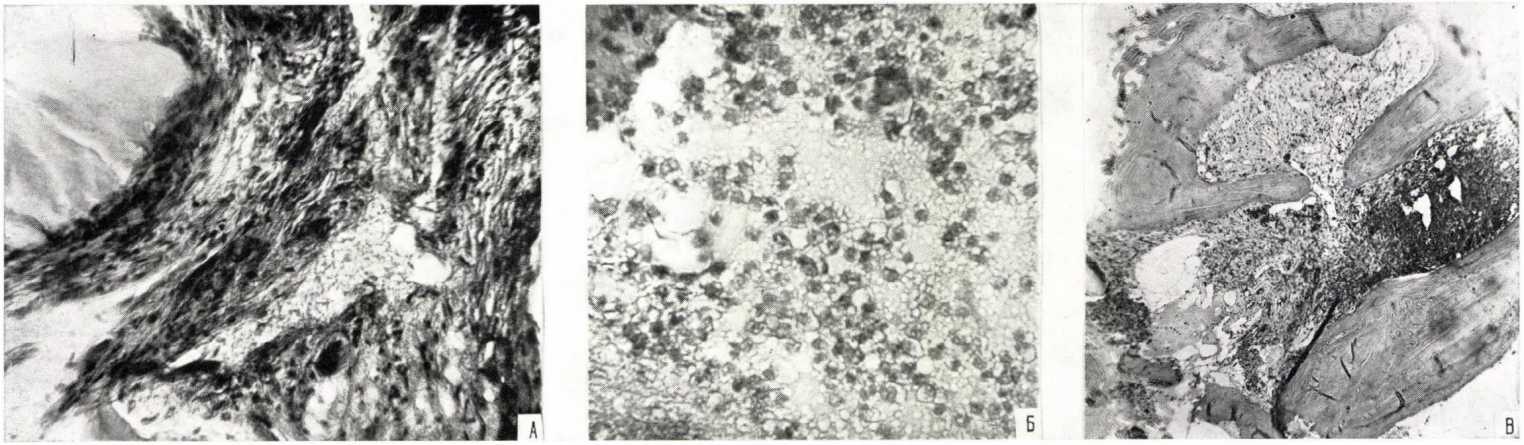


Рис. 1. Трепанобиопсия: А) до лечения; Б) после лечения; В) неравномерность изменений в костном мозгу

В статье представлен десятилетний опыт лечения миелобромолом больных миелофиброзом в различных стадиях заболевания. Выявлена эффективность миелобромола, разработаны показания для его применения с учетом выявленного минимального побочного действия миелобромола на лейкотромбо-и эритропоэз.

В результате терапии миелобромолом состояние больных улучшалось, проходила слабость, улучшался аппетит, увеличивался вес, возвращалась трудоспособность. Резистентности к миелобромолу не наблюдалось, что позволило проводить повторные курсы терапии.

После первого курса лечения миелобромолом у всех 65 больных было достигнуто клинико-гематологическое улучшение (табл. 4). В связи с обострением заболевания повторный курс проведен 43 больным также с положительным терапевтическим эффектом, за исключением двух больных; одному лечение миелобромолом прекращено из-за снижения числа тромбоцитов (при дозе 3000), а у другого больного лечебная доза 5375 мг не дала никаких улучшений.

Три курса лечения миелобромолом получил 31 больной, у 29 эффект был положительный. У двух клинико-гематологического улучшения не получено (из-за быстрого снижения числа тромбоцитов и лейкоцитов).

Четыре курса лечения получили 25 больных: у 24 с положительным эффектом. У одного больного улучшения не наступило.

При проведении последующих курсов лечения (до 10) у всех получен положительный эффект.

Надо отметить, что 5 больных, у которых миелобромол не дал положительного эффекта, находились в тяжелом состоянии, четверо из них в терминальной стадии.

После первого курса лечения миелобромолом средняя ремиссия была более длительной (15 месяцев), чем после последующих (10—7 мес), тогда как доза миелобромола была примерно одинаковой (6600—5750 мг). Максимальный срок ремиссии — 7 лет (дальнейшее наблюдение прервано), минимальный — 2 недели — 3 месяца, средняя продолжительность ремиссии — 15 месяцев (табл. 4).

Надо иметь в виду, что в период лечения или вскоре после окончания курса из-за повышенного распада миелокариоцитов может развиться уремия и повышенное суточное выделение мочевой кислоты.

У четырех больных в период лечения развилась почечная колика, у восьми больных — признаки подагры: артриты с выраженным болевым синдромом, лихорадкой, воспалительным процессом, а у одной больной — с поражением кожи. У всех этих больных выявлено значительное повышение мочевой кислоты в сыворотке крови и моче. Принимая во внимание возможность возникновения подобных осложнений в процессе лечения, рекомендуется назначать, особенно при повторных курсах, цитратные смеси, щелочи *per os*, аллопуринол.

Как видно из приведенных данных, миелобромол является эффективным средством лечения больных миелофиброзом. Препарат сокращает размеры печени и селезенки, снимает оссалгии, клинические проявления геморрагической тромбоцитемии и полиглобулии. Миелобромол оказывал действие на фиброретикулярную ткань, миелоидную гиперплазию, гиперплазированный тромбо- и эритропоэз. При этом наиболее чувствительным

Таблица 4

Результаты лечения миелобромолом больных миелофиброзом

Курс лечения	Количество больных			Средняя доза препарата на курс, *мг*	Осложнения			Геморрагии	Средняя продолжительность ремиссии, *мес*
	всего	в состоянии ремиссии	без эффекта		Лейкопения (до 2000)	Тромбоцитопения (ниже 100 000)	Панцитопения		
I	65	65	0	6300	9	12	5	1	15,0
II	43	41	2	6325	4	9	1	1	10,0
III	31	29	2	6600	2	7	1	—	10,0
IV	25	24	1	6000	2	8	1	1	8,0
V	16	16	—	5750	2	2	—	—	10,8
VI	12	12	—	5750	0	2	—	—	7,0
VII	6	6	—	6450	1	0	1	—	8,4
VIII	6	6	—	6250	1	1	—	—	6,0
IX	4	4	—	6500	0	0	—	—	6,0

к действию миелобромола оказался тромбопоэз; для подавления гиперплазированного тромбоцитопоэза было достаточно в среднем 4000 мг, эритропоэза 7125 мг, а фиброретикулярной пролиферации 6300 мг.

После отмены препарата через две недели развивается депрессия лейко- и тромбопоэза, которая обычно быстро и самостоятельно восстанавливалась. Гипоплазия гемопоэза развивалась редко (у восьми больных из 65); в подобных случаях назначение кортикостероидов и анаболических гормонов в сочетании с гемотрансфузиями способствовало купированию депрессии гемопоэза.

Резистентности к миелобромолу не наблюдалось, за исключением двух больных, которые находились в крайне тяжелом состоянии, в терминальной стадии заболевания. Отсутствие резистентности к миелобромолу позволяет проводить повторные курсы лечения, также с положительным терапевтическим эффектом (до 10 курсов). Более длительная ремиссия наблюдается после первого курса лечения (табл. 4).

Миелобромол оказался эффективным даже после безуспешного применения миелосана, допана, рентгенотерапии и P^{32}.

Поддерживающее лечение миелобромолом проведено восьми больным в дозе 125—250 мг, в зависимости от числа лейкоцитов и тромбоцитов, через день или два в неделю. Однако длительное наблюдение за больными показало, что поддерживающие дозы не способствовали удлинению ремиссий. Кроме того, лечение поддерживающими дозами требует довольно частого контроля количества лейкоцитов и тромбоцитов, что не всегда осуществимо из-за дальности расстояний. Поэтому в дальнейшем мы отказались от проведения поддерживающей терапии.

Из 65 больных умерло 32, за пятью наблюдение преравно и за 28 наблюдение продолжается.

Из 32 умерших больных у 6 лечение было начато в терминальной стадии на 3—15—13—10—20-й год течения заболевания; четверо из них после проведенного лечения прожили еще в течение 1—4—5 лет.

Выводы

1. Миелобромол является эффективным средством лечения миелофиброза.

2. Миелобромол оказывает медленное цитостатическое действие не только на элементы гемопоэза, но и на фиброзную ткань. Как правило, через две недели после окончания курса лечения развивается депрессия лейко- и тромбопоэза, которые спустя некоторое время проходят.

3. Угнетение гемопоэза развивается редко, что является показанием для назначения гемотрансфузий, стероидных и анаболических гормонов.

4. Ремиссия, достигнутая лечением миелобромолом, продолжается в среднем 15 месяцев (средняя курсовая доза 6300 мг). Поддерживающее лечение миелобромолом предотвращает рецидив и не удлиняет продолжающейся ремиссии.

5. Ценным качеством миелобромола является отсутствие резистентности к препарату, что позволяет применять его для повторных курсов лечения.

Литература

1. Дульцин, М. С., Климова, Н. Ф.: Лечение больных идиопатическим миелофиброзом миелобромолом. Haematologia vol. 1, №3, стр. 276, 1967 г.
2. Климова, Н. Ф.: Лечение миелобромолом больных идиопатическим миелофиброзом в эритремической фазе. Пробл. гемат., №1, стр. 50 (1970 г.)

Correspondence: Dr. N. F. Klimova, Central Institute of Haematology and Blood Transfusion, Clinical Research Department, Novozykovsky 4, A-167, Moscow.

Ten Years Experience with Myelobromol Treatment of Myelofibrosis

The data of 65 patients treated with Myelobromol are reported. Myelobromol is an effective drug against the steadily progressing splenomegaly and hepatomegaly followed by abdominal discomfort, thrombocythaemia and polyglobulia with enlarged spleen and liver. The daily dose was 250 mg for two or four weeks, then 125 mg for another two weeks. The drug

exerted a slow cytotoxic effect on myelopoiesis, thrombopoiesis and erythrocytopoiesis, and also on fibrosis. After 15 days treatment leucopoiesis and thrombopoiesis were depressed temporarily. In eight cases pancytopenia resulted. The patients were given prednisone and androgen therapy and were treated with blood transfusions. The effect was positive in all the eight cases. There were no toxic side effects. As no drug resistance developed, treatment could be repeated later. The remission achieved after the first treatment lasted 15 months on the average. After repeated administration it lasted 7—10 months.

Haematologia 11 (1–2), pp. 73–77 (1977)

Nonsynthetizing Multiple Myeloma

J. AMIR, W. B. FORMAN, Ann RASSIGA, G. M. BERNIER

Division of Hematology-Oncology, Cleveland V. A. Hospital and Department of Medicine. Case Western Reserve School of Medicine, Cleveland, Ohio, USA

(Received October 5, 1976)

A case of multiple myeloma with no monoclonal protein synthesis is reported. The patient had hypogammaglobulinemia, bone marrow invasion, osteolytic lesions, and plasma cell tumors. The absence of protein synthesis has been demonstrated in bone marrow culture with tritiated leucine. No evidence of immunoglobulin production was found. Bone marrow or plasma cell tumor biopsy may be the only method of diagnosis in such cases.

Multiple myeloma is a malignant proliferation of plasma cells with invasion of the bone marrow, production of monoclonal protein or free light chains, found in the serum or urine and osteolytic lesions [1, 2]. These pathognomonic findings are seen in the vast majority of patients. However, in the last few years a number of patients have been described who have clinical multiple myeloma without the presence of a monoclonal protein [3–9]. These have been termed nonsecretory multiple myeloma. The present report deals with a patient with typical clinical multiple myeloma but with no paraproteinemia and Bence - Jones proteinuria. There was no evidence of intracellular M protein production.

Case Report

R. C., a 50-year-old Caucasian male, presented with a painless mass on the occipital region of the skull of two weeks' duration. There was no previous trauma, no fever and no weight loss. The patient complained of low back pain of several months' duration.

On physical examination the cardiovascular and pulmonary systems were normal. There was no adenopathy, hepatomegaly or splenomegaly. A soft non-tender mass, 20 cm in length and 10 cm in width, was palpated on the occipital area of the skull. There was mild tenderness over the lower lumbar region.

Laboratory data included a hematocrit of 41 %, white cell count of 5,500/cu. mm, with a normal differential count, platelet count of 350,000/cu.mm. Urinalysis showed a pH of 5.5, specific gravity 1.019 with 0–3 white cells per high power field. There were no plasma cells in the urinary sediment. There was proteinuria with 900 mg excreted in 24 hours. No Bence–Jones protein was found

by heat test. Electrophoresis of the concentrated urine did not reveal any monoclonal protein. The liver and kidney function tests were normal. The erythrocyte sedimentation rate was 12 mm in the first hour (Westergreen). The total plasma protein was 6.7 g/100 ml with albumin 4.3 and globulin 2.4 g/100 ml. Quantitative immunoglobulin levels by radial diffusion were: IgG 830 mg/100 ml, IgA 22 mg/100 ml and IgM 75 mg/100 ml. (Normal values: IgG 600–1400, IgA 100–350, IgM 50–150 mg/100 ml.) Skeletal roentgenograms showed numerous punched out bony lesions in the skull (Fig. 1), ribs, clavicles, thoracolumbar vertebrae, pelvis and right femur as well as widespread osteoporosis. A liver and spleen scan was normal. Bone marrow biopsy and aspiration from the iliac crest revealed a hypercellular marrow with 22% immature plasma cells. A biopsy from the mass in the skull showed numerous immature plasma cells (Fig. 2).

Five ml of bone marrow were cultured in Eagle's minimum essential medium less leucine, containing 20% decomplemented fetal calf serum and ^{3}H-L-leucine at 37 °C in 5% CO_2 for 96 hours. The supernatant was separated from the cells by centrifugation. The cells were washed and lysed by freezing and thawing. Aliquots from the supernatant and the lysed cells were then incubated for one hour with different monospecific rabbit antisera directed against the five heavy chains. Normal rabbit sera were used as controls. Following that a gamma G fraction of goat antiserum directed to rabbit gamma G was mixed with the in-

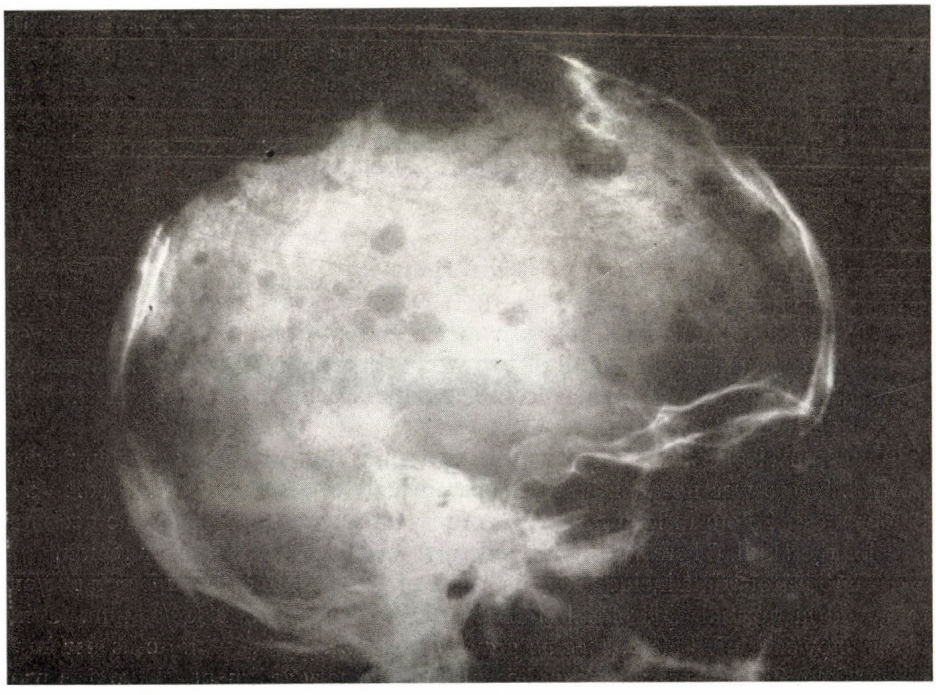


Fig. 1. Skull X-ray demonstrating huge osteolytic lesion and numerous smaller lesions

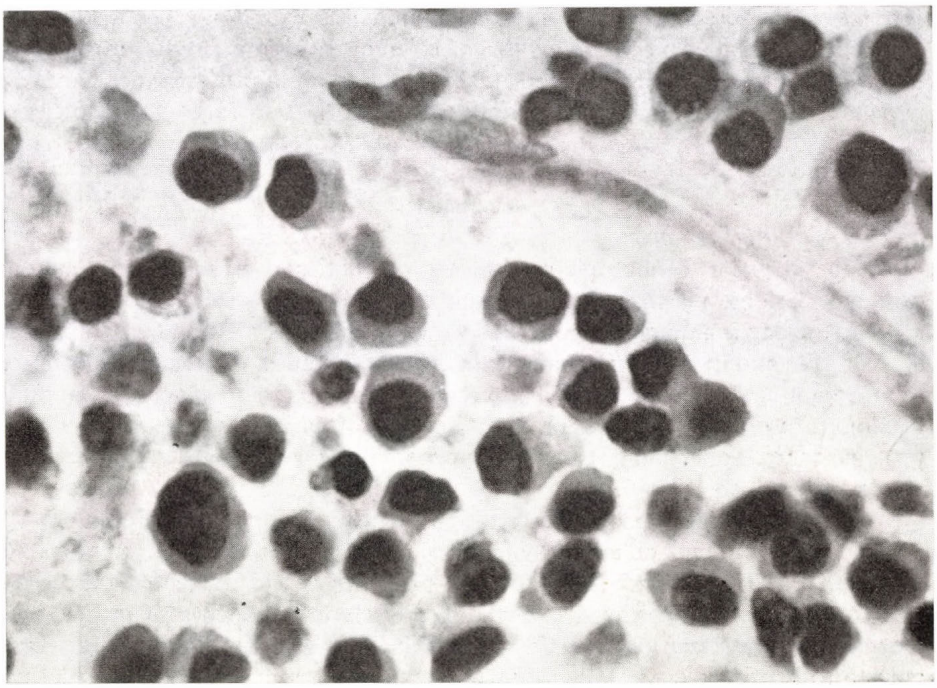


Fig. 2. Biopsy from skull mass demonstrating numerous immature plasma cells (Hematoxylin-eosin, original magnification ×680)

cubated samples. The precipitate was then counted in a Packard scintillation counter. No incorporation of leucine over background was observed in any sample. Thus, the patient's plasma cells were unable to synthetize any identifiable M protein.

A diagnosis of multiple myeloma was made and the patient was started on melphalan and prednisone. The large skull mass was irradiated with 4000 rads. The lesion regressed completely but left a bony defect. The patient felt well for 10 months while maintained on melphalan. During that period of time numerous examinations of his serum and concentrated urine did not reveal any abnormal proteins. A year following the date of diagnosis the patient complained of weight loss, severe low back pain, cough and fever. On admission the patient was found to be cachectic with a fever of 40 °C. Rales were audible at both lung bases. There were decreased breath sounds over the left lung. There was no adenopathy or hepatosplenomegaly. A soft nontender mass 5 cm in diameter was noted on the left chest wall. A biopsy revealed numerous immature plasma cells. Chest roentgenograms were consistent with pneumonia and pleural effusion. Repeated blood culture yielded no bacteria. The patient exhibited a markedly rapid downhill course over the next two months. He developed hypercalcemia (15 mg per

100 ml) with obtundation, severe constipation and vomiting. Treatment with mithramycin resulted in the reduction of calcium to 11 mg per 100 ml in three days. The pulmonary infection did not respond to antibiotic treatment and the patient expired. Permission for autopsy was denied.

Comment

The diagnosis of multiple myeloma in our patient was based on numerous osteolytic lesions, plasma cell infiltration of the bone marrow and plasma cell tumours. Using immunoelectrophoresis we were unable to detect any paraprotein in the serum and in the concentrated urine. The production of monoclonal protein or free light chains is one of the basic requirements for the diagnosis of multiple myeloma. In different series approximately 98% of patients secreted monoclonal proteins or free light chains [10–12]. It appears that the incidence of nonsecretory myelomas is 1 to 2% [6, 9].

Of the 34 reported cases of multiple myeloma without M protein [3–9, 13–19], only 10 had detailed immunological studies of their plasma cells [6, 8, 9, 13, 14, 18]. On the basis of these studies two groups of patients emerged. The first group, comprising 8 patients, clearly showed evidence of intracellular plasma cell production of monoclonal protein [8, 9, 13, 18]. These patients were able to synthetize the M protein but were unable to secrete it into the plasma. Therefore the condition should properly be called nonsecretory myeloma. These patients presented with bone pains and had osteolytic lesions and severe osteoporosis. The second group consisted of two previously published cases and our patient. Here the plasma cells did not exhibit any evidence of M protein synthesis. Curiously, two of these three patients presented with large plasma cell tumours of the skull in addition to osteolytic lesions and osteoporosis. Our patient had a moderate bone marrow infiltration with plasma cells while the other patient with plasma cell tumour had a normal bone marrow. In all three patients the immunoglobulin levels were normal or moderately decreased. As no monoclonal protein could be demonstrated in the plasma cells of these three patients, their condition should be classified as nonsynthetizing myeloma. Although it is possible that our present immunological tools did not allow to identify abnormal proteins in the plasma cells of these patients, it is plausible that the abnormal cells in these patients are not plasma cells. Gach [6] suggested in his case that these cells have developed from reticulum cells and have a "plasmacytoid" structure but do not possess the capacity to synthetize protein as do the plasma cells derived from lymphoblasts. If this is the case then these patients do not have plasma cell myeloma. It is important to accumulate further data on the subject, thus we suggest that every patient with multiple myeloma without M protein should undergo elaborate immunological studies.

References

1. Osserman, E. F., Fahey, J. L.: Plasma cell dyscrasias: Current clinical and biochemical concepts. *Amer. J. Med. 44*, 256 (1968).
2. Ruiz, F. G., Alexanian, R.: Multiple myeloma and microglobulinemia. *Postgrad. Med. 55*, 179 (1974).
3. Coltman, C. A. Jr.: Multiple myeloma without a paraprotein. *Arch. intern. Med. 120*, 687 (1967).
4. Forssman, O., Nillssen, G.: A case of myeloma with flaming plasma cells but no significant M-component in serum or urine. *Acta med. scand. 181*, 33 (1967).
5. Leffler, H., Knopp, A., Krecke, H. J.: Cases of multiple myeloma (plasmacytoma) "without paraprotein". *Germ. med. Mth. 12*, 226 (1967).
6. Gach, J., Simar, L., Salmon, J.: Multiple myeloma without M-type proteinemia. *Amer. J. Med. 50*, 835 (1971).
7. Azar, H. A., Zarrie, E. C., Pham, T. D., Yannopoulos, K.: "Nonsecretory" plasma cell myeloma. Observations of seven cases with electron microscope studies. *Amer. J. clin. Path. 58*, 618 (1972).
8. Menkes, C. J., Herreman, G., Preud'Homme, J. L., Godeau, P., Delbarre, F.: Myeloma à plasmacytes non excrétants. *Nouv. Presse méd. 1*, 306 (1972).
9. Arend, W. P., Adamson, J. W.: Nonsecretory myeloma. Immunofluorescent demonstration of paraprotein within bone marrow plasma cells. *Cancer 33*, 721 (1974).
10. Carbone, P. P., Kellerhouse, L. E., Gehan, E. A.: Plasmacytic myeloma. A study of the relationship of survival to various clinical manifestations and anomalous protein type in 112 patients. *Amer. J. Med. 42*, 937 (1967).
11. Nordenson, N. G.: Myelomatosis. A clinical view of 310 cases. *Acta med. scand. Suppl. 445*, 173 (1966).
12. Osserman, E. F., Takatsuki, K.: Plasma cell myeloma. Gamma globulin synthesis and structure. *Medicine 42*, 357 (1963).
13. Hurez, D., Preud'Homme, J. L., Seligmann, M.: Intracellular "monoclonal" immunoglobulin in non-secretory human myeloma. *J. Immunol. 104*, 263 (1970).
14. River, G. L., Tewksbury, D. A., Fudenberg, H. H.: "Nonsecretory" multiple myeloma. *Blood 40*, 204 (1972).
15. Lorriaux, A., Mizon, J. P., Gentit, E., Lemay, B.: Les myélomes multiples sans anomalies globuliniques décelables. *Lille Méd. 16*, 1420 (1971).
16. Rossini, A., Lucchelli, P. D.: Myeloma multiplo plasmocitario non secernente. *Haemat. lat. 7*, 335 (1964).
17. Kim, I., Harley, J. B., Weksler, B.: Multiple myeloma without paraproteins. *Amer. J. med. Sci. 264*, 267 (1972).
18. Delbarre, E., Siguier, F., Godeau, P., Saporta, L.: La maladie de Kahler à plasmocytes "non excrétants". *Ann. Med. intern. 121*, 537 (1970).
19. Szücs, J.: Multiple myeloma without paraprotein. *Haematologia 4*, 97 (1970).

Correspondence: Dr. J. Amir, Cleveland V. A. Hospital, Department of Hematology-Oncology, 10701 East Boulevard, Cleveland, Ohio 44106

Haematologia 11 (1–2), pp. 79–83 (1977)

Serum Levels of IgA, IgM, IgD and IgE in IgG Paraproteinaemias

M. TICHÝ, Z. HRNČÍŘ, J. ŠIMŠA

Second Department of Medicine, Faculty of Medicine, Charles University, and Medical Research Institute, Hradec Králové, Czechoslovakia

(Received August 30, 1976)

The dependence of serum levels of polyclonal IgA, IgM, IgD and IgE on the amount of IgG paraprotein was studied in a series of 58 IgG paraproteinaemias. A significant correlation was found for IgA ($r = -0.4782$, $p = 0.0007$), for IgM ($r = -0.3296$, $p = 0.0237$) and for IgD ($r = -0.3589$, $p = 0.0143$) in the whole series of IgG paraproteinaemias and in the subgroup of myeloma paraproteinaemias ($n = 34$) for IgM ($r = -0.4276$, $p = 0.0207$) and for IgD ($r = -0.4384$, $p = 0.0196$)

Introduction

Some patients with paraproteinaemia show reduced levels of polyclonal immunoglobulins in serum. This is characteristic of multiple myeloma and, to a lesser degree, also of Waldenström's macroglobulinaemia [2, 4, 8]. So far, attention has been paid mainly to the deficiency of polyclonal immunoglobulins of the classes M, A and G in patients with multiple myeloma [2]. Less attention has been paid to the deficit of polyclonal immunoglobulins in non-myeloma paraproteinaemias [5], to the deficit of IgD and IgE in myeloma [3, 7], and non-myeloma IgG paraproteinaemias and to correlation analyses of the amount of IgG paraprotein on the one hand and polyclonal immunoglobulins of the individual classes on the other [7].

Material and Subjects

The series under investigation consisted of 58 patients with IgG paraproteinaemia, 27 women and 31 men of 63 years mean age (ranging between 38 and 88). Thirty-four patients had multiple IgG myeloma meeting the criteria of NCI [9], the remaining 24 patients with solid tumours, haemoblastoses and liver cirrhosis had facultative non-myeloma IgG paraproteinaemias. IgG paraproteinaemias in healthy subjects were not included in the series. From the point of view of the antigenic type of the light chains, light chains kappa were found in 36 cases and light chains lambda in 19 cases, while in 3 cases, the antigenic type of the light

chains could not be identified. The control group consisted of 29 blood donors of corresponding age in whom the presence of paraprotein in the serum was excluded.

Methods

IgG paraprotein was demonstrable by immunoelectrophoresis with monospecific rabbit antisera (Sevac, Prague). The amount of paraprotein was determined from electrophoretograms on cellulose acetate (Cellogel, Chemetron, Italy). Total protein was determined by the biuret reaction. Polyclonal immunoglobulins of the classes A and M were determined quantitatively by radial immunodiffusion using the IDP kits (Sevac, Prague), polyclonal immunoglobulin of class D by the kits Immuno-Plate, Human IgD test (Hyland), and polyclonal immunoglobulin of class E by the radioimmunoassay Phadebas IgE test (Pharmacia).

Results were evaluated by the non-paired *t*-test and by the method of correlations and regressions using a Hewlett Packard 9830A computer.

Table 1

Basic statistical data for IgG paraproteins

	Series tested	No.	Mean	S.D.	95% confidence interval
IgA mg/100 ml	Control group	29	348.3	134.4	296 —400
	IgG paraproteins	58	252.6	153	212 —293
	IgG myelomatosis	34	192.5	126.6	148 —236.6
	Non-myelom. IgG parap.	24	337.7	146.6	274.5 —400.8
IgM mg/100 ml	Control group	29	121.6	38.5	107 —136.5
	IgG paraproteins	58	72.6	42.9	61.2 — 84
	IgG myelomatosis	34	69.9	50.3	52 — 87.5
	Non-myelom. IgG parap.	24	76.4	28.8	64 — 88.8
IgD mg/100 ml	Control group	29	10.1	6.3	7.6 — 12.5
	IgG paraproteins	57	7.6	4.5	6.4 — 8.8
	IgG myelomatosis	33	7.8	5.1	6 — 9.6
	Non-myelom. IgG parap.	24	7.3	3.3	5.8 — 8.7
IgE U/ml	Control group	29	125.9	104.4	74.4 —177.5
	IgG paraproteins	52	36.9	47.8	23.5 — 50.3
	IgG myelomatosis	30	26	33.5	13.2 — 38.7
	Non-myelom. IgG parap.	22	47.1	59.5	20.1 — 74
Paraprotein IgG g/100 ml	IgG paraproteins	48	2.37	1.38	1.96— 2.8
	IgG myelomatosis	29	2.93	1.3	2.4 — 3.4
	Non-myelom. IgG parap.	19	1.55	0.99	1.06— 2 04

Results

Basic statistical data for the whole series of IgG paraproteins, for the subgroups of myeloma and non-myeloma IgG paraproteinaemias and for the control group are presented in Table 1, and the results of statistical evaluation in Table 2. A comparison of the test groups and the subgroup of IgG myelomas with the control group using the non-paired *t*-test revealed significantly lower values for IgA, IgM and IgE. In the subgroup of non-myeloma IgG paraprotein-

Table 2

Results of statistical evaluation

Non-paired *t*-test		IgA	IgM	IgD	IgE
Control group: whole group (N = 29)	IgG paraproteins (N = 58)	$p < 0.01$*	$p < 0.01$*	$p > 0.05$	$p < 0.01$*
	IgG myelomatosis (N = 34)	$p < 0.01$*	$p < 0.01$*	$p > 0.05$	$p < 0.02$*
	non-myelom. IgG paraproteins (N = 24)	$p > 0.05$	$p < 0.01$*	$p > 0.05$	$p < 0.01$*
IgG myelomatosis: non-myelom. (N = 34)	IgG paraproteins (N = 24)	$p < 0.01$*	$p > 0.05$	$p > 0.05$	$p > 0.05$

* Value statistically significant at $p < 0.05$

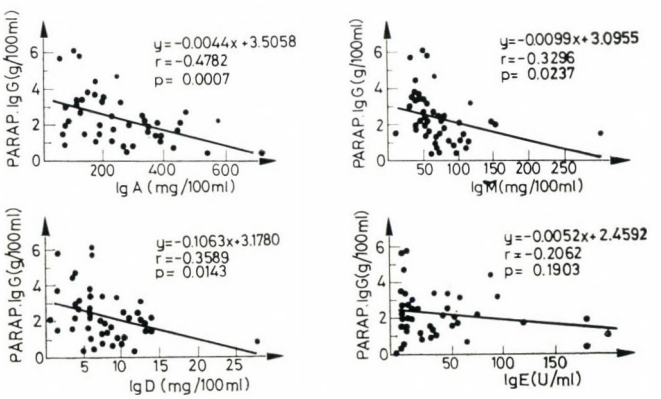


Fig. 1. Regression analysis of the relationship of the levels of paraproteins IgG and immunoglobulins A, M, D and E in the whole group of IgG paraproteinaemias

aemias, significantly lower values for IgM and IgE were found on comparison with the control group.

Significant correlation and indirectly proportional linear regression were found in the whole series between the amount of IgG paraprotein and the amount of polyclonal IgA, IgM and IgD; the values for IgE were not significant statistically (Fig. 1). In the subgroup of myeloma IgG paraproteinaemias, significant correlation and indirectly proportional linear regression were observed between the amount of IgG paraprotein and the amount of polyclonal IgM and IgD; for IgA, these values were on the border of significance (Fig. 2). In the subgroup of non-myeloma IgG paraproteinaemias, no significant correlations were found between the amount of IgG paraproteinaemia and the amount of polyclonal IgA, IgM, IgD and IgE.

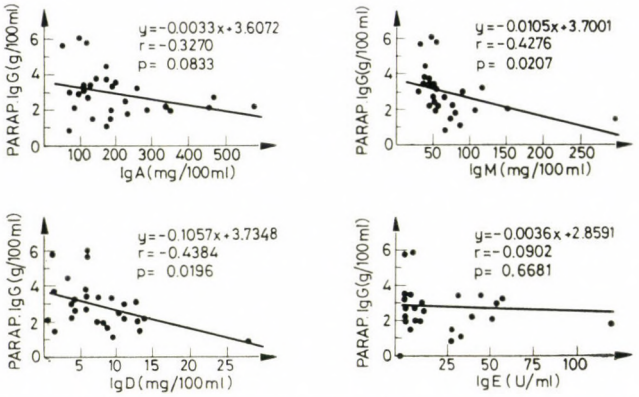


Fig. 2. Regression analysis of the relationship of the levels of paraproteins IgG and immunoglobulins A, M, D and E in the subgroup of myeloma paraproteinaemias

Discussion

The amount of paraprotein in serum is proportional to the mass of cells producing monoclonal immunoglobulin. This means that the highest paraprotein levels are found most frequently in advanced myelomas [6]. An opposite trend can be expected in the serum of these patients in polyclonal immunoglobulins. This is suggested by studies focussed mainly on immunoglobulins of the main classes. In the present study we have confirmed the significant decrease in polyclonal IgA, IgM and IgE in IgG paraproteinaemias and demonstrated in addition that the level of polyclonal IgA was significantly depressed only in IgG myelomas, but never in non-myeloma IgG paraproteinaemias. The levels of polyclonal IgA were significantly different in these groups (Table 2). These relations are particularly evident from the results of correlation and regression analyses. In the whole series, there was a significant correlation between the amount of IgG paraprotein and the levels of polyclonal IgA, IgM and IgD. The situation was similar in the

subgroup of myeloma IgG paraproteinaemias. On the other hand, no significant relations were demonstrated in the subgroup of non-myeloma IgG paraproteinaemias. The levels of polyclonal immunoglobulins in myeloma and non-myeloma IgG paraproteinaemias can be influenced by an increase in fractional catabolism [8]. It follows from some studies that deficient formation of polyclonal immunoglobulins in myelomas may be due to the suppressive effect of an inhibitor of cellular origin [1].

References

1. Broder, S., Humphrey, R., Durm, M., Blackman, M., Meada, B., Goldman, C., Strober, W., Waldmann, T.: Impaired synthesis of polyclonal (non-paraprotein) immunoglobulins by circulating lymphocytes from patients with multiple myeloma. *New Engl. J. Med. 293*, 887 (1975).
2. Cwynarski, M. T., Cohen, S.: Polyclonal immunoglobulin deficiency in myelomatosis and macroglobulinaemia. *Clin. exp. Immunol. 8*, 237 (1971).
3. Grove, D. I., Burston, T. O., Forbes, I. J.: Serum IgE levels in paraproteinaemia. *Int. Arch. Allergy 49*, 564 (1975).
4. Jones, S. E., Durie, B. G. M., Salmon, S. E.: Immunologic aspects of the hematologic neoplasma. *Postgrad. Med. 54*, 209 (1973).
5. Ritzmann, S. E., Loukas, D., Sakai, H., Daniels, J. C., Levin, W. C.: Idiopathic (asymptomatic) monoclonal gammopathies. *Arch. intern. Med. 135*, 95 (1975).
6. Salmon, S. E.: Immunoglobulin synthesis and tumor kinetics of multiple myeloma. *Semin. Hemat. 10*, 135 (1973).
7. Schwarz, S., Gabl, F., Pastner, D., Huber, H., Braunsteiner, H.: Immunoglobulin E bei chronischer lymphatischer Leukämie und monoklonalen Gammopathien. *Z. Immun.-Forsch. 148*, 196 (1974).
8. Snapper, I., Kahn, A.: Myelomatosis. S. Karger, Basel 1971, p. 380.
9. Bash, Ch. M., Rosenbaum, E. M.: Multiple myeloma. *Cardif. Med. 110*, 1 (1969).

Correspondence: Dr. M. Tichý, Charles University, Second Department of Medicine, Pospíšilova tř., 500 36 Hradec Králové, Czechoslovakia

Haematologia 11 (1—2), pp. 85—91 (1977)

A Simple Allogeneic Test System for the Study of Antibody-Dependent Cellular Cytotoxicity (ADCC) in Man

I. LÁNG, B. FEKETE, P. GERGELY, Gy. PETRÁNYI

Second Department of Medicine, Semmelweis University Medical School, Budapest, Hungary

(Received October 18, 1976)

A simple allogeneic test system for the study of ADCC activity of human mononuclear peripheral blood leucocytes (MPBL) is presented. The test uses human Rh-positive erythrocytes as target cells coated with specific human IgG antibody. The effector function of human MPBL is demonstrated after depletion of most of the monocytes and B lymphocytes on nylon wool column. The test system seems to be useful in the clinical study of ADCC activity in man.

Introduction

Cytolysis of antibody-coated target cells by Fc-receptor-bearing lymphoid cells (antibody-dependent cellular cytotoxicity, ADCC) is a widely studied phenomenon (for reviews, see [14, 17]). The nature of effector cells is not well defined. They are Fc receptor-bearing [5, 10, 11] non-T lymphocytes [16, 21], K cells [18] that lack easily demonstrable surface Ig. The effector activity of different cell types depends on the system used [13, 19]. For human ADCC investigations it is advantageous to use an allogeneic test system where both target cells and anti-target antibody are of human origin. Previous attempts to use an indicator system of human erythrocytes coated with specific IgG antibody against natural target cell surface antigens have been unsuccessful in demonstrating human lymphocytic killer activity [6, 8, 9, 19]. On the other hand, Urbaniak [20] showed that lymphoid-cell-dependent (K cell) lysis may also play a role in destroying IgG-sensitized human erythrocytes.

In the following, we present a simple allogeneic test system for the study of ADCC in man, where target cells are specific human IgG antibody-coated human erythrocytes.

Materials and Methods

Target cells

Human 0 Rh-positive erythrocytes treated with human anti-D antiserum were used as target cells. Two to three ml heparinized venous blood was drawn from healthy 0 Rh-positive blood donors. The erythrocytes were washed three times in phosphate-buffered saline (PBS) before use, and the cell count was adjusted

to 10^9/ml in TC 199 medium containing 10% heat-inactivated calf serum absorbed against target cells. 10^8 erythrocytes were incubated with 100 μCi (^{51}Cr) sodium chromate (Isotope Institute, Budapest, Hungary; specific activity 2 mCi/ml) and 500 μl undiluted, heat-inactivated human anti-D antiserum in a final volume of 0.8 ml. After incubation under constant agitation in a 37 °C water bath for 90 min, the cells were washed three times with TC 199, and reconstituted with medium (6×10^3 erythrocytes/μl). In some of the experiments human erythrocytes labelled with ^{51}Cr in the absence of anti-D antiserum were also used.

Lymphocyte-dependent antibody (LDA)

Sera from healthy volunteer blood donors hyperimmunized with 0 Rh-positive human erythrocytes (State Institute for Haematology and Blood Transfusion, Budapest, Hungary) were used. The titre of hyperimmune antiserum was 1 : 16,384 in most of the experiments. In some cases antisera with 1 : 512–2048 titre were used. Antisera were stored at −20 °C and thawed immediately before use.

Effector cells

Thirty ml heparinized venous blood was drawn from healthy blood donors. The blood was allowed to pass through nylon wool column (containing 1.3 g nylon wool), then separated on Ficoll-Uromiro [2]. In some experiments only Ficoll-Uromiro separation was applied. The lymphocytes were suspended in TC 199 and their concentration was adjusted to $1.2-2.4 \times 10^7$ cells/ml. Viability of cells was over 98% as judged by trypan blue exclusion. After combined nylon wool and Ficoll-Uromiro separation, 97 to 100% of cells were lymphocytes; monocyte contamination was less than 2%.

Characterization of lymphocytes

Complement receptor-bearing lymphocytes were detected by EAC rosette formation according to the method of Bianco et al. [1]. EA rosettes were formed according to the method of Brain and Marston [3]. The details of spontaneous rosette formation were described elsewhere [4]. Lymphocyte surface immunoglobulin-bearing cells (SIg) were identified by staining with FITC-labelled goat anti-human immunoglobulin (Hyland, Los Angeles, USA) at 4 °C for 45 min. After three washings with PBS, the cells were inspected in a Leitz Orthoplan fluorescence microscope equipped with a vertical illuminator.

ADCC assay

3×10^4 target cells were incubated in glass test tubes with $3 \times 10^4 - 2.4 \times 10^6$ lymphocytes giving an effector/target cell ratio of 1 : 1–80 : 1. Total volume was adjusted with medium to 0.05 ml/tube. Tubes were centrifuged at 1300

r.p.m. for 10 min immediately before incubation to produce a close contact between target and effector cells. Control tubes contained 3×10^4 target cells and 1.2×10^5 unlabelled human erythrocytes in the same amount of medium. Additional controls included tubes with labelled target cells without anti-D antiserum treatment. The samples were incubated at 37 °C for 2 to 16 hr. The reaction was terminated by the addition of 0.1 ml cold medium. The tubes were centrifuged at 2500 r.p.m. for 10 min, 0.1 ml of the supernatant was transferred into separate tubes, 0.1 ml TC 199 was added to the pellet, and the radioactivity of the supernatants and pellets was measured in a Nuclear-Chicago gamma counter. All determinations were made in triplicate. Maximum release was determined by treatment of the target cells with 4% saponin. The percentage of chromium release was calculated according to the formula:

$$1.5 \times \frac{\text{c.p.m supernatant}}{\text{c.p.m. pellet} + \text{c.p.m. supernatant}} \times 100$$

Cytotoxicity index (CI) was expressed as per cent specific release of ^{51}Cr:

$$CI = \frac{\text{test release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Mean spontaneous release was 8.94 ± 0.42 (ranges, 5.5 to 11.5%). Cytotoxicity was considered positive only if test release was greater than spontaneous release ($p < 0.01$) and CI was greater than 5%.

Results

Requirement of specific anti-target LDA to induce ADCC

Rh-positive and -negative human erythrocytes and chicken erythrocytes were labelled with ^{51}Cr and incubated with either human anti-D or rabbit anti-chicken erythrocyte antiserum. The ability of these sera to induce lysis of the target cells by effector cells was tested. Effector/target cell ratio was 40 : 1, incubation time 6 hr. Results are shown in Table 1. Rh-positive human erythrocytes were lysed by effector cells only if coated with anti-D antiserum, while chicken erythrocytes were lysed if coated with anti-chicken erythrocyte antiserum. No target cell destruction was observed in the absence of specific anti-target antibody. Cytotoxicity was influenced by the origin and titre of anti-D sera. In every experiment the same anti-D-target combination displaying the highest cytotoxicity index was used.

Effect on cytotoxicity of incubation time

ADCC activity of MPBL of healthy donors was determined at intervals of 1 to 16 hr. Effector/target cell ratio was 30 : 1. Three hours incubation resulted in a marked cytotoxicity, which increased with further incubation (Fig. 1), reaching a plateau between 12 and 15 hr.

Table 1

Specificity of LDA in the induction of target cell lysis by human lymphoid cells

Target cells	LDA		
	human anti-D antiserum	rabbit anti-chicken erythrocyte antiserum	culture medium only
0 Rh-positive human erythrocytes	+	−	−
0 Rh-negative human erythrocytes	−	−	−
Chicken erythrocytes	−	+	−

+ = CI > 10%; − = CI < 1%

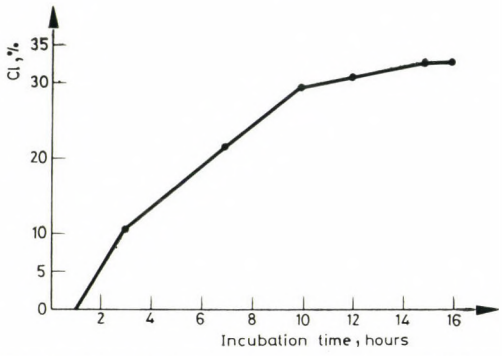


Fig. 1. Effect of incubation period on human ADCC activity

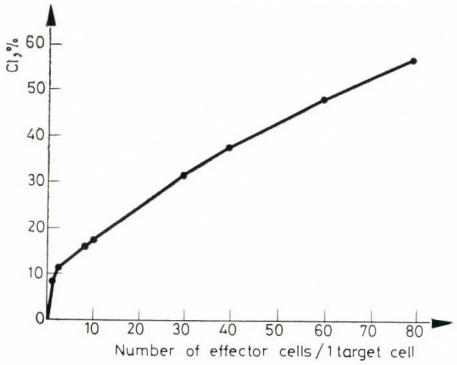


Fig. 2. Effect of effector cell/target cell ratio on human ADCC activity

Effect on cytotoxicity of effector/target cell ratio

Incubation time was 12 hr. Results are shown in Fig. 2. Significant cytotoxicity was observed already at an effector/target cell ratio 2 : 1, and there was a continuous increase on increasing the ratios.

Effect on cytotoxicity of lymphocyte separation

Using nylon wool column, the percentage of B cells determined by EAC rosette formation and surface Ig staining was considerably reduced (Table 2). There was an enrichment in the proportion of T cells, and the percentage of SIg-negative FC receptor-positive cells increased. After nylon wool column separation there was a considerable increase in cytotoxicity ($p < 0.01$).

Table 2

Effect of nylon wool separation on lymphocyte populations and ADCC (mean±SE; n: 24)

Separation	Rosettes (per cent)				SIg cells (per cent)	Cytotoxicity CI (per cent)
	E	EA		EAC		
		sIg-neg.	sIg-pos.			
Ficoll-Uromiro	71.9±1.0	7.2±0.7	18.6±1.9	11.2±0.9	18.6±1.8	32.9±0.3
Nylon wool and Ficoll-Uromiro	89.8±0.6	16.5±1.3	3.2±0.3	0.5±0.2	3.2±0.3	46.2±0.3+

+ $p < 0.01$

Cytotoxic activity of healthy subjects

The cytotoxic activity of 16 healthy individuals was tested after nylon wool–Ficoll-Uromiro separation continued for 12 hr. Each subject tested exerted cytotoxicity under these conditions giving a mean value of 8.38±0.63% (range, 5.11 to 12.82, effector/target cell ratio 4 : 1) and 38.74±0.76% (range, 31.24 to 49.93, effector/target cell ratio 40 : 1), respectively.

Discussion

For evaluation of changes in the number and/or the activity of human killer cell population under pathological conditions it is essential to have a well-defined standard test system, i.e. sensitive target cells coated with specific anti-target IgG antibody. One of the common test systems uses chicken erythrocytes coated with specific anti-chicken erythrocyte antiserum [17, 18]. It has several well-known advantages though it offers a xenogeneic system. Dextran-coated

human erythrocytes seem to be appropriate targets if coated with anti-dextran antibodies, though dextran represents an artificial component, and the routine use of this test involves immunization of volunteers with dextran [12]. It has been shown that human erythrocyte ADCC target systems have very poor, if any, ability to be attacked by human lymphocytes [7, 8, 9, 15, 19]. On the other hand, Urbaniak [20] demonstrated that lymphoid-cell-dependent (K cell) lysis might also play a role in destroying human erythrocytes coated with anti-D. The differences in these results may reflect the complexity of the Rh system.

In our experiments we presented evidence that human Rh-positive erythrocytes coated with human anti-D IgG antibody offer a suitable test system for the clinical study of human ADCC activity. The nylon-wool-separated MPBL of normal subjects showed a significant cytotoxicity in this system as early as after 3 hr incubation and at an effector/target cell ratio of 4 : 1. In some cases cytotoxicity could be observed also at 2 : 1 effector/target cell ratio. Results were well reproducible. Cytotoxicity increased with incubation time and with the number of effector cells. Depletion of most of the monocytes and B cells resulted in an increase in cytotoxicity. We have therefore concluded that the main effector cells in this system are Fc receptor-positive SIg-negative MPBL. The activity of the few monocytes remaining after combined nylon wool and Ficoll-Uromiro separation cannot fully be excluded.

The method presented might be useful in clinical studies of ADCC activity in man.

References

1. Bianco, C., Patrick, R., Nussenzweig, V.: A population of lymphocytes bearing a membrane receptor Ag-Ab. Complement complexes. *J. exp. Med. 132*, 701 (1970).
2. Büyum, A.: Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest. Suppl. 21*, 97 (1968).
3. Brain, P., Marston, R. H.: Rosette formation by human T and B lymphocytes. *Europ. J. Immunol. 3*, 6 (1973).
4. Fekete, B., Szegedi, Gy., Gergely, P., Szabó, G., Petrányi, Gy.: Technical problems of spontaneous rosette formation. A suggestion for the standardization of the method. *Haematologia 8*, 353 (1974).
5. Gelfand, E. W., Resch, K., Prester, M.: Antibody-mediated target cell lysis by non-immune cells. Characterization of the antibody and effector cell population. *Europ. J. Immunol. 2*, 419 (1972).
6. Hayward, A. R.: Non-specific induction of cytotoxicity by human lymphocytes with human IgG. *Immunology 26*, 61 (1974).
7. Hinz, C. F., Chickosky, J. F.: Proceedings of the Sixth Leukocyte Culture Conference. Academic Press, New York 1972, p. 699.
8. Holm, G.: Lysis of antibody treated human erythrocytes by human leucocytes and macrophages in tissue culture. *Int. Arch. Allergy 43*, 671 (1972).
9. Holm, G., Hammarström, S.: Haemolytic activity of human blood monocytes. Lysis of human erythrocytes treated with anti-A serum. *Clin. exp. Immunol. 13*, 29 (1973).
10. Kedar, E., Ortiz de Landazuri, M., Fahey, J.: Enzymatic enhancement of cell-mediated cytotoxicity. *J. Immunol. 112*, 26 (1974).

11. Larsson, A., Perlmann, P.: Study of Fab and $F(ab')_2$ from rabbit IgG for capacity to induce lymphocyte-mediated target cell destruction *in vitro*. *Int. Arch. Allergy 43*, 80 (1972).
12. Larsson, A., Pisarri-Salsano, S., Öhlander, C., Natvig, J. B., Perlmann, P.: Destruction of dextran-coated target cells by normal human lymphocytes and monocytes. Induction by a human anti-dextran serum with IgG antibodies restricted to the IgG2 subclass. *Scand. J. Immunol. 4*, 241 (1975).
13. MacDonald, H. R., Bonnard, G. D., Sordat, B., Zawodnik, S. A.: Antibody-dependent cell-mediated cytotoxicity: Heterogeneity of effector cells in human peripheral blood. *Scand. J. Immunol. 4*, 487 (1975).
14. MacLennan, I. C. M.: Antibody in the induction and inhibition of lymphocyte cytotoxicity. *Transplant. Rev. 13*, 67 (1972).
15. MacLennan, I. C. M., Loewi, G.: Effect of specific antibody to target cells on their specific and non-specific interactions with lymphocytes. *Nature (Lond.) 219*, 1067 (1968).
16. Möller, G., Svehag, S. E.: Specificity of lymphocyte-mediated cytotoxicity induced by *in vitro* antibody-coated target cells. *Cell. Immunol. 4*, 1 (1972).
17. Perlmann, P., Holm, G.: Cytotoxic effects of lymphoid cells *in vitro*. *Advanc. Immunol. 11*, 117 (1969).
18. Perlmann, P., Perlmann, H.: Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes. *Cell. Immunol. 1*, 300 (1970).
19. Perlmann, P., Perlmann, H., Larsson, A., Wahlin, B.: Antibody dependent cytolytic effector lymphocytes (K cells) in human blood. *J. Reticuloendothel. Soc. 17*, 241 (1975).
20. Urbaniak, S. J.: Lymphoid-cell-dependent (K-cell) lysis of human erythrocytes sensitized with Rhesus alloantibodies. *Brit. J. Haemat. 33*, 409 (1976).
21. Van Boxel, J. A., Stobo, J. D., Paul, W. E., Green, I.: Antibody dependent lymphoid cell-mediated cytotoxicity: No requirement for thymus-derived lymphocytes. *Science 175*, 194 (1972).

Correspondence: Dr. I. Láng, Second Department of Medicine, Semmelweis University Medical School, Szentkirályi utca 46, H-1088 Budapest, Hungary

Haematologia 11 (1—2), pp. 93—102 (1977)

Cell-Mediated Immunity in Subjects Immunized with HLA Antigens

P. ALFÖLDY, Éva GYÓDI, E. DOMBI, T. TÓTH, Susan R. HOLLÁN

National Institute of Haematology and Blood Transfusion, Budapest, and Blood Transfusion Service, County Hospital, Győr, Hungary

(Received March 11, 1976)

Production of cellular immune reaction in subjects immunized against HLA antigens was studied by the capillary migration inhibition test *in vitro* and by the skin test *in vivo*. The majority of the immunized subjects (10 out of 13) developed a cell-mediated immunity to the donor antigens. Production of HLA antibodies was demonstrable in a smaller proportion of the cases. The complementary nature of humoral and cellular responsiveness was borne out by the present observations. The result of the skin test performed simultaneously with the migration inhibition test correlated well with the result *in vitro*.

The capillary migration inhibition test is one of the *in vitro* procedures suitable for the demonstration of cell-borne immunity [1, 2, 3]. The reaction has the following mechanism as its basis: In the presence of the specific antigen the lymphocytes of the sensitized body produce a factor inhibitory to the migration of leukocytes [3].

Inhibition of migration by transplantation antigens in the allograft reaction has been the subject of extensive studies in laboratory animals [4, 5]. Beside the issue of organ transplantation [6, 7, 8] there are few studies in which the experimentally induced immune response to human (HLA) antigens has been tested by this method [9].

In the present study migration inhibition tests have been performed at intervals of five months, on two occasions altogether, in 13 subjects sensitized against HLA antigens. Parallel with these investigations, the skin test and the state of antibody production was also studied. It has been sought by these studies to ascertain the relationships between the three parameters.

Material and Method

Test subjects and immunization

The 13 immunized recipients as well as the immunizing donors were blood donor volunteers from the Blood Transfusion Service of Győr-Sopron County Hospital. They were typed in the framework of the "Workshop" held in Budapest in 1972 with the participation of eight laboratories of six countries when the following HLA antigens were identified [10]:

Table 1

HLA types, immunization schemes and parameters of antibody production in donor–recipient pairs immunized for the purpose of HLA antibody production

Serial number	HLA types*		Immunization**		Antibody	
	Donor	Recipient	Intradermal	Transfusion	Spec.	Titre
1	A2, A3-B7, *B12*	A2, A3-B7, B7, BW16	++++ ++ +	+ + + ±	B12	1 : 8
2	A2, A3-B7, *BW16*	A2, A3-B7, BW15	++++ ++ +	+ + + +		
3	A1, A9-B5, *B18*	A9, A11-BW15, BW21	++++ + +	++++++++++ + + +		
4	A2, AW30-*B18*, BW35	A2, AW30-BW35, BW15	++++ + +	++++ ++++++ + + +		
5	A1, A2-B8, *BW15*	A1, A2-B8	++++ + ++	++++++++++ + + +		
6	A1, A28-B8, BW35	A10, A28-B14, BW40		+++++++++ + + +		
7	A2, A11-B18, *BW22*	A2, A10-B18		+++ ++++++ + + +	A11	1 : 4
8	A2, A11-BW35, *BW22*	A1, A2-BW35	++++ + +	++++++++++ + + +		
9	A1, A2-B5, *BW22*	A1, A2-B13, BW35	++++ + +	++ ++++++++ + + ±	B7 + BW22	1 : 2
10	A2, AW32-B12, BW40	A2, A9-B12, B27		+++++++++++ +		
12	A9, *AW32*-B5, B27	A9, A10-B7, BW15		+++++++ + + + ±	AW32+B27	1 : 2
13	A2, *AW30*-BW35, BW15	A11, A28-BW35, BW15		+++++++++ + + +		
14	A2, A10-B18, *7 new*	A2, A11-B18, BW22		+++++++++ + + +		

Data for No. 11 were incomplete

* According to the 1975 nomenclature

** + = single antigeneic stimuli during a period of 31 months

± = antibody production

Basic HLA incompatibility between donor and recipient for the induction of antibody production in italics

Locus A: A1, A2, A3, A9 (AW23, AW24), A10 (AW25, AW26), A11, A28, A29, AW30, AW31, AW32
Locus B: B5, B7, B8, B12, B13, BW40, B14, BW15, BW16, BW17, B18, BW21, BW22, B27

The majority of the donor–recipient pairs was incompatible for one or two, a few of them for three antigens (Table 1). Pairing of the subjects was prompted by the objective of obtaining from the recipients monospecific antisera suited for HLA typing.

For the immunization of the recipients, intradermal injections of 1 to 2×10^7 freeze-stored donor lymphocytes were given at first weekly, later monthly (1, 8, 23, Table 1). From the 16th month onward a leukocyte suspension from 250 ml donor blood was used for immunization, the suspensions being transfused at monthly intervals for another 15 months, in a few cases with interruptions. Some of the recipients received transfusions only.

HLA antibody titration

The recipient sera were studied for HLA antibodies at each immunization (see Table 1) by the standard NIH lymphocyte cytotoxicity micromethod [14]. The sera which revealed titres of at least 1 : 2, 1 : 4 against the corresponding donor, were further studied on a phenotyped lymphocyte panel of 30 members.

Migration test

The migration test was performed at 26 and 31 months after immunization, by the capillary leukocyte migration technique described by Söborg and Bendixen [3].

The leukocytes were separated from heparinized recipient blood by addition of 1% dextran of 250,000 mol. weight (Pharmacia, Uppsala) for the acceleration of erythrocyte sedimentation, then the residual erythrocytes in the supernatant were haemolyzed by adding a 0.83% solution of ammonium sulphate. The medium for cell migration was TC 199 (Difco) nutrient supplemented with 10% foetal calf serum, containing also 100 U/ml penicillin and 100 μg/ml streptomycin. Antigen was added to the test medium, not to the control chambers. The migration inhibitory activity of the antigens was tested on plastic planchettes mounted with six parallel capillary tubes for each antigen. After incubation at 37 °C for 24 hours the migration planchettes were placed in a photographic enlarger, the images of the migration areas were projected, outlined, subjected to planimetry and the cell migration values were calculated on the basis of the following formula expressing the inhibition in per cent of the control values:

$$\text{Migration } \% = \frac{\text{mean planimetric figure in the presence of the antigen}}{\text{mean planimetric figure in the antigen-free control medium}} \times 100$$

Only migration values of less than 80% were regarded as inhibitory.

Preparation of antigen

The leukocytes were separated from heparinized donor blood as described for the migration test. The leukocyte suspensions were adjusted in TC 199 nutrient to 20×10^6 cells per ml, divided into 2 ml portions and stored at $-20\,^{\circ}C$. On the day of utilization the cell suspensions were thawed and the cells were homogenized in an ice bath by exposure to ultrasound. The homogenisate thus obtained was added to the nutrient providing the medium for migration, in doses based on the adjusted cell counts. The antigen doses used corresponded to 1×10^6, 3×10^6 and 9×10^6 donor cells, referred to in the following as "low", "medium" and "high".

Skin test

Of the homogenisate corresponding to 2×10^6 and 0.2×10^6 donor cells, 0.1 ml was injected intradermally into the cubital surface of the forearm. Two diameters of the erythematous area at right angles to each other were measured at 48 and 72 hours and the intensity of the reaction was defined by the mean of the two values. As a control, the same amount of nutrient was injected intradermally 50 mm from the test injection, and all antigen preparations were tested in two non-immunized volunteers.

Results

1. *Antibody production*

Antibodies appeared in the 14th month of immunization in the sera of 3 recipients, somewhat later in further 2, and one recipient displayed a transitory antibody level. Antibody production was observed after the first transfusion in recipient No. 8 (see Table 1).

2. *Cell-mediated immunity*

a) *Testing of the antigen preparations*

All antigen preparations were subjected to the leukocyte migration test, using leukocytes of young healthy male donors who before had never received a transfusion. The preparations revealed no toxic or migration inhibitory effect in the control subjects, with the exception of high doses of antigens 6 and 8 (with 9×10^6 cells per ml) which caused a slight migration inhibition (migration index 72 to 78%). These effects of the antigens were taken into consideration when calculating the values for specific migration inhibition.

A comparative study of two antigens prepared at different times for their migration-inhibitory activity is shown in Fig. 1. The first antigen was prepared

two days, the second five months before the test; this latter was stored at −20 °C before testing. With the exception of high doses of preparation 2, the inhibitory potency of the earlier and of the recent preparation showed complete parallelism.

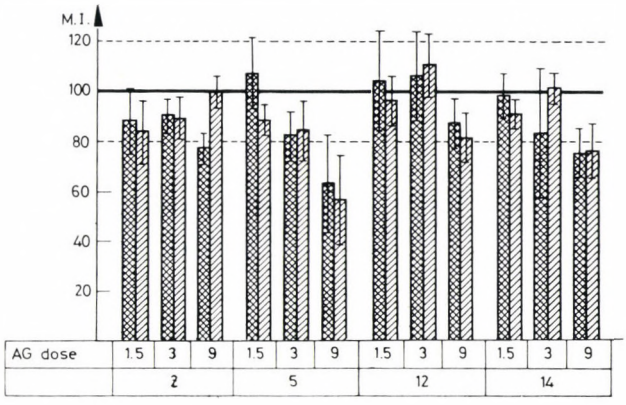


Fig 1. Comparison of two antigen preparations for migration inhibition at two different points of time. The earlier preparations (double hatched column) have been stored at −20 °C for 5 months. AG dose: the nutrient medium contained a homogenisate of 1.5 ×, 3 × and 9×10^6 donor cells per ml; 2, 5, 12, 14 are serial numbers of the antigens (donor–recipient pairs); each column represents mean of 6 capillaries

b) *Leukocyte migration inhibition tests*

The first migration inhibition test performed 26 months after immunization revealed in 8 out of 10 cases a significant inhibition in the chambers containing the specific donor antigen at high concentrations (Fig. 2). At medium concentrations the antigen proved inhibitory to migration in 3 cases. No inhibition was obtained with any of the doses in recipients 7 and 12.

In the second test carried out 31 months after immunization, the antigen at high concentrations inhibited migration in all cases in which the previous test had been positive (Fig. 2). The allogeneic antigen failed to inhibit the leukocytes of recipients 7 and 12 this time too, and recipient 13, who had not participated in the previous test, proved also negative. The medium dose was found inhibitory in 5 cases, the low dose in 3 cases.

c) *Skin test*

Skin sensitivity of the recipients to the allogeneic antigens was tested 7 days after the second migration inhibition test. All antigen preparations were tested a) in the subjects whose cell served for antigen preparation (autocontrols); b) in 2 non-immunized healthy male volunteers. All control subjects proved negative, nor did intradermal injections of 0.1 ml of the TC 199 nutrient serving for the preparation of the antigens cause any reaction in the controls or in the immunized subjects. On the other hand, the immunized subjects exhibited a skin reaction

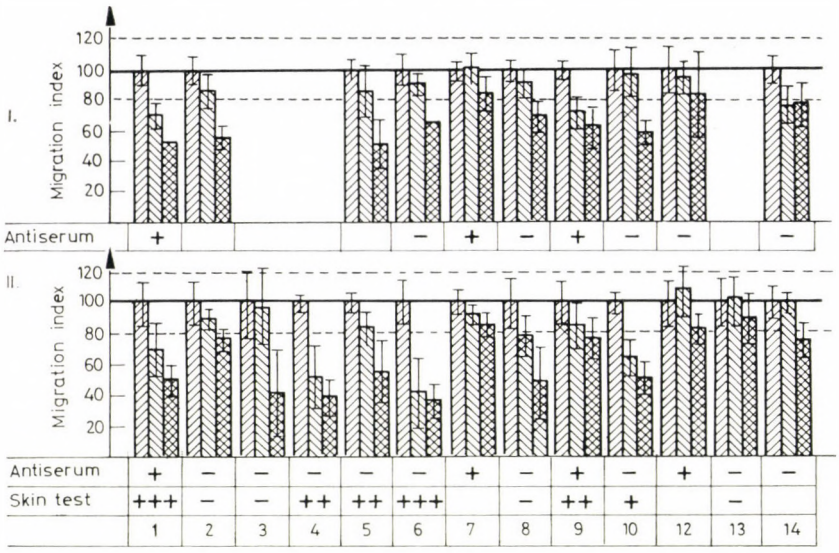


Fig. 2. Cell-mediated reaction-leukocyte migration, antibody production and skin test in subjects immunized with HLA antigens. The columns are in proportion to the mean cell migration ±SD. I, first migration inhibition, 26 months after immunization; II, second test, 31 months after immunization; ▨ migration in control medium, in the presence of ▧ medium dose (3×10^6 cells per ml) and (double hatcahed column) high dose (9×10^6 cells per ml) antigen; anti-HLA antibody production 25 and 31 months after immunization is marked with +

Table 2

Skin reaction of recipients immunized with HLA antigens

No.	36 hr	48 hr		72 hr		Evaluation
		Dose of antigen (cell number)				
		2×10^6	0.2×10^6	2×10^6	0.2×10^6	
1	+	16	6	5	−	+++
2	−	−	−	−	−	−
3	−	−	−	−	−	−
4	+	10.5	−	−	−	++
5	+	10	−	−	−	++
6	+	14.5	11.5	10	9	+++
7	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
8	−	−	−	−	−	−
9	+	6.5	−	12	−	++
10	+	7	−	−	−	+
12	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.
13	−	−	−	−	−	−
14	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.

Mean diameter of erythema (mm)

of variable intensity to the homologous antigens (Table 2). In 7 of the 10 test subjects the skin reaction appeared as early as 36 hours. In case 8 the reaction faded by the 48th hour, in the others the erythema varied in intensity and duration. The reaction attained its maximum intensity in recipient 6, where the erythema was still apparent 72 hours after the administration of a low dose.

Discussion

For migration inhibition tests with transplantation antigens, simple ultrasonic homogenization of the antigen-carrying cells [16, 19] was found suitable for the production of antigen preparations. Preparations thus obtained in amounts equivalent to 9×10^6 cells still failed to affect the migration of non-sensitized leukocytes. The slight inhibitory effect of two antigen preparations at high dose levels indicated, however, that this antigen concentration is at the borderline of toxicity.

Wood et al. [8], in their migration inhibition tests, found human leukocytes to have preserved their antigenicity 3 months after ultrasonic homogenization and storage at $-20\,^{\circ}C$. Svehag et al. [15] found ultrasonic mouse cell homogenisates still active in the migration test after storage at $-70\,^{\circ}C$ for one year. In the present study it has been ascertained by testing of leukocyte preparations stored at $-20\,^{\circ}C$ for 5 months against freshly prepared antigens that the antigenicity was not affected by storage. This is important in migration inhibition tests in kidney-transplanted patients where the donor antigen stored at low temperatures serves for repeated testing of the recipient over months.

On the evidence of our tests in 13 subjects hyperimmunized with HLA antigens we have found, in conformity with other workers [16], that the results of the leukocyte migration inhibition tests are reproducible (Fig. 2). The test was performed twice within five months and the same recipients (Nos 7 and 12) were found negative on both occasions, moreover a further recipient (No. 13) who had not been tested earlier also proved negative in the second test.

On the evidence of the migration inhibition tests 10 out of the 13 immunized subjects developed a cell-mediated sensitivity to the donor antigens. Antibody production against the donor antigens was noted in 4 out of the 13 cases. The two reactions were not congruent. Inhibition of migration was associated with antibody production in some cases, and with none in some others.

Repeated blood transfusions involve the production of humoral as well as of cellular immunity. In parallel studies of polytransfused subjects, D'Apice [16] and other workers [17] found that the migration test was a more sensitive indicator of sensitization than lymphocytotoxicity. On the other hand, in some cases negative migration inhibition was found together with antibody production. The authors quoted above assume a parallelism between the production of humoral and cellular sensitization, and regard the absence of migration inhibition in seropositive cases as a false negative result attributable in all likelihood to

poor cellular sensitization. Since, as demonstrated by Söborg [18], poor sensitization in the presence of low antigen concentrations manifests itself frequently in a simulation of migration, the nature of the negative results can be found out by the application of different antigen doses. In the present material too, we had two antigen-producing recipients with negative migration inhibition, and in spite of the use of three different antigen doses in the migration test, no significant stimulative effect was demonstrable. We have thus to attribute the negative results to a true deficiency of cellular sensitization.

Serial blood transfusions need not result in antibody production in every case. It has been demonstrated by Opelz et al. [4] that, parallel with the number of transfusions the proportion of the cytotoxic antibody procedures increases among the recipients, to attain 50% after 30 transfusions. Our findings concerning antibody-production are compatible with these data. It has furthermore been shown that the serum may lose its earlier cytotoxic activity, a phenomenon termed "secondary unresponsiveness" [4, 19, 20]. It is partly this phenomenon, partly the differences in the sensitivity of the various methods which, according to D'Apice, seem to account for the pattern characterized by inhibition of migration with a complete lack of antibody production [16]. We are, however, not justified in incriminating the phenomenon of "secondary unresponsiveness" for our own findings of this kind, since, on the evidence of the cytotoxic antibody tests repeated at monthly intervals over the entire period of immunization, the disappearance of antibody production was confined to a sole recipient (No. 2).

Dual sensitization, i.e. migration inhibition associated with antibody production, occurred in 2 cases of the present material (Nos 1 and 9). Recipient 12 deserves particular notice because he developed humoral sensitivity in the course of the five months between the two migration inhibition tests which, however, remained negative. In opposition to the authors quoted above, we tend to regard our results as indicative of a complementary relationship between the humoral and cellular immune responses.

The results of the skin tests performed 31 months after immunization, i.e. simultaneously with the second migration inhibition test, were in agreement with the results *in vitro*. The positive skin tests were always consistent with the results of the migration tests, i.e. a positive skin reaction was invariably associated with migration inhibition. The negative result in recipient No. 13 was also in agreement with the negative skin test. The lesser sensitivity of the skin test may account for its occasional failure to indicate the sensitization reflected in the inhibition of migration [22]. Our results agree with the data published by Kostiala et al. [23] who found a parallelism between the migration test and the 24-hr skin reaction in guinea-pigs, while passive haemagglutinin, haemolysin and cytophilic antibody production were unrelated to these reactions.

Further studies on a larger scale will have to decide whether the migration inhibition and the skin test are related in intensity. In any case, the most intensive skin reaction and at the same time the strongest inhibition of migration were found in recipient No. 6, and this was the only case in which even low doses of

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

the antigen proved inhibitory to migration. (In this case there was a donor–recipient incompatibility for three antigens.)

Our recent studies of immunized subjects for Ia antibodies, i.e. those formed against the newly defined specificities of the histocompatibility system HLA identified by serological techniques on B lymphocytes, also seem to support our claim. The recipients who failed to produce HLA bodies in response to immunization (Fig. 2, Nos 2, 3, 4, 5, 6, 8, 10, 13, 14) did form Ia antibodies. The relationships between Ia antibodies and cell-mediated immunity are, however, still poorly understood.

On the grounds of our findings we regard the leukocyte migration test as suitable for the demonstration of cellular sensitization to human transplantation antigens.

References

1. David, J. R., Al Askari, S., Lawrence, H. S., Thomas, L.: Delayed hypersensitivity *in vitro*. I. The specificity of inhibition of cell migration by antigens. *J. Immunol. 93*, 264 (1964).
2. David, J. R., Lawrence, H. S., Thomas, L.: Delayed hypersensitivity *in vitro*. II. Effect of sensitive cells on normal cells in the presence of antigen. *J. Immunol. 93*, 274 (1964).
3. Söborg, M., Bendixen, G.: Human lymphocyte migration as a parameter of hypersensitivity. *Acta med. scand. 181*, 247 (1967).
4. Opelz, G., Mickey, M. R., Terasaki, P. J.: Blood transfusions and unresponsiveness to HL-A. *Transplantation 16*, 649 (1973).
5. Weir, D. M., Suckling, D. E. J.: Macrophage migration inhibition induced by tissue antigen in guinea pigs. *Clin. exp. Immunol. 8*, 791 (1971).
6. Falk, R. E., Guttmann, R. D., Beaudoin, J. D.: Leukocyte migration *in vitro* and its relationship to human renal allograft rejection and enhancement. *Transplantation 13*, 461 (1972).
7. Richmond, D. E., Doak, P. B., North, J. D. K.: Inhibition of leucocyte migration as an index of rejection in renal transplant recipients. *Clin. exp. Immunol. 15*, 17 (1973).
8. Wood, R. F. M., Gray, A. L., Briggs, J. D., Bell, P.R. F.: The prediction of acute rejection in human renal transplantation using the leukocyte migration test. *Transplantation 16*, 41 (1973).
9. Falk, E. R., Thorsby, E., Möller, E., Möller, G.: In vitro assay of cell-mediated immunity: the inhibition of migration of sensitized human lymphocytes by HL-A antigens. *Clin. exp. Immunol. 5*, 445 (1970).
10. Petrányi, Gy., Iványi, P., Hollán, Zs.: Relations of HL-A and Rh systems to immune reactivity. Joint report of the results of "HL-A and Immune Response" Workshop. *Vox Sang. (Basel) 26*, 470 (1974).
11. Curtoni, E., S., Scudeller, G., Mattiuz, P. L., Savi, M., Cepellini, R.: Anti HL-A antibody evolution in recipients of planned transfusions. *Tissue Antigens 2*, 415 (1972).
12. Ferrara, G. B., Tosi, R., Azzolina, G., Carminati, G., Kissmeyer-Nielsen, F.: The production of anti HL-A cytotoxic antisera through planned immunization by intravenous injection of small aliquots of whole blood, I. Immunization of unrelated recipients. *Tissue Antigens 2*, 359 (1972).
13. Zsdánszky, K., Dóbiás, Gy., Langfelder, M., Margittay, E., Nemák, P.: Donorok immunizálása emberi leukocyta suspensióval anti-HL-A serumok nyerése céljából. *Orv. Hetil. 114*, 915 (1973).

14. Teresaki, P. I.: Microdroplet lymphocyte cytotoxicity test. Manual of Tissue Typing Techniques *4*, 50 (1972).
15. Svehag, S.-E., Schilling, W.: Solubilization of species-specific antigens and murine alloantigens by pulsed high frequency sonic energy. *Scand. J. Immunol. 2*, 115 (1973).
16. D'Apice, A. J. P., Tait, B. D., Morris, P. J.: The leucocyte migration technique as an assay of presensitization to human transplantation antigens. *Clin. exp. Immunol. 19*, 407 (1975).
17. Garovoy, M. R., France, V., Zschaeck, D., Carpenter, C. B., Strom, T. B., Meril, J. P.: Direct lymphocyte-mediated cytotoxicity as an assay of presensitization. *Llancet 1*, 573 (1973).
18. Söborg, M.: *In vitro* migration of peripheral human leukocytes in cellular hypersensitivity. *Acta med. scand. 184*, 135 (1968).
19. Dossetor, J., B., Olson, L. A.: Evidence of latent sensitisation to HL-A antigens. *Transplantation 13*, 574 (1972).
20. Ferrara, G. B., Tosi, R., Azzolina, G., Carminati, G., Largo, A.: In: Proc. Int. Symposium on HL-A Reagents. Karger, Basel 1973, p. 20.
21. Maehara, K. T., McIvor, K. L.: Migration inhibition of alloimmune mouse macrophages by soluble transplantation antigens. *Cell. Immunol. 15*, 11 (1975).
22. Rédei, A., Keserü, G., Frigyesi, A.: Macrophage migration inhibition as an *in vitro* correlate of cutaneous delayed skin reaction. II. Investigations on BCG vaccinated guinea pigs. *Acta microbiol. Acad. Sci. hung. 20*, 275 (1973).
23. Kostiala, A. A. I., Kosumen, T. U.: Delayed hypersensitivity, macrophage migration, and antibodies in guinea-pigs immunized with diphtheria toxoid. I. Immunization with antigen-antibody precipitates. *Scand. J. Immunol. 1*, 143 (1972).

Correspondence: Dr. P. Alföldy, National Institute of Haematology and Blood Transfusion, Daróczi út 24, H-1113 Budapest, Hungary

Haematologia 11 (1—2), pp. 103—110 (1977)

Antagonistic Effect of Heparin and a Water-Soluble Glucocorticoid on the Peripheral Lymphocyte Count in Mice

I. JÓKAY, Erika KARCZAG

Microbiological Research Group, Hungarian Academy of Sciences, Budapest, Hungary

(Received August 16, 1976)

The effect of heparin and prednisolone succinate on the peripheral white blood cell count of mice has been studied 3 hours after injecting the drugs. Heparin in low doses did not influence the cell count but in higher doses (20 to 400 U) elicited marked lymphocytosis. When prednisolone and heparin were administered together, heparin (5 to 10 U) markedly inhibited the prednisolone-induced lymphopenia.

The effect on the lymphocyte count of prednisolone combined with a large dose of heparin depends on the doses of the drugs. The antagonism seems to be non-competitive in nature.

The PMN count shows only minor changes and the reaction of eosinophils to heparin is less pronounced. The antagonistic effect was manifest on the spleen weight.

Introduction

The antagonistic action of heparin and hydrocortisone (or cortisone) on the peripheral white blood cell count has been observed in man, dogs and rats [7, 9, 15]. As this antagonism may have a regulatory role in some physiological processes, we have studied it in detail. We present data that heparin is able to antagonize the lymphopenic effect of a water-soluble glucocorticoid in mice. As a similar antagonism was reported earlier on the peripheral eosinophil cell count [7], the number of these cells too was registered.

Materials and Methods

BALB/c female inbred mice 11 to 13 weeks of age were used. Two lots of commercial heparin preparations were employed: Unless otherwise stated the heparin contained 100 U per mg (Lot No. 3289, Nutritional Biochemical Corp.), the other preparation (Lot No. 5043, ICN Pharmaceuticals) contained 131 U per mg of heparin. Both preparations were free from preservatives. Prednisolone succinate was purchased from Organon (Di-Adreson-F aquosum) and solutions were prepared immediately before use.

Unless otherwise stated, heparin and prednisolone were administered subcutaneously.

When both drugs were injected they were in the same solution. Control animals were injected with saline solution. The animals received a standard diet ad libitum before the experiments. Three hours after the injection, the mice were bled from the tail artery to a tube containing 10 μl of 0.1 M ethylenediaminetetraacetic acid neutralized with NaOH (ph = 7.2). Total leucocyte count and differential counts were performed, and the ratio of polymorphonuclear cells (PMN) to the mononuclear (lymphoid) cells was determined. The eosinophil cells were counted directly according to Szmuk [1]: 50 μl blood was diluted with 0.2 ml of eosin-methylene blue solution (9 volumes of eosin solution were mixed with 1 volume of methylene blue solution before use) and 20 minutes later the eosinophils were counted in the total field of a Buerker chamber.

Student's *t*-test was used for statistical analysis. The difference was considered significant when $p < 0.05$.

Results

Table 1 shows the effect on the leucocyte count of graded doses of heparin. Ten U of heparin had no effect, but larger doses markedly increased the number of both lymphoid and eosinophil cells; the per cent increase was greater in the case of lymphocytes.

Table 1

Effect on WBC count of graded doses of heparin

Treatment+ (units of heparin)	Cell count per μl blood $\pm$SE			
	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
0	6.6±0.7	4.9±0.6	1.6±0.2	82±27
10	6.2±0.8	3.9±0.5	2.2±0.2	64±5
25	10.6±0.9*	8.4±0.7*	2.2±0.2	100±24
50	13.0±1.0*	11.1±1.0*	1.8±0.2	127±37
100	17.9±1.8*	14.8±1.6*	3.0±0.4*	164±37
400	23.9±0.9*	20.8±1.4*	3.0±0.5*	200±13*

+ 4—5 mice in each group
* Significant differences to the control

Table 2 presents the dose-response relationship after the injection of prednisolone. Even 2 μg of prednisolone was effective in diminishing both the lymphoid and eosinophil count. After reaching a maximum fall in the lymphoid cell count, no further decrease was observed on increasing the dose of prednisolone.

Using different routes of administration, prednisolone had an inconsistent effect on the PMN (and Eo) counts, but it reduced the lymphoid cell count ir-

Table 2

Effect on WBC count of graded doses of prednisolone

Treatment+ (μg Pr)	Cell count per μl blood ±SE			
	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
0	7.6±0.5	5.8±0.4	1.8±0.2	114±13
2	6.0±0.4*	4.3±0.3*	1.6±0.2	65±15*
5	5.3±0.6*	3.8±0.5*	1.4±0.2	56±16*
10	4.3±0.5*	3.2±0.2*	1.0±0.1*	45±3*
25	2.7±0.4*	1.7±0.2*	1.0±0.1*	38±5*
50	3.2±0.2*	2.2±0.2*	0.9±0.1*	29±5*

+ 4 mice in each group
* Significant differences to the control

respective of the route of administration (Table 3). Heparin antagonized the effect of prednisolone, and this effect proved to be more pronounced on lymphoid cells. As in separate experiments heparin proved equally effective when given subcutaneously or intraperitoneally, in further experiments the subcutaneous route of administration was used.

Table 4 shows the effect on the WBC of graded doses of heparin and a constant dose of prednisolone. Five U of heparin, which *per se* did not influence

Table 3

Effect on WBC count of prednisolone and heparin by various routes of administration

Treatment+		Cell count per μl blood ±SE			
Pr (μg)	H (units)	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
—	— IP	7.5±0.4	6.1±0.4	1.3±0.1	101±21
100	— IP	5.5±0.4	2.6±0.4	2.8±0.2	33± 9
100	50 IP	7.5±0.6*	6.2±0.8*	1.3±0.2*	55±14
100	— SC	2.9±0.1	2.2±0.1	0.7±0.1	28± 6
100	50 SC	7.4±0.7*	6.3±0.3*	1.1±0.2	36± 7
100	— IV	3.6±0.4	2.0±0.3	1.5±0.2	6± 3
100	50 IV	6.9±1.3*	4.2±0.2*	2.6±1.1	14± 5

+ 4 mice in each group
* Significant differences between the heparin-treated groups and the respective controls.
IP = intraperitoneal
SC = subcutaneous
IV = intravenous

the leucocyte count, inhibited the effect of prednisolone on the lymphoid cells, while only 10 U of heparin were effective in inhibiting the decrease of eosinophil count caused by prednisolone.

Table 4

Effect on WBC count of graded doses of heparin and a constant dose of prednisolone

Treatment+		Cell count per μl blood $\pm$SE			
Pr (μg)	H (units)	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
—	—	7.5$\pm$0.8	5.4$\pm$0.6	2.1$\pm$0.2	155$\pm$33
200	—	3.9$\pm$0.6	2.6$\pm$0.4	1.3$\pm$0.1	28$\pm$11
200	5	7.4$\pm$0.3*	5.4$\pm$0.4*	1.9$\pm$0.1*	33$\pm$11
200	10	8.1$\pm$1.1*	5.9$\pm$0.6*	2.2$\pm$0.2*	83$\pm$14*
200	20	8.7$\pm$0.6*	6.7$\pm$0.5*	1.9$\pm$0.2*	40$\pm$10
200	50	9.9$\pm$1.4*	8.5$\pm$0.9*	1.4$\pm$0.3	37$\pm$12

+ 5 mice in each group

* Significant differences between the heparin-treated groups and the prednisolone-treated control group. All the values of the latter group showed significant differences against the saline-treated controls.

From the above data it was assumed that the sensitivity to prednisolone depends on the presence of heparin in the blood. This seems to be true at least in the case of lymphoid cells (Table 5). Small doses of heparin which do not exert any significant influence on the lymphoid cell count prevented the effect of 200 μg prednisolone. On the other hand, when prednisolone was given in the presence of a large dose of heparin inducing in itself lymphocytosis, a decrease of the

Table 5

Sensitivity to prednisolone at different heparin levels

Treatment+		Cell count per μl blood $\pm$SE			
Pr (μg)	H (units)	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
—	—	5.7$\pm$0.4	4.5$\pm$0.4	1.1$\pm$0.1	62$\pm$12
200	—	2.6$\pm$0.2*	1.8$\pm$0.4*	0.8$\pm$0.2	12$\pm$ 2*
—	10	6.4$\pm$0.8	5.0$\pm$0.7	1.3$\pm$0.2	88$\pm$24
200	10	5.2$\pm$0.3	4.1$\pm$0.3	1.0$\pm$0.1	28$\pm$ 6*
—	100	17.1$\pm$1.6	14.9$\pm$1.5	2.3$\pm$0.2	107$\pm$15
200	100	10.7$\pm$0.6*	9.4$\pm$0.6*	1.2$\pm$0.2*	45$\pm$10*

+ 5 mice in each group

* Significant differences between the prednisolone-treated groups and the respective controls

lymphocyte count could be observed, but not below the normal level. In contrast to lymphocytes this change in sensitivity did not seem to be valid for the eosinophils, as prednisolone effected about the same fall in the eosinophil count independently of the heparin level.

The effect of small doses of heparin given together with different doses of prednisolone was also studied. In this experiment a heparin preparation containing 131 U per mg was used. Table 6 shows that 20, and even 10 U of heparin by themselves caused an increase in the number of circulating lymphoid cells

Table 6

Effect on WBC count of heparin with different doses of prednisolone**

Treatment+		Cell count per μl blood $\pm$SE			
Pr (μg)	H (units)	WBC ($\times 10^3$)	Ly ($\times 10^3$)	PMN ($\times 10^3$)	Eosinophil
—	—	9.2$\pm$0.5	7.2$\pm$0.5	2.0$\pm$0.2	139
—	10	11.0$\pm$0.8	9.3$\pm$1.0	1.8$\pm$0.2	216
—	20	12.8$\pm$0.9*	10.5$\pm$0.7*	2.0$\pm$0.4	157
20	—	4.5$\pm$0.6	3.7$\pm$0.5	0.8$\pm$0.2	36
20	10	6.2$\pm$0.4*	4.9$\pm$0.2*	1.3$\pm$0.3	58
20	20	6.5$\pm$0.6*	5.7$\pm$0.5*	0.7$\pm$0.2	40
200	—	4.7$\pm$0.5	3.4$\pm$0.4	1.4$\pm$0.2	72
200	10	6.3$\pm$0.2*	5.2$\pm$0.2*	1.1$\pm$0.2	94
200	20	7.2$\pm$0.8*	5.8$\pm$0.6*	1.4$\pm$0.2	58

+ 6 mice per group; the eosinophil counts are from the pooled samples

* Significant differences between the heparin-treated groups and the respective control groups

** In this experiment a heparin preparation (131 units per mg, ICN) different from those used in the other experiments was employed

Table 7

Effect on spleen weight of prednisolone and heparin

Group	Treatment+		Spleen weight (mg) $\pm$SE 3 hours after treatment
	Pr (μg)	H (units)	
1	—	—	115.3$\pm$5.4
2	200	—	96.2$\pm$5.0*
3	200	100	110.6$\pm$4.4*
4	—	100	129.5$\pm$7.0

+ 10 mice in each group

* Significant differences: group 2 vs. group 1; group 3 vs. group 2

and inhibited the lymphopenic effect of 20 to 200 μg doses of prednisolone in a similar degree. It can also be seen that smaller doses of heparin resulted consistently in higher levels of eosinophil cells than the larger doses.

Table 7 shows that the prednisolone-heparin antagonism can also be demonstrated by measuring the spleen weight even 3 hours after administration of the drugs. Prednisolone lowered, while heparin by itself slightly increased the spleen weight but no change occurred after injecting the two drugs simultaneously.

Discussion

Heparin is the best known representative of endogenous glucosaminoglycan polysulphates which may have regulatory activity [11, 12]. The lymphocytosis-inducing effect of heparin [2, 10, 17–19] and its effect antagonistic to some glucocorticoids [7, 9, 15] on the peripheral leucocyte count have been described in species other than mice. Beside heparin other sulphated polysaccharides such as dextran sulphate have a lymphocytosis-promoting effect [17, 18].

In contrast to the sulphated polysaccharides, glucocorticoid hormones cause lympho- and eosinopenia [6].

The results presented show that heparin and a water-soluble glucocorticoid have antagonistic effects on the peripheral lymphoid cells in mice. Such doses of heparin (5 to 10 U) do not influence the lymphocyte count significantly but markedly protect the prednisolone-induced fall of the lymphoid cell count. Heparin in large doses induces a rapid and marked lymphocytosis and antagonizes the lymphopenic effect of prednisolone.

Inhibition of the recirculation of lymphocytes [8] from blood into some lymphoid organs as was shown by Bradfield and Born in rats [2] offers a possible explanation of the lymphocytosis-inducing effect of heparin.

The antagonism between heparin and prednisolone on the lymphoid cell count seems to be non-competitive in nature, as 10 to 20 U of heparin equally inhibited the lymphopenic effect of 20 to 200 μg prednisolone.

The observation that a relatively low dose of heparin can alter the glucocorticoid sensitivity of lymphocytes may be of interest.

The slight differences observed by us using two heparin preparations may reflect either the inhomogeneities in the preparations or a real difference between heparins derived from different sources or, else, the lack of appropriate standardization procedures [11].

As it was shown, glucocorticoids cause a redistribution of lymphocytes in the lymphoid organs [5, 14]. It is conceivable that heparin acts by inhibiting this redistribution the first step of which is a disappearance of the glucocorticoid-sensitive lymphocyte population from the circulation. The change in spleen weights observed even 3 hours after injecting prednisolone or heparin, may also reflect this redistribution process.

Another question is whether heparin influences the lymphocytes directly or (at least partly) in an indirect way, by generating some substances. The latter possibility cannot be excluded, since heparin was shown to activate the complement system [16], thus it probably generates a number of complement-derived factors. This is also true for dextran sulphate which induces lymphocytosis [17] and activates the alternate pathway of the complement system [3].

The reaction of eosinophils to heparin was less pronounced and less consistent than that of lymphoid cells. The question therefore arises whether the effect on eosinophils is mediated by the heparin itself or it is due to some contaminants present in the heparin preparation.

Recent data indicate a selective eosinophil chemotactic activity of histamine [4] which may be present in the heparin preparation as a contaminant. As the latter possibility has not been ruled out, the effect on eosinophils, not being the main scope of this paper, will not be discussed further.

The antagonism between heparin and glucocorticoids on the peripheral lymphocyte count may have a significance in the regulation of the blood lymphocyte level in both normal and pathological conditions. It must, however, be admitted that prolonged heparinaemia is infrequent under natural conditions [13].

The antagonistic action of heparin and prednisolone on the peripheral lymphocyte count may be only one example of the antagonism between some glucocorticoids and heparin or other glucosaminoglycan polysulphates. Further studies are required to clarify whether this antagonism is valid for other effects of glucocorticoids.

*

We thank Prof. I. Földes for helpful discussions. The excellent technical assistance of Mrs. Anikó Vajda and Mr. Á. Zsigmondy is appreciated.

References

1. Bálint, P. (Ed.): Klinikai laboratóriumi diagnosztika. 3rd ed. Medicina, Budapest 1962, p. 184.
2. Bradfield, J. W. B., Born, G. V. R.: Inhibition of lymphocyte recirculation by heparin. *Nature (Lond.) 222*, 1183 (1969).
3. Burger, R., Hadding, U., Schorlemmer, H. U., Brade, V., Bitter-Suermann, D.: Dextran sulphate: A synthetic activator of C3 via the alternate pathway. I. Influence of molecular size and degree of sulphation on the activation potency. *Immunology 29*, 549 (1975).
4. Clark, R. A. F., Gallin, J. I., Kaplan, A. P.: The selective eosinophil chemotactic activity of histamine. *J. exp. Med. 142*, 1462 (1975).
5. Cohen, J. J.: Thymus-derived lymphocytes sequestered in the bone marrow of hydrocortisone-treated mice. *J. Immunol. 108*, 841 (1972).
6. Dougherty, T. F., White, A.: Influence of adrenal secretion on blood elements. *Science 98*, 367 (1943).
7. Godlowsky, Z. Z.: Prevention of hormonal eosinopenia and lymphopenia by inhibition of clotting in blood. *Brit. med. J. 1*, 854 (1951).

8. Gowans, J. L.: The recirculation of lymphocytes from blood to lymph in the rat. *J. Physiol. (Lond.) 146*, 54 (1959).
9. Hamilton, L. H.: Heparin-induced block of the leucocyte response to cortisone. *Endocrinology 61*, 392 (1952).
10. Jansen, C. R., Cronkite, E. P., Mather, G. C., Nielsen, N. O., Rai, K., Adamik, E. R., Sioe, C. R.: Studies on lymphocytes. II. The production of lymphocytosis by intravenous heparin in calves. *Blood 20*, 443 (1962).
11. Jaques, L. B.: Biological actions of heparin. *Cienc. e Cult. 26*, 141 (1974).
12. Jorpes, J. E.: Heparin. Its Chemistry, Physiology and Application in Medicine. Oxford University Press, London 1939.
13. Koller, F.: The physiological function of heparin. *Thrombos. Diathes. haem. (Stuttg.) 33*, 17 (1975).
14. Levine, M. A., Claman, H. N.: Bone marrow and spleen: Dissociation of immunologic properties by cortisone. *Science 167*, 1515 (1970).
15. Paluska, D. J., Hamilton, L. H.: Effect of heparin on leucocyte response to hydrocortisone injections. *Amer. J. Physiol. 204*, 1103 (1963).
16. Rent, R., Ertel, N., Eisenstein, R., Gewurcz, H.: Complement activation by interaction of polyanions and polycations. I. Heparin-protamine induced consumption of complement. *J. Immunol. 114*, 120 (1975).
17. Sasaki, S.: Production of lymphocytosis by polysaccharide polysulphates (heparinoids). *Nature (Lond.) 214*, 1041 (1967).
18. Sasaki, S., Suchi, T.: Mobilization of lymphocytes from lymph nodes and spleen by polysaccharide polysulphate. *Nature (Lond.) 216*, 1013 (1967).
19. Vallent, K., Stekker, K., Fonyódi, S.: Über die Wirkung der Heparin-Behandlung auf die Serum-Eiweissfraktionen und auf das qualitative Blutbild in postoperativen septischen Zuständen. *Acta chir. Acad. Sci. hung. 6*, 165 (1965).

Correspondence: Dr. I. Jókay, Microbiological Research Group, Hungarian Academy of Sciences, Pihenő út 1, H-1529 Budapest, Hungary

Haematologia 11 (1—2), pp. 111—114 (1977)

Serum Lysozyme Activity in Some Myeloproliferative Diseases

Krystyna MERKIEL, J. PROKOPOWICZ, M. BIELAWIEC, Beata KIERSNOWSKA

Department of Clinical Diagnostics, Haematology Clinic, Medical School, Białystok, Poland

(Received March 25, 1977)

Serum lysozyme activity has been determined in patients suffering from myeloproliferative diseases, chronic myelogenous leukaemia (CML), acute myelogenous leukaemia (AML), chronic lymphatic leukaemia (CLL) and pancytopenia (P).

Lysozyme activity was tested in undiluted and tenfold diluted sera. Increased lysozyme activity was found in patients with CML and CLL, whereas there was no change in patients with AML and P. Dilution of sera enhanced lysozyme activity. These data may indicate the presence of inhibitor in the sera tested. The diagnostic significance of the presented findings is discussed.

It is generally known that in patients with myeloproliferative diseases, immunity to bacterial infections decreases [12]. Despite many investigations, the problem is still unelucidated. It is commonly accepted that immature forms of granulocytes have a diminished phagocytic activity [13].

Among the many factors participating in the mechanism of immunity, there are some unspecified ones such as lysozyme (muramidase E.C.3.2.I.17.). Lysozyme acts on mucopolysaccharides in the bacterial wall, where it splits the middle link of the tetrasaccharide N-acetylglucosamine – N-acetyl muramic acid – N-acetylglucosamine – N-acetyl muramic acid [14]. As a result, the structure of bacterial wall is damaged and it becomes increasingly susceptible to bactericidal agents. On the other hand, lysozyme is known as a factor stimulating the early stages of phagocytosis [9].

The main sources of lysozyme in blood are monocytes and granulocytes [4]. The enzyme has also been shown in tissue macrophages, some organs and body fluids [14]. There is an interrelationship between plasma lysozyme activity and the leukocyte count [1].

The purpose of the present investigations was to estimate the serum lysozyme activity in patients suffering from myeloproliferative diseases.

Material and Method

Patients. The investigations were carried out on 33 subjects; 7 with CML, 4 with AML, 16 with CLL, and 6 with P.

The diagnosis was based on clinical data, peripheral blood findings, bone marrow picture and in some cases on the histology of lymph nodes. Cytochemical reactions were done for differential diagnosis of the leucocytes.

The *control group* consisted of 30 healthy subjects of the same age groups as the tested group.

Serum lysozyme activity was determined turbidometrically according to Smolelis and Hartsell [14]. The activity was tested in undiluted serum and in serum diluted tenfold with 0.067 M Sörensen buffer pH 6.2, a system optimum for lysozyme activity [4]. The results were expressed in International Units (IU) per ml. A standard curve was constructed by means of a commercial egg white lysozyme preparation (Koch-Light Laboratories, Ltd.).

Micrococcus lysodeicticus, 24-hour agar cultures were used.

Results

Figure 1 shows lysozyme activity in undiluted sera. In the control group lysozyme activity was 200 to 500 IU/ml, mean 300 IU/ml, in patients with AML lysozyme activity was close to normal, despite numerous cells containing lysozyme.

In patients with CML, the activity was nearly double the normal; the difference was statistically significant ($0.05 > p > 0.025$). In the patients with CLL, the lysozyme activity was somewhat lower, while in the patients with P, the activity was normal.

Figure 2 shows lysozyme activity in diluted sera. In the control sera, mean lysozyme activity was 600 IU/ml. In AML patients, it was nearly four times higher than in the controls ($p < 0.001$) and the same was observed in the CML

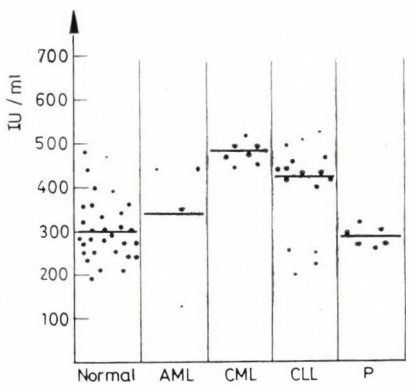


Fig. 1. Lysozyme activity in undiluted sera

patients ($0.002 > p > 0.001$). In these two groups, some cases even displayed a sevenfold increase in activity.

In the diluted sera of CLL patients a more than twofold increase of activity was found in comparison to the normal value, while in diluted sera from patients with P, lysozyme activity was similar to that observed in their undiluted sera.

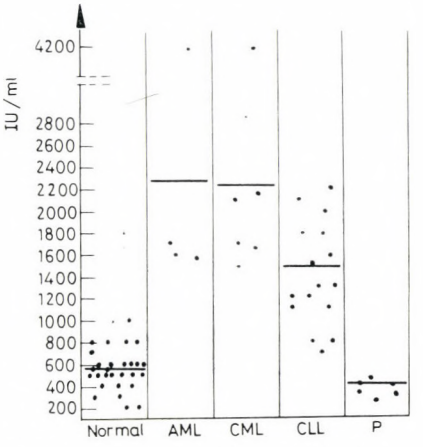


Fig. 2. Lysozyme activity in tenfold diluted sera

Discussion

Our results are in line with the data [2, 5, 12, 16] reporting an increased serum lysozyme activity in patients with CML. In contrast to those authors [5, 11] who found a decreased activity in CLL patients, in our CLL patients serum lysozyme activity displayed a twofold increase. The discrepancy may be due to variations in the peripheral granulocyte and monocyte counts observed in some of our patients, these cells being the main source of the enzyme.

In patients with AML some authors [6] found no increase or a moderate rise of lysozyme activity in undiluted sera.

The significant increase in lysozyme activity in our patients was probably due to the presence of lysozyme inhibitors, observed even in normal sera by Tepper et al. [17]. Dilution of the serum then abolished the inhibitory activity. Such lysozyme inhibitory substances are heparin, DNA and some other compounds [6]. On the other hand, in the course of myeloproliferative diseases such substances may be released from damaged leucocytes.

So it is reasonable to suppose that the high lysozyme activity in sera is successfully masked by inhibitors. Increased serum lysozyme activity in patients with myeloproliferative diseases may be a compensatory factor in decreased antibacterial mechanisms observed in these diseases.

Estimation of serum lysozyme activity may be of diagnostic value in leukaemias, especially for differentiating pancytopenia from AML, considering its

micromyeloblastic form. Lysozyme activity is evidently different in these two diseases. An increased lysozyme activity in patients with AML supports the finding that myeloblasts are rich in lysozyme [1], but a real lysozyme activity can only be shown in diluted sera.

References

1. Assamer, H., Schmalz, F., Braunstliner, H.: Die diagnostische und prognostische Bedeutung der Muramidase (Lysozym); Bestimmung in Leukozyten-Lysaten, Serum und Harn von Leukämie-Patienten. *Klin. Wschr. 49*, 587 (1971).
2. Catovsky, D., Galton, D. A. G., Griffin, Ch., Hoffbrand, A. V., Szur, L.: Serum lysozyme and vitamin B_{12}-binding capacity in myeloproliferative disorders. *Brit. J. Haemat. 21*, 661 (1971).
3. Catovsky, D., Galton, D. A. G.: Lysozyme activity and nitroblue tetrazolium reduction in leukaemic cells. *J. clin. Path. 26*, 60 (1973).
4. Daniels, J. C., Fukushima, M., Larson, D. L., Abston, S., Ritzman, S. E.: Studies on muramidase (lysozyme). I. Serum and urine muramidase activity in burnt children. *Texas Rep. Biol. Med. 29*, 13 (1971).
5. Fink, M. E., Finch, S. C.: Serum muramidase and granulocyte turnover. *Proc. Soc. exp. Biol. (N.Y.) 127*, 365 (1968).
6. Hankiewicz, J., Swierczek, E.: Lizozym — zagadnienia biochemiczne i kliniczne. *Post. Hig. Med. Dosw. 5*, 609 (1976).
7. Hansen, N. E.: Plasma lysozyme — a measure of neutrophil turnover. *Ser. Hemat. 7*, 7 (1974).
8. Harrison, J. F., Swingler, M.: The effect of serum macromolecules on the lysis of Micrococcus lysodeicticus cells by lysozyme. *Clin. chim. Acta 31*, 149 (1971).
9. Jolles, P., Sternberg, M., Mathé, G.: The relationship between serum lysozyme levels and the blood leukocytes. *Israel J. med. Sci. 1*, 445 (1965).
10. Klockars, M., Roberts, P.: Stimulation of phagocytosis by human lysozyme. *Acta haemat. (Basel) 55*, 289 (1976).
11. Levi, J. A., Soeden, J. B., Vincent, P. C., Gunz, F. W.: Studies of muramidase in haematological disorders: serum and marrow muramidase in leukaemia. *Pathology 5*, 59 (1973).
12. Noble, R. E., Fudenberg, H. H.: Lekocyte lysozyme activity in myelocytic leukemia. *Blood 30*, 465 (1967).
13. Sbarra, A. J., Shirley, W., Rotman, J. S.: The role of the phagocyte in host-parasite interactions. III. The phagocytic capacity of leukocytes from myeloproliferative and other neoplastic disorders. *Cancer Res. 25*, 1199 (1965).
14. Smolelis, A. N., Hartsell, S. E.: The determination of lysozyme. *J. Bact. 58*, 731 (1949).
15. Szmigielski, St., Ludwicka, H.: Rola lizozymu (muramidazy) w diagnostyce hematologicznej. *Diagn. Lab. 8*, 141 (1972).
16. Szmigielski, St., Oksińska, A., Ostojska, J., Moneta, J.: Serum lysozyme (muramidase) as functional test of granulopoiesis in the course of cytostatic therapy. *Folia haemat. (Lpz.) 101*, 935 (1974).
17. Tepper, T., Meijer, S., Brouwer-Pijpen, G.: Reliability of enzymatic lysozyme determinations. *J. clin. Path. 29*, 170 (1976).

Correspondence: Dr. K. Merkiel, Department of Clinical Diagnostics, Institute of Physiology and Biochemistry, Medical School, M. C. Skłodowskiej 24A, 15-276 Białystok, Poland

Haematologia 11 (1—2), pp. 115—126 (1977)

Итоги экспериментального и клинического изучения нового противолейкозного препарата гексафосфамида

Обзор литературы и собственные данные

Е. И. ХОМЧЕНОВСКИЙ

Центральный институт гематологии и переливания крови
Министерства здравоохранения СССР, Москва
(директор — член-корр. АМН СССР проф. О. К. Гаврилов)

(Поступило 4, 12, 1974)

1. Новый противолейкозный препарат гексафосфамид обладает в эксперименте на животных относительно невысокой токсичностью и при введении терапевтических доз вызывает подавление нормального гемопоэза, преимущественно гранулоцитопоэза.

2. Гексафосфамид подавляет рост ряда экспериментальных лейкозов: гемоцитобластоза αA мышей C_{57}, ретикулеза-гемоцитобластоза крыс, лейкоза Л-1210 мышей ДВА/2, лимфолейкоза мышей AKR — ЦОЛИПК № 8 и № 30, а также перевиваемых сарком крыс и мышей.

3. Гексафосфамид обладает более высокой избирательной способностью противолейкозного действия в эксперименте при сравнении с Тио ТЭФ.

4. Гексафосфамид является эффективным цитостатическим средством для лечения развернутой стадии хронического миелолейкоза.

5. Гексафосфамид эффективен при развившейся устойчивости к другим активным цитостатическим препаратам (миелосан, миелобромол, допан, рентгеновские лучи).

6. Гексафосфамид обладает выраженным противоопухолевым действием при сравнительно нерезком лейкопеническом влиянии, поэтому препарат целесообразно назначать при вариантах хронического миелолейкоза с выраженной спленомегалией и относительно невысоким лейкоцитозом.

7. Лечение гексафосфамидом следует начинать с дозы 10—20 мг ежедневно; в случае необходимости можно увеличивать интервал между введениями препарата от 1 до 6 дней. Общие дозы препарата и продолжительность лечения определяются индивидуально, в зависимости от темпа снижения количества лейкоцитов и уменьшения размеров селезенки.

8. Периодическое проведение «поддерживающего» лечения небольшими дозами гексафосфамида с учетом характера стабильности показателей лейко-и тромбоцитопоэза способствует закреплению и удлинению продолжительного лечебного эффекта.

9. Гексафосфамид не эффективен при развитии бластного криза.

Химиотерапия лейкозов за последние годы стала на прочную основу и быстро развивается как в экспериментальном, так и в клиническом направлении. Создано большое количество препаратов, часть из которых вошла в повседневную лечебную практику.

Настоящая работа посвящена изучению специфичности и широты терапевтического действия в эксперименте и клинике одного из наиболее активных новых этилениминов (*N,N'*-диэтилен-*N''*-циклогексил-триамидо-

тиофосфата) — гексафосфамида, синтезированного в результате выполнения программы изучения этилениминных производных (И. Л. Кнунянц и соавт, 1962; С. Э. Зурабян 1965).

Гексафосфамид — белый кристаллический порошок, нерастворимый в воде, растворим в бензоле, спирте, эфире, ацетоне и других органических растворителях. Температура плавления 96,5—97 °С.

Противолейкозная активность гексафосфамида в эксперименте была установлена Е. И. Хомченовским и соавторами в 1964, 1967, 1968 годах; выявлена также высокая противолейкозная активность препарата в клинике Е. И. Хомченовский, Т. П. Невская, Т. А. Чернцова, В. М. Сусова [1968].

Исследование токсичности производилось путем введения в желудок взвеси препарата в 1%-ном крахмальном клейстере. Токсичность изучалась на мышах линии C_{57} черных и АФБ и крысах линии *Wistar* по методу Литчфилд—Уилкоксона ($P = 0{,}05$).

На мышах линии C_{57} $ЛД_{50}$ составляла 105 мг/кг (66 ÷ 166,9). $ЛД_{10}$ равнялась 61 мг/кг. $ЛД_{50}$ на мышах АФБ составляла 125 мг/кг. При однократном введении максимально переносимых и токсических доз гексафосфамида, а также доз, в 2—3 раза больших $ЛД_{50}$, не наблюдалось симптомов «острой токсичности» непосредственно после введения. Симптомы интоксикации возникали лишь спустя 2—7 дней после введения доз и выражались в снижении веса тела, потере аппетита и подавлении гемопоэза.

При изучении действия гексафосфамида на нормальное кроветворение у животных установлено, что в максимально переносимых дозах препарат вызывал у мышей и крыс относительно небольшое и быстро восстанавливающееся угнетение гемопоэза. Отмечено более сильное подавление препаратом гранулоцитопоэза, по сравнению с лимфопоэзом. Количество гемоглобина и эритроцитов, даже при максимальных дозах, снижалось незначительно. При многократном введении терапевтических доз гексафосфамида также отмечено более сильное подавление гранулоцитопоэза.

Регенерация кроветворения у крыс наступала через 2—3 недели после отмены введений гексафосфамида.

Изучение действия препарата на сердечно-сосудустую систему, почки, надпочечники, легкие, желудочно-кишечный тракт, нервную систему, терморегуляцию не выявило каких-либо грубых морфологических изменений или нарушений их функций.

Противолейкозная активность гексафосфамида изучалась на мышах и крысах с перевиваемыми лейкозами. Установлена высокая активность препарата, особенно при лейкозе Швец крыс. Результаты этих исследований суммированы в табл. 1.

При начале лечения через 24 часа после перевивки гексафосфамид вызывал полную регрессию подкожной лейкозной опухоли у крыс, и при микроскопическом исследовании в органах, костном мозгу и в крови крыс не было обнаружено лейкозных изменений. Наблюдение в течение трех месяцев за излеченными животными этой серии показало полное отсутствие

Таблица 1

Действие гексафосфамида на перевиваемые лейкозы мышей и крыс при многократном введении

Штамм лейкоза	Срок начала лечения после инокуляции	Доза, мг/кг	К-во введений	Процент торможения	Увеличение продолжительности жизни	Изменения веса тела, в граммах оп/контр.	Вес селезенки, в мг. оп/контр.	Количество лейкоцитов в мм³ к концу эксперимента оп/контр.	Критерий достоверности P
Лейкоз Швец	24 часа	30,0	11	100		±10,0—48,6	1066/1056	5100/14600	0,001
Лейкоз Швец	7-й день	30,0	10		242**	—	—	—	0,001
Гемоцитобластоз LAH	24 час	10,0	7		96**	—	362/1130	—	0,005
Лейкемия 1210 асцитная Ф.	24 час	20,0	8		62*	—0,4/±2,1	117/228	2400/69900	0,004
Лейкемия 1210 подкожная Ф	5-й день	10,0	10		18**	—0,92/—2,29	303/414	9670/62900	0,4
Лейкоз Lel	24 часа	12,5	7		31*	—0,3/±0,4	1054/730		0,16
Лейкоз Lel	24 часа	6,0		37*		—1,6/±0,4	1054/730	—	0,16
Лимфолейкоз ЦОЛИПК № 8	24 часа	10,0	7	71		±0,1/±3,1	100/200	6350/17070	0,005
Лейкоз ЦОЛИПК № 30	24 часа	10,0	10	79		—1,2/1,4	—	4000/19050	0,001
Сакома Иенсена	24 день	50,0	10	99		—17,9—23,1	325/510	3670/19650	0,001
Саркома Иенсена	4-й день	30	10	96		—8,5/—23,1	350/510	5120/19650	0,001
Саркома 180	24 часа	20	11	59		—2,4/±1,2	100/197	—	0,005

* Увеличение продолжительности жизни мышей с асцитными формами лейкозов.

** Увеличение продолжительности жизни мышей и крыс при подкожной перевивке лейкозов.

у них каких- либо изменений, характерных для лейкоза. В то же время все контрольные крысы погибли на 14—18-й день после инокуляции при явлениях генерализованного лейкоза с поражением печени, почек, лимфоузлов, легких, большим количеством бластов в костном мозгу и наличии патологических лейкозных клеток в периферической крови. У крыс с лейкозом отмечалось значительное понижение количества эритроцитов и гемоглобина, составляющих в контрольной группе, в среднем 4 200—3 500 000 в мм^3 эритроцитов и 9,5—6,2 г% гемоглобина соответственно, а у крыс, получавших 11 введений препарата в дозе 30 мг/кг веса тела — 5 800 000 в мм^3 и 11,1 г% с последующим увеличением гемоглобина до нормы. В другой серии экспериментов с этим лейкозом изучалась продолжительность жизни крыс при начале лечения на 7—11-й дни после перевивки; когда подкожные лейкозные опухоли достигали размера 17,2 мм и более, явления генерализации были резко выражены. При этом гексафосфамид вызывал увеличение продолжительности жизни животных более чем на 242%. Большинство крыс этой серии прожили более 2—3 месяцев без признаков лейкоза и были забиты для гистологического исследования (рис. 1).

Препарат вызывал увеличение продолжительности жизни мышей с острым лейкозом Л-1210 на 62%. При этом в группах, подвергнутых лечению,

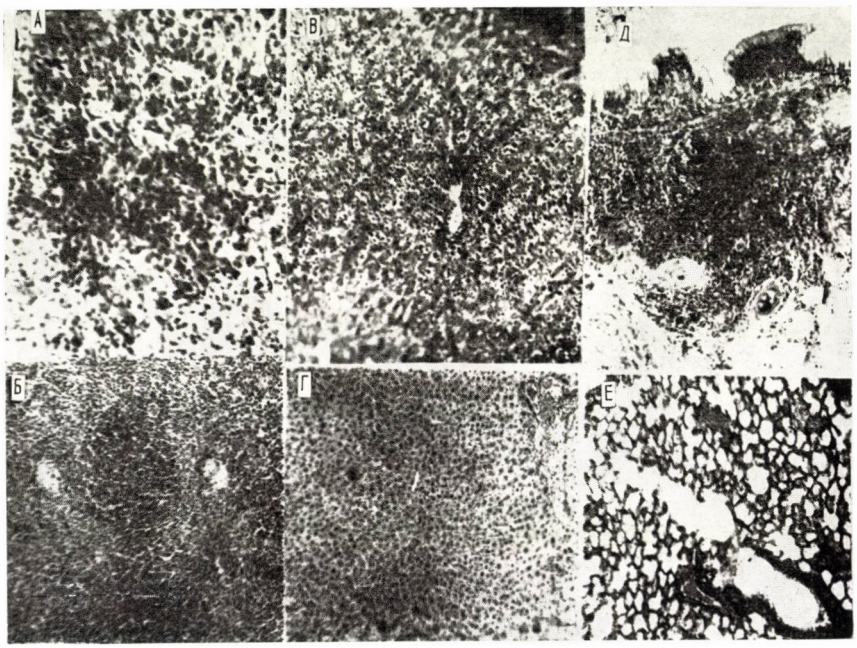


Рис. 1. Гистологические изменения во внутренних органах крыс с лейкозом Щвец в контроле и после курса лечения гексафосфамидом в дозе 20 мг/кг: А — селезенка, В — печень, Д — легкое крыс, подвергшихся контролю; Б — селезенка, Г — печень, Е — легкое крыс подвергшихся лечению гексафосфамидом

наблюдалось увеличение продолжительности жизни мышей, снижение количества лейкоцитов и «бластов» в периферической крови и костном мозгу и уменьшение инфильтрации внутренних органов.

У мышей с гемоцитобластозом ЛА при подкожной перевивке наблюдалось увеличение продолжительности жизни на 96%, полное торможение роста подкожной лейкозной опухоли, снижение веса селезенки в три раза по сравнению с контролем.

При асцитной форме гемоцитобластоза ЛА увеличение продолжительности жизни было еще большим и при оптимальной дозе равнялось 150%.

Препарат также угнетал и развитие других лейкозов: ЦОЛИПК №8 на 71%, ЦОЛИПК № 30 на 79%.

Гексафосфамид оказался активным препаратом и в отношении опухолей. Проводилось изучение действия препарата на саркому Иенсена и саркому Крокер-180. На саркоме Иенсена в дозе 50 мг/кг (суммарная доза 500 мг/кг веса тела) при введении через рот препарат вызывал 99% торможения роста опухоли, а 30 мг/кг —96%. (Суммарная доза 300 мг/кг.)

Препарат тормозил рост саркомы-180 на 59%.

После установления высокой противолейкозной активности гексафосфамида были поставлены специальные опыты по сравнительной количественной оценке его действия с широко применяемым в клинике препаратом Тио ТЭФ. Опыты проводились на двух перевиваемых лейкозах: гемо-

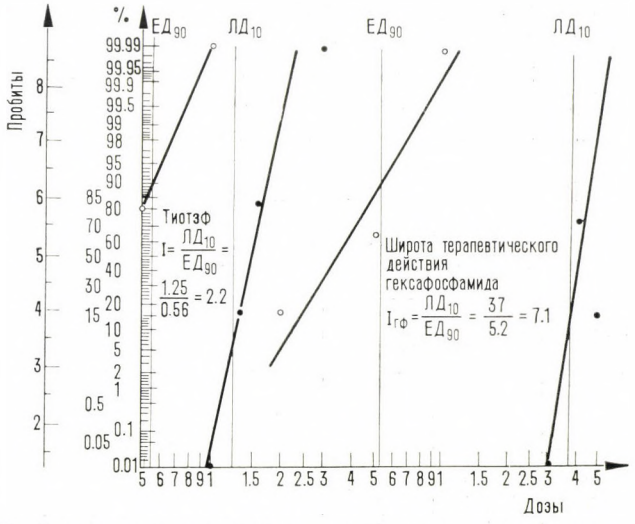


Рис. 2. Пробит-анализ терапевтического действия гексафосфамида и ТиоТЭФ. Широта терапевтического действия характеризуется расстоянием между вертикальными линиями, определяющими $ЛД_{10}$ и $ЕД_{90}$.

Опыт на крысах с лейкозом Швец. Лечение начато на 5-й день после подкожной перевивки при хорошо измеримых опухолях.

Гексафосфамид вводился зондом в желудок. ТиоТЭФ-внутрибрюшинно.

цитобластозе мышей ЛА и лейкозе Швец крыс. Для оценки эффективности сравнивались не только величины максимального эффекта, вызываемого препаратом, но и индексы, в которых сопоставлялись определенные величины токсичности и противолейкозной активности, характеризующие широту терапевтического действия препарата.

ТиоТЭФ вызывал увеличение средней продолжительности жизни у мышей с гемоцитобластозом ЛА максимум на 26%, в то время как гексафосфамид в оптимальной дозе в 10 мг/кг на 150%. При этом наблюдалось значительно меньшее поражение органов лейкозными инфильтратами; изменения со стороны крови были также значительно менее выраженными, чем при контроле.

Сравнительное изучение широты терапевтического действия гексафосфамида и ТиоТЭФ показало, что терапевтический индекс у гексафосфамида равен при ретикулезе-гемоцитобластозе крыс 7,1 по сравнению с 2,2 для ТиоТЭФ (рис. 2).

Специфическая противолейкозная активность гексафосфамида значительно превышала активность многих препаратов, за исключением лейкерана (табл. 2).

Результаты клинического изучения гексафосфамида.

В результате первой фазы клинического изучения гексафосфамида установлена его высокая противолейкозная активность при хроническом миелолейкозе, хорошая переносимость при введении через рот (Дульцин М. С. и соавт. 1968; Хомченовский Е. И., Невская Т. П., Чернцова Т. А., Сусоева В. М., 1968). Это дало возможность перейти ко второй фазе клинического изучения. П. М. Воронцов (1971), Я. И. Выговская и др. (1971),

Таблица 2

Действие на лейкоз Швец крыс различных противолейкозных агентов при многократном введении на 5-й день после перевивки

Препарат	Доза, мг/кг	Путь введения	Число введений	Средняя продолжительность жизни, *дни*	% излечения
Контроль	—	—	—	16,8 (15,1÷18,5)	0
Милеран	2		10	19,5 (19,4÷19,5)	0
6—МП	20	вн/бр	10	17,1 (11,5÷22,7)	0
Метотрексат	0,2	вн/бр	6	15,5 (13,7÷17,2)	0
Колхамин	1	вн/бр	10	11,6 (9,0÷14,2)	0
Циклофосфамид	8	вн/бр	10	>90*	83,4
Лейкеран	2	per os	15	>90*	100
Гексафосфамид	10	per os	10	>90*	100

* Выживание крыс, забитых без признаков лейкоза.

Т. П. Невская (1971), А. С. Погосян и Д. С. Айдинян (1971), О. А. Садыхов и др. (1971), В. В. Шильников и В. П. Ражева (1971), Т. П. Невская, Р. А. Мокеева и Ф. Э. Файнштейн (1971) и другие исследователи изучили действие препарата на 329 больных, 315 из которых составляли больные с хроническим миелолейкозом и 14 с другими злокачественными заболеваниями крови — с эритремией, хроническим лимфолейкозом, лимфогранулематозом (табл. 3).

Таблица 3

Сводные результаты терапевтической эффективности гексафосфамида при хроническом миелолейкозе

Форма лейкоза	Общее число больных	Результаты					
		Из них подлежит оценке	Выраженный объективный эффект	Незначительное объективное улучшение	Субъективное улучшение	Без эффекта	Не подлежит оценке
Хронический миелолейкоз	315	309	245	37	1	26	6
	в%	100	79,3	12,0	0,3	8,4	

Как видно из табл. 3—5, клиническое изучение показало высокую активность гексафосфамида при хроническом миелолейкозе, однако препарат не обладает большой активностью в отношении хронического лимфолейкоза, лимфогранулематоза, полицитемии.

Анализ суммарных данных, приведенных в табл. 3, показывает, что из 309 больных, которые подлежат оценке, выраженный терапевтический эффект получен у 245 больных (79,3%), незначительное объективное улучшение у 37 (12%), только субъективное улучшение у 1 (0,3%) и не получено эффекта в 26 случаях —8,4%.

Клиническое изучение показало, что общая суточная доза препарата колебалась от 10 до 40 мг, в редких случаях до 60 мг, общую курсовую дозу варьировали от 240 до 4 000 мг.

Продолжительность непрерывного лечения у больных, ранее не подвергавшихся лечению, колебалась от 16 до 113 дней, у тяжелых больных с выраженной резистентностью к другим препаратам достигала 240 дней.

Терапевтическое действие препарата выявлялось у больных обычно в одни и те же сроки. Как правило, после начала лечения у больных наблюдалась непродолжительная фаза раздражения кроветворения, что выражалось в повышении количества лейкоцитов на 5—7-й день лечения. С 7—14-го дня улучшалось самочувствие больных, начинало снижаться количество лейкоцитов, в основном незрелых гранулоцитов в периферической крови, уменьшались размеры селезенки (рис. 3, табл. 5, 6). Обращает на себя внимание то, что при лечении гексафосфамидом наблюдается значительное

Haematologia 11, 1977

Таблица 4

Колебания показателей красной и белой крови до и после лечения гексафосфамидом*

Гемоглобин, в единицах		Лейкоциты, мм³		Тромбоциты, мм³		Содержание незрелых форм гранулоцитов, %		Палочкоядерные нейтрофилы, %		Сегментоядерные нейтрофилы, %	
Колебания и средние цифры		Колебания и средние цифры		Колебания и средние цифры		Колебания и средние цифры		Колебания и средние цифры		Колебания и средние цифры	
до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения
52—82	62—81	62 500—380 000	6 600—45 000	100 000—870 000	90 000—600 000	27,5—76	1—28,5	12,5—46	5—41	6,5—44	26—63
67,0	74	173 900	20 850	231 500	309 000	41,9	12,7	29,7	20,9	22,5	41,4

* По данным профессора С. И. Шермана и старшего научного сотрудника К. М. Абдулкадырова (Ленинградский институт гематологии и переливания крови).

Таблица 5

Результаты лечения гексафосфамидом больных хроническим миелолейкозом(*)

Больной	Возраст (в годах)	Общая доза в мг	Гемоглобин в г%		Эритроциты в млн		Лейкоциты в тыс		Миелобласты в %		Промиелоциты в %		Миелоциты в %		Юные нейтрофилы в %		Палочко ядерные нейтрофилы в %		Сегменто-ядерные нейтрофилы в %		Лимфоциты в %		Моноциты в %		Тромбоциты в тыс		Размеры селезенки по Курлову в см		Печень ниже реберной дуги в см	
			до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения
Р. А.	34	1110	12.4	12.0	3.92	4.20	410	13	16	—	14	1	9	6	9	6	21	17	27	49	1	14	1	6	352	1134	$19\frac{28}{21}$	У реберного края	9	1
О. Т.	48	420	10.0	10.0	3.70	3.45	31	5	1	—	2	—	8	1	5	3	37	25	32	44	10	24	1	2	111	158	$15\frac{24}{18}$	$3\frac{12}{7}$	5	1
Б. Е.	37	2340	12.0	10.0	3.75	3.85	210	25.1	15	0.5	8	4.5	9	10.5	11	18	29	26	18	30.5	2	3.5	—	0.5	375	327	$13\frac{25}{18}$	$3\frac{12}{5}$	4	У реберного края
К. М.	65	1170	8.0	10.2	2.93	3.90	238	7.3	5	—	11	—	22	4	20	6	19	20	13	49	3	12	1	1	131	159	$22\frac{30}{24}$	$6\frac{12}{10}$	4	У реберного края
Р. Н.	35	1020	12.8	13.0	4.05	4.14	142	13.8	—	—	1	—	18	10	13	9	32	25	21	34	6	18	—	3	259	269	$13\frac{27}{25}$	$7\frac{16}{10}$	3	1
С. Л.	17	1140	8.4	12.0	3.48	4.00	288	26	2	0.5	4	0.5	15	4	19	8	28	23	27	56	3	4	2	3	332	328	$16\frac{25}{20}$	$8\frac{17}{10}$	4	2
К. О.	37	810	9.4	11.2	4.02	3.61	190	3.1	2	—	4	—	17	1	11	4	34	4	23	54	2	28	3	7	274	573	$3\frac{11}{8}$	не определялась	2	У реберного края
С. М.	61	1320	6.0	9.8	1.93	3.45	260	5.8	34	—	3	—	10	—	5	—	20	21	11	57	4	13	—	4	135	227	$17\frac{27}{18}$	$12\frac{15}{11}$	4	3
О. В.	55	880	9.0	13.2	3.01	4.48	275	4	5	—	8	1	16	1	17	—	27	5	20	63	1	28	—	2	427	400	$28\frac{36}{22}$	$12\frac{20}{8}$	5	2
М. П.	16	750	8.4	14.0	2.21	4.43	533	7.5	10	—	18	—	17	—	11	—	20	3	18	57	2	31	—	5	170	132	$20\frac{28}{15}$	не определялась	3	1
Среднее		1096	9.4	11.5	3.29	3.95	258	11.1	9	0.1	7.3	0.7	14	3.8		5.4	26.7	16.9	21	49.4	3.4	17.6	0.8	3.4	256.6	370.0				

(*) По данным д-ра П. М. Воронцова (Белорусский НИИ гематологии и переливания крови).

Таблица 6

Результаты лечения гексафосфамидом больных миелолейкозом с выраженным клиническим улучшением. (Из работы Е. И. Хомченовского, Т. А. Чернцовой, В. М. Сусоевой 1968 год ЦОЛИПК)

№№ п/п	Ф. И. О. больных	Возраст	Общая доза в мг	Срок лечения	Срок поддерж. лечения и ремиссия (в мес)	Гемоглобин (г%)		Эритроциты в млн		Лейкоциты в тыс		Недифференцированные в %		Незрелые гранулоциты в %		Зрелые гранулоциты в %		Тромбоциты в тыс		Размеры селезенки по Курлову в см		Печень ниже реберной дуги в см	
						до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения
1.	Т. Д. В.	40	1460	50	15	10,8	13,4	3,33	4,10	101	16	—	—	33	9	58	73	346	139	$5\frac{16}{10}$	—	1—2	0
2.	Б. А. М.	33	1530	150	9	8,4	13,0	3,20	5,00	124	5,4	8	4	29	—	45	53	271	127	$15\frac{24}{18}$	—	3—6	0
3.	К. А. П.	57	1670	45	4	8,2	9,6	3,06	3,50	255	6	26	2	25	3	40	84	475	445	$10\frac{18}{9}$	$4\frac{13}{6}$	14	8
4.	В. Г. А.	67	240	21	4	11,4	12,0	4,00	4,35	155	5	9	—	16	—	71	69	316	468	$21\frac{28}{21}$	$6\frac{14}{8}$	3—4	0
5.	Т. В. В.	40	4000	96	14	8,6	13,0	3,00	5,00	360	30	3	—	40	10	50	65	420	217	$25\frac{36}{22}$	$5\frac{16}{8}$	1—2	1
6.	С. К. В.	51	980	22	13	11,6	13,8	4,20	4,50	132	7	—	—	33	4	28	70	576	473	$4\frac{14}{10}$	—	3—4	0
7.	Р. М. Ф.	60	400	23	5,5	13,8	13,0	4,73	4,20	85	4	12	—	34	4	51	86	247	456	$18\frac{24}{21}$	—	5—6	3—4
8.	У. Т. С.	60	300	14	4	9,4	12,4	3,20	4,20	215	1,6	1,5	—	36	1	53	59	207	138	$21\frac{31}{10}$	$3\frac{12}{7}$	8—9	3—4
9.	Б. Г. К.	25	780	22	5	10,6	12,4	3,50	4,27	243	13	5	—	46	12	35	63	287	354	$6\frac{18}{13}$	$2\frac{15}{9}$	1.5	0
10.	С. Н. П.	66	1790	40	6	9,8	13,0	2,80	4,0	282	120	4	—	57	18	30	70	900	700	$8\frac{17}{10}$	—	4	2
11.	В. Е. П.	50	700	30	4	9,0	11,0	3,00	4,00	161	14	4	—	37	20	50	70	500	450	$20\frac{30}{17}$	$10\frac{18}{12}$	1	0
12.	К. Р. А.	21	700	30	1	11.2	12.3	3.50	4.00	210	130	3	—	38	36	57	52	175	200	$3\frac{11}{3}$	—	0	0
13.	К. И. П.	30	700	50	7	7,0	13,0	2,00	4,00	142	2	18	—	37	2	32	90	220	408	$30\frac{40}{23}$	$17\frac{25}{17}$	4	4
	Среднее		1172,1	45,6	7	10,0	12,4	3,35	4,00	189,6	27,2	7,2	0,2	35,5	9,2	46,2	69,5	380	352				

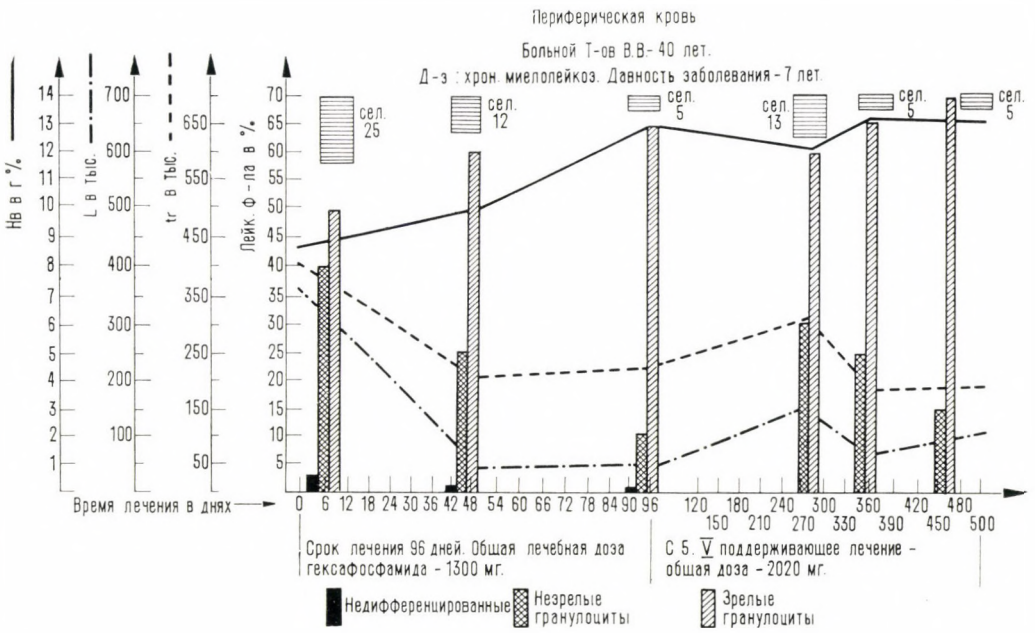


Рис. 3. Гемограмма больного во время основного и поддерживающего курса лечения гексафосфамидом.
Обозначения на рисунке.

уменьшение размеров селезенки при относительно небольшом снижении числа лейкоцитов. Снижение числа лейкоцитов в периферической крови до 20 000—30 000 мм^3, сопровождающееся параллельным уменьшением размеров селезенки требовало прекращения лечения гексафосфамидом. У многих больных после отмены препарата при указанных цифрах лейкоцитов наблюдалось дальнейшее снижение числа лейкоцитов от трех дней до двух недель. При слишком быстром темпе снижения числа лейкоцитов возникала необходимость в удлинении интервалов между введениями препарата или уменьшении дозы. Введение гексафосфамида вызывало отчетливые сдвиги в состоянии кроветворения в сторону его нормализации и сокращение размеров печени и селезенки (табл. 4 – 6).

Количество гемоглобина и число эритроцитов повышалось в более поздние сроки лечения; с 13 по 40-й день. У ряда больных положительная динамика показателей красной крови наблюдалась только к концу курса лечения, иногда на 30—60-й день. Препарат вызывает относительное увеличение содержания лимфоцитов в периферической крови с 3,4 до 17,6 после лечения (табл. 5).

На тромбоцитопоэз гексафосфамид действовал не одинаково: так, при повышенном числе тромбоцитов наблюдалось снижение уровня тромбоци-

тов, при тромбоцитопении у большинства больных (7 из 12) — повышение числа тромбоцитов, у трех больных с тромбоцитопенией их число оставалось на прежнем уровне и только у двух больных отмечено усугубление тромбоцитопении. Из 38 больных с нормальным числом тромбоцитов у большинства [28] при приеме гексафосфамида это число не менялось, у 6 отмечено повышение уровня тромбоцитов, а у четырех — снижение (Т. П. Невская, 1971).

В результате проведенного лечения препаратом 263 больных ремиссия до трех месяцев наблюдалась у 48 (18,2%), от 3 до 6 месяцев у 50 (19%), от 6 до 12 месяцев у 78 (29,6%), более длительные ремиссии — 12 месяцев и более – наблюдались у 87 (33%) больных.

При получении полной клинической и гематологической ремиссии лечение не возобновлялось до тех пор, пока число лейкоцитов крови не повышалось до 9 000—17 000 в $мм^3$ и более.

Применение поддерживающего лечения после окончания основного курса увеличивало время ремиссии (Т. П. Невская, 1971).

Поддерживающая терапия применялась путем введения 10—20 мг препарата в день 1—2 раза в неделю. В единичных случаях применялось ежедневное введение дозы в 10 мг или 10—20 мг один раз в 10 дней.

В результате применения подобной тактики лечения гексафосфамидом из 17 нелеченных ранее больных длительность ремиссии составляла у 14 больных от 7 до 56 месяцев причем у 10 из них со сроком наблюдения от 21 до 56 месяцев, ремиссии еще не окончились. Длительность улучшения у трех больных составляла 6, 10, 43 месяца. Рецидив заболевания в этих случаях протекал, как правило, по типу бластного криза (Невская Т. П., Мокеева Р. А. ФайнштейнФ. Э. и др. 1971).

Важно подчеркнуть, что гексафосфамид оказался эффективным при развитии резистентности к другим противолейкозным препаратам: миелосану (милерану), миелобромолу, допану и рентгенотерапии.

Из 113 больных, резистентных к предшествующей терапии, выраженное объективное улучшение получено у 74 больных (65,6%), незначительное объективное улучшение у 23 (20,3%), только у двух (1,1%) субъективное улучшение и у 14 больных (12,3%) эффекта получено не было. Эти результаты показывают, что гексафосфамид может найти применение в случаях возникновения резистентности к известным высоко активным препаратам, таким, как миелосан, миелобромол, допан.

Наряду с терапевтическим действием, у гексафосфамида в ряде случаев отмечаются и побочные эффекты. Побочное действие препарата в значительной степени зависит от индивидуальной чувствительности больных, от предшествующей терапии и от правильности применяемой схемы введения препарата. Побочные эффекты наблюдались у 49 (17%) из 315 больных при различных курсовых дозах: от 80 до 1 400 мг.

Основным побочным эффектом является подавление костно-мозгового кроветворения. Наиболее часто наблюдалась лейкопения у 36 (73,4%) из

49 больных с побочным действием, тромбоцитопения у 23 больных (43,6%) и прочие побочные эффекты у 5 больных, что составляло 10,2% от числа наблюдаемых побочных эффектов.

При многократном введении гексафосфамида может возникнуть суммация и в некоторых случаях кумуляция токсического эффекта. Поэтому введение препарата следует прекратить при снижении числа лейкоцитов до 30 000 и возобновить его лишь после возникновения явной тенденции к увеличению числа лейкоцитов. При приближении числа лейкоцитов к указанным минимально-допустимым цифрам целесообразно увеличить интервалы между введениями и проводить чаще анализы крови. Учитывая миелодепрессивные свойства гексафосфамида, не следует брать на лечение гексафосфамидом больных с небольшим количеством лейкоцитов.

Применяя кинетический метод исследования, установили, что биологический эффект гексафосфамида в дозе 20 мг соответствует дозе миелосана в 8 мг, миелобромола в 250 мг и рентгенооблучения области селезенки 100 р один раз в 2—4 дня.

Литература

. Воронцов П. М.: Изучение терапевтической эффективности гексафосфамида при лечении больных хроническим миелолейкозом. В сб. «Патогенез, лечение и эпидемиология лейкозов» Рига, 1971, 236—239.

. Выговская Я. И., Голотенко И. И., Мазурок А. А.: Об эффективности гексафосфамида при лечении больных хроническим миелолейкозом. Там же, 239—240.

3. Дульцин М. С., Зарецкий И. И., Хомченовский Е. И.: Итоги экспериментального и клинического изучения противолейкозного препарата гексафосфамида. Материалы Первой Всесоюзной конференции по химиотерапии злокачественных опухолей, Рига, 1968, 576—577.

4. Егоров Л. В.: Изучение обратимости специфического действия гексафосфамида на экспериментальных животных. В сб. «Патогенез, лечение и эпидемиология лейкозов», Рига, 1971, 232—234.

5. Зарецкий И. И. и соавт.: Новый противолейкозный препарат гексафосфамид, его химические свойства и результаты экспериментального изучения. В сб. «Патогенез, лечение и эпидемиология лейкозов», Рига, 1971, 229—231.

6. Зурабян С. Э.: Исследования в области новых канцеролитических соединений. Автореферат кандидатской диссертации, Ереван, 1965.

7. Кнунянц И. Л., Кильдишева О. В., Голубева Н. Е., Зурабян С. Э.: Диэтиленимидофосфорильные и диэтиленимидотиофосфорильные производные аминокислот и пептидов. Доклады АН СССР, 1962, 142, № 2, 370—375.

8. Невская Т. П.: Поддерживающее лечение гексафосфамидом больных хроническим миелолейкозом. В сб. «Патогенез, лечение и эпидемиология лейкозов», Рига, 1971, 247—249.

9. Невская Т. П., Мокеева Р. А., Файнштейн Ф. Э.: Пятилетний опыт применения гексафосфамида при хроническом миелолейкозе. «Современные проблемы лейкозологии и трансфузиологии, Тбилиси», 1971, 234—236.

10. Погосян А. С., Айдинян Д. С.: Лечение хронических миелолейкозов гексафосфамидом. В сб. «Патогенез, лечение и эпидемиология лейкозов», Рига, 1971, 242—247.

11. Садыхов О. А., Кочарлинская Д. А.: Результаты применения гексафосфамида при хроническом миелолейкозе. Там же, 241—242.
12. Хомченовский Е. И., Невская Т. П., Чернцова Т. А., Сусоева В. М.: Экспериментальное и клиническое изучение противолейкозной активности *N*, *N'*, диэтилен-*N''*-цикло гексилтриамидотиофосфата. «Проблемы гематологии и переливания крови», 1968, № 10, 49—56.
13. Хомченовский Е. И.: Сравнительное количественное изучение противолейкозной активности гексафосфамида, известных противолейкозных препаратов и рентгеновских лучей. «Бюллетень экспериментальной биологии и медицины», 1968, № 4, 90—98.
14. Хомченовский Е. И., Одинокова В. А., Смирнова Г. С., Алешинская Э. Е., Никольская Б. С.: Экспериментальные данные о новых противолейкозных препаратах. 41 Пленум Ученого Совета ЦОЛИПК. Тезисы докладов, 49—50, М., 1964.
15. Шильников В. В., Ражева В. П.: Ближайшие и отдаленные результаты лечения гексафосфамидом больных хроническим миелолейкозом. В сб. «Патогенез, лечение и эпидемиология лейкозов», Рига, 1971, 241—242.

Correspondence: Dr. E. Khomchenovsky, Institute of Hematology and Blood Transfusion, Novozykovsky 4, A–167, Moscow, U.S.S.R.

Experimental and Clinical Study of a New Antileukaemic Drug: Hexaphosphamide

Hexaphosphamide has moderate toxicity [in mice C_{57} LD_{50} = 105(66 ÷ 166.9) mg/kg]. MPD in normal rodents suppressed haemopoiesis, especially granulocytopoiesis. The drug possesses pronounced activity on transplantable leukaemias: it ensures complete regression of rat reticulosis-haemocytoblastosis if treatment is begun on the 5th day after transplantation (T.I. LD_{10}/ED_{90} = 7.1; ThioTEPA T.I. = 2.2). Hexaphosphamide improves the haematological picture and increases the life-span of mice with different leukaemia strains: La–150%; L-1210–62%; ZOLIPK N 8–71%; ZOLIPK N 30–79%. Hexaphosphamide has no gastrointestinal toxicity. In patients suffering from different forms of leukaemia, the drug had a beneficial effect especially in chronic myeloleukosis. Of 315 treated patients with chronic myeloleukosis 309 were evaluated. A good therapeutic effect was observed in 79.3%, a moderate effect in 12%, subjective improvement in 0.3%, and no effect in 8.4%. The therapeutic dose varied from 10 to 40 mg daily, the total dose was 240 to 4000 mg. Duration of treatment depends on the susceptibility of the haemopoietic system and varies from 16 to 113 days. In resistant patients duration of treatment reaches 240 days. Hexaphosphamide is an effective cytostatic drug for treatment of cases with acquired resistance to busulfan, myelobromol, dopan, X-rays. Of 113 patients resistant to previous therapy, a good response was achieved in 65.5%, a slight response in 20.3%, subjective improvement in 1.1%, and no effect in 12.3%. The most important side effect of hexaphosphamide is an individual susceptibility of haemopoiesis of some patients which is difficult to predict. Hexaphosphamide is ineffective in the treatment of blastic crisis of chronic myelosis.

Haematologia 11 (1—2), pp. 127—132 (1977)

Infusion of Physiological Saline Supplemented with Adenine, Pyruvate and Phosphate

W. RUDOWSKI, J. DASZYŃSKI, J. SCHIER,
Natalia KOWALCZYK, A. GACZKOWSKI, S. GÓRALSKI

Institute of Haematology, Warsaw, Poland

(Received October 9, 1976)

After controlled bleeding 20 ml/kg enriched infusion fluid containing 0.005 mol/l adenine, 0.1 mol/l pyruvate, 0.1 mol/l dibasic sodium phosphate and 0.15 mol/l sodium chloride was given to 14 dogs. Three dogs received after bleeding the same infusion fluid without adenine. One dog received only dextran after blood withdrawal and served as control. Before and after bleeding and 1, 4, 24, 48 and 72 hours after infusion the levels of 2,3-DPG, ATP and P_{50} index were determined.

After infusion of enriched fluid with and without adenine the level of 2,3-DPG and the value of P_{50} index increased. No rise in the ATP level was observed.

The properties of major phosphate esters and their significance in oxygen affinity for haemoglobin in erythrocytes have been stressed first by Benesch and Chanutin [2, 3]. Their studies concerned mainly the role of 2,3-diphosphoglycerate (2,3-DPG); its presence in the erythrocytes influenced the oxygen equilibrium in oxygen transfer by haemoglobin. A high level of 2,3-DPG in erythrocytes caused a shifting of the oxygen curve to the right and an increase in the P_{50} index.

During preservation of erythrocytes, a fall of the 2,3-DPG level and a rise in the haemoglobin oxygen affinity curve are observed. It is possible to regenerate *in vitro* the active major phosphate esters in erythrocytes by incubating them with fluids containing nucleosides, pyruvate and phosphate [7].

Attempts have also been made to raise the 2,3-DPG level *in vivo* by administration of fluids containing these compounds to experimental animals. Satisfactory results were obtained pointing to the possibility of influencing the haemoglobin dissociation curve by pharmacological means, raising the levels of 2,3-DPG and ATP [8, 11].

The purpose of the present investigations was to establish whether infusion fluids containing no nucleosides can raise the level of phosphate esters and to increase in this way the ability of oxygen equilibrium maintenance by haemoglobin.

Material and Methods

The investigations were carried out on 14 healthy mongrel dogs weighing 10 to 27 kg, aged 18 to 24 months. General anaesthesia with 25 mg/kg hexobarbital was applied and after its induction indwelling catheters were introduced

through the femoral blood vessels into the aorta and inferior vena cava. The sensor of a flowmeter was introduced into the contralateral femoral vessel for determination of flow velocity by the Doppler method. Blood samples were taken for haematocrit value determination before removing 20 ml per kg of body weight of blood. The duration of blood withdrawal was about 15 min. After an average stabilization period of 15 min, 10 dogs were infused with an equal saline enriched with 0.005 mol/l adenine, 0.1 mol/l sodium pyruvate, 0.1 mol/l dibasic sodium phosphate and 0.5 mol/l sodium chloride, pH 7.0 and 420 mOsm/kg. Three dogs received saline containing all these components with the exception of adenine. One dog was given only dextran; this animal served as control.

During the first day of observation the dogs were given sedating doses of propiomarine, a drug used in veterinary medicine as a neuropletic agent, tranquillizer and for premedication before anaesthesia. Before and after bleeding as well as 1, 4, 24, 48 and 72 hours after fluid infusion the following determinations were made in erythrocytes:

1. 2,3-Diphosphoglycerate level (2,3-DPG) according to Bartlett [1];
2. adenosine triphosphate (ATP) level by chromatography [5];
3. oxyhaemoglobin dissociation by calculating the P_{50} index, i.e. partial pressure of oxygen at which haemoglobin is saturated to 50% [9].

The haematocrit was also determined. One of the 14 experimental dogs received only dextran and served as control.

Results and Discussion

After bleeding, hypovolaemia ensued with an about 50% reduction of blood flow and anuria. These changes disappeared after infusion of enriched fluid but 2 to 3 hours after infusion the flow rate dropped again but only to the lower normal range. Urine secretion reappeared 15 to 30 min after the onset of infusion and after a brief phase of polyuria reduced urine secretion developed lasting to the end of direct observation.

The ATP level before bleeding was 1.6 ± 0.6 μmol/g Hb and it showed no significant change after the termination of bleeding, or after infusion of the enriched fluid.

In 3 dogs receiving fluid without adenine the ATP level was similar as after infusion of adenine-containing fluid and showed no significant deviation from its normal value. On the other hand, in the control dog receiving dextran the ATP level fell after bleeding and was lowest one hour after dextran infusion. In the following stages the ATP level returned gradually to its normal value. The results of ATP determinations are shown in Table 1.

The concentration of 2,3-DPG changed somewhat in the various stages of the experiment. The starting concentration was 17.5 ± 2.2 μmol/g Hb and in the first hour after fluid infusion it increased by 12%; the difference was at the borderline

Table 1

Adenosine triphosphate (ATP) level in μmol/g of Hb before and after infusion of enriched fluids

No. of dog N = 10	Before bleeding	After bleeding	After infusion of fluid with adenine				
			1 hr	4 hrs	24 hrs	48 hrs	72 hrs
$\bar{x}$	1.60	1.76	1.94	1.66	1.80	1.84	1.94
$\pm\delta$	0.60	0.29	0.34	0.32	0.41	0.27	0.62
No. of dogs	Before bleeding	After bleeding	After infusion of fluid without adenine				
1	1.17	0.93	1.02	1.50	1.18	1.67	1.26
2	1.54	1.39	1.60	1.40	1.73	1.44	1.32
3	1.30	1.35	1.66	1.38	1.42	1.07	1.12
Control dog	Before bleeding	After bleeding	After dextran infusion				
1	1.33	0.76	0.54	0.94	1.11	1.86	1.71

Table 2

2,3-Diphosphoglycerate (2,3-DPG) level in μmol of Hb after infusion

No. of dogs N = 10	Before bleeding	After bleeding	After infusion of fluid with adenine				
			1 hr	4 hrs	24 hrs	48 hrs	72 hrs
$\bar{x}$	17.50	16.27	19.86	18.46	20.30	19.77	20.26
$\pm\delta$	2.20	2.16	1.91	1.77	2.20	2.88	2.95
No. of dogs	Before bleeding	After bleeding	After infusion of fluid without adenine				
1	15.46	12.88	12.79	14.48	17.45	18.61	17.47
2	14.96	14.13	14.26	14.53	16.43	16.66	15.86
3	14.52	12.54	13.75	15.37	17.67	17.52	16.78
Control dog	Before bleeding	After bleeding	After dextran infusion				
1	12.8	11.5	12.5	13.9	13.8	11.1	12.3

of statistical significance. On the other hand, a statistically significant rise in 2,3-DPG concentration was found 24 hours after fluid infusion. It was significant in relation to the value before as well as after blood withdrawal. After 48 and 72 hours the level of 2,3-DPG increased and the differences were statistically significant in relation to the initial value.

Infusion of fluid without adenine to 3 dogs had a similar effect on the 2,3-DPG level. After a transient fall following bleeding and one hour after infusion the concentration increased reaching its peak value 24 and 72 hours following infusion. In the control dog dextran infusion was followed by a decrease of the 2,3-DPG level. This was particularly evident after bleeding and at the three successive determinations after 1, 4 and 24 hours. These results are shown in Table 2.

The basic function of erythrocytes in the organism is transport of gases and their exchange. This process depends on the ability of haemoglobin to reversible binding of oxygen and its efficiency depends, among other factors, on the values of partial oxygen and carbon dioxide pressures, pH in the tissues and haemoglobin affinity for oxygen. The affinity depends on the presence of major phosphate esters, mainly 2,3-DPG, and on the presence of ATP. The measure of haemoglobin affinity for oxygen is the partial pressure (P_{50}) at which haemoglobin is saturated to 50% [2].

In the case of the dogs studied, the P_{50} index was 13.7 ± 1.3 at the beginning of the experiment; after bleeding it fell significantly and after one hour it increased also significantly, even in relation to the preceding value. A significant rise was observed in the P_{50} index in the next determinations. Infusion of adenine-free fluid had no evident effect on the P_{50} index in the 3 dogs.

The values obtained in various stages of the experiment were very similar to those in the group receiving fluid with adenine. In the control dog the P_{50} value rose less steeply than in the dogs receiving the infusion fluid. A rise of P_{50} was observed after dextran infusion, particularly after 4 hours, but it did not exceed 8% of the initial value. These results are summarized in Table 3.

The enriched infusion fluid contained no such active substances as adenosine or inosine the role and ability of which to be incorporated into the metabolic cycle of erythrocytes are well known [4, 8]. Adenine in blood preserving fluids ensures, however, that the ATP level in the preserved erythrocytes remain high. Adenine participates in the metabolic cycle of erythrocytes and in the presence of 5-phosphoribosilo-1-phosphate ATP synthesis is the end result [11]. Our studies failed to corroborate this observation since no rise in ATP level was observed after infusion of adenine-containing fluid. Fluctuations of ATP concentration in the erythrocytes were similar after infusion of fluid with and without adenine. The rise of the 2,3-DPG level and of the P_{50} index observed after infusion of fluid with and without adenine suggests the responsibility of pyruvate and phosphate present in the fluid; being an intermediate product of glycolysis it could activate the functions of erythrocytes. Phosphate present in the fluid increased the glucose-6-phosphate concentration, and activated hexokinase (EC.2.7.1.1.) and phosphofructokinase (EC.2.7.1.2.) [6, 12].

Table 3

P_{50} value before and after infusion

No. of dogs N = 10	Before bleeding	After bleeding	After infusion of fluid with adenine				
			1 hr	4 hrs	24 hrs	48 hrs	72 hrs
$\bar{x}$	13.7	11.8	14.7	14.2	14.1	13.3	13.3
$\pm\delta$	1.3	1.8	1.3	1.3	1.4	1.8	0.9
No. of dogs	Before bleeding	After bleeding	After infusion of fluid without adenine				
1	15.4	12.4	13.8	14.7	14.1	13.6	13.3
2	15.6	13.2	15.2	14.9	16.0	15.7	15.5
3	16.5	11.2	14.7	16.5	17.0	16.2	17.2
Control dog	Before bleeding	After bleeding	After dextran infusion				
1	12.8	11.5	12.5	13.9	13.8	11.1	12.3

In the presently described experimental model it was not possible to use other fluid compositions since purine nucleoside phosphorylase (EC.4.4.2.1.) and the enzyme which metabolizes nucleosides to uric acid are absent in the erythrocytes of the dog.

Conclusions

1. Adenine added to infusion fluid containing sodium pyruvate, phosphate and sodium chloride had no significant influence on the values of ATP, 2,3-DPG, and P_{50}.

2. Infusion of fluid with sodium pyruvate, phosphate and sodium chloride without adenine had a significantly favourable effect on 2,3-DPG and ATP levels and P_{50} value in dogs in hypovolaemic shock.

References

1. Bartlett, G. R.: Phosphorus assay on column chromatography. *J. Biol. Chem. 234*, 466 (1959).
2. Benesch, R., Benesch, R. E.: The effect of organic phosphates from human erythrocyte on the allosteric properties of hemoglobin. *Biochem. biophys. Res. Commun. 26*, 162 (1967).
3. Chanutin, A., Curnisch, R. R.: Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. *Arch. Biochem. 121*, 96 (1967).

4. Lowy, B. A., Williams, M. K.: Studies on the metabolism of adenosine and adenine in stored and fresh human erythrocytes. *Blood 27*, 623 (1966).
5. Mills, G. C., Summers, L. B.: The metabolism of nucleotides and other phosphate esters in erythrocytes during *(in vitro)* incubation at 37 °C. *Arch. Biochem. Biophys. 84*, 7 (1959).
6. Minakami, S., Yoshikawa, H.: Inorganic phosphate and erythrocyte glycolysis. *Biochem. biophys. Acta (Amst.) 99*, 175 (1965).
7. Oski, F. A., Travis, S. F., Miller, L. D., Delivoria-Papadopoulos, M., Caunon, E.: The *in vitro* restoration of red cell 2,3-diphosphoglycerate levels in banked blood. *Blood 37*, 52 (1971).
8. Oski, F. A., Sugerman, H., Miller, L. D.: Experimentally induced alterations in the affinity of hemoglobin for oxygen. I. *In vitro* restoration of erythrocyte 2,3-diphosphoglycerate and its relationship to erythrocyte purine nucleoside phosphorylase activity in a variety of species. *Blood 39*, 522 (1972).
9. Rossi-Fenelli, A., Antonini, E.: Studies on the oxygen and carbon monoxide equilibria of human myoglobin. *Arch. Biochem. Biophys. 77*, 478 (1958).
10. Sugerman, H. J., Pollock, T. W., Rosato, E. F., Delivoria-Papadopoulos, M., Miller, L. D., Oski, F. A.: Experimentally induced alterations in affinity of hemoglobin for oxygen. II. *In vivo* effect of inosine, pyruvate, and phosphate on oxygen hemoglobin affinity in rhesus monkey. *Blood 39*, 525 (1972).
11. Simon, E. R.: Adenine and purine nucleosides in human red cell preservation: A review. *Transfusion 7*, 3 (1967).
12. Williamson, J. R.: General features of metabolic control as applied to the erythrocyte in red cell metabolism and function. *Advanc. exp. med. Biol. 6*, 117 (1970). Ed. G. J. Brewer. Plenum Press, New York.

Correspondence: Prof. W. Rudowski, Institute of Hematology, ul. Chocimska 5, 00-957 Warsaw, Poland

Haematologia 11 (1—2), pp. 133—137 (1977)

Immune Haemolytic Anaemia after Exchange Transfusion

E. FAGIOLO

Istituto di Clinica Medica, Università Cattolica del Sacro Cuore, Rome, Italy

(Received August 30, 1976)

Two cases of immune haemolytic anaemia in newborn infants who had received exchange transfusion on the first day of life because of hyperbilirubinaemia probably due to a conjugation defect, are reported. The first baby, born in the 30th week of gestation presented erythrocyte-fixed IgM antibodies + C, neutropenia, and circulating leucocytotoxic antibodies. The second baby, born at term to a diabetic mother showed erythrocyte-fixed IgG + IgM antibodies + C and a selective IgA deficit. In both cases the anaemia improved and the Coombs test became negative spontaneously in the 4th and 5th months of life, respectively.

These haematologic alterations are similar to a graft versus host (GVH) reaction due to the persistence in the neonate blood of the donor's immunocompetent cells.

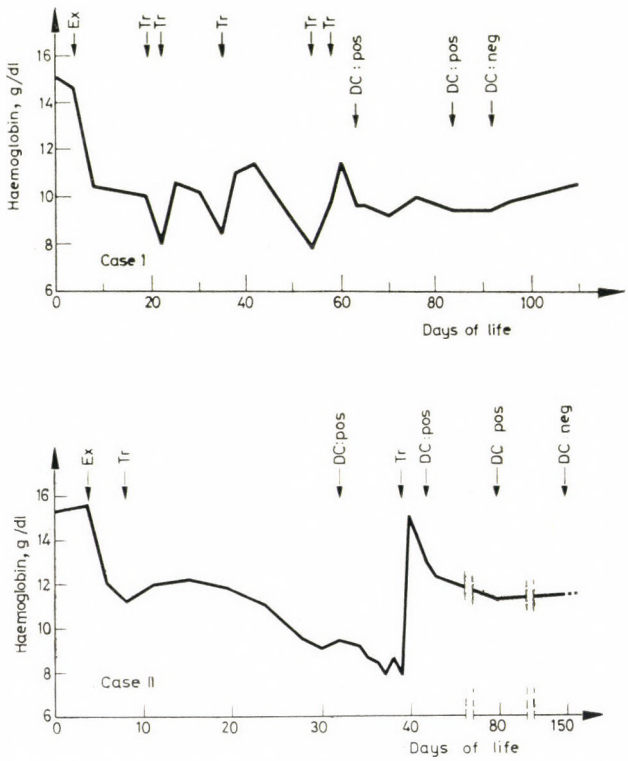
Immune haemolytic anaemia in the newborn who had received intrauterine or neonatal fresh blood transfusions has rarely been described. Case reports on this subject are particularly interesting because haemolytic anaemia seems to be associated with some clinical and pathologic manifestations of graft versus host (GVH) reaction, and has therefore been interpreted as due to the persistence of the donor's immunocompetent cells in the recipient's blood.

In this paper two cases of immune haemolytic anaemia following an exchange transfusion carried out in the first days of life because of non-haemolytic hyperbilirubinaemia probably due to conjugation defects, are described.

Case 1. M. R., a male infant born in the 30th week of gestation. Blood group A Rh(D)-negative. Mother's blood group AB Rh(D)-negative. The direct Coombs test with umbilical cord erythrocytes was negative. On the first day of life laboratory tests showed Hb 15 g/100 ml, erythrocytes 4,700,000/μl, haematocrit 49%, white blood cells 6200/μl. On the second day of life an increasing icterus without any modification in the erythrocyte count or Hb was noted. On the fourth day of life in view of a bilirubinaemia 17.8 mg/100 ml an exchange transfusion of 400 ml of fresh 0 Rh(D)-negative blood collected in acid–citrate dextrose was performed. On the following days a progressive reduction in erythrocytes and Hb occurred. The baby then received 5 transfusions of 40—50 ml packed, non-washed 0 Rh(D)-negative erythrocytes resulting in little and inconstant benefit. On the 63rd day of life laboratory tests showed: erythrocytes 3,200,000/μl, Hb 9.6 g/100 ml, reticulocytes 85,000/μl, white blood cells 4600/μl with neutro-

phils 35%, eosinophils 3%, lymphocytes 56%, monocytes 6%, platelets 450,000/μl. The direct Coombs test was positive (IgM + C); indirect Coombs test and agglutination with enzyme-treated erythrocytes were negative. Antileucocyte antibodies were searched for [1]: the patient's serum was cytotoxic for allogenic granulocytes and lymphocytes of 4 samples out of 8 tested. Serum immunoglobulin levels were IgG 100 mg/100 ml, IgA 19 mg/100 ml, IgM 27 mg/100 ml. Blood transfusions were discontinued on the 60th day of life. The direct Coombs test became negative on the 93rd day. The anaemia progressively improved while leucopenia persisted. At 110 days when the child was discharged from the hospital the laboratory tests showed Hb 10.5 g/100 ml, erythrocytes 3,900,000/μl, white blood cells 3400/μl with neutrophils 38%, eosinophils 2%, lymphocytes 60% and monocytes 3% (Fig. 1).

Case 2. C. S., a female infant of a diabetic mother was born in the 35th week of gestation. The child's blood group was B Rh(D)-positive, the mother's was 0 Rh(D)-positive. The direct Coombs test on umbilical cord erythrocytes



Figs 1 and 2. Haemoglobin (Hb g/dl) variations and transfusion schedule. Ex: exchange transfusion, 400 ml whole blood. Tr: transfusion 40–50 ml packed erythrocytes. DC: direct Coombs test

was negative. No immune anti-B antibodies were detected in the mother's serum. The child's weight at birth was 2200 g. Laboratory tests on the first day of life showed Hb 15.3 g/100 ml, erythrocytes 4,200,000/μl, white blood cells 16,500/μl, haematocrit 51%, reticulocytes 280,000 μl, platelets 320,000/μl. The peripheral blood smear showed 5 orthochromatic erythroblasts out of 100 nucleated cells. Bilirubinaemia was 11.5 mg/100 ml at birth, and 16.5 mg/100 ml on the third day of life. The baby therefore was given an exchange transfusion of 400 ml 0 Rh(D)-negative fresh blood collected in acid-citrate dextrose. Phototherapy was started. On the following days a progressive reduction in Hb and erythrocyte count was evident. Two transfusions of 40–50 ml packed, non-washed group 0 Rh(D)-negative erythrocytes were performed on the 9th and 32nd days of life. Other transfusions were not done because cross-matching gave mostly positive results. Out of 6 samples of blood assayed for cross-matching, 4 were positive in agglutination in saline, indirect Coombs test and agglutination with enzyme-treated erythrocytes. Complete and incomplete erythrocyte antibodies were therefore present. At 32 days Hb was 9.8 g/100 ml, erythrocytes 3,400,000/μl, reticulocytes 90,000/μl, platelets 240,000/μl. Bone marrow aspiration revealed erythroid hyperplasia. The direct Coombs test was positive (IgA + IgM + C); the indirect Coombs test was negative; agglutination with enzyme-treated erythrocytes was positive. On the 43rd day of life, when the child was discharged from the hospital, laboratory tests showed Hb 12.2 g/100 ml, erythrocytes 4,200,000/μl, reticulocytes 40,000/μl; the Coombs test was still positive and remained so at 80 days. Control tests performed in the fifth month of life showed Hb 11.4 g/100 ml and erythrocytes 4,100,000/μl. Serum immunoglobulin levels were IgG 300 mg/100 ml, IgA 5 mg/100 ml, IgM 30 mg/100 ml; the direct Coombs test was negative (Fig. 2).

Discussion

Immune haemolytic anaemia in the newborn who has received blood transfusions during intrauterine life or soon after birth is of great interest in the study of immune tolerance in the foetal and neonatal periods. In the two cases the symptoms were referable to a Coombs-positive haemolytic anaemia associated in the first case with granulocytopenia, lymphocytopenia and leucocytotoxic antibodies. In both cases the Coombs test became negative and the anaemia improved spontaneously after 4 months and 6 months, respectively. In the first case, haemolytic crises started about two weeks after the exchange transfusion; in the second case a marked drop in erythrocyte count occurred on the 20th day of life. It was not possible to define when erythrocyte antibodies had first appeared, because the Coombs test was carried out on the 60th and 32nd days of life, to explain the haemolytic anaemia and in the second case also for positive cross-matching.

Antibodies present in the 0 group blood transfused at birth could not be the cause of haemolytic anaemia in these cases.

The anti-A, anti-B natural antibody titre was low, 1/32 and 1/8 respectively, and immune antibodies were absent. Moreover, the presence of IgA and IgM antibodies fixed on the neonate's erythrocytes up to the eightieth day of life could not be related to exchange transfusions because immunoglobulins of this type have a half-life of 5–6 days. As a consequence, the haemolytic anaemia as well as the leucopenia in the first case seemed to be associated with a GVH reaction, in accordance with data in the literature.

Hathaway et al. [3] described two children aged 2 and 3½ months presenting necrotic vaccinia, erythemato-papular rash, pancytopenia and bone marrow aplasia one week after they had received a fresh blood transfusion. Autopsy in both cases showed a lymphocyte depletion in the lymph nodes, spleen, intestinal wall and thymus; in the thymus no Hassal's corpuscles were found. In one case the clinical picture and family history suggested a diagnosis of Swiss-type agammaglobulinaemia.

Nayman et al. [5] observed a newborn with haemolytic anaemia due to Rh incompatibility treated with intrauterine blood transfusion, 6 exchange transfusions in the first 80 hours of life, and 5 other blood transfusions in the first 7 weeks of life to overcome the persisting anaemia. In the eighth week of life the baby developed vomiting and diarrhoea and then anaemia became worse. The bone marrow was hypocellular with a marked decrease in the granuloblastic line. The direct Coombs test was always positive. A cytogenetic study in the child's blood showed the presence of a lymphocyte population resembling that of the donor of the intrauterine blood transfusions. Necropsy demonstrated a diffuse lymphocyte depletion and thymic atrophy.

Parkman et al. [7] described two newborns presenting with haemolytic anaemia due to Rh incompatibility. They were therefore treated with intrauterine exchange transfusions. On the twelfth day of life they showed an erythematous papular rash, hypothermia, anaemia, thrombocytopenia, and bone marrow aplasia. Necropsy revealed a marked lymphocyte depletion in the lymph nodes and thymus; the latter displayed a normal structure. Karyotype analysis suggested that the origin of the lymphocytes causing the GVH reaction was an exchange transfusion donor in both cases.

Similar findings were made experimentally. Intrauterine injection of spleen cells in the rabbit causes a Coombs-positive haemolytic anaemia [6, 8].

The factors conditioning the survival of the transfused lymphocytes in the newborn's blood are not clear; in fact, the newborn is capable of a modest response to bacterial and viral antigens and lymphocyte blast transformation [9]. It is usually capable of rapidly eliminating the lymphocytes introduced by exchange transfusion [2]; only in a few cases was their persistence for many months demonstrated in children treated with intrauterine transfusion of maternal lymphocytes [4].

Failure to reject transfused lymphocytes may be due to several factors: The degree of histocompatibility between the donor and the recipient, the number of viable transfused lymphocytes, the incomplete development of the immune

system, still relatively tolerant, and congenital or acquired immune deficiencies. The latter possibility seems to have occurred in our and some of the reported cases. The lymphocyte depletion observed, being a typical aspect of the GVH reaction, makes it difficult to prove the diagnosis of immune deficiency. In the two cases described in this paper, the spontaneous disappearance of erythrocyte antibodies and the improvement of anaemia indicate that transfused lymphocytes can be eliminated during development of the immune system.

References

1. Engelfriet, C. P., Britten, A.: Cytotoxic antibodies against leucocytes. *Vox Sang. (Basel) 11*, 334 (1966).
2. Galle, K., Schwanitz, G., Grosse, K.: Chromosomenuntersuchungen bei Neugeborenen nach Austauschtransfusion mit gegengeschlechtlichem Blut. *Blut 31*, 307 (1975).
3. Hathaway, W. E., Githeus, J. H., Blackburn, W. R., Fulginiti, V., Kempe, C. H.: Aplastic anemia, histiocytosis and erythrodermia in immunologically deficient children. *New Engl. J. Med. 273*, 953 (1965).
4. Hutchinson, D. L., Turner, J. H., Schlesinger, E. R.: Persistence of donor cells in neonates after fetal and exchange transfusion. *Amer. J. Obstet. Gynec. 109*, 281 (1971).
5. Nayman, J. L., Punnett, H. H., Lischner, H. W. M., Destiné, M. L., Arey, J. B.: Possible graft-versus-host reaction after intrauterine transfusion for Rh erythroblastosis fetalis. *New Engl. J. Med. 281*, 697 (1969).
6. Oliner, H., Schwartz, R., Dameshek, W.: Studies in experimental autoimmune disorders. I. Clinical and laboratory features of autoimmunization (runt disease) in the mouse. *Blood 17*, 20 (1961).
7. Parkman, R., Mosier, D., Umansky, I., Cochran, W., Carpenter, C. B., Rosen, F. S.: Graft-versus-host disease after intrauterine and exchange transfusions for hemolytic disease of the newborn. *New Engl. J. Med. 290*, 359 (1974).
8. Porter, A. K.: Immunohemolysis: a feature of secondary disease and runt disease in the rabbit. *Ann. N. Y. Acad. Sci. 87*, 391 (1960).
9. Yu, V. Y. H., Waller, C. A., Maclennan, I. C. M., Baum, J. D.: Lymphocyte reactivity in pregnant women and newborn infants. *Brit. med. J. 1*, 428 (1975).

Correspondence: Dr. E. Fagiolo, Istituto di Clinica Medica, Università Cattolica del Sacro Cuore. Via della Pineta Sacchetti 526, 00168 Roma, Italy

Haematologia 11 (1—2), pp. 139—147 (1977)

H-Excess A_1 and A_{int} Variants among the Bulgarian Population

P. PRODANOV

Institute of Haematology and Blood Transfusion, Sofia, Bulgaria

(Received March 25, 1977)

During the investigation of the subgroups of 45,895 A blood group persons, H-excess A_1 and A_{int} variants were found in 0.359%. These variants were subdivided into three categories provisionally named A_{1H}, A_{1Hint} and A_{intH} according to their most typical isoserological characteristics. The results of genealogical investigation are presented. An attempt was made to explain the existence of these atypical subgroups.

The unusual ratio of A : H antigens in the subgroups of some blood group A subjects attracted the attention of a number of authors [2, 3, 4, 9, 10, 13, 15, 16, 19] in the last decade. Race and Sanger [19] reported two cases of A_{int} with unusually high agglutinability with anti-H reagents. Bird found racial differences in the frequency of similar H-excess A_{int} [2]. In a detailed study, Brain reported an extremely high frequency of similar phenotypes among the Bantus [4]. We also described three similar cases observed in Czechoslovakia and designated them provisionally A_{intH} [9, 10].

In 1973, we reported on the A_{1H} variant [13] named so because of its equally high agglutinability of red cells with anti-A, anti-A_1 as well as with anti-H reagents. A year later Sathe and Bhatia [15] also presented the results of the investigation of A_1 high H variants.

The purpose of this report is to present some further studies on H-excess A_1 and A_{int} variants among the Bulgarian population.

Materials and Methods

The blood groups of 106,981 Bulgarians from district Haskovo were investigated. The subgroups of 45,895 of them who belonged to blood group A were also analyzed.

Investigation of subgroups was carried out in test tubes which were centrifuged at 1000 r.p.m. after 15 minutes at room temperature. For the routine test we used anti-A_1 and anti-H lectins from *Dolichos biflorus* and *Laburnum alpinum* respectively. The red cells with discrepancy in A : H ratio were analyzed additionally with human anti-A_1 serum and anti-H of *Ononis spinosa*, *Ulex europaeus* and eels. The secretor status was studied by the classical inhibition method

with different dilutions of the saliva. Agglutinability was studied quantitatively by means of calculations of the percentage of agglutinated red cells by the method of Fillitti-Wurmser [1] and Salmon [13] in our own modification [8, 9].

The method and the way of standardization of the reagents have been described in detail [8, 9].

The results were analyzed graphically by projecting the antigen strength (in per cent of the agglutinability) on the abscissa, and the frequency (in per cent of individuals for each per cent of agglutinability) on the ordinate. The obtained curve was termed a curve of frequency as a function of the antigen strength (FfAS curves).

These curves have three parameters which are convenient for comparison: (1) The length of the curve illustrating the limits of variability of the antigen strength; (2) the height of the curve illustrating the frequency of bloods for each of the different per cent of agglutinability; (3) the character of the curve shape.

Results

During the investigation of the subgroups of 45,895 persons belonging to blood group A we found discrepancies of the A : H ratio in 0.359% of the cases (Table 1). The subjects of the present report are H-excess A_1 and A_{int} phenotypes which constitute 0.242% of the Bulgarian population. We subdivided these cases into three groups, provisionally named A_{1H}, A_{1Hint} and A_{intH} in accordance with their most typical isoserological characteristics.

Table 1

Frequency of A subtypes

	Group	N	Per cent
Subgroup	A_1	39 582	86.245
	A_2	6 148	13.396
H-excess	A_{1H}	1	0.002
	A_{1Hint}	58	0.127
	A_{intH}	52	0.113
Intermediary	A_{int}	48	0.105
H-deficient	A_{Hm}	5	0.010
	A_h	1	0.002

Variant A_{1H}. The red cells of persons with A_{1H} phenotype reacted with anti-A and anti-A_1 reagents as strongly as A_1 control red cells did, and their reactivity with anti-H was identical to the reactivity of the control 0 red cells. It was therefore impossible to determine the subgroups (Table 2). During the quantitative studies

Table 2

Reaction of H-excess erythrocytes with anti-A_1 and anti-H

Reagents	Erythrocytes					
	A_1	A_2	A_{1H}	A_{1Hint}	A_{intH}	0
Anti-A	++++	++++	++++	++++	+++	−
Anti-A_1 (human)	++++	−	−+++	++++	++	−
Anti-A_1 (*Dolichos bifl.*)	++++	−	++++	++++	++	−
Anti-H (*Ulex europaeus*)	− or ±	+++	++++	+++	++±	++++
Anti-H (*Laburnum alp.*)	− or ±	+++	++++	++±	+++±	++++
Anti-H (*Ononis spinosa*)	− or ±	+++	++++	++±	+++±	++++
Anti-H (eel)	− or ±	+++	++++	++±	++++	++++

(Table 3) the red cells of A_{1H} persons showed agglutinability with anti-A and anti-A_1 reagents, varying the limits of agglutinability established for A_1 red cells, while the agglutinability with anti-H was similar as the agglutinability of 0 red cells.

Variant A_{1Hint}. Red cells of this type showed agglutinability with anti-A and anti-A_1 reagents similarly ($p > 0.05$) to the agglutinability of A_1 and A_{1H} red cells with the same reagents. In contrast to A_1 red cells, the anti-H reagents agglutinated them like A_2 ($p > 0.05$) but not so strongly ($p < 0.01$) as 0 and

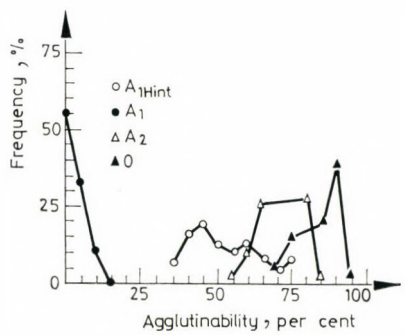


Fig. 1. FfAS curves for A_{1Hint}, A_1, A_2 and 0 erythrocytes with anti-H reagent

Table 3

Percentage

Group	N	Anti-A			
		range	mean $\bar{x}$	$S_{\bar{x}}$	SD
A_1	50	90—100	97.8	0.42	1.9
A_{1H}	4	94—98	97.5		
A_{1Hint}	58	90—99	96.2	0.26	0.8
A_{intH}	52	51—97	87.3	1.41	4.6
A_2	63	80—100	94.5	0.48	3.8
0	40	0	0	0	0

A_{1H} red cells (Tables 2, 3). The ratios are illustrated by the anti-H FfAS curve of A_{1Hint} which in its major part occupies an intermediary position when compared to the curves for A_1 on the one hand, and A_2 and 0 on the other (Fig. 1).

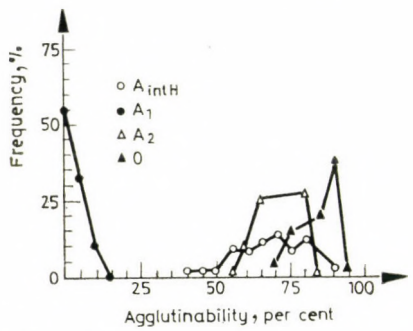


Fig. 2. FfAS curves for A_{intH}, A_1, A_2 and 0 erythrocytes with anti-H reagent

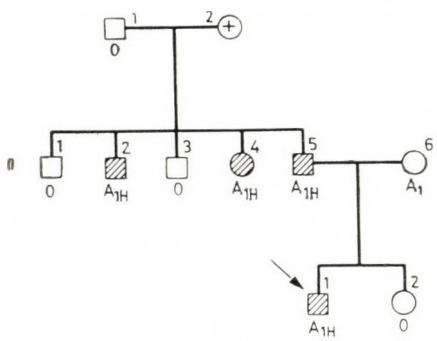


Fig. 3. Fam. R.T.

agglutination

Anti-A_1				Anti-H			
range	mean $\bar{x}$	$S_{\bar{x}}$	SD	range	mean $S_{\bar{x}}$	$\bar{x}$	SD
93—100	97.8	0.42	1.9	0—11	4.1	0.35	1.1
95—99	98.5			94—99	98.7		
82—99	97.9	0.48	1.6	30—79	51.1	1.39	4.6
20—78	33.4	1.58	5.9	38—95	74.9	1.74	5.7
0	0	0	0	45—60	52.6	0.40	1.3
0	0	0	0	75—99	85.7	0.88	2.9

Variant A_{intH}. In this category 52 cases were classified whose agglutinability with anti-A and especially with anti-A_1 reagents was considerably ($p < 0.001$) lower than that of A_1, A_{1H}, A_{1Hint}. At the same time their agglutinability with anti-H was stronger ($p < 0.001$) than that of A_2 and A_{1Hint} red cells, but in most cases not so strong as the agglutinability of 0 and A_{1H} red cells (Tables 2, 3, Fig. 2).

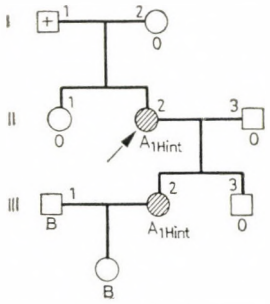


Fig. 4. Fam. D.T.

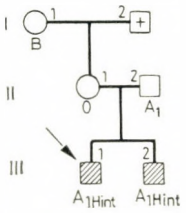


Fig. 5. Fam. P.P.

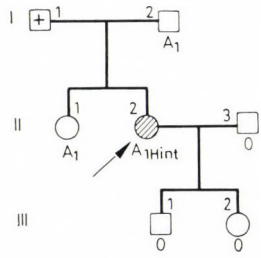


Fig. 6. Fam. Y.Z.

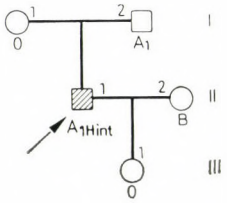


Fig. 7. Fam. G. T.

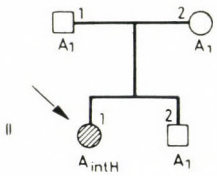


Fig. 8. Fam. P. M.

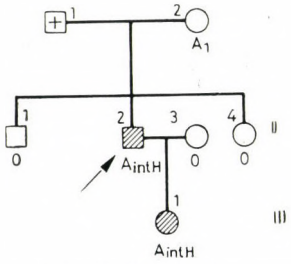


Fig. 9. Fam. T.T.

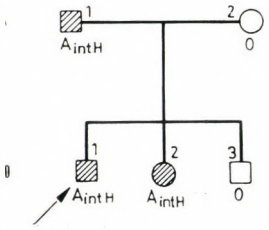


Fig. 10. Fam. J.N.

The sera of all the investigated persons of A_{1H}, A_{1Hint} or A_{intH} phenotype contained only the expected anti-B agglutinins with titres usually not exceeding 1 : 64. Normal quantities of A and H substances were found in their saliva when they were secretors.

The results of part of our genealogical investigations of H-excess variants are shown in Figs 3 – 12. During the investigation of A_{1H} variant (Fig. 3) we found three relatives of the propositus who had the same isoserological peculiarities. Figs 4 – 12 show the results for the A_{1Hint} and A_{intH} phenotypes. The mating $A_{intH} \times B$ (Figs 11, 12) resulted in A_{intH} and A_2B children and $A_2B \times B$ in A_{intH} and A_2B children.

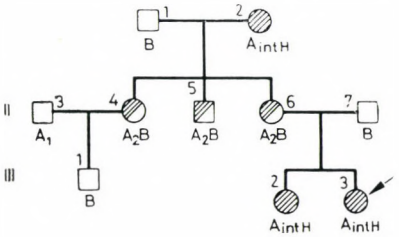


Fig. 11. Fam. S.N.

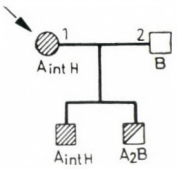


Fig. 12. Fam. A.D.

Discussion

According to the results we could subdivide H-excess A_1 and A_{int} phenotypes into three main categories taking into consideration their most typical isoserological peculiarities. The first and the least frequent (0.002%) A_{1H} category of red cells was identical in agglutinability to A_1 red cells when investigated with anti-A and anti-A_1 reagents. At the same time the agglutinability with anti-H reagents was similar to 0 control red cells. In order to illustrate the equal antigen ratio A_1 : H we named this H-excess A_1 category A_{1H}.

The second category A_{1Hint} agglutinated with anti-A and anti-A_1 reagents like A_1 and A_{1H} red cells did. This category could, however, be agglutinated with anti-H reagents as strongly as A_2 control red cells, but weaker than 0 and A_{1H} red cells. As their agglutinability with anti-H occupied an intermediate position between A_1 on the one hand, and 0 and A_{1H} on the other, we named this category A_{1Hint}.

The third category differed from the other two by its weaker agglutinability with anti-A and particularly with anti-A_1 reagents. At the same time these red

cells were agglutinated by anti-H reagents stronger than A_2 red cells and weaker than 0 red cells. That is the reason why for this category we preserved the symbol A_{intH} proposed by us before [9, 10].

We assume that the cases described by Race and Sanger [19], Bird [2] and Brain [4] could be classified as A_{intH} because of their reduced agglutinability with anti-A_1 reagents and their agglutinability with anti-H which was higher than that of A_2 control red cells.

The A_1 high H phenotypes described by Sathe and Bhatia [15] could be classified in the A_{1Hint} category in view of their equally high agglutinability with anti-A and anti-A_1 reagents and agglutinability with anti-H reagents approximating that of A_2 control red cells.

Thus, H-excess variants of A_1 and A_{int} occur among Caucasians though not so frequently than among the South African Bantus [4], Maharastrians [2], Indians [15], etc. These differences are evidently of anthropological interest.

At this stage it is difficult to explain the existence of H-excess phenotypes without biochemical studies. In order to find some theoretical explanation we based our assumptions on the genetical investigations of Jacob and Monod [7] concerning the *Escherichia coli* system. We also took into consideration the assumption of Solomon [18] and Hrubiško [5, 6] about the existence of Zz genes which regulate Hh production of Hh structural genes in lipid-soluble form only.

We have assumed by analogy that A_{1H} phenotype can be a result of a constitutive mutation of the regulator/operator system (Zz) of Hh structural genes [7, 13]. Consequently, the structural H gene produces abnormal quantities of fucosyl-transferases and H substance in lipid-soluble form.

The normal A_1 gene uses part of these quantities necessary for the synthesis of A_1 specificity and the remaining part, the "excess" of H probably causes the unusual parallel reactivity of the red cells with anti-H reagents. When the mutation affects the operator gene, the inheritance is dominant and when the regulator gene is affected, the inheritance is recessive.

A_{1Hint} phenotypes which contain a comparatively smaller quantity of H substance could be a result of incomplete mutations in the same operator/regulator systems.

Though the "excess" H substance is typical of A_{intH} red cells as well, it is difficult to assume that they should result from a combination of A_{int} allele and at the same time a constitutive mutation of the operator/regulator system of Hh genes. It is more probable that they are incomplete mutations of the regulator/operator system (Yy) of A genes. These mutations cause a partial blockage of the synthesis of lipid-soluble A specificity. As a result, great quantities of basic H substance, produced by the normal Hh gene, fail to be converted into A_1.

The change of A_{intH} into A_2B and its reappearance in the third generation as A_{int} in the families A.D. and S.H. could be explained as a result of interaction with the stronger gene B [9, 10, 12, 17]. Though it is a rare type of inheritance, it should be taken into consideration in paternity.

Regardless of the reasons for the formation of H-excess phenotypes, the existence of such anomalies should be taken into consideration in further attempts to form an entire genetic conception for ABO, Hh systems and their variants.

References

1. Fillitti-Wurmser, S., Jaquot-Armand, J.: Étude quantitative de l'isohémagglutination. *C. R. Soc. Biol. (Paris) 140*, 469 (1946).
2. Bird, G. W. G.: An intermediate in Maharastrian blood donors. *Vox Sang. (Basel) 9*, 629 (1964).
3. Booth, P. B., Sturrock, S.: Variations of H, A and B antigen strengths among Melanesians. *Vox Sang. (Basel) 31*, 211 (1976).
4. Brain, P.: Subgroups of A in the South African Bantu. *Vox Sang. (Basel) 11*, 686 (1966).
5. Hrubisko, M., Mergancova, O.: New variant of the blood group system ABO/H: the variant H_m. Congress of Czechoslovak Society of Haematology, Prague, 1965.
6. Hrubisko, M., Mergancova, O.: Beobachtungen über Varianten des Blutgruppensystems ABO. III. Die neuen Varianten 0_{Hm} und A_{Hm}. *Blut 13*, 278 (1966).
7. Jacob, F., Monod, J.: Genetic regulatory mechanism in the synthesis of proteins. *J. molec. Biol. 3*, 318 (1961).
8. Landsteiner, K., Levine, P.: Differentiation of a type of human blood by means of normal animal serum. *J. Immunol. 18*, 87 (1930).
9. Prodanov, P.: Quantitative aspects of blood group system ABO. Thesis, Bratislava, 1968.
10. Prodanov, P.: Étude quantitative du système de groupes sanguine ABO. I. Étude quantitative des antigènes A_1, A_i, A_2, B normaux et des types d'interactions entre eux. *Nouv. Rev. franç. Hémat. 1*, 19 (1970).
11. Prodanov, P., Hrubisko, M., Beranova, G., Calkovska, Z., Velvartova, M., Dankova, A.: Étude quantitative du système de groupes sanguins ABO. II. Paramètres quantitatifs de certaines catégories faibles des complexes agglutinogènes A et B et des phénotypes "Bombay" $A_h^{A_1}$, A_h et B_h rares. Étude quantitative des phénotypes 0_{Hm}, A_{Hm} et $A_{Hm}B$. *Nouv. Rev. franç. Hémat. 1*, 31 (1970).
12. Prodanov, P.: Gene interactions of AB phenotypic level. Proc. XIIth Congress Int. Soc. Blood Transfusion, Moscow, 1972.
13. Prodanov, P.: A new variant of blood group system ABO(H) : A_{1H}. 2nd Meeting Europ. African Divisi on, Int. Soc. Haematology, Prague, 1973. Abstr. 2, 322.
14. Salmon, Ch., Hautenauve, M.: Étude thermodynamique de l'anticorps anti-B des sujets de phénotype A_x. *Nouv. Rev. franç. Hémat. 1*, 847 (1961).
15. Sathe, M., Bhatia, H. M.: Serology and genetics of the A_1 high H subgroup. *Vox Sang. (Basel) 26*, 374 (1974).
16. Ssebabi, E. C. T.: A study of red cell H-content in Ugandan Africans and Europeans of group 0. *Vox Sang. (Basel) 30*, 204 (1976).
17. Ssebabi, E. C. T.: Action of African B gene on A_1, subgroup of A. *Vox Sang. (Basel) 30*, 308 (1976).
18. Solomon, J. M., Waggoner, R., Leyshon, W.: A quantitative immunogenetic study of gene suppression involving A_1 and H antigens of the erythrocyte without affecting secreted blood group substances. The ABH phenotypes A_m^h and 0_m^h. *Blood 25*, 470 (1965).
19. Race, R. R., Sanger, R.: Blood Groups in Man. Blackwell, Oxford 1962.

Correspondence: Dr. P. Prodanov, ul. Oboriste 28, Haskovo, Bulgaria.

Haematologia 11 (1—2), pp. 149—154 (1977)

Effect of F-Leurosine (formyl-leurosine) on Platelet Production in Mice

F. KRIZSA, I. CSERHÁTI

Second Department of Medicine, University Medical School, Szeged, Hungary

(Received May 14, 1976)

F-leurosine (formyl-leurosine) was found to exert a thrombocytopoietic effect in CFLP mice. The effect was most pronounced in a dose of 1.0 mg/kg. The increased activity of the megakaryocyte system was not due to thrombocytopenia. The preparation induced in the bone marrow an increasing megakaryocyte formation (increase of the megakaryocyte index, a shift to the left in the qualitative composition of the giant cells, increase of the ^{75}Se-methionine incorporation). F-leurosine seems to exert its effect through a direct stimulation of the megakaryocyte system, which might be utilized in therapy.

The Vinca rosea alkaloids vincristine (VC) and vinblastine (VB), due to their mitosis-inhibiting properties, are widely used in the therapy of tumours and haematologic diseases. In certain doses both drugs have a thrombocytosis-stimulating effect. This is important, because they have a favourable effect on megakaryocytopoiesis, and by inducing thrombocytosis allow an insight into the mechanism of platelet production.

F-leurosine (F-L), a third alkaloid of Vinca rosea, has been recommended for therapeutic purposes only recently. Being less toxic than the other two vinca alkaloids, it may have a more favourable therapeutic effect. In a series of mouse experiments we have studied the effect of F-L on the megakaryocyte system, to establish its supposed stimulating effect.

Material and Method

In the experiments 2—3 months old male mice of the CFLP strain of an average weight of 30 g, kept on a standard diet, were used.

The required dose of F-L (Richter Co., Ltd., Budapest) dissolved in the solvent supplied by the makers, was applied intraperitoneally in a volume of 0.25 ml. The thrombocyte count was determined in a drop of blood taken from the tail-vein, using the phase-contrast microscope method of Fisher and Germer [3]. The megakaryocyte content of the bone marrow was determined by our own method [5]. The essence of the method is to measure the bone marrow blown out from both femurs by air, weighing and suspending it in a given quantity of

propylene glycol, and determining the number of giant cells in a Bürker-chamber. The obtained ratio is suitable for expressing the megakaryocyte content of the bone marrow. For the qualitative study of the cells of the megakaryocyte system we prepared smears from the bone marrow of 10 mice and after staining according to May-Grünwald-Giemsa we classified 100 giant cells in each smear. Megakaryoblasts and promegakaryocytes were ranged in Group I, the immature cells forming no thrombocytes in Group II, the mature megakaryocytes forming thrombocytes in Group III and the senile giant cells having frequently bare nuclei only, in Group IV.

Incorporation of ^{75}Se-methionine (Se-M) in the thrombocytes was determined by the method introduced by Penington [8]. We administered 2 μCi isotope to each mouse intraperitoneally (i.p.) in 0.2 ml 0.9% NaCl solution. After 48 hours the thrombocytes were separated from 0.5 ml blood and their radioactivity was determined with a liquid-scintillating device of automatic relay (Packard).

The thrombopoietic activity of serum was studied by the mouse test [10], by administering 0.3 ml serum from the blood of the examined (donor) mice to the recipient group consisting of 4 to 5 mice. The result was considered positive if the average thrombocyte count of the recipient group increased by at least 30% by the 5th day.

Results

We studied the effect on the circulating thrombocytes of F-L in doses from 0.1 to 16 mg/kg. The effect of each dose was determined in 10 to 15 mice. Carrying out the test 6 hours after administering F-L in doses between 0.1 and 2.0 mg/kg we did not notice any effect of the drug. In higher doses we found a moderate thrombocyte-reducing effect which was most marked (33%) with 16 mg/kg.

To appraise the thrombopoietic effect, taking into consideration the megakaryocyte generation time, the peripheral thrombocyte count was studied systematically on the 5th day. In our dose-interval the drug displayed the most significant thrombocytopoietic effect in a dose of 1.0 mg/kg, increasing the thrombocyte count by nearly 60% by the 5th day. After doses of 0.5 mg/kg the effect was moderate, whereas a dose of 0.1 mg/kg had no effect. After doses of 4 to 16 mg/kg, no thrombocytopenia could be noticed on the 5th day (Fig. 1).

Following the administration of F-L in a dose of 1.0 mg/kg the peripheral thrombocyte count began to increase on the 3rd day, reached its maximum on the 5th day to decrease then gradually to the initial value by the 10th day (Fig. 2).

F-L in doses of 1.0 mg/kg increased the megakaryocyte index (Fig. 3) 48 to 72 hours after its administration. This was a consequence of the appearance of young cells, causing a shift to the left in the qualitative composition of the megakaryocyte system (Table 1). (Of course our megakaryocyte index method does not give any information about the possible marrow mass changes during the experiments.)

Studying the effect of F-L on megakaryocytopoiesis with ^{75}Se-methionine, we observed changes in the peripheral thrombocyte count. The drug displayed the most intensive thrombopoietic effect in a dose of 1.0 mg/kg, whereas doses

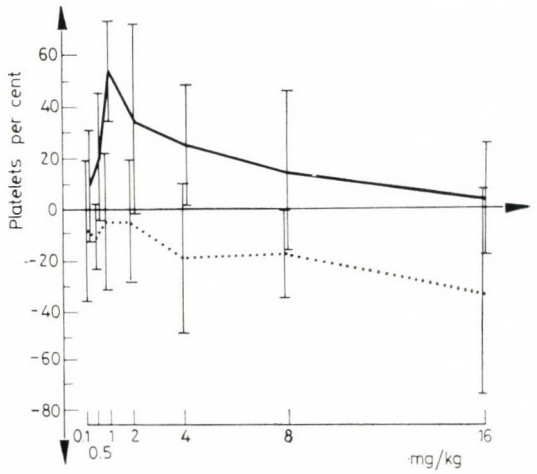


Fig. 1. Effect of different doses of F-L on the peripheral thrombocyte count in the 6th hour (· · · ·) and on the 5th day (———) after a single dose. Values represent the mean ±S.D. for 10 to 15 mice

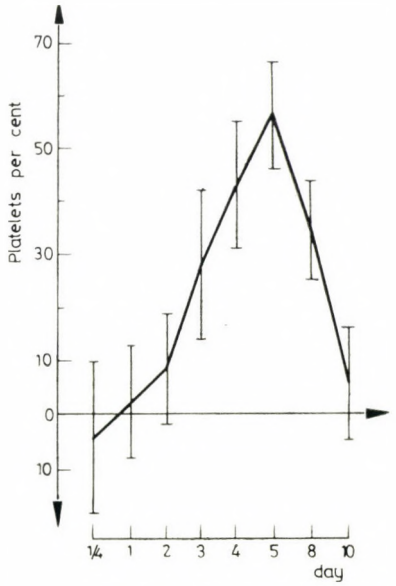


Fig. 2. Effect of a single dose of 1.0 mg/kg F-L on the peripheral platelet count. Values represent the mean ±S.D. for 10 mice

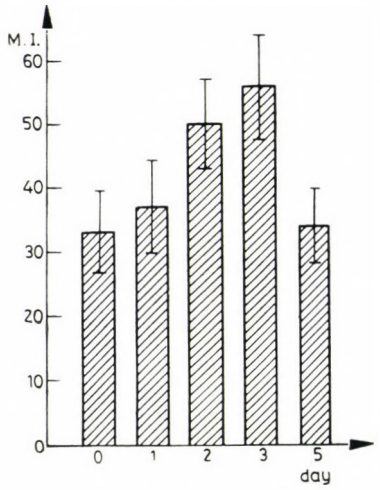


Fig. 3. Effect of a single dose of 1.0 mg/kg F-L on megakaryocyte index of the bone marrow. Each group represents the average of 10 mice and the S.D.

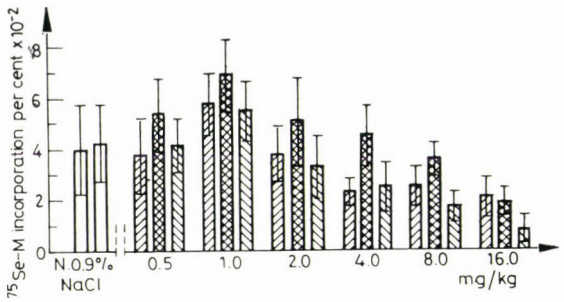


Fig. 4. 48 hours ^{75}Se-methionine intake 24 ▨, 48 ▩ and 96 ▧ hours after F-L administration. Each column gives the average of 5 to 8 mice, and the S.D.

Table 1

Effect of a single dose of 1.0 mg/kg F-L on the qualitative distribution of giant cells (estimated on the basis of 100 cells in 10 mice each on each day of the experiment)
(For classification, see text)

Group	0	1	2	3	5	10
	day					
I	3.8	13.5	16.3	22.1	3.9	3.5
II	16.1	25.9	42.7	40.1	19.2	15.4
III	78.8	58.9	38.5	35.1	75.5	79.9
IV	1.3	1.7	2.5	2.7	1.4	1.2

of 8 and 16 mg/kg showed a moderate selenium-incorporation-reducing effect (Fig. 4).

To clarify whether any humoral substance (thrombopoietin) had a role in thrombocytosis, F-L was administered in doses of 1.0 mg/kg and after 6, 12, 24, and 48 hours the effect of the serum was studied in the recipient mice for the peripheral thrombocyte count. No increase in the thrombocyte count was caused by the serum of the treated donors.

Discussion

The thrombocytosis causing effect of periwinkle alkaloids has been proved by Robertson and McCarthy [13] in men, by Robertson et al. [11, 12] in the rat, by Rák [9] and our team in mice [6].

Klener et al. [4] observed in the rat that VB and VC stimulated the megakaryocyte system while according to Odell et al. [7] they have a suppressive effect. The thrombocytosis-inducing mechanism of VC and VB is not clear; we have observed an increased activity of the megakaryocyte system following a temporary increase in thrombocytopenia and the thrombopoietin level after a single dose of VC. In animals pretreated with antithrombocyte serum, Rák [9] found an increased ^{75}Se-methionine incorporation with a normal thrombocyte count. Choi et al. [1] administering VC to rats assumed that the cause of thrombocytosis was an increased compensatory inflow following the inhibition of the giant cell precursors. Ebbe et al. [2] interpreted the thrombocytosis caused by VC as a mechanism compensating the cytotoxic effect exerted upon the megakaryocytes. None of the teams have studied the thrombopoietin level.

According to our knowledge the effect of F-L on the thrombocyte system has not been studied. The thrombocyte-formation-increasing mechanism of F-L as observed by us differs from that of VC in that it is not preceded by a reduction of the peripheral cell count or by the inhibition of megakaryocyte maturation. After a single dose of F-L we could notice an increased thrombopoietic activity in the serum. This also suggests the occurrence of a direct stimulatory effect of the drug on giant cells. If this were to prove to be the case in clinical trials, the application of F-L might be beneficial in human therapy.

*

We are indebted to Gedeon Richter Co. Ltd. for supplies of F-L and to Mrs. I. László and Miss Á. Böröcz for skilful technical assistance.

References

1. Choi, S., Simone, J. V., Edwards, C. C.: Effect of vincristine on platelet production. In: Platelets: Production, Function, Transfusion and Storage. M. Baldini, S. Ebbe (eds). Grune and Stratton, New York 1974, pp. 51—61.

2. Ebbe, S., Howard, D., Phalen, E. Stohlman, F.: Effect of vincristine on normal and stimulated megakaryocytopoiesis in the rat. *Brit. J. Haemat. 29*, 593 (1975).
3. Fischer, W., Germer, W. D.: Über eine einfache Methode der direkten phasenoptischen Thrombozytenzählung. *Röntgen-Lab. Praxis 10*, 49 (1957).
4. Klener, P., Donner, L., Houskova, J.: Thrombocytosis in rats induced by vinblastine. *Haemostasis 1*, 73 (1972).
5. Krizsa, F., Dobó, M., Zsilinszky, E.: Eine Methode zur Megakaryozytenzählung im Knochenmark der Maus. *Z. med. Labortechnik 7*, 370 (1966).
6. Krizsa, F., Kovács, Z., Dobay, E.: Effect of vincristine on the megakaryocyte system in mice. *J. Med. 4*, 12 (1973).
7. Odell, T. T., Jefferson, M., Jackson, C. M., Reiter, R. S.: Effects of vinblastine on megakaryocytes and platelets of rats. *Exp. Hemat. 10*, 36 (1966).
8. Penington, D. G.: Assessment of platelet production with ^{75}Se-selenomethionine. *Brit. med. J. 4*, 782 (1969).
9. Rák, K.: Effect of vincristine on platelet production in mice. *Brit. J. Haemat. 22*, 617 (1972).
10. Rák, K., Lehoczky, D., Krizsa, F., Cserháti, I., Kelemen, E.: Über die Thrombozytose verursachende Wirkung des Serums hämatologischer Kranken bei Mäusen. *Fol. haemat. (Lpz.) N. F. 7*, 8 (1963).
11. Robertson, J. H., Crozier, F. H., Woodend, B. E.: The effect of vincristine on the platelet count in rats. *Brit. J. Haemat. 19*, 331 (1970).
12. Robertson, J. H., Crozier, F. H., Woodend, B. E.: Vincristine-induced thrombocytosis studied with ^{75}Se-selenomethionine. *Acta haemat. (Basel) 47*, 356 (1972).
13. Robertson, J. H., McCarthy, G. M.: Periwinkle alkaloids and the platelet count. *Lancet 2*, 353 (1969).

Correspondence: Dr. F. Krizsa, University Medical School, Second Department of Medicine, Korányi S. rkp. 8—10, H-6701 Szeged, Hungary

Haematologia 11 (1—2), pp. 155—161 (1977)

Effect of Lanthanum Ion on Platelet Electrophoretic Mobility

G. KOSZTOLÁNYI, K. JOBST, I. KÁDAS

Department of Paediatrics and Clinical Chemistry Centre, University Medical School and County Hospital, Pécs, Hungary

(Received January 10, 1977)

The effect of trivalent La ions on the electrophoretic mobility of washed human platelets has been studied. The mobility of the human platelet in 0.145 M sodium chloride at pH 7.2 was $-0.84 \pm 0.06\ \mu$ per sec per volt per cm with an isoelectric point approximately at pH 3.4. La^{3+} at a concentration corresponding to the physiological level of Ca^{2+} changed the sign of the electrophoretic mobility of the platelets at pH 5.5 and pH 4.0, but had only a faint or practically no effect at pH 3.0 and pH 2.0. It is assumed that La^{3+} exerts its effect when bound to the phosphate groups on the surface.

Introduction

The physico-chemical background of platelet aggregation is not known in detail, in spite of its importance in thrombus formation. It is generally accepted that the platelet surface charge has an important role in platelet aggregation. In the phenomenon that no platelet aggregation occurs physiologically, the electrostatic force of repulsion between the identically (negatively) charged platelets is certainly implicated, whereas a decrease in their negative charge will enhance the tendency of the platelets to aggregate [3, 8, 11, 12, 16]. That is why many papers deal with the surface charge of platelets suspended in plasma or isotonic electrolyte solutions.

Cell electrophoresis is a method suitable for gaining information about the cell surface [1]. The electrophoretic mobility (EPM) of cells is determined by their surface charge density, i.e. the number and sign of the ionizing groups in the outermost layer of the membrane, and by the nature of the ions adsorbed to the surface. Electrophoretic studies combined with enzyme treatment have shown that the surface of the human platelet is amphoteric, at physiological pH values it has a negative surface charge, a negative EPM, which under identical experimental conditions represents an easily reproducible value [8, 12, 16].

Hampton and Mitchell [6] and others [2, 13, 16, 18] have shown that at low concentration various aggregating agents, e.g. ADP, which plays a primary role in physiological aggregation, increase the EPM of platelets, whereas at higher concentration they decrease it. The effect is poorly understood. The presence of

Ca^{2+} and fibrinogen seems to be essential for both these biphasic mobility changes and the aggregation induced by ADP *in vitro* [2, 6, 13, 16].

In connection with Ca^{2+} it has often been suggested that it exerts its effect when bound to the platelet membrane [3, 4, 6, 7, 16].

The platelet aggregation induced by ADP *in vitro* can be inhibited by the trivalent cation lanthanum [7, 14]. According to Holmsen et al. [7] this effect is not exerted by an interaction with Ca^{2+} but to a probably intracellular direct cell-damaging action or, according to O'Brien [14], to a competitive inhibition. Several observations [5, 7, 10] indicate, however, that by its stronger affinity La^{3+} is able to block the binding sites for Ca^{2+}.

In our earlier investigations [9], Ca^{2+} at physiological concentrations (order of magnitude 10^{-3} M) did not influence the EPM of washed platelets. In the present study the effect of La^{3+} on platelet EPM was observed under various experimental conditions.

Materials and Methods

Blood from normal subjects was collected by antecubital puncture into sodium citrate (1 volume to 9 volumes of blood), centrifuged at 300 r.p.m. for 8 minutes at 23 °C; the platelet-rich plasma obtained was diluted tenfold with PBS solution (0.145 M sodium chloride adjusted to pH 7.2 with 0.1 M phosphate buffer) containing 1% Na_2EDTA, and centrifuged at 3000 r.p.m. for 15 minutes. The supernatant was discarded and the platelets were washed three times in the above solution. All procedures were carried out in siliconized equipment. Some of the washed platelets were suspended in 0.145 M sodium chloride buffered to pH 7.2 with sodium bicarbonate, and the EPM of the control cells was determined. To study the mobility – pH relationship, platelet suspensions were prepared in 0.145 M sodium chloride adjusted to pH 4.0, pH 3.0 and pH 2.0 with 0.1 N hydrochloric acid, and the EPM of platelets was measured in these electrolytes. To study the effect of La^{3+}, a stock solution of 2×10^{-2} M was prepared from $LaCl_3 \cdot 6H_2O$ in 0.145 M sodium chloride. From the stock solution a series o different La^{3+} concentrations, each 2 ml in volume, was prepared by dilution with 0.145 M sodium chloride. To each of the 2 ml La^{3+} solutions was added 2 ml of a platelet suspension in 0.145 M sodium chloride (5×10^8 cells per ml), and then the pH value of each solution was adjusted. This was followed by incubation at room temperature for 10 minutes, after which the EPM of the platelets was measured in an OPTON cytopherometer at 4 mA – occasionally 5 or 6 mA – current intensity at 25 °C. After measurement, the specific resistance of each suspending electrolyte was determined. In each sample the migration of at least 50 cells was measured in each direction. The measurements were made within 4 hours of the collection of blood.

Results

1. *Human platelet EPM as a function of pH*

Figure 1 shows the pH – EPM relationship. Our results agreed well with those of other authors [2, 3, 8, 11, 16, 18, 19]. The EPM of the washed human platelet at pH 7.2 was -0.84 ± 0.06 μ per second per volt per cm, which is indicative of a

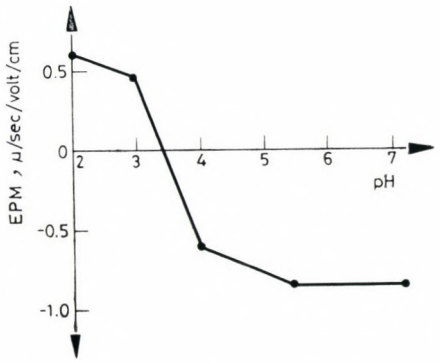


Fig. 1. pH-dependent changes in electrophoretic mobility of washed human blood platelets suspended in 0.145 M sodium chloride

negative surface charge. At pH 3.0 and pH 2.0 platelet migration occurred in the opposite direction, i.e. the EPM had a positive sign. Thus in this pH range cations predominate on the platelet surface. The form of the curve suggests that the platelet surface has its isoelectric point around pH 3.4.

2. *Effect of La^{3+} on platelet EPM*

Since at pH 7.2 $LaCl_3$ forms a precipitate, the effect of La^{3+} was studied at pH 5.5 and below. (Fig. 1 shows that the platelet EPM measured at pH 5.5 did not differ from that measured at pH 7.2.) Low concentration of La^{3+} did not alter the mobility (Fig. 2), but on increasing the concentration, the EPM gradually decreased; then, at a concentration of 10^{-3} M, its sign changed, i.e. the platelets obtained positive charge. The effect appeared immediately and did not change in 4 hours and was not affected by the field strength. The positivity of the surface could, however, be abolished; on washing the platelets in 0.145 M sodium chloride three times, the mobility was restored to the initial value. La^{3+} elicited a small degree of aggregation which did not interfere with the electrophoretic measurements: most of the platelets remained isolated.

3. *Effect of La^{3+} on platelet EPM as a function of pH*

The effect of the highest La^{3+} concentration was examined at different pH values (Fig. 3). Below pH 4.0 the positive surface charge decreased gradually and the mobility estimated at pH 2.0 was almost identical with that of untreated

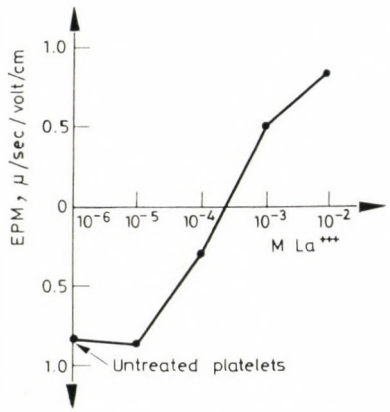


Fig. 2. Effect of La^{3+} at different concentrations on the electrophoretic mobility of human blood platelets suspended in 0.145 M sodium chloride at pH 5.5

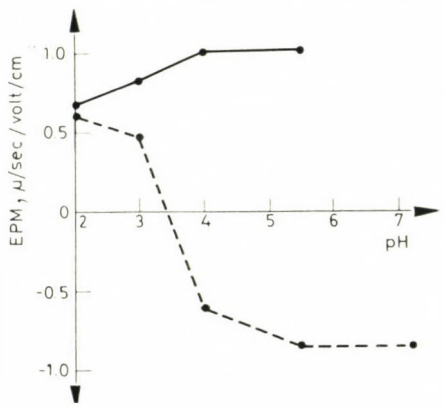


Fig. 3. Electrophoretic mobility of washed human blood platelets as a function of pH, ———— after treatment with 10^{-2} M La^{3+} and – – – – after treatment with 0.145 M sodium chloride

platelets. It was therefore assumed that the change in EPM in response to La^{3+} is elicited by surface groups whose dissociation decreases below pH 4.0 and is so reduced at pH 2.0 that La^{3+} cannot bind to the surface.

4. *Effect of La^{3+} on the EPM of fixed human red blood cells (RBC)*

In response to 10^{-2} M La^{3+}, the EPM of human RBC fixed in paraldehyde (-1.19 ± 0.04 μ per second per volt per cm), like platelet mobility, changed its sign and became positive. The rate of migration towards the cathode, however, decreased gradually after turning on the current and, after 2 to 3 minutes exposure

to the field strength, the cells again began to move towards the anode, and slowly the original EPM was restored. After 24 hours incubation the same phenomenon was observed.

5. *The in vivo effect of rat platelets treated with La^{3+} in vitro*

Rat blood platelets were treated with 10^{-2} M La^{3+} in the above manner, which rendered their sign of mobility positive. An intravenous injection of 1 ml of the platelets treated in this way was given to each of two rats weighing 200 g, while two control rats received a platelet suspension not treated with La^{3+}. The animals were sacrificed by decapitation, one La^{3+}-treated rat and its control after 5 hr, and the other pair after 24 hr, and their lungs, livers, spleens and kidneys were studied histologically. No differences were noted between the La^{3+}-treated animals and their controls; there were no microthrombi or platelets adhering to the endothelium.

Discussion

In the present experiments La^{3+} exerted a marked, concentration-dependent effect on the EPM of platelets. At concentrations corresponding to the physiological Ca^{2+} level (10^{-3} M) La^{3+} changed the sign to the opposite both of the platelet zeta-potential and of the platelet surface charge. The same effect of La^{3+} was observed with Ehrlich ascites tumour cells by Smith [17]. Such a change in zeta-potential may be accounted for by the presence of cations showing strong surface adsorption. Under our experimental conditions, La^{3+} must therefore have been bound by the platelet surface.

The electrophoretic change was reversible: La^{3+} adsorption could be abolished by washing. This suggested that the cell membrane had not been damaged irreversibly, or at least no change affecting the electrokinetic properties had occurred. The result obtained *in vivo*, i.e. the failure of La^{3+}-treated, positively charged platelets to adhere to the endothelium, was also ascribed to that fact. It may be assumed that the adsorption of La^{3+} was abolished intravascularly, and the platelet surface again obtained a negative charge.

On the basis of the behaviour of the fixed RBC, we believe that no stronger adsorption of La^{3+} to their surface could develop. What may have taken place was only a loose electrostatic interaction between the negative surface and the positive ions, and this was abolished by the electric field. Thus, it may be assumed that La^{3+} was adsorbed to those groups on the platelet surface which were not present on the surface of the RBC. Experiments with neuramidase and other enzymatic treatment have shown that the EPM of the RBC is determined almost solely by the terminal COOH groups of the sialic acid present in the RBC surface layer [15], while in the case of platelets other anionic groups can also be detected [12]. According to present knowledge, the most important are the phosphate

groups [4, 11, 12] whose ability to form chelate is well known. La^{3+} may possibly be adsorbed to these phosphate groups.

According to Brossmer and Patscheke [4] there are 10^{6-7} surface phosphate groups per platelet. Accepting this calculation, the La^{3+} concentration at which there is one ion to every surface phosphate group in a given platelet suspension, can be reckoned. Under our experimental conditions this La^{3+} concentration at which blocking of the surface phosphate groups may be regarded as complete, was inside the range ($10^{-2}-10^{-6}$ M) chosen by us. It is unlikely that in response to La^{3+} the molecules in the uppermost platelet surface layer would rearrange themselves, because the electrophoretic changes observed were reversible. The changed mobility and the net positive charge of platelets may therefore arise from the proportion of other charged (amino, carboxyl) groups taking place on the surface after blocking the phosphate groups and/or from the free La^{3+} charge.

References

1. Ambrose, E. J. (ed.): Cell Electrophoresis. Churchill, London 1965.
2. Betts, J. J., Betts, J. P., Nicholson, J. T.: Significance of ADP, plasma and platelet concentration in platelet electrophoretic studies. *Nature (Lond.) 219*, 1280 (1968).
3. Born, G. V. R.: Current ideas on the mechanism of platelet aggregation. *Ann. N. Y. Acad. Sci. 201*, 4 (1972).
4. Brossmer, R., Patscheke, H.: Über Rezeptoren an der Oberfläche menschlicher Thrombozyten. *Hoppe-Seylers Z. physiol. Chem. 349*, 1242 (1968).
5. Foreman, J. C., Mongar, J. L.: Dual effect of lanthanum on histamine release from mast cells. *Nature New Biol. 240*, 255 (1972).
6. Hampton, J. R., Mitchell, J. R. A.: Effect of aggregating agents on the electrophoretic mobility of human platelets. *Brit. med. J. 1*, 1074 (1966).
7. Holmsen, H., Whann, J., Day, H. J.: Inhibition by lanthanum ions of ADP-induced platelet aggregation. *Experientia (Basel) 27*, 451 (1971).
8. Kirschmann, C., Katchalsky, A., De Vries, A.: Electrochemical study of the platelet surface. *Arch. Biochem. 80*, 140 (1959).
9. Kosztolányi, G.: Unpublished data, 1973.
10. Levinson, Ch., Mikiten, T. M., Smith, Th. C.: Lanthanum-induced alterations in cellular electrolytes and membrane potentials in Ehrlich ascites tumor cells. *J. Cell Physiol. 79*, 299 (1972).
11. Mehrishi, J. N.: Phosphate groups (receptors?) on the surface of human blood platelets. *Nature (Lond.) 226*, 452 (1970).
12. Mehrishi, J. N.: The human blood platelets as a molecular mosaic. Its role in aggregation and thrombus formation. *Thrombos. Diathes. haemorrh. (Stuttg.) 26*, 370 (1971).
13. Nicholls, D. G., Hampton, J. R.: Density gradient separation of human platelets from plasma and the role of plasma in ADP-induced platelet electrophoretic mobility changes. *Thrombos. Diathes. haemorrh. (Stuttg.) 27*, 425 (1972).
14. O'Brien, J. R.: Some effects of mucopolysaccharide stains on platelet aggregation. *J. clin. Path. 23*, 784 (1970).
15. Seaman, G. V. F., Cook, G. M. W.: Modification of the electrophoretic behaviour of the erythrocyte by chemical and enzymatic methods. In: Cell Electrophoresis. Ed. E. J. Ambrose. Churchill, London 1965, p. 48.
16. Seaman, G. V. F., Vassar, P. S.: Changes in the electrokinetic properties of platelets during their aggregation. *Arch. Biochem. 117*, 10 (1966).

17. Smith, Th. C.: The effect of lanthanum on electrophoretic mobility and passive cation movements of the Ehrlich ascites tumor cells. *J. Cell Physiol. 87*, 47 (1975).
18. Turpie, A. G. G., McNicol, G. P., Douglas, A. S.: Platelet electrophoresis: effect of defibrination by ancrod (Arvin). *Cardiovasc. Res. 6*, 101 (1972).
19. Zerial, A., Wilkins, D. J.: Electrophoretic behaviour of some human blood cells. *Experientia (Basel) 28*, 1435 (1972).

Correspondence: Dr. G. Kosztolányi, Department of Paediatrics, University Medical School, Pécs, H-7623 Pécs, Hungary

Haematologia 11 (1—2), pp. 163—168 (1977)

Effect of Collagen on Thrombin Inactivation by Antithrombin-III and Heparin

Gy. BLASKÓ, Judith BÉDI, R. MACHOVICH, L. Á. PÁLOS

First Department of Medicine, Postgraduate Medical School, Budapest, Hungary

(Received April 14, 1976)

Thrombin inactivation by antithrombin-III and heparin has been found to decrease in the presence of collagen, whereas thrombin activity and the rate of thrombin inactivation by antithrombin-III alone are not affected. Albumin, at the same concentration as collagen, does not influence either thrombin activity or thrombin inactivation by antithrombin-III or by antithrombin-III plus heparin.

It has been shown that heparin increases the inactivation of thrombin in serum [3, 7, 16, 17]. It is generally accepted that heparin acts on this reaction by accelerating the thrombin-antithrombin-III complex formation [1, 16, 21]. The exact role of heparin in this reaction has not been elucidated, but several suggestions have been made [8, 16].

This heparin-activated reaction is influenced by several factors (cations, polar and non-polar groups, proteins, etc.) [4, 10, 11, 15]. Among these factors, the effect of collagen on thrombin inactivation by antithrombin-III and heparin has now been studied.

Materials and Methods

Collagen (non-soluble, from bovine Achilles tendon) was purchased from Boehringer GmbH, Mannheim, FRG, bovine albumin was obtained from Human Institute for Serobacteriological Production and Research, Budapest, Hungary. Both were dialyzed overnight at 0 °C against 0.05 M Tris-HCl buffer pH 7.3, containing 0.002 M NaCl, before experimentation. Fibrinogen (human, lyophilized, Grade L) was the product of AB Kabi, Stockholm, Sweden. Heparin (beef lung of 125 IU/mg specific activity) was obtained from Chemical Works of Gedeon Richter Ltd. and the other chemicals from Reanal Factory for Laboratory Chemicals, Budapest, Hungary.

Thrombin (specific activity about 200 NIH units per mg protein) was partially purified from bovine thrombin, Topostasin® (Hoffmann-La Roche, Basel, Switzerland), by DEAE-cellulose chromatography according to the method of Yin and Wessler [20].

Thrombin activity was assayed at 37 °C, in 0.5 ml final volume containing 5 μg thrombin protein, 5 μmoles Tris-HCl buffer pH 7.3, 1 μmole NaCl and 1000 μg fibrinogen. The clotting time was registered. Determinations were made in duplicate.

Antithrombin-III was partially purified from human plasma as published earlier [9].

Antithrombin-III activity was measured in a final volume of 0.4 ml containing 5 μg thrombin protein, 60 μg antithrombin-III protein, 5 μmoles Tris-HCl buffer pH 7.3, and 1 μmole NaCl. After incubation at 37 °C for periods between 0 and 5 min, 0.1 ml fibrinogen solution (1000 μg protein) was added and the clotting time was measured.

Protein was determined according to Lowry et al. [6].

Heparin concentrations were determined by their effect on the thrombin–antithrombin-III reaction. A standard calibration curve for heparin was constructed with commercial heparin.

The amount of heparin bound to collagen was calculated from free heparin of a collagen–heparin mixture. One mg collagen was mixed with different concentrations of heparin in 2.0 ml final volume containing 100 μmoles Tris-HCl buffer pH 7.3 and 4 μmoles NaCl. Thereafter it was centrifuged at 10,000 r.p.m. for 10 min in a Beckman J 21 B centrifuge and the heparin content of the supernatant was determined.

Results

Bovine collagen (0.8 mg per ml, non-soluble) or heparin (0.02 IU/ml) did not influence the thrombin activity during a 0 to 10 min incubation period. The addition of 60 μg per ml of antithrombin-III alone resulted in the prolongation of the clotting time of thrombin from 10 sec to 26 sec after 3 min incubation. When 0.02 IU/ml of heparin was added to the thrombin–antithrombin-III reaction mixture, clotting time was further increased to 75 sec. As to the effect of collagen on the kinetics of thrombin inactivation by antithrombin-III, it can be seen from Fig. 1 that thrombin inactivation without heparin was not altered in the presence of collagen at the above concentration, whereas the decrease of the clotting time from 75 sec to 40 sec indicated a decrease of the heparin effect.

The change of thrombin inactivation by antithrombin-III was also measured as a function of heparin and collagen concentration (Fig. 2). The reaction mixtures were incubated at 37 °C for 3 min in the presence of heparin and collagen at different concentrations. When thrombin was incubated with antithrombin-III alone, 0.006 IU/ml of heparin already increased the clotting time to about 38 sec. To obtain the same clotting time in the presence of 2 mg/ml of collagen, about 0.01 IU/ml of heparin was necessary. A further decrease of clotting time was measured on increasing the concentration of collagen. At 8 mg/ml collagen concentration, approximately 0.02 IU/ml of heparin was needed to restore the clotting time to 38 sec.

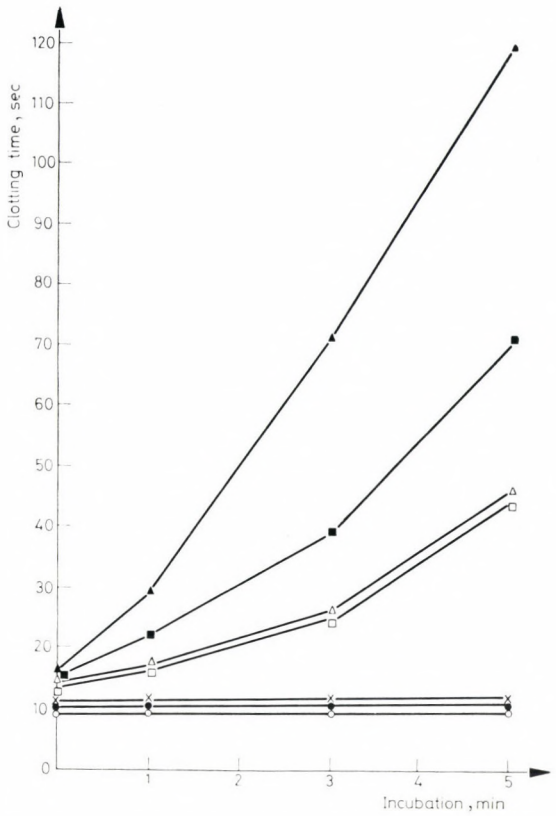


Fig. 1. Effect of collagen on thrombin inactivation by antithrombin-III and heparin as a function of time. 10 μg/ml of thrombin protein was incubated either alone, or with 0.8 mg/ml collagen, or with 120 μg/ml antithrombin-III, or with antithrombin-III plus heparin (0.02 IU/ml). ○———○ thrombin alone, ●———● thrombin plus heparin, ×———× thrombin plus collagen, △———△ thrombin plus antithrombin-III, □———□ thrombin plus antithrombin-III plus collagen, ▲———▲ thrombin plus antithrombin-III plus heparin, ■———■ thrombin plus antithrombin-III plus heparin in the presence of collagen

According to these findings, collagen seemed to neutralize the heparin effect on the thrombin–antithrombin-III reaction. Since the effect might be the consequence of a heparin–collagen interaction [21], it appeared worth-while to investigate the binding of heparin to collagen. Therefore, at different heparin concentrations we incubated with three different quantities of collagen at pH 7.3. After 10 min standing at 37 °C, the mixtures were centrifuged and the supernatants tested for heparin content. As shown in Table 1, 1 mg collagen can bind approximately 0.48 IU of heparin.

Since heparin is a highly sulphated polysaccharide with strong negative charges, it interacts with several proteins. Such a bound form of heparin may,

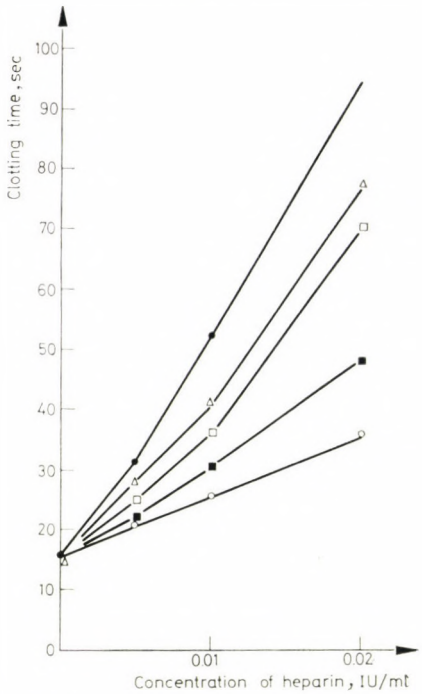


Fig. 2. Effect of collagen on thrombin inactivation by antithrombin-III as a function of heparin concentration. Experiments were carried out as described in Materials and Methods, except that incubation was done at 37 °C for 3 min. 10 μg/ml thrombin was incubated with 80 μgl/m antithrombin-III and heparin, in the presence of various collagen concentrations: no collagen ●——●, 1 mg/ml △——△, 2 mg/ml □——□, 4 mg/ml ■——■, 8 mg/ml ○——○

Table 1

Binding of heparin to collagen

Collagen concentration (mg/ml)	Added heparin (IU/ml)		
	0.1	0.5	5.0
0.5	—	0.03—0.04	
1.0	—	0.02	
10.0	—	—	0.03

Results are presented as IU/ml of free heparin.

however, maintain its biological effect on blood coagulation. To obtain some information in this context, we have examined a plasma protein, albumin, from this point of view. Bovine albumin, at concentrations between 16 μg and 8 mg per ml caused no alteration of either thrombin activity or thrombin inactivation by antithrombin-III, or antithrombin-III plus heparin (Fig. 3).

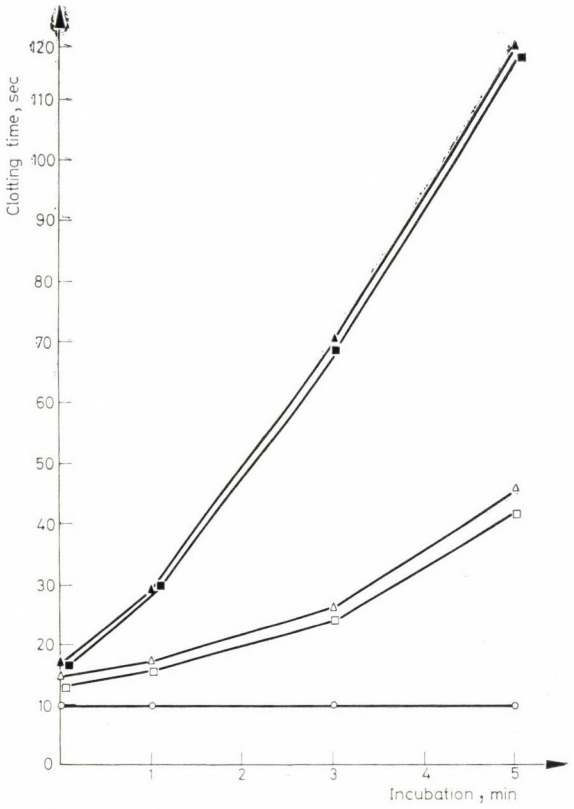


Fig. 3. Thrombin inactivation by antithrombin-III and heparin in the presence of albumin. Experiments were carried out as described in Materials and Methods, except that, instead of collagen, albumin was used at a concentration of 16 μg/ml, 80 μg/ml, 160 μg/ml, 800 μg/ml and 8 mg/ml protein. The concentration of thrombin, antithrombin-III and heparin was the same as described in Fig. 1. ○———○ thrombin plus albumin, △———△ thrombin plus antithrombin-III, □———□ thrombin plus antithrombin-III plus albumin, ▲———▲ thrombin plus antithrombin-III plus heparin, ■———■ thrombin plus antithrombin-III plus heparin plus albumin

Discussion

Thrombin inactivation by antithrombin-III may be influenced by several factors. Among the possible factors, the effect of non-soluble collagen was studied since it may play a role in some pathological blood coagulation [2, 19].

According to our findings, thrombin activity or thrombin inactivation by antithrombin-III was not affected by collagen, whereas enzyme inactivation by antithrombin-III plus heparin decreased in the presence of collagen. The results suggest that heparin binds to collagen and in this bound form heparin fails to accelerate the thrombin–antithrombin-III reaction.

On the other hand, plasma albumin affects neither thrombin activity nor enzyme inactivation by antithrombin-III and heparin. It is supposed that albumin does not bind heparin or the albumin-bound form of heparin retains its biological function. The first suggestion agrees with the data of Marciniak [12].

*

We thank Mrs. Theresa Fazekas for excellent technical assistance.

References

1. Abilgaard, U.: Inhibition of the thrombin-fibrinogen reaction by heparin and purified cofactor. *Scand. J. Haemat. 5*, 440 (1968).
2. Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F.: The effect of platelet-collagen reaction and blood coagulation on hemostasis. *J. clin. Invest. 46*, 1053 (1961).
3. Gerendás, M., Pálos, L. Á., Csefkó, I.: Heparin effect and thrombin inactivation. *Ann. Inst. Biol. Pervest. hung. 19*, 191 (1948).
4. Godal, H. C.: The assay of heparin in thrombin system. *Scand. J. clin. Lab. Invest. 13*, 153 (1961).
5. Jorpes, J. E.: Heparin in the Treatment of Thrombosis. Oxford Univ. Press, London 1946.
6. Lowry, O. H., Rosebrough, N. J., Farr, H. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem. 193*, 265 (1951).
7. Lyttleton, J. W.: Antithrombin activity of human plasma. *Biochem. J. 58*, 8 (1954).
8. Machovich, R.: Mechanism of action of heparin through thrombin on blood coagulation. *Biochim. biophys. Acta (Amst.) 412*, 13 (1975).
9. Machovich, R., Blaskó, Gy., Pálos, L. Á.: Action of heparin on thrombin-antithrombin reaction. *Biochim. biophys. Acta (Amst.) 379*, 193 (1975).
10. Machovich, R., Blaskó, Gy., Himer, Á., Szikla, K.: Effect of sodium and potassium ions on the activity of human antithrombin-heparin cofactor. *Thrombos. Res. 7*, 305 (1975).
11. Machovich, R.: Effect of calcium phosphate on thrombin and on its sensitivity to heparin and antithrombin-III. *Thrombos. Res. 9*, 123 (1976).
12. Marciniak, E.: Binding of heparin *in vitro* and *in vivo* to plasma proteins. *J. Lab. clin. Med. 84*, 344 (1974).
13. Németh-Csóka, M., Kajtár, J., Kajtár, M.: Biological significance of helical conformation of acid polysacharides. *Connect. Tissue Res. 3*, 207 (1975).
14. Pálos, L. Á.: Protecting effect of heparin on the inactivation of thrombin by heat. *Proc. Soc. exp. Biol. (N.Y.) 71*, 471 (1949).
15. Pálos, L. Á., Blaskó, Gy., Machovich, R.: Some properties of human progressive antithrombin. *Acta physiol. Acad. Sci. hung.* In press.
16. Rosenberg, R. D., Damus, P. S.: The purification and mechanism of action of human antithrombin-heparin cofactor. *J. biol. Chem. 248*, 6490 (1973).
17. Seegers, W. H., Warner, E. D., Brinkhous, K. M., Smith, H. P.: Heparin and the antithrombin activity of plasma. *Science 96*, 300 (1942).
18. Thaler, E., Schmer, G.: A simple two-step isolation procedure for human and bovine antithrombin II/III (heparin cofactor): A comparison of two methods. *Brit. J. Haemat. 31*, 233 (1975).
19. Wilner, G. D., Nossel, H. L., Leroy, E. C.: Aggregation of platelets by collagen. *J. clin. Invest. 47*, 2616 (1968).
20. Yin, E. T., Wessler, S.: Bovine thrombin and activated factor X. Separation and purification. *J. biol. Chem. 243*, 112 (1968).
21. Yin, E. T., Wessler, S., Stoll, P. J.: Identity of plasma-activated factor X inhibitor with antithrombin III and heparin cofactor. *J. biol. Chem. 246*, 3712 (1971).

Correspondence: Dr. Gy. Blaskó, First Department of Medicine, Postgraduate Medical School, Szabolcs u. 35, H-1389 Budapest, Hungary

Haematologia 11 (1–2), pp. 169–176 (1977)

Fibrinogen Metabolism in Cyanotic Congenital Heart Disease

B. GOLDSCHMIDT, J. SIEGLER, Mariann SCHMIDT

Second Department of Paediatrics, Semmelweis University Medical School, Budapest, Hungary

(Received October 25, 1976)

The metabolism and *in vivo* kinetics of fibrinogen labelled with radioactive iodine was studied in children with cyanotic congenital heart disease. The patients had a significantly lowered plasma fibrinogen pool, shortened fibrinogen half-life and increased fractional catabolic rate of fibrinogen compared with healthy children. The average plasma fibrinogen and absolute catabolic rate of fibrinogen did not differ from control values. The shortened fibrinogen half-life together with the correcting effect of anticoagulation with heparin indicated that fibrinogen was consumed by chronic disseminated intravascular coagulation. Inhibition of the fibrinolytic system with epsilon-aminocaproic acid in three cyanotic patients had no influence on the fibrinogen half-life in two of them but resulted in its prolongation in one patient.

In children with cyanotic congenital heart disease (CCHD) clotting disturbances are frequently observed. Some authors consider the abnormal coagulation to represent a chronic form of disseminated intravascular coagulation (DIC) [1, 4, 6, 7]. Other authors, however, deny this [5]. Primary hyperfibrino-(geno)lysis following hypoxaemia may also be a causative factor [6, 7, 10]. In some cases blood coagulation parameters were normal or near normal, hence, on the basis of these data the pathogenesis could not be clarified. Since fibrinogen acts as substrate in both the coagulation and the fibrinolytic system, it was attempted to clarify the mechanism of the coagulation abnormalities in CCHD by studying the kinetics of fibrinogen combined with anticoagulation or antifibrinolytic therapy.

Material and Methods

Examinations were performed in 10 children with compensated CCHD. No signs suggestive of haemorrhage or thrombosis could be detected. None of the patients had an elevated plasma level of fibrin monomers tested by the protamine paracoagulation test or ethanol gelation test. The control group consisted of 8 haematologically normal children, who had serious neurological damage with mental retardation. None of the children had any medication for 10 days before and during the study. Clinical and haematological details of the children are shown in Tables 1 and 2.

Table 1

Clinical and haematological data of patients

Case No.	Age (year)	Sex	Diagnosis	Body weight (kg)	Ht (per cent)	SaO_2 (per cent)	Plasma volume (ml/kg)	PT (per cent)	ECL (min)	FDP (μg/ml)
1	5	M	F−T	15	69	85	47	60	180	5
2	14	F	F−T	33	61	79	42	55	180	5
3	4	M	Tr	13	60	71	69	70	150	10
4	5	M	F−P	15	56	79	32	60	60	30
5	11	M	Tr	20	72	71	41	65	135	10
6	3	F	F−T	9	66	78	39	50	180	10
7	4	M	F−T	13	66	61	43	75	90	20
8	7	M	F−T	16	81	58	37	62	150	30
9	6	M	F−P	17	82	75	37	68	135	20
10	6	M	F−T	15	72	67	26	70	105	40
Mean ±SD							41.3±11	64		18

F = female; M = male; F−T = tetralogy of Fallot; F−P = pentalogy of Fallot; Tr = transposition of great vessels

Table 2

Clinical and haematological data, metabolism and distribution of fibrinogen in healthy children

Case No.	Age, (year)	Sex	Body weight (kg)	Ht (per cent)	Plasma volume (ml/kg)	Fibrinogen					
						plasma level (mg/ml)	IFP (mg/kg)	T/2 (days)	C_1	FCR	ACR (mg/kg/day)
1	4	M	17	42	46	2.20	101	2.7	0.65	0.39	39
2	8	M	25	45	75	1.90	142	2.5	0.64	0.43	61
3	5	M	16	42	40	3.18	127	1.5	0.60	0.77	98
4	8	M	26	46	35	2.50	87	2.2	0.72	0.44	38
5	10	F	25	45	32	2.78	89	2.8	0.68	0.36	32
6	15	F	51	44	57	2.16	123	2.7	0.52	0.49	60
7	12	M	36	40	76	2.15	163	2.0	0.46	0.75	122
8	11	M	35	45	50	2.22	111	2.3	0.70	0.43	48
Mean ±SD				44	51± 18	2.38± 0.41	118± 21	2.3± 0.43	0.62± 0.09	0.51± 0.14	62± 32

Ht = haematocrit; T/2 = half life of fibrinogen; C_1 = linear terminal portion of plasma radioactivity extrapolated to the ordinate; FCR = fractional catabolic rate constant; ACR = absolute catabolic rate of fibrinogen. SD = standard deviation

Fibrinogen catabolism was studied by the method of Collen et al. [3]. A commercial product of ^{125}I-fibrinogen (Joliot Curie Institute, Budapest) was used. The mean total clottability of the material *in vitro* was 70 to 80%, the specific activity 100 μCi/mg. To block thyroid ^{125}I uptake every child received 20 mg of Lugol-solution daily before the injection of labelled fibrinogen and then for 5 days. Then 1.2 to 2.0 μCi/kg body weight of ^{125}I-fibrinogen was applied intravenously. Blood sampling was done at 10, 20 and 30 min, and later, at 24, 48 and 72 hr and subsequently at daily intervals. To determine fibrinogen concentration, fibrin was purified by washing in water, alcohol and ether after plasma coagulation by thrombin. Radioactivity of all the purified fibrin samples was measured with a scintillating counter at the end of each experiment. The activity was expressed in mg of fibrinogen. The activity 30 min after application of the ^{125}I-fibrinogen was taken as 100%. Half-life (T/2) of fibrinogen was obtained by graphical analysis from regression lines to fit the value obtained more than 48 hr after injection. The *fractional catabolic rate constant* (FCR) was obtained by extrapolation according to Sterling as modified by McFarlane [12]. The *absolute catabolic rate* (ACR) was obtained by multiplying the fractional catabolic rate with the plasma fibrinogen pool. The *intravascular plasma fibrinogen pool* (IFP) was estimated by dividing the total injected radioactivity by the plasma radioactivity in the 30 min sample. *Plasma volume* was obtained from total injected radioactivity divided by radioactivity of 1 ml of plasma in the 30 min sample. Radioactivity over the thyroid gland was measured by a Geiger–Müller tube.

Plasma fibrinogen concentration was determined by gravimetry. *Fibrin(ogen) degradation products* (FDP) were determined by the haemagglutination inhibition test described by Merskey et al. [13] (FDP-Kit, Wellcome Reagent, Ltd., Beckenham, Kent, Great Britain). *Euglobulin clot lysis* (ECL) was determined by the method of Kaulla and Schultz [9]. *Prothrombin complex activity* (PT) was determined by the method of Quick, as modified by Schultze [14].

Arterial oxygen saturation (SaO_2) was determined with an Atlas type oxymeter. *Haematocrit* (Ht) was determined in microhaematocrit tubes.

Plan of study. Two separate studies were performed. 1. *Anticoagulation* was performed starting 5 days after injecting the labelled fibrinogen and continued for 5 days by giving heparin intravenously every 4 to 6 hr. The daily amount of heparin was adjusted individually to maintain uniform PT. 2. *Inhibition of the fibrinolytic system* was produced by oral administration of 0.1 g/kg body weight of epsilon-aminocaproic acid (EACA) every 6 hr for 5 days starting 5 days after injection of labelled fibrinogen. Patients with increased ECL and increased FDP titre were selected for the study.

The control group received no anticoagulant or antifibrinolytic therapy.

Statistical evaluation was done with Student's *t*-test.

Results

The radioactivity over the thyroid gland changed parallel with the activity of ^{125}I-fibrinogen in plasma. The findings for fibrinogen metabolism are shown in Tables 2 and 3.

Table 3

Metabolism and distribution of fibrinogen in children with cyanotic congenital heart disease

Case No.	Fibrinogen plasma conc. (mg/ml)	IFP (mg/kg)	T/2 (days)	C_1	FCR	ACR (mg/kg/day)
1	2.06	97	1.4	0.60	0.82	79
2	1.93	91	1.3	0.70	0.76	69
3	2.06	142	1.4	0.73	0.69	98
4	2.63	84	1.6	0.68	0.64	54
5	2.55	100	2.0	0.70	0.49	49
6	1.92	75	1.3	0.75	0.71	53
7	2.26	97	2.0	0.58	0.60	58
8	2.30	92	1.5	0.66	0.70	64
9	1.63	60	1.8	0.65	0.59	35
10	2.63	68	1.6	0.70	0.62	42
Mean $\pm$SD	2.18	91	1.6	0.67	0.66	60
	0.34	22	0.3	0.05	0.09	18
Normal	(n = 8)					
Mean $\pm$SD	2.38	118	2.3	0.62	0.51	62
	0.41	21	0.4	0.09	0.14	32
p	>0.05	<0.01	<0.001	>0.05	<0.05	>0.05

IFP = intravascular fibrinogen pool; T/2 = half life of fibrinogen; C_1 = the linear terminal portion of the plasma radioactivity extrapolated to the ordinate; FCR = fractional catabolic rate constant; ACR = absolute catabolic rate of fibrinogen; p = significance between patients and control group

Plasma fibrinogen concentration was stable both in the patients and in the controls. The mean was 218 ± 34 mg/100 ml in the patient group and 238 ± 41 mg/100 ml in the control group. The difference was not significant statistically ($p > 0.05$).

The *plasma fibrinogen pool* was lower in the cyanotic than in the control group. The mean values were 91 ± 22 mg/kg and 118 ± 21 mg/kg. The difference was significant statistically ($p < 0.01$).

The *half-life* of ^{125}I-fibrinogen was shortened in the cyanotic group as compared to the control group. The mean T/2 was 1.6 ± 0.3 days and 2.3 ± 0.43 days, respectively; the difference was significant statistically ($p < 0.001$).

The *mean fractional catabolic rate* of 0.66 ± 0.09 was significantly higher ($p < 0.05$) in the cyanotic patients than in the controls (0.51 ± 0.15).

The *absolute catabolic rate* of fibrinogen in the cyanotic group (60 ± 18 mg/kg/day) did not differ from control values (62 ± 32 mg/kg/day).

Effect of anticoagulation on fibrinogen catabolism. Heparin was given to 4 patients with CCHD (patients 1, 6, 8 and 9). There was a subnormal activity of prothrombin complex (50 to 75%), a normal ECL (over 120 min) and a normal or a little higher level of FDP (5 to 30 μg/ml) in the patients prior to the study. The half-life of fibrinogen was shortened in every case to 1.3 to 1.8 days.

Heparin administration induced a prolongation of the fibrinogen half-life in every case (Table 4, Fig. 1). The PT was between 22 and 35%, ECL was normal and the concentration of FDP decreased in the normal range during therapy. Plasma fibrinogen concentration did not change.

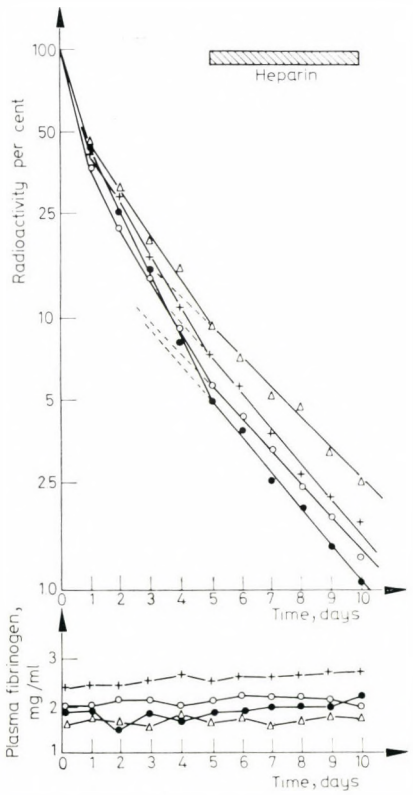


Fig. 1. Fibrinogen metabolism in four children with CCHD before and during anticoagulation. Upper curves show the elimination of ^{125}I-fibrinogen from the plasma. The lower curves show the plasma fibrinogen concentration

Table 4

Effect of anticoagulation on catabolism of fibrinogen in patients with cyanotic congenital heart disease

Case No.	Half-life of fibrinogen (days)	
	before heparin	after heparin
1	1.4	2.5
6	1.3	2.2
8	1.5	2.3
9	1.8	2.5

Effect of fibrino(geno)lysis inhibition on fibrinogen catabolism. Fibrinolytic inhibition by EACA was employed in 3 children with CCHD (patients 4, 7 and 10). They had shortened ECLs (60 to 105 min) and elevated FDP levels (20 to 40

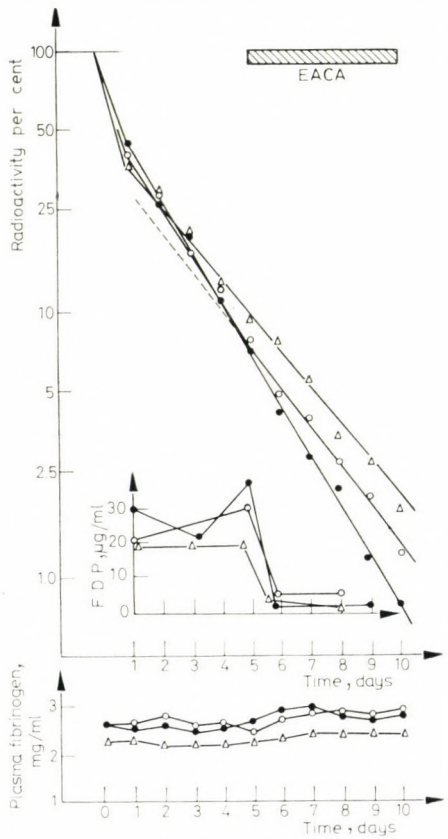


Fig. 2. Fibrinogen metabolism in three children with CCHD before and during antifibrinolytic treatment. Upper curves show the elimination from plasma of ^{125}I-fibrinogen, middle curves the serum FDP concentration, lower curves the plasma fibrinogen concentration

μg/ml) prior to therapy, pointing to an increased fibrino(geno)lytic activity. The fibrinogen half-life was shorter in these patients (1.6 to 2.0 days) than in the controls. Antifibrinolytic therapy prolonged the ECLs and normalized the FDP levels. Fibrinogen half-life was not affected in 2 cases (patients 7 and 10) and was prolonged in one (Table 5, Fig. 2). No change in plasma fibrinogen concentration was seen in any of the cases.

Table 5

Effect of inhibition of the fibrinolytic system on the catabolism of fibrinogen in patients with cyanotic congenital heart disease

Case No.	Half-life of fibrinogen (days)	
	before EACA	after EACA
4	1.6	2.0
7	2.0	2.0
10	1.6	1.6

Discussion

Fibrinogen half-life was shorter in the control cases than generally reported, although some lower values have also been mentioned [2, 8, 12]. Experiments *in vitro* showed that the ^{125}I had split from a portion of the marked fibrinogen earlier, and this may have been the cause of the variance. The T/2 time influences the other parameters of metabolism in an inverse proportion. Consequently, the control values for FCR and ACR were higher in our cases than those mentioned in the literature. These circumstances do not affect the usefulness of the method or the judgement of differences between the patients and controls.

The plasma fibrinogen pool was significantly smaller, the fibrinogen half-life shorter and the fractional catabolic rate of fibrinogen higher in children with CCHD than in the normal controls. There were no significant differences between the two groups in the plasma fibrinogen concentration and in the absolute catabolic rate of fibrinogen. The shorter T/2 and higher FCR indicate that the catabolism of fibrinogen is faster in CCHD than physiologically. This could mean that fibrinogen destruction is accelerated via DIC [11, 15, 16] or an enhanced fibrino(geno)lytic pathway [15]. The plasma fibrinogen concentration was constant, i.e. its synthesis and catabolism were in equilibrium. The total amount of fibrinogen catabolized (and synthetized) daily in the cyanotic patients did not differ from that in the controls, despite the significantly enhanced FCR. The cause of this is to be sought in the decreased plasma volume (high haematocrit) and in the low fibrinogen plasma pool. This moreover suggests that the hepatic fibrinogen synthetizing capacity is not exhausted by the increased consumption in compensated CCHD.

In vivo intravascular coagulation or fibrino(geno)lysis are not essential pathways in fibrinogen catabolism under physiological conditions [3]. The finding of a shortened fibrinogen life-span which can be corrected by heparin points to the suspended activation of the coagulation system in CCHD. Moreover the inefficacy of EACA on the shortened fibrinogen life-span in two cases with hyperfibrinolysis suggests that an enhanced breakdown of fibrinogen would not be the prime cause of the accelerated fibrinogen destruction. On the other hand, the prolongation of plasma radioactivity half-life during EACA therapy in one case out of three must have been due to primary hyperfibrinolysis, i.e. without DIC.

References

1. Gaille, Y., Blanc, Th., Monties, J. R., Goudard, A., Henry, E.: Syndrome de coagulation intravasculaire disséminée chronique et cardiopathie congénitale cyanogène. *Presse méd. 78*, 1571 (1970).
2. Christensen, L. K.: The turnover of plasma fibrinogen. *Acta med. scand. 162*, 407 (1958).
3. Collen, D., Tytgat, G. N., Claeys, H., Piessens, R.: Metabolism and distribution of fibrinogen. I. Fibrinogen turnover in physiological conditions in humans. *Brit. J. Haemat. 22*, 681 (1972).
4. Dennis, L. H., Stewart, J. L., Conrad, M. E.: A consumption coagulation defect in congenital cyanotic heart disease and its treatment with heparin. *J. Pediat. 71*, 407 (1967).
5. Ekkert, H., Gilchrist, G. S.: Coagulation studies in congenital heart disease. *Lancet 2*, 280 (1968).
6. Goldschmidt, B.: Das fibrinolytische Enzymsystem bei cyanotischen Kindern mit angeborenen Herzfehlern. *Mschr. Kinderheilk. 116*, 140 (1968).
7. Goldschmidt, B.: Blutkoagulationsstörungen bei Kindern mit angeborenen Herzfehlern. *Mschr. Kinderheilk. 118*, 450 (1970).
8. Hammond, J. D. S., Verel, D.: Observations on the distribution and biological half-life of human fibrinogen. *Brit. J. Haemat. 5*, 451 (1959).
9. Kaulla, K. N., Schultz, R. I.: Methods for the evaluation of human fibrinolysis. *J. clin. Path. 29*, 104 (1958).
10. Kontras, S. B., Sirak, H. D., Newton, W. A.: Hematologic abnormalities in children with congenital heart disease. *J. Amer. med. Ass. 195*, 611 (1966).
11. Künzer, W., Karitzky, D., Pringsheim, W.: Atemnotsyndrom und disseminierte intravasale Gerinnung. *Dtsch. med. Wschr. 95*, 2141 (1970).
12. McFarlane, A. S.: *In vivo* behaviour of I^{131}-fibrinogen. *J. clin. Invest. 42*, 346 (1963).
13. Merskey, L., Kleiner, G. J., Johnson, A. J.: Quantitative estimation of split products of fibrinogen in human serum: Relation to diagnosis and treatment. *Blood 28*, 1 (1966).
14. Schultze, H. E.: Neue Erkenntnisse über das Prothrombin und das Accelerator-Globulin von Seegers. *Dtsch. med. Wschr. 75*, 607 (1950).
15. Tytgat, G. N., Collen, D., Vermylen, J.: Metabolism and distribution of fibrinogen. II. Fibrinogen turnover in polycythaemia, thrombocytosis, haemophilia A, congenital afibrinogenaemia and during streptokinase therapy. *Brit. J. Haemat. 22*, 701 (1972).
16. Wardle, E. N.: Intravascular coagulation in experimental acute renal failure. Assessment by radio-fibrinogen technique. *Thrombos. Diathes. haemorrh. (Stuttg.) 29*, 579 (1973).

Correspondence: Dr. B. Goldschmidt, Second Department of Paediatrics, Semmelweis University Medical School, Tűzoltó u. 7—9, H-1094 Budapest, Hungary

Haematologia 11 (1–2), pp. 177–187 (1977)

Antihaemophilic Factor (Factor VIII) from Human Granulocytes*

M. SZMITKOWSKI, J. PROKOPOWICZ, Krystyna SZMITKOWSKA

Department of Clinical Diagnostics, Institute of Physiology and Biochemistry, Medical School, Białystok, Poland

(Received November 30, 1976)

Factor VIII has been isolated and purified from human granulocytes using chromatography on Sephadex G-200 and DEAE-Sephadex A-50. The final preparation was purified 60-fold. Some properties of this partly purified factor VIII were compared with the human plasma AHF preparation. Optimum pH activity and the highest activity after incubation at different pH values was found in buffer at pH 7.0. The preparation was thermolabile and had a molecular weight of about 214,000. Absorption curves indicated that it is a protein exhibiting four fractions in polyacrylamide gel disk electrophoresis. AHF preparations from plasma and granulocytes were almost identical in the characteristics tested.

Introduction

The antihaemophilic factor (factor VIII) participates in the phase of intrinsic blood coagulation. There is much information on factor VIII (AHF) in plasma, but few facts are known on the distribution of the factor in the kidney [6, 7], liver [29] and spleen [21, 29]. Szpilman et al. [27] found procoagulant factor VIII activity in the granulocytes of these cells. Zacharski et al. [32] reported an increase of factor VIII activity in leukocyte cultures. There are data to indicate the presence of factor VIII activity in granulocytes.

We wish to report on the isolation and purification of human antihaemophilic factor from human granulocytes, and on the properties of this factor as compared with factor VIII.

Materials

Granulocytes were collected from human purulent pleural exudates.

Human plasmas deficient in factors VII, VIII, IX and XI were obtained from patients with hereditary deficiencies of these factors.

Standard normal plasma was prepared from plasma of 20 healthy individuals.

* Supported in part by the Polish Academy of Sciences.

Anti-VIII serum was kindly supplied by L. Uszyński and S. Lopaciuk, Warsaw. It was prepared by immunization of rabbits with highly purified (8000- to 10,000-fold) factor VIII and Freud's adjuvant. The factor was obtained by separation from fibrinogen by means of defibrase [17]. The antiserum adsorbed on plasma from a patient with von Willebrand's disease showed in agar gel immunodiffusion a single line with normal plasma and with cryoprecipitate. This adsorbed antiserum inhibited the factor VIII activity of normal plasma.

Methods

Protein concentration was determined by a Unicam SP 500 apparatus and calculated as follows: protein concentration mg/ml = $1.45 \times A_{280} - 0.74 \times A_{260}$.

Factor VIII activity was determined by the modified one-stage kaolin–cephalin method of Langdell et al. [15]:

0.05 ml of factor VIII-deficient plasma (factor VIII less than 1%);
0.05 ml of homogenate, fractions or plasma;
0.05 ml of kaolin;
0.05 ml of cephalin solution;
10 min incubation at 37 °C;
0.05 ml of 1/30 M $CaCl_2$.

The time required to form a clot after the addition of calcium chloride was recorded with a stop watch. The results were calculated from a standard curve. It was arbitrarily accepted that standard plasma contains 100 U of factor VIII in 1 ml of plasma.

Factor IX and XI activity was determined by the one-stage kaolin–cephalin method of Langdell et al. [15].

Factor VII activity was determined by the method of Koller et al. [14].

Thrombin activity in fraction III after incubation with thrombin and $Al(OH)_3$ treatment was detected by the clotting of a standard 0.3% fibrinogen solution.

Immunological identification of AHF from granulocytes and plasma after initial purification was performed by the method of Mancini et al. [18] in Fahey and McKelvay's modification [10].

Granulocytes were separated from purulent pleural exudates by centrifugation, washed four times with chilled 0.9% NaCl, and homogenized in a Potter homogenizer in a solution of 0.34 M saccharose and 0.001 M $MnCl_2$ at final concentration. The homogenate was fractionated by the method of Cohn and Hirsch [8]. The most active fraction III containing ribosomes, mitochondria and fragments of Golgi apparatus was used for further procedures.

Initial purification. Fraction III possessing 1.9 U of factor VIII activity/mg protein was treated according to Barrow et al. [4] omitting dialysis and 0.1 ml

of aluminium hydroxide gel was added to 5 ml of fraction III. After centrifugation, 0.01 ml thrombin (100 U/ml) was added to 5 ml of the supernatant. The system was incubated for 30 minutes in a water bath at 37 °C. The final concentration of thrombin was 0.2 μg/ml. After centrifugation, 0.1 ml of aluminium hydroxide gel was added to every 10 ml of supernatant, the centrifugation was repeated and the sediment was discarded. The supernatant was used for further purification.

Chromatography on Sephadex G-200. Sephadex G-200 was prepared and packed in a 35 cm long column 2.3 cm in diameter as indicated by the manufacturer. Chromatography of 3.5 ml of the preparation was carried out at 4 °C, at a flow rate of 0.5 ml/min. Five ml fractions were collected using a fraction collector. The following buffers were used for stepwise elution:

1. Tris-HCl buffer 0.05 M, pH 7.6, with $MnCl_2$ at 1 mM final concentration;
2. Tris-HCl buffer 0.05 M, pH 7.6, with 0.1 M NaCl and 1 mM $MnCl_2$;
3. Tris-HCl buffer 0.05 M, pH 7.6, with 0.5 M NaCl and 1 mM $MnCl_2$.

Chromatography on DEAE-Sephadex A-50. Sephadex was prepared as indicated by the manufacturer and packed in a 15 cm long column 2 cm in diameter. The active fractions from the previous column were used in this procedure. Elution was performed with Tris-HCl buffer 0.05 M, pH 7.6, and a linear gradient of NaCl from 0.25 M to 1.0 M. Three ml fractions were collected at 4 °C.

Molecular weight determinations on Sephadex G-200 of granulocyte and plasma AHF after chromatography on Sephadex G-200 was performed five times by the method of Andrews [2]. As standard marker substances cytochrome C, haemoglobin, ovalbumin, human albumin, plasmin, plasminogen and apoferritin were used. Elution was performed with Tris-HCl buffer 0.05 M, pH 7.6. The void volume (29 ml) of the column (length, 35 cm; diameter, 2.3 cm) was determined by means of blue dextran.

pH optimum determination for AHF activity. Partly purified preparations (i.e. after incubation with thrombin and adsorption on aluminium hydroxide gel) of AHF were diluted with Britton and Robinson's (0.04 M, pH 2 – 12) buffers. The final pH of the tested system were 4, 5, 6, 7, 8, 9 and 10.

Incubation at different pHs. The above preparations were diluted with Britton and Robinson's (0.04 M, pH 2 – 12) buffers in 1 : 1 proportion and the final pH of the system tested was 3, 4, 5, 6, 7, 8, 9, 10 and 11. Factor VIII was incubated for 60 minutes before activity determination.

The effect of temperature on factor VIII activity. Preparations of AHF after Sephadex G-200 chromatography were incubated in water bath at 20, 37 or 50 °C for 30 min.

Determination of the absorption curve in ultraviolet light was done using a Unicam SP 800 spectrophotometer for preparations obtained after Sephadex G-200 chromatography.

Polyacrylamide gel disk electrophoresis. Electrophoresis was carried out on 7.5% gel in Tris-glycine buffer pH 8.3.

AHF was purified from plasma using the same method as from granulocytes. The final product after initial purification and chromatography on Sephadex G-200 was purified 24-fold.

Results

Table 1 illustrates the results of initial purification of the antihaemophilic factor. The final preparation was purified sixfold in comparison to the homogenate. It should be stressed that we have observed a twofold increase in activity of the factor after incubation with thrombin. This fact was in good agreement with the high yield obtained at this step. During preparation we have discarded over 60% of the protein. Up to the last step of the preparation, in these solutions factor IX and XI activity was also found. The final product was free from thrombin activity.

Figure 1 represents the chromatographic pattern of the supernatant obtained during preparation on Sephadex G-200. In the fraction with highest activity we found 41 U per ml. The whole activity was localized in one sharp peak and eluted with buffer 1.

Figure 2 shows the results of chromatography on DEAE-Sephadex A-50. In the peak with highest activity we found 16 U per ml. The whole activity was located in one narrow peak in five fractions. The activity was eluted at 0.7 M NaCl concentration.

The chromatographic purification of the antihaemophilic factor is presented in Table 2. We obtained 39-fold purification by Sephadex G-200, and a 60-fold one by DEAE-Sephadex A-50. The specific activities were 31.3 and 48, respectively. The yield was about 17%.

Figure 3 shows the immunological characteristics of AHF. We obtained a distinct precipitation between anti-AHF serum and the AHF preparation iso-

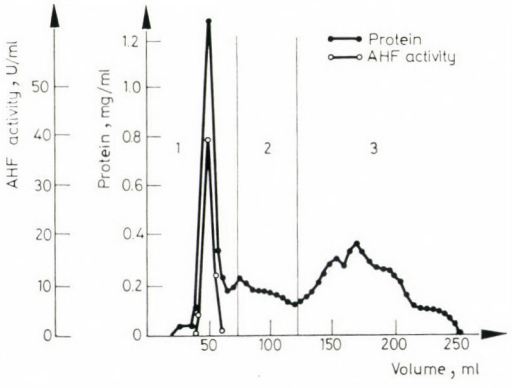


Fig. 1. Column chromatography of granulocyte AHF preparation after initial purification on Sephadex G-200

Table 1

Purification of antihaemophilic factor from human granulocytes

Step of preparation	Volume, ml	Protein, mg/ml	Antihaemophilic activity		Purification	Total antihaemophilic activity, units	Yield, per cent
			U/ml	U/mg of protein			
Homogenate + 0.34 M saccharose and 0.001 M $MnCl_2$	60.0	30.0	24.0	0.8	1.0	1440	100
Fraction III	50.0	13.0	24.7	1.9	2.4	1235	86
Fraction III treated with $Al(OH)_3$	49.5	12.0	25.2	2.1	2.6	1249	87
Fraction III incubated with thrombin	49.0	11.5	48.4	4.2	5.3	2370	164
Fraction III treated with $Al(OH)_3$	49.0	11.0*	52.8*	4.8*	6.0*	2590*	180*

* Up to this step factor VIII activity was contaminated by factors IX and XI

lated from granulocytes after initial purification. In control plasmas factor VIII was identified with this procedure.

Fraction III, after the second $Al(OH)_3$ treatment, corrected the prolonged clotting time of factor IX and XI-deficient plasmas. Factor VIII active fractions after chromatography on Sephadex G-200 and DEAE-Sephadex A-50 did not correct the prolonged clotting time of factor VII, IX and XI-deficient plasmas. The same results were obtained by Saba et al. [24].

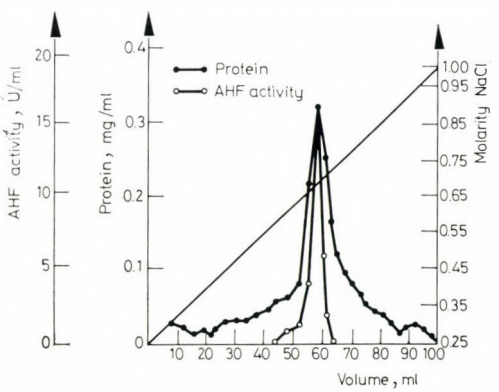


Fig. 2. Column chromatography on DEAE-Sephadex A-50 of AHF active fraction from column chromatography on Sephadex G-200

Table 2

Chromatographic purification of antihaemophilic factor from human granulocytes

Column chromatography	Recovery of total protein, per cent	Total recovery of antihaemophilic activity, per cent	Specific activity, U/mg of protein	Purification
Sephadex G-200	97	145	31.3	39
DEAE-Sephadex A-50	50	17	48.0	60

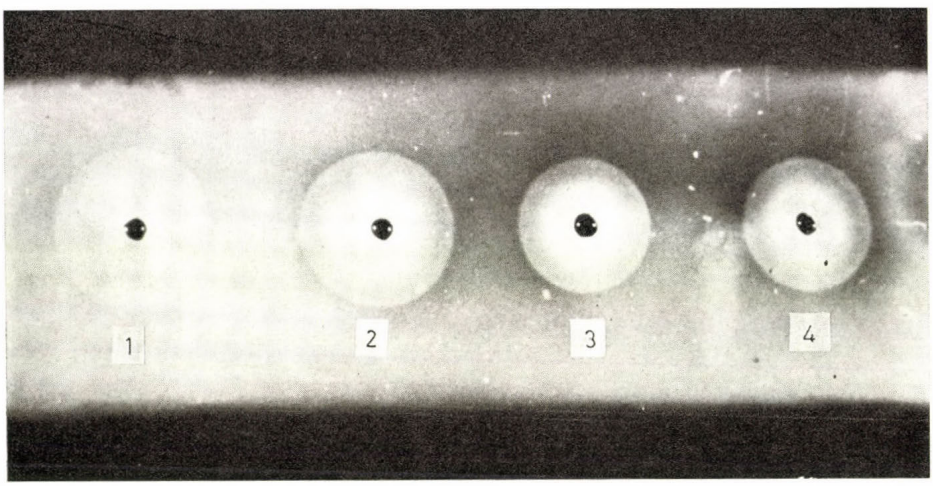


Fig. 3. Immunological identification of AHF. Precipitation zones between anti-factor VIII serum and AHF preparation. 1 and 2 — AHF from plasma, 3 and 4 — AHF from granulocytes

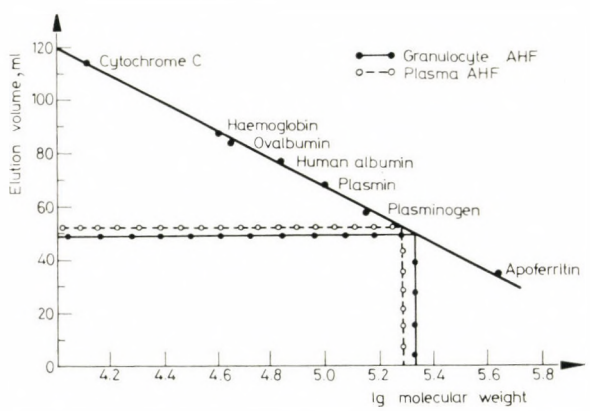


Fig. 4. Molecular weight determination of granulocyte and plasma AHF on Sephadex G-200

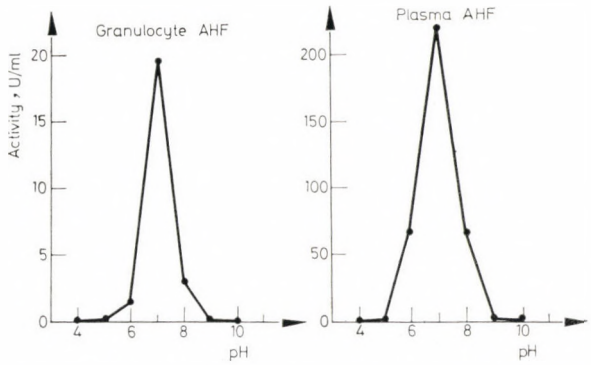


Fig. 5. Optimum pH determination of granulocyte and plasma AHF preparations (after initial purification)

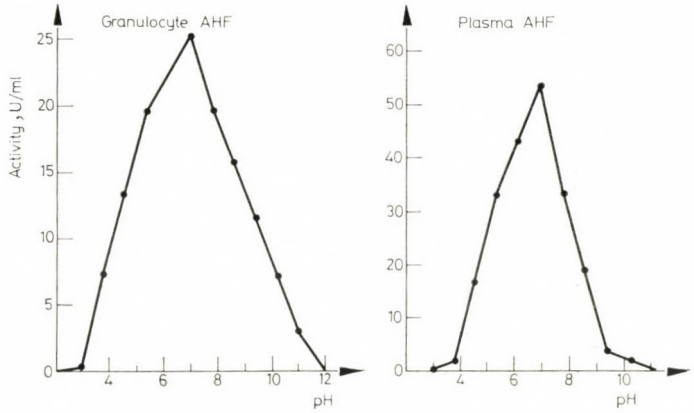


Fig. 6. Determination of granulocyte and plasma AHF activity after 60 min incubation at various pHs

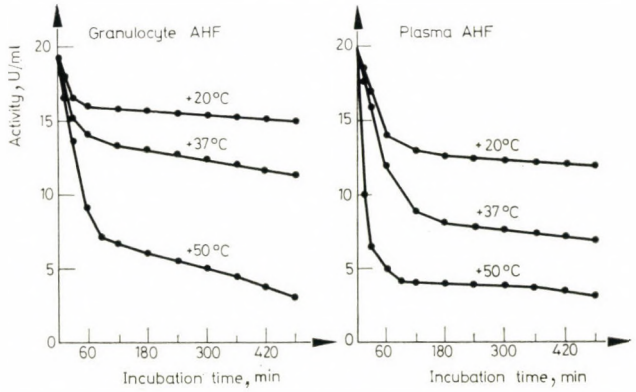


Fig. 7. Effect of temperature on granulocyte and plasma AHF activity

Figure 4 shows that the factor VIII preparation from granulocytes had a molecular weight of 214,000 and that of factor VIII obtained from plasma, 195,000.

The optimum pH values for factor VIII activity are shown in Fig. 5. The AHF preparation from granulocytes and plasma after initial purification had the highest activity at pH 7.0.

Incubation of the factor VIII preparation in buffers of different pH showed the highest activity at pH 7.0 (Fig. 6).

As Fig. 7 shows, factor VIII from plasma was slightly more thermolabile than that from granulocytes.

Light absorption curves were almost identical with preparations from granulocytes and plasma. These curves indicate that the tested preparations are proteins (Fig. 8).

The factor VIII preparation after chromatography on Sephadex G-200 exhibited five fractions, and after DEAE-Sephadex A-50, four fractions (Fig. 9).

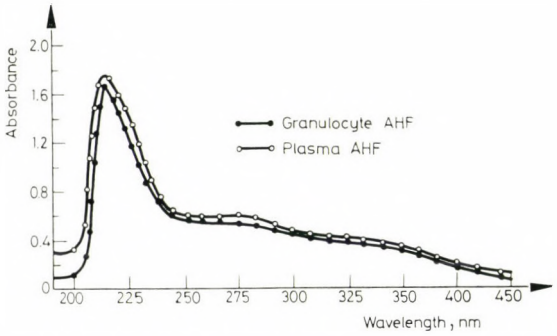


Fig. 8. Absorption curves of granulocyte and plasma AHF preparations after Sephadex G-200 column chromatography



Fig. 9. Polyacrylamide gel disk electrophoresis. Granulocyte AHF preparation purified on Sephadex G-200 (1) and on DEAE-Sephadex A-50 (2)

Discussion

Factor VIII from human granulocytes was purified 60-fold over the initial homogenate. Granulocytes washed several times with 0.9% NaCl seem to be free from clotting factors IX and XI adsorbed on their surface, in good agreement with the findings of Rapaport et al. [22] who successfully liberated clotting factors by several washings from rabbit granulocytes.

In leukocytes we found about 95% granulocytes. All purification steps were performed at +4 °C. Under these conditions we may expect that nearly all factor VIII activity will preserve Mn^{2+}ions exhibiting stabilizing activity on antihaemophilic globulin [4]. It was therefore not surprising that we should have found such a low yield after column chromatography where we could not introduce $MnCl_2$, as elution of DEAE-Sephadex A-50 might be disturbed by the chloride anion. It is generally accepted that thrombin activates factor VIII, but prolonged activation may degrade it [20, 31].

Both preparations showed the highest activity at pH 7.0 and their thermolability was about the same. The absorption curves were nearly identical, indicating that the preparations contained protein. Molecular weights as determined for granulocyte and plasma factor VIII differed only by 19,000 which may be accepted as experimental error.

Several authors have shown that in the presence of some buffers, saline [28, 30] and thrombin [23] AHF of 2,000,000 molecular weight is able to dissociate into two fragments, a low about 200,000 molecular weight (LMW) one, and a high molecular weight (HMW) one. These reagents were used during our purification procedure. It is interesting to note that LMW factor VIII exhibited procoagulant activity [23]. Our results indicate that the isolated proteins are also LMW where the activity of factor VIII is located.

As a final product of highest purity, Johnson et al. [12, 13] presented two fractions of factor VIII. Our preparation of granulocyte factor VIII showed four fractions on disk gel electrophoresis. This was due either to the low purity or it may indicate that AHF is not a homogeneous protein. On the basis of published data and our findings we cannot state whether these small fragments possessed factor VIII activity.

Many data are indicating a relationship between the leukocyte count and the factor VIII activity of plasma [1, 5, 25]. Procoagulant activity of the granulocytes has been described by many authors pointing to the role of these cells in coagulation [16, 19]. Increase of factor VIII activity in plasma after injection of adrenalin is associated with a rise in granulocyte count [5, 25]. Distribution of factor VIII activity in the granulocytes has been described by Szpilman et al. [27]. Published findings indicate the presence of this factor in the granulocytes, a fact supported by our immunological findings.

There is thus no doubt that leukocytes are a source of AHF. Other experiments performed by us have shown that peripheral leukocytes contained about 15% of plasma AHF [26].

Antonioli et al. [3] found that leukocytes in blood amount to only about 0.3% of these cells in the organism. So we can expect that antihaemophilic activity in many organs may be connected with a local destruction of leukocytes. Granulocytes also seem to be a source of the antihaemophilic activity of plasma.

References

1. Amundsen, M. A., Spittel, J. A., Tompson, J. H., Owen, C. A.: Hypercoagulability associated with malignant disease and with the postoperative state: Evidence for elevated levels of antihemophilic globulin. *Ann. intern. Med. 58*, 608 (1963).
2. Andrews, P.: Molecular-sieve chromatography. *Brit. med. Bull. 22*, 109 (1966).
3. Antonioli, J. A., Vannotti, A.: Métabolisme du granulocyte dans l'inflammation. *Schweiz. med. Wschr. 97*, 113 (1967).
4. Barrow, E. M., Amos, S., Graham, J. B.: Certain biochemical properties of human AHF (Factor VIII) separated from fibrinogen with manganous chloride and thrombin. *J. Lab. clin. Med. 168*, 803 (1966).
5. Barrow, E. M., Dejanov, I. I., Ingram, G. I. C., Knights, S. F.: The rise in factor VIII after administration of adrenalin. *Coagulation 2*, 203 (1969).
6. Barrow, E. M., Graham, J. B.: Antihemophilic factor activity isolated from kidneys of normal and hemophilic dogs. *Amer. J. Physiol. 220*, 1020 (1971).
7. Barrow, E. M., Graham, J. B.: Kidney antihemophilic factor. Partial purification and some properties. *Biochemistry 7*, 3917 (1968).
8. Cohn, Z. A., Hirsch, J. G.: The specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. *J. exp. Med. 112*, 983 (1960).
9. Dixon, M., Webb, E. C.: Enzymes. Spottiswoods Ballantyne and Co., London, Colchester 1959.
10. Fahey, J. W., McKelvey, E.: Quantitative determination of serum immunoglobulins in antibody agar plates. *J. Immun. 94*, 84 (1965).
11. Hjort, P., Rapaport, S. I., Owen, P. A.: A simple, specific one-stage prothrombin assay using Russell viper venom in cephalin suspension. *J. Lab. clin. Med. 46*, 89 (1955).
12. Johnson, A. J., Newman, J., Howell, M. B.: The preparation and some properties of a clinically useful, high purity, human antihemophilic factor (AHF). *Bibl. Haemat. 29*, 1109 (1968).
13. Johnson, A. J., Newman, J., Howell, M. B., Puszkin, S.: Purification of antihemophilic factor (AHF) for clinical and experimental use. *Thrombos. Diathes. haemorrh. (Stuttg.) Suppl. 26*, 377 (1967).
14. Koller, F., Loeliger, A., Duckert, F.: Experiments on a new clotting factor (Factor VII). *Acta haemat. (Basel) 6*, 1 (1951).
15. Langdell, R. D., Wagner, R. H., Brinkhous, K. M.: Effect of antihemophilic factor on one-stage clotting test. *J. Lab. clin. Med. 41*, 637 (1953).
16. Lisiewicz, J.: Z badań nad tromboplastyczną aktywnością leukocytów prawidłowych. *Acta physiol. pol. 2*, 287 (1966).
17. Łopaciuk, S., Latałło, Z. S., Bykowska, K., Daszyński, J.: Separation of human antihaemophilic factor (AHF, Factor VIII) from fibrinogen by means of defibrase. *Folia haemat. (Lpz.) 102*, 671 (1975).
18. Mancini, G., Carbonara, A. O., Herremans, J. F.: Immunological quantitation of antigens by single radial immunodiffusion. *Immunochemistry 2*, 235 (1965).
19. Niemetz, J.: Coagulant activity of leukocytes. *J. clin. Invest. 45*, 307 (1972).
20. Ozge-Anwar, A. H.: Slow activation of factor VIII by thrombin at low temperature. *Scand. J. Haemat. 7*, 5 (1970).

21. Pool, J. G.: Antihemophilic globulin (AHG, factor VIII) activity in spleen. *Fed. Proc. 25*, 317 (1966).
22. Rapaport, S. I., Hjort, P. F.: The blood clotting properties of rabbit peritoneal leukocyte *in vitro*. *Thrombos. Diathes. haemorrh. (Stuttg.) 17*, 22 (1967).
23. Rick, E. M., Hoyer, L. W.: Activation of low molecular weight fragment of antihemophilic factor (Factor VIII) by thrombin. *Nature (Lond.) 252*, 404 (1974).
24. Saba, H. I., Herion, J. C., Walker, R. I., Roberts, H. R.: The procoagulant activity of granulocytes. *Proc. Soc. exp. Biol. (N.Y.) 142*, 614 (1973).
25. Steel, C. M., French, E. B., Aitchison, W. R. C.: Studies on adrenalin-induced leukocytosis in normal man. I. The role of the spleen and of the thoracic duct. *Brit. J. Haemat. 21*, 413 (1971).
26. Szmitkowski, M.: Comparative studies on the content of antihemophilic globulin in the plasma and leukocytes. Proc. Second Meeting of the European Division of the International Society of Haematology. Prague 1973. Abstracts 1, 219.
27. Szpilman, H., Prokopowicz, J., Niewiarowski, S.: Distribution of procoagulant activity in the subcellular fractions of human granulocytes. *Experientia (Basel) 25*, 77 (1961).
28. Thelin, G. M., Wagner, R. H.: Sedimentation of plasma antihemophilic factor. *Arch. Biochem. 95*, 70 (1961).
29. Webster, W. R., Raddick, R. L., Roberts, H. R., Penick, G. D.: Release of factor VIII (antihaemophilic factor) from perfused organs and tissues. *Nature (Lond.) 213*, 1146 (1967).
30. Weiss, H. J., Phillips, L. L., Rosner, W.: Separation of subunits of antihemophilic factor (AHF) by agarose gel chromatography. *Thrombos. Diathes. haemorrh. (Stuttg.) 27*, 212 (1972).
31. Weiss, H. J., Kochwa, S.: Molecular forms of antihaemophilic globulin in plasma cryoprecipitate after thrombin activation. *Brit. J. Haemat. 18*, 89 (1970).
32. Zacharski, L. R., Bovine, E. J. W., Titus, J. W., Owen, C. A.: Synthesis of antihemophilic factor (Factor VIII) by leukocytes: Preliminary report. *Mayo Clin. Proc. 43*, 617 (1968).

Correspondence: Dr. M. Szmitkowski, Department of Clinical Diagnostics, Institute of Physiology and Biochemistry, Medical School, M. C. Skłodowskiej 24A, Białystok, Poland

Book Reviews

Clinical Tumor Immunology. Eds: J. Wybran, M. J. Staquet. Pergamon Press, Oxford—New York 1976. 282 pages. Price: US $ 25.—

The Symposium on Clinical Tumor Immunology organized by the European Organization for Research on Treatment of Cancer (EORTC) in Brussels, May 26—29, 1975, was mainly educational. This book which contains the main lectures and the abstracts of presentations gives an up-to-date review on the general topics and some important special subjects of clinical tumor immunology.

The first part deals with the mechanism in tumor immunity including the biological activity of thymic hormone, the role of macrophages and the immune cellular mechanism at the site of the tumor. This part summarizes remarkable data concerning the new aspects of macrophage activities, especially the possible role of their secretory function, affecting the growth of other cells (Lejeune).

The possible role of immunological defects in cancer is still an unanswered question. The value of tests suitable for characterizing the immune system is also a subject of discussion. Part of the book is devoted to the usefulness of immunological tests in cancer patients. The active T-rosette test (Wybran and Fudenberg) proved to be an index of the cellular immune competence of the cancer patients. The mechanism of cell-mediated cytotoxicity *in vitro* and its relevance to tumor immunity (Cerottini and Brunner), the character of K cells and their possible role in human neoplastic diseases (MacLennan) are discussed. Critical evaluation of lymphocyte stimulation tests for detection of tumor-positive reactivity is given by Vánky et al. The advantages of leucocyte migration inhibition test in controlling the tumor-directed cellular immunity in malignant melanoma patients are summarized by Cochran et al. The last paper of the series deals with the usefulness of migration inhibition as well as lymphocyte stimulation assays in detection of cell-mediated immunity against tumor-associated antigens in breast carcinoma. Von Kleist summarizes the basic rules to be respected before the investigations of carcinoembryonic antigen, alpha-feto-protein and alpha-2-hepatic ferroglobulin in human cancer. A brief summary of the association between HLA antigens and malignant diseases is given by Govaerts.

The introduction of cell surface phenotyping has opened new vistas in the characterization of leukemias. The results of investigations of cell markers showed a new classification of these diseases. Data based on cell surface phenotyping of leukemic cells (Greaves et al.), classification of B cell proliferations and non-Hodgkin lymphomas (Brouet et al.) as well as leukemias and hematocarcinomas (Delpomme et al.) are the topics of the next chapter. The importance of the problems of immunotherapy is underlined by the size of the next part of the book. Data obtained in more than 100 patients with resectable lung cancer seem to be very promising (Amery). "Immune" RNA appears again in the presentation of Pilch et al. In a brilliant essay six questions about transfer factor are answered (Fudenberg) emphasizing its definite immunologic and clinical effects in humans, leaving open, however, the mechanism of its action. Immunotherapy

of cancer, the combination of specific stimulus and ECG, the effect of combined chemotherapy and active immunotherapy are discussed by several authors (Mathé; Whitehouse; Gutterman et al.).

The book is very useful for immunologists who are interested in the problems of tumor immunology and for oncologists who would like to be informed about the latest results in clinical tumor immunology. The critical discussions of methodological and therapeutical problems will be a help in their practical work.

J. Gergely

Abnormal Haemoglobin and Thalassaemia. Diagnostic Aspects. Ed. by R. M. Schmidt. Academic Press, New York—San Francisco—London 1975. 371+LVI Pages. Price: $19.75.

This volume is a publication of the International Committee for Standardization in Haematology Expert Panel on Abnormal Haemoglobin and Thalassaemia. In the introduction the editor informs the readers on the contents of the volume: The 18 papers published represent the individual contributions of the members of the Panel held in 1974.

The authors of the first chapter stress that the separation of standard haemoglobin fractions into separate strips is largely dependent on the used solid medium, the buffer, voltage and current intensity. Fluctuation of separation may be due to the varying quality of cellulose acetate papers. Frequently, different series of paper produced by the same manufacturer will give different resolution of haemoglobins. Commercially available pathological haemoglobin standards are not reliable.

Six papers are involved in the estimation of Hb A_2 fraction. Authors recommend cellulose acetate paper for quantitative assay. Peculiarly enough none of the authors refers to chromatography. One of the chapters reports the results of control examinations performed in 150 laboratories. Cellulose acetate electrophoresis was used in these trials. Three successive trials improved the efficiency (accuracy) of these laboratories, although even so 30% of the participants reported results greater than ± 2 S.D.

Four chapters discuss the estimation of Hb F. One of the authors describes the automated techniques. The "classical" alkaline denaturalization, the various modifications of this method, and the Kleihauer-Betke slide technique and experience related to these are discussed by authors who have studied these subjects for many years. Unfortunately, other methods, for example column chromatography, are not referred to. It is well known that in cases of high values the alkaline denaturalization method gives values lower than the real ones.

Two sections discuss the diagnostics of abnormal haemoglobin. The method of oxygen saturation is also described. Some chapters discuss comprehensive diagnostic problems, for example the detection of Hb Lepore and the research of haemoglobinopathies in developing countries.

A proposal for standardized information for complete reporting of abnormal haemoglobins is also presented in the volume. A chapter deals with the question: when are haemoglobin structural studies indicated? The answer is that they are indicated when you have an unknown haemoglobin, sufficient resources and interest to determine the structure. The author supports his view with many examples.

The last chapter presents a list of known haemoglobin substitutions and deletions until 19th March, 1975.

This book is not a laboratory manual containing descriptions of precisely elaborated techniques. The individual opinion of authors with great experience in the field compensates for the deficiency. On the whole, the volume presents to the haematologist and clinical worker an enormous amount of information. In several cases the single chapters overlap each other and certain inaccuracies can also be discovered in the papers.

The preface is a homage to Professor Lehmann, one of the pioneers of haemoglobin research and the President of the International Committee for Standardization in Haematology. A list of his papers published between 1935 and 1974 is presented. 479 papers are listed, which have been written by himself or with various coworkers. This life work certainly deserves great honour.

B. Ringelhann

Le Facteur Stabilisant de la Fibrine (F.S.F.) et son déficit congénital (Fibrin-stabilizing factor and its congenital deficiency). By J. Favre-Gilly, J. P. Pradier, J. P. Thouvrez, J. Belleville. 260 pages with 12 illustrations. J. Tixier et Fils, Lyon 1974.

This manual discusses the fibrin-stabilizing factor (FSF) designated as factor XIII in the international nomenclature. The volume consists of two main parts. The first is involved in the discovery of FSF, its physical and chemical properties, and the role of FSF in the formation of the stable fibrin structure. Direct and indirect methods of determining FSF are discussed in detail. They are based on the observation that in FSF deficiency the polymerized fibrin clot is soluble in urea, Na-monoiodoacetate or monochloroacetic acid. The degree of dissolution depends on the FSF activity.

The physiological and pathological changes of FSF and the associated secondary FSF deficiencies are also discussed in the first part.

The second and more extensive part of the volume presents a detailed analysis of FSF deficiencies occurring in the period 1960 to 1971: The first congenital FSF deficiency described by Duchert in 1960, and the 46 cases reported in the literature until 1971 including the authors' own observations. The case reports include a description of the clinical picture, laboratory findings and analysis of the family history. This part is followed by a detailed analysis of the 70 FSF deficiencies known until now, including the clinical and laboratory findings, the analysis of inheritance and family analysis. Finally, problems of diagnostics and therapy are discussed. Besides whole blood, plasma Cohn I fraction cryoprecipitate and factor XIII concentrate are mentioned as therapeutic possibilities.

At the end of the volume, 216 literary references are listed.

S. Elődi

The Distribution of the Human Blood Groups and other Polymorphisms. 2nd ed. By A. E. Mourant, A. C. Kopec, K. Domaniewska-Sobczak. Oxford University Press, London—New York—Toronto 1976. XV + 1055 pages, 38 figures, cloth. Price: £ 55.—

Monographs, manuals, encyclopaedias are indispensable aids for both the scientist and the practitioner. This second edition of the book by Mourant et al. is also one of these indispensable aids, presenting information to anthropologists, experts working in the field of population genetics, and doctors involved in the study of blood groups.

The volume contains data of antigen-frequencies for all the populations examined in the world. Collection of data for this book was terminated in 1969, but data on new blood group distributions reported in 1970 have also been included.

Collection of data for the first edition was terminated in 1953—54. This second edition presents information on the new genetic markers described since then but, unfortunately, the genetic markers of immunoglobulin (Gm, Inv, etc.) and the transplantation antigens are not included in the book. Still, we agree with the authors in that inclusion of these data would have added to the bulk of the large volume.

Data for several countries, for Hungary among others, have greatly increased. The white spots previously designating Asia and the Asiatic areas of the Soviet Union have disappeared.

The first part of the volume is involved in the biochemistry, serology and genetics of the various blood group antigens. A substantial completion compared to the first edition are the directives given in the 13th chapter on how to calculate gene-frequency in blood group systems other than AB0 and Rh.

In the second part of the volume the authors divide the population of the world into seven geographical regions. This part gives a short description of each region and the gene-frequency of the blood groups of their population. A separate chapter discusses the migrant and mixed populations.

The 25th chapter, based on present knowledge, attempts to synthetize the hypotheses on blood group and other biochemical polymorphisms. The authors attempt to terminate the dogmatic separation of the two current hypotheses (natural selection against random genetical processes). Namely,

random genetical processes studied on isolated populations are difficult to extrapolate to panmictical populations. On the other hand, direct verification of the effect of natural selection still remains to be supported, although there are several observations revealing associations between blood groups and diseases.

Finally, the knowledge on blood groups accumulated since 1954 is summarized.

The third part of the book demonstrates in tables the distribution of blood groups in various populations. Their arrangement is uniform. The place of observation, the population at issue, the authors and the number of observations, absolute value of the distribution of phenotypes, gene-frequency, chi-square test, goodness of fit and where necessary additional remarks are presented.

The volume contains 36 gene-atlases, which excellently illustrate the data presented in the tables.

The authors refer to many thousand publications which also proves the gigantic size of their work.

The volume is edited very carefully. The single chapters and their subdivisions are arranged so as to make orientation very easy in the various subjects. The subject index, the list of populations in alphabetic order, the author index are also a great aid to the reader.

The authors have set themselves an enormous task, which they have fulfilled very successfully. The volume is of high scientific value.

T. Tauszik

Struktur und Funktion der Erythrozyten. VII. Internationales Symposium. (Structure and Function of Red Blood Cells, 7th International Symposium.) Eds: S. Rapoport, F. Jung. Akademie Verlag, Berlin 1975. 684 pages. Price: DM 130.—

The Berlin symposia on red blood cells have become a traditional meeting of outstanding experts. Held every third year since 1955, these symposia have enjoyed great success due to the efforts and competence of the Organizing Committee headed by Drs. S. Rapoport and F. Jung.

This volume of the 7th Symposium held in August 1973 comprises the full text of 117 papers and the round-table discussions. As on previous occasions, several up-to-date topics have been treated.

I. Model studies on glycolysis and glycolytic enzymes (17 papers).

II. Structure and function of haemoglobins (27 papers).

III. Interaction between haemoglobin and anions (12 papers).

IV. Enzymopathies and metabolic defects (12 papers).

V. Cell membrane metabolism (11 papers).

VI. Blood preservation (6 papers).

VII. Maturation of the erythron (25 papers).

VIII. Metabolism of reticulocytes. Non-glycolytic metabolic processes (7 papers).

About half of the contributions came from universities and research institutes of the German Democratic Republic. The rest of the papers were presented by scientists from 17 countries. A degree of disproportion and unevenness is probably inevitable in any multiauthored work. In the case of this book it might have been due to the fact that every paper submitted had been accepted and was published.

Among the material of value mention should be made of the series of essays centered around model studies on glycolysis written by researchers from Rapoport's school of biochemistry. Of great interest are the new results in blood conservation reported by Swedish and Japanese authors, further the review of American scientists on stimulators and inhibitors influencing erythroblast maturation *in vitro*.

G. Gárdos

Trends in Haematology. J. B. Chatterjea memorial volume. Ed. by N. N. Sen. Associate ed.: A. K. Basu. J. B. Chatterjea Memorial Committee (School of Tropical Medicine, Calcutta 75), 1975. 506 pages, 90 figures, 67 tables. Price: $ 60.—

This volume comprises essays written on the invitation of the Chatterjea Memorial Committee. It pays a tribute to the memory of J. B. Chatterjea (1919—1972), late Director of the Haematology Unit of the Indian Council of Medical Research and Vice President of the International Society of Haema-

tology. Accordingly, the first pages are devoted to the commemoration of his work and personality. Thereafter a series of 24 reviews sheds light on the horizons of haematology treated in the broadest sense. Immunological, biochemical, cytochemical, genetic, clinical and molecular aspects are all considered. European, American, Japanese, Indian, Australian haematologists contribute to the volume, among them distinguished experts of the discipline like Caen, Castaldi, Biggs, Beutler, Goldberg, de Gruchy, Hollán, Jaffé, Mathé, Waldenström, Weatherall and others. Haematologists from India deal with the local variants of haemoglobins and the AB0 system including the Bombay Oh phenotypes, as well as with nutritional anaemia in India and other countries.

Blood coagulation, fibrinolysis and platelet function are treated first. Then after the problems of immunochemistry, immunohaematology and blood preservation, attention is directed to the fields of haemoglobin research and erythropoiesis. The survey is completed by essays on leukaemias, classification and therapy. The volume ends with the bibliography of J. B. Chatterjea; the list of his 372 publications clearly manifests his contribution to the progress of contemporary medicine.

A great number of illustrations facilitates the understanding of the recent advances in haematology. The variety of topics, the conciseness and clarity of the selected essays represent a fitting tribute to the memory of Professor Chatterjea, the eminent scientist and clinician in haematology.

Ilma Szász

International Histological Classification of Tumours: No. 14. Histological and Cytological Typing of Neoplastic Diseases of Haematopoietic and Lymphoid Tissues. Ed. by G. Mathé, H. Rapaport in collaboration with G. T. O'Conor, H. Torloni and haematologists and pathologists of five countries. World Health Organization, Geneva 1976. 45 pages, illustrated. Price: $ 30.—

Classification of haematopoietic and lymphoid neoplastic diseases has been in the focus of interest in the past twenty years. Although the main morphological types had been described at the beginning of this century, new methods introduced in diagnosis necessitated a new approach to the problem. It was with this aim in mind that WHO had established the International Reference Centre in 1962. Haematologists and pathologists of five countries participated in the extensive work that enabled the editors to compile the impressive collection of "typical" illustrations for the proposed new classification.

The classification is based on the morphological appearance of malignant cells, as compared to their presumptive normal progenitors. A translation of the classification into French, Russian and Spanish is also included in the book to aid pathologists in relating the new entities to their existing nomenclature.

Diagnosis of the tumours is based on both histological and pathological findings. The neoplasms are grouped in two major categories, one with systemic involvement, the other with localized tumour.

The first group comprises the acute and chronic leukaemias and related lympho- or myeloproliferative diseases. Apart from the description of morphological features characterizing the various subtypes, cytochemical and immunological markers are also referred to in differential diagnosis.

The second group is the tumours of the lymphoid tissues. As the authors themselves point out, it is impossible at present to give a scientifically accurate and clinically tested classification of the non-Hodgkin lymphomas. With the introduction of new cytochemical and immunological markers a number of new systems have been proposed in the literature. This book combines some of these suggested nomenclatures and contains excellent photographs that illustrate the characteristic features of the given subtypes. Inevitably, however, there are diseases that are difficult to categorize and that are interpreted differently in other classification systems. This book should be a useful source of references for most clinicians and pathologists involved in the care of haematological and oncological patients.

T. Révész

Abstracts

Supersaturation in sickle cell hemoglobin solutions. J. Hofrichter, Ph. D. Ross, W. A. Eaton (Laboratories of Chemical Physics and Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md.). *Proc. nat. Acad. Sci. (Wash.) 73*, 3035 (1976).

Kinetic inhibition of the gelation of haemoglobin S is compared to the change in haemoglobin S solubility, when the solubility is altered by carbon monoxide, pH, or urea. By means of a new technique, delay time and the extent of gelation are measured on the same sample. Delay time, t_d, was found to be proportional to a high power (30—40) of the haemoglobin S solubility. Together with the previously reported concentration dependence, this result demonstrates that the rate is proportional to a high power of the supersaturation, S, defined as the ratio of the total haemoglobin S concentration to the equilibrium solubility. The results obey the supersaturation equation $t_d^{-1} = \gamma S^n$, where γ is an empirical constant (about 10^{-7} sec^{-1}) and n is about 35. The supersaturation equation may account for the kinetics of cell sickling and is therefore used to estimate the increase in delay time for sickling necessary to produce significant clinical benefit to patients with sickle cell disease.

G. Gárdos

Inhibition of erythrocyte sickling by cystamine, a thiol reagent. W. Hassan, Y. Beuzard, J. Rosa (Unité de Recherches sur les Anémies, Hôpital Henri Mondor, Créteil, France). *Proc. nat. Acad. Sci. (Wash.) 73*, 3288 (1976).

Incubation of sickle cells with cystamine, a thiol reagent, resulted in the formation of an intracellular S-ethylamine derivative. The rate of the reaction depended on the cystamine concentration, the temperature, and the duration of incubation. The cystamine-treated cells displayed a marked inhibition of sickling under hypoxic conditions, a decrease in their mean corpuscular haemoglobin concentration, and a significant increase in their affinity to oxygen. The oxygen affinity of these cells was less dependent on their mean corpuscular haemoglobin concentration than that of untreated sickle cells. The minimum gelling concentration of S-ethylamine deoxyhaemoglobin S was slightly increased. Cystamine did not affect the intracellular pH or the 2,3-diphosphoglycerate level. The exact contribution of the interrelated factors in cystamine inhibition of sickling (changes in oxygen affinity, mean corpuscular haemoglobin concentration, and minimum gelling concentration) has yet to be determined.

G. Gárdos

Water proton magnetic resonance studies of normal and sickle erythrocytes. Temperature and volume dependence. A. Zipp, T. L. James, I. D. Kuntz, S. B. Shohet (School of Pharmacy, Department of Pharmaceutical Chemistry, University of California, San Francisco, Calif. and School of Medicine, Department of Clinical Pathology and Laboratory Medicine, and the Cancer Research

Institute, University of California, San Francisco, Calif.). *Biochim. biophys. Acta (Amst.) 428*, 291 (1976).

The temperature and cell volume dependence of the NMR water proton linewidth, spin-lattice and spin-spin relaxation times have been studied for normal and sickle erythrocytes as well as haemoglobin A and haemoglobin S solutions. Upon deoxygenation, the spin-spin relaxation time (T_2) decreases by a factor of 2 for sickle cells and haemoglobin S solutions but remains relatively constant for normal cells and haemoglobin A solutions. The spin-lattice relaxation time (T_1) shows upon deoxygenation no significant change for normal and sickle packed red cells. Studies of the change in NMR linewidth, T_1 and T_2, as the cell hydration is changed indicate that these parameters are affected only slightly by a 10 to 20% cell dehydration. This suggests that the reported 10% cell dehydration observed with sickling is not important in the altered NMR properties. Low temperature studies of the linewidth and T_1 for oxy- and deoxy haemoglobin A and haemoglobin S solutions suggest that the "bound" water possesses similar properties for all four species. The low temperature linewidth ranges from about 250 Hz at $-15\,^{\circ}C$ to 500 Hz at $-36\,^{\circ}C$ and analysis of the NMR curves yield hydration values near 0.4 g water/g haemoglobin for all four species. The low temperature T_1 data display a minimum at $-35\,^{\circ}C$ for measurements at 44.4 MHz and $-50\,^{\circ}C$ for measurements at 17.1 MHz and are similar for oxy- and deoxy haemoglobin A and haemoglobin S. These similarities in the low temperature NMR data for oxy- and deoxy haemoglobin A and haemoglobin S suggest a hydrophobically driven sickling mechanism. The room temperature and low temperature relaxation time data for normal and sickle cells are interpreted in terms of a three-state model for intracellular water. In the context of this model the relaxation time data imply that type III, or irrotationally bound water, is altered during the sickling process.

Ilma Szász

Aging of human erythrocytes. Differential sensitivity of young and old erythrocytes to hemolysis induced by peroxide in the presence of thyroxine. R. Walls, K. S. Kumar, P. Hochstein (Department of Pharmacology, University of Southern California School of Medicine, Los Angeles, Calif.). *Arch. Biochem. Biophys. 174*, 463 (1976).

Human erythrocytes were separated according to cell age using albumin density gradients. In the presence of 100 mg/dl glucose young cells are able to protect themselves effectively against thyroxine-peroxide-induced haemolysis; old cells exhibited less protection. Haemolysis in heterogeneous populations is preceded by lipid peroxidation, K^+ leak and decreased filtrability of the cells. Hydroxy radical scavengers partially inhibited haemolysis while superoxide dismutase had no effect. It is postulated that the differential sensitivity of young and old erythrocytes to thyroxine-peroxide-induced metabolic and morphological alterations may play a role in the recognition and removal of senescent cells from the circulation.

Ilma Szász

(^{51}Cr) sodium chromate incorporation into the soluble protein fraction of the human erythrocyte: Binding not associated with the hemoglobin monomeric subunit. E. M. Skrabut, N. Catsimpoolas, J. P. Crowley, C. R. Valeri (Department of Hematology, Boston University Medical Center, Boston, Mass., and Naval Blood Research Laboratory, Chelsea, Mass.). *Biochem. biophys. Res. Commun. 69*, 672 (1976).

The distribution of radioactive chromium in proteins isolated in the soluble fraction of the human erythrocyte was studied utilizing sodium dodecyl sulphate polyacrylamide gel electrophoresis. The radioactive label was associated with the haemoglobin monomeric subunit and, unexpectedly, with another soluble fraction protein. This protein was approximately 6% of the total isolated protein, contained approximately 20% of the radioactivity, and had an estimated molecular weight of 26,700 daltons.

G. Gárdos

Response of the glycolysis of human erythrocytes to the transition from the oxygenated to the deoxygenated state at constant intracellular pH. I. Rapoport, H. Berger, S. M. Rapoport, R. Elsner, G. Gerber (Institut für Physiologische und Biologische Chemie der Humboldt-Universität zu Berlin, 104 Berlin, DDR.) *Biochim. biophys. Acta (Amst.) 428*, 193 (1976).

The time course of the rate of glycolysis of human erythrocytes and of some metabolites was determined before and after rapid deoxygenation at constant intracellular pH. For this purpose stripped deoxygenated haemoglobin was used as a rapid oxygen acceptor. Deoxygenation caused a 26% increase of the glycolytic rate. Glucose-6-phosphate is decreased while the adenine nucleotides and 2,3-bisphosphoglycerate remain constant. Fructose 1,6-bisphosphate and the triose phosphates decrease transiently before rising. The data can be explained by increased binding of phosphocompounds to deoxygenated as compared with oxygenated haemoglobin; thereby the control enzymes hexokinase and phosphofructokinase are affected. It is concluded that under physiological conditions changes in the oxygenation of haemoglobin per se alter the glycolytic rate.

G. Gárdos

Studies on adenine and adenosine metabolism by intact human erythrocytes using high performance liquid chromatography. B. M. Dean, D. Perrett (The Medical Professorial Unit, Dunn Laboratories, St. Bartholomew's Hospital, London, U.K.). *Biochim. biophys. Acta (Amst.) 437*, 1 (1976).

Adenine and adenosine metabolism has been studied in intact human erythrocytes in vitro using high performance liquid chromatography, isotopic labelling and electrophoresis. Their metabolism to nucleotides was controlled by phosphoribose diphosphate synthesis which was phosphate dependent. Adenosine formed hypoxanthine or IMP depending upon P_i concentration, but adenosine kinase and deaminase activities were not affected by P_i levels. Free [^{14}C] adenine and [^{14}C] hypoxanthine were found in cellular extracts. Rapid interconversions occurred to give a 10 : 1 : 0.1 distribution for ATP : ADP : AMP. Marked decomposition of ATP to ADP and AMP occurred during incubation in plasma and Earle's media in air on nitrogen, but ATP levels remained stable in phosphate buffers and in the presence of oxygen. At physiological P_i (1 mM) adenosine kinase activity grossly exceeded adenine phosphoribosyltransferase activity. The latter was approximately 7-fold of hypoxanthine phosphoribosyltransferase activity. These differences decreased with increasing P_i levels. No significant increase in corresponding nucleotides was obtained on incubation with high levels (0.5 mM) of adenine, guanine or guanosine at physiological P_i. ATP increased by 10% independently of the substrate employed and significant amounts of IMP and GTP were formed from adenosine and guanosine, respectively. The existence of a bound intracellular pool of ATP is suggested.

Ilma Szász

A normal level of adenosine deaminase activity in the red cell lysates of carriers and patients with severe combined immunodeficiency disease. P. P. Trotta, E. M. Smithwick, M. E. Balis (Laboratory of Cell Metabolism, Memorial Sloan-Kettering Cancer Center, New York, N. Y.). *Proc. nat. Acad. Sci. (Wash.). 73*, 104 (1976).

The red cell lysates of two children with severe combined immunodeficiency disease (SCID) exhibited a practically total absence of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) when standard volumes were assayed. Under these conditions the parents exhibited depressed specific activity except for one of the mothers, whose lysate showed a normal activity. Upon storage of the lysate at 4 °C, a significant activity appeared in one of the SCID children, and the activity of the heterozygous carriers was stimulated. With a sensitive spectrophotometric assay based on conversion of inosine to uric acid, it was shown that the specific enzymatic activity in each of the SCID patients increased progressively as the volume of lysate assayed was lowered.

With the smallest amount of lysate this specific activity was in the normal range. Similarly, the specific activity of each of the parents' lysates increased to normal and in one case, to double the normal, as smaller volumes were assayed. Activity in the SCID patient was inhibited by 2-fluoroadenosine and N^6-methyladenosine deaminase. The lysate from the SCID patient was also shown to inhibit adenosine deaminase partially purified from a normal individual. The results are interpreted in terms of a genetically programmed production of an adenosine deaminase inhibitor in at least one variant of the severe combined immunodeficiency disease.

G. Gárdos

Asymmetry and transposition rates of phosphatidylcholine in rat erythrocyte ghosts. B. Bloj, D. B. Zilversmit (Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, N. Y.). *Biochemistry 15*, 1277 (1976).

Purified phospholipid exchange protein from beef heart cytosol was used to accelerate the exchange of phospholipids between labelled sealed ghosts and phosphatidylcholine/cholesterol liposomes. The purified protein accelerates the transfer of phosphatidylcholine and, to a lesser degree, that of sphingomyelin, phosphatidylinositol, and lysophosphatidylcholine. The presence of exchange protein did not accelerate the exchange of phospholipids between intact red blood cells and liposomes, but 75% of the phosphatidylcholine of sealed ghosts was readily available for exchange. The remaining 25% was also exchangeable but at a slower rate. When the exchange was assayed between inside-out vesicles and liposomes, 37% of the phosphatidylcholine was readily available, and 63% was exchanged at a slower rate. These results are consistent with an asymmetric distribution of phosphatidylcholine in isolated erythrocyte membrane fractions. The sum of the forward and backward transposition of phosphatidylcholine between the inner and outer layers of sealed ghost membranes amounts to 11% per hour, and the half-time for equilibration is 2.3 hr. Significantly lower values are obtained for the inside-out vesicles (half-time for equilibration: 5.3 hr). The results suggest that, during formation of the vesicles, the asymmetry of phosphatidylcholine is partially preserved, but structural changes occur in the membrane that affect the rate of membrane transposition of phosphatidylcholine.

Ilma Szász

Studies on the hemolytic and hydrolytic actions of phospholipases against mammalian erythrocyte membranes. R. Taguchi, H. Ikezawa (Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya, Japan). *Arch. Biochem. Biophys. 173*, 538 (1976).

The haemolytic actions of three kinds of phospholipase C on horse and sheep erythrocytes were studied in relation to their hydrolytic activities on the phospholipid components of these red cells. *Clostridium novyi (oedematiens)* type A phospholipase C haemolysed horse red cells by hydrolysing phosphatidylcholine. The enzyme failed to lyse sheep cells or to hydrolyse phospholipid under the same conditions, although it hydrolysed both sphingomyelin and phosphatidylethanolamine in the phospholipid mixture extracted from sheep red cells. *Clostridium perfringens* phospholipase C haemolysed not only horse red cells by hydrolysing phosphatidylcholine but also sheep red cells by hydrolysing sphingomyelin. Sphingomyelin of the sheep red cell membrane was hydrolysed 10 times faster by the enzyme than that of the horse red cell membrane. *Pseudomonas aureofaciens* phospholipase C haemolysed horse red cells by attacking phosphatidylcholine and phosphatidylethanolamine. The enzyme did not attack sheep red cells but hydrolysed phosphatidylethanolamine in the extracted phospholipid mixture from sheep cells. The haemolytic activity of phospholipase C depends not only on the enzyme and the asymmetric distribution of phospholipids in the erythrocyte membrane but also on the accessibility of the enzyme to the phospholipids on the surface of the membranes. Haemolysis by phospholipase C is of the not-cold type.

B. Sarkadi

Subunit structure of human erythrocyte glycophorin A. H. Furthmayr, V. T. Marchesi (Department of Pathology, Brady Memorial Laboratory, Yale University, New Haven, Conn.). *Biochemistry 15*, 1137 (1976).

Glycophorin A is a sialoglycoprotein isolated from human erythrocyte membranes which seems to exist in the form of stable dimeric complexes in the presence of sodium dodecyl sulphate. When analysed by dodecyl sulphate acrylamide electrophoresis, the molecule forms two PAS-stainable bands (PAS-1 and PAS-2) which are reversibly interconvertible. This change in electrophoretic mobility is dependent on the concentration of dodecyl sulphate, the use of Tris-buffer systems, the protein concentration in the incubation mixture, and the duration and temperature of incubation before electrophoresis. Reducing agents do not influence the results. Chromatography of the sialoglycopeptides on Sepharose columns in dodecyl sulphate before and after heat treatment gave similar results. A small hydrophobic peptide (T-6) derived from glycophorin A was able to prevent reassociation of the monomeric subunits back to the higher molecular weight form. This peptide was able to bind to the subunit of glycophorin A, but not to the high molecular weight complex. These results are consistent with a model of glycophorin A composed of two subunits which can dissociate and reassociate in the presence of detergents. These subunits may interact via the hydrophobic portions of the polypeptide chains.

G. Gárdos

Reducible components in the proteins of human erythrocyte membrane. A. J. Bailey, S. P. Robins, M. J. A. Tanner (A.R.C. Meat Research Institute, Langford, Bristol, Department of Animal Husbandry, University of Bristol, Langford; and Department of Biochemistry, University of Bristol, England). *Biochim. biophys. Acta (Amst.) 434*, 51 (1976).

In contrast to a previous report, no collagen or elastin-type cross-links derived from lysine-aldehydes were detected in human erythrocyte membranes. The major reducible components of erythrocyte membranes were shown to be hexosyllysines. From their structure it is clear that these components cannot act as cross-links between the protein subunits of the membrane. The components were also shown to be present in varying proportions in human serum albumin and haemoglobin. Whether the hexose attachments have any physiological significance or are artifacts of the analytical procedure has not yet been demonstrated. One other major reducible component was present but, although unidentified, this compound was shown to be unrelated to any of the known lysine-aldehyde-derived cross-links of collagen and elastin. A minor acid component was identified as glycosylvaline derived from the N-terminus of the β chain of haemoglobin A_{1c} and not a lysine-aldehyde precursor of the collagen cross-links.

Ilma Szász

Studies on the interaction of propranolol with erythrocyte membranes. D. V. Godin, Th. W. Ng, J. M. Tuchek (Department of Pharmacology, University of British Columbia Faculty of Medicine, Vancouver, British Columbia, Canada). *Biochim. biophys. Acta (Amst.) 436*, 757 (1976).

The interaction of propranolol with erythrocyte membranes at concentrations stabilizing intact erythrocytes against hypotonic haemolysis produced corresponding perturbations in membrane protein and particularly membrane phospholipid components as monitored by increases in the reactivity of membrane amino and sulfhydryl groups towards trinitrobenzenesulfonic acid and 5,5'-dithio-bis (2-nitrobenzoic acid), respectively. Membrane-propranolol interactions were also analysed in terms of alterations produced in the kinetic properties of membrane enzymes. These experiments provided evidence that propranolol-induced perturbations were sufficiently generalized for influencing the activity of enzymatic processes associated with both inner and outer membrane surfaces. Configurational changes in membrane

phospholipids were implicated in these effects of propranolol, which included alterations in functionally significant membrane–cation interactions. It is suggested that the findings described may provide a basis for understanding of molecular aspects of membrane stabilization in other systems.

Ilma Szász

Effect of propranolol and nitroglycerine on hemoglobin-oxygen affinity. G. J. Gross, D. C. Warltier, H. F. Hardman (Department of Pharmacology, The Medical College of Wisconsin, Milwaukee, Wis.). *Europ. J. Pharmacol. 36*, 267 (1976).

The effect of propranolol on the oxyhaemoglobin dissociation curve (P-50) of coronary venous blood was studied in isolated canine hearts. Intravenous administration of propranolol (0.5, 5.0 mg/kg) produced no significant change in P-50 over 4 hr. Following propranolol, an intracoronary infusion of nitroglycerine (10 μg/min) increased P-50. Addition of propranolol (5×10^{-5} M to 1×10^{-4} M) to central venous blood *in vitro* produced a significant increase in P-50. These data indicate that propranolol increases P-50 *in vitro* but does not increase P-50 *in vivo*, whereas nitroglycerine increases P-50 *in vivo* but not *in vitro*.

Ilma Szász

Partial purification of a membrane protein from human erythrocytes involved in glucose transport. A. Kahlenberg (Laboratory of Membrane Biochemistry, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada). *J. biol. Chem. 251*, 1582 (1976).

In an attempt to determine which membrane proteins are essential in the stereospecific uptake of d-glucose, isolated human erythrocyte membranes were exposed to a variety of reagents capable of selectively extracting various membrane proteins. These reagents included EDTA, lithium 3,5-diiodosalicylate, sodium iodide, and 2,3-dimethylmaleic anhydride. Selective elution of spectrin and components 2.1, 2.2, 2.3, 4.1, 4.2, 5, and 6, representing 65% of the ghost protein, had no effect on the uptake of d-glucose. All of the sugar transport proteins are associated with a membrane residue consisting of the proteins of Bands 3, 4.5 and 7, the periodic acid-Schiff-sensitive glycoproteins, and ghost phospholipids. Specific cross-linking of the proteins of Band 3 of ghosts by the catalysed oxidation of intrinsic sulfhydryl groups with the o-phenanthroline cupric ion complex inhibits d-glucose uptake and alters the relative electrophoretic mobility of Band 3 proteins in sodium dodecyl sulfate-polyacrylamide-agarose gels. This uptake activity and the relative mobility of Band 3 proteins are recovered upon reversal of the cross-linking reaction by reduction with 2-mercaptoethanol. These results and other observations indicate that the d-glucose transport protein is an intrinsic component of the hydrophobic structure of the erythrocyte membrane and may be associated with the proteins of Band 3 which are glycoproteins spanning the membrane bilayer. It is proposed that d-glucose transport occurs through a water-filled channel formed by specific subunit aggregates of the transport proteins in the erythrocyte membrane rather than by rotation of the protein within the plane of the membrane.

Ilma Szász

Binding characteristics of M and L isoantibodies to high and low potassium sheep red cells. P. K. Lauf, W. W. Sun (Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N. C.). *J. Memb. Biol. 28*, 351 (1976).

Binding of highly purified ^{125}I labelled M and L antibodies, both belonging to the immunoglobulin G class, was studied in high potassium (HK) and low potassium (LK) sheep red cells. Anti-M and anti-L bound specifically to M and L antigen positive HK and LK red cells, respectively. Nonspecific binding was higher for anti-L to HK cells than for anti-M to LK cells. Once bound, the M and L antibodies were capable of inducing complement dependent immune haemolysis. Only 75—100 and 500—750

molecules of anti-M and anti-L immunoglobulins were required to haemolyse 50% of HK (MM) and LK (LL) red cells, suggesting that the M and L antigens may be clustered on the surface of these cells. Equilibrium binding studies revealed that the maximum number of M sites is $3-6\times10^3$ in HK (MM) and $1.5-4\times10^3$ in LK (LM) cells. In comparison, the number of L antigens is slightly lower in LK cells, about $1.2-1.8\times10^3$ in LL and less in LM (LK) red cells. The number of M and L antigens is therefore more than an order of magnitude larger than that of the Na^+K^+ pumps measured previously in these cells by ^{3}H-ouabain binding, thus precluding a quantitative correlation between M and L antigens and the Na^+K^+ pumps different in the three genetic types of sheep red cells. The binding affinities of both anti-M and anti-L could not be described by a single equilibrium dissociation constant indicating heterogeneous antibody populations and/or variability in the antigenic sets of individual HK or LK cells. The pronounced heterogeneity of antigens and/or antibodies in the M and L systems was reflected in the antibody association kinetics which exhibited a remarkable temperature dependence. The data suggest that the correlation between the M and L antigens and the Na^+K^+ pump molecules is more complex than that reported previously for goat red cells.

G. Gárdos

Perturbational effects of inorganic cations on human erythrocyte membranes. D. V. Godin, M. Garnett (Department of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, Canada). *J. Memb. Biol. 28*, 143 (1976).

The perturbational effects of monovalent and divalent cations on human erythrocyte membranes were analyzed by examining their influence on kinetic and structural characteristics of trinitrobenzenesulfonic acid (TNBS) incorporation into the amino groups of protein and phospholipid structural components. The stimulatory effect of monovalent cations on TNBS incorporation, which were size-independent and attributed to nonspecific membrane alterations resulting from ionic strength factors, contrasted with the more pronounced stimulatory properties of divalent cations which were markedly size-dependent. These stimulatory effects of cations on TNBS incorporation were associated with alterations not only in rate but also in activation energy of incorporation. Changes in activation energy produced by divalent cations paralleled their ability to perturb membrane protein components and probably reflected changes in probe permeation. The rate of TNBS incorporation exhibited a dependence on divalent cation ionic radius which paralleled ion-induced perturbations in the labelling of the membrane amino phospholipid, phosphatidylethanolamine. Divalent cations differed in relative extent and in the characteristics of protein and phospholipid perturbation. Alkaline earth cations behaved as a rather homogeneous group while Ni^{++}, Co^{++} and Mn^{++} constituted a second heterogeneous group. The influence of monovalent and divalent cations on the haemolytic behaviour of intact erythrocytes closely paralleled their effects on TNBS incorporation into isolated membranes. It is suggested that TNBS incorporation may provide a valuable means of analyzing functionally relevant cation-induced alterations in biological membranes in general.

G. Gárdos

The effect of external calcium and lanthanum on platelet calcium content and on the release reaction. L. S. Robblee, D. Shepro (Boston University Biology Department and School of Medicine, Boston, Mass.). *Biochim. biophys. Acta (Amst.) 436*, 448 (1976).

Calcium compartments in calf platelets were studied using a lanthanum washout procedure to distinguish between surface-bound calcium and intracellular calcium. The calcium content of calf platelets ranges from 20 to 60 nmol/10^9 platelets and is sensitive to the calcium concentration of the suspending medium. With 1 mM calcium in the medium, calcium uptake is rapid and reaches the steady state in 1–2 min. Results obtained with the lanthanum procedure indicate that it is the surface compart-

ment which is most affected by the extracellular calcium concentration. The surface compartment appears to be saturable and is highly exchangeable. Although the total calcium as well as the calcium content of the surface and internal compartments are variable, the ratio of calcium in either compartment to the total saturated calcium is quite constant. The data indicate that 68–85% of the platelet calcium is located internally. Thrombin produces an immediate release of platelet calcium and labelled serotonin and an increase in the $^{45}Ca^{2+}$ uptake of both the surface and internal compartments. The release reaction is not dependent upon exogenous calcium or an influx of exogenous calcium since it occurs even in the presence of ethylene-glycol-bis-(β-aminoethylether)-N,N'-tetraacetic acid. Lanthanum, however, inhibits the release reaction, probably by blocking the surface calcium site and reducing the mobility of endogenous platelet calcium.

B. Sarkadi

Direct evidence for intracellular divalent cation redistribution associated with platelet shape change. G. C. Le Breton, R. J. Dinerstein, L. J. Roth, H. Feinberg (Department of Pharmacology, University of Illinois College of Medicine, Chicago, Ill., and Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Ill.). *Biochem. biophys. Res. Commun. 71*, 362 (1976).

Chlortetracycline was used as a fluorescent probe to monitor shifts in divalent cation distribution when blood platelets were induced to change shape. Human platelets in their native plasma were incubated with 50 μM chlortetracycline at 25 °C. It was found that when platelet shape change was stimulated by ADP or the divalent cation ionophore A23187, a significant decrease occurred in platelet-chlortetracycline fluorescence. This fluorescence shift was consistent with the time course for the change in platelet shape. ATP, which inhibits ADP-induced shape change, also inhibited the decrease in platelet-chlortetracycline fluorescence.

B. Sarkadi

Metabolic aspects of the secretion of stored compounds from blood platelets. IV. Effects of ionophore X537A on washed platelets. E. H. Mürer, K. Davenport, H. J. Day (SCOR Thrombosis Center, Temple UHSC, Philadelphia, Pa.). *Biochim. biophys. Acta (Amst.) 428*, 369 (1976).

X537A at concentrations below 10 μM can liberate platelet serotonin from washed human platelets without inducing the platelet release reaction. Up to 100% of serotonin preabsorbed by the platelets can be liberated before initiation of the release reaction. Concentrations of X537A above 10 μM initiate the platelet release reaction, with a maximum release of adenine nucleotides and platelet factor 4 antigen comparable to that obtained with 1.25 units thrombin/ml. The changes in ATP metabolism at the concentration necessary for X537A-induced release are more profound than those in platelets exposed to concentrations of thrombin or A23187 giving the same degree of realese, and approach those seen with A23187 at high concentrations. At concentrations where serotonin is liberated but no adenine nucleotide or platelet factor 4 antigen is released, brief incubation causes no change in the level of metabolic ATP. In Na^+ medium the release increases with prolonged incubation to a level which seems to be characteristic of a particular ionophore concentration. Replacing Na^+ with K^+ in the incubation medium produces maximum release for a particular concentration after short-time incubation, and no further increase is seen. When platelets are incubated with antimycin and glucose so that anaerobic metabolism is predominant, and then exposed to X537A, a marked sparing effect on ATP utilization can be observed in K^+ medium, and an increased ATP breakdown in Na^+ medium. The shift in monovalent cation does not affect the amount liberated and released. It is suggested that the increased speed of release induction and of serotonin liberation reflects a direct effect of the ion change on the ionophore action, possibly by increasing the speed of X537A accumulation in the platelet membrane.

B. Sarkadi

From the International Literature of Haematology

Acta Haematologica (Basel) **55** (1976) No. 6

Hemopoietic recovery in bone marrow of lethally irradiated rats following parabiosis. I. Granulopoiesis. *Seno, S., Fang Chao Hui, Himei, S., Hsueh, C. L., Nakashima, Y.* (Department of Pathology, Okayama University Medical School, Okayama, Japan), p. 321

Reconstitution capacity of bone marrow cells from nude mice in radiation chimeras. *Tao, T. W., Floersheim, G. L.* (Reprint requests: Prof. G. L. Floersheim, Laboratory for Transplantation Biology, Kantonsspital, CH-4056 Basel, Switzerland), p. 332

Characterization of blast cells in acute nonlymphoid leukemias by consecutive cytochemical reactions. *Grusovin, G. D., Castoldi, G. L.* (Clinica Medical Generale, Università di Ferrara, I-44100 Ferrara, Italy), p. 338

Malignant lymphoma with plasmacytoid differentiation and polycional gammopathy. *Guardia, J., Pedreira, J. D., Vidal, M. T., Roca, A.* (Departments of Internal Medicine and Pathology, Ciudad Sanitaria de la Seguridad Social, Universidad Autónoma, Barcelona 17, Spain), p. 346

Splenunculectomy in recurrent thrombocytopenia. *Spero, J. A., Lewis, J. H., Hasiba, U., Gumerman, L. W.* (Central Blood Bank of Pittsburgh, Pittsburgh, Pa. 15219), p. 354

Multiple myeloma with terminal erythroleukemia. *Meytes, D., Seligsohn, U., Ramot, B.* (Reprint requests: Prof. B. Ramot, Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel), p. 358

Acta Haematologica (Basel) **56** (1976) No. 1

The distribution of B and T lymphocytes in the peripheral blood of patients with Hodgkin's disease. *Many, A., Biniaminov, M., Rosenthal, E., Aghai, E., Ramot, B.* (Reprint requests: Prof. B. Ramot, Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel), p. 1

La réserve de granulocytes médullaires chez des patients soumis à l'hémodialyse périodique. *Buscarini, L.* (Reparto Medicina, Ospedale Civile, Fiorenzuola d'Arda, Italy), p. 7

Cytoplasmic connections between erythroblasts in megaloblastic anaemia. *Rozman, C., Woessner, S.* (Hospital Clinico y Provincial, Barcelona-11, Spain), p. 10

Erythrocyte 2,3-diphosphoglycerate and adenosine-triphosphate in cretins living at high altitude. *Adam, W. H.* (The Johns Hopkins University, International Center for Medical Research, Baltimore, Md. 21205), p. 14

Interrelations between AB0 blood group, plasminogen, α_1-antitrypsin, α_2-macroglobulin and the platelet count in blood donors. *Dawson, A., Allardyce, M., Allan, T. M., Ogston, D., Kerridge, D. F., Lewis, H. B. M.* (Reprint requests: Dr. D. Ogston, Department of Medicine, University of Aberdeen, Aberdeen AB9 2ZD, Scotland), p. 19

Factor X Friuli coagulation disorder. Report of a newly recognized patient and some considerations on the frequency of the disease in the Friuli area. *Girolami, A., Falomo, F., De Marco, L., Patrassi, G.* (Istituto di Semeiotica Medica, Padova, Italy), p. 27

Fetal erythropoiesis and dyserythropoiesis in juvenile chronic myeloid leukaemia. *Gahr,*

M., Jentsch, E., Schröter, W. (Reprint requests: Prof. W. Schröter, Universitätskinderklinik, D-34 Göttingen, BRD), p. 39

Glutathionreduktasemangel mit Membrandefekt bei hereditärer hämolytischer Anämie. *Pohl, A., Bugajer-Gleitman, H. E., Lachmann, D., Moser, K.* (I. Medizinische Universitätsklinik, A-1090 Wien, Österreich), p. 47

Favism in a Portuguese family due to a deficient glucose-6-phosphate dehydrogenase variant of "Canton" or "Canton-like" type. *Kahn, A., Marie, J., Desbois, J.-C., Boivin, P.* (Hôpital Beaujon, F-92110, Clichy, France), p. 58

Acta Haematologica (Basel) **56** (1976) No. 2

Granulocyte function in untreated acute and chronic granulocytic leukemia. *Pinkerton, P. H., Robinson, J. B.* (Sunnybrook Hospital, Toronto, Ont. M4N 3M5, Canada), p. 65

Response of blood and bone marrow neutrophils to the nitroblue-tetrazolium test in children. *Cassimos, C., Sklavunu-Zurukzoglu, S., Catriu, D., Nanas, C.* (Pediatric Clinic, AHEPA Hospital, Thessaloniki, Greece), p. 73

Progression and prognosis of chronic myelogenous leukemia in the acute stage: Hematologic and cytogenetic aspects. *Motomura, S., Ogi, K., Kaneko, S.* (Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan), p. 78

Residues 124 and 125 (H2 and H3) of the human haemoglobin δ-chain. *Lehmann, H., Casey, R.* (Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QR, England), p. 84

Versuch einer Stadieneinteilung der Osteomyelosklerose nach ferrokinetischen Befunden. *Neumann, E.* (I. Medizinische Universitätsklinik, A-1090 Wien, Österreich), p. 89

Platelet and leukocyte antibodies in autoimmune hemolytic anemia. *Fagiolo, E.* (Istituto di Clinica Medica, Università Cattolica del Sacro Cuore, I-00168 Roma, Italy), p. 97

Colony formation by canine hemopoietic cells in vitro. Inhibition by polymorphonuclear leukocytes. *Kovács, P., Bruch, C., Fliedner, T. M.* (Reprint requests: Dr. T. M. Fliedner, Abteilung für Klinische Physiologie, Universität Ulm, M24 309, D-7900 Ulm/Donau, BRD), p. 107

Glucose-6-phosphate dehydrogenase Ferrara. A new variant of G-6-PD identified in Northern Italy. *Carandina, G , Moretto, E., Zecchi, G., Conighi, C.* (Ospedale Civile Borselli, I-44012 Bondeno Ferrara, Italy), p. 116

Cytoplasmic fibrils in plasma cell leukemia. *Ogawa, M., Preston, R. A., Carson, M. D., Komiyama, A., Spicer, S. S.* (Veterans Administration Hospital, Charleston, S. C. 29403), p. 123

Acta Haematologica (Basel) **56** (1976) No. 3

Cytochemical studies on T and B lymphocytes and lymphoblasts with special reference to acid phosphatase. *Wehinger, H., Möbius, W.* (Universitätskinderklinik, D-78 Freiburg i. Br., BRD), p. 129

Meningeal relapse after long-term remission in acute childhood lymphocytic leukemia. *Feldges, A. J., Nussbaumer, A.* (Ostschweizer Säuglings- und Kinderspital, 9000 St. Gallen, Switzerland), p. 137

Splenectomy, iron overload and liver cirrhosis in β-thalassemia major. *Okon, E., Levij, I. S., Rachmilewitz, E. A.* (Reprint requests: Prof. I. S. Levij, Department of Pathology, Hadassah University Hospital, Jerusalem, Israel), p. 142

The effect of ionophore on platelet aggregation in von Willebrand's disease and in congenital afibrinogenemia. A comparison with Ristocetin. *Girolami, A., Fabris, F., de Marco, L., Peruffo, R.* (Istituto di Semeiotica Medica, Padova, Italy), p. 151

Studies on human platelets stored at 20–22 °C without agitation. *Vainer, H., Prost-Dvojaković, R. J., Jaisson, F., Letohic, F.* (Institut de Recherches sur les Leucémies, Hôpital Saint-Louis, Paris, France), p. 160

Congenital enzymopenic methaemoglobinaemia. Clinical and biochemical study of a family with three homozygotes. *Mast, A., Milo, R., Junien, C., Leroux, A.,*

Krishnamorrthy, R., Wajcman, H., Labie, D., Kaplan, J. C. (Department of Internal Medicine, Section of Pneumology, Akademisch Ziekenhuis, B-9000 Gent, Belgium), p. 174

A case with both hemoglobins C and N-Baltimore. *Johnson, C., Powars, D., Schroeder, W. A.* (LAC-USC Medical Center, General Hospital 14610, Los Angeles, Calif. 90033), p. 183

Monoclonal gammopathy of IgA and IgE type in a case of chronic lymphatic leukaemia. *Baenkler, H. W.* (Institut für Klinische Immunologie, D-8520 Erlangen, BRD), p. 189

Acta Haematologica (Basel) **56** (1976) No. 4

Proliferation characteristics of lymphoid cells of human bone marrow in health and in chronic renal failure. *Kempgens, U., Mayer, M., Schlosshardt, S., Müller, U., Queisser, W.* (I. Medizinische Klinik, Fakultät für Klinische Medizin, D-6800 Mannheim, BRD), p. 193

ε-Amino-caproic acid in the management of acute promyelocytic leukaemia. *Keane, T.J., Gorman, A. M., O'Connell, L., G. Fennelly, J. J.* (Reprint requests: Dr. L. G., O'Connell, St. Vincent's Hospital, Dublin 4, Ireland), p. 202

Immunoglobulin in idiopathic thrombocytopenic purpura in childhood. *Khalifa, A. S., Lusher, J. M., Cejka, J., Zuelzer, W. W.* (Ain Shams Faculty of Medicine, Cairo, Egypt), p. 205

The effects of late thymectomy on the immunosuppressive action of cortisone and testosterone in rats immunized to sheep red blood cells. *Aschkenasy, A.* (Centre National de la Recherche Scientifique, Centre Marcel Delépine, F-45 Orléans, France), p. 212

Erythropoietin level and thrombocytopoiesis in mice. *Krizsa, F., Cserháti, I.* (Second Department of Medicine, University Medical School, H-6701 Szeged, Hungary), p. 221

A drug-induced haemolytic anaemia due to Hb Torino (α43(CD1) Phe → Val). Second finding in an Italian family. *Sansone, G., Sciarratta, G. V., Lang, A., Lorkin, P. A., Lehmann, H.* (Reprint requests: Prof. H. Lehmann, University of Cambridge, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge CB2 2QR, England), p. 225

Haemoglobin Lepore Boston in a Spanish family. *Gimferrer, E., Baiget, M., Darbre, P. D., Lehmann, H.* (Servicio de Hematologia, Hospital de la Santa Cruz y San Pablo, Barcelona, Spain), p. 234

β^+-Thalassaemia, haemoglobin S and hereditary elliptocytosis in a Zaïrian family. Ischaemic costal necroses in a child with sickle cell β^+-thalassaemia. *Van Ros, G., Seynhaeve, V., Fiasse, L.* (Department of Haematology, Institute of Tropical Medicine, B-2000 Antwerp, Belgium), p. 241

Blood (New York) **47** (1976) No. 4

Granulopoiesis in severe congenital neutropenia. *Amato, D., Freedman, M. H., Saunders, E. F.* (Reprint requests: Dr. E. F. Saunders, Department of Pediatrics, Hospital for Sick Children, Toronto, Ontario, Canada), p. 531

Granulocyte function in experimental human endotoxemia. *Territo, M. C., Golde, D. W.* (Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 539

Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. *DeChatelet, L. R., Shirley, P. S., Johnston, R. B. Jr.* (Department of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C. 27103), p. 545

Chediak-Higashi syndrome: Reversal of increased susceptibility to infection by bone marrow transplantation. *Kazmierowski, J. A., Elin, R. J., Reynolds, H. Y., Durbin, W. A., Wolff, S. M.* (NIAID, Bethesda, Md. 20014), p. 555

The anemia of chronic renal failure and chronic diseases: In vitro studies of erythropoiesis. *Wallner, S. F., Kurnick, J. E., Ward, H. P., Vautrin, R., Alfrey, A. C.* (Denver Veterans Administration Hospital, Denver, Col. 80220), p. 561

Stereospecific tissue uptake and nuclear accumulation of testosterone in the development of the mouse erythropoietic spleen.

Hadjian, A. J., Spivak, J. L., Kowarski, A., Dickerman, H. W., Migeon, C. J. (Reprint requests: Dr. H. W. Dickerman, Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. 12201), p. 571

Effect of erythropoietin on chromosomal protein synthesis. *Spivak, J. L.* (Clayton Laboratories, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205), p. 581

Regeneration of blood-forming organs after autologous leukocyte transfusion in lethally irradiated dogs. II. Distribution and cellularity of the marrow in irradiated and transfused animals. *Calvo, W., Fliedner, T. M., Herbst, E., Hügl, E., Bruch, C.* (Reprint requests: Dr. T. M. Fliedner, Department of Clinical Physiology, University of Ulm, Oberer Eselberg, 79 Ulm /Donau, FRG), p. 593

In vitro colony-forming cells in the marrow of leukemic and preleukemic mice. *Hays, E. F., Craddock, C. G., Haskett, D., Newell, M.* (UCLA, Laboratory of Nuclear Medicine, Los Angeles, Calif. 90024), p. 603

Acquired XO/XY clones in bone marrow of a patient with paroxysmal nocturnal hemoglobinuria (PNH). *Whang-Peng, J., Knutsen, T., Lee, E. C., Leventhal, B.* (Cytogenetic Oncology Section, National Cancer Institute, Bethesda, Md. 20014), p. 611

Delay time of gelation: A possible determinant of clinical severity in sickle cell disease. *Eaton, W. A., Hofrichter, J., Ross, P. D.* (Laboratory of Chemical Physics, NIH, Bethesda, Md. 20014), p. 621

Mechanism of dilutional anemia in massive splenomegaly. *Hess, C. E., Ayers, C. R., Sandusky, W. R., Carpenter, M. A., Wetzel, R. A., Mohler, D. N.* (Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Va. 22901), p. 629

Studies on γ-glutamyl transpeptidase in human and rabbit erythrocytes. *Srivastava, S. K., Awashi, Y. C., Miller, S. P., Yoshida, A., Beutler, E.* (Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550), p. 645

Abnormal parachloromercuriphenylsulfonate-sensitive cation channel in the erythrocytes of hereditary spherocytosis. *Kitao, T., Hattori, K., Takeshita, M.* (Department of Internal Medicine, Kanazawa University School of Medicine, Kanazawa, Japan), p. 651

Immunologic evidence that the properties of human antihemophilic factor (factor VIII) are attributes of a single molecular species. *Ratnoff, O. D., Slover, C. C., Poon, M.-C.* (Department of Medicine, University Hospital of Cleveland. Cleveland, Ohio 44106), p. 657

Activation of the coagulation system in polycythemia vera. *Carvalho, A., Ellman, L.* (Harvard Medical School, Department of Medicine, Massachusetts General Hospital, Boston, Mass. 02114), p. 669

Preferential involvement of chromosomes No. 8 and No. 21 in acute leukemia and preleukemia. *Yamada, K., Furusawa, S.* (Department of Genetics, Clinical Research Institute, National Medical Center Hospital, Tokyo 162, Japan), p. 679

Premature chromosome condensation in human leukemia. *Williams, D. M., Scott, C. D., Beck, T. M.* (Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah 84132), p. 687

Polyamine concentrations in bone marrow aspirates of children with leukemia and other malignancies. *Rennert, O., Miale, T., Shukla, J., Lawson, D., Frias, J.* (Department of Pediatrics. Box J-296, University of Florida College of Medicine, Gainesville, Fla. 32610), p. 695

Blood (New York) **47** (1976) No. 5

Chromosomal banding patterns in acute nonlymphocytic leukemia. *Rowley, J. D., Potter, D.* (University of Chicago, Chicago, Ill. 60637), p. 705

Decrease and altered distribution of human T antigen on chronic lymphatic leukemia cells of T type, suggesting a clonal origin. *Thiel, E., Rodt, H., Huhn, D., Thierfelder, S.* (Institut für Hämatologie der GSF, 8 München 2, BRD), p. 723

Unregulated growth of murine leukemic cells and suppression of normal granulocyte growth in diffusion chamber cultures. *Miller, A. M., Marmor, J. B., Page, P. L., Russell, J. L., Robinson, S. H.* (Reprint

requests: Dr. S. H. Robinson, Harvard Medical School, Department of Medicine, Beth Israel Hospital, Boston, Mass. 02215), p. 737

Treatment of advanced non-Hodgkin's lymphomas with favorable histologies: Preliminary results of a prospective trial. *Portlock, C. S., Rosenberg, S. A., Glatstein, E., Kaplan, H. S.* (Reprint requests: Dr. S. A. Rosenberg, Division of Oncology, Stanford University, School of Medicine, Stanford, Calif. 94305), p. 747

Specific release of neutrophilic- and eosinophilic-stimulating factors from sensitized lymphocytes. *Ruscetti, F. W., Cypess, R. H., Chervenick, P. A.* (University of Pittsburgh School of Medicine, Department of Medicine, Pittsburgh, Pa. 15261), p. 757

Isolation and in vitro differentiation of human erythroid precursor cells. *Kim, H. C., Marks, P. A., Rifkind, R. A., Maniatis, G. M., Bank, A.* (Reprint requests: Dr. A. Bank, Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, N. Y. 10032), p. 767

Separation of the erythropoietin-responsive progenitors BFU-E and CFU-E in mouse bone marrow by unit gravity sedimentation. *Heath, D. S., Axelrad, A. A., McLeod, D. L., Shreeve, M. M.* (Reprint requests: Dr. A. A. Axelrad, University of Toronto, Toronto, Ont., Canada M5S1A8), p. 777

Aplastic anemia: Failure of patient leukocytes to stimulate allogeneic cells in mixed leukocyte culture. *Mickelson, E. M., Fefer, A., Thimas, E. D.* (Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 793

Effect of vitamin B_{12} and folic acid deficiencies on neutrophil function. *Kaplan, S. S., Basford, R. E.* (University of Pittsburgh Medical School, Pittsburgh, Pa. 15261), p. 801

Pyruvate kinase deficiency and leg ulcers. *Müller-Soyano, A., de Roura, E. T., Duke, P.-R., De Acquatella, G. C., Arends, T., Guinto, E., Beutler, E.* (Reprint requests: Prof. E. Beutler, City of Hope Medical Center, Duarte, Calif. 91010), p. 807

Trisomy 8 in the bone marrow associated with high red cell glutathione reductase activity. *de la Chapelle, A., Vuopio, P., Icén, A.* (Folkhälsan Institute of Genetics, SF-00101 Helsinki 10, Finland), p. 815

Hemoglobin Pyrgos $\alpha_2\beta_2^{83\ (EF7)\ Gly \rightarrow Asp}$: A new hemoglobin variant in double heterozygosity with hemoglobin S. *Tatsis, B., Sofroniadou, K., Stergiopoulos, C. I.* (Department of Hematology, Queens Hospital Center, Long Island Jewish-Hillside Medical Center, Jamaica, N. Y.), p. 827

Phagocytosis in human platelets: Localization of acid phosphatase-positive phagosomes following latex uptake. *Lewis, J. C., Maldonado, J. E., Mann, K. G.* (Mayo Clinic, Rochester, Minn. 55901), p. 833

The pH dependence of quantitative ristocetin-induced platelet aggregation: Theoretical and practical implications. A new device for maintenance of platelet-rich plasma pH. *Coller, B. S., Franza, B. R. Jr., Gralnick, H. R.* (Hematology Service, Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Md. 20014), p. 841

The effect of experimental hematoma on serum levels of fibrinogen-related antigen. *Cade, J., Regoeczi, E., Hirsh, J., Buchanan M.* (Reprint requests: Dr. E. Regoeczi, McMaster University Medical Center, Hamilton, Ont. L8S4J9, Canada), p. 855

Colony-stimulating factor in patients with chronic neutropenia. *Wewerka, J. R., Dale, D. C.* (Reprint requests: Dr. D. C. Dale, Department of Medicine, RG 20, University of Washington, School of Medicine, Seattle, Wash. 98195), p. 861

Experimental *Pseudomonas* pneumonia in leukopenic dogs: Comparison of therapy with antibiotics and granulocyte transfusions. *Dale, D. C., Reynolds, H. Y., Pennington, J. E., Elin, R. J., Herzig, G. P.* (Department of Medicine, RG-20, University of Washington, School of Medicine, Seattle, Wash. 98195), p. 869

Blood (New York) **47** (1976) No. 6

Enrichment of erythrocytes of fetal origin from adult-fetal blood mixtures via selective hemolysis of adult blood cells: An aid to antenatal diagnosis of hemoglobinopathies. *Boyer, S. H., Noyes, A. N., Boyer, M. L.* (The Johns Hopkins Univer-

sity Medical School, Baltimore, Md. 21205), p. 883

Decreased α globin mRNA in nucleated red cell precursors in α thalassemia. *Natta, C. L. Ramirez, F., Wolff, J. A., Bank, A.* (Reprint requests: Dr. A. Bank, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 899

Decreased life span and membrane damage of carbamylated erythrocytes in vitro. *Lane, T. A., Burka, E. R.* (Reprint requests: Dr. E. R. Burka, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pa.), p. 909

A family with red cell pyrimidine 5'-nucleotidase deficiency. *Ben-Bassat, I., Brok-Simoni, F., Kende, G., Holtzmann, F., Ramat, B.* (Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel), p. 919

Protein-mediated uptake of vitamin B_{12} by isolated mitochondria. *Gams, R. A. Ryel, E. M. Ostroy, F.* (Hematology Division, UAB Medical Center, University Station, Birmingham, Ala. 35294), p. 923

Complement-mediated granulocyte dysfunction in paroxysmal nocturnal hemoglobinuria. *Craddock, P. R. Fehr J., Jacob, H. S.* (Department of Medicine, Mayo Memorial Building, University of Minnesota, Minneapolis, Minn. 55455), p. 931

Platelet function in the Chediak-Higashi syndrome. *Buchanan, G. R., Handin, R. I.* (Reprint requests: Dr. R. I. Handin, Peter Bent Brigham Hospital, Boston, Mass. 02115), p. 941

Defective platelet function following the administration of penicillin compounds. *Brown, C. H., III, Bradshaw, M. W., Natelson, E. A., Alfrey, C. P., Jr., Williams, T. W. Jr.* (85 West Miller St., Orlando, Fla. 32806), p. 949

Platelet inhibition by sodium nitroprusside, a smooth muscle inhibitor. *Saxon, A., Kattlove, H. E.* (Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 957

Distribution of phospholipids, fatty acids, and platelet factor 3 activity among subcellular fractions of human platelets. *Broekman, M. J., Handin, R. I., Derksen, A., Cohen, P.* (Reprint requests: Dr. P. Cohen, Department of Nutrition, Harvard School of Public Health, Boston, Mass. 02115), p. 963

Antibodies to factor VIII. V. Patterns of immune response to factor VIII in hemophilia A. *Allain, J.-P., Frommel, D.* (Centre pour Enfants Hémophiles, 78940 La Queue-lez-Yvelines, France), p. 973

Use of prothrombin complex concentrates in the treatment of a hemophilic patient with an inhibitor of factor VIII. *Sonoda, T., Solomon, A., Krauss, S., Cruz, P., Jones, F. S., Levin, J.* (Reprint requests: Dr. A. Solomon, University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 983

Detection of soluble intermediates of the fibrinogen-fibrin conversion using erythrocytes coated with fibrin monomers. *Largo, R., Heller, V., Straub, P. W.* (Reprint requests: Dr. P. W. Straub, Division of Hematology, Department of Medicine, Kantonsspital, 8091 Zürich, Switzerland), p. 991

Chemotherapy of the blastic phase of chronic granulocytic leukemia: Hypodiploidy and response to therapy. *Canellos, G. P., DeVita, V. T., Whan-Peng, J., Chabner, B. A., Schein, P. S., Young, R. C.* (Reprint requests: Dr. R. C. Young, Medicine Branch, National Cancer Institute, Bethesda, Md. 20014), p. 1003

Immune function at diagnosis in relation to response to therapy in acute lymphocytic leukemia of childhood. *Jose, D. G., Ekert, H., Colebatch, J., Waters, K., Wilson, F., O'Keefe, D.* (Royal Children's Hospital Research Foundation, Parkville, 3052 Victoria, Australia), p. 1011

Periodic oscillation of blood leukocytes, platelets, and reticulocytes in a patient with chronic myelocytic leukemia. *Chikkappa, G., Borner, G., Burlington, H., Chanana, A. D., Cronkite, E. P., Öhl, S., Pavelec, M., Robertson, J. S.* (Medical Research Center, Brookhaven National Laboratory, Upton, N. Y. 11973), p. 1023

Increase in circulating stem cells following chemotherapy in man. *Richman, C. M., Weiner, R. S., Yankee, R. A.* (Sidney Farber Cancer Center, Boston, Mass. 02111), p. 1031

Assessment of erythrocytic and granulocytic colony formation in an in vivo plasma clot diffusion chamber culture system.

Steinberg H. N., Handler, E. S., Handler, E. E. (Reprint requests: Dr. E. S. Handler, Department of Biological Sciences, Hunter College, New York, N. Y. 10021), p. 1041

Blood (New York) **48** (1976) No. 1

The role of cytogenetics in hematology. *Rowley, J. D.* (University of Chicago, Box 420, Chicago, Ill. 60637), p. 1

Acute nonlymphocytic leukemia in adults: Correlations with Q-banded chromosomes. *Golomb, H. M., Vardiman, J., Rowley, J. D.* (University of Chicago Hospitals, Chicago, Ill. 60637), p. 9

Accelerated myeloblast destruction and abnormal lysosome disruption in cultured bone marrow: Association with indolent acute myelogenous leukemia. *Branda, R. F., Jacob, H. S., Doublas, S. D., Moldow, C. F., Puumala, R. R.* (Department of Medicine, University of Minnesota Hospitals, Minneapolis, Minn. 55455), p. 23

Insulin binding of acute lymphocytic leukemia cells. *Esber, E. C., Buell, D. N., Leikin, S. L.* (Reprint requests: Dr. S. L. Leikin, Children's Hospital National Medical Center, Washington, D. C. 20009), p. 33

κ and λ light chain disease: Survival rates and clinical manifestations. *Shustik, C., Bergsagel, D. E., Pruzanski, W.* (Reprint requests: Dr. W. Pruzanski, Wellesley Hospital, Toronto, Ont., Canada M4Y 1J3, p. 41)

Familial deficiency of glutathione reductase in human blood cells. *Loos, H., Roos, D., Weening, R., Houwerzijl, J.* (Central Laboratory, The Netherlands Red Cross Blood Transfusion Service, 9190 Amsterdam, The Netherlands), p. 53

Severe aplastic anemia: A prospective study of the effect of early marrow transplantation on acute mortality. *Camitta, B. M., Thomas, E. D., Nathan, D. G., Santos, G., Gordon-Smith, E. C., Gale, R. P., Rappeport, J. M., Storb, R.* (Milwaukee Children's Hospital, Milwaukee, Wis. 53233), p. 63

The pathogenesis of aplastic anemia: A defective pluripotent hematopoietic stem cell with inappropriate balance of differentiation and self-replication. *Boggs, D. R., Boggs, S. S.* (Department of Medicine, Division of Hematology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15261), p. 71

Role of copper in mitochondrial iron metabolism. *Williams, D. M., Loukopoulos D., Lee, G. R., Cartwright, G. E.* (University Hospital, Salt Lake City, Utah 81432), p. 77

Evidence that functional subunits of antihemophilic factor (factor VIII) are linked by noncovalent bonds. *Poon, M.-C., Ratnoff, O. D.* (Reprint requests: Dr. O. D. Ratnoff, Department of Medicine, University Hospital of Cleveland, Cleveland, Ohio 44106), p. 87

Immunosuppressive therapy of factor VIII inhibitors. *Hultin, M. B., Shapiro, S. S., Bowman, H. S., Gill, F. M., Andrews, A. T., Martinez, J., Eyster, M. E., Sherwood, W. C.* (Reprint requests: Dr. S. S. Shapiro, Cardeza Foundation, Jefferson Medical College, Philadelphia, Pa. 19107), p. 95

Evaluation of the role of in vivo proteolysis (fibrinogenolysis) in prolonging the thrombin time of human umbilical cord fibrinogen. *Galanakis, D. K., Mosesson, M. W.* (Downstate Medical Center, Brooklyn, N. Y. 11203), p. 109

Effect of heterologous antibody on human platelets. *Colman, R. W., Schreiber, A. D.* (Reprint requests: Dr. A. D. Schreiber, University of Pennsylvania School of Medicine, Hematology-Oncology Section, Philadelphia, Pa. 19104), p. 119

Separation of megakaryocytes from mouse bone marrow by density gradient centrifugation. *Nakeff, A., Floeh, D. P.* (Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Mo. 63110), p. 133

Identification of monocytes in suspensions of mononuclear cells. *Rothbarth, P. H., Hendriks-Sturkenboom, I., Ploem, J. S.* (Department of Immunology, Het Wilhelmina Kinderziekenhuis, Utrecht, The Netherlands), p. 139

Degranulation and abnormal bactericidal function of granulocytes procured by reversible adhesion to nylon wool. *Klock, J. C., Bainton, D. F.* (Cancer Research Institute, University of California, San Francisco, Calif. 94143), p. 149

Blut (München) **32** (1976) No. 5

Arzneimittelbedingte Thrombozytopenien. *Miescher, P. A., Nydegger, U.* (Division d'Hématologie et Centre de Transfusion Sanguine, Hôpital Cantonal, CH-1211 Geneva 4, Switzerland), p. 329

Drei Fälle von Eosinophilenleukämie mit atypischer Granulation in Eosinophilen und Neutrophilen. *Ruzicka, F., Pawlowsky, J., Erber, A., Nowotny, H.* (No address), p. 337

Elektronische Zählung von Thrombozyten aus dem Vollblut. *Adam, W., Schulz, J., Kratt, E., Nitsche, H.-J., Kubanek, B.* (Deutsche Klinik für Diagnostik, 6200 Wiesbaden, BRD), p. 347

Inhibitory activity of medium-dialyzed transfer factor on lymphocyte blastogenesis. *Mueller-Eckhardt, C., Ritts, R. E. Jr.* (Abteilung für Klinische Immunologie und Transfusionsmedizin, Universität Gießen, D-6300 Gießen, BRD), p. 353

Sex difference and the influence of endotoxin and hypertransfusion in spleen colony formation. *Stevenson, A. F. G., Duplan, J.-F.* (Laboratorium für experimentelle Radiologie, 8042 Neuherber bei München, BRD), p. 361

An auto-anti-B in an A_1B person. Serological studies. *Finke, M., Sachs, V., Vollert, B., Lopez, M., Salmon, C., Hoppe, H. H., Fischer, K.* (Reprint requests: Prof. Dr. V. Sachs, Abteilung für Bluttransfusionswesen, Klinikum der Universität Kiel, D-2300 Kiel, BRD), p. 371

Leukämieviren. *Hehlmann, R.* (Medizinische Poliklinik der Universität, D-8000 München 2, BRD), p. 365

Blut (München) **32** (1976) No. 6

Arzneimittelbedingte Agranulozytosen. *Gross, R., Hellriegel, K.-P.* (Medizinische Universitätsklinik, 5000 Köln 41, BRD), p. 409

Combined deficiency of factor V and factor VIII. A report of another case. *Girolami, A., Violante, N., Cella, G., Patrassi, G.* (Istituto di Semeiotica Medica dell'Università, 105 Padova, Italy), p. 415

Mißbildungen, Anomalien und Variationen bei Patienten mit schwerer Eisenmangelkrankheit. *Reimann, F., Erdogán, G.* (Istanbul-Ayaz Pasa, Medizinische Fakultät Çapa der Universität Istanbul, Istanbul, Türkei), p. 423

Ultrastructure of chronic megakaryocytic-granulocytic myelosis. *Thiele, J., Georgii, A., Vykoupil, K.-F.* (Pathologisches Institut der Medizinischen Hochschule Hannover, 3000 Hannover 61, BRD), p. 433

Haematological values for the Finnish reindeer. *Timisjärvi, J., Reinilä, M., Järvensivu, P.* (Department of Physiology, University of Oulu, SF-90220 Oulu 22, Finland), p. 439

Biologische Aktivität verschiedener Intrinsicfaktor-Präparationen im Schillingtest. *Neumann, E.* (I. Medizinische Universitätsklinik, A-1090 Wien, Österreich), p. 443

Experimentelle Untersuchungen zur Populationsgenetik der 3. Komplementkomponente (C3) und der Serumkomponente Transferrin (Tf) in der Bevölkerung Nordbayerns. *Sittner, G., Wiebecke, D., Trenkel, K.* (Chirurgische Universitätsklinik, 8700 Würzburg, BRD), p. 447

Blut (München) **33** (1976) No. 1

Arzneimittelbedingte Panmyelopathien. *Heimpel, H., Kern, P.* (Abteilung Hämatologie im Zentrum für Innere Medizin der Universität, 7900 Ulm/Donau, BRD), p. 1

Colony growth of mouse bone marrow cells in agar contained in glass capillaries. *Maurer, H. R., Henry, R.* (Pharmazeutisches Institut der Freien Universität Berlin, West-Berlin 33), p. 11

Ribosome-Lamellae Complex in chronic lymphatic leukaemia. *Woessner, S., Rozman, C.* (Postgraduate School of Haematology, 143 Barcelona-11, Spain), p. 23

Phospho-accepting proteins in bovine sera. *Kinzel, V., Kühler, D.* (Institut für Experimentelle Pathologie, D-6900 Heidelberg, BRD), p. 29

Von Willebrand-Jürgens-Syndrom mit einer Variante des Faktor VIII-assoziierten Antigens. *Böttcher, D., Hasler, K., Sutor, A. H., Mair, D.* (Medizinische Universitätsklinik, D-7800 Freiburg, BRD), p. 33

Decrease of normotest/thrombostest discrepancy in non-contacted coumarin plasma after glass or ellagic acid activation.

Girolami, A., Patrassi, G., Burul, A. (University of Padua Medical School, Padua, Italy), p. 41

Effect of osmotic pressure, ionic strength and dibutyryl cyclic adenosine monophosphate on the adhesion of hen erythrocytes. *Imre, S., Szilágyi, T., Tóth, S.* (Institute of Pathophysiology, University Medical School, H-4012 Debrecen, Hungary), p. 49

Blut (München) **33** (1976) No. 2

Toxische hämolytische Anämien. *Kleihauer, E., Kohne, E.* (Abteilung für Kinderheilkunde der Universität, 7900 Ulm/Donau, BRD), p. 73

Detection of carriers of hemophilia by hemorrhagometry. *Jesdinsky-Buscher, C., Sutor, A. H.* (Reprint requests: Dr. A. H. Sutor, Universitäts-Kinderklinik, 7800 Freiburg i. Br., BRD), p. 83

Zur Standardisierung der ADP-induzierten Thrombozytenaggregation. *von Voss, H., Petrich, C., Tomaschoff, J., Göbel, U.* (Universitäts-Kinderklinik II, 4000 Düsseldorf, BRD), p. 89

An immunological investigation of factor VIII associated antigen in combined factor V and factor VIII deficiency. *Girolami, A., Borsato, N., Patrassi, G., Sticchi, A.* (University of Padua Medical School, Institute of "Semeiotica Medica", 105 Padua, Italy), p. 97

Zur Bedeutung der PAS-positiven Myeloblastenleukämie. *Abbrederis, K., Schmalzl, F., Braunsteiner, H.* (Medizinische Universitätsklinik, 6020 Innsbruck, Österreich), p. 103

Kein Hinweis für eine Koppelung der Rh- und Gm-Loci am Chromosom 1. *Mayr, W. R., Prokop, O.* (Institut für Blutgruppenserologie, A-1090 Wien, Österreich), p. 109

Blut (München) **33** (1976) No. 3

Erythropoietin. *Goldwasser, E.* (Department of Biochemistry, University of Chicago, Chicago, Ill. 60637), p. 135

T-Zell-Leukämien des Erwachsenen. *Huhn, D., Rodt, H., Thiel, E., Grosse-Wilde, H., Fink, U., Theml, H., Jäger, G., Steidle, C., Thierfelder, S.* (Medizinische Klinik der Universität München, 8000 München 2, BRD), p. 141

Starting procedures for the isolation and purification of granulocyte chalone activities. *Maurer, H. R., Weiß, G., Laerum, O. D.* (Institut für Pharmazie der Freien Universität Berlin, West-Berlin 33), p. 161

Erythroid colony formation (CFU_e) in fetal liver and adult bone marrow and spleen from the mouse. *Rich, I. N., Kubanek, B.* (Abteilung für Hämatologie der Universitäts-Klinik Ulm, D-7900 Ulm/Donau, (BRD), p. 171

Österreichische Gesellschaft für Hämatologie. Kongreß 1976, Feldkirch, 30. September bis 1. Oktober 1976, p. 181

British Journal of Haematology (Oxford) **33** (1976) No. 2

Annotation. The plasma transport of vitamin B_{12}. *Allen, R. H.* (Divisions of Hematology and Oncology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo. 63110), p. 161

Mouse red-cell rosettes in B-lymphoproliferative disorders. *Catovsky, D., Cherchi, M., Okos, A., Hedge, U., Galton, D. A. G.* (M. R. C. Leukaemia Unit, Royal Postgraduate Medical School, London, W. 12, 0HS, England), p. 173

Analysis of treatment in childhood leukaemia. II. Timing and the toxicity of combined 6-mercaptopurine and methotrexate maintenance therapy. *MacLennan, I. C. M., Kay, H. E. M., Festenstein, M., Smith, P. G.* (Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 179

Surface sulphydryl groups and phagocytosis-associated oxidative metabolic changes in human polymorphonuclear leucocytes. *Tsan, M.-F., Newman, B., McIntyre, P. A.* (Divisions of Hematology and Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Md. 21205), p. 189

Surface sulphydryl groups and hexose monophosphate pathway activity in resting human polymorphonuclear leucocytes. *Tsan, M.-F., Newman, B., Chusid, M. J., Wolff, S. M., McIntyre, P. A.* (Divisions

of Hematology and Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Md. 21205), p. 205

Activation of intravascular coagulation by endotoxin: the significance of granulocytes and platelets. *Berghaus-Müller, G., Bohn, E., Höbel, W.* (Department of Medicine, Justus Liebig-Universität, 63 Gießen, FRG), p. 213

Immunoradiometric assay of factor VIII related antigen, with observations in 32 patients with von Willebrand's disease. *Ruggeri, Z. M., Mannucci, P. M., Jeffcoate, S. L., Ingram, G. I. C.* (Haemophilia and Thrombosis Centre, Ospedale Policlinico, University of Milan, Milan, Italy), p. 221

Three approaches to the radioimmunoassay of human β-thromboglobulin. *Bolton, A. E., Ludlam, C. A., Moore, S., Pepper, D. S., Cash, J. D.* (MRC Radioimmunoassay Team, Royal Infirmary, Edinburgh EH1 2QW, Scotland), p. 233

Studies on the liberation of β-thromboglobulin from human platelets *in vitro*. *Ludlam, C. A., Cash, J. D.* (Department of Haematology, University Hospital of Wales, Cardiff CF4 3XW, Wales), p. 239

A familial abnormality of circulating vitamin B_{12} binding proteins: Occurrence in a family of high serum concentrations of transcobalamin II. *Kane, S. P., Hoffbrand, A., Allen, R. H., Neale, G.* (Department of Gastroenterology, Charing Cross Hospital, London, W6 8RF, England), p. 249

Inhibitory effect of eggs on vitamin B_{12} absorption: Description of a simple ovalbumin ^{57}Co-vitamin B_{12} absorption test. *Doscherholmen, A., McMahon, J., Ripley, D.* (Veterans Administration Hospital, Minneapolis, Minn. 55417), p. 261

The effect of some anticonvulsant drugs on depletion of folate in rats on a vitamin B_{12}-deficient diet. *Williams D. L., Spray, G. H.* (Reprint requests: Dr. G. H. Spray, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 273

Rheological properties of red cells in haemoglobin Köln disease. *Tillmann, W., Labitzke, N., Schröter, W.* (Reprint requests: Dr. W. Schröter, Department of Paediatrics, University of Göttingen, 34 Göttingen, FRG), p. 279

Erythrocyte membrane modification in malignant diseases of myeloid and lymphoreticular tissues. I. Tn-polyagglutination in acute myelocytic leukaemia. *Bird, G. W. G., Wingham, J., Pippard, M. J., Hoult, J. G., Melikian, V.* (West Midlands Regional Blood Transfusion Service, Edgbaston, Birmingham B15 2SG, England), p. 289

Erythrocyte membrane modification in malignant disease of myeloid and lymphoreticular tissues. II. Erythrocyte "mosaicism" in acute erythroleukaemia. *Bird, G. W. G., Wingham, J., Chester, G. H., Kidd, P., Payne, R. W.* (West Midlands Regional Blood Transfusion Service, Edgbaston, Birmingham B15 2SG, England), p. 295

The relationship between total red cell volume, plasma volume and venous haematocrit. *Bentley, S. A., Lewis, S. M.* (Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 301

Correspondence. Detection and measurement of factor VIII antibodies in an agarose gel: a modified method. *Rainsford, S. G., Hall, A.* (Lord Mayor Treloar College, Froyle, Alton, Hants, England), p. 309

British Journal of Haematology (Oxford) **33** (1976) No. 3

Annotation. Functions of the eosinophil leucocyte. *Kay, A. B.* (South-East Scotland Regional Blood Transfusion Service, Royal Infirmary, Edinburgh, Scotland), p. 313

Immunological classification of acute lymphoblastic leukaemias: Evaluation of its clinical significance in a hundred patients. *Brouet, J.-C., Valensi, F., Daniel, M.-T., Flandrin, G., Preud'Homme, J.-L., Seligmann, M.* (Laboratory of Immunochemistry and Immunopathology, Research Institue on Blood Diseases, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 319

Nuclear DNA-polymerase estimation in human leukaemic myeloblasts. *Wantzin, G. L., Karle, H., Killmann, S.-A.* (Division of Haematology, Department of Medicine A., Rigshospitalet 9, DK-2100 Copenhagen, Denmark), p. 329

Disseminated intravascular coagulation caused by the carpet viper *(Echis carinatus)*: Trial of heparin. *Warrell, D. A., Pope, H. M., Prentice, C. R. M.* (The Radcliffe Infirmary, Oxford OX2 6HE, England), p. 335

Quantitative determination of factor-VIII protein by two-stage gel electrophoresis. *Weinstein, M. J., Deykin, D., Davie, E. W.* (Boston Veterans Administration Hospital, Boston, Mass. 02130), p. 343

Severe hereditary haemolytic anaemia in dogs treated by marrow transplantation. *Weiden, P. L., Storb, R., Graham, T. C., Schroeder, M.-L.* (Division of Oncology, Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 357

Haemolytic anaemia due to glucose-6-phosphate dehydrogenase (G6PD) deficiency: Demonstration of two new biochemical variants, G6PD Hamm and G6PD Tarsus. *Gahr, M., Bornhalm, D., Schröter, W.* (Universitätskinderklinik, D-3400 Göttingen, BRD), p. 363

The role of disulphide bonds in Heinz body attachment to membranes. *Chan, E., Desforges, J. F.* (Reprint requests: Dr. J. F. Desforges, New England Medical Center Hospital, Boston, Mass. 02111), p. 371

The effects of acute bleeding on acid-base balance, erythropoietin (Ep) production and *in vivo* P_{50} in the rat. *Miller, M. E., Rörth, M., Stohlman, F. Jr., Valeri, C. R., Lowrie, G., Howard, D., McGilvray, N.* (St. Elizabeth's Hospital, Brighton, Mass. 02135), p. 379

Haemopoietic progenitor cells in prenatal congenitally anaemic "flexed-tailed" *(f/f)* mice. *Cole, R. J., Regan, T.* (Developmental Genetics Group, School of Biological Sciences, University of Sussex, Falmer, Brighton, England), p. 387

Control of haemopoietic stem cell proliferation by cells in DNA synthesis. *Necas, E., Neuwirt, J.* (Institute of Pathological Physiology, Faculty of General Medicine, Charles University, 128 53 Prague 2, Czechoslovakia), p. 395

Biological variation in the heterogeneous distribution of haemoglobin F among erythrocytes. *Bhattacharya, S. P., Anyaibe, S. I., Headings, V. E.* (Division of Medical Genetics, Howard University College of Medicine, Washington, D. C. 20059), p. 401

Lymphoid cell dependent (K-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. *Urbaniak, S. J.* (South-East Scotland Regional Blood Transfusion Service, Royal Infirmary, Edinburgh EH3 9HB, Scotland), p. 409

In vivo plasma and urine folate binding after ingestion of ^{3}H-folic acid and ^{14}C-methyl-folate. *Retief, F. P., du P. Heyns, A., Oosthuizen, M., van Reenen, O. R.* (Department of Internal Medicine, University of Orange Free State, Bloemfontein, Republic of South Africa), p. 415

Patterns of food iron absorption in iron-deficient white and Indian subjects and in venesected haemochromatic patients. *Bezwoda, W. R., Disler, P. B., Lynch, S. R., Charlton, R. W., Torrance, J D., Derman, D., Bothwell, T. H., Walker, R. B., Mayet, F.* (Reprint requests: Prof. T. H. Bothwell, Department of Medicine, Medical School, Johannesburg 2001, South Africa), p. 425

The abundance of ferritin in yolk-sac derived red blood cells of the embryonic mouse. *Theil, E. C.* (Department of Biochemistry, North Carolina State University, Raleigh, N. C. 27607), p. 437

British Journal of Haematology (Oxford) **33** (1976) No. 4

Annotation. The treatment of plasma cell myeloma. *Bergsagel, D. E.* (Ontario Cancer Institute, Toronto, Canada M4X 1K9), p. 443

Proposals for the classification of the acute leukaemias. French-American-British (FAB) Co-operative Group. *Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R., Sultan, C.* (Reprint requests: Prof. D. A. G. Galton, MRC Leukaemia Unit, Royal Postgraduate Medical School, London W12 0HS, England), p. 451

T cell variant of chronic lymphocytic leukaemia with chromosome abnormality and defective response to mitogens. *Nowell, P., Jensen, J., Winger, L., Daniele, R., Growney, P.* (Departments of Pathology and Medicine, School of Medicine, Uni-

versity of Pennsylvania, Philadelphia, Pa. 19174), p. 459

Intercellular contacts between erythroid precursors in the bone marrow in dyserythropoiesis. *Frisch, B., Lewis, S. M., Swan, M.* (Reprint requests: Dr. S. M. Lewis, Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 469

Chelation studies with 2,3-dihydroxybenzoic acid in patients with β-thalassaemia major. *Peterson, C. M., Graziano, J. H., Grady, R. W., Jones, R. L., Vlassara, H. V., Canale, V. C., Miller, D. R., Cerami, A.* (Rockefeller University, New York, N. Y. 10021), p. 477

The use of Chang cells cultured *in vitro* to evaluate potential iron chelating drugs. *White, G. P., Jacobs, A., Grady, R. W., Cerami, A.* (Reprint requests: Prof. A. Jacobs, Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 487

The uptake of ferric iron by rat liver ferritin *in vivo* and *in vitro*. *Hoy, T. G., Harrison, P. M.* (Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 497

Iron mobilization in the pregnant rat. *Hershko, C., Cohen, H., Zajicek, G.* (Department of Haematology, Hadassah University Hospital, Jerusalem, Israel), p. 505

Effect of iron deficiency and desferrioxamine on DNA synthesis in human cells. *Hoffbrand, A. V., Ganeshaguru, K., Hooton, J. W. L., Tattersall, M. H. N.* (Department of Haematology, Royal Free Hospital, Hampstead, London NW3 2QG, England), p. 517

Thymidine kinase in megaloblastic anaemia. *Hooton, J. W. L., Hoffbrand, A. V.* (Department of Haematology, The Royal Free Hospital, Hampstead, London NW3 2QG, England), p. 527

HL-A groups in pernicious anaemia. *Chanarin, I., Knight, S., O'Brien, J., James, D.* (Section of Haematology, Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, England), p. 539

Factor V in an industrial population. *Borozović, M., Chakrabarti, R., Stirling, Y., Fenton, S., North, W. R. S., Meade, T. W.* (Reprint requests: Dr. T. W. Meade, MCR-DHSS Epidemiology and Medical Care Unit, Harrow, Middlesex HA1 3UJ, England), p. 543

Bleeding from self-administration of phenindione: a detailed case-study. *Gover, P. A., Ingram, G. I. C., Cork, M. S., Holland, L., Hopkins, R. P., Callaghan, P., Barkhan, P., Shearer, M. J.* (Reprint requests: Prof. G. I. C. Ingram, Department of Haematology, St. Thomas' Hospital, London SE1 7EH, England), p. 551

Acquired von Willebrand syndrome with inhibitors both to factor VIII clotting activity and ristocetin-induced platelet aggregation. *Stableforth, P., Tamagnini, G. L., Dormandy, K. M.* (Reprint requests: Dr. K. M. Dormandy, Haemophilia Centre, Royal Free Hospital, London NW3 2QG, England), p. 565

Sheehan's syndrome with complete bone marrow aplasia: Long-term results of substitution therapy with hormones. *Ferrari, E., Ascari, E., Bossolo, P. A., Barosi, G.* (First Medical Clinic, A. Ferrata, The University, Pavia, Italy), p. 575

Banked blood microfiltration. I. Microfilter composed of five polyurethane foam layers with graded pore size. *Guidoin, R. G., Gaylor, J. D. S., Borsanyi, J.-P.* (Laboratory of Experimental Surgery, Department of Surgery, University of Laval, Quebec City G1K7P4, Canada), p. 583

Banked blood microfiltration. II. Dacron-wool microfilter. *Guidoin, R. G., Gilchrist, T.* (Laboratory of Experimental Surgery, Department of Surgery, University of Laval, Quebec City G1K7P4, Canada), p. 591

British Journal of Haematology (Oxford) **34** (1976) No. 1

Annotation. Metabolic consequences of iron overload. *Jacobs, A.* (Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 1

Anti-Wr^b, and other autoantibodies responsible for positive direct antiglobulin tests in 150 individuals. *Issitt, P. D., Pavone, B. G., Goldfinger, D., Zwicker, H., Issitt, C. H., Tessel, J. A., Kroovand, S. W., Bell, C. A.* (Paul I. Hoxworth Blood Center, Univer-

sity of Cincinnati, Cincinnati, Ohio 45267), p. 5

Another example of an antibody reacting optimally with *p* red cells. *Issitt, C. H., Duckett, J. B., Osborne, B. M., Gut, J. B., Beasley, J.* (Paul I. Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio 45267), p. 19

Identification of haemoglobin S in red cells and normoblasts, using fluorescent anti-Hb S antibodies. *Papayannopoulou, T., McGuire, T. C., Lim, G., Garzel, E., Nute, P. E., Stamatoyannopoulos.* (Division of Hematology, Department of Medicine, University of Washington, Seattle, Wash. 98195), p. 25

Quantitation of ineffective erythropoiesis from the incorporation of [^{15}N] delta-aminolaevulinic acid and [^{15}N] glycine into early labelled bilirubin. I. Normal subjects. *Samson, D., Halliday, D., Nicholson, D. C., Chanarin, I.* (Reprint requests: Dr. I. Chanarin, Section of Haematology, M. R. C. Clinical Research Centre, Harrow, Middlesex HA1 3UJ, England), p. 33

Quantitation of ineffective erythropoiesis from the incorporation of [^{15}N] delta-aminolaevulinic acid and [^{15}N] glycine into early labelled bilirubin. II. Anaemic patients. *Samson, D., Halliday, D., Nicholson, D. C., Chanarin, I.* (Reprint requests: Dr. I. Chanarin, Section of Haematology, M. R. C. Clinical Research Centre, Harrow, Middlesex HA1 3UJ, England), p. 45

Unbalanced globin chain synthesis in erythroid precursor cells of heterozygous α-thalassaemia. *Steinberg, M. H., Coleman, M., Dreiling, B.* (VA Hospital, Jackson, Miss. 39216), p. 55

Simultaneous inheritance of mutant isoenzymes of erythrocyte pyruvate kinase associated with chronic haemolytic anaemia. *Paglia, D. E., Gray, G. R., Growe, G. H., Valentine, W. N.* (Division of Surgical Pathology, Center for the Health Sciences, University of California, Los Angeles. Calif. 90024), p. 61

A convenient and reproducible filtration technique for the determination of erythrocyte flexibility. *Baar, S.* (MRC Industrial Injuries and Burns Unit, Birmingham Accident Hospital, Birmingham B15 1NA, England), p. 69

Blood leucocyte enzymes. III. Diurnal rhythm of activity in isolated lymphocytes of normal subjects and chronic lymphatic leukaemia patients. *Ramot, B., Brok-Simoni, F., Chweidan, E., Ashkenazi, Y. E.* (Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel), p. 79

Primary leucocyte alkaline phosphatase deficiency in an adult with repeated infections. *Repine, J. E., Clawson, C. C., Brunning, R. D.* (Pulmonary Division, Department of Medicine, University of Minnesota, Minneapolis, Minn. 55455), p. 87

Defective neutrophil function in chronic granulocytic leukaemia. *El-Maalem, H., Fletcher, J.* (Reprint requests: Dr. J. Fletcher, City Hospital, Nottingham, England), p. 95

The cryopreservation of leukaemia cells: Morphological and functional changes. *Lowenthal, R. M., Park, D. S., Goldman, J. M., Th'ng, K. H., Hill, R. S., Whyte, G.* (Department of Medicine, Royal Hobart Hospital Clinical School, Hobart, Tasmania 7000, Australia), p. 105

Remission maintenance therapy for meningeal leukaemia: Intrathecal methotrexate and dexamethasone versus intrathecal craniospinal irradiation with a radiocolloid. *Muriel, F. S., Schere, D., Barengols, A., Eppinger-Helft, M., Braier, J. L., Pavlovsky, S., Macchi, G. H., Guman, L.* (Hospital de Niños, Servicio de Hematologia, Buenos Aires, Argentina), p. 119

Christmas disease (haemophilia B) in a girl with deletion of the short arm of one X-chromosone (functional Turner syndrome). *Spinelli, A., Schmid, W., Straub, P. W.* (Reprint requests: Prof. P. W. Straub, Department of Haematology, Kantonsspital, Zürich, Switzerland), p. 129

Evidence that collagen releases human platelet constituents by two different mechanisms. *Huzoor-Akbar, Ardlie, N. G.* (Reprint requests: Dr. N. G. Ardlie, Department of Clinical Science, The John Curtin School of Medical Research, Australian National University, Canberra A. C. T. 2601, Australia), p. 137

Different antiplatelet antibody specificities in immune thrombocytopenic purpura. *Donnell, R. L., McMillan, R., Yelenosky, R. J., Longmire, R. L., Lightsey, A. L.*

(Reprint requests: Dr. R. McMillan, Department of Hematology/Oncology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 147

British Journal of Haematology (Oxford) **34** (1976) No. 2

Adenosine deaminase and immune function. *Van der Weyden, M. B., Kelley, W. N.* (Department of Medicine, Monash University Medical School, Alfred Hospital, Melbourne 3181, Victoria, Australia), p. 159

Blast crisis of chronic myeloid leukaemia (CML). I. Presentation simulating acute lymphoid leukaemia (ALL). *Beard, M. E. J., Durrant, J., Catovsky, D., Wiltshaw, E., Amess, J. L., Brearley, R. L., Kirk, B., Wrigley, P. F. M., Jánossy, G., Greaves, M. F., Galton, D. A. G.* (Reprint requests: Prof. D. A. G. Galton, Medical Research Council Leukaemia Unit, Royal Postgraduate Medical School, London W.12 0HS, England), p. 167

Blast crisis of chronic myeloid leukaemia (CML). II. Cell surface marker analysis of "lymphoid" and myeloid cases. *Jánossy, G., Greaves, M. F., Révész, T., Lister, T. A., Roberts, M., Durrant, J., Kirk, B., Catovsky, D., Beard, M. E. J.* (ICRF Tumour Immunology Unit, Department of Zoology, University College London, London WC1E 6BT, England), p. 179

Alpha-thalassaemia trait in various racial groups in the United Kingdom: Characterization of a variant of alpha-thalassaemia in Indians. *Walford, D. M., Deacon, R.* (Department of Haematology, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, England), p. 193

Studies on the proportion and synthesis of haemoglobin G Philadelphia in red cells of heterozygotes, a homozygote, and a heterozygote for both haemoglobin G and α-thalassaemia. *Milner, P. F., Huisman, T. H. J.* (Sickle Cell Center, Medical College of Georgia, Augusta, Ga. 30902), p. 207

Iron uptake and ferritin synthesis by peripheral blood leucocytes from normal subjects and patients with iron deficiency and the anaemia of chronic disease. *Summers, M. R., Jacobs, A.* (Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 221

Chelate mediated transfer of iron from transferrin to desferrioxamine. *Pollack, S., Aisen, P., Lasky, F. D., Vanderhoff, G.* (Department of Medicine, Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 231

Iron absorption in the rat: The search for possible intestinal mucosal carriers. *Halliday, J. W., Powell, L. W., Mack, U.* (Department of Medicine, University of Queensland, Royal Brisbane Hospital, Brisbane, Australia), p. 237

Depressed phagocytic function exhibited by polymorphonuclear leucocytes from chronically iron deficient rabbits. *Likhite, V., Rodvien, R., Crosby, W. H. Jr.* (Harvard Medical School, Boston, Mass. 02115), p. 251

A comparison of platelet size, platelet count, and platelet ^{35}S incorporation as assays for thrombopoietin. *McDonald, T. P.* (University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 257

Activation of platelets by platelet activating factor (PAF) derived from IgE-sensitized basophils. IV. PAF does not activate platelet factor 3 (PF_3). *Henson, P. M., Landes, R. R.* (Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 269

Fragmentation haemolysis in patients with severe diabetic angiopathy. *Brunning, R. D., Jacob, H. S., Brenckman, W. D., Jimenez-Pasquau, F., Goetz, F. C.* (Reprint requests: Dr. H. S. Jacobs, University of Minnesota Hospitals, Minneapolis, Minn. 55455), p. 283

Binding of thrombin to human platelets and its possible significance. *Ganguly, P., Sonnichsen, W. J.* (Laboratory of Hematology, St Jude Children's Research Hospital, Memphis, Tenn. 38101), p. 291

Factor VIII and human platelet aggregation. I. Evidence that the platelet aggregating activity of bovine factor VIII is a property of its "carrier protein" subunit. *Bottecchia, D., Vermylen, J.* (Reprint requests: Dr. J. Vermylen, Academisch Ziekenhuis St Rafaël, B-3000 Leuven, Belgium), p. 303

Factor VIII and human platelet aggregation. II. Characteristics of aggregation of human platelets by bovine factor VIII. *Bottecchia, D., Vermylen, J.* (Reprint requests: Dr. J. Vermylen, Academisch Ziekenhuis St Rafaël, B-3000 Leuven, Belgium), p. 313

Factor VIII and human platelet aggregation. III. Further studies on aggregation of human platelets by neuraminidase-treated human factor VIII. *Vermylen, J., Bottecchia, D., Szpilman, H.* (Academisch Ziekenhuis St Rafaël, B-3000 Leuven, Belgium), p. 321

Factor VIII and human platelet aggregation. IV. Effect of proteolytic enzymes on platelet aggregation by factor VIII. *Vermylen, J., Bottecchia, D.* (Academisch Ziekenhuis St. Rafaël, B-3000 Leuven, Belgium), p. 331

Correspondence: PICA: A compulsion caused by iron deficiency. *Crosby, W. H.* (Division of Hematology/Oncology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 341

Immunoglobulin bound calcium and ultrafiltrable serum calcium in myeloma. *Soria, J., Soria, C., Dao, C., James, J.-M., Bousser, J., Pasquier-Bilski, G.* (Department of Haematology, Hôtel-Dieu, Parvis Notre-Dame, Paris 4, France), p. 343

British Journal of Haematology (Oxford) **34** (1976) No. 3

Plasma and whole blood viscosity. *Phillips, M. J., Harkness, J.* (Department of Haematology, Taunton and Somerset Hospital, Taunton, Somerset, England), p. 347

An epidemiological study of the haemostatic and other effects of oral contraceptives. *Meade, T. W., Brozović, M., Chakrabarti, R., Howarth, D. J., North, W. R. S., Stirling, Y.* (MRC-DHSS Epidemiology and Medical Care Unit, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, England), p. 353

Platelet density and size: the interpretation of heterogeneity. *Penington, D. G., Lee, N. L. Y., Roxburgh, A. E., McGready, J. R.* (University of Melbourne, Department of Medicine, St Vincent's Hospital, Melbourne 3065, Victoria, Australia), p. 365

Clinical experience with transfusion of cryopreserved platelets. *Schiffer, C. A., Aisner, J., Wiernik, P. H.* (Baltimore Cancer Research Center, University of Maryland Hospital, Baltimore, Md. 21201), p. 377

The utilization of a new strength citrate anticoagulant during centrifugal platelet pheresis. I. Assessment of donor effects. *Mishler, J. M., Janes, A. W., Lowes, B., Farfan, C., Emerson, P. M.* (Department of Haematology, Gibson Laboratories, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 387

Preparation and storage of platelet concentrates. I. Factors influencing the harvest of viable platelets from whole blood. *Slichter, S. J., Harker, L. A.* (Puget Sound Blood Center, Seattle, Wash. 98104), p. 395

Preparation and storage of platelet concentrates. II. Storage variables influencing platelet viability and function. *Slichter, S. J., Harker, L. A.* (Puget Sound Blood Center, Seattle, Wash. 98104), p. 403

Evidence for a splenic release factor of platelets in chronic blood loss plasma of rabbits. *Weintraub, A. H., Khan, I., Karpatkin*, S. (Reprint requests: Dr. S. Karpatkin, Department of Medicine, New York University Medical School, New York, N. Y. 10016), p. 421

Abnormal fibrin polymerization in liver disease. *Green, G., Thomson, J. M., Dymock, I. W., Poller, L.* (Reprint requests: Dr. L. Poller, WHO Collaborating Centre for Anticoagulant Control Reagents, Withington Hospital, Manchester M20 8LR, England), p. 427

An inhibitor of stem cell proliferation in normal bone marrow. *Lord, B. I., Mori, K. J., Wright, E. G., Lajtha, L. G.* (Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England), p. 441

Terminal deoxynucleotidyl transferase measurements in the differential diagnosis of adult leukaemias. *Hutton, J. J., Coleman, M. S.* (Medical Service, Veterans Administration Hospital, Lexington, Ky. 40507), p. 447

Identification of human mononuclear cells by anti-monocyte serum. *Stuart, A. E., Young, G. A., Grant, P. F.* (Department of Pathology, University Medical School, Edinburgh EH8 9AG, Scotland), p. 457

Quantitative studies of splenic erythropoiesis in polycythaemia vera and myelofibrosis. *Pettit, J. E., Lewis, S. M., Williams, E. D., Grafton, C. A., Bowring, C. S., Glass, H. I.* (Department of Haematology, Royal Free Hospital, London NW3 2QG, England), p. 465

Solubilization, partial purification and radioassay for the intrinsic factor receptor from the ileal mucosa. *Cotter, R., Rothenberg, S. P.* (Reprint requests: Dr. S. P. Rothenberg, Department of Medicine, New York Medical College, New York, N. Y. 10029), p. 477

Folic acid metabolism in normal, folate deficient and alcoholic man. *Lane, F., Goff, P., McGuffin, R., Eichner, E. R., Hillman, R. S.* (Reprint requests: Dr. R. S. Hillman, Health Sciences Learning Resources Center, University of Washington, Seattle, Wash. 98195), p. 489

Effect of pyridoxal 5'-phosphate on the oxygen affinity of human erythrocytes. *Maeda, N., Takahashi, K., Aono, K., Shiga, T.* (Second Department of Physiology, Ehime University, School of Medicine, Ehime, Japan 791-02), p. 501

Autoimmune haemolytic anaemia in children. *Zupańska, B., Ławkowicz, W., Górska, B., Kozłowska, J., Ochocka, M., Rokicka-Milewska, R., Derulska, D., Ciepielewska, D.* (Institute of Haematology, 00-791 Warsaw, Poland), p. 511

Experimental Hematology (Copenhagen) **4** (1976) No. 4

Competitive proliferation in the hematopoietic tissues of irradiated hybrid mice engrafted with parental bone marrow and spleen. *Muramatsu, S., Monnot, P., Duplan, J. F.* (National Institute of Radiological Sciences, Anagawa, Chiba-Shi, Japan), p. 188

The effect of thymectomy on the competitive potential of C57BL/6 bone marrow graft. *Volf, D., Kastelan, A., Dolejsková-Hauptfeld, V.* (Department of Animal Physiology, Faculty of Natural Sciences and Mathematics, University of Zagreb, 41000 Zagreb, Yugoslavia), p. 201

Effect of hemic cells on hybrid resistance. *Lemeneva, L. N., Chertkov, J. L.* (Reprint requests: Prof. J. L. Chertkov, Central Institute of Hematology and Blood Transfusion, Moscow A-167, USSR 125167), p. 209

Experience with second marrow transplants. *Wright, S. E., Thomas, E. D., Buckner, C. D., Clift, R. A., Fefer, A., Neiman, P. E., Storb, R.* (Division of Oncology (111), Veterans Administration Hospital, Salt Lake City, Utah 84113), p. 221

On the mechanism of erythropoietin-induced differentiation. XIV. The apparent effect of etiocholanolone on initiation of erythropoiesis. *Gross, M., Goldwasser, E.* (Department of Pathology, University of Chicago, Chicago, Ill. 60637), p. 227

The HLA linkage group. *van Rood, J. J., van Leeuwen, A., Termijtelen, A., Keuning, J. J.* (Department of Immunohaematology, Building 23, University Hospital, Leiden, The Netherlands), p. 237

Characteristics of B-lymphocyte colony growth *in vitro*. *Metcalf, D.* (Walter and Eliza Hall Institute, Royal Melbourne Hospital, Victoria, Australia), p. 243

Nonbursal origin of humoral immunity: Immune capacity and cytomorphological changes in chickens bursectomized as 52- to 64-hour-old embryos. *Janković, B. D., Isaković, K., Marković, B. M., Rajčević, M., Knežević, Z.* (Immunology Research Center, 1100 Belgrade, Yugoslavia), p. 246

Treatment of bone marrow aplasia by bone marrow graft after conditioning with antilymphocyte globulin. Long term results. *Mathé, G., Schwarzenberg, L.* (Institut de Cancérologie et d'Immunogénétique, Hôpital Paul Brousse, 948 00 Villejuif, France) p. 256

Experimental Hematology (Copenhagen) **4** (1976) No. 5

Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Friedenstein, A. J., Gorskaja, U. F., Kulagina, N. N.* (Immunomorphological Laboratory, N. F. Gamaleya Institute for Epidemiology and Microbiology, Academy of Medical Sciences, Moscow, USSR), p. 267

Thymus dependency of bone marrow stem cell proliferation in response to certain

antigens. *Frindel, E., Leuchars, E., Davies, A. J. S.* (Institut de Radiobiologie Clinique, Institut Gustave Roussy, 94800 Villejuif, France), p. 275

Techniques for induction of neutropenia and granulocytosis in rats. *Popovic, V., Schaffer, R., Popovic, P.* (Department of Physiology, Emory University School of Medicine, Atlanta, Ga. 30322), p. 285

The influence of steroids on erythropoiesis in mouse fetal liver cell cultures: Relevance of cell cycle state. *Dunn, C. D. R., Napier, J. A. F.* (University of Tennessee, Memorial Research Center, Knoxville, Tenn. 37920), p. 289

Effect of androgens on maturation and metabolism of erythroid tissue. *Molinari, P. F., Esber, H. J., Snyder, L. M.* (Division of Hematology/Oncology, Saint Vincent Hospital, Worcester, Mass. 01610), p. 301

Effects of radiation on hematopoietic stroma. *Fried, W., Chamberlin, W., Kedo, A., Barone, J.* (Division of Hematology, Michael Reese Medical Center, Chicago, Ill. 60616), p. 310

Bone marrow stem cells in high and low lines of Swiss mice. *Frindel, E., Lepault, F.* (Institut Gustave Roussy, 94800 Villejuif, France), p. 315

Time-lapse characterization of erythrocytic colony-forming cells in plasma cultures. *Cormack, D.* (Division of Histology, Department of Anatomy, University of Toronto, Toronto, Ont. M5S 1A8, Canada), p. 319

Folia Haematologica (Leipzig) **103** (1976) No. 2

Studies on the lymphocyte subpopulations in chronic lymphocytic leukaemia. *Hołowiecki J., Stella, B., Lutz, D., Krawczyk, M.* (Silesian Medical Academy, Institute of Internal Medicine, 40-029 Katowice, Poland), p. 153

The ultrastructure of formed white blood elements (neutrophils) in schizophrenia. *Naneishvili, B. R., Zurabashvili, Z. A.* (Institute of Psychiatry, Tbilisi, USSR), p. 160

Das Säure–Basen-Gleichgewicht im Verlauf lymphoblastischer Leukämien der Maus. *Schneiberg, K., Rytwiński, K., Bartnikowa, W.* (Institut für Onkologie, Gliwice, Polen), p. 166

Quantitative and morphological analysis of bone marrow associated with bone induction phenomenon in mice. *Włodarski, K., Jakóbisiak, M., Kossakowska, A. E.* (Department of Histology and Embryology, Institute of Biostructure, Medical School, 02-004 Warsaw, Poland), p. 177

Die zerebrale Verträglichkeit der "Meningosis-Prophylaxe" mit ^{198}Au-Radiogold an Hand von EEG-Befunden. *Metz, O., Häßler, A.* (Universitäts-Kinderklinik, DDR-69 Jena), p. 183

Das Vorkommen von Heinz-Körpern in den Erythrozyten von im niederen Dosisbereich strahlenexponiert arbeitenden Personen. *Jacobsohn, E., Arndt, D., Schüttmann, W., Konopatzky, H.* (Staatliches Amt für Atomsicherheit und Strahlenschutz der DDR, HA Strahlenschutzmedizin, DDR-1157 Berlin), p. 191

Degrees of impairment of cell-mediated immune reactivity in Hodgkin's disease. *Petrov, M.* (Institute of Haematology and Blood Transfusion, Sofia, 56 Bulgaria), p. 201

Zur Frage der Proliferation der Knochenmarkzellen beim Di-Guglielmo-Syndrom. *Urasiński, I.* (Hämatologische Klinik, Szczecin, Polen), p. 211

Changes in the blood parameters of an air-breathing fish during different respiratory conditions. *Singh, B. R., Thakur, R. N., Yadav, A. N.* (Postgraduate Department of Zoology, Bihar University, Muzaffarpur, Bihar, India), p. 216

Untersuchungen zur Morphologie des Blutes vom europäischen Aal *(Anguilla anguilla)*. I. Die Erythrozyten und ihre Entwicklungsstadien. *Kreutzmann, H.-L.* (Sektion Biologie der Universität Rostock, DDR-25 Rostock), p. 226

Untersuchungen über die "freien" Erythrozyten bei Neugeborenen. *Libich, M., Màjský, A.* (Bezirkstransfusionsstation, Most, ČSR), p. 236

Der Nachweis von HL-A-Antigenen an Thrombozyten unter verschiedenen Konditionen. *Bube, F. W., Seegers, D., Siebel, E., Heumann, H.* (Institut für Transfusionsmedizin, Krankenhaus Merheim, BRD-5 Köln 91), p. 240

The influence of azathioprine on the be-

haviour of blood platelets in vitro. *Bogdanikowa, B., Myśliwiec, M., Bielawiec, M., Kiersnowska-Rogowska, B.* (Institute of Internal Diseases, Medical Academy Białystok, Białystok, Poland), p. 248

Die Reaktivität des antikoagulatorischen Systems desympathisierter Tiere unter den Bedingungen eines durch akustische Reizung hervorgerufenen Streß. *Kudrjashov, B. A., Rodionow, I. M., Ljapina, L. A., Muchammedow, A.* (Laboratorium für Physiologie und Biochemie der Blutgerinnung, Moskauer Staatliche Universität Moskau W-234, UdSSR), p. 252

Failure of tranexamic acid to influence the ellagic acid-induced hypercoagulable state. *Girolami, A., Cella, G., Burul, A., Zucchetto, M.* (Istituto di Semeiotica Medica, Università di Padova, Padova, Italy), p. 261

Relations between fibrinogen degradation products and heparinocytes. *Frick, G., Frick, U.* (Klinik für Innere Medizin, Wilhelm-Pieck-Universität Rostock, DDR-25 Rostock), p. 271

Haemostasis (Basel) **5** (1976) No. 2

Further studies of the interaction between ristocetin and fibrinogen. *Goh, A. T. W., Firkin, B. G.* (Department of Medicine, Alfred Hospital, Monash University, Prahran, 3181 Victoria, Australia), p. 57

The appearance of schistocytes in the peripheral blood in correlation to the degree of disseminated intravascular coagulation. An experimental study in rats. *Heyes, H., Köhle, W., Slijepcevic, B.* (Universitäts-Frauenklinik, D-79 Ulm, BRD), p. 66

Haemostasis in the microvasculature of the rabbit mesentery: effects of some pharmacological agents of current interest in haemostasis and thrombosis. *Bergqvist, D., Arfors, K.-E.* (Reprint requests: Dr. K.-E. Arfors, Experimental Medicine, Pharmacia AB, S-751 25 Uppsala, Sweden), p. 74

Inhibition of platelet aggregation by acetylsalicylic acid and other inhibitors. *Seuter, F.* (Bayer AG, Institut für Pharmakologie, D-56 Wuppertal 1, BRD), p. 85

Adhesion of human blood platelets to glass and polymer surfaces. II. Demonstration of the presence of a natural platelet adhesion inhibitor in plasma and serum. *Mohammad Fazal, S., Hardison, M. D., Chuang, H. Y. K., Mason, R. G.* (Department of Pathology, The Memorial Hospital, Pawtucket, R. I. 02860), p. 96

Effect of Amadori-type sugar derivatives of poly-L-lysine on platelet aggregation. *Mester, M., Kraska, B., Crisba, J., Mester, L.* (Institut de Chimie des Substances Naturelles, CNRS, F.-91190 Gif-sur-Yvette, France), p. 115

Haemostasis (Basel) **5** (1976) No. 3

Induction of a labile chicken plasma coagulation factor by choline: Dose-response relations, specificity, and effects of protein synthesis inhibitors. *Sørbye, O.* (Departments of Physiology and Biochemistry, Dental Faculty, University of Oslo, Blindern, Oslo 3, Norway), p. 121

Sonography: A method for localization of hematomas in hemophiliacs. *Nowotny, C., Niessner, H., Thaler, E., Lechner, K.* (I. Medizinische Universitäts-Klinik, A-1097 Vienna, Austria), p. 129

Occurrence of C1 inactivator and other proteinase inhibitors in euglobulin fractions and their influence on fibrinolytic activity. *Kluft, C.* (Gaubius Institute, Health Organization TNO, Leiden, The Netherlands), p. 136

Pretreatment with heparin in thrombolytic therapy with urokinase. Heparin-urokinase therapy. *Bizzi, B., Leone, G., Accorrà, F.* (Department of Internal Medicine, Università Cattolica del Sacro Cuore, I-Roma, Italy), p. 147

Platelet-collagen interaction. The influence of native and modified collagen (type I) on the aggregation of human platelets. *Balleisen, L., Marx, R., Kühn, K.* (I. Medizinische Universitätsklinik, D-8000 München 2, BRD), p. 155

Stimulation of human platelet aggregation by phospholipase-A and a saline "extract" of a polyurethane. *Iatridis, S. G., Iatridis, P. G., Tsiala-Paraschou, E., Markidou, S. G., Ragatz, B. H.* (Department of Physiology, Indiana University School of Medicine, Gary, Ind. 46408), p. 165

Ionophore A 23187: Some effects on platelet

aggregation and clot retraction. *Bottecchia, D., Fantin, G., Gruppo, P., Nassuato, G.* (Istituto di Fisiologia Umana, Università di Padova, Padova, Italy), p. 176

Factor-VIII-related antigen and cold-insoluble globulin in leukemias and carcinomas. *Bruhn, H. D., Heimburger, N.* (I. Medizinische Universitäts-Klinik, Kiel, BRD), p. 189

Nouvelle Revue Française d'Hématologie (Blood Cells) (Paris) **16** (1976) No. 1

Les myélofibroses aiguës ou subaiguës. *Brière, J., Castro-Malaspina, H., Brière, J. F., Bernard, J.* (Clinique des Maladies du Sang, Hôpital Saint-Louis, F.-75475 Paris Cedex 10, France), p. 3

Un cas d'instabilité majeure de l'hémoglobine: L'hémoglobine Bicêtre. *Allard, C., Mohandas, N., Wajcman, H., Krisnnamoorthy, R.* (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, 94270 Kremlin-Bicêtre, France), p. 23

Inhibitory activity in human marrow plasma directed against mouse marrow cell proliferation: Reduced in subjects with decreased granulopoiesis. *Grammens, G. L. Brennan, J. K., Lichtman, M. A.* (Reprint requests: Dr. J. K. Brennan, 601 Elmwood Ave., Rochester, N. Y. 14642), p. 37

Comparaison du métabolisme de deux préparations de fer injectable (fer-sorbitol et fer-polymaltose) avec le métabolisme du fer transferrinique et du fer hémoglobinique. *Dresch, C., Boulard, M., Najean, Y.* (Centre de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, 75575 Paris Cedex 15, France), p. 47

Anémies réfractaires avec myéloblastose partielle. Analyse d'un protocole, groupant 79 cas. I. Caractères cliniques et évolution sous androgénothérapie. *Najean, Y., Pecking, A., Broquet, M.* (Centre de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, 75575 Paris Cedex 15, France), p. 67

Anémies réfractaires avec myéloblastose partielle. Analyse d'un protocole, portant sur 77 cas. II. Résultats de l'examen des biopsies médullaires. *Duhamel, G., Muratore, R., Bryon, P.* (No address), p. 81.

Nouvelle Revue Française d'Hématologie (Paris) **16** (1976) No. 2

Toxoplasmose et leucémie myéloïde chronique. *Beauvais, B., Garin, J. F., Larivière, M., Languillat, G.* (Laboratoire de Parasitologie-Mycologie, U.E.R. Lariboisière-Saint-Louis, F. 75270 Paris Cedex 06, France), p. 169

Maladie de Niemann-Pick de l'adulte suivie de la naissance à l'âge de 26 ans. Forme viscérale pure avec surcharge en sphingomyéline et déficit en sphingomyélinase. *Brière, J., Calman, F., Lageron, A., Hinglais, N., Emerit, J., Bernard, J.* (Clinique des Maladies du Sang, Hôpital Saint-Louis, F. 75475 Paris Cedex 10, France), p. 185

Transfusion de sang sélectionné sur base du phénotype HL-A. Estimation des possibilités offertes par une population de 2,380 donneurs. *Bouillenne, Cl., Leclercq, P., Brocteur, J., André, A.* (Laboratoire des Groupes Sanguins et de Transfusion, B-4020 Liège, Belgique), p. 203

L'immunisation anti-HL-A des polytransfusés en fonction de la composition antigénique des sangs transfusés. *Bouillenne, Cl., Leclercq, P., Brocteur, J., Servais, M., Bustin, J., André, A.* (Université de Liège, Laboratoire des Groupes Sanguins et de Transfusion, B-4020 Liège, Belgique), p. 209

The erythrocyte-granulocyte rosette. An immune cellular interaction found in some cases of autoimmune hemolytic anemias. *Marmont, A. M., Raffo, M.* (Division of Haematology and Clinical Immunology, Ospedale Generale Regionale, Genova, Italy), p. 221

Détergents et précipitation des lipoprotéines sériques par des immunsérums. *Burstein, M., Legmann, P.* (Centre National de Transfusion Sanguine, F.-75739 Paris Cedex 15, France), p. 229

The erythrocytic line in refractory anemia (preleukemia or hemopoietic dysplasia) and myelomonocytic leukemia. II. Some unusual morphologic variants of the polychromatic normoblasts and reticulocytes. *Maldonado, J. E., Mandon, P.* (Mayo Clinic, Rochester, Minn. 55901), p. 239

Comparaison en double aveugle de la vitamine E au placebo dans la prévention de

l'anémie de l'enfant de faible poids de naissance à la 7^E semaine de vie: Essai thérapeutique. *Fermanian, J., Salmon, D., Olive, G., Zambrowski, S., Rossier, A., Caldera, R.* (C. H. U. Pitié-Salpêtrière, F.-75634 Paris Cedex 13, France), p. 245

Volumes and cellularity in populations of hematopoietic clones. *Reincke, U., Cronkite, E. P. Hinkelmann, K.* (MRC, Brookhaven National Laboratory, Long Island, New York, N. Y. 11973), p. 255

Groupe française d'étude sur l'hémostase et la thrombose. Toulouse, 6 mars 1976, p. 273

History of hematology. How the mouse leukemia virus was discovered. A talk with Ludwik Gross. *Bessis, M.* (Institut de Pathologie Cellulaire, INSERM, Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France), p. 287

Revue Française de Transfusion et Immuno-Hématologie (Paris) **19** (1976) No. 1

Antigènes AB0 et enzymes. Introduction. *Salmon, Ch.* (Centre National de Transfusion Sanguine, 75571 Paris Cedex 12, France), p. 11

Classification of weak variants of blood-group A. *Moore, B. P. L.* (Canadian Red Cross Blood Transfusion Service, Toronto 5, Canada M4Y 1H6), p. 25

Étude quantitative et thermodynamique des phénotypes érythrocytaires "A faible". *Cartron, J. P.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex, 15 France), p. 35

L'hétérogénéité de la distribution cellulaire des antigènes A érythrocytaires. Étude au microscope électronique. *Gourdin, M.-F., Reyes, F., Lejonc, J. L., Mannoni, P., Breton-Gorius, J., Dreyfus, B.* (Unité de Recherches sur les Anémies INSERM-U. 91, Hôpital Henri Mondor, 94010 Créteil, France), p. 55

Étude de propriétés α-N-acétylgalactosaminyltransférasiques des sérums de sujets A et "A faible". *Cartron, J. P.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 67

Les phénotypes B faibles B_3, B_x, B_{el}. Classification pratique proposée. *Salmon, Ch.* (Centre National de Transfusion Sanguine, 75571 Paris Cedex 12, France), p. 89

Activités glycosyltransférasiques sériques associées à la biosynthèse des antigènes de groupes sanguins A et B. Application à l'étude de sujets B normaux et Cis AB. *Badet, J.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 105

Le groupe sanguin Cis AB. Étude quantitative et qualitative des antigènes ABH. *Lopez, M.* (Centre National de Transfusion Sanguine, Établissement Saint-Antoine, 75571 Paris Cedex 12, France), p. 117

L'antigène B acquis. *Gerbal, A., Ropars, C.* (Service d'Immunologie, Centre National de Transfusion Sanguine, 75015 Paris, France), p. 127

Le renforcement allélique. *Salmon, Ch., Cartron, J. P.* (Centre National de Transfusion Sanguine, Service d'Immunologie, 75012 Paris Cedex, France), p. 145

Deficient H phenotypes. *Hrubisko, M.* (Department of Haematology and Blood Transfusion, 80100 Bratislava, Czechoslovakia), p. 157

Heterogeneity of anti-H lectin. *Bird, G. W.G.* (Regional Blood Transfusion Centre, Birmingham B15 25G, England), p. 175

Antigénicité I des hématies Bombay. *Doinel, C.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 185

Aspects biochimiques des antigènes de groupes sanguins. *Ropars, C., Cartron, J. P.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 193

Distribution of the antigens A, D and c on red cell membranes. *Hughes-Jones, N. C., Romano, E.* (MRC Experimental Haematology Unit, St. Mary's Hospital, Paddington, London, England), p. 207

Biological and clinical significance of differences between RBC membrane (Rh) and non-membrane (ABH, MN, P) antigenic sites: Illegitimate AB0, M—N(T), P (Tj^a) antigens in malignancy. *Levine, Ph.* (Ortho Research Foundation, Laboratories of Immuno-Hematology, Raritan, N. J.), p. 213

Tn-polyagglutination. *Bird, G. W. G.* (Regional Blood Transfusion Centre, Birmingham B14 25G, England), p. 231

Polyagglutinabilité due à l'antigène Hempas.

Rochant, H., Gerbal, A. (Unité de Recherche sur les Anémies, U. 91, INSERM, Hôpital Henri Mondor, F.-9400 Créteil, France), p. 239

Mixtures, mosaics and modifications. *Bird, G. W. G.* (Regional Blood Transfusion Centre, Birmingham B15 25G, England), p. 247

Le système Atri. Système immunogénétique lymphocytaire associé au système de groupe AB0 et au système de sécrétion ABH. *Marcelli-Barge, A., Poirier, J. C., Benajam, A., Dausset, J.* (Laboratoire d'Immuno-Hématologie, Centre Hayem, Hôpital Saint-Louis, 75475 Paris Cedex 10 France), p. 253

Revue Française de Transfusion et Immuno-Hématologie (Paris) **19** (1976) No. 2

Nécrologie. Alain Gerbal. *Salmon, Ch.* (No address)

Élimination par la chaleur des fausses réactions positives lors de la détection de l'antigène Australie par les tests d'hémagglutination passive. *Tran-My-Hoang, Leplus, P.* (Centre de Secteur de Transfusion Sanguine, Hôpital Bicêtre, 94-Le Kremlin-Bicêtre, France), p. 285

A propos d'un nouveau cas d'auto-agglutination en milieu albumineux chez une donneuse de sang. *Guimbretière, J., Bignon, J. D., Guimbretière, L., Peyrat, M. A., Toscer, M.* (Centre Régional de Transfusion Sanguine, 44011 Nantes Cedex, France), p. 297

Virus de l'hépatite B et pathologie associée. *Chicot, D.* (Centre de Transfusion Sanguine du Groupe Hospitalier Pitié-Salpêtrière, 75634 Paris Cedex 13, France), p. 307

Le paludisme post-transfusionnel en France. *Ranque, J.* (Centre de Transfusion Sanguine, 13005 Marseille, France), p. 315

Expression clinique du paludisme transfusionnel. *Chataing, B.* (Centre de Transfusion Sanguine, 01700 Miribel, France), p. 327

Prévention du paludisme post-transfusionnel par séro-dépistage des porteurs latents de plasmodium parmi les donneurs de sang. *Ambroise-Thomas, P.* (Service de Parasitologie, Faculté de Médecine de Grenoble, 38700 La Tronche, France). p. 331

Résultats d'une enquête sur le paludisme post-transfusionnel en France entre 1960 et 1974. *Saleun, J. P., Baret, M., Dauriac, J.* (Centre Départemental de Transfusion Sanguine, 29200 Brest, France), p. 337

Essai de dépistage sérologique des porteurs de *Plasmodium* parmi les donneurs de sang du C. T. S. de Marseille. *Faugère, B., Ranque, J.* (Centre de Transfusion Sanguine, 13005 Marseille, France), p. 349

Essai de dépistage systématique du paludisme. Expérimentation sur 1,500 donneurs de sang. *Courtois, F., Farragi, M., Richard-Lenoble, D., Gentilini, M.* (Centres de Transfusion de l'Hôpital Louis Mourier de Colombes, La Pitié-Salpêtrière de Paris, 92700 Colombes, France), p. 357

Sérologie transfusionnelle du paludisme par un test d'immunofluorescence indirecte utilisant l'antigène *Plasmodium berghei. Gentilini, M., Richard-Lenoble, D., Reviron, J., Farragi, M.* (Centre Hospitalier Pitié-Salpêtrière, 75013 Paris, France), p. 363

A propos de deux observations de paludisme transfusionnel. Essai de prévention associant un test d'immunofluorescence indirecte aux critères de sélection clinique. *Besson, P., Robert, J. F., Reviron, J., Richard-Lenoble, D., Gentilini, M.* (Centre de Transfusion, Hôpital Saint-Louis, 75010 Paris, France), p. 369

A propos d'une anémie sur prothèse valvulaire aortique. *Jaulmes, B., Terrier, E., Bernadou, A.* (Centre de Transfusion de l'Hôpital Broussais, Paris, France), p. 375

Scandinavian Journal of Haematology (Copenhagen) **16** (1976) No. 5

Extremely long duration of chronic myeloid leukaemia with Ph^1 negative and Ph^1 positive bone marrow cells. *Brandt, L., Mitelman, F., Panani, A., Lenner, H. C.* (Department of Internal Medicine, University Hospital, S-221 85 Lund, Sweden), p. 321

A comparison of mice in rebound-thrombocytosis with platelet-hypertransfused mice for the assay of thrombopoietin. *McDonald, T. P., Clift, R., Nolan, C., Tribby, I. I. E.* (University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 326

Arabinosyl cytosine in chronic myeloid leu-

kaemia: Evidence for high cytokinetic sensitivity of myeloblasts. *Baccarani, M., Santucci, A. M., Tura, S., Killmann, S.-A.* (Servizio di Ematologia, Ospedale S. Orsola, 40138 Bologna, Italy), p. 335

Acute myeloid leukaemia complicating a breast cancer after mastectomy and radiotherapy. *Gardais J.* (Laboratoire d'Hématologie et d'Immuno-Pathologie, Centre Hospitalier Régional, F. 49036 Angers, France), p. 353

A colchicine-sensitivity test for leukaemic lymphocytes. *Schrek, R., Messmore, H. L., Knospe, W. H., Stefani, S. S.* (Veterans Administration Hospital, Hines, Ill. 60141) p. 357

G banding analysis of complex aneuploidy in a case of erythroleukaemia. *Philip, P.* (Department of Medicine A, Rigshospitalet, DK-2100 Copenhagen, Denmark), p. 365

Immunosuppressive treatment in haemophiliacs with inhibitors to factor VIII and factor IX. *Nilsson, I. M., Hedner, U.* (Coagulation Laboratory Allmänna Sjukhuset, 21401 Malmö, Sweden), p. 369

Streptokinase resistance test in a group of Mediterranean people and its possible variations as regards sex and age. *Aznar, J., Delgado, F., Estelles, A., Gilabert, J.* (Reprint requests: Dr. J. A. Lucea, Departamento de Biopatologia Clinica, Valencia, Spain), p. 383

A case of gamma heavy chain disease associated with autoimmune haemolytic anaemia: Clinical, haematological, immunological and pathological details. *Shirakura, T., Kobayashi, Y., Murai, Y., Inoue, T., Imamura, Y.* (Division of Hematology, Tokyo Metropolitan Geriatric Hospital, Itabashi-ku, Tokyo, Japan), p. 387

Circulating micromegakaryocytes signaling blast transformation of chronic leukaemia. *Udomratn, U., Steinberg, M. H., Dreiling, B. J., Lockhard, V.* (Reprint requests: Dr. M. H. Steinberg, V. A. Hospital, Jackson, Miss. 39216), p. 394

Scandinavian Journal of Haematology (Copenhagen) **17** (1976) No. 1

Platelet consumption and plasma concentration of platelet factor 4 (PF-4). *Gjesdal, K., Abrahamsen, A. F.* (Medical Department A, Aker Hospital, Oslo 5, Norway), p. 5

Familial polycythaemia vera: A study of 3 sisters. *Manoharan, A., Garson, O. M.* (Reprint requests: Dr. O. M. Garson, University of Melbourne, Department of Medicine, St. Vincent's Hospital, Fitzroy 3065, Victoria, Australia), p. 10

Chromosome banding studies in Philadelphia chromosome positive myeloid leukaemia. *Lawler, S. D., O'Malley, F., Lobb, D. S.* (Royal Marsden Hospital, London SW3 6JJ, England), p. 17

Heparin elimination in uraemic patients on haemo-dialysis. *Teien, A. N., Bjørnson, J.* (Medical Department A, Aker Sykehus, Oslo 5, Norway), p. 29

Restoration of defective cellular immunity by Levamisole in a patient with immunoblastic lymphadenopathy. *Ellegaard, J., Boesen, A. M.* (Medical Department II. Aarhus Amtssygehus, DK-8000 Aarhus C, Denmark), p. 36

Atypical intraerythrocyte pyruvate kinase in some haematological diseases. *Hanel, H. K., Philip, P.* (Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark), p. 44

Spontaneous and oxalate-induced radial segmentation of the nuclei of lymphocytes from peripheral blood from patients with chronic lymphocytic leukaemia. *Valmin, K.* (Clinic of Medicine, St. Sigfrid's Hospital, S-351 01 Växjö, Sweden), p. 53

The oxalate-induced radial segmentation of the nuclei in peripheral blood lymphocytes of different size. *Söderström, U.-B., Norberg, B., Brandt, L.* (Reprint requests: Dr. B. Norberg, Department of Internal Medicine, University Hospital, S-221 85 Lund, Sweden), p. 57

Neutrophil defect in multiple myeloma. Studies on intraneutrophilic lysozyme in multiple myeloma and malignant lymphoma. *Karle, H., Hansen, N. E., Plesner, T.* (Division of Haematology, Department of Medicine A, Rigshospitalet 9, DK-2100 Copenhagen, Denmark), p. 62

Preparation of concentrated platelet suspensions by dehydration against polyethyleneglycol 20,000. *Akkerman, J. W. N., Gorter, G., Sixma, J. J., Wester, J.* (Department of Haematology, University Hospital, Utrecht, The Netherlands), p. 71

Adrenaline: Further discussion of its role in mobilization of neutrophils. *Mishler, J. M., Sharp, A. A.* (Department of Haematology, Gibson Laboratories, Radcliffe Infirmary, Oxford, England), p. 78

Scandinavian Journal of Haematology (Copenhagen) **17** (1976) No. 2

The use of cytogenic abnormalities in bone marrow cells for mapping by gene dosage. *de la Chapelle, A.* (Folkhälsan Institute of Genetics, SF-00101 Helsinki 10, Finland), p. 81

Nonsecretory myelomatosis without intracellular immunoglobulin. Immunofluorescent and ultramicroscopic studies. *Stavem, P., Frøland, S. S., Haugen, H. P., Lislerud, A.* (Section of Haematology, Medical Department A, Rikshospitalet, Oslo, Norway), p. 89

Testicular function after radiotherapy to inverted "Y" field for malignant lymphoma. *Asbjørnsen, G., Molne, K., Klepp, O., Aakvaag, A.* (General Department, Norwegian Radium Hospital, Oslo, Norway), p. 96

Isochromosome 17 in a case of chronic erythroleukaemia. *Najfeld, V.* (General Medical Research, Veterans Administration Hospital, Seattle, Wash. 98108), p. 101

Autophagia in myeloid precursors. An explanation for neutropenia in Chediak-Higashi syndrome? *Oberling, Fr., Lang, J. M., Juif, J., Luckel, J. C.*, (Laboratoire de Microscopie Electronique, Centre de Transfusion Sanguine, 67000 Strasbourg, France), p. 105

Plasma iron and erythrocytic glutathione peroxidase activity. A possible mechanism for oxidative haemolysis in iron deficiency anaemia. *Cellerino, R., Guidi, G., Perona, G.* (Istituto di Patologia Medica II, Policlinico di Borgo Roma, 37100 Verona, Italy), p. 111

Effect of hyperlipidaemic diet and clofibrate treatment on rat erythrocyte lipids. *Pelliniemi, T.-T., Viikari, J., Nieminen, L.* (Department of Haematology, Turku University Hospital, SF-20520 Turku 51, Finland), p. 117

Polyploidy of the bone marrow. *Borgström, G. H., Vuopio, P., de la Chapelle, A.* (Folkhälsan Institute of Genetics, 00101 Helsinki 10, Finland), p. 123

Autoradiographic evidence for reutilization of DNA catabolites by granulocytopoiesis in the rat. *Gerecke, D., Gross, R.* (Medizinische Universitätsklinik Köln, D-5000 Köln 41, BRD), p. 132

Ultrastructural aspects of crystal-like inclusions in a case of IgA plasma cell proliferation. *Cordier, A. C., Scolari, L., Vaerman, J.-P., Dutrieux-Fauchet, M.-C., Dutrieux, C., Haumont, S.* (Department of Histology, University of Louvain, 1200 Brussels, Belgium), p. 143

Scandinavian Journal of Haematology (Copenhagen) **17** (1976) No. 3

The pathogenesis of spleen mediated phenomena in chronic myeloid leukaemia and agnogenic myeloid metaplasia: A non abscopal mechanism. *Adler, S. S.* (Section of Hematology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill. 60612), p. 153

The measurement of ^{59}Fe clearance from the plasma. *Cavill, I., Ricketts, C., Napier, J. A. F., Jacobs, A., Trevett, D., Bishop, R. D.* (Department of Haematology, Welsh National School of Medicine, University Hospital of Wales, Cardiff, Wales), p. 160

Thrombin-induced platelet prostaglandin and cyclic AMP production and a possible intrinsic modulation of platelet function. *Droller, M. J.* (Stanford University Medical Center, Stanford, Calif. 94305), p. 167

Erythrocyte enzymes in myelomatosis. *Hansen, O. P., Hanel, H. K.* (Frostjernevej 5, DK-2880 Bagsvaerd, Denmark), p. 179

Chromosome studies in untreated polycythaemia vera. *Westin, J., Wahlström, J., Swolin, B.* (Department of Medicine II, Sahlgren's Hospital, S-413 45 Göteborg, Sweden), p. 183

Chromosome abnormalities after chlorambucil therapy of polycythaemia vera. *Westin, J.* (Department of Medicine II, Sahlgren's Hospital, S-413 45 Göteborg, Sweden), p. 197

Platelet function and plasma free fatty acids during acute myocardial infarction and severe angina pectoris. *Gjesdal, K.* (Depart-

ment of Medicine, Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway), p. 205

The peripheral platelet count in response to intravenous infusion of isoprenaline. *Olsson, L.-B., Kutti, J., Lundborg, P., Fredén, K.* (Reprint requests: Dr. J. Kutti, Department of Medicine III, Sahlgren's Hospital, 411 33 Göteborg, Sweden), p. 213

Fasting (acute energy deprivation) in man: Effect on polymorphonuclear granulocyte functions, plasma iron and serum transferrin. *Palmblad, J.* (Laboratory for Clinical Stress Research, S-104 01 Stockholm, Sweden). p. 217

Bone marrow metastases from prostatic cancer-marked cytolytic effect after only a few days of treatment with diethylstilbestrol. *Bjørneklett, A., Stavem, P., Ly, B., Egeberg, O.* (Medical Department A, Rikshospitalet, Oslo, Norway), p. 227

Unclassified haemolytic anaemia with splenomegaly and erythrocyte cation abnormalities. A disease of the spleen? *Bernard, J. F., Bournier, O., Renoux, M., Charron, D., Boivin, P.* (Hôpital Beaujon, 92110 Clichy, France), p. 231

Seminars in Hematology (New York) **13** (1976) No. 2

Foreword. *Castle, W. B.* (22 Irving Street, Brookline, Mass. 02146), p. 87

Thomas Hale Ham, p. 91

Thomas Hale Ham, The Teacher. An appreciation. *Jaffé, E. R.* (Department of Medicine, Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 97

Hyperosmolality: A factor in ethanol-related platelet dysfunction? *Cowan, D. H., Graham, R. C. Jr., Shook, P.* (Department of Medicine, Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109), p. 103

Hepatocellular carcinoma and erythrocytosis. *Davidson, C. S.* (Clinical Research Center, Cambridge, Mass. 02142), p. 115

Scanning electron microscopy of erythrocyte deformation: The influence of a calcium ionophore, A23187. *White, J. G.* (Department of Pediatrics, University of Minnesota Medical Center, Minneapolis, Minn. 55455), p. 121

Effect of pyridoxine plus chronic phlebotomy on the function and morphology of bone marrow and liver in pyridoxine-responsive sideroblastic anemia. *Hines, J. D.* (Department of Medicine, Case Western Reserve University at Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109), p. 133

Criteria for the differentiation of dysfibrinogenemic states. *Ratnoff, O. D., Forman, W. B.* (Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106), p. 141

From man to molecule and back to mankind. *Castle, W. B.* (Veterans Administration Hospital, West Roxbury, Mass.), p. 159

Thrombosis and Haemostasis (Stuttgart) **35** (1976) No. 1

Editorial. The Journal Thrombosis and Haemostasis. *Biggs, R.* (Oxford Haemophilia Centre, Churchill Hospital, Oxford, England), p. 1

Introduction to the Vth Congress of the International Society on Thrombosis and Haemostasis held in Paris, July, 1975. *Caen, J. P.* (No address), p. 3

Professor J. P. Soulier, President of the Vth Congress of the International Society on Thrombosis and Haemostasis Paris 1975, p. 4. (No address)

What can be done and what is worth doing: Thoughts on limits and priorities. Third Shirley A. Johnson Memorial Lecture. *Born, G. V. R.* (Department of Pharmacology, University of Cambridge, Cambridge CB2 2QD, England), p. 6

Introduction for the First Wright-Schulte lecture before the Vth Congress of the International Society on Thrombosis and Haemostasis. *Wright, I. S.* (No address), p. 24

First Wright-Schulte lecture. Prophylaxis and treatment of thrombosis. Present state and future aspects. *Stormorken, H.* (Institute for Thrombosis Research, Rikshospitalet, Oslo 1, Norway), p. 26

Lipids as triggering factors in thrombosis. *Nordøy, A.* (Department of Medicine, Institute of Clinical Medicine, University of Tromsø, 9L2 Tromsø, Norway), p. 32

Clinical trials in thrombosis: Secondary prevention of myocardial infarction. *Klimt, C. R., Doub, P. H., Doub, N. H.* (University of Maryland, Division of Clinical Investigation, Baltimore, Md.), p. 49

Streptokinase and urokinase in the treatment of pulmonary thromboemboli. From a National Cooperative Study. *Bell, W. R.* (Department of Medicine, Division of Hematology, The Johns Hopkins University School of Medicine and Hospital, Baltimore, Md. 21205), p. 57

Inhibition of injury induced thromboatherosclerotic lesions by anti-platelet serum in rabbits. *Moore, S. Friedman, R. J., Singal, D. P., Gauldie, J., Blajchman, M. A., Roberts, R. S.* (Department of Pathology and Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Ontario, L8S 3J9, Canada), p. 70

Isolation and preliminary characterization of a vitamin K dependent peptide from human prothrombin. *Elion, J., Benarous, R., Labie, D.* (Institut de Pathologie Moléculaire, Paris 14e, France), p. 82

Evidence for an ester bond between thrombin and heparin cofactor. *Owen, W. G., Penick, G. D., Yoder, E., Poole, B. L.* (Department of Pathology, University of Iowa, Iowa City, Iowa 52242), p. 87

Biological control of factor VII. *Nemerson, Y.* (Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn. 06510), p. 96

Specificity of antisera to human fibrinopeptide A used in clinical fibrinopeptide A assays. *Nossel, H. L., Butler V. P., Wilner, G. D., Canfield, R. E., Harfenist, E. J.* (Departments of Medicine and Pathology, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 101

Immunologic studies in von Willebrand's disease. *Sultan, Y., Simeon, J., Maisonneuve, P., Caen, J. P.* (Laboratoire d'Hémostase et de Thrombose Expérimentale, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 110

The platelet-endothelial cell. VIII axis. *Nachman, R. L., Jaffe, E. A.* (Division of Hematology, Department of Medicine, The New York Hospital Cornell Medical Center, New York, N. Y.), p. 120

Platelet adhesion, release and aggregation in flowing blood: Effects of surface properties and platelet function. *Baumgartner, H. R., Muggli, R., Tschopp, T. B., Turitto, V. T.* (Department of Experimental Medicine, F. Hoffmann-La Roche and Co., Ltd., CH-4002 Basel, Switzerland), p. 124

Role of surface glycoproteins in human platelet function. *Nurden, A. T., Caen, J. P.* (Nuffield Institute of Comparative Medicine, The Zoological Society of London, London NW1 4RY, England), p. 139

In vitro and in vivo functions of thrombin-treated platelets. *Reimers, H.-J., Kinlough-Rathbone, R. L., Cazenave, J.-P., Senyi, A. F., Hirsh, J., Packham, M. A., Mustard, J. F.* (Department of Pathology, McMaster University, Hamilton, Ontario, Canada), p. 151

Comparison of two types of endothelial cells in long term culture. *Sedlak, B. J., Booyse, F. M., Bell, S., Rafelson, M. E. Jr.* (Department of Biological Sciences, Smith College, Northampton, Mass. 01060), p. 167

Studies on the thromboplastic effect of human plasma lipoproteins. *Sandberg, H., Andersson, L.-O.* (AB KABI, Research Department, Biochemistry, Fack S-104 25 Stockholm, Sweden), p. 178

Importance of protease inhibition in studies on purified factor VIII (antihaemophilic factor). *Beck, E. A., Bachmann, P., Barbier, P., Furlan, M.* (Central Haematology Laboratory, Inselspital, CH-3010 Berne, Switzerland), p. 186

The dissociation of factor VIII by reducing agents, high salt concentration and affinity chromatography. *Peake, I. R., Bloom, A. L.* (Department of Haematology, University Hospital of Wales, Cardiff, Wales), p. 191

The effects of Hepes buffer on clotting tests, assay of factors V and VIII and on the hydrolysis of esters by thrombin and thrombokinase. *Roberts, P. S., Hughes, H. N., Fleming, P. B.* (Division of Medical Oncology, Department of Medicine, Medical College of Virginia, Richmond, Va.), p. 202

Purification of human factor IX by chromatography of a coagulation factor concentrate. *Suomela, H.* (Finnish Red Cross Blood Transfusion Service, 00310 Helsinki 31, Finland), p. 211

Study of a proposed international standard for blood coagulation factor IX. *Brozović, M., Kirkwood, T. B. L., Robertson, I.* (National Institute for Biological Standards and Control, Hampstead, London NW3 6RB, England), p. 222

Effect of platelet antiserum on the precipitation of soluble fibrin by endotoxin. *Müller-Berghaus, G., Kramer, W.* (Department of Medicine, Justus-Liebig-Universität Gießen, D-63 Gießen, BRD), p. 237

Platelet aggregation by thimerosal. Role of ADP and SH groups. *Leone, G., Boni, P., Vincenti, A.* (Laboratory of Blood Coagulation, Department of Internal Medicine, Catholic University, Rome, Italy), p. 249

Report of the Subcommittee on Standards, 1975. *Johnson, A. J.* (Department of Medicine, New York University Medical Center, New York, N. Y. 10016), p. 258

Glycoproteins of mammalian platelet membranes. *Cierniewski, C., Krajewski, T., Wodzinowská, B.* (Department of Biochemistry, University of Lódż, Lódż, Poland), p. 264

Thrombosis and Haemostasis (Stuttgart) **35** (1976) No. 2

An appreciation. *Brinkhous, K. M.* (No address), p. 269

Cryoprecipitate and the haemophiliac. *Massie, R.* (No address), p. 272

The estimation of the number of binding sites for antibody on antigen molecules with reference to factor VIII and its antibody. *Pool, J., Biggs, R., Miller, R. G.* (Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford OX3 7LK, England), p. 274

Molecular variant of factor VII. *Briet, E., Loeliger, E. A., van Tilburg, N. H., Veltkamp, J. J.* (Hemostasis and Thrombosis Research Unit, University Hospital, Leiden, The Netherlands), p. 289

The effect of antithrombin III on the activity of the coagulation factors VII, IX and X. *Osterud, B., Miller-Andersson, M., Abildgaard, U., Prydz, H.* (Institute of Medical Biology, University of Tromsø, Tromsø, Norway), p. 295

Fibrinogen and its derivatives: Cofactors in the intrinsic generation of thrombin. *Triantaphyllopoulos, D. C., Ryan, L. T.* (American National Red Cross, Blood Research Laboratory, Bethesda, Md. 20014), p. 305

The clotting of a snake *(Crotalus viridis Helleri)* plasma and its interaction with various snake venoms. *Denson, K. E. W.* (Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford, England), p. 314

Studies on chemically modified fibrinogen. *Kloczewiak, M., Wegrzynowicz, Z., Mathias, F. R., Heene, D. L., Zajdel, M.* (Department of Medicine, Justus-Liebig-University, Gießen, FRG), p. 324

Enzymatic removal of ADP from plasma: Unaltered platelet adhesion but reduced aggregation on subendothelium and collagen fibrils. *Tschopp, Th. B., Baumgartner, H. R.* (Department of Experimental Medicine, F. Hoffmann-La Roche and Co., Ltd., CH-4002 Basel, Switzerland), p. 334

The young platelet population in children with cyanotic congenital heart disease. *Goldschmidt, B., Sørland, S. J.* (Second Department of Paediatrics, Semmelweis Medical University, H-1094 Budapest, Hungary), p. 342

Increased protein synthesis by human platelets during phagocytosis of latex particles in vitro. *Bessler, H., Agam, G., Djaldetti, M.* (Department of Medicine "B" and Hematology Clinic, Hasharon Hospital, Petah-Tiqva, Israel), p. 350

The effect of halogenate or halogenate free acid on human, rat and guinea pig platelet aggregation. *Minsker, D. H., Jordan, P. T., Kling, P., MacMillan, A., Hucker, H. B., Tocco, D. J.* (Merck Institute for Therapeutic Research, West Point, Pa. 19486), p. 358

Metabolic properties of human platelet membranes. II. Thrombin-induced phosphorylation of membrane lipids and demonstration of phosphorylating enzymes in the platelet membrane. *Kaulen, H. D., Gross, R.* (Medizinische Universitäts-Klinik, D-5000 Köln 41 — Lindenthal, BRD), p. 364

Treatment of amyloidosis associated factor X deficiency. *Spero, J. A., Lewis, J. H.,*

Hasiba, U., Ellis, L. D. (Department of Medicine, University of Pittsburgh, Pittsburgh, Pa. 15219), p. 377

Changes in human amniotic fluid fibrinolytic inhibitor levels during pregnancy. *Aznar, J., Hernandez, A., Parrilla, J. J., Estelles, A., Gilabert, J.* (Department of Clinical Pathology and Department of Obstetrics and Gynecology, Ciudad Sanitaria "La Fe", Valencia, Spain), p. 382

Various prothrombin complex concentrates and their effect on coagulation and fibrinolysis in vivo. *Hedner, U., Nilsson, I. M., Bergentz, S.-E.* (Coagulation Laboratory, University of Lund, Allmänna Sjukhuset, Malmö, Sweden), p. 386

The effect of beta adrenergic blockade upon exercise-induced changes in blood coagulation and fibrinolysis. *Britton, B. J., Wood, W. G., Smith, M., Hawkey, C., Irving, M. H.* (Surgical Unit, St. Bartholomews Hospital, London, E.C. 1, England), p. 396

The effects of therapy with oestriol succinate and ethinyl oestradiol on the haemostatic mechanism in post-menopausal women. *Davies, T., Fieldhouse, G., McNicol, G. P.* (University Department of Medicine, General Infirmary, Leeds LS1 3EX, England), p. 403

Blood coagulation and fibrinolysis in thyroid disease. *Farid, N. R., Griffiths, B. L., Collins, J. R., Marshall, W. H., Ingram, D. W.* (General Hospital, Newfoundland, Canada A1C 5S7), p. 415

Comparative sensitivity of two hemagglutination inhibition immunoassays for fibrinolytic degradation products in human serum. *Chi, D. S., Chen, J. P.* (University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 423

Studies on the action mechanism of the antihemostatic effect of iodopeptides. *Golub, A. L., Mende, T. J.* (Department of Biochemistry, University of Miami, School of Medicine, Miami, Fla. 33152), p. 437

Chemorheological studies of the effect of heparin on the course of coagulation. *Overholser, K. A., Baysinger, C. B., Harris, T. R., Deveau, T.* (Station B, Vanderbilt University, Nashville, Tenn. 37235), p. 447

Effects of endotoxin in cortisone-treated rabbits with a hereditary deficiency of the sixth component of complement (C6 deficiency). *Stafford, B. T., Rapaport, S. I., Shen, S. M.-C.* (Department of Medicine, University of Southern California, School of Medicine, Los Angeles, Calif.), p. 460

Report of the task force on heparin standardization. *Bangham, D. R.* (National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England), p. 472

Report of the task force on blood clotting zymogens and zymogen intermediates. *Jackson, C. M.* (Department of Biological Chemistry, School of Medicine, Washington University, St. Louis, Mo. 63110), p. 474

Report of the task force on standards of inhibitors of blood coagulation and fibrinolysis. *Nilsson, I. M.* (Coagulation Laboratory, Malmö General Hospital, Malmö, Sweden), p. 475

Report of the task force on standardization of thrombin and thrombin-like enzymes. *Aronson, D. L.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20034), p. 477

Report of the task force on preparation of factor Xa (bovine) PRP (ICTHR). *Jackson, C. M., Henriksen, R. A., Peng, Ch. W., Yin, E. T.* (Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Mo. 63110), p. 479

Report of the expert panel on oral anticoagulant control. *Ingram, G. I. C.* (Department of Haematology, St. Thomas' Hospital, London, England), p. 483

Heparin and ristocetin-induced platelet aggregation. *Pekçelen, Y., Inceman, S.* (Department of Hematology, Clinic of Internal Medicine, Istanbul Medical Faculty, Capa, Istanbul, Turkey), p. 485

Clinical use of factor IX concentrates. *Bidwell, E., Rizza, C. R., Dike, G. W. R., Snape, T. J.* (Oxford Haemophilia Centre, Churchill Hospital, Oxford OX3 7LK, England), p. 488

Re: Tranexamic acid and bleeding: a double-blind cross-over study on three brothers with Christmas disease (factor IX deficiency). *Beck, E. A.* (Central Haematology Laboratory, Inselspital, CH-3010 Bern, Switzerland), p. 492

Drugs with antithrombotic effect: Experimental study. *Coeugniet, E.* (Medical

Department TA, Laboratory of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark), p. 495

Thrombosis and Haemostasis (Stuttgart) **35** (1976) No. 3

Acquired "storage pool disease" of platelets. *Zahavi, J.* (Medical Department B, Ichilov Municipal Medical Center, Tel-Aviv, Israel), p. 501

Introduction to the Symposium held during the 3rd Meeting of the European and African Division of the International Society of Haematology in London 1975. *Biggs, R.* (Haemophilia Center, Oxford, England), p. 508

Management of haemophilia in Sweden. *Nilsson, I. M.* (Coagulation Laboratory, University of Lund, Allmänna Sjukhuset, Malmö, Sweden), p. 510

Management of haemophilia in Greece. *Mandalski, T.* (2nd Regional Blood Transfusion Centre, "Vassilefs Pavlos" Hospital, Athens, Greece), p. 522

Haemophilia care in Italy. *Mannucci, P. M., Ruggeri, Z. M.* (Hemophilia and Thrombosis Centre Angelo Bianchi Bonomi, 3rd Institute of Clinical Medicine, University of Milano, 20122 Milano, Italy), p. 531

Management of hemophilia in Spain. *Martin-Villar, J., Ortega, F., Magallon, M.* (Ciudad Sanitaria de la Seguridad Social "La Paz", Madrid, Spain), p. 537

Home care of hemophilia in West Germany. *Brackmann, H.-H., Hofmann, P., Etzel, F., Egli, H.* (Institut für Experimentelle Hämatologie und Bluttransfusionswesen, Universität Bonn, Bonn, BRD), p. 544

Management of hemophilia in France. *Allain, J.-P.* Centre pour Enfants Hémophiles, Croix-Rouge Française, 78940 La Queue les Yvelines, France), p. 553

The management of haemophilia in the United Kingdom. *Rizza, C. R.* (Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford, England), p. 559

Haemophilia in Australia. *Rickard, K. A.* (International Haemophilia Treatment and Training Centre, Royal Prince Alfred Hospital, Sydney N. S. W. 2050, Australia) p. 566

Hageman factor: Its structure and modes of activation. *Revak, S. D., Cochrane, C. G.* (Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 570

Purification and properties of human factor IX_a. *Váradi, K., Elődi, S.* (Reprint requests: Dr. S. Elődi, Department of Blood Coagulation, National Institute of Haematology and Blood Transfusion, 1113 Budapest, Hungary), p. 576

Four heparin preparations: Anti Xa potentiating effect of heparin after subcutaneous injection. *Johnson, E. A., Kirkwood, T. B. L., Stirling, Y., Perez-Requejo, J. L., Ingram, G. I. C., Bangham, D. R., Brozović, M.* (National Institute for Biological Standards and Control, Hampstead, N.W. 3, England), p. 586

On the value of the activated partial thromboplastin time (APTT) in monitoring heparin therapy. *Teien, A. N., Abildgaard, U.* (Medical Department A, Aker Hospital, Oslo, Norway), p. 592

Alteration of plasma antithrombin III levels in ischemic heart disease. *Yue, R. H., Gertler, M. M., Starr, T., Koutrouby, R.* (Cardiovascular Research Institute of Rehabilitation Medicine, New York University Medical Center, New York, N. Y. 10016), p. 598

The influence of the thyroid function on the metabolic rate of prothrombin, factor VII, and factor X in the rat. *van Oosterom, A. T., Mattie, H., Hermens, W. T., Veltkamp, J. J.* (Haemostasis and Thrombosis Research Unit, Division of Haematology and Division of Clinical Pharmacology, University Hospital, Leiden, The Netherlands), p. 607

Reactivity of fibrinogen crosslinking sites in the absence of thrombin. *Seelich, T., Furlan, M., Beck, E. A.* (Central Hematology Laboratory, Inselspital, CH-3010 Bern, Switzerland), p. 620

Fibrinogen/fibrin degradation products in serum of patients with idiopathic thrombocytopenic purpura: Elevated levels during severe thrombocytopenic phase of the disease. *Nagasawa, T., Kono, I., Sakurai, T., Kashiwagi, H.* (First Department of Internal Medicine, Tokyo Medical and Dental University School of Medicine, Tokyo 113, Japan), p. 628

Effect of thrombin on phosphorylation of

platelet membrane proteins. *Steiner, M.* (The Memorial Hospital, Pawtucket, R. I. 02860), p. 635

Plasmin inactivation in plasma. *Haselager, E. M., Goote, T. M., Vreeken, J.* (Department of Internal Medicine, Wilhelmina Gasthuis, Amsterdam, The Netherlands), p. 643

Biological properties of dialysable peptides derived from plasma digestion of bovine fibrinogen preparations. *Buczko, W., de Gaetano, G., Franco, R., Donati, M. B.* (Department of Pharmacology, Medical School, Białystok, Poland), p. 651

The variability of platelet-collagen interaction. Its influence on aspirin-induced inhibition of platelet aggregation. *Sack, E. S., Quiroga, E.* (Hematology Section and Fundacion Viviana Luckhaus, Hospital Juan A. Fernández, Buenos Aires, Argentina, South America), p. 658

On the measurement of spontaneous platelet aggregation. The platelet aggregation test III. Methods and first clinical results. *Breddin, K., Grun, H., Krzywanek, H. J., Schremmer, W. P.* (Department of Angiology, Center of Internal Medicine, University of Frankfurt a. M., FGR), p. 669

Fibrinogen response to turpentine and endotoxin in busulfan-treated rabbits. *Rapaport, S. I., Zivelin, A.* (Medical Service (111), V. A. Hospital, San Diego, Calif. 92161), p. 692

Spontaneous platelet aggregation in arterial insufficiency: Mechanisms and implications. *Wu, K. K., Hoak, J. C.* (Rush Presbyterian St. Luke's Medical Center, Chicago, Ill. 60612), p. 702

Oxygen consumption in platelets of newborn infants before and after stimulation by thrombin. *Del Principe, D., Mancuso, G., Menichelli, A., Maretto, G., Sabetta, G.* (Institute of Pediatrics, University of Rome Medical School, Rome, Italy), p. 712

Aggregation of platelets and inert particles induced by thrombin. *Chao, F. C., Tullis, J. L., Conneely, G. S., Lawler, J. W.* (Center for Blood Research, Department of Medicine, New England Deaconess Hospital, Boston, Mass.), p. 717

Effects of dextran on the molecular structure and tensile behaviour of human fibrin. *Dhall, T. Z., Bryce, A. J., Dhall, D. P.* (Reprint requests: Dr. D. P. Dhall, Department of Surgery, Woden Valley Hospital, Canberra A. C. T., Australia), p. 737

Report of the Working Party on Animal Models. *Dodds, W. J.* (Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. 12201), p. 746

Report of the task force on the clinical use of factor IX concentrates. *Ménaché, D.* (Service Central d'Immunologie et Hématologie, Hôpital Beaujon, 92-Clichy, France), p. 748

Therapeutic range with BCTR. *Barkhan, P.* (Guy's Hospital, London, SE1 9RT, England), p. 751

Interaction of ristocetin and von Willebrand factor. *Jenkins, C. S. P., Meyer, D., Larrieu, M. J.* (Institut de Pathologie Celluraire, Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France), p. 752

Thrombosis and Haemostasis (Stuttgart) **36** (1976) No. 1

The functional defects of hereditary dysfibrinogens. *Marder, V. J.* (Department of Medicine, Temple University Health Sciences Center, Philadelphia, Pa.), p. 1

Comparison of the actions of thrombin and the thrombin-like venom enzymes ancrod and batroxobin. *Aronson, D. L.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20014), p. 9

Molecular size distribution of fibrinogen derivatives formed *in vitro* and *in vivo*: a chromatographic study. *Donati, M. B., Verhaeghe, R., Culasso, D. E., Vermylen, J.* (Laboratory of Haemostasis and Thrombosis Research, "Mario Negri" Institute, 62-20157 Milano, Italy), p. 14

γ-Dimerization, α-polymerization, and plasmin degradation of human fibrin. Effect of various inhibitors of factor XIII on the patterns in SDS-electrophoresis and crossed immunoelectrophoresis. *Feddersen, J. C., Gormsen, J.* (Coagulation Laboratory, Sundby Hospital, DK-2300 Copenhagen S, Denmark), p. 27

Fibrin formation: The role of the fibrinogen-fibrin monomer complex. *Brass, E. P., Forman, W. B., Edwards, R. V., Lindan, O.* (Reprint request: Dr. W. B. Forman, Chemical Engineering Department, Hema-

tology/Oncology Section, Cleveland Veterans Administration Hospital, Case Western Reserve University, Cleveland, Ohio), p. 37

Analysis of the disappearance curve of labelled fibrinogen at the time of hyperfibrinogenemia in rabbits with acute or chronic intravascular coagulation. *Hasegawa, H., Watanabe, N., Nagata, H., Murao, M.* (First Department of Medicine, Hokkaido University School of Medicine, Hokkaido, Japan), p. 49

Scanning electron microscopic and electrophoretic studies on clotting factor adsorbents. *Deacon, S. L. M., Howell, R. M.* (Department of Biochemistry, Queen Elizabeth College, Campden Hill, London, W8 7AH, England), p. 60

The recovery of factor VIII from fresh-frozen indated and outdated human plasma. *Whitman, D. E., Switzer, M. E., McKee, P. A.* (Department of Medicine and Biochemistry, Duke University Medical Center, Durham, N. C. 27710), p. 71

Family studies of patients with reduced ristocetin aggregation and abnormalities of factor VIII and/or platelet function. *Ekert, H., Ananthakrishnan, R., Muntz, R. H., Dowling, S., D'Souza, S.* (Research Foundation, Royal Children's Hospital, Parkville, Victoria, 3052, Australia), p. 78

Failure of immunosuppression in a severe haemophilia B patient with specific antibody. *Allain, J.-P., Frommel, D.* (Centre pour Enfants Hémophiles, Croix-Rouge Française, F.-78940 La Queue-les-Yvelines, France), p. 86

Properties of human tissue thromboplastins from brain, lung, arteries and placenta. *Gonmori, H., Takeda, Y.* (Reprint requests: Dr. Y. Takeda, Department of Medicine, University of Colorado School of Medicine, Denver, Col. 80220), p. 90

The activation of human factor X in sodium citrate: the role of factor VII. *Aronson, D. L., Mustafa, A. J.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20014), p. 104

Bleeding in uremic patients after carbenicillin. *Andrassy, K., Weischedel, E., Ritz, E., Andrassy, T.* (Ludolf-Krehl-Klinik, 6900 Heidelberg, BRD), p. 115

Platelet adhesiveness and fibrinolysis after recent cerebro-vascular accidents and their relationship with subsequent deep venous thrombosis of the legs. *Warlow, C. P., Rennie, J. A. N., Ogston, D., Douglas, A. S.* (National Hospitals for Nervous Diseases, Maida Vale Hospital, London W. 9, England), p. 127

Microvascular haemostasis and the effect of local stimulation and inhibition of platelet function. An experimental study in rabbits. *Bergqvist, D., Arfors, K.-E.* (Reprint requests: Dr. K.-E. Arfors, Pharmacia AB, Experimental Medicine, S-75104 Uppsala 1, Sweden), p. 133

The effect of defibrinogenation on pulmonary embolism in dogs. *Meissner, A. G., O'Sullivan, G., Macbeth, R. A.* (Institute of Haematology, Department of Surgery, Warsaw, Poland), p. 140

Venous thrombosis in suspected pulmonary embolism: incidence detectable by Doppler ultrasound. *Barnes, R. W., Kinkead, L. R., Wu, K. K., Hoak, J. C.* (Division of Thoracic and Cardiovascular Surgery, University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242), p. 150

Low-dose heparin and deep-vein thrombosis after total hip replacement. *Mannucci, P. M., Citterio, L. E., Panajotopoulos, N.* (Via Pace 15, 20122 Milano, Italy), p. 157

Comparison between a modified ethanol gelation test and protamine sulfate test. Experimental studies. *Zielinsky, A., Altman, R., Rouvier, J.* (Departamento de Docencia e Investigación, U. A. M. I. Rivadavia, Peralta Ramos, Las Heras 2670, Buenos Aires, Argentina), p. 165

Inhibition of fibrinolysis in the burned-infected rat: Antiplasmin induced by burn wound infection. *Andes, W. A., Lindberg, R. B., McEuen, D. D., Baron, J. P.* (United States Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234), p. 173

Platelet preservation. II. The response of human platelet suspensions to hypotonic stress. *Odink, J.* (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 182

Platelet preservation. III. The influence of dimethylsulfoxide and cooling conditions or serotonin uptake velocity and response to hypotonic stress of human platelets. *Odink, J., Sprokholt, R.* (Central Labora-

tory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 192

Newborn platelet dysfunction: a storage pool and release defect. *Corby, D. G., Zuck, T. F.* (Clinical Investigation Service, Fitzsimons Army Medical Center, Denver, Col.), p. 200

The use of a chelating ion-exchange resin to evaluate the effects of the extracellular calcium concentration on adenosine diphosphate induced aggregation of human blood platelets. *Heptinstall, S.* (University Department of Medicine, General Hospital, Nottingham, NG1 6HA, England), p. 208

The effect of dimethyl sulfoxide on *in vitro* platelet function. *Schiffer, C. A., Whitaker, C. L., Schmukler, M., Aisner, J., Hilbert, S. L.* (Baltimore Cancer Research Center, University of Maryland Hospital, Baltimore, Md. 21201), p. 221

The prothrombin time test: Effect of varying citrate concentration. *Ingram, G. I. C., Hills, M.* (Haematology Department, St. Thomas' Hospital and Medical School, London SE1 7EH, England), p. 230

Reference method for the one-stage prothrombin time test on human blood. International Committee for Standardization in Hematology. *Ingram, G. I. C., Hills, M.* (Haematology Department, St. Thomas' Hospital and Medical School, London SE1 7EH, England), p. 237

Registry of prospective clinical trials. Second Report. *Verstraete, M.* (Department of Medical Research, K. U. L. Academisch Ziekenhuis, B. 3000 Leuven, Belgium), p. 239

General classification system for hemorrhagic and thrombotic disorders. *Lechner, K., Barrett, D. A., Veltkamp, J. J.* (I. Medizinische Universitätsklinik, Vienna, Austria), p. 251

Report of the Working Party on Platelets. *Day, H. J., Holmsen, H., Zucker, M. B.* (Specialized Center for Thrombosis Research, Temple University, Philadelphia, Pa. 19140), p. 263

A micro-technique for determining platelet aggregability in whole blood. *Sivertsen, U.* (Institute for Experimental Research in Surgery, University of Copenhagen, 2100 Copenhagen Ø, Denmark), p. 277

The effects of drugs on platelet function tests. *de Gaetano, D., Donati, M. B., Garattini, S.* (Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy), p. 281

Qualitative platelet abnormality due to absent collagen-induced aggregation. *Mielke, C. H., Jr., Rodvien, R.* (Institutes of Medical Sciences, Pacific Medical Center, San Francisco, Calif.), p. 283

A comparison of two methods for measurement of von Willebrand's factor (ristocetin cofactor). *Dowling, S. V., Ekert, H.* (Department of Haematology, Royal Children's Hospital, Parkville, Victoria 3052, Australia), p. 284

ADP, ristocetin, bovine factor VIII and thrombasthenic platelets. *Miragliotta, G., Remuzzi, G., Minetti, B., de Gaetano, G.* (Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche "Mario Negri", 62-20157 Milano, Italy), p. 286

Heparin and blood coagulation. *Sas, G., Pepper, D. S.* (Postgraduate Medical School, H-1135 Budapest, Hungary), p. 289

Platelet aggregation by thimerosal. *Caen, J. P.* (Hôpital Lariboisière, 75475 Paris Cedex 10, France), p. 292

Lymphocyte membrane modifications induced by acetylsalicylic acid and dipyridamole. *Coeugniet, E., Dirksen, A.* (Laboratory of Clinical Immunology, Medical Department TA, Rigshospitalet, University Hospital, Copenhagen, Denmark), p. 293

Detection and prevention of a fibrinolysis after an extracorporeal circulation in cardiac surgery. *Doutremepuich, C., Baudet, E., Chauve, A., Coqueran, J., Fontan, F.* (Laboratoire de Chirurgie Cardiaque, Hôpital du Tondu, Bordeaux, France), p. 296

Thrombosis Research (New York) **8** (1976) No. 3

On physiological microthromboembolization as the primary platelet function: Elimination of invaded particles from the circulation and its pathogenic significance. *Copley, A. L., Witte, S.* (Laboratory of

Biorheology, Polytechnic Institute of New York, Brooklyn, N. Y. 11201), p. 251

Pathologic cells as procoagulant substance of disseminated intravascular coagulation syndrome in acute promyelocytic leukemia. *Sakarugawa, N., Takahashi, K., Hoshiyama, M., Jimbo, C., Matsuoka, M., Onishi, Y.* (First Department of Internal Medicine, Niigata University School of Medicine, Niigata, Japan), p. 263

Properties of a partially purified preparation of a circulating plasminogen activator. *Ogston, D., Bennett, B., Mackie, M.* (Department of Medicine, University of Aberdeen, Aberdeen, Scotland), p. 275

Alterations of hemostasis associated with cardiopulmonary bypass. *Bick, R. L., Schmalhorst, W. R., Abregast, N. R.* (Bay Area Hematology/Oncology Medical Group, Santa Monica, Calif. 90404), p. 285

Kinetics of the formation of the factor X activating enzyme of the blood coagulation system. *Váradi, K., Hemker, H. C.* (Laboratory of Blood Coagulation, National Institute of Haematology and Blood Transfusion, H-1113 Budapest, Hungary), p. 303

Factor VIII (Willebrand) antigen and ristocetin-Willebrand factor in pigs with von Willebrand's disease. *Fass, D. N., Brockway, W. J., Owen, C. A. Jr., Bowie, E. J. W.* (Mayo Clinic and Mayo Foundation, Section of Hematology Research, Rochester, Minn. 55901), p. 319

Detection and measurement of low levels of prothrombin. Use of a procoagulant from *Echis carinatus* venom. *Franza, B. R. Jr., Aronson, D. L.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md.), p. 329

Purification of factor XII (Hageman factor) from human plasma. *Chan, J. Y. C., Movat, H. Z.* (Department of Pathology, University of Toronto, Toronto M5S 1A8, Canada), p. 337

Clot-inducing substances present in snake venoms with particular reference to *Echis carinatus*. *Denson, K. W. E.* (John Radcliffe Hospital, Oxford, England), p. 351

Suloctidil: A novel inhibitor of platelet aggregation in human beings. *de Gaetano, G., Miragliotta, G., Roncucci, R., Lansen, J., Lambelin, G.* (Laboratory for Haemostasis and Thrombosis Research, "Mario Negri" Institute for Pharmacological Research, 20157 Milan, Italy), p. 361

^{125}I labeling of fibrinogen by the chloramine-T method without protein denaturation. *Lazewatsky, J. L., Murray, B. W.* (Department of Nuclear Engineering, Massachusetts Institute of Technology, Cambridge, Mass. 02139), p. 373

Actin and myosin from blood platelets or muscle are potent aggregating agents. *Cohen, I., Kaminski, E., Glaser, T.* (Rogoff-Wellcome Medical Research Institute, Hemostasis Unit, Tel-Aviv University Medical School, Beilinson Medical Center, Petah Tikva, Israel), p. 383

The effect of inhibition of prostaglandin synthesis on microvascular leakage. *Arfors, K.-E., Arturson, G., Bergqvist, D., Sensjö, E.* (Department of Experimental Medicine, Pharmacia AB, Uppsala, Sweden), p. 393

The effect of leukopenia versus thrombocytopenia on endotoxin induced intravascular coagulation. *Lipinski, B., Gurewich, V.* (Vascular Laboratory, Lemuel Shattuck Hospital, Tufts University School of Medicine, Boston, Mass.), p. 403

Assay of heparin in plasma using a chromogenic substrate for activated factor X. *Teien, A. N., Lie, M., Abildgaard, U.* (Medical Department A, Aker Hospital, Oslo, Norway). p. 413

Inhibition of the platelet release reaction and platelet prostaglandin synthesis by furosemide. *Ingerman, C. M., Smith, J. B., Silver, M. J.* (Cardeza Foundation and Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pa. 19107), p. 417

Comments on the activation of Hageman factor (factor XII) by collagen. *Niewiarowski, S.* (Specialized Center for Thrombosis Research, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 421

Thrombosis Research (New York) **8** (1976) No. 4

Fibrinogen subunit degradation in vitro: Studies on human fibrinogen heterogeneity. *Scully, M. F., Lane, D. A., Thomas, D. P., Kakkar, V. V.* (Department of Surgery, King's College Hospital Medical School, London SE5 8RX, England), p. 427

Influence of time, temperature and platelet concentration on dog platelet aggregation. *Brackett, D. J., Schaefer, C. F., Gunn, C. G.* (Department of Medicine, University of Oklahoma, Health Sciences Center, Oklahoma City, Okla. 73190), p. 441

Subcellular distribution of fibrinogen and factor XIII in human blood platelets. *Lopaciuk, S., Lovette, K. M., McDonagh, J., Chuang, H. Y. K., McDonagh, R. P.* (Reprint requests: Dr. J. McDonagh, Department of Pathology, University of North Carolina, School of Medicine, Chapel Hill, N. C. 27514), p. 453

Adriamycin and fibrinolysis. *Bick, R. L., Fekete, L. F., Wilson, W. L.* (Bay Area Hematology/Oncology Medical Group, St. John's Hospital and Health Science Center, Santa Monica, Calif.), p. 467

The effect of D_2O and chlortetracycline on ADP-induced platelet shape change and aggregation. *Le Breton, G. C., Sandler, W. C., Feinberg, H.* (Department of Pharmacology, University of Illinois, College of Medicine, Chicago, Ill.), p. 477

Generation of a thrombin-like activity in late pregnancy. *van Royen, E. A., ten Cate, J. W.* (Department of Haematology, Wilhelmina Gasthuis, Amsterdam-West, The Netherlands), p. 487

Release of a cell growth promoting factor from human platelets. *Busch, C., Wasteson, Å., Westermark, B.* (Department of Pathology and Institute of Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden), p. 493

Induction of generalized Shwartzman reaction (GSR) in irradiated rabbits by a single injection of endotoxin. *Wronowski, T., Uchańska-Dudzińska, B., Teisseyre, E., Kopeć, M.* (Department of Radiobiology and Health Protection, Institute of Nuclear Research, Warsaw 03-195, Poland), p. 501

Inhibition of plasmin by normal and anti-plasmin-depleted human plasma. *Edy, J., De Cock, F., Collen, D.* (Laboratory of Blood Coagulation, Department of Medical Research, University of Leuven, Leuven, Belgium), p. 513

Prekallikrein and plasminogen proactivator: Absence of plasminogen proactivator in Fletcher factor deficient plasma. *Venneröd, A. M., Laake, K.* (Department of Pharmacology, Institute of Pharmacy, University of Oslo, Oslo, Norway), p. 519

Evidence that electrical and mechanical stimulation can induce reptilase-clot retraction. *Fantin, G. P., Bottecchia, D., Nassuato, G., Gruppo, P., Zatti, P.* (Istituto di Fisiologia Umana, Università di Padova, 35100 Padova, Italy), p. 523

Polylysine aggregation of human blood platelets. *Tiffany, M. L., Penner, J. A.* (Department of Internal Medicine, Simpson Memorial Institute, University of Michigan, Ann Arbor, Mich. 48109), p. 529

Thrombosis Research (New York) **8** (1976) No. 5

The dynamic inter-relationship between factor VIII and von Willebrand factor. *Koutts, J., Gude, N., Firkin, B. G.* (Monash University, Department of Medicine, Alfred Hospital, Prahran, 3181 Victoria, Australia), p. 533

Relationship of "new" vitamin K-dependent protein C and "old" autoprothrombin II-A. *Seegers, W. H., Novoa, E., Henry, R. L., Hassouna, H. I.* (Department of Physiology, Wayne State University, School of Medicine, Detroit, Mich.), p. 543

Experimental results to the deposition hypothesis of atherosclerosis. *Müller-Mohnssen, H.* (Institute of Biology, Department of Physiology, D-8042 Neuherberg/Munich, FRG), p. 553

Immunological characterization of early fibrinogen degradation products. Radioimmunoassay of fragments released from the A α-chain of fibrinogen. *Blombäck, M., Blombäck, B., Holmquist, H.* (Department of Blood Coagulation Research, Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden), p. 567

Double labeling of platelets with ^{51}chromium and ^{14}C-serotonin. *Rådegran, K.* (Surgical Research Laboratory, Thoracic Clinics, Karolinska Sjukhuset, 104 01 Stockholm 60, Sweden), p. 579

New evidence for the activation of factor XII by epinephrine. *Zubairov, D. M., Popova, L. G.* (Kazan Medical Institute, Kazan, USSR), p. 587

Influence of humoral shock factors on in vitro aggregation of dog platelets. *Bridenbaugh, G. A., Lefer, A. M.* (Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pa. 19107), p. 599

Interaction of fragments of fibrinogen with insolubilized fibrin monomers (activated fibrinogen). *York, L. L., Blombäck, B.* (Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden), p. 607

Thrombin-like enzymes of snake venoms: Actions on prothrombin. *Pirkle, H., Markland, F. S., Theodor, I.* (Department of Pathology, College of Medicine, University of California, Irvine, Calif. 92717), p. 619

"Fibrinolytic potential": The response to a 5 minute venous occlusion test. *Walker, I. D., Davidson, J. F., Hutton, I.* (Department of Haematology, Glasgow Royal Infirmary, Glasgow, Scotland), p. 629

Disulfide bridges in NH_2-terminal part of human fibrinogen. *Blombäck, B., Hesse B., Hogg, D.* (Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden), p. 639

Heparin precipitable fraction (HPF) from dermatological patients. II. Studies on the non-clottable proteins. Identification of cold insoluble globulin as the main non-clottable component. *Fyrand, O., Solum, N. O.* (Institute for Thrombosis Research, University of Oslo, Oslo, Norway), p. 659

The effects of ancrod anticoagulation on renal function in sheep. *Olivet, R. T., Kaye, M. P., Knox, F. G.* (Cardiovascular Surgical Research Laboratory, Mayo Clinic and Mayo Foundation, Rochester, Minn. 55901), p. 673

Probabilistic classification of hemophilia A carriers by discriminant analysis. *Elston, R. C., Graham, J. B., Miller, C. H. Reisner, H. M., Bouma, B. N.* (University of North Carolina, Chapel Hill, N. C. 27514), p. 683

Changes of prothrombin assayed by the method using *Echis carinatus* venom in experimental disseminated intravascular coagulation and a clinical case. *Sakuragawa, N., Takahashi, K., Hoshiyama, M., Jimbo, C., Niiya, K., Matsuoka, M., Ohnishi, Y.* (Department of Medicine, Niigata University School of Medicine, Niigita, Japan), p. 697

Platelet glycocalicin: A single receptor for platelet aggregation induced by thrombin or ristocetin. *Okumura, T., Jamieson, G. A.* (American National Red Cross, Blood Research Laboratory, Bethesda, Md. 20014), p. 701

Fibrin clot retraction by a rat rhabdomyosarcoma cell line. *Donati, M. B., Dolfini, E., Morasca, L., de Gaetano, G.* (Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy), p. 707

Two dimensional immunoelectrophoretic studies in anti-thrombin III deficiency. *Gomperts, E. D., Feesey, M., van der Walt, J. D.* (Department of Haematology, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa), p. 713

Platelet membrane glycoproteins: Alteration during storage of human platelet concentrates. *George, J. N.* (Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284), p. 719

The role of leukocytes and platelets in the precipitation of fibrin in vivo: Mechanisms of the generation of microclots from soluble fibrin. *Müller-Berghaus, G., Eckhardt, T., Kramer, W.* (Department of Medicine, Justus-Liebig-Universität, 63 Gießen, FRG), p. 725

Thrombosis Research (New York) **8** (1976) No. 6

A simplified method for isolation of the S-carboxymethyl derivative chains of normal fibrinogen and fibrinogen Cleveland I. *Forman, W. B., Haday, M.* (Hematology/Oncology Section, Veterans Administration Hospital, Cleveland, Ohio 44106), p. 731

Fibrinogen structure after spraying on mice: Assessing the electron microscopic basis for a trinodular model. *Mosesson, M. W.*

(Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 737

A new two-stage functional assay for antithrombin-III (heparin cofactor): Clinical and laboratory evaluation. *Bick, R. L., Kovacs, I., Fekete, L. F.* (Bay Area Hematology/Oncology Medical Group, Santa Monica, Calif.), p. 745

Importance of anticoagulant concentration for the activation of some human blood clotting factors. *Honorato, R., Novoa, E., Cicković, L., Ivanović, N.* (Laboratorio de Investigaciones Hematológicas, Departamento de Medicina Experimental, Universidad de Chile, Casilla 6539, Santiago 4, Chile), p. 757

Influence of glass and anticoagulant concentration on improvement of factor X deficiency tests. *Honorato, R., Ivanović, N., Novoa, E.* (Laboratorio de Investigaciones Hematológicas, Departamento de Medicina Experimental, Universidad de Chile, Casilla 6539, Santiago 4, Chile), p. 769

Selective destruction of the platelet aggregation component in bovine factor VIII preparations by reduction. *Brown, J. E., Baugh, R., Hougie, C.* (Department of Pathology, M-012, University of California, San Diego, La Jolla, Calif. 92093), p. 777

Effect of bovine high molecular weight kininogen and its fragments on Fitzgerald trait plasma. *Waldmann, R., Scicli, A. G., McKregor, R. K., Carretero, O. A., Abraham, J. P., Kato, H., Han, Y. N., Iwanaga, S.* (Department of Medicine, Henry Ford Hospital, Detroit, Mich.), p. 785

The effect of aspirin on in vivo platelet function in humans. *Fleischman, A. I., Bierenbaum, M. L., Stier, A.* (New Jersey State Department of Health, Montclair, N. J.), p. 797

Characterization of two direct fibrinogenolytic activities and of one proteolytic inhibitor activity in the human prostate. *Brassinne, C., Coune, A., Nijs, M., Tagnon, H. J.* (Service de Médecine et Laboratoire d'Investigation Clinique, Institut Jules Borde, Bruxelles, Belgium), p. 803

Phagocytic and catabolic function of the reticuloendothelial system in dogs subjected to defibrinogenation. *Ahlgren, T., Berghem, L., Lagergren, H., Lahnborg, G., Schildt, B.* (National Defence Research Institute, Department 4, Sundbyberg, Sweden), p. 819

On the proteolytic inhibitory effect of human fetal and neoplastic tissues. *Fornasari, P. M., Pandolfi, M., Åstedt, B.* (Department of Clinical Medicine Adolfo Ferrata, University of Pavia, Pavia, Italy), p. 829

New alternative scheme for human blood coagulation. *Honorato, R., Ivanović, N., Novoa, E.* (Laboratorio de Investigaciones Hematológicas, Departamento de Medicina Experimental, Universidad de Chile, Casilla 6539, Santiago 4, Chile), p. 837

Intravascular coagulation in hyperlipidemia. *Carvalho, A. G., Lees, R. S., Vaillancourt, R. A., Cabral, R. B., Weinberg, R. M., Colman, R. W.* (Coagulation Research Laboratory and Cardiac Unit, Massachusetts General Hospital, Boston, Mass. 02114), p. 843

The anticoagulant effect of heparin sulfate and dermatan sulfate. *Teien, A. N., Abildgaard, U., Höök, M.* (Medical Department A, Aker Hospital, Oslo, Norway), p. 859

Brief communication. Effect of Evans blue on blood platelets and microvascular hematostasis. *Görög, P., Kovács, I. B.* (EGYT Pharmacochemical Works, Department of Pharmacology, H-1475 Budapest, Hungary), p. 869

Immunochemical distinction between the inhibitors of plasminogen activation and antiplasmin in human plasma. *Hedner, U., Collen, D.* (Coagulation Laboratory, Allmänna Sjukhuset, Malmö, Sweden), p. 875

Thrombin adsorption to surfaces and prevention with polyethylene glycol 6,000. *Wasiewski, W., Fasco, M. J., Martin, B. M., Detwiler, T. C., Fenton, J. W. II.* (Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 881

Experimental studies on coagulation-fibrinolytic activity of white blood cells influenced by endotoxin. *Sakuragawa, N., Takahashi, K., Yoshiyama, M., Jimbo, C., Niiya, K., Matsuoka, M., Ohnishi, Y.* (Departments of Medicine and Pathology, Niigata University School of Medicine, Niigata, Japan), p. 887

Thrombosis Research (New York) **8** (1976) Suppl. No. II

Foreword. *Copley, A. L.* (Laboratory of Biorheology, Polytechnic Institute of New York, Brooklyn, N. Y. 11201), p. V

Viscosity and viscoelasticity of blood systems

Thixotropic parameters of whole human blood. *Huang, C. R., Fabisiak, W.* (Department of Chemical Engineering and Chemmistry, New Jersey Institute of Technology, Newark, N. J. 97102), p. 1

Hemorrheology in hyper- and hypoviscosity syndrome. *Ohshima, N.* (Institute of Basic Medicine, University of Tsukuba, Ibaraki, Japan), p. 9

Hemorrheological studies on the pathogenesis of diabetic microangiopathy. *Isogai, Y., Iida, A., Mochizuki, K., Abe, M.* (No address), p. 17

Relationship between fibrinogen and blood viscosity. *Matsuda, T., Murakami, M.* (Tokyo Metropolitan Institute of Gerontology, Department of Physiology, Tokyo, Japan), p. 25

Linear viscoelasticity of suspensions of spherical shell structures and erythrocyte membrane. *Sakanishi, A., Mitaku, S., Takano, Y.* (Department of Physics, Faculty of Science, University of Tokyo, Tokyo, Japan), p. 35

Dynamic viscoelasticity of fibrin gels: Dependence on ionic strength. *Kaibara, M., Fukada, E.* (Biopolymer Physics Laboratory, Institute of Physical and Chemical Research, Saitama 351, Japan), p. 45.

Rheological measurements of fibrin gels during clotting. *Fukada, E., Kaibara, M.* (Biopolymer Physics Laboratory, Institute of Physical and Chemical Research, Saitama 351, Japan), p. 49

Thrombin: Activation and inhibition

Activation of bovine prothrombin by an activator isolated from *Echis carinatus* venom. *Morita, T., Iwanaga, S., Suzuki, T.* (Division of Plasma Proteins, Institute for Protein Research, Osaka University, Osaka-565, Japan), p. 59.

A new series of synthetic thrombin-inhibitors (OM-inhibitors) having extremely potent and selective action. *Okamato, S., Hijikata, A., Ikezawa, K., Kinjo, K., Kikumoto, R., Tonomura, S., Tamao, Y.* (Department of Physiology, Kobe University School of Medicine, Kobe, Japan), p. 77

Fibrinolysis

Plasminogen activator releasing by the K^+ rich solution. *Izaki, S., Kitaguchi, H.* (Department of Physiology, Kobe University School of Medicine, Kobe, Japan), p. 91

Some aspects of the dose-response relation in urokinase administration. *Matsuo, O., Rokushima, Y.* (Department of Physiology, Miyazaki Medical College, Miyazaki, Japan), p. 97

Enhancement of fibrinolytic and thrombolytic potential in the rat by an anabolic steroid, furazabol. *Abiko, Y., Kumada, T.* (Research Institute and Toshihiko Kumada Research Institute, Daiichi Seiyaku Co. Ltd., Tokyo 132, Japan), p. 107

Defibrination syndrome as drastic deviation of coagulation and fibrinolysis balance and its therapeutic management. *Abe, T., Kazama, M.* (Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan), p. 115

Plasmatic clotting and/or blood cellular clumping in clinical conditions, experimental animals, and in perfused organs

Quantitation of von Willebrand factor and an antibody inhibitor. *Brinkhous, K. M., Culp, H. R.* (Department of Pathology, University of North Carolina, Chapel Hill, N. C.), p. 125

Change of the molecular mass of fibrinogen in circulating blood, as an index of the hypercoagulable states. *Kazama, M., Abe, T.* (Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan), p. 133.

The coagulation system during placental separation. *Soma, H.* (Tokyo Medical College, Department of Obstetrics and

Gynecology, Shinjuku, Tokyo, Japan), p. 143

In vitro perfusion of human reproductive organs. *Tojo, S., Fukunishi, H.* (Department of Obstetrics and Gynecology, Kobe University School of Medicine, Kobe 650, Japan), p. 149

Anticoagulant release during shock. *Maki, M., Nagayama, M., Oda, T., Shinagawa, S.* (Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, Hirosaki, Japan), p. 159

Integrated analysis of hemorrhagic-thrombotic tendency. *Yasunaga, K.* (Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan), p. 165

The effects of an alpha blocking agent on the respiratory distress syndrome with disseminated intravascular coagulation in the newborn infants and experimental animals. *Yamada, K., Shirahata, A., Meguro, T.* (Department of Pediatrics, Keio University Hospital and Research Project of Hematology, Ogikubo Hospital, Tokyo, Japan), p. 179

Clumping (reversible aggregation and irreversible agglutination) of blood cellular elements

Electrochemical interactions between erythrocyte surfaces. *Chien, S.* (Laboratory of Hemorrheology, Department of Physiology, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 189

Platelet retention in glass bead columns: Its mechanism and the effect of plasma proteins. *Tsukada, T.* (Department of Hematologic Research, Toranomon General Hospital, Tokyo, Japan), p. 203

Hyperaggregability of platelets in thromboembolic disorders. *Yamazaki, H., Sano, T., Asano, T., Hidaka, H.* (Third Department of Internal Medicine, Tokyo Medical and Dental University, School of Medicine, Yushima, Tokyo, Japan), p. 217

Role of blood platelet in the formation of thrombus. *Maekawa, T., Kobayashi, N., Arai, H., Takeuchi, T.* (Third Department of Internal Medicine, Gunma University School of Medicine, Maebashi, Japan 371), p. 227

Electrochemical features of platelet interactions. *Seaman, G. V. F.* (Department of Neurology, University of Oregon Health Science Center, Portland, Ore. 97201), p. 235

The blood vessel wall, hemorrheology and hemodynamics

Characteristic endothelial junctions in different segments of the vascular system. *Simionescu, M., Simionescu, N., Palade, G. E.* (Yale University School of Medicine, Section of Cell Biology, New Haven, Conn. 06510), p. 247

Structural-functional correlates in the transendothelial exchange of water-soluble macromolecules. *Simionescu, N., Simionescu, M., Palade, G. E.* (Yale University School of Medicine, Section of Cell Biology, New Haven, Conn. 06510), p. 257

A theoretical analysis of material transport across a capillary wall. *Murata, T.* (Department of Physics, Tokyo Metropolitan University, Setagaya-ku, Tokyo, Japan), p. 271

Endothelial damage and thrombosis: A scanning and transmission electron microscopic study. *Sumiyoshi, A., Tanaka, K.* (Department of Pathology, Faculty of Medicine, Kyushu University, Fukuoka, Japan), p. 277

A theoretical estimation of the plasma layer in arteriolar blood flow. *Matunobu, Y.* (Department of Physics, Keio University, Yokohama, Japan), p. 287

Studies on the orifice flow through stenotic vessels and mitral valve orifice. *Taniguchi, K.* (Second Department of Internal Medicine, School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan), p. 295

A theoretical approach to the effect of shear stress on the development of atheroma. *Oka, S.* (Department of Physiology, Kyorin University School of Medicine, Shinkawa, Mitaka, Tokyo, Japan), p. 305

Interaction of blood cells with vessel walls in microcirculation. *Fung, Y.-C. B.* (University of California, San Diego, Calif. 92037), p. 315

Thrombogenesis

Fibrinogen and fibrin formation. *Blombäck, B., Hogg, D. H., Gårdlund, B., Hessel, B., Kudryk, B.* (Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden), p. 329

Metabolic and kinetic study of thrombogenesis: Regulatory mechanism in early step of platelet plug formation. *Kuramoto, A., Taketomi, Y., Nakagawa, J., Uchino, H.* (Department of Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima 734, Japan), p. 347

Plasmatic arterionecrosis and its thrombotic occlusion. *Ooneda, G., Yoshida, Y., Suzuki, K., Shinkai, H.* (Second Department of Pathology, School of Medicine, Gunma University, Maebashi, Japan), p. 357

Rheological observation on the deformation of thrombus. *Kikuchi, A.* (Okaira, Memorial Tokyo Hitachi Hospital, Tokyo, Japan), p. 365

Disturbance of blood flow as a factor of thrombus formation. *Azuma, T., Fukushima, T.* (Department of Physiology, Shinshu University Medical School, Matsumoto, Japan), p. 375

New aspects of blood clotting and thrombosis: Investigations with the method of rheostimulation. *Hartert, H. H.* (Saar-Universität und Städtisches Krankenhaus, Kaiserslautern, BRD), p. 381

Polymolecular layers of fibrinogen systems and the genesis of thrombosis. *Copley, A. L., King, R. G.* (Laboratory of Biorheology, Department of Life Science and Bioengineering, Polytechnic Institute of New York, Brooklyn, N. Y. 11201), p. 393

Thrombosis Research (New York) **9** (1976) No. 1

Methods for correcting inhibitory effects of fibrinogen degradation products in fibrinogen determinations. *Alving, B. M., Bell, W. R.* (Division of Hematology, Department of Medicine, The Johns Hopkins Medical Institutions, Baltimore, Md.), p. 1

Radiolabeled ATIII as a probe for the detection of activation of blood coagulation in vivo. *Chandra, S., Bang, N. U., Marks, C.* (Lilly Laboratory for Clinical Research, Indiana University School of Medicine, Indianapolis, Ind.), p. 9

Fibrinogen Detroit. An abnormal fibrinogen with non-functional NH_2-terminal polymerization domain. *Kudryk, B., Blombäck, M.* (Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden), p. 25

Changes in plasma cold-insoluble globulin concentration during experimental Rocky Mountains spotted fever infection in Rhesus monkeys. *Mosher, D. F.* (United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. 21701), p. 37

Defibrinogenation treatment in patients suffering from severe intermittent claudication. A controlled study. *Martin, M., Hirdes, E., Auel, H.* (Aggertalklinik, Clinic for Vascular Diseases, Engelskirchen-Köln, FRG), p. 47

Technique and laboratory control of subcutaneously administered defibrase. *Martin, M., Auel, H.* (Aggertalklinik, Clinic for Vascular Diseases, Engelskirchen-Köln, FRG), p. 59

Intravenous arachidonate in the mouse: A model for the evaluation of antithrombotic drugs. *Kohler, C., Wooding, W., Ellenbogen, L.* (Cardiovascular-Renal Disease Therapy Research Section, Lederle Laboratories, Pearl River, N. Y. 10965), p. 67

Defibrinogenation as an alternative to heparinization during prolonged extracorporeal circulation in the dog. *Berglin, E., Hansson, H.-A., Teger-Nilsson, A.-C., William-Olsson, G.* (Departments of Thoracic Surgery, Clinical Chemistry, Internal Medicine II and Histology, University of Göteborg, Göteborg, Sweden), p. 81

Detection of thrombin-antithrombin III complex by crossed immunoelectrophoresis. *Sas, G., Pepper, D. S.* (Reprint requests: Dr. D. S. Pepper, S. E. Scotland Regional Blood Transfusion Centre, Royal Infirmary, Edinburgh, Scotland), p. 95

Transfusion (Philadelphia) **16** (1976) No. 2

Blood collection and use by AABB institutional members (1973). *Hemphill, B. M.*

(American Association of Blood Banks, San Francisco, Calif.), p. 91

Antigen site densities and ultrastructural distribution patterns of red cell Rh antigens. *Masouredis, S. P., Sudora, E. J., Mahan, L., Victoria, E. J.* (University Hospital Blood Bank, University of California, San Diego, Calif.), p. 94

Distribution of progesterone and its effect on human blood during storage. *DeVenuto, F., Wilson, S. M.* (Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 107

Studies of the recovery and the cost of low-glycerol cryopreserved human red blood cells. *Bowman, H. S., Oski, F. A., Reihart, J., Simmonds, M. A., Cunningham, R. K.* (Milton S. Hershey Medical Center, Harrisburg, Pa. 17101), p. 113

Effect of chilling on platelet cyclic adenosine 3 : 5-monophosphate and adenylate cyclase activity. *Zappia, G. C., Steiner, M., Ando, Y., Baldini, M.* (Division of Hematologic Research, Memorial Hospital, Pawtucket, R. I.), p. 122

Exchange transfusion in Reye's syndrome with saline-washed red blood cells. *Casper, J. T., Varma, R. R., Lewis, J. D., Harrington, G. J., Aster, R. H.* (Milwaukee Blood Center, Inc., Milwaukee, Wisc. 53233), p. 130

Fatal pulmonary hypersensitivity reaction to HL-A incompatible blood transfusion: Report of a case and review of the literature. *Wolf, C. F. W., Canale, V. C.* (Blood Bank, New York Hospital, New York, N. Y. 10021), p. 135

Hepatitis B virus and hepatitis B surface antigen in human albumin products. *Hoofnagle, J. H., Barker, L. F., Thiel, J., Gerety, R. J.* (Reprint requests: Dr. R. J. Gerety, Bureau of Biologics, FDA, Bethesda, Md. 20014), p. 141

The quality of over- and undercollected blood for transfusion. *Button, L. N., Orlina, A. R., Kevy, S. V., Josephson, A. M.* (Children's Hospital Medical Center, Boston, Mass. 02115), p. 148

Plasmapheresis-induced hemodilution and its effects on serum constituents. *Friedman, B. A., Schork, M. A., Alm. S. K., Jones, A. S., Oberman, H. A.* (University of Michigan Medical School, Ann Arbor, Mich. 48104), p. 155

Optimum centrifugation conditions for the preparation of platelet and plasma products. *Kahn, R. A., Cossette, I., Friedman, L. I.* (American National Red Cross, Blood Research Laboratory, Bethesda, Md. 20014), p. 162

Blood bank reagents: Some problems related to preservatives and dyes. *Howard, P. L.* (Department of Pathology, University of Vermont, Burlington, Vt. 05401), p. 166

The safety of dexamethasone and hydroxyethyl starch in the multiply leukapheresed donor. *Mishler, J. M., Moser, A. M., Carter, J. B.* (Medical Affairs Laboratory, McGaw Laboratories, Santa Ana, Calif. 92711), p. 170

Chloramphenicol antibody causing interference in antibody detection and identification tests. *Beattie, K. M., Ferguson, S. J., Burnie, K. L., Barr, R. M., Urbaniak, S. T., Atherton, P. J.* (Michigan Community Blood Center, Detroit, Mich. 48201), p. 174

Some components of the kinin system in Cohn's unheated human plasma proteins. *Morichi, S., Izaka, K.* (Central Laboratory, Green Cross Corporation, Osaka, Japan 534), p. 178

Donor and nondonor motivations. *Leibrecht, B. C., Hogan, J. M., Luz, G. A., Tobias, K. I.* (Biomedical Stress Department, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 182

Transfusion (Philadelphia) **16** (1976) No. 3

Single donor platelet transfusions: Scientific, legal and ethical considerations. *Wieckowicz, M.* (Puget Sound Blood Center, Seattle, Wash.), p. 193

Purification and use of the C3d subunit of C3. *Moore, J. A., Michael, J. M., Chaplin, H.* (Washington University School of Medicine, Department of Preventive Medicine, St. Louis, Mo. 63110), p. 200

Survival of transfused factor VIII in hemophilic patients treated with epsilon aminocaproic acid. *Weiss, A. E., Webster, W. P., Strike, L. E., Brinkhous, K. M.* (Coagulation Laboratory, Good Samaritan Hospital, Cincinnati, Ohio 45267), p. 209

The fate of bacteria in frozen red cells. *Kahn, R. A., Meryman, H. T., Syring, R. L., Flinton, L. J.* (American National Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 215

The acquisition of granulocytes by leukapheresis: A comparison of continuous flow centrifugation and filtration leukapheresis in normal and corticosteroid-stimulated donors. *MacPherson, J. L., Nusbacher, J., Bennett, J. M.* (Reprint requests: Dr. J. Nusbacher, Rochester Regional Red Cross Blood Program, Rochester, N. Y.), p. 221

Platelets and factor VIII as functions of blood collection time. *Reiss, R. F., Katz, A. J.* (Reprint requests: Dr. A. J. Katz, Connecticut Red Cross Blood Center Program, Farmington, Conn. 06032), p. 229

Increasing whole blood oxygen affinity during rapid exchange transfusion: a potential hazard. *White, J. M., White, Y. S., Buskard, N., Gillies, I. D. S.* (Kings College Hospital Medical School, London S. E. 4, England), p. 232

The prevalence of hepatitis-B antigen (HB_sAg) and its antibody (anti-HB_s) among London hemophiliacs and blood donors from London and two tropical areas. *Islam, M. N., Banatvala, J. E.* (Department of Virology, St. Thomas' Hospital and Medical School, London SE1 7EH, England), p. 237

Further evidence of heterogeneity within the Kidd blood group system. *Humphrey, A. J., Morel, P. A.* (Irwin Memorial Blood Bank, San Francisco Medical Society, San Francisco, Calif. 94118), p. 242

Autologous blood transfusion in coronary artery bypass surgery. *Cove, H., Matloff, J., Sacks, H. J., Sherbecoe, R., Goldfinger, D.* (Cedars-Sinai Medical Center, Los Angeles, Calif. 90029), p. 245

Adenine metabolism during and after exchange transfusions in newborn infants with CPD-adenine blood. *Kreuger, A.* (Department of Clinical Chemistry, Blood Center, University Hospital, Uppsala, Sweden), p. 249

The little red bucket. Temporary intramural blood storage. A simple inexpensive method. *Byerly, C. T. C., Klein, R. E.* (J. H. Thomas Memorial Blood Bank, Alachua General Hospital, Gainesville, Fla.), p. 253

Leukapheresis of patients with chronic granulocytic leukemia (CGL), using the Haemonetics blood processor. *Huestis, D. W., Price, M. J., White, R. F., Inman, M.* (University of Arizona College of Medicine, Tucson, Ariz. 85724), p. 255

Eluate analysis of anti-Bg^a associated renal allograft rejection. *Frist, S., Wenz, B.* (Department of Pathology, Montefiore Hospital and Medical Center, Bronx, N. Y. 10467), p. 261

Determination of Rh blood group of fetuses in abortions by suction curettage. *Greendyke, R. M., Banzhaf, J. C., LaFerla, J. J.* (Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.), p. 267

Blood. Legend laden liquid of life. *Myhre, B. A.* (Department of Pathology, UCLA School of Medicine, Los Angeles, Calif.), p. 270

Effect of prostaglandin E-1 upon microaggregate and fibrin formation in stored blood. *Harp, J. R., Marshall, B. E., Wurzel, H. A., Miller, A. S.* (Temple University Health Science Center, Philadelphia, Pa. 19140), p. 277

Transfusion (Philadelphia) **16** (1976) No. 4

Use of a low-ionic-strength medium in manual tests for antibody detection. *Moore, H. C., Mollison, P. L.* (Baptist Memorial Hospital, Memphis, Tenn. 38103), p. 291

The significance of red cell bound complement components in development of standards and quality assurance for the anti-complement components of antiglobulin sera. *Garratty, G., Petz, L. D.* (Hematology-Immunology Research Unit, Institutes of Medical Sciences, Pacific Medical Center, San Francisco, Calif. 94115), p. 297

Rhesus monkey platelet kinetics and storage. *Button, L. N., Jacobson, M. S., Kevy, S. V.* (Children's Hospital Medical Center, Boston, Mass. 02115), p. 307

Statewide support of thrombocytopenic patients with AB0 matched single donor platelets. *Reiss, R. F., Katz, A. J.* (Depart-

ment of Pathology, State University Hospital, Brooklyn, N. Y. 11203), p. 312

Frozen autologous platelets in the supportive care of patients with leukemia. *Schiffer, C. A., Buchholz, D. H., Aisner, J., Wolff, J. H., Wiernik, P. H.* (Section of Medical Oncology, National Cancer Institute, Baltimore Cancer Research Center, Baltimore, Md. 21201), p. 321

Fate in humans of the plasticizer, di-2-ethylhexyl phthalate, arising from transfusion of platelets stored in vinyl plastic bags. *Rubin, R. J., Schiffer, C. A.* (Department of Environmental Medicine, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md. 21205), p. 330

Ultrastructural damage of leukocytes procured by the Leukopak: Vulnerability of leukocytes to mechanical injury. *Ts'Ao, C., Ruder, E. A.* (Northwestern University School of Medicine, Chicago, Ill. 60611), p. 336

Preparation of antihemophilic factor from indated plasma. *Wickerhauser, M.* (American National Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 345

Anti-Rd (Radin) following the transfusion of a Radin positive unit of blood. *Winn, L. C., Eska, P. L., Grindon, A. J.* (Blood Bank, The Johns Hopkins Hospital, Baltimore, Md. 21205), p. 351

Additional studies concerning the metabolism of packed erythrocytes in CPD adenine. *Bensinger, T. A., Zuck, T. F.* (Blood Research Division, Department of Surgery, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 353

Delayed overt hemolytic transfusion reaction due to anti-U antibody. *Rothman, I. K., Alter, H. J., Strewler, G. J.* (Blood Bank Department, National Institutes of Health, Bethesda, Md. 20014), p. 357

Disseminated intravascular coagulation and blood component therapy. *Bick, R. L., Schmalhorst, W. R., Fekete, L.* (Bay Area Hematology/Oncology Medical Group, Santa Monica, Calif. 90404), p. 361

A walking blood donor program for seriously ill premature infants. *Hattersley, P. G., Goetzman, B. W., Gross, S., Blankenship, W. J.* (Sacramento Medical Center, University of California, Davis, Calif.), p. 366

Optimizing recovery of platelets in platelet rich plasma by the simple strategy. *Reiss, R. F., Katz, A. J.* (Connecticut Red Cross Blood Center, Farmington, Conn. 06032), p. 370

Motivation of voluntary plasmapheresis donors. *Cataldo, J. F., Cohen, E., Morganti, J. B.* (Reprint requests: Dr. E. Cohen, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 375

The maximum surgical blood order schedule and surgical blood use in the United States. *Friedman, B. A., Oberman, H. A., Chadwick, A. R., Kingdon, K. I.* (University of Michigan Medical School, University Hospital, Ann Arbor, Mich. 48104), p. 380

Vox Sanguinis (Basel) **31** (1976) No. 3

The HLA polymorphism and susceptibility to disease. *Mittal, K. K.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20014), p. 161

Cold lymphocytotoxins. Chromatographical study. *Mayer, S., Hauptmann, G., Tongio, M. M.* (Institut d'Hématologie, Hôpital Civil Strasbourg, Centre de Transfusion Sanguine, F-67000 Strasbourg, France), p. 174

Expression of genetic markers of erythrocyte immunoglobulin G autoantibodies in autoimmune hemolytic anemia. *Le Petit, J. C., Rivat, L., François, N., Ropartz, C., Brizard, C. P.* (Centre Départemental de Transfusion Sanguine, 42022 Saint-Étienne Cedex, France), p. 183

Gas chromatographic trace analysis of β-propiolactone in sterilized serum proteins. *Pruggmayer, D., Stephan, W.* (Battelle-Institut e. V., 6 Frankfurt/Main 90, BRD), p. 191

Physical and chemical changes of ACD-preserved blood: A comparison of blood in glass bottles and plastic bags. *Sasakawa, S., Tokunaga, E.* (Central Blood Center, Japanese Red Cross, Tokyo 150, Japan), p. 199

Variations of H, A and B antigen strengths among Melanesians. *Booth, P. B., Sturrock, S.* (Immunohaematology Department, Christchurch, New Zealand), p. 221

Hepatitis B antibody detection in solid-phase radioimmunoassay. *Artaz, C., Voyat, O., Rosso-Chioso, M.* (Centro Transfusionale–Maternità, I-11100 Aosta, Italy), p. 222

International Forum, p. 225

Vox Sanguinis (Basel) **31** (1976) No. 4

Preparation of red cells coated with C4 and C3 subcomponents and production of anti-C4d and anti-C3d. *Freedman, J., Mollison, P. L.* (MRC Experimental Haematology Unit, St. Mary's Hospital Medical School, London W2 1PG, England), p. 241

A study of the Ag factors in a British (West Midland) population. *Walton, K. W., Valente, A. J.* (Reprint requests: Dr. A. J. Valente, Department of Experimental Pathology, Rheumatism Research Wing, University of Birmingham, Birmingham B15 2TJ, England), p. 258

Agglutination of group A_x erythrocytes by anti-A sera (group B). *Mohn, J. F., Plunkett, R. W., Cunningham, R. K.* (Blood Group Research Unit, Department of Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, N. Y. 14214), p. 271

An unusual Rh immunization. *Leonard, G. L., Ellisor, S. S., Reid, M. E., Sanchez, P. D., Tippett, P.* (Reprint requests: Dr. S. S. Ellisor, Reference Laboratory, Central California Red Cross Blood Center, San José, Calif. 95110), p. 275

Studies on new process procedures in plasma fractionation on an industrial scale. I. Determination of the pH value of ethanol containing protein solutions at low temperatures. *Friedli, H., Fournier, E., Volk, T., Ingold, W., Meier, R., Kistler, P.* (Reprint requests: Dr. P. Kistler, Zentrallaboratorium, Blutspendedienst SRK, CH-3000 Bern, Switzerland), p. 277

Studies on new process procedures in plasma fractionation on an industrial scale. II. Experiments in concentrating dilute albumin solutions using hollow fibre ultrafiltration. *Friedli, H., Fournier, E., Volk, T., Kistler, P.* (Reprint requests: Dr. P. Kistler, Zentrallaboratorium, Blutspendedienst SRK, CH-3000 Bern, Switzerland), p. 283

Studies on new process procedures in plasma fractionation on an industrial scale. III. Removal of precipitates by filtration through a horizontal leaf filter with centrifugal cleaning (Funda filter), as an alternative to tubular-bowl centrifuges. *Friedli, H., Mauerhofer, M., Faes, A., Kistler, P.* (Reprint requests: Dr. P. Kistler, Zentrallaboratorium, Blutspendedienst SRK, CH-3000 Bern, Switzerland), p. 289

International Forum. Can a national all-voluntary blood transfusion service by adequate blood component therapy cover actual and future needs of AHF? p. 296

Vox Sanguinis (Basel) **31** (1976) No. 5

Electron microscopic observations on the H antigen sites of human erythrocytes using ferritin antibody conjugates. *Matsukura, Y.* (Department of Legal Medicine, Niigata University School of Medicine, Niigata, Japan), p. 321

Detection of an antigen in albumin subjected to repeated heating. *Cohen, P., Roelands, J. F.* (Bureau of Biologics, Food and Drug Administration, Bethesda, Md. 20014), p. 332

The rare blood group antigen, Wu. *Kornstad, L., Howell, P., Jørgensen, J., Larsen, A. M. H., Wadsworth, L. D.* (Statens Institutt for Folkehelse, Oslo 1, Norway), p. 337

The Vel blood group in Northern Sweden. *Cedergren, B., Giles, C. M., Ikin, E. W.* (Umeå Hospital Blood Bank, S-901 85 Umeå, Sweden), p. 344

Kx antigen, the McLeod phenotype, and chronic granulomatous disease: Further studies. *Marsh, W. L., Øyen, R., Nichols, M. E.* (Lindsley F. Kinball Research Institute of the New York Blood Center, New York, N. Y. 10021), p. 356

Km (1) [Inv(l)] typing of saliva and semen. *Davie, M. J., Kipps, A. E.* (Reprint requests: Dr. A. E. Kipps, Central Research Establishment, Berkshire, RG7 4PN, England), p. 363

Fresh blood — a myth or a real need? International Forum. No address, p. 368

The search for standard HLA reagents. *Albert, E., Curtoni, E. S., Dausset, J.,*

Engelfriet, C. P., Govaerts, A., Jeannet, M., Kissmeyer-Nielsen, F., van Rood, J. J., Seidl, S., Mayr, W. R., Tovey, G. H. (Kinderpoliklinik der Universität München, München, BRD), p. 380

Report of the Committee on Clinical Immunology of the International Union of Immunological Societies (IUIS). No address, p. 387

Comment on undegraded human 7S-immunoglobulin for intravenous use. *Wadsworth, C.H.* (Department of Immunology, Institute of Medical Microbiology and Department of Pediatrics, University of Göteborg, Göteborg, Sweden), p. 394

Comments on the Comment on Undegraded 7S Immunoglobulin for Intravenous Use by C. H. Wadsworth. *Stephan, W.* (Biotest-Serum-Institut GmbH, Frankfurt am Main, BRD), p. 397

Summarizing thoughts on "Comments" on the Comment. *Wadsworth, C. H.* (Department of Immunology, Institute of Medical Microbiology and Department of Pediatrics, University of Göteborg, Göteborg, Sweden), p. 399

MAGYAR
TUDOMÁNYOS AKADÉMIA
KÖNYVTÁRA

News Items

Lady Tata Memorial Trust

International Scholarships and Fellowships for Research on Leukaemia and Allied Conditions

Academic Year 1978–1979

The Trustees of the Lady Tata Memorial Trust invite applications for Fellowships and Scholarships for research on leukaemia, in the Academic Year beginning 1st October 1978. In view of the affinity between leukaemia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration. The Trustees specially wish to encourage studies of the leukaemogenic viruses in animals, the epidemiology and natural history of leukaemia, and the immunogenetic aspects.

The awards offered by the Trust are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad.

Fellowships are offered at rates appropriate to the age, seniority, and experience of the candidates, and to the Institution at which the work will be done. Fellowships are tenable for 3 years, with provision for annual increments, bench expenses and superannuation, and scholarships are tenable for 1 year in the first instance, renewable up to a maximum of 3 years.

Forms of application may be obtained by writing to Mrs Miglierina, Secretary for Continental Europe of the Lady Tata Memorial Trust, Laboratoire d'Immunochimie, Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, 2, place du Dr. Fournier, 75475 Paris Cedex 10, France. Applications must be submitted before March 31st, 1978 and awards will be announced by the Trustees in June.

Announcements

The 6th Jean Julliard Prize, which was established by the International Society of Blood Transfusion in memory of its first Secretary General, will be awarded during the XVth International Congress of Blood Transfusion to be held in Paris (France), from July 23rd to 29th, 1978.

The prize is reserved for investigators under 40 years of age in recognition of recently completed scientific work on blood transfusion and related subjects.

In order to qualify, candidates must forward six copies of an unpulished manuscript or recently published papers to the Secretary General, Dr. F. Josso – 6, rue Alexandre-Cabanel, 75739 Paris Cedex 15, France –, *before the 15th of April, 1978*.

The prize will be awarded during the Congress. The value of the prize is 3 000 Swiss francs.

Further information shall be requested from the Secretary General.

*

The XXIVth annual meeting of the International Committee on Thrombosis and Haemostasis will be held at the University of Leuven, Belgium, on July 20th-22th, 1978.

The different subcommittees will meet on Thursday, July 20 (morning and afternoon) and on Saturday, July 22.

Thursday evening and Friday morning are devoted to plenary sessions on the design of clinical trials in thrombosis.

All meetings are open and interested physicians and scientists are welcome.

Further information and the registration form can be obtained from the office of the Secretary General of the I. C. T. H., Dr. K. M. Brinkhous (Preclinical Educational Building, 228-H, Chapel Hill, N. C. 27514, U. S. A.) or by contacting Dr. M. Verstraete and Dr. J. Vermylen, Center for Thrombosis and Vascular Research, Department of Medical Research, Campus Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium.

Card Indexes

G. Astaldi, G. P. Bagnara, M. A. Brunelli, D. Karanovic, J. Karanovic, R. Scorza, Ü. Topuz

Experimental and clinical investigations on stem cell take and colony formation. Haematologia *11*, 11 (1977).

First, lymphocyte transplantation into total body-irradiated rats is discussed. The effect on spleen colony formation caused by the transplantation of untreated lymphocytes, as well as of lymphocytes previously incubated with PHA, or with PHA plus L-asparaginase, or with lymphokines, was studied. Then the effect of the urinary colony-stimulating factor *in vitro*, and the *in vitro* feeder-layer activity of leucocytes on colony formation of human and mice bone marrow cells in haematological diseases are dealt with. The injection of rat lymphocytes previously incubated for 24 hours with PHA resulted in a higher number and a larger size of colonies in the spleen of the recipient rats. Lymphocytes preincubated with lymphokines gave rise to the formation of spleen colonies which were larger than those developing after the injection of untreated lymphocytes. When the lymphocytes were previously incubated with PHA plus L-asparaginase, PHA failed to stimulate colony formation in the spleen. The phenomenon is explained by assuming that PHA, as an aspecific stimulator of cell division, initiated the division of CFUs, thus the CFUs content of the preincubated samples increased, resulting in an increase in the number of colonies formed after the transplantation of lymphocytes pretreated with PHA. Another possible explanation is that CFUs division, or their spleen take is enhanced by the immunocompetent lymphocytes activated by PHA, either directly or via soluble mediators produced or released by immunocompetent lymphocytes such as lymphokines. The study of colony-forming cells and colony-stimulating activity in primary myelofibrosis showed an increase in the number of circulating CFUc in this condition, and an abnormal density of these cells reaching a peak below 1.062. The lowering of CSA in the first two peripheral blood gradient fractions agrees with the observation in the same fractions of a high percentage of CFUc at the expense of the CSC population. Thus, double cell population seems to exist in PM. One is greatly abnormal with a low specific density and high plating efficiency, whereas the other population is almost normal, showing a higher specific density and a lower plating efficiency.

A. Mód, V. Harsányi, M. Jakschitz, S. R. Hollán

The treatment of infections in granulocytopenic patients with granulocyte transfusions. Haematologia *11*, 31 (1977).

Twenty-three severely neutropenic patients were given 31 courses of AB0 and HL-A-compatible granulocyte-rich plasma transfusions between 1974—76 at the National Institute of Haematology and Blood Transfusion. The criteria for the effectiveness of therapy were disappearance of fever and survival for over 3 weeks after granulocyte transfusion. Of the 28 evaluated cases granulocyte transfusions were effective in 16 and ineffective in 12 cases. The effectiveness of granulocyte transfusion was favourably influenced by early introduction of therapy and continuation for several days.

K. Šulc, J. Neuwirt, T. Trávníček, P. Kobylka, E. Radikovská

Bone marrow cell separation on Ficoll gradient. Haematologia *11*, *41* (1977).

Cell fraction from rat and murine bone marrow were obtained by sedimentation at unit gravity. A linear gradient of polymerized sucrose (Ficoll) was used and some properties of individual cell fractions were studied. The 3rd cell fraction containing the lymphoblasts and lymphocytes contained also stem cells.

T. Fleischmann, F. Krizsa

Chromosomes in malignant lymphomas (Study on short-term lymph node cultures). Haematologia *11*, 47 (1977).

In 15 cases, chromosome studies were performed in short-term lymph node cultures, using the banding technique. Clonal aberrations were found in two cases of Hodgkin's disease and in two of lymphosarcoma. In agreement with earlier observations, the cases of lymphosarcoma with abnormal karyotypes proved hyperdiploid. No chromosomal aberrations were found in three cases of reactive lymphoreticular hyperplasia.

N. F. Klimova

Ten years experience with Myelobromol treatment of myelofibrosis. Haematologia *11*, 57 (1977).

The data of 65 patients treated with Myelobromol are reported. Myelobromol is an effective drug against the steadily progressing splenomegaly and hepatomegaly followed by abdominal discomfort, thrombocythaemia and polyglobulia with enlarged spleen and liver. The daily dose was 250 mg for two or four weeks, then 125 mg for another two weeks. The drug exerted a slow cytotoxic effect on myelopoiesis, thrombopoiesis and erythrocytopoiesis, and also on fibrosis. After 15 days treatment leucopoiesis and thrombopoiesis were depressed temporarily. In eight cases pancytopenia resulted. The patients were given prednisone and androgen therapy and were treated with blood transfusions. The effect was positive in all the eight cases. There were no toxic side effects. As no drug resistance developed, treatment could be repeated later. The remission achieved after the first treatment lasted 15 months on the average. After repeated administration it lasted 7—10 months.

J. Amir, W. B. Forman, A. Rassiga, G. M. Bernier

Nonsynthetizing multiple myeloma. Haematologia *11*, 73 (1977).

A case of multiple myeloma with no monoclonal protein synthesis is reported. The patient had hypogammaglobulinemia, bone marrow invasion, osteolytic lesions, and plasma cell tumors. The absence of protein synthesis has been demonstrated in bone marrow culture with tritiated leucine. No evidence of immunoglobulin production was found. Bone marrow or plasma cell tumor biopsy may be the only method of diagnosis in such cases.

M. Tichý, Z. Hrnčíř, J. Šimša

Serum levels of IgA, IgM, IgD and IgE in IgG paraproteinaemias. Haematologia *11*, 79 (1977).

The dependence of serum levels of polyclonal IgA, IgM, IgD and IgE on the amount of IgG paraprotein was studied in a series of 58 IgG paraproteinaemias. A significant correlation was found for IgA ($r = -0.4782$, $p = 0.0007$), for IgM ($r = -0.3296$, $p = 0.0237$) and for IgD ($r = -0.3589$, $p = 0.0143$) in the whole series of IgG paraproteinaemias and in the subgroup of myeloma paraproteinaemias ($n = 34$) for IgM ($r = -0.4276$, $p = 0.0207$) and for IgD ($r = -0.4384$, $p = 0.0196$).

I. Láng, B. Fekete, P. Gergely, Gy. Petrányi

A simple allogeneic test system for the study of antibody-dependent cellular cytotoxicity (ADCC) in man. Haematologia *11*, 85 (1977).

A simple allogeneic test system for the study of ADCC activity of human mononuclear peripheral blood leucocytes (MPBL) is presented. The test uses human Rh-positive erythrocytes as target cells coated with specific human IgG antibody The effector function of human MPBL is demonstrated after depletion of most of the monocytes and B lymphocytes on nylon wool column. The test system seems to be useful in the clinical study of ADCC activity in man.

P. Alföldy, É. Gyódi, E. Dombi, T. Tóth, S. R. Hollán

Cell-mediated immunity in subjects immunized with HLA antigens. Haematologia *11*, 93 (1977).

Production of cellular immune reaction in subjects immunized against HLA antigens was studied by the capillary migration inhibition test *in vitro* and by the skin test *in vivo*. The majority of the immunized subjects (10 out of 13) developed a cell-mediated immunity to the donor antigens. Production of HLA antibodies was demonstrable in a smaller proportion of the cases. The complementary nature of humoral and cellular responsiveness was borne out by the present observations. The result of the skin test performed simultaneously with the migration inhibition test correlated well with the result *in vitro*.

I. Jókay, E. Karczag

Antagonistic effect of heparin and a water-soluble glucocorticoid on the peripheral lymphocyte count in mice. Haematologia *11*, 103 (1977).

The effect of heparin and prednisolone succinate on the peripheral white blood cell count of mice has been studied 3 hours after injecting the drugs. Heparin in low doses did not influence the cell count but in higher doses (20 to 400 U) elicited marked lymphocytosis. When prednisolone and heparin were administered together, heparin (5 to 10 U) markedly inhibited the prednisolone-induced lymphopenia. The effect on the lymphocyte count of prednisolone combined with a large dose of heparin depends on the doses of the drugs. The antagonism seems to be non-competitive in nature. The PMN count shows only minor changes and the reaction of eosinophils to heparin is less pronounced. The antagonistic effect was manifest on the spleen weight.

K. Merkiel, J. Prokopowicz, M. Bielawiec, B. Kiersnowska

Serum lysozyme activity in some myeloproliferative diseases. Haematologia *11*, 111 (1977).

Serum lysozyme activity has been determined in patients suffering from myeloproliferative diseases, chronic myelogenous leukaemia (CML), acute myelogenous leukaemia (AML), chronic lymphatic leukaemia (CLL) and pancytopenia (P). Lysozyme activity was tested in undiluted and tenfold diluted sera. Increased lysozyme activity was found in patients with CML and CLL, whereas there was no change in patients with AML and P. Dilution of sera enhanced lysozyme activity. These data may indicate the presence of inhibitor in the sera tested. The diagnostic significance of the presented findings is discussed.

E. I. Khomchenovsky

Experimental and clinical study of a new antileukaemic drug: Hexaphosphamide. Haematologia *11*, 115 (1977).

Hexaphosphamide has moderate toxicity [in mice $C_{57}LD_{50} = 105$ (66 ÷ 166.9) mg/kg]. MPD in normal rodents suppressed haemopoiesis, especially granulocytopoiesis. The drug possesses pronounced activity on transplantable leukaemias: it ensures complete regression of rat reticulosis-haemocytoblastosis if treatment is begun on the 5th day after transplantation (T.I. $LD_{10}/ED_{90} = 7.1$; ThioTEPA T.I. = 2.2). Hexaphosphamide improves the haematological picture and increases the life-span of mice with different leukaemia strains: La–150%; L-1210 – 62%; ZOLIPK N 8 – 71%; ZOLIPK N 30 – 79%. Hexaphosphamide has no gastrointestinal toxicity. In patients suffering from different forms of leukaemia the drug had a beneficial effect especially in chronic myeloleukosis. Of 315 treated patients with chronic myeloleukosis 309 were evaluated. A good therapeutic effect was observed in 79.3%, a moderate effect in 12%, subjective improvement in 0.3%, and no effect in 8.4%. The therapeutic dose varied from 10 to 40 mg daily, the total dose was 240–4000 mg. Duration of treatment depends on the susceptibility of the haemopoietic system, and varies from 16 to 113 days. In resistant patients duration of treatment reaches 240 days. Hexaphosphamide is an effective cytostatic drug for treatment of cases with acquired resistance to busulfan, mitobromitol, dopan, X-rays. Of 113 patients resistant to previous therapy, a good response was achieved in 65.5%, a slight response in 20.3%, subjective improvement in 1.1% and no effect in 12.3%. The most important side effect of hexaphosphamid is an individual susceptibility of haemopoiesis of some patients which is difficult to predict. Hexaphosphamide is ineffective in the treatment of blastic crisis of chronic myelosis.

W. Rudowski, J. Daszyński, J. Schier, N. Kowalczyk, A. Gaczkowski, S. Góralski

Infusion of physiological saline supplemented with adenine, pyruvate and phosphate. Haematologia *11*, 127 (1977).

After controlled bleeding 20 ml/kg enriched infusion fluid containing 0.005 mol/l adenine, 0.1 mol/l pyruvate, 0.1 mol/l dibasic sodium phosphate and 0.15 mol/l sodium chloride was given to 14 dogs. Three dogs received after bleeding the same infusion fluid without adenine. One dog received only dextran after blood withdrawal and served as control. Before and after bleeding and 1, 4, 24, 48 and 72 hours after infusion the levels of 2,3-DPG, ATP and P_{50} index were determined. After infusion of enriched fluid with and without adenine the level of 2,3-DPH and the value of P_{50} index increased. No rise in the ATP level was observed.

E. Fagiolo

Immune haemolytic anaemia after exchange transfusion. Haematologia *11*, 133 (1977).

Two cases of immune haemolytic anaemia in newborn infants who had received exchange transfusion on the first day of life because of hyperbilirubinaemia probably due to a conjugation defect, are reported. The first baby, born in the 30th week of gestation presented erythrocyte-fixed IgM antibodies + C, neutropenia, and circulating leucocytotoxic antibodies. The second baby, born at term to a diabetic mother showed erythrocyte-fixed IgG + IgM antibodies + C and a selective IgA deficit. In both cases the anaemia improved and the Coombs test became negative spontaneously in the 4th and 5th months of life, respectively. These haematologic alterations are similar to a graft versus host (GVH) reaction due to the persistence in the neonate blood of the donor's immunocompetent cells.

P. Prodanov

H-excess A_1 and A_{int} variants among the Bulgarian population. Haematologia *11*, 139 (1977).

During the investigation of the subgroups of 45,895 A blood group persons, H-excess A_1 and A_{int} variants were found in 0.359%. These variants were subdivided into three categories provisionally named A_{1H}, A_{1Hint} and A_{intH} according to their most typical isoserological characteristics. The results of genealogical investigation are presented. An attempt was made to explain the existence of these atypical subgroups.

F. Krizsa, I. Cserháti

Effect of F-leurosine (formyl-leurosine) on platelet production in mice. Haematologia *11*, 149 (1977).

F-leurosine (formyl-leurosine) was found to exert a thrombocytopoietic effect in CLFP mice. The effect was most pronounced in a dose of 1.0 mg/kg. The increased activity of the megakaryocyte system was not due to thrombocytopenia. The preparation induced in the bone marrow an increasing megakaryocyte formation (increase of the megakaryocyte index, a shift to the left in the qualitative composition of the giant cells, increase of the ^{75}Se-methionine incorporation). F-leurosine seems to exert its effect through a direct stimulation of the megakaryocyte system, which might be utilized in therapy.

G. Kosztolányi, K. Jobst, I. Kádas

Effect of lanthanum ion on electrophoretic platelet mobility. Haematologia *11*, 155 (1977).

The effect of trivalent La ions on the electrophoretic mobility of washed human platelets has been studied. The mobility of the human platelet in 0.145 M sodium chloride at pH 7.2 was -0.84 ± 0.06 μ per sec per volt per cm with an isoelectric point approximately at pH 3.4. La^{3+} at a concentration corresponding to the physiological level of Ca^{2+} changed the sign of the electrophoretic mobility of the platelets at pH 5.5 and pH 4.0, but had only a faint or practically no effect at pH 3.0 and pH 2.0. It is assumed that La^{3+} exerts its effect when bound to the phosphate groups on the surface.

Gy. Blaskó, J. Bédi, R. Machovich, L. Á. Pálos

Effect of collagen on thrombin inactivation by antithrombin-III and heparin. Haematologia *11*, 163 (1977).

Thrombin inactivation by antithrombin-III and heparin has been found to decrease in the presence of collagen, whereas thrombin activity and the rate of thrombin inactivation by antithrombin-III alone are not affected. Albumin, at the same concentration as collagen does not influence either thrombin activity or thrombin inactivation by antithrombin-III or by antithrombin-III plus heparin.

B. Goldschmidt, J. Siegler, M. Schmidt

Fibrinogen metabolism in cyanotic congenital heart disease.
Haematologia *11* 169 (1977).

The metabolism and *in vivo* kinetics of fibrinogen labelled with radioactive iodine were studied in children with cyanotic congenital heart disease. The patients had a significantly lowered plasma fibrinogen pool, shortened fibrinogen half-life and increased fractional catabolic rate of fibrinogen compared with healthy children. The average plasma fibrinogen and absolute catabolic rate of fibrinogen did not differ from control values. The shortened fibrinogen half-life together with the correcting effect of anticoagulation with heparin indicated that fibrinogen was consumed by chronic disseminated intravascular coagulation. Inhibition of the fibrinolytic system with epsilon-aminocaproic acid in three cyanotic patients had no influence on the fibrinogen half-life in two of them but resulted in its prolongation in one patient.

M. Szmitkowski, J. Prokopowicz, K. Szmitkowska

Antihaemophilic factor (factor VIII) from human granulocytes.
Haematologia *11*, 177 (1977).

Factor VIII has been isolated and purified from human granulocytes using chromatography on Sephadex G-200 and DEAE-Sephadex A-50. The final preparation was purified 60-fold. Some properties of this partly purified factor VIII were compared with the human plasma AHF preparation. Optimum pH activity and the highest activity after incubation at different pH values was found in buffer at pH 7.0. The preparation was thermolabile and had a molecular weight of about 214.000. Absorption curves indicated that it is a protein exhibiting four fractions in polyacrylamide gel disk electrophoresis. AHF preparations from plasma and granulocytes were almost identical in the characteristics tested.

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to the advancement in all fields related to haematology and blood transfusion. Papers in English, French, German and Russian are accepted on the condition that they have not been previously published or accepted for publication.

Manuscripts with a clear carbon copy should be sent to the Editor-in-Chief

Susan R. Hollán, M. D.
National Institute of Haematology
and Blood Transfusion
Daróczi út 24
1113 Budapest, Hungary.

If in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will speed publication. Although every effort will be made to guard against loss, it is advised that authors retain copies of all material which they submit. The editorial board reserves the right to make literary corrections.

Manuscripts should be typed double-spaced on one side of good quality paper with proper margins and bear the title of the paper, name, address and degrees of the author together with the name of the hospital, laboratory or institute where the work has been carried out. The name and full postal address of the author who will be responsible for reading proofs should also be given. An abstract of 50 to 100 words should precede the text of the paper. The paper should not exceed 15 pages including tables and references. The approximate location of tables and figures should be indicated in the margin.

References. Only papers closely related to the author's work should be referred to. The citations should include the name of the author and/or the reference number in parenthesis. A list of numbered references should follow the end of the manuscript.

References to periodicals should mention: (1) Lame(s) and initials of the author(s); (2) title of paper; (3) international abbreviation of the periodical; (4) volume; (5) number of the first page; (6) year of publication in parenthesis. Thus: 14. Bean, W., Mills, A.: Coronary occlusion, heart failure and environmental temperature. *Amer. Heart J. 16*, 701 (1938).

References to books should include: (1) author(s)' name; (2) title; (3) publisher; (4) place and year of publication. Thus: 8. Alsted, G.: The incidence of peptic ulcer in Denmark. Danish Science Press Ltd., Copenhagen 1953.

Illustrations should be selected carefully and only up to a quantity required. Black-and-white photographs should be in the form of glossy prints. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each print. Cloured illustrations should be given only if indispensable. Legends should be brief and attached on a separate sheet. Tables, each bearing a title, should be self-explanatory and numbered consecutively.

Authors will receive page proofs which must be sent back by return mail.

Authors are entitled to 50 reprints free of charge.

Reviews of the Hungarian Academy of Sciences are obtainable at t following addresses:

AUSTRALIA

C.B.D. Library and Subscription Service
Box 4886, G.P.O.
Sydney, N.S.W. 200zff

Cosmos Book and Record Shop
145 Acland Street
St. Kilda 3182, Vic.

Globe and Co.
694—696 George Street
Sydney 2000 N.S.W.

AUSTRIA

Globus
Vertrieb Ausländischer Zeitschriften
Höchstädtplatz 3
A-1200 Wien XX

BELGIUM

„Du Monde Entier" S.A.
Rue du Midi 162
B-1000 Bruxelles

Office International de Librairie S.A.
Avenue Marnix 30
B-1050 Bruxelles

BULGARIA

Direkzia R.E.P.
11 pl. Slaveikov
Sofia

CANADA

Pannonia Books
P. Box 1017
Postal Station „B"
Toronto, Ont. M5T 2T8

CHINA

CNPICOR
Periodical Division
P.O.B. 88
Peking

CZECHOSLOVAKIA

Mad'arská Kultura
Narodní Trida 22
11566 Praha I

Postova Novinova Sluzba — dovoz tisku
Vinohradská 46
Praha 2

Postova Novinova Sluzba — dovoz tlace
Leningrádská 14
Bratislava

DENMARK

Munksgaard's Boghandel
Norregade 6
dK-1165 Kobenhavn K

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
SF-00100 Helsinki 10

FRANCE

Office International de Documentation et Librairie
48, rue Gay-Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Zeitungsvertriebsamt
Strasse der Pariser Kommune 3—4
1004 Berlin

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
7000 Stuttgart I
Postfach 46

GREAT BRITAIN

Blackwell's Periodicals Department
P.O. Box 40
Hythe Bridge Street
Oxford OX1 -EU

Bumpus Haldane and Maxwell Ltd.
Cowper House
Olney, Bucks MK46 4BN

Collet's Holdings Limited
Denington Estate
Wellinborough, Northants NN8 2QT

W. M. Dawson and Sons Ltd.
Cannon House
Folkestone, Kent

H. K. Lewis and Co. Ltd.
136 Gower Str., P.O.B. 66
London WC1E 6BS

HOLLAND

Martinus Nijhoff
P.O. Box 269
Den Haag
Swets and Zeitlinger
Keizersgracht 487
Amsterdam C

ITALY

Licosa
Via Lamarmora 45
Casella Postale 552
50121 Firenze

JAPAN

Igaku Shoin Ltd.
Foreign Department
Tokyo International
P.O. Box 5063
Tokyo

Maruzen Co. Ltd.
P.O. Box 5050
Tokyo International 100-31
Nauka Ltd .
Yasutomi Bldg. 5F
2—12,
Kanda Jinbocho, Chiyoda-ku
Tokyo 101

NORWAY

Tanum-Cammermeyer
Karl Johansgt. 41—43
Oslo I

POLAND

B.K.W.Z. Ruch
ul. Wronia 23
00840 Warszawa

ROMANIA

D.E.P.
Bucuresti
D.E.P.
Oradea

SOVIET UNION

Mezhdunarodny pochtamt
tseh-4
Moscow
Pochtamt-Import
Leningrad

SWEDEN

Almqvist and Wiksell Förlag A.B.
Box 2120
S-103 13 Stockholm 2

Wennegren-Williams AB
Subscription Department
Nordenflychtsvägen 70
Fack
S-104 25 Stockholm 30

SWITZERLAND

Karger Libri A.G.
Petersgraben 31
CH-4011 Basel

USA

Ebsco Industries, Inc.
1st Ave. North AT 13th Street
Birmingham, Alabama 35201
FAM Book Service
69th Fifth Avenue
New York, N.Y. 10003

Intercontinental Medical Book Corporation
381 Park Avenue South
New York, N.Y. 10016

J. B. Lippincott Company
East Washington Square
Philadelphia, Pa. 19105
Stechert-Macmillan, Inc.
7520 Westfield Avenue
Pennsauken, N.J. 08110

YUGOSLAVIA

Forum
Vojvode Misica broj 1
21000 Novi Sad

Jugoslovenska Knjiga
Terazije 27
11000 Beograd

Prosveta Export and Import
Teraizje 1611
11001 Beograd

Index: 826861

HU ISSN 0017—6559

345.871

VOLUME 11 · NUMBER 3—4 · 1977

HAEMATOLOGIA

International Quarterly of Haematology

EDITOR-IN-CHIEF:
S. R. HOLLÁN

EDITOR:
I. BERNÁT

Editorial Board:
G. A. ALEXEIEFF (Moscow)
A. ANDRÉ (Liège)
A. ANTONINI (Rome)
V. APATEANU (Bucharest)
R. ARNAUD (Tours)
G. ASTALDI (Tortona)
I. BARTA (Pécs)
G. BAST (Rostock)
H. BEGEMANN (Munich)
E. BENEDEK (Budapest)
J. BERNARD (Paris)
E. BEUTLER (Duarte, California)
H. BRAUNSTEINER (Innsbruck)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Odense)
O. K. GAVRILOV (Moscow)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
T. J. GREENWALT (Washington, D.C.)
A. HÄSSIG (Bern)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
E. KELEMEN (Budapest)
P. J. LAH (Ljubljana)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Freiburg/Br.)
P. L. MOLLISON (London)
A. E. MOURANT (London)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Debrecen)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM† (Liège)
W. RUDOWSKI (Warsaw)
G. RUHENSTROTH-BAUER (Munich)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Pavia)
A. VIDEBAEK (Copenhagen)
A. S. WIENER† (Brooklyn, N. Y.)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

HAEMATOLOGIA

is an international quarterly publishing original papers on haematology. It also provides the reader with complex and up-to-date information on both research and clinical practice. A General Survey, an Open Forum, Book Reviews, Abstracts of more important papers from other periodicals and a Documentation of the well-known and the less accessible journals are to serve this purpose.

Haematologia is published at quarterly intervals, the four issues per year make up a volume of about 500 pages.

Subscription price: $ 36.— per volume (per year). Orders may be placed with

KULTURA

Foreign Trading Company

Budapest 62. P.O.B. 149

or with its representatives abroad, listed on the verso of the cover.

VOLUME 11 · NUMBER 3—4

HAEMATOLOGIA

INTERNATIONAL QUARTERLY OF HAEMATOLOGY

EDITOR-IN-CHIEF:
S. R. HOLLÁN

EDITOR:
I. BERNÁT

MEMBERS OF THE EDITORIAL BOARD:

G. A. ALEXEIEFF (Moscow) · A. ANDRÉ (Liège) · A. ANTONINI (Rome) · V. APATEANU (Bucharest) · R. ARNAUD (Tours) · G. ASTALDI (Tortona) · I. BARTA (Pécs) · G. BAST (Rostock) · H. BEGEMANN (Munich) · E. BENEDEK (Budapest) · J. BERNARD (Paris) · E. BEUTLER (Duarte, California) · H. BRAUNSTEINER (Innsbruck) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle, Washington) · L. GARBY (Odense) · O. K. GAVRILOV (Moscow) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest) · F. GRÁF (Budapest) · A. GRAFFI (Berlin) · T. J. GREENWALT (Washington, D. C.) · A. HÄSSIG (Bern) · L. P. HOLLÄNDER (Basel) · J. HOŘEJSÍ (Prague) · M. HRUBIŠKO (Bratislava) · E. KELEMEN (Budapest) · P. J. LAH (Ljubljana) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Freiburg/Br.) · P. L. MOLLISON (London) · A. E. MOURANT (London) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Debrecen) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM† (Liège) · W. RUDOWSKI (Warsaw) · G. RUHENSTROTH-BAUER (Munich) · V. SERAFIMOV-DIMITROV (Sofia) · I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIC (Belgrade) · E. STORTI (Pavia) · A. VIDEBAEK (Copenhagen) · A. S. WIENER † (Brooklyn, N. Y.)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES
BUDAPEST 1977

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója
Műszaki szerkesztő: Zacsik Annamária
A kézirat nyomdába érkezett: 1978. I. 27. – Terjedelem: 21,35 (A/5) ív, 26 ábra
78.5464 Akadémiai Nyomda, Budapest – Felelős vezető: Bernát György

Contents

Ishiyama, H., Okuyama, K., Masuda, K., Ozaki, J., Yasuda, J.: Modification of the biological activity of anti-D immunoglobulin caused by heat aggregation 251
Gergely, P., Bakács, T.: Mitogenicity of fetal calf serum 259
Simon, G., Lengyel, A., Szücs, A.: Erythrocyte acetylcholinesterase activity is not affected by adenovirus-induced haemagglutination 265
Freidin, N., Mandel, E. M., Djaldetti, M.: Hypercalcaemia, hyperphosphataemia and cytoplasmic inclusions in a patient with chronic lymphocytic leukaemia 269
Zywicka-Łopaciuk, H., Janowska-Wieczorek, A., Romejko, M., Swencz-Szczepanik, K., Kryzyżanowska-Woźniakowska, J., Michalski, R., Pawelski, S.: Occurrence of malignant neoplasms in patients with chronic lymphatic leukaemia 279
Heilmann, E., Nordiek, R.: Beziehungen zwischen den Lymphozytensubpopulationen und der Stadieneinteilung der chronischen lymphatischen Leukämie von Rai 289
Legutko, L.: Relationship between serum copper and bone marrow blasts in acute leukaemia 297
Dintenfass, L.: Some aspects of haemorrheology of metastasis in malignant melanoma 301
Louwagie, A. C.: I. Increase in bone marrow CFU-S and CFU-C after *in vivo* phytohaemagglutinin (PHA) administration in mice 309
Louwagie, A. C.: II. Mechanism of increased colony formation of CFU-S and CFU-C after *in vivo* PHA administration in mice 321
Boda, D., Eck, E.: Favourable effect of storage at 0 °C on the O_2-transport function of heparinized blood 331
Heilmann, E., Vogel, M.: Hämolyse nach extrakorporaler Blutbestrahlung des Menschen? 335
Bláha, M., Kačerovský, J., Hrnčíř, Z., Nožička, Z., Špaček, J., Široký, O.: Systemic mastocytosis. Report of a case 349
Nowicka, J., Kotschy, M., Chwistecki, K., Jeleń, M.: Disseminated intravascular coagulation (DIC) during superacute haemolysis in a patient with ovarian dermatoid cyst treated with rifampicin 359

Book Reviews 365

Abstracts 369

From the International Literature of Haematology 377

News Item 479

Contents of Volume 11 481

Author Index 483

Subject Index 485

Haematologia 11 (3—4), pp. 251—258 (1977)

Modifications of the Biological Activity of Anti-D Immunoglobulin Caused by Heat Aggregation

H. ISHIYAMA, K. OKUYAMA, K. MASUDA, J. OZAKI, J. YASUDA

Laboratory of Blood Products, Department of General Biologics Control, National Institute of Health, Tokyo, Japan

(Received October 20, 1977)

Some of the biological activities known to be confined to certain anti-D sera such as Ripley could be simulated using heat-treated human anti-D Ig preparations. Thus, human red cells sensitized with heat-treated anti-D Ig were found to be capable of causing Fc-receptor on lymphocyte surfaces and of fixing complement. In its other effects such as reactivity to rheumatoid factor and to anti-allotype agglutinators, heat-treated anti-D Ig seemed inferior to such anti-D sera as Ripley.

It is a generally accepted concept that physicochemical treatments may confer upon Ig molecules biological activity similar to that of antibodies complexed with the corresponding antigens. For example, aggregated Ig molecules are known to be capable of fixing complement, just as antigen-antibody complexes do [7].

On the other hand, not all the anti-D sera, when bound to D-positive human red cells, exhibit complement-fixing or rheumatoid-factor agglutination activity with the exception of Ripley and a few other antisera [4, 10].

Antigen-antibody complexes and aggregated IgG share another common feature, both being capable of binding to a site on the lymphocyte membrane through the Fc-receptors. This finding was brought to light by inhibition experiments on rosette-formation [2, 11]. Here again, human red cells sensitized with only a few anti-D sera such as Ripley can effectively form rosettes around certain lymphocytes.

The present report describes our efforts at studying whether heat treatment could confer on commercial anti-D human Ig preparations the activities possessed by certain anti-D sera such as Ripley. Four parameters were chosen for this purpose, *viz.* Fc-rosette formation, complement consumption, and reactivity to rheumatoid factors and to allotypic antisera.

Materials and Methods

Anti-D antibody. Four batches of anti-D human immunoglobulin (abbreviated as anti-D Ig; KABI, Stockholm, Sweden) were used either in an untreated state or after being aggregated by heat treatment. Protein content was 100 mg/ml. The potency was titrated using the conventional albumin suspension technique [9]. Before these four batches were chosen, two batches of the same Ig preparation and

ten anti-D sera of individual origin, kindly supplied by Tokyo University Hospital's Blood Transfusion Service, had been tested for their usefulness in Fc-rosette formation.

Aggregated anti-D Ig. Anti-D Ig preparations diluted 1 : 10 were kept at 63 °C for 20 min under continuous shaking. Immediately before use, the samples were centrifuged at 1000 *g* for 30 min to remove deposits. By means of gel filtration on Sephadex G-200 column, all heat-treated Ig preparations were found to contain aggregated Ig.

Sensitization of erythrocytes. A suspension of human group 0, D(Rho)-positive red cells at a concentration of 10^8 cells/ml was prepared from pilot samples of blood supplied by the Japanese Red Cross Central Blood Centre in Tokyo. The red cell suspension was mixed with an equal volume of anti-D Ig, diluted 1 : 10 with saline, and the sensitization was carried out at 37 °C for one hour. The cells were suspended at the final concentration of 10^8 cells/ml after being washed three times with physiological saline.

Lymphocyte preparation. Human lymphocytes were isolated from the peripheral blood of a healthy individual using Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradients and were suspended in Hanks balanced salt solution at a concentration of 2×10^6 cells/ml.

Fc-rosette formation. Hundred μl of lymphocyte suspension were incubated with an equal volume of anti-D-coated erythrocyte suspension at 37 °C for 20 min. The test mixture was then centrifuged at 400 *g* for 5 min to enhance the reaction. A drop from this mixture was mounted on an eosinophil counter (Kayagaki Irika-kogyo Co., Tokyo, Japan; 0.2 mm depth) and examined microscopically. About 400 to 600 lymphocytes were examined in each sample and lymphocytes surrounded by four or more erythrocytes were read as "rosettes".

Complement fixation. The ability of anti-D-coated human red cells to fix complement was tested using the conventional method [8]. In the first phase, sensitized human erythrocytes (1×10^9 cells/ml) were incubated with one CH_{50} of reconstituted guinea-pig complement (Nankai Kagaku Co., Tokyo, Japan) in a water bath at 37 °C for 45 min. The reaction mixture was centrifuged at 4000 r.p.m. for 5 min to separate the supernatant fluid from the red cells, and the residual complement activity in the supernatant fluid was determined in the second phase by incubation with ovine red cells sensitized with the optimum dose of rabbit anti-sheep erythrocyte serum (Toshiba Kagaku Co., Tokyo, Japan). At the end of the second incubation time, the OD_{540} of the supernatant fluid was determined. From this value the haemolysis percentage was calculated.

Rheumatoid factor (RF). From patients with rheumatoid arthritis, four sera reacting positively to sensitized ovine red cell agglutination (RAHA) as well as to FII-latex fixation (RA) were used in a tube agglutination test together with human red cells sensitized with anti-D Ig.

Anti-allotypic sera. Anti-G1m(1), anti-G1m(2), anti-G3m(12) or (5) and anti-Km(1), kindly supplied by Dr. H. Geiger (Behring Institute, Marburg/Lahn, GFR), were used in the tube agglutination test following the enclosed directions.

Results

Fc-rosette formation

With most of the anti-D sera and with the majority of anti-D Ig, the rate of rosette formation was found to be very low. No correlation was observed between the rate of rosette formation and the anti-D titre. All the anti-D Ig preparations had shown titres not lower than 1 : 2000 in the indirect antiglobulin test. The anti-D titre of individual sera in the indirect antiglobulin test was within the range of 1 : 8 to 1 : 516. The titre of a serum showing exceptionally high rosette formation rate (18.8%) was 1 : 128. We arbitrarily selected four out of the six batches of anti-D Ig, with rosette-forming rates of 9.0%, 3.8%, 2.0% and 0, respectively, for use in subsequent experiments.

The potency of the four batches of anti-D Ig before and after heat treatment was measured by titration using the conventional albumin suspension technique to prevent the titres in the antiglobulin test being influenced by an antigenic modification of Ig as well as by a possible decrease in antibody activity. No change in antibody titre was observed with the anti-D Ig preparations that could be traced to heat treatment, so far as titration using the albumin suspension technique was concerned.

The effect of heat aggregation on the ability of the anti-D Ig preparations to form Fc-rosettes is shown in Fig. 1. An increase in the rosette formation rate was seen to have occurred with all the four batches used, although the enhancing effect differed in intensity with each of the four batches.

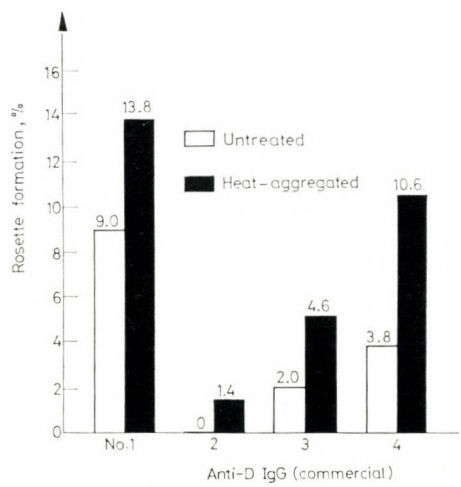


Fig. 1. Rosette formation by anti-D-coated RBC

Complement consumption

Table 1 shows residual complement activity after incubation with human erythrocytes coated with untreated or heat-aggregated anti-D Ig. As the OD_{540} value with completely lysed ovine erythrocyte suspension was 0.75, the haemolysis percentage could readily be calculated. The OD_{500} values obtained for each of the test mixtures were divided by 0.75 and multiplied by 100. They are shown in the right column of Table 1. As one CH_{50} of complement was used in this experiment, a residual haemolytic activity of approximately 50% was interpreted as "no consumption of complement".

Table 1

Residual haemolytic complement activity after treatment with anti-D-coated RBC

Anti-D Ig		OD_{540}	Residual complement activity, per cent
No. 1	Untreated (U)	0.32	42.7
	Aggregated (A)	0.06	8.0
No. 2	U	0.38	50.7
	A	0.08	10.7
No. 3	U	0.18	24.0
	A	0.16	21.3
No. 4	U	0.21	28.0
	A	0.17	22.7

With batches 1 and 2, considerable differences in the consumption of complement were observed with red cells coated with untreated and heat-aggregated Ig. The differences were not so remarkable with the remaining two batches of anti-D Ig, which were found to have consumed nearly 50% of the added complement, even in the untreated state.

Reactivity to RF

A twofold dilution series prepared with each of the four RF-containing sera was tested against human red cells sensitized with either untreated or heat-aggregated anti-D Ig.

The results are given in Table 2. With the first two batches (Nos 1 and 2), heat treatment resulted in a two to fourfold increase in agglutination titre in two or three of the four RF-containing sera. With the other samples of anti-D Ig (Nos 3 and 4), however, practically no increase in titre was observed, with the exception of the combination of RF serum NT and anti-D No. 4.

Table 2

Reactivity to rheumatoid factor of anti-D-Ig-coated RBC

Sera from RA patients		Agglutination titre							
		Anti-D human immunoglobulin							
RA titre	RAHA titre	No. 1		No. 2		No. 3		No. 4	
		Untreated	Aggregated						
		(U)	(A)	(U)	(A)	(U)	(A)	(U)	(A)
NA 8	80	4	4	2	2	2	2	8	4
SM 8	640	8	32	4	8	4	4	4	4
NT 1	40	2	4	2	2	<2	<2	<2	2
MO 8	320	16	32	8	16	8	8	8	8

Reactivity against allotypic antisera

Erythrocyte suspension sensitized with each of the four untreated anti-D Ig was weakly agglutinated by anti-G1m(1) and anti-G1m(2) sera. No enhancement of agglutination could be observed when heat-aggregated anti-D Ig, instead of the untreated preparations, was used for sensitization of the same red cells. No agglutination by anti-G3m(5) or anti-Km(1) serum was observed with any of the red cell suspensions, whether these red cells were coated with untreated or with heat-treated anti-D Ig preparations.

Physicochemical properties of heat-treated Ig

Gel filtration analysis showed that all the heat-treated anti-D Ig had two protein peaks. One peak, which appeared with untreated anti-D, too, was thought to correspond to 7S IgG. The other protein peak eluted prior to the former was shown to be composed of aggregated IgG by the results of ultracentrifugal analysis. Both fractions were specifically reactive in gel immunodiffusion against anti-human IgG serum.

There was a rough correlation between the biological activity of the anti-D Ig preparations and the content of the aggregates formed on heat treatment. The extent of aggregate formation was highest in No. 1 and lowest in No. 4. Nevertheless, No. 3 formed almost as much aggregate as No. 2, whereas the former was less active than the latter.

Discussion

Fc-receptors on human lymphocyte membranes have been found on various kinds of cells related to the immune system such as on most of the B-lymphocytes, on certain T-lymphocytes and on undefined lymphocyte-like (UL) cells [2]. In

general, the antigen-antibody complex and aggregated Ig are thought to bind to Fc-receptors with greater affinity than does native Ig [1]. Human erythrocytes sensitized with some exceptional anti-D sera such as Ripley appear to bind to UL cells, but not to B- or T-lymphocytes [3]. Some other features of red cells sensitized with Ripley and a few other anti-D sera are that they can fix complement, and can also be agglutinated by RF and by most of the anti-allotypic sera [5, 12, 14]. Since these three activities are thought to be parallel, they are attributed to some particular structure formed or exposed only in a few anti-D sera as a result of combination with a corresponding antigen.

As to the kinds of biological activity that can be conferred to anti-D Ig by heat treatment, results are summarized in Table 3.

Table 3

Reactivity of red cells sensitized with anti-D immunoglobulin modified by heat aggregation

Anti-D	Rosette formation	Consumption of complement	RF	Allotype antibodies
No. 1	+	+	+	−
No. 2	+	+	+	−
No. 3	+	−	−	−
No. 4	+	−	−	−

+ : enhancement by heat-treatment of anti-D Ig
− : no enhancement by heat-treatment of anti-D Ig

First, it was found that more rosette-forming cells occurred when red cells had been sensitized with heat-treated anti-D Ig than when the cells had been coated with untreated anti-D from the same batches. The order of rosette-forming rates remained, however, the same with the four batches of anti-D Ig during heat treatment. Thus, heat-aggregation may have merely enhanced the activity of the anti-D Ig already present in the untreated state. It remains to be determined if the rosette-forming cells reactive to heat-aggregated Ig alone are UL-cells, B-cells, or both.

Second, with two of the four batches of anti-D Ig, consumption of guinea-pig complement was observed only when the red cells were sensitized with heat-aggregated Ig. As the classical complement fixation technique was used in our experiments, our results do not seem to corroborate the commonly accepted assumption that anti-D-coated red cells are never lysed by complement. In a previous study [6] it was shown that the inhibitory effect of human Ig preparations on Fc-rosette formation around lymphocytes does not always run parallel with the anti-complementary activity of the preparations, even though both activities are known to be localized in the Fc-portion.

Third, with anti-D Ig Nos 1 and 2, reactivity to RF was somewhat enhanced by heat treatment. The enhancing effect was more marked with the sera of higher RAHA titre than with those of lower RAHA titre.

Vyas et al. [13] reported that heat treatment enhanced G1m(1) antigenicity when G1m(1+) protein was adsorbed to red cells by $CrCl_3$ treatment. This was not the case in our experiment in which allo-anti-D Ig was used for sensitizing red cells.

Thus, in three of the biological activities studied, two out of the four heat-treated anti-D Ig (Nos 1 and 2) were always found to be superior to the other two, although even the best one seemed inferior to Ripley. The biological activity did not seem to be correlated with the anti-D titre. Nor could the variation be attributed to the allotypes of the anti-D molecules, since no enhanced reactivity to anti-allotypic antibodies was observed with any of the anti-D Ig.

No interpretation would, therefore, seem to be feasible other than the assumption that the variation in biological activity must have been inherent to the anti-D molecules contained in each of the individual sera or preparations. Although the antigen-binding sites are known to be located in the variable region, the extent of conformational modification exerted on the Fc-portion when the anti-D molecules are bound to the antigens may vary with antibody molecules in each individual sera. Such a modification may be supplemented, but cannot be exactly duplicated, by the aggregation caused by physicochemical treatments.

References

1. Dickler, H. B.: Studies on the human lymphocyte receptor for heat-aggregated or antigen-complexed immunoglobulin. *J. exp. Med. 140*, 508 (1974).
2. Dickler, H. B.: Lymphocyte receptors for immunoglobulin. *Adv. Immunol. 24*, 167 (1976).
3. Frøland, S. S., Wisløff, F., Michaelsen, T. E.: Human lymphocytes with receptors for IgG. A population of cells distinct from T- and B-lymphocytes. *Int. Arch. Allergy 47*, 124 (1974).
4. Fudenberg, H. H., Kunkel, H. G.: Specificity of the reaction between rheumatoid factors and gamma globulin. *J. exp. Med. 114*, 257 (1961).
5. Harboe, M., Müller-Eberhard, H. J., Fudenberg, H., Polley, M. J., Mollison, P. L.: Identification of the components of complement participating in the antiglobulin reaction. *Immunology 6*, 412 (1963).
6. Ishiyama, H., Okuyama, K., Masuda, K., Yasuda, J.: Effect of human immunoglobulin preparations on Fc-rosette formation by anti-D-coated erythrocytes. *Z. Immun.-Forsch.* In press.
7. Ishizaka, K., Ishizaka, T.: Biologic activity of aggregated γ-globulin. II. A study of various methods for aggregation and species differences. *J. Immunol. 85*, 1631 (1960).
8. Kabat, E. A., Mayer, M. M.: Experimental Immunochemistry. 2nd ed. Charles Thomas, Springfield, Ill. 1961, p. 214.
9. Ministry of Health and Welfare, Japanese Government: Minimum Requirements for Biological Products 1971. (English edition 1973.)
10. Mollison, P. L.: Blood Transfusion in Clinical Medicine. 5th ed. Blackwell Scientific Publ., Oxford 1972, p. 281.
11. Ramasamy, R., Richardson, N. E., Feinstein, A.: The specificity of the Fc receptor on murine lymphocytes for immunoglobulins of the IgG and IgM classes. *Immunology 30*, 851 (1976).

12. Vos, G. H., Vos, D.: Antiglobulin antibodies and anticomplementary factors in hepatitis B antigenaemia. *Acta haemat. (Basel) 52*, 345 (1974).
13. Vyas, G. N., Fudenberg, H. H., Pretty, H. M., Gold, E. R.: A new rapid method for genetic typing of human immunoglobulin. *J. Immunol. 100*, 274 (1968).
14. Waller, M., Lawler, S. D.: A study of the properties of the Rhesus antibody (ri) diagnostic for the rheumatoid factor and its application to Gm grouping. *Vox Sang. (Basel) 7*, 591 (1962).

Correspondence: Junichi Yasuda, M. D., Laboratory of Blood Products, Department of General Biologics Control, National Institute of Health, 10—35, 2-chome, Kamiosaki, Shinagawa-ku, Tokyo, Japan

Haematologia 11 (3–4), pp. 259–263 (1977)

Mitogenicity of Fetal Calf Serum

P. GERGELY, T. BAKÁCS

Second Department of Medicine, Semmelweis University Medical School, Budapest, Hungary
and Department of Tumour Biology, Karolinska Institutet, Stockholm, Sweden

(Received June 15, 1977)

The mitogenic effect of fetal calf serum (FCS) has been studied. Human lymphocytes, especially cord blood cells, undergo blastogenesis in media supplemented with FCS. The mitogenic effect is exerted on non-T (B and null) lymphocytes, but not on T cells. Bovine serum albumin (BSA) successfully substitutes for FCS in mitogen-induced lymphocyte blastogenesis. Unlike FCS, it has no mitogenic properties.

Fetal calf serum (FCS) is widely used in tissue cultures. The lymphocytes do not grow in protein-free media, therefore they have to be supplemented with some kind of serum. FCS-containing media seem to be suitable for studying mitogenic responses of lymphocytes because of the high rate of blastogenesis. FCS, however, is blastogenic for human lymphocytes [6, 7, 13] and a similar effect was observed on mouse lymphoid cells [2, 12]. Thus, FCS may interfere with other mitogens making difficult to evaluate minor blastogenic effects.

In the present work we studied the effect of FCS on the blastogenesis of different lymphocyte fractions and also the possibility of its substitution by serum albumin.

Materials and Methods

Lymphocytes from the peripheral blood of healthy adults and from cord blood were separated on Ficoll-Isopaque gradient [1]. Macrophages were removed by carbonyl iron treatment. Subsequent processing of the cells was carried out by spontaneous rosette gradient separation [3, 4, 5]. Cells from different fractions were resuspended in RPMI 1640 containing either 10% FCS (Gibco), or 2 mg/ml [11] bovine serum albumin (BSA, Sigma, Fraction V). The cells were cultured on Falcon microplates (Falcon Plastics), 0.2×10^6 cells per well, at 37 °C in 5% CO_2 for 5 days. Sixteen hours before harvesting 1 μCi ^{3}H-thymidine (Amersham, specific activity: 5 Ci/mM) was added to each well. The cultures were sucked off with the aid of a multiple culture harvester (Skatron, Lierbyen, Norway) to glass fibre filters. After drying, the filters were placed into vials containing scintillation fluid. Isotope determinations were made by a Packard Scintillation Counter Model 3320, and expressed as c.p.m. 2-Mercaptoethanol (2-ME,

Sigma) was used in a concentration of 5×10^{-5} M/ml. Phytohaemagglutinin (PHA, Leukoagglutinin, Pharmacia) was used in a concentration of 1 μg/ml.

B cells were identified by membrane fluorescence after staining with FITC-labelled polyvalent goat anti-human immunoglobulin (Hyland).

Viability of cells was tested by trypan blue exclusion after 24 hr incubation. No toxic effect of BSA was observed.

Results

Cord blood lymphocytes had a very high "spontaneous" DNA synthesis rate in the presence of 10% FCS (Table 1). Apart from individual variations, the rate was usually higher than that of lymphocytes from adults. Purified T cells did not show this high rate of blastogenesis. The high values of Cases 1 and 2 of cord blood may have been due to separation failure, as B cell contamination was marked in these cases. Though the frequency of B (SIg) cells was considerably higher in cord blood than in adult peripheral blood, the individual differences cannot be ascribed to such variations. There was no clear-cut correlation between B cell percentage and DNA synthesis rate.

Purified T cells showed a significantly lower blastogenic response ($p < 0.005$) to FCS than either unfractionated or non-T (B plus null) cells (Table 2). Thymidine incorporation rate was equally low in all three fractions tested in the presence

Table 1

Lymphocyte DNA synthesis in the presence of 10% FCS

Donor No.	Unfractionated lymphocytes		T cell enriched fraction	
	^{3}H-thymidine incorporation	SIg cells per cent	^{3}H-thymidine incorporation	SIg cells per cent
PB 1	3 577*	18.0	452*	1.5
2	2 398	11.5	234	0.5
3	4 331	8.5	562	0.5
4	2 906	10.5	463	0.5
5	10 022	12.5	550	1.0
CB 1	24 895	24.0	1987	2.0
2	7 861	27.0	1313	3.0
3	24 380	28.5	414	1.0
4	16 014	32.0	620	1.0
5	4 943	25.0	444	0.5

* in cpm (mean of triplicate samples)
SIg: surface immunoglobulin
PB: peripheral (adult) blood
CB: cord blood

of 2 mg/ml BSA. 2-ME increased blastogenesis in each group. The most striking differences ($p < 0.01$) were observed in the T cell enriched fractions, as well as in T depleted and unfractionated populations in the presence of BSA.

The PHA-induced blastogenesis in FCS- and BSA-containing medium is compared in Table 3. For the sake of simplicity, mean values (± SEM) of 15 peripheral (adult) bloods are given. Both types of medium promoted blastogenesis, albeit in a different degree. Although the absolute figures for thymidine incorporation were considerably lower ($p < 0.01$) in the BSA-supplemented medium, because of the much lower background of controls the stimulation index was significantly higher ($p < 0.01$). 2-ME, enhancing DNA synthesis both in controls and in PHA-containing samples, resulted in a lower stimulation index in FCS-containing samples, while stimulation indices did not change when BSA-supplemented medium was used.

Table 2

Effect of BSA and FCS on DNA synthesis of lymphocyte fractions (peripheral blood, n: 15, mean cpm ± SEM)

Lymphocyte fraction	Treatment			
	FCS	FCS+2-ME	BSA	BSA+2-ME
Unfractionated	3228± 612	5121±1210	632±102	1425±210
T cell depleted	4230±1004	6915±1550	751± 95	1120±150
T cell enriched	352± 67	1280± 238	483± 75	1821±456

Table 3

Blastogenic response to PHA in medium containing FCS or PHA (peripheral blood, n: 15, mean ± SEM)

Medium	^{3}H-thymidine incorporation		S.I.*
	control	PHA	
FCS	3505± 822	90 647± 8 484	25.9
FCS + 2-ME	6512±1262	106 917±14 171	16.4
BSA	773± 118	30 029± 6 807	38.8
BSA + 2-ME	1286± 290	50 285± 9 015	39.1

* stimulation index (cpm treated/cpm control)

Discussion

FCS has a mitogenic effect on human lymphocytes. This is in line with other observations [6, 7, 13]. This mitogenic activity is exerted on non-T (B and/or null) cells, but not on purified T cells. It is likely that surface Ig-positive B cells respond to FCS, since blastogenesis was more pronounced in cord blood lympho-

cytes among which B cells figure in a higher proportion. A strict correlation between B cell frequency and ^{3}H-thymidine incorporation was not, however, established. The majority of SIg-negative, E-rosette-negative (= null) cells belong to the B cell compartment [8], so that their mitogenic response ought to be similar as that of B cells. The higher rate of blastogenesis in cord blood lymphocytes may also be caused by factors other than the B cell/T cell ratio [9, 10].

BSA is practically free of mitogenicity but highly purified BSA preparations do not allow a sufficient growth of lymphoid cells (I. Ernberg, personal communication).

2-Mercaptoethanol increased blastogenesis both in FCS- and BSA-containing samples; the increase was highest in T cell samples. The PHA-induced blastogenesis was equally impressive in both types of medium. Due to the much lower rate of spontaneous DNA synthesis in controls containing BSA instead of FCS, stimulation indices are much higher. The mitogen reactivity and viability of human lymphocytes in cultures (RPMI 1640) containing BSA at higher concentrations (4 to 6 mg/ml) is even more favourable (M. Steinitz, personal communication). BSA can thus replace FCS for the growth of mitogen-activated human lymphocytes. Apart from being free of mitogenic or suppressive substances, it has the advantage over human serum that it can be standardized. Whether BSA can be used in other systems (e.g. MLC or antigenic stimulation) requires further study. Polet and Spieker-Polet [11], however, reported favourable results with BSA in MLC, but not with PPD.

References

1. Böyum, A.: Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest. 21*, Suppl. *97*, 77 (1968).
2. Coutinho, A., Möller, G., Andersson, J., Bullock, W. W.: In vitro activation of mouse lymphocytes in serumfree medium: Effect of T and B cell mitogens on proliferation and antibody synthesis. *Europ. J. Immunol. 3*, 299 (1973).
3. Gergely, P., Bakács, T., Cornain, S., Klein, E.: Fc receptors on human blood B lymphocytes. *Clin. exp. Immunol. 28*, 99 (1977).
4. Gergely, P., Ernberg, I.: Blastogenic response and EBNA induction in human lymphocytes by Epstein-Barr virus only requires B cells but not macrophages. *Cancer Letters 2*, 217 (1977).
5. Gergely, P., Bakács, T.: Izolált lymphocyta populációk mitogen reaktivitása. A phytohaemagglutininre adott blastos transformatio. *Kísérl. Orvostud.* (In press.)
6. Johnson, G. J., Russell, P. S.: Reaction of human lymphocytes in culture to components of the medium. *Nature (Lond.) 208*, 343 (1965).
7. Kreeftenberg, J. G., Leerling, M. F., Loggen, H. G.: B- and T-cell markers on human lymphoblasts after stimulation with mitogens or antigens. *Clin. exp. Immunol. 22*, 121 (1975).
8. MacDermott, R. P., Chess, L., Schlossman, S. F.: Immunologic functions of isolated human lymphocyte subpopulations. V. Isolation and functional analysis of a surface Ig negative, E rosette negative subset. *Clin. Immunol. Immunopathol. 4*, 415 (1975).

9. Page Faulk, W., Goodman, J. R., Maloney, M. A., Fudenberg, H. H., Yoffey, J. M.: Morphology and nucleoside incorporation of human neonatal lymphocytes. *Cell. Immunol. 8*, 166 (1973).
10. Prindull, G., Prindull, B., Ron, A., Yoffey, J. M.: Cells in spontaneous DNA synthesis in cord blood of premature and full-term newborn infants. *J. Pediat. 86*, 773 (1975).
11. Polet, H., Spieker-Polet, H.: Serum albumin is essential for in vitro growth of activated human lymphocytes. *J. exp. Med. 142*, 949 (1975).
12. Vogt, A., Mishell, R. O., Dutton, R. W.: Stimulation of DNA synthesis in cultures of mouse spleen cell suspensions by bovine transferrin. *Exp. Cell Res. 54*, 195 (1969).
13. Zielske, J. V., Golub, S. H.: Fetal calf serum-induced blastogenic and cytotoxic responses of human lymphocytes. *Cancer Res.* 36, 3842 (1976).

Correspondence: Dr. P. Gergely, Second Department of Medicine, Semmelweis University Medical School, Szentkirályi u. 46, H-1088 Budapest

Haematologia 11 (3—4), pp. 265—268 (1977)

Erythrocyte Acetylcholinesterase Activity is not Affected by Adenovirus-Induced Haemagglutination

G. SIMON, ANNA LENGYEL, ANNA SZÜCS

Institute of Pathophysiology and Institute of Microbiology, Semmelweis University Medical School, Budapest, Hungary

(Received May 12, 1977)

No correlation was found between adenovirus-induced haemagglutination and AChE activity of human RBCs as ascertained with three different types of virus. The haemagglutination titre of the adenoviruses did not decrease when RBCs with AChE inhibited by dichlorvos or eserine were used. The receptors involved in active haemagglutination by adenoviruses thus seem to differ from those acting in passive haemagglutination. The AChE inactivating effect of substances such as tannic acid and glutaraldehyde, among others, might be due to a non-specific damage of the RBC membrane.

The surface of the human red blood cell (RBC) membrane possesses specific structures, called receptors, which interact with certain types of adenoviruses [10]. Attachment to such structures of the fibre part of the capsid of these adenoviruses causes the direct agglutination of RBC. The structure and exact localization within the RBC surface of the adenovirus-binding receptors (ABR) have not been elucidated.

Of the few enzymes identified in the human RBC membrane, acetylcholinesterase (AChE) is also located on the cell surface [1, 2] but, similarly as the ABR, its exact localization within the surface is not known. The present report deals with experiments in which a possible interrelationship between ABR and AChE has been explored.

Materials and Methods

RBCs were treated with extracts of adenovirus prototype 9 and 10 as well as of a type 8 strain isolated from a patient with epidemic keratoconjunctivitis. Adenovirus types 8 and 10 were grown in HEp-2 cell cultures, type 9 in MASA cell cultures. For culturing, Parker's solution containing 3% rabbit serum was used. RBC incubated with saline and uninfected cell culture extracts served as controls in each experiment.

Fresh, pooled, normal blood specimens were used in all experiments, with sodium citrate as anticoagulant. The blood was centrifuged at 2500 *g* and the RBCs were washed with physiological saline three times for 10 min. Washed and packed RBCs were incubated with cell culture extracts containing different virus

doses and washed with saline in each experiment under the same conditions. Haemagglutination (HA) was performed in Takátsy's microtitrator.

In different experiments RBCs were tested by adding increasing virus doses to the system, and the maximum virus quantity bound to the RBC was determined. This quantity was called "saturation quantity". The amounts of virus applied in different experiments were expressed in percentage of the saturation quantity. When the amount of virus added to the system exceeded the saturation quantity, a certain proportion of viral haemagglutinin remained unbound in the medium, while each binding site of the RBC surface was saturated.

Erythrocyte AChE activity was determined by the method of Ellman et al. [3, 9] using acetylthiocholine as substrate and dithio-bis-nitrobenzoic acid as colour reagent. Since there are individual activity variations, all results were expressed in percentage of the respective controls, consisting of RBCs treated with saline alone.

In the second experimental series, 10^{-6} M dichlorvos (DDVP) and 10^{-5} M eserine salicylate were used to inhibit AChE activity. The RBCs were preincubated with the inhibitors at 25 °C for 20 min. After removing the excess inhibitor by centrifugation, the RBCs were washed with saline and exposed to virus preparations as indicated above and their agglutinability was established. In other experiments the cells were not washed before treatment with viruses.

Results

All virus concentrations applied caused haemagglutination.

AChE activity in the maintaining solution, in the tissue control and in the washing saline was constantly below 1% of that of the normal RBC. In the supernatants, too, AChE activity was rather low.

As shown in Table 1, the treatment with the three adenovirus types did not influence AChE activity of the RBC. In case of types 9 and 10, the virus

Table 1

Effect of adenovirus-induced haemagglutination on red blood cell acetylcholinesterase activity

Virus type	Virus quantity*	AChE activity of RBC**	Virus quantity*	AChE activity of RBC**	Virus quantity*	AChE activity of RBC**	Virus quantity*	AChE activity of RBC**
8	1	94	4	94				
9	1.1	100	4.3	98	14.8	100	120	97
10	1.3	100	5.1	102.4	33.3	96.7	133.3	94.5

* expressed in percentage of the saturation quantity

** expressed in percentage of the AChE activity of saline-normal RBC control

quantities applied varied within a wide range, while of type 8 comparatively smaller amounts were used because of the low infectivity titre of this virus type [8].

As it is seen in Table 2 when AChE activity was inhibited to more than 80% with eserine or more than 65% with dichlorvos, there was no change in RBC agglutinability by either type of adenovirus tested. (The significant difference in haemagglutination titre of viruses between one set of experiments and the other, as shown in Table 2, was due to individual differences between the blood samples.)

Table 2

Effect of acetylcholinesterase inhibition on the agglutinability of red blood cells by adenovirus

Adeno-virus type	Untreated	Preincubated with inhibitor		Preincubated with inhibitor and washed with saline	
	haemagglutination titre of the virus	AChE activity in percentage of normal RBC activity	haemagglutination titre of the virus	AChE activity in percentage of normal RBC activity	haemagglutination titre of the virus
		Eserine-treated			
8	48	18	48	20	48
9	192	18	192	20	192
10	256	18	256	20	256
		DDVP-treated			
8	8	32	8	49	8
9	32	32	32	49	32
10	48	32	48	49	48

Discussion

The experiments clearly proved that three different types of human adenovirus capable of agglutinating human RBC [10] did not affect the AChE activity of RBCs. Nor did we find any diminution in the agglutinability of RBCs whose AChE was inhibited either by organophosphate, dichlorvos or by the carbamate eserine.

Certain chemical agents (tannic acid, glutaraldehyde) that sensitize RBCs to passive HA are known to decrease AChE activity [4, 5, 6, 7]. In this case a considerable damage might occur. In the case of direct adenovirus HA the membrane may, however, not be impaired, only the fibre of the virus particle attaches to the appropriate "receptors". These receptors may not be in the proximity of the AChE molecules on the surface, as according to our experiments there is no functional relationship between the two structures.

References

1. Brodbeck, U., Ott, P., Wiedmer, T.: Comparative studies on the molecular properties of purified AChE from human erythrocytes and from the electric organ of *Electrophorus electricus*. *Croatica chem. Acta 47*, 201 (1975).
2. Ciliv, G., Özand, P. T.: Human erythrocyte acetylcholinesterase purification, properties and kinetic behavior. *Biochim. biophys. Acta (Amst.) 284*, 136 (1972).
3. Ellman, G. L., Courtney, K. D., Andres, V. Jr., Featherstone, R. M.: A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol. 7*, 88 (1961).
4. Herz, F., Kaplan, E.: Effects of tannic acid on erythrocyte enzymes. *Nature (Lond.) 217*, 1258 (1968).
5. Herz, F., Kaplan, E.: Surface alterations and acetylcholinesterase activity of human red cells. *Proc. Soc. exp. Biol. (N. Y.) 134*, 437 (1970).
6. Herz, F., Kaplan, E.: Modification of the erythrocyte membrane by glutaraldehyde: Effect on acetylcholinesterase. *Proc. Soc. exp. Biol. (N. Y.) 140*, 270 (1972).
7. Herz, F., Kaplan, E.: *In vitro* modifications of red cell acetylcholinesterase activity. *Brit. J. Haemat. 26*, 165 (1974).
8. Lengyel, A., Nász, I.: Soluble components of adenovirus type 8. *J. Virol. 6*, 406 (1970).
9. MacQueen, J., Plant, B., Borges, J., Anido, O.: Manual colorimetric methods for pseudocholinesterase and red cell (true) cholinesterase. *Clin. Chem. 17*, 481 (1971).
10. Rosen, L.: Hemagglutination-inhibition technique for typing adenoviruses. *Amer. J. Hyg. 71*, 120 (1960).

Correspondence: Dr. G. Simon, Institute of Pathophysiology, Semmelweis University Medical School, Hőgyes E. u. 9, H-1092 Budapest

Haematologia 11 (3–4), pp. 269–278 (1977)

Hypercalcaemia, Hyperphosphataemia and Cytoplasmic Inclusions in a Patient with Chronic Lymphocytic Leukaemia*

N. FREIDIN, E. M. MANDEL, M. DJALDETTI**

Department of Medicine "B" and Electron Microscopy Unit, Hasharon Hospital, Petah-Tiqva, and Tel-Aviv University Medical School, Tel-Aviv, Israel

(Received June 12, 1977)

The history of a patient with chronic lymphocytic leukaemia (CLL), known for ten years, is presented. During the terminal stage of the disease, she developed hypercalcaemia and hyperphosphataemia. The peripheral blood and lymph node lymphocytes contained crystalline inclusions in the dilated cisterns of the endoplasmic reticulum. The possible mechanism of hypercalcaemia and hyperphosphataemia and the significance of the crystalline inclusions are discussed.

Hypercalcaemia is frequent in malignant disease [13] but a rare complication of chronic lymphocytic leukaemia (CLL) [17]. Similarly to other neoplasms of the haematopoietic system, hypercalcaemia in CLL is usually secondary to the involvement and destruction of the bones. Only one patient with Hodgkin's disease showed an increased level of parathyroid-like hormone [15]. Hyperphosphataemia secondary to massive destruction of lymphocytes and hypercalcaemia was reported in Burkitt lymphoma [3] and acute lymphoblastic leukaemia [18].

We report a patient with CLL who developed hypercalcaemia and hyperphosphataemia during the terminal stage of the illness. In addition, crystalline inclusions were found in the dilated cisterns of the rough endoplasmic reticulum of peripheral blood and lymph node lymphocytes.

Case Report

A 72-year-old female was diagnosed as suffering from CLL for the last ten years. In December, 1974, she was hospitalized in our Department because of erysipelas of the left hand. The patient was pale and cachectic. Generalized lymphadenopathy was found, the lymph nodes were rubbery, not tender and adherent. The liver and the spleen were firm and palpable 7 and 8 cm below the costal margin, respectively.

* This work was supported by a grant from the Chief Scientist's Bureau, Ministry of Health, Israel.

** Established Investigator of the Chief Scientist's Bureau, Ministry of Health, Israel.

Laboratory examinations showed: Haemoglobin 6.7 g/dl, white blood cells 27,000/cu.mm, lymphocytes 90%, segmented neutrophils 10%, platelet count 27,000/cu.mm. The blood urea was 34 mg/dl, creatinine 1.0/dl, uric acid 6.4 mg/dl, alkaline phosphatase 3.0 Bessey Lowry units, serum calcium 9.1 mg/dl and phosphorus 3.5 mg/dl. Serum albumin was 4.1 g/dl, globulin 2.0 g/dl. Paper

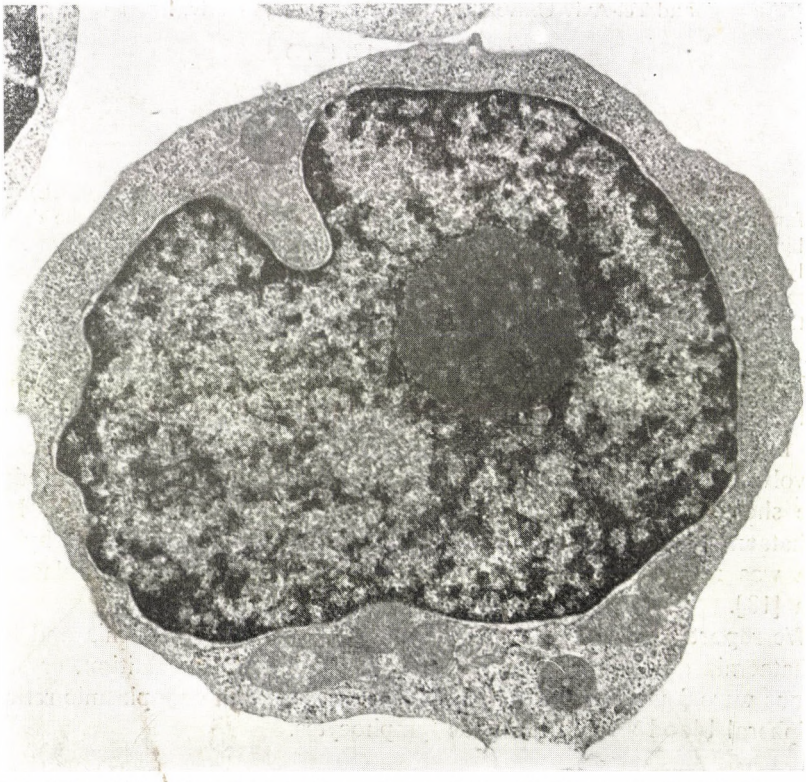


Fig. 1. Peripheral blood lymphocyte. Scanty heterochromatin, large, round nucleolus. ×18,580

electrophoresis of the proteins showed 6% gamma-globulin. Immunoelectrophoresis revealed IgG 120 mg/dl, IgA 25 mg/dl and IgM 50 mg/dl. Bone marrow aspiration biopsy showed massive infiltration by mature lymphocytes. Cells of the myeloid series or megakaryocytes were not found.

X-ray examination of the lumbar spine showed osteoporosis and partial collapse of the L_3 vertebra.

The patient responded well to treatment with antibiotics and was discharged. She was followed-up in the outpatient clinic and treated with daily doses of 4 mg chlorambucil and 30 mg prednisone. During 1975, she was hospitalized several times because of respiratory tract infections and low back pain. X-ray examination

of the spine showed collapse of D_6, L_3, L_4 and osteoporosis. The patient was hospitalized again in February, 1976, because of confusion of several days duration. On examination she was in a poor general condition, pale and cachectic. Generalized lymphadenopathy was found. The liver and spleen were firm and not tender, 10 and 12 cm below the costal margin, respectively. The thoracic and lumbar spine were painful on pressure.

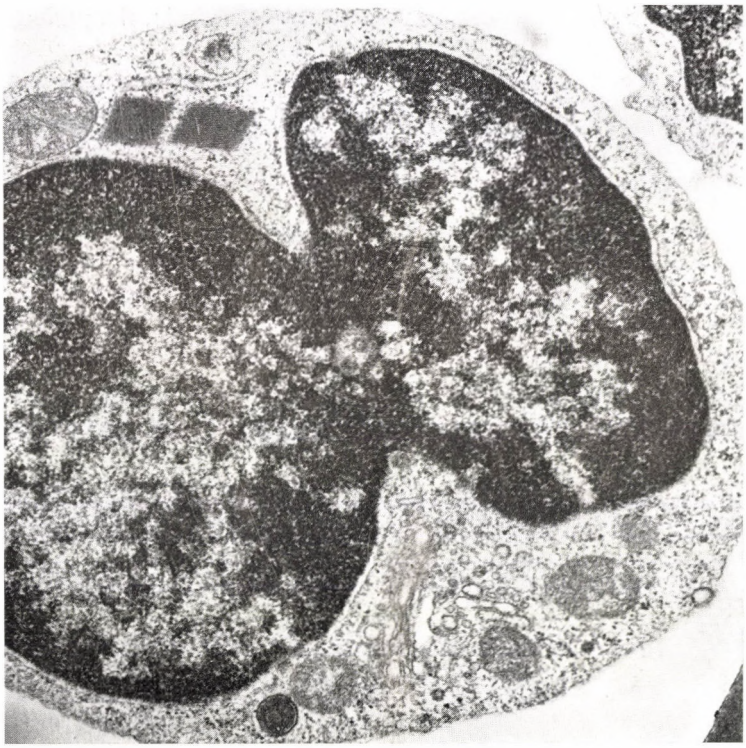


Fig. 2. Peripheral blood lymphocyte with "dumbbell"-shaped nucleus. Two crystalline inclusions in the cytoplasm. × 24,000

The laboratory tests showed: Haemoglobin 9.3 g/dl, white blood cells 200,000/cu.mm with 94% mature lymphocytes, 6% segmented neutrophils. Platelet count was 40,000/cu.mm. The blood urea was 140 mg/dl, creatinine 1.4 mg/dl, uric acid 7.4 mg/dl, calcium 13 mg/dl, phosphorus 4.1 mg/dl, alkaline phosphatase 6.9 Bessey Lowry units. Twenty-four-hour urine excretion of calcium and phosphorus was 250 mg and 1500 mg, respectively. The creatinine clearance was 35 cc/min. The cyclic AMP level in the urine was normal and the total reabsorption of phosphorus (TRP), 94% (normal values 85 to 94%).

The patient was treated with saline infusions, furosemide and prednisone 60 mg daily. The blood calcium level returned to normal after four days. The

treatment was continued with intravenous administration of nitrogen mustard 5 mg daily for six days, but the white blood cell count increased to 650,000/cu. mm and the blood phosphorus to 7.2 mg/dl. The patient developed severe congestive heart failure and died.

On autopsy, generalized enlargement of the lymph nodes as well as enlarged liver and spleen were found. Light microscopy examination showed an almost complete replacement of the bone marrow by a homogeneous infiltration with immature lymphocytes. The bone trabeculae were very thin, the periosteum and the bone itself were infiltrated by lymphocytes. The lymph nodes and the spleen architecture were completely destroyed and replaced by immature lymphocytes. Calcification was seen in the renal tubules. The parathyroid glands were normal.

Electron microscopy

Buffy coat obtained from 10 ml heparinized venous blood and small pieces of a lymph node biopsy specimen were fixed in cold 1% glutaraldehyde in phosphate buffer pH 7.4, post-fixed in 1% osmium tetroxide, dehydrated in graded

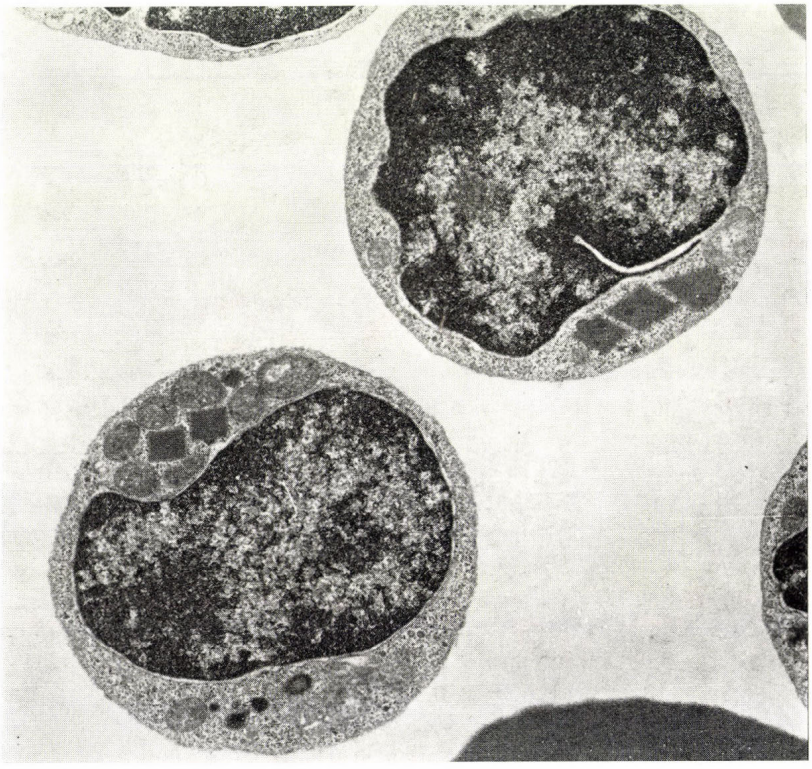


Fig. 3. Two peripheral blood lymphocytes containing crystalline inclusions. ×12,950

alcohol and embedded in Epon 812. Thin sections cut with an LKB ultratome III were examined with a Philips 300 electron microscope.

The peripheral blood lymphocytes were in different stages of maturation. The lymphoblasts (Fig. 1) showed a high nucleo-cytoplasmic ratio. The nucleus possessed little heterochromatin and a large round nucleolus. There was no endoplasmic reticulum in the cytoplasm. The mitochondria were round and small.

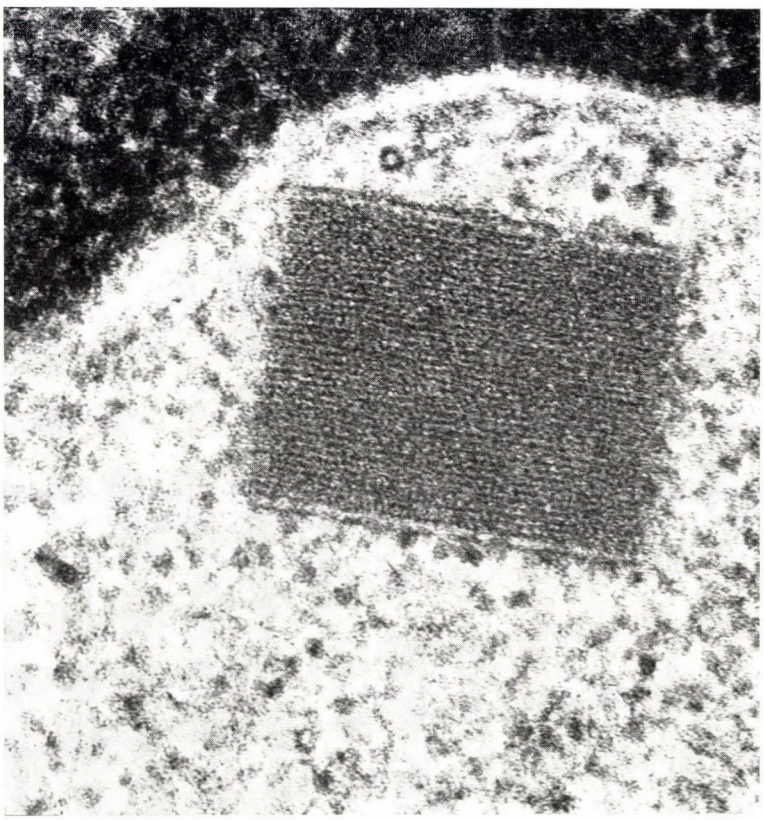


Fig. 4. Crystalline inclusion at high magnification revealing its internal structure and surrounding membrane. ×154,900

The more mature lymphocytes had bizarre-shaped nuclei (Fig. 2), the nucleo-cytoplasmic ratio being still high. Very often, nuclear pockets were detected. The most conspicuous finding was a single (Fig. 2) or several (Fig. 3) crystals, up to 4 in a single cell, rectangular or triangular in shape, according to the plane of section. On higher magnification they were composed of parallel arrays with a lattice-like structure (Fig. 4) and were located in dilated cisterns of the rough endoplasmic reticulum. Golgi formations and centrioles were detected occasion-

ally. The mitochondria were usually located in the vicinity of the nuclear invagination.

The lymph node was composed of younger and more mature lymphocytes. Already under low magnification (Fig. 5) cytoplasmic crystals, similar to those described in the peripheral lymphocytes, were detected. In this case too, they were located in dilated cisterns of the rough endoplasmic reticulum (Fig. 6) with numerous ribosomes on the cisternal membrane (Fig. 7).

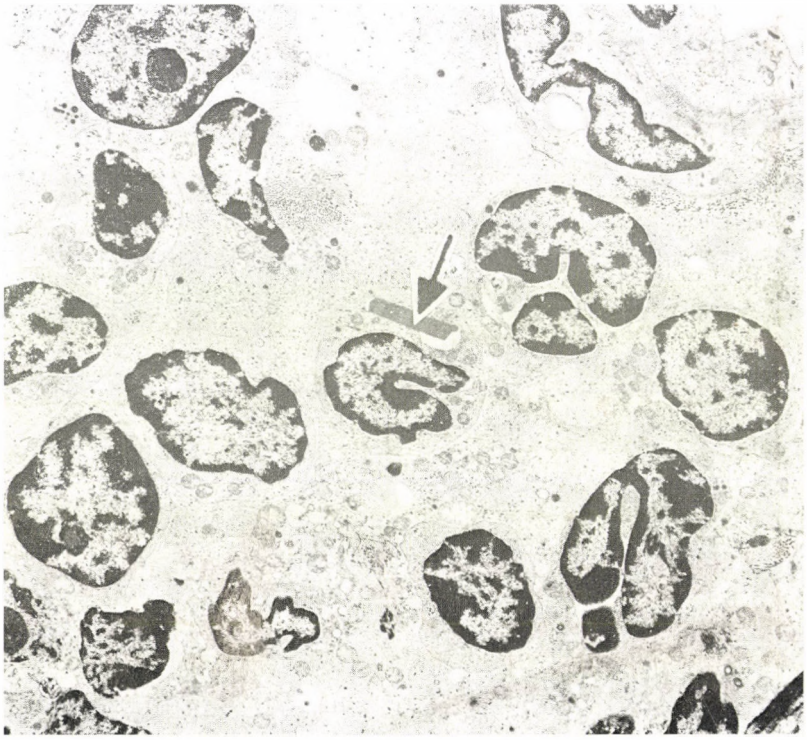


Fig. 5. Electron micrograph of a lymph node. A lymphocyte with a crystalline inclusion located in a dilated cistern of endoplasmic reticulum is marked by an arrow. × 5000

Discussion

There are only few reports on hypercalcaemia complicating CLL. Jordan [13] reviewed 123 cases of leukaemia and found hypercalcaemia in two patients with acute leukaemic and in one patient with acute lymphoblastic leukaemia. Thirty-two patients suffering from CLL had a normal calcium level. David et al. [8] reviewed the diagnostic spectrum of hypercalcaemia and described a patient with CLL who developed recurrent hypercalcaemia. The calcium level of this patient

on his first admission was 16.0 mg/dl with a normal phosphorus level; at that time, leukopenia of 2725/cu.mm was present. The hypercalcaemia developed during treatment with triamcinolone 8 mg daily and it decreased after intravenous administration of 300 mg of hydrocortisone. Autopsy revealed advanced osteoporosis, normal parathyroid glands and no metastatic calcifications. Silva and Wilson [16] reported a patient with CLL and hypercalcaemia associated with

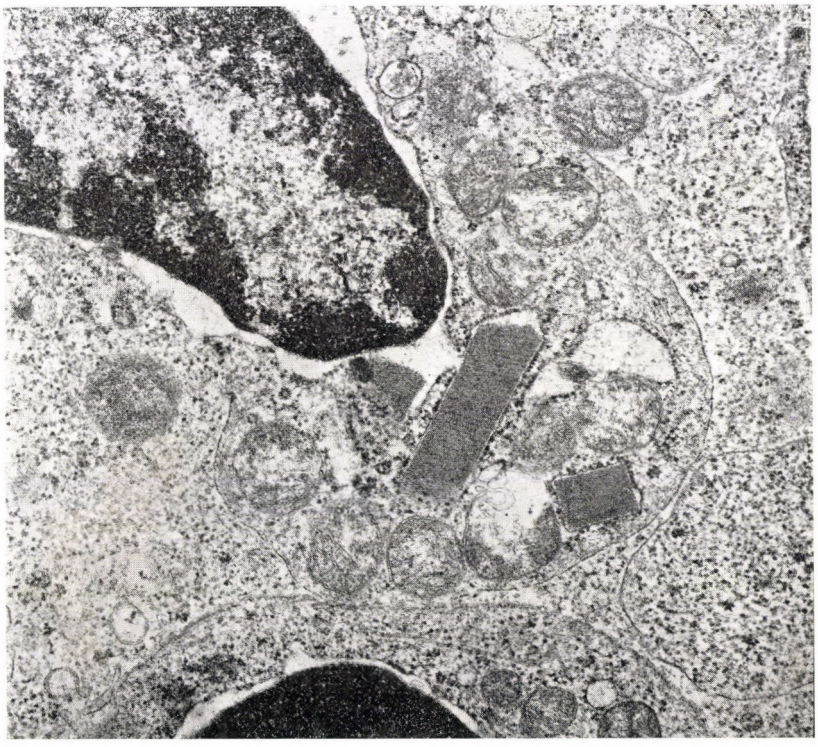


Fig. 6. A lymph node lymphocyte containing two crystalline inclusions. Dilated perinuclear membrane and cisterns of rough endoplasmic reticulum. ×25,000

renal failure and hyperphosphataemia. The hypercalcaemia was resistant to treatment and was corrected temporarily only after the CLL had been controlled. Autopsy revealed CLL with abnormal lymphosarcoma-type cells infiltrating the lymph nodes, spleen, liver, kidneys and lungs. Extensive calcium deposits were found in the lungs.

In the present case, the hypercalcaemia developed during the terminal stage of the disease while the patient was treated with prednisone 30 mg daily and chlorambucil 4 mg daily. There was severe osteoporosis and bone destruction. Leukaemic infiltrates were detected in the bone marrow and periosteum. It is

conceivable that the hypercalcaemia was secondary to bone destruction by the leukaemic process. The high phosphorus level in the blood, the normal level of cyclic 5 AMP in the urine, the TRP of 94% and the normal parathyroid glands found at autopsy did not support the possibility that the hypercalcaemia was secondary to hyperparathyroidism.

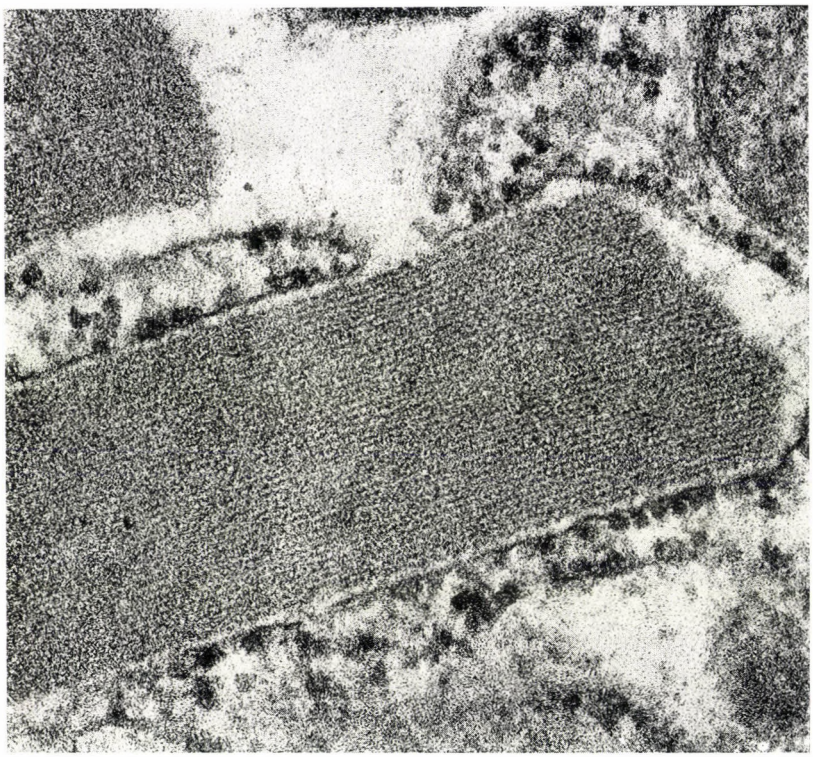


Fig. 7. High power view of the crystalline inclusion shown in Fig. 6. The surrounding membrane of the rough endoplasmic reticulum is clearly seen. × 135,000

During the patient's last admission the blood phosphorus level rose from 4.1 mg/dl to 7.2 mg/dl just before death. This phenomenon occurred with a rapid proliferation and exacerbation of the leukaemia, when the total white blood cell count rose from 200,000 to 650,000/cu.mm. The hyperphosphataemia could not be explained by the slight renal failure accompanied by high excretion of phosphorus in the urine. According to Goldsmith [11] hyperphosphataemia can be expected in renal failure with a creatinine clearance of 35 cc/min. It can be assumed that the hyperphosphataemia in our patient was secondary to the rapid destruction of lymphocytes by the drugs administered.

Hyperphosphataemia was described by Zusman et al. [18] in 6 patients with ALL and by Brereton et al. [3] in 2 patients with Burkitt lymphoma. They

related the hyperphosphataemia to the outflow of phosphate from the lymphocytic cells destroyed by the drugs; the patients developed symptomatic hypocalcaemia to the hyperphosphataemia.

Intracytoplasmic inclusions have been described in patients with CLL [2, 4, 5, 6, 7, 12], as well as with lymphoreticular malignancies [1, 9, 10, 14]. In the majority, the inclusions were located in cisterns of the endoplasmic reticulum, although in a few instances they were surrounded by membranes of ribosomes [7, 12]. Immunofluorescent studies characterized these crystals as IgA-lambda [5], IgM-lambda [12] or IgM-kappa [11].

The ultrastructures of the crystalline inclusions in our case were similar to those described in other patients with CLL. They were located in cisterns of the rough endoplasmic reticulum. The low immunoglobulin level in the patients supports the suggestion of Cawley et al. [5] that the peripheral and lymph node lymphocytes which contain intraplasmic crystalline immunoglobulin inclusions do not secrete them in significant amounts.

*

The technical assistance of Mrs. P. Fishman and the photographic work of Mr. J. Sadovnik and Mrs. M. Gilden are highly appreciated.

References

1. Argani, I., Kipkie, G. F.: The cellular origin of macroglobulins. A study of the protein-secreting cells in Waldenström's disease. *Lab. Invest. 7*, 720 (1965).
2. Bernard, J., Bessis, M., Soulier, J. P., Thiéry, J. P.: Étude au microscope électronique d'une leucémie lymphoïde avec cristaux intra-cytoplasmiques. *Rev. Hémat. 14*, 227 (1959).
3. Brereton, H. D., Anderson, T., Johnson, R. E., Schein, P. S.: Hyperphosphatemia and hypocalcemia in Burkitt lymphoma. *Arch. intern. Med. 135*, 307 (1975).
4. Brunning, R. D., Parkin, J.: Ultrastructural studies of parallel tubular arrays in human lymphocytes. *Amer. J. Path. 78*, 59 (1975).
5. Cawley, J. C., Barker, C. R., Britchford, R. D., Smith, J. L.: Intracellular IgA immunoglobulin crystals in chronic lymphocytic leukaemia. *Clin. exp. Immunol. 13*, 407 (1973).
6. Clark, C., Rydell, R. E., Kaplan, M. E.: Frequent association of IgMλ with crystalline inclusions in chronic lymphatic leukemic lymphocytes. *New Engl. J. Med. 289*, 113 (1973).
7. Cohnen, G., Douglas, S. D., König, E., Brittinger, G.: Crystalloid intracytoplasmic inclusions in lymphocytes of a patient with chronic lymphocytic leukemia. *Mount Sinai J. Med. 11*, 249 (1973).
8. David, N. J., Verner, J. V., Engel, F. L.: The diagnostic spectrum of hypercalcemia. Case reports and discussion. *Amer. J. Med. 33*, 88 (1962).
9. De Man, J. C. H., Meiners, W. B. H.: Crystals of protein nature in the cytoplasm of lymphatic cells in a case of lymphoreticular malignancy. *Blood 20*, 492 (1962).
10. Goldberg, A. F.: An unusual lymphomatous disease associated with intracytoplasmic crystals in lymphoplasmacytoid cells. *Blood 16*, 1693 (1960).
11. Goldsmith, R. S.: Differential diagnosis of hypercalcemia. *New Engl. J. Med. 274*, 674 (1966).

12. Hurez, D., Flandrin, G., Preud'homme, J. L., Seligmann, M.: Unreleased intracellular monoclonal macroglobulin in chronic lymphocytic leukaemia. *Clin. exp. Immunol. 10*, 223 (1972).
13. Jordan, G. W.: Serum calcium and phosphor abnormalities in leukemia. *Amer. J. Med. 41*, 381 (1966).
14. Mennemeyer, R., Hammar, S. P., Cathey, W. J.: Malignant lymphoma with intracytoplasmic IgM crystalline inclusions. *New Engl. J. Med. 291*, 960 (1974).
15. Moses, A. M., Spencer, H.: Hypercalcemia in patients with malignant lymphoma. *Ann. intern. Med. 59*, 531 (1963).
16. Silva, S. N., Wilson, D. M.: Hypercalcemia associated with chronic lymphocytic leukemia. *Postgrad. Med. 53*, 129 (1973).
17. Wintrobe, M. M., Lee, G. R., Boggs, D. R., Bitchell, T. C., Athens, J. W., Foerster, J.: Clinical Hematology. 7th ed. Lea and Febiger, Philadelphia 1974, p. 1692.
18. Zusman, J., Brown, D. M., Nesbit, M. E.: Hyperphosphoremia, hyperphosphaturia and hypocalcemia in acute lymphoblastic leukemia. *New Engl. J. Med. 289*, 1335 (1973)

Correspondence: Prof. M. Djaldetti, Department of Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel

Haematologia 11 (3–4), pp. 279–287 (1977)

Occurrence of Malignant Neoplasms in Patients with Chronic Lymphatic Leukaemia

HALINA ZYWICKA-ŁOPACIUK, ANNA JANOWSKA-WIECZOREK, MARIA ROMEJKO, KRYSTYNA SWENZ-SZCZEPANIK, JADWIGA KRZYŻANOWSKA-WOŹNIAKOWSKA, R. MICHALSKI, S. PAWELSKI

Medical Clinic of the Institute of Haematology and Cancer Control and Epidemiology Department of the Institute of Oncology, Warsaw, Poland

(Received September 26, 1977)

In a group of 280 patients with chronic lymphatic leukaemia, a coexistence of malignant tumours diagnosed simultaneously with the leukaemia was found in 14 cases or 5.0%. No significant difference was observed between the development of the second malignant neoplasm in the patients studied and the expected incidence in the general population, taking into account also the sex and age of patients and their place of residence. No statistically significant relation was found between the duration of CLL and the occurrence of second malignancies. No significant correlation was found between the total dose of cytostatic drugs, corticosteroids or radiotherapy and the incidence of cancer.

Whipham [21] had first called attention to the coexistence of leukaemia and carcinoma. Since then, the occurrence of malignant neoplasm in chronic lymphatic leukaemia (CLL) patients was the subject of many clinical studies [1, 3, 4, 6, 7, 9, 11, 13, 14, 15, 17, 19] as well as pathological analyses [8, 11, 15]. Until now it has not, however, been established whether in patients with CLL the risk of a second neoplasm was greater than the expected number. The divergence of the results is due to differences in the selection of patients and to difficulties in finding a comparable control group, among others. The increased frequency of another neoplasm in CLL has been explained by genetic factors [6], by the action of a multipotential oncogenic virus [14, 19], by the weakening of immunological mechanisms following the development of a proliferative process, or by the immunosuppressive treatment applied [9, 13, 19].

The purpose of the present study was to establish whether the duration of CLL had a role in tumour development and whether immunosuppressive treatment increased the risk of another malignancy.

Selection of Patients and Results of Studies

The present study was based on an analysis of 280 patients with CLL from the population of Warsaw, observed in the period 1955 to 1975. Only patients living in Warsaw were considered since this allowed a statistical comparison of the frequency of a second malignant process in these patients with the data of the Institute of Oncology concerning the whole population of Warsaw [10].

Coexistence of a malignant solid tumour was observed in 25 cases, i.e. in 8.9% of all the studied ones.

The sites of these malignant tumours are shown in Table 1. In 12 cases the diagnosis of malignant tumour preceded that of CLL, in 2 cases the tumour was diagnosed simultaneously, and in 12 cases during the disease. In one case the coexistence of three malignant processes was found.

Table 1

Time relationship between the diagnosis of malignant neoplasm and chronic lymphatic leukaemia

Site of neoplasm	Diagnosis of neoplasm (No. of cases)		
	before leukaemia diagnosis	at the same time	after leukaemia diagnosis
Skin cancer	2		2
Digestive tract cancer	1	1	1
Lung cancer			4
Kidney cancer			1
Breast cancer	3		
Cervical and uterine corpus cancer	3	1	2
Prostatic and testicular cancer	2*		
Hepatoma			2*
Laryngeal cancer	1		
Total	12	2	12
		14	

* in 1 patient 3 malignant processes were found: prostatic cancer, hepatoma, leukaemia.

Table 2

Patients with CLL and subsequently diagnosed cancer

Patients	Total	Sex		Sex ratio	Age in years at time of leukaemia diagnosed			Survival rate after leukaemia diagnosis, per cent			
								up to 5 years		up to 10 years	
		M	F		from	to	mean	M	F	M	F
CLL	280	148	132	1.1	24	84	63.8	53.5	60.6	23.3	38.2
CLL and co-existent cancer	14	8	6	1.3	29	84	60.9	37.5	100.0	25.0	50.0

The further clinical and statistical analysis concerns only the patients in whom CLL and malignant tumour were diagnosed simultaneously or with a

Table 3

Patients with malignant tumour recognized at the time of CLL diagnosis or later

No.	Sex	Age at the time of CLL diagnosis	Year of diagnosis		Site of neoplasm	Histological type of tumour
			CLL	tumour		
1.	F	61	1958	1970	liver hepatoma	hepatocellular carcinoma
2.	F	54	1960	1965	uterine corpus	adenocarcinoma
3.	F	69	1961	1969	skin	spinocellular carcinoma
4.	F	62	1961	1961	uterine corpus	adenocarcinoma
5.	F	57	1964	1975	uterine cervix	planoepithelial and intermediocellular, partly spinocellular keratoblastic carcinoma
6.	F	66	1964	1966	skin	carcinomatous keratosis senilis papillaris
7.	M	77	1957	1960	lung	bronchogenic planoepithelial carcinoma
8.	M	35	1961	1967	kidney	clarocellular carcinoma
9.	M	29	1963	1973	lung	bronchogenic macrocellular anaplastic carcinoma
10.	M	76	1963	1940 1965	prostate liver hepatoma	— partly solid adenocarcinoma
11.	M	64	1968	1970	lung	bronchogenic planoepithelial carcinoma
12.	M	64	1969	1972	lung	bronchogenic microcellular carcinoma
13.	M	55	1971	1972	rectum	mucocellular, partly gelatinous microfocal carcinoma
14.	M	84	1974	1974	stomach	cubocellular tubular carcinoma

difference not exceeding 3 months, or in whom the tumour was detected after the diagnosis of CLL. These patients accounted for 5.0% of the whole material.

Table 2 includes the data on sex and age, and survival calculated according to Berkson and Gage [2]. The age of the patients at the time of carcinoma diagnosis ranged from 39 to 84 years, mean 65.5 years.

Table 3 shows the sites of tumours and their histological type. In one case (patient No. 10) three malignant processes were found: Prostatic carcinoma diagnosed 23 years before the onset of CLL and a primary liver cancer detected two years after the diagnosis of CLL.

In 13 cases the diagnosis of malignant tumour was made during the life of the patient and in one case it was recognized post mortem (case No. 7). Two

patients (Nos. 4 and 8) are being followed up at the Institute of Haematology and 12 died. In one case (No. 2) the exact cause of death could not be established. In 5 cases (Nos. 1, 9, 10, 12, 13) the malignant tumour was the direct cause of death, in 3 cases (Nos. 7, 11, 14) death was due to both CLL and the tumour. In 2 cases in which skin cancer had been diagnosed previously (cases 3 and 6) and in another case (No. 8) with a history of renal carcinoma removed surgically five years before death, infectious complications of CLL were the direct cause of death. In these cases autopsy failed to demonstrate malignant lesions.

The frequency of carcinoma in the patients with CLL was compared to the expected incidence of malignant neoplasm in the Warsaw population. The analysis was based on the cases of malignant tumour recorded at the Institute of Oncology in the years 1963 to 1972. Their comparative analysis by the method of Watson [20] in the modification of Manusow and Weinerman [13] included the data on sex and age of the patients. Statistical analysis was done by the method of Poisson.

Table 4

Incidence of malignant tumour in patients with CLL per 100 patient-years

	Total	Males	Females
Leukaemia patients	280	148	132
Patient-years	1352.7	686.4	666.3
Patients with CLL and tumour	14	8	6
Incidence index in 100 patient-years	1.0	1.2	0.9

Table 5

Relationship between malignant tumour incidence and survival time

Survival after diagnosis of CLL (months)	No. of patients	Patients with malignant tumour	
		No. of patients	percent
1—24	70	3	4.3
25—60	100	5	5.0
61—120	82	3	3.7
>120	28	3	10.7
Total	280	14	5.0

The ratio of the observed to the expected incidence was in males 8/7.953 = = 1.006 ($p < 0.55$), in females 6/5.750 = 1.043 ($p < 0.52$) and in the whole group 14/13.118 = 1.067 ($p < 0.45$).

This comparison shows that in the analysed group of patients with CLL the frequency of another malignant neoplasm was statistically not significantly different from the incidence of these tumours in the general population.

Table 4 shows the incidence of a second malignant tumour calculated per 100 patient-years of observation. The incidence coefficient was calculated for all cases and for each sex separately.

Table 5 shows the relationship between the duration of CLL and the occurrence of the second malignancy. The survival time was reckoned since the moment of diagnosis of CLL until death, or to the last follow-up, or to the development

Table 6

Treatment of patients with CLL without malignant tumour and of patients with CLL before the tumour diagnosis

		No. of patients	
		without tumour (237 cases)	with tumour (14 cases)
Without treatment		31	5
Treatment applied			
Chlorambucil (total dose in mg)	<500	43	4
	500— 1000	57	1
	1000— 3000	75	2
	3000— 5000	23	1
	5000—10000	11	1
	>10000	2	—
Cyclophosphamide (total dose in mg)	1000— 3000	4	—
	3000— 6000	9	—
	6000—10000	2	—
	>10000	7	1
Other drugs: vinblastine, vincristine, nitrogen mustard, anathioprime, Cytostasan, procarbazine		16	—
Prednisone (total dose in mg)	<1000	26	—
	1000— 5000	56	4
	5000—10000	26	—
	10000—20000	17	—
	>20000	4	—
Radiotherapy		4	1

of the second malignancy. In the group with a survival of 2 years, the frequency of a second malignancy was 4.2%. In the groups with 2 to 5 years, 5 to 10 and over 10 years survival the frequency was 5.0%, 3.7% and 10.7%, respectively. The differences were not significant statistically ($\chi^2 = 2.32$; $\chi^2_\alpha = 0.05 = 7.82$).

No significant correlation was found between the total dose of cytostatics and corticosteroids administered or of the radiotherapy applied and the frequency of malignant tumours. The data in Table 6 indicate that in the group of 237 patients with repeated intensive treatment, no malignant tumour was observed. In a group of 14 patients with malignant tumour coexistence with CLL 5 patients had (cases 4, 11, 12, 13, 14) had no treatment in the period preceding the diagnosis of the tumour, in 4 patients (cases 2, 7, 8, 10) the total dose of chlorambucil was less than 500 mg, in 3 patients (cases 1, 3, 6) it ranged from 500 to 3000 mg. In 2 cases (Nos. 2 and 6) prednisone was administered simultaneously. Intensive treatment was given in 2 cases only: Case 9 (chlorambucil, cyclophosphamide, prednisone) and case 5 (chlorambucil, prednisone). The patient with three malignant processes (case 10) was treated surgically and by radiotherapy.

Discussion

In the 280 patients with CLL a malignant tumour was found in 25, i.e. 8.9%. In 14 cases, i.e. 5.0%, malignancy was demonstrated simultaneously with the diagnosis of CLL or during its course. This group was analysed further clinically and statistically.

Reports in the literature (Table 7) show concerning the frequency of coexistent CLL and malignant neoplasm considerable differences ranging from 4.1 to 34.4% [1, 3, 4, 6, 7, 9, 11, 13, 14, 15, 17, 19]. The divergence is due, among other factors, to differences in the selection of the analysed material. In the reported material all cases of neoplasm were taken into account [3, 4, 6, 7, 9, 11] or only neoplasms diagnosed simultaneously or after the diagnosis of CLL were considered [1, 13, 14, 17]. Some reports failed to include cases of asymptomatic carcinoma found accidentally during clinical investigation or autopsy [1, 14, 17]. To evaluate more exactly the incidence of another neoplasm it is necessary to consider the years of observation.

In the material analysed by us the incidence per 100 patient-years was 1.03, lower than that found by Berg [1] and Stavraky et al. [17] and much below the value found by Manusow and Weinerman [13]. No significant difference was observed in the sex-dependent incidence, although this figure was somewhat higher in males than in females.

The problem whether the incidence of another malignant neoplasm is higher in patients with CLL than in a given population remains open. In the present investigations no significant difference was observed between the incidence of another malignant neoplasm in our patients and the expected incidence in the population, taking into account the age and sex of patients and the place of residence.

Table 7

Coexistence of CLL and malignant neoplasm (data from the literature)

Authors		No. of patients with leukaemia	Incidence of malignant tumour	
			No. of patients	per cent
Moertel, Hagedorn	(1957)	739	31 (8)*	4.2
Lawrence, Donald	(1959)	162	14 (7)	8.6
Faber, Borum	(1962)	760	31 (11)	4.1
Bousser et al.	(1964)	253	17 (4)	6.7
Gunz, Angus	(1965)	86	19 (16)	22.0
Berg	(1967)	381	19 (11)**	5.0
Hyman	(1969)	209***	72 (16)	34.4
Stavraky et al.	(1970)	258	13 (5)**	5.0
Hansen	(1973)	189	31 (14)	16.4
Manusow, Weinerman	(1975)	102	16 (7)**	15.6
Moayeri et al.	(1976)	191	41 (13)**	21.4
Quaglino et al.	(1976)	258	14 (3)	5.4
Present study		180	25 (4)	8.9
			14 (2)**	5.0

* in parentheses — number of patients with skin and lip cancer

** only cases of cancer diagnosed simultaneously or after diagnosis of leukaemia are included

*** patients with chronic lymphatic leukaemia and lymphosarcoma

In the analyses based on similar criteria the results obtained by Stavraky et al. [17] agreed with our results, while Manusow and Weinerman [13] and Moayeri et al. [14] considered the risk of a second malignant neoplasm in patients with CLL to be threefold, and Gunz and Angus [6] fourfold. According to Berg [1] and Gunz and Angus [6], after ruling out skin cancer, the incidence of malignant tumours in patients with CLL is near or equal to the expected incidence in a given age group. On the other hand, Manusow and Weinerman [13] and Moayeri et al. [14] pointed out that even after having ruled out skin cancer the incidence of malignant tumours in these patients was twice as high as the expected one for a given population.

In accordance with the opinion of many authors, skin cancer is the most frequently observed malignant lesion in patients with CLL and its incidence is 4 to 13 times higher than in the general population [1, 6, 7, 13]. According to the same authors, this incidence is overestimated in view of the insufficient notification of skin cancer [3, 7, 14, 17]. In the opinion of Bousser et al. [3] in patients with carcinoma coexistent with CLL the localization of the malignant tumour and the clinical course of CLL show no characteristic features.

The present results seem to show that the occurrence of a second malignancy was related to the duration of CLL survival (4.4% up to 10 years, and 10.7% over 10 years). Nevertheless, this observation cannot be considered a proof as the differences failed to be significant statistically (by the chi square test).

It is well known that malignant neoplasms have an increased incidence in cases of immunodeficiency [18]. Certain authors believe that in CLL a disorder involving a defect of one or more lymphoid cell subpopulations, the immunological deficiency may exert a direct influence on the process of carcinogenesis [9, 13, 19] and it was suggested that immunosuppressive treatment applied in CLL may increase the incidence of cancer [9, 13].

Some statistics indicated that immunosuppressant drugs increase the frequency of malignant neoplasms, especially during long-term immunosuppressive treatment following organ transplantation [5, 16]. There is, however, no reliable evidence that for this an insufficiency of immune mechanisms would be responsible. On the other hand, Leibowitz and Schwartz [12] consider that strong immunosuppressants administered to patients with autoimmune diseases do not cause any significant risk of neoplasm development.

In the present study in patients with CLL no correlation was observed between the total dose of immunosuppressants and the incidence of malignant neoplasm. Out of 14 patients with cancer coexisting with CLL 9, i.e. 64%, had no treatment or had received only a small total dose of chlorambucil prior to the diagnosis of cancer. Only 2 patients had been treated intensively. Moayeri et al. [14] have also observed that most of the patients who developed a second neoplasm had not had any intensive chemotherapy.

References

1. Berg, J. W.: The incidence of multiple primary cancers. I. Development of further cancers in patients with lymphomas, leukemias and myeloma. *J. nat. Cancer Inst. 38*, 741 (1967).
2. Berkson, J., Gage, R. P.: Calculation of survival rates for cancer. *Mayo Clin. Proc. 25*, 270 (1950).
3. Bousser, J., Zittoun, R., Simony, B., Schaison, G.: L'association d'une léucose lymphoïde à un cancer épithélial. *Sem. Hôp. Paris 40*, 2721 (1964).
4. Faber, M., Borum, K.: Leukaemia and a malignant tumour in the same patient. *Brit. J. Haemat. 8*, 313 (1962).
5. Fahey, J. L.: Cancer in the immunosuppressed patient. *Ann. intern. Med. 75*, 310 (1971).
6. Gunz, F. W., Angus, H. B.: Leukemia and cancer in the same patient. *Cancer (Philad.) 18*, 145 (1965).
7. Hansen, M. M.: Chronic lymphocytic leukaemia. *Scand. J. Haemat. Suppl. 18*, 105 (1973).
8. Helbig, W., Voigt, K. G.: Zur Frage der Syntropie von Leukämie und Carcinom. *Arch. Geschwulstforsch. 21*, 213 (1963).
9. Hyman, G. A.: Increased incidence of neoplasia in association with chronic lymphocytic leukaemia. *Scand. J. Haemat. 6*, 99 (1969).
10. Koszarowski, T., Gadomska, H., Wronkowski, Z., Romejko, M.: Cancer in Poland, City of Warsaw and Selected Rural Areas. 1963–1972 Polish Medical Publishers, Warsaw 1977.
11. Lawrence, J. H., Donald, W. G.: The incidence of cancer in chronic leukemia and in polycythemia vera. *Amer. J. med. Sci. 237*, 488 (1959).
12. Leibowitz, S., Schwartz, R. S.: Malignancy as a complication of immunosuppressive therapy. *Advanc. intern. Med. 17*, 95 (1971).

13. Manusow, D., Weinerman, B. H.: Subsequent neoplasia in chronic lymphocytic leukemia. *J. Amer. med. Ass. 232*, 267 (1975).
14. Moayeri, H., Han, T., Stutzman, L., Sokal, J. E.: Second neoplasms with chronic lymphocytic leukemia. *N. Y. State J. Med. 76*, 378 (1976).
15. Moertel, C. G., Hagedorn, A. B.: Leukemia or lymphoma and coexistent primary malignant lesions: A review of the literature and a study of 120 cases. *Blood 12*, 788 (1957).
16. Penn, I., Halgrimson, C. G., Starzl, T. E.: *De novo* malignant tumors in organ transplant recipients. *Transplant. Proc. 3*, 773 (1971).
17. Stavraky, K. M., Watson, T. A., White, D. F., Miles, E. M.: Chronic lymphocytic leukemia and subsequent cancer in the same patient. *Cancer (Philad.) 26*, 410 (1970).
18. Steffen, J.: Immunological factors in the origin and development of neoplasm. *Nowotwory 25*, 123 (1975).
19. Quaglino, D., Lusvarghi, E., Picciinini, L., Ubaldo di Prisco, A., Guerzoni, O., Mauri, C.: The association between chronic lymphocytic leukaemia and a solid tumour: A survey study of 258 cases of chronic lymphocytic leukaemia covering an eleven-year period. *Haematologica 61*, 456 (1976).
20. Watson, T. A.: Incidence of multiple cancer. *Cancer (Philad.) 6*, 365 (1953).
21. Whipham, T.: Splenic leukaemia with carcinoma. *Trans. path. Soc. London 29*, 313 (1878).

Correspondence: Dr. H. Zywicka-Łopaciuk, Institute of Hematology, ul. Chocimska 5, 00-957 Warsaw, Poland

Haematologia 11 (3–4), pp. 289–295 (1977)

Beziehungen zwischen den Lymphozytensubpopulationen und der Stadieneinteilung der chronischen lymphatischen Leukämie von Rai

Edelgard HEILMANN, R. NORDIEK

Medizinische Poliklinik der Westfälischen Wilhelms-Universität Münster, BRD

(Eingegangen am 8. Dezember 1977)

In der vorliegenden Arbeit wurden T- und B-Lymphozyten von 36 Patienten mit chronischer lymphatischer Leukämie (CLL) studiert. Je 12 Patienten erhielten extrakorporale Blutbestrahlung (ECIB) oder Chlorambucil, 12 blieben ohne Therapie. Die Patienten wurden der von Rai und Mitarb. proponierten Stadieneinteilung entsprechend in Gruppen eingestuft. In allen Gruppen waren signifikant erhöhte B- und T-Zellzahlen zu beobachten. Es wurde bewiesen, daß keine Beziehungen zwischen den Lymphozytenwerten und dem Schweregrad der Erkrankung vorliegen. Unter ECIB verminderte sich die Anzahl der B-Lymphozyten signifikanterweise, während die der T-Lymphozyten unbeeinflußt blieb.

Nach allgemein übereinstimmender Meinung handelt es sich bei der chronischen lymphatischen Leukämie (CLL) in der Regel um eine B-Zellerkrankung, bei der nach Dameshek [7] eine Akkumulation immuninkompetenter Lymphozyten vorliegt, deren Rezirkulation über den Ductus thoracicus nach Huber und Mitarb. [16] sowie Bremer und Mitarb. [4] gestört ist.

Der klinische Verlauf der Erkrankung ist sehr variabel und hängt von verschiedenen Faktoren ab. Die Stadieneinteilung der CLL von Rai und Mitarb. [23] ermöglicht es, mit Hilfe von klinischen Parametern einen Hinweis auf die Prognose zu erlangen.

Für die Verlaufskontrolle zur Beurteilung eines erfolgreichen therapeutischen Vorgehens erscheint zudem noch das immunologische Verhalten im Einzelfalle den Krankheitsverlauf entscheidend zu bestimmen. Während sich über das Verhalten der B-Lymphozyten bei CLL zahlreiche Mitteilungen finden, liegen nur wenige über die T-Lymphozyten vor.

In der vorliegenden Studie wurde versucht, Beziehungen zwischen dem Krankheitsstadium der CLL und den Lymphozytensubpopulationen unter verschiedenen therapeutischen Bedingungen aufzuzeigen.

Patientengut und Methodik

Zur Untersuchung gelangten 36 Patienten mit CLL im Alter zwischen 49 und 80 Jahren. Zwölf Patienten wurden mittels extrakorporaler Blutbestrahlung (ECIB), 12 mit Chlorambucil behandelt, 12 blieben ohne Therapie. Zwölf gesunde Probanden der gleichen Altersstufe dienten als Kontrolle.

Die Diagnose war bei den Patienten von 1 Monat bis zu 5 Jahren vor Einleitung der Therapie gestellt worden.

Von den mit ECIB behandelten Fällen wurden nur diejenigen berücksichtigt, die nicht mit Zytostatika vorbehandelt waren. Die ECIB wurde nach einer früher beschriebenen Methode durchgeführt [13]. Die Untersuchungsergebnisse wurden vor und nach 10 ECIB ausgewertet.

Bei den mit Chlorambucil behandelten Patienten lag die Medikation zwischen 1 und 12 mg pro Tag.

Die Patienten wurden der Stadieneinteilung von Rai und Mitarb. [23] in 5 Gruppen eingestuft.

Die Lymphozyten des peripheren Blutes wurden mittels des Dichtegradienten mit Lymphoprep® (Nyegaard) gewonnen. Die Darstellung der B-Lymphozyten erfolgte mit fluoreszeinmarkierten Antihumanglobulinen [1, 3, 15]. T-Lymphozyten wurden im Rosettentest nach Zusatz von Neuraminidase nachgewiesen [17, 26]. Die statistische Auswertung der Ergebnisse erfolgte mit Hilfe des *t*-Testes.

Ergebnisse

Die Verteilung der Patienten unter Berücksichtigung der verschiedenen Behandlungsformen entsprechend den Stadien von Rai geht aus Tabelle 1 hervor. Während in der Gruppe der unbehandelten Patienten das Stadium II vorherrscht, finden sich unter den mit Chlorambucil behandelten Fällen vorwiegend die Stadien II und III. Vor der ECIB lagen die meisten Patienten im Stadium IV, nach dessen Durchführung im Stadium I. Die Mittelwerte der Lymphozytensubpopulationen des peripheren Blutes in den einzelnen Gruppen gehen aus Tabelle 2 hervor. In allen Fällen bestehen signifikant höhere T- und B-Lymphozyten als bei den Normalpersonen, wie die summarische Auswertung ergibt. Die Anzahl der T-Lymphozyten beträgt bei den CLL-Patienten durchschnittlich 2,400 pro μl und liegt damit signifikant höher ($p < 0.001$) als bei den Normalpersonen (1,300 pro

Tabelle 1

Stadieneinteilung nach Rai [23] bei Patienten mit chronischer Lymphadenose unter verschiedenen Therapie-Formen

Therapie / Stadium	unbehandelt	Chloram-bucil	ECIB	
			vor	nach
0	1	—	—	3
I	—	—	1	6
II	7	4	2	—
III	2	5	2	2
IV	2	3	7	1

Tabelle 2

Mittelwerte der T- und B-Lymphozyten im peripheren Blut (pro μl) bei Patienten mit chronischer Lymphadenose unter verschiedenen Therapieformen

Therapie / Stadium	Unbehandelt		Chlorambucil		ECIB vor		ECIB nach	
	T	B	T	B	T	B	T	B
0	2,700	16,900	—	—	—	—	2,090	16,800
I	—	—	—	—	5,400	52,900	1,720	11,320
II	2,628	35,771	1,350	18,475	2,150	25,250	—	—
III	1,484	62,050	1,880	25,060	1,500	15,400	1,720	3,250
IV	3,100	37,000	3,133	37,400	2,041	42,457	—	40,600
$\bar{x}$	2,478	37,930	2,121	26,978	2,727	34,002	1,843	17,992

Normalpersonen: T-Lymphozyten 1,300
B-Lymphozyten 600

μl). Die höchsten Lymphozytenzahlen finden sich in der Gruppe der unbehandelten Patienten, die niedrigsten ergeben sich nach ECIB. Der Abfall der Gesamtlymphozyten unter ECIB ist auf eine hochsignifikante ($p < 0.0001$) Verminderung der B-Lymphozyten von 34,002 auf 17,992 pro μl zurückzuführen, während sich die T-Lymphozyten nicht signifikant von durchschnittlich 2.727 auf 1,843 pro μl reduzieren ließen.

Die Anzahl der T-Lymphozyten nach ECIB unterscheidet sich nicht von den Patienten unter zytostatischer Therapie.

Die Zuordnung der Lymphozytenpopulation zu den einzelnen Stadien der CLL unter Berücksichtigung der verschiedenen Therapienformen geht aus Abb. 1 hervor.

Bei Betrachtung der Mittelwerte der T- und B-Lymphozyten aller Behandlungsgruppen ergibt sich, daß keine Beziehungen zwischen der Höhe der einzelnen Lymphozytensubpopulationen einerseits und dem Krankheitsstadium andererseits bestehen.

In der Gruppe der mit Chlorambucil behandelten Patienten zeigt sich, daß mit zunehmender Schwere des Stadiums die T-Lymphozyten eine ansteigende Tendenz erkennen lassen, wie dieses auch bei den B-Lymphozyten zu beobachten ist.

Die niedrigsten Werte für T- und B-Lymphozyten finden sich nach ECIB.

Bei einer Aufgliederung der T-Lymphozyten in die Gruppen behandelter und nicht behandelter Patienten zeigt sich, daß keine Beziehungen zwischen der Schwere der Erkrankung und der Höhe der T-Zellzahlen bestehen. In den Stadien III und IV findet sich jedoch nur jeweils 1 unbehandelter Patient.

Eine Besonderheit ergab sich bei einem mit ECIB behandelten Patienten im Stadium IV, der unter dieser Therapie eine schwere Pneumonie entwickelte. T-Lymphozyten waren zu diesem Zeitpunkt nicht nachweisbar.

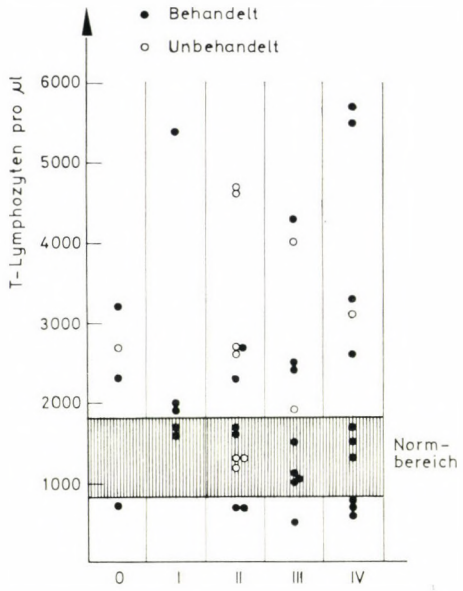


Abb. 1. T-Lymphozyten bei Patienten mit chronischer Lymphadenose in den Stadien nach Rai [23]

Diskussion

Zahlreiche Untersuchungen über die CLL haben ergeben, daß der Verlauf dieser Erkrankung sehr unterschiedlich ist. Neben aktiven, progredienten Formen gibt es sehr stabile Verläufe, bei denen die Lymphozytenzahlen über Monate und Jahre konstant bleiben. Die Aktivität der CLL wurde bislang vorwiegend nach klinischen Kriterien festgelgt. Die von Rai und Mitarb. [23] vorgeschlagene Klassifikation ermöglicht eine gewisse Hilfe, die verschiedenen Verlaufsformen der CLL einzuteilen und einen Therapiebeginn festzulegen. Dabei wurden bislang immunologische Differenzierungen der Lymphozyten nicht berücksichtigt.

Immunologische Untersuchungen zur Klassifizierung der Zellpopulationen haben ergeben, daß es sich bei der CLL in der Regel um eine Proliferation der B-Zellen handelt [6, 8, 10, 11, 21, 24]. Nur in seltenen Fällen sind CLL-Formen vom T-Zell-Typ beschrieben worden [19]. Hinsichtlich des klinischen Verlaufs sind zwei Formen bekannt, die eine mit aktivem Verlauf bei steigenden Lymphozytenzahlen, während die stabilen Verlaufsformen über Jahre hin konstante hämatologische Parameter und klinische Befunde zeigen.

Nach Untersuchungen von Han und Mitarb. [10] bestehen gute Beziehungen zwischen dem klinischen Stadium der CLL und der T- und B-Zellzahl. Die Autoren zeigten, daß der prozentuale und absolute Anteil der B-Lymphozyten bei aktiven Verlaufsformen signifikant höher lag als bei Patienten in kompletter bzw. partieller Remission. Demgegenüber fanden Shohat und Joshua [25], daß Patienten mit den höchsten und niedrigsten absoluten B-Zellen sich als therapieresistent erwiesen. Frühere Untersuchungen [11, 12] hatten ergeben, daß bei Patienten mit CLL nach ECIB ein signifikanter Abfall der B-Lymphozyten nachweisbar ist.

Die in dieser Studie vorgelegten Ergebnisse zeigen sowohl in allen Behandlungsgruppen als auch in allen Stadien der CLL eine signifikante Erhöhung der absoluten B-Zellen. Die niedrigsten Werte fanden sich nach ECIB.

Auf den niedrigen Anteil an T-Lymphozyten ist in zahlreichen Studien [6, 8, 15, 27] hingewiesen worden. Einige Autoren sahen die Anzahl der T-Zellen als vermindert an.

Dellon [9] machte darauf aufmerksam, daß die absolute T-Zellzahl wichtiger ist als der prozentuale Anteil. Die von uns gefundene Erhöhung der absoluten T-Lymphozyten stimmt mit den Mitteilungen von Astaldi und Mitarb. [2], Rabin und Whiteside [22], Mellstedt und Petterson [20] sowie Holowiecki und Mitarb. [14] überein. Als Ursachen für die Erhöhung der T-Zellen diskutieren die letzterwähnten Autoren eine Proliferation beider Lymphozytensubpopulationen, eine Reaktion der T-Zellen gegen die leukämischen B-Zellen bzw. eine Störung des Gleichgewichtes zwischen T- und B- Zellen.

Die Beobachtung von Catovsky und Mitarb. [5], daß unbehandelte Patienten mit stationärer Verlaufsform der CLL höhere T-Lymphozyten aufweisen als behandelte Fälle mit Progredienz, scheint bei Betrachtung der Mittelwerte der T-Zellen mit den Ergebnissen bei unseren Patienten übereinzustimmen.

Unter Berücksichtigung der einzelnen Krankheitsstadien ergibt sich jedoch, daß keine Beziehungen zwischen der Höhe der T-Zellen und dem Schweregrad der Erkrankung bestehen. Die Streuung der Ergebnisse ist in den einzelnen Gruppen zu groß, um daraus eine feste Beziehung abzulesen. Wir kommen somit zu dem Schluß, daß erhöhte T-Zellzahlen nicht in allen Fällen als Ausdruck eines stationären Verlaufes anzusehen sind, wir haben aber den Eindruck, daß die erniedrigten T-Zellzahlen als Folge der Therapie anzusehen sind. Jønsson [18] beobachtete unter zytostatischer Therapie eine Abnahme der Zahl der T- und B-Lymphozyten bei Zunahme der sogenannten O-Zellen, die sich mit Hilfe von konventionellen immunologischen Markierungen nicht darstellen lassen, eine Erscheinung, die der Autor darauf zurückführt, daß Zytostatika die immunologischen Funktionen der Lymphozyten zerstören. Die Frage, ob die bei einem unserer Patienten nicht nachweisbaren T-Zellen als Ursache oder Folge der Pneumonie einerseits oder der ECIB anderseits anzusehen ist, kann nicht sicher entschieden werden. Nach Abheilung der Pneumonie konnten unter Fortführung der ECIB wieder T-Zellen nachgewiesen werden.

Die Beobachtung, daß unter ECIB die Anzahl der B-Zellen hoch signifikant

dezimiert wird, ist durch die eingangs angeführten pathophysiologischen Veränderungen der CLL zu erklären, bei der eine Störung der Rezirkulation der B-Lymphozyten vorliegt, die zudem aufgrund ihrer gestörten Immunfunktion durch die ECIB selektiv geschädigt werden können, während die T-Zellen sich im Gegensatz zur zytostatischen Therapie weitgehend der Bestrahlung entziehen können.

Die Erfahrung, daß es möglich ist, mit Hilfe der ECIB das Stadium der CLL zurückzustufen, gibt die Hoffnung, die Prognose dieser Erkrankung möglicherweise entscheidend zu beeinflussen.

Literatur

1. Aisenberg, A. C., Bloch, K. J.: Immunoglobulin on the surface of neoplastic lymphocytes. *New Engl. J. Med. 287*, 272 (1972).
2. Astaldi, G., Astaldi, G. C. B., Paleani Vettori, P., Astaldi, A. Jr.: Spleen irradiation and B/T cell relationship in chronic lymphatic leukaemia. *Lancet 1*, 529 (1975).
3. Auity, F., Coombs, R. R. A., Cerottini, J. C.: Identification, enumeration and isolation of B and T lymphocytes from human peripheral blood. *Scand. J. Immunol. 3*, 521 (1974).
4. Bremer, K., Schick, P., Wack, O., Theml, H., Brass, B., Heimpel, H.: Rezirkulation von Lymphozyten bei Patienten mit malignen lymphatischen Systemerkrankungen. *Blut 24*, 215 (1972).
5. Catovsky, D., Miliani, E., Okos, A., Galton, D. A. G.: Clinical significance of T cells in chronic lymphatic leukaemia. *Lancet 2*, 751 (1974).
6. Cooper, D. A., Petts, V., Luckhurts, J. C.: T and B cell population in blood and lymph node in lymphoproliferative disease. *Brit. J. Cancer 31*, 550 (1975).
7. Dameshek, W.: Chronic lymphatic leukemia — an accumulative disease of immunologically incompetent lymphocytes. *Blood 29*, 566 (1967).
8. Davis, S.: Hypothesis: Differentiation of the human lymphoid system based on cell surface markers. *Blood 45*, 871 (1975).
9. Dellon, A. L.: Percent T cells: An ambiguous reporting technique. *Lancet 1*, 749 (1974).
10. Han, T., Moayesi, H., Minowda, J.: T and B lymphocytes in chronic lymphocytic leukemia; correlation with clinical and immunological status of the disease. *J. nat. Cancer Inst. 57*, 477 (1976).
11. Heilmann, E., Wannenmacher, M., Nordiek, R., Vogel, M.: Extrakorporale Blutbestrahlung zur Frühbehandlung der chronischen Lymphadenose. *Med. Klin. 71*, 1340 (1976).
12. Heilmann, E., Nordiek, R.: Der Einfluß der extrakorporalen Blutbestrahlung auf die T- und B-Lymphozyten des peripheren Blutes bei chronischer Lymphadenose. *Folia haemat. (Lpz.) 104*, 340 (1977).
13. Heilmann, E., Wannenmacher, M., Nordiek, R. Castrup, W., Vogel, M.: Die extrakorporale Blutbestrahlung mittels ^{137}Cs zur Behandlung der chronischen Lymphadenose. *Med. Welt 28*, 120 (1977).
14. Holowiecki, J., Stella, B., Krawezyk, M., Rudzka, E.: T-lymphocyte subpopulation in the blood of patients with chronic lymphatic leukaemia. *Acta haemat. pol. 7*, 111 (1976).
15. Huber, C., Dworzak, E., Fink, U., Michlmayr, G., Braunsteiner, H., Huber, H.: Receptor sites for aggregated gammaglobulin (AGG) on lymphocytes in lymphoproliferative disease. *Brit. J. Haemat. 27*, 643 (1974).
16. Huber, Ch., Huber, H., Menne, H. D., Asamer, H., Flad, H. D.: Gestörte B-Zellrezirkulation bei chronischer Lymphadenose. *Verh. dtsch. Ges. inn. Med. 79*, 547 (1973).

17. Jondal, M., Holm, G., Wigzell, H.: Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. exp. Med. 136*, 207 (1972).
18. Jønsson, V.: Influence of prednisone and cytostatics on human blood B-, T- and O-lymphocytes in diseases. *Scand. J. haemat. 15*, 109 (1975).
19. Lille, J., Desplaces, A., Meeus, L., Saracino, R. T.: Thymus-derived proliferating lymphocytes in chronic lymphatic leukaemia. *Lancet 2*, 263 (1973).
20. Mellstedt, H., Petterson, D.: Lymphocyte subpopulations in chronic lymphatic leukaemia. *Scand. J. Immunol. 3*, 303 (1974).
21. Peter, C. R., Mackenzie, M. R., Galssy, F. J.: T and B cell origin of some non-Hodgkin's lymphomas. *Lancet 2*, 686 (1974).
22. Rabin, B. S., Whiteside, T. L.: Percent T cells. *Lancet 1*, 46 (1976).
23. Rai, K. R., Sawitsky, A., Cronkite, G. P., Chanana, A., Levy, R. N., Pasternak, B. S.: Clinical staging in chronic lymphatic leukemia. *Blood 46*, 219 (1975).
24. Rudders, R. A.: B lymphocyte subpopulations in chronic lymphocytic leukemia. *Blood 47*, 229 (1976).
25. Shohat, B., Joshua, H.: Formation of mouse red cell rosette by lymphocytes from patients with chronic lymphatic leukemia and assessment of T cell function. *Clin. Immunol. 6*, 389 (1976).
26. Wybran, J., Carr, M. C., Fudenberg, H. H.: The human rosette forming cell as a marker of a population of thymus derived cells. *J. clin. Invest. 51*, 2537 (1972).
27. Wybran, J., Fudenberg, H. H.: Thymus derived rosette forming cells in various human disease status. *J. clin. Invest. 52*, 1026 (1973).

Korrespondenz: Fr. Priv.-Doz. Dr. E. Heilmann, Medizinische Poliklinik der Westfälischen Wilhelms-Universität, Westring 3, 4400 Münster, BRD

Correlations between Lymphocyte Subpopulation and Stage of Disease in Chronic Lymphatic Leukaemia of Rai

T- and B-lymphocytes have been studied in 36 patients with chronic lymphatic leukaemia (CLL). Each 12 cases remained untreated or were treated with chlorambucil or extracorporeal irradiation of the blood (ECIB). The patients were classified according to the staging system proposed by Rai et al. In all groups we found significantly elevated B- and T-cells. There was no correlation between the stage of the disease and the proportion of lymphocytes. B-lymphocytes were reduced significantly by ECIB, whereas T-lymphocytes have not been influenced.

Haematologia 11 (3—4), pp. 297—300 (1977)

Relationship between Serum Copper and Bone Marrow Blasts in Acute Leukaemia

L. LEGUTKO

Second Paediatric Department, Medical Academy in Kraków, Kraków, Poland

(Received August 15, 1977)

Investigation of serum copper level (SCL) and bone marrow obtained on the same day from 45 children with acute lymphoblastic leukaemia (ALL) showed a highly significant correlation between SCL and marrow blasts. The increase of SCL was associated with the progress of ALL and the elevation of the marrow parablast percentage. The highest mean SCL level was observed in cases displaying full marrow involvement with concomitant hyperleukocytosis and/or leukaemic tumours. These observations suggest that SCL can be useful in the management of children with acute leukaemia.

Earlier studies showed that the serum copper level (SCL) could be used as an index of the activity and of the efficacy of therapy in lymphoproliferative diseases and leukaemias in children and adults [2, 5—9, 15—20].

The cause of the elevation of SCL in acute leukaemia has not been defined. Some authors suggest that the increase runs parallel to the rise in the concentration of copper-containing oxidases in neoplastic proliferation [3, 5]. Increase of SCL in acute leukaemia is usually preceded by an intensification of coeruloplasmin synthesis or may result from an increase of copper absorption in the digestive tract [5, 7, 8, 11].

The present study was undertaken to determine the possible correlation of SCL with the bone marrow parablast cells (BMPC) in children with acute lymphoblastic leukaemia (ALL).

Material and Methods

SCL was determined in 45 children with ALL (age 2 to 15 years) [1, 10]. On the same day the haemoglobin level, red cell and thrombocyte counts, white cell count, bone marrow examination, liver tests such as bilirubin concentration, transaminases, and serum protein were performed. The total number of SCL determinations was 233. As a control group, 59 healthy children, 31 boys and 28 girls, in the same age range were studied. The normal values in these boys and girls showed no statistically significant difference.

SCL was estimated in blood serum deproteinized with 20% trichloroacetic acid, by means of a Hilger—Watts, Model H 1170 atomic absorption spectro-

photometer [4, 8, 10, 13]. The sample readings were compared with the standard curve and expressed in μl of Cu per dl of serum [8, 10, 13].

The SCL values were analyzed in relation to the degree of the disease activity (Table 1). Arithmetic means were compared by Student's (t) or Welch's (W) tests

Table 1

Serum copper values and marrow parablast percentage in relation to the degree of disease activity

Group		I	II	III	IV
Marrow parablasts (per cent)		0–5	6–25	26–100	26–100*
Number of observations (of children)		83 (45)	76 (41)	53 (26)	21 (17)
Cu	$\bar{x}$ (μg/dl)	133.2	172.9	209.3	254.5
	SD	±20.9	±27.1	±19.9	±42.8
Parablasts	$\bar{x}$ (per cent)	3.2	13.9	74.6	90.3
	SD	±1.51	±5.5	±19.6	±9.6

$\bar{x}$ — arithmetic mean
±SD — standard deviation
Group I — complete remission [1, 12]. Results of SCL excluded in extra-marrow relapses and bacterial or viral infections
Group II — imminent relapse or improvement during treatment [1, 12]
Group III — 1st bone marrow involvement or relapse
Group IV — marrow involvement with peripheral blood hyperparablastosis from 21 to 231 thousand/mm^3 and leukaemic infiltration of testes, kidneys, spleen, mediastinal tumours and hepatosplenomegaly*

[8, 14]. The relationship between SCL and percentage of BMPC was examined by the double correlation tests; coefficients of double correlation (r) between SCL and BMPC were verified by t test according to Fisher's formula [8].

Results

SCL values observed in complete remission ($\bar{x}=132.2\pm20.9$ μg/dl) appeared to be statistically higher ($p < 0.001$) than those obtained in the controls ($\bar{x}=116.4\pm$ ±19.9 μg/dl).

The increase of SCL and that of the marrow paralymphoblast count ran a convergent course of leukaemia proliferation (Table 1). The compared SCL values and mean marrow blast counts were significantly different ($p < 0.001$) in every stage (I to IV) of the disease. The highest mean value for SCL, 254.5 μl/dl, was reached in marrow involvement with accompanying peripheral blood hyperparablastosis and/or organ localization of acute leukaemia* (Group IV – Table 1).

The coefficients of correlation (r) between SCL and BMPC in Groups I, II, III and IV were statistically significant. They showed positive values in Groups II ($r = +0.51$, $p < 0.02$); III ($r = +0.70$, $p < 0.001$); and IV ($r = +0.46$, $p < 0.05$), beside *r* in complete remission (Group I) which was negative and also significant statistically ($r = -0.72$, $p < 0.001$).

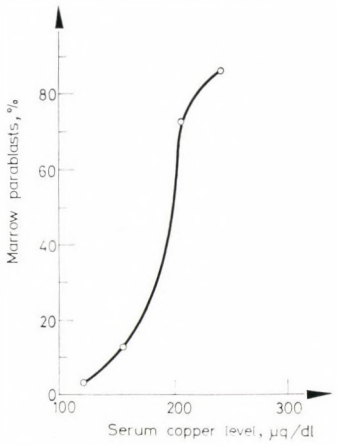


Fig. 1. Correlation between serum copper level and marrow paralymphoblast percentage in acute leukaemia (see Table 1)

Discussion

Tessmer et al. [18] and Hrgovcic et al. [5, 6] found in children with acute leukaemia a high degree of correlation between SCL determined at the beginning of ALL and the marrow parablast count. The increase of SCL in remission (Table 1) confirms the data in the literature according to which SCL in remission of acute leukaemia is higher than that observed in healthy children [20].

The increase in mean SCL values corresponded almost proportionally to the mean marrow blast count with the progress of leukaemia. The disease reached a significantly high level in marrow involvement with accompanying peripheral hyperblastosis or in the presence of leukaemic tumours (Group IV), i.e. in the case of an unfavourable prognosis [1, 12]. It was not the result solely of the near-maximum leukaemic proliferation but also of the general affection of organs by neoplastic tissue similarly as in malignant lymphogranulomatosis [5, 7, 16]. The coefficients of correlation between SCL and BMPC were statistically significant. Its lower value in full marrow involvement with concomitant peripheral blood hyperleukocytosis and/or organ localizations of ALL may result from the neoplastic infiltration of the liver which may additionally disturb copper metabolism.

Increased SCL in children with acute lymphoblastic leukaemia shows a positive correlation with the disease activity. Therapy and resulting improvement cause a decrease of SCL values to normal.

Thus, SCL may offer some information on the degree of marrow paralymphoblast proliferation and allow to reduce the frequency of bone marrow biopsy.

References

1. Aur, R. J. A., Pinkel, D.: Total therapy of acute lymphocytic leukemia. *Progr. clin. Cancer 5*, 155 (1973).
2. Delves, H. T., Alexander, F. W., Lay, H.: Copper and zinc concentration in the plasma of leukaemic children. *Brit. J. Haemat. 24*, 525 (1973).
3. Evans, A. E., Blore, J.: Oxygen consumption of human leukocytes from blood, lymph nodes in patients with leukemia. *Proc. Soc. exp. Biol. (N. Y.) 142*, 1118 (1973).
4. Hilger and Watts. Report No. ZA206. The Determination of Gold, Iron and Copper in Serum. London 1971.
5. Hrgovcic, M., Tessmer, C. F., Brown, B. W., Wilbur, J. R., Mumford, D. M., Thomas, F. B., Shullenberger, C. C., Taylor, G.: Serum copper studies in the lymphomas and acute leukemias. *Progr. clin. Cancer 5*, 121 (1973).
6. Hrgovcic, M., Tessmer, C. F., Thomas, F. B., Ong, P. S., Gamble, J. F., Shullenberger, C. C.: Serum copper observations in patients with malignant lymphomas. *Cancer 32*, 1512 (1973).
7. Jelliffe, A. M.: Value of fluctuations in the serum copper level in the control of patients with Hodgkin's disease. Int. Symp. Hodgkin's Dis. Stanford, Calif., March 20—23, 1972, pp. 325—326.
8. Legutko, L.: Żelazo i miedź w surowicy krwi u dzieci chorych na ostrą białaczkę. Praca doktorska. Akademia Medyczna, Kraków 1976.
9. Massi, M., Vecchi, V., Vivarelli, F., Paolucci, P.: La ceruloplasminemia nella leucosi acuta linfoblastica e nel morbo di Hodgkin della infanzia. *Minerva pediat. 27*, 1223 (1975).
10. Parker, M. M., Humoller, F. L., Mahler, D. J.: Determination of copper and zinc in biological material. *Clin. Chem. 13*, 40 (1967).
11. Pekarek, R. S., Powanda, M. C., Wannermacher, R. W. Jr.: The effect of leucocytic endogenous mediator (LEM) on serum copper and ceruloplasmin concentrations in the rat. *Proc. Soc. exp. Biol. (N. Y.) 141*, 1029 (1972).
12. Pochedly, C.: The Child with Leukemia. Charles C. Thomas, Springfield, Ill. 1973, pp. 196, 209, 211.
13. Przesmycki, J., Król, B.: Oznaczanie miedzi w osoczu metodą spektrofotometrii atomowo-absorpcyjnej (SAA). *Diagn. Lab. 9*, 283 (1973).
14. Remington, R. D., Schork, M. A.: Statistics with Applications to the Biological and Health Sciences. Englewood Cliffs, Prentice-Hall, Inc. 1970.
15. Tessmer, C. F., Hrgovcic, M., Brown, B. W., Wilbur, J., Thomas, F. B.: Serum copper correlation with bone marrow. *Cancer 29*, 173 (1972).
16. Tessmer, C. F., Hrgovcic, M., Wilbur, J.: Serum copper in Hodgkin's disease in children. *Cancer 31*, 303 (1973).
17. Tessmer, C. F., Hrgovcic, M., Thomas, F. B., Fuller, L. M., Castro, J. R.: Serum copper as an index of tumor response to radiotherapy. *Radiology 106*, 635 (1973).
18. Tessmer, C. F., Hrgovcic, M., Thomas, F. B., Wilbur, J., Mumford, D. M.: Long-term serum copper studies in acute leukemia in children. *Cancer 30*, 358 (1972).
19. Thorling, E. B., Thorling, K.: The clinical usefulness of serum copper determination in Hodgkin's disease. *Cancer 38*, 225 (1976).
20. Волосянко, Р. П.: Содержание железа и меди в сыворотке при некотопых заболеваниях крови у детей. *Педятрия 50*, 66 (1971).

Correspondence: Dr. L. Legutko, Krowoderskich Zuchów 23/103 St., 31-271 Kraków, Poland

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

Haematologia 11 (3—4), pp. 301—307 (1977)

Some Aspects of Haemorrheology of Metastasis in Malignant Melanoma

L. DINTENFASS

Haemorrheology Department, Medical Research, Kanematau Memorial Institute, Sydney Hospital and Department of Medicine, University of Sydney, Sydney, Australia

(Received November 22, 1976)

A study of 125 patients with malignant melanoma, of which 30 succumbed to metastases, indicated that plasma viscosity and aggregation of red cells are significantly elevated in patients with metastases. Plots of the intervals between the time of the viscosity test and the time of death against plasma viscosity showed a significant negative correlation; while plots of the same intervals against blood viscosity showed a significant positive correlation.

Viscosity functions (plasma viscosity *vs.* globulin) showed a significant ($P < 0.005$) difference between patients with metastases and patients free from metastases. A significant difference was noted in some functions between patients of A and 0 blood groups.

Introduction

In an earlier report [1] it was noted that the occurrence of metastases in malignant melanoma is associated with an elevation of plasma viscosity. At the time of this report, the number of deceased was only 4, and the number of survivors 26, for which the haemorrheological data were available. A larger number of patients was followed over the subsequent years. Between 1972 and 1975 this study covered 125 patients with malignant melanoma, of which 30 died of metastases of melanoma into heart, brain, liver or kidney.

The aim of the present study was to define some values of the blood viscosity factors in patients, and to explore a hypothesis that metastasis is associated with a significant change in the volume of the blood viscosity factors or blood viscosity functions.

Material and Methods

All patients were Caucasians, mainly of Irish, Scottish and English ancestry. Men outnumbered women only fractionally in this study although among the deceased, men exceeded women by a ratio of 2.5 to 1. Mean age of patients who survived was 40 ± 13 years for females; and the mean age of patients who died from metastases was 48 ± 14 years for males, and 39 ± 16 years for females. Treatment was by surgery.

Blood was obtained by clean venipuncture using disposable needles and plastic syringes. The first portion of blood was used immediately for the study of artificial thrombus formation in the Variable-Frequency-Thromboviscometer (VFTV), the principles of which have been documented [2, 3, 4]. Blood viscosity was determined by means of a rotational rhombospheroid viscometer [5, 6, 7, 8] using blood anticoagulated with EDTA. Plasma viscosity was achieved by the Harkness-Coulter Automatic Capillary Viscometer [9] using 0.3 mm diameter capillary, the test being carried out at 37 °C. Aggregation of red cells was estimated [6, 8, 10] from the erythrocyte sedimentation rates, using EDTA blood without any further dilution with citrate, and employing Westergreen tubes; experimental ESR's were corrected for plasma viscosity (that is, multiplied by plasma viscosity in centipoise) and adjusted to the constant haematocrit of 30%.

Rigidity of red cells was estimated using term 'Tk' of the blood viscosity equation, "$\eta_r = (1 - \mathrm{HTk})\ \mathrm{exp.} = 2.5$" in which H is the haematocrit, given as a volume fraction, and η_r is the relative viscosity of blood, i.e. the viscosity of blood divided by plasma viscosity [6, 8, 11, 12].

Results

Thirty patients, of which 22 were males, died from metastases. Survivors were composed of even groups of men and women.

Results are shown in four tables. Table 1 shows that plasma viscosity and aggregation of red cells were significantly elevated in patients who subsequently succumbed to metastases.

Table 2 shows a series of regressions for the following blood viscosity functions: Blood viscosity measured at 180 sec^{-1} *vs.* haematocrit, the relative viscosity

Table 1

Viscosity of plasma and aggregation of red cells in patients who died with melanoma metastases and in survivors

	n	$\bar{x} \pm SD$	t	P
i. Plasma viscosity, cP				
Deceased	27	1.336 ± 0.166	3.00	<0.0025
Surviving	85	1.234 ± 0.106		
ii. Aggregation of red cells, mm/hr				
Deceased	30	203 ± 101	2.09	<0.025
Surviving	89	159 ± 95		

Note: tests carried out at 37 °C; Student's *t*-test used.

of blood *vs.* haematocrit, plasma viscosity *vs.* globulin level, aggregation of red cells *vs.* apparent viscosity of the artificial red-white thrombus, and rigidity of red cells *vs.* apparent viscosity of the artificial red-white thrombus. These regressions

Table 2

A comparison of patients who died of melanoma metastases and of survivors, by means of linear regressions exploring the correlations between blood viscosity and haematocrit, plasma viscosity and globulin level, aggregation of red cells or rigidity of red cells and the apparent viscosity of artificial red-white thrombi

Patients	Linear regression				Significance			
	n	a	b	$S_{y.x}$	r	F_1	F_2	P
i. Blood viscosity *vs.* haematocrit								
Deceased	30	1.78	0.082	0.86	0.569	8.52	2.69	<0.005
Surviving	95	0.41	0.106	0.53	0.681			
ii. Relative viscosity of blood *vs.* haematocrit								
Deceased	25	0.85	0.078	0.98	0.530	2.00	4.98	<0.001
Surviving	88	0.65	0.078	0.45	0.624			
iii. Plasma viscosity *vs.* globulin								
Deceased	27	0.91	0.124	0.16	0.375	6.94	2.95	<0.005
Surviving	78	0.93	0.096	0.09	0.446			
iv. Aggregation of red cells *vs.* apparent viscosity of artificial red-white thrombus								
Deceased	22	147.8	9.55	88.7	0.491	3.56	13.1*	<0.005
Surviving	74	140.9	4.23	98.3	0.215			
v. Rigidity of red cell (Tk) *vs.* apparent viscosity of artificial red-white thrombus								
Deceased	22	1.0	−0.007	0.16	−0.237	0.0	2.3	<0.05
Surviving	74	1.0	−0.001	0.10	−0.064			

Note: the linear regression equation is $y = a + bx$; $S_{y.x}$ is an error of estimate or a standard deviation from regression; r is the correlation coefficient; P is the significance of differences between regressions obtained from F distribution, in which F_1 corresponds to elevation, and F_2 to residual variance; the relative viscosity of blood is the viscosity of whole blood divided by the viscosity of plasma.

* Slope of regressions.

All statistical calculations, and especially the comparison of linear regressions, were carried out after G. W. Snedecor and W. G. Cochran (Statistical Methods 6th ed., The Iowa State University Press, 1972).

were compared for the deceased and survivor groups, and in each pair of regressions a significant difference was found between the viscosity functions in deceased and in survivors.

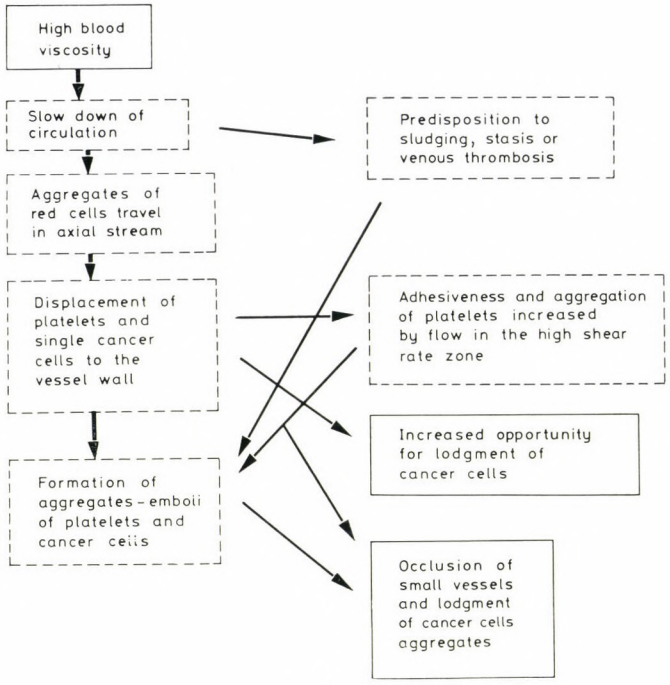


Fig. 1

Table 3

The interval (y) between viscosity test and death *vs.* plasma viscosity (1) and blood viscosity at shear rate of 180 sec^{-1} (2)

Patients	n	a	b	$S_{y.x}$	r	P	$P_{comp.}$
1. *vs.* plasma viscosity							
Deceased	25	28.1	−15.5	5.8	−0.4358	<0.05	
2. *vs.* blood viscosity							
Deceased	30	−6.56	3.0	7.7	0.377	<0.05	
Males only	22	−9.17	3.4	8.1	0.437	<0.05	NS
Females only	8	5.12	0.8	7.5	0.046	NS	

The linear regression used is $y = a + bx$; $S_{y.x}$ is an error of estimate or sample standard deviation from regression; P is the significance of the correlation coefficient r.

The period between the time of viscosity test and the time of death ranged from one month till 30 months, the mean and standard deviation being 9 ± 8 months. Table 3 shows plots of the intervals between tests and deaths against plasma viscosity and blood viscosity at shear rate of 180 sec^{-1}. With the approach of death there appeared to be a significant elevation of plasma viscosity and a significant decrease in the viscosity of blood.

Table 4 shows a comparison of patients with blood groups A and 0. The linear regression showed a significant difference between A and 0 blood groups for aggregation of red cells *vs.* fibrinogen level in patients who survived.

Fibrinogen levels were 447 ± 200 for the metastasis group, and 446 ± 145 mg/dl for the surviving group.

Table 4

Correlations in which the effect of AB0 blood groups appears to be of importance

	Linear regression				Significance		
	n	a	b	$S_{y.x}$	r	F	P
Aggregation of red cells as a function of fibrinogen in survivors							
Blood group A	26	−57	0.61	97	0.61	4.4	<0.01
Blood group 0	49	1	0.40	63	0.66		

Note: significance of differences between regressions was obtained by comparison of elevations, slopes, and residual variance. The regression equation is: $y = a + bx$; $S_{y.x}$ is an error of estimate or standard deviation from regression.

Discussion

Although the frequency of malignant melanoma appears to be the same in men as in women, it is striking that the frequency of metastases (and thus death) is more than two-and-half times higher in men than in women. While the mean age at which melanoma was diagnosed is about the same in men and women, death from metastases takes place in older men (mean, 48 years) but in younger women (mean, 39 years). The reason is obscure, although it is possible that it is linked to the observation of Hersey et al. [13] that pregnancy is a protecting factor against death from melanoma.

Plasma viscosity is significantly elevated in patients with melanoma metastases. Plasma viscosity in survivors is only slightly elevated when compared with the normal values (1.229 + 0.086 cP [8]). A significant elevation of aggregation of red cells on metastasis cannot be ascribed to anaemia as the assessment of aggregation included an adjustment to a constant haematocrit of 30%. Aggregation of red cells in both groups of patients is higher than normal. A similar observation on aggregation of red cells in a study of malignant breast cancer was

reported by Riley [14] and on lung and bowel cancer by Dintenfass and Forbes [15].

The fact that there are significant rheological differences in patients with and without metastases is documented by means of five pairs of linear regressions which relate blood viscosity with haematocrit, relative viscosity of blood with haematocrit, plasma viscosity with the level of globulin, and aggregation of red cells or rigidity of red cells with the apparent viscosity of artificial red-white thrombus. While a comparison of each pair of regressions might not look too convincing (notwithstanding its statistical significance), the very fact that a series of such comparisons yields the same result should inspire confidence into the reality of these observations.

There were significant changes in the level of some viscosity factors as the time of death from metastases was approaching. Plasma viscosity appears to increase, at $P < 0.05$. On the other hand, the viscosity of blood appeared to decrease, at $P < 0.05$, although this effect was not significant in women.

It has been suggested [1, 8] that on elevation of some viscosity factors (especially, aggregation of red cells and plasma viscosity) in melanoma metastasis might represent not only a significant if incidental association, but that a definite vicious circle might exist in which an elevation of viscosity factors may promote metastasis and, in its turn, is reinforced by the subsequent successful metastasis. A hydrodynamic model was suggested in which an elevation of aggregation of red cells leads to the displacement of freely floating cancer cells from the axial region towards the vessel wall where these will undergo buffeting and collisions with the vessel wall and platelets, with an increasing chance of embolization and lodgement [8]. Recent reports of Gastpar [16, 17] suggest that not only an abnormal co-aggregation of platelets with the circulating cancer cells promotes metastasis, but that an inhibition of platelet aggregation inhibits also cancer cell lodgement, at least in rats.

In order to evaluate the effects of metastasis on the rheology of blood, all the types of viscosity factors must be evaluated. Interpretation of some of these factors is most complex, and its complexity appears to be increased by the genetic aspects.

A comparison of linear regressions of such viscosity functions as aggregation of red cells *vs.* fibrinogen level indicates that there exists a definite and significant difference between patients of blood groups A and 0. The very fact that a significant difference exists in blood rheology between patients of AB0 blood groups in ischaemic heart disease, renal failure, hypertension, etc., is well documented [18, 19, 20].

While the increasing rheological complexity of metastasis formation will demand further studies, future explorations should lead to establishment of a firm rationale for rheological diagnosis and prognosis of some form of metastases.

*

I am indebted to Professor G. W. Milton, Head of Melanoma Clinic, for access to patients under his care, and to Mrs. Sylvia Kammer, for excellent technical assistance.

References

1. Dintenfass, L., Milton, G. W.: Blood viscosity factors and prognosis in malignant melanoma. Effect of AB0 blood groups. *Med. J. Aust. 1*, 1091 (1973).
2. Dintenfass, L., Rozenberg, M. C.: The influence of the velocity gradient on *in vitro* blood coagulation and artificial thrombosis. *J. Atheroscler. Res. 5*, 276 (1965).
3. Dintenfass, L.: Dynamics of blood coagulation. Introducing a new coagulation factor "velocity gradient". *Haematologia 1*, 387 (1967).
4. Dintenfass, L., Sharp, A.: Dynamic blood coagulation, thrombus formation and degradation in patients with peripheral vascular diseases (arteriosclerosis, including diabetic). *Ann. Surg. 170*, 984 (1969).
5. Dintenfass, L.: A coaxial rhombo-spheroid viscometer: a further development of the cone-in-cone viscometer. *Biorheology 6*, 33 (1969).
6. Dintenfass, L.: Blood Microrheology, Viscosity Factors in Blood Flow, Ischaemia and Thrombosis. Butterworths, London 1971.
7. Dintenfass, L.: Blood viscosity in healthy men, measured in rhombospheroid viscometer on EDTA blood. A comparison with the cone-in-cone viscometer data and those of Copley. *Biorheology 11*, 397 (1974).
8. Dintenfass, L.: Rheology of Blood in Diagnostic and Preventive Medicine. Butterworths, London 1976.
9. Harkness, J.: A new instrument for the measurement of plasma viscosity. *Lancet 2*, 280 (1963).
10. Dintenfass, L.: Significant effect of AB0 blood groups on the aggregation of red cells in patients with diabetes mellitus. Role of fibrinogen and serum proteins. *Microvasc. Res. 7*, 326 (1974).
11. Dintenfass, L.: Internal viscosity of the red cell: problems associated with definition of plasma viscosity and effective volume of red cells in the blood viscosity equation. *Biorheology 12*, 253 (1975).
12. Dintenfass, L.: The internal viscosity of the red cell and the structure of the red cell membrane. Considerations of the liquid crystalline structure of the red cell interior and membrane from rheological data. *Molecular Crystals-Liquid Crystals 8*, 101 (1969).
13. Hersey, P., Morgan, G., Stone, D. E., McCarthy, W. H., Milton, G. W.: Previous pregnancy as a protective factor against death from melanoma. *Lancet 1*, 451 (1977).
14. Riley, V.: Breast cancer patients: substance in blood causing acceleration of erythrocyte sedimentation rate. *Science 191*, 86 (1976).
15. Dintenfass, L., Forbes, C. D.: Effect of fibrinogen on aggregation of red cells and on apparent viscosity of artificial thrombi in haemophilia, myocardial infarction, thyroid disease, cancer and control systems: effect of AB0 blood groups. *Microvasc. Res. 9*, 107 (1975).
16. Gastpar, H.: Inhibition of cancer cell stickiness by anticoagulants, fibrinolytic drugs, and pyrimido-pyrimidine derivatives. *Hemat. Rev. 3*, 1 (1972).
17. Gastpar, H.: Platelet cancer cell interaction in metastasis formation: a possible therapeutic consequence in metastasis prophylaxis. 16th Int. Congress Hematology, Kyoto, 1976. Abstracts 8—92, p. 343.
18. Dintenfass, L., Forbes, C. D.: GOT, GPT and LDH levels and blood viscosity factors after recent myocardial infarction. Effect of AB0 blood groups. *Haematologia 8*, 323 (1974).
19. Dintenfass, L.: The role of AB0 blood groups in blood rheology of cardiovascular disorders. *Angiology 24*, 442 (1973).
20. Dintenfass, L.: The role of AB0 blood groups in dynamic coagulation and thrombus formation in vascular disease. *Haematologia 5*, 205 (1971).

Correspondence: Dr. L. Dintenfass, University of Sydney, Department of Medicine, Sydney, N. S. W. 2006, Australia

Haematologia 11 (3—4), pp. 309—320 (1977)

I. Increase in Bone Marrow CFU-S and CFU-C after *in vivo* Phytohaemagglutinin (PHA) Administration in Mice*

A. C. LOUWAGIE

Laboratory for Physiopathology-Haematology, Department of Medical Research, University of Leuven, Leuven, Belgium

(Received January 6, 1977)

An absolute increase in bone marrow CFU-S was observed within 4 hours after intraperitoneal administration of 3 mg PHA-M or 250 μg PHA-P. At that time, there was no increase in spleen CFU-S, while a pronounced decrease in the number of circulating CFU-S was noted. A two to fourfold increase in bone marrow CFU-C was found within 12 hours after intraperitoneal administration of a similar dose of PHA-M or PHA-P. Addition of PHA-M or PHA-P at small concentrations resulted in a marked inhibition of granulocytic colony formation whereas macrophage colony growth became depressed only at very high concentrations.

Introduction

Since Nowell [25] made the remarkable observation that phytohaemagglutinin (PHA) possesses mitogenic properties, this agent has been used as a therapeutic tool in aplastic anaemia [2, 12, 15, 17, 18, 28]. Further trials, however, failed to fulfil expectations.

Nevertheless, experimental data suggest that *in vivo* administration of PHA might induce an enhanced proliferation of CFU-S. An increased endogenous colony formation was reported [4, 5, 9, 21]. Treatment of donor mice was found to cause an increase in the number of circulating CFU-S [24] and a rise in the number of splenic CFU-S [7, 30]. Concerning the effect on the bone marrow CFU-S potential conflicting data have been reported. Antal et al. [1] observed an increase after intravenous administration of 35 μg/g PHA-P, while Scaro et al. [30] found little if any effect when mice were treated intravenously with 0.05 to 0.30 ml of their own PHA preparation. Further, Curry and Trentin [9] observed a slight decrease in bone marrow CFU-S, when mice were treated intraperitoneally with 20 μg/g PHA-P for three consecutive days.

It is difficult to compare these data, as the forms and dosage of PHA used and the route of administration were different. The purpose of the present study was to define the effect of a single administration of 3 mg PHA-M or 250 μg PHA-P on the bone marrow CFU-S and CFU-C count and on the spleen and peripheral blood CFU-S potential. As changes in the number of progeny cells might have an influence on stem cells, the effect of PHA-M on peripheral blood and the cellularity of the bone marrow and spleen were also studied.

* This work was supported by FWGO contract No. 1217.

Material and Methods

Adult C_3H mice, aged 10 to 12 weeks, were used. Leucocytes and nucleated bone marrow and spleen cells were counted twice using a Bürker chamber. The differential counts were made on 500 nucleated cells. The low monocyte count was grouped together with the lymphocytes.

CFU-S was counted according to the excolonization technique of Till and McCulloch [31]. The recipient mice received a total body irradiation of 850 r from a Maxitron General Electric apparatus as X-ray source operating at 250 kV and 25 mA. The dose rate was 24 r/min. A half value layer of 1 mm copper and 1 mm aluminium was used, 5×10^4 bone marrow or 5×10^5 spleen cells were given within 4 hours of irradiation. The number of recipient mice was between 6 and 14 in each individual experiment. Results are given for individual experiments and were combined for the controls and at 4 hours after PHA administration.

Phytohaemagglutinin (Difco) was administered intraperitoneally as intravenous administration of 3 mg PHA-M resulted in an immediate high mortality, in a marked decrease in the packed red cell volume and in an increase in spleen weight of the surviving animals. PHA-M and PHA-P were given in a dose of 3 mg and 250 μg, respectively. Lower dosages did not result in a significant increase in CFU-S. Administration of doses of less than 6 mg PHA-M or 500 μg PHA-P did not result in a more pronounced increase in bone marrow CFU-S than after administration of 3 mg PHA-M or 250 μg PHA-P. After higher doses a decrease in bone marrow cellularity, an increase in spleen weight and ascites were observed.

The *in vitro* colony-forming cells, the CFU-C, were assessed by an *in vitro* culture technique similar to that described by Ichikawa et al. [19, 32]. Bone marrow cells were collected in Ham's F 12 medium [16]. 2.5×10^4 bone marrow cells were cultured in a medium containing 2% methylcellulose (Mc 4000 cP) (Fluka). Calf serum, inactivated for 2 hours at 56 °C, was used in a concentration of 20%. The supernatant of a mouse fibroblast culture, clone NCTC-C (Flow), used as a source of colony-stimulating factor, was added in a concentration of 10% of the culture, containing penicillin 1200 U/ml and streptomycin 120,000 μg/ml. The lysate was prepared by addition of 2 ml distilled water to 1 ml blood followed by centrifugation at 3000 c.p.m. for 5 minutes to remove the cell debris. This bone marrow cell suspension was placed in 35 mm Falcon-Petri dishes and cultured for 7 days in a 10% humidified Bekso incubator at 37 °C with a constant flow of air containing 10% CO_2. Four to five cultures were prepared similarly.

The number of colonies was assessed on the seventh day as at that time granulocytic colony formation was maximal. Colonies were counted using an inverted microscope. Only cell aggregates with 50 or more cells were considered a colony.

Results are expressed as their mean and standard error, each time indicating the number of experimental animals used. Significance levels between results

were calculated by Student's t test. As administration of PHA could influence the cellularity and stem cell number, the obtained t value was tested against the two-tailed t value corresponding to a two-tailed probability $P = 0.05$. Thus only values of $P \leq 0.05$ were considered significant statistically. The standard deviation (SD_{wi}) and standard error (SE_{wi}) within groups of animals treated with the same batch were calculated according to Croxton [8]:

$$SD_{wi} = \sqrt{SD^2_{wi}} = \sqrt{\frac{SE^2 \cdot n1\ (n1 - 1) + SE^2 \cdot n2\ (n2 - 1) + \ldots.}{(n1 - 1) + (n2 - 1) + \ldots.}}$$

$$SE_{wi} = \sqrt{\frac{SD^2_{wi}}{N}}$$

n: number of animals in each group,
N: total number of animals treated with the same batch.

Results

The first part of the experiments dealt with the influence of intraperitoneal administration of 3 mg PHA-M on packed red cell volume, reticulocyte number, leucocytes, and number of circulating CFU-S. The second part was related to the effect of PHA-M on bone marrow and spleen cellularity, the bone marrow CFU-S and CFU-C and the spleen CFU-S number. The third part dealt with *in vitro* colony formation in the presence of PHA.

Peripheral blood

Packed red cell volume and reticulocyte count were determined up to 9 days after PHA-M administration (Fig. 1). A significant decrease in the packed red cell volume was observed at 24 hours. This was followed by an increase to slightly subnormal levels on day 3. A progressive increase in reticulocytes, being significant on the second day, was noted. This was followed by a pronounced decrease on the third day after which the values remained low.

No significant change in packed red cell volume and reticulocyte count were observed in saline-treated mice.

The leucocytes were counted at the same time intervals after PHA-M administration. A significant increase in the total leucocyte count was observed 4 hours after PHA administration, which was due to an increase in granulocytes. At that time and even at 24 hours there was a marked decrease in lymphocytes. Using two other batches of PHA-M, various changes in granulocyte numbers were observed in the first 24 hours. There was consistently an immediate and pronounced decrease in lymphocytes which was followed by a return to normal values on day 2. Numerous "lymphoblast-like" cells were observed on the second

and third days. In the saline-treated controls, only small fluctuations in the leucocyte, granulocyte and lymphocyte counts were observed.

The number of CFU-S present in circulating blood was determined at 4 hours, 1, 2 and 3 days after PHA-M administration. Each time 5 donor mice were killed and their blood was pooled. Each of the irradiated recipient mice

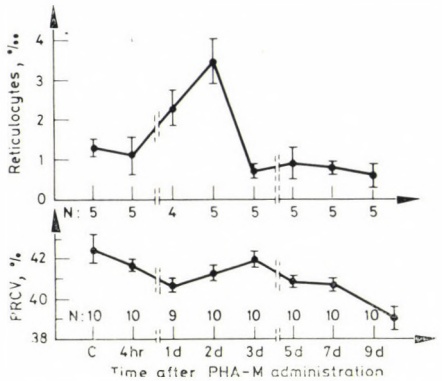


Fig. 1. Packed red cell volume (PRCV) and reticulocyte counts in controls and PHA-M treated mice. N: number of animals. Vertical bars indicate ± standard error of the mean (SEM). Student's *t* test: PRCV: control — days 1, 5, 7 and 9; $P < 0.05$; reticulocyte count: control — days 2 and 3; $P < 0.05$

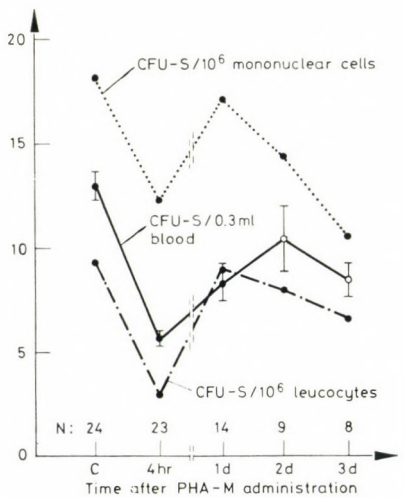


Fig. 2. Circulating CFU-S number (± 1 SEM) in controls and in PHA-M treated donor mice, expressed per 0 3 ml blood injected, or per 10^6 leucocytes or 10^6 mononuclear cells. Student's *t* test: CFU-S/0.3 ml blood: control — 4 hr and days 1 and 3; $P < 0.005$; control — day 2; $0.05 < P < 0.10$

received 0.3 ml of this pooled blood by intravenous injection. Within 4 hours after PHA-M administration, there was a pronounced drop in CFU-S number to below 50% of the control value (Fig. 2). Thereafter there was a progressive rise, but even on the second and third days, the CFU-S number remained subnormal.

Calculating the CFU-S number per 10^6 leucocytes or 10^6 mononuclear cells present in the circulating blood of the PHA-M-treated mice, the decrease in CFU-S, observed at 4 hours after PHA-M administration, was more pronounced than the observed drop in mononuclear cells.

Bone marrow

Cellularity

A slight but not significant decrease in total femoral cellularity was observed from day 5 onwards after the administration of 3 mg PHA-M. The differential count revealed an absolute increase (47.9%) in the erythroblast number 4 hours after PHA administration, being statistically significant for $0.05 < P < 0.10$. This increase was followed by a progressive decrease to below control values on the second day. Subsequently there was a second rise, most pronounced on the seventh day.

The total number of granulocytes and granulocyte precursors was decreased 4 hours after PHA-M administration. Differential counts showed that modifications in the cellularity of this compartment were predominantly due to fluctuations in the number of bone marrow granulocytes and not of the granulocyte precursors.

The lymphocyte count was slightly increased at 4 hours and decreased to values around those of the controls on days 1 to 3. From day 5 onwards, subnormal values were observed.

CFU-S and CFU-C numbers

The effect of PHA on the femoral bone marrow CFU-S and CFU-C potential was studied till 72 hours after administration of 3 mg PHA-M or 250 μg PHA-P.

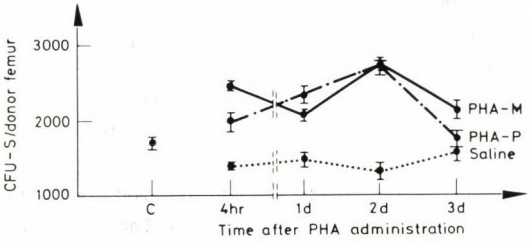


Fig. 3. Femoral CFU-S number at different intervals after saline, respectively PHA-M and PHA-P administration. Student's t test: after PHA-M and PHA-P treatment: spleen colony numbers were significantly different from the controls (untreated or saline treated) for $P < 0.05$ except for day 3

A significant increase in the total bone marrow CFU-S count was observed within 4 hours, reaching a maximum value on the second day (Fig. 3). Using saline-treated animals as a control, no significant changes occurred in bone marrow cellularity and CFU-S number. In order to eliminate potential influence of differences in dosage and of different batches, other experiments were carried out, which gave the following results.

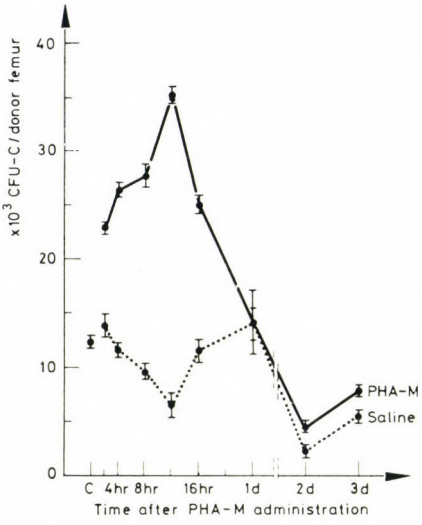


Fig. 4. Femoral bone marrow CFU-C number (± 1 SEM) in untreated controls (C) and at different time intervals after saline, respectively PHA-M treatment *in vivo*. Student's *t* test: the CFU-C number observed at 2, 4, 8, 12 and 16 hours after PHA-M treatment was statistically different from the untreated and saline treated controls for $P < 0.001$

(1) Doses below 3 mg PHA-M or 250 μg PHA-P caused no significant increase in CFU-S 4 hours after administration. Doses of 6 mg PHA-M or 500 μg PHA-P resulted in a significant increase in bone marrow CFU-S, which was not more pronounced than after administration of 3 mg PHA-M or 250 μg PHA-P.

(2) Next to the PHA-M batch four batches were used. A significant increase in CFU-S per 5×10^4 grafted bone marrow cells was observed 4 hours after PHA-M administration. For one out of the four batches, this was not absolute since a marked decrease in bone marrow cellularity was observed.

(3) In another experiment it was shown that the increase in CFU-S was due to the intactness of the PHA molecule and not to the administration of a foreign substance. PHA was denatured by exposure to 100 °C for 30 minutes since it was shown that this procedure results in a loss of mitogenic activity [22]. Administration of heat-incubated PHA-M to donor mice before sacrifice and cell transfer did not result in an increase in CFU-S.

CFU-C

The bone marrow CFU-C potential was evaluated at different time intervals after a single administration of 3 mg PHA-M. The results are presented in Fig. 4; they showed an immediate and pronounced but short increase in CFU-C. The maximum value was reached 12 hours after PHA-M treatment. This increase was followed by a progressive decrease on the second day. Similar results were obtained after administration of 250 μg PHA-P.

Denaturation of PHA by exposure to 100 °C resulted in a marked loss of its stimulating effect.

Spleen

Cellularity

A significant decrease in spleen cellularity was noted 4 hours after PHA-M administration, which was followed by a progressive rise to values slightly above the control on days 2 and 3. During the subsequent days, a progressive decrease was observed.

In contrast to its effect on the bone marrow, there was no immediate increase but a slight decrease in the spleen erythroblast number. A progressive increase in erythroblasts was noted from day 2 onwards, becoming statistically significant on day 7.

The differential count revealed that, also for the spleen, changes in the total number of myeloid cells were predominantly due to fluctuations in the granulocytes.

A significant decrease in lymphocytes was noted at 4 hours which was followed by a progressive increase and values above those of the controls were observed from days 2 to 5, subsequently a gradual decrease was noted. Numerous lymphoblast-like cells were noted on the second and third days.

CFU-S number

A significant increase in CFU-S per 5×10^5 grafted spleen cells was observed 4 hours after administration of 3 mg PHA-M or 250 μg PHA-P. This,

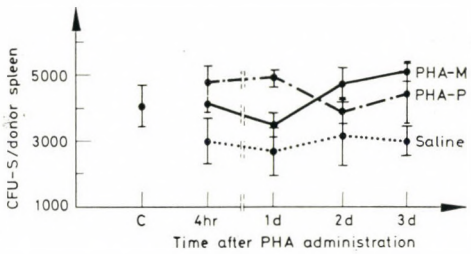


Fig. 5. Spleen CFU-S number ± 1 SEM at different time intervals after saline, respectively PHA-M and PHA-P administration

however, was not an absolute increase in total spleen CFU-S since at the same time there was a decrease in spleen cellularity. A significant increase in the total spleen CFU-S count was only observed 3 days after PHA administration and was nearly equal to the rise of spleen cellularity (Fig. 5).

In vitro colony formation in the presence of PHA

A markedly decreased *in vitro* colony formation was noted after addition of even small amounts of PHA-M or PHA-P (Fig. 6). There was, however, a marked difference between granulocytic and macrophage colony growth. Granulocytic

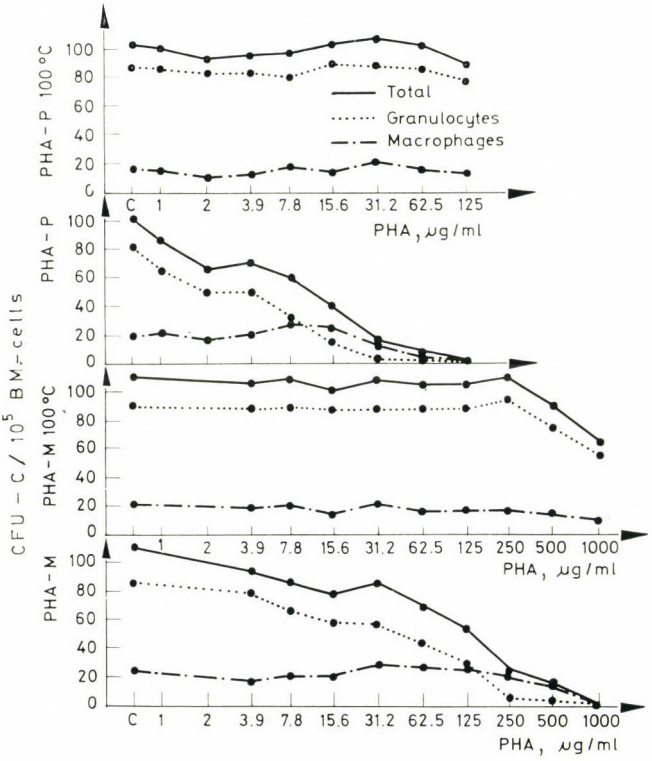


Fig. 6. *In vitro* bone marrow granulocytic and macrophage colony growth in the presence of different concentrations of untreated or heat incubated PHA-M or PHA-P

colony formation was significantly decreased after the addition of PHA-M at a concentration of 2 µg. Macrophage colony growth was severely inhibited only at very high concentrations.

Similarly as *in vivo*, the effect of heat-incubated PHA was also studied. Heat-incubated PHA caused a decrease in colony formation only at very high concentrations.

Discussion

Intraperitoneal administration of 3 mg PHA-M or 250 μg PHA-P was found to elicit an immediate and absolute increase in bone marrow CFU-S and CFU-C potential. There was a slight, not significant increase in spleen CFU-S count and a pronounced decrease in the number of circulating CFU-S. Exposure of PHA-M to heat resulted in a loss of its *in vivo* potentiating effect, suggesting that the effect of PHA-M is related to its intact structure and not to some foreign substance. Addition of PHA *in vitro* resulted in a marked inhibition of granulocytic or colony growth which was not observed when heat-exposed PHA was used.

Regarding the effect of PHA on the CFU-S *in vivo*, these data are not in full agreement with those in the literature. Difference might, however, be due to differences in the doses used and in the route of administration.

First of all we observed a decrease in the number of circulating CFU-S, while Micklem [24] observed an increase. This author, using a similar dosage PHA-M, treated his mice intravenously. Many of them died and there was a toxic effect in the surviving animals such as hepatic necrosis.

Secondly we observed an immediate and significant increase in bone marrow CFU-S. Finally there was no significant increase in spleen CFU-S during the first 24 hours after PHA administration. The increase in total spleen CFU-S number noted on the second and third days was comparable to the increase in spleen cellularity.

Antal et al. [1], administering 35 μg/g PHA-P intravenously, observed a marked decrease in bone marrow cellularity; an increase in the total number of CFU-S was only found several days later when the bone marrow cellularity rose again. Scaro et al. [30] observed an increase in spleen CFU-S 48 to 96 hours after PHA administration but found no increase in bone marrow CFU-S. The latter authors administered an own PHA preparation intravenously to protein-fasted donor mice and used recipient mice rendered aplastic by cytostatic drugs. Curry and Trentin [9] observed a slight decrease in bone marrow CFU-S when mice were treated intraperitoneally with 20 μg/g PHA-P for three consecutive days.

Cerny [7] reported a significant increase in spleen CFU-S concentration 24 hours after the intraperitoneal administration of 200 μg PHA-P, which was ascribed to the release of potentiating factors by spleen lymphocytes. No information was given regarding the influence of this dose on total spleen cellularity.

From the data reported it may be concluded that intravenous administration of PHA-M or PHA-P has a toxic effect on donor mice. Further, it is conceivable that intraperitoneal administration of higher doses of PHA-P given as a single dose or divided in repeated injections, may also have a toxic effect. As in the preliminary experiments, doses of 500 μg PHA-P – being about 20 μg/g – re-

sulted in a marked decrease in bone marrow cellularity, an increase in spleen weight and on higher doses in ascites.

A two to fourfold increase in bone marrow CFU-C was observed within 24 hours after PHA administration. In contrast to its effect on the bone marrow CFU-S, the immediate increase in CFU-C was more pronounced but was no more present two days after PHA administration.

In vitro addition of PHA resulted in a marked depression of colony growth *in vitro*. An inhibition of colony formation after the addition of PHA has been described and this inhibitory effect could be abolished by incubation of PHA either with N-acetyl-D-galactosamine [27] or with periodate [6].

Our data further showed that PHA had a different effect on the granulocytic and macrophage colony formation. Severe inhibition of granulocytic colony formation was observed after small amounts of PHA-M or PHA-P, while macrophage colony growth was severely inhibited only at very high concentrations.

It was concluded that the intraperitoneal administration of low – nontoxic – doses of PHA-M or PHA-P predominantly results in an immediate increase in the bone marrow CFU-S and CFU-C potential. Different possibilities have to be considered to explain this phenomenon. First of all a temporary accumulation of stem cells, secondly a stem cell multiplication and finally an increased lodging of CFU-S in the recipient spleens and an increased colony formation *in vitro* due to the release of colony-stimulating factors.

A temporary accumulation of CFU-S due to a maturation arrest of the progeny cells has to be considered, since 4 hours after PHA-M administration there was an about 50% increase in bone marrow erythroblasts. On a theoretical basis it seems, however, unlikely that after such a short time interval this can result in a 40 to 50% increase in CFU-S, since Lajtha et al. [20] showed that only a small fraction of CFU-S are in DNA phase at any one time and the duplication time for CFU-S is much longer.

The immediate increase in bone marrow erythroblasts has to be ascribed to a temporary accumulation followed by an enhanced release, as there was a significant increase in reticulocytes three days after PHA administration. An increase in CFU-C due to a temporary accumulation is less likely since there were no changes in the number of granulocyte precursor cells. An increase in CFU-C due to an increased generation of CFU-C from the CFU-S compartment seems also unlikely, first of all since there is no decrease but rather an increase in bone marrow CFU-S, secondly 4 to 12 hours is too short a time to result in such an increase.

Since an accumulation of stem cells is less likely, an increase in CFU-S after PHA administration might be due to an alteration in the kinetic state of the CFU-S resulting in stem cell multiplication, as Cerny [7] demonstrated the release of factors that are capable to stimulate CFU-S proliferation. In the absence of stem cell replication, an increase in CFU-S can be due to an augmented lodging in the recipient spleen as has been reported after treatment of donor mice with antigen or endotoxin [3, 13].

In the absence of a CFU-C replication, an enhanced *in vitro* colony formation can be due to the release of additional colony-stimulating factors. Release of substances with colony-stimulating activity by monocytes [10, 11] and by lymphocytes [14, 23, 26, 29] after exposure to plant lectins has been demonstrated.

*

The author wishes to thank Prof. E. Kelemen (Budapest, Hungary) for valuable advices, and to Mrs. Suzy Van Baelen-Nelemans and Mr. Paul Pollaris for excellent technical assistance.

References

1. Antal, S., Fehér, I., Gallyas, A.: Effect of phytohaemagglutinin in subtoxic doses on haemopoiesis in mice. I. Effect on normal mice. *Haematologia 7*, 319 (1973).
2. Aksoy, M., Erden, S., Dincol, K.: Phytohaemagglutinin in refractory anaemia. *Lancet 2*, 1464 (1966).
3. Barnes, D. W., Loutit, J. F.: Effects of irradiation and stimulation on circulating haemopoietic stem cells of the mouse. *Nature (Lond.) 213*, 1142 (1967).
4. Boggs, D. R., Marsh, J. C., Cartwright, G. E., Wintrobe, M. M.: The effect of foreign plasma injections on formation of endogenous hematopoietic colonies. *Exp. Hemat. 12*, 49 (1967a).
5. Boggs, D. R., Marsh, J. C., Chervenick, P. A., Bishop, C. R., Cartwright, G. E., Wintrobe, M. M.: Factors influencing hematopoietic spleen colony formation in irradiated mice. II. The effect of foreign materials. *J. exp. Med. 126*, 851 (1967b).
6. Burns, M. V., Lozzio, B. B.: Inhibition of marrow granulocytic colony formation by phytohaemagglutinin. *J. Cell Physiol. 82*, 277 (1973).
7. Cerny, J.: Stimulation of bone marrow haemopoietic stem cells by a factor from activated T cells. *Nature (Lond.) 249*, 63 (1974).
8. Croxton, F.: Elementary Statistics with Applications of Medicine and the Biological Sciences. Dover Publications Inc., New York 1959.
9. Curry, J. L., Trentin, J. J.: Hemopoietic spleen colony studies. IV. Phytohemagglutinin and hemopoietic regeneration. *J. exp. Med. 126*, 819 (1967).
10. Eaves, A. C., Bruce, W. R.: Colony-stimulating activity following exposure of human blood leukocytes to endotoxin, phytohemagglutinin and concanavalin A. *Exp. Hemat. 1*, 264 (1973).
11. Eaves, A. C., Bruce, W. R.: *In vitro* production of colony-stimulating activity. I. Exposures of mouse peritoneal cells to endotoxin. *Cell Tiss. Kinet. 7*, 19 (1974).
12. Fleming, A. F.: Phytohaemagglutinin. *Lancet 2*, 647 (1964).
13. Fred, S. S., Smith, W. W.: Induced changes in transplantability of hemopoietic colony forming cells. *Proc. Soc. exp. Biol. (N. Y.) 128*, 364 (1968).
14. Golde, P. W., Cline, M. J.: Regulation of human bone marrow leucopoiesis. *Brit. J. Haemat. 26*, 235 (1974).
15. Grunwald, H., Taub, R. N., Wong, G. M., Kiossoglou, K. A., Dameshek, W.: Phytohaemagglutinin in treatment of aplastic anaemia. *Lancet 1*, 962 (1965).
16. Ham, R. G.: Clonal growth of mammalian cells in a chemically defined synthetic medium. *Proc. nat. Acad. Sci. (Wash.) 56*, 488 (1965).
17. Humble, J. G.: *In vivo* action of phytohaemagglutinin in severe human aplastic anaemia. *Nature (Lond.) 198*, 1313 (1963).
18. Humble, J. G.: The treatment of aplastic anaemia with phytohaemagglutinin. *Lancet 1*, 1345 (1964).

19. Ichikawa, Y., Pluznik, D. H., Sachs, L.: *In vitro* control of the development of macrophage and granulocytic colonies. *Proc. nat. Acad. Sci. (Wash.) 56*, 488 (1966).
20. Lajtha, L. G., Pozzi, L. V., Schofield, R., Fox, M.: Kinetic properties of haemopoietic stem cells. *Cell Tiss. Kinet. 2*, 39 (1969).
21. Lopez, A., Lozzio, B. B.: Effect of phytohemagglutinin on hematopoietic depression produced by radiation. *Exp. Hemat. 22*, 90 (1972).
22. Machado, E., Lozzio, B. B., Chernoff, A. I.: Functional and cellular alteration produced by phytohaemagglutinin. II. Depression of antibody formation and proliferation of lymphopoietic system. *Arch. Path. 88*, 111 (1969).
23. McNeill, T. A.: Release of bone marrow colony stimulating activity during immunological reactions *in vitro*. *Nature New Biol. 244*, 175 (1973).
24. Micklem, H. S.: Effect of phytohemagglutinin-M on the spleen-colony-forming capacity of mouse lymph node and blood cells. *Transplantation 4*, 732 (1966).
25. Nowell, P. C.: Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res. 20*, 462 (1960).
26. Parker, J. W., Metcalf, D.: Production of colony-stimulating factors in mitogen-stimulated lymphocyte cultures. *J. Immunol. 112*, 502 (1974).
27. Pluznik, D. H., Guttmann, R., Steckelman, I.: Sensitivity to cytotoxic action of PHA of normal hemopoietic stem cells and leukemic cells. *Exp. Hemat. 1*, 262 (1973).
28. Retief, F. P., Wasserman, H P., Hofmeyer, N. H.: Phytohaemagglutinin in aplastic anaemia. *Lancet 2*, 1343 (1964).
29. Ruscetti, F. W., Chervenick, P. A.: Release of colony-stimulating activity from thymus-derived lymphocytes. *J. clin. Invest. 55*, 520 (1975).
30. Scaro, J. L., Carrera, M. A., Teruel, J. E., Alvarez Prado de Trombolesi, R.: Phytohaemagglutinin and haemopoietic stem cells in the mouse. *Acta haemat. (Basel) 46*, 275 (1971).
31. Till, J. E , McCulloch, E. A.: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res. 14*, 213 (1961).
32. Worton, R. G., McCulloch, E. A., Till, J. E.: Physical separation of hemopoietic stem cells forming colonies in culture. *J. Cell Physiol. 74*, 171 (1969).

Correspondence: Dr. A. C. Louwagie, Laboratory for Physiopathology-Haematology, Department of Medical Research, University of Leuven, Leuven, Belgium

Haematologia 11 (3–4), pp. 321–330 (1977)

II. Mechanism of Increased Colony Formation of CFU-S and CFU-C after *in vivo* PHA Administration in Mice*

A. C. LOUWAGIE

Laboratory for Physiopathology-Haematology, Department of Medical Research, University of Leuven, Leuven, Belgium

(Received January 6, 1977)

In vivo administration of 3 mg PHA-M results in an increase in spleen colonies due to an augmented seeding of CFU-S into the spleen. An increase in the number of CFU-S in the developing nine-day spleen colonies also occurred together with a slight increase in the number of CFU-S in DNA-S phase 24 hours after PHA-M administration. The increased colony formation was due to an increased number of CFU-C from the early culture period onwards but could not be explained by an increase in the number of CFU-C in DNA/S phase. The relase of colony-stimulating substances was proved but this only partially accounted for the observed rise in bone marrow CFU-C.

Introduction

In a previous study it was shown that intraperitoneal administration of PHA resulted in a significant increase in the number of bone marrow CFU-S and CFU-C. The increase in CFU-S was less pronounced but lasted two days, while there was a two to fourfold increase in CFU-C within 24 hours after PHA administration followed by a sharp decrease during the following days [14].

The present study was aimed at clarifying the mechanisms which might explain the immediate rise of CFU-S and CFU-C. Since an increase in stem cell numbers due to a maturation arrest of the progeny cells seems unlikely, the increase in the bone marrow CFU-S and CFU-C potential may have been due to a stimulated stem cell multiplication or to an increased *in vivo* and *in vitro* colony formation without an increased stem cell replication. Both possibilities have been considered. The first part of the study is concerned with the increase in CFU-S while the second set of experiments dealt with the increase in CFU-C number.

The data presented revealed an increased lodging of CFU-S in the spleen as well as an increase in the number of CFU-S present in the developing nine-day spleen colonies. Release into the peripheral blood of substances with colony-stimulating activity has been proved.

* This work was supported by FWGO contract No. 1217.

Material and Methods

CFU-S number was determined according to the excolonization technique of Till and McCulloch [20]. Recipient mice received a total body irradiation of 850 r, the dose rate being 24 r/min (for further details see [14]).

The *in vitro* colony-forming cells, the CFU-C, were assessed by an *in vitro* culture technique similar to that described by Ichikawa et al. [11] and Worton et al. [21].

The number of stem cells reaching the spleen was determined 24 hours after cell transfer, since at this stage the spleen reaches its smallest volume and the number of spleen colonies observed on the ninth day corresponding to the amounts of CFU-S present in the spleen [13]. Primary recipient mice were grafted with 2×10^6 bone marrow cells derived from saline or PHA-M-treated donor mice. Twenty-four hours after cell transfer, their spleen was removed and the number of CFU-S present in these spleens was assessed by injection of 4.2 to 5×10^6 spleen cells into a secondary group of irradiated recipient mice. The number of CFU-S injected in the primary recipient mice was calculated from the spleen colony number in mice grafted with 5×10^4 bone marrow cells.

The number of CFU-C present in the developing spleen colonies nine days after cell transfer was determined according to Schofield and Lajtha [19]. Primary recipients were grafted with 10^5 bone marrow cells derived from donor mice treated with either 0.3 ml saline or 3 mg PHA-M 4 hours before sacrifice and cell transfer. On day 9 the spleens were removed and a homogeneous spleen cell suspension was prepared. Secondary recipients were grafted with 10^6 cells of this spleen cell suspension and the number of developing spleen colonies was counted on the ninth day. From the number of spleen colonies observed in the secondary recipients, the number of CFU-S present in the primary recipient spleen was calculated.

The number of stem cells in the phase of DNA synthesis [5] was determined by the *in vitro* thymidine suicide technique [3, 12]. The bone marrow was collected either in cold Hanks or Fisher's medium and diluted to a concentration of 5×10^5 bone marrow cells/ml. These cell suspensions were prewarmed in a rotating water bath at 37 °C for 10 minutes. To one of the cell suspensions 200 μC C_3H methyl-6-thymidine (CEN, SK, Mol, Belgium) of 22 Ci/mM specific activity was added. Cold thymidine was used as a control in the CFU-C assay. After 30 min incubation, the cell suspensions were diluted with cold Fisher's medium to a final concentration of 2.5×10^5/ml.

Results

CFU-S

Since 4 hours after PHA administration there was a significant increase in bone marrow CFU-S, experiments were performed to find out if the increase was due to an augmented number of CFU-S present in the cell suspension at the

time of cell-grafting or, in the absence of a CFU-S replication, to an increase in the amount of CFU-S lodging in the spleen.

In a last experiment the number of CFU-S present in the developing spleen colonies was determined for mice grafted with bone marrow derived from saline or PHA-M treated donor mice.

Number of CFU-S lodging in the spleen

The number of CFU-S reaching the spleen was determined in recipient mice 24 hours after grafting of bone marrow cells derived from donor mice treated with 3 mg PHA-M 4 hours before sacrifice. The primary recipient mice were grafted with 2×10^6 bone marrow cells and the secondary recipient mice with either 4.2 or 5×10^6 primary recipient spleen cells. Spleen cellularity of the primary recipients was the same in mice grafted with bone marrow derived from saline

Table 1

Fraction "f" of transplanted bone marrow CFU-S present in the spleen when donor mice were treated with either 0.3 ml saline or 3 mg PHA-M

Haematopoietic cells

5×10^4 BM-cells
X-ray ↓
mice
↓ 9 d
spleen colonies
saline PHA-M
571623 582982

2×10^6 BM-cells
↓ X-ray
primary recipients
↓ 24 hours
spleen cell suspension
↓ X-ray
secondary recipients
↓ 9 d.
spleen colonies

N	29	70	42
Mean	8.97	14.16	13.38
SE	0.47	0.32	0.39

ratio: PHA-M/saline: I. 58 (571623)
I. 49 (582982)

Donor mice (primary recipients)				Recipient mice			
		spleen cellularity ($\times 10^6$)	injected cell ($\times 10^6$)	Spleen colonies on day 9 N	Mean	SE	Ratio PHA-M / saline
571623	saline	24.9	5.00	13	12.51	0.90	1.37
	PHA-M	23.0	5.00	9	17.11	2.09	
					$t = -6.19$	$P<0.001$	
582982	saline	24.6	4.20	21	10.24	0.54	1.61
	PHA-M	23.9	4.20	17	16.51	0.91	
					$t = -2.26$	$0.025<P<0.05$	

and in the PHA-M treated primary donor mice. The number of CFU-S injected was calculated by counting the spleen colonies in another group of irradiated recipient mice grafted with 5×10^4 bone marrow cells.

The results (Table 1) showed that there was a significant increase in the number of CFU-S from the primary recipient spleens when the primary graft has been derived from PHA-treated mice. Compared to the control group, the factor of increase was 1.37 and 1.61 for the first and second experiment, respectively. This factor of increase was of an almost similar magnitude as the difference in the number of spleen colonies observed on day 9 after grafting 5×10^4 bone marrow cells derived from either PHA-M or saline-treated mice, the ratio being 1.58 and 1.49 for the first and second experiment, respectively.

Number of CFU-S in DNA-S phase

The number of bone marrow CFU-S in DNA synthesis was assessed at 4 and 24 hours after *in vivo* administration of 3 mg PHA-M. Using the *in vitro* thymidine suicide technique, no increase in the number of CFU-S in DNA-S phase was observed at 4 hours. Bone marrow derived from mice treated with PHA-M 24 hours previously contained an increased number of CFU-S in S phase as there was an increased CFU-S loss after *in vitro* exposure to tritiated thymidine. Compared to the saline-treated control group the factor of increase was 2.51 and 2.35 in the first and second experiment, respectively. The differences in CFU-S loss between the saline-treated mice and the groups of PHA-M-treated mice were not significant statistically.

CFU-S number present in the nine-day spleen colonies

Secondary recipient mice were grafted with 10^6 spleen cells derived from primary recipient mice on day 9 after cell transfer. In two similar experiments a higher number of CFU-S was found in the developing nine-day spleen colonies when primary donor mice were treated with PHA-M 4 hours before sacrifice and cell-grafting (Table 2).

The number of developing spleen colonies/10^5 grafted bone marrow cells was calculated from the data given in Table 2. The total number of CFU-S present in the primary recipient spleen (Table 2B, line 4) was computed from the spleen cellularity of the primary recipients (line 2) and the number of colonies/10^7 spleen cells (line 3).

The increase in CFU-S in the developing spleen colonies of the primary recipients (line 5) was computed from the number of spleen colonies observed in the primary recipient on the ninth day (line 1) and the total number of CFU-S recovered from these primary spleens (line 4). The ratio between the PHA-M and saline-treated groups was 1.39 and 1.52 in experiments 1 and 2, respectively (line 6).

From the above experiments it may be concluded that

(1) the increase in the number of colonies observed on day 9 is of similar magnitude as the increase in number of CFU-S which can be recovered from the recipient spleens 24 hours after cell transfer;

(2) the increased seeding of CFU-S into the spleen cannot be explained by an increase in the number of stem cells present in the cell inoculum at the time of cell-grafting, a faster replication of some CFU-S may, however, occur in the recipient mice within the first 24 hours after cell-grafting;

(3) there was an increase in the number of CFU-S present in the nine-day spleen colonies, suggesting an increased CFU-S renewal, an increase in CFU-S may, however, also occur if there is a reduced differentiation of the CFU-S.

CFU-C

The increase in CFU-C can be due either to a CFU-C multiplication or to an increased *in vitro* colony formation due to the release of substances with colony-stimulating activity.

Number of colonies at different intervals after cell-plating

The number of developing colonies was studied at different intervals after cell-plating. Since the maximum increase was found at 12 hours after PHA-M administration, donor mice were treated with either 0.3 ml saline or 3 mg PHA-M 12 hours before sacrifice.

As Table 3 shows, after a 4-day culture period there was a significant increase in the number of developing colonies, indicating that the increase in CFU-C is due to a difference in the number of potential *in vitro* colony-forming cells at the onset of the cell culture. As regards the difference in colony number on the fourth and seventh day, the ratio PHA-M/saline was slightly higher on the fourth day. This might indicate that after *in vivo* PHA-M treatment, *in vitro* colony-forming cells reach faster the stage of a visible colony.

Number of CFU-C in DNA-S phase

The number of CFU-C in DNA synthesis was determined 12 hours after administration of 3 mg PHA-M since at that time there was a maximum increase in CFU-C. In two experiments, no major influence on the kinetic state of the CFU-C was found.

Colony-stimulating factors

Since there is no increase in the number of CFU-C in the inoculum at the time of cell-plating, the increased *in vitro* colony formation might be due to an increase in colony-stimulating factors. Therefore, the influence of *in vivo* PHA-M administration on (1) the release into the peripheral blood of substances with

Table 2

Increase in CFU-S/primary spleen colony

	Exp. 1				Exp. 2			
	saline		PHA-M		saline		PHA-M	
A. No. of BM cells injected: 5×10^4; No. of spleen colonies on day 9	9.83	(1.01)	15.88	(0.58)	11.00	(0.41)	14.05	(0.86)
B. Primary recipients grafted with 10^5 BM cells (prim. donor); (1) number of spleen colonies; +	19.66		31.76		22.00		28.90	
(2) number of spleen cells $\times 10^7$ on day 9	3.08		4.96		3.06		5.40	
Secondary recipients grafted with 10^6 spleen cells (prim. recipient — secondary donor); (3) number of colonies/10^7 spleen cells; +	45.00	(3.4)	62.80	(2.9)	64.30	(0.63)	73.00	(0.87)
(4) total number of CFU-S/donor spleen; (2) × (3)	138.57		311.43		196.80		394.34	
(5) increase in CFU-S/primary spleen colony: (4): (1) (on day 9);	7.05		9.81		8.95		13.64	
(6) ratio PHA-M/saline	9.81/7.05=1.39				13.64/8.95=1.52			

( ) standard error
+ data computed from results given in A.

colony-stimulating activity, and (2) the elaboration of CSF by cells present in the cell cultures was investigated.

Bone marrow cells obtained from PHA-M-treated donor mice were plated in the presence of their own whole blood lysate or lysate originating from saline-treated donor mice. As a control, bone marrow from saline-treated donor mice

Table 3

In vitro colony growth of bone marrow cells of mice treated 12 hr previously with saline or PHA-M

	Colonies/10^5 BM-cells			
	day 4	day 7	day 10	
PHA-M				factor of increase in colony number between day 4 — day 7 for
Mean	77.6	265.6	338.4	PHA-M: 4.71
SE	11.36	9.93	12.04	saline: 5.44
Saline				ratio of colony number PHA-M/saline on
Mean	27.2	148.0	153.6	day 4: 2.85
SE	1.50	6.32	7.76	day 7: 2.47

Table 4

Influence of the origin of the lysate on *in vitro* colony growth of bone marrow cells obtained from mice treated 12 hr previously with 0.3 ml saline or 3 mg PHA-M

Marrow (M)	Lysate (L)	Colonies/10^5 BM cells					
		Exp. 1*		Exp. 2		Exp. 3	
		Mean	SE	Mean	SE	Mean	SE
Saline	Saline	141.6	13.48	72.0	6.57	64.8	6.74
Saline	PHA-M	199.2+	13.82	106.4+	4.83	73.6	12.04
PHA-M	PHA-M	307.2	16.90	264.8	5.43	273.6	9.00
PHA-M	Saline	241.3	23.59	173.6++	7.86	241.6++	10.25

* number of plates: 3

+ ^{t}M-saline and L-saline versus M-saline and L-PHA-M: $P<0.05$

++ ^{t}M-saline and L-PHA versus M-PHA and L-saline: $P<0.001$

was cultured in the presence of their own lysate or lysate obtained from PHA-M-treated donor mice. Since the time of PHA-M administration might be of importance, donor mice received PHA-M either 4 or 12 hours before plating the cells. Lysate derived from mice treated with PHA-M 4 hours before sacrifice did not enhance colony formation *in vitro*.

On the other hand, *in vitro* colony formation was significantly increased in two out of the three experiments made with lysate derived from mice treated with PHA-M 12 hours previously (Table 4).

These data thus show that PHA-M when given to mice 12 hours before sacrifice results in the release of colony-stimulating substances in peripheral blood. Still, this accounts only partially for the observed increase in CFU-C as bone

marrow derived from saline-treated mice, in the presence of lysate of PHA-M-treated mice, shows lower colony formation than in the reverse situation. The possibility of an enhanced colony growth due to a continuous CSF production by cells present in the bone marrow inoculum was envisaged since Chan and Metcalf [6] and Moore et al. [16] demonstrated that mononuclear cells present in the bone marrow can produce and release CSF. Bone marrow cells from saline or PHA-M-treated donor mice were therefore cultured in the presence or absence of whole blood lysate and/or of exogenous CSF. In cultures of bone marrow cells obtained from PHA-M-treated mice and cultured in the presence of their own lysate without addition of external CSF, colony formation was moderate which was not observed in the control experiment. In the absence of lysate and exogenous CSF, colony formation was observed only with marrow originating from PHA-treated donor mice.

Discussion

The data presented demonstrate that PHA treatment of donor mice results in an increased number of spleen colonies and also in an increase in the number of CFU-S present in the developing nine-day spleen colonies. The experiment showed an increase in the number of CFU-S lodging in the spleen, in a magnitude similar to the increase in the number of nine-day spleen colonies.

An increased seeding in the spleen has been observed when donor mice were treated with antigen or endotoxin [1, 9]. An increase in CFU-S due to an increased colony-forming efficiency means that higher numbers of the transplanted CFU-S reach the spleen, and one has to assume that PHA-M treatment of donor mice may cause changes in the cell surface which render CFU-S more sticky to the spleen. Although an increase in the number of CFU-S in DNA-S phase at the time of the cell transfer could not be demonstrated, one cannot exclude the possibility of a faster replication of some CFU-S in the recipient mice in the first 24 hours and before settling in the spleen. In addition, a single stem cell may produce more than one colony upon cell transplantation. In fact, Barnes et al. [2] showed that bone marrow cells from irradiated donor mice can produce more than one colony. The possibility that PHA-M treatment of donor mice has a similar effect on the CFU-S cannot be excluded.

The data further showed that PHA-M treatment results in an increase in the number of CFU-S present in the developing nine-day spleen colonies. This can be due to an increased CFU-S replication or to reduced CFU-S differentiation. An increased CFU-S replication is most likely since spleen cellularity is markedly increased when the recipient mice were grafted with bone marrow cells obtained from PHA-M-treated donor mice. Further, the release of factors potentiating stem cell proliferation has been demonstrated by Cerny [4] and Cerny et al. [5] after exposure to phytohaemagglutinin *in vivo* and *in vitro*.

The pronounced increase in CFU-C cannot be ascribed to an augmented seeding from the CFU-S compartment. Firstly, four to twelve hours is too short

a time to result in such a CFU-C increase; secondly, if this would be the case one should expect a decrease in the CFU-S number and that would be in contradiction to the observations. The only possibility which can be considered is a commitment of CFU-S toward CFU-C differentiation but with a temporary persistence of their capacity to form spleen colonies. In this instance, the self-renewal of CFU-S would have been reduced but no such reduction was observed (Table 2).

The increase in CFU-C could not be explained by differences in the number of CFU-C present in the cell suspension at the time of plating. The release of substances with colony-stimulating activity has been proved, and CSF production by exposure to plant lectins of monocytes [7, 8] and of lymphocytes [10, 15, 17, 18] has been demonstrated. Still, the observed increase in CFU-C can only partially be explained by a release of CSF into peripheral blood, since bone marrow cells from PHA-treated donor mice and cultured in the presence of lysate obtained from saline-treated mice, show a higher colony formation than in the reverse situation. Most likely, PHA given to donor mice has stimulated some cells present in the cell suspension to production and elaboration of additional CSF, since bone marrow cells derived from PHA-treated mice cultured in the absence of lysate and exogenous CSF showed some colony formation. In fact, Chan and Metcalf [6] and Moore et al. [16] and many others have shown that mononuclear cells present in the bone marrow can produce and release substances with colony-stimulating activity.

The release of CSF material in peripheral blood and the stimulation of bone marrow cells to CSF production may account for the increased colony formation *in vitro*.

*

The author wishes to thank Prof. E. Kelemen (Budapest, Hungary) for valuable advices, and to Mrs. Suzy Van Baelen-Nelemans and Mr. P. Pollaris for excellent technical assistance.

References

1. Barnes, D. W. H., Loutit, J. E.: Effects of irradiation and antigenic stimulation on circulating haemopoietic stem cells of the mouse. *Nature (Lond.) 213*, 1142 (1967).
2. Barnes, D. W. H., Evans, E. P., Ford, C. E., West, B. J.: Spleen colonies in mice: karyotypic evidence of multiple colonies from single cells. *Nature (Lond.) 219*, 518 (1968).
3. Becker, A. J., McCulloch, E. A., Siminovitch, L., Till, J. E.: The effect of differing demands for blood cell production in DNA synthesis by hemopoietic colony-forming cells of mice. *Blood 26*, 296 (1965).
4. Cerny, J.: Stimulation of bone marrow haemopoietic stem cells by a factor from activated T cells. *Nature (Lond.) 249*, 63 (1974).
5. Cerny, J., Waner, E. B., Rubin, A. S.: T cell products activating stem cells: Further studies on the origin and action of the factor(s). *J. Immunol. 115*, 513 (1975).
6. Chan, S. H., Metcalf, D.: Local production of colony-stimulating factor within the bone marrow. Role of non-hemopoietic cells. *Blood 40*, 646 (1972).

7. Eaves, A. C., Bruce, W. R.: Colony-stimulating activity following exposure of human blood leukocytes to endotoxin, phytohemagglutinin and concanavalin A. *Exp. Hemat. 1*, 264 (1973).
8. Eaves, A. C., Bruce, W. R.: *In vitro* production of colony-stimulating activity. I. Exposure of mouse peritoneal cells to endotoxin. *Cell Tiss. Kinet. 7*, 19 (1974).
9. Fred, S. S., Smith, W. W.: Induced changes in transplantability of hemopoietic colony-forming cells. *Proc. Soc. exp. Biol. (N. Y.) 128*, 364 (1968).
10. Golde, D. W., Cline, M. J.: Regulation of human bone marrow leucopoiesis. *Brit. J. Haemat. 26*, 235 (1974).
11. Ichikawa, Y., Pluznik, D. H., Sachs, L.: *In vitro* control of the development of macrophage and granulocytic colonies. *Proc. nat. Acad. Sci. (Wash.) 56*, 488 (1966).
12. Lajtha, L. G., Pozzi, L. V., Schofield, R., Fox, M.: Kinetic properties of haemopoietic stem cells. *Cell Tiss. Kinet. 2*, 39 (1969).
13. Lord, B. L.: The relationship between spleen colony production and spleen cellularity. *Cell Tiss. Kinet. 4*, 211 (1971).
14. Louwagie, A. C.: I. Increase in bone marrow CFU-S and CFU-C after *in vivo* phytohaemagglutinin (PHA) administration in mice. *Haematologia 11*, 309 (1977).
15. McNeill, T. A.: Release of bone marrow colony-stimulating activity during immunological reactions *in vitro*. *Nature New Biol. 244*, 175 (1973).
16. Moore, M. A. S., Williams, N., Metcalf, D.: Purification and characterisation of the *in vitro* colony-forming cell in monkey hemopoietic tissue *J. Cell Physiol. 79*, 283 (1972).
17. Parker, J. W., Metcalf, D.: Production of colony-stimulating factors in mitogen-stimulated lymphocyte cultures. *J. Immunol. 112*, 502 (1974).
18. Ruscetti, F. W., Chervenick, P. A.: Release of colony-stimulating activity from thymus-derived lymphocytes. *J. clin. Invest. 55*, 520 (1975).
19. Schofield, R., Lajtha, L. G.: Graft size consideration in the kinetics of spleen colony development. *Cell Tiss. Kinet. 2*, 147 (1969).
20. Till, J. E., McCulloch, E. A.: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res. 14*, 213 (1961).
21. Worton, R. G., McCulloch, E. A., Till, J. E.: Physical separation of hemopoietic stem cells forming colonies in culture. *J. Cell Physiol. 74*, 171 (1969).

Correspondence: Dr. A. C. Louwagie, Laboratory for Physiopathology-Haematology, Department of Medical Research, University of Leuven, Leuven, Belgium

Haematologia 11 (3—4), pp. 331—334 (1977)

Favourable Effect of Storage at 0 °C on the O_2 Transport Function of Heparinized Blood*

(Short Communication)

D. Boda, E. Eck

Department of Paediatrics, University Medical School, Szeged, Hungary

(Received December 21, 1976)

The biological value of heparinized blood was found to decrease less on storage at 0 °C than on storage at +4 °C. At 0 °C the P_{50} value decreases substantially later and less, the blood is less acidotic, and the UV absorption of the dialysate is less enhanced than at +4 °C. The findings justify a further search for the conditions suitable for the widespread practical application of storage at 0 °C.

Recent investigations have revealed that the O_2 transport function of blood depends on the metabolism of the red blood cells (RBC), and that the O_2 affinity of haemoglobin is determined by the 2,3-diphosphoglycerate (2,3-DPG) content of the blood [2]. Subsequently, in the research into blood preservation the behaviour of the O_2 transport function has become a primary factor in the assessment of the biological value of stored blood [6, 8]. On this basis, a number of new stabilizers and additives have been recommended and even the regeneration of outdated blood has become possible [9].

It has been found that when heparinized blood is kept in melting ice at 0 °C, its O_2 transport function deteriorates significantly slower than during the usual storage at +4 °C. Our results are reported in the present paper.

Material and Methods

Blood obtained from healthy donors was mixed with 12 μg/ml of heparin, and distributed in 5 ml portions into sterile Wassermann tubes sealed with rubber membranes. Half of the tubes were placed in a glass container, which in turn was immersed in broken ice in a Dewar flask, and the flask was stored in a refrigerator at +4 °C. The melted ice was replaced when necessary. The other tubes were stored in the same way, with the difference that supplementary cooling in ice was omitted.

One tube each was opened on the 1st, 2nd, 3rd, 4th, 7th, 14th and 27th days, and the following data were determined: Acid–base equilibrium, haematocrit, plasma haemoglobin content, UV absorption of the plasma dialysate, P_{50}

* Supported by the Scientific Research Council, Hungarian Ministry of Health (4-24-0301-01-1/B).

Table 1

Data for blood samples stored at +4 °C and 0 °C. Mean ± S.D. ( ): No. of blood samples

Duration of storage	P_{50}, mm Hg	2–3 DPG, μM/ml RBC	UV absorption of plasma dialysate		pH	Standard bicarbonate, mEq/l	Blood RBC, %
			210 nm	240 nm			
	Storage at +4 °C						
0 hr	(7) 24.51 ± 2.71	(7) 3.25 ± 0.71	(7) 0.134 ± 0.057	(7) 0.048 ± 0.021	(7) 7.42 ± 0.10	(7) 20.59 ± 1.43	(7) 41.79 ± 3.46
24 hr	(7) 21.80 ± 3.30	(7) 3.72 ± 0.56	(7) 0.162 ± 0.045	(7) 0.069 ± 0.010	(7) 7.39 ± 0.097	(7) 17.26** ± 1.76	(7) 41.90 ± 3.75
48 hr	(6) 24.50 ± 1.52	(7) 3.81 ± 1.09	(7) 0.223 ± 0.035	(7) 0.085 ± 0.015	(7) 7.38** ± 0.15	(7) 17.14** ± 1.74	(7) 42.39 ± 4.33
72 hr	(7) 23.71 ± 1.68	(7) 3.27 ± 1.68	(7) 0.238 ± 0.036	(7) 0.087 ± 0.014	(7) 7.35** ± 0.16	(7) 15.19** ± 1.23	(7) 41.61 ± 4.16
96 hr	(7) 20.37 ± 3.61	(7) 2.12 ± 1.61	(6) 0.229 ± 0.022	(6) 0.098 ± 0.009	(7) 7.28** ± 0.20	(7) 14.04** ± 1.44	(7) 41.89 ± 3.38
1 wk	(6) 13.77 ± 1.18	(5) 1.46 ± 1.39	(7) 0.34 ± 0.06	(7) 0.125 ± 0.032	(7) 7.15** ± 0.08	(7) 13.32** ± 1.47	(7) 41.69 ± 3.66
2 wk	(7) 12.93 ± 1.27	(3) 0.64 (1.5–0.2)	(6) 0.44 ± 0.12	(6) 0.157 ± 0.030	(7) 7.26** ± 0.21	(7) 12.64** ± 2.38	(7) 43.74 ± 3.64
4 wk	(7) 12.94 ± 0.98	(2) 1.10 ± 0.56	(7) 0.52 ± 0.12	(7) 0.195 ± 0.45	(5) 7.30** ± 0.13	(5) 11.74** ± 1.27	(5) 42.50 ± 2.78
	Storage at 0 °C						
0 hr	(7) 24.51 ± 2.71	(7) 3.25 ± 0.71	(7) 0.134 ± 0.057	(7) 0.048 ± 0.021	(7) 7.42 ± 0.10	(7) 20.59 ± 1.43	(7) 41.79 ± 3.46
24 hr	(7) 22.74 ± 2.84	(7) 4.64 ± 1.70	(7) 0.181 ± 0.051	(7) 0.071 ± 0.020	(7) 7.41 ± 0.033	(7) 19.60 ± 1.29	(7) 39.00 ± 1.41
48 hr	(5) 25.40 ± 0.76	(7) 3.22 ± 1.23	(7) 0.245 ± 0.062	(7) 0.103 ± 0.030	(7) 7.43 ± 0.04	(7) 19.26 ± 2.41	(7) 39.71 ± 2.73
72 hr	(7) 26.17* ± 0.96	(7) 4.39 ± 1.50	(7) 0.23 ± 0.05	(7) 0.097 ± 0.022	(7) 7.44 ± 0.04	(7) 18.33 ± 0.96*	(7) 41.57 ± 6.09
96 hr	(5) 25.78* ± 1.87	(5) 3.76 ± 1.95	(5) 0.21 ± 0.04	(5) 0.091 ± 0.014	(5) 7.34 ± 0.06	(5) 16.76 ± 0.72*	(5) 43.60 ± 7.85
1 wk	(7) 23.81* ± 3.38	(7) 2.51 ± 1.68	(7) 0.29 ± 0.06	(7) 0.119 ± 0.022	(7) 7.31* ± 0.06	(7) 14.99 ± 1.82	(7) 39.14 ± 7.23
2 wk	(7) 19.64* ± 3.66	(7) 2.18 ± 1.61	(7) 0.32* ± 0.06	(7) 0.131 ± 0.020	(6) 7.33 ± 0.07	(6) 13.45 ± 2.51	(6) 38.58 ± 5.47
4 wk	(7) 13.56 ± 1.35	(2) 1.25 ± 0.64	(7) 0.38* ± 0.12	(7) 0.147 ± 0.057	(5) 7.36 ± 0.08	(5) 10.48 ± 0.95	(5) 36.20 ± 9.26

* Compared to storage at +4 °C, the difference was significant statistically ($P < 0.05$)
** Compared to the 0 hr value, the difference was significant statistically ($P < 0.05$)

value (the pO_2 value of 50% saturated blood, in mm Hg, at pH 7.40, at 37 °C), and its 2,3-DPG content.

The acid–base data were determined according to Astrup et al. [1].

UV absorption of the plasma dialysate was measured by our own method [4]. Briefly, 50 μl plasma is dialyzed in 2.5 ml isotonic NaCl for 60 min and the spectrum is recorded in the range 200–350 μm with a Specord UV-Vis recording photometer. In the present study, the extinctions of a dialysate corresponding to a 1 : 50 plasma dilution were measured at 210 and 240 μm.

The P_{50} value of blood was determined by our own mixing technique procedure [3].

2,3-DPG content of RBC was determined with the Sigma No. 665 colorimetric enzymatic kit.

Results

Results for 10 blood samples stored at 0 °C and for 10 controls stored at +4 °C are shown in Fig. and Table 1. It is seen that on storage, the 2,3-DPG content decreased less, UV absorption at 210 and 240 μm increased less and the blood was less acidotic. The haematocrit value and the plasma haemoglobin content did not change as compared to the control.

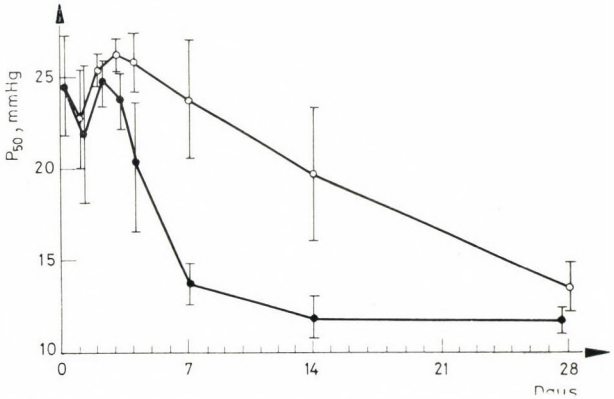


Fig. 1. P_{50} value of blood stored at +4 °C (●) and at 0 °C (○) (mean ± S.D.)

Discussion

The results indicate that storage at 0 °C is accompanied by a significantly smaller decrease in the biological value of blood than if the storage temperature is +4 °C. All our data indicate that this has to be ascribed to the stronger metabolism inhibitory effect of the lower temperature.

As to the UV absorption of the dialysate, it was shown earlier that the change in absorption is due to the formation of metabolites of unknown chemical structure in blood during storage [4]. The present results too showed that UV absorption

is a good indicator of the ageing of heparinized blood and of the associated decrease in its biological value.

Our results obviously suggest the adoption of storage at 0 °C. Technically, this is a simple procedure. In a Dewar flask kept in the refrigerator at +4 °C the ice melts slowly, the functionally more advantageous procedure can be carried out without special additives. Before the widespread application of the procedure it is, however, necessary to control other data indicative of the biological value of preserved blood. The possibility of application is limited by the fact that the favourable effect of storage at 0 °C was observed only with heparinized blood preparations; no essential advantage of storage at 0 °C was found with ACD as stabilizer. Thus, it will have to be clarified whether or not this was simply a consequence of the low pH of the ACD blood preparation.

Few data in the literature deal with blood storage at temperatures lower than +4 °C. Jones [5] suggested that storage at +4 °C was advantageous because of the absence of the bactericidal effect of blood at lower temperatures. At that period, however, the O_2 transport function did not feature among the criteria for assessing blood. More recently, Neville et al. [7] found that the decrease of ATP and 2,3-DPG content of CPD blood was slower at 0 to 2 °C than at +4 °C.

Storage at 0 °C appears risky in view of the danger of freezing and the resulting haemolysis. In our preliminary experiments haemolysis was indeed more marked on storage at 0 °C if the tubes were immersed directly into melting ice. If the blood tubes were placed in a separate container, haemolysis during storage was less than at +4 °C.

References

1. Astrup, P., Siggard-Andersen, O., Jørgensen, K., Engel, K.: The acid-base metabolism. A new approach. *Lancet 1*, 1035 (1960).
2. Benesch, R., Benesch, R. R., Yu, C I.: Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin. *Proc. nat. Acad. Sci. (Wash.) 59*, 526 (1968).
3. Boda, D.: Egyszerű mikromódszer a vér O_2-affinitásának (P_{50}) meghatározására. *Kísérl. Orvostud. 26*, 257 (1974).
4. Boda, D., Gál, Gy., Eck, E.: Ultraviolettspektrophotometrische Untersuchung des Serumdialysats von chronisch intermittierend mit der künstlichen Niere behandelten Patienten. *Z. Urol. 70*, 345 (1977).
5. Jones, N. C. H.: Storage of red cells at temperatures between +10 °C and −20 °C. *Brit. J. Haemat. 4*, 249 (1958).
6. McConn, R., Derrick, J. B.: The respiratory function of blood. Transfusion and blood storage. *Anaesthesiology 36*, 119 (1972).
7. Neville, J. L., Bensinger, T. A., Zuck, T. F.: Effect of temperature on blood storage. *Transfusion 16*, 517 (1976).
8. Schafer, A. W., Tange, L. L., Welch, M. H., Guenter, C. A.: 2,3-Diphosphoglycerate in red cells stored in acid-citrate-dextrose and citrate-phosphate-dextrose. Implications regarding delivery of oxygen. *J. Lab. clin. Med. 77*, 430 (1971).
9. Valeri, C. R., Zaroulis, C. G.: Rejuvenation and freezing of outdated stored human red cells. *New Engl. J. Med. 287*, 1307 (1972).

Correspondence: Dr. D. Boda, Department of Paediatrics, University Medical School, Korányi fasor 18, H-6725 Szeged, Hungary

Haematologia 11 (3–4), pp. 335–348 (1977)

Hämolyse nach extrakorporaler Blutbestrahlung des Menschen?

EDELGARD HEILMANN, M. VOGEL

Medizinische Poliklinik der Westfälischen Wilhelms-Universität, Münster, BRD

(Eingegangen am 14. März, 1977)

Bei 20 Patienten mit chronischer Lymphadenose wurden vor und nach Durchführung von extrakorporalen Blutbestrahlungen Untersuchungen zur Frage der Hämolyse durchgeführt. Freies Hämoglobin, Haptoglobin und Hämopexin erfuhren durch die Bestrahlung keine Änderung, so daß bei dem angewandten Verfahren die akkumulierte Transitdosis keine Hämolyse verursacht.

Schon im Jahre 1921 teilte Heymans [43] mit, daß man eine Leukopenie erzeugen kann, indem man das Blut in einem extrakorporalen Kreislauf mit ionisierenden Strahlen behandelt.

In verschiedenen Studien wurde eine selektive Strahlenempfindlichkeit der Lymphozyten festgestellt [11, 12, 13, 14, 15, 86, 87, 88, 91, 99, 100]. Diese soll auf einer Störung der Sauerstoffutilisation, einer Schädigung der Zellenzyme oder einer Störung des Kohlenhydratstoffwechsels der Lymphozyten beruhen [5, 38, 98].

In den frühen sechziger Jahren erarbeitete eine Forschergruppe um Cronkite in ausgedehnten Versuchen an Kälbern, daß man mit der extrakorporalen Blutbestrahlung eine langandauernde Lymphopenie hervorrufen kann [20, 21, 22, 23, 24, 25, 26].

Zum ersten Mal wandten Lajtha und Mitarb. im Jahre 1962 die extrakorporale Blutbestrahlung beim Menschen zur Therapie verschiedener Leukämieformen an [53, 54, 55, 56].

Durch die extrakorporale Blutbestrahlung erhielt man neue Erkenntnisse über die Kinetik der Lymphozyten und deren Störungen bei der chronischen lymphatischen Leukämie [18, 24, 25, 28, 29, 30, 31, 33, 39, 51, 52, 61, 67, 72, 76, 80, 81, 82, 84].

Klinisch findet die Methode Anwendung zur Immunsuppression vor Organtransplantationen [16, 23, 92, 101, 102, 105], zur Behandlung der akuten myeloischen und der chronisch lymphatischen Leukämie [1, 2, 3, 4, 14, 15, 32, 35, 37, 44, 45, 48, 49, 50, 62, 64, 68, 93, 97]. Neben großen immobilen Strahlern wie ^{60}Co und ^{137}Cs wurden auch kleine portable Strahler mit ^{90}Sr als Quelle entwickelt [6, 7, 28, 65, 66, 70, 71, 90, 104]. Das therapeutische Prinzip der extrakorporalen Blutbestrahlung bei der chronisch lymphatischen Leukämie beruht auf der hohen Strahlensensibilität der leukämischen Lymphozyten [32, 85], die

durch radioaktive Strahlung selektiv geschädigt werden. Durch Zerstörung der im peripheren Blut zirkulierenden Lymphozyten, deren Akkumulation im Krankheitsgeschehen der chronischen Lymphadenose eine zentrale Bedeutung zukommt [8, 27], kann man auch weitere Lymphozyten aus den lymphatischen Bildungsorganen ins Blut drainieren [10, 17, 19, 40, 41, 58, 60, 62, 74, 79].

Einen dieses Verfahren limitierenden Faktor stellt die Schädigung der Erythrozyten mit Ausbildung einer Hämolyse dar. Da diesbezügliche Studien vorwiegend an Tiermodellen durchgeführt wurden und bei Rückschlüssen auf den Menschen keine befriedigende Antwort gegeben haben, untersuchten wir das Problem der intravasalen Hämolyse beim Menschen.

Patientengut und Methodik

Zur Untersuchung gelangten 20 Patienten im Alter zwischen 46 und 79 Jahren, bei denen wegen einer chronischen Lymphadenose in der Zeit von Oktober 1975 bis Januar 1977 extrakorporale Blutbestrahlungen (ECIB) durchgeführt wurden.

Die Behandlungen erfolgten in allen Fällen ambulant. Das extrakorporale Kreislaufsystem wurde durch eine venovenöse Punktion beider Armvenen des Patienten angeschlossen.

Als Bestrahlungsgerät wurde eine 500 Ci Caesium-137-Quelle (Fa. Buchler & Co., Braunschweig) benutzt, mit der pro Sitzung eine Transitdosis von 330 bis 900 rad verabfolgt wurde. Die Bestrahlungsdauer betrug 50 bzw. 100 Minuten bei einem Blutfluß von 80 – 100 ml/min und wurde wöchentlich bzw. alle 4 Wochen durchgeführt. Es wurde eine Transitdosis von minimal 14,200 bis maximal 36,000 rad appliziert. Bei den Patienten wurde vor und unmittelbar nach den Bestrahlungen 8 ml Venenblut zur Bestimmung von freiem Hämoglobin, Hämopexin und Haptoglobin im Serum entnommen. Die Blutproben wurden 10 Minuten lang bei 300 Umdrehungen pro Minute zentrifugiert und die gewonnenen Serumproben bei 2 bis 4 °C im Kühlschrank bis zur Bestimmung gelagert. Es wurden die Mittelwerte vor und nach insgesamt 10 ECIB berücksichtigt.

Die Bestimmungen des freien Hämoglobins wurden photometrisch durchgeführt. Hämopexin und Haptoglobin im Serum wurden mit Hilfe der M-Partigen Immundiffusionsplatten (Behring-Werke, Marburg) nach dem Prinzip der radialen Immundiffusion nach Mancini und Mitarb. [59] ermittelt.

Als Kontrollen dienten Serumproben von 10 gesunden Probanden. Es wurden in allen Fällen Doppelbestimmungen durchgeführt.

Ergebnisse

Die Mittelwerte und Standardabweichungen des freien Hämoglobins im Serum vor und nach insgesamt 10 ECIB der einzelnen Patienten gehen aus Tabelle 1 hervor.

Der Mittelwert und die Standardabweichung des freien Hämoglobins im Serum betrugen bei den 10 gesunden Kontrollpersonen 42,3 ± 3,6 mg/dl.

Aus Abb. 1 gehen die Mittelwerte und Standardabweichungen des freien Hämoglobins im Serum der 20 Patienten vor und nach ECIB hervor. Die darge-

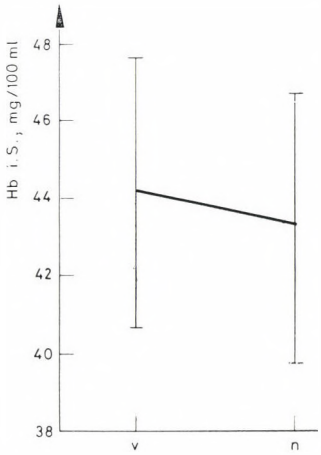


Abb. 1. Mittelwerte und Standardabweichungen des freien Hämoglobins im Serum (Hb i.S.) vor (v) und nach (n) 10 extrakorporalen Blutbestrahlungen bei 20 Patienten mit chronischer Lymphadenose

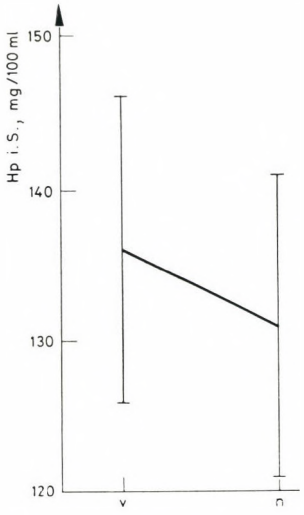


Abb. 2. Mittelwerte und Standardabweichungen des Haptoglobins im Serum (Hp i.S.) vor (v) und nach (n) 10 extrakorporalen Blutbestrahlungen bei 20 Patienten mit chronischer Lymphadenose

stellten Ergebnisse weichen nicht signifikant voneinander ab. Es ergibt sich ebenfalls kein signifikanter Unterschied zu den Kontrollpersonen.

Die Ergebnisse der Mittelwerte und Standardabweichungen der Bestimmungen des Haptoglobins im Serum der einzelnen Patienten gehen aus Tabelle 2 hervor.

Bei den 10 gesunden Kontrollpersonen ergab sich ein Mittelwert des Haptoglobins im Serum von 158, bei einer Standardabweichung von 12,8 mg/dl.

Tabelle 1

Mittelwerte und Standardabweichungen des freien Hämoglobins im Serum (Hb i.S.) bei 20 Patienten mit chronischer Lymphadenose vor und nach 10 extrakorporalen Blutbestrahlungen

Pat. Nr.	Hb i.S. (mg/dl)	
	vor	nach
1	35,4 ± 0,5	30,2 ± 0,5
2	38,7 ± 0,4	35,1 ± 0,3
3	42,5 ± 0,5	42,7 ± 0,5
4	69,2 ± 0,4	65,6 ± 0,5
5	62,8 ± 0,5	64,8 ± 0,6
6	34,8 ± 0,4	28,7 ± 0,3
7	31,8 ± 0,3	36,2 ± 0,4
8	48,6 ± 0,4	44,8 ± 0,3
9	23,0 ± 0,2	22,4 ± 0,2
10	25,0 ± 0,3	24,7 ± 0,3
11	39,8 ± 0,4	42,3 ± 0,4
12	41,4 ± 0,4	43,2 ± 0,4
13	29,8 ± 0,2	27,6 ± 0,2
14	31,7 ± 0,2	28,8 ± 0,2
15	28,5 ± 0,2	30,8 ± 0,2
16	40,5 ± 0,4	39,1 ± 0,4
17	62,0 ± 0,5	63,3 ± 0,5
18	39,3 ± 0,4	41,7 ± 0,4
19	42,4 ± 0,3	38,3 ± 0,3
20	52,2 ± 0,3	53,4 ± 0,4

Abbildung 2 zeigt die Ergebnisse der Mittelwerte und Standardabweichungen des Haptoglobins im Serum vor und nach ECIB bei den Patienten. Die Ergebnisse weichen nicht signifikant voneinander ab.

Es ergibt sich ebenfalls kein signifikanter Unterschied zu den Kontrollpersonen.

Die Ergebnisse der Mittelwerte und Standardabweichungen der Hämopexin-Bestimmungen im Serum (Hx i.S.) vor und nach den ECIB der untersuchten 20 Patienten gehen aus Tabelle 3 hervor.

Der Mittelwert und die Standardabweichung des Hämopexins im Serum betrugen bei den 10 gesunden Kontrollpersonen 104 ± 16,3 mg/dl Serum.

Tabelle 2

Mittelwerte und Standardabweichungen des Haptoglobins im Serum (Hp i.S.) bei 20 Patienten mit chronischer Lymphadenose vor und nach 10 extrakorporalen Blutbestrahlungen

Pat. Nr.	Hp i.S. (mg/dl)	
	vor	nach
1	96±4,8	76±5,7
2	120±7,0	136±6,8
3	125±5,2	144±6,0
4	220±8,4	200±7,6
5	230±9,2	240±9,4
6	144±4,2	120±4,1
7	126±3,8	130±4,0
8	180±6,4	158±6,2
9	160±4,8	168±4,6
10	168±4,4	144±4,2
11	152±4,0	160±3,8
12	134±5,2	116±4,8
13	144±4,8	136±4,6
14	154±4,2	164±4,4
15	106±3,8	100±3,6
16	130±3,4	120±4,2
17	140±4,2	160±4,4
18	180±6,3	156±6,2
19	86±6,2	86±8,0
20	152±3,6	144±4,0

Abbildung 3 stellt die Mittelwerte und Standardabweichungen des Hämopexins im Serum vor und nach ECIB der 20 Patienten mit chronischer Lymphadenose dar.

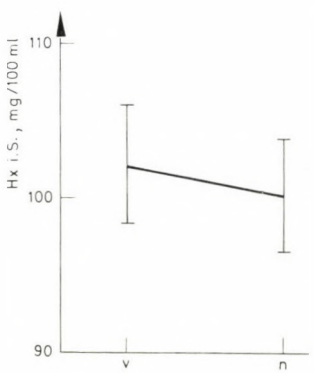


Abb. 3. Mittelwerte und Standardabweichungen des Hämopexins im Serum (Hx i.S.) vor (v) und nach (n) 10 extrakorporalen Blutbestrahlungen bei 20 Patienten mit chronischer Lymphadenose

Tabelle 3

Mittelwerte und Standardabweichungen des Hämopexins im Serum (Hx i.S.) bei 20 Patienten mit chronischer Lymphadenose vor und nach 10 extrakorporalen Blutbestrahlungen

Pat. Nr.	Hx i.S. (mg/dl)	
	vor	nach
1	92±3,8	80±2,9
2	100±4,2	108±5,4
3	90±3,2	74±3,0
4	98±3,0	80±2,9
5	148±4,0	144±4,2
6	114±2,8	128±2.1
7	120±2,6	112±2,4
8	128±2,8	120±2,6
9	96±2,4	90±2,2
10	108±2,6	96±2,4
11	80±2,2	80±2,1
12	92±3,0	100±3,2
13	108±3,4	108±3,3
14	120±3,6	104±3,2
15	90±2,9	104±3,2
16	120±3,1	116±3,3
17	110±3,0	118±3,2
18	124±3,6	120±4,2
19	110±4,1	118±4,0
20	90±3,0	92±3,2

Es ergab sich kein signifikanter Abfall der Meßwerte. Die Hämopexinwerte der Patienten weichen ebenfalls nicht signifikant von denen der Kontrollpersonen ab.

Diskussion

Die extrakorporale Blutbestrahlung hat inzwischen Eingang in die routinemäßig durchführbaren Behandlungsmethoden der chronischen Lymphadenose gefunden. Experimentelle Untersuchungen von Lajtha und Pellerin hatten gezeigt, daß die Dauer der Behandlung nur durch die Strahlenempfindlichkeit der Erythrozyten begrenzt wird [54, 69].

Durch Versuche an eineiigen Kälberzwillingen konnten Schnappauf und Mitarb. zeigen, daß die Strahlenempfindlichkeit der Erythrozyten von deren Lebensalter abhängt [83].

Die Autoren wiesen nach, daß eine Dosis von 50,000 rad 140 Tage alte Kälbererythrozyten mehr schädigt als eine Dosis von 100,000 rad 12 Tage alte Erythrozyten. Diese Tierbefunde konnte Andersen beim Menschen bestätigen [2].

Junge Erythrozyten erwiesen sich beim Menschen als strahlenresistenter als die Gesamterythrozytenpopulation.

Als Mechanismus der Erythrozytenschädigung durch radioaktive Strahlen nimmt Schiffer [82] eine Membranschädigung an, die zu erhöhtem Natriumeinstrom in die Zelle bei gleichzeitigem Verlust von intrazellulärem Kalium führt. Bresciani [9] stellte nach *in vitro* Bestrahlung der Erythrozyten ein Absinken der ATPase-Aktivität fest. Sauerbier [75] diskutiert eine schlagartig einsetzende Inaktivierung von Erythrozytenenzymen. Bei einem Versuch mit Kaninchen starben bis zu 100% der roten Blutkörperchen bereits am zweiten Tag der Bestrahlung. Schiffer [82] bemerkte außerdem, daß bei Dosen bis 100,000 rad die osmotische Resistenz der Erythrozyten sogar zunimmt und erst ab einer Dosis von 200,000 rad signifikant abfällt.

Aus den vorliegenden Literaturbeispielen, die eine Vielzahl von Gründen für eine Schädigung der Erythrozyten anführen, geht hervor, daß die genaue Ätiologie des Strahlenschadens der Erythrozyten von radioaktiven Strahlen im Bereich bis zu 100,000 rad noch nicht sicher bekannt ist. Das Ausmaß des Strahlenschadens hängt nicht nur vom Alter der Zellen ab. Andersen [1, 2, 3], Begemann [8], Honetz [47, 48], Lajtha [56, 57], Meuret [62, 63], Safier [73], Schiffer [78], Schnappauf [83], Sipe [89], Storb [94, 95, 96] und Weeke [101, 102, 103] und ihre Mitarbeiter stellten fest, daß die Schädigung der Erythrozyten dosisabhängig ist. Im Laufe der extrakorporalen Blutbestrahlung akkumulieren die roten Blutkörperchen die Transitdosen, mit denen das Blut bestrahlt wird, um die Lymphozyten abzutöten.

Andersen [2] markierte die Erythrozyten von seinen sechs mittels extrakorporaler Blutbestrahlung behandelten chronischen Lymphadenose-Patienten mit ^{51}Cr und ^{59}Fe. Die normale Halbwertszeit der mit ^{51}Cr markierten Erythrozyten beträgt 26 bis 30 Tage [42]. Bei Patienten mit chronisch lymphatischer Leukämie ist sie laut Frischauf [34] auch ohne extrakorporale Blutbestrahlung verkürzt.

Vor der extrakorporalen Blutbestrahlung betrug die Halbwertszeit bei 6 Patienten im Mittel 14,8 Tage. Nach der extrakorporalen Blutbestrahlung mit Gesamttransitdosen zwischen 45,000 und 75,000 rad betrug sie nur noch 9 Tage. Bereits nach Erreichen von Dosen zwischen 18,500 and 34,000 rad stellte Andersen [2] eine zunehmende ^{51}Cr-Aktivitätsminderung fest, was Ausdruck einer verkürzten Erythrozytenlebensdauer ist.

Bei 3 Patienten wurde außerdem ein Abfall der ^{59}Fe-Aktivität festgestellt. Bei der Auswertung der Resultate mit der ^{51}Cr-Markierungsmethode ist zu beachten, daß erstens nicht sicher festgestellt ist, ob der ^{51}Cr-Aktivitätsverlust bei CLL-Patienten ähnlich ist wie bei Normalpersonen. Zweitens stellte Stohlmann [92] fest, daß die Bestrahlung von radioaktiv markierten Zellen deren ^{51}Cr-Aktivität direkt beeinflußt. Andersen [2] zog den Schluß, daß es zwar bei den 6 Patienten zu einer Verkürzung der Erythrozytenüberlebensdauer gekommen ist, aber der strahleninduzierten Hämolyse mißt er wenig klinische Bedeutung bei. Hollard und Mitarb. [45] wiesen bei Kälbern nach ECIB eine Hyperfibrino-

genämie nach, die beim Menschen bislang nicht beobachtet wurde. Schiffer [78] teilt mit, daß von 16 behandelten Patienten nur bei einem ein erhöhter Bedarf an Bluttransfusionen festzustellen war.

Diese Auffassung wird durch unsere Befunde unterstützt. Frühere Untersuchungen an Hämodialyse-Patienten hatten ergeben, daß das freie Hämoglobin, Haptoglobin und Hämopexin empfindliche Parameter zum Nachweis einer intravasalen Hämolyse darstellen. Die entsprechenden Untersuchungen bei den Patienten mit chronischer Lymphadenose haben bei normalen Ausgangswerten vor der extrakorporalen Blutbestrahlung keine Änderung der Parameter erfahren.

Sowohl unmittelbar als auch nach Durchführung von 10 ECIB konnte kein Anstieg des freien Hämoglobins bzw. kein Abfall des Haptoglobins und des Hämopexins im Serum nachgewiesen werden. Nach Untersuchungen von Gehrmann [36] reicht die Hp-Konzentration des Serums normalerweise aus, um 100 bis 140 mg/dl Hb zu binden. Unter steady-state Bedingungen findet man im Serum 100 bis 300 mg Hp/dl. Es erfolgt eine konstante Elimination des Hp–Hb-Komplexes aus dem Blutstrom von 0.5 bis 0.8 g pro Stunde. Sowohl bei einer Störung der Haptoglobin-Bildung in der Leber als auch bei einer Hämolyse mit gesteigertem Verbrauch an Haptoglobin im Serum kann vermehrt freies Hämoglobin nachweisbar sein. Die ECIB führt bei Patienten mit chronischer Lymphadenose nicht zu Zeichen einer intravasalen Hämolyse. Unsere Befunde stehen in Übereinstimmung mit den Mitteilungen anderer Autoren [2, 8, 47, 48, 62, 63, 77], die mit Gesamttransitdosen bis zu 84,000 rad arbeiteten und keine Hämolyseerscheinungen beobachteten. Sie wiesen darauf hin, daß eine kontinuierliche extrakorporale Blutbestrahlung nicht so effektiv ist, wie eine fraktionierte Bestrahlung. Diese Beobachtungen wurden auch von Parmentier [68], Storb [95], Thomas [98] und ihren Mitarbeitern und von Weeke [100, 104] bestätigt.

Diese Tatsache hat erhebliche Bedeutung für die praktische Durchführung der ECIB. Denn man kann mit der fraktionierten Bestrahlung, wie sie auch von uns durchgeführt wurde, einerseits den Strahlenschaden an den Erythrozyten möglichst gering halten, andererseits erreicht man mit dieser Bestrahlungsart auch den Zweck der extrakorporalen Blutbestrahlung, nämlich einen möglichst hohen Lymphozytenabfall, am besten.

Betrachtet man die bei dieser Behandlungsart akkumulierte Transitdosis der Erythrozyten, so ist zu berücksichtigen, daß die normale Erythrozytenlebensdauer 100–120 Tage beträgt. Diese ist bei den meisten Patienten mit chronisch lymphatischer Leukämie, wie die ^{51}Cr- und ^{59}Fe-Markierungstests von Frischauf [34] zeigen, noch verkürzt.

Der Beobachtungszeitraum unserer Studien betrug etwa 500 Tage. Sämtliche Erythrozyten, die im Oktober 1975 bestrahlt wurden und die Dosis akkumulierten, waren im Januar 1977 nicht mehr vorhanden. Man kann davon ausgehen, daß im März sogar schon die 3. Erythrozytengeneration seit Bestrahlungsbeginn im Blut der Patienten kreiste. Somit ist die akkumulierte Strahlendosis nach jeweils etwa 100 Tagen neu zu berechnen.

Diese Tatsache eröffnet gute Perspektiven für die Langzeitbehandlung der chronischen Lymphadenose mittels extrakorporaler Blutbestrahlung. Berechnet man die Dosis alle 100 Tage neu, so bleiben wir bisher weit unter der von Andersen als kritisch angesehenen Strahlendosis von 25,000 rad.

In der Weise, wie die extrakorporale Blutbestrahlung also von uns angewandt wird, ist keine Nebenwirkung im Sinne einer Hämolyse zu befürchten, die Anlaß zum Abbruch der Therapie der chronisch lymphatischen Leukämie mittels extrakorporaler Blutbestrahlung sein könnte.

Literatur

1. Andersen, V., Sorensen, S. F., Killmann, S. A.: Traitement de la leucémie lymphoïde chronique par l'irradiation extra-corporelle du sang. *Nouv. Rev. franç. Hémat. 8*, 709 (1968).
2. Andersen, V., Karle, H., Dige-Petersen, H.: Haemolysis in patients with chronic lymphocytic leukaemia during extracorporal irradiation of the blood. *Scand. J. Haemat. 7*, 222 (1970).
3. Andersen, V., Weeke, E., Killmann, S. A.: Extracorporal irradiation of the blood: Clinical applications. *Strahlentherapie 148*, 603 (1974).
4. Barnes, B. A., Brownell, G. L., Flex, M. R.: Irradiation of the blood: Method for reducing lymphocytes in blood and spleen. *Science 145*, 1188 (1964).
5. Bartowiak, J., Klyszejko-Stefanowicz, L., Wierzbicki, R.: Selective extraction of histones from normal and irradiated calf lymph nodes. *Int. J. Radiat. Biol. 20*, 173 (1971).
6. Begemann, H., Loose, W., Trepel, F., Pichlmayer, H.: Behandlung der chronisch lymphatischen Leukämie durch extrakorporale Blutbestrahlung. *Klin. Wschr. 46*, 673 (1968).
7. Begemann, H., Czempiel, H., Fink, U., Pichlmayer, H., Rastetter, H., Trepel, F., Wernecke, G.: Therapie der chronischen Lymphadenose. In: Chemo- und Immuntherapie der Leukosen und malignen Lymphome. Herausg.: Bohman, Wien 1969. S. 242.
8. Begemann, H., Theml, H., Fink, U.: Die Therapie der chronisch lymphatischen Leukämie. In: Leukämie und maligne Lymphome. Herausg.: A. Stacher. Urban und Schwarzenberg, Wien 1973. S. 293.
9. Bresciani, F., Aunichio, F., Fiore, C.: Mechanism of X-ray inhibition of sodium pump in human erythrocytes. *Nature (Lond.) 196*, 186 (1962).
10. Binet, J. L., Villeneuve, B., Becat, R., Logeais, Y., Laudat, P., Methey, J.: Temps du passage dans le canal thoracique des lymphocytes du sang de la leucémie lymphoïde chronique. *Nouv. Rev. franç. Hémat. 8*, 621 (1968).
11. Binet, J. L., Villeneuve, B., Vaugier, G., Bernard, J.: Extracorporal irradiation of blood in four cases of chronic lymphocytic leukaemia by ultraviolet light. *Brit. J. Haemat. 17*, 406 (1969).
12. Boggs, D. R., Soffermann, S. A., Wintrobe, M. M., Cartwright, G. E.: Factors influencing the duration of survival of patients with chronic lymphocytic leukemia. *Amer. J. Med. 40*, 243 (1966).
13. Bradfield, J. W. B., Born, G. V. R.: Inhibition of lymphocyte recirculation by heparin. *Nature (Lond.) 222*, 1183 (1969).
14. Bullimore, J. A., Chan, B. W. B., Hayhoe, F. G. J.: Changes in the leukaemic blast cells following extracorporal irradiation of the blood. *Brit. J. Haemat. 17*, 407 (1969).
15. Chan, B. W. B., Hayhoe, F. G. J., Bullimore, J. A.: Effect of extracorporal irradiation of the blood on bone marrow activity in acute leukaemia. *Nature (Lond.) 221*, 972 (1969).

16. Chanana, A. D., Brecher, G., Cronkite, E. P., Joel, D., Schnappauf, H.: The influence of extracorporal irradiation of the blood and lymph on skin homograft rejection. *Radiat. Res. 27*, 330 (1966).
17. Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R., Singer, S., Sipe, C. R.: Studies on lymphocytes. III. Effects of extracorporal irradiation of the circulating blood upon lymphoreticular organs in the calf. *Blood 24*, 241 (1964).
18. Cottier, H., Ruchti, C., Sordat, B., Cronkite, E. P.: Irradiation extra-corporelle du sang circulant: Moyens d'étude de circulation et recirculation lymphocytaires chez le veau. *Nouv. Rev. franç. Hémat. 8*, 679 (1968).
19. Cottier, H., Stoner, R. D., Sipe, C. R., Chanana, A. D., Joel, D. D., Cronkite, E. P.: Differential depletion of lymphoreticular organs by extracorporal irradiation of the circulating blood and its significance for immunological responsiveness. *Brit. J. Haemat. 17*, 412 (1969).
20. Cronkite, E. P., Jansen, C. R., Mather, G., Adamik, E. R., Sipe, C.: The induction of prolonged lymphopenia by extracorporal irradiation of the blood. *Radiat. Res. 16*, 586 (1962).
21. Cronkite, E. P., Jansen, C. R., Mather, G., Adamik, E. R., Nielsen, N., Usenik, E. A., Sipe, C. R.: Studies on lymphocytes. I. Lymphopenia produced by prolonged extracorporal irradiation of circulating blood. *Blood 20*, 203 (1962).
22. Cronkite, E. P., Jansen, C. R., Rai, K. R., Cottier, H., Fliedner, T. M.: The combined application of lymph duct drainage and extracorporal irradiation of the blood in the study of lymphopoesis. In: Proc. Guinness Symposium on Cell Proliferation. Trinity College, Dublin, Ireland, August, 1962. Blackwell Scientific Publications, Oxford 1962. S. 125–156.
23. Cronkite, E. P.: Extracorporal irradiation of the blood in the study of lymphocyte proliferation and function. In: Grundlagenforschung in ihrer Bedeutung für die klinische Medizin. Herausg.: W. Keiderling. Schattauer, Stuttgart 1965. S. 221.
24. Cronkite, E. P., Chanana, A. D., Schnappauf, H. P.: Extracorporal irradiation of blood and lymph in animals: Its effects on homograft and lymphoma. *New Engl. J. Med. 272*, 456 (1965).
25. Cronkite, E. P.: Depletion lymphocytaire par l'irradiation extra-corporelle du sang et de la lymphe. *Nouv. Rev. franç. Hémat. 8*, 643 (1968).
26. Dameshek, W.: Chronic lymphocytic leukemia: An accumulative disease of immunologically incompetent lymphocytes. *Blood 29*, 566 (1967).
27. Dawson, K. B., Field, F. O., Smithers, D. W., Janski, M. N.: Preliminary notes on extracorporal irradiation of the blood in lympho-reticular neoplasia. *Brit. J. Haemat. 40*, 319 (1967).
28. Ewers, W. T.: Treatment of leukaemia by extracorporal irradiation of the blood with a conventional x-ray machine. *Brit. J. Haemat. 17*, 406 (1969).
29. Field, E. O., Dawson, K. B., Hammersley, P. A.: The value of isotopic labelling studies in the treatment of chronic lymphocytic leukaemia by extracorporal irradiation of the blood. *Brit. J. Haemat. 17*, 409 (1969).
30. Field, E. O., Dawson, K. B., Peckham, M. J., Hammersley, P. A., Cooling, C. I., Morgan, R. C., Smithers, D. N.: The response of chronic lymphocytic leukemia to treatment by extracorporal irradiation of the blood as assessed by isotope-labelling procedures. *Blood 36*, 87 (1970).
31. Field, E. O., Sharpe, H. B. A., Dawson, K. B., Andersen, V., Killmann, S. A.: Turnover rate of normal blood lymphocytes and exchangeable pool size in man, calculated from analysis of chromosomal aberrations sustained during extracorporal irradiation of the blood. *Blood 39*, 39 (1972).
32. Fliedner, T. M., Meuret, G.: Die extrakorporale Blutbestrahlung. *Klin. Wschr. 49*, 895 (1971).
33. Fodor, J.: Die Veränderungen der peripheren leukämischen Lymphocyten während

der Strahlenbehandlung und die klinischen Symptome der indirekten Strahlenwirkung. *Strahlentherapie 149*, 162 (1975).
34. Frischauf, H., Honetz, H., Keibl, E.: Erythrokinetische Untersuchungen mit Fe^{59} und Cr^{51} bei chronischen Lymphadenosen. *Blut 18*, 149 (1968).
35. Fröhlich, D., Bock, O.: Ergebnisse der Langzeitbehandlung chronischer Lymphadenosen mit extrakorporaler Blutbestrahlung. *Verh. dtsch. Ges. inn. Med. 79*, 524 (1973).
36. Gehrmann, G.: Hämolyse und hämolytische Anämien. Georg Thieme Verlag, Stuttgart 1969.
37. Gerhardt, P.: Über die Wirkung ionisierender Strahlen auf den Kohlenhydratstoffwechsel der Leukozyten. *Strahlentherapie 138*, 602 (1969).
38. Goodman, W., Hodgson, G. S.: Evidence for stem cells in the peripheral blood of mice. *Blood 19*, 702 (1962).
39. Gowans, J. C.: The life history of lymphocytes. *Brit. med. Bull. 15*, 50 (1959).
40. Greenberg, M. L., Chanana, A. D., Cronkite, E. P., Schiffer, C. M., Stryckmans, P. A.: Extracorporal irradiation of the blood in man: Radiation resistance of circulating platelets. *Radiat. Res. 35*, 147 (1968).
41. Heilmann, E., Wannenmacher, M., Castrup, W., Nordiek, R., Vogel, M.: Extrakorporale Blutbestrahlung zur Frühbehandlung der chronischen Lymphadenose. *Med. Klin. 71*, 1340 (1976).
42. Heilmeyer, L.: Handbuch der Inneren Medizin. 2. Band: Blut und Blutkrankheiten. Springer, Heidelberg 1968. S. 146.
43. Heymans, F.: Iso, hyper et hypothermisation des mammifères par calorification et frigorification du sang de la circulation carotidojugulaire anastomosée. *Arch. int. Pharmacodyn. 25*, 1 (1921).
44. Hollard, D., Suscillon, M., Patet, J., Schaerer, R.: Cytological and cytogenetic study of lymphocytes during treatment of chronic lymphocytic leukaemia by extracorporal irradiation of the blood. *Brit. J. Haemat. 17*, 410 (1969).
45. Hollard, D., Suscillon, M., Barnique, J., Benard, Y., Darnault, J., de Choudens, H., Herbaut, Y., Latreille, R., Limongny, A., Miguet, B., Rambaud, F.: Problèmes techniques posés par l'utilisation experimentale d'une irradiation en circulation extracorporelle. *Nouv. Rev. franç. Hémat. 8*, 1673 (1968).
46. Hollard, D., Bernabid, Y., Laurent, M., Kalodie, L., Schaerer, R., Suscillon, M.: Hyperfibrinogenaemia after extracorporal irradiation of the blood in calves. *Brit. J. Haemat. 17*, 410 (1969).
47. Honetz, N., Frischauf, H., Madl, W., Mittermayer, K., Heumann, E.: Zum Einfluß der extrakorporalen Blutbestrahlung auf Klinik und Lymphocytenfunktion bei chronisch lymphatischer Leukämie. *Verh. dtsch. Ges. inn. Med. 79*, 530 (1973).
48. Honetz, N., Cless-Bernert, T., Frischauf, H., Madl, W., Mittermayer, K.: Über die Behandlung der chronischen Lymphadenose mittels extrakorporaler Blutbestrahlung. In: Leukämie und maligne Lymphome. Herausg.: A. Stacher. Urban und Schwarzenberg, Wien 1973. S. 314.
49. Hornung, G., Hornung, C., Brecher, G., Deckner, K., Schmidt, C. G.: Extrakorporale Blutbestrahlung bei Leukämien. *Med. Welt 23*, 1397 (1972).
50. Jansen, C. R., Cronkite, E. P., Mather, G. M., Nielsen, N. D., Rai, K. R., Adamik, R., Sipe, C. R.: Studies on lymphocytes. II. Lymphocytosis by intravenous heparin in calves. *Blood 20*, 443 (1962).
51. Jansen, C. R., Roos, B. A., Hugo, N., van der Sprey, H. J.: Thoracic duct lymphocytes which have received 1000 rads *in vitro* do not recirculate. *Brit. J. Haemat. 17*, 411 (1969).
52. Karlsson, B. R.: On the equivalence of two models for the blood dose calculation in extracorporal irradiation techniques. *Radiat. Res. 39*, 112 (1969).
53. Kuhl, O. A., Rippberger, W., Huszach, D. W., Cronkite, E. P.: Cobalt 60 medical fluid irradiator: Design, irradiation dose, volume, flow-rate. Considerations and usefulness. *Nucleonics 22*, 79 (1964).

54. Lajtha, L. G., Lewis, C. L., Oliver, R., Cunning, A. J., Sharpe, A. B., Calender, S.: Extracorporal irradiation of the blood: A possible therapeutic measure. *Lancet 1*, 353 (1962).
55. Lajtha, L. G., Garret, J. V., Turner, L., Gilberg, G. W., Halnan, K. E., Easson, E. C., Nutall, P. M.: Extracorporal irradiation of the blood in leukaemic patients. *Brit. J. Haemat. 16*, 39 (1969).
56. Lajtha, L. G., Garret, J. V.: Some conclusions from treatment of human leukaemias with extracorporal irradiation of the blood. *Brit. J. Haemat. 17*, 403 (1969).
57. Lajtha, L. G.: Strahlenbiologische Grundlagen der Leukämiebehandlung. *Verh. dtsch. Ges. inn. Med. 79*, 289 (1973).
58. Little, J. R., Brecher, G., Bradley, T. R., Rose, S.: Determination of lymphocyte turnover by continuous infusion of ^{3}H-thymidine. *Blood 19*, 236 (1962).
59. Mancini, O., Carbonara, A. O., Hermans, J. F.: Immunochemical quantification of antigens by single radial immunodiffusion. *Immunochemistry 2*, 235 (1965).
60. Marsaglia, G., Thomas, E. D.: The radiation dose accumulated by blood during extracorporal irradiation. *Radiat. Res. 25*, 269 (1965).
61. Mauer, A. M., Fisher, V. M.: *In vivo* study of cell kinetics in acute leukaemia. *Nature (Lond.) 197*, 456 (1963).
62. Meuret, G., Schütz, W., Harriss, E. B., Fliedner, T. M., Hoelzer, D., Afkam, J., Obrecht, P., Musshoff, K., Heinze, V., Tourkantonis, A.: Die Behandlung der chronisch lymphatischen Leukämie durch extrakorporale Blutbestrahlung unter Verwendung konventioneller Strahlentherapiegeräte. *Strahlentherapie 137*, 429 (1969).
63. Meuret, G., Fliedner, T. M., Schütz, W., Öhl, N., Afkam, J., Obrecht, P., Musshoff, K.: Erfahrungen mit der extrakorporalen Blutbestrahlung bei der Behandlung der chronisch lymphatischen Leukämie. *Klin. Wschr. 49*, 899 (1971).
64. Musshoff, K.: Möglichkeiten und Indikationen der Strahlentherapie chronischer Leukämien. *Dtsch. med. Wschr. 95*, 1018 (1970).
65. Oldendorf, W. H.: Beta radiation of the circulating blood by an implanted shielded Y^{90} source. *J. nucl. Med. 5*, 883 (1964).
66. Oliver, R., Shepstone, B. J.: Extracorporeal irradiation of the blood: Mathematical problem of dosimetry. *Brit. J. Haemat. 10*, 181 (1964).
67. Pankerd, T. A. J., Clifton, J. C., Emery, E. W., Provan, J., Souhami, R.: Extracorporeal irradiation in acute leukaemia in adults. *Brit. J. Haemat. 17*, 405 (1969).
68. Permentier, C., Chauvel, P., Girard, J. F., Tubiana, M.: La radiothérapie des leucémies lymphoïdes chroniques. *Strahlentherapie 148*, 579 (1974).
69. Pellerin, P., Remy, M. L.: Irradiation extra-corporelle massive et unique du sang chez le rat à doses supérieures à 500.000 rad. *Nouv. Rev. franç. Hémat. 8*, 657 (1968).
70. Pichelmayer, H., Frey, K. W., Trepel, F.: Eine tragbare Strontium-90 Quelle zur Bestrahlung des extrakorporal zirkulierenden Blutes. *Klin. Wschr. 45*, 550 (1967).
71. Pompidou, A., Veyrat, M., Le Go, R., Malarbet, J. L., Prudhomme, J.: Modification du taux des lymphocytes sanguines chez le lapin après irradiation extra-corporelle répétée au césium 137. Action comparée de l'héparine et de l'acénocoumarol. *Biomédicine 23*, 370 (1975).
72. Ruchti, C., Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R.: Studies on lymphocytes. XVII. Differential lymphocyte depletion in lymphoreticular organs of the calf during extracorporeal x-irradiation of the circulating blood. *Cell Tissue Kinet. 3*, 301 (1970).
73. Safier, S., Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R., Wagner, H. P.: Studies on lymphocytes. VI. Evidence showing different generation times for cytologically different lymphoid cell lines in the thoracic duct of the calf. *Blood 30*, 301 (1967).
74. Sasaki, S., Suchi, T.: Mobilization of lymphocytes from lymph nodes and spleen by polysaccharide polysulphate. *Nature (Lond.) 216*, 1013 (1967).
75. Sauerbier, H.: Untersuchungen über die Einwirkung von Roentgenstrahlen auf Erythro-

cyten und ihre Bedeutung für die Ausbildung einer Anämie nach Ganzkörperbestrahlung. *Strahlentherapie 107*, 468 (1958).

76. Scherer, E., Makoski, H.-Br.: Die extrakorporale Blutbestrahlung mit dem Telecäsiumgerät. II. Mitteilung: Morphologische Studien bei Patienten mit chronischer Lymphadenose. *Strahlentherapie 152*, 32 (1976).
77. Schick, P., Theml, H., Kaboth, W., Czempiel, H., Begemann, H., Fliedner, T. M., Maillig, B., Zellner, J., Bremer, H., Boch, O.: Einflüsse von extrakorporaler Blutbestrahlung und Milzbestrahlung auf *in vitro* markierte und autotransfundierte Blutlymphocyten von CLL-Patienten. In: Leukämie und maligne Lymphome. Herausg.: A. Stacher. Urban und Schwarzenberg, Wien 1973. S. 302.
78. Schiffer, L. M., Atkins, H. L., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Johnson, H. A., Robertson, J. S., Stryckmans, R. A.: Extracorporeal irradiation of the blood (ECIB) in man. II. Treatment of acute myelotic leukemia. *Blood 31*, 17 (1968).
79. Schiffer, L. M., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Joel, D. D., Schnappauf, H. P., Stryckmans, P. A.: Extracorporeal irradiation of the blood. *Semin. Haemat. 3*, 154 (1966).
80. Schiffer, L. M., Atkins, H. L., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Johnson, H. A., Robertson, J. S., Stryckmans, P. A.: Extracorporeal irradiation of the blood in humans: Effects upon erythrocyte survival. *Blood 27*, 831 (1966).
81. Schiffer, L. M., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Okuyama, S., Rai, K. R., Robertson, J. S., Stryckmans, P. A., Vincent, P. C.: Lymphocyte kinetics in chronic lymphocytic leukaemia studied by ECIB. *Brit. J. Haemat. 17*, 408 (1969).
82. Schiffer, L. M.: Conference summary: International symposium on extracorporeal irradiation of the blood. *Brit. J. Haemat. 17*, 413 (1969).
83. Schnappauf, H. P., Joel, D. D., Chanana, A. D., DiGiacomo, R., Cronkite, E. P.: The influence of *in vitro* irradiation of blood on red cell survival after auto- and homotransfusion of cells labelled with iron-59 or chromium-51. *Radiat. Res. 25*, 646 (1965).
84. Schoen, H. P.: Strahlentherapie der chronischen Leukosen. In: Leukämie. Herausg.: R. Gross, van de Loo, J. Springer, Heidelberg 1972. S. 595.
85. Schrek, R., Leithold, S. L., Friedman, I. A., Best, W. R.: Clinical evaluations of an *in vitro* test for radiosensitivity of leukemic lymphocytes. *Blood 20*, 432 (1962).
86. Schrek, R., Stefani, S.: Cytotoxic effects of x-rays on normal and leukemic lymphocytes. *Ann. N. Y. Acad. Sci. 13*, 1033 (1964).
87. Sharpe, H. B. A., Dolphin, G. W., Dawson, K. B., Field, E. O.: Chromosomal aberration in lymphocytes from an extracorporeally irradiated patient. *Lancet 2*, 1338 (1967).
88. Shepard, J. S., Ferrari, J.: Stage sensitivity and dose response in human leucocytes. *Radiat. Res. 44*, 855 (1970).
89. Sipe, C. R., Chanana, A. D., Cronkite, E. P., Joel, D. D., Schnappauf, H. P.: The influence of varying doses and repetitive short sessions of extracorporeal irradiation of the blood on the production of lymphopenia. *Radiat. Res. 25*, 684 (1965).
90. Slatkin, D. N., Robertson, J. S.: Extracorporeal irradiation of blood by beta-emitting isotopes: Principles of dose calculations. *Radiat. Res. 44*, 846 (1970).
91. Slatkin, D. N., Jansen, C. R., Cronkite, E. P., Robertson, J. S.: Extracorporeal irradiation of the blood: Calculation of the radiation dose. *Radiat. Res. 19*, 409 (1963).
92. Stohlmann, F. S., Brecher, J. G., Schneidermann, M., Cronkite, E. P.: The hemolytic effect of ionizing radiations and its relationship to the hemorrhagic phase of radiation injury. *Blood 12*, 1061 (1957).
93. Stoner, R. D., Cottier, H., Sipe, C. R., Chanana, A. D., Joel, D. D., Cronkite, E. P.: The effect of extracorporeal irradiation of circulating blood and thoracic duct lymph on tetanus antitoxin responses in calves. *Radiat. Res. 37*, 539 (1969).
94. Storb, R., Epstein, R. B., Rudge, H., Bryant, J., Thomas, E. D.: Marrow engraftment by allogenic leucocytes in lethally irradiated dogs. *Blood 30*, 805 (1967).

95. Storb, R., Epstein, R. B., Buckner, C. D., Thomas, E. D.: Treatment of chronic lymphocytic leukemia by extracorporeal irradiation. *Blood 31*, 490 (1968).
96. Storb, R., Ragde, H., Thomas, E. D.: Extracorporeal irradiation of the blood in baboons. *Radiat. Res. 38*, 43 (1969).
97. Stryckmans, P. A., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Schiffer, L. M.: Studies on lymphocytes. X. Influence of extracorporeal irradiation of the blood on lymphocytes in chronic lymphocytic leukaemia. Apparent correlation with RNA-turnover. *Radiat. Res. 37*, 118 (1969).
98. Thomas, E. D., Epstein, R. B., Eschbach, J. W., Prager, D., Buckner, C. D., Marsaglia, G.: Treatment of leukemia by extracorporeal irradiation. *New Engl. J. Med. 273*, 6 (1965).
99. Wagner, R., Meyerriecks, N., Berman, C. Z.: *In vitro* effects of X-radiation on white blood cells and blood platelets. *Blood 12*, 365 (1957).
100. Weeke, E.: Extracorporeal irradiation of the blood: Effect of varying transit doses on the degree and rate of development of lymphopenia. *Acta med. scand. 191*, 455 (1972).
101. Weeke, E.: Lymphocyte transformation test before, during and after ECIB. *Acta med. scand. 192*, 271 (1972).
102. Weeke, E., Thayssen, J. H.: ECIB: Clinical results after necrokidney transplantation. *Acta med. scand. 193*, 181 (1973).
103. Weeke, E., Andersen, V., Dige-Petersen, H., Karle, H.: Survival of DF ^{32}P-labelled erythrocytes of uraemic patients during ECIB. *Acta med. scand. 194*, 285 (1973).
104. Weeke, E.: ECIB: Further investigations on the effect of varying transit dose, blood flow rate and frequency of treatment on the development of lymphopenia in uraemic patients. *Acta med. scand. 195*, 149 (1974).
105. Weeke, E., Thayssen, J. H.: The effect of ECIB in necrokidney transplantation: A 3 years follow-up study. *Acta med. scand. 195*, 485 (1974).

Anschrift: Priv.-Doz. Dr. Edelgard Heilmann, Medizinische Poliklinik, Wilhelms-Universität, Westring 3, 44-Münster, BRD.

Haemolysis after Extracorporeal Blood Irradiation of Man?

Haemolysis was studied before and after extracorporeal irradiation of the blood of 20 chronic lymphadenosis patients. Free haemoglobin, haptoglobin and haemopexin were not affected by irradiation, indicating that no haemolysis was caused by the transit dose accumulated during the applied procedure.

Haematologia 11 (3—4), pp. 349—357 (1977)

Systemic Mastocytosis

Report of a Case

M. Bláha, J. Kačerovský, Z. Hrnčíř, Z. Nožička, J. Špaček, O. Široký

Second Department of Medicine and Institute of Pathology, Charles University, Faculty of Medicine, Hradec Králové, Czechoslovakia

(Received January 5, 1977)

The case of a 20-year-old woman with systemic mastocytosis is reported. The disorder was not preceded by a clinically diagnosed local form of the disease; fibrous changes in organs, bone changes, eosinophilia and increased serotonin, heparin or histamine levels had not been observed. Clinical symptomatology was typical and the patient died one year after the first signs of the disease.

Mastocytes are cells derived from the reticuloendothelial system. They are normally present in the skin and tissues, but not in blood. They contain numerous basophilic granules and produce heparin, histamine and other substances such as serotonin and those of slow reaction [8]. Their biological function is not quite clear.

Proliferation of mastocytes gives rise to mastocytosis. Clinically, the disease appears in two forms.

1. Exclusively cutaneous form occurs mainly in children and has been known for more than hundred years [7]. It affects exclusively the skin and is usually termed urticaria pigmentosa. About 700 cases have been described of this chronic disease [14]. Improvement or complete disappearance of the skin efflorescences is frequently observed after puberty [12].

2. Generalized malignant disease of the reticuloendothelial system, part of which are mastocytes. Systemic mastocytosis (SM) is a rare disorder. In about 10% of the cases it develops after several years presence of the localized skin form [10]. SM has repeatedly been described in the last twenty years [1, 2, 4, 6, 13—15]. Its course and signs vary to such an extent that two cases with an identical course have never been described [13]. Every new observation is therefore a valuable contribution to the exploration of this uncommon disease. An early diagnosis is also important in view of the fact that good results have recently been obtained with combined cytostatic therapy.

Case Report

J.P., a 20-year-old laboratory technician in a tinned-food factory, had been free of symptoms until March, 1975. Then she had developed low back pain and had been observed in a district hospital for 14 days, with negative findings. Shortly

afterwards she had had severe headaches accompanied by dark red, frequently map-like efflorescences on the skin of the whole body appearing at irregular intervals and seemingly independently of other circumstances. At the same time, she had experienced heat flushes, palpitation, dyspnoea, pain in the weight-bearing joints and had run temperatures of 38 to 39 °C. The symptoms had lasted from several minutes to one day, occurring with variable intensity and absolute irregularity from once a month to three times a week. Between the attacks she had felt healthy except for psychic lability caused mainly by fear from further attacks.

In July and September, 1975, she had been in two district hospitals. The available medical and laboratory examinations gave negative results. On September 25, 1975, she was admitted to the Second Department of Medicine of our university hospital. The somatic finding was normal except for a slight hepatosplenomegaly. The liver was felt 2 cm below the costal margin in the midclavicular line, and the spleen 2 cm during inspiration. The headaches lasting 15 to 20 min and waking the patient even at night, were repeatedly observed. A map-like, dark red exanthem appeared on the whole body, accompanied by restlessness and a 20–30 torr drop in blood pressure.

In spite of numerous tests, it was impossible to arrive at a diagnosis. After discharge of the patient on November 21, 1975, painful vomitings occurred in December. Protracted flushes of the usual character, dyspnoea, tachycardia, hypotension, nausea, vomiting, intensive substernal pains and delusions appeared. An acute abdominal episode was excluded in a district hospital and the patient was transferred to our Department in December, 1975, where she was under treatment until the end of January, 1976. There were severe dysphagia and retrosternal pains with massive melaena and haematemesis on the following day. Vasography of the abdominal vessels and fibroscopy of the stomach failed to reveal the source of bleeding. Splenomegaly became more pronounced and signs of hypersplenism with pancytopenia developed. Splenectomy was therefore performed in February, 1976. Appendectomy and biopsy of the liver and mesenteric lymph nodes were carried out simultaneously. The histological finding in the nodes and the appendix was non-contributory. The spleen and the liver were abundantly infiltrated by cells the cystoplasm of which contained metachromatic granules different in size. The diagnosis of systemic mastocytosis, presumed on the basis of clinical signs, was confirmed. The postoperative course was regular and convalescence uneventful. Laboratory findings are presented in Table 1.

On April 9, 1976, diffuse abdominal pain and arthralgia in both the upper and lower limbs appeared, and a few hours later she had flushes with the above-mentioned complaints and then haematemesis followed by hypotension. The shock could not be managed therapeutically and during repeated haematemesis the patient died on the subsequent day. One day before death, the patient received cyclophosphamide in a dose of only 50 mg for fear from the liberation of mediators from disintegrating tumour cells and deepening of the crisis. There was no time for the planned gradual increase in the dose using the CVP (cyclophosphamide + vincristine + prednisone) protocol.

Table 1

Laboratory tests

Normal	Pathological
Urine + urinary sediment, creatinine, urea nitrogen, creatinine clearance, quantitative bacteriuria, minerals in urine and serum	FW, occult bleeding in stools, blood gases, hypersensitivity to histamine (skin test)
X-ray examination of heart, lungs, stomach, vertebral column and pelvis, irrigoscopy, gastroscopy, rectoscopy, grammagraphy of liver, ECG ——→	repolarization changes
Glycaemia, liver function tests, alkaline and acid phosphatase, GOT, GPT, protein electrophoresis, BSP test, cholesterol →	decreased
latex fixation test, SCAT, sialic acid, BWR, Fe in plasma	
Organ antibodies, ANF, LE cells, immuno-electrophoresis, ^{131}I accumulation, spirometry, EEG, gastric chemism ——→	high basal secretion
porphyrinuria	
Neurological, gynaecological and ophthalmological examinations	
Vanillic-mandelic acid, serotonin, hydroxyindoleacetic acid, latex-histamine test, challenge test with norepinephrine and hot tea	
Coombs test, complete coagulation examination except for →	thrombin time and factors V, VII, X, XI
Protamine tolerance, alkaline phosphorylase in leukocytes;	
thrombocytes ——→	thrombocytopenia
leukocytes ——→	leukopenia
Sternal puncture	
Histologic examination of bone marrow from the sternum according to Weitz, trepanobiopsy from the ilium	anaemia

Note: Only intermittent pathological findings are marked with arrows.

Survey of pathological findings

FW ranged between 20/39 and 34/64.
Occult bleeding in stools repeatedly positive.
Blood gases after attack: pH, 7.33; pCO_2, 27.5 mEq/l; pO_2, 59.5 mEq/l; BE −11.
ECG: non-specific ST changes during and immediately after flushes.
Cholesterol, 120—160 mg/dl.
Gastric chemism: sample of basal secretion pH 2.0, volume 105 ml. Further samples pH 1—2, volume ranging from 15 to 30 ml. Total volume 241 ml, output 15.07 mEq/l.
Blood picture on admission: haematocrit, 30.5%; Hb, 10.08 g/dl; Hb, 33%; thrombocytes, 81,000, 93,000, 130,000, 150,000, 91,000. Leucocytes ranged from 3000 to 5000 in 1 cu.mm, the differential count was always normal.
Thrombin time on Dec. 23, 1975: 19.8″—19.6″ (control, 14.0″—14.2″)
Factor V on Dec. 23, 1975: 27.0″—28.2″ (control, 27.0″—28.2″)
on Jan. 15, 1976: 36.2″—37.8″ (control, 32.2″—31.4″)
on Apr. 9, 1976: 14.0″—14.2″ (control, 35.2″—35.4″)

Factor VII on Jan .15, 1976: 1′46″—1′45″ (control, 67.8″—68.8″)
on Apr. 9, 1976: 61.0″—60.0″ (control, 35.2″—35.4″)
Factor X on Dec. 19, 1975: 31.0″—31.6″ (control, 25.4″—26.2″)
on Dec. 23, 1975: 35.2″—35.4″ (control, 24.8″—24.4″)
on Jan. 6, 1976: 27.0″—27.4″ (control, 26.4″—27.2″)
Factor I on Dec. 23, 1975: 29.8″—37.8″ (control, 24.8″—25.8″)
on Jan. 6, 1976: 34.8″—33.8″ (control, 27.4″—28.0″)
Peroperative liver and spleen biopsy: mast cell infiltrates

After death, samples of liver tissue and bone marrow from the iliac bone and the femur for electron microscopic examination were collected within two hours. For electron microscopy, small blocks of liver parenchyma were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, postfixed in 1% osmic acid in the same buffer, dehydrated and embedded in Durcupan. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope. The preparation showed sinuses with mast cells containing characteristic granules, confirming the diagnosis.

Post mortem examination carried out 37 hr after death revealed pronounced hepatomegaly (3600 g) with ascites (1000 ml) and hydrothorax (200 ml). In both the fundus ventriculi and bulbus duodeni there was a small mucous erosion with a haemorrhagic base. The stomach and the small intestine were filled with haemorrhagic secretion. Large amounts of aspirated gastric contents were found in the lungs. After formol fixation, the tissues were embedded in paraffin. In addition to haematoxylin-eosin staining, toluidine blue, cresyl violet, gentian violet-oxalic acid and the Giemsa stain were used for the detection of metachromatic granules.

Histology revealed an extensive infiltration of the liver parenchyma by mast cells which not only filled the sinuses but also penetrated into the portobiliary spaces. A similar mast cell infiltration, though arranged in small clusters, was found in the femoral bone marrow which exhibited a haematopoietic character. Smaller mast cell infiltrations were observed mainly perivascularly in the myocardium and periglomerularly in the kidneys. Lower nephron nephrosis was also seen.

Mast cell infiltration in the liver was more extensive in the post-mortem specimen than in the biopsy material. This was obviously due to the progression of the disease. A similar progression was observed in some other organs, e.g. the bone marrow, where post-mortem the infiltration was pronounced whereas in the biopsy performed a few months earlier the bone marrow finding was negative.

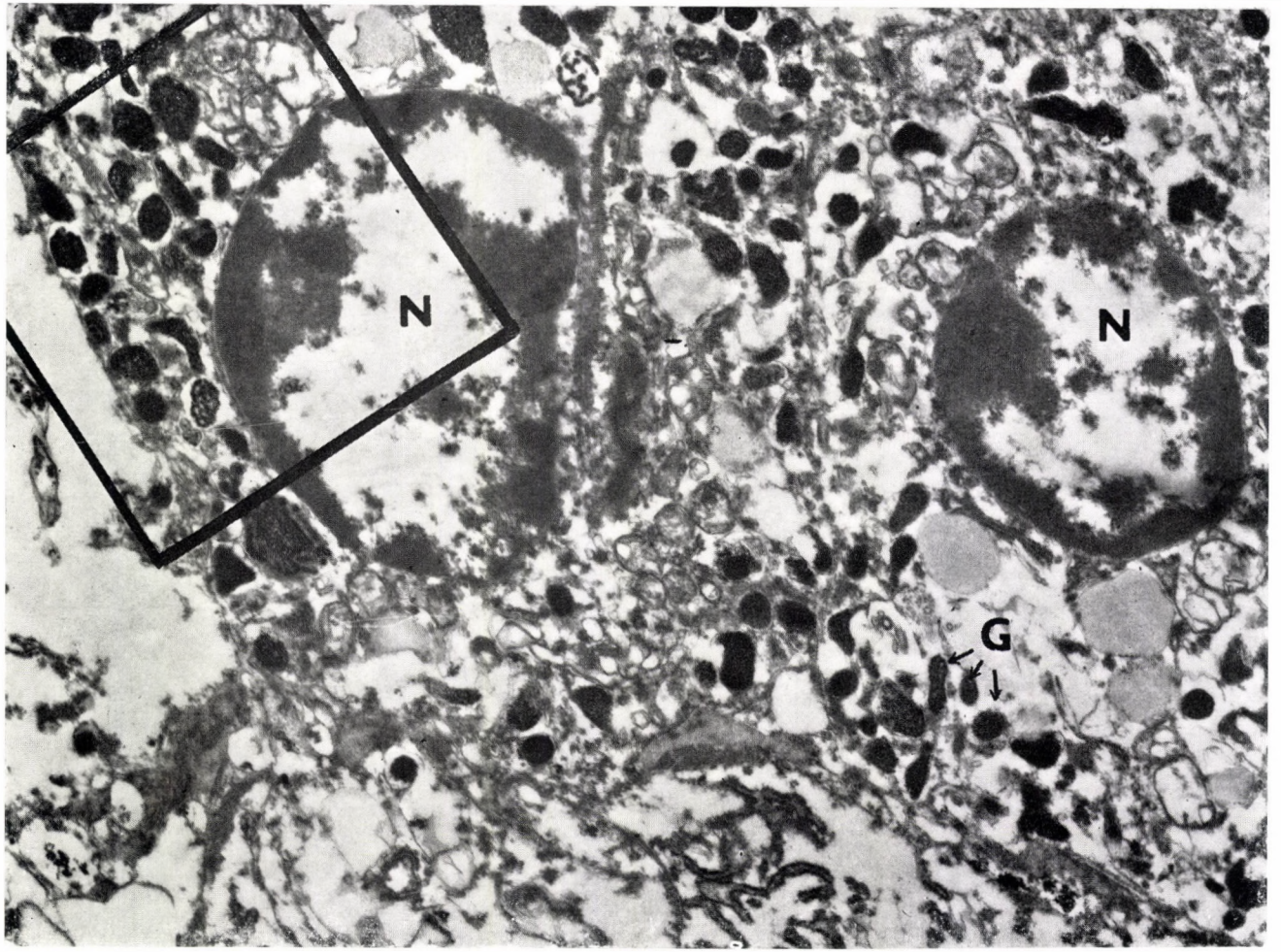


Fig. 1. Electron micrograph, ×13,800. In two mast cells, found in the portal sinus of the liver, nuclei (N) and numerous granules in the cytopaslm (G). The marked field is shown in Fig. 2 under higher power



Fig. 2. Electron micrograph, ×41,000. In addition to part of the nucleus of a mast cell (N) it shows numerous finely granular, coarsely granular and completely dense granules (G)

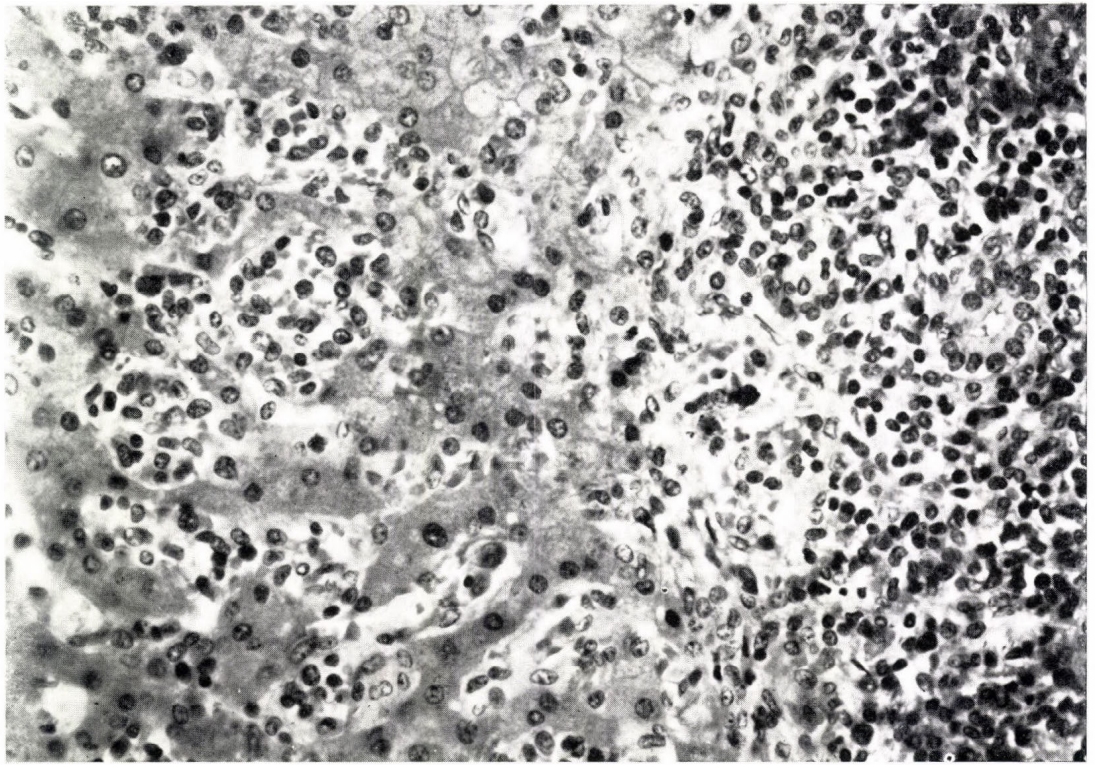


Fig. 3. Liver tissue with massive mast-cell infiltration of the portobiliary space. Mast cells between the liver trabecules in the sinusoids. The mast cells were identified using toluidine blue, not shown in the black-and-white photograph. Haematoxylin-eosin staining, × 250

Discussion

Polysystemic proliferation of mast cells, besides being a disease entity (systemic mastocytosis), is an excellent model for estimating the role of mastocytes. It has been shown experimentally that these cells play an essential role in the regulation of a number of vital processes [11].

The signs of systemic mastocytosis follow from the pathophysiology of the mast cells. Overproduction and increased release of histamine give rise to itching, dermatographism, fever, headaches, swelling of the nasal mucosa, dyspnoea due to bronchial spasms to attacks of bronchial asthma [10]. When greater amounts of histamine are liberated (friction of skin, heat, exertion), a generalized vasomotor reaction – "flush" – may occur during which an increased level of histamine can be demonstrated in blood and of its metabolites in urine [14]. Hyperacidity, hypersecretion of gastric juices and vomiting [14], increased intestinal motility and diarrhoea [3] occur in the digestive tract. Exceptionally, even a so-called histamine shock may develop [5]. Our patient J.P. presented only some of the signs, which is the usual case [13]. The variability of symptoms is probably connected with the functional immaturity and atypical morphology and secretion of the mast cells.

Overproduction of heparin and mucopolysaccharides is a less frequent finding, as also an increased production of histidine, N-acetylneuraminic acid, lipoprotein lipase and urinary taurine [13]. In our patient coagulation disturbances were observed, these, however, were not clear-cut and permanent. The low cholesterol level, influenced also by heparin [10], was only transient. Eosinophilia is commonly associated with mastocytosis and histamine is considered to be an eosinotactic substance [4]. It is believed that eosinophils phagocytize mast cell granules [16], but eosinophilia did not occur in our patient.

Heparin and/or hyaluronic acid are in close connection with the formation of connective tissue in the skin, liver – where even cirrhosis may develop –, spleen and elsewhere [3]. The same mechanism is perhaps responsible for the various bone changes observed in the X-ray picture simultaneously with osteosclerotic and osteolytic findings [6, 13]. These were not observed in our patient.

The role of serotonin in mastocytosis is unclear. Its production may cause changes similar to carcinoid [14]. From this aspect, it would be of interest to study the similarity of these two diseases in their clinical and humoral manifestations. Differential diagnosis may cause difficulties. In our patient normal values for serotonin in plasma and 5-hydroxy-indoleacetic acid in urine were obtained repeatedly and no mast cell infiltration could be demonstrated in the sternal and iliac bone marrow but this seems obvious after liver, lymph node and spleen biopsy. At the same time it was clear from the beginning that splenomegaly is not part of the carcinoid picture.

The therapeutic approach is based on the fact that SM is regarded as a haemoblastosis similar as the non-Hodgkin lymphomas. It is a malignant proliferation of reticuloendothelial cells. Combinations of cytostatics, such as the

CVP protocol have therefore been applied and partial remissions could be achieved [4]. Some authors recommend to begin with a small monotherapeutic dose to avoid a sudden disintegration of the tumour and development of "histamine shock" [10]. The effect of therapy could not be assessed in our patient because of the short duration of treatment. In general, however, the effectiveness of treatment is one of the reasons why attention should be paid to early diagnosis.

References

1. Bartelheimer, W., Lux, G., Ottenjan, R., Elster, K.: Mastozytose im Intenstinaltrakt. *Z. Gastroent. 12*, 608 (1974).
2. Debray, C., Leymarios, J., Cerf, M., Bosquet, L., Marche, C., Boivin, P., Kahn, A., Husson, J. M.: Mastocytose digestive. *Arch. Mal. App. dig. 62*, 411 (1973).
3. Ellis, J. M.: Urticaria pigmentosa; report of a case with autopsy. *Arch. Path. 48*, 426 (1949).
4. Gagnon, J. H., Kalz, F., Kadri, A. M., Graefe, I. V.: Mastocytosis: unusual manifestations; clinical and radiologic changes. *Canad. med. Ass. J. 112*, 1329 (1975).
5. Hamrin, B.: Release of histamine in urticaria pigmentosa. *Lancet 2*, 867 (1957).
6. Müller, I., Straňák, V.: Patologická zlomenina obratle u pigmentosní urticarie. *Acta chir. orthop. traum. čech. 41*, 52 (1974).
7. Nettleship, E.: Rare forms of urticaria. *Brit. med. J. 11*, 323 (1969).
8. Riely, J. F.: The Mast Cell. Livingstone, Edinburgh and London 1959.
9. Rodriguez, F. J. M.: Mastocytosis systemica. *Sangre (Barcelona) 19*, 445 (1974).
10. Sagher, F., Even-Paz, Z.: Mastocytosis and Mast Cell. Year Book Publishers, Chicago 1967.
11. Selye, H.: The Mast Cells. Butterworth, Washington, D. C. 1965.
12. Sideny, N. K., Winkelmann, R. K.: Course of urticaria pigmentosa in children. *Arch. Derm. 86*, 116 (1962).
13. Tempka, T., Stypulowski, C., Guminska, M., Chlap, Z.: Systemic proliferation of mast cell (mastocytosis in adult age) with special localisation in the digestive tract. *Acta med. pol. 14*, 291 (1973).
14. Tlustý, L., Hub, M., Čiháček, J.: Příspěvek k obrazu mastocytosy dospělých. *Vnitř. Lék. 16*, 595 (1970).
15. Vetter, D., Amman, R., Deyhle, P., Schmid, M.: Mastozytose und urticarielle Veränderungen im Magen-Darm-Trakt: Endoskopische Befunde bei 3 Fällen. *Z. Gastroent. 12*, 607 (1974).
16. Welsh, R. A., Greer, J. C.: Phagocytosis of mast cell granule by the eosinophilic leukocytes in the rat. *Amer. J. Path. 35*, 103 (1959).

Correspondence: Dr. M. Bláha, Second Department of Medicine, Charles University, Faculty of Medicine, Hradec Králové, Czechoslovakia

Haematologia 11 (3—4), pp. 359—364 (1977)

Disseminated Intravascular Coagulation (DIC) during Superacute Haemolysis in a Patient with Ovarian Dermatoid Cyst Treated with Rifampicin

JADWIGA NOWICKA, MARIA KOTSCHY, K. CHWISTECKI, M. JELEŃ

Departments of Haematology, Angiology, and Cardiology, Institute of Internal Diseases, and Department of Pathomorphology, Institute of Biostructure, School of Medicine, Wrocław, Poland

(Received March 18, 1976)

A 53-year-old female patient with ovarian dermatoid cyst and lung tuberculosis had been treated with rifampicin. During repeated rifampicin treatment she developed an acute haemolytic syndrome with haemorrhagic diathesis. Laboratory findings showed that it was caused by disseminated intravascular coagulation. Death ensued despite intensive administration of heparin and Trasylol. It is assumed that the repeated rifampicin application had provoked massive haemolysis by an allergic mechanism leading to thrombofibrinolytic haemorrhagic diathesis.

Introduction

Haemolytic syndrome is a rare complication of rifampicin treatment and occurs mainly during intermittent therapy [4, 5, 6, 10, 12, 14]. The syndrome may be an allergic reaction due to the repeated administration of rifampicin [4, 5, 10]. There are reports of disorders in blood coagulation during rifampicin therapy, but cases of DIC are extremely rare [7, 12, 16].

Report of a Case

A 53-year-old woman was admitted because of severe pain in the abdomen and lumbar area, progressive weakness, and restlessness. The abdominal and lumbar pains had appeared on the day before admission. They became stronger in the morning hours and were accompanied by anuria. One day before admission she had taken a 450 mg dose of rifampicin.

Ten years ago the patient had suffered from pulmonary tuberculosis. She had retired in 1965 because of chronic cor pulmonale and cirrhosis of the upper lobe of the right lung. Until 1971 she had positive cultures for *M. tuberculosis*. Beside other drugs she had been treated with rifampicin. This treatment was repeated twice during the years 1972—1974. Three years ago she had been found to have an ovarian cyst.

On the admission the patient was in acute distress, lying on her side with the knees bent. She was restless, had tachycardia and tachypnoea, the liver was

tender and enlarged extending 4 cm below the right costal margin. Above the symphysis a tumour of fist size was palpable. Blumberg's and Goldflamm's signs were positive. Pelvic examination revealed a tumour in the Douglas space extending most probably from the ovary. Blood pressure was 120/80 mm Hg.

Laboratory data

Hb, 15.4 g/dl; RBC, 4,679,000; WBC, 14,200. In the smear eryhtrocyte shadows were found. Differential count: 4% band cells, 77% polymorphonuclear neutrophils, 2% eosinophils, 16% lymphocytes, 2% monocytes. Reticulocyte count, 10%. Bleeding time 3 min 22 sec; total bilirubin, 9.0 mg/dl; free bilirubin, 3.0 mg/dl; SGOT (King) 10.0 U, SGPT (King), 35.0 U; urea, 50.0 mg/dl; creatinine, 1.5 mg/dl. Her blood was A_1 Rh plus (CcDEe/Kell/-) negative. The direct Coombs test with fresh and trypsinized red blood cells was negative. The pepsin test was also negative. Antibodies were determined in native plasma because the blood sample failed to show spontaneous coagulation.

ECG: P pulmonale, signs of chronic coronary ischaemia. Three hours after admission petechiae appeared, the platelet count amounted to 22,000/μl. The blood failed to clot, the serum was brownish in appearance, and free haemoglobin was 680.0 mg/dl. Repeated estimations of bilirubin could not be performed because of haemolysis. Nor could further examinations of blood morphology be performed.

As massive intravascular haemolysis was suspected, the blood coagulation system was examined.

Coagulation study

(a) Materials

1. Human red cells "0" (Rh−), coated with human fibrinogen. 2. Oxolated blood plasma, $BaCO_3$ plasma and serum of patient and control. 3. Human fibrinogen-antiserum obtained from rabbits by "Wytwórnia Surowic i Szczepionek", Warszawa. 4. 0.5% human fibrinogen "Kabi". 5. 1% bovine thrombin (400 μ/ml). 6. 1% thromboplastin from rabbit brain. 7. 0.05% cephalin from calf brain. 8. 4% casein. 9. "Distreptase" produced by "Wytwórnia Surowic i Szczepionek", Warszawa. 10. Folin-Ciocalteu tyrosine reagent. 11. Stypven Russel Viper Venom, Bourroughs Wellcome. 12. Kaolin. 13. Calcium chloride, 0.025 M.

(b) Methods

1. Plasma recalcification, cephalin, kaolin–cephalin, stypvencephalin, prothrombin and thrombin time. The tests were performed in oxalate plasma without and with addition of 0.5% fibrinogen. 2. Plasma thromboplastin generation into the Biggs-Douglas test [1]. 3. Factors V and VIII, IX and X were estimated in

plasma thromboplastin generation test according to Biggs and Douglas [1]. Prothrombin was also tested in stage II, according to Kowarzyk et al. [9]. 5. Serum prothrombin time. 6. Euglobulin clot lysis time. 7. Fibrinogen content was measured by the tyrosine method, using the Folin-Ciocalteu reagent. 8. Plasminogen content was determined using streptokinase and casein according to Bruhn et al. [2]. Plasminogen was expressed in Christensen units (1 U = 450 μg of tyrosine split during one hour at 37 °C). 9. Proteolytic activity of plasma was determined as plasminogen on casein activation without streptokinase. 10. Fibrinogen/fibrin split products were estimated in serum with the haemagglutination method of Merskey et al. [13]. 11. Antithrombin III activity was tested in serum according to von Kaulla [8].

(c) Results

Table 1 shows that the patient's plasma failed to clot on the addition of calcium ions, cephalin, brain thromboplastin or thrombin. In the presence of exogenous fibrinogen a long thrombin time was observed.

Table 1

Coagulation data

	Patient plasma		Control plasma
	without	with fibrinogen	
Platelet count	22,000		245,000
Plasma recalcification time, sec.	>600	>600	85
Plasma cephalin time, sec.	>600	>600	73
Plasma kaolin—cephalin time, sec.	>600	—	42
Stypvencephalin time, sec.	>600	>600	19.4
Plasma thromboplastin time, sec.	> 90	16.8	8.3
Plasma prothrombin time, sec.	>600	>600	12.5
Serum prothrombin time, sec.	—	>600	53
Thrombin time, sec.	>600	75	7
Euglobulin clot lysis time, hr	No clot	24	3
Fibrin split products, μg/ml	500	—	3
Proteolytic activity, per cent of normal	200	—	100
Plasminogen, U. Cas/ml per cent	0	—	1.84
Fibrinogen, g/dl	0	—	0.31
Prothrombin, U/ml	0	—	277
Factor V + VIII in $BaCO_3$ plasma, per cent of normal	0	—	100
Factor IX + X in serum, per cent of normal	15	—	100

The patient's plasma did not possess any prothrombin, factors V, VIII, fibrinogen or plasminogen activity, but showed increased proteolytic activity on casein. High concentrations of fibrin split products were found in the serum. The laboratory findings were suggestive of an acute DIC with secondary fibrinolysis.

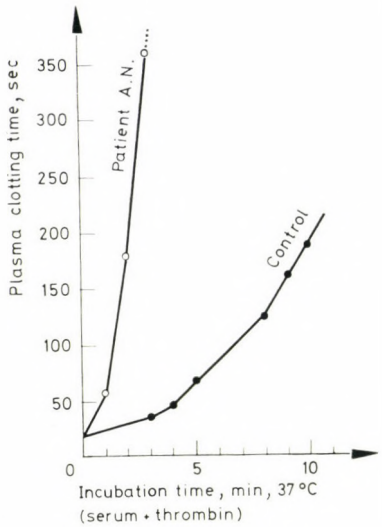


Fig. 1. Antithrombin activity of serum during different incubation times with thrombin

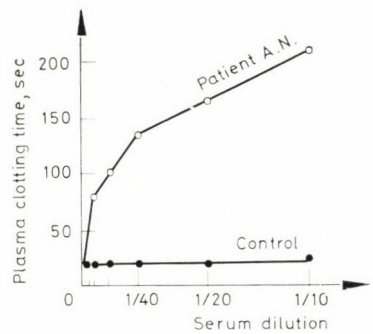


Fig. 2. Antithrombin activity in different concentrations of serum

Figure 1 illustrates the antithrombin activity of a control serum and of the patient's serum during incubation with thrombin. Antithrombin activity was higher in the patient's serum than in the control serum, and increased during incubation.

Figure 2 shows the antithrombin activity of the control serum and of the patient's serum at different concentrations. Incubation time of the serum and

thrombin mixture lasted one min. At concentrations at which the control serum had no influence on thrombin time, the patient's serum inhibited significantly the thrombin activity.

The high antithrombin activity of the patient's serum was due to the presence of increased amounts of fibrin split products.

After collection of blood samples for the coagulation studies, treatment was started with 100,000 U of Trasylol and 100 mg heparin.

In the fifth hour of hospitalization, cardiac arrest ensued; resuscitation for two hours was ineffective.

Necropsy revealed teratoma of both ovaries, caseous tuberculosis of the lungs, bronchopneumonia, numerous skin haemorrhages. Both kidneys were enlarged and had a smooth red surface with dark red spots. The cut surface displayed numerous small, dark red foci similar to those on the surface of the kidneys. The calyces, pelvis and ureters were normal. On haematoxylin and eosin-stained sections the cortex was hyperaemic and extravasations of blood were seen particularly in the glomeruli and their closest vicinity. The vessels were thickened and resembled the "wire loop" seen in lupus erythematosus. There were few normal glomeruli. In the cortex, large foci of recent tubular necrosis were seen. The tubular epithelium was hydropic and degenerated in the vicinity of some slightly changed glomeruli. In the pelvis inflammatory infiltrations were seen, composed of mononuclear lymphocyte-like cells. Weigert staining for fibrin was negative.

PAS stained the glomerular loops, especially thickened ones, intensely red, the contents of some tubuli in the medulla, and of some in the cortex, were similar in colour. Prussian-blue staining for haemosiderin and Best carmine stained for glycogen were negative. In liver specimens only hyperaemia was observed.

Discussion

Haemolysis may be the cause of DIC in cases of incompatible blood transfusion and paroxysmal nocturnal haemoglobinuria. It is rare in other types of haemolytic anaemias. Coagulation is initiated by a factor present in the erythrocyte stroma. Intact erythrocytes as well as free haemoglobin do not give rise to DIC [15]. In the present case, the plasma free haemoglobin level was increased hundredfold and blood smears contained numerous erythrocyte shadows.

In this patient, there was a coincidence of two pathological factors which predispose to intravascular haemolysis, the teratomas and rifampicin treatment, although dermatoid ovarian cysts rather cause chronic autoimmunologic anaemia [3]. In our case, haemolytic symptoms developed rapidly, shortly after rifampicin had been given for the third time and showed therefore the features of allergic haemolysis. Haemolysis proceeded at such a pace that in the first blood tests performed one hour after admission no decrease in blood haemoglobin and RBC, and no increase of the reticulocyte count was found.

The fibrinolytic haemorrhagic diathesis manifested itself later, an indication that it was secondary to the haemolysis. The decrease of the platelet count, fibrinogen and plasminogen, and the increase of fibrin split products pointed to the dominant role of coagulation and the secondary activation of fibrinolysis in the aetiology of the haemorrhagic diathesis. The prolonged fibrinolysis time showed that the blood was poor in plasminogen activator which had perhaps been consumed during DIC or adsorbed to tissues. In drug allergy, haemolysis with subsequent DIC is a very rare occurrence.

References

1. Biggs, R., Douglas, M. S.: The thromboplastin generation test. *Clin. Path. 6*, 23 (1953).
2. Bruhn, H. D., Müller, L., Duckert, F.: Quantitative determination of plasminogen. A caseinolytic method. *Thrombos. Diathes. haemorrh. (Stuttg.) 23*, 101 (1970).
3. De Bruyère, M., Sokal, D., Deveirille, J. M., Fauchet-Dutrieux, M. Ch., de Spa, V.: Autoimmune haemolytic anaemia and ovarian tumour. *Brit. J. Haemat. 20*, 83 (1971).
4. Germann, H. J., Heppe-Seyler, G. F., Boesken, W. H.: Akutes Nierenversagen nach Rifampicin. *Dtsch. med. Wschr. 99*, 1455 (1974).
5. Hasse, W., Pehle, H. D., Warnicke, F., Wiek, K.: Hämolytische Krise durch Rifampicin. *Prax. Pneumol. 25*, 466 (1971).
6. Herrmann, H., Eule, H.: Immunological and haematological investigations on patients with adverse reactions during treatment with RMP plus INH once weekly. *Scand. J. resp. Dis. 84 (Suppl.)* 87 (1973).
7. Jaroszewicz, W.: Chemioterapia gruźlicy. PZWL, Warszawa 1974.
8. Kaulla, K. N. von: Liver in the regulation of fibrinolytic activity. *Lancet 1*, 1046 (1964).
9. Kowarzyk, H., Marciniakówna, E., Kotschy, M., Czerwińska, B.: Równoważnik trombinowy jednostek protrombiny i modyfikacja dwustopniowege puiomu protrombiny. *Postepy Hig. Med. dośw. 14*, 95 (1960).
10. Krönig, B., Fiegel, P., Weihrauch, Th., Höffler, D., Jahnecke, J., Arndt-Hanser, A.: A case of severe repeated immunological reactions to rifampicin treatment. *Europ. J. clin. Pharmacol. 5*, 53 (1972).
11. Kruś, S.: Patomorfologia nerek. PZWL, Warszawa 1973.
12. Mattson, K.: Side effects of rifampicin. A clinical study. *Scand. J. resp. Dis. 82 (Suppl.)* (1973).
13. Merskey, C., Kleiner, G. J., Johnson, A. J.: Quantitative estimation of split products of fibrinogen in human serum, relation to diagnosis and treatment. *Blood 1*, 28 (1966).
14. Möhring, K., Asbach, H. W., Schubothe, H., Weber, S.: Hämolytische Krise mit akutem Nierenversagen unter Rifampicin-Behandlung. *Dtsch. med. Wschr. 99*, 1458 (1974).
15. Mac, D., Surgenor, N.: Erythrocytes and blood coagulation. *Thrombos. Diathes. haemorrh. (Stuttg.) 32*, 274 (1974).
16. Zierski, M., Kucharska, A., Wisłowska-Orłowska, B.: Side effects of rifampicin used intermittently in chronic pulmonary tuberculosis. *Gruźlica 41*, 1139 (1973).

Correspondence: Dr. J. Nowicka, Akademia Medyczna, Instytut Chorób Wewnętrznych, Klinika Hematologiczna, ul. Pasteura 4, 50-367 Wrocław, Poland

Book Reviews

Artificial Organs and Cardiopulmonary Support Systems. Ed. by F. T. Rapaport and J. P. Merrill. Grune and Stratton, New York 1971, 1972. Price: $15.—

The book contains the proceedings of the Transplantation Society's Symposium and is actually a reprint of the December 1971 issue (Volume III, Number 4) of the quarterly journal *Transplantation Proceedings*.

The first section deals with the present status, future prospects and biological considerations of the artificial kidney. It is stressed again that patients with chronic renal failure should be treated with dialysis early rather than after marked symptoms had appeared. Cadaver organ transplantation should be the ultimate goal for renal allografting, but in many instances the artificial kidney may be a permanent solution. The improvement of uraemia, the control of blood pressure offer the patient an acceptable alternative to death. The ideal artificial kidney should utilize a membrane that is non-traumatic to blood, non-thrombogenic, the flow pass should be short and contain a small volume, so that no priming with blood or transfusions are necessary. The use of frozen blood appears to minimize the risk of sensitizing the patient to human transplant antigens and to acquiring hepatitis. The paper on the biological considerations of regular dialysis deals with the pros and cons of treatment. Among the consequences anaemia, acute pericarditis and the occasional mild symptoms of peripheral and central nervous system dysfunction are mentioned.

The next section on membrane oxygenators reviews the history of the technique. It is pointed out that silicon copolymers are the most effective, being permeable to oxygen and CO_2 but not to water.

In the section on artificial hearts, several ingenious devices are described. Special emphasis is given to the problem of thromboembolism and disseminated intravascular coagulation. Experience with a non-thrombogenic expandable balloon assist device that can be inserted into the aorta is outlined. The importance of the early use of balloon pumping in cardiogenic shock is stressed. The use of a convenient prepackaged membrane oxygenator which is easily sterilized and quickly utilized seems promising. The counter-pulsation blood pumping technique is based on a hydraulic system. A striking phenomenon was the development of coronary collateral circulation observed in experimental animals. This section includes a general review of the problems of clotting and of thrombogenic surfaces. The concept is accepted that thromboembolism and lodging of small clots in remote sites may account for the decrease in platelet count and disseminated intravascular coagulation.

Section III is devoted to alimentary and metabolic life-support techniques, such as the intravenous infusion of a total nutrient solution in patients with absent or non-functioning small bowel. A series of papers deal with the treatment of liver failure by various techniques. Most speakers agreed that extracorporeal application of an intact pig or baboon liver was the treatment of choice, although exchange transfusion could add some advantages. It was agreed in the discussion that many of the patients eventually died of septicaemia. Of all the techniques described, the only truly successful one was the transplantation of an auxiliary liver,

especially in instances where regeneration of the patient's liver might be expected.

In the final section, methods of resurfacing denuded skin areas by autografting and the use of artificial membranes are being dealt with. The problem of organ banking is reviewed from several points. An interesting discussion of the technique called xenobanking is presented. In this technique a kidney would be transferred to another species and maintained until ready for transplantation.

Finally, the various techniques of organ freezing and rethawing are being discussed. It was agreed that with the exception of skin and possibly small bowel, organ preservation has not yet progressed to the point where it would be possible to establish organ banks.

Summarizing the proceedings, J. P. Merrill points out that with the exception of artificial kidney and in special cases the intraarterial balloon pumping device, there are at present no adequate substitutes for normal organ function, as exemplified by a successful transplant.

Susan R. Hollán

Disorders of Hemostasis in Surgery. Ed. by W. J. Rudowski. The University Press of New England, Hanover, New Hampshire 1977. 463 pages.

This volume contains more than its title indicates. Besides discussing the disorders of hemostasis in surgery, the reader is presented with an extensive review of the pathomechanism of congenital and acquired disorders of hemostasis and the treatment of various bleeding disorders.

In chapters 1 and 2, S. Łopaciuk discusses the physiological and biochemical aspects of the three main components of hemostasis: vessel wall, thrombocytes and the two enzyme systems: coagulation and fibrinolysis. Further, the basic principles of the diagnostics of bleeding disorders are discussed. Chapter 2 by S. Łopaciuk and L. Uszynski is closely linked to the last (21st) chapter which presents the laboratory methods used in the diagnostics of bleeding disorders. As the subjects of the second and last chapters, *viz.* the methods, their sensitivity, the possibilities of combination and evaluation of the results, are very closely related, it seems somewhat unfortunate that they are so far apart in the volume.

The third, fourth and fifth chapters deal with the bleeding disorders due to disturbances of the three main components of hemostasis: the quantitative and functional disorders of thrombocytes (L. Konopka), the lack of coagulation factors (J. M. Ziemsky), and the injuries of the vessel wall (L. Uszynski).

The sixth chapter (M. Szczepanski) reviews the pathogenesis, clinical and laboratory diagnostics and the therapy of disseminated intravascular coagulation (DIC). DIC is not an independent disease but a secondary hemorrhagic diathesis developing in several conditions. Therefore, DIC is discussed also in other chapters: in chapter 7 (R. Scharf) as a bleeding complication occurring after incompatible blood transfusion; in chapter 8 (M. Palester-Chlebowczyk, W. Sitkowski) during extracorporeal circulation and hypothermia; in chapter 12 (H. Zywicka-Łopaciuk), as a complication of tumorous diseases and in chapter 13 (M. Szczepanski) in surgical infections.

Chapter 9 by J. M. Ziemsky discusses the problems of secondary hemorrhagic diathesis related to liver disease and liver transplantation.

Chapter 11 by L. Uszynski deals with the bleeding disorders linked to myeloproliferative disease.

Three chapters discuss the effect of drugs on hemostasis. In chapter 10, M. Szczepanski reviews the bleeding complications of anticoagulant and thrombolytic therapy. Chapter 15 by H. Zywicka discusses the effect on hemostasis of drugs such as acetylsalicylic acid, corticosteroids, dextran, antibiotics, etc. Chapter 18 (J. M. Ziemski) describes the effects of vitamin K, epsilon aminocaproic acid and Trasylol (aprotinin).

In addition, chapters 8 and 13 include also other surgical aspects; chapter 14 (W. Rudowski, R. Scharf) discusses hemorrhagic shock; chapter 19 (J. Jastrzebski) the problems of anaesthesia, and chapter 20 (W. Rudowski) the possibilities of local hemostasis. The modern treatment of bleeding disorders also includes aimed replacement therapy: the possibilities of applying therapeutic blood preparations are discussed in chapter 17 (J. M. Ziemski).

The aim of the volume is to offer extensive information and guide to clinicians working in the field. Today the single branches of science are all extremely specialized, and more and more manuals are pubished dealing with one single condition. Volumes discussing and reviewing a wider range of problems are rather scarce. The many points and various aspects of the problems discussed in the book are an advantage and the authors being experts in their subjects, present a concise and up-to-date summary of each topic.

*Susan Előd*i

Immunologische Arbeitsmethoden (Immunological Methods). Ed. by H. Friemel. Gustav Fischer Verlag, Jena 1976. 496 pages, 116 illustrations, 3 colour photographs, 15 tables. Price: M 75.—

The volume consists of 59 chapters in which 59 authors discuss the methods most frequently used in humoral and cellular immunology. The book is a practical guide discussing the principles, the technique and errors of the methods, their field of application and the principle of evaluation of the results.

The aim of the volume is to present the laboratory worker precise and reproducible descriptions of the immunological methods, thus shortening the time of their introduction into the laboratory. A further aim is their standardization in order to allow a comparison of results reported by various laboratories and to facilitate a more extensive use of immunological procedures in medical practice and research.

The methods are classified as follows.

Methods for observing the humoral immune response: Immunodiffusion, immunoelectrophoresis, antibodies to bacterial antigens, latex agglutination, complement fixation, etc.

Methods for examining the cellular immune response: Lymphocyte cytotoxicity test, macrophage migration test, lymphocyte transformation, etc.

Separation, preparation and fractionation of immunoglobulin components.

Separation, preparation and fractionation of cells and factors (lymphocyte, transplantation antigens, transfer factor, etc.) involved in cellular immunity.

Preparation and application of immunosorbents, radioactive labelling of proteins and its use (radioimmune assay) are briefly reviewed.

Production of human ABO test serum and anti-D serum are also discussed.

Each chapter is followed by the most important literary references related to the development of the method discussed.

The volume is an extremely useful manual for all laboratory workers involved in the field of humoral and cellular immunology.

Judith Lajos

Arbeitsmethoden der inneren Medizin und verwandter Gebiete. Bd. VI. Mikroskopische Untersuchungsmethoden und Arbeitsmethoden auf verschiedenen Spezialgebieten. Lfg. 1. Allgemeine und morphologische hämatologische Methoden (Practical Methods in Internal Medicine and Related Fields. Vol. 6. Special microscopic examinations and techniques. Part 1. General and morphological methods in haematology.) Hrsg. R. Emmrich. Bearbeitet von E. Conrad, W. Helbig, K. Röder, D. Schneider, H. Schwenke, P. Schwenke und W. Storch. G. Fischer Verlag, Jena, 1976. 596 Seiten, 226 Abbildungen, 52 Tabellen. Preis: M 82.—

This part of the famous series is an indispensable manual for the haematologist working at the bed-side or in the laboratory. The bulk of the book contains an imposing collection of laboratory procedures and techniques with due consideration to the clinical features. The eight chapters written by outstanding experts deal with several aspects of their topics. The general, routine and special blood examination methods are treated comprising all subject matters related to the red blood cell, the white blood cell and the platelet systems. Novel trends as e.g. the analysis of leukocyte concentrates are also discussed. Aspiration biopsy of the bone marrow, the lymph node and the spleen is presented from a practical approach, too. It is stressed that unstained preparation should be sent to the haematologist, in order to apply the staining method of his choice. The section dealing with the evalua-

tion of the findings describes the determination of absolute marrow cell count, further the haematocrit examination of the marrow. Bartelheimer's bone needle biopsy and Burkhardt's myelotomy, their possible complications, the preparation of the biopsy material, further its microscopic and cytologic examination methods are described in detail. An important chapter is devoted to cytochemical methods in haematology, another to the technique and clinical value of the immunofluorescence assay. The review on radiological examinations in haemoblastosis is of practical importance.

The structure of the book is clear-cut, the material is arranged in a systematic manner. Beside giving a historical survey on earlier examination methods, it treats the present-day principles, the instruments and reagents to be used and the performance of the examinations. The normal values and the clinical importance of the pathological findings are also indicated.

As the manuscripts of the chapters were prepared in 1973 or earlier, they do not treat the latest developments. For the same reason, of the many hundred references closing each chapter, practically none cites literature published after 1970.

Katalin Natonek
and *E. Benedek*

Abstracts

The role of calcium in transferrin and iron uptake by reticulocytes. D. Hemmaplardh, E. H. Morgan (Department of Physiology, University of Western Australia, Nedlands, Australia). *Biochim. biophys. Acta (Amst.) 468*, 423 (1977).

The possible role of calcium in the uptake of transferrin and iron by rabbit reticulocytes was investigated by altering cellular calcium levels by the use of the chelating agents EDTA and ethyleneglycol-bis-(β-aminoethylether)-N,N'-tetraacetic acid (EGTA) and the ionophores, A23187 and X537A. Incubation of reticulocytes with EDTA or EGTA at 4 °C had no effect on transferrin and iron uptake but incubation at 37 °C resulted in an irreversible inhibition associated with decreased adsorption of transferrin to the cells and evidence of inactivation or loss of the transferrin receptors. Transferrin and iron uptake were also inhibited when the cells were incubated with A23187 or X537A. In the case of A23187, the action was exerted primarily on the temperature-sensitive stage of transferrin uptake and was associated with loss of cellular K^+ and decrease in cell size. The effect was stronger when Ca^{2+} was added to the incubation medium than in its absence. X537A produced a more marked inhibition of iron uptake than of transferrin uptake, associated with a reduction in cellular ATP concentration. The action of X537A was unaffected by the presence of Ca^{2+} in the incubation medium.

The results obtained with EDTA and EGTA indicate that cell membrane Ca^{2+} is required for the integrity or binding of transferrin receptors to the reticulocyte membrane. No evidence was obtained from the experiments with ionophores that an increase of cellular Ca^{2+} affects transferrin and iron uptake directly. The inhibition caused by A23187 was mainly due to a reduction in cell size resulting from increased membrane permeability to K^+ and that caused by X537A appeared to result from an inhibition of energy metabolism and ATP production.

A. Egyed

Identification of transferrin receptors in reticulocytes. F. M. van Bockxmeer, E. H. Morgan (Department of Physiology, University of Western Australia, Nedlands, Australia). *Biochim. biophys. Acta (Amst.) 468*, 437 (1977).

Experiments were performed to obtain definite evidence for the presence on reticulocytes of membrane receptors for transferrin. Rabbit reticulocytes were incubated with ^{125}I-labelled rabbit transferrin. The transferrin taken up by the cells was solubilized using the non-ionic detergent, Teric 12A9 (polyoxyethylene [$\bar{n} = 9$] dodecyl alcohol). The soluble extracts of the cells were examined by gel filtration and a transferrin-binding moiety of 275,000 approximate molecular weight was identified. This binding moiety was found only in reticulocytes, not in mature erythrocytes. The membrane component could bind transferrin but no other plasma proteins. Only transferrin could displace bound transferrin from the complex. Rabbit transferrin was bound more strongly than human transferrin. The binding of transferrin to the component was reversible

and saturable. It is concluded that the transferrin-binding component identified in the reticulocyte stroma is a true physiological receptor for transferrin.

A. Egyed

Mobile carrier ionophores for Fe(II). S. P. Young, B. D. Gomperts (Department of Clinical Haematology and Experimental Pathology, University College Hospital Medical School, London, U. K.). *Biochim. biophys. Acta (Amst.) 469*, 281 (1977).

A23187 and certain other carboxylate ionophores are capable of transferring Fe(II) but not Fe(III) across phospholipid bilayers (liposomes) and red cell membranes. A23187 is able to transfer Fe(II) from ferritin loaded liposomes when allied with a suitable redox couple and sink. The affinity of A23187 for Fe(II) is approximately five orders of magnitude greater than for Ca^{2+}, as judged by two phase extraction techniques.

A. Egyed

NADPH-flavin reductase in human erythrocytes and the reduction of methemoglobin through flavin by the enzyme. T. Yugisui T. Matsuki, K. Tanishima, M. Takeshita, Y. Yoneyama (Department of Biochemistry, School of Medicine, and Department of Medical Technology, School of Paramedicine, Kanazawa University, Kanazawa, Japan). *Biochem. biophys. Res. Commun. 76*, 174 (1977).

A NADPH-dehydrogenase of human erythrocytes was exhaustively purified to a homogeneous protein by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Studies on the specificity for the electron acceptor of the enzyme suggest that flavins serve as the natural and direct electron acceptor. The enzyme showed a broad specificity for flavins and the Michaelis constants for flavins were estimated to be 5×10^{-5} M for both FMN and riboflavin. Rapid reduction of methaemoglobin by the enzyme in the presence of flavin was demonstrated, and the reduction was explained by the reduction of flavin by the enzyme, and subsequent non-enzymatic reduction of methaemoglobin by the reduced flavin. The therapeutic significance of flavins is discussed with reference to the flavin reductase activity in hereditary methaemoglobinaemia.

Ilma Szász

Hemolysis of human erythrocytes by a transient electric field. K. Kinosita, Jr., T. Y. Tsong (Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA). *Proc. nat. Acad. Sci. (Wash.) 74*, 1923 (1977).

Exposure of human erythrocytes under isotonic conditions to a high voltage pulse of a few kV/cm leads to total haemolysis of the red cells. The present experiments demonstrate that the haemolysis is due to the effect of the electric field. Neither the effect of current nor the extent of rapid Joule-heating of the suspending medium shows a direct correlation with the haemolysis. Voltage pulsation of the erythrocyte suspension can induce a transmembrane potential across the cell membrane and, at a critical point, it either opens up or creates pores in the red cells. In isotonic saline the pores are small. They allow the passage of potassium and sodium ions but not of sucrose and haemoglobin molecules. The pores are larger under low ionic conditions and permit permeation of sucrose molecules, but under no circumstances can haemoglobin leak out under the direct effect of the voltage pulse. Kinetic measurements indicate that the haemolysis of red cells follows a stepwise mechanism: leakage of ions leads to an osmotic imbalance which in turn causes a colloidal haemolysis of the red cells. Other effects of the voltage pulsation are also discussed.

Ilma Szász

Preparation of uniform haemoglobin-free human erythrocyte ghosts in isotonic solution. F. Schneeweiss, U. Zimmermann, M. Saleemuddin (Institute of Chemistry, Institute II: Biophysical Chemistry, Nuclear Research Center Jülich, Jülich, FRG). *Biochim. biophys. Acta (Amst.) 466*, 373 (1977).

A method is described for the preparation of haemoglobin-free human erythrocyte ghosts in isotonic solutions using the dielectric

breakdown technique. In this single haemolytic procedure, almost complete removal of haemoglobin (< 0.1 %) was achieved by exposing the erythrocytes suspended in phosphate-buffered isotonic KCl solution at 0 °C to three consecutive electrical field pulses of 16 kV/cm in the presence of 10 mM EDTA; EDTA was used to prevent electrical haemolysis. Haemolysis is induced by subsequent dilution with isotonic and isoionic solution to lower the EDTA concentration. Haemolysis is complete after 5 min; the cells are centrifuged, washed and resuspended in a solution of the same composition and osmolarity, containing 4 mM $MgCl_2$, but no EDTA. The resealing process, carried out at 37 °C, was complete in about one hour. Measurements of size distribution of the ghost cells in the hydrodynamically focussing Coulter Counter at varying field strengths in the orifice revealed that the ghost population was nearly uniform. The mean (modal) volume of the ghost cells was 110 to 120 μm^3 when suspended in phosphate-buffered NaCl solution. The apparent breakdown voltage was about 1.3 V.

G. Gárdos

Decreased surface charge and accelerated senescence of red blood cells following neuraminidase treatment. S. A. Landaw, T. Tenforde, J. C. Schooley (Donner Laboratory, University of California, Berkeley, Calif.). *J. Lab. clin. Med. 89*, 581 (1977).

Female LAF_1 mice were given single or repeated injections of *V. cholerae* neuraminidase and the effects on circulating RBC surface charge and life-span were determined. Intravenous injection of neuraminidase caused a rapid decrease by approximately 14 % in RBC surface charge and survival of such treated cells was reduced by approximately one fifth by virtue of an acceleration of senescence. When RBCs were treated *in vitro* with neuraminidase, a comparable (14 to 17 %) reduction in surface charge was observed. Such cells, when injected into intact mice, showed a similar acceleration of senescence. When neuraminidase was injected intravenously into splenectomized mice, RBC survival was similar as of the controls. Intravenous injection of neuraminidase one hour before injection of labelled RBCs did not alter RBC survival, nor did it accelerate the clearance of carbon particles by the RES. These results indicate that neuraminidase accelerates senescence in treated mouse erythrocytes by acting on the RBCs and not by activating the RES. The absence of this effect in splenectomized mice implicates the spleen as the sensor of the induced alterations in surface charge. These results and those recently reported for treated RBCs in the dog, rat, rabbit and man suggest that at least a portion of the phenomenon of RBC senescence may be related to the loss of RBC surface charge.

Ilma Szász

Rearrangements of integral membrane components during in vitro aging of sheep erythrocyte membranes. H. U. Lutz, A. J. Lomant, P. McMillan, E. Wehrli (Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, USA, and Abteilung Cytologie, Institut für Pflanzenbiologie, Universität Zürich, Zürich, Switzerland). *J. Cell Biol. 74*, 389 (1977).

In vitro aged sheep erythrocytes and sheep erythrocyte ghosts spontaneously release vesicles that consist of long protrusions affixed to flattened head-like structures. The intramembraneous particles seen on the protoplasmic face of freeze-fracture electron micrographs of vesicle protrusions are arranged in paired particle rows. On the equivalent fracture face of head-like structures, the particle density is low; if particles are present, they are clustered along the rim of the flattened head-like structure and at the junction with the protrusion. The released vesicles are depleted of the intramembraneous particles seen on the exoplasmic face of ghosts but retain almost exclusively particles of the protoplasmic face. Correspondingly, the exoplasmic face of ghosts that have released vesicles reveals a 28 % higher density of intramembraneous particles than that of fresh ghosts. Purified vesicles are depleted of spectrin but retain integral membrane proteins, with one of an apparent mol. wt. of 160,000 accounting for nearly 50 % of the total protein. When vesicles are modified with the cleavable cross-linking reagent

[^{35}S] dithiobis (succinimidyl propionate) at 0 °C, the 160,000 mol. wt. protein is rapidly converted to disulphide-linked dimers and higher oligomers. Exposure of intact ghosts to the reagent in the same way fails to yield equivalent polymers. A comparison of the morphological and biochemical aspects of ghosts and vesicles suggests that a marked rearrangement of membrane proteins accompanies the supramolecular redistribution of intramembraneous particles during spontaneous vesiculation. The results also suggest that the paired particles of the protoplasmic face of vesicle protrusions are arranged in paired particle helices and contain the 160,000 mol. wt. protein as dimers.

B. Sarkadi

Decreased iodination of the red cell surface following phospholipase C treatment. E. Reichstein, R. Blostein (Division of Haematology, Royal Victoria Hospital, and Department of Experimental Medicine, McGill University, Montreal, Quebec, Canada). *Biochim. biophys. Acta (Amst.) 468*, 502 (1977).

Human red blood cells were treated with phospholipase C from *Clostridium welchii*. Lipase concentrations which produced $<1\%$ haemolysis and $10-15\%$ hydrolysis of the membrane phospholipids reduced markedly ($>80\%$) the accessibility of membrane proteins to the external surface as measured by lactoperoxidase-catalysed iodination.

Ilma Szász

Effect of glutaraldehyde treatment on enzyme-loaded erythrocytes. J. Deloach, S. Peters, O. Pinkard, R. Glew, G. Ihler (Department of Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh, Pa.). *Biochem. biophys. Acta (Amst.) 496*, 507 (1977).

In principle, enzyme-loaded erythrocytes can be used as a vehicle for enzyme replacement therapy in lysosomal storage diseases. Glutaraldehyde treatment renders these erythrocytes more resistant to lysis without inactivating the enzymes that have been entrapped inside them. Glutaraldehyde treatment does not prevent ingestion of enzyme-loaded erythrocytes by macrophages *in vitro* so that these cells can be used to deliver enzymes to lysosomes. *In vivo*, the glutaraldehyde-treated cells are quickly removed from the circulation by the spleen or the liver. The degree of glutaraldehyde treatment allows the erythrocytes to be targeted either to the spleen (low glutaraldehyde concentrations) or to the liver (high glutaraldehyde concentrations).

G. Gárdos

Arrangement of phosphatidylserine and phosphatidylethanolamine in the erythrocyte membrane. G. V. Marinetti (Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.). *Biochim. biophys. Acta (Amst.) 465*, 198 (1977).

Cross-linking of phosphatidylethanolamine and phosphatidylserine in the erythrocyte membrane with the reagent difluorodinitrobenzene was studied as a function of temperature, time and concentration of difluorodinitrobenzene. The optimal extent of cross-linking of phosphatidylethanolamine to phosphatidylserine and phosphatidylserine to phosphatidylserine was expressed as molar ratios of these three different cross-linked species. The experimental results were compared to different models of a phospholipid monolayer containing phosphatidylethanolamine and phosphatidylserine in which phosphatidylserine was arranged primarily as singles (having 6 phosphatidylethanolamine neighbours) as clusters of dimers, trimer and tetramers or as large cluster. In the various model monolayers each lipid component has 6 neighbours. The models which are consistent with the experimental results are those in which phosphatidylserine and phosphatidylethanolamine occur in small clusters in a non-random array.

Ilma Szász

Lateral mobility of human erythrocyte integral membrane proteins. V. Fowler, D. Branton (Biological Laboratories, Harvard University, Cambridge, Massachusetts, USA). *Nature (Lond.) 268*, 23 (1977).

Fluorescein isothiocyanate-labelled integral membrane proteins are mobile in the membranes of human erythrocytes that have

been fused (and haemolyzed) by Sendai virus or polyethyleneglycol. Minimum diffusion coefficients are of the order of 10^{-11} cm^2 s^{-1} at 37 °C. This mobility is reduced several-fold at room temperature, not detected at 0 °C, and is significantly greater in fresh than in aged blood. Mobility was assessed by observing the spread of fluorescence on labelled cells which had been fused with unlabelled cells; neither intramembrane particle aggregation nor spectrin release occurred during the process.

B. Sarkadi

Inhibition of erythrocyte sickling in vitro by dl-glyceraldehyde. A. M. Nigen, J. M. Manning (The Rockefeller University, New York, N. Y.). *Proc. nat. Acad. Sci. (Wash.) 74*, 367 (1977).

dl-Glyceraldehyde at concentrations between 5 and 20 mM reduces the sickling of S/S erythrocytes even in the complete absence of oxygen; at 10 mM glyceraldehyde the increase in the number of normal cells ranges from 20 to 40%. The inhibition of sickling was both concentration- and time-dependent and was not reversed by repeated washings with buffer. Incubation of erythrocytes with increasing concentrations of glyceraldehyde resulted in a small increase in the oxygen affinity, a moderate reduction in the Hill coefficient, a substantial increase in the minimum gelling concentration, and modification of up to two lysine residues per haemoglobin molecule.

Ilma Szász

On the mechanism of ATP-induced shape change in human erythrocyte membranes. I. The role of the spectrin complex. M. P. Sheetz, S. J. Singer (Department of Biology, University of California at San Diego, La Jolla, California, USA). *J. Cell Biol. 73*, 638 (1977).

Human erythrocyte ghosts have been shown by scanning electron microscopy to undergo ATP-dependent changes in shape. Under appropriate conditions the ghosts prepared from normal disk-shaped intact cells adopt a highly crenated form, which in the presence of Mg-ATP at 37 °C is slowly converted to the disk shape and eventually to the cup shape. These changes are not observed with other nucleotides or with 5'-adenylyl imidodiphosphate. Anti-spectrin antibodies, incorporated along with the Mg-ATP into ghosts in amounts less than equivalent to the spectrin, considerably accelerate the changes in shape observed with Mg-ATP alone. The Fab fragments of these antibodies are, however, ineffective. It is concluded that the structural effect produced by ATP is promoted by the cross-linking of spectrin by its antibodies, and may therefore be some kind of polymerization or network formation involving the spectrin complex on the cytoplasmic face of the membrane. The factors that contribute to the shape of the ghost and of the intact erythrocyte are discussed in the light of these findings.

Ilma Szász

Effects of methylprednisolone on the physical properties of the human red cell. P. W. Rand, E. Lacombe, N. D. Barker, G. L. Kallechey (Research Department, Maine Medical Center, Portland, Maine). *J. Lab. clin. Med. 89*, 1241 (1977).

Prompted by evidence suggesting preserved red cell deformability in heart surgery patients pretreated with pharmacologic dosages of methylprednisolone, *in vitro* experiments were performed to examine the ability of similar levels of methylprednisolone and hydrocortisone to modify erythrocyte membrane changes produced by metabolic depletion or membrane-active compounds. The variables measured included cell morphology, blood viscosity, membrane deformability, osmotic fragility, red cell cholesterol, and glycolytic intermediates. In incubated samples, methylprednisolone partially prevented the transition of disks to echinocytes, the rise in whole blood viscosity, the decrease in membrane deformability, and the loss of red cell cholesterol which accompany ATP depletion, but it had no apparent effect on red cell glycolysis. The drug also inhibited esterification of cholesterol in cell-free serum. In unincubated samples to which lysolecithin was added, methylprednisolone partially prevented and reversed morphologic and rheologic responses without affecting membrane cholesterol. Hydrocortisone demonstrated similar properties. Possible mechanisms for these actions are discussed. The concept is advanced that preserved blood fluidity may con-

tribute to the beneficial response to these drugs in certain clinical conditions.

Ilma Szász

The role of tightly bound phospholipid in the activity of erythrocyte acetylcholinesterase. G. Beauregard, B. D. Roufogalis (Laboratory of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B. C., Canada). *Biochem. biophys. Res. Commun. 77*, 211 (1977).

Acetylcholinesterase was extracted from bovine erythrocyte ghosts in a hypotonic (25 mOsm) phosphate buffer (pH 7.4) and solubilized with Lubrol WX (2 mg/ml). Solubilization resulted in a decrease of the partial specific volume of the acetylcholinesterase from 0.895 ml/g in the particulate fraction to 0.793 ml/g in the Lubrol WX-solubilized fraction. Both the particulate fraction and the solubilized fractions gave Arrhenius plots with a discontinuity in the activation energy at 20 °C. Phospholipase A_2 or C did not abolish the discontinuity. Treatment with 1.77 M NaCl resulted in a linear Arrhenius plot of intermediate activation energy. The high salt treatment removed the phospholipid previously not extracted by chloroform/methanol. The extracted phospholipid corresponded to cardiolipin on one- and two-dimensional silica gel chromatography. Addition of cardiolipin to a high salt-treated preparation of acetylcholinesterase restored the discontinuity in the Arrhenius plot. It is concluded that the physical state of cardiolipin tightly bound to acetylcholinesterase by ionic interactions, modulates the catalytic activity of acetylcholinesterase.

B. Sarkadi

Localization of the phlorizin site on Na, K-ATPase in red cell membranes. A. Nakagawa, M. Nakao (Tokyo Metropolitan Research Laboratory of Public Health, Shinjuku-ku, Tokyo, and Department of Biochemistry, Tokyo Medical and Dental University, School of Medicine, Bunkyo-ku, Tokyo, Japan). *J. Biochem. 81*, 1511 (1977).

Phlorizin at 2×10^{-4} M inhibited Na^+- and Rb^+-activated ATPase activities in human red cell membrane by 43%. It inhibited the ^{86}Rb uptake activity of erythrocytes by only 15%. ^{86}Rb uptake into resealed ghosts was inhibited strongly when phlorizin and ATP were preloaded in the ghosts before resealing. Na, K-ATPase activity in the resealed ghosts was also inhibited in the presence of phlorizin inside but not outside the ghosts. These findings suggest that the phlorizin site is located inside the cell.

G. Gárdos

pH-Dependent changes of 2,3-bisphosphoglycerate in human red cells during transitional and steady states in vitro. I. Rapoport, H. Berger, R. Elsner, S. Rapoport (Institut für Physiologische und Biologische Chemie, Humboldt-Universität, Berlin, DDR). *Eur. J. Biochem. 73*, 421 (1977).

A systematic study of the pH-dependent changes in the range 6.6—7.4 of 2,3-bisphosphoglycerate and the adenine nucleotides was performed in the presence and absence of glucose during transitional and steady states. The results indicate that 2,3-bisphosphoglycerate phosphatase breaks down 2,3-bisphosphoglycerate almost independently of the pH at a rate of 480 μmol 2,3-bisphosphoglycerate $\times$ 1 cells^{-1} $\times$ h^{-1}. 2,3-Bisphosphoglycerate mutase is practically completely inhibited below pH 6.9. An increased K_m 2,3-bisphosphoglycerate is postulated. The 2,3-bisphosphoglycerate level in the presence of glucose reaches a pH-dependent steady state after about 18 hr in keeping with theoretical predictions. The constancy of adenine nucleotides in the presence of glucose regardless of the rate of glycolysis indicates a close coupling between ATP formation and breakdown. The decline of the sum of adenine nucleotides appears to be determined by the rate of AMP breakdown. The share of the 2,3-bisphosphoglycerate bypass in the steady state decreases from 24% at pH 7.4 to 12% at pH 7.04. The initially decreased glycolysis at low pH values increases in long-term experiments with lower 2,3-bisphosphoglycerate levels. The formation of pyruvate corresponds to the breakdown of 2,3-bisphosphoglycerate after consumption of an unknown reducing substance.

G. Gárdos

Interaction of local anesthetics with the transport system of glucose in human erythrocytes. L. Lacko, B. Wittke, I. Lacko (Gustaw-Embden-Zentrum der biologischen Chemie, Abteilung für physikalische Biochemie, D-6000 Frankfurt/Main 70, GFR). *J. Cell Physiol. 92*, 257 (1977).

Local anaesthetics inhibit the exchange transport of glucose in human erythrocytes. All compounds tested showed a competitive inhibition except lidocaine and tolycaine causing a non-competitive one. Moreover, the transport system can bind two inhibitor molecules to one transport site as described for tetracaine and oxybuprocaine.

G. Gárdos

The influence of anesthetics on glycerol and K^+ translocation across the membrane of red blood cells. A. W. de Bruijne, J. van Steveninck (Sylvius Laboratories, Laboratory for Medical Chemistry, Leiden, Netherlands). *Biochem. Pharmacol. 26*, 779 (1977).

A wide variety of chemically unrelated anaesthetics protects red blood cells agtinst osmotic haemolysis. The protective effects of most anaesthetics are additive, suggesting common rather than separate mechanisms of action. Other properties shared by these anaesthetics are their inhibition of facilitated diffusion of glycerol into human erythrocytes and the stimulation of passive transport of this solute in bovine red blood cells. With respect to their influence on passive K^+ translocation, anaesthetics can be divided into two groups. Neutral and anionic anaesthetics cause in isotonic NaCl solutions a considerable K^+ loss and in hypotonic NaCl solutions a K^+ leakage which decreases rapidly with increasing cell volume. Cationic anaesthetics have the opposite effect; no K^+ loss occurs at the optimal protective concentration, whereas at higher concentrations a K^+ leakage occurs which increases with increasing cell volume. The possible significance of these differences in action is discussed.

G. Gárdos

The influence of anaerobiosis on human erythrocyte metabolism. W. E. Marshall, J. M. Goldinger, A. Omachi (Department of Physiology and Biophysics, University of Illinois at the Medical Center, Chicago, Ill.). *Proc. Soc. exp. Biol. (N. Y.) 154*, 356 (1977).

Anaerobiosis caused a small increase in lactate production when oxygenated and deoxygenated human erythrocytes had similar initial and final pH values. The change was attributed to phosphofructokinase activation. Under physiological conditions, pyruvate kinase also appeared to be activated, as judged by the marked decrease in 3-phosphoglycerate concentration. When the pH rise associated with deoxygenation was allowed to take place, the increase in lactate production was threefold.

G. Gárdos

Active and passive cation transport and L antigen heterogeneity in low potassium sheep red cells. Evidence against the concept of leak-pump interconversion. P. K. Lauf, B. J. Stiehl, C. H. Joiner (Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina, USA). *J. gen. Physiol. 70*, 221 (1977).

Several lines of experimental evidence are presented suggesting that the L antigens in low potassium (LK) sheep red cells are associated with separate Na^+K^+ pump and K^+ leak pathways. The stimulating effect of anti-L_p on K^+ pump flux is distinct from the action of anti-L on K^+ leak flux, implying that K^+ leak transport sites may not be converted into active pumps by the L antiserum. Treatment of LK red cells with trypsin completely abolished both the stimulation of K^+ pump flux and the enhancement of the rate of ouabain-binding brought about by anti-L. That this effect is due to a total destruction of the L_p determinant associated with the LK pump was evident from the complete failure of anti-L_p to bind to trypsinized LK red cells. The L_p antigen can effectively be protected against the trypsin attack by prior incubation with anti-L, indicating that the sites for antibody-binding and trypsin action may be closely adjacent

at the structural level. Trypsin treatment, however, did not interfere with anti-L_1 reducing ouabain-insensitive K^+ leak influx, nor did it prevent binding of anti-L_{1s}, the haemolytically active L antibody, which is probably identical with anti-L_1. The functional independence of the L_p and L_1 sites was supported by the observation that anti-L_1 still reduced K^+ leak influx in LK cells with experimentally induced high potassium concentrations, at which K^+ pump flux is fully suppressed, whether or not anti-L_p was binding to the L_p antigen associated with the LK pump.

G. Gárdos

Active transport of 5-hydroxytryptamine by plasma membrane vesicles isolated from human blood platelets. G. Rudnick (Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut, USA). *J. biol. Chem. 252*, 2170 (1977).

Plasma membrane vesicles isolated from human platelets accumulate 5-hydroxytryptamine when an electrical potential (interior negative) or an Na^+ gradient (out > in) is imposed across the vesicle membrane. Kinetic studies revealed a K_m of 0.5 μM for the transport process. Uptake was strongly inhibited by tricyclic antidepressants and by ionophores such as gramicidin which catalyze transmembrane exchange of Na^+ for K^+. Transport is absolutely dependent upon external Na^+ and Cl^- and is only mildly, if at all, inhibited by reserpine, cinanserin, ouabain, or arsenate. Experiments are presented which suggest that a single positive charge crosses the vesicle membrane with each molecule of 5-hydroxytryptamine. The results provide direct evidence for Na^+-coupled active 5-hydroxytryptamine transport by the platelet plasma membrane.

B. Sarkadi

From the International Literature of Haematology

Acta Haematologica (Basel) **56** (1976) No. 5

Hb Helsinki: a variant with a high oxygen affinity and a substitution at a 2,3-DPG binding site (β 82 [EF6] Lys → Met). *Ikkala, E., Koskela, J., Pikkarainen, P., Rahiala, E.-L., El-Hazmi, M. A. F., Nagai, K., Lang, A., Lehmann, H.* (Reprint requests: Prof. H. Lehmann, Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QR, England), p. 257

Hypochromie der Erythrozyten bei heterozygotem Hämoglobin E (β 26 Glu → Lys). *Keller, P., Kohne, E.* (I. Medizinische Klinik der Städtischen Krankenanstalten, D-75 Karlsruhe, BRD), p. 276

Regulation of the β- and δ-hemoglobin genes. A family with hereditary persistent fetal hemoglobin and β-thalassemia. *Rotschild, H., Bickers, J., Marcus, R.* (Department of Medicine, L. S. U. School of Medicine, New Orleans, La. 70112), p. 285

Persisting production of out-of-cycle blasts during methotrexate therapy in acute leukaemia. *Pagliardi, G. L., Gabutti, V., Gavosto, F.* (Reprint requests: Prof. F. Gavosto, Istituto di Semeiotica Medica, I-10133 Torino, Italy), p. 292

Evaluation d'une méthode d'étude des agrégats plaquettaires par fixation au formol. *Stoltz, J. F.* (Centre Régional de Transfusion Sanguine, F-54500 Vandoeuvre les Nancy, France), p. 303

Platelet aggregation and adhesiveness in classical factor X deficiency and in the abnormal factor X (factor X Friuli) coagulation disorder. *Girolami, A., Fabris, F., Peruffo, R., Coser, P.* (Istituto di Semeiotica Medica, Padova, Italy), p. 307

Platelet aggregation in congenital factor XIII deficiency. *Özsoylu, S., Hicsönmez, G.* (Children's Hospital, Hacettepe University, Ankara, Turkey), p. 314

Acta Haematologica (Basel) **56** (1976) No. 6

Proliferation of leukemic blood cells in short-term liquid cultures. Relevance to the differential diagnosis of acute myeloblastic and lymphoblastic leukemia. *Bakkeren, J. A. J. M., de Vaan, G. A., Hillen, H. F. P.* (Department of Pediatrics, St. Radboud Ziekenhuis, University of Nijmegen, Nijmegen, The Netherlands), p. 321

Red cell membrane protein abnormality in paroxysmal nocturnal hemoglobinuria and in *in vitro* induced PNH-like erythrocytes. *Kotsifopoulos, P. N.* (Professorial Medical Unit, Evangelismos Hospital, Athens 140, Greece), p. 328

Screening for abnormal hemoglobins in the Middle East: New data on hemoglobin S and the presence of hemoglobin C in Saudi Arabia. *Gelpi, A. P., King, M. C.* (Palo Alto Medical Clinic, Palo Alto, Calif.), p. 334

Failure of isogeneic marrow engraftment in a patient with acute leukemia. *Weber, W., Speck, B., Cornu, P., Nissen, C., Groff, P., Burri, H. P., Buckner, C. D.* (Reprint requests: Dr. Bruno Speck, Kantonsspital, CH-4004 Basel, Switzerland), p. 338

Unusual clonal evolution in a case of chronic myelogenous leukemia. *Whang-Peng, J., Broder, S., Lee, E., Young, R. C.* (National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014), p. 345

Primary fibrinolysis and spontaneous rupture of the spleen in acute lymphoblastic leukemia. *Gafter, U., Mandel, E. M., Weiss, S., Djaldetti, M.* (Reprint requests: Dr. M. Djaldetti, Hasharon Hospital, Petah-Tiqva, Israel), p. 355

Acta Haematologica (Basel) **57** (1977) No. 1

Electron microscope and high resolution autoradiographic studies of megaloblastic erythropoiesis. *Wickramasinghe, S N., Bush, V.* (MRC Experimental Haematology Unit, St. Mary's Hospital Medical School, London W2, England), p. 1

Idiopathic acquired refractory sideroblastic anemia: Banded chromosome analysis in six patients. *Bitran, J., Golomb, H. M., Rowley, J. D.* (University of Chicago, Chicago, Ill. 60637), p. 15

Sideroblast score: A sensitive indicator of iron deficiency and hypoproliferative anemia. *Baumgartner-Stäubli, R., Beck, E. A.* (Reprint requests: Prof. E. A Beck, Hämatologisches Zentrallabor, Inselspital, CH-3010 Bern, Switzerland), p. 24

Quantitation of Hb A_2 with DE-52 microchromatography in whole blood as screening test for β-thalassemia heterozygotes. *Galanello, R., Melis, M. A., Muroni, P., Cao, A.* (Reprint requests: Prof. A. Cao, Clinica Pediatrica, I-09100 Cagliari, Sardinia, Italy), p. 32

Inherited erythrocyte pyruvate kinase deficiency: Studies on 15 members of two related families. *Dachà, M., Canestrari, F., Bossù, M., Ferrini, P. L. R., Fornaini, G.* (Istituto di Chimica Biologica, Università di Urbino, I-61029 Urbino, Italy), p. 37

Familial thrombosis due to antithrombin III deficiency in a Greek family. *Stathakis, N. E., Papayannis, A. G., Antonopoulos, M., Gardikas, C.* (Reprint requests: Dr. C. Gardikas, Evangelismos Hospital, Athens 140, Greece), p. 47

Tetraploid cell line in a girl with acute leukaemia. *Foadi, M. D.* (Department of Haematology, Charing Cross Hospital, London W6 8RF, England), p. 55

Acta Haematologica (Basel) **57** (1977) No. 2

Effect of blood transfusion on the immune response of children with thalassaemia. *Kanakoudi-Tsakalidis, F., Spyroglou, K., Tzafi, R., Cassimos, C.* (Paediatric Clinic, Aristotle University, Thessaloniki, Greece), p. 65

A simplified one-step procedure for the simultaneous determination of complement receptor lymphocytes and lymphocytes with membrane-bound immunoglobulins. *Winterleitner, H., Rotter, M., Knapp, W.* (Institut für Immunologie der Universität Wien, A-1090 Wien, Austria), p. 74

Histogenesis of myeloid metaplasia in the spleen. *Iyengar, B.* (Department of Pathology, Maulana Azad Medical College, New Delhi, India), p. 81

Circulating platelet aggregates and thrombocytopenia induced by intravenous infusion of arachidonic and lauric acids in guinea pigs. *Rafflenbeul, D., Zbinden, G.* (Reprint requests: Prof. G. Zbinden, Institut für Toxikologie der ETH und Universität Zürich, CH-8603 Schwerzenbach b. Zürich, Switzerland), p. 87

Transferrin-immune complex disease. *Westerhausen, M., Meuret, G.* (Reprint requests: Dr. G. Meuret, Medizinische Klinik C, Kantonsspital, CH-9006 St. Gallen, Switzerland), p. 96

Haemoglobin Shepherds Bush (β 74 [E 18] Gly $\rightarrow$ Asp) in an Italian family. *Sansone, G., Sciarratta, G. V., Genova, R., Darbre, P. D., Lehmann, H.* (Reprint requests: Prof. H. Lehmann, University of Cambridge, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge CB2 2QR, England), p. 102

Interaction of haemoglobin E with α-thalassaemia and haemoglobin Constant Spring. *Ganesan, J., Lie-Injo, L. E., Ng, T. S., George, R.* (Division of Haematology, Institute for Medical Research, Kuala Lumpur, Malaysia), p. 109

Red cell pyruvate kinase deficiency: Adverse effect of oral contraceptives. *Kendall, A. G., Charlow, G. F.* (Division of Haematology, Department of Medicine, Royal Victoria Hospital and McGill University Clinic, Montreal, Quebec H3A 1A1, Canada), p. 116

Glucose-6-phosphate dehydrogenase Velletri. A new variant with reduced activity in a patient with congenital non-spherocytic haemolytic anaemia. *Mandelli, F., Amadori,*

S., De Laurenzi, A., Kahn, A., Isacchi, G., Papa, G. (Cattedra di Ematologia dell'Università degli Studi di Roma, 00161 Roma, Italy), p. 121

Acta Haematologica (Basel) **57** (1977) No. 3

Freeze-fracture of circulating human eosinophils. *Douglas, S. D., Ooka, M. P.* (Department of Medicine, University of Minnesota Medical School, Mayo Memorial Building, Minneapolis, Minn. 55455), p. 129

Circulating anticoagulant against factor XII and platelet antibodies in systemic lupus erythematosus. *Gandolfo, G. M., Afeltra, A., Amoroso, A., Biancolella, F., Ferri, G. M.* (Institute of Medical Pathology I, University of Rome, Rome, Italy), p. 135

Congenital Heinz body haemolytic anaemia due to haemoglobin Perth in a Nama child seemingly aggravated by the high nitrate content of the water supply. *Grové, S. S., Jenkins, T., Kamuzora, H. L., Lehmann, H.* (Reprint requests: Prof. H. Lehmann, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge CB2 2QR, England), p. 143

Infectious mononucleosis in Hodgkin's disease. A further case report. *Davidson, R. J. L., Lessels, S. E.* (Haematology Unit, Department of Pathology, University Medical Buildings, Aberdeen AB9 2ZD, Scotland), p. 152

A study of folic-acid-binding protein in normal subjects. *Gorst, D. W., Courtis, M., Delamore, I. W.* (Department of Clinical Haematology, Manchester Royal Infirmary, Manchester MI3 9WL, England), p. 156

Membrane deformability of erythrocytes with glucose-6-phosphate dehydrogenase Hamburg. *Tillmann, W., Gahr, M., Labitzke, N., Schröter, W.* (Reprint requests: Prof. W. Schröter, Universitäts-Kinderklinik, D-3400 Göttingen, BRD), p. 162

A simple and rapid platelet calcium determination. Normal values and results in May-Hegglin anomaly. *Creter, D., Rubinstein, I., Menache, R.* (Clinical Laboratories, Hasharon Hospital, Petah-Tiqva, Israel), p. 168

Chronic idiopathic myelofibrosis. A reversible disease? *Kelemen, E., Krasznai, G., Endes, P., Szinay, G.* (I. Department of Medicine, Semmelweis University Medical School, H-1083 Budapest, Hungary), p. 171

In vivo behaviour of neuraminidase-treated rabbit erythrocytes and reticulocytes. *Balduini, C. L., Ricevuti, G., Sosso, M. C., Ascari, E., Brovelli, A., Balduini, C.* (Reprint requests: Prof. C. Balduini, Istituto di Chimica Biologica, Facoltà di Scienze, I-27100 Pavia, Italy), p. 178

Burkitt's lymphoma cell leukemia. *Acar, S., Tekinalp, G., Özsoylu, Ş., Çevik, N., Yaşar, H.* (Hacettepe Children's Hospital, Ankara, Turkey), p. 188

Acta Haematologica (Basel) **57** (1977) No. 4

Effects of dipyridamole on sodium and potassium content of human red blood cells. *Vettore, L., De Matteis, M. C., Del Conte, G.* (Istituto di Patologia Medica, Università di Padova, Policlinico di Borgo Roma, 37100 Verona, Italy), p. 193

Peripheral blood lymphocytes in systemic lupus erythematosus. Relation to activity. *Michalevicz, R., Many, A., Ramot, B.* (Reprint requests: Prof. B. Ramot, Institute of Hematology, The Chaim Sheba Medical Center, Tel Hashomer, Israel), p. 199

Cold agglutinins in a case of chronic lymphatic leukaemia. A study of the lymphocyte surface. *Greally, J. F., Whelan, C. A., O'Connell, L., O'Gorman, D.* (Department of Immunology, School of Pathology, Trinity College, Dublin 2, Ireland), p. 206

Peptichemio: A new oncolytic drug in combination with vincristine and prednisolone in the treatment of non-Hodgkin lymphomas. *Carella, A. M., Bacigalupo, A., Santini, G., Giordano, D., Damasio, E. E., Fusco, F. A., Rossi, E., Marmont, A. M.* (Division of Haematology and Clinical Immunology, Ospedale Regionale S. Martino, Genova, Italy), p. 211

Inherited platelet abnormalities associated with low factor VIII activity in the same family. *Ponari, O., Civardi, E., Megha, A., Pini, M., Poti, R., Dettori, A. G.* (Reprint requests: Prof. A. G. Dettori, 5. Divisione Medica, Ospedali Riuniti, Parma, Italy), p. 225

Chemotherapeutic remissions in Wistar/Furth rat acute myelogenous leukemia: A model for human AML. *Greenberger, J. S., Bocaccino, C. A., Szot, S. J., Moloney, W. C.* (Reprint requests: Dr. W. C. Moloney, Peter Bent Brigham Hospital, Boston, Mass. 02115), p. 233

Influence of cytostatics on some platelet functions *in vitro*. V. Hydroxyurea. *Kubisz, P., Pařizek, M., Kováčová, J., Cronberg, S.* (Reprint requests: Dr. S. Cronberg, Almänna Sjukhuset, S-214 01 Malmö, Sweden), p. 242

Scanning electron microscopic study of leukemic human B lymphocytes. *Fischer, K., Cohnen, G., Szaniawski, W., Brittinger, G.* (Reprint requests: Prof. G. Brittinger, Hämatologische Abteilung, Medizinische Universitätsklinik, D-43 Essen, BRD), p. 247

Acta Haematologica (Basel) **57** (1977) No. 5

Preparation, purification and *in vitro* properties of a serum against human lymphoblastic leukemia-associated antigens. *Pandolfi, F., Luzi, G., Tozzi, M. C., Aiuti, F.* (Università degli Studi di Roma, III Cattedra di Clinica Medica Generale, I-00161 Roma, Italy), p. 257

Postoperative platelet function in patients on small subcutaneous doses of heparin. *Economopoulos, T. C., Papayannis, A. G., Stathakis, N. E., Arapakis, G., Gardikas, C.* (Reprint requests: Prof. C. Gardikas, Evangelismos Hospital, Athens 140, Greece), p. 266

Imidazole carboxamide (DTIC) in the treatment of advanced lymphomas. Efficacy of DTIC in cases which fail to respond to conventional chemotherapeutic combination. *Klener, P., Donner, L.* (Second Department of Medicine, Division of Haematology, Charles University Hospital, 128 08 Prague, Czechoslovakia), p. 272

Large granules and lysosomal fusion in human Chediak-Higashi white blood cells. *Rozenszajn, L. A., David, E. B., Sela, S. B.* (Hematologic Laboratories, Meir Hospital, Kfar Saba, Israel), p. 279

Antihaemophilic factor A activity, F VIII-related antigen and von Willebrand factor in hepatic cirrhosis. *Baele, G., Matthijs, E., Barbier, F.* (Department of Internal Medicine, Akademisch Ziekenhuis, B-9000 Ghent, Belgium), p. 290

Chronic myelogenous leukemia with elevated leukocyte alkaline phosphatase, positive indirect Coombs' test, neutrophilic leukocytosis and unusual cytogenetical findings. *Grinblat, J., Mammon, Z., Lewitus, Z., Joshua, H.* (Medical Department E, Beilinson Hospital, Petah Tiqva, Israel), p. 298

Erythrocyte γ-aminolaevulinic acid dehydratase, urinary porphyrins and porphyrin precursors in iron deficiency anaemia. *Chalevelakis, G., Lyberatos, C., Manopoulos, D., Pyrovolakis, J., Gardikas, C.* (Reprint requests: Prof. C. Gardikas, Professorial Memorial Unit, Evangelismos Hospital, Athens, Greece), p. 305

Acta Haematologica (Basel) **57** (1977) No. 6

B and T cell markers of bone marrow and peripheral blood lymphoid cells in patients with paraproteinaemia. *Husz, S., Krizsa, F., Dobozy, A., Hunyadi, J., Simon, N.* (Department of Dermatology, University Medical School, H-6701 Szeged, Hungary), p. 321

Lymphocyte reactivity in healthy subjects and cancer patients. *Haasz, R., Robinson, E.* (Reprint requests: Dr. E. Robinson, Department of Oncology, Ramban University Hospital, Haifa, Israel), p. 331

Acquired pyruvate kinase deficiency with hemolysis in preleukemia. *Helmstädter, V., Arnold, H., Blume, K. G., Uhl, N., Hunstein, W.* (Medizinische Universitätspoliklinik, D-6900 Heidelberg, BRD), p. 339

Anti-γ-globulin activity, DNA and antibodies to DNA in nonlupoid cryoglobulinemias. *Invernizzi, F., Pioltelli, P., Gavazzeni, G., Riboldi, P. S., Zanussi, C.* (IV. Medical Clinic, University of Milan, I-20123 Milano, Italy), p. 344

Combined G-6PD and 6-PGD deficiency in a Hindu boy. *Dash, S., Bhagwat, A. G.* (Department of Haematology, Postgraduate Institute of Medical Education and Research, Chandigarh-160011, India), p. 351

Pyridoxal-5-phosphate-resistant sideroblastic anaemia with trisomy 8 mosaicism in

the bone marrow. *Penchas, S., Zlotnick, A., Cohen, R., Cohen, M. M.* (Department of Medicine A, Hadassah University Hospital, Jerusalem, Israel), p. 354

A case of lambda type tetramer Bence—Jones proteinemia. *Kozuru, M., Benoki, H., Sugimoto, H., Sakai, K., Ibayashi, H.* (Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan), p. 359

In memory of Professor Heinrich Lüdin. *Moeschlin, S.* (Solothurn, Switzerland), p. 366

Acta Haematologica (Basel) **58** (1977) No. 1

In vitro labelling of platelets. *Olsson, L.-B., Kutti, J., Weinfeld, A.* (Reprint requests: Prof. J. Kutti, Department of Medicine III, Sahlgren's Hospital, 411 33 Gothenburg, Sweden), p. 3

Platelet antibodies in different forms of chronic thrombocytopenia. *Gandolfo, G. M., Afeltra, A., Amendolea, M. A., Mannella, E., Masala, C.* (Istituto di Patologia Speciale Medica I, Policlinico Umberto I, Roma, Italy), p. 10

Congenital deficiency of factor XIII with normal subunit S and lack of subunit A. Report of a new family. *Girolami, A., Burul, A., Sticchi, A.* (Istituto di Semeiotica Medica, University of Padua Medical School, Padua, Italy), p. 17

Effect of bleeding on *in vivo* and *in vitro* colony-forming hemopoietic cells. *Pannacciulli, I. M., Massa, G. G., Saviane, A. G., Ghio, R. L., Bianchi, G. L., Bogliolo, G. V.* (Istituto Scientifico di Medicina Interna, I-16132 Genoa, Italy), p. 27

Refractory anemia with hyperplastic bone marrow: Subclassification based on responsiveness to erythropoietin *in vitro*. *Stathakis, N. E., Gidari, A. S., Levere, R. D.* (Reprint requests: Dr. R. D. Levere, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 34

Waldenström-like immunocytic lymphoma with IgG serum M component. *Resegotti, L., Palestro, G., Coda, R., Dolci, C., Poggio, E. Leonardo, E.* (Ospedale Maggiore della SS. Annunziata, I-12038 Savigliano CN, Italy), p. 38

Increased serum folate-binding capacity. A familial trait. *Muckerheide, M. M., Raich, P. C., Carlson, I. H.* (Reprint requests: Dr. P. C. Raich, University Hospitals, Madison, Wisc. 53706), p. 45

Biochemical studies on the leukocytes in Chediak-Higashi syndrome. *Zabucchi, G., Cramer, R., Soranzo, M. R., Tamaro, P., Panizon, F.* (Istituto di Patologia Generale, Università di Trieste, I-34127 Trieste, Italy), p. 50

Sea-blue histiocyte syndrome in Thai siblings. *Chainuvati, T., Piankijagum, A., Viranuvatti, V., Silverstein, M. N.* (Department of Medicine, Siriraj Hospital, Bangkok 7, Thailand), p. 58

Acta Haematologica (Basel) **58** (1971) No. 2

An *in vitro* demonstration of the ability of human bone marrow stromal elements to sustain granulocytopoiesis. *Altman, A. J.* (Department of Pediatrics, Division of Hematology-Oncology, University of Connecticut Health Center, Farmington, Conn. 06032), p. 65

Regulation of human hemopoietic stem cell proliferation by syngeneic thymus-derived lymphocytes. *Barr, R. D., Whang-Peng, J., Perry, S.* (Reprint requests: Dr. S. Perry, National Institutes of Health, Building 1, Room 111, Bethesda, Md. 20014), p. 74

Sensitization of stabilized fibrin to urea dispersion by undiluted plasma and serum. *de Cataldo, F., Baudo, F.* (Centro di Ematologia, Ospedale Maggiore Ca'Grande, Milano, Italy), p. 79

Binding of deoxyribonucleic acid to the surface of human platelets. *Clejan, L., Menahem, H.* (Reprint requests: Dr. H. Menahem, Municipal Governmental Medical Center, Ichilov Hospital, Tel-Aviv—Jaffa, Israel), p. 84

Metoprolol and the peripheral platelet count. *Kutti, J., Bergström, A.-L., Lundborg, P.* (Department of Medicine III, Sahlgren's Hospital, S-411 33 Gothenburg, Sweden), p. 89

Classic hemophilia A in a female. *Joist, J. H., Bouhasin, J. D., Roodman, S.* (Hemostasis and Thrombosis Laboratory, Division of Laboratory Medicine, Washington University School of Medicine, Barnes Hospital, St. Louis, Mo. 63110), p. 94

Nonspecific esterase activity in "hairy cells". *Kass, L.* (Department of Internal Medicine, Simpson Memorial Institute, University of Michigan, Ann Arbor, Mich. 48109), p. 103

Acute promyelocytic leukemia: Results of therapy and analysis of 13 cases. *Ruggero, D., Baccarani, M., Guarini, A., Gugliotta, L., Gobbi, M., Ricci, P., Zaccaria, A., Lauria, F., Tomasini, I., Fiacchini, M., Santucci, M. A., Tura, S.* (Servizio di Ematologia, Ospedale S. Orsola, 40138 Bologna, Italy), p. 108.

Multiple myeloma associated with Kaposi sarcoma. *Mandel, E. M., Lask, D., Gafter, U., Weiss, S., Kende, L., Djaldetti, M.* (Department of Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel), p. 120

Acta Haematologica (Basel) **58** (1977) No. 3

Thrombocytopenic purpura as the sole manifestation of recurrence in Hodgkin's disease. *Weitzman, S., Dvilansky, A., Yanai, I.* (Reprint requests: Dr. A. Dvilansky, Soroka Medical Center, Department of Medicine A, Ben Gurion University of the Negev, Beersheba, Israel), p. 129

Incorporation of H^3-leucine in the mouse kidney in thrombocytopenia. Attempt to demonstrate thrombopoietin production. *Krizsa, F., Cserháti, I., Halász, N., Joó, F.* (Second Department of Medicine, University Medical School, H-6701 Szeged, Hungary), p. 134

Oral contraceptives, anti-thrombin III and fibrinolytic activity in Africans. *Greig, H. B. W.* (Department of Haematology, Faculty of Medicine, University of Natal, Congella 4013, Durban, South Africa), p. 138

Permeability of membrane to potassium in hypochromic red cells with different specific density. *Vettore, L., De Matteis, M. C., Antonini, L.* (Istituto di Patologia Medica 2° Università di Padova, Policlinico di Borgo Roma, 37100 Verona, Italy), p. 145

Red cell metabolism and severe neonatal jaundice in West Malaysia. *Lie-Injo, L., E., Virik, H. K., Lim, P. W., Lie, A. K., Ganesan, J.* (Hooper Foundation, University of California, San Francisco, Calif. 94143), p. 152

Proportions of mouse erythrocyte rosette-forming lymphocytes, immunoglobulin-bearing cells and E rosettes in patients with lymphoproliferative diseases. *Dobozy, A., Hunyadi, J., Husz, S., Kenderessy, A. Sz., Simon, N.* (Department of Dermatology and Venerology, University Medical School, H-6701 Szeged, Hungary), p. 161

Atypical B cell dyscrasia with Bence–Jones proteinuria and intracellular retention of γ-chains. *Chenais, F., Virella, G., Young, C. D., Liu, P., Whittle, T. S. Jr.* (Reprint requests: Dr. G. Virella, Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, S. C. 29401), p. 166

Scanning and transmission electron microscopy study on the plasma cells of a patient with multiple myeloma. *Djaldetti, M., Fishman, P.* (Department of Medicine B, Hasharon Hospital, Petah-Tiqva, Israel), p. 173

Susceptibility to autoxidation of lipids of paroxysmal nocturnal hemoglobinuria (PNH)-like red cells. *Kalafatas, P., Voulgaris, E., Vorias, N., Kotsifopoulos, P.* (Professorial Medical Unit, Evangelismos Hospital, Athens, Greece), p. 181

Acta Haematologica (Basel) **58** (1977) No. 4

Results of three years' experience with the deoxyuridine suppression test. *Wickramasinghe, S. N., Saunders, J. E.* (Department of Haematology, St. Mary's Hospital Medical School, London W2 1PG, England), p. 193

Transfer of bovine J blood-group activity to human erythrocytes *in vitro*. *Krötlinger, F., Gallasch, E., Thiele, O. W.* (Reprint requests: Dr. O. W. Thiele, Physiologisch-Chemisches Institut der Universität, D-3400 Göttingen, BRD), p. 207

Refractory sideroblastic anemia secondary to autoimmune hemolytic anemia. *Celada, A., Farquet, J. J., Muller, A. F.* (Department of Medicine, Cantonal Hospital, CH-1206 Geneva, Switzerland), p. 213

A new approach to the diagnosis of β°-thalassaemia. *Chalevelakis, G., Thomopoulos, D., Ladas, S., Pyrovolakis, J., Lyberatos, C., Stathakos, D.* (Research Unit, Professorial Department of Medicine, Evan-

gelismos Hospital, Athens, Greece), p. 217

Haemoglobin H disease and pregnancy in a Malaysian woman. *Ong, H. C., White, J. C., Sinnathuray, T. A.* (UKM Unit, Hospital Bersalin, Kuala Lumpur, Malaysia), p. 229

Cytogenetic studies in acute leukaemias. Prognostic implications of chromosome imbalances. *Alimena, G., Annino, L., Balestrazzi, P., Montuoro, A., Dallapiccola, B.* (Cattedra di Ematologia, I-00161 Roma, Italy), p. 234

Circulating anticoagulant against factor XI and thrombocytopenia with platelet aggregation inhibition in systemic lupus erythematosus. *Leone, G., Accorra, F., Boni, P.* (Istituto di Clinica Medica, Università Cattolica, I-00168 Rome, Italy), p. 240

Combined factor VII and factor VIII deficiency due to a casual association of heterozygosis for factor VII deficiency and hemophilia A^1. *Girolami, A., Dal Bo Zanon, R., Fabris, F., Franzoso, R.* (Istituto di Semeiotica Medica, Padova, Italy), p. 246

Acta Haematologica (Basel) **58** (1977) No. 5

Acquired aplastic anaemia in adults. I. A retrospective analysis of 40 cases: Single factors influencing the prognosis. *Haak, H. L., Hartgrink-Groeneveld, C. A., Eernisse, J. G., Speck, B., van Rood, J. J.* (Isolation Ward, University Hospital, Leiden, The Netherlands), p. 257

Congenital hypoplastic anaemia with unusual dyserythropoietic features. A case report. *Neri, A.* (Ospedale Generale Regionale, 89053 Cantona di Reggio Calabria, Italy), p. 278

Fetal hemoglobin and α-fetoprotein in various malignancies. *Chudwin, D. S., Rucknagel, D. L., Scholnik, A. P., Waldmann, T. A., McIntire, K. R.* (Reprint requests: Prof. D. L. Rucknagel, University of Michigan Medical School, Department of Human Genetics, Ann Arbor, Mich. 48109), p. 288

Hemophiliac with hemolytic anemia resulting from factor VIII concentrate. *Soni, N. S., Patel, A. R., Vohra, R. M., Shah, P. C.* (Reprint requests: Dr. A. R. Patel, Division of Hematology, Hektoen Institute, Chicago, Ill. 60612), p. 294.

Further observations on the incidence and properties of lymphocytotoxins in leukaemia. *Dickson, A., Birchmore, B.* (University College Hospital Medical School, Department of Clinical Haematology, London, WC1E 6HX, England), p. 298

Macroglobulinemia of Waldenström associated with severe osteolytic lesions. *Krausz, Y., Zlotnick, A.* (Department of Medicine A, Hadassah University Hospital, Jerusalem, Israel), p. 307

Effect of cancer chemotherapy drugs on platelet aggregation in children. *Hicsönmez, G., Büyükpamukcu, M.* (Hacettepe University, School of Medicine, Ankara, Turkey), p. 312

A tentative classification of factor VIII deficiency in two groups. *Girolami, A., Burul, A., Fabris, F., Betterle, C.* (University of Padua Medical School, Institute of Semeiotica Medica, Padova, Italy), p. 318

Acta Haematologica (Basel) **58** (1977) No. 6

Systemic mastocytosis: a case report. Cytological, cytochemical and ultrastructural considerations. *Woessner, S., Lafuente, R., Pardo, P., Rosell, R., Rozman, C., Sans-Sabrafen, J.* (Department of Hematology and Clinical Oncology, Hospital de la Cruz Roja, University of Barcelona, Barcelona 13, Spain), p. 321

Bone marrow morphology in patients on regular haemodialysis treatment. *Sjögren, U., Thysell, H.* (Department of Internal Medicine, University Hospital, S-221 85 Lund, Sweden), p. 332

Acquired aplastic anaemia in adults. II. Conventional treatment: Retrospective study in 40 patients. *Haak, H. L., Hartgrink-Groeneveld, C. A., Guiot, H. F. L., Speck, B., Eernisse, J. G., van Rood, J. J.* (Isolation Ward, University Hospital, Leiden, The Netherlands), p. 339

Fletcher factor deficiency. Detection of a severe case in a population survey. *Essien, E. M., Ebhota, M. I.* (Department of Haematology, University College Hospital, Ibadan, Nigeria), p. 353

American Journal of Hematology (New York) **1** (1976) No. 1

The activities of uroporphyrinogen synthetase and cosynthetase in congenital erythropoietic porphyria (CEP). *Miyagi, K., Petryka, Z. J., Bossenmaier, I., Cardinal, R., Watson, C. J.* (Reprint requests: Dr. C. J. Watson, Northwestern Hospital, Minneapolis, Minn. 55407), p. 3

Sickle β-thalassemia: Identical twins differing in severity implicate nongenetic factors influencing course. *Joishy, S. K., Griner, P. F., Rowley, P. T.* (Reprint requests: Dr. P. T. Rowley, Department of Medicine, University of Rochester Medical Center, Rochester, N. Y. 14642), p. 23

Clinical, hematologic and biosynthetic studies in sickle cell-β^0-thalassemia: A comparison with sickle cell anemia. *Steinberg, M. H., Dreiling, B. J.* (VA Hospital, Jackson, Miss. 39216), p. 35

Zinc in the treatment of homozygous sickle cell anemia: Studies in an animal model. *Schoomaker, E. B., Brewer, G. J., Oelshlegel, F. J. Jr.* (Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich. 48104), p. 45

The effect of thrombin on the uptake and transformation of arachidonic acid by human platelets. *Russell, F. A., Deykin, D.* (Reprint requests: Dr. D. Deykin, Boston VA Hospital, Jamaica Plain, Mass. 02130), p. 59

In vivo dissociation of factor VIII (AHF) activity and factor VIII-related antigen in von Willebrand's disease. *Lian, E. Ch.-Y., Deykin, D.* (VA Hospital, Miami, Fla. 33125), p. 71

Effects of single and combined chemotherapeutic agents on hemopoietic stem cells in mice. *Jenkins, V. K., Costanzi, J. J., Ellis, H. N.* (Department of Radiology, University of Texas Medical Branch, Galveston, Texas 77550), p. 79

Platelet injury during phototherapy. *Maurer, H. M., Haggins, J. C., Still, W. J. S.* (Medical College of Virginia, Virginia Commonwealth University, Richmond, Va. 23298), p. 89

Treatment of refractory thrombocytopenic purpura with cyclophosphamide. *Verlin, M., Laros, R. K. Jr., Penner, J. A.* (Reprint requests: Dr. J. A. Penner, Simpson Memorial Institute, University of Michigan Medical Center, Ann Arbor, Mich. 48104), p. 97

Arteriovenous shunts in the human spleen. *Barnhart, M. I., Baechler, C. A., Lusher, J. M.* (Department of Physiology, Wayne State University School of Medicine, Detroit, Mich. 48201), p. 105

Idiopathic thrombocytopenic purpura in Hodgkin's disease after splenectomy. *Julia, A., Miller, S. P.* (Reprint requests: Dr. S. P. Miller, Bethpage Medical Building, Bethpage, N. Y. 11714), p. 115

A view of the current status of antisickling therapy. *Brewer, G. J.* (Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich. 48104), p. 121

The diagnosis of beta-thalassemia trait: A review. *Rowley, P. T.* (Division of Genetics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. 14642), p. 129

American Journal of Hematology (New York) **1** (1976) No. 2

Festschrift in honor of Wolf W. Zuelzer, M. D. *Lusher, J. M., Brown, A. K.* (No address)

Long-term cytogenetic studies in acute leukemia of children; the nature of relapse. *Zuelzer, W. W., Inoue, S., Thompson, R. I., Ottenbreit, M. J.* (Reprint requests: Dr. S. Inoue, The Child Research Center of Michigan, Detroit, Mich. 48201), p. 143

IMFRA (intermittent intrathecal methotrexate and fractional radiation) plus chemotherapy in childhood leukemia. *Zuelzer, W. W., Ravindranath, Y., Lusher, J. M., Sarnaik, Sh., Considine, B. Jr.* (Reprint requests: Dr. Y. Ravindranath, Children's Hospital of Michigan, Detroit, Mich. 48201), p. 191

Chemotherapy of acute lymphoblastic leukemia in children. *Haghbin, M.* (Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 201

T and B lymphocytes in leukemia therapy. *Esber, E., DiNicola, W., Movassaghi, N., Leikin, S. L.* (Reprint requests: Dr. S. L. Leikin, Children's Hospital National

Medical Center, Washington, D. C. 20009), p. 211

Identification of T cell lymphoma tumor antigens on human T cell lines. *Kaplan, J., Tilton, J., Peterson, W. D. Jr.* (Children's Hospital of Michigan, Detroit, Mich. 48201), p. 219

Bilateral interstitial pneumonia in acute lymphoblastic leukemia. *Iyer, R., Ravindranath, Y., Kulkarni, R., Philippart, A., Reed, J. O., Brough, A. J., Zuelzer, W. W.* (Reprint requests: Dr. Y. Ravindranath, Children's Hospital of Michigan, Detroit, Mich. 48201), p. 225

Identification of cells in culture. *Stulberg, C. S., Peterson, W. D. Jr., Simpson, W. F.* (The Child Research Center of Michigan, Detroit, Mich. 48201), p. 237

The human spleen as revealed by scanning electron microscopy. *Barnhart, M. I., Lusher, J. M.* (Wayne State University School of Medicine, Department of Physiology, Detroit, Mich. 48201), p. 243

A prophylactic transfusion program for children with sickle cell anemia complicated by CNS infarction. *Lusher, J. M., Haghighat, H., Khalifa, A. S.* (Children's Hospital of Michigan, Detroit, Mich. 48201), p. 265

Red cell values on the first postnatal day during the last 16 weeks of gestation. *Zaizov, R., Matoth, Y.* (Beilinson Hospital, Petah-Tikva, Israel), p. 275

AB0 hemolytic disease of the newborn, without hyperbilirubinemia. *Kaplan, E., Herz, F., Scheye, E.* (Department of Pediatrics, Montefiore Hospital, Bronx, N. Y. 10467), p. 279

American Journal of Hematology (New York) **1** (1976) No. 3

Thalassemia intermedia caused by heterozygosity for both β-thalassemia and hemoglobin Saki [β14(All)Leu—Pro]. *Milner, P. F., Corley, C. C., Pomeroy, W. L., Wilson, J. B., Gravely, M., Huisman, T. H. J.* (Comprehensive Sickle Cell Medical College of Georgia, Augusta, Ga. 30902), p. 283

A filtration model for study of leukocyte transit in the microcirculation. *Tanner, L. McK., Scott, R. B.* (Reprint requests: Dr. R. B. Scott, Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, Va. 23298), p. 293

Leukocyte function in familial Mediterranean fever. *Territo, M. C., Peters, R. S., Cline, M. J.* (Reprint requests: Dr. M. J. Cline, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 307

Actions of ristocetin on platelets. *Koutts, J., Stott, L., Firkin, B. G.* (Laboratoire de Coagulation, Institut de Pathologie Cellulaire, Hôpital de Bicêtre, 94270 Le Kremlin Bicêtre, France), p. 313

Combined deficiencies of factor VIII (AHF) and factor XI (PTA). *Lian, E. C.-Y., Deykin, D., Harkness, D. R.* (Department of Medicine, Veterans Administration Hospital, Miami, Fla. 33125), p. 319

Immunologic studies of human plasma cells. *Beaulieu, R., Pirofsky, B., Davies, G. H.* (Reprint requests: Dr. B. Pirofsky, University of Oregon Health Sciences Center, Portland, Ore. 97201), p. 325

Quantitative microchromatographic determination of hemoglobin F in patients with hemoglobins S and/or C. *Schroeder, W. A., Evans, L., Grussing, L., Abraham, E. C., Huisman, T. H. J., Lam, H., Shelton J. B.* (Reprint requests: Dr. T. H. J. Huisman, Sickle Cell Center, Medical College of Georgia, Augusta, Ga. 30902), p. 331

Post-transfusion thrombocytopenic purpura: A case report. *Howard, J. E., Glassberg, A. B., Perkins, H. A.* (Reprint requests: Dr H. A. Perkins, Irwin Memorial Blood Bank, San Francisco, Calif. 94118), p. 339

Hereditary antithrombin III deficiency and thromboembolic disease. *Filip, D. J., Eckstein, J. D., Veltkamp, J. J.* (Center for Disease Control, Hematology Division, Atlanta, Ga. 30333), p. 343

Pathogenesis of polycythemia vera. New concepts. *Golde, D. W., Cline, M. J.* (Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 351

Current status of post-transfusion hepatitis. *Conrad, M. E.* (Division of Hematology and Oncology, University of Birmingham, Ala. 35294), p. 357

American Journal of Hematology (New York) **1** (1976) No. 4

Inhibition of murine granulopoiesis *in vitro* by dexamethasone. *Golde, D. W., Bersch, N., Quan, S. G., Cline, M. J.* (Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 369

Alteration of colony-stimulating factor output, endotoxemia, and granulopoiesis in cyclic neutropenia. *Greenberg, P. L. Baz, I., Levin, J., Andrews, T. M.* (Department of Medicine, Stanford University School of Medicine, Stanford, Calif. 94305), p. 375

Myelotoxicity of vincristine-prednisone therapy in treatment of chronic myelogenous leukemia in blastic transformation. *Stein, R. S., Roth, D. G.* (Milwaukee County Medical Complex, Milwaukee, Wis. 53226), p. 387

Staging laparotomy and splenectomy: Treatment and complications of Hodgkin's disease in children. *Lanzkowsky, P., Shende, A., Karayalcin, G., Aral, I.* (Department of Pediatrics, Long Island Jewish-Hillside Medical Center, New Hyde Park, N. Y. 11040), p. 393

Prognostic significance of surface marker analysis in childhood non-Hodgkin's lymphoproliferative malignancies. *Coccia, P. F., Kersey, J. H., Gajl-Peczalska, K. J., Krivit, W., Nesbit, M. E.* (Department of Pediatrics, University of Minnesota, Health Sciences Center, Minneapolis, Minn. 54555), p. 405

Effect of sialidase on the viability of erythrocytes in circulation. *Aminoff, D., Bell, W. C., Fulton, I., Ingebrigsten, N.* (Simpson Memorial Institute, University of Michigan Medical Center, Ann Arbor, Mich. 48109), p. 419

Two new glucose 6-phosphate dehydrogenase variants associated with congenital nonspherocytic hemolytic anemia found in Japan: GD(−) Tokushima and GD(−) Tokyo. *Miwa, S., Ono, J., Nakashima, K., Abe, S., Kageoka, T., Shinohara, K., Isobe, J., Yamaguchi, H.* (Third Department of Internal Medicine, Yamaguchi University School of Medicine, Yamaguchi-ken 755, Japan), p. 433

Effect of antiserum on transplantable hematopoietic colony-forming units during Rauscher leukemia development. *OKunewick, J. P., Phillips, E. L., Brozovich, B.* (Cancer Research Unit, Clinical Radiation Therapy Research Center, Allegheny General Hospital, Pittsburgh, Pa. 15212), p. 443

Erythropoietic precursors in mice with phenylhydrazine-induced anemia. *Hara, H., Ogawa, M.* (Veterans Administration Hospital, Charleston, S. C. 29403), p. 453

Specific radioimmunochemical identification and quantitation of hemoglobins A_2 and F. *Garver, F. A., Jones, C. S., Baker, M. M., Altay, G., Barton, B. P., Gravely, M., Huisman, T. H.* (Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Ga. 30902), p. 459

Predictive value of cross-matching for transfusion of platelet concentrates to alloimmunized recipients. *Filip, D. J., Duquesnoy, R. J., Aster, R. H.* (Reprint requests: Dr. R. H. Aster, Milwaukee Blood Center, Milwaukee, Wis. 53233), p. 471

In vivo and *in vitro* effects of thrombin and plasmin on human factor VIII (AHF). *Lian, E. C.-Y., Nunez, R. L., Harkness, D. R.* (Department of Medicine, Veterans Administration Hospital, Miami, Fla. 33125), p. 481

Evaluation of survival data for chronic myelocytic leukemia. *Sokal, J. E.* (Roswall Memorial Institute, Department of Medicine B, Buffalo, N. Y. 14263), p. 493

Loss of the Y chromosome from bone marrow cells of males with myeloproliferative disorders. Report of two cases and review of the literature. *DiLeo, P. E., Müller, H., Obrecht, J.-P., Speck, B., Bühler, E. M., Stalder, G. R.* (Reprint requests: Dr. H. Müller, Abteilung Genetik, Universitätsklinik Basel, CH-4005 Basel, Switzerland), p. 310

Blood (New York) **48** (1976) No. 2

Factor XI activity and factor XI antigen in homozygous and heterozygous factor XI deficiency. *Rimon, A., Schiffman, S., Feinstein, D. I., Rapaport, S. I.* (Reprint requests: Dr. S. Schiffman, Department of Medicine, University of Southern California School of Medicine, Los Angeles, Calif. 90033), p. 165

Decreased nucleotide and serotonin storage associated with defective function in Chediak-Higashi syndrome cattle and human platelets. *Bell, T. G., Meyers, K. M., Prieur, D. J., Fauci, A. S., Wolff, S. M., Padgett, G. A.* (Reprint requests: Dr. G. A. Padgett, Department of Pathobiology, College of Veterinary Medicine, Washington State University, Pullman, Wash. 99163), p. 175

Potassium uptake and release by human blood platelets. *Wiley, J. S., Kuchibhotla, J., Shaller, C. C., Colman, R. W.* (Haematology Department, Cancer Institute, Melbourne, Victoria 3000, Australia), p. 185

Phospholipid transfer between plasma and platelets *in vitro*. *Joist, J. H., Dolezel, G., Lloyd, J. V., Mustard, J. F.* (Hemostasis and Thrombosis Laboratory, Washington University School of Medicine, Barnes Hospital, St. Louis, Mo. 63110), p. 199

Heterogeneity of immunologic markers and surface morphology in childhood lymphoblastic lymphoma. *Jaffe, E. S., Braylan, R. C., Frank, M. M., Green, I., Berard, C. W.* (National Institutes of Health, Bethesda, Md. 20014), p. 213

Further characterization of the circulating cell in chronic lymphocytic leukemia. *Schultz, E. F., Davis, S., Rubin, A. D.* (Reprint requests: Dr. A. D. Rubin, Division of Oncology/Immunology, St. Joseph's Hospital and Medical Center, Paterson, N. J. 07503), p. 223

A comparison of granulopoiesis in culture from blood and marrow cells of nonleukemic individuals and patients with acute leukemia. *Tebbi, K., Rubin, S., Cowan, D. H., McCulloch, E. A.* (Reprint requests: Dr. E. A. McCulloch, Ontario Cancer Institute, Toronto, Ont. M4X 1K9, Canada), p. 235

DNA histogram analysis of human hemopoietic cells. *Barlogie, B., Spitzer, G., Hart, J. S., Johnston, D. A., Büchner, T., Schumann, J., Drewinko, B.* (Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030), p. 245

Spinal cord disease in hereditary spherocytosis: Report of two cases with a hypothesized common mechanism for neurologic and red cell abnormalities. *McCann, S. R., Jacob, H. S.* (Reprint requests: Dr. H. S. Jacob, Mayo Building, University of Minnesota Hospital, Minneapolis, Minn. 55455), p. 259

The conformational requirements for the mechanical precipitation of hemoglobin S and other mutants. *Roth, E. F. Jr., Elbaum, D., Bookchin, R. M., Nagel, R. L.* (Department of Medicine, Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 265

Molecular and cellular effects of antisickling concentrations of alkylureas. *Elbaum, D., Roth, E. F. Jr., Neumann, G., Jaffé, E. R., Bookchin, R. M., Nagel, R. L.* (Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 273

Fetal hemopoiesis in diffusion chamber cultures. III. The effect of neutropenia. *Symann, M., Quesenberry, P., Fontebuoni, A., Howard, D., Ryan, M., Stohlman, F. Jr.* (Reprint requests: Dr. P. Quesenberry, Department of Medicine, St. Elizabeth's Hospital, Brighton, Mass. 02135), p. 283

A diffusible stimulator of eosinophilopoiesis produced by lymphoid cells as demonstrated with diffusion chambers. *Miller, A. M., McGarry, M. P.* (Reprint requests: Dr. M. P. McGarry, Department of Biological Resources, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 293

Evidence for early recruitment of granulocyte precursors during high-dose methotrexate infusions in mice. *Pinedo, H. M., Chabner, B. A., Zaharko, D. S., Bull, J. M.* (Reprint requests: Dr. J. M. Bull, National Cancer Institute, Bethesda, Md. 20014), p. 301

The biochemical basis of nitroblue tetrazolium reduction in normal humans and chronic granulomatous disease polymorphonuclear leukocytes. *Baehner, R. L., Boxer, L. A., Davis, J.* (James Whitcomb Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Ind. 46202), p. 309

In vitro function and post-transfusion survival of granulocytes collected by continuous-flow centrifugation and by filtration leukapheresis. *McCullough, J., Weiblen, B. J., Deinard, A. R., Boen, J., Fortuny, I. E., Quie, P. G.* (University of Minnesota Hospitals Blood Bank, Minneapolis, Minn. 55455), p. 315

Blood (New York) **48** (1976) No. 3

5-azacytidine (NSC 102816): A new drug for the treatment of myeloblastic leukemia. *Vogler, W. R., Miller, D. S., Keller, J. W.* (Emory University, Atlanta, Ga. 30322), p. 331

Human acute myelomonocytic leukemia: Serologic studies with simian antisera. *Mohanakumar, T., Miller, D. S., Metzgar, R. S.* (Department of Microbiology and Immunology, Duke University Medical Center, Durham, N. C. 27710), p. 339

Granulopoiesis in chronic myeloid leukemia. II. Serial cloning of blood and bone marrow cells in agar culture. *Olafsson, T., Olsson, I.* (Reprint requests: Dr. I. Olsson, Research Department 2, E-blocket, University Hospital, S-221 85 Lund, Sweden), p. 351

Leukocyte density and volume in normal subjects and in patients with acute lymphoblastic leukemia. *Zipursky, A., Bow, E., Seshadri, R. S., Brown, E. J.* (Department of Pediatrics, McMaster University Medical Centre, Hamilton, Ont. Canada L8S 4J9), p. 361

Regulation of bone marrow cell growth in diffusion chambers: The effect of adding normal and leukemic (CML) polymorphonuclear granulocytes. *Böyum, A., Lövhaug, D., Boecker, W. R.* (Norwegian Defense Research Establishment, Division for Toxicology, N-2007 Kjeller, Norway), p. 373

Successful bone marrow transplantation against mixed lymphocyte culture barrier. *Feig, S. A., Opelz, G., Winter, H. S., Falk, P. M., Neerhout, R. C., Sparkes, R., Gale, R. P.* (Department of Pediatrics, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 385

Antibody-induced von Willebrand's disease: A newly defined inhibitor syndrome. *Handin, R. I., Martin, V., Moloney, W. C.* (Peter Bent Brigham Hospital, Boston, Mass. 02115), p. 393

Human marrow erythropoiesis in culture. I. Characterization of methylcellulose colony assay. *Ogawa, M., Parmley, R. T., Bank, H. L., Spicer, S. S.* (Veterans Administration Hospital, Charleston, S. C. 29403), p. 407

Erythropoietic events in cultured embryonic mouse spleen. *Bessler, H., Nothi, I., Fishman, P., Djaldetti, M.* (Reprint requests: Prof. M. Djaldetti, Department of Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel), p. 419

Factors which affect erythropoiesis in partially nephrectomized and sham-operated rats. *Anagnostou, A., Vercellotti, G., Barone, J., Fried, W.* (Reprint requests: Dr. W. Fried, Division of Hematology, Michael Reese Medical Center, Chicago, Ill. 60616), p. 425

Delayed erythropoiesis in irradiated rats grafted with syngeneic marrow: Effects of cytotoxic drugs and iron-deficiency anemia. *Rodday, P., Bennett, M., Vitale, J. J.* (Mallory Institute of Pathology, Boston City Hospital, Boston, Mass. 02118), p. 435

Evaluation of the iron status of a population. *Cook, J. D., Finch, C. A., Smith, N. J.* (Division of Hematology, Department of Medicine, University of Kansas Medical Center, Kansas City, Kans. 66103), p. 449

Increased potassium transport and ouabain binding in human Rh_{null} red blood cells. *Lauf, P. K., Joiner, C. H.* (Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N. C. 27710), p. 457

Distribution of spermidine and spermine in blood from cystic fibrosis patients and control subjects. *Cohen, L. F., Lundgren, D. W., Farrell, P. M.* (National Institutes of Health, Bethesda, Md. 20014), p. 469

Blood (New York) **48** (1976) No. 4

What's in a name? *Levy, R. I., Zuelzer, W. W.* (Department of Health, Education and Welfare, National Institutes of Health, Bethesda, Md. 20014), p. 481

Allogeneic marrow grafting for treatment of aplastic anemia: A follow-up on long-term survivors. *Storb, R., Thomas, E. D., Buckner, C. D., Clift, R. A., Fefer, A., Fernando, L. P., Giblett, E. R., Johnson, F. L., Neiman, P. E.* (Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 485

Establishment of erythropoiesis following bone marrow transplantation in a patient with congenital hypoplastic anemia

(Diamond-Blackfan syndrome). *August, C. S., King, E., Githens, J. H., McIntosh, K., Humbert, J. R., Greensheer, A., Johnson, F. B.* (Reprint requests: Dr. J. H. Githens, Department of Pediatrics, University of Colorado Medical Center, Denver, Colo. 80220), p. 491

A clinical study of the lupus anticoagulant. *Schleider, M. A., Nachman, R. L., Jaffe, E. A., Coleman, M.* (The New York Hospital-Cornell Medical Center, New York, N. Y. 10021), p. 499

Acquired storage pool disease in platelets during disseminated intravascular coagulation. *Pareti, F. I., Capitanio, A., Mannucci, P. M.* (Hemophilia and Thrombosis Center Angelo Bianchi Bonomi, University of Milano, Milano, Italy), p. 511

A platelet abnormality in the Chediak-Higashi syndrome of man. *Costa, J. L., Fauci, A. S., Wolff, S. M.* (National Institute of Mental Health, NIH Clinical Center, Bethesda, Md. 20014), p. 517

Immunologic characterization of canine factor VIII. *Benson, R. E., Dodds, W. J.* (Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. 12201), p. 521

Fibrinolysis in normal plasma and blood: Evidence for significant mechanisms independent of the plasminogen-plasmin system. *Moroz, L. A., Gilmore, N. J.* (Royal Victoria Hospital, Montreal, P. Q. Canada H3A 1A), p. 531

Relationships between thrombopoiesis and erythropoiesis: With studies of the effects of preparations of thrombopoietin and erythropoietin. *Evatt, B. L., Spivak, J. L., Levin, J.* (Hematology Division, The Johns Hopkins Hospital, Baltimore, Md. 21205), p. 547

Comparison of normal and chronic lymphocytic leukemia lymphocyte surface IG determinants using peroxidase-labeled antibodies. II. Quantification of light chain determinants in atypical lymphocytic leukemia. *Dighiero, G., Follezou, J. Y., Roisin, J. P., Ternynck, T., Binet, J. L.* (Reprint requests: Dr. J. Y. Follezou, Département d'Hématologie, Faculté de Médicine Pitiè-Salpétrière, Paris 75013, France), p. 559

Acquired angioedema with lymphoproliferative disorder: Association of C1 inhibitor deficiency with cellular abnormality. *Schreiber, A. D., Zweiman, B., Atkinson P., Goldwein, F., Pietra, G., Atkinson, B., Abdou, N. I.* (Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pa. 19104), p. 567

Granulocyte function in chronic granulocytic leukemia. II. Bactericidal capacity, phagocytic rate, oxygen consumption, and granule protein composition in isolated granulocytes. *Olofsson, T., Odeberg, H., Olsson, I.* (Research Department 2, University Hospital, S-221 85 Lund, Sweden), p. 581

Duplication of part of the long arm of chromosome 1 in marrow cells of a treated case of myelomatosis. *Spriggs, A. I., Holt, J. M., Bedford, J.* (Laboratory of Clinical Cytology, Churchill Hospital, Oxford, England), p. 595

Some observations of the hematopoietic status *in vivo* and *in vitro* on mice of genotype $S1/S1^d$. *Wilson, F. D., O'Grady, L.* (Radiobiology Laboratory, University of California, Davis, Calif. 95616), p. 601

Synthesis of cobalamin coenzymes by human lymphocytes *in vitro* and the effect of folates and metabolic inhibitors. *Quadros, E. V., Matthews, D. M., Hoffbrand, A. V., Linnell, J. C.* (Reprint requests: Dr. J. C. Linnell, Department of Experimental Chemical Pathology, Vincent Square Laboratories, London SW1V 2RH, England), p. 609

Blood (New York) **48** (1976) No. 5

Abnormal hemoglobins in a quarter million people. *Schneider, R. G., Hightower, B., Hosty, T. S., Ryder, H., Tomlin, G., Atkins, R., Brimhall, B., Jones, R. T.* (Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas 77550), p. 629

A new alkali-resistant hemoglobin α_2^1 Oxford γ_2^F in a Sicilian baby girl with homozygous β^0 thalassemia. *Schilirò, G., Musumeci, S., Pizzarelli, G., Russo, A., Marinucci, M., Bruni, E., Russo, G.* (Pediatric Clinic of the University, 95125 Catania, Italy), p. 639

Hemoglobin Mequon β41 (C7) phenylalanine → tyrosine. *Burkert, L. B., Sharma,*

V. S., Pisciotta, A. V., Ranney, H. M., Bruckheimer, S. (Reprint requests: Dr. A. V. Pisciotta, Milwaukee County General Hospital, Milwaukee, Wisc. 53226), p. 645

Causal mechanisms of multiple acquired red cell enzyme defects in a patient with acquired dyserythropoiesis. *Kahn, A., Cottreau, D., Boyer, C., Marie, J., Galand, C., Boivin, P.* (INSERM U24, Hôpital Beaujon, 92110 Clichy, France), p. 653

Red cell membrane deformability: New data. *Bull, B. S., Brailsford, J. D.* (Loma Linda University, School of Medicine, Loma Linda, Calif. 92354), p. 663

Reticulocyte size in nutritional anemias. *Clarkson, D. R., Moore, E. M.* (Department of Medicine, University of South Alabama, College of Medicine, Mobile, Ala. 36617), p. 669

Marrow regeneration after mechanical depletion. *Brecher, G., Tjio, J. H., Smith, W. W., Haley, J. E.* (University of California, San Francisco, School of Medicine, San Francisco, Calif. 94143), p. 679

Specific chromosomal aberrations in polycythemia vera. *Zech, L., Gahrton, C., Killander, D., Franzén, S., Haglund, U.* (Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institutet, S-104 01 Stockholm 60, Sweden), p. 687

Chromosomes and causation of human cancer and leukemia. XXI. Cytogenetically unusual cases of leukemia. *Sonta, Sh., Oshimura, M., Sandberg, A. A.* (Reprint requests: Dr. A. A. Sandberg, Roswell Park Memorial Institute, Department of Health-State of New York, Buffalo, N. Y. 14263), p. 697

Lymphosarcoma cell leukemia: The contribution of cell surface study to diagnosis. *Aisenberg, A. C., Wilkes, B.* (Huntington Laboratories, Massachusetts General Hospital, Boston, Mass. 02114), p. 707

Human lymphocyte motility: Normal characteristics and anomalous behavior of chronic lymphocytic leukemia cells. *Jarvis, C. S., Snyderman, R., Cohen, H. J.* (Reprint requests: Dr. H. J. Cohen, Hematology-Oncology Section, VA Hospital, Duke University Medical Center, Durham, N. C. 27705), p. 717

A method for the recognition and separation of human blood monocytes on density gradients. *Loos, H., Blok-Schut, B., van Doorn, R., Hoksbergen, R., de la Rivière, A. B., Meerhof, L.* (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 731

Size distribution, electronic recognition, and counting of human blood monocytes. *Loos, H., Blok-Schut, B., Kipp, B., van Doorn, R., Meerhof, L.* (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 743

Surface markers of human eosinophils. *Gupta, S., Ross, G. D., Good, R. A., Siegal, F. P.* (Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 755

Stimulation of megakaryocytopoiesis by acute thrombocytopenia in rats. *Odell, T. T., Murphy, J. R., Jackson, C. W.* (Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830), p. 765

Mechanisms involved in enhancement of plasma fibrinolytic activity by chloroform. *Moroz, L. A., Gilmore, N. J.* (Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1), p. 777

Blood (New York) **48** (1976) No. 6

A simplified *in vitro* classification for prognosis in adult acute leukemia: The application of *in vitro* results in remission-predictive models. *Spitzer, G., Dicke, K. A., Gehan, E. A., Smith, T., McCredie, K. B., Barlogie, B., Freireich, E. J.* (Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030), p. 795

Monocyte-mediated antibody-dependent cellular cytotoxicity: A clinical test of monocyte function. *Poplack, D. G., Bonnard, G. D., Holiman, B. J., Blaese, R. M.* (Pediatrics Oncology Branch, National Institutes of Health, Bethesda, Md. 20014), p. 809

Aplastic anemia treated by allogeneic bone marrow transplantation: A report on 49 new cases from Seattle. *Storb, R., Thomas, E. D., Weiden, P. L., Buckner, C. D.,*

Clift, R. A., Fefer, A., Fernando, L. P., Giblett, E. R., Goodell, B. W., Johnson, F. L., Lerner, K. G., Neiman, P. E., Sanders, J. E. (Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 817

Fetal erythropoiesis following bone marrow transplantation. *Alter, B. P., Rappeport, J. M., Huisman, T. H. J., Schroeder, W. A., Nathan, D. G.* (Division of Hematology-Oncology, Children's Hospital Medical Center, Boston, Mass. 02115), p. 843

Steroids and hematopoiesis. III. The response of granulocytic and erythroid colony-forming cells to steroids of different classes. *Singer, J. W., Adamson, J. W.* (Reprint requests: Dr. J. W. Adamson, Hematology Research Laboratory, Veterans Administration Hospital, Seattle, Wash. 98108), p. 855

Kinetics of mobilization of neutrophils and their marrow pool in protein-calorie deficiency. *Suda, A. K., Mathur, M., Deo, K., Deo, M. G.* (Department of Pathology, All India Institute of Medical Sciences, New Delhi 110016, India), p. 865

Phosphorylation in erythrocyte membranes from abnormally shaped cells. *Greenquist, A. C., Shohet, S. B.* (Cancer Research Institute, University of California Medical Center, San Francisco, Calif. 94143), p. 877

Human red cell protein kinase in normal subjects and patients with hereditary spherocytosis, sickle cell disease, and autoimmune hemolytic anemia. *Beutler, E., Guinto, E., Johnson, C.* (City of Hope Medical Center, Duarte, Calif. 91010), p. 887

Stabilization of the shape of sickled cells by calcium and A23187. *Clark, M. R., Greenquist, A. C., Shohet, S. B.* (Reprint requests: Dr. S. B. Shohet, Cancer Reseearch Institute, University of California, San Francisco School of Medicine, San Francisco, Calif. 94143), p. 899

Total folate binding capacity of normal human plasma, and variations in uremia, cirrhosis, and pregnancy. *Colman, N., Herbert, V.* (Reprint requests: Dr. V. Herbert, Hematology and Nutrition Laboratory, Veterans Administration Hospital, Bronx, N. Y. 10568), p. 911

The effect of chelating agents on iron mobilization in Chang cell cultures. *White, G. P., Jacobs, A., Grady, R. W., Cerami, A.* (Reprint requests: Dr. A. Jacobs, Department of Haematology, Welsh National School of Medicine, University Hospital of Wales, Cardiff CF4 4XN, Wales, United Kingdom), p. 923

Characterization of human anti-factor VIII antibodies purified by immune complex formation. *Lavergne, J. M., Meyer, D., Reisner, H.* (Reprint requests: Dr. D. Meyer, Institut de Pathologie Cellulaire (INSERM U 143), Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France), p. 931

Fitzgerald factor (high molecular weight kininogen) clotting activity in human plasma in health and disease in various animal plasmas. *Saito, H., Goldsmith, G., Waldmann, R.* (Department of Medicine, University Hospital of Cleveland, Cleveland, Ohio 44106), p. 941

Evidence suggesting the regulation of coagulation factor levels in rabbits by a transferable plasma agent. *Friedman, E. W., Karpatkin, M., Karpatkin, S.* (Reprint requests: Dr. S. Karpatkin, Department of Medicine, New York University Medical Center, New York, N. Y. 10016), p. 949

In memoriam: Robert Inslee Weed, 1928—1976. *La Celle, P. L., Lichtman, M. A.* (No address)

Blood (New York) **49** (1977) No. 1

Letter from the editor. *Jaffé, E. R.*

Acquired granulocyte abnormality during drug allergic reactions: Possible role of complement activation. *Bowers, T. K., Craddock, P. R., Jacob, H. S.* (Department of Medicine, University of Minnesota School of Medicine, Minneapolis, Minn.), p. 3

Improvement of Chediak—Higashi leukocyte function by cyclic guanosine monophosphate. *Boxer, L. A., Rister, M., Allen, J. M., Baehner, R. L.* (Indiana University School of Medicine, J. W. Riley Hospital for Children, Indianapolis, Ind. 46202), p. 9

Hairy cell leukemia: B-lymphocyte and phagocytic properties. *Utsinger, P. D., Yount, W. J., Fuller, C. R., Logue, M. J., Lampkin, B. C., Granger, S.* (Division of Rheumatology and Clinical Immunology, Univer-

sity of North Carolina, School of Medicine, Chapel Hill, N. C. 27514), p. 19

Bone marrow cellularity determination: Comparison of the biopsy, aspirate, and buffy coat. *Gruppo, R. A., Lampkin, B. C., Granger, S.* (Children's Hospital Research Foundation, Hematology-Oncology Division, Cincinnati, Ohio 45229), p. 29

Cytofluorometric analysis of the kinetics of lymphocyte transformation after phytohemagglutinin stimulation: Comparison with the kinetics of thymidine incorporation. *Utsinger, P. D., Yount, W. J., Fallon, J. G., Logue, M. J., Fuller, C. R., Elliott, D.* (Division of Rheumatology and Clinical Immunology, University of North Carolina, School of Medicine, Chapel Hill, N. C. 27514), p. 33

Changes in hemopoiesis during the course of acute LCM virus infection in mice. *Bro-Jørgensen, K., Knudtzon, S.* (Institute of Medical Microbiology, University of Copenhagen, 2100 Copenhagen Ø, Denmark), p. 47

Thrombocytic suppression of megakaryocyte production from stem cells. *Goldberg, J., Phalen, E., Howard, D., Ebbe, S., Stohlman, F. Jr.* (Reprint requests: Dr. S. Ebbe, St. Elizabeth's Hospital, Brighton, Mass. 02135), p. 59

Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Corash, L., Tan, H., Gralnick, H. R.*, (Hematology Service, Clinical Center, National Institutes of Health, Bethesda, Md. 20014), p. 71

Labeling of the releasable adenine nucleotides of washed human platelets. *Reimers, H.-J., Packham, M. A., Mustard, J. F.* (Department of Pathology, McMaster University, Hamilton, Ontario L8S 4J9, Canada), p. 89

Inhibition and potentiation of platelet function by lysolecithin. *Joist, J. H., Dolezel, G., Cucuianu, M. P., Nishizawa, E. E., Mustard, J. F.* (Hemostasis and Thrombosis Laboratory, Division of Laboratory Medicine, Washington University School of Medicine, Barnes Hospital, St. Louis, Mo. 63110), p. 101

Increased membrane binding of erythrocyte catalase in hereditary spherocytosis and in metabolically stressed normal cells. *Allen, D. W., Cadman, S., McCann, S. R., Finkel, B.* (Hematology Section, Veterans Administration Hospital, Minneapolis, Minn. 55417), p. 113

Applicability of an enzymatic quantitation of methylmalonic, propionic, and acetic acids in normal and megaloblastic states. *Frenkel, E. P., Kitchens, R. L.* (Department of Internal Medicine, University of Texas Health Science Center, Southwestern Medical School, Dallas, Texas 75235), p. 125

Immunologic differences in human isoferritins: Implications for immunologic quantitation of serum ferritin. *Hazard, J. T., Yokota, M., Arosio, P., Drysdale, J. W.* (Reprint requests: Dr. J. W. Drysdale, Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Mass. 02111), p. 139

Effect of induced fever on serum iron and ferritin concentrations in man. *Elin, R. J., Wolff, S. M., Finch, C. A.* (National Institutes of Health, Clinical Center, Bethesda, Md. 20014), p. 147

Blood (New York) **49** (1977) No. 2

Prothrombin complex concentrates: Potentially thrombogenic materials and clues to the mechanism of thrombosis *in vivo*. *White, G. C. II., Roberts, H. R., Kingdon, H. S., Lundblad, R. L.* (Division of Hematology, University of North Carolina, Chapel Hill, N. C. 27614), p. 159

Relationship between the anticoagulant and antithrombin effects of heparin in experimental venous thrombosis. *Chiu, H. M., Hirsh, J., Yung, W. L., Regoeczi, E., Gent, M.* (Reprint requests: Dr. J. Hirsh, Department of Pathology, McMaster University Medical Centre, Hamilton, Ont., Canada L8S 4J9), p. 171

Effect of propranolol on platelet function. *Weksler, B. B., Gillick, M., Pink, J.* (Department of Medicine, New York Hospital-Cornell Medical Center, New York, N. Y. 10021), p. 185

Induction of thrombocytopenia by thrombopheresis in man: Patterns of recovery in normal subjects during ethanol ingestion and abstinence. *Sullivan, L. W., Adams, W. H., Liu, Y. K.* (Reprint requests: Dr. W. H. Adams, Department of Medi-

cine, Boston City Hospital, Boston, Mass. 02118), p. 197

Rabbit factor VIII: Identification of size heterogeneity. *Rick, M. E., Wampler, D. E., Hoyer, L. W.* (Department of Medicine, University of Connecticut Health Center, Farmington, Conn. 06032), p. 209

Demonstration of granulocytic protease in plasma of patients with acute leukemia and septicemia with coagulation defects. *Egbring, R., Schmidt, W., Fuchs, G., Havemann, K.* (Department of Medicine, University Hospital, D-3550 Marburg/Lahn, FRG), p. 219

Association of decreased membrane protein phosphorylation with red blood cell spherocytosis. *Matsumoto, N., Yawata, Y., Jacob, H. S.* (Reprint requests: Dr. H. S. Jacob, Department of Medicine, University of Minnesota Medical School, Minneapolis, Minn. 55455), p. 233

The molecular lesion of hereditary spherocytosis (HS): A continuing enigma. *Valentine, W. N.* (Department of Medicine, University of California, Center for the Health Sciences, Los Angeles, Calif. 90024), p. 241

G-6-PD Long Prairie: A new glucose-6-phosphate dehydrogenase mutant exhibiting normal sensitivity to inhibition by NADPH and accompanied by nonspherocytic hemolytic anemia. *Johnson, G. J., Kaplan, M. E., Beutler, E.* (Hematology Section, Medical Service, Veterans Administration Hospital, Minneapolis, Minn. 55417), p. 247

Hematopoietic stem cells: Effect of preirradiation, bleeding, and erythropoietin on thrombopoietic differentiation. *Goodman, R., Grate, H., Hannon, E., Hellman, S.* (Joint Center for Radiation Therapy, Boston, Mass. 02115), p. 253

Enhancement of colony-stimulating activity production by lithium. *Harker, W. G., Rothstein, G., Clarkson, D., Athens, J. W., Macfarlane, J. L.* (Reprint requests: Dr. G. Rothstein, University of Utah College of Medicine, Salt Lake City, Utah 84132), p. 263

Interacting cell populations in cultures of leukocytes from normal or leukemic peripheral blood. *McCulloch, E. A., Till, J. E.* (Ontario Cancer Institute, Toronto, Ontario M4X 1K9, Canada), p. 269

Ph^1-positive acute lymphocytic leukemia with chromosome 7 abnormalities. *Mandel, E. M., Shabtai, F., Gafter, U., Klein, B., Halbrecht, I., Djaldetti, M.* (Reprint requests: Dr. M. Djaldetti, Department of Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel), p. 281

Effect of cytochalasin B on human monocyte binding and sphering of IgG-coated human erythrocytes. *Herskowitz, B., Guerry, D. IV, Cooper, R. A., Schreiber, A. D.* (Reprint requests: Dr. A. D. Schreiber, Hematology-Oncology Section, Department of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104), p. 289

IgE myeloma with osteoblastic lesions. *Rogers, J. S. II, Spahr, J., Judge, D. M., Varano, L. A., Eyster, M. E.* (Reprint requests: Dr. M. E. Eyster, The Milton S. Hershey Medical Center, Hershey, Pa.), p. 295

Blood volume in monoclonal gammopathy. *Alexanian, R.* (Department of Medicine, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025), p. 301

Formation of lipid inclusions in normal human leukocytes. *Lutas, E. M., Zucker-Franklin, D.* (Reprint requests: Dr. D. Zucker-Franklin, Department of Medicine, New York University Medical Center, New York, N. Y. 10016), p. 309

Blood (New York) **49** (1977) No. 3

Combination chemotherapy ("CHOP-Bleo") in advanced (non-Hodgkin) malignant lymphoma. *Rodriguez, V., Cabanillas, F., Burgess, M. A., McKelvey, E. M., Valdivieso, M., Bodey, G. P., Freireich, E. J.* (Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030), p. 325

Immunodeficiency in patients with non-Hodgkin lymphomas. *Jones, S. E., Griffith, K., Dombrowski, P., Gaines, J. A.* (Section of Hematology and Oncology, Department of Medicine, University of Arizona College of Medicine, Tucson, Ariz. 85724), p. 335

Electrolyte and acid-base disturbances in the management of leukemia. *O'Regan, S.,*

Carson, S., Chesney, R. W., Drummond, K. N. (Reprint requests: Dr. K. N. Drummond, Department of Nephrology, Montreal Children's Hospital, Montreal, Quebec, Canada), p. 345

Changing erythrocyte populations in juvenile chronic myelocytic leukemia: Evidence for disordered regulation. *Dover, G. J., Boyer, S. H., Zinkham, W. H., Kazazian, H. H. Jr., Pinney, D. J., Sigler, A.* (The Johns Hopkins Hospital, Department of Pediatrics, Baltimore, Md. 21205), p. 355

The Philadelphia chromosome in human macrophages. *Golde, D. W., Burgaleta, C., Sparkes, R. S., Cline, M. J.* (Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 367

T and B lymphocyte antigen-positive null cell leukemias. *Kaplan, J., Ravindranath, Y., Peterson, W. D. J.* (Children's Hospital of Michigan, Detroit, Mich. 48201), p. 371

Multiple myeloma: Quantitative staging and assessment of response with a programmable pocket calculator. *Salmon, S. E., Wampler, S. B.* (Section of Hematology and Oncology, Department of Internal Medicine, University of Arizona College of Medicine, Tucson, Ariz. 85724), p. 379

Semicontinuous flow centrifugation for the pheresis of immunocompetent cells and stem cells. *Weiner, R. S., Richman, C. M., Yankee, R. A.* (Division of Medical Oncology, Department of Medicine, University of Florida College of Medicine, J. Hillis Miller Health Center, Gainesville, Fla. 32610), p. 391

Polycythemia vera: Hormonal modulation of erythropoiesis *in vitro*. *Golde, D. W., Bersch, N., Cline, M. J.* (Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 399

In vitro stimulation of primate hemoglobin synthesis by L-thyroxine. *Fuhr, J. E., Gengozian, N., Overton, M.* (University of Tennessee Memorial Research Center for the Health Sciences, Knoxville, Tenn. 37920), p. 407

Proliferation of diffusion chamber colony-forming units (CFUD) in cultures of normal human bone marrow in diffusion chambers in mice. *Jacobson, N.* (Laboratories of Experimental Hematology, Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 415

Congenital neutropenia: An intrinsic cell defect demonstrated by electron microscopy of soft agar colonies. *Zucker-Franklin, D., L'Esperance, P., Good, R. A.* (New York University Medical Center, School of Medicine, New York, N. Y. 10016), p. 425

Hydrogen peroxide production and killing of *Staphylococcus aureus* by human polymorphonuclear leukocytes. *Tsan, Min-Fu, Douglas, K. H., McIntyre, P. A.* (Division of Nuclear Medicine and Hematology, The Johns Hopkins Medical Institutions, Baltimore, Md. 21205), p. 437

Effect of cyanide on NADPH oxidation by granules from human polymorphonuclear leukocytes. *DeChatelet, L. R., McPhail, L. C., Shirley, P. S.* (Department of Biochemistry, The Bowman Gray School of Medicine, Winston-Salem, N. C. 27103), p. 445

Free erythrocyte porphyrin: Hemoglobin ratios, serum ferritin, and transferrin saturation levels during treatment of infants with iron-deficiency anemia. *Thomas, W. J., Koenig, H. M., Lightsey, A. L. Jr., Green, R.* (Reprint requests: Dr. H. M. Koenig, MC USN, Naval Regional Medical Center, San Diego, Calif. 92134), p. 455

Intraerythrocyte adaptation (2,3 DPG, P_{50}) in thalassemia minor. *Pearson, H. A., Motoyama, E., Genel, M., Kramer, M., Zigas, C. J.* (Yale University School of Medicine, Department of Pediatrics, New Haven, Conn. 06510), p. 463

Glucose-6-phosphate dehydrogenase deficiency and red cell glutathione peroxidase. *Beutler, E.* (Division of Medicine, City of Hope Medical Center, Duarte, Calif. 91010), p. 467

Effects of ascorbic acid on glucose-6-phosphate dehydrogenase-deficient erythrocytes: Studies in an animal model. *Udomratn, T., Steinberg, M. H., Campbell, G. D. Jr., Oelshlegel, F. J. Jr.* (Reprint requests: Dr. M. H. Steinberg, VA Hospital, Jackson, Miss. 39216), p. 471

Techniques for demonstration for the specificity of circulating anticoagulants against antihemophilic factor (factor VIII), with studies of two cases possibly related to

diphenylhydantoin therapy. *Poon, M.-Ch., Saito, H., Ratnoff, O. D., Forman, W. B., Wisnieski, J.* (Reprint requests: Dr. O. D. Ratnoff, Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106), p. 477

Migration of transfused granulocytes in leukopenic dogs. *Appelbaum, F. R., Norton, L., Graw, R. G. Jr.* (Experimental Hematology Section, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014), p. 483

Blood (New York) **49** (1977) No. 4

Gamma heavy chain disease: Clinical aspects and characterization of a deleted, noncovalently linked γ1 heavy chain dimer (BAZ). *Faguet, G. B., Barton, B. P., Smith, L. L., Garver, F. A.* (Department of Medicine, Medical College of Georgia, Augusta, Ga. 30902), p. 495

Blood viscosity in Waldenström macroglobulinemia. *MacKenzie, M. R., Lee, T. K.* (Section of Hematology and Oncology, University of California, Davis, Sacramento Medical Center, Sacramento, Calif. 95817), p. 507

One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Thomas, E. D., Buckner, C. D., Banaji, M., Clift, R. A., Fefer, A., Flournoy, N., Goodell, B. W., Hickman, R. O.* et al. (Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 511

Ultrastructural cytochemical analysis of blastic transformation of chronic myelocytic leukemia. *Palakavongs, P., Teichberg, S., Vinciguerra, V., Degnan, T. J., Sinlaratana, P.* (Reprint requests: Dr. V. Vinciguerra, Division of Oncology, North Shore University Hospital, Manhasset, N. Y. 11030), p. 535

Ph^1-positive childhood leukemias: Spectrum of lymphoid-myeloid expressions. *Forman, E. N., Padre-Mendoza, T., Smith, P. S., Barker, B. E., Farnes, P.* (Division of Pediatric Hematology-Oncology, Rhode Island Hospital, Providence, R. I. 02902), p. 549

Treatment of juvenile chronic myeloid leukemia with sequential subcutaneous cytarabine and oral mercaptopurine. *Lilleman, J. S., Harrison, J. F., Black, J. A.* (Department of Hematology, The Children's Hospital, Sheffield, S10 2TH, England), p. 559

Systemic mastocytosis in a patient with polycythemia vera treated with radioactive phosphorus. *Eagan, J. W. Jr., Baughman, K., Miller, S., Conley, C. L., Eggleston, J. C.* (Department of Pathology, The Johns Hopkins Hospital, Baltimore, Md. 21205), p. 563

Stimulation by human placental conditioned medium of hemopoietic colony formation by human marrow cells. *Burgess, A. W., Wilson, E. M. A., Metcalf, D.* (Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, 3050 Victoria, Australia), p. 573

cGMP stimulation of stem cell proliferation. *Oshita, A. K., Rothstein, G., Lonngi, G.* (Reprint requests: Dr. G. Rothstein, University of Utah College of Medicine, Salt Lake City, Utah 84132), p. 585

Colony formation *in vitro* by mouse blood monocytes. *Hsiu-san Lin* (Mallinckrodt Institute of Radiology, Washington University Medical School, St. Louis, Mo. 63110), p. 593

Cell size and palmitate-1-^{14}C turnover of rabbit marrow fat. *Trubowitz, S., Bathija, A.* (Hematology Section, Veterans Administration Hospital, East Orange, N. J. 07019), p. 599

Carriers with excessively low factor VIII procoagulant activity (VIII AHF): A study of two unrelated families with mild hemophilia A. *Eyster, M. E., Ladda, R. L., Bowman, H. S.* (The Milton S. Hershey Medical Center, Hershey, Pa. 17033), p. 607

Demonstration and mode of action of an inhibitor for activated Hageman factor (factor XIIa) of the intrinsic blood coagulation pathway from *Schistosoma mansoni*. *Tsang, V. C. W., Damian, R. T.* (Department of Zoology, University of Georgia, Athens, Ga. 30602), p. 619

Significance of the intact polypeptide chains of human fibrinogen in ADP-induced platelet aggregation. *Niewiarowski, S., Budzynsky, A. Z., Lipinski, B.* (Specialized

Center for Research in Thrombosis, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 635

Effect of aspirin on thrombogenesis and on production of experimental aortic valvular *Streptococcus viridans* endocarditis in rabbits. *Levison, M. E., Carrizosa, J., Tanphaichitra, D., Schick, P. K., Rubin, W.* (The Medical College of Pennsylvania, Philadelphia, Pa. 19129), p. 645

Circulation of donor lymphocytes after blood transfusion in man. *Schechter, G. P., Whang-Peng, J., McFarland, W.* (Hematology Research Section, VA Hospital, Washington, D. C. 20422), p. 651

Erythrocyte membrane plaques from rats with magnesium deficiency. *Elin, R. J., Tan, H. K.* (National Institutes of Health, Clinical Center, Bethesda, Md. 20014), p. 657

Blood (New York) **49** (1977) No. 5

Current status of bone marrow transplantation for aplastic anemia and acute leukemia. *Thomas, E. D., Fefer, A., Buckner, C. D., Storb, R.* (Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104), p. 671

Pretreatment cytokinetic studies in 94 children with acute leukemia. Relationship to other variables at diagnosis and to outcome of standard treatment. *Murphy, S. B., Aur, R. J. A., Simone, J. V., George, S., Mauer, A. M.* (St. Jude Children's Research Hospital, Memphis, Tenn. 38101), p. 683

Abnormalities in granule formation in acute myelogenous leukemia. *Bainton, D. F., Friedlander, L. M., Shohet, S. B.* (Department of Pathology, University of California School of Medicine, San Francisco, Calif. 94143), p. 693

Characteristics of blast crisis in chronic granulocytic leukemia. *Rosenthal, S., Canellos, G. P., DeVita, V. T. Jr., Gralnick, H. R.* (Reprint requests: Dr. H. R. Gralnick, Hematology Service, Clinical Pathology Department, Clinical Center, NIH, Bethesda, Md. 20014), p. 705

Group-specific human granulocyte antigens on a chronic myelogenous leukemia cell line with a Philadelphia chromosome marker. *Drew, S. I., Terasaki, P. I., Billing, R. J., Bergh, O. J., Minowada, J., Klein, E.* (Department of Surgery, School of Medicine, University of California, Los Angeles, Calif. 90024), p. 715

Variations in lymphocyte counts four hours after administration of hydrocortisone in patients with chronic lymphocytic leukemia. *Dighiero, G., Vaugier, G., Charron, D., d'Athis, P., Binet, J.-L.* (Reprint requests: Prof. J. L. Binet, Département d'Hématologie, Groupe Hôpitalier Pitié-Salpêtrière, 75013 Paris, France), p. 719

Evidence for differentiation of human leukemic blood cells in diffusion chamber culture. *Hoelzer, D., Kurrle, E., Schmücker, H., Harriss, E. B.* (Abteilung Hämatologie, Zentrum für Innere Medizin u. Kinderheilkunde, Universität Ulm, 7900 Ulm, BRD), p. 729

Leukemic reticuloendotheliosis: "Hairy cell leukemia", functional and structural features of the abnormal cell in a patient with profound leukocytosis. *Boldt, D. H., Speckart, S. F., MacDermott, R. P., Nash, G. S., Valeski, J. E.* (Reprint requests: Dr. S. F. Speckart, Department of Hematology, WRAIR, Washington, D. C. 20012), p. 745

Combination chemotherapy of advanced non-Hodgkin lymphoma with bleomycin, adriamycin, cyclophosphamide, vincristine, and prednisone (BACOP). *Skarin, A. T., Rosenthal, D. S., Moloney, W. C., Frei, E. III* (Sidney Farber Cancer Center, Boston, Mass. 02115), p. 759

Depressed *in vitro* lymphocyte response to PHA in patients with Hodgkin disease in continuous long remissions. *Case, D. C. Jr., Hansen, J. A., Corrales, E., Young, C. W., Dupont, B., Pinsky, C. M., Good, R. A.* (Reprint requests: Dr. J. A. Hansen, Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 771

Antibody response to intravenous immunization following splenic tissue autotransplantation in Sprague-Dawley rats. *Schwartz, A. D., Dadash-Zadeh, M., Goldstein, R., Luck, S., Conway, J. J.* (University of Maryland Hospital, Department of Pediatrics, Baltimore, Md. 21202), p. 779

Detection of sickle α- or β°-thalassemia by studies of globin biosynthesis. *Kim, H. C.,*

Weierbach, R .G., Friedman, S., Schwartz, E. (Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, Pa. 19104), p. 785

Detection of factor VIII inhibitors with the partial thromboplastin time. *Lossing, T. S., Kasper, C. K., Feinstein, D. I.* (Reprint requests: Dr. C. K. Kasper, Department of Medicine, University of Southern California School of Medicine, Los Angeles, Calif. 90007), p. 793

Circulating anticoagulant in a family with prolonged bleeding time and factor VIII deficiency. *Diez-Ewald, M., Lian, E. C.-Y., Nunez, R., Deykin, D., Harkness, D. R.* (Reprint requests: Dr. E. C.-Y. Lian, Department of Medicine, Veterans Administration Hospital, Miami, Fla. 33125), p. 799

Heterogeneity of factor VIII antibodies: Further immunochemical and biologic studies. *Hultin, M. B., London, F. S., Shapiro, S. S., Yount, W. J.* (Reprint requests: Dr. S. S. Shapiro, Cardeza Foundation, Jefferson Medical College, Philadelphia, Pa. 19107), p. 807

Factor V activity of platelets: Evidence for an activated factor V molecule and for a platelet activator. *Østerud, B., Rapaport, S. I., Lavine, K. K.* (Research Service (VI51), San Diego Veterans Administration Hospital, San Diego, Calif. 92161), p. 819

Eosinophil colony formation in semisolid cultures of human bone marrow cells. *Dresch, C., Johnson, G. R., Metcalf, D.* (Reprint requests: Dr. G. R. Johnson, Walter and Eliza Hall Institute, Cancer Research Unit, Royal Melbourne Hospital, Victoria, Australia), p. 835

Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. *Asano, S., Urabe, A., Okabe, T., Sato, N., Kondo, Y., Ueyama, Y., Chiba, S., Ohsawa, N., Kosaka, K.* (Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113, Japan), p. 845

Blood (New York) **49** (1977) No. 6

Human marrow cells capable of erythropoietic differentiation *in vitro:* Definition of three erythroid colony responses. *Gregory, C. J., Eaves, A.C.* (Cancer Foundation, Vancouver, B. C. V5Z 3J3, Canada), p. 855

Factors controling stem cell recirculation. III. Effect of the thymus on the migration and differentiation of hemopoietic stem cells. *Petrov, R. V., Khaitov, R. M., Aleinikova, N. V., Gulak, L. V.* (Radiobiology Department, Institute of Biophysics, USSR Ministry of Health, Moscow 123182, USSR), p. 865

Effect of corticosteroids on lymphocyte activation. *Yu, D. T. Y.* (UCLA School of Medicine, Division of Rheumatology, Rehabilitation Center, Los Angeles, Calif. 90024), p. 873

Prognostic classification of Hodgkin disease in pathologic stage III, based on anatomic considerations. *Desser, R. K., Golomb, H. M., Ultmann, J. E., Ferguson, D. J., Moran, E. M., Griem, M. L., Vardiman, J., Miller, B., Oetzel, N., Sweet, D., Lester, E. P., Kinzie, J. J., Blough, R.* (Reprint requests: Dr. J. E. Ultmann, Department of Medicine, Surgery, Radiotherapy, Pathology, Nursing and Biomedical Computation, University of Chicago, Chicago, Ill.), p. 883

Cell marker analysis in acute monocytic leukemias. *Koziner, B., McKenzie, S., Straus, D., Clarkson, B., Good, R. A., Siegal, F. P.* (Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 895

Marrow culture studies in adult acute leukemia at presentation and during remission. *Vincent, P. C., Sutherland, R., Bradley, M., Lind, D., Gunz, F. W.* (Medical Research Department, Kanematsu Memorial Institute, Sydney Hospital, Sydney, N. S. W. 2000 Australia), p. 903

Chronic myeloid leukemia: Physical and functional similarities to acute leukemia. *Senn, J. S., Price, G. B.* (Sunnybrook Medical Centre, Room 2040, Toronto, Ontario, Canada), p. 913

Induction of serum stimulation and plasma cell proliferation during chemotherapy of multiple myeloma. *Karp, J. E., Burke, P. J., Humphrey, R. L.* (Johns Hopkins Comprehensive Cancer Center, Baltimore, Md. 21205), p. 925

Relationship of contact activation cofactor

(CAC) procoagulant activity to kininogen. *Schiffman, S., Lee, P., Feinstein, D. I., Pecci, R.* (Department of Medicine, University of Southern California School of Medicine, Los Angeles, Calif. 90033), p. 935

Lipid A as the biologically active moiety in bacterial endotoxin (LPS)-initiated generation of procoagulant activity by peripheral blood leukocytes. *Niemetz, J., Morrison, D. C.* (Division of Hematology, Veterans Administration Hospital, Bronx, N. Y. 10468), p. 947

Role of transferrin in determining internal iron distribution. *Pootrakul, P., Christensen, A., Josephson, B., Finch, C. A.* (Reprint requests: Prof. C. A. Finch, Division of Hematology RM-10, Department of Medicine, School of Medicine, University of Washington, Seattle, Wash. 98195), p. 957

Red cells in sickle cell crisis: Observations on the pathophysiology of crisis. *Rieber, E. E., Veliz, G., Pollack, S.* (Department of Medicine, Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 967

Frequencies of thalassemia in American blacks. *Pierce, H. I., Kurachi, S., Sofroniadou, K., Stamatoyannopoulos, G.* (Madigan Army Medical Center, Tacoma, Wash. 98431), p. 981

Circulating antibody to transcobalamin II causing retention of vitamin B_{12} in the blood. *Carmel, R., Tatsis, B., Baril, L.* (University of Southern California, School of Medicine, Los Angeles, Calif. 90033), p. 987

Reticulocyte motility and form: Studies on maturation and classification. *Mel, H. C., Prenant, M., Mohandas, N.* (Division of Medical Physics, Donner Laboratory, University of California, Berkeley, Calif. 94720), p. 1001

American Board of Internal Medicine guidelines for training in hematology and medical oncology. No address, p. 1011

Blood Cells (Paris) **3** (1977) No. 1

Section I — Methods for Evaluation of Red Cell Deformability

Intravascular rheology of erythrocytes in man. *Brånemark, P.-I., Bagge, U.* (Laboratory of Experimental Biology, Department of Anatomy, University of Göteborg, S-400 33 Göteborg 33, Sweden), p. 11

The aspiration of red cell membrane into small holes: New data. *Brailsford, J. D., Korpman, R. A., Bull, B. S.* (Reprint requests: Dr. B. S. Bull, School of Allied Health Professions and School of Medicine, Loma Linda University, Loma Linda, Calif. 92354), p. 25

Red cell membrane deformability: an examination of two apparently disparate methods of measurement. *Bull, B. S., Brailsford, J. D., Korpman, R. A.* (School of Medicine, Loma Linda University, Loma Linda, Calif. 92354), p. 39

Basic principles of the "filterability test", (FT) and analysis of erythrocyte flow behavior. *Teitel, P.* (Department of Physiology, Technical University, 5100 Aachen, FRG), p. 55

Principles and techniques for assessing erythrocyte deformability. *Chien, S.* (Laboratory for Hemorrheology, Department of Physiology, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 71

Viscometric techniques and the rheology of blood. *Phillips, W. M.* (College of Engineering, The Pennsylvania State University, University Park, Pa. 16802), p. 101

Section II — Biochemical Basis for Red Cell Shape and Deformability

Possible roles for membrane protein phosphorylation in the control of erythrocyte shape. *Shohet, S. B., Greenquist, A. C.* (University of California San Francisco, School of Medicine, Cancer Research Center, San Francisco, Calif. 94142), p. 115

Human red cell protein kinase in normal subjects and patients with hereditary spherocytosis, sickle cell disease, and autoimmune hemolytic anemia. *Beutler, E., Guinto E., Johnson, C.* (City of Hope Medical Center, Duarte, Calif.), p. 135

Role of ATP depletion on red cell shape and deformability. *Feo, C., Mohandas, N.* (Institut de Pathologie Cellulaire, INSERM U. 48, Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 153

The effects of ATP depletion on the response

of erythrocytes to shear stress. *Tadano, K., Hellums, J. D., Lynch, E. C., Peck, E. J., Alfrey, C. P.* (Biomedical Engineering Laboratory, Rice University, Houston, Texas 77001), p. 163

Effect of protein modification on erythrocyte membrane mechanical properties. *Heusinkveld, R. S., Goldstein, D. A., Weed, R. I., LaCelle, P. L.* (Division of Radiation Oncology, Arizona Medical Center, University of Arizona, Tucson, Ariz. 85724), p. 175

Antibody-induced spherocytic anemia. I. Changes in red cell deformability. *Mohandas, N., de Boisfleury, A.* (Cancer Research Institute, University of California, San Francisco, Calif. 94143), p. 187

Antibody-induced spherocytic anemia. II. Splenic passage and sequestration of red cells. *de Boisfleury, A., Mohandas, N.* (Cancer Research Institute, University of California, San Francisco, Calif. 94143), p. 197

Red cell deformability changes in hemolytic anemias estimated by diffractometric methods (ektacytometry). Preliminary results. *Allard, C., Mohandas, N., Bessis, M.* (Reprint requests: Dr. N. Mohandas, Cancer Research Institute, University of California, San Francisco, Calif. 94143), p. 209

Blood Cells (Paris) **3** (1977) No. 2

Section I — Sickle Cell Rheology

Laser diffraction patterns of sickle cells in fluid shear fields. *Bessis, M., Mohandas, N.* (Institut de Pathologie Cellulaire, (INSERM U.-48), Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 229

Deformability of normal and sickle erythrocytes in a pressure-flow filtration system. *Lessin, L. S., Kurantsin-Mills, J., Weems, H. B.* (Division of Hematology and Oncology, Department of Medicine, The George Washington University Medical Center, Washington, D. C. 20037), p. 241

Microvascular blood flow of sickled erythrocytes. A dynamic morphologic study. *Klug, P. P., Lessin, L. S.* (The George Washington University Medical Center, Washington, D. C. 20037), p. 263

Oxygen delivery to muscle cells during capillary occlusion by sickled erythrocytes. *LaCelle, P. L.* (Departments of Medicine and Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. 14642), p. 273

Rheology of sickle cells and erythrocyte content. *Chien, S.* (Laboratory of Hemorrheology, Department of Physiology, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 283

Experimentally-induced alterations in the kinetics of erythrocyte sickling. *Zarkowsky, H. S., Hochmuth, R. M.* (Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Mo. 63110), p. 305

Section II — General Theories of Red Cell Shape Structure and Rheology

The red cell shape as an indicator of membrane structure. Ponder's rule reexamined. *Korpman, R. A., Dorrough, D. C., Brailsford, J. D., Bull, B. S.* (School of Medicine and School of Allied Health Professions, Loma Linda University, Loma Linda, Calif. 92354), p. 315

Erythrocyte membrane elasticity, fragmentation and lysis. *LaCelle, P. L., Evans, E. A., Hochmuth, R. M.* (Department of Medicine and Radiation Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. 14642), p. 335

Tank tread motion of red cell membranes in viscometric flow: Behavior of intracellular and extracellular markers (with film). *Fischer, T., Schmid-Schönbein, H.* (Abteilung Physiologie, Medizinische Fakultät der RWTH Aachen, D-5100 Aachen, BRD), p. 351

Theoretical aspects and clinical applications of the blood viscosity equation containing a term for the internal viscosity of the red cell. *Dintenfass, L.* (Hemorrheology Unit, Medical Research, Kanematsu Memorial Institute, Sydney Hospital, Sydney, Australia), p. 367

Hemolysis thresholds in microporous structures. *Blackshear, P. L. Jr., Anderson, R. J.* (Department of Mechanical Engineering, University of Minnesota, Minneapolis, Minn. 55455), p. 377

Effect of radio contrast media on the red blood cell. An *in vitro* study on human erythrocytes. *Aspelin, P., Schmid-Schönbein H.* (Department of Radiology, University of Malmö, Malmö, Sweden), p. 397

Effects of storage on the respiratory function and flexibility of red blood cells. *Sirs, J. A.* (Department of Biophysics, St. Mary's Hospital Medical School, University of London, Paddington, W.2, England), p. 409

Section III

Rheological methods. *Chien, S.* (Department of Physiology, Columbia Medical Center, New York, N. Y. 10032), p. 427

The implications of rheology for red cell membrane structure. *Bull, B.* (Department of Pathology, School of Medicine, Loma Linda University, Loma Linda, Calif. 92354), p. 431

Clinical applications. *Brecher, G.* (Department of Laboratory Medicine, University of California, San Francisco, Calif. 94143), p. 437

Blood Cells (Paris) **3** (1977) No. 3

Leukemic cell surveillance. *Bernard, J. Bessis, M.* (Clinique des Maladies du Sang, Hôpital Saint Louis, F-75010 Paris, France), p. 445

Section I — Regulatory Mechanisms of Leukemic Cell Populations

Proliferation regulators in haemopoietic cell populations. *Lord, B. I., Mori, K. J., Wright, E. G., Lajtha, L. G.* (Reprint requests: Dr. L. Lajtha, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England), p. 451

Cellular factors regulating granulopoiesis in myeloid leukemia. *Berthier, R., Douady, F., Hollard, D.* (Centre d'Études Nucléaires, Laboratoire d'Hématologie, DRF 85X 38041 Grenoble Cedex, France), p. 461

Diversity in proliferation patterns of acute lymphoid leukemias. *Wagner, H. P., Giesen, L., Müller, J., Cottier, H.* (Institute for Clinical Cancer Research, University of Bern, CH-3004 Bern, Switzerland), p. 475

Colony-inhibiting activity of polymorphonuclear cells in chronic myeloid leukemia. *Stryckmans, P. A., Peyer, A., Collard, E., Mattelaer, M., Debusscher, L.* (Institut Jules Bordet, B-1000 Bruxelles, Belgium), p. 493

The L 5222 acute leukemia in rats: Observations on cellular characteristics and on its influence on normal hematopoiesis. *Fliedner, T. M., Hoelzer, D.* (Abteilung für Klinische Physiologie, Zentrum für Klinische Grundlagenforschung, Universität Ulm, D-7900 Ulm Donau, BRD), p. 505

Studies on the regulation of leukemic cell production in human acute leukemia. *Hoelzer, D., Fliedner, T. M.* (Abteilung für Klinische Physiologie, Zentrum für Klinische Grundlagenforschung, Universität Ulm, D-7900 Ulm, BRD), p. 519

Pathogenesis and blastic transformation on chronic myeloid leukemia as consequences of Ph^1-positive stem cell hyperplasia: A unifying concept. *Pedersen, B.* (Institute of Cancer Research, Radiumstationen, DK-8000 Aarhus C, Denmark), p. 535

Selective proliferation during the preneoplastic state: an example in liver. *Solt, D., Farber, E.* (Department of Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8), p. 553

Relevance of the BN leukemia as a model for human acute myeloid leukemia. *van Bekkum, D. W., Hagenbeek, A.* (Radiobiological Institute TNO, Rijswijk, The Netherlands), p. 565

The limits of the cytogenetic method in acute leukemia. *Zuelzer, W. W.* (Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md. 20014), p. 581

Section II — Immunity and Leukemic Cell Surveillance

Genetically determined resistance mechanisms in leukemia. *Schwartz, R. S., Datta, S. K.* (Hematology Service, New England Medical Center Hospital, Boston, Mass. 02111), p. 591

Plasma-mediated leukemic cell destruction: Current status. *Kassel, R. L., Old, L. J., Day, N. K., MacEwen, E. G., Hardy, W. D. Jr.* (Animal Medical Center and Memorial Sloan-Kettring Cancer Center, Sloan-Kettering Institute, Rye, N. Y. 10580), p. 605

Thymic hormone and T-cell maturation in acute leukemia. *Astaldi, G., Cavder, A., Topuz, Ü.* (Frazione Centro, I-27044 Canneto Pavese [PV], Italy), p. 623

Chronic lymphocytic leukemia: Concepts and observations. *Chandra, P., Chanana, A. D., Chikkappa, G., Cronkite, E. P.* (Medical Research Center, Brookhaven National Laboratory, Upton, N. Y. 11973), p. 637

Surveillance of chronic lymphocytic leukemia. *Binet, J. L., Dighiero, G., Leporrier, M., Follezou, J. Y., Charron, D., Debré, P.* (Département d'Hématologie, CHU Pitié-Salpêtrière [ERA CNRS 500], F-75634 Paris Cedex 13, France), p. 651

Human leukaemia associated antigens. I. Definition with patients' sera. *Guibout, C., Doré, J.-F., Mathé, G.* (Institut de Cancérologie et d'Immunogénétique, F-94800 Villejuif, France), p. 655

Human leukaemia associated antigens. II. Recognition by normal human sera. *Bertoglio, J., Doré, J.-F., Guibout, C.* (Reprint requests: Dr. J.-F. Doré, Laboratoire d'Immunologie et de Cancérologie Expérimentale, Centre Léon Bérard, 69373 Lyon Cedex 2, France), p. 663

Section III — Leukemic Cell Surveillance by the Physician

Supervision of treatment of acute leukemias: Remarks. *Bernard, J.* (Clinique des Maladies du Sang, Hôpital Saint Louis, 75010 Paris, France), p. 675

A new technique for surveillance of response to chemotherapy in leukemia. *Ross, D. W.* (Department of Laboratory Medicine, University of California, San Francisco, Calif. 94143), p. 677

Relevance of cell size to the monitoring of treatment efficacy in acute leukemia and chronic lymphocytic leukemia. *Binet, J. L., Dighiero, G., Cayre, Y., de Montaut, F., Couty, M. C.* (Département d'Hématologie, CHU Pitié-Salpêtrière [ERA CNRS 500], 75634 Paris Cedex 13, France), p. 687

Section IV — Discussion

Alternatives to surveillance. *Brecher, G.* (University of California, School of Medicine, Department of Laboratory Medicine, San Francisco, Calif. 94143), p. 693

Summing up. *Bernard, J.* (Clinique des Maladies du Sang, Hôpital Saint-Louis, F-75010 Paris, France), p. 695

Blut (München) **33** (1976) No. 4

Congenital dysfibrinogenemias: Molecular abnormalities of fibrinogen. *Mammen, E. F.* (College of Pharmacy and Allied Health Professions, Wayne Street University, Detroit, Mich. 48202), p. 229

Fraktionierung von normalem menschlichem Knochenmark auf Albumin-Gradienten. *Jehn, U., Oerkermann, H., Heller, A., Hötzel, J.* (Medizinische Universitäts-Klinik Köln, 5000 Köln 41, BRD), p. 235

Selenkonzentration und Aktivität der Glutathionperoxydase im Lysat menschlicher Erythrozyten. *Schmidt, K., Heller, W.* (Laboratorium der Chirurgischen Universitätskliniken, 7400 Tübingen, BRD), p. 247

Zur Frage der Lymphozytopenie beim Pseudo-Lupus Erythematodes-Syndrom. *Genth E., Hartl, W., Hanning, H., Steffens, E.* (Rheumaklinik, Landesbad Aachen, D-5100 Aachen, BRD), p. 253

Vitamin B^{12}-Stoffwechsel menschlicher Granulozyten. *Pfeiffer, R.* (Innere Universitätsklinik und Poliklinik [Tumorforschung], D-4300 Essen 1, BRD), p. 265

Das Rh_{null}-Syndrom. *Seidl, S.* (Institut für Immunhämatologie, Klinikum der Universität, 6000 Frankfurt/Main 71, BRD), p. 271

Technical improvements in heat-ethanol isolation of serum albumin. *Schneider, W., Wolter, D., McCarty, L. J.* (Blutspendedienst des Deutschen Roten Kreuzes, Institut Hagen, D-5800 Hagen, BRD), p. 275

Veränderungen des κ/λ-Verhältnisses der menschlichen Serumimmunoglobuline im

Verlaufe der Entwicklung. *Skvaril, F., Barandun, S., Kuffer, F., Probst, M.* (Institut für Klinisch-Experimentelle Tumorforschung, Tiefenauspital, CH-3004 Bern, Schweiz), p. 281

Blut (München) **33** (1976) No. 5

Clones of antibody-forming cells in microcultures. *Lefkovits, I.* (Basel Institute for Immunology, CH-4005 Basel, Switzerland), p. 297

Re-entry of resting leukaemic blood cells into proliferation in human acute leukaemia during diffusion chamber culture. *Hoelzer, D., Schmücker, H., Kurrle, E.* (Abteilung für Hämatologie, Zentrum für Innere Medizin und Kinderheilkunde, Universität Ulm, 7900 Ulm/Donau, BRD), p. 301

Lysosomal acid phosphatase: Differences between normal and chronic lymphocytic leukaemia T and B lymphocytes. *Meusers, P., König, E., Fink, U., Brittinger, G.* (Hämatologische Abteilung der Medizinischen Universitätsklinik, D-8000 München 80, BRD), p. 313

A_{mh}, eine Bombay-Variante mit Kryptantigen H. *Gallasch, E., Fintelmann, E., Mack, R.* (Hygiene-Institut der Universität, D-3400 Göttingen, BRD), p. 319

Untersuchungen zum Polymorphismus der menschlichen erythrozytären Esterase D. *Wiebecke, D.* (Chirurgische Universitätsklinik, Abteilung Transfusionsmedizin und Immunhämatologie, 8700 Würzburg, BRD), p. 329

Plasmazelleinschlüsse bei Morbus Pompe (Glycogenose Typ II). Zweite Beobachtung einer neuen Anomalie. *Pralle, H., Löffler, H.* (Reprint requests: Prof. Dr. H. Löffler, Medizinische Klinik der Universität, 6300 Gießen, BRD), p. 332

SI-Einheiten in der Hämatologie. *Stamm, D.* (Abteilung für Klinische Chemie des Max-Planck-Instituts für Psychiatrie, D-8000 München 40, BRD), p. 333

Blut (München) **33** (1976) No. 6

The D locus (LD) antigens of the HLA complex. *Bach, F. H.* (Immunobiology Research Center, University of Wisconsin, Madison, Wis. 53706), p. 353

Acute lymphoblastic leukemia: A study of 25 cases by scanning electron microscopy. *Polliack, A., Froimovici, M., Pozzoli, E., Lambertenghi-Deliliers, G.* (Department of Haematology, Hadassah University Hospital, Jerusalem, Israel), p. 359

Erfahrungen bei der Langzeitbehandlung des angeborenen Faktor XIII-Mangels mit Faktor XIII-Konzentrat. *Egbring, R., Andrassy, K., Havemann, K., Fucks, G., Ruf, B., Schander, K., Trobisch, H.* (Medizinische Klinik, 3550 Marburg/Lahn, BRD), p. 367

Isolierung und Charakterisierung des schwangerschaftsassoziierten α_2-Glykoproteins (α_2 PAG). *Bohn, H., Winckler, W.* (Behringwerke AG, 3550 Marburg/Lahn, BRD), p. 377

Neutrophil marrow release. *Meuret, G., Bammert, J., Gessner, U.* (Medizinische Klinik C, Katonsspital, CH-9007 St. Gallen, Switzerland), p. 389

Blut (München) **34** (1977) No. 1

Experience with incompatible maternal donors for bone marrow transplantation. *Feig, S. A., Falk, P. M., Neerhout, R. C., Sparkes, R., Gale, R. P., Opelz, G., Cline, M. J., Fahey, J., Smith, G., Sarna, G., Territo, M., Young, L., Langdon, E. A., Fawzi, F.* (Department of Pediatrics, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 1

Prognosis in acquired aplastic anemia. An approach in the selection of patients for allogeneic bone marrow transplantation. *Hellriegel, K.-P., Züger, M., Gross, R.* (Medizinische Universitätsklinik, D-5000 Köln 41, BRD), p. 11

Neutrophil marrow egress in polycythemia vera. *Meuret, G., Heibges, M.* (Medizinische Klinik C, Kantonsspital, CH-9007 St. Gallen, Switzerland), p. 19

Factor VIII coagulant activity and factor VIII-related antigen released from isolated perfused human spleens. *Pabst, R., Heyes, H., Rasche, H., Schick, P., Trepel, F.* (Abteilung für Funktionelle und Angewandte Anatomie [4120], Medizinische Hochschule Hannover, D-3000 Hannover 61, BRD), p. 27.

In vivo study of platelet kinetics by ^{75}Se-methionine in different haematological

disorders. *Müller, U., Kulinna, J., Lüber, J., Hebestreit, H.-P., Mayer, M., Kempgens, U., Queißer, W.* (Reprint requests: Prof. Dr. W. Queißer, I. Medizinische Klinik, Krankenanstalten, D-6800 Mannheim 1, BRD), p. 31

Reappraisal of the clinical and etiological significance of immunoglobulin deviations in autoimmune hemolytic anemia ("warm type"). *Mueller-Eckhardt, Ch., Möhring, F., Kretschmer, V., Höbel, W., Löffler, H.* (Abteilung Klinische Immunologie und Transfusionsmedizin, Universität Gießen, D-6300 Gießen, BRD), p. 39

A modified method for measuring the filtrability of erythrocytes. *Imre, S.* (Institute of Pathophysiology, University Medical School, H-4012 Debrecen, Hungary), p. 49

Blut (München) **34** (1977) No. 2

Granulopoietic studies in acute lymphocytic leukemia in children. *Mangalik, A., Robinson, W. A., Holton, C. P.* (Department of Pediatrics, University of Colorado Medical Center, Denver, Col. 80220), p. 77

Growth kinetics by scanning of granulocytic cell colonies in glass capillaries. *Maurer, H. R., Henry, R.* (Pharmazeutisches Institut der Freien Universität Berlin, D-1000 Berlin, 33, BRD), p. 89

"Fibrinogen Hannover", ein weiteres atypisches Fibrinogen. *Barthels, M., Sandvoß, G.* (Department für Innere Medizin der Medizinischen Hochschule, 3000 Hannover, BRD), p. 99

Fibrinogen Marburg. A new genetic variant of fibrinogen. *Fuchs, G., Egbring, R., Havemann, K.* (Medizinische Universitäts-Poliklinik, Städtisches Krankenhaus, 7980 Ravensburg, BRD), p. 107

Clinical and cytogenetic observations during a six-year period in an adult with Fanconi's anaemia. *Schroeder, T. M., Drings, P., Beilner, P., Buchinger, G.* (Institut für Anthropologie und Humangenetik, 6900 Heidelberg, BRD), p. 119

The changes of erythrocyte shape following action of different substances altering Mg^{++}-dependent ATPase activity (actomyosin-like protein). *Mirčevová, L., Viktora, L., Šimonová, A.* (Institute of Haematology and Blood Transfusion, 128 20 Prague 2, Czechoslovakia), p. 133

Zur Frage der Konditionierbarkeit der Veränderungen der Thrombozytenzahl und anderer Komponenten des menschlichen Kapillarblutes. *Freund, J. L., Regan, H.* (Experimentelle Streßforschung der Max-Planck-Gesellschaft im Psychologischen Institut, J.-Gutenberg-Universität, D-6500 Mainz, BRD), p. 139

Qualitätskontrolle und Standardisierung der Prothrombinzeitbestimmung zur Kontrolle oraler Antikoagulation. *Loeliger, E. A., van Dijk-Wierda, C. A., Roos, J.* (Academisch Ziekenhuis, Afdeling Haemostase-en Thromboseonderzoek, Leiden, Nederland), p. 147

Internationales Symposium "Recent Trends in the Immunological Diagnosis of Leukemias and Lymphomas", Neuherberg/München, 28. bis 30. Oktober 1976, p. 159

Blut (München) **34** (1977) No. 3

Transplantation of fetal liver cells in the treatment of severe combined immunodeficiency disease. *Löwenberg, B., Vossen, J. M. J. J., Dooren, L. J.* (Radiobiological Institute T. N. O., Rijswijk (Z. H.), The Netherlands), p. 181

Selective deficiency of IgG and IgA as a first stage defect in B cell differentiation. *Bricarelli, F. D., DeLanglade, E., LaFauci, M. F., Moscatelli, P.* (Cattedra di Genetica Umana dell'Università, 16128 Genova, Italy), p. 197

Relative and absolute numbers of E- and EAC-rosette-forming cells in lymphoid organs. *Pabst, R., Kaupp, E., Trepel, F.* (Abteilung für Funktionelle und Angewandte Anatomie (4120), Medizinische Hochschule Hannover, D-3000 Hannover 61, BRD), p. 201

Mycoplasma pneumoniae infection complicated by paroxysmal cold hemoglobinuria with anti-P specificity of biphasic hemolysin. *Boccardi, V., D'Annibali, S., Di Natale, G., Girelli, G., Summonti, D.* (Istituto di Patologia Medica I dell'Università di Roma, 00100 Rome, Italy), p. 211

Platelet function, blood coagulation and fibrinolysis in Behcet's syndrome.

Stathakis, N. E., Economopoulos, T. C., Papayannis, A. G., Thomopoulos, D. (Reprint requests: Prof. Dr. A. G. Papayannis, Evangelismos Hospital, Athens 140, Greece), p. 215

Determination of the bacteriostatic capacity of neutrophils and monocytes in mixed cell populations. *Meuret, G., Westerhausen, M., Senn, H., Pagon, S.* (Medizinische Klinik C, Kantonsspital, CH-9006 St. Gallen, Switzerland), p. 223

Establishment of peripheral lymphoid cell cultures from patients with Hodgkin's disease depending on Epstein-Barr-virus-reactivity and cellular immunity. *Diehl, V., Johansson, B.* (Abteilung für Hämatologie-Onkologie, Medizinische Hochschule, 3000 Hannover, BRD), p. 227

The utilization of a new strength citrate anticoagulant during centrifugal plateletpheresis. II. Assessment of *in vitro* platelet function. *Mishler, J. M., Borberg, H., Reuter, H., Gross, R.* (Department of Haematology, Gibson Laboratory, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 237

Blut (München) **34** (1977) No. 4

Histokompatibilität und Thrombozytentransfusion. *Mueller-Eckhardt, C.* (Institut für Klinische Immunologie und Transfusionsmedizin der Justus-Liebig-Universität, Giessen, 6300 Lahn-Giessen, BRD), p. 261

Zur Problematik des Konduktorinnen-Nachweises bei der Hämophilie A: Fehlermöglichkeiten bei der Bestimmung des Faktor VIII-assoziierten Antigens. *Salfner, B., Göbel, U., Kokemohr, H., von Voss, H., Petrich, C.* (Reprint requests: Dr. U. Göbel, Medizinische Einrichtungen der Universität Düsseldorf, Kinderklinik, 4000 Düsseldorf, BRD), p. 271

Verminderte Flexibilität der Erythrozyten von Neugeborenen. *Tillmann, W., Wagner, D., Schrötter, W.* (Universitäts-Kinderklinik, 3400 Göttingen, BRD), p. 271

Growth of bone marrow cells of normal and Rauscher virus infected mice in suspension cultures stimulated by CSA. *Seidel, H. J., Iturriza, R. G.* (Abteilung für Klinische Physiologie, Universität Ulm, 7900 Ulm/Donau, BRD), p. 289

Immunochemical and ultrastructural investigations in a patient with cold-agglutinine-active monoclonal IgMK-gammopathy. *Ottó, Sz., Börzsönyi, M., Eckhardt, S., Mohay, A., Gergely, J., Kellner, R.* (National Oncological Institute, H-1525 Budapest, Hungary), p. 299

Langzeittherapie mit Anti-Hunde-Lymphozyten-Globulin vom Pferd ohne Sensibilisierung gegen Pferdeprotein. *von Scheel, J., Duswald, K.-H., Ring, J. Seifert, J., Scholz, S., Brendel, W.* (Institut für Chirurgische Forschung, Chirurgische Klinik der Universität, 8000 München 2, BRD), p. 305

Disorders of the mononuclear phagocyte system. An analytical review. *Meuret, G.* (Department of Internal Medicine, Section of Hematology and Oncology, Kantonsspital, CH-9006 St. Gallen, Switzerland), p. 317

Cyclic 3',5'-adenosine monophosphate level and adenylatecyclase activity in human blood platelets during storage in ACD solution. *Lammerant-Guillemare, E., Corcelle-Cerf, F., Lammerant, J.* (Reprint requests: Prof. J. Lammerant, School of Medicine, Department of Physiology, B-5000 Namur, Belgium), p. 329

Blut (München) **34** (1977) No. 5

Recent progress in the immunological characterization of leukaemic cells. *Greaves, M. F.* (Membrane Immunology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Field, London, England), p. 349

Occurrence of S^u in a German family. *Mauff, G., Pulverer, G., Hummel, K., Spielmann, W., Bender, K.* (Hygiene-Institut der Universität, D-5000 Köln 41, BRD), p. 357

Lack of inhibition of ellagic acid induced hypercoagulable state and lack of thrombosis in the dog after PAMBA administration. *Girolami, A., Brunetti, A., Cella, G., Pedrazzoli, S.* (University of Padua, Medical School, Institute of "Semeiotica Medica", 105 Padova, Italy), p. 363

Differenzierungspotenz peripherer Blutzellen von Patienten mit akuter Leukämie. *Hoelzer, D., Hellriegel, K. P., Schmücker, H., Kurrle, E.* (Zentrum für Innere Medizin

und Kinderheilkunde, 7900 Ulm, BRD), p. 371

Possible implications of reducing substances on the development of leukemia. *Lohmann, W., Löffler, H.* (Institut für Biophysik der Universität, D-6300 Giessen, BRD), p. 376

Proliferationshemmung der AKR-Mäuseleukämie *in vivo* mit Kalbsmilzextrakt (Lymphozytenchalon). *Heidemann, E., Ganser, H., Podgornik, N., Rutsch, H., Wilms, K., Wörz, F.* (Medizinische Universitätsklinik, D-7400 Tübingen, BRD), p. 381

Verstärkung der intravasalen Hämolyse durch Daunorubicin bei der Rattenleukämie L 5222. *Dietrich, M., Finkl, F., Rasche, H., Pflieger, H., Hoelzer, D.* (Bernhard-Nocht-Institut für Schiffs- und Tropenkrankheiten, 2000 Hamburg 4, BRD), p. 384

Human E-rosette forming lymphocytes exhibiting antibody-dependent cytotoxic activity. *Saal, J.-G., Rieber, E. P., Hadam, M., Riethmüller, G.* (Abteilung für Experimentelle Chirurgie und Immunologie der Chirurgischen Universitätsklinik, 7000 Tübingen, BRD), p. 388

Vorkommen von Tartrat-resistenter saurer Phosphatase in verschiedenen Zelltypen des lympho-retikulären und hämopoetischen Systems. *Schaefer, H.-E., Hellriegel, K.-P., Fischer, R.* (Pathologisches Institut der Universität, 5000 Köln 41, BRD), p. 393

Effekt von Kurzzeit-Suspensionskulturen hämatopoetischer Zellen auf die Mitoserate bei Frühstadien unreifzelliger Leukämien: Eine Methode zur Verbesserung der Präparationsbedingungen für zytogenetische Untersuchungen. *Hellriegel, K.-P., Heit, W., Byrne, P.* (Medizinische Universitätsklinik, 5000 Köln 41, BRD), p. 398

Granulocytic colony forming cells *in vitro*: I. Patients with drug induced agranulocytosis. *Heit, W., Kern, P., Heimpel, H., Kubanek, B.* (Zentrum für Innere Medizin der Universität, 7900 Ulm, BRD), p. 403

Granulocytic colony forming cells *in vitro*: II. Patients with aplastic anaemia. *Kern, P., Heit, W., Kubanek, B., Heimpel, H.* (Zentrum für Innere Medizin der Universität, 7900 Ulm, BRD), p. 406

Die Beeinflussung des exogenen Gerinnungssystems durch Endotoxin-stimulierte Monozyten. *Hiller, E., Saal, J. G., Griffiths, G. W.* (Medizinische Universitäts-Klinik, 7400 Tübingen, BRD), p. 409

Ein neues Konzept der Hämatopoese im Säugetierorganismus während der Nachgeburtsphase: Nachweis mehrerer Volumenpopulationen der Erythrozyten bei Ratten, Mäusen, Meerschweinchen, Kaninchen, Schafen und Ziegen. *Valet, G., Hanser, G., Ruhenstroth-Bauer, G.* (Max-Planck-Institut für Biochemie, Abteilung für Experimentelle Medizin, D-8033 Martinsried bei München, BRD), p. 413

Drugs, genes and methemoglobinemia. *Stanulović, M., Popović, K., Borota, R., Cvetić, L., Efremov, G.* (Medicinski fakultet, 2100 Novi Sad, Yugoslavia), p. 419

Blut (München) **34** (1977) No. 6

Nekrologia. Hans Hermann Bennhold. *Bock, H. E.* (Tübingen, BRD), p. 437

EDV-Einsatz zur organisatorischen Unterstützung von Blutspendezentralen in Akutkrankenhäusern. *Kretschmer, V., Haase, H., Pönighaus, K.-H.* (Abteilung für Klinische Immunologie und Transfusionsmedizin der Justus-Liebig-Universität Gießen, 6300 Lahn-Gießen, BRD), p. 441

Erythropoese bei Eisenmangel. *Dörmer, P., Lau, B.* (Institut für Hämatologie, 8000 München 2, BRD), p. 453

Cytochemical observations in preleukemic states and remission of acute leukemia. *Heller, A., Gross, R.* (Medizinische Klinik der Universität Köln, 5000 Köln 41, BRD), p. 465

In vitro-Untersuchungen mit menschlichem Transferfaktor. *Rainer, H., Moser, K.* (I. Medizinische Universitätsklinik, 1090 Wien, Österreich), p. 471

Kälte-lymphozytotoxische Antikörper (CoCoCy) in Seren von Cervix-Karzinom-Patientinnen (CCa). *Müller, N., Koenig, U. D.* (Institut für Experimentelle Hämatologie und Bluttransfusionswesen der Universität Bonn, 5300 Bonn-Venusberg, BRD), p. 479

Blut (München) **35** (1977) No. 1

RNA-dependent DNA polymerase (reverse transcriptase). *Bauer, G.* (Max-Planck-

Institut für Biochemie, Abteilung Virusforschung, D-8033 Martinsried bei München, BRD), p. 3

Transmission and scanning electron microscopy study on plasma cell leukemia. *Klein, B., Lewinski, U., Shabtai, F., Freidin, N., Djaldetti, M.* (Reprint requests: Prof. Dr. M. Djaldetti, Department of Internal Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel), p. 11

Myelomonozytäre Leukämie: Klinische, zytologische und zytogenetische Studien bei akuten, subakuten und chronischen Verlaufsformen. *Mende, S., Fülle, H.-H., Knuth, A., Weißenfels, I.* (II. Innere Abteilung, Krankenhaus Moabit, D-1000 Berlin, 21, BRD), p. 21

Erythroid stem cells in Rauscher virus infected mice. *Opitz, U., Seidel, H.-J., Rich, I. S.* (Abteilung für klinische Physiologie der Universität, D-7900 Ulm/Donau, BRD), p. 35

Proliferation characteristics of erythroblasts in chronic renal failure. *Kempgens, U., Mayer, M., Schautt, B., Queisser, W.* (I. Medizinische Klinik, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, D-6800 Mannheim 1, BRD), p. 45

Hematologic and immunologic studies in dogs given nitrogen mustard (HN3). *Abb, J., Netzel, B., Rodt, H., Grosse-Wilde, H., Weger, N., Thierfelder, S.* (Institut für Hämatologie, Abteilung Immunologie, D-8000 München 2, BRD), p. 51

PHA and endotoxin stimulation of human lymphocytes separated by albumin density gradient centrifugation. *Biermann, A., v. Keyserlingk, D. G., Boll, I.* (Institut für Anatomie der Freien Universität Berlin, I. Innere Abteilung des Städtischen Krankenhauses, D-1000 Berlin-Neukölln, BRD), p. 57

Übereinstimmung von Zytostatikaeffekten *in vivo* und im Kurzzeittest *(in vitro)* bei der Leukämie L 1210. *Volm, M., Hummel, H., Mattern, J.* (Deutsches Krebsforschungszentrum Heidelberg, Institut für Experimentelle Pathologie, D-6900 Heidelberg 1, BRD), p. 65

The use of RA 233 in the preparation of platelets, with the blood cell separator. *Beyer, J.-H., Hilgard, P.* (Reprint requests: Dr. P. Hilgard, Innere Klinik und Poliklinik (Tumorforschung), D-4300 Essen 1, BRD), p. 75

Blut (München) **35** (1977) No. 2

In memoriam A. S. Wiener. *Speiser, P.* (Institut für Blutgruppenserologie der Universität, A-1090 Wien, Österreich), p. 93

Prinzipien der Zytostatika-Testung. *Klein, H. O.* (I. Medizinische Universitätsklinik Köln, D-5000 Köln 41, BRD), p. 97

Kulturverhalten weißer Blutzellen bei Patienten mit Haarzell-Leukämie in der Diffusionskammer. *Tigges, F.-J., von Heyden, H. W., Waller, H. D.* (Medizinische Universitätsklinik Tübingen, Abteilung Innere Medizin II, D-7400 Tübingen, BRD), p. 103

Hämatologische Veränderungen bei Anorexia nervosa. *Kubanek, B., Heimpel, H., Paar, G., Schoengen, A.* (Abteilung Innere Medizin und Hämatologie, Universität von Ulm, D-7900 Ulm, BRD), p. 115

Erythropoetin in Serum und Harn von Gesunden unter hypoxischer Stimulation und hypoxischer Stimulation nach Androgengabe (Fluoxymesteron). *Schulz, E.* (Medizinische Universitäts-Poliklinik und Medizinische Klinik, D-5000 Köln-Merheim, BRD), p. 125

Die elektrophoretische Beweglichkeit der Blutlymphozyten bei chronischer lymphatischer Leukämie. *Schubert, J. C. F., Schopow, K., Walther, F.* (Zentrum der Inneren Medizin der Johann-Wolfgang-Goethe-Universität, D-6000 Frankfurt/M., 70, BRD), p. 135

Allogeneic bone marrow transplantation in conventional mice: I. Effect of antibiotic therapy on long term survival of allogeneic chimeras. *Heit, H., Heit, W., Kohne, E., Fliedner, T. M., Hughes, P.* (Zentrum für Klinische Forschung der Universität, Abteilung Klinische Physiologie, D-7900 Ulm/Donau, BRD), p. 143

Blut (München) **35** (1977) No. 3

Erythrocyte serology of some malignant diseases. *Bird, G. W. G.* (The West Midlands Regional Blood Transfusion Service,

Regional Blood Transfusion Centre, Edgbaston, Birmingham B15 2SG, England), p. 165

Immunhämolyse unter Penicillintherapie. *In-vitro*-Ergebnisse unter Verwendung isolierter Monozyten. *Denz, H., Spath, P., Huber, H.* (Reprint requests: Dr. H. Huber, Krankenhaus der Barmherzigen Schwestern, A-4020 Linz, Austria), p. 171

Extrakorporale Asparaginasetherapie bei Allergie und toxischen Nebenwirkungen. *Pralle, H., Löffler, H.* (Zentrum für Innere Medizin der Justus-Liebig-Universität, D-6300 Gießen, BRD), p. 179

Glucosephosphate isomerase deficiency type Liège: A new variant with congenital nonspherocytic hemolytic anemia. *Arnold, H., Dodinval-Versie, J., Lambotte, C., Löhr, G. W., van der Hofstadt, J.* (Medizinische Universitätsklinik, D-7800 Freiburg, BRD), p. 187

Cryopreservation of blood mononuclear leukocytes and stem cells suspended in a large fluid volume. A preclinical model for a blood stem cell bank. *Fliedner, T. M., Körbling, M., Calvo, W., Bruch, Ch., Herbst, E.* (Department of Clinical Physiology, University of Ulm, D-7900 Ulm/Donau, BRD), p. 195

Comparative haematological changes after 15 MeV neutron- and X-irradiation. I. Differential counts in peripheral blood and bone marrow. *Birkenmayer, E., Stevenson, A. F. G., Messerschmidt, O.* (Reprint requests: Dr. A. F. G. Stevenson, Laboratorium für Experimentelle Radiologie, D-8042 Neuherberg bei München, BRD), p. 203

Die Beschreibung einer einfachen Filtrationstechnik zur Erfassung der Erythrozyten-Verformbarkeit in der Schwangerschaft. *Heilmann, L., Kurz, E.* (Frauenklinik im Universitätsklinikum der Gesamthochschule Essen, D-4300 Essen, BRD), p. 213

Human lymphocyte culture: Intrinsic factors influencing the quality of chromosome preparations. *Chaudhuri, J. P., Ludwig, E., Labouvie, C.* (Fraunhofer-Gesellschaft, Laboratorium für Experimentelle Radiologie, D-8402 Neuherberg bei München, BRD), p. 223

Isolierung und Charakterisierung eines 7S-Beta$_1$-Globulins aus menschlichen Erythrozyten. *Haupt, H., Bohn, H.* (Behringwerke, AG, D-3550 Marburg, BRD), p. 229

Untersuchungen der Gerinnungsproteine im Brandblaseninhalt bei schweren Verbrennungen. *Burkhardt, H., Zellner, P. R.* (Krankenhaus Bruchsal, D-7520 Bruchsal, BRD), p. 241

Apparent "inhibition" as tested by means of the dilution curve system in patients with clotting defect due to liver damage. *Girolami, A., Dal Bo Zanon, R., Sticchi, A.* (University of Padua Medical School, Institute of "Semeiotica Medica", Padova, Italy), p. 247

Elektronische Blutplättchenzählung unter besonderer Berücksichtigung von Thrombozytopenien. *Kuse, R., Burmeister, H., Hausmann, K.* (Hämatologische Abteilung des Allgemeinen Krankenhauses St. Georg, D-2000 Hamburg 1, BRD), p. 253

Blut (München) **35** (1977) No. 4

Interferons: Anti-neoplastic drugs? *Strander, H.* (Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm, Sweden), p. 277

A test with dexamethasone in acute leukemia. *Bohinjec, J.* (Interna klinika I, Klinicni Center, 61000 Ljubljana, Yugoslavia), p. 289

Isoenzym-Muster saurer Phosphatasen aus normalen und leukämischen weißen Blutzellen. *Heyden, H.-W. von, Weber, R., Nerke, O., Saal, J. G., Stein, W.* (Medizinische Universitätsklinik Tübingen, Abteilung Innere Medizin, D-7400 Tübingen, BRD), p. 295

Isolierung, Charakterisierung und quantitative immunologische Bestimmung von zwei neuen Serumproteinen (HPG-1 und HPG-2). *Bohn, H., Winckler, W.* (Behringwerke AG, D-3550 Marburg/Lahn, BRD), p. 305

Zur Ätiologie der Thrombozytopenie bei der Vinylchlorid-Krankheit. *Heusermann, U., Stutte, H.-J.* (Institut für Pathologie der Universität Kiel, D-2300 Kiel, BRD), p. 317

The role of Mg^{++}-ATPase (actomyosin-like protein) in maintaining the biconcave shape of erythrocytes. *Mircevová, L.* (Institute of Haematology and Blood Trans-

fusion, 12820 Prague, Czechoslovakia), p. 323

Abstracts. Österreichische Gesellschaft für Hämatologie und Onkologie, Deutsche Gesellschaft für Hämatologie Gemeinsamer Kongreß 1977, p. 329

Blut (München) **35** (1977) No. 5

Die Beeinflussung der Kollagen-induzierten Plättchen-Aggregation durch fünf käuflich erhältliche Heparinpräparate. *Kronik, G., Deutsch, E.* (I. Medizinische Universitätsklinik Wien, A-1090 Wien, Österreich), p. 363

The relation between baseline and 150 min platelet counts in response to selective beta-1-receptor blockade. An experimental study on healthy subjects. *Fredén, K., Kutti, J., Lundborg, P., Vilén, L.* (Reprint requests: Dr. J. Kutti, Department of Medicine III, Sahlgren's Hospital, S-411 33 Göteborg, Sweden), p. 373

Thrombophorese I. Einfluß der Zentrifugendrehzahl auf Thrombozytenausbeute, Thrombozytenverteilung im Zellseparator und Ausbreitungsfähigkeit der Thrombozyten. *Wysk, J., Schüttler, W., Stangel, W., Deicher, H.* (Reprint requests: Prof. H. Deicher, Department Innere Medizin, Medizinische Hochschule Hannover, D-3000 Hannover 61, BRD), p. 377

Thrombophorese II. Auswirkung der Thrombozytenseparation beim Blutspender. *Wysk, J., Marx, D., Schüttler, W., Avenarius, H.-J., Stangel, W., Eckert, G., Poliwoda, H., Deicher, H.* (Reprint requests: Prof. H. Deicher, Department Innere Medizin, Medizinische Hochschule Hannover, D-3000 Hannover 61, BRD), p. 387

Isoenzym-Muster der sauren Phosphatasen von Epstein-Barr-Virus-DNS enthaltenden, permanent wachsenden lymphoiden Zell-Linien. *von Heyden, H.-W., Weber, R., Stuckstedte, H., Saal, J.-G., Fresen, K. O.* (Medizinische Universitätsklinik Tübingen, D-7400 Tübingen, BRD), p. 395

The clinical efficacy of granulocyte transfusions: Studies on the oral cavity. *Arnold, R., Pflieger, H., Dietrich, M., Heimpel, H.* (Departments für Innere Medizin, Kinderheilkunde und Dermatologie der Universität Ulm, D-7900 Ulm, BRD), p. 405

An improved modification of cycle filtration leucopheresis. *Kretschmer, V., Mueller-Eckhardt, Ch.* (Institut für Klinische Immunologie und Transfusionsmedizin, Klinikum ker Justus-Liebig-Universität Gießen, D-3600 Gießen, BRD), p. 415

Blut (München) **35** (1977) No. 6

Evidence for monoclonal proliferation in prolymphocytic leukemia of T-cell origin. A cytogenetic and quantitative immunoautoradiographic analysis. *Thiel, E., Bauchinger, M., Rodt, H., Huhn, D., Theml, H., Thiefelder, S.* (Institut für Hämatologie, Abteilung für Immunologie, GSF, D-8000 München 2, BRD), p. 427

Ultrastruktur der erythroiden Zellen bei einer kongenitalen dyserythropoetischen Anämie Typ II. *Biermann, A., von Keyserlingk, D. G.* (Institut für Anatomie der Freien Universität Berlin, D-1000 Berlin 33, BRD), p. 437

Significance of complement activation in autoimmune haemolytic anaemia of "warm type". *Kretschmer, V., Mueller-Eckhardt, Ch.* (Institut für Klinische Immunologie und Transfusionsmedizin, Klinikum der Justus-Liebig-Universität, D-6300 Gießen, BRD), p. 447

Ein neuer Fall von schwerem Faktor XIII-Mangel. Untersuchungen zum Verhalten der Untereinheiten und des Turnover des fibrinstabilisierenden Faktors. *Zimmermann, R., Ehlers, W., Manz, F., Meinrenken, W., Egbring, R., Gemmeke, H.* (Medizinische Universitätsklinik Heidelberg, Ludolf-Krehl-Klinik, D-6900 Heidelberg 1, BRD), p. 457

A sensitive latex agglutination method for the detection of soluble fibrin in plasma. *Müller, U. P., Largo, R., Straub, P. W.* (Reprint requests: Prof. P. W. Straub, Medizinische Universitätsklinik, Inselspital, CH-3010 Bern, Switzerland), p. 465

British Journal of Haematology (Oxford) **34** (1976) No. 4

Annotation. Bone marrow transplantation. *Kay, H. E. M.* (Department of Haema-

tology, Royal Marsden Hospital, London, S. W. 3, England), p. 521

The Negro variety of hereditary persistence of fetal haemoglobin is a mild form of thalassaemia. *Charache, S., Clegg, J. B., Weatherall, D. J.* (Reprint requests: Prof. Dr. D. J. Weatherall, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 527

Globin synthesis in normal human bone marrow. *Chalevelakis, G., Clegg, J. B., Weatherall, D. J.* (Reprint requests: Dr. J. B. Clegg, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 535

Ultrastructure of sickling and unsickling in time-lapse studies. *Hahn, J. A., Messer, M. J., Bradley, T. B.* (Reprint requests: Dr. M. J. Messer, Hematology Section, 151A, Veterans Administration Hospital, San Francisco, Calif. 94121), p. 559

Serum "uracil + uridine" levels in pernicious anaemia. *Parry, T. E., Blackmore, J. A.* (Department of Haematology, Llandough Hospital, Glam CF6 1XX, Wales, U. K.), p. 567

Serum "uracil + uridine" levels before and after vitamin B_{12} therapy in pernicious anaemia. *Parry, T. E., Blackmore, J. A.* (Department of Haematology, Llandough Hospital, Glam CF6 1XX, Wales, U. K.), p. 575

Studies with the folate binding protein in chronic granulocytic leukaemia cells. I. Synthesis and release of binder by cells in short-term culture. *Da Costa, M., Rothenberg, Sh. P.* (Reprint requests: Dr. Sh. P. Rothenberg, Department of Medicine, New York Medical College, New York, N. Y. 10029), p. 581

Microcytosis, anisocytosis and the red cell indices in iron deficiency. *England, J. M., Ward, S. M., Down, M. C.* (MRC Experimental Haematology Unit, St Mary's Hospital Medical School, London, W. 2, England), p. 589

Indium chloride scintigraphy: An index of severity in patients with aplastic anaemia. *McNeil, B. J., Rappeport, J. M., Nathan, D. G.* (Department of Radiology, Harvard Medical School, Boston, Mass. 02115), p. 599

Studies on calcium transport and calcium-dependent adenosine triphosphatase activity of erythrocyte membranes in hereditary spherocytosis. *Zail, S. S., van den Hoek, A. K.* (Department of Haematology, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa), p. 605

The heterogeneity of erythrocyte antigen distribution in human normal phenotypes: an immunoelectron microscopy study. *Reyes, F., Gourdin, M. F., Lejonc, J. L., Cartron, J. P., Gorius, J. B., Dreyfus, B.* (I.N.S.E.R.M. U.91, CHU Henri Mondor, 94010 Créteil, France), p. 613

Differential expression of cell surface binding sites for cholera toxin in acute and chronic leukaemias. *Révész, T., Greaves, M. F., Capellaro, D., Murray, R. K.* (Reprint requests: Dr. M. F. Greaves, Department of Zoology, University College London, London WC1E 6BT, England), p. 623

Two biochemical markers in lymphocyte subpopulations. *Huang, A. T., Logue, G. L., Engelbrecht, H. L.* (Division of Hematology and Oncology, Department of Medicine, Duke University Medical Center, Durham, N. C. 27710), p. 631

Megakaryocytes and the heterogeneity of circulating platelets. *Penington, D. G., Streatfield, K., Roxburgh, A. E.* (University of Melbourne, Department of Medicine, St Vincent's Hospital, Melbourne 3065, Victoria, Australia), p. 639

Relationship of factor VIII-like antigen (VIII AGN) and clot promoting activity (VIII AHF) as measured by one- and two-stage assays in patients with liver disease. *Rogers, J. S., Eyster, M. E.* (Reprint requests: Dr. M. E. Eyster, The Milton S. Hershey Medical Center, Hershey, Pa. 17033), p. 655

Correspondence. Heterogeneity of ferritin from red blood cells. *Brown, J. E., Theil, E. C.* (Department of Biochemistry, North Carolina State University, Raleigh, N. C. 27607), p. 663

Effect of aspirin on monoamine oxidase in human platelet-rich plasma. *Norstrand, I. F., Druck, P., Glantz, M. D.* (Brooklyn VA Hospital, Brooklyn College of the City University of New York, New York, N. Y. 11209), p. 665

British Journal of Haematology (Oxford) **35** (1977) No. 1.

Red cell membrane injury in sickle cell anemia. *Palek, J.* (Hematology Research Laboratory, Division of Hematology, Saint Vincent Hospital, Worcester, Mass.), p. 1

Monocyte recruitment in tuberculosis and sarcoidosis. *Schmitt, E., Meuret, G., Stix, L.* (Reprint requests: Dr. G. Meuret, Medizinische Klinik C, Kantonsspital, CH-9006 St. Gallen, Switzerland), p. 11

Neutrophil accumulation *in vivo* following the administration of chemotactic factors. *Jones, D. G., Richardson, D. L., Kay, A. B.* (Reprint requests: Dr. A. B. Kay, South-East Scotland Regional Blood Transfusion Service, Royal Infirmary, Edinburgh, Scotland), p. 19

Adaptation of marrow sinus wall to fluctuation in the rate of cell delivery: Studies in rabbits after blood-letting. *Tavassoli, M.* (Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 25

Ferrokinetics and erythropoiesis in man: Red-cell production and destruction in normal and anaemic subjects. *Cavill, I., Ricketts, C., Napier, J. A. F., Jacobs, A.* (Department of Haematology, University Hospital of Wales, Cardiff CF4 4XN, Wales), p. 33

Ferrokinetics and erythropoiesis in man: An evaluation of ferrokinetic measurements. *Ricketts, C., Cavill, I., Napier, J. A. F., Jacobs, A.* (Reprint requests: Dr. I. Cavill, Department of Haematology, University Hospital of Wales, Cardiff CF4 4XN, Wales), p. 41

Benzene inhibition of *in vitro* rabbit reticulocyte haem synthesis at delta aminolaevulinic acid synthetase: Reversal of benzene toxicity by pyridoxine. *Freedman, M. L., Wildman, J. M., Rosman, J., Eisen, J., Greenblatt, D. R.* (Department of Medicine, New York University School of Medicine, New York, N. Y. 10016), p. 49

Aplastic anaemia. Histological investigation of methacrylate embedded bone marrow biopsy specimens: Correlation with survival after conventional treatment in 15 adult patients. *te Velde, J., Haak, H. L.* (Pathological Laboratory, University Hospital, Leiden, The Netherlands), p. 61

Myosin in cultured human endothelial cells. *Moore, A., Jaffe, E. A., Becker, C. G., Nachman, R. L.* (Division of Hematology, Department of Medicine, Cornell University Medical College, New York, N. Y. 10021), p. 71

Factor-VIII activity and antigen, platelet count and biochemical changes after adrenoceptor stimulation. *Ingram, G. I. C., Vaughan Jones, R., Hershgold, E. J., Denson, K. W. E., Perkins, J. R.* (Department of Haematology, St Thomas's Hospital, London SE1 7EH, England), p. 81

Spectrum of von Willebrand's disease: a study of 100 cases. *Mannucci, P. M.* (International Training Centre Angelo Bianchi Bonomi, World Federation of Haemophilia, 20122 Milano, Italy), p. 101

Platelet antibodies in thrombocytopenic patients. *Hedge, U. M., Gordon-Smith, E. C., Worlledge, S.* (Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 113

Platelet antithrombins: Role of thrombin binding and the release of platelet fibrinogen. *Watanabe, K., Chao, F. C., Tullis, J. L.* (Center for Blood Research, Boston, Mass. 02115), p. 123

Effects of cephalothin and penicillin G on platelet function *in vitro*. *Cazenave, J.-P., Guccione, M. A., Packham, M. A., Fraser Mustard, J.* (Department of Pathology, Faculty of Medicine, McMaster University, Hamilton, Ontario, Canada L8S 4J9), p. 135

Haematologic profile of natural populations: Red cell parameters. *Kelly, A., Munan, L.* (Epidemiology Laboratory, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada), p. 153

Recommendations for abnormal haemoglobin standard, p. 161

British Journal of Haematology (Oxford) **35** (1977) No. 2

Annotation. Biochemical abnormalities in chronic erythraemic myelosis. *Kass, L.* (Department of Internal Medicine, University of Michigan, Ann Arbor, Mich. 48109), p. 169

Haemoglobin Radcliffe ($\alpha_2 \beta_2^{99(G1)Ala}$): A high oxygen-affinity variant causing familial polycythaemia. *Weatherall, D. J., Clegg, J. B., Callender, S. T., Wells, R. M. G., Gale, R. E., Huehns, E. R., Perutz, M. F., Viggiano, G., Ho, C.* (Nuffield Department of Clinical Medicine, The Radcliffe Infirmary, Oxford OX2 6HE, England), p. 177

Studies of the influence of cyclic nucleotides on *in vitro* haemoglobin synthesis. *Brown, J. E., Adamson, J. W.* (Hematology Research Laboratory, Veterans Administration Hospital, Seattle, Wash. 98108), p. 193

Congenital dyserythropoietic anaemia, types I and II: Aberrant pattern of erythrocyte membrane proteins in CDA II, as revealed by two-dimensional polyacrylamide gel electrophoresis. *Anselstetter, V., Horstmann, H.-J., Heimpel, H.* (Institut für Physiologische Chemie der Universität Erlangen-Nürnberg, 8520 Erlangen, BRD), p. 209

Reversal of ineffective erythropoiesis in pernicious anaemia following vitamin B_{12} therapy. *Samson, D., Halliday, D., Chanarin, I.* (Reprint requests: Dr. I. Chanarin, Department of Haematology, Northwick Park Hospital and Clinical Research Centre, Harrow, Middlesex, England), p. 217

Effect of hypertransfusion on granulopoiesis in bone marrow depression: Studies in the irradiated mouse. *Firkin, F. C., Hays, E. F., Cline, M. J.* (University of Melbourne, Department of Medicine, St. Vincent's Hospital, Fitzroy, Victoria 3065, Australia), p. 225

Biphasic thrombopoietic response to severe hypobaric hypoxia. *Jackson, C. W., Edwards, C. C.* (St. Jude Children's Hospital, Memphis, Tenn. 38101), p. 233

Neutrophil function in bone marrow transplant recipients. *Territo, M. C., Gale, R. P., Cline, M. J.* (Department of Medicine, Division of Hematology-Oncology, Center for the Health Sciences, University of California, Los Angeles, Calif. 90024), p. 245

Bone marrow culture studies in refractory cytopenia and "smouldering leukaemia". *Milner, G. R., Testa, N. G., Geary, C. G., Dexter, T. M., Muldal, S., MacIver, J. E., Lajtha, L. G.* (Reprint requests: Dr. C. G. Geary, Manchester Royal Infirmary, Manchester M13 9WL, England), p. 251

The immunotherapy of acute myelogenous leukaemia using intravenous BCG. *Whittaker, J. A., Slater, A. J.* (Department of Haematology, University Hospital of Wales, Cardiff CF4 4XN, Wales, United Kingdom), p. 263

Defective platelet lipid peroxidation in myeloproliferative disorders: A possible defect of prostaglandin synthesis. *Keenan, J. P., Wharton, J., Shepherd, A. J. N., Bellingham, A. J.* (Department of Haematology, University of Liverpool, Nuffield Unit of Medical Genetics, Liverpool L69 3BX, England), p. 275

Membrane changes in human platelets induced by lipopolysaccharide endotoxin. *Hawiger, J., Hawiger, A., Steckley, S., Timmons, S., Cheng, C.* (Coagulation and Thrombosis Laboratory, Vanderbilt University Hospital, Nashville, Tenn. 37232), p. 285

Acquired dysfibrinogenaemia in acute and chronic liver disease. *Lane, D. A., Scully, M. F., Thomas, D. P., Kakkar, V. V., Woolf, I. L., Williams, R.* (Thrombosis Research Unit, King's College Hospital Medical School, London SE5 8RX, England), p. 301

Inherited sialoglycoprotein deficiencies in human erythrocytes of type En(a-). *Annstee, D. J., Barker, D. M., Judson, P. A., Tanner, M. J. A.* (South West Regional Blood Transfusion Centre, Southmead, Bristol BS10 5ND, England), p. 309

Mucosal iron-binding proteins in mice with X-linked anaemia. *Valberg, L. S., Sorbie, J., Ludwig, J.* (Department of Medicine, University Hospital, London, Ontario N6G 2K3, Canada), p. 321

International Committee for Standardization in Haematology. Recommended methods for red-cell enzyme analysis. *Beutler, E., Blume, K. G., Kaplan, J. C., Löhr, G. W., Ramot, B., Valentine, W. N.* (Division of Medicine, City of Hope National Medical Center, Duarte, Calif. 91010), p. 331

Correspondence. HLA matching in autoimmune thrombocytopenic purpura. *Goebel, K. M., Hahn, E., Havemann, K.* (Department of Medicine, University of Marburg, D-3550 Marburg, FRG), p. 341

British Journal of Haematology (Oxford) **35** (1977) No. 3

Annotation. α-thalassaemia in the United Kingdom. *Walford, D. M.* (Department of Haematology, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, England), p. 347

Splenic red cell pooling in hairy cell leukaemia. *Lewis, S. M., Catovsky, D., Hows, J. M., Ardalan, B.* (Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 351

Immunoglobulin synthesis in hairy cell leukaemia. *Golde, D. W., Stevens, R. H., Quan, S. G., Saxon, A.* (Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 359

Combined T and B lymphocyte marker test in lymphoproliferative disorders. *Chapel, H. M., Ling, N. R.* (Department of Experimental Pathology, Medical School, University of Birmingham, Birmingham B15 2TJ, England), p. 367

Evidence for rapid loss of newly synthesized haemoglobin S molecules in sickle cell anaemia and sickle cell trait. *DeSimone, J., Adams, J. G. III, Shaeffer, J.* (Center for Genetics, University of Illinois College of Medicine, Chicago, Ill. 60612), p. 373

Altered haem-haem interaction and tissue-oxygen supply: A theoretical analysis. *Neville, J. R.* (Letterman General Hospital, San Francisco, Calif. 94129), p. 387

Abnormal folate polyglutamate ratios in untreated pernicious anaemia corrected by therapy. *Perry, J., Chanarin, I.* (Section of Haematology, Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, England), p. 397

Pathophysiological changes in serum erythropoiesis stimulating activity. *Napier, J. A. F., Dunn, C. D. R., Ford, T. W., Price, V.* (Department of Haematology, The Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 403

The early detection of remission in acute myelogenous leukaemia by *in vitro* cultures. *Spitzer, G., Dicke, K. A., McCredie, K. B., Barlogie, B.* (Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030), p. 411

Clinical course and survival after elective splenectomy in 19 patients with primary myelofibrosis. *Mulder, H., Steenbergen, J., Haanen, C.* (Reprint requests: Dr. C. Haanen, Division of Haematology, Department of Internal Medicine, University of Nijmegen, 6800 Nijmegen, The Netherlands), p. 419

Factor XI and platelets: Evidence that platelets contain only minimal factor XI activity and antigen. *Schiffman, S., Rimon, A., Rapaport, S. I.* (USC School of Medicine, Los Angeles, Calif. 90033), p. 429

The conformation of fibrinogen and fibrin: An electron microscopic study. *Blakey, P. R., Groom, M. J., Turner, R. L.* (Reprint requests: Dr. R. L. Turner, Postgraduate School of Medical and Surgical Sciences, University of Bradford, Bradford BD7 1DP, England), p. 437

Specific interaction of HLA antibodies (eluates) with washed platelets. *Heinrich, D., Stephinger, U., Mueller-Eckhardt, C.* (Department of Internal Medicine, Justus Liebig-University, D-6300 Giessen, FRG), p. 441

Isolation and characterization of an actin-like protein from membranes of human red cells. *Ohnishi, T.* (Department of Biological Chemistry, Hahnemann Medical College, Philadelphia, Pa. 19102), p. 453

A circadian variation of Melphalan (L-phenylalanine nitrogen mustard) toxicity to murine bone marrow: Relevance to cancer treatment protocols. *Simpson, H. W., Stoney, P. J.* (University Department of Pathology, Royal Infirmary, Glasgow G4 0SF, Scotland), p. 459

Depression of the platelet count after inoculation of mice with L1210 or L5178Y cells. *Hacker, M., Roberts, D., Jackson, C.* (Reprint requests: Dr. D. Roberts, St. Jude Children's Research Hospital, Memphis, Tenn. 38101), p. 465

Correspondence. Thrombopoietin assays. *Karpatkin, S.* (School of Medicine, New York University Medical Center, New York, N. Y. 10016), p. 473

The role of erythrocyte membrane-associated proteolytic activity in immune haemolysis and autolysis. *Scott, G. K.* (Department

of Biochemistry, University of Auckland, Auckland, New Zealand), p. 474

Pica: A hypothesis. *von Bonsdorff, B.* (Hespiragatan 5, 00260 Helsingfors-26, Finland), p. 476

British Journal of Haematology (Oxford) **35** (1977) No. 4

Annotation. Automated differential leucocyte counting: The present state of the art. *Bentley, S. A., Lewis, S. M.* (Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 481

Haemophilia treatment in the United Kingdom from 1969 to 1974. *Biggs, R.* (Oxford Haemophilia Centre, Churchill Hospital, Oxford, England), p. 487

Non-invasive screening for venous thromboembolic disease. *Gordon, Y. B., Cooke, E. D., Bowcock, S. A., Ratky, S. M., Pilcher, M. F., Chard, T.* (Department of Reproductive Physiology, Medical College of St. Bartholomew's Hospital, London, E.C.1, England), p. 505

Thromboxane synthesis and the platelet release reaction in Bernard-Soulier syndrome, thrombasthenia Glanzmann and Hermansky-Pudlak syndrome. *Malmstein, C., Kindahl, H., Samuelsson, B., Levy-Toledano, S., Tobelem, G., Caen, J. P.* (Department of Chemistry, Karolinska Institutet, S-104 01, Stockholm, Sweden), p. 511

Abnormal platelet function in Chediak-Higashi syndrome. *Boxer, G. J., Holmsen, H., Robkin, L., Bang, N. U., Boxer, L. A., Baehner, R. L.* (Lilly Laboratory for Clinical Research, Indianapolis, Ind. 46202), p. 521

Binding of factor VIII to platelets in the presence of ristocetin. *Zucker, M. B., Kim, S.-J., McPherson, J., Grant, R. A.* (New York University Medical Center, New York, N. Y. 10016), p. 535

Defibrination of normal human blood *in vitro:* a method giving a high recovery of untraumatized cells. *Bray, P. J., Ford, D., Gledhill, R. M., Grimes, A. J.* (Reprint requests: Dr. A. J. Grimes, Department of Haematology, St. Thomas' Hospital and Medical School, London SE1 7EH, England), p. 551

Immune haemolytic anaemia and renal failure induced by streptomycin. *Letona, J. M.-L., Barbolla, L., Frieyro, E., Bouza, E., Gilsanz, F., Fernández, M. N.* (Reprint requests: Dr. L. Barbolla, Servicio de Hematologia, Clinica Puerta de Hierro, Madrid-35, Spain), p. 561

Red cell metabolic and membrane features in haemolytic anaemia of alcoholic liver disease (Zieve's syndrome). *Goebel, K. M., Goebel, F. D., Schubotz, R., Schneider, J.* (Medizinische Poliklinik, Universität Marburg, Marburg/Lahn, BRD), p. 573

The correlation of cold agglutinin titrations in saline and albumin with haemolytic anaemia. *Garratty, G., Petz, L. D., Hoops, J. K.* (Hematology and Immunology Unit, Pacific Medical Center, San Francisco, Calif. 94120), p. 587

Analysis of treatment in childhood leukaemia. III. Independence of lymphopenia induced by irradiation and by chemotherapy. *Waller, C. A., MacLennan, I. C. M., Campbell, A. C., Festenstein, M., Kay, H. E. M.* (Reprint requests: Dr. I. C. M. MacLennan, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 597

Granulocytic progenitor cells in aplastic anaemia. *Kern, P., Heimpel, H., Heit, W., Kubanek, B.* (Clinical Department, Bernhard-Nocht Institute for Nautical and Tropical Diseases, D-2000 Hamburg 4, FRG), p. 613

Stimulation of human haemopoietic cells by colony stimulating factors: Adherent cell dependent colony stimulating activity in human serum. *Francis, G. E., Berney, J. J., Hoffbrand, A. V.* (Department of Haematology, The Royal Free Hospital, Hampstead, London NW3 2QG, England), p. 625

Effect of testosterone and oestradiol on erythropoietin production *in vitro*. *Zucali, J. R., Mirand, E. A.* (Department of Biological Resources, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 639

The anaemia of chronic disorders: Studies of iron reutilization in the anaemia of experimental malignancy and chronic inflammation. *Zarrabi, M. H., Lysik, R., DiStefano, J., Zucker, S.* (VAH, Northport, N. Y. 11768), p. 647

An investigation into the development and

fate of neutrophil giant metamyelocytes using the techniques of electron microscopy and high resolution autoradiography. *Wickramasinghe, S. N., Bush, V.* (Department of Haematology, St. Mary's Hospital Medical School, Paddington, London W.2, England), p. 659

Abstracts of papers presented at a Joint Meeting of the British Society of Haematology and the Netherlands Haematology Society held in Amsterdam, 14—16 October 1976, p. 665

British Journal of Haematology (Oxford) **36** (1977) No. 1

Annotation. The measurement of granulocyte kinetics. *Vincent, P. C.* (Clinical Haematology Unit, Medical Research Department, Sydney, N. S. W. 2000, Australia), p. 1

Thymoma, pure red cell aplasia, pernicious anaemia and candidiasis: a defect in immunohomeostasis. *Robins-Browne, R. M., Green, R., Katz, J., Becker, D.* (Department of Microbiology, South African Institute for Medical Research, Johannesburg 2000, South Africa), p. 5

HLA antigens in Addisonian pernicious anaemia: Absence of an HLA and disease association. *Wright, J. P., Callender, S. T. E., Grumet, F. C., Payne, R. O., Taylor, K. B.* (Departments of Medicine and Pathology, Stanford University School of Medicine, Stanford, Calif. 94305), p. 15

Reversible C_3 hypocomplementaemia in megaloblastic anaemia due to vitamin B_{12} deficiency. *Horton, M. A., Burman, J. F.* (Department of Haematology, St. Bartholomew's Hospital, London EC1A 7BE, England), p. 23

The effect of thiamphenicol on the production of immature red blood cells under anaemic conditions. *Nijhof, W., Wierenga, P. K., Kardaun, S.* (Laboratory of Physiological Chemistry, State University Groningen, Groningen, The Netherlands), p. 29

Erythroid hypoplasia in myelofibrosis: a feature associated with blastic transformation. *Bentley, S. A., Murray, K. H., Lewis, S. M., Roberts, P. D.* (Department of Haematology, Royal Postgraduate Medical School, London W12 0HS, England), p. 41

The effect of endotoxin on circulating lymphocytes in normal man. *Gale, R. P., Opelz, G., Golde, D. W.* (Department of Medicine, UCLA School of Medicine, Los Angeles, Calif. 90024), p. 49

Inhibition of leukaemia blast cell motility as a test for specific antibody in acute adult myelogenous leukaemia. *Katz, J., Currie, G. A., Oliver, R. T. D.* (Reprint requests: Dr. R. T. D. Oliver, ICRF Medical Oncology Unit, St. Bartholomew's Hospital, London E.C.1, England), p. 59

Adenosine deaminase (ADA) activity in lymphocytes of normal individuals and patients with chronic lymphatic leukaemia. *Ramot, B., Brok-Simoni, F., Barnea, N., Bank, I., Holtzmann, F.* (Institute of Hematology, The Chaim Sheba Medical Centre, Tel-Hashomer, Israel), p. 67

New evidence relating to the nature and origin of the hairy cell of leukaemic reticuloendotheliosis. *Burns, G. F., Cawley, J. C., Barker, C. R., Goldstone, A. H., Hayhoe, F. G. J.* (Department of Haematological Medicine, University Clinical School, Cambridge CB2 2QL, England), p. 71

The role of endocytosis in transferrin uptake by reticulocytes and bone marrow cells. *Hemmaplardh, D., Morgan, E. H.* (Reprint requests: Dr. E. H. Morgan, Department of Physiology, University of Western Australia, Nedlands, Western Australia 6009), p. 85

Evidence that stimulation of megakaryocytopoiesis by low dose vincristine results from an effect on platelets. *Jackson, C. W., Edwards, C. C.* (St. Jude Children's Research Hospital, Memphis, Tenn. 38101), p. 97

Evidence that washed human platelets possess factor-X activator activity. *Semeraro, N., Vermylen, J.* (Reprint requests: Dr. J. Vermylen, Laboratory of Blood Coagulation, A. Z. St. Rafael, 3000 Leuven, Belgium), p. 107

Assay and characterization of the factor in porcine and bovine plasma which aggregates human platelets. *Evans, R. J., Austen, D. E. G.* (Reprint requests: Dr. D. E. G. Austen, Oxford Haemophilia Centre, Churchill Hospital, Oxford, England), p. 117

A CRM-positive variant of factor-VII de-

ficiency and the detection of heterozygotes with the assay of factor-like antigen. *Mazzucconi, M. G., Mandelli, F., Mariani, G., Briët, E., Veltkamp, J. J.* (Institute of Haematology, University of Rome, 00161 Rome, Italy), p. 127

SDS polyacrylamide gel characterization of serum FDP produced in response to ancrod and streptokinase/plasminogen infusions in man. *Lane, D. A., Robbins, P. A., Rampling, M. W., Kakkar, V. V.* (Thrombosis Research Unit, King's College Hospital Medical School, London SE5 8RX, England), p. 137

Correspondence. Stimulation of CFU-E growth *in vitro* by erythrocyte lysate. *Bateman, A. E., Pollock, K.* (Biology School, Sussex University, Falmer, Brighton, England), p. 150

Improved factor VIII concentrate of intermediate purity. *Ellis, D., Pettet, N., Williams, J. D., Maycock, W. D'A., Smith, J. K., Evans, D. R., Bidwell, E.* (Blood Products Laboratory, The Lister Institute of Preventive Medicine, Elstree, Herts WD6, 3AX, England), p. 150

British Journal of Haematology (Oxford) **36** (1977) No. 2

Annotation. Polycythaemia and high affinity haemoglobins. *Stephens, A. D.* (Department of Haematology, St. Bartholomew's Hospital, London EC1A 7BE, England), p. 153

Comparison of haematological features of the β° and β° thalassaemia traits in Jamaican Negroes. *Millard, D. P., Mason, K., Serjeant, B. E., Serjeant, G. R.* (Reprint requests: Dr. G. R. Serjeant, Laboratories Jamaica, University of the West Indies, Mona, Kingston 7, Jamaica W. I.), p. 161

Reduced ferrochelatase activity: a defect common to porphyria variegata and protoporphyria. *Becker, D. M., Viljoen, J. D., Katz, J., Kramer, S.* (Reprint requests: Dr. S. Kramer, Department of Haematology, South African Institute for Medical Research, Johannesburg 2000, South Africa), p. 171

The effect of anticonvulsant drugs on thymidine and deoxyribosenucleic acid synthesis by human marrow cells. *Taguchi, H., Laundy, M., Reid, C., Reynolds, E. H., Chanarin, I.* (Reprint requests: Dr. I. Chanarin, Department of Haematology, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, England), p. 181

Cobalamine dependent methionine synthesis and methyl-folate-trap in human vitamin D_{12} deficiency. *Sauer, H., Wilmanns, W.* (Department of Haematology, Immunology and Oncology, Robert-Bosch-Hospital, D-7000 Stuttgart 50, FRG), p. 189

Characterization of serum ferritin in iron overload: Possible identity to natural apoferritin. *Arosio, P., Yokota, M., Drysdale, J. W.* (Reprint requests: Dr. J. W. Drysdale, Department of Biochemistry, Tufts University School of Medicine, Boston, Mass. 02111), p. 199

Intracellular iron transport in rat intestinal epithelium: Biochemical and ultrastructural observations. *Humphrys, J., Walpole, B., Worwood, M.* (Reprint requests: Dr. M. Worwood, Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XW, Wales), p. 209

Haematological changes associated with the McLeod phenotype of the Kell blood group system. *Wimer, B. M., Marsh, W. L., Taswell, H. F., Galey, W. R.* (Reprint requests: Dr. W. L. Marsh, New York Blood Center, New York, N. Y. 10021), p. 219

Simultaneous presence of receptors for complement and sheep red blood cells on human fetal thymocytes. *Stein, H., Müller-Hermelink, H. K.* (Department of Pathology, University of Kiel, D-2300 Kiel I, FRG), p. 225

Acid esterase in human lymphoid cells and leukaemic blasts: a marker for T lymphocytes. *Kulenkampff, J., Janossy, G., Greaves, M. F.* (Reprint requests: Dr. G. Janossy, Membrane Immunology Group, Imperial Cancer Research Fund, London WC2A 3PX, England), p. 231

Effect of hyperoxia on superoxide anion and hydrogen peroxide production of polymorphonuclear leucocytes and alveolar macrophages. *Rister, M., Baehner, R. L.* (Reprint requests: Dr. R. Baehner, Division of Pediatric Hematology-Oncology, James W. Riley Hospital for Children, Indianapolis, Ind. 46202), p. 241

Development of neutrophilia by serially increasing doses of dexamethasone. *Mishler, J. M., Emerson, P. M.* (IV. Department of Haematology, Gibson Laboratories, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 249

Studies of von Willebrand factor: Effects of different kinds of carbohydrate oxidases, SJ-inhibitors and some other chemical reagents. *Fukui, H., Mikami, S., Okuda, T., Murashima, N., Takase, T., Yoshioka, A.* (Department of Pediatrics, Nara Medical College, Kashihara City, Nara, Japan), p. 259

Changes in electrophoretic mobility of human factor VIII-related antigen: Evidence for subunit structure. *Ekert, H., Chavin, S. I.* (Reprint requests: Dr. S. I. Chavin, MRC Research Laboratory, Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford OX3 7LJ, England), p. 271

Metabolism and function of human platelets washed by albumin density gradient separation. *Walsh, P. N., Mills, D. C. B., White, J. G.* (Specialized Center for Thrombosis Research, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 281

British Journal of Haematology (Oxford) **36** (1977) No. 3

Annotation. Rheology and blood coagulation. *McIntire, L. V.* (Biomedical Engineering Laboratory, Department of Chemical Engineering, Rice University, Houston, Texas 77001), p. 299

Autologous bone marrow repopulation following high dose cyclophosphamide and allogeneic marrow transplantation in aplastic anaemia. *Territo, M. C.* (Department of Medicine, University of California Center for the Health Sciences, Los Angeles, Calif. 90024), p. 305

An unusual form of chronic neutropenia in a father and daughter with hypogammaglobulinaemia. *Mentzer, W. C. Jr., Johnston, R. B. Jr., Baehner, R. L., Nathan, D. G.* (Department of Pediatrics, San Francisco General Hospital, San Francisco, Calif. 94110), p. 313

Intravascular phagocytosis in the rabbit bone marrow: A possible fate of normal senescent red cells. *Tavassoli, M.* (Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 323

The Duffy blood group determinants: Their role in the susceptibility of human and animal erythrocytes to *Plasmodium knowlesi* malaria. *Mason, S. J., Miller, L. H., Shiroishi, T., Dvorak, J. A., McGinniss, M. H.* (Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.), p. 327

Activated T-cells in autoimmune haemolytic anaemia. *Parker, A. C., Stuart, A. E., Dewar, A. E.* (Department of Haematology, Royal Infirmary, Edinburgh, Scotland, Great Britain), p. 337

The Philadelphia chromosome (Ph^1) in adults presenting with acute leukaemia: a comparison of Ph^1+ and Ph^1- patients. *Bloomfield, C. D., Peterson, L. C., Yunis, J. J., Brunning, R. D.* (Mayo Building, University of Minnesota Hospital, Minneapolis, Minn. 55455), p. 347

Chronic lymphatic leukaemia: Correlation of immunofluorescent characteristics and clinical features. *Hamblin, T., Hough, D.* (Department of Haematology, Royal Victoria Hospital, Bournemouth, Dorset, England), p. 359

Basophilic chronic granulocytic leukaemia with hyperhistaminaemia. *Rosenthal, S., Schwartz, J. H., Canellos, G. P.* (Reprint requests: Dr. J. H. Schwartz, St. Vincent Hospital, Worcester, Mass. 01610), p. 367

Occurrence of acute leukaemia in myeloproliferative disorders. *Rosenthal, D. S., Moloney, W. C.* (Department of Medicine, Division of Hematology, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Mass. 02115), p. 373

The role of iron in the regulation of hepatic transferrin synthesis. *Morton, A. G., Tavill, A. S.* (Department of Biochemistry, University of Edinburgh Medical School, Edinburgh EH8 9AG, Scotland, Great Britain), p. 383

Serum ferritin in haemochromatosis: Changes in the isoferritin composition during venesection therapy. *Halliday, J. W., McKeering, L. V., Tweedale, R., Powell, L. W.* (Reprint requests: Prof. L. W.

Powell, Department of Medicine, University of Queensland, Brisbane 4029, Australia), p. 395

Aspects of folate metabolism in renal failure. *Retief, F. P., du P. Heyns, A., Oosthuizen, M., van Reenen, O. R.* (Department of Internal Medicine, University of the Orange Free State, Bloemfontein, Republic of South Africa), p. 405

Incorporation of $^{32}PO_4$ into phospholipids of blood platelets. *Leung, N. L., Kinlough-Rathbone, R. L., Mustard, J. F.* (Department of Pathology, McMaster University, Hamilton, Ontario L8S 4J9, Canada), p. 417

Impairment by heparin of primary haemostasis and platelet [^{14}C]5-hydroxytryptamine release. *Heiden, D., Mielke, C. H. Jr., Rodvien, J.* (Pacific Medical Center, S-704 San Francisco, Calif. 94120), p. 427

Abstracts of papers presented at the Annual General Meeting of the British Society of Haematology held in London, 24 and 25 March 1977, p. 437

British Journal of Haematology (Oxford) **36** (1977) No. 4

Annotation. Chromosomes in haematology. *Lawler, S. D.* (Royal Marsden Hospital, London SW3 6JJ, England), p. 455

Heterocellular hereditary persistence of fetal haemoglobin (heterocellular HPFH) and its interaction with β-thalassaemia. *Wood, W. G., Weatherall, D. J., Clegg, J. B., Hamblin, T. J., Edwards, J. H., Barlow, A. M.* (Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford, England), p. 461

Decreased deformability of erythrocytes in haemolytic anaemia associated with glucosephosphate isomerase deficiency. *Schröter, W., Tillmann, W.* (Department of Paediatrics, University of Göttingen, D-3400 Göttingen, FRG), p. 475

Mechanisms of red cell destruction mediated by noncomplement binding IgG antibodies: the essential role *in vivo* of the Fc part of IgG. *von dem Borne, A. E. G. Kr., Beckers, D., Engelfriet, C. P.* (Department of Haematology, Binnengasthuis, University of Amsterdam, Amsterdam, The Netherlands), p. 485

Proliferation of erythroid colonies in semisolid agar. *Horland, A. A., Wolman, S. R., Murphy, M. J. Jr., Moore, M. A. S.* (Department of Pathology, New York University Medical Center, New York, N. Y. 10016), p. 495

The effect of androstanes on granulopoiesis *in vitro* and *in vivo*. *Francis, G. E., Berney, J. J., Bateman, S. M., Hoffbrand, A. V.* (Department of Haematology, The Royal Free Hospital, Hampstead, London NW3 2QG, England), p. 501

A to O bone marrow transplantation in severe aplastic anaemia: Dynamics of blood group conversion and demonstration of early dyserythropoiesis in the engrafted marrow. *Marmont, A. M., Damasio, E. E., Bacigalupo, A., Giordano, D., Rossi, E., Reali, G., Gay, A., Dagna-Bricarelli, F., Brema, F., Carella, A. M., Santini, G.* (Division of Haematology and Clinical Immunology, Ospedale Generale Regionale, 16132 Genova, Italy), p. 511

Transferrin receptors in developing murine erythroid cells. *Nunez, M. T., Glass, J., Fischer, S., Lavidor, L. M., Lenk, E. M., Robinson, S. H.* (Reprint requests: Dr. J. Glass, Department of Medicine and Thorndike Laboratory, Harvard Medical School at Beth Israel Hospital, Boston, Mass. 02215), p. 519

Iron deficiency in pregnant women in Ibarapa District of Western Nigeria. *Oluboyede, O. A., Topley, E., Ogunbode, O.* (Department of Haematology, University College Hospital, Ibadan, Nigeria), p. 527

The detection of granulocyte alloantibodies with an indirect immunofluorescence test. *Verheugt, F. W. A., von dem Borne, A. E. G. Kr., Décary, F., Engelfriet, C. P.* (Department of Immunohaematology, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 533

Lymphocyte abnormalities in myeloma. *Sewell, R. L.* (Imperial Cancer Research Fund Medical Oncology Unit, St. Bartholomew's Hospital, London, E. C. 1, England), p. 545

Microvascular occlusive lesions in primary thrombocythaemia. *Singh, A. K., Wetherley-Mein, G.* (Reprint requests: Prof. G. Wetherley-Mein, Department of Haematology, St. Thomas' Hospital and Medical

School, London SE1 7EH, England), p. 553

Adenosine diphosphate induced platelet aggregation and release reaction in heparinized platelet rich plasma and the influence of added citrate. *Heptinstall, S., Mulley, G. P.* (University Department of Medicine, General Hospital, Nottingham NG1 6HA, England), p. 565

In vitro thrombogenicity tests of factor IX concentrates. *Pepper, D. S., Banhegyi, D., Howie, A., Cash, J. D.* (South-East Scotland Regional Blood Transfusion Centre, Royal Infirmary, Edinburgh 3, Scotland), p. 573

Thrombin activation of factor VIII: the effect of inhibitors. *Rick, M. E., Hoyer, L. W.* (Division of Hematology, Department of Medicine, University of Connecticut Health Center, Farmington, Conn. 06032), p. 585

Experimental Hematology (Copenhagen) **4** (1976) No. 6

Contribution of granulocytopenia to endotoxin sensitivity of mice irradiated or undergoing graft-versus-host reaction. *Walker, R. I., Sheil, J. M.* (Experimental Pathology Department, Armed Forces Radiobiology Research Institute, Bethesda, Md. 20014), p. 329

Inhibition by mouse serum of hemopoietic colony formation *in vitro*. *Metcalf, D., Russell, S.* (Walter and Eliza Hall Institute of Medical Research, Melbourne, 3050 Victoria, Australia), p. 339

Mitigation of graft-versus-host disease in mice with xenogeneic antithymocyte serum and complement. *Volf, D., Sensenbrenner, L. L., Santos, G. W.* (The Johns Hopkins Oncology Center, B2-South, Baltimore City Hospitals, Baltimore, Md. 21224), p. 354

Changes in erythropoiesis and renal ultrastructure during exposure of mice to hypoxia. *Dunn, C. D. R., Jarvis, J. H., Napier, J. A. F.* (Department of Haematology, The Welsh National School of Medicine, Cardiff CFA 4XW, Wales, United Kingdom), p. 365

Weakening of genetic resistance. II. The effect of injection of the macrophage toxic agents silica and carragheenan and the cytostatic drugs cyclophosphamide, busulphan and vinblastin. *Buurman, W. A., van Bruggen, I.* (Department of Cellular Biology and Genetics, Erasmus University, Rotterdam, The Netherlands), p. 382

Experimental Hematology (Copenhagen) **5** (1977) No. 1

Red blood cell selection in chimeric mice. *West, J. D.* (Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 1

Changes in the immunocompetence and surface markers of lymphocytes in stored blood. *Khan, A., Wakasugi, K., Hill, N., Komezi, T., Osamura, S.* (Department of Immunotherapy, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235), p. 8

Rosette forming cells in the spleens of mice with lymphoid and myeloid leukemia. *Gabrilovac, J., Boranić, M.* ("R. Boskovic" Institute, 41001 Zagreb, Yugoslavia), p. 13

Hematologic effects of a single dose of methylnitrosourea. *Seidel, H. J.* (Department of Clinical Physiology, Center of Basic Clinical Research, University of Ulm, 79 Ulm/Donau, FRG), p. 19

Erythroid precursor fusion induced by Sendai virus. *Djaldetti, M., Bessler, H., Fishman, P., Apostolov, K.* (Department of Medicine "B" and Electron Microscopy Laboratory, Hasharon Hospital, Petah-Tiqva, Israel), p. 27

Erythropoietin in renal and hepatic lymph of conscious ewes. *Murphy, M. J. Jr., MacPherson, H. G., Camiscoli, J. F., Gordon, A. S.* (Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021), p. 41

Recovery of hematologic competence without engraftment following attempted bone marrow transplantation for aplastic anemia: Report of a case with diffusion chamber studies. *Sensenbrenner, L. L., Steele, A. A., Santos, G. W.* (Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205), p. 51

Phytohemagglutinin response of murine spleen cells during pregnancy and inhibi-

tion of normal phytohemagglutinin response by pregnancy- or post-partum serum. *Ruppert, B. L., Richie, E. R.* (Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030), p. 59

Predominance of granulocytopoiesis in bone marrow grafts in the omenta of mice treated with erythropoietin. *Meck, R. A., Laissue, J. A.* (Department of Pathology, University of Kentucky Medical Center, Lexington, Ky. 40506), p. 69

Inhibition of CFU entry into cycle by a bone marrow extract. *Frindel, E., Guigon, M.* (Institut de Radiobiologie Clinique, Institut Gustave-Roussy, 94800 Villejuif, France), p. 74

Experimental Hematology (Copenhagen) **5** (1977) No. 2

Colony-stimulating factor (CSF) in oestrogen-stimulated spayed murine uteri. *Rosendaal, M.* (Biological Research Unit, Cancer Institute, Melbourne 3000, Australia), p. 79

Cell-free granulocyte colony inhibiting activity derived from human polymorphonuclear neutrophils. *Broxmeyer, H. E., Moore, M. A. S., Ralph, P.* (Memorial Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021), p. 87

Stimulation of erythropoiesis in a grafted animal by mouse fetal liver culture media. *Zucali, J. R., McGarry, M. P., Mirand, E. A.* (Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 103

Bone marrow transplantation for an infant with neutrophil dysfunction. *Camitta, B. M., Quesenberry, P. J., Parkman, R., Boxer, L. A., Stossel, T. P., Cassady, J. R., Rappeport, J. M., Nathan, D. G.* (Milwaukee Children's Hospital, Milwaukee, Wis. 53233), p. 109

Replacement of serum by hemolysate as growth promoter for murine leukemic and normal hemopoietic progenitor cells in culture. *Rothmann, J., Hertogs, C. F., Pluznik, D. H.* (Reprint requests: Dr. D. H. Pluznik, Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel), p. 117

Colony growth of normal human bone marrow in agar-gel. *Entringer, M. A., Robinson, W. A., Kurnick, J. E.* (University of Colorado Medical Center, Denver, Col. 80220), p. 125

Radiation induced hemopoiesis in adult mouse liver. *Testa, N. G., Hendry, J. H.* (Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England), p. 136

Erythropoietic precursors in mice under erythropoietic stimulation and suppression. *Hara, H., Ogawa, M.* (Reprint requests: Dr. M. Ogawa, Veterans Administration Hospital, Charleston, S. C. 29403), p. 141

An estimate of the extent of depletion of colony-stimulating factors in culture. *Rosendaal, M.* (Biological Research Unit, Cancer Institute, Melbourne 3000, Australia), p. 149

Effect of furosemide on plasma fibrinolytic activity and urokinase excretion. *Chohan, I. S., Singh, I., Vermylen, J., Verstraete, M.* (Medical Research Department, Laboratory of Blood Coagulation, University of Leuven, Leuven, Belgium), p. 153

Experimental Hematology (Copenhagen) **5** (1977) No. 3

Editor's Comments, p. 159

Erythropoietic precursors in murine blood. *Hara, H., Ogawa, M.* (Department of Medicine, Medical University of South Carolina, Charleston, S. C. 29401), p. 161

Granulocyte tranfusion in recovery of neutropenic rats from induced *E. coli* toxicemia. *Popovic, V., Schaffer, R., Popovic, P.* (Department of Physiology, Emory University School of Medicine, Atlanta, Ga. 30322), p. 166

Quantitative aspects of an *in vivo* diffusion chamber assay for normal human hemopoietic colony forming units. *Jacobsen, N., Fauerholdt, L.* (Laboratory of Experimental Hematology, Memorial Sloan-Kettering Cancer Center, New York, N. Y. 10021), p. 171

Mobilization of hematopoietic stem cells (CFU-C) into the peripheral blood of man by endotoxin. *Cline, M. J., Golde, D. W.* (Department of Medicine, University of California School of Medicine, Los Angeles, Calif. 90024), p. 186

Effects of chronic hypoxia on platelet production in mice. *Langdon, J. R.,*

McDonald, T. P. (Reprint requests: Dr. T. P. McDonald, University of Tennessee, Memorial Research Center, Knoxville, Tenn. 37920), p. 191

Growth and differentiation of normal and leukemic human bone marrow cells cultured in diffusion chambers. *Steele, A. A., Sensenbrenner, L. L., Young, M. G.* (Reprint requests: Dr. L. L. Sensenbrenner, The Johns Hopkins Comprehensive Cancer Center, Baltimore, Md. 21205), p. 199

Reduced extrarenal erythropoietin production in rats treated with isoproterenol. *Zangheri, E. O.* (Cátedra de Fisiologia, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, 5500 Mendoza, Argentina, South America), p. 211

Resistance to parental, allogeneic and xenogeneic hemopoietic grafts in irradiated mice. *Lotzovà, E.* (University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas 77030), p. 215

Experimental Hematology (Copenhagen) **5** (1977) No. 4

The role of the submandibular glands in extrarenal erythropoietin production in mice. *Zangheri, E. O., Lopez, O. I., Honorato, L. E., Puscama, L. O., Rodriguez, M. E., Reta, E.* (Cátedra de Fisiologia, Universidad Nacional de Cuyo, 5500 Mendoza, Argentina, South America), p. 237

Growth and differentiation of rat bone marrow stem cells in diffusion chamber culture. *Kurrle, E., Hoelzer, D., Harriss, E. B.* (Universität Ulm, Zentrum für Innere Medizin, 7900 Ulm/Donau, BRD) p. 241

Factors affecting erythroid colony growth (CFU-E) from human marrow. *Freedman, M. H., Saunders, E. F.* (Division of Hematology, Hospital for Sick Children, Toronto, Ontario, M5G 1X5, Canada), p. 250

The interaction of erythropoietin with fetal liver cells. I. Measurement of proliferation by tritiated thymidine incorporation. *Fredrickson, T. N., Smith, K. A., Cornell, C. J., Jasmin, C., McIntyre, O. R.* (Department of Pathobiology, U-89, University of Connecticut, Storrs, Conn. 06268) p. 254

Some aspects of iron metabolism during erythropoietic depression in the mouse. *Aggio, M. C., Fernández, L. M., Bruzzo, M. T.* (Laboratorio de Hematologia, Hospital "Dr. José Penna", 8000 Bahia Blanca, Argentina, South America), p. 266

The effect of peripheral blood contamination on colony yield from human bone marrow aspirates. *Gordon, M. Y., Douglas, I. D. C.* (Institute of Cancer Research, Sutton, Surrey SM2 5PX, England), p. 274

Mesenteric hemopoietic colonies: Occurrence in BALB/c mice after transplantation of syngeneic normal or leukemic hemopoietic cells. *Umar, M. H., van Griensven, L. J. L. D.* (Reprint requests: Dr. van Griensven, L. J. L. D., Department of Experimental Pathology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands), p. 281

Hematologic changes and thrombopoietin production in mice after X-irradiation and platelet-specific antisera. *McDonald, T. P., Cottrell, M., Clift, R.* (University of Tennessee, Memorial Research Center, Knoxville, Tenn. 37920), p. 291

Radiation-induced enlargement of granulocytic and macrophage progenitor cells in mouse bone marrow. *Metcalf, D., Johnson, G. R., Wilson, J.* (Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, 3050 Victoria, Australia), p. 299

Attempts to purify hemopoietic stem cell enrichment in bone marrow by use of glass wool filtration. *Davis, M. L., Woodard, D. A., Pesto, M., Toya, R. E.* (Division Radiation Biology, University of Alabama Medical Center, Birmingham, Ala. 35294), p. 310

Induction of proliferation of haemopoietic stem cells in culture. *Löwenberg, B., Dicke, K. A.* (Radiobiological Institute TNO, Rijswijk, The Netherlands), p. 319

Experimental Hematology (Copenhagen) **5** (1977) No. 5

The interaction of erythropoietin with fetal liver cells. II. Inhibition of the erythropoietin effect by interferon. *Smith, K. A.,*

Fredrickson, N., Mobraaten, L. E., DeMaeyer, E. (Darthmouth-Hitchcock Medical Center, Hanover, NH 03755), p. 333

Effects of fibroblasts on the growth of erythroid progenitor cells *in vitro*. *Ghio, R., Bianchi, G., Löwenberg, B., Dicke, K. A., Ajmar, F.* (Department of Hematology School of Medicine, University of Genova, I-16132 Genova, Italy), p. 341

Haemopoietic recovery in spleen and marrow after transplantation of bone marrow from either normal or hydroxyurea treated mice. *Hasthorpe, S., Hodgson, G. S.* (Biological Research Unit, Cancer Institute, Melbourne 3000, Australia), p. 348

Modulation of hemopoiesis by prostaglandins. *Kurland, J., Moore, M. A. S.* (Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021), p. 357

Factors regulating yolk sac hematopoiesis in diffusion chambers: Various types of sera, cyclophosphamide, irradiation and long-term culture. *Weinberg, S. R., Stohlman, F. Jr.* (St. Elizabeth's Hospital of Boston, Boston, Mass. 02135), p. 374

Erythropoietin, thrombopoietin and colony stimulating factor in fetal mouse liver culture media. *Zucali, J. R., McDonald, T. P., Gruber, D. F., Mirand, E. A.* (Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 385

Identification of erythropoietin producing cells in fetal mouse liver cultures. *Gruber, D. F., Zucali, J. R., Mirand, E. A.* (Reprint requests: Dr. J. R. Zucali, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 392

Temporal transition in the site of rat erythropoietin production. *Gruber, D. F., Zucali, J. R., Wleklinski, J., LaRussa, V., Mirand, E. A.* (Reprint requests: Dr. J. R. Zucali, Department of Biological Resources, Roswell Park Memorial Institute, Buffalo, N. Y. 14263), p. 399

Hydroxyurea (HU) in experimental hematology. I. Characterization of *in vitro* and *in vivo* effects on progenitor and transit cells. *Benestad, H. B., Warhuus, K., Reikvam, Å.* (Institute of Physiology, University of Oslo, Oslo 1, Norway), p. 408

Hydroxyurea (HU) in experimental hematology. II. Similarities and dissimilarities between HU and ^{3}H-thymidine killing. *Benestad, H. B.* (Institute of Physiology, University of Oslo, Oslo 1, Norway), p. 415

In vivo synchronization of haemopoietic stem cells with hydroxyurea. *Hodgson, G., Blackett, N.* (Biological Research Unit, Cancer Institute, Melbourne 3000, Victoria, Australia), p. 423

Experimental Hematology (Copenhagen) **5** (1977) No. 6

Analysis of receptor mechanisms involved in the hemopoietic effects of androgens: Use of the *Tfm* mutant. *Byron, J. W.* (Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, Md. 21201), p. 429

Frozen human bone marrow from normal individuals: A possible control for the culture of human bone marrow in agar. *Grande, M., Berthier, R., Hollard, D.* (Via Vitt. Emanuele, 52, 74011 Catellaneta, Taranto, Italy), p. 436

Tritiated thymidine incorporation and cell-mediated lympholysis as correlates of acute graft-versus-host reaction. *Sharkis, S. J., Strong, D. M., Ahmed, A., Sell, K. W.* (The Johns Hopkins University School of Medicine, Oncology Center, Baltimore City Hospitals, Baltimore, Md. 21224), p. 443

Serum half-life and organ distribution of radiolabeled colony stimulating factor in mice. *Burgess, A. W., Metcalf, D.* (Cancer Research Unit, Walter and Eliza Hall Institute of Medical Research, P. O. Royal Melbourne Hospital, Parkville, Victoria 3050, Australia), p. 456

Studies on the mechanism of allogeneic disease in mice. I. Influence of bone marrow T lymphocytes on the differentiation and proliferation of hemopoietic stem cells. *Burek, É., Plavljanić, Dj., Šlamberger, S., Vitale, B.* (Reprint requests: Dr. B. Vitale, Institut "Rudjer Bošković", 41001 Zagreb, Yugoslavia), p. 465

Phagocytosis of "old" red blood cells by macrophages from syngeneic mice *in vitro*. *Knyszynski, A., Leibovich, S. J., Danon, D.* (Section of Biological Ultrastructure, The Weizmann Institute of Science, Rehovot, Israel), p. 480

The statistical distribution of colony counts in the spleen colony assay. *Mossman, K. L.* (Department of Radiology, Georgetown University, Medical Center, Washington, D. C.), p. 487

Effect of a low dose of vincristine on platelet production in mice. *Mandel, E.M., Bessler, H., Djaldetti, M.* (Reprint requests: Prof. M. Djaldetti, Department of Medicine "B", Hasharon Hospital, Petah-Tiqva, Israel), p. 499

Identification of distinctive stromal elements in erythroid and neutrophil granuloid spleen colonies: Light and electron microscopic study. *La Pushin, R. W., Trentin, J. J.* (Reprint requests: Dr. J. J. Trentin, Division of Experimental Biology, Baylor College of Medicine, Houston, Texas 77030), p. 505

Analysis of populations of macrophage-granulocyte progenitor cells stimulated by activities in mouse lung-conditioned medium. *Williams, N., Jackson, H.* (Sloan-Kettering Institute for Cancer Research, Department of Developmental Hematopoiesis, Rye, New York 10580), p. 523

Myeloid membrane markers. I. Expression of marker antigens specific for murine granulocytes and their precursor cells. *Jäger, G., Hoffmann-Fezer, G., Thiel, E., Ruppelt, W., Thierfelder, S.* (Institut für Hämatologie, GSF, Munich, BRD), p. 535

Oxymetholone and erythropoiesis: Failure to detect an effect in fetal mouse liver cell culture. *Dunn, C. D. R., Napier, J. A. F., Ford, T. W., Price, V. A.* (University of Tennessee, Memorial Research Center, Knoxville, Tenn. 37920), p. 546

A technique for staining haemopoietic colonies in agar cultures. *Bol, S., van den Engh, G., Visser, J.* (Radiobiological Institute TNO, Rijswijk, The Netherlands), p. 551

Folia Haematologica (Leipzig) **103** (1976) No. 3

X. Erfurter Konferenz über Hämostase u. Thrombose, "Synthetische Fibrinolytika, Thrombolytische Therapie mit Streptokinase", Erfurt 23. und 24. Mai 1975, p. 289

Eröffnung der Konferenz und Begrüßung, p. 291

Biochemisch-pharmakologische Grundlagen der antithrombotischen Therapie. *Markwardt, F.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 293

Synthetische Fibrinolytika. Eigene Erfahrung und Vorschläge. *von Kaulla, K. N.* (Coagulation Laboratories, University of Colorado Medical Center, Denver, Col. 80220), p. 313

Fibrinolysis induction by synthetic organic acids. Chemical structure and biological activity relationship. *Čepelák, V., Roubal, Z., Kuchař, M.* (Department of Clinical Haematology and Clinical Pharmacology, Faculty Hospital Plzeň, 30599 Plzeň, Czechoslovakia), p. 343

Synthetische Verbindungen mit fibrinolytischer und thrombolytischer Aktivität. I. Teil: Pharmakologische Betrachtungen. *Desnoyers, P.* (Abteilung für Gefäßpharmakologie und Hämostase, F.-92150 Suresnes, France), p. 351

Synthetische Verbindungen mit fibrinolytischer und thrombolytischer Aktivität. 2. Teil: Untersuchungen zum Wirkungsmechanismus. *Desnoyers, P., Samama, M.* (Abteilung für Gefäßpharmakologie und Hämostase, F.-92150 Suresnes, France), p. 357

Oral fibrinolytic therapy. General review. *Samama, M.* (Faculty of Medicine, Hôtel-Dieu, 75004 Paris, France), p. 363

Phenformin and ethyloestrenol in recurrent deep venous thrombosis (DVT). *Hedner, U., Nilsson, I. M., Isacson, S.* (Koagulationslaboratoriet Allmänna Sjukhuset, 21401 Malmö, Sweden), p. 372

The influence of long-term therapy with phenformin plus metanabol on blood fibrinolytic activity, platelet adhesiveness and lipid metabolism in patients with arteriosclerosis. *Perzanowski, A., Bielawiec, M., Myśliwiec, M.* (Reprint requests: Dr. M. Bielawiec, Institute of Internal Medicine, Medical Academy Białystok, Poland), p. 381

Influence of some organic and inorganic ions on fibrinolysis *in vitro*. *Haverkate, F., Kluft, C.* (Gaubius Institute TNO, Leiden, The Netherlands), p. 389

Die Gewebekultur als ein Testsystem für das Studium der Fibrinolyseaktivatoren. *Åstedt, B.* (Frauenklinik, Almänna Sjukhuset, 21401 Malmö, Schweden), p. 399

Über die Freisetzung von Plasminogenaktivatoren. *Klöcking, H.-P., Bock, G., Drawert, J., Hinsenbrock, K.-P. Kaiser, B., Sedlařik, U.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 404

Fibrindegradationsprodukte (FDP) und fibrinolytische Aktivität des Blutes bei einigen akuten und chronischen Krankheiten. *Kotschy, M.* (Klinik für Angiologie des Institutes für Innere Krankheiten, Wrocław, Polen), p. 425

Mikrobiologisch-immunologische Aspekte der Streptokinase-gewinnung und -anwendung. *Köhler, W., Gerlach, D.* (Zentralinstitut für Mikrobiologie u. Experimentelle Therapie, DDR-69 Jena), p. 431

Biochemische Aspekte der Streptokinasewirkung. *Landmann, H.* (VEB Arzneimittelwerk Dresden, DDR-8122 Radebeul), p. 437

Zur Pharmakologie des Streptokinasepräparates Awelysin. *Klöcking, H.-P.* (Institut für Pharmakologie u. Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 445

Klinik der thrombolytischen Therapie mit Streptokinase. *Vogel, G., Huyke, R.* (Medizinische Klinik der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 456

Gerinnungsphysiologische Erscheinungen und klinische Nebenwirkungen bei Streptokinasetherapie. *Vinazzer, H.* (Blutgerinnungslaboratorium Linz, A-4020 Linz-Donau, Österreich), p. 468

Thrombolytische Therapie in der Pädiatrie. *Weinmann, G., Giertler, U., Schwela, S.* (Kinderklinik der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 472

Thrombolytische Behandlung in der Schwangerschaft und unter der Geburt. *Brandt, M., Niedner, W.* (Frauenklinik der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 477

Zur Anwendung von Fibrinolytika in der Augenheilkunde. *Lohse, K., Weller, P.* (Reprint requests: Dr. P. Weller, II. Medizinische Universitäts-Klinik, DDR-402 Halle/Saale), p. 483

Folia Haematologica (Leipzig) **103** (1976) No. 4

The amoebocytic corpuscles in the circulating fluid of the *lamelli*-branches, *Indonaia caerulia* and *Parreysia (Parreysia) favidens. Singh, K., Narain, A. S.* (Reprint requests: Dr. A. S. Narain, Department of Zoology, University of Gorakhpur, Gorakhpur 273001, U.P. India), p. 497

Blood of the common Indian weaver birds *Ploceus philippinus* and *Ploceus benghalensis* (Linnaeus). *Banerjee, V., Banerjee, M.* (Department of Zoology, Science College, Patna University, Patna 5, India), p. 506

Zur Problematik der Behandlung unreifzelliger Leukosen im höheren Lebensalter. *Trux, F., Fink, R., Wutke, K.* (Medizinische Klinik, Medizinische Akademie Erfurt, DDR-50 Erfurt), p. 516

Blutverlust und Tetrachlorkohlenstoffintoxikation. *Richter, C., Pankow, D., Ponsold, W.* (Reprint requests: Dr. W. Ponsold, Martin-Luther-Universität, DDR-40 Halle), p. 523

Susceptibility to volume stress in *Colisa fasciatus. Agrawal, U., Srivastaka, A. K.* (Department of Zoology, University of Gorakhpur, Gorakhpur 273001 U.P. India), p. 530

Intravitale Lebenszeit und Verformbarkeit schwach amidinierter Rattenerythrozyten. *Halbhuber, K.-J., Unger, J., Geyer, G.* (Anatomisches Institut, Friedrich-Schiller-Universität Jena, DDR-69 Jena), p. 534

Mechanische Resistenz amidinierter Humanerythrozyten. *Käska, M., Halbhuber, K.-J., Geyer, G.* (Reprint requests: Prof. Dr. G. Geyer, Anatomisches Institut, Friedrich-Schiller-Universität, DDR-69 Jena), p. 541

Die Wirkung der chemisch mittels Äthylpalmitat herbeigeführten Splenektomie auf die Bildung von Heteroagglutininen bei der Ratte. *Sebestik, V., Májský, A., Ptmesilová, I., Jelinek, J.* (Institut für Hämatologie und Bluttransfusion Prag, 12820 Prag 2, Tschechoslowakei), p. 547

Filtrability of erythrocytes in "hypersplenic" rats. *Brabec, V.* (Institute of Haematology and Blood Transfusion, 12820 Prague 2, Czechoslovakia), p. 552

Der Einfluß von Inosin, anorganischem Phosphat und Pyruvat auf die Glykolysemetabolite in Erythrozyten unter Kon-

servierungsbedingungen. *Hasart, E., Roigas, H., Roth, W.* (Reprint requests: Dr. H. Roigas, Bezirksinstitut für Blutspende- und Transfusionswesen Berlin, DDR-113 Berlin), p. 559

Über fibrinolytische Eigenschaften eines Heparin-Ocrase-Komplexes. *Kudrjashov, B. A., Liapina, L. A., Klöcking, H.-P.* (Laboratorium für Physiologie und Biochemie der Blutgerinnung, Moskauer Staatliche Lomonossow-Universität, Moskau W-234, UdSSR), p. 573

Mikromethode für die Bestimmung von Hitzenfibrinogen. *Rogner, G.* (Kinderklinik des Stadtkrankenhauses, Dresden-Neustadt, DDR-8023 Dresden), p. 583

Untersuchungen an Kryopräzipitaten. 6. Mitteilung: Osmolalität und elektrolytische Leitfähigkeit. *Uteg, K.-H., Sander, W.* (Bezirksinstitut für Blutspende- und Transfusionswesen Schwerin, DDR-27 Schwerin), p. 589

Folia Haematologica (Leipzig) **103** (1976) No. 5

Übersicht. Ontogenie des Sauerstofftransportes im Blut. *Bartels, H.* (Physiologisches Institut der Medizinischen Hochschule Hannover, D-3 Hannover 61, BRD), p. 609

T cells, B cells and intermediate forms in the newborn studied by scanning electron microscopy and phosphatase marker. *Lisiewicz, J., Dobrowolski, J., Bryniak, C.* (Hematological Clinic, Cracow, Poland), p. 621

Formation of multinucleated giant cells from circulating blood cells cultivated in diffusion chambers. *Levander, H. G.* (Department of Internal Medicine, University Hospital, Uppsala, Sweden), p. 631

Veränderungen der zellulären Immunreaktion im Verlauf von akuten lymphatischen Leukämien. *Aurich, G.* (Universitäts-Kinderklinik und Poliklinik "Jussuf Ibrahim", DDR-69 Jena), p. 635

Mehrfache, 2- bis 10malige Asparaginase-Behandlung bei Kindern mit lymphoproliferativen Erkrankungen. *Legutko, L., Zajaczkowski, J., Halikowska-Łacka, E., Pituch, A., Cyklis, R.* (Second Pediatric Clinic, Institute of Pediatrics, 30-663 Cracow, Poland), p. 644

Ergebnisse der Polychemotherapie und Immuntherapieversuche bei akuten Leukosen. *Kruse, H., Reddemann, H., Zastrow, J., Eggers, G., Blau, H.-J., Griefahn, B.* (Universitäts-Kinderklinik, DDR-22 Greifswald), p. 651

Möglichkeiten zur Beeinflussung von Varizellen-Herpes zoster-Infektionen bei Leukosen im Kindesalter. *Exadaktylos, P., Grävinghoff, J.* (Universitäts-Kinderklinik und Poliklinik, DDR-40 Halle/Saale), p. 660

Zur Transplantabilität von Knochenmark und Milzzellen nach Filtration durch Silongewebe. *Fiala, J.* (Institut für Hämatologie und Bluttransfusion, 128 20 Prag 2, ČSSR), p. 672

Inhibition of normal granulopoiesis by cytostatic agents. *Szmigielski, S., Jeljaszewicz, J.* (Reprint requests: Dr. J. Jeljaszewicz, National Institute of Hygiene, Department of Bacteriology, 00-791 Warsaw, Poland), p. 678

Untersuchungen zur Morphologie des Blutes vom europäischen Aal *(Anguilla anguilla)*. II. Untersuchungen zur Granulopoese. *Kreutzmann, H.-L.* (Sektion Biologie der Wilhelm-Pieck-Universität Rostock, DDR-25 Rostock), p. 686

Verhalten der Heparinozyten nach Goldtherapie. *Frick, G., Frick, U., Engelmann, C., Preussner, S., Konrad, H., Nowotny, P., Keysser, M., Bast, G.* (Klinik für Innere Medizin des Bereiches Medizin der Universität, DDR-25 Rostock), p. 701

Zur Thrombozytenkinetik vor und nach Goldtherapie bei Rheumatoidarthritis. *Preussner, S., Konrad, H., Frick, G., Nowotny, P., Engelmann, C., Bast, G.* (Klinik für Innere Medizin der Universität Rostock, DDR-25 Rostock), p. 707

Zur Fibrinolyse vor und nach Goldtherapie bei Rheumathoidarthritis. *Frick, U., Frick, G., Preussner, S., Konrad, H., Nowotny, P., Keysser, M., Bast, G.* (Chirurgische Universitätsklinik, DDR-25 Rostock), p. 715

Die Beeinflussung der Leukopoese und der Granulozytenphagozytose vor und nach Goldtherapie bei Rheumatoidarthritis. *Nowotny, P., Konrad, H., Preussner, S., Bast, G., Keysser, M., Günther, I.* (Abteilung für Hämatologie der Klinik für Innere Medizin des Bereiches Medizin,

Universität Rostock, DDR-25 Rostock), p. 719

Changes in erythropoiesis during the course of polycythaemia vera. *Burger, T., Schmelzer, M.* (First Department of Medicine, University Medical School, H-7643 Pécs, Hungary), p. 726

Herstellung eines angereicherten Faktor-VIII-Präparates. *Bukurestliev, A., Angelova, Z., Lissitschkov, T.* (Institut für Hämatologie und Bluttransfusion, Sofia 56, Bulgarien), p. 742

The "Echis Carinatus Venom" prothrombin assay in coumarin treated patients. A comparison with one-stage and immunological assays. *Girolami, A., Brunetti, A., Patrassi, G., Borsato, N.* (Istituto di Semeiotica Medica dell'Università di Padova, Padova, Italy), p. 746

Ergebnisse in der Behandlung der immunthrombozytolytischen Purpura (ITP). *Rák, K.* (II. Medizinische Klinik der Universität Debrecen, H-4012 Debrecen, Ungarn), p. 758

Die Bestimmung der Thrombozytenzahl im plättchenreichen Plasma. *Maeß, M., Wendt, F., Rudolf, R., Tädling, A.* (Chirurgische Klinik, DDR-10 Berlin), p. 765

Über das Vorkommen von antithrombozytären und antileukozytären Antikörpern bei Hämophilie-A-Patienten nach Transfusion von Kryopräzipitaten. *Uteg, K.-H., Májsky, A.* (Bezirksinstitut für Blutspende- und Transfusionswesen Schwerin, DDR-27 Schwerin), p. 771

Folia Haematologica (Leipzig) **103** (1976) No. 6

Übersicht. Angeborene und erworbene Varianten des Plasmafibrinogens. *Krantz, S., Lober, M.* (Physiologisch-Chemisches Institut der Ernst-Moritz-Arndt-Universität Greifswald, DDR-22 Greifswald), p. 793

Untersuchungen über die Beeinflussung der zellulären Immunreaktion bei Kindern mit akuten lymphatischen Leukosen in Abhängigkeit von der Art der zytostatischen Therapie. *Aurich, G.* (Universitäts-Kinderklinik und Poliklinik "Jussuf Ibrahim", DDR-69 Jena), p. 807

Beeinflussung der Lymphozytenfunktion bei akuten lymphatischen Leukosen unter der Immuntherapie. *Aurich, G.* (Universitäts-Kinderklinik und Poliklinik "Jussuf Ibrahim", DDR-69 Jena), p. 817

Zur Frage der Fähigkeit zur Antikörperbildung bei der akuten Leukose im Kindesalter. *Blau, H.-J., Drescher, U., Kurmaschow, W. I., Koschel, I. W., Fokina, T. W., Schkarenkowa, L. W.* (Kinderklinik des Bereiches der Wilhelm-Pieck-Universität Rostock, DDR-25-Rostock 1), p. 826

Zytogenetische Untersuchungen als Beitrag zur Diagnostik von Präleukämie und Leukämierezidiv im Kindesalter. *Kirchner, M., Hofmann, A.* (Universitäts-Kinderklinik Jena, DDR-69 Jena), p. 831

Klinische und zytologische Unterschiede bei akuten lymphatischen und akuten undifferenzierten Leukämien des Erwachsenen. *Abbrederis, K., Schmalzl, F.* (Medizinische Universitätsklinik Innsbruck, A-6020 Innsbruck, Österreich), p. 844

Der Einfluß exogener Alkalisierung auf zytokinetische Vorgänge und die Überlebensrate von Mäusen mit lymphoblastischer Leukämie. *Schneiberg, K., Rytwiński, K., Bartnikowa, W.* (Onkologisches Institut Gliwice, Gliwice, Polen), p. 852

Präfinale klinische Befunde und Autopsieergebnisse von 82 Kindern mit akuter lymphatischer Leukose (ALL) bei unterschiedlichem Therapieregime. *Reddemann, H., Griefahn, B., Heidecke, G.* (Universitäts-Kinderklinik Greifswald, DDR-22 Greifswald), p. 860

Die spontane Lymphozytentransformationsrate bei Risikoneugeborenen. *Wiersbitzky, S., Dierschke, R.* (Universitäts-Kinderklinik der Ernst-Moritz-Arndt-Universität Greifswald, DDR-22 Greifswald), p. 873

The human blood platelet: Its derivation from the red blood cell. A morphologic study. *Leiter, S. S.* (1301 Westbury Drive, Philadelphia, Pa. 19151), p. 878

Über die Relevanz des Schizozytennachweises für die Erkennung der Rejektion bei nierentransplantierten Patienten. *Bube, F. W., Hoer, P. F., Siebel, E.* (Institut für Transfusionsmedizin der Stadt Köln, D-5 Köln 91, BRD), p. 883

Die Formabhängigkeit der Dunkelfeld-Streulichtintensität menschlicher Erythrozyten. *Scheven, Ch., Geyer, G.* (Anatomisches Institut der Friedrich-Schiller-Universität, DDR-69 Jena), p. 893

Zur "Zuckeragglutinabilität". *Halbhuber, K.-J., Geyer, G.* (Anatomisches Institut der Friedrich-Schiller-Universität Jena, DDR-69 Jena), p. 900

Iron absorption in gastric and duodenal pathology in patients with iron deficiency anaemias. *Ryabov, S. I., Ryss, E. S.* (Department of Propaedeutics of Internal Diseases, First Medical Institute of Leningrad, Leningrad P-89, USSR), p. 906

Hb Lepore and (haemo-) blastoma. *Quattrin, N., Mastrobouni, A., Laudisio, F. L., Brancaccio, V., Pagnini, D.* (Department of Haematology, Social Centre for Genotypical Haemoglobinopathies, Naples, Italy), p. 915

Autologe Transfusion von Erythrozyten mit erhöhter bzw. erniedrigter 2,3-Diphosphoglyzerat-Konzentration. Überlebensrate, Überlebenszeit und Hämoglobin-Sauerstoff-Affinität. *Hasart, E., Roigas, H., Lun, A., Seefeld, B., Strauss, D., Roth, W.* (Bezirksinstitut für Blutspende- und Transfusionswesen Berlin, DDR-113 Berlin), p. 920

Untersuchungen über den Nachweis eines Gerinnungsfaktor VIII- oder IX-Mangels mit Hilfe der partiellen Thromboplastinzeit. *Lutze, G.* (Zentrallabor der Medizinischen Akademie Magdeburg, DDR-301 Magdeburg), p. 933

Isolation of non-modified gamma globulin for intravenous use. *Schneider, W., Wolter, D., McCarty, L. J.* (German Red Cross Blood Transfusion Service, D-58 Hagen/Westf., FRG), p. 938

Folia Haematologica (Leipzig) **104** (1977) No. 1

Übersicht. Hämorrhagische Diathese als Folge einer akuten Einwirkung von ionisierenden Strahlen. *Krantz, S., Lober, M.* (Physiologisch-chemisches Institut, DDR-22 Greifswald), p. 1

Der Lysozymgehalt der Leukozyten bei verschiedenen Leukoseformen. *Jaworkowsky, L., Grant, H.* (Zentrales wissenschaftliches Laboratorium des Rigaer Medizinischen Instituts, Riga, USSR), p. 11

Die Kinetik der basophilen Granulozyten des Menschen. *Arndt, D., Parwaresch, M. R.* (Abteilung Radiologie, Strahlenbiologisches Labor, D-2300 Kiel, BRD), p. 24

Juvenile pernicious anemia associated with intestinal nodular lymphoid hyperplasia and immune deficiency state. Report of a case. *Ambrus, M., Horváth, L., Bajtai, G., Paál, M., Papp, T., Kádas, I.* (Bezirks-Institut für Blutspende- und Transfusionswesen, H-7621 Pécs, Hungary), p. 39

Über die Korrelation des klinischen und anatomischen Bildes bei der Meningosis im Kindesalter. *Hrodek, O., Hyniová, H., Svatý, J., Karpenko, A.* (II. Kinderklinik der Karls-Universität, 15006 Prag 5-Motol, ČSSR), p. 48

Klinische Erfahrungen mit der Langzeittherapie beim generalisierten Plasmozytom. *Sakalová, A., Gazová, S., Makaiová, I.* (Klinik für Hämatologie und Bluttransfusion, Bratislava, ČSSR), p. 57

Zur Frage der suppressiven Wirkung von antilymphozytärem Globulin (ALG) auf die Bildung von Anti-ALG-Antikörpern. *Bednarik, T., Cajthamlová, H.* (Institut für Hämatologie und Bluttransfusion, 12820 Prag 2, ČSSR), p. 64

Haematological studies in relation to environmental temperature and different periods of breeding cycle in an air breathing fish, *Heteropneustes fossilis. Pandey, B. N.* (Department of Zoology, Gaya College, Gaya, Bihar, India), p. 69

Some aspects of haematology of an Indian grass snake *Natrix stolata* in relation to sex and size. *Choubey, B. J., Singh, N. K.* (Postgraduate Department of Zoology, Bhagalpur University, Bhagalpur, 7 Bihar, India), p. 75

Agglutinationsverhalten roter Blutzellen nach experimenteller Glykokalyxalteration. *Halbhuber, K.-J., Geyer, G., Feuerstein, H.* (Anatomisches Institut der FSU Jena, DDR-69 Jena), p. 85

Alcianblauinduzierte Mischzellagglutination. Kurze Originalmitteilung. *Halbhuber, K.-J., Geyer, G.* (Anatomisches Institut der Friedrich-Schiller Universität Jena, DDR-69 Jena), p. 98

Ein Fall von Morbus Osler mit gleichzeitig bestehender Thrombozytopenie und einem Faktor VIII-Inhibitor. *Gola, A., Kotschy, M., Nowicka, J., Kaiser, A.* (Klinika Hematologiczna, 53-374 Wrocław, Polen), p. 102

Präzisionskontrolle bei einer einstufigen Bestimmung des Gerinnungsfaktors VIII im Plasma. *Lutze, G.* (Zentrallabor der Medizinischen Akademie Magdeburg, DDR-301 Magdeburg), p. 109

Heat-ethanol isolated serum albumin: Discussion. *Schneider, W., Wolter, D., McCarty, L. J.* (German Red Cross Blood Transfusion Service North-Rhine Westfalia, Hagen Institute, D-5800 Hagen, FRG), p.116

Anti-HBbrno: A new CIEP hepatitis B marker, more sensitive than HBsAg radioimmunoassay. *Pintera, J., Vacl, J.* (Department of Clinical Biochemistry and Immunochemistry, Institute of Blood Transfusion and Haematology KUNZ, 657 20 Brno, ČSSR), p. 123

Folia Haematologica (Leipzig) **104** (1977) No. 2

Übersicht. Leukotaxisdefekte der neutrophilen Granulozyten und Monozyten. *Morenz, J.* (Institut für Medizinische Mikrobiologie und Epidemiologie der Medizinischen Akademie Magdeburg, DDR-301 Magdeburg), p. 153

Ein Testsystem zur Messung der zellulären RNS-Synthese von humanem Knochenmark. *Daene, A., Coutelle, Ch., Thierbach, V., Holle, M., Raderecht, Ch., Roigas, H.* (Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR, Bereich Bioregulation, DDR-1115 Berlin-Buch), p. 193

Activity of beta-glucuronidase in peripheral blood lymphocytes of patients with cancer of the larynx. *Gierek, T., Lisiewicz, J., Kuśnierczyk, W., Pilch, J.* (Clinic of Laryngology, Silesian Academy of Medicine, 40-027 Katowice, Poland), p. 202

Intracellular enzymatic response of lymphocytes and neutrophils in patients with cancer of the larynx. *Gierek, T., Lisiewicz, T., Pilch, J.* (Clinic of Laryngology, Silesian Academy of Medicine 40-027 Katowice, Poland), p. 208

A peculiar lymph node anti-carcinoma reaction. *Popa, G., Dobrescu, G., Hanganu, E.* (Clinic of Medical Emergency, 6600 Iassy, Romania), p. 216

Zur Anwendung der sterilen Pflege in der Hämatologie. *Bláha, M., Günther, I., Vanásek, J., Merka, V., Mazák, J., Nowotny, P., Brijmohan, R. R.* (Purkynje Medizinisches Forschungsinstitut, Hradec Kralove, Czechoslovakia), p. 222

Untersuchungen zur Herstellung hämatologischer und zytologischer Präparate mit der Technik der Zentrifugalpräparation. *Aurich, G., Schorcht, E., Plenert, W.* (Universitäts-Kinderklinik, DDR-69 Jena), p. 229

The transient HLA antigenic loss dependent on hormonal state of organism in a healthy woman. *Májský, A., Jakoubková, J.* (Institute of Haematology and Blood Transfusion, 128 20 Prague 2, Czechoslovakia), p. 237

Die Anämia bei chronischer Hämodialyse. *Helbig, W., Aschenbach, H., Löbe, J., Gottschild, D.* (Medizinische Klinik der Karl-Marx-Universität, DDR-701 Leipzig zig), p. 242

Über Einsatzmöglichkeiten von amidinierten Humanerythrozyten als Agglutininadsorbens bei Hämagglutinationsreaktionen. *Halbhuber, K.-J., Geyer, G.* (Anatomisches Institut der Friedrich-Schiller-Universität Jena, DDR-69 Jena), p. 258

Hämagglutination mit Gal-N-Ac-Rezeptoren der Hepatozytenmembran. *Halbhuber, K.-J., Geyer, G.* (Anatomisches Institut der Friedrich-Schiller-Universität Jena, DDR-69 Jena), p. 265

Three cases of hemoglobin O Arab. *Penev, M. N., Panova, D. Y., Ivanov, B. N.* (Research Institute of Internal Medicine, and Pharmacology, Sofia, 1431, Bulgaria), p. 271

A-like Spezifität bei Tn-aktivierten Erythrozyten der Blutgruppe B. *Kurteva, B.* (Institut für Hämatologie und Bluttransfusion Sofia, Sofia, Bulgaria), p. 277

Glass activation-induced decrease or disappearance of normotest-thrombotest discrepancy in congenital coagulation disorders of the prothrombin complex. *Girolami, A., Patrassi, G., Fabris, F.* (Istituto di Semeiotica Medica dell'Università di Padova, Padova, Italy), p. 283

Folia Haematologica (Leipzig) **104** (1977) No. 3

Übersicht. Seltene Indikationen zur Splenektomie. *Stobbe, H.* (I. Medizinische Klinik der Charité, DDR-104 Berlin), p. 305

Über die HL-A-Spezifität der Immunreaktion bei Parablastenleukosen. *Nedelkova, M., Iliev, D., Serafimov-Dimitrov, W.* (Institut für Hämatologie und Bluttransfusion, Sofia 56, Bulgarien), p. 324

Time-conditioned coordination of dehydrogenase activity in blood cells in infantile acute leukemia. *Kachergene, N. B., Koshel, I. V., Nartsissov, R. P.* (Academy of Medical Sciences of USSR, Institute of Pediatrics, Moscow 117296, USSR), p. 330

Der Einfluß der extrakorporalen Blutbestrahlung auf die T- und B-Lymphozyten des peripheren Blutes bei chronischer Lymphadenose. *Heilmann, E., Nordick, R.* (Medizinische Poliklinik Münster, D-4400 Münster/Westf., BRD), p. 340

Morphologische, zytochemische, autoradiographische und quantitative Untersuchungen der Zellen in Lymphozytenkulturen unter Zusatz von Tuberkulin. *Drescher, J., Merk, K., Mau, G.* (Städtische Kinderklinik, D-29 Oldenburg/Oldb., BRD), p. 349

Über den Fall eines IgD-Plasmozytoms mit ungewöhnlicher neurologischer Symptomatik. *Kubát, R., Švehla, F., Horáček, J.* (Medizinische Abteilung, Krankenhaus OUNZ, 360 73 Karlovy Vary, ČSSR), p. 366

Zytoplasmavakuolen und Kinetik von Knochenmarkzellen bei Alkoholismus, Leberzirrhose und Anämien. *Boll, I., Weingärtner, K. R.* (Krankenhaus Neukölln, D-1 Berlin-West 47, BRD), p. 376

Der Einfluß von Lagerung und Kurzzeitkultivierung auf die Repopulationsfähigkeit von Stammzellen aus Mäuseknochenmark. *Viktora, L., Hermanová, E.* (Institut für Hämatologie und Bluttransfusion Prag, 2, ČSSR), p. 388

Conductance of the cytoplasm of erythrocytes as a criterion for evaluating the quality of blood preservation. *Krupa, J., Kalinowski, M., Adamiak, E.* (Institute of Automations and Radiocommunications, Merchant Navy Academy, 81-962 Gdynia, Poland), p. 396

Der Einfluß von AcD-AG-Stabilisator auf die Überlebenszeit und Oberflächenaktivität von flüssigkeitskonservierten Plättchenkonzentraten im Vergleich zu Konzentraten aus Frischblut. *Preußner, S., Frick, G., Konrad, H.* (Klinik für Innere Medizin der Wilhelm Pieck Universität Rostock, DDR-25 Rostock), p. 406

Untersuchungen zur Diagnostik des Vitamin B_{12}-Mangels. *Heilmann, E., Poblotzki, F.* (Medizinische Poliklinik der Universität Münster, D-440 Münster/Westf., BRD), p. 420

Lysis of red blood cells in some haemolytic anaemias — the lytic effect of *Agkistrodon piscivorus* venom *in vitro*. *Brabec, V., Kornalík, F.* (Institute of Haematology and Blood Transfusion, 128 20 Prague 2, ČSSR), p. 428

Quantitative Untersuchungen des ABO-Blutgruppensystems. Quantitative Veränderungen der Agglutinabilität der Antigene A, B and H in Beziehung zum Alter der Probanden. *Prodanov, P.* (Institut für Hämatologie und Bluttransfusion, Sofia 56, Bulgarien), p. 436

Untersuchungen an Familien mit Morbus Osler. *Hadnagy, Cs., Kovalszky, P.* (II. Medizinische Universitätsklinik, Tirgu-Mures, Rumänien), p. 452

Thrombotest mixing experiments in congenital coagulation disorders of the prothrombin complex and in coumarin treated patients. An additional evidence against the presence of an inhibitor in the latter. *Girolami, A., Patrassi, O., Cella, G.* (Istituto di Semeiotica Medica dell'Università a Padova, Padova, Italy), p. 463

Folia Haematologica (Leipzig) **104** (1977) No. 4

Glückwunschadresse zum 60. Geburtstag von Prof. Dr. med. M. Hrubisko. *Stobbe, H.* (Hämatologische Abteilung, I. Medizinische Klinik des Bereiches Medizin (Charité), Humboldt-Universität Berlin, DDR-104 Berlin), p. 482

Zur Pathogenese der chronischen Lymphadenose (Teil I). *Schwenke, H., Emmrich, F.* (Medizinische Klinik, Bereich Medizin der Karl-Marx-Universität, DDR-701 Leipzig), p. 497

Zur Pathogenese der chronischen Lymphadenose (Teil II). *Emmrich, F., Schwenke, H.* (Medizinische Klinik, Bereich Medizin der Karl-Marx-Universität, DDR-701 Leipzig), p. 504

On the presence of lysozyme in the nuclei of leukocytes. *Jaworkowsky, L. I.,*

Puddubnais, H. V., Grant, H. J. (Central Research Laboratory, Institute for Medicine, Riga, UdSSR), p. 518

Untersuchungen zur Zellproliferation bei akuten Leukämien unter zytostatischer Behandlung. *Urasínski, I., Kordecki, H.* (Hämatologische Klinik der Medizinischen Akademie in Szczecin, 71-163 Szczecin, Polen), p. 523

Karyotype 45, XX, $-11,2q^+$ of bone marrow cells in a case of di Guglielmo syndrome. *Izakovič, V., Šteruská, M., Vojtaššák, J.* (Department of Clinical Genetics, Institute for Postgraduate Medical Education, 80953 Bratislava, Czechoslovakia), p. 533

Untersuchungen zur Morphologie des Blutes vom europäischen Aal *(Anguilla anguilla)*. III. Beobachtungen an monozytären und lymphatischen Zellen. *Kreutzmann, H.-L.* (Wilhelm-Pieck-Universität Rostock, DDR-25 Rostock), p. 538

The inhibitory effect of methyl palmitate on heteroagglutinin formation in rats. *Šebestík, V., Májský, A., Potměšilová, I., Jelínek, J.* (Institute of Haematology and Blood Transfusion, 12 820 Prague 2, Czechoslovakia), p. 558

Smooth muscle autoantibodies in normal rabbit sera. Cross-reaction with human platelets as demonstrated by immunofluorescence. *Betterle, C., Fabris, F., de Prete, G. F., Bersani, G., Girolami, A.* (Reprint requests: Prof. A. Girolami, Istituto di Semeiotica Medica dell'Universita di Padova, Padova, Italy), p. 561

Der Vitamin-B_{12}-Spiegel im Serum in verschiedenen Altersstufen. *Heilmann, E., Bartling, K.* (Medizinische Poliklinik der Westfälischen Wilhelms-Universität, D-44 Münster, BRD), p. 569

Zu den Ursachen intra- und postoperativer Verbrauchskoagulopathien sowie postoperativer Thromboembolien. *Frick, G., Frick, U., Borchert, K., Lange, G.* (Klinik für Innere Medizin der Wilhelm-Pieck-Universität, DDR-25 Rostock), p. 575

Folia Haematologica (Leipzig) **104** (1977) No. 5

In memoriam Professor Dr. Sc. Mihály Gerendás. *Gaertner, H.* (Department of Medicine, E. Biernacki City Hospital, 31-061 Cracow, Poland), p. 593

Das Thromboplastin. Eine kurzgefaßte Übersicht neuer Ergebnisse. *Kase, F., Klir, P.* (Zentrallabor der Poliklinik, 400 01 Usti n. L., ČSSR), p. 596

Übersicht. Das Haptoglobin bei verschiedenen Erkrankungen. *Heilmann, E.* (Medizinische Poliklinik der Westfälischen Wilhelms-Universität Münster, D-44 Münster, BRD), p. 608

Comparative ultrastructural morphometric studies of micromyeloblastoid and lymphoblastoid paramyeloblasts. *Dobreva, A., Meshkov, T.* (Research Institute of Haematology and Blood Transfusion, Sofia-Darwenitza 56, Bulgaria), p. 619

Lysozyme activity in serum of patients suffering from leukaemia in the course of treatment. *Merkiel, K., Bielawiec, M., Kiersnowska, B., Prokopowicz, J.* (Department of Biochemistry, Institute of Physiology and Biochemistry, Medical School of Białystok, 15-276 Białystok, Poland), p. 627

Preservation of rat bone marrow with dimethylsulphoxide at $-150\,^{\circ}C$. *Nikolov, Ch., Detcheva, Z.* (Institute of Haematology and Blood Transfusion, Sofia 56, Bulgaria), p. 633

Rezidivfreie Phasen und Überlebenszeiten bei Kindern mit zytochemisch unterschiedlich reagierenden akuten lymphatischen Leukämien. *Kruse, H., Reuter, G.* (Kinderklinik der Wilhelm-Pieck-Universität, DDR-25 Rostock), p. 639

Anämie bei weiblichen Jugendlichen. *Heilmann, E., Menke, A., Rethmeier, H.-J., Seelbach, W.* (Medizinische Poliklinik der Universität Münster, D-44 Münster, BRD), p. 643

Geophagia sideropenica. *Hadnagy, Cs., Andreicut, S., Binder, P.* (II. Medizinische Universitätsklinik, Tg.-Mures, Rumänien), p. 648

Hepatogene Polyglobulien und Polyzythämien. *Hadnagy, Cs., Sass, Gy., Szabó, Z.* (II. Medizinische Universitätsklinik, Tg.-Mures, Rumänien), p. 656

Platelet adhesiveness and aggregation in combined factor V and factor VIII deficiency and in combined factor VII and factor VIII deficiency. *Girolami, A., de Marco, L., Fabris, F., Casonato, A.* (Istituto di Semeiotica Medica, University of Padova, Padova, 105 Italy), p. 663

Apparent decrease of "inhibition" in the dilution curve system with the increase of anticoagulation. A paradox that is against the presence of inhibitors in coumarin treated patients. *Girolami, A., Fabris, F., dal Bo Zanon, R., Cappellato, G.* (Istituto di Semeiotica Medica, University of Padova, 105 Padova, Italy), p. 670

Ein Arbeitsverfahren zur Gewinnung von sterilem Coombs-Serum. *Rojahn, J.* (Bezirksinstitut für Blutspende- und Transfusionswesen Leipzig, DDR-7021 Leipzig), p. 677

Folia Haematologica (Leipzig) **104** (1977) No. 6

XI. Erfurter Konferenz über Hämostase und Thrombose, "Disseminierte intravaskuläre Gerinnung (DIC)", Erfurt, 20. und 21. Mai 1977.

Eröffnung der Konferenz und Begrüßung. *Markwardt, F.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 691

Begrüßung der Gäste der XI. Erfurter Konferenz über Hämostase und Thrombose am 20. 5. 1977 in Molsdorf durch den Rektor der Medizinischen Akademie. Erfurt. *Usbeck, W.* (No address), p. 693

Pharmakologische Beeinflussung der disseminierten intravaskulären Gerinnung (DIC). Stand und Entwicklungstendenzen. *Markwardt, F.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 697

Die Rolle von Antithrombin bei gestörter Hämostase. *Róka, L., Bleyl, H.* (Institut für Klinische Chemie der Justus-von-Liebig-Universität, D-63 Gießen, BRD), p. 707

Die Rolle des Heparins bei der Thrombininhibition durch Antithrombin. *Bleyl, H., Róka, L.* (Institut für Klinische Chemie und Pathobiochemie, Universitätsklinik, D-6300 Gießen, BRD), p. 719

Untersuchungen zur Reaktionsweise des Heparins. *Stürzebecher, J.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 731

Disseminierte intravaskuläre Gerinnung: Antithrombin III und Heparin. *Thaler, E.* (I. Medizinische Universitätsklinik Wien, A-1090 Wien, Österreich), p. 740

Neue Aspekte der Heparintherapie. *Nagy, I., Losonczy, H., Hergert, K., Szilágyi, C.* (I. Medizinische Universitätsklinik, H-7643 Pécs, Ungarn), p. 751

Subkutane Anwendung von Heparin in der inneren Medizin. *Losonczy, H., Nagy, I.* (I. Medizinische Universitätsklinik, H-7643 Pécs, Ungarn), p. 761

Niederdosierte Heparintherapie bei per- und postoperativen Verbrauchsreaktionen der Blutgerinnung. *Fischer, M.* (Krankenhaus der Stadt Wien-Lainz, A-1130 Wien, Österreich), p. 769

Prophylaxe und Heparinbehandlung der Schwangerschaftskoagulopathie. *Glavanakova, V., Deutschinoff, A.* (Institut für Geburtshilfe und Frauenheilkunde, Sofia, Bulgarien), p. 778

Erste klinische Erfahrungen mit dem kleinmolekularen synthetischen Thrombininhibitor 4-Amidinophenylbrenztraubensäure (APPA). *Vogel, G., Huyke, R., Heerklotz, I.* (Medizinische Klinik der Medizinischen Akademie, DDR-50 Erfurt), p. 785

Diaplazentare Hemmung der DIC im fetalen Organismus durch 4-Amidinophenylbrenztraubensäure. *Niedner, W.* (Frauenklinik der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 789

Verbrauchskoagulopathie und Fibrinolyse. *Lasch, H. G.* (Justus Liebig-Universität, D-63 Gießen, BRD), p. 794

Fibrinolytische Therapie bei DIC im Kindesalter. *Weißbach, G., Lenk, H., Braun, W., Domula, M., Friedel, D.* (Kinderklinik der Karl-Marx-Universität, DDR-705 Leipzig), p. 801

Einfluß von Ocrase auf die thrombininduzierte DIC bei Ratten. *Nowak, G.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 810

Die Wirkung von Antikoagulantien und Hemmstoffen der Thrombozytenaggregation auf die disseminierte intravaskuläre Gerinnung (DIC). *Lakin, K. M., Makarov, V. A., Petruchina, G. N.* (,Medizinisch Stomatologisches Institut "N. A. Semashko", Moskau, UdSSR) p. 816

Über den Einfluß von α-Rezeptorenblockern auf adrenerge Reaktionen bei der disseminierten intravaskulären Gerinnung (DIC). *Glusa, E.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 819

Disseminierte intravaskuläre Gerinnung (DIC) bei exogenen Vergiftungen. *Klöcking, H.-P., Nowak, G., Markwardt, F.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 825

Inhibition of experimental arterial and venous thrombosis by Ecarin, a defibrinating enzyme. *Kornalik, F.* (Institute of Pathophysiology, Charles University, 128 53 Prague 2, Czechoslovakia), p. 833

Zur Diagnostik der DIC. *Koller, F.* (Department für Innere Medizin des Kantonsspitals Basel, Basel, Schweiz), p. 839

Rundtischgespräch über ausgewählte Probleme der Diagnostik der disseminierten intravasalen Gerinnung. *Vogel, G.* (Institut für Pharmakologie und Toxikologie der Medizinischen Akademie Erfurt, DDR-50 Erfurt), p. 851

Zur Methodik der Antiheparin- und Heparinmessung. *Domula, M., Weißbach, G.* (Kinderklinik der Karl-Marx-Universität, DDR-705 Leipzig), p. 865

Fibrin stabilizing factor (factor XIII) consumption as an indicator of disseminated intravascular coagulation (DIC). *Zuch, A., Kloczko, J., Bielawiec, M., Myśliwiec, M., Grabowski, R., Buluk, K.* (Reprint requests: Prof. M. Bielawiec, Haematology Clinic, Institute of Internal Medicine, Medical School, Białystok, Poland), p. 874

Zum Einsatz der partiellen Thromboplastinzeit und der Thrombinzeit in der Diagnostik der disseminierten intravasalen Gerinnung. *Frick, U., Frick, G.* (Chirurgische Klinik des Bereiches Medizin der Wilhelm-Pieck-Universität Rostock DDR-25 Rostock), p. 879

Haemostasis (Basel) **5** (1976) No. 4

Rat liver macrophages will not phagocytose fibrin during disseminated intravascular coagulation. *Emeis, J. J., Lindeman, J.* (Gaubius Institute, Health Research Organization, TNO, Leiden, The Netherlands), p. 193

Correlations between radioiodine uptake and blood viscosity factors in hypothyroid and hyperthyroid states. *Dintenfass, L.* (Department of Medicine, University of Sydney, Sydney 2006, Australia), p. 211

Purification of a plasminogen activator inhibitor indistinguishable from α_1-antitrypsin and an urokinase inhibitor in pregnancy plasma. *Clemmenses, I., Thorsen, S., Müllertz, S.* (Department of Clinical Chemistry, Hvidovre Hospital, DK-2650 Hvidovre, Denmark), p. 218

Binding of ^{14}C-ADP by thrombasthenic platelet membranes. *Legrand, C., Caen, J. P.* (Department of Hemostasis and Experimental Thrombosis, Hôpital Lariboisière, F-75010 Paris, France), p. 231

Dose-dependent influence of acetylsalicylic acid on platelet functions and plasmatic coagulation factors. *Loew, D., Vinazzer, H.* (Blood Coagulation Laboratory, A-4020 Linz, Austria), p. 239

Combined administration of low dose heparin and aspirin as prophylaxis of deep vein thrombosis after hip joint surgery. *Schöndorf, T. H., Hey, D.* (Zentrum für Innere Medizin, Universitätskrankenhaus, D-6300 Giessen, BRD), p. 250

Changes in coagulation factors following acute myocardial infarction in man. *Rennie, J. A. N., Ogston, D.* (Department of Medicine, University of Aberdeen, Aberdeen AB9 2ZD, Scotland), p. 258

Haemostasis (Basel) **5** (1976) No. 5

Antifactor Xa activity measured with amidolytic methods. *Ødegård, O. R., Lie, M., Abildgaard, U.* (Medical Department A, Aker Hospital, Oslo 5, Norway), p. 265

Von Willebrand's syndrome studies on a variant factor VIII. *Hill, F. G. H., Chan, M. C. K., Hardisty, R. M.* (Department of Haematology, Hospital for Sick Children, London, England), p. 276

Evaluation of hypercoagulability in septic abortion. *Graeff, H., Ernst, E., Bocaz, J. A., von Hugo, R., Hafter, R.* (I. Frauenklinik der Universität München, D-8000 München 2, BRD), p. 285

Differences in the reactivities of human urokinase and the porcine tissue plasmin-

ogen activator. *Thorsen, S., Astrup, T.* (Department of Clinical Chemistry, Hvidovre Hospital, DK-2650 Hvidovre, Denmark), p. 295

Inhibition of human platelet aggregation by the proteolytic effect of streptokinase role of the human factor-VIII-related protein. *Castillo, R., Maragall, S., Alvarez-Guisasola, J., Casals, F., Profitós, J., Ordinas, A.* (Servicio de Hemoterapia, Hospital Clinico y Provincial, Barcelona 11, Spain), p. 306

A technique for the evaluation of platelet-collagen adhesiveness *in vitro*. *Aznar, J., Jimenez, C., Villa, P.* (Departmento de Biopatologia Clinica, Ciudad Sanitaria "La Fe", Valencia, Spain), p. 318

Haemostasis (Basel) **5** (1976) No. 6

Purification and immunochemical characterization of human plasma factor XIII. *Kazama, M., McDonagh, J., Wagner, R. H., Langdell, R. D., McDonagh, R. P.* (Reprint requests: Dr. J. McDonagh, Department of Pathology, University of North Carolina, School of Medicine, Chapel Hill, N. C. 27514), p. 329

Coagulation defect in experimental trypanosomial infection. *Essien, E. M., Ikede, B. O.* (Department of Haematology, University College Hospital, Ibadan, Nigeria), p. 341

Treatment of thrombosis by sequential therapy with ancrod followed by streptokinase: clinical pharmacology and rheology. *Forbes, C. D., Barbenell, J., Prentice, C. R. M.* (University Department of Medicine, Royal Infirmary, Glasgow G4 OSF, Scotland), p. 348

Distribution and variation of fibrinolytic activity in the walls of human arteries and veins. *Hegt, V. N.* (Gaubius Instituut, Health Research Organization, TNO, Leiden, The Netherlands), p. 355

Haemostasis (Basel) **6** (1977) No. 1

Fibrin(-ogen) interactions with plasmin. *Gaffney, P. J.* (National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England), p. 2

Heparin, heparin-activated enzymes and platelets. *Ham, J. M., Lawrence, J. C.* (Department of Surgery, Prince of Wales Hospital, Randwick, N. S. W., 2031 Australia), p. 26

Use of rabbit antisera in the preparation of factor-XII-free platelet-rich and platelet-poor plasma. *Muntz, R. H., Castaldi, P. A.* (Department of Haematology, Austin Hospital, Heidelberg, Victoria 3084, Australia), p. 35

The role of factor XI in the coagulant activity of platelets. *Connellan, J. M., Castaldi, P. A., Muntz, R. H.* (Department of Haematology, Austin Hospital, Melbourne, Victoria 3084, Australia), p. 41

Studies on the chemistry of human platelet factor 4. *Morgan, F. J., Chesterman, C. N., McGready, J. R., Begg, G. S.* (St. Vincent's School of Medical Research, Melbourne, Victoria 3065, Australia), p. 53

Platelet activation in haemostasis. Role of thrombin and other clotting factors in platelet-collagen interaction. *Huzoor-Akbar, Ardlie, N. G.* (Reprint requests: Dr. N. G. Ardlie, Department of Clinical Science, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia), p. 59

Activation of plasminogen as a feature in its assay. *Gaffney, P. J., Brasher, M., Lord, K., Kirkwood, T. B. L.* (National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England), p. 72

Haemostasis (Basel) **6** (1977) No. 2

Determination of tissue thromboplastin activity. *Wijngaards, G., Hemker, H. C.* (Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands), p. 89

Amidolytic assay of thrombin bound to α_2-macroglobulin in plasma. *Musumeci, V., Landolfi, R., Bizzi, B.* (Istituto di Clinica Medica, Policlinico A. Gemelli, I-00168 Rome, Italy), p. 98

Alteration of rat bone marrow megakaryocytes following administration of cytosine arabinoside, daunomycin and hydroxyurea. *Jerushalmy, Z., Patya, M., Pinkhas, J., de Vries, A.* (Rogoff-Wellcome Medical

Research Institute, Beilinson Hospital, Petah-Tiqva, Israel), p. 110

Comparable inhibition of aggregation of PRP of neonates and adults by aspirin. *Chung-hsin Ts'ao* (Department of Pathology, Northwestern University McGay Medical Center, Chicago, Ill. 60611), p. 118

Ditazole and platelets. I. Effect of ditazole on human platelet function *in vitro*. *de Gaetano, G., Tonolli, M. C., Bertoni, M. P., Roncaglioni, M. C.* (Laboratory for Haemostasis and Thrombosis Research, Istituto di Richerche Farmacologiche "Mario Negri", I-20157 Milano, Italy), p. 127

Impaired fibrin formation in advanced cirrhosis. *Dettori, A. G., Ponari, O., Civardi, E., Megha, A., Pini, M., Poti, R.* (5a Divisione Medica e Centre per le Malattie Emostatiche, Ospedali Riuniti di Parma, I-43100 Parma, Italy), p. 137

Haemostasis (Basel) **6** (1977) No. 3

Qualitative changes in fibrinogen following exposure to agents used for preparation of fibrin monomers. *Brosstad, F., Godal, H. C.* (Haematological Research Laboratory, Department IX, Ullevål Hospital, University Clinic, Oslo, Norway), p. 149

Factor VIII deficient plasma for laboratory tests prepared from normal plasma and a human antibody. *Exner, T., Rickard, K. A., Speers, S.* (Haematology Department, Royal Prince Alfred Hospital, Sydney NSW 2050, Australia), p. 157

The effect of heparin on the interaction of factor VIII and human platelets *in vitro*. *de Marco, I., Fabris, F., Molaro, G., Girolami, A.* (University of Padua Medical School, Institute of "Semeiotica Medica", 35100 Padova, Italy), p. 163

Clotting factors and platelets. Immunofluorescence evidence that fibrinogen and factor VIII are present in human washed platelets, whereas prothrombin complex factors and factor XIII are lacking. *Better, C., Fabris, F., de Marco, L., del Prete, G. F., Girolami, A.* (Institute of "Semeiotica Medica", University of Padua Medical School, 35100 Padova, Italy), p. 171

Human blood platelet elastase and proelastase. Activation of proelastase and release of elastase after adhesion of platelets to collagen. *Legrand, Y., Pignaud, G., Caen, J. P.* (INSERM U-150, Thrombose Expérimentale et Hémostase, Hôpital Lariboisière, F-75010 Paris, France), p. 180

Ditazole and platelets. II. Effect of ditazole on *in vivo* platelet aggregation and bleeding time in rats. *de Gaetano, G., Cavenaghi, A. E., Stella, L.* (Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche "Mario Negri", I-20157 Milan, Italy), p. 190

Plasma and platelet phospholipids in normal subjects and in patients with chronic arterial obliterations. *Bruhn, H. D., Jipp, P., Ravens, K. G.* (I. Medizinische Universitätsklinik Kiel, 23 Kiel, BRD), p. 197

Blood platelet membranes symposium. *Caen, J., Hardisty, R. M.* (No address), p. 203

Haemostasis (Basel) **6** (1977) No. 4

Generation of coagulation factors V, XI, and XII, by the isolated rat liver. *Owen, C. A. Jr., Bowie, E. J. W.* (Mayo Clinic, Rochester, Minn. 55901), p. 205

Some characteristics of various fibrin monomer preparations made from dissolved fibrin clots. *Brosstad, F., Godal, H. C., Kierulf, P.* (Haematological Research Laboratory, Department IX, Ullevål Hospital, University Clinic, Oslo, Norway), p. 213

Preparation of fibrin monomers from human fibrinogen in urea, using soluble or insolubilized thrombin. *Brosstad, F., Godal, H. C., Kierulf, P.* (Haematological Research Laboratory, Department IX, Ullevål Hospital, University Clinic, Oslo, Norway). p. 225

Dissolution of noncovalently bonded fibrin in the presence of bromide ions. *Dyr, J. E., Vorlová, Z., Vodrážka, Z.* (Institute of Hematology and Blood Transfusion, 128 20 Prague, Czechoslovakia), p. 236

Effect of hypovolemic shock on the generalized Shwartzman reaction. *Howes, E. L. Jr., McKay, D. G.* (Department of Pathology, San Francisco General Hospital, San Francisco, Calif. 94110), p. 244

Low dose heparin in major surgery: Clinical relevance of plasma heparin concentra-

tions. *Kruse-Blinkenberg, H. O., Bech-Jansen, P., Jensen, J., Schmidt, A., Gormsen, J.* (Reprint requests: Dr. J. Gormsen, Kommunehospitalet Ø, DK-1399 Copenhagen K, Denmark), p. 252

Ditazole and platelets. III. Effect of ditazole on tumor-cell induced thrombocytopenia and on bleeding time in mice. *Mussoni, L., Poggi, A., Donati, M. B., de Gaetano, G.* (Reprint requests: Dr. G. de Gaetano, Laboratory for Haemostasis and Thrombosis Research, Istituto di Ricerche Farmacologiche Mario Negri, I-20157 Milan, Italy), p. 260

Influence of acetylsalicylic acid and paracetamol on menstrual blood loss. *Petruson, B., Hahn, L., Korsan-Bengtsen, K., Hallberg, L.* (ENT-Department, University of Göteborg, Sahlgren's Hospital, S-41345 Göteborg, Sweden), p. 266

Haemostasis (Basel) **6** (1977) No. 5

Partial purification of the protein moiety of porcine tissue thromboplastin. *Wijngaards, G., Hemker, H. C., van Deenen, L. L. M.* (Gaubius Institute, Health Research Organization, TNO, Leiden, The Netherlands), p. 269

Photometric assay of platelet factor 4 with a chromogenic substrate. *Vinazzer, H.* (Blood Coagulation Laboratory, A-4020 Linz, Austria), p. 283

Variations in blood coagulation, fibrinolysis, platelet function and various plasma proteins during the menstrual cycle. *Cederblad, G., Hahn, L., Korsan-Bengtsen, K., Pehrsson, N. G., Rybo, G.* (Reprint requests: Dr. K. Korsan-Bengtsen, Department of Medicine II, University of Göteborg, S-413 45 Göteborg, Sweden), p. 294

Inhibition of the fibrinolytic system in Behcet's disease? *Asbeck, F., Meyer-Boernecke, D., van de Loo, J.* (Medizinische Universitätsklinik, D-4400 Münster, BRD), p. 303

Ditazole activity and its interaction with urokinase on experimental thrombosis. *Caprino, L., Borrelli, F., Cafiero, C., Gandolfo, G. M.* (Department of Pharmacology, Research Laboratories, Istituto Farmacologico Serono, 00176 Rome, Italy), p. 310

A histochemical study of fibronolytic activity and inhibition of plasmin in the lungs of some animal species. *Smokovitis, A., Astrup, T.* (Reprint requests: Dr. T. Astrup, Gaubius Institute, Health Research Organization, TNO, Leiden, The Netherlands), p. 318

Haemostasis (Basel) **6** (1977) No. 6

The anticoagulant effect of heparin + thrombin III using purified fibrinogen substrate. *Andersen, P., Godal, H. C.* (Haematological Research Laboratory, Department IX, Ullevål Hospital, University Clinic, Oslo, Norway), p. 329

The antiheparin effect of α_1-acid glycoprotein and platelet material, evaluated by the heparin thrombin clotting time. *Andersen, P., Godal, H. C.* (Haematological Research Laboratory, Ullevål Hospital, University Clinic, Oslo, Norway), p. 339

Procoagulant activity of activated monocytes. *Hiller, E., Saal, J. G., Riethmüller, G.* (Medizinische Universitätsklinik, D-74 Tübingen, BRD), p. 347

Elimination of inhibition in euglobulin fibrinolysis by use of flufenamate: Involvement of Cl-inactivator. *Kluft, C.* (Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands), p. 351

Heparin-induced release of plasminogen activator. *Markwardt, F., Klöcking, H.-P.* (Institut für Pharmakologie und Toxikologie, Medizinische Akademie Erfurt, DDR-50 Erfurt), p. 370

Nouvelle Revue Française d'Hématologie, Blood Cells (Paris) **16** (1976) No. 3

Anomalie chromosomique nouvelle dans une leucémie myéloïde chronique. *Berger, R., Gyger, M., Bussel, A.* (Centre de Recherches de Biologie du Développement Foetal et Néo-natal, Hôpital Port-Royal, F-75014 Paris, France), p. 309

Fetal hemopoiesis in diffusion chamber cultures: II. Cell proliferation and differentiation. *Symann, M., Quesenberry, P.* (Université Catholique de Louvain, Unité de Recherches sur les Maladies du Sang, Bruxelles, Belgium), p. 321

Fréquence de la thalassémie dans le Sud-Est de la France. *Dujardin, P., Soummer, A. M., Cassuto, J. P., Clastrier, J., Chambon, P., Audoly, P.* (Hôpital de Cimiez, 06031 Nice Cedex, France), p. 329

Étude de l'immunisation foeto-maternelle au moyen du test LDA. *Lévy, S., Tongio, M.-M., Mayer, S.* (Centre de Transfusion Sanguine, 67085 Strasbourg, France), p. 337

Purification of rabbit anti-human fibrinogen antibodies. Cross reactivity of mammalian fibrinogen. *Burstein, M., Legmann, P.* (Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 345

Red cells adhering to a glass surface: Deformation in a well-defined fluid shear field. *Mohandas, N.* (Cancer Research Institute, University of California, San Francisco, Calif. 94143), p. 357

IIe Congrès Français d'Hématologie, Le Touquet, France, 24—27 Mai 1976, Symposium sur les Thrombopathies Constitutionnelles et Acquises, p. 363

Nouvelle Revue Française d'Hématologie, Blood Cells (Paris) **17** (1976) No. 1—2

Preleukemic states. *Bernard, J.* (Clinique des Maladies du Sang, Hôpital Saint-Louis, F-75010 Paris, France), p. 5

The preleukemic syndrome: Clinical and laboratory features, natural course, and management. *Linman, J. W., Bagby, G. C. Jr.* (Division of Hematology and Medical Oncology, University of Oregon Health Sciences Center, Portland, Ore. 97201), p. 11

Preleukemic states. I. Definition and classification. II. Refractory anemia with an excess of myeloblasts in the bone marrow (smoldering acute leukemia). *Dreyfus, B.* (Clinique Hématologique et Unité de Recherches sur les Anémies, I.N.S.E.R.M. U. 91, Hôpital Henri Mondor, F-94010 Créteil, France), p. 33

Preleukemic states in atomic bomb survivors in Japan. *Kamada, N., Uchino, H.* (Department of Internal Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan), p. 57

Acute myeloblastic leukemia after immunodepressive therapy for primary nonmalignant disease. *Tchernia, G., Mielot, F., Subtil, E., Parmentier, C.* (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 67

Preleukemia: Does it exist? *Killmann, S.-A.* (Department of Medicine A, Rigshospitalet, University Hospital of Copenhagen, DK-2100 Copenhagen, Denmark), p. 81

Prediction of relapse and remission in AML by marrow culture criteria. *Moore, M. A. S.* (Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021), p. 109

In vitro colony growth of acute myelogenous leukemia. *Dicke, K. A., Spitzer, G., Cork, A., Ahearn, M. J.* (Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025), p. 125

The use of the Robinson *in vitro* agar culture assay in adult acute leukemia. *Spitzer, G., Dicke, K. A., Gehan, E. A., Smith, T., McCredie, K. B.* (Department of Developmental Therapeutics, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025), p. 139

Significance of PHA induced clonogenic cells in chronic myeloid leukemia and early acute myeloid leukemia. *Spitzer, G., Schwarz, M. A., Dicke, K. A., Trujillo, J. M., McCredie, K. B.* (Departments of Developmental Therapeutics and Laboratory Medicine, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025), p. 149

An approach to human preleukemia using cell culture studies. *Senn, J. S., Price, G. B., Mak, T. W., McCulloch, E. A.* (Sunnybrook Medical Centre, Toronto, Ontario M4N 3M5, Canada), p. 161

Comparative electron-microscopic study of the erythrocyte line in refractory anemia (preleukemia) and myelomonocytic leukemia. *Maldonado, J. E., Maigne, J., Lecoq, D.* (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 167

Cytochemical and ultrastructural studies of aberrant granules in the neutrophils of two patients with myeloperoxidase deficiency during a preleukemic state. Relationship to abnormal bactericidal activity.

Breton-Gorius, J., Coquin, Y., Vilde, J. L., Dreyfus, B. (Unité de Recherches sur les Anémies. INSERM U. 91, Hôpital Henri Mondor, F-94010 Créteil, France), p. 187

Blood group changes in preleukemic states. *Salmon, Ch.* (Centre National de Transfusion Sanguine, F-75571 Paris Cedex 12, France), p. 211

The cellular distribution of erythrocyte and normoblast A_1 and A antigens in normal and preleukemic states. An immunoelectron microscopy study. *Gourdin, M. F., Reyes, F., Lejonc, J. L., Breton-Gorius, J., Mannoni, P., Dreyfus, B.* (Reprint requests: Dr. F. Reyes, I.N.S.E.R.M. U. 91, Hôpital Henri Mondor, F-94010 Créteil, France), p. 221

Abnormal distribution of erythrocytes A_1 antigens in preleukemia as demonstrated by an immunofluorescence technique. *Rochant, H., Tonthat, H., Henri, A., Titeux, M., Dreyfus, B.* (Unité de Recherches sur les Anémies, I.N.S.E.R.M. U. 91, Hôpital Henri Mondor, F-94010 Créteil, France), p. 237

Cell membrane antigens associated with human adult acute leukemia. *Hollinshead, A. C.* (Division of Hematology and Oncology, Department of Medicine, George Washington University, Medical Center, Washington, D. C. 20037), p. 257

Notions about human stem cells. *Cronkite, P., Feinendegen, L. E.* (Medical Research Center, Brookhaven National Laboratory, Upton, N. Y. 11973), p. 269

Preleukemic states in the light of the leukemia cytokinetics. *Baserga, A.* (Clinica Medica Universitaria, 203 I-44100 Ferrara, Italy), p. 285

The role of lymphoid and haematopoietic target cells in viral lymphomagenesis of C57BL/Ka mice. I. Susceptibility to viral replication. *Lieberman, M., Kaplan, H. S.* (Cancer Biology Research Laboratory, Department of Radiology, Stanford University School of Medicine, Stanford, Calif. 94305), p. 291

The role of lymphoid and haematopoietic target cells in vital lymphomagenesis of C57BL/Ka mice. II. Neoplastic transformation of bone marrow-derived cells in the thymic microenvironment. *Kaplan, H. S., Liebermann, M.* (Cancer Biology Research Laboratory, Department of Radiology, Stanford University School of Medicine, Stanford, Calif. 94305), p. 301

Oncogenesis in human acute leukemia. *Barr, R. D., Perry, S.* (National Cancer Institute, Bethesda, Md. 20014), p. 319

Problems relating to immunoselection of leukemias. *Pedrinis, E., Zimmermann, A., Bertschmann, M., Hess, M. W., Cottier, H.* (Institute of Pathology, University of Bern, CH-3000 Bern, Switzerland), p. 329

Nouvelle Revue Française d'Hématologie, Blood Cells (Paris) **17** (1976) No. 3

A catalogue of white blood cell movements (normal and pathologic). *Bessis, M., Boisfleury, A. de* (Institut de Pathologie Cellulaire, INSERM Unité 48, Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 365

Influence of surface on white cell movements. *Lajtha, L. G.* (Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, England), p. 411

Motility of acute human leukemia cells: A study by time-lapse cinematography and scanning electron microscopy. *Haemmerli, G., Felix, H.* (Division of Cancer Research, Institute of Pathology, University of Zürich, CH-8050 Zürich, Switzerland), p. 145

Locomotion, necrotaxis and phagocytosis in normal and leukemic myeloblasts. *Tchernia, G., Grange, M. J., Gombaud-Saintonge, G.* (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, F-94270 Kremlin-Bicêtre, France), p. 431

Mobilization of leukemic cells from tissue stores by synthetic polyanions: Experimental and clinical data. *Hagenbeek, A., Brommer, E. J. P., Martens, A. C. M., van Bekkum, D. W.* (Radiobiological Institute TNO, Rijswijk, The Netherlands), p. 437

Cell movement as related to oncogenesis. *Barski, G., Leon, B., le François, D., Belehradek, J. Jr.* (Laboratoire de Culture de Tissus et de Virologie, Institut Gustave-Roussy, F-94800 Villejuif, France), p. 453

Remarks on locomotion of normal and neoplastic white blood cells in the organism. *Sträuli, P.* (Division of Cancer Research,

Institute of Pathology, University of Zürich, CH-8050 Zürich, Switzerland), p. 467

On the nomenclature of white cell movements. *Brecher, G.* (University of California, Department of Laboratory Medicine, School of Medicine, San Francisco, Calif. 94143), p. 473

Granulocyte rheology. *Bagge, U.* (Laboratory for Experimental Biology, Department of Anatomy, University of Göteborg, S-400 33 Göteborg 33, Sweden), p. 481

The filterability of normal and leukemic human leukocytes. *Lichtman, M. A., Kearney, E. A.* (Department of Medicine, University of Rochester School of Medicine, Rochester, N. Y.), p. 491

The shape and surface morphology of human leukocytes *in vitro:* Effect of temperature, metabolic inhibitors and agents that influence membrane structure. *Lichtman, M. A., Santillo, P. A., Kearney, E. A., Roberts, G. W., Weed, R. I.* (Department of Medicine, University of Rochester School of Medicine, Rochester, N. Y.), p. 507

Alteration in the chloroform quenching of red cell membrane native protein fluorescence following incubation with malonaldehyde and other crosslinking agents. *Goldstein, B. D., Falk, G. W., Benjamin, L. J., McDonagh, E. M.* (Departments of Medicine and Environmental Medicine, New York University, Medical Center, New York, N. Y. 10016), p. 535

Primary and secondary granule contents and bactericidal capability of neutrophils in acute leukaemia. *Odeberg, H., Olofsson, T., Olsson, I.* (Reprint requests: Dr. I. Olsson, Research Department 2, University Hospital, S-221 85 Lund, Sweden), p. 543

Improving manual differential white cell counts with cytochemistry. *Ornstein, L., Ansley, H., Saunders, A.* (Cell Research Laboratory, Mount Sinai School of Medicine, New York, N. Y. 10029), p. 557

Surface morphology and ultrastructure of murine granulocytes and monocytes in long-term liquid culture. *Allen, T. D., Dexter, T. M.* (Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M2O 9BX, England), p. 591

Nouvelle Revue Française d'Hématologie, Blood Cells (Paris) **18** (1977) No. 1

Theodor Boveri et la théorie chromosomique de la cancérogénèse. *de Grouchy, J.* (C. N. R. S., Hôpital des Enfants-Malades, 75730 Paris Cedex 15, France), p. 1

Aspects du métabolisme du fer dans la malnutrition protéo-énergétique de l'enfant. *Fondu, P., Bouton, J. M., de Maertelaere-Laurent, E., Mandelbaum, I. M.* (Hôpital Saint-Pierre, B 1000 Bruxelles, Belgium), p. 5

Localisations cérébro-méningées des leucémies aiguës myéloblastiques de l'adulte. Etude clinique et anatomo-pathologique de 15 cas. *Renoux, M., Dhermy, D., Bernard, J.-F., Brousse, N., Hénin, D., Amar, M., Boivin, P.* (Service d'Hématologie Clinique, Hôpital Beaujon, 92110 Clichy, France), p. 23

Un nouveau cas d'hémoglobine J Capetown α92 (FG4) Arg $\rightarrow$ Gln. *Gacon, G., Amegnizin, K. E., Belkhodja, O., Denis, P., Krishnamoorthy, R., Labie, D., Lefrançois, R., Michel, Y., Pasquis, P., Wajcman, H.* (Laboratoire d'Hémobiologie, Hôpital Broussais, 75014 Paris, France), p. 35

Identification immunologique et cytochimique combinée des cellules nucléées du sang périphérique humain normal. *Alarion, A., Claudy, A. L., Faure, M., Thivolet, J.* (Laboratoire d'Immunopathologie, Clinique Dermatologique, Hôpital Edouard-Herriot, 69374 Lyon Cedex 2, France), p. 45

Action des glutaryl-lécithines sur les globules rouges humains. *Dousset, N., Quan, D. Q., Douste-Blazy, L.* (Unité de Recherches I. N. S. E. R. M., C. H. R. Purpuran, F-31052 Toulouse Cedex, France), p. 53

Culture des cellules souches granulocytaires au cours du neuroblastome de l'enfant. *Tchernia, G., Parmentier, C., Morel-Brossel, S., Morardet, N., Schweisguth, O.* (Institut Gustave-Roussy, 94800 Villejuif, France), p. 63

IIe Congrès Français d'Hématologie, 24—27 Mai 1976, p. 75

Nouvelle Revue Francaise d'Hématologie, Blood Cells (Paris) **18** (1977) No. 2

Genes and geography. *Waldenström, J. G.* (Karolinska Institutet, 10401 Stockholm 60, Sweden), p. 275

Pluripotential and committed hemopoietic stem cells. A hypothesis. *Brecher, G.* (Department of Laboratory Medicine, University of California, San Francisco), p. 285

Traitement des leucémies aiguës. *Jacquillat, Ct., Weil, M., Auclerc, M. F., Schaison, G., Holland, J. F.* (Institut de Recherche sur les Leucémies et les Maladies du Sang, Centre G.-Hayem, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 293

Excès d'HLA-A2 et d'homozygotes HLA-A2 parmi les malades atteints d'aplasie médullaire et maladie de Fanconi. *Dausset, J., Gluckman, E., Lemarchand, F., Nunez-Roldan, A., Contu, L., Hors, J.* (Unité INSERM U-93, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 315

Hématologie, génétique et ethnologie. *Ruffié, J.* (Centre d'Hémotypologie, C. H. U. de Purpan, 31300 Toulouse, France), p. 325

Geographical distribution of HBs Ag in Africa. *Soulier, J.-P., Couroucé-Pauty, A.-M.* (C. N. T. S., 75015 Paris, France), p. 331

L'hétérogénéité des leucémies lymphoïdes humaines révélée par l'étude immunologique des marqueurs de membrane. *Seligmann, M., Brouet, J.-C., Preud'homme, J.-L.* (Laboratoire d'Immunochimie et d'Immunopathologie, Institut de Recherches sur les Leucémies et les Maladies du Sang, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 339

La lymphographie dans la leucémie lymphoïde chronique. *Binet, J. L., Nizet, M. G., Dighiero, G., Grellet, J.* (Départements d'Hématologie et de Radiologie, C. H. U. Pitié-Salpêtrière, F-75013 Paris, France), p. 351

La dystrophie thrombocytaire hémorragipare congénitale. Syndrome de Bernard-Soulier. De la description clinique (1948) à l'ère moléculaire (1977). *Caen, J.-P., Tobelem, G., Lévy-Tolédano, S., Nurden, A.* (Laboratoire d'Hémostase et de Thrombose Expérimentale, Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, 75474 Paris Cedex 10, France), p. 365

Syndrome de Willebrand. A propos de 48 observations. *Larrieu, M. J., Meyer, D., Ardaillou, N.* (Institut de Pathologie Cellulaire, Hôpital de Bicêtre, F-94270 Le Kremlin-Bicêtre, France), p. 371

Étude de la multiplication des virus murins de type C dans des lignes cellulaires derivées du tératocarcinome de la souris. *Periès, J., Debons-Guillemin, M. C., Canivet, M., Emanoil-Ravicovitch, R., Tavitian, A., Boiron, M.* (Institut de Recherches sur les Leucémies, Centre Hayem, Hôpital Saint-Louis, 75475 Paris Cedex 10, France), p. 383

L'enseignement assisté par ordinateur en hématologie. *Varet, B., Lévy, J.-P.* (Département d'Hématologie, C. H. U. Cochin, Université Paris V, Paris, France), p. 391

Éléments du pronostic des dysplasies hématopoïétiques. *Dresch, C., Faille, A., Glogowski, A., Najean, Y.* (Centre Recherches sur les Maladies du Sang, Hôpital Saint Louis, F-75475 Paris Cedex 10, France), p. 401

La recherche scientifiqie, le rêve et la poésie. *Bessis, M.* No address, p. 415

Hommages à Jean Bernard

Courte note. Qui n'a rien d'hématologique sur Jean Bernard. *Hamburger, J.* No address, p. 425

Jean Bernard écrivain. *Wolff, E.* No address, p. 429

Le poète Jean Bernard. *Buchet, E.* No address p. 439

Jean Bernard, médecin et philosophe de sciences. *Escoffier-Lambiotte, C.* No address, p. 441

Un page ignorée, mais exemplaire, de la vie de Jean Bernard. *Milliez, P.* No address, p. 445

Jean Bernard et l'hématologie Argentine. *Pavlovsky, A.* No address, p. 451

Symposium sur la maladie de Hodgkin. Le Touquet, France, 24—27 Mai 1976, p. 457

Revue Française de Transfusion et Immuno-Hématologie (Paris) **19** (1976) No. 3

Recherche comparative des substances de groupes A, B, H, dans les stromas délipidés et non délipidés. Etudes de la membrane érythrocytaire. *Le Bec, A., Waitz, R.* (Centre de Transfusion Sanguine de Strasbourg, 67085 Strasbourg, France), p. 399

L'antigène Cad dans la population française. *Gerbal, A., Lopez, M., Chassaigne, M., Genetet, B., Selva, J., Yvart, J., Salmon, Ch.* (Reprint requests: Dr. M. Lopez, Service d'Immunologie, Centre National de Transfusion Sanguine, 75012 Paris, France), p. 415

Groupage et détection d'anticorps dans le système PL^A (plaquettaire). Application à l'étude des thrombopénies néonatales par allo-immunisation foeto-maternelle PL^{A1}. *Soulier, J. P., Patereau, C.* (Centre National de Transfusion Sanguine, 75015 Paris, France), p. 431

Fréquence des hépatites après circulation extra-corporelle. *Aufeuvre, J. P., Lexuan, T. C., Simonneau, M., Terrier, E., Jaulmes, B.* (Centre de Transfusion Sanguine, Hôpital Broussais, 75014 Paris, France), p. 449

Etude comparative des techniques de dépistage des porteurs d'antigène HBs. *Simonneau, M., Ayed, K., Terrier, E., Jaulmes, B.* (Centre de Transfusion Sanguine, Hôpital Broussais, 75674 Paris Cedex 14, France), p. 461

Le polymorphisme du facteur B de la properdine (C3PA, GBG, Bf) et la liaison histocompatibilité-complément. *Hauptmann, G., Tongio, M. M., Mayer, S.* (Centre de Transfusion Sanguine, 67085 Strasbourg Cedex, France), p. 471

A propos d'une exsanguino-transfusion. *Chassaigne, M., Saint-Paul, B.* (Centre Départemental de Transfusion Sanguine, 78000 Versailles, France), p. 487

Transfusion de plaquettes. *Mannoni, P., Rodet, M., Beaujean, F.* (Centre Départemental de Transfusion, Hôpital Henri-Mondor, 94010 Créteil, France), p. 489

Revue Française de Transfusion et Immuno-Hématologie (Paris) **19** (1976) No. 4

Hemoglobin function in stored blood. XVII. Maintenance of red cell 2,3 DPG (function) and ATP (viability) for six weeks in ACD or CPD-adenine-inosine-methylene blue. *Dawson, R. B.* (University of Maryland Hospital, Baltimore, Md. 21201), p. 527

Modifications de la membrane érythrocytaire au cours de la conservation du sang. Influence de la progestérone. *Ciavatti, M., Jouvenceaux, A., Chataing, B., Micel, G.* (Laboratoire d'Immuno-Hématologie, Centre de Transfusion Sanguine, Hôpital Edouard-Herriot, 69374 Lyon, France), p. 539

Purification d'une fraction de beta lipoprotéines riche en Lp (a). *Dubarry, M., Moullec, J.* (Reprint requests: Dr. J. Moullec, Centre National de Transfusion Sanguine, 75739 Paris Cedex 15, France), p. 555

Nouvelles données sur le role de l'immigration dans la distribution géographique de l'antigène HBs. *Cazal, P., Lemaire, J. M., Robinet-Levy, M.* (Centre de Transfusion Sanguine de Montpellier, 3400 Montpellier, France), p. 563

Nouvelle enquête sur la diffusion familiale de l'antigène HBs. *Cazal, P., Lemaire, J. M., Robinet-Levy, M.* (Centre de Transfusion Sanguine de Montpellier, 3400 Montpellier, France), p. 569

Hépatite B et rapport de masculinité. *Cazal, P., Lemaire, J.-M., Robinet-Levy, M.* (Centre de Transfusion Sanguine de Montpellier, 3400 Montpellier, France), p. 577

Age et sexe des porteurs chroniques de l'antigène HBs parmi 130.000 donneurs de sang. *Cazal, P., Lemaire, J.-M., Robinet-Levy, M.* (Centre de Transfusion Sanguine Montpellier, 3400 Montpellier, France), p. 583

Dosage du di (ethyl-2 hexyl) phtalate (D.E.H.P.) dans les poches de matières plastiques et les flacons de verre destinés à la conservation du sang. *Friocourt, M. P., Picart, D., Saleun, J. P., Bardou, L. G., Floch, H. H.* (Centre de Transfusion Sanguine de Brest, 29200 Brest, France), p. 589

Techniques utilisées par les centres de transfusion français pour le dépistage de l'antigène Australie (HBs). *Saint-Paul, B., Chassaigne, M.* (Centre Départemental de Transfusion Sanguine, 78000 Versailles, France), p. 597

Présomption de paludisme transfusionnel. *Andrianjafy, M., Thuillier, M., Gandrille, M. C.* (Reprint requests: Dr. M. C. Gandrille, Poste de Transfusion, Hôpital Boucicaut, 75015 Paris, France), p. 603

La prévention de l'immunisation foeto-maternelle rhésus par les immunoglobulines anti-D. *Huchet, J., Pinon, F.* (Centre d'Hémobiologie Périnatale, 75012 Paris, France), p. 605

Revue Française de Transfusion et Immuno-Hématologie (Paris) **20** (1977) No. 1

Le Professeur Robert André, 1904—1976. *Soulier, J.-P., Salmon, Ch.* (Centre National de Transfusion Sanguine, 75739 Paris 15, France), p. 9

Le complex HLA considéré comme une unité immunologique. *Dausset, J.* (INSERM, U. 93, Hôpital Saint-Louis, Paris, France), p. 15

La liaison génétique HLA-complément. *Hauptmann, G., Tongio, M. M., Mayer, S.* (Centre de Transfusion, F-67085 Strasbourg Cedex, France), p. 19

Microtechnique de traitement des lymphocytes par la neuraminidase. Intérêt pour la recherche des anticorps lymphocytotoxiques. *de Mouzon, A., Ohayon, E., Ducos, J.* (Centre de Transfusion, C.H.U. Purpan, F-31052 Toulouse Cedex, France), p. 23

HLA and disease. *Svejgaard, A.* (Blood Grouping Department, State University Hospital, Copenhagen, Denmark), p. 27

L'antigène HL-A B 27 dans la maladie de Crohn associée à la spondylarthrite ankylosante et dans une population de sujets normaux. *de Bruyère, M., Nagant de Deuxchaisnes, Ch., Fiasse, R., Huaux, J. P., de Vogelaer, J.-P.* (Services d'Hématologie, de Rheumatologie et de Gastro-entérologie, Cliniques Universitaires Saint-Pierre, Louvain, Belgique), p. 31

Etude des antigènes d'histocompatibilité dans une fratrie comportant deux cas de spondylarthrite ankylosante. *Muller, J. Y., Prou, O., D'Ambrosio, A. M., Halle, L.* (Centre National de Transfusion Sanguine, 75015 Paris, France), p. 35

HLA et maladie de Behcet. *Godeau, P., Torre, D., Campinchi, R., Bloch-Michel, E., Schmid, M., Nunez-Roldan, A., Hors, J., Dausset, J.* (Hôpital de la Pitié, 75013 Paris, France), p. 43

Les antigènes d'histocompatibilité dans l'hémochromatose idiopathique. *Fauchet, R., Simon, M., Genetet, B., Bourel, M.* (Centre Régional de Transfusion, 35000 Rennes, France), p. 47

Répartition et transmission des antigènes HLA dans la maladie de Willebrand HLA. *Goudemand, J., Mazurier, G., Parquet, A.* (Centre Régional de Transfusion Sanguine, Lille, France), p. 48

Rôle du système HLA dans le transfusion de plaquettes et de leucocytes. *Mannoni, P., Rodet, M., Braco, Ch.* (Centre Départemental de Transfusion de Val-de-Marne, Hôpital Henri-Mondor, 94010 Créteil, France), p. 49

Problèmes généraux posés par la greffe de moelle osseuse. *Jeannet, M.* (Laboratoire d'Immunologie et Transplantation, CH-1211 Genève 4, Suisse), p. 57

Greffe de moelle osseuse dans les aplasies médullaires. *Gluckman, E., Bussel, A., Bernard, J., Dausset, J.* (Service d'Hématologie-Immunologie, Hôpital Saint-Louis, 75010 Paris, France), p. 61

Greffe de moelle dans les déficits immunitaires. *Griscelli, S., Durandy, A., Herzog, C., Mozziconacci, P.* (Clinique Médicale des Enfants, Hôpital Necker-Enfants Malades, 75730 Paris Cedex 15, France), p. 65

Greffe de moelle dans les aplasies et dans les leucémies. La problème de la réaction du greffon contre l'hôte et de la maladie secondaire. *Schwarzenberg, L.* (Institut de Cancérologie, Hôpital Paul-Brousse, 94800 Villejuif, France), p. 73

Greffe de moelle osseuse. Résultats à Bâle. *Speck, B., Cornu, P., Groff, P., Sartorius, J., Nissen, C., Jeannet, M.* (Service d'Hématologie, Hôpital Universitaire, Bâle, Suisse), p. 73

Séance sur l'alpha 1 antitrypsine, p. 83

Séance sur les controles immunologiques systématiques chez les donneurs de sang: détection d'anticorps acquis, p. 109

Séance sur les progrès récents dans les groupes érythrocytaires, p. 165

Séance sur la surveillance médicale des donneurs, p. 225

Revue Française de Transfusion et Immuno-Hématologie (Paris) **20** (1977) No. 2

Syphilis et transfusion sanguine. *Garretta, M., Paris-Hamelin, A., Muller, A., Vaisman, A.* (Centre National de Transfusion Sanguine, 91400 Orsay-Courtaboeuf, France), p. 287

Dépistage systématique des gammapathies monoclonales dans une population de 3.758 sujets de race noire (Sénégal). *Fine, J. M., Fine, A., Lambin, P.* (Laboratoire d'Immunochimie, Centre National de Transfusion Sanguine, 75015 Paris, France), p. 309

Dépistage et titrage des anticorps antitétaniques par une méthode d'hémagglutination passive automatisée. *Duvillers-Montreuil, M., Goudemand, M.* (C. R. T. S. de Lille, 59000 Lille, France), p. 315

Etudes sur l'adhésion des polynucléaires aux fibres de nylon au cours des leucophérèses par filtration. *Malinvaud, G., Gaillard, S., Gualde, N.* (Laboratoire d'Hématologie-immunologie Maladie du Sang, C. H. U. Dupuytren, 87100 Limoges, France), p. 327

Fréquences des phénotypes de la s. G. P. T. et de la fraction C'_3 du complément dans la population parisienne. *Seger, J., Salmon, D.* (C. N. T. S. Etabl. Saint-Antoine, 75571 Paris Cedex 12, France), p. 331

Poches de transfusion sanguine. *Friocourt, M. P., Picart, D., Saleun, J. P., Bardou, L., Floch, H. H.* (Centre de Transfusion, Sanguine, Faculté de Médecine et Laboratoire de Biochimie, 29279 Brest Cedex, France), p. 337

Les pseudo anti-N des hémodialysés chroniques. *Bizot, M., Coves, F., Rubio, F.* (Centre de Transfusion de Montpellier, B. P. 1213, 3400 Montpellier, France), p. 345

Une immunisation anti-D après transfusion de concentrés leuco-plaquettaires. *Catoni-Charpentier, M. P.* (Centre de Transfusion de Secteur Broussaus, Cochin, Saint-Vincent-de-Paul, Paris 14, France), p. 349

Glycoprotéines membranaires: Les glycoproteines des érythrocytes. *Picard, J.* (Laboratoire de Biochimie Médicale, C. H. U. Saint-Antoine, 75571, France), p. 351

Un nouvel exemple d'anti-Do^b. *Yvart, J., Cartron, J., Fouillade, M. T., Gerbal, A., Murat, J., Salmon, Ch.* (Banque de Sang, Hôpital Saint-Joseph, 75014 Paris, France), p. 395

Revue Française de Transfusion et Immuno-Hématologie (Paris) **20** (1977) No. 3

Pour une réhabilitation scientifique de la presse médicale française. *Leger, L.* (No address), p. 415

Propriétés et indications d'une nouvelle préparation d'immunoglobulines (IgGAM) enrichie en IgA et IgM. *Blatrix, C., Thebault, J., Steinbuch, M.* (Hôpital Intercommunal de Créteil, 9400 Créteil, France), p. 419

Antigène Fy^x: Etude quantitative chez les sujets $Fy^bFy^x_y$, Fy^aFy^x et Fy^xFy^a provenant de deux nouvelles familles. *Habibi, B., Fouillade, M. T., Levanra, I., Beige, D., Federspiel, M., Salmon, C.* (Centre National de Transfusion Sanguine, 75571 Paris Cedex 12, France), p. 427

Effets cliniques de la transfusion des leucocytes prélevés par filtration en flux continu. *Malinvaud, G., Gaillard, S., Gualde, N.* (C. H. Dupuytren, Laboratoire d'Hématologie-Immunologie, 87000 Limoges, France), p. 439

Trois sujets A_{end} Cad dans une famille française. *Lopez, M., Gerbal, A., Girard-Debord, M., Salmon, C.* (Centre National de Transfusion Sanguine, 75012 Paris, France), p. 457

Dépistage et titrage des anticorps antitétaniques par hémagglutination passive. *Benamon, D., Garretta, M., Muller, A.* (Centre National de Transfusion Sanguine, 75015 Paris, France), p. 467

Technique de désagglutination différentielle spécifique du système ABO. *Garretta, M., Muller, A., Jacquet, D.* (Centre National de Transfusion Sanguine, 91300 Orsay, France), p. 473

Préparation du sang déleucocyté par une méthode de sédimentation-filtration.

Ehrsam, A., Coval, G., Béteul, H. (Centre de Transfusion Sanguine, 01700 Miribel, France), p. 483

Techniques d'étude d'un dialysat leucocytaire total et de ses fractions. *Allary, M., Bonnin, A., Benyamin, Y., Cornille, A., Dufer, J., Fabre, G., Gamet, J., Hainaut, J., Lesourd, B., Saint-Blancard, J., Sarrouy, J., Wanono, G.* (Centre de Transfusion Sanguine des Armées, 92140 Clamart, France), p. 487

Hyperbilirubinémie chez le nouveau-né par transfusion de sang de longue conservation. *Crégut, R.* (Centre d'Hémobiologie Périnatale, 75012 Paris, France), p. 493

Les compléments en immuno-hématologie. *Habibi, B.* (Centre National de Transfusion Sanguine, 75012 Paris, France), p. 947

Letters to the editor, p. 525

Revue Française de Transfusion et Immuno-Hématologie (Paris) **20** (1977) No. 4

Répercussions de la hausse vertigineuse du coût des soins médicaux sur les Services de Transfusion Sanguine. *Hassig, A.* (Laboratoire Central du Service de Transfusion Sanguine de la Croix-Rouge Suisse, CH-3000 Berne 22, Suisse), p. 559

Etude quantitative et thermodynamique de l'antigène B dans une famille comportant des sujets de phénotype B_3 et AB_3. *Chassaigne, M., Lopez, M., Krawczynska, S., Saint-Paul, B.* (Centre Départemental de Transfusion Sanguine et d'Hématologie de Versailles, I. N. S. E. R. M., U 76, 78000 Versailles, France), p. 565

Fréquences dans la population parisienne des gènes correspondant au polymorphisme de l'adénosine déaminase, de la 6-phosphogluconate déshydrogénase et de l'estérase D. *Seger, J.* (Centre National de Transfusion Sanguine, Hôpital Saint-Antoine, 75012 Paris, France), p. 575

Etude *in vitro* et *in vivo* d'une solution d'hémoglobine utilisée comme substitut du sang. *Teisseire, B., Loisance, D., Soulard, C., Hérigault, R.* (Hôpital Henri Mondor, 94010 Créteil, France), p. 585

Titrages et dépistages rapides simultanés des anticorps anti-tétaniques et anti-HBs par la technique de Laurell. *Mesnier, F., Piquet, Y., Moulinier, J.* (Service d'Immunochimie, C. T. S. de Bordeaux, B. P. 24, 33035 Bordeaux Cedex, France), p. 593

Méthode de cryoconservation des plaquettes à 196 °C. Application cliniques. *Hervé, P., Masse, M., Potron, G., Kieffer, Y., Carbillet, J. P., Bidet, A., Bosset, J. F., Peters, A.* (Centre Régional de Transfusion Sanguine, B. P. 1181, 25003 Besançon Cedex, France), p. 603

Symposium sur les groupes sanguins. *Rouger, Ph.* (Centre National de Transfusion Sanguine, 75571 Paris Cedex 12, France), p. 617

Maladie hémolytique du nouveau-né par anticorps anti-Miltenberger. *Alcalay, D., Tanzer, J.* (Centre Départemental de Transfusion Sanguine, 86201 Poitiers Cedex, France), p. 623

Les allohémagglutinines du système ABO. *Rouger, Ph., Salmon, Ch.* (Centre National de Transfusion Sanguine, Service d'Immunologie, 75571 Paris Cedex 12, France), p. 627

Lettres à la rédaction, p. 659

Scandinavian Journal of Haematology (Copenhagen) **17** (1976) No. 4

Abnormal lymphocyte populations in pure red cell aplasia. *Frøland, S. S., Wisløff, F., Stavem, P.* (Institute of Immunology and Rheumatology, Oslo 1, Norway), p. 241

Autopsy findings in leukaemia. *Chi, C. H., Lagerlöf, B., Lantz, B., Reizenstein, P.* (Reprint requests: Dr. B. Lagerlöf, Department of Pathology, Karolinska Hospital, Stockholm, Sweden), p. 251

Heparin cofactor activity and antithrombin III concentration in plasma related to age and sex. *Ødegård, O. R., Fagerhol, M. K., Lie, M.* (Medisinsk avd. A, Aker Sykehus, Oslo 5, Norway), p. 258

Infusion of human and canine factor VIII in dogs with von Willebrand's disease: Studies of the von Willebrand and factor VIII synthesis stimulating factors. *Bouma, B. N., Dodds, W. J., van Mourik, J. A., Sixma, J. J., Webster, W. P.* (Department of Haematology, University Hospital, Utrecht, The Netherlands), p. 263

Adenylate-rich sequences of heterogeneous nuclear RNA from normal and chronic

lymphocytic leukaemia lymphocytes. *Månsson, P. E., Deutsch, A., Brandt, L.* (Wallenberlab, Dach 7031, S-220 07 Lund 7, Sweden), p. 276

Platelet production capacity at intervals after acute thrombocytopenia. *Jackson, C. W., Edwards, C. C.* (St. Jude Children's Research Hospital, Memphis, Tenn. 38101), p. 285

Erythropoiesis inhibiting factor[s] (EIF). The specificity and toxicity of urinary EIF studied *in vivo* and *in vitro*. *Lindemann, R., Laerum, O. D.* (Department of Pediatrics, Rikshospitalet, Oslo 1, Norway), p. 293

Lymphocyte purification from normal human peripheral blood by filtration through columns of cotton wool. *Barr, R. D., Perry, S.* (Haematology Unit, Department of Pathology, University of Aberdeen, Aberdeen, AB9 2ZD, Scotland), p. 300

Protection of rats by phospholipase C from *Bacillus cereus* against the effects of intravenous infusions of purified tissue thromboplastin. *Giercksky, K.-E., Bjørklid, E.* (Institute of Medical Biology, University of Tromsø, 9001 Tromsø, Norway), p. 305

Effects of anticonvulsant drugs on the synthesis of DNA and protein by human bone marrow cells *in vitro*. *Wickramasinghe, S. N., Saunders, J., Williams, G.* (Department of Haematology, St. Mary's Hospital Medical School, London W2, England), p. 312

Scandinavian Journal of Haematology (Copenhagen) **17** (1976) No. 5

Sclerosing reticulum cell sarcoma following prolonged treatment with azathioprine for idiopathic thrombocytopenic purpura. *Nord, E., Douer, D., Kessler, E., Pinkhas, J., de Vries, A.* (Reprint requests: Prof. J. Pinkhas, Department of Internal Medicine D, Beilinson Medical Center, Petah Tiqva, Israel), p. 321

Aplastic anaemia with "hot pockets". *Kansu, E., Erslev, A. J.* (Reprint requests: Dr. A. J. Erslev, Cardeza Foundation for Hematologic Research, Thomas Jefferson University, Philadelphia, Pa. 19107), p. 326

Response of uraemic bone marrow cells to erythropoietin *in vitro*. *Urabe, A., Chiba, S., Kosaka, K., Takaku, F.* (Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113, Japan), p. 335

Membrane bound IgG on erythroblasts in pure red cell aplasia following thymectomy. *Björkholm, M., Holm, G., Mellstedt, H., Carlberger, G., Nisell, J.* (Department of Medicine, Seraphimer Hospital, S-112 83 Stockholm, Sweden), p. 341

Deaths from severe megaloblastic anaemia in hospitalised patients. *Lawson, D. H., Parker, J. L. W.* (Medical Division, Royal Infirmary, Glasgow, G4 OSF, Scotland), p. 347

The morphology of dyserythropoiesis in a patient with acute erythroleukaemia associated with multiple myeloma. *Zwaan, F. E., den Ottolander, G. J., Brederoo, P., van Zwet, Th. L., Te Velde, J., Willemze, R.* (Isolation Pavilion, University Hospital Leiden, The Netherlands), p. 353

Interaction between normal and leukaemia human cells in agar culture. *Knudtzon, S., Mortensen, B. T.* (Department of Internal Medicine, Finsen Institute, DK-2100 Copenhagen, Denmark), p. 369

The effect of syngeneic peripheral blood cells on the formation of colonies by normal human bone marrow cells in diffusion chambers in mice. *Elmgreen, J., Jacobsen, N., Knudtzon, S.* (Reprint requests: Dr. N. Jacobsen, Laboratories of Experimental Hematology, Memorial Sloan-Kettering Cancer Center, New York, 10021, N. Y.), p. 379

Confirmation of the existence of human serum leukaemia-associated antigens (LAA). *Berg, K., Stavem, P., Harris, R., Noer, G., Molne, K.* (Institute of Medical Genetics, University of Oslo, Oslo 3, Norway), p. 388

Pernicious anaemia and lymphoproliferative disease. *Parker, A. C., Bennett, M.* (Department of Haematology, Royal Infirmary, Edinburgh EH3 9YW, Scotland, United Kingdom), p. 395

Scandinavian Journal of Haematology (Copenhagen) **18** (1977) No. 1

Assays for thrombopoietin. *McDonald, T. P.* (University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 5

Leukaemia-associated antigen (LAA) in proliferative blood disorders. *Stavem, P., Berg, K., Noer, G.* (Reprint requests: Dr. K. Berg, Institute of Medicel Genetics, University of Oslo, Blindern, Oslo 3, Norway), p. 13

Syndrome of Shwachman and leukaemia. *Huijgens, P. C., van der Veen, E. A., Meijer, S., Muntinghe, O. G.* (Department of Internal Medicine, Free University Hospital, Amsterdam, The Netherlands), p. 20

The peripheral blood in chronic granulocytic leukaemia. Study of 50 untreated Philadelphia-positive cases. *Spiers, A. S. D., Bain, B. J., Turner, J. E.* (Division of Medical Oncology, Boston University Medical Center, Boston, Mass. 02118), p. 25

Garcia's disease. Cyclic thrombocytopenic purpura in a child and abnormal platelet count in his family. *Aranda, E., Dorantes, S.* (Reprint requests: Dr. S. Dorantes, Laboratorio de Investigación en Hematologia, Hospital Infantil de México, México 7 D.F., México), p. 39

Fatty acid composition of adipose cells in red and yellow marrow: A possible determinant of haematopoietic potential. *Tavassoli, M., Houchin, D. N., Jacobs, P.* (L. C. Jacobson Blood Center, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 47

A modified technique for combination of cytochemistry and radioautography of haemic cells. *Benestad, H. B.* (Institute of Physiology, University of Oslo, Oslo 1, Norway), p. 54

Increased potassium permeability induced *in vitro* by menadione in normal human red cells. *Vettore, L., de Matteis, M. C., Falezza, G. C.* (Istituto Patologia Medica 2° dell'Università di Padova, Policlinico di Borgo Roma, 37100 Verona, Italy), p. 61

Light green crystals in May-Grünwald and Giemsa-stained bone marrow macrophages in patients with myeloid leukaemia. *Stavem, P., Ly, B., Bjørneklett, A.* (Section of Haematology, Medical Department A, Rikshospitalet, Oslo, Norway), p. 67

Serum myeloperoxidase and lactoferrin in neutropenia. *Olofsson, T., Olsson, I., Venge, P., Elgefors, B.* (Reprint requests: Dr. I. Olsson, Research Department 2, University Hospital, S-221 85 Lund, Sweden), p. 73

Scandinavian Journal of Haematology (Copenhagen) **18** (1977) No. 2

Long-term use of phenytoin: Effects on whole blood and red cell folate and haematological parameters. *Weber, T. H., Knuutila, S., Tammisto, P., Tontti, K.* (Medix Laboratories Ltd., SF 00101 Helsinki 10, Finland), p. 81

Antifactor Xa activity in thrombophilia. Studies in a family with At-III deficiency. *Ødegård, O. R., Abildgaard, U.* (Medical Department A, Aker Sykehus, Oslo 5, Norway), p. 86

Immunologic similarities of thrombopoietin from different sources. *McDonald, T. P., Cottrell, M., Nolan, C., Walasek, O.* (University of Tennessee Memorial Research Center, Knoxville, Tenn. 37920), p. 91

Detection of anti-red blood cell antibodies by treatment of the cells with neuraminidase. *Katz, A., Klajman, A., Yaretzky, A., Steiner, Z. P., Knyszynski, A.* (Reprint requests: Prof. A. Klajman, Medical Department B, Meir Hospital, Kfar-Saba, Israel), p. 98

The role of granulocytes in colony stimulation by human white blood cells in agar cultures. Enhancement and inhibition of CSA. *Heit, W., Kern, P., Heimpel, H., Kubanek, B.* (Division of Haematology, Department of Internal Medicine, University of Ulm, D-7900 Ulm/Donau, FRG), p. 105

Myeloperoxidase and lactoferrin of blood neutrophils and plasma in chronic granulocytic leukaemia. *Olofsson, T., Olsson, I., Venge, P.* (Reprint requests: Dr. I. Olsson, Research Department 2, University Hospital, S-221 85 Lund, Sweden), p. 113

The effect of adrenaline, insulin and hydrocortisone on human peripheral blood lymphocytes studied by cell surface markers. *Eriksson, B., Hedfors, E.* (Reprint requests: Dr. E. Hedfors, Department of Medicine, Karolinska Hospital, S-10401 Stockholm, Sweden), p. 121

Bone marrow cell culturing in double diffusion chambers. *Pfeffer, P., Bøyum, A.* (Reprint requests: Dr. A. Bøyum, Norwegian Defence Research Establishment, Division of Toxicology, N-2007 Kjeller, Norway), p. 129

Blood B and T lymphocytes and *in vitro* cellular immune reactivity in untreated human malignant lymphomas and other malignant tumors. *Heier, H. E., Klepp, R., Gundersen, S., Godal, T., Normann, T.* (Laboratory for Haematology and Lymphology, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway), p. 137

DNA synthesis in unstimulated blood lymphocytes of patients with untreated malignant lymphomas or other malignant tumors. *Heier, H. E., Godal, T.* (Laboratory for Haematology and Lymphology, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway), p. 149

Microcalorimetric measurements of heat production in erythrocytes of patients with chronic uraemia. *Monti, M.* (Department of Internal Medicine, University Hospital, S-221 85 Lund, Sweden), p. 154

Trisomy 8 in acute myeloid leukaemia: A non-random event. Lack of correlation with prognosis and cytokinetic parameters. *Philip, P., Wantzin, G. L., Jensen, M. K., Drivsholm, A.* (Department of Medicine A, Rigshospitalet, DK-2100 Copenhagen, Denmark), p. 163

In vitro platelet stain preventing (degranulating) effect of various substances. Study of mechanism of action. *Stavem, P., Kjaerheim, Å.* (Medical Department A, Rikshospitalet, Oslo, Norway), p. 170

Scandinavian Journal of Haematology (Copenhagen) **18** (1977) No. 3

On the function of normal B and T lymphocytes as deduced from lymphocytokinetic investigations. *Christensen, B. E., Jønsson, V.* (Medical Department C, Gentofte University Hospital, DK-2900 Hellerup, Denmark), p. 177

Distribution of B, T, and O lymphocytes in blood and tissues of normal humans reflecting a kinetic model. *Jønsson, V., Christensen, B. E.* (Medical Department C, Gentofte University Hospital, DK-2900 Hellerup, Denmark), p. 185

The incidence and characteristics of acute myeloid leukaemia arising in Hodgkin's disease. *Larsen, J., Brincker, H.* (Reprint requests: Dr. H. Brincker, Department of Oncology and Radiotherapy, Odense University Hospital, 5000 Odense, Denmark), p. 197

Surface features of Sezary cells: A scanning electron microscopy study of 5 cases. *Polliack, A., Djaldetti, M., Reyes, F., Biberfeld, P., Daniel, M. T., Flandrin, G.* (Department of Haematology, Hadassah University Hospital, Jerusalem, Israel), p. 207

Hepatitis type B in haemophiliacs. Relation to the source of clotting factor concentrates. *Holsteen, V., Skinhøj, P., Cohn, J.* (Reprint requests: Dr. P. Skinhøj, Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark), p. 214

The effect of phospholipase C on rat blood platelets *in vivo*. *Giercksky, K.-E.* (Institute of Medical Biology, University of Tromsø, Tromsø, Norway), p. 219

Effects of hydroxyurea pretreatment of donor rats on growth of haematopoietic diffusion chamber colonies in mice. *Ben-Ishay, Z., Sharon, S.* (Department of Anatomy, Hebrew University-Hadassah Medical School, Jerusalem, Israel), p. 226

Constant post-irradiation repopulation rates and linear relationship between cellular blood response and number of transplanted bone marrow cells in inbread mice. *Petersen, B. H.* (Norwegian Defence Research Establishment, Division for Toxicology, N-2007 Kjeller, Norway), p. 235

Extracorporeal perfusion treatment with immobilized L-asparaginase. *Langem, S., Hydén, H.* (Reprint requests: Prof. H. Hydén, Institute of Neurobiology, S-400 33 Göteborg, Sweden), p. 244

The Hermansky-Pudlak syndrome. Evidence for a lowered 5-hydroxytryptamine content in platelets of heterozygotes. *Gerritsen, S. M., Akkerman, J. W. N., Nijmeijer, B., Sixma, J. J., Witkop, C. J., White, J.* (Department of Intensive Care, University Hospital Nijmegen, The Netherlands), p. 249

Scandinavian Journal of Haematology (Copenhagen) **18** (1977) No. 4

Kinetics of small lymphocytes in normal and nude mice after splenectomy. *Hougen, H. P., Hansen, F., Kehn Jensen, E., Röpke, C.* (Reprint requests: Dr. C. Röpke, University of Copenhagen, Anatomy Department A, DK-2200 Copenhagen N, Denmark), p. 257

ADP-induced refractory state of platelets *in vitro*. II. Functional and ultra studies on gel filtered platelets. *Holme, S., Sixma, J. J., Wester, J., Holmsen, H.* (Cardeza Foundation for Hematologic Research, Philadelphia, Pa. 19107), p. 267

Splenectomy in chronic lymphocytic leukaemia. *Egelund Christensen, B., Hansen, M. M., Videbaek, A. A.* (Medical Department C, Gentofte Hospital, 2900 Hellerup, Denmark), p. 279

Chronic myelocytic leukaemia presenting in blastic transformation. Two cases primarily diagnosed as acute myelocytic leukaemia. *Worm, A.-M., Pedersen-Bjergaard, J.* (Reprint requests: Dr. J. Pedersen-Bjergaard, The Finsen Institute, Department of Internal Medicine, DK-2100 Copenhagen, Denmark), p. 288

Blastic transformation of chronic myelocytic leukaemia. Clinical manifestations, prognostic factors and results of therapy. *Pedersen-Bjergaard, J., Worm, A.-M., Hainau, B.* (The Finsen Institute, Department of Internal Medicine, DK-2100 Copenhagen, Denmark), p. 292

Cytogenetic studies in preleukaemia using the G-banding staining technique. *Panani, A., Papayannis, A. G., Kyrkou, K., Gardikas C.* (Reprint requests: Prof. C. Gardikas, Evangelismos Hospital, Athens 140, Greece), p. 301

Inverse relationship between splenomegaly and stem cell compartment size in mice treated with nitrogen mustard. *Jensen, R. H., Sharp, J. G., Zajic, G. H., Anderson, R. W.* (Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105), p. 309

Function of neutrophils in preleukaemia. *Ruutu, P., Ruutu, T., Vuopio, P., Kosunen, T. U., de la Chapelle, A.* (Department of Bacteriology and Immunology, University of Helsinki, 00290 Helsinki 29, Finland), p. 317

The haemostatic effect of ^{51}Cr-labelled blood platelets. An experimental study in the rabbit. *Björnson, J., Aursnes, I.* (Department VII, Ullevål Hospital, Oslo, Norway), p. 326

The relationship between normotest and thrombotest in patients on oral anticoagulant therapy. *Refsum, N., Arnesen, H., Gilhuus-Moe, C. C., Godal, H. C.* (Nyegaard and Co., Oslo 4, Norway), p. 333

Chronic monocytic leukaemia. A prolonged survival. *Amir, J., Djaldetti, M.* (Cleveland V. A. Hospital, Cleveland, Ohio 44106), p. 337

In vitro detection of cell-mediated immunity to antigens associated with Hodgkin's granulomas. *Andersen, E.* (Medical Department C, Gentofte Hospital, DK-2900 Hellerup, Denmark), p. 343

Streptokinase resistance in medical patients in Oslo. *Arnesen, H., Rygh, M., Ly, B., Jakobsen, E.* (Medical Department VIII, Ullevål Hospital, Oslo, Norway), p. 348

Scandinavian Journal of Haematology (Copenhagen) **18** (1977) No. 5

Increased potassium permeability by calcium in hypochromic red blood cells. *Vettore, L., de Matteis, M. C., Falezza, G. C.* (Istituto Patologia 2° dell'Università di Padova, Policlinico di Borgo Roma, 37100 Verona, Italy), p. 353

Characterization of the cobalamins attached to transcobalamin I and transcobalamin II in human plasma. *Nexø, E.* (Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark), p. 358

Immunologic profile of patients with cured Hodgkin's disease. *Björkholm, M., Holm, G., Mellstedt, H.* (Department of Medicine, Serafimerlasarettet, S-112 83 Stockholm, Sweden), p. 361

Increased ATP-ase activity of lymphocytes after rosette formation with sheep red blood cells. *Kragballe, K., Ellegaard, J.* (Department of Medicine and Clinical Haematology, Aarhus Amtssygehus, DK-8000 Aarhus, Denmark), p. 369

In vitro culture of leukaemic cells from 81 patients with acute leukaemia. *Knudtzon,*

S. (Department of Internal Medicine, Finsen Institute, DK-2100 Copenhagen, Denmark), p. 377

Vitamin B_{12} metabolism in myelomatosis. *Hansen, P. O., Drivsholm, A., Hippe, E.* (Department of Internal Medicine, Section of Haematology, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark), p. 395

Comparison of biochemical properties of human serum B_{12} binders derived from Sephadex and DEAE-cellulose chromatography. *Kumar, S., Meyer, L. M., Gams, R. A.* (Perinatal Laboratory, Christ Hospital, Oak Law, Ill. 60453), p. 403

Ph^1-negative eosinophilic leukaemia with trisomy 8. Case report and review of cytogenetic studies. *Weinfeld, A., Westin, J., Swolin, B.* (Department of Medicine II, Sahlgren's Hospital, University of Göteborg, S-413 45 Göteborg, Sweden), p. 413

Resistance to activated F IX concentrate (FEIBA). *Stenbjerg, S., Jørgensen, J.* (Blood Bank and Blood Grouping Laboratory, University Hospital, DK-8000 Aarhus C, Denmark), p. 421

Increase of proliferative activity of leukaemic blast cells from human peripheral blood in liquid culture. *Kurrle, E., Schmücker, H., Hoelzer, D.* (Department of Medicine, Clinical Research Centre, University of Ulm, D-79 Ulm/Donau, FRG), p. 427

Discrimination of B, T and null lymphocytes by esterase cytochemistry. *Higgy, K. E., Burns, G. F., Hayhoe, F. G. J.* (Reprint requests: Prof. F. G. J. Hayhoe, Department of Haematological Medicine, Cambridge CB2 2QL, England), p. 437

A case of T-cell lymphoma with Sézary cells in the blood and bone marrow, accompanied by peripheral T and B lymphocytosis. *Manthorpe, R., Jønsson, V., Christensen, B. E., Hesselvik, M., Egeberg, J., Videbaek, Aa.* (Medical Department C, Gentofte University Hospital, DK-2900 Hellerup/Copenhagen, Denmark), p. 449

Seminars in Hematology (New York) **13** (1976) No. 3

Hereditary spherocytosis: A study of splenectomized persons. *Schilling, R. F.* (University of Wisconsin Medical School, Madison, Wis. 53706), p. 169

Hemoglobin CC erythrocytes: Decreased intracellular pH and decreased O_2 affinity-anemia. *Murphy, J. R.* (Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106), p. 177

Shortened survival of autotransfused ^{51}Cr-labeled erythrocytes in infants with severe iron-deficiency anemia. *Rasch, C. A., Cotton, E. K., Griggs, R. C., Harris, J. W.* (Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109), p. 181

Hemolytic anemia in premature infants: Relationship to vitamin E, selenium, glutathione peroxidase, and erythrocyte lipids. *Gross, S.* (Division of Hematology and Oncology, Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106), p. 187

Acid hemolysis revisited. *Hinz, C. F. Jr.* (University of Connecticut School of Medicine, Farmington, Conn. 06032), p. 201

Scurvy. *Wallerstein, R. O., Wallerstein, R. O. Jr.* (Children's Hospital, San Francisco, Calif. 94118), p. 211

Exchange transfusions in the treatment of thrombotic thrombocytopenic purpura. *Bukowski, R. M., Hewlett, J. S., Harris, J. W., Hoffman, G. C., Battle, J. D. Jr., Silverblatt, E., Yang, I-Yen* (Department of Hematology and Medical Oncology, Cleveland Clinic, Cleveland, Ohio 44106), p. 219

Lack of influence of oral contraceptives on serum folate, hematologic values, and uterine cervical cytology. *Ross, C. E., Stone, M. K., Reagan, J. W., Wentz, W. B., Kellermeyer, R. W.* (Reprint requests: Dr. R. W. Kellermeyer, Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106), p. 233

Plasma cell asynchrony in myeloma: Correlation of light and electron microscopy. *Bernier, G. M., Graham, R. C. Jr.* (University Hospitals of Cleveland, University Circle, Cleveland, Ohio 44106), p. 239

Seminars in Hematology (New York) **13** (1976) No. 4

Foreword. *Swisher, S. N.* (Department of Medicine, Michigan State University, East Lansing, Mich. 48824), p. 247

Clinical aspects of autoimmune hemolytic anemia. *Pirofsky, B.* (University of Oregon Health Sciences Center, Portland, Ore. 97201), p. 251

Coombs-negative immune hemolytic anemia. *Gilliland, B. C.* (University of Washington School of Medicine, Seattle, Wash. 98195), p. 267

Immunologic mechanisms in autoimmune hemolytic disease. *Logue, G., Rosse, W.* (Durham Veterans Administration Hospital, Durham, N. C. 27705), p. 277

Serologic profile in autoimmune hemolytic disease: Pathophysiologic and clinical interpretations. *Lalezari, P.* (Division of Immunohematology, Department of Medicine, Montefiore Hospital and Medical Center, Bronx, N. Y. 10467), p. 291

Transfusion therapy for autoimmune hemolytic anemia. *Rosenfield, R. E., Jagathambal* (Department of Pathology, Mount Sinai School of Medicine, City University of New York, New York, N. Y. 10029), p. 311

Drug therapy of autoimmune hemolytic anemia. *Murphy, S., LoBuglio, A. F.* (Reprint requests: Dr. A. F. LoBuglio, University Hospital N-1022, Columbus, Ohio 43210), p. 323

The role of the spleen and splenectomy in autoimmune hemolytic disease. *Bowdler, A. J.* (Department of Medicine, College of Human Medicine, Michigan State University, East Lansing, Mich. 48824), p. 335

Animal models of autoimmune hemolytic disease. *Bull, R. W.* (Department of Human Medicine, College of Human Medicine, Michigan State University, East Lansing, Mich. 48824), p. 349

Seminars in Hematology (New York) **14** (1977) No. 1

Introduction. *Muller-Eberhard, U.* (Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 1

The clinical biochemistry of iron. *Worwood, M.* (Department of Haematology, Welsh National School of Medicine, Cardiff, Wales, Great Britain), p. 3

The iron-binding function of transferrin in iron metabolism. *Aisen, P., Brown, E. B.* (Department of Biophysics and Medicine, Albert Einstein College of Medicine, New York, N. Y. 10461), p. 31

Ferritin: An iron-storage molecule. *Harrison, P. M.* (Department of Biochemistry, The University, Sheffield, S1O 2TN, England), p. 55

Human isoferritins in normal and disease states. *Drysdale, J. W., Adelman, T. G., Arosio, P., Casareale, D., Fitzpatrick, P., Hazard, J. T., Yokoto, M.* (Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Mass. 02111), p. 71

Iron overload. Clinical and pathologic aspects. *Jacobs, A.* (Department of Haematology, Welsh National School of Medicine, Cardiff CF4 4XN, Wales, Great Britain), p. 89

Iron overload: Clinical and pathologic aspects in pediatrics. *O'Brien, R. T.* (Department of Pediatrics, Yale University School of Medicine, New Haven, Conn. 06510), p. 115

Chelation therapy for the treatment of thalassemia. *Graziano, J. H., Cerami, A.* (Department of Pediatric Hematology, Cornell University Medical Center, New York, N. Y. 10021), p. 127

Seminars in Hematology (New York) **14** (1977) No. 2

Hemochromatosis: The unsolved problems. *Crosby, W. H.* (Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 135

Enzymatic formation and cellular regulation of heme synthesis. *Gidari, A. S., Levere, R. D.* (Division of Hematology and Oncology, Department of Medicine, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 145

Porphyrin and iron metabolism in sideroblastic anemia. *Bottomley, S. S.* (Veterans Administration Hospital, Oklahoma City, Okla. 73104), p. 169

Experimental porphyrias as models for human hepatic porphyrias. *De Matteis, F., Stonard, M.* (Toxicology Unit, Medical Research Council Laboratories, Carshalton, Surrey, England), p. 187

Modern spectroscopic and chromatographic techniques for the analysis of porphyrin

on a microscale. *Jackson, A. H.* (Department of Chemistry, University College, Cardiff CF1 1XL, Wales, Great Britain), p. 193

Erythropoietic porphyrias: Current mechanistic, diagnostic, and therapeutic considerations. *Poh-Fiztpatrick, M. B.* (Department of Dermatology, Columbia University College of Physicians and Surgeons, New York, N. Y. 10032), p. 211

Interaction of porphyrin with proteins. *Koskelo, P., Muller-Eberhard, U.* (Third Department of Medicine, University Central Hospital, SF-00290 Helsinki 29, Finland), p. 221

Porphyrin metabolism in porphyria cutanea tarda. *Elder, G. H.* (Department of Medical Biochemistry, Welsh National School of Medicine, Cardiff CF4 4XN, Wales, Great Britain), p. 227

Hepatic siderosis and porphyria cutanea tarda: Relation of iron excess to the metabolic defect. *Felsher, B. F., Kushner, J. P.* (Veterans Administration Hospital, Long Beach, Calif. 90822), p. 243

Treatment of porphyria cutanea tarda by phlebotomy. *Ippen, H.* (Kliniken der Universität Göttingen, Hautklinik und Poliklinik, 34 Göttingen, BRD), p. 253

Conclusion. *Muller-Eberhard, U.* (Department of Biochemistry, Scripps Clinic and Research Foundation, University of California at San Diego, La Jolla, Calif.), p. 261

Seminars in Hematology (New York) **14** (1977) No. 3

Introduction. *Mibashan, R. S.* (Haemophilia Centre, Department of Haematology, King's College Hospital Medical School, London, England), p. 263

The hemostatic plug. *Sixma, J. J., Wester, J.* (University Hospital, Department of Haematology, Utrecht, The Netherlands), p. 265

Coagulation pathways: Interrelationships and control mechanisms. *Bennett, B.* Department of Medicine, Medical School, Aberdeen, Scotland), p. 301

Molecular genetics of factor VIII and its disorders. *Bloom, A. L., Peake, I. R.* (Department of Haematology, Welsh National School of Medicine, Cardiff, Wales, United Kingdom), p. 319

Hemostatic failure in clinical medicine. *Bowie, E. J. W., Owen, C. A., Jr.* (Mayo Clinic, Rochester, Minn. 55901), p. 341

Oral anticoagulant therapy: The biosynthetic basis. *Suttie, J. W.* (Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisc. 53706), p. 365

Seminars in Hematology (New York) **14** (1977) No. 4

Developments and problems in the management of hemophilia. *Jones, P.* (Newcastle Haemophilia Centre, Department of Haematology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, England), p. 375

Blood coagulation and the inflammatory response. *Zimmerman, T. S., Fierer, J., Rothberger, H.* (Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 391

Hypercoagulability. *Hirsh, J.* (Department of Pathology, McMaster University Medical Centre, Hamilton, Ontario L8 4J9, Canada), p. 409

Biologic actions of heparin. *Rosenberg, R. D.* (Sidney Farber Cancer Institute, Beth Israel Hospital, Harvard Medical School, Boston, Mass. 02215), p. 427

Thrombosis and Haemostasis (Stuttgart) **36** (1976) No. 2

A new era in the laboratory evaluation of coagulation and fibrinolysis. *Stormorken, H.* (Institute for Thrombosis and Research, Rikshospitalet, Oslo 1, Norway), p. 299

Some observations on the preparation of platelet-rich plasma. *Woods, B.P., Dennehy, A., Clarke, N.* (Department of Pathology, University College, Dublin, 2 Ireland), p. 302

Effects of matrix contact during gel filtration of human platelets in plasma. *Lindon, J. N., Rodvien, R., Waugh, D. F.* (Pacific Medical Center, San Francisco, Calif.), p. 311

Platelet dysfunction in migraine: Effect of self-medication with aspirin. *Deshmukh,*

S. V., Meyer, J. S., Mouche, R. J. (Department of Neurology, Baylor College of Medicine, Houston, Texas 77030), p. 319

Effects of fasting on plasma and platelet-free fatty acids and platelet function in healthy males. *Gjesdal, K., Nordøy, A., Wang, H., Bernsten, H., Mjøs, O. D.* (Medical Department A, Aker Hospital, Oslo 5, Norway), p. 325

Sedimentation and adhesion of platelets onto a horizontal glass surface. *Ruckenstein, E., Marmur, A., Rakower, S. R.* (Faculty of Engineering and Applied Sciences, School of Medicine, State University of New York at Buffalo, Buffalo, N. Y. 14214), p. 334

Competitive inhibition by lithium and hydrogen ions of the effect of calcium on the aggregation of rabbit platelets. *Anderson, E. R., Foulks, J. G.* (Department of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada), p. 343

Reactions of polylysine with human platelets in plasma and in suspensions of washed platelets. *Guccione, M. A., Packham, M. A., Kinlough-Rathbone, R. L., Perry, D. W., Mustard J. F.* (Reprint requests: Dr. M. A. Packham, Department of Biochemistry Faculty of Medicine University of Toronto, Toronto, Ontario, Canada M5S 1A8), p. 360

Effects of bupranolol, a new β-blocker, on platelet functions of rabbit and humans *in vitro*. *Umetsu, T., Sanai, K., Kato, T.* (Research Laboratory, Kaken Chemical Co., Ltd., Bunkyo-ku, Tokyo, Japan), p. 376

Inhibition of ADP-induced platelet aggregation by adenosine tetraphosphate. *Harrison, M. J., Brossmer, R.* (Institut für Biochemie II, Universität Heidelberg, D-6900 Heidelberg, BRD), p. 388

Effect of platelet age on adhesiveness to collagen and platelet surface charge. *Castellan, R. M., Steiner, M.* (Division of Hematologic Research, The Memorial Hospital, Pawtucket and Brown University, Providence, R. I.), p. 392

Inhibition of human and animal platelet adhesiveness to glass bead columns by adenosine, dipyridamole, chlorpromazine and acetylsalicylic acid. *Fujitani, B., Tsuboi, T., Takeno, K., Yoshida, K., Shimizu, M.* (Research Laboratory, Dainippon Pharmaceutical Co., Ltd., Osaka 564, Japan), p. 401

Release, aggregation and lysis of human platelets by antilymphocyte globulin and antiplatelet serum. *Lekas, N., Rosenberg, J. C.* (Wayne State University, Department of Surgery, Detroit, Mich. 48201), p. 411

Platelet aggregation following defibrination with ancrod. *Slade, C. L., Andes, W. A., Mason, A. D., Jr.* (United States Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam, Houston, Texas 78234), p. 424

Physico-chemical explanation of blood cell adhesion in thrombus formation. *Marmur, A., Ruckenstein, E., Rakower, S. R.* (Department of Chemical Engineering, Technion, Haifa, Israel), p. 430

An immunological method for detection of the carrier of hemophilia B. *Matsuoka, M., Ito, M., Takahashi, K., Sakuragawa, N.* (Department of Internal Medicine, Niigata University School of Medicine, Niigata, Japan), p. 441

Enhancement of fibrinolytic and thrombolytic potential in the rat by treatment with an anabolic steroid, furazabol. *Kumada, T., Abiko, Y.* (Research Institute, Daiichi Seiyaku Co., Ltd., Tokyo 132, Japan), p. 451

Effects of ions on ADP-induced aggregation of bovine or human platelets. *Waugh, S., Lindon, J. N.* (Massachusetts Institute of Technology, Cambridge, Mass. 02139), p. 465

Hyperaggregability of platelets in thromboembolic disorders. *Zahavi, J.* (Department of Medicine B, Ichilov Municipal Government Hospital, Tel-Aviv, Israel), p. 479

Absorption of heparin by kaolin. *Barrowcliffe, T. W., Sims, G. E. C.* (NIBSC, Holly Hill, Hampstead, London NW3 6RB, England), p. 483

British comparative thromboplastin therapeutic range. *Poller, L.* (W. H. O. Collaborating Centre for Anticoagulant Control Reagents, Withington Hospital, Manchester M20 8LR, England), p. 485

Linear regression technique. *Duncan, B. McL., Lloyd, J. V.* (Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, Australia), p. 486

Fibre inadequately replaced by cellulose or

other forms of roughage in animal experimental diets. *Trowell, H.*, (Woodgreen, Fordingbridge, Hampshire SP6 2AZ, England), p. 489

Thrombosis and Haemostasis (Stuttgart) **36** (1976) No. 3

Severe antithrombin III deficiency in an infant associated with multiple arterial and venous thromboses. *Mendelsohn, G., Gomperts, E. D., Gurwitz, D.* (Reprint requests: Dr. E. D. Gomperts, Department of Haematology, The South African Institute for Medical Research, Johannesburg 2000, South Africa), p. 495

Inactivation of α- and β-thrombin by antithrombin-III and heparin. *Machovich, R., Blaskó, Gy., Borsodi, A.* (Postgraduate Medical School, First Department of Medicine, H-1389 Budapest, Hungary), p. 503

Studies of an inhibitor of the thrombin-fibrinogen reaction localized in rat liver microsomes. Interference with the polymerization step. *Helgeland, L.* (Department of Biochemistry, University of Oslo, Blindern, Norway), p. 509

Prothrombin determination by means of a chromogenic peptide substrate. *Axelsson, G., Korsan-Bengtsen, K., Waldenström, J.* (Department of Medicine II, University of Göteborg, Sahlgren's Hospital, S-413 45 Göteborg, Sweden), p. 517

Effect of the root extract of *Fagara zanthoxyloides* on blood coagulation. *Essien, E. M., Okogun, J. I.* (Department of Haematology, University College Hospital, Ibadan, Nigeria), p. 525

The influence of ethanol feeding on the activities of factors II, VII, VIII, IX and X in rats. *Mørland, J., Holm, T., Prydz, H.* (Institute of Medical Biology, University of Tromsø, 9000 Tromsø, Norway), p. 532

Bleeding disorder with abnormal wound healing, acid-soluble clots and normal factor XIII. *Ragaz, S., Kemp, G., Furlan, M., Beck, E. A.* (Central Haematology Laboratory, Inselspital, CH-3010 Bern, Switzerland), p. 537

Plasma factor XIII and platelet factor XIII in hyperlipaemia. *Cucuianu, M. P., Miloszewski, K., Porutiu, D., Losowsky, M. S.* (Reprint requests: Dr. K. Miloszewski, Department of Medicine, St. James's University Hospital, Leeds, LS9 9TF, England), p. 542

On the reliability of plasminogen measurement employing the proactivator-activator converting method. *Martin, M., Auel, H.* (Aggertalklinik, Clinic for Vascular Diseases, Engelskirchen-Cologne, FRG), p. 551

Studies on activator formation in human plasma with streptokinase. III. Investigation of activator kinetics in undiluted plasma in terms of urokinase equivalents. *Martin, M., Auel, H.* (Aggertalklinik, Clinic for Vascular Diseases, Engelskirchen-Cologne, FRG), p. 566

Clottability and cross-linking reactivity of fibrin(ogen) following differential release of fibrinopeptides A and B. *Furlan, M., Seelich, T., Beck, E. A.* (Central Haematology Laboratory, Inselspital, CH-3010 Bern, Switzerland), p. 582

Demonstration of reactivity to airborne and food allergens in cutaneous vasculitis by variations in fibrinopeptide A and other blood coagulation, fibrinolysis and complement parameters. *Theorell, H., Blombäck, M., Kockum, C.* (Department of Allergy, Karolinska Sjukhuset, Stockholm, Sweden), p. 593

The effect of the fibrinogen concentration and the leukocyte count on intravascular fibrin deposition from soluble fibrin monomer complexes. *Gurewich, V., Lipinski, B., Hyde, E.* (Vascular Laboratory, Lemuel Shattuck Hospital, Boston, Mass. 02130), p. 605

Thrombin elaboration in endotoxin-induced intravascular fibrin deposition. A leukocyte dependent process distinct from systemic hypercoagulability. *Lipinski, B., Gurewich, V., Hyde, E.* (Vascular Laboratory, Lemuel Shattuck Hospital, Boston, Mass. 02130), p. 615

Inhibitory effect of acetylsalicylic acid on human platelet function in normal volunteers and in women using a combined oral contraceptive regime. *Baele, G., Thiery, M., Vermeulen, A., Barbier, F.* (Department of Medicine, University Hospital, B-9000 Ghent, Belgium), p. 623

Effect of aspirin on platelet phospholipids. *Valles, J., Aznar, J., Santos, M. T.* (Reprint

requests: Dr. J. Aznar, Department of Clinical Pathology, Ciudad Sanitaria "La Fe", Valencia, Spain), p. 628

Impairment of ADP-induced platelet aggregation by hashish components. *Levy, R., Schurr, A., Nathan, I., Dvilanski, A., Livne, A.* (Reprint requests: Prof. A. Livne, Ben-Gurion University of the Negev, Beer-Sheva, Israel), p. 634

A new therapy in post-traumatic intravascular coagulation? *Giercksky, K. E., Otnaess, A.-B., Bjørklid, E., Prydz, H.* (Institute of Medical Biology, University of Tromsø, Tromsø, Norway), p. 641

Recurrent thromboembolism with spontaneous platelet aggregation. *Scrobohaci, M.-L., Cunescu, V., Orha, I.* (Center of Cardiology, Bucharest, Romania), p. 645

Platelets in the urine: Further evidence. *Sutor, A. H., Ketelsen, U. P., Schindera, F.* (Universitäts-Kinderklinik, Freiburg i. Br., BRD), p. 647

An inverse relation between platelet factor 4 in the platelet and in the plasma. *O'Brien, J. R., Etherington, M. D.* (Central Laboratory, St. Mary's General Hospital, Portsmouth, England), p. 649

A method for the removal of platelet aggregating activity of commercial apyrase. *Whigham, K. A. E., Drummond, A. H., Edgar, W., Prentice, C. R. M.* (University Department of Medicine, Royal Infirmary, Glasgow, Scotland), p. 652

Re: The standardization of anticoagulant control. *Poller, L., Thomson, J. M.* (National Reference Laboratory for Anticoagulant Control Reagents, Withington Hospital, University Hospital of South Manchester, Manchester, M20 8LR, England), p. 654

Thrombosis and Haemostasis (Stuttgart) **37** (1977) No. 1

Platelet interaction with collagen fibrils in flowing blood. I. Reaction of human platelets with α-chymotrypsin-digested subendothelium. *Baumgartner, H. R.* (Pharmaceutical Research Department, F. Hoffmann-La-Roche and Co., Ltd., CH-4002 Basel, Switzerland), p. 1

Platelet interaction with collagen fibrils in flowing blood. II. Impaired adhesion-aggregation in bleeding disorders. A comparison with subendothelium. *Baumgartner, H. R., Tschopp, T. B., Weiss, H. J.* (Pharmaceutical Research Department, F. Hoffmann-La-Roche and Co., Ltd., CH-4002 Basel, Switzerland), p. 17

The effect of phospholipase C on platelet factor 3 in human blood platelets. *Otnaess, A.-B., Prydz, H.* (Reprint requests: Dr. H. Prydz, MRC Cell Pathology Unit, Northwick Park Clinical Research Centre, Harrow, Middlesex, England), p. 29

The effect of intravenous adenosine diphosphate on the number of circulating platelets in experimental animals: Inhibition by prostaglandin E_1, dipyridamole, SH-869 and VK-774. *Holmes, I. B., Smith, G. M., Freuler, F.* (Biological and Medical Research Division, Sandoz Ltd., CH-4002 Basel, Switzerland), p. 36

In vivo labeling of platelets with ^{75}Se-selenomethionine in patients with hepatic cirrhosis and thrombocytopenia. *Mayer, M., Herrmann, I., Kempgens, U., Queisser, W.* (First Department of Internal Medicine, University of Heidelberg, Heidelberg, FRG), p. 47

Influence of cytotoxic drugs on platelet functions and coagulation *in vitro*. IV. Melphalan. *Klener, P., Kubisz, P., Suranová, J.* (Second Department of Medicine, Charles University Hospital, 12808 Prague 2, Czechoslovakia), p. 53

Induction of the platelet release reaction by 1,3-dimethyl-5-aminoadamantane, a new adamantane derivative. *Haacke, U., Wesemann, W.* (Institut für Physiologische Chemie II, Philipps-Universität, Marburg/Lahn, BRD), p. 62

Half-life of platelet factor 4 (PF-4) in plasma and platelets from *Macaca mulatta*. *Gjesdal, K., Pepper, D. S.* (Medical Department A, Aker Hospital, Oslo, Norway), p. 73

Kinetic aspects of the interaction of blood clotting enzymes. VII. The relation between clotting time and prothrombin concentration. *Hemker, H. C., Vermeer, C., Govers-Riemslag, J.* (Department of Biochemistry, Biomedical Centre, State University Limburg, Maastricht, The Netherlands), p. 81

A comparison of coagulation factor replacement with and without prednisolone in the treatment of haematuria in haemo-

philia and Christmas disease. *Rizza, C. R., Kernoff, P. B. A., Matthews, J. M., McLennan, C. R., Rainsford, S. G.* (Reprint requests: Dr. P. B. A. Kernoff, University of Leeds, Department of Medicine, General Infirmary, Leeds, England), p. 86

Localization of tissue thromboplastin in the human brain. *Bjørklid, E., Storn-Mathisen, J., Storm, E., Prydz, H.* (Institute of Medical Biology, University of Tromsø, Tromsø, Norway), p. 91

Comparison between British comparative thromboplastin (BCT) and a factor II-VII-X determination method (Simplastin A) based on fresh plasma samples from dicoumarol-treated patients. *Korsan-Bengtsen, K., Johnsen, M., Pehrsson, N.-G.* (Blood Coagulation Laboratory, Medical Department, Sahlgren's Hospital, University of Göteborg, S-413 45 Göteborg, Sweden), p. 98

Effects of t-AMCHA and EACA on the plasma kallikrein system. *Nakahara, M.* (Department of Orthopaedics, Sapporo Medical College, Sapporo, Japan), p. 104

Studies of the effects of estradiol, progesterone, cortisol, thrombophlebitis, and typhoid vaccine on synthesis and catabolism of antithrombin III in the dog. *Kobayashi, N., Takeda, Y.* (Reprint requests: Dr. Y. Takeda, Department of Medicine, University of Colorado, School of Medicine, Denver, Col. 80220), p. 111

The reversible antithrombin activity of incubated plasma. *Gomperts, E. D., Stavridis, J. C., Mibashan, R. S.* (Department of Haematology, School of Pathology, University of Witwatersrand, Johannesburg, South Africa), p. 123

Immunoelectrophoretic studies on the kinetics of plasmic degradation of human fibrinogen. *Braude, R., Altman, R., Rouvier, J., Aguilar, N.* (Docencia e Investigación, Hospital U.A.M.I., Las Heras 2670, Buenos Aires, Argentina), p. 136

Soluble fibrin complexes: Separation as a function of pH and characterization. *Benabid, Y., Concord, E., Suscillon, M.* (Laboratoire d'Hématologie, Institut de Recherche Fondamentale, Centre d'Études Nucléaires de Grenoble, 85 X Grenoble, France), p. 144

A comparative *ex vivo* study of plasminogen activators and proteases for fibrinolytic activity and side effects in rabbits. *Janik, B. A., Papaioannou, S. E.* (Searle Laboratories, Chicago, Ill. 60680), p. 154

Intermittent plasminogen-streptokinase treatment of deep vein thrombosis. *Scully, M. F., Lane, D. A., Sagar, S., Thomas, D. P., Kakkar, V. V.* (Thrombosis Research Unit, King's College Hospital Medical School, London SE5 8RX, England), p. 162

Oxygen-induced consumptive coagulopathy and its enhancement by lead acetate. *Kiesow, L. A., Shapiro, S., Lindsley, B. F., Bless, J. W.* (Department of Experimental Medicine, Naval Medical Research Institute, Bethesda, Md. 20014), p. 170

Alterations in human-platelet serotonin uptake following the addition of thrombin or A 23187. *Costa, J. L., Murphy, D. L.* (Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20014), p. 177

Thrombosis and Haemostasis (Stuttgart) **37** (1977) No. 2

Evaluation of a new anti-fibrinogen-coated latex particle agglutination test in the measurement of serum fibrin degradation products. *Marder, V. J., Cruz, G. O., Schumer, B. R.* (Department of Medicine and the Specialized Center of Research in Thrombosis, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 183

Soluble fibrin complexes in early fibrin digests. *Nanninga, L. B.* (Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550), p. 192

The half-life of plasmic degradation products of human fibrinogen in rabbits. *Ardaillou, N., Dray, L., Budzynski, A. Z., Marder, V. J., Larrieu, M. J.* (U. 143, J. P. C. Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France), p. 201

A new method for the determination of plasma activators of fibrinolysis. *Margalit, R., Gidron, E., Shalitin, Y.* (Reprint requests: Dr. E. Gidron, Department of Medicine B, Rotschild University Hospital, Haifa, Israel), p. 210

In vitro and *in vivo* measurement of total antiplasmin activity. *Matsuo, O., Mihara, H.* (Department of Physiology, Miyazaki Medical College, 5200 Kihara, Miyazaki-ken, 889-16, Japan), p. 216

Controlled trial of the sequential use of streptokinase and ancrod in the treatment of deep vein thrombosis of lower limb. *Tibbutt, D. A., Chesterman, C. N., Williams, E. W., Faulkner, T., Sharp, A. A.* (Worcester Royal Infirmary, Ronkswood Branch, Worcester, England), p. 222

Comparative studies of coagulative and fibrinolytic faculties between the Japanese monkey and the human. *Suzuki, K., Egawa, H., Hashimoto, S.* (Department of Clinical Pathology, Kansai Medical University, Osaka 570, Japan), p. 233

Some factors influencing metabolism and distribution of fibrinogen in man and rabbits. *Chen, Y.-H., Reeve, E. B.* (Department of Medicine, Veterans Administration West Side Hospital, Chicago, Ill.), p. 243

Assay of the esterase activity of thrombin, plasmin and trypsin with a chromogenic substrate *p*-nitrobenzyl *p*-toluenesulfonyl-L-arginine. *Aogaichi, T., Plaut, G. W. E.* (Reprint requests: Dr. G. W. E. Plaut, Department of Biochemistry, School of Medicine, Temple University, Philadelphia, Pa. 19140), p. 253

The stability of glutardialdehyde-stabilized ^{35}S-heparinized surfaces in contact with blood. *Larsson, R. L., Hjelte, M.-B., Eriksson, J. C., Lagergren, H. R., Olsson, P.* (Aminkemi AB, Institute of Technology, Karolinska Sjukhuset, Stockholm, Sweden), p. 262

Prevention of platelet adhesion and aggregation by a glutardialdehyde-stabilized heparin surface. *Olsson, P., Lagergren, H., Larsson, R., Rådegran, K.* (Surgical Research Laboratory, Thoracic Clinics, Karolinska Sjukhuset, Stockholm, Sweden), p. 274

Kinetic parameters of platelet aggregation as an expression of platelet responsiveness. *Rossi, E. C., Louis, G.* (Department of Medicine, Northwestern University School of Medicine, Chicago, Ill. 60611), p. 283

Properties of washed human platelets. *Kinlough-Rathbone, R. L., Mustard, J. F., Packham, M. A., Perry, D. W., Reimers, H.-J., Cazenave, J. P.* (Department of Pathology, McMaster University, Hamilton, Ontario L8S 4J9, Canada), p. 291

Binding of collagen by canine blood platelets. *Kay, W. W., Swanson, R., Chong, G., Kurylo, E., Bharadway, B. B.* (Department of Biochemistry and Microbiology, University of Victoria, Victoria, B. C. V8W 242, Canada), p. 309

Binding of anti-actin autoantibodies to platelets. *Bouvier, C. A., Gabbiani, G., Ryan, G. B., Badonnel, M.-C., Majno, G., Lüscher, E. F.* (Reprint requests: Dr. G. Gabbiani, Department of Pathology, University of Geneva, CH-1211 Geneva, 4, Switzerland), p. 321

Enhancement of platelet sensitivity to ADP-aggregation by isometric exercise in arteriosclerotic patients and its prevention. *Sano, T., Motomiya, T., Yamazaki, H., Shimamoto, T.* (Department of Internal Medicine, Aoyama Tokyo Metropolitan Hospital, Tokyo 150, Japan), p. 329

Cyanide insensitive oxidase in platelets of newborn infant. *Del Principe, D., Menichelli, A., Damiano, A. M., Coppola, L., Sabetta, G.* (Institute of Pediatrics, Medical School, University of Rome, Rome, Italy), p. 339

Ethanol-induced microaggregate formation in pig and rabbit blood. An *in vitro* study. *Elmér, O., Göransson, G., Saku, M., Bengmark, S.* (Department of Surgery, University of Lund, S 221 85 Lund, Sweden), p. 344

International Committee Communications. XXII Annual Meeting of the International Committee on Thrombosis and Haemostasis. *Brinkhous, K. M.* (Department of Pathology, University of North Carolina, Chapel Hill, N. C. 27514), p. 351

Observations of a high molecular weight platelet protein released by thrombin. *Lawler, J. W., Chao, F. C., Pao-Hsien Fang* (Sidney Farber Cancer Institute, Jimmy Fund Building, Boston, Mass. 02115), p. 355

A different organization of bound carbohydrate within cat platelet membranes. *Nurden, A.* (Unité 150 de l'INSERM, Hôpital Lariboisière, Paris Cedex 10, France), p. 358

Abnormal factor VIII related antigen (FVIIIRAG) in von Willebrand's disease

(vWd): Decreased precipitation by concanavalin A. *Peake, I. R., Bloom, A. L.* (Department of Haematology, University Hospital of Wales, Cardiff, CF4 4XW, Wales), p. 361

Preparation of factor IX concentrate from platelet-rich plasma. *Smith, J. K., Snape, T. J., Bidwell, E., Gunson, H. H.* (Plasma Fractionation Laboratory, Regional Transfusion Centre, Churchill Hospital, Oxford, England), p. 363

Assay of F. VIII-related antigen in a variant of von Willebrand's disease. *Over, J., Vlooswijk, H. A. A., Sixma, J. J.* (Department of Haematology, University Hospital, Utrecht, The Netherlands), p. 367

Thrombosis and Haemostasis (Stuttgart) **37** (1977) No. 3

Editorial. History of research on thrombosis and haemostasis. *Beck, E. A.* (Central Haematology Laboratory, Inselspital, CH-3010 Berne, Switzerland), p. 373

The chemistry of blood coagulation: a summary by Paul Morawitz (1905). *Beck, E. A.* (Central Haematology Laboratory, Inselspital, CH-3010 Berne, Switzerland), p. 376

Enzymatic properties of 5'-AMP deaminase in platelet lysates. *Holmsen, H., Østvold, A.-C., Pimentel, M. A.* (Institute for Thrombosis Research, University of Oslo, Oslo-1, Norway), p. 380

Effects of thrombin, chymotrypsin and aggregated gamma-globulins on the proteins of the human platelet membrane. *Podolsak, B.* (Universitäts-Kinderklinik, D-8700 Würzburg, BRD), p. 396

Calcium electrode — a method for the continuous monitoring of the platelet release reaction. *Robblee, L. S., Kornstein, L. B., Shepro, D.* (Reprint requests: Dr. D. Shepro, Department of Biology, Boston University, Boston, Mass. 02215), p. 407

Potentiation by α and inhibition by β-adrenergic stimulations of rat platelet aggregation. A comparative study with human and rabbit platelets. *Yu, S. K., Latour, J. G.* (Reprint requests: Dr. J. G. Latour, Institut de Cardiologie de Montréal, Montreal H1T 1C8, Quebec, Canada), p. 413

Cigarette-smoking effect on platelet function. *Grignani, G., Gamba, G., Ascari, E.* (Istituto Clinica Medica I°, Policlinico S. Matteo, 27100 Pavia, Italy), p. 423

ADPase activity of normal and atherosclerotic human aorta intima. *du P. Heyns, A., Badenhorst, C. J., Retief, F. P.* (Department of Haematology, University of the Orange Free State, Bloemfontein 9301, Republic of South Africa), p. 429

The plasminogen activator of the arterial wall. *Donner, L., Klener, P., Roth, Z.* (Reprint requests: Prof. L. Donner, Department of Medicine, Charles University, Division of Haematology, Prague 2, Czechoslovakia), p. 436

Marathon run I: Effects on blood coagulation and fibrinolysis. *Mandalaki, T., Dessypris, A., Louizou, C., Bossinakou, I., Panayotopoulou, C., Antonopoulou, A.* (Second Regional Blood Transfusion Centre of Athens, Hospital Vassilefs Pavlovs, Athens, Greece), p. 444

Marathon run II: Effects on platelet aggregation. *Dimitriadou, C., Dessypris, A., Louizou, C., Mandalaki, T.* (Second Regional Blood Transfusion Centre of Athens, Hospital Vassilefs Pavlovs, Athens, Greece), p. 451

A method for the measurement of fibrinolytic activity based on one dimensional diffusion using small glass tubes. II. *Yasukouchi, T., Fujine, M., Kohn, S., Sakurama, S., Morioka, T., Mikami, A., Iju, M., Nakagawa, S.* (Second Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo 060, Japan), p. 456

A simple and practical method for isolation of an early plasmin degradation product of human fibrinogen. *Takagi, K., Kawai, T.* (Department of Clinical Pathology, Jichi Medical School, Tichigi-Ken, Japan), p. 464

Characterization of serum fibrinogen degradation products using gel chromatography and radioimmunoassay. *Ratky, S. M., Sykes, A., Cooke, E. D., Gordon, Y. B.* (Department of Reproductive Physiology, St. Bartholomew's Hospital Medical College, London, England), p. 471

Early venous thrombosis. A scanning electron microscopic study. *Day, T. K., Cowper, S. V., Kakkar, V. V., Clark, K. G. A.* (Kings College Hospital, London S. E. 5, England), p. 477

Use of ^{125}I-fibrinogen kinetic data to detect disseminated intravascular coagulation and deposition of fibrin in patients with metastatic cancer. *Robson, E. B., Murawski, G. F., Bettigole, R. E.* (188CC Annex, Clinical Center, E. J. Meyer Memorial Hospital, Buffalo, N. Y. 14215), p. 484

Interlaboratory oral anticoagulant quality assessment by the Netherlands Federation of Thrombosis Services. *van Dijk-Wierda, C. A., Hermans, J., Loeliger, E. A., Roos, J.* (Bureau of the Netherlands Federation of Thrombosis Services, Red Cross Hospital, The Hague, The Netherlands), p. 509

Factor VIII inhibitor associated with chlorpromazine-induced hepatic injury. *Glazier, R. L., Crowell, E. B. Jr.* (McGuire Clinic, Richmond, Va. 23229), p. 523

Fibrinolytic, factor VIII and pulse rate responses to repeated adrenaline infusion followed by haemorrhage. *Britton, B. J., Giddings, J. C., Brooks, L., Bloom, A. L.* (Nuffield Department of Surgery, Radcliffe Infirmary, University of Oxford, Oxford, England), p. 527

Spectrophotometric determination of factor Xa generation in factor IX concentrates. *Pepper, D. S., Banhegyi, D., Howie, A.* (South East of Scotland Regional Blood Transfusion Service, Royal Infirmary, Edinburgh EH3 9HB, Scotland), p. 535

Studies on the tissue thromboplastin during the coagulation-fibrinolytic process. Ultrastructural changes. *Hasegawa, H., Nagata, H., Murao, M.* (First Department of Medicine, Hokkaido University School of Medicine, Sapporo, 060, Japan), p. 541

Inherited bleeding syndromes in Iraq. *Al-Mondhiry, H. A. B.* (Department of Medicine, College of Medicine, Baghdad University, Medical City, Baghdad, Iraq), p. 549

Purification and properties of a neutral protease from soil bacterium WM 122. *Papaioannou, S. E., Marshek, W. J.* (Department of Biological Research, Searle Laboratories, Chicago, Ill. 60680), p. 556

International Committee Communications. Report of the Working Party on Animal Models. *Owe, C. A., Dodds, W. J.* (Department of Biochemistry, Mayo Clinic, Rochester, Minn. 55901), p. 566

Letters to the Editor. Diagnostic value of the thrombotest dilution curve. *Girolami, A.* (University of Padua Medical School, Institute of Semeiotica Medica, Padua, Italy), p. 568

The choice of water for coagulation tests. *Kaelin, A. C., Andes, W. A.* (Section of Hematology, Tulane University School of Medicine, New Orleans, La.), p. 570

Factor VII concentrate. *Dike, G. W. R., Griffiths, D., Tadman, A. J.* (Plasma Fractionation Laboratory, Churchill Hospital, Oxford OX3 7LJ, England), p. 572

Dilution with normal plasma in a heparin assay. *de Swart, C. A. M., Sixma, J. J., Akkerman, J. W. N., Nijmeijer, A.* (Department of Haematology, University Hospital, Utrecht, The Netherlands), p. 575

Demonstration of thrombopoietin production after plasma infusion in a patient with congenital thrombopoietin deficiency. *McDonald, T. P., Green, D.* (University of Tennessee Memorial Research Center, Tennessee and Northwestern University Medical School, Chicago, Ill.), p. 577

Platelet elastase and leukocyte elastase are two different entities. *Legrand, Y., Cane, J. P., Robert, L., Wautier, J. L.* (Unité de Recherches sur la Thrombose et l'Hémostase de l'INSERM, Hôpital Lariboisière, F-75475 Paris Cedex 10, France), p. 580

Thrombosis Research (New York) **9** (1976) No. 2

Evaluation of antithrombotic properties of suloctidil in comparison with aspirin and dipyridamole. *Gurewich, V., Lipinski, B.* (Vascular Laboratory, Lemuel Shattuck Hospital, Tufts University School of Medicine, Boston, Mass.), p. 101

Inhibitory effect of colchicine and vinblastine on reptilase clot retraction. *Kubisz, P., Cronberg, S.* (University Hospital, Oran, Algeria), p. 109

Hydrolysis of fibrinogen Paris I by plasmin. *Mosesson, M. W., Denninger, M. H., Menaché, D.* (Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 115

Effect of calcium phosphate on thrombin and on its sensitivity to heparin and

antithrombin-III. *Machovich, R.* (Postgraduate Medical School, First Department of Medicine, H-1389 Budapest, Hungary), p. 123

Flow patterns in a platelet adhesion test cell. Implications for adhesion measurement. *Day, R. C., Feuerstein, I. A., Brash, J. L.* (Department of Chemical Engineering and Pathology, McMaster University, Hamilton, Ontario, Canada L8S 4L7), p. 133

The influence of drugs on disseminated intravascular coagulation (DIC). I. Effects of antifibrinolytics and fibrinolytics on thrombin-induced DIC in rats. *Markwardt, F., Nowak, G., Meerbach, W., Rüdiger, K.-D.* (Institute of Pharmacology and Toxicology, Medical Academy, Erfurt, GDR), p. 143

Soluble fibrin complexes in coronary disease. *Anaya-Galindo, R., Shattil, S.J., Shelburne, J. C., Colman, R. W.* (Hematology-Oncology and Cardiology Sections, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104), p. 153

The identification of accelerated coagulability. *Caprini, J. A., Zuckerman, L., Cohen, E., Vagher, J. P., Lipp, V.* (Department of Surgery, Evanston Hospital and Vogelback Computing Center, Northwestern University, Medical School, Evanston, Ill.), p. 167

Potentiation of the thrombin induced platelet release reaction by fibrin. *Niewiarowski, S., Millman, M.* (Departments of Medicine and Physiology, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 181

A method for characterising serum fibrinogen and fibrin degradation products. *Lane, D. A., Scully, M. F., Kakkar, V. V.* (Thrombosis Research Unit, Department of Surgery, King's College Hospital Medical School, London, SE5 8RX, England), p. 191

Brief Communication. Daunomycin and fibrinolysis. *Bick, R. L., Murano, G., Fekete, L., Wilson, W. L.* (Bay Area Hematology Oncology Clinical and Research Laboratories, Santa Monica, Calif.), p. 201

"Low-dose heparin". Significance of subcutaneous injection. *Jaques, L. B.* (Hematosis-Thrombosis Research Unit, Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO), p. 205

The serial thromboplastin dilution test: A presumptive test for hypercoagulability. *Wolf, P., Williams, S.* (MRC External Scientific Staff and Department of Haematology, Dudley Road Hospital, Birmingham, England), p. 209

Thrombosis Research (New York) **9** (1976) No. 3

Inhibition of the activated Cls subunit of the first component of complement by antithrombin III in the presence of heparin. *Ogston, D., Murray, J., Crawford, G. P. M.* (Department of Medicine, University of Aberdeen, Aberdeen, Scotland), p. 217

Type III collagen and probably not type I collagen aggregates platelets. *Hugues, J., Hérion, F., Nusgens, B., Lapière, Ch. M.* (Department of Medicine, Section Hematology, University of Liège, Hôpital de Bavière, B-4000 Liège, Belgium), p. 223

Estrogen incubation of fibrinogen: Failure to demonstrate soluble fibrin monomer complexes. *Stemberger, A., Hafter, R., Graeff, H., Hörmann, H.* (Max-Planck-Institut für Biochemie, 8033 Martinsried/München, BRD), p. 233

Plasma heparin levels after administration of calcium and sodium salts of heparin. *Thomas, D. P., Sagar, S., Stamataksi, J. D., Maffei, F. H. A., Erdi, A., Kakkar, V. C.* (Thrombosis Research Unit, King's College Hospital Medical School, London SE5 8RX, England), p. 241

Adsorption on glass and polyethylene from solutions of fibrinogen and albumin. *Brash, J. L., Davidson, V. J.* (Departments of Chemical Engineering and Pathology, McMaster University, Hamilton, Ontario, Canada), p. 249

Digestion of amidinated and phenylglyoxal treated fibrinogen by plasmin. *Kang, E. P., Triantaphyllopoulos, D. C.* (American National Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 261

Spontaneous aggregation of low molecular weight factor VIII and its prevention by

2 mM $CaCl_2$. *Sussman, I. I., Weiss, H. J.* (Queens Hospital Center, Long Island Jewish Hillside Medical Center, Jamaica, N. Y.), p. 267

Experience of extensive vascular surgery on defibrase-defibrinogenated patients. *Olsson, P., Blombäck, M., Egberg, N., Ekeström, S.* (Thoracic Surgical Clinic, Karolinska Sjukhuset, Stockholm, Sweden), p. 277

Human thrombin: Amino acid sequence of the autocatalytically removed 13 residue Al peptide. *Walz, D. A., Hewett-Emmett, D., Reuterby, J., Seegers, W. H.* (Department of Physiology, Wayne State University, School of Medicine, Detroir, Mich.), p. 289

High levels of factor VIII. Related antigen in cadaveric blood. *Hargreaves, L. N. McF., Todd, A. S.* (Department of Pathology, University of Dundee, Dundee DD1 4HN, Scotland), p. 301

Thrombosis Research (New York) **9** (1976) No. 4

Regulation of the thrombin time in cirrhosis. *Braunstein, K. M., Kinard, H. B., Hepfer, T. W., Craig, W. R., Eurenius, K.* (Department of Medicine, Medical University of South Carolina, Charleston, S.C.), p. 309

The exclusion of the cerebral circulation: Effect on the "in vivo" platelet aggregation. *Aragno, R., Doni, M. G.* (Institute of Human Physiology, University of Padova, Padova, Italy), p. 319

Studies on the degradation of fibrinogen Gießen I. *Bleyl, H., Krause, W. H.* (Department of Clinical Chemistry and Pathobiochemistry, Justus Liebig University, 6300 Gießen, FRG), p. 329

Inhibition of human platelet aggregation by phospholipase-A. *Iatridis, S. G., Iatridis, P. G., Markidou, S. G., Ragatz, B. H.* (Departments of Physiology and Biochemistry, University of Athens, School of Medicine, Athens, Greece), p. 335

Indium-111 labeled platelets: Studies on preparation and evaluation of *in vitro* and *in vivo* functions. *Thakur, M. L., Welch, M. J., Joist, J. H., Coleman, R. E.* (Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Mo. 63110), p. 345

Studies on chemically modified fibrinogen. II. Physicochemical properties of maleylated fibrinogen. *Kloczewiak, M., Wegrzynowicz, Z., Matthias, F. R., Heene, D. L.* (Institute of Nuclear Research, 03-195 Warsaw, Poland), p. 359

Studies on human plasma antithrom bin isolated on heparin gel. *Medén-Britth, G., Miller-Andersson, M., Olsson, P.* (Surgical Research Laboratory, Thoracic Clinics, Karolinska Sjukhuset, Stockholm, Sweden), p. 369

Effect of dextran on clotting time: Steric exclusion accelerates fibrin monomer polymerisation. *Rampling, M. W., Lane, D. A., Kakkar, V. V.* (Department of Biophysics, St. Mary's Hospital Medical School, London, W. 2, England), p. 379

Factors affecting platelet cyclic GMP levels during aggregation induced by collagen and by arachidonic acid. *Davies, T., Davidson, M. M. L., McClenaghan, M. D., Say, A., Haslam, R. J.* (Department of Pathology, McMaster University, Hamilton, Ontario, Canada), p. 387

Isolation of an active derivative produced from native streptokinase by dog plasmin. *Reddy, K. N. N., Kline, D. L.* (Department of Physiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio), p. 407

Formation of prostaglandin D_2 during endoperoxide-induced platelet aggregation. *Smith, J. B., Ingerman, C. M., Silver, M. J.* (Cardeza Foundation and Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pa. 19107), p. 413

Purity and genuinity of human fibrinogen. *Godal, H. C., Kierulf, P.* (Hematological Research Laboratory, Department IX, University Clinic, Ullevål Hospital, Oslo, Norway), p. 419

Thrombosis Research (New York) **9** (1976) No. 5

Interaction between factor XII (Hageman factor), high molecular weight kininogen and prekallikrein. *Chan, J. Y. C., Habal, F. M., Burrowes, C. E., Movat, H. Z.* (Division of Experimental Pathology, Department of Pathology, University of Toronto, Toronto, Ontario M55 1A8, Canada), p. 423

Effect of diet-induced hyperlipidemia on *in vitro*-function of rabbit platelets. *Joist, J. H., Dolezel, G., Kinlough-Rathbone, R.* (Department of Pathology, McMaster University, Hamilton, Ontario, Canada), p. 435

Studies on cold insoluble globulin in dermatological patients. I. Immunochemical quantitation in citrated plasma from patients with increased amounts of heparin precipitable fraction (HPF). *Fyrand, O., Solum, N. O.* (Institute for Thrombosis Research, University of Oslo, Rikshospitalet, Oslo, Norway), p. 447

Inactivation and binding of human plasma kallikrein by antithrombin III and heparin. *Venneröd, A. M., Laake, K., Solberg, A. K., Strömland, S.* (Department of Pharmacology, Institute of Pharmacy, University of Oslo, Oslo, Norway), p. 457

In vivo platelet function in diabetes mellitus. *Fleischmann, A. I., Bierenbaum, M. L., Stier, A., Somol, H., Watson, P. B.* (Division of Community Health Service, New Jersey State Department of Health, Montclair, N. J.), p. 467

Cross-immuno reactivities of mammalian fibrinogens. *Lewis, J. H.* (Department of Medicine, University of Pittsburgh, Pittsburgh, Pa)., p. 473

Isolation of residual fibrinogen from a supernatant of plasma precipitated with 2.1 M glycine. *Jilg, W., Priess, H., Hörmann, H.* (Max-Planck-Institut für Biochemie, München-Martinsried, BRD), p. 479

Platelet-thrombin interactions as assessed by affinity chromatography. *Workman, E. F. Jr., White, G. C. II., Lundblad, R. L.* (Department of Pathology, Medicine and Biochemistry, University of North Carolina, Chapel Hill, N. C. 27514), p. 491

The effect of leucocytic pyrogen on rabbit fibrinogen. A third view. *Bocci, V., Muscettola, M., Pacini, A., Pessina, G. P.* (Istituto di Fisiologia Generale, Università di Siena, 53100 Siena, Italy), p. 505

An experimental model of *in vivo* fibrinolysis. *Sasaki, Y., Morita, T.* (Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., Toda-shi, Saitama, Japan), p. 513

No effect of factor Xa or tissue thromboplastin and factor VII on the activation of plasminogen. *Wiman, B., Holm, T., Prydz, H.* (Department of Medical Chemistry, University of Umea, Umea, Sweden), p. 523

On the characterization of acquired inhibitors to Ristocetin induced platelet aggregation found in patients with von Willebrand's disease. *Egberg, N., Blombäck, M.* (Blood Coagulation Laboratory, Karolinska Sjukhuset, Stockholm, Sweden), p. 527

Thrombosis Research (New York) **9** (1976) No. 6

The *in vivo* increase of factor VIII activity by 2,3-diphosphoglycerate. *Iatridis, P. G., Iatridis, S. G., Ragatz, B. H.* (Department of Physiology, Northwest Center for Medical Education, Indiana University School of Medicine, Gary, Ind. 46408), p. 533

Cryofibrinogen in the plasma of patients with skin ulcerative lesions on the legs: A complex of fibrinogen and cold insoluble globulin. *Matsuda, M., Saida, T., Hasegawa, R.* (Hemostasis and Thrombosis Research Division, Institute of Hematology, Jichi Medical School, Tochigi-Ken, Tokyo, Japan), p. 541

Simultaneous aggregation and release from platelets monitored continuously in plasma. *Kronick, P. L., Jimenez, S. A.* (Franklin Institute Research Laboratories, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.), p. 553

Purification and activation of human factor X: Cooperative effect of Ca^{++} on the activation reaction. *Kosow, D. P.* (American National Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 565

Anticoagulant properties of heparin fractionated by affinity chromatography on matrix-bound antithrombin III and by gel filtration. *Andersson, L.-O., Barrowcliffe, T. W., Holmer, E., Johnson, E. A., Sims, G. E. C.* (National Institute for Biological Standards and Control, Holly Hill, London, England), p. 575

Antagonism by Suloctidil of arterial thrombus formation in rats. *Roba, J., Bourgain, R., Andries, R., Claeys, M., Van Opstal, W., Lambelin, G.* (Continental PHARMA, Research Laboratories, B-1830 Machelen, Belgium), p. 585

The interaction of platelets with a tritium-labelled heparin. *Shanberge, J. N., Kambayashi, J., Nakagawa, M.* (Department of Pathology, Mount Sinai Medical Center, Milwaukee, Wis. 53201), p. 595

Effect of brinase (protease I from *Aspergillus oryzae*) on the elimination of fibrin from the lungs and pulmonary damage in rats with induced intravascular coagulation and inhibited fibrinolysis. *Diffang, C., Saldeen, T.* (Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden), p. 611

Influences on the formation and structure of fibrin. *Carlin, G., Wik, K. O., Arfors, K.-E., Saldeen, T., Tangen, O.* (Department of Experimental Medicine, Pharmacia AB, Uppsala University, Uppsala, Sweden), p. 623

Synthetic inhibitors of serine proteinases. XIV. Inhibition of factor Xa by derivatives of benzamidine. *Stürzebecher, I., Markwardt, F., Walsmann, P.* (Institute of Pharmacology and Toxicology, Medical Academy, Erfurt, GDR), p. 637

Aspirin effects on human platelets: A scanning electron microscope study. *Lewis, J. C., Hagedorn, A. B.* (Department of Pathology and Anatomy, Mayo Clinic and Mayo Foundation, Rochester, Minn. 55901), p. 647

Influence of thrombin-induced disseminated intravascular coagulation on RES functions in rabbits. *Lahnborg, G., Berghem, L., Lagergren, H., Schildt, B.* (Department of Surgery, Serafimerlasarettet, S-112 83 Stockholm, Sweden), p. 653

Smoking and platelet labile aggregation stimulating substance (LASS) synthesizing activity. *Horns, D. J., Gerrard, J. M., Rao, G. H. R., Krivit, W., White, J. G.* (Department of Pediatrics, University of Minnesota Medical Center, Minneapolis, Minn. 55455), p. 661

Comparison of methods of measuring loss of cytoplasmic constituents from platelets. *Kinlough-Rathbone, R. L., Packham, M. A., Mustard, J. F.* (Department of Pathology, McMaster University, Hamilton, Ontario, Canada), p. 669

The effects of inhibitors on the catalytic conversion of urokinase. *Soberano, M. E., Ong, E. B., Johnson, A. J.* (Department of Medicine and Pathology, New York University School of Medicine, New York, N. Y. 10016), p. 675

Thrombosis Research (New York) **10** (1977) No. 1

Release of platelet factor 4 (PF4) measured by a Polybrene assay in response to *in vitro* platelet damage. *Levine, Sh. P., Wohl, H., Marzec, U., Bernstein, E. F., Kroener, J.* (Departments of Medicine and Surgery, University of California, San Diego, Calif.), p. 1

Mechanical trauma effect on clot structure formation. *Glover, C. J., McIntire, L. V., Brown, C. H. III, Natelson, E. A.* (Reprint requests: Dr. L. V. McIntire, Biomedical Engineering Laboratory, Rice University, Houston, Texas 77001), p. 11

The use of an immunoradiometric assay for factor VIII related antigen in the study of atypical von Willebrand's disease. *Peake, I. R., Bloom, A. L.* (Department of Haematology, University Hospital of Wales, Cardiff, Wales, United Kingdom), p. 27

Effects of trypsin on the platelet-aggregating activity of mouse tumor cells. *Gasic, G. J., Gasic, T. B., Jimenez, S. A.* (Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19174), p. 33

Effect of platelet antiserum on the activation of intravascular coagulation by endotoxin. *Kramer, W., Müller-Berghaus, G.* (Department of Medicine, Justus Liebig-Universität, Giessen, FRG), p. 47

Human platelet glycoproteins. Further evidence that the "GP I band" from whole platelets contains three different polypeptides one of which may be involved in the interaction between platelets and factor VIII. *Solum, N. O., Hagen, I., Peterka, M.* (Institute for Thrombosis Research, University of Oslo, Rikshospitalet, Oslo, 1, Norway), p. 71

^{3}H-heparin and antithrombin III in the isolated liver perfusion. *Losito, R., Barlow, G., Lemieux, E.* (Department of Medicine, Centre Hospitalier Universitaire, Sherbrooke, P. Q., Canada), p. 83

Effects of clofibrate on platelets and evidence of the involvement of platelet lipids in platelet function. *Huzoor-Akbar, Ardlie, N. G.* (Department of Clinical Science,

The John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T. 2601, Australia), p. 95

The ratio of factor VIII-related antigen and factor VIII biological activity as an index of hypercoagulability and intravascular clotting. *Denson, K. W. E.* (John Radcliffe Hospital, Oxford, England), p. 107

Localization and distribution of fibrinolysis inhibition in the walls of human arteries and veins. *Hegt, V. N.* (Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands), p. 121

The effects of pCO_2 and pH on platelet shape change and aggregation for human and rabbit platelet-rich plasma. *Tang, S. S., Frojmovic, M. M.* (Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6), p. 135

Independence of intravascular generation of fibrin monomer and fibrin deposition on canine jugular veins. *Stewart, G. J., Lynch, P. R.* (Specialized Center for Thrombosis Research, Departments of Medicine and Physiology, Temple Health Sciences Center, Philadelphia, Pa. 19140), p. 147

Antithrombin III immunoprecipitation pattern. *Mackie, I., Howarth, D., Brozović, M.* (Coagulation Laboratory, Northwick Park Hospital, Harrow, Middlesex, England), p. 159

Effect of exercise on F VIII-complex: Proportional increase of ristocetin cofactor (von Willebrand factor) and F VIII-AGN, but disproportional increase of F VIII-AHF. *Stibbe, J.* (Division of Haemostasis and Thrombosis Research, Department of Haematology, University Hospital Rotterdam-Dykzigt, Rotterdam, The Netherlands), p. 163

Platelet factor 4-like activity in the initial stages of atherosclerosis in pigeons. *Fuster, V., Lewis, J. C., Kottke, B. A., Ruiz, C. E., Bowie, E. J. W.* (Mayo Clinic and Mayo Foundation, Rochester, Minn.), p. 169

Skin fibroblasts from a patient with Glanzmann's thrombasthenia do not induce clot retraction. *Donati, M. B., Balconi, G., Remuzzi, G., Borgia, R., Morasca, L., de Gaetano, G.* (Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy), p. 173

The D : E complex as a discrete plasmic cleavage product of fibrinogen. *Plow, E. F., Cierniewski, C., Edgington, T. S.* (Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037), p. 175

Reappraisal of the gel sieving properties of plasma protransglutaminase (factor XIII). *Bannerjee, D., Mosesson, M. W.* (Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 183

Thrombin time prolongation in cirrhosis in relation to enhanced fibrinogen degradation. *Lipinski, B., Gurewich, V., Lipinska, I.* (Vascular Laboratory, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, Mass. 02135), p. 185

Thrombosis Research (New York) **10** (1977) No. 2

Roles of polymer and ε-amino groups in polylysine-platelet interaction. *Mohammad, S. F., Chuang, H. Y. K., Mason, R. G.* (Department of Pathology, Memorial Hospital, Pawtucket, R. I.), p. 193

Production and properties of *Staphylococcus aureus* (strain Newman D_2C) with uniform clumping factor activity. *Miller, K. D., Hetrick, D. L., Bielefeldt, D. J.* (Departments of Medicine and Microbiology, University of Miami, School of Medicine, Miami, Fla. 33152), p. 203

The specific interaction between plasminogen and fibrin. A physiological role of the lysine binding site in plasminogen. *Wiman, B., Wallén, P.* (Department of Medical Chemistry, Umeå University, S-901 87 Umeå, Sweden), p. 213

The purification of human acarboxy prothrombin. Characterization of its derivatives after thrombin cleavage. *Guillin, M.-C., Aronson, D. L., Bezeaud, A., Menaché, D., Schlegel, N., Amar, M.* (Service Central d'Immunologie et Hématologie, Hôpital Beaujon, 92110 Clichy, France), p. 223

Deep venous thrombosis and multiple regression analysis of the influence from various clinical factors on the fibrinogen half-life in surgical patients, using ^{125}I-fibrinogen. *Forsberg, K., Törngren, S.* (Division of Hematology, Karolinska Hospital, Stockholm, Sweden), p. 235

Solution stability of bovine fibrinogen. *Weathersby, P. K., Horbett, T. A., Hoffman, A. S.* (Department of Chemical Engineering, University of Washington, Seattle, Wash. 98195), p. 245

An abnormal fibrinogen in a Venezuelan family. *de Bosch, N. B., Arocha-Piñango, C. L., Soria, J., Soria, C., Rodríguez, A., Rodríguez, S.* (Banco Municipal de Sangre, Caracas, Venezuela, South America), p. 253

The activation of factor X evaluated by using synthetic substrates. *Suomela, H., Blombäck, M., Blombäck, B.* (Finnish Red Cross Transfusion Service, Helsinki, Finland), p. 267

The effect of a new non-steroidal anti-inflammatory agent, sulindac, on platelet function. *Green, D., Given, K. M., Chunghsin Ts'ao, Whipple, J. P., Rossi, E. C.* (Sections of Hematology, Northwestern University Medical School, Chicago, Ill.), p. 283

Heparin-induced platelet aggregation in burn patients. *Mims, J. A., Sarji, K. E., Kleinfelder, J. S., Eurenius, K.* (Department of Medicine, Medical University of South Carolina, Charleston, S. C. 29401), p. 291

Effect of thrombin and ADP on a membrane bound cyclic and dependent kinase of human platelets. Studies on normal platelets and those from patients with von Willebrand's disease. *Smith, D., Damus, P. S.* (Center for the Health Sciences, University of California at Los Angeles, Los Angeles, Calif.), p. 301

Activation of factor XII (Hageman factor): Enhancing effect of a potentiator. *Chan, J. Y. C., Burrowes, C. E., Movat, H. Z.*, (Division of Experimental Pathology, Department of Pathology, University of Toronto, Toronto, Ontario, M55 1A8, Canada), p. 309

Characteristics and thrombogenicity of prothrombin complex concentrates. *Sakuragawa, N., Takahashi, K., Hoshiyama, M., Niiya, K., Itoh, M., Matsuoke, M., Ohnishi, Y.* (Department of Medicine, Niigata University School of Medicine, Niigata, Japan), p. 315

Contact activation of factor XI: Evidence that the primary role of contact activation cofactor (CAC) is to facilitate the activation of factor XII. *Schiffman, S., Pecci, R., Lee, P.* (Department of Medicine and Biochemistry, University of Southern California Medical School, Los Angeles, Calif. 90033), p. 319

Thrombosis Research (New York) **10** (1977) No. 3

Platelet function in sickle cell anemia. *Gruppo, R. A., Glueck, H. I., Granger, S. M., Miller, M. A.* (Departments of Pediatrics and Medicine, University of Cincinnati College of Medicine, Cincinnati General Hospital, Cincinnati, Ohio), p. 325

Presence of a low molecular weight plasma fibrinogen stimulator and inhibitor in human urine. *Wentland, S. H., Carlson, T. H., Leonard, B. D., Fradl, D. C., Reeve, E. B.* (University of Colorado Medical Center, Denver, Col. 80262), p. 337

Polyphloretine phosphatase as an inhibitor of platelet adhesion. Comparison with acetylsalicylic acid and dipyridamole. *Olsson, P., Schalin, L., Lagergren, H., Swedenborg, J.* (Surgical Research Laboratory, Thoracic Clinics, Karolinska Hospital, S-104 01 Stockholm, Sweden), p. 349

Affinity chromatography and quantitation of soluble fibrin from plasma. *Matthias, F. R., Reinicke, R., Heene, D. L.* (Department of Medicine, Justus Liebig University, Giessen, FRG), p. 365

The activity of γ-linolenate and dihomo-γ-linolenate methyl esters *in vitro* and *in vivo* on blood platelet function in non-human primates and in man. *Sim, A. K., McCraw, A. P.* (Division of Biochemistry, Inveresk Research International, Musselburgh, Midlothian, Scotland), p. 385

Evaluation of an amidolytic heparin assay method: Increased sensitivity by adding purified antithrombin III. *Teien, A. N., Lie, M.* (Medical Department A, Aker Hospital, Oslo, Norway), p. 399

Degradation of human fibrinogen by plasmin: A kinetic study. *Regañon, E., Aznar, J., Vila, V.* (Department of Clinical Pathology, Ciudad Sanitaria La Fe, Valencia, Spain), p. 411

The formation of a fibrinogen-activator complex by the action of streptokinase. *Chesterman, C. N., Cederholm-Williams,*

S. A., Allington, M. J., Sharp, A. A. (Department of Medicine, St. Vincent's Hospital, Fitzroy 3065, Australia), p. 421

Dissociation of human factor VIII by *Rhizopus lipase. Furlan, M., Jakab, T., Beck, E. A.* (Central Hematology Laboratory, Inselspital, Bern, Switzerland), p. 431

Lipolytic versus proteolytic degradation of human factor VIII. *Furlan, M., Jakab, T., Beck, E. A.* (Central Hematology Laboratory, Inselspital, Bern, Switzerland), p. 445

The extract from the tissue of gastric cancer as procoagulant in disseminated intravascular coagulation syndrome. *Sakuragawa, N., Takahashi, K., Hoshiyama, M., Jimbo, C., Ashizawa, K., Matsuoka, M., Ohnishi, Y.* (Department of Medicine and Department of Pathology, Niigata University School of Medicine, Niigata, Japan), p. 457

The relationship between shape change and release reaction of lectin-stimulated platelets. Effects of cytochalasin B and prostaglandin E_1. *Patscheke, H., Wörner, P., Brossmer, R.* (Institut für Biochemie II, Universität Heidelberg, Heidelberg, BRD), p. 465

Evidence that platelet density and volume are not related to aging. *Boneau, B., Corberand, J., Plante, J., Bierme, R.*, (Laboratoire d'Hémostase, Centre de Transfusion Sanguine, 3210-31052 Toulouse Cédex, France), p. 475

Thrombin-like enzyme from the venom of *Crotalus adamanteus* (Eastern diamondback rattlesnake). *Markland, F. S., Pirkle, H.* (Department of Biochemistry and Cancer Research Institute, University of Southern California, School of Medicine, Los Angeles, Calif. 90033), p. 487

A new method for the preparation of artificial factor II reagents from normal human and bovine plasma. *Vermeer, C., Soute, B. A. M., Hemker, H. C.* (Department of Biochemistry, State University Limburg, Maastricht, The Netherlands), p. 495

Disordered "fibrinolytic potential" in coronary heart disease. *Walker, I. D., Davidson, J. F., Hutton, I., Lawrie, T. D. V.* (Department of Haematology and University Department of Medical Cardiology, Glasgow Royal Infirmary, Glasgow, Scotland), p. 509

Effect of the calcium antagonist TMB-6 on intracellular calcium redistribution associated with platelet shape change. *Le Breton, G. C., Dinerstein, R. J.* (Department of Pharmacology, University of Illinois College of Medicine, Chicago, Ill. 60612), p. 521

Effect of Suloctidil on bleeding time and *in vivo* platelet aggregation in rats. *de Gaetano, G., Cavenaghi, A. E.* (Laboratory of Cardiovascular Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy), p. 525

Effect of Brinase and thrombolysin on the electrochemical nature of the cardiovascular system of experimental animals (dogs and rabbits). *Sawyer, P. N.* (Electrochemical and Biophysical Laboratories, Department of Surgery and Surgical Research, State University of New York, Downstate Medical Center, Brooklyn, N. Y. 11203), p. 531

Thrombosis Research (New York) **10** (1977) No. 4

Effect of the portacaval shunt in the rat on the *in vivo* platelet responsiveness to ADP. *Doni, M. G., Aragno, R., Vassanelli, P.* (Institute of Human Physiology and Surgical Pathology, University of Padova, Padova, Italy), p. 539

Problems in the assay of thrombin using synthetic peptides as substrates. *Gaffney, P. J., Lord, K., Brasher, M., Kirkwood, T. B. L.* (National Institute for Biological Standards and Control, Holly Hill, London NW3 6RB, England), p. 549

Radiolabel incorporation associated with tissue factor production in cultured human fibroblasts and smooth muscle cells *Pitlich, F. A., Valentyn-Benz, M.* (Departments of Internal Medicine and Molecular Biophysics and Biochemistry, Yale Medical School, New Haven, Conn. 06510), p. 557

Composition of soluble fibrin complexes in pre-eclampsia. *Edgar, W., McKillop, C., Howie, P. W., Prebtice, C. R. M.* (University Department of Medicine, Royal Infirmary, Glasgow, G4 OSF, Scotland), p. 567

Fragments D. Correlation between structure and biological activity. *Dray-Attali, L.,*

Larrieu, M. J. (Department of Coagulation, Institut de Pathologie Cellulaire, Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France), p. 575

Evaluation of three methods used to identify accelerated coagulability. *Cohen, E., Caprini, J., Zuckerman, L., Vagher, P., Robinson, B.* (Vogelback Computing Center, Northwestern University and Evanston Hospital, Evanston, Ill.), p. 587

Inhibition of fibrinolytic activity of plasmin by suramin (Antrypol) and Trypan Blue. *Moroz, L. A.* (Harry Webster Thorp Laboratories, McGill University Clinic, Montreal, Quebec, Canada H3A 1A1), p. 605

Immunofluorescent detection of α_1-antitrypsin in platelets and megakaryocytes. *Nalli, G., Cattaneo, G., Malamani, G. D., Majolino, I., Fornasari, P. M., Almasio, P., Piovella, F., Ascari, E.* (Department of Clinical Medicine "A. Ferrata", University of Pavia, Policlinico S. Matteo, 27100 Pavia, Italy), p. 613

Bleeding time in rats is prolonged by aspirin. *Minsker, D. H., Kling, P. J.* (Merck Institute for Therapeutic Research, West Point, Penn. 19486), p. 619

A tanned red cell haemagglutination immuno assay for the estimation of antibodies directed against defibrase. *Soria, J., Soria, C., Vinazzer, H., Stocker, K.* (Haematology Department, Hôtel-Dieu, Paris 4, France), p. 623

Quantitation of *in vivo* platelet aggregation by platelet aggregate ratio method. *Burns, T. S., Saunders, R. N.* (Department of Biological Research, Searle Laboratories, Chicago, Ill. 60680), p. 629

Thrombosis Research (New York) **10** (1977) No. 5

Procoagulant activity of thrombosthenin preparations. *Muszbek, L., Fésüs, L., Szabó, T., Hársfalvi, J.* (Department of Pathophysiology, University Medical School, H-4012 Debrecen, Hungary), p. 635

The inactivation of thrombin and plasmin by antithrombin III in the presence of Sepharose-heparin. *Hatton, M. W. C., Regoeczi, E.* (Plasma Protein Research Laboratory, McMaster University Medical Centre, Hamilton, Canada L8S 4J9), p. 645

Action of insolubilized homologous anti-factor VIII antibodies on factor VIII-related antigen. *Mazurier, C., Kacem, M., Parquet-Gernez, A., Goudemand, M.* (Laboratoire d'Hémostase, Centre Régional de Transfusion Sanguine, 59000 Lille, France), p. 661

Studies on tissue culture produced plasminogen activator. I. Preliminary observations and the enhancing effect of colchicine and other antimitotic agents. *Hull, R. N., Cherry, W. R., Huseby, R. M., Clavin, S. A.* (Lilly Research Laboratories, Indianapolis, Ind. 46206), p. 669

Studies on tissue culture plasminogen activator. II. The detection and assay of urokinase and plasminogen activator from LLC-PK$_1$ cultures (porcine) by the synthetic substrate Nα-benzyloxycarbonyl-glycyl-glycylarginyl-4-methoxy-2-naphthylamide. *Huseby, R. M., Clavin, S. A., Smith, R. E., Hull, R. N., Smithwick, E. L. Jr.* (Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind. 46202), p. 679

Human fibrinogen. Amino acid sequence of fragment E and of adjacent structures in the Aα- and Bβ-chains. *Gårlund, B.* (Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden), p. 689

Incorporation of ^{125}I-fibrinogen in circulating soluble fibrin monomer complexes during hypercoagulability. *von Hugo, R., Hafter, R., Stein, B., Stemberger, A., Rjosk, H., Graeff, H.* (I. Frauenklinik der Universität, 8000 München 2, BRD), p. 703

Estimation and characterization of soluble fibrin monomer complexes during endotoxin-induced intravascular coagulation. *Hafter, R., Müller-Berghaus, G., von Hugo, R., Graeff, H.* (I. Frauenklinik der Universität, 8000 München 2, BRD), p. 711

Antithrombin III (AT-III) as a diagnostic aid in disseminated intravascular coagulation. *Bick, R. L., Dukes, M. L., Wilson, W. L., Fekete, L. F.* (Bay Area Hematology-Oncology Medical Group, 901 Santa Monica, Calif. 90404), p. 721

Comparison of precipitating antisera against normal and abnormal factor IX. *Ørstavik, K. H.* (Institute of Medical Genetics,

University of Oslo, Blindern, Oslo 3, Norway), p. 731

Collagen-induced platelet aggregation: Evidence of several mechanisms for the induction of platelet release by collagen. *Nyman, D.* (Department of Blood Coagulation Disorders, Karolinska Institutet, Stockholm, Sweden), p. 743

Threshold phenomena in blood fibrinolysis. *Matsuo, O., Mihara, H.* (Department of Physiology, Miyazaki Medical College, Miyazaki, Japan), p. 753

Production of plasminogen activator activity in factor XII-deficient plasma. *Kluft, X., Jenks, R. L., Astrup, T.* (Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands), p. 759

Calcium dependent and independent release of plasminogen activator from the vascular wall. *Izaki, S., Kitaguchi, H.* (Department of Physiology, Kobe University School of Medicine, Kobe, Japan), p. 765

Further comments on the failure of collagen to activate factor XII. *Zacharski, L. R., Rosenstein, R.* (Veterans Administration Hospital, White River Junction, VT 05001, Hanover, N. H. 03755), p. 771

Prolonged thrombin clotting times in liver disease. *Lane, D. A., Scully, M. F., Kakkar, V. V.* (Thrombosis Research Unit, King's College Hospital Medical School, London SE5 8RX, England), p. 773

Thrombosis Research (New York) **10** (1977) No. 6

Intermediate product I of prothrombin proteolysis as a natural factor of the anticoagulating system stimulation. *Kudrjashov, B. A., Yurieva, G. Yu., Strukova, S. M., Solodenko, N. M., Lyapina, L. A.* (Department of Human and Animal Physiology, Moscow State University, Moscow, U. S. S. R.), p. 775

Influence of dihydroergotoxine on platelet aggregation in rabbits. *Markwardt, F., Glusa, E., Barthel, W.* (Institute of Pharmacology and Toxicology Medical Academy, Erfurt, GDR), p. 783

The influence of metabolites on the assay of platelet serotonin. *Rao, G. H. R., Friedland, M., Gerrard, J. M., White, J. G.* (Departments of Pediatrics and Laboratory Medicine and Pathology, University of Minnesota Medical Center, Minneapolis, Minn. 55455), p. 791

Protective effect of calcium in the plasmin degradation of fibrinogen and fibrin fragments D. *Haverkate, F., Timan, G.* (Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands), p. 803

Inactivation of α- and β-thrombin by heat defibrinated human plasma. Effect of heparin. *Orakzai, S. A., Machovich, R.* (Reprint requests: Dr. R. Machovich, Postgraduate Medical School, First Department of Medicine, 1389 Budapest, Hungary), p. 813

Partial purification and some properties of the tissue plasminogen activator from the human myometrium. *Soszka, T.* (Institute of Obstetrics and Gynecology, Medical School, Białystok, Poland), p. 823

Effects of sulphinpyrazone and dipyridamole on capillary bleeding time in man. *Weston, M. J., Rubin, M. H., Langley, P. G., Westaby, S., Williams, R.* (King's College Hospital and Medical School, London, SE5, England), p. 833

On the interaction between ristocetin and the ristocetin cofactor (RCOF). *Floyd, M., Burns, W., Green, D.* (Reprint requests: Dr. D. Green, Department of Medicine, Northwestern University Medical School, Chicago, Ill. 60611), p. 841

Inhibition of plasminogen binding to fibrin by α_2-plasmin inhibitor. *Moroi, M., Aoki, N.* (Institute of Hematology and Department of Medicine, Jochi Medical School, Yakushiji, Tochigi-ken 329-04, Japan), p. 851

A heparin-degrading endoglycosidase from rat spleen. *Höök, M., Pettersson, I., Ögren, S.* (Department of Medical and Physiological Chemistry, Royal Veterinary College, Biomedical Center, S-751 23 Uppsala, Sweden), p. 857

Thrombocytin. A novel platelet activating enzyme from *Bothrops atrox* venom. *Niewiarowski, S., Kirby, E. P., Stocker, K.* (Specialized Center for Thrombosis Research, Departments of Medicine, Physiology and Biochemistry, Temple University Health Sciences Center, Philadelphia, Pa. 19140), p. 863

Transfusion (Philadelphia) **16** (1976) No. 5

Blood collection and use by AABB institutional members (1974). *Hemphill, B. M.* (AABB, San Francisco, Calif.), p. 393

The phenotypes En(a–), Wr(a–b–), and En(a+), Wr(a+b–), and further studies on the Wright and En blood group system. *Issitt, P. D., Pavone, B. G., Wagstaff, W., Goldfinger, D.* (Paul I. Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio 45267), p. 396

Identification of a C4 subcomponent on C3d-coated erythrocytes. *Michael, J. M., Moore, J. A., Chaplin, H. Jr.* (Washington University School of Medicine, St. Louis, Mo. 63110), p. 408

Hemolytic transfusion reactions caused by failure of commercial antiglobulin reagents to detect complement. *Sherwood, G. K., Haynes, B. F., Rosse, W. F.* (Duke University Medical Center, Durham, N. C.), p. 417

Hemagglutination inhibition studies of water soluble blood group substances recovered from the erythrocytes of classical Bombay O_h subjects. *Vos, G. H., Moores, P. P.* (Experimental Immunology Unit, Natal Institute of Immunology, Durban, South Africa), p. 421

Characterization of three new apparently related high frequency antigens. *Well, R. F., Korn, G., Hafleigh, B., Grumet, F. C.* (Transfusion Service, Stanford University Hospital, Stanford, Calif. 94305), p. 427

Serologic activity of fatty acid dependent antibodies in albumin-free systems. *Beck, M. L., Edwards, R. L., Pierce, S. R., Hicklin, B. L., Bayer, W. L.* (Community Blood Center, Greater Kansas City, Kansas City, Mo. 64111), p. 434

A standardized technique for efficient platelet and leukocyte collection using the model 30 blood processor. *Aisner, J., Schiffer, C. A., Wolff, J. H., Wiernik, P. H.* (National Cancer Institute, Baltimore Cancer Research Center, Baltimore, Md. 21201), p. 437

Blood preservation using metabolic regulators and nutrients: XXI. Further studies on pyruvate and DHA (dihydroxyacetone). *Dawson, R. B.* (Blood Bank and Transfusion Service, Department of Pathology, University of Maryland Hospital Blood Bank, Baltimore, Md.), p. 446

Blood storage. XXII. Improvement in red blood cell 2,3-DPG levels at six weeks by 20 mM PO_4 in CPD-adenine-inosine. *Dawson, R. B.* (Blood Bank and Transfusion Service, Department of Pathology, University of Maryland Hospital Blood Bank, Baltimore, Md.), p. 450

Plateletpheresis residues: A source of large quantities of human blood lymphocytes. *Segel, G. B., Lichtman, M. A., Gordon, B. R., MacPherson, J. L., Nusbacher, J.* (Departments of Pediatrics, Medicine, Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, N. Y.), p. 455

Fibrinolysis and disseminated intravascular coagulation in open heart surgery. *Umlas, J.* (Harvard Medical School, Department of Pathology, Mount Auburn Hospital, Cambridge, Mass. 02138), p. 460

Use and analysis of saline washed red blood cells. *O'Connor Wooten, M. J.* (Wesley Medical Center, Wichita, Ka. 67214), p. 464

A comparison of a low ionic strength saline medium with routine methods for antibody detection. *Wicker, B., Wallas, C. H.* (University of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City, Ka. 66103), p. 469

Transfusion-induced malaria from an asymptomatic carrier. *Najem, G. R., Sulzer, A. J.* (Department of Preventive Medicine and Community Health, New Jersey Medical School, Newark, N. J. 07103), p. 473

Sterile connector. *Tenczar, F. J.* (Anatomic and Clinical Pathology, Ltd., Evergreen Park, Ill.), p. 477

Problems in immunohematology. No. 2. *Wiener, A. S.* (64, Rutland Road, Brooklyn, N. Y.), p. 483

Transfusion (Philadelphia) **16** (1976) No. 6

A comparison of methods to wash liquid-stored red blood cells and red blood cells frozen with high or low concentrations of glycerol. *Contreras, T. J., Valeri, C. R.* (Naval Blood Research Laboratory, Boston, Mass. 02118), p. 539

Development and use of pediatric frozen red cell packs. *Staples, J. W., Fritz, G. E.* (Pacific Northwest Red Cross, Portland, Ore. 97207), p. 566

Metabolic changes during platelet storage. *Rock, G., Figueredo, A.* (Canadian Red Cross BTS, Ottawa, Ontario K1S 3E2 Canada), p. 571

Quantitative and thermodynamic studies of erythrocytic AB0 antigens. *Salmon, Ch., Lopez, M., Cartron, J. P., Bouguerra, A.* (National Blood Transfusion Centre, Paris, France), p. 580

Removal of HB_sAg from blood *in vitro*. I. Effects of washing alone, glycerol addition and removal, and glycerolization, freezing, and washing. *Contreras, T. J., Valeri, C. R.* (Naval Blood Research Laboratory, Boston, Mass. 02118), p. 594

Preferential decrease in thymus dependent lymphocytes during storage at 4 C in anticoagulant. *Grunow, J. E., Lubet, R. A., Ferguson, M. J., Gaulden, M. E.* (Department of Pediatrics, University of Oklahoma Medical School, Oklahoma City, Oklahoma), p. 610

Preparation of cryoprecipitated factor VIII concentrates. *Slichter, S. J., Counts, R. B., Henderson, R., Harker, L. A.* (Reprint requests: Dr. L. A. Harker, Division of Hematology, Harborview Medical Center, Seattle, Wash. 98104), p. 616

Freezing and deglycerolizing sickle-trait red blood cells. *Meryman, H. T., Hornblower, M.* (American National Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 627

A second example of anti-Lu 14. *Marsh, W. L., Øyen, R., Gruber, D., Rosso, M., Cooper, J.* (Lindsley F. Kimball Research Institute of the New York Blood Center, New York, N. Y. 10021), p. 633

The use of frozen blood in neonatal exchange transfusion. *Umlas, J., Gootblatt, S.* (Mount Auburn Hospital, Cambridge, Mass.), p. 636

Cryoprecipitates as a source of fibrinogen in treatment of disseminated intravascular coagulation (DIC). *Hattersley, P. G., Kunkel, M.* (University of California at Davis, Sacramento Medical Center, Sacramento, Calif. 95817), p. 641

Clinical importance of the rare erythrocyte antibody anti-Jr^a. *Kendall, A. G.* (Transfusion Service, Royal Victoria Hospital, Montreal 112, P. Q. Canada), p. 646

Transfusion (Philadelphia) **17** (1977) No. 1

The purification of red cells for transfusion by freeze-preservation and washing. V. Red cell recovery and residual leukocytes after freeze-preservation with high concentrations of glycerol and washing in various systems. *Crowley, J. P., Wade, P. H., Wish, C., Valeri, C. R.* (USNR, Naval Blood Research Laboratory, Boston, Mass. 02118), p. 1

Viability and function of platelets frozen at 2 to 3 C per minute with 4 or 5 per cent DMSO and stored at −80 C for 8 months. *Spector, J. I., Yarmala, J. A., Marchionni, L. D., Emerson, C. P., Valeri, C. R.* (Naval Blood Research Laboratory, Boston, Mass. 02118), p. 8

A method for radioactive antiglobulin testing with ^{125}I labeled anti-IgG. *Jenkins, D. E. Jr., Moore, W. H., Hawiger, A.* (Nashville Regional Red Cross Blood Center, Nashville, Tenn. 37203), p. 16

A simple method for the cryopreservation of human lymphocytes at −80 C. *Simon, J. D., Flinton, L. J., Albala, M. M.* (Division of Clinical Hematology, Rhode Island Hospital, Providence, R. I. 02902), p. 23

Anti-A_1 in the plasma of platelet concentrates causing a hemolytic reaction. *Zoes, C., Dube, V. E., Miller, H. J., Vye, M. V.* (Evanston Hospital, Blood Bank, Evanston, Ill. 60201), p. 29

Clinical value of washed-platelet concentrates in patients with non-hemolytic transfusion reactions. *Silvergleid, A. J., Hafleigh, E. B., Harabin, M. A., Wolf, R. M., Grumet, F. C.* (Stanford University School of Medicine, Palo Alto, Calif. 94304), p. 33

The uptake and egress of adenine from human red blood cells *in vitro*. *Moore, G. L. Ledford, M. E.* (Blood Research Division, Department of Surgery, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 38

Detection of HBsAG in coagulation factor IX-concentrates. *O'Connor, M. L., Meek, E. S., Simmons, A.* (University of Iowa

Hospitals and Clinics, Iowa City, Iowa 52242), p. 44

Independence of Wright from many other blood group systems. *Pavone, B. G., Issitt, P. D., Wagstaff, W.* (Paul I. Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio 45267), p. 47

Variation in cryoprecipitate production. *Rock, G., Tittley, P.* (Canadian Red Cross BTS, Ottawa, Ontario, Canada KIS 3E2), p. 50

Experimental induction of caprylate-dependent albumin antibodies. *Hossaini, A. A., Hazeghi, K., Amiri, P.* (Medical College of Virginia, Virginia Commonwealth University, Richmond, Va. 23298), p. 54

In vivo stability of cryoprecipitate fibrinogen. *Sherman, L. A.* (Blood Bank, Barnes Hospital, Division of Laboratory Medicine, St. Louis, Mo. 63110), p. 59

Component preparation record. *Frohman, E. M.* (Blood Bank, Tripler Army Medical Center, APO, San Francisco, Calif. 96438), p. 62

Rapid *in vivo* destruction of Yt (a+) erythrocytes in a recipient with anti-Yt^a. *Ballas, S. K., Sherwood, W. C.* (Thomas Jefferson University, Philadelphia, Pa. 19107), p. 65

Factors affecting the efficiency of filtration leukapheresis. *Katz, A. J., Houx, J., Morse, E. E.* (Connecticut Red Cross Blood Center, University of Connecticut School of Medicine, Farmington, Conn. 06032), p. 67

Decreased accumulation of phthalate plasticizer during storage of blood as packed cells. *Miripol, J. E., Stern, I. J.* (Travenol Laboratories, Inc., Morton Grove, Ill. 60053), p. 71

Chemical analysis of a 30-year-old bottle of lyophilized plasma. *Fu, P., Myhre, B.* (Department of Pathology, UCLA School of Medicine, Harbor General Hospital, Torrance, Calif.), p. 73

Self-administered blood donor medical history. *Katz, A., Bray, J., Pelton, T., Caton, C.* (Connecticut Red Cross Blood Center, University of Connecticut School of Medicine, Farmington, Conn. 06032), p. 75

Transfusion (Philadelphia) **17** (1977) No. 2

Acute hemolytic transfusion reactions. A fresh look at pathogenesis and considerations regarding therapy. *Goldfinger, D.* (Department of Clinical Pathology, Cedars-Sinai Medical Center, Los Angeles, Calif.), p. 85

Oxygen consumption, platelet aggregation and release reactions in platelets freeze-preserved with dimethylsulfoxide. *Spector, J. I., Skrabut, E. M., Valeri, C. R.* (Naval Blood Research Laboratory, Boston, Mass. 02118), p. 99

A rapid method for the preparation of high potency auto and alloantibody eluates. *Jenkins, D. E., Jr., Moore, W. H.* (Nashville Red Cross Blood Center, Nashville, Tenn.), p. 110

Changes in agglomeration of human red blood cells in liquid storage in CPD media. *Meyerstein, N., Mazor, D., Dvilansky, A.* (Ben-Gurion University of the Negev, Beersheba, Israel), p. 115

A review of blood donor motivation and recruitment. *Oswalt, R. M.* (Skidmore College, Saratoga Springs, N. Y.), p. 123

In vitro function of granulocytes isolated from blood of normal volunteers using continuous-flow centrifugation in the IBM-aminco celltrifuge and adhesion-filtration leukapheresis using nylon fiber. *Wade, P. H., Skrabut, E. M., Vinciguerra, L., Valeri, C. R.* (Naval Blood Research Laboratory, Boston, Mass. 02118), p. 136

Hydroxyethyl starch and prednisone as adjuncts to granulocyte collection. *Bearden, J. D. III, Coltman, C. A. Jr., Ratkin, G. A.* (Hematology-Oncology Staff, Wilford Hall USAF Medical Center, Lackland, AFB, Texas 78236), p. 141

Effect of initial storage at room temperature on human red blood cell ATP, 2,3-DPG, and viability. *Chapman, R. G., Rettberg, W. A. H., Dougherty, S.* (University of Colorado Medical Center, Denver, Col. 80262), p. 147

Citrate anticoagulants for plateletpheresis. *Huestis, D. W., Fletcher, J. L., White, R. F., Price, M. J.* (Department of Pathology, University of Arizona, College of Medicine, Tucson, Ariz. 85724), p. 151

Use of dextran 75 as a macromolecular agent in centrifugal leukapheresis. *Huestis, D. W.* (University of Arizona, College of Medicine, Tucson, Ariz. 85724), p. 156

Abdominal pain in donors during filtration leukapheresis. *Wiltbank, T. B., Nusbacher, J., Higby, D. J., McPherson, J. L.* (De-

partment of Hematology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. 14607), p. 159

The type and screen: A safe alternative and supplement in selected surgical procedures. *Boral, L. I., Henry, J. B.* (Department of Pathology, Wilford Hall Medical Center, Lackland Air Force Base, Texas), p. 163

Red blood cell survival studies in patients with anti-Ch^a, anti-Yk^a, anti-Ge, and anti-Vel. *Tilley, C. A., Crookston, M. C., Haddad, S. A., Shumak, K. H.* (Department of Laboratory Hematology, Toronto General Hospital, Toronto, Ontario, Canada M5G 1L7), p. 169

Plasma exchange for cold agglutinin hemolytic anemia. *Taft, E. G., Propp, R. P., Sullivan, S. A.* (Hematology Laboratory, Blood Bank, Albany Medical Center Hospital, Albany, N. Y. 12208), p. 173

An example of anti-Jr^a. *Tritchler, J. E.* (Naval Regional Medical Center, Department of Laboratory Medicine, San Diego, Calif. 92134), p. 177

Graphs to facilitate the computation of patient response to platelet concentrates or antihemophiliac concentrate. *Myhre, B. A., Nakasako, Y.* (Harbor General Hospital Campus, Torrance, Calif. 90509), p. 179

Transfusion (Philadelphia) **17** (1977) No. 3

Quality control. How much is enough? *Guy, L. R., Neitzer, G. M., Klein, R. E.* (Southwestern Medical School, University of Texas Health Science Center, Dallas, Texas), p. 183

A rapid method for determining the sterility of frozen-reconstituted blood. *Myhre, B. A., Nakasako, Y. Y., Schott, R.* (UCLA School of Medicine, Harbor General Hospital Campus, Torrance, Calif.), p. 195

Platelet preservation. V. Survival, serotonin uptake velocity, and response to hypotonic stress of fresh and cryopreserved human platelets. *Odink, J., Brand, A.* (University Hospital, Department of Obstetrics and Gynaecology, Leiden, The Netherlands), p. 203

CPD-adenine as a blood preservative. Studies *in vitro* and *in vivo*. *Messeter, L., Ugander, L., Monti, M., Lundh, B., Löw, B.* (Blood Bank, University Hospital, Lund, Sweden), p. 211

A highly specialized and motivated volunteer population: platelet donors. *Wendlandt, G. McG., Lichtiger, B.* (Department of Laboratory Medicine, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030), p. 218

How donors and nondonors view people who do not give blood. *Rados, D. L.* (Rutgers Graduate School of Business Administration, Newark, N. J. 07102), p. 221

Is blood a commodity? *Kellner, A.* (New York Blood Center, New York, N. Y. 10021), p. 225

The opsonic activity of stored blood. *McClellan, M. A., Alexander, J. W.* (Department of Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267), p. 227

Hemagglutination inhibition in capillary tubes. A simple and reliable test for HBsAg. *Milner, L. V., Ellis, J. L., Viljoen, M. H., Marimuthu, T., Godefroy, J.* (Natal Blood Transfusion Service, Durban 4000, South Africa), p. 233

Blood storage. XXIII. 2,3-DPG maintenance for six weeks in a CPD-adenine-inosine preservative with and without methylene blue. *Dawson, R. B.* (University of Maryland School of Medicine, Baltimore, Md. 21201), p. 238

Blood storage. XXIV. Red blood cell 2,3-DPG and ATP maintenance for six weeks in CPD-adenine with higher phosphate, pyruvate and dihydroxyacetone. *Dawson, R. B.* (University of Maryland School of Medicine, Baltimore, Md. 21201), p. 242

Blood storage. XXV. Ascorbic acid (vitamin C) and dihydroxyacetone (DHA) maintenance of 2,3-DPG for six weeks in CPD-adenine. *Dawson, R. B.* (University of Maryland School of Medicine, Baltimore, Md. 21201), p. 248

Platelet transfusion survey in a regional blood program. *Silva, V. A., Miller, W. V.* (Henry Ford Hospital, Detroit, Mich. 48202), p. 255

Hemoglobin screening in prospective blood donors. A comparison of methods. *Avoy, D. R., Canuel, M. L., Otton, B. M.,*

Mileski, E. B. (Central California Regional Red Cross Blood Program, San Jose, Calif. 95110), p. 261

Unacceptable variability of hemoglobin estimation on samples obtained from ear punctures. *Coburn, T. J., Miller, W. V., Parrill, W. D.* (Northeast Regional Red Cross Blood Program, Boston, Mass. 02115), p. 265

Exchange transfusion in sickle cell disease using a continuous-flow blood cell separator. *Kernoff, L. M., Botha, M. C., Jacobs, P.* (Department of Haemotology, Groote Schuur Hospital, Cape Town, South Africa), p. 269

Tn-polyagglutinability of red blood cells and acquired A-like specificity. *Kourteva, B. T., Manolova, V. R., Mitova, D. K.* (Department of Immunohematology, Institute of Hematology and Blood Transfusion, Sofia 56, Bulgaria), p. 272

Genetic independence of the Lutheran and Diego blood group loci. *Buchanan, D. I., Makeli, D., Marsh, S., Gangopadhyay, K. C.* (Canadian Red Cross Blood Transfusion Service, Alberta T5K 1E2, Canada), p. 277

A chemical refrigerant. *McPeak, D. W., Shulthise, A. J., Camp, F. R. Jr.* (Louisville Regional Red Cross Blood Center, Louisville, Ky. 40201), p. 280

An elapsed time-temperature monitor for blood storage. *Harris, G. E., Cloud, S., Myhre, B. A.* (Los Angeles County Harbor General Hospital, Department of Pathology, Torrance, Calif.), p. 282

Simplified quality assurance of floor model centrifuges. *Tregellas, W. M., Bourland, D. L.* (Blood Services Consultation Services, Phoenix, Ariz. 85007), p. 285

Correspondence, p. 288

Transfusion (Philadelphia) **17** (1977) No. 4

Blood group alloantibodies. An assessment of some laboratory practices. *Giblett, E. R.* (Puget Sound Blood Center, Seattle, Wash. 98104), p. 299

The introduction of adenine fortified blood preservatives: Introduction and an interpretation of its history. *Swisher, S. N.* (Department of Medicine, College of Human Medicine, Michigan State, East Lansing, Mich. 48824), p. 309

Adenine in blood banking. *Simon, E. R.* (Division of Blood and Blood Products, Bureau of Biologics, Bethesda, Md. 20014), p. 317

Toxicology and pharmacology of adenine in animals and man. *Warner, W. L.* (Cutter Laboratories, Inc., Berkeley, Calif.), p. 326

Adenine: Chemistry, analytical methods, sources, purity, and specifications. *Bartlett, G. R.* (Laboratory for Comparative Biochemistry, San Diego, Calif. 92109), p. 333

Biology of free and combined adenine; distribution and metabolism. *Bartlett, G. R.* (Laboratory for Comparative Biochemistry, San Diego, Calif. 92109), p. 339

Metabolism by the rabbit of intravenously administered adenine. *Bartlett, G. R.* (Laboratory for Comparative Biochemistry, San Diego, Calif. 92109), p. 351

Formation of oxyadenine metabolites in the rabbit after intravenous administration of adenine. *Bartlett, G. R.* (Laboratory for Comparative Biochemistry, San Diego, Calif. 92109), p. 358

Metabolism by man of intravenously administered adenine. *Bartlett, G. R.* (Laboratory for Comparative Biochemistry, San Diego, Calif. 92109), p. 367

The *in vivo* survival of red blood cells stored in modified CPD with adenine: Report of a multiinstitutional cooperative effort. *Zuck, T. F., Bensinger, T. A., Peck, C. C., Chillar, R. K., Beutler, E., Button, L. N., McCurdy, P. R., Josephson, A. M., Greenwalt, T. J.* (Department of Surgery, Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 374

Enhanced solubility of 2,8 dihydroxyadenine (DOA) in human urine, *Peck, C. C., Bailey, F. J., Moore, G. L.* (Blood Research Division, Department of Surgery, Letterman Army Institute of Research, Presidio of San Francisco, Calif.), p. 383

Reduction of postperfusion cytomegalovirus-infections following the use of leukocyte depleted blood. *Lang, D. J., Ebert, P. A., Rodgers, B. M., Boggess, H. P., Rixse, R. A.* (Department of Pediatrics, Duke University Medical Center, Durham, N. C. 27710), p. 391

Transfusion (Philadelphia) **17** (1977) No. 5

Blood collection and use by AABB institutional members (1975). *Hemphill, B. M.* (Irwin Memorial Blood Bank, San Francisco Medical Society, San Francisco, Calif.), p. 403

Continuous- and semicontinuous-flow blood centrifugation systems: Therapeutic applications, with plasma-, platelet-, lympha-, and eosinapheresis. *Pineda, A. A., Brzica, S. M. Jr., Taswell, H. F.* (Department of Laboratory Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minn.), p. 407

Relative efficiency and interchangeability of Huggins and American Red Cross cell freezing procedures. *Hornblower, M., Meryman, H. T.* (American National Red Cross Blood Research Laboratory, Bethesda, Md.), p. 417

Error rate, precision, and accuracy in immunohematology. *Grindon, A. J., Eska, P. L.* (Atlanta Regional Red Cross Blood Program, Atlanta, Ga. 30324), p. 425

Evaluation of a large-scale frozen blood program. *Szymanski, I. O., Carrington, E. J.* (Blood Bank, University of Massachusetts Medical Center, Worcester, Mass. 01605), p. 431

A simplified procedure for deglycerolizing red blood cells frozen in a high glycerol concentration. *Meryman, H. T., Hornblower, M.* (American Red Cross Blood Research Laboratory, Bethesda, Md. 20014), p. 438

Formaldehyde in the pathogenesis of hemodialysis-related anti-N antibodies. *White, W. L., Miller, G. E., Kaehny, W. D.* (Department of Pathology, University of Colorado Medical Center, Denver, Col. 80220), p. 443

Ultrafiltrable adenosine triphosphate and 2,3-diphosphoglycerate concentrations in cold-stored human erythrocytes. *Marshall, W. E., Rassaian, N., Greenwald, L. S., Omachi, A.* (Department of Physiology and Biophysics, University of Illinois, Medical Center, Chicago, Ill. 60612), p. 448

Studies on 4 °C stored frozen-reconstituted red blood cells. *Myhre, B. A., Nakasako, Y. Y., Schott, R.* (UCLA School of Medicine, Harbor General Hospital, Torrance, Calif. 90509), p. 454

Consequences of prior alloimmunization during granulocyte transfusion. *Appelbaum, F. R., Trapani, R. J., Graw, R. G. Jr.* (Experimental Hematology Section, Pediatric Oncology Branch, National Cancer Institute, Bethesda, Md.), p. 460

Preservation of human granulocytes. II. Characteristics of granulocytes obtained by counterflow centrifugation. *Lionetti, F. J., Hunt, S. M., Lin, P. S., Kurtz, S. R., Valeri, C. R.* (Center for Blood Research, Tufts University School of Medicine, Boston, Mass.), p. 465

Military blood banking. Blood transfusion aboard a naval hospital ship receiving multiple casualties in a combat zone, a controled medical environment. *Monaghan, W. P., Levan, D. R., Camp, F. R. Jr.* (Blood Bank, National Naval Medical Center, Bethesda, Md. 20014), p. 473

An empirical approach to projecting regional blood need. *Krowka, M. J., Schwartz, J. P.* (School of Medical Sciences, Mackay Science Building, University of Nevada, Reno, New. 89507), p. 477

Microaggregate content and flow rates of packed red blood cells. *Reiss, R. F., Katz, A. J.* (Division of Blood Bank-Hematology, Department of Pathology, State University Hospital, Brooklyn, N. Y. 11203), p. 484

Chronic liver dysfunction in multitransfused hemophiliacs. *Hasiba, U. W., Spero, J. A., Lewis, J. H.* (University of Pittsburgh, Department of Medicine, Pittsburgh, Pa. 15261), p. 490

Liver dysfunction in patients with hemophilia A, B, and von Willebrand's disease. *Hilgartner, M. W., Giardina, P.* (Cornell University Medical College-New York Hospital, New York, N. Y. 10021), p. 495

Failure of postfiltration leukapheresis plasma to increase granulocyte yields. *Schiffer, C. A., Aisner, J.* (Clinical Oncology Branch, Baltimore Cancer Research Center, Baltimore, Md. 21201), p. 500

Evaluation of liquid scintillation counting solutions for radioimmunoassay of hepatitis-associated antigen (HBsAg). *Hicks, E. J., Oei, T. O., Nordschow, C. D., Hughes, B. J.* (Department of Clinical

Pathology, Indiana University Medical Center, Indianapolis, Ind. 46202), p. 503

Large scale preparation of leukocyte concentrates and further purification of lymphocytes and granulocytes with the IBM 2991 cell processor. *Brutel de la Rivière, A., Verhoef-Karssen, P. R., de Wit, J. J. F. M., Loos, J. A., Prins, H. K.* (Binnengasthuis, Department of Surgery, Amsterdam, The Netherlands), p. 509

Discontinuous flow centrifugation leukapheresis and neutrophil function. *Glasser, L.* (Department of Pathology, College of Medicine, University of Arizona, Tucson, Ariz. 85724), p. 513

Autoimmune hemolytic anemia with anti Jk^a specificity in a patient taking Aldomet. *Patten, E., Beck, C. E., Scholl, C., Stroope, R. A., Wukasch, C.* (Blood Bank, The University of Texas Medical Branch, Galveston, Texas 77550), p. 517

Blood typing in disputed paternity cases. Capabilities of American laboratories. *Polesky, H. F., Krause, H. D.* (Minneapolis War Memorial Blood Bank, Minneapolis, Minn. 55404), p. 521

Hemoglobin function in stored blood. XIX. Inosine maintenance of 2,3-DPG for 35 days in a CPD-adenine preservative. *Dawson, R. B.* (Blood Research Laboratory, Department of Pathology, School of Medicine, University of Maryland, Baltimore, Md. 21202), p. 525

Transfusion (Philadelphia) **17** (1977) No. 6

Autoantibodies mimicking alloantibodies. *Issitt, P. D., Zellner, D. C., Rolih, S. D., Duckett, J. B.* (Paul I. Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio 45267), p. 531

A "normal" individual with a positive direct antiglobulin test. Case complicated by pregnancy and unusual autoantibody specificity. *Henry, R. A., Weber, J., Pavone, B. G., Issitt, P. D.* (Reprint requests: Dr. B. G. Pavone, Paul I. Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio 45267), p. 537

Partial plasma exchange, an adjunct in therapy to complex clinical problems. *Laningham, J. E. T.* (Department of Pathology, Moore Memorial Hospital, Pinehurst, N. C. 28374), p. 547

Total and partial blood exchange in the rat with hemoglobin prepared by crystallization. *De Venuto, F., Moores, W. Y., Zegna, A. I., Zuck, T. F.* (Letterman Army Institute of Research, Presidio of San Francisco, Calif. 94129), p. 555

An evaluation of several blood transfusion filters. *Kennedy, P. S., Solis, R. T., Scott, M. A., Wilson, R. K.* (Department of Medicine, Baylor College of Medicine, Houston, Texas 77030), p. 563

Hemolytic disease of the newborn due to anti-PP_1P^k (anti-Tj^a). *Levene, C., Sela, R., Rudolphson, Y., Nathan, I., Karplus, M., Dvilansky, A.* (Reference Laboratory for Blood Groups and Immunohematology, Government Central Laboratories, Jerusalem, Israel), p. 569

Comments on hemolytic disease of newborn due to anti-PP_1P^k (anti-Tj^a). *Levine, P.* (Ortho Diagnostics, Raritan, N. J.), p. 573

Risk factors in transmission of non-A, non-B posttransfusion hepatitis. The role of hepatitis B antibody in donor blood. *Conrad, M. E., Knodell, R. G., Bradley, E. L. Jr., Flannery, E. P., Ginsberg, A. L.* (Division of Hematology and Oncology, University of Alabama, Birmingham, Al. 35294), p. 579

Automated donor-recipient identification systems as a means of reducing human error in blood transfusion. *Sherer, P. B., Chambers, R. W., Taswell, H., Altshuler, C., Aster, R., Covino, K., Gordon, S., Gajewski, M., Smith, A., Horrigan, S., Pfaff, K.* (National Heart and Lung Institute, National Institutes of Health, Bethesda, Md.), p. 586

Causes of disqualification in a volunteer blood donor population. *Farrales, F. B., Stevenson, A. R., Bayer, W. L.* (Community Blood Center of Greater Kansas City, Kansas City, Mo. 64111), p. 598

Cost effectiveness of use of frozen blood to alleviate blood shortages. *Cumming, P. D., Kendall, K. E., Pegels, C. C., Seagle, J. P.* (Comprehensive Health Planning Council, Buffalo, N. Y.), p. 602

Adult outpatient transfusion clinic in a hospital-based blood bank. *Boral, L. I., Crowley, K. M., Henry, J. B.* (USAF, MC, Pathology Department, Wilford Medical Center, Lackland AFB, Texas, 78236), p. 607

The effect of prior whole blood transfusion on subsequent granulocyte support in leukopenic dogs. *Westrick, M. A., Debelak-Fehir, K. M., Epstein, R. B.* (North Carolina Baptist Hospital, Winston-Salem, N. C. 27103), p. 611

Changes of pulmonary function following transfusion of stored blood. *Takaori, M., Nakajo, N., Ishii, T.* (Department of Anesthesiology, Kawasaki Medical School, Kurashiki City, Okayama, 701 Japan), p. 615

Occurrence of anti-Wr^a in blood donors and in selected patient groups, with a note on the incidence of the Wr^a antigen. *Greendyke, R. M., Banzhaf, J. C.* (University of Rochester School of Medicine and Dentistry, Rochester, N. Y. 14642), p. 621

Evaluation of passive hemagglutination techniques in detection of Australia antigen (HBsAg). *Scalise, G., Mura, M. S., Delia, S.* (Infectious Diseases Department, University of Sassari, Sassari, Italy), p. 625

Synthesis of ITP and ATP and regneration of 2,3-DPG in human erythrocytes incubated with adenosine, pyruvate, and inorganic phosphate. *Zachara, B.* (Department of Biochemistry, Institute of Physiology and Biochemistry, Medical Academy, 90647 Lódz, Poland), p. 628

Effect of hydroxyethyl starch on platelet function following granulocyte collection using the continuous flow cell separator. *Farrales, F. B., Belcher, C., Summers, T., Bayer, W. L.* (Community Blood Center of Greater Kansas City, Kansas City, Mo. 64111), p. 635

Selection of compatible platelet donors: A prospective evaluation of three crossmatching techniques. *Wu, K. K., Hoak, J. C., Koepke, J. A., Thompson, J. S.* (Coagulation and Thrombosis Unit, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill. 60612), p. 638

Plasma exchange for mushroom poisoning. *Mercuriali, F., Sirchia, G.* (Transplantation Immunology and Blood Transfusion Service, Ospedale Policlinico, Milan, Italy), p. 644

Fetal cell counting as an evaluation of Rh prophylaxis with antenatally administered anti-Rh_0(D) gamma globulin. *Harrison, K. L.* (Pathology Department, The Royal Women's Hospital, Brisbane 4029 Australia), p. 647

A simple method for freezing reagent erythrocytes in liquid nitrogen. *Behzad, O., Lee, C. L.* (Mid America Regional Red Cross Blood Program, Rush Medical College, Chicago, Ill.), p. 650

Undetectable ABO isoagglutinin in a patient with chronic myelocytic leukemia. *Ogata, H., Hasegawa, S.* (University of Shinshu Medical School Hospital, Matsumoto, Nagano-ken, Japan), p. 651

Nontraumatic therapeutic phlebotomy. *Inwood, M. J., Thomson, S.* (Department of Blood Transfusion, St. Joseph's Hospital, London, Ontario, Canada), p. 655

The relationship between donor-recipient lymphocytotoxicity and the transfusion response using HLA-matched platelet concentrates. *Herzig, R. H., Terasaki, P. I., Trapani, R. J., Herzig, G. P., Graw, R. G. Jr.* (Case-Western Reserve, School of Medicine, Department of Medicine, Cleveland, Ohio 44118), p. 657

A practical blood bank workbench. *Milam, J. D.* (Blood Bank and Transfusion Service, St. Luke's Episcopal Hospital, Houston, Texas), p. 662

Vox Sanguinis (Basel) **31** (1976) No. 6

Application of a high sensitivity aggregate-haemagglutination test for the diagnosis of autoimmune haemolytic anaemia with a negative direct antiglobulin test. *Idelson, L. I., Koyfman, M. M., Gorina, L. G., Olovnikov, A. M.* (Central Institute for Advanced Medical Studies, Department of Haematology, Central Hospital, No. 2 Moscow B-150, USSR), p. 401

Specificity and possible origin of anti-N antibodies developed by patients undergoing chronic haemodialysis. *Boettcher, B., Nanra, R. S., Roberts, T. K., Mallan, M., Watterson, C. A.* (Department of Biological Sciences, University of Newcastle, Newcastle, NSW 2308, Australia), p. 408

Sterilized hepatitis B (surface) antigen for production of specific antisera. *Stephan, W., May, G.* (Biotest-Serum-Institut GmbH, D-6000 Frankfurt/Main, BRD), p. 416

Preparation of human immunoglobulin by ammonium sulfate precipitation. *Habeeb, A. F. S., Francis, R. D.* (Department of Microbiology, University of Alabama, School of Medicine, Birmingham, Ala. 35294), p. 423

Subtypes of hepatitis B surface antigen in native-born and immigrant Israelis. *Sandler, S. G., Polesky, H. F., Shouval (Steigbuegel), D., Hanson, M., Olson, C.* (Reprint requests: Dr. H. F. Polesky, Minneapolis War Memorial Blood Bank, Minneapolis, Minn. 55404), p. 435

Automated irregular antibody screening on a modified 15-channel blood-grouping machine. *Cotton, R., Ray, T. C.* (South West Regional Blood Transfusion Centre, Southmead, Bristol BS 10 5ND, England), p. 440

Collection of lymphocytes on a continuous-flow blood cell separator. *Pentycross, C. R., Walden, P. A. M., Bagshawe, K. D.* (Department of Medical Oncology, Charing Cross Hospital (Fulham), London W6 8RF, England), p. 446

False-positive Rose-Waaler rheumatoid factor titre given by anti-ruminant IgM antibody. *Johnson, P. M., Reading, C. A., Holborow, E. J., Holdstock, D. J.* (Institute of Immunology and Rheumatology, Rikshospitalet University Hospital, Oslo 1, Norway), p. 451

International Computer Workshop in Forensic Serology and Human Genetics, September 29—30, 1975, Bonn, p. 456

Vox Sanguinis (Basel) **31** (1976) Suppl. 1

Increased whole blood viscosity on cooling in a patient with cold hemoagglutinin disease. *Girolami, A., Cella, G., Patrassi, G.* (University of Padova Medical School, Institute of Semeiotica Medica, Padova, Italy), p. 1

In vivo and *in vitro* activation of T-antigen receptors on leukocytes and platelets. *Hysell, J. K., Hysell, J. W., Nichols, M. E., Leonardi, R. G., Marsh, W. L.* (St. Joseph Mercy Hospital, Ann Arbor, Mich. 48104), p. 9

Reevaluation of the polyvinylpyrrolidone sedimentation test in the diagnosis of AB0 hemolytic disease of the newborn. *Shilo, R., Gutman, R., Ascari, D., Schwartz, J.* (Reprint requests: Dr. R. Gutman, Bacteriological Laboratory, "Zahalon" Government Hospital, Jaffa, Israel), p. 16

Collection of peritoneal exudate cells from small laboratory animals. *Casciato, D. A., Goldberg, L. S., Bluestone, R.* (691/111A, VA Wadsworth Hospital Center, Los Angeles, Calif. 90073), p. 25

Complement levels in relation to certain antibodies in polytransfused thalassaemic patients. *Economidou, J., Pathouli, C., Hadziyannis, S., Fostiropoulos, G., Constantoulakis, M.* (Hellenic Red Cross Blood Research Laboratory, Athens 109, Greece), p. 32

A Swiss family showing independent segregation of the Luthern and Swann genes. *Metaxas, M. N., Metaxas-Buehler, M.* (Blood Group Reference Laboratory, Swiss Red Cross, CH-8001 Zurich, Switzerland), p. 39

Observations on the antiglobulin tests. II. C4 components on erythrocytes. *Stratton, F., Rawlinson, V. I.* (Regional Blood Transfusion Centre, Manchester M1 3BP, England), p. 44

Contributions to the optimal use of human blood. VIII. Stability of blood coagulation factor VIII during collection and storage of whole blood and plasma. *Vermeer, C., Soute, B. A. M., Ates, G., Hellings, J. A., Brummelhuis, H. G. J.* (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), p. 55

The efficacy of vaccinial immune globulin. A 15-year study. *Feery, B. J.* (Virology Research Laboratory, Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia), p. 68

The H specificities of butanol extracts of human erythrocytes. *Lambert, R. M., Zelenski, S. K.* (Blood Group Research Unit, Department of Microbiology, School of Medicine, State University of New York at Buffalo, Buffalo, N. Y. 14214), p. 77

Purification of hepatitis B surface antigen using polyethylene glycol, pepsin and Tween 80. *Nath, N., Mazzur, S., Ledman, R., Fang, C. T.* (Hepatitis Testing Laboratory, Blood Research Laboratory, American National Red Cross, Bethesda, Md. 20014), p. 84

The Coté serum (anti-Kell), an antibody defining a new variant in the Kell system. *Guevén, R. M., Taliano, V., Waldmann, O.* (Canadian Red Cross Blood Transfusion Service, Montréal, H1W 1B2, Canada), p. 96

Vox Sanguinis (Basel) **32** (1977) No. 1

A comparison of different methods of screening blood donations for HBsAg. *Barbara, J. A. J., Howell, D. R., Cleghorn, T. E., Cameron, C. H., Briggs, M., Dane, D. S.* (North London Blood Transfusion Centre, Edgware, Middlesex HA8 9BD, England), p. 4

Genetic control of immune responsiveness in man. *Mackay, I. R., Whittingham, S., Tait, B.* (The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia), p. 10

Volume limitations of plasmapheresis. *Lundsgaard-Hansen, P.* (University Department of Experimental Surgery, Inselspital, CH-3010 Bern, Switzerland), p. 20

Increase in strength of red cell Bg^a antigen following infectious mononucleosis. *Morton, J. A., Pickles, M. M., Darley, J. H.* (Reprint requests: Dr. M. M. Pickles, Department of Pathology, The Gibson Laboratories, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 26

Expression of red cell Bg^a antigen in children. Lack of correlation with previous EB virus infection. *Morton, J. A., Hill, F. G. H.* (The Radcliffe Infirmary, Oxford, England), p. 38

Haemostasis disorders in open heart surgery with extracorporeal circulation. Importance of the platelet function and the heparin neutralization. *Moriau, M., Masure, R., Hurlet, A., Debeys, C., Chalant, C., Ponlot, R., Jaumain, P., Servaye-Kestens, Y., Ravaux, A., Louis, A., Goenen, M.* (Laboratory of Hemostasis and Thrombosis Research, Clinique Universitaires St.-Pierre, B-3000 Leuven, Belgium), p. 41

Properdin factor B (Bf) polymorphism in Norway. *Teisberg, P., Olaisen, B.* (Reprint requests: Dr. B. Olaisen, Rettsmedisinsk Institutt, Rikshospitalet, Oslo 1, Norway), p. 52

Two Japanese families with P^k members. *Nakajima, H., Yokota, T.* (Department of Forensic Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan), p. 56

Vox Sanguinis (Basel) **32** (1977) No. 2

Autoimmune haemolytic anaemia with the unusual combination of both IgM and IgG autoantibodies. *Freedman, J., Newlans M.* (MRC Experimental Haematology Unit, St. Mary's Hospital Medical School, London W2 1PG, England), p. 61

Naturally occurring human antibodies with specificity for light chains of immunoglobulins. *Scherz, R., Pflugshaupt, R., Bütler, R.* (Central Laboratory, Blood Transfusion Service SRC, CH-3000 Berne 22, Switzerland), p. 69

Weak anti-IgA antibodies with limited specificity and nonhemolytic transfusion reactions. *Koistinen, J., Leikola, J.* (Reprint requests: Dr. J. Leikola, Finnish Red Cross Blood Transfusion Service, SF-00310 Helsinki 31, Finland), p. 77

Association between HLA and red cell antigens. IV. Further studies of haemagglutinins in cytotoxic HLA antisera. *Nordhagen, R.* (National Institute of Public Health, SIFF, Oslo, 1, Norway), p. 82

Use of discriminant analysis in relating maternal anti-D levels to the severity of haemolytic disease of the newborn. *Morley, G., Gibson, M., Eltringham, D.* (Regional Blood Transfusion Centre, Edgbaston, Birmingham, B15 2SG, England), p. 90

Selective depression of blood group antigens associated with hereditary ovalocytosis among Melanesians. *Booth, P. B., Serjeantson, S., Woodfield, D. G., Amato, D.* (Immunohaematology Department, Christchurch Hospital, Christchurch, New Zealand), p. 99

Biochemical genetics of MN. *Walker, M. E., Rubinstein, P., Allen, F. H. Jr.* (New York Blood Center, New York, N. Y. 10021), p. 111

Yet more *Salvia* agglutinins. *Bird, G. W. G., Wingham, J.* (Regional Blood Transfusion Service, Edgbaston, Birmingham B15 2SG, England), p. 121

Vox Sanguinis (Basel) **32** (1977) No. 3

Radioimmunoassay inhibition method for confirming the specificity of positive hepatitis B surface antigen reactions and for survey of antibodies to the antigen. *Forghani, B., Schmidt, N. J., Lennette, E. H.* (Reprint requests: Dr. E. H. Lennette, Chief Viral and Rickettsial Disease Laboratories, State of California Department of Health, Berkeley, Calif. 94704), p. 125

Serial infusion effects of hydroxyethyl starch on ESR, blood typing and crossmatching and serum amylase levels. *Janes, A. W., Mishler, J. M., Lowes, B.* (Department of Haematology, Gibson Laboratories, Radcliffe Infirmary, Oxford OX2 6HE, England), p. 131

Warm IgM anti-I^T causing autoimmune haemolytic anaemia. *Freedman, J. Newlands, M., Johnson, C. A.* (MRC Experimental Haematology Unit, St. Mary's Hospital Medical School, London W2 1PG, England), p. 135

Preparation of human immunoglobulin free of plasmin and anticomplement activities. *Habeeb, A. F. S. A., Francis, R. D.* (Department of Microbiology, University of Alabama School of Medicine, Birmingham Ala. 35294), p. 143

Effect of fragmentation of tetanus immune globulin (human) on neutralization of tetanus toxin. *Kneapler, D., Cohen, P.* (Reprint requests: Dr. P. Cohen, Merieux Institute, Miami, Fla. 33169), p. 159

Mitogenic effect of syngeneic peritoneal fluids and their relation to lymphocyte trapping. *Frost, P.* (Wayne State University Medical School, Department of Immunology and Medicine, Detroit, Mich. 48201), p. 165

Development of an intravenous γ-globulin with Fe activities. I. Preparation and characterization of S-sulfonated human γ-globulin. *Masuho, Y., Tomibe, Y., Matsuzawa, K., Ohtsu, A.* (Teijin Institute for Biomedical Research, Tokyo 191, Japan), p. 175

Processes to increase the yields of normal serum albumin. *Wye, E. J., Kim, M. K.* (Plasma Products Division, Connaught Laboratories Limited, Willowdale, Ontario, Canada), p. 182

A monitoring and alarm system for use with a Technicon 15-channel blood grouping machine. *Duddin, R.* (Regional Blood Transfusion Centre, Birmingham B15 2SG, England), p. 185

Vox Sanguinis (Basel) **32** (1977) No. 4

The use of IgM anti-D coated cells in the deliberate immunisation of Rh-negative male volunteers. *Lee, D., Flowerday, M. H. E., Tomlinson, J.* (Blood Transfusion Centre, Lancaster, England), p. 189

"VA", a new type of erythrocyte polyagglutination characterized by depressed H receptors and associated with hemolytic anemia. I. Serological and hematological observations. *Graninger, W., Rameis, H., Fischer, K., Poschmann, A , Bird, G W. G., Wingham, J., Neumann, E.* (Institute of Blood Group Serology, University of Vienna, A-1090 Vienna, Austria), p. 195

"VA", a new type of erythrocyte polyagglutination characterized by depressed H receptors and associated with hemolytic anemia. II. Observations by immunofluorescence, electron microscopy, cell electrophoresis and biochemistry. *Graninger, W., Poschmann A., Fischer, K., Schedl-Giovanonni, I., Hörandner, H., Klaushofer, K.* (Institute of Blood Serology, University of Vienna, A-1090 Vienna, Austria), p. 201

Colton blood groups in Canadian Caucasians: Frequencies, inheritance and linkage analysis. *Lewis, M., Kaita, H., Chown, B., Giblett E. R., Anderson J.* (Rh Laboratory and Department of Pediatrics, University of Manitoba, Winnipeg, Man., R3E OL8, Canada), p. 208

Anti-Lu14: A Lutheran antibody defining the products of an allele at the Lu8 blood group locus. *Judd W. J. Marsh, W. L., Øyen, R., Nichols, M. E., Allen, F. H. Jr., Contreras, M., Stroup, M.* (Reprint requests: Dr. W. L. Marsh, New York Blood Center, New York, N. Y. 10021), p. 214

A further example of Kp^a/K^o exhibiting depression of some Kell group antigens. *Ford, D. S., Knight, A. E., Smith, F.* (New Zealand Blood Transfusion Service (Otago Region), Clinical Service Centre, Dunedin, New Zealand), p. 220

Subtypes of HB_sAG in Eastern and South-Eastern Europe. *Madaliński, K., Holland, P. V., Moraczewska, Z., Kalinowska, B., Alter, H. J.* (Department of Immunopathology, State Institute of Hygiene, 00-791 Warsaw, Poland), p. 224

An immunological investigation of haemophilia B with a tentative classification of the disease into five variants. *Girolami, A., Sticchi, A., Burul, A., Dal Do Zanon, R.* (University of Padova, Medical School, Institute of "Semeiotica Medica", Padova, Italy), p. 230

Polymer distribution in human serum albumin powders prepared by lyophilization or acetone drying. *Solli, N. J., Bertolini, M. J.* (Cutter Laboratories Inc., Berkeley, Calif. 947 10), p. 239

Quantitative determination of albumin dimer in albumin preparations by combined starch-gel electrophoresis and immunoprecipitation. *Skoog, B., Granstrand, B.* (AB Labi, Research Department, Analytical Chemistry, S-104 25 Stockholm, Sweden), p. 242

International Forum, p. 247

Vox Sanguinis (Basel) **32** (1977) No. 5

Identification of sialosylparagloboside as the erythrocyte receptor for an "anti-p" antibody. *Schwarting, G. A., Marcus, D. M., Metaxas, M.* (Reprint requests: Dr. D. M. Marcus, Albert Einstein College of Medicine, Bronx, N. Y. 10461), p. 257

Studies on the biosynthetic pathway of human P erythrocyte antigen using genetic complementation tests between fibroblasts from rare p and P^k phenotype donors. *Fellous, M., Gerbal, A., Nobillot, G., Weils, J.* (Laboratoire d'Immunohématologie, Centre Hayem, Hôpital Saint-Louis, 75475 Paris, Cedex 10, France), p. 262

The identity of Kamhuber and Far antigens. *Giles, C. M.* (MRC Blood Group Reference Laboratory, London SW1W 8QJ, England), p. 269

Three antibodies of the MNSs system and their association with the Miltenberger complex of antigens. I. Anek serum. *Chandanayingyong, D., Pejrachandra, S., Poole, J.* (Reprint requests: Miss J. Poole, MRC Blood Group Reference Laboratory, London SW1W 8QJ, England), p. 272

Three antibodies of the MNSs system and their association with the Miltenberger complex of antigens. II. Raddon and Lane sera. *Webb, A., Giles, C. M.* (Reprint requests: Dr. C. M. Giles, MRC Blood Group Reference Laboratory, London SW1W 8QJ, England), p. 274

Three antibodies of the MNSs system and their association with Miltenberger complex of antigens. III. Anek, Raddon and Lane antisera in relation to each other and the Miltenberger complex. *Giles, C. M., Chandanayingyong, D., Webb, A. J.* (MRC Blood Group Reference Laboratory, London SW1W 8QJ, England), p. 277

Erythrocyte autoantibody with unusual specificity. *Bird, G. W. G., Wingham, J.* (Regional Blood Transfusion Service, Birmingham, B15 2SG, England), p. 280

H, A and A_1 reactivity in two Polynesian groups. *Booth, P. B., Cartwright, L.* (Immunohaematology Department, Christchurch, New Zealand), p. 283

Development of an intravenous γ-globulin with Fc activities. II. Reconversion of S-sulfonated human γ-globulin into the original γ-globulin. *Masuho, Y., Timibe, K., Watanabe, T., Fukumoto, Y.* (Teijin Institute for Biomedical Research, Hino, Tokyo 191, Japan), p. 290

High prevalence of *e* antigen among healthy blood donors carrying hepatitis B surface antigen in Japan. *Tachibana, C. F., Baba, K., Fukuda, M., Imai, M., Miyakawa, Y., Mayumi, M.* (Reprint requests: Dr. M. Mayumi, Immunology Division, Jochi Medical School, Tochigi-ken 329-04, Tokyo, Japan), p. 296

Normal lymphocyte transfer reaction in humans and its possible mechanism. *Saha, K., Mittal, M. M.* (Department of Bacteriology, Govind Ballabh Pant Hospital, New Delhi, India), p. 300

International Forum. Does a relationship exist between massive blood transfusions and the adult respiratory distress syndrome? If so, what are the best preventive measures? p. 311

Letter to the Editor. Serologic facts and serologic languages. *Hirschfeld, J.* (Statens Rättskemiska Laboratorium, S-10401 Stockholm, 60, Sweden), p. 321

Vox Sanguinis (Basel) **32** (1977) No. 6

A comparative analysis of methods suitable for detection of platelet alloantibodies. Inhibition of ^{14}C-serotonin uptake, ^{14}C-serotonin release and microcomplement fixation. *Heinrich, D., Kessler, C., Mueller-Eckhardt, C.* (Department of Internal Medicine, Justus Liebig University, D-6300 Giessen, FRG), p. 325

Distribution of IgG subclasses in commercial and some experimental γ-globulin preparations. *Skvaril, F., Probst, M., Audran, R., Steinbuch, M.* (Institute for Clinical and Experimental Cancer Research, University of Bern, CH-3004 Bern, Switzerland), p. 335

Blood chimeric twins. An example of blood cell chimerism. *Hosoi, T., Yahara, S., Kunitomo, K., Saji, H., Ohtsuki, Y.* (U. Youngchaiyud, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand), p. 339

International Forum, p. 346

News Item

Le IVe Congrès Française d'Hématologie et le XIe Congrès National de Transfusion Sanguine

se tiendront à Strasbourg du 25 au 29 juin 1979

au

nouveau Palais de la Musique et des Congrès.

Pour tous renseignements s'adresser au:

Congrès de Transfusion et d'Hématologie
Centre de Transfusion Sanguine
10 rue Spielmann
67085 STRASBOURG Cedex, France.
Téléphone: (88) 36.08.38.

Contents

Volume 11

Rosenfield, R. E.: In memoriam Alexander S. Wiener 5

Astaldi, Ü., Bagnara, G. P., Brunelli, M. A., Karanovic, D., Karanovic, J., Scorza, R., Topuz, Q.: Experimental and clinical investigations on stem cell take and colony formation 11

Mód, A., Harsányi, V., Jakschitz, M., Hollán, S. R.: The treatment of infections in granulocytopenic patients with granulocyte transfusions 31

Šulc, K., Neuwirt, J., Trávniček, T., Kobylka, P., Radikovská, E.: Bone marrow cell separation on Ficoll gradient 41

Fleischmann, T., Krizsa, F.: Chromosomes in malignant lymphomas 47

Климова, Н. Ф.: Десятилетний опыт лечения миелобромолом больных миелофиброзом 57

Amir, J., Forman, W. B., Rassiga, A., Bernier, G. M.: Nonsynthetizing multiple myeloma 73

Tichý, M., Hrnčiř, Z., Šimša, J.: Serum levels of IgA, IgM, IgD and IgE in IgG paraproteinaemias 79

Láng, I., Fekete, B., Gergely, P., Petrányi, Gy.: A simple allogeneic test system for the study of antibody-dependent cellular cytotoxicity (ADCC) in man 85

Alföldy, P., Dombi, É., Tóth, T., Gyódi, É., Hollán, S. R.: Cell-mediated immunity in subjects immunized with HLA antigens 93

Jókay, I., Karczag, E.: Antagonistic effect of heparin and a water-soluble glucocorticoid on the peripheral lymphocyte count in mice 103

Merkiel, K., Prokopowicz, J., Bielawiec, M., Kiersnowska, B.: Serum lysozyme activity in some myeloproliferative diseases 111

Хомченовский, Е. И.: Итогу экспериментального и клинического ияучения нового противолейкозного препарата гексафозфамида 115

Rudowski, W., Daszýnski, J., Schier, J., Kowalczyk, V., Gaczkowski, A., Góralski, S.: Infusion of physiological saline supplemented with adenine, pyruvate and phosphate 127

Fagiolo, E.: Immune haemolytic anaemia after exchange transfusion 133

Prodanov, P.: H-excess A_1 and A_{int} variants among the Bulgarian population 139

Krizsa, F., Cserháti, I.: Effect of F-leurosine (formyl-leurosine) on platelet production in mice 149

Kosztolányi, G., Jobst, K., Kádas, I.: Effect of lanthanum ion on platelet electrophoretic mobility 155

Blaskó, Gy., Bédi, J., Machovich, R., Pálos, L. Á.: Effect of collagen on thrombin inactivation by antithrombin-III and heparin 163

Goldschmidt, B., Siegler, J., Schmidt, M.: Fibrinogen metabolism in cyanotic congenital heart disease 169

Szmitkowski, M., Prokopowicz, J., Szmitkowska, K.: Antihaemophilic factor (factor VIII) from human granulocytes 177

Book Reviews 189

Abstracts 195

From the International Literature of Haematology 203

News Items 247

Ishiyama, H., Okuyama, K., Masuda, K., Ozaki, J., Yasuda, J.: Modifications of the biological activity of anti-D immunoglobulin caused by heat aggregation 251
Gergely, P., Bakács, T.: Mitogenicity of fetal calf serum 259
Simon, G., Lengyel, A., Szücs, A.: Erythrocyte acetylcholinesterase activity is not affected by adenovirus-induced haemagglutination 265
Freidin, N., Mandel, E. M., Djaldetti, N.: Hypercalcaemia, hyperphosphataemia and cytoplasmic inclusions in a patient with chronic lymphocytic leukaemia 269
Zywicka-Łopaciuk, H., Janowska-Wieczorek, A., Romejko, M., Swencz-Szczepanik, K., Kryzyzanowska-Wozniakowska, J., Michalski, R., Pawelski, S.: Occurrence of malignant neoplasms in patients with chronic lymphatic leukaemia 279
Heilmann, E., Nordiek, R.: Beziehungen zwischen den Lymphozytensubpopulationen und der Stadieneinteilung der chronischen lymphatischen Leukämie von Rai 289
Legutko, L.: Relationship between serum copper and bone marrow blasts in acute leukaemia 297
Dintenfass, L.: Some aspects of haemorrheology of metastasis in malignant melanoma 301
Louwagie, A. C.: I. Increase in bone marrow CFU-S and CFU-C after *in vivo* phytohaemagglutinin (PHA) administration in mice 309
Louwagie, A. C.: II. Mechanism of increased colony formation of CFU-S and CFU-C after *in vivo* PHA administration in mice 321
Boda, D., Eck, E.: Favourable effect of storage at 0°C on the O_2-transport function of heparinized blood 331
Heilmann, E., Vogel, M.: Hämolyse nach extrakorporaler Blutbestrahlung des Menschen? 335
Bláha, M., Kačerovský, J., Hrnčíř, Z., Nožička, Z., Špaček, J., Široký, O.: Systemic mastocytosis. Report of a case 349
Nowicka, J., Kotschy, M., Chwistecki, K., Jelen, M.: Disseminated intravascular coagulation (DIC) during superacute haemolysis in a patient with ovarian dermatoid cyst treated with rifampicin 359
Book Reviews 365
Abstracts 369
From the International Literature of Haematology 377
News Item 479
Contents of Volume 11 481
Author Index 483
Subject Index 485

Author Index

A

Alföldy, P. 93
Amir, J. 73
Astaldi, G. 11

B

Bagnara, G. P. 11
Bakács, T. 259
Bédi, J. 163
Bernier, G. M. 73
Bielawiec, M. 111
Bláha, M. 349
Blaskó, Gy. 163
Boda, D. 331
Brunelli, M. A. 11

C

Chwistecki, K. 359
Cserháti, I. 149

D

Daszýnski, J. 127
Dintenfass, L. 301
Djaldetti, N. 269
Dombi, É. 93

E

Eck, E. 331

F

Fagiolo, E. 133
Fekete, B. 85
Fleischmann, T. 47
Forman, W. B. 73
Freidin, N. 269

G

Gaczkowski, A. 127
Gergely, P. 85, 259
Goldschmidt, B. 169
Góralski, S. 127
Gyódi, É. 93

H

Harsányi, V. 31
Heilmann, E. 289, 335
Hollán, S. R. 31, 93
Hrnčiř, Z. 79, 349

I

Ishiyama, H. 251

J

Jakschitz, M. 31
Janowska-Wieczorek, A. 279
Jelen, M. 359
Jobst, K. 155
Jókay, I. 103

K

Kačerovský, J. 349
Kádas, I. 155
Karanovic, D. 11
Karanovic, J. 11
Karczag, E. 103
Khomchenovsky, E. I. 115
Kiersnowska, B. 111
Klimova, N. F. 57
Kobylka, P. 41
Kosztolányi, G. 155
Kotschy, M. 359
Kowalczyk, V. 127
Krizsa, F. 47, 149
Krzyzanowska-Wozniakowska, J. 279

L

Láng, I. 85
Legutko, L. 297
Lengyel, A. 265
Louwagie, A. C. 309, 321

M

Machovich, R. 163
Mandel, E. M. 269
Masuda, K. 251
Merkiel, K. 111
Michalski, R. 279
Mód, A. 31

N

Neuwirt, J. 41
Nordiek, R. 289
Nožička, Z. 349

O

Okuyama, K. 251
Ozaki, J. 251

P

Pálos, L. Á. 163
Pawelski, S. 279
Petrányi, Gy. 85
Prodanov, P. 139
Prokopowicz, J. 111, 177

R

Radikovská, E. 41
Rassiga, A. 73
Romejko, M. 279
Rosenfield, R. E. 5
Rudowski, W. 127

S

Schier, J. 127
Schmidt, M. 169
Scorza, R. 11
Siegler, J. 169
Simon, G. 265
Šimša, J. 79
Široký, O. 349
Špaček, J. 349
Šulz, K. 41
Swencz-Szczepanik, K. 279
Szmitkowska, K. 177
Szmitkowski, M. 177
Szücs, A. 265

T

Tichý, M. 79
Topuz, Ü. 11
Tóth, T. 93
Trávniček, T. 41

V

Vogel, M. 335

Y

Yasuda, J. 251

Z

Zywicka-Łopaciuk, H. 279

Subject Index

Acetylcholinesterase in erythrocytes 265
Adenine in infusion fluid 127
Adenovirus-induced haemagglutination 265
Allogeneic test system 85
Anaemia, immune haemolytic 133
Antibody-dependent cellular cytotoxicity 85
Antigen A 139
— H 139
— HLA 93
Antihaemophilic factor in human granulocytes 177
Antithrombin III, effect on thrombin inactivation 163

Blood groups, isoserological characteristics 139
— storage 331
Bone marrow blasts in leukaemia 297
— — cell separation 41
— —, colony formation 309, 321

Chromosome studies in lymphomas 47
Collagen, role in thrombin inactivation 163
Colony formation of lymphocytes 11, 309, 321
Copper level of serum in leukaemia 297
Cyanotic congenital heart disease 169
Cytoplasmic inclusions in chronic lymphatic leukaemia 269

Disseminated intravascular coagulation (DIC) 359

Exchange transfusion 133

Factor VIII in human granulocytes 177
Fibrinogen metabolism 169
Ficoll gradient for bone marrow cell separation 41
Formyl-leurosine effect on platelet production 149

Granulocytes, antihaemophilic factor in 177
— transfusion 31
Granulocytopenia 31

Haemagglutination, adenovirus-induced 265
Haemolysis and disseminated intravascular coagulation (DIC) 359
— — irradiation 335
— — rifampicin 359
Haemorrheology in malignant melanoma 301
Heat aggregation of anti-D immunoglobulin 251
Heparin, effect on lymphocyte count 103
—, — — thrombin inactivation 163
Hexaphosphamide therapy 115
HLA antigens 93
Hodgkin's disease 47
Hypercalcaemia in chronic lymphatic leukaemia 269
Hyperphosphataemia in chronic lymphatic leukaemia 269

IgG paraproteinaemia 79
Immune haemolytic anaemia 133
— reaction, cellular 93
Immunoglobulins, anti-D 251
— in paraproteinaemias 79
Infusion fluid with adenine, pyruvate and phosphate 127
Irradiation of blood, extracorporeal 335
Isoserological characteristics of A blood group persons 139

Lanthanum ion, effect on platelet electrophoretic mobility 155
Leucocyte migration test 93

Leukaemia, acute lymphoblastic 297
— and lymphocyte subpopulations 289
—, chronic lymphatic 269, 279, 289
—, serum copper level in 297
—, therapy with hexaphosphamide 115
Lymphocytes, colony formation 11, 309, 321
— count, peripheral 103
—, effect of phytohaemagglutinin (PHA) on 11
—, subpopulations in leukaemia 289
Lymphomas, malignant 47
Lymphosarcoma 47
Lysozyme activity in serum 111

Malignant tumours and chronic lymphatic leukaemia 279
Mastocytosis, systemic 349
Melanoma, malignant 301
Mitogenicity of fetal calf serum 259
M protein in multiple myeloma 73
Myelobromol treatment of myelofibrosis 57
Myelofibrosis 11, 57
Myeloma, multiple 73
Myeloproliferative diseases 111

Oxygen transport function of blood 331

Paraproteinaemia, IgG 79
Phosphate in infusion fluid 127
Phytohaemagglutinin (PHA) effect on lymphocytes 11, 309, 321
Platelet electrophoretic mobility 155
— production in mice 149
Prednisolone, effect on lymphocyte count 103
Pyruvate in infusion fluid 127

Rifampicin and haemolysis 359

Serum copper level in leukaemia 297
—, fetal calf 259
Skin sensitivity to allogeneic antigens 93
Spleen, colony formation in 11, 309, 321
Stem cell population 11

Thrombin inactivation 163
Transfusion, exchange 133
—, granulocyte 31
Tumours, malignant and chronic lymphatic leukaemia 279

Viscosity of blood in malignant melanoma 301

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

Therapie der Blutstillungsstörungen

Anleitung zur Diagnose hämorrhagischer Diathesen und zur Therapie mit Hämostyptika und Blutbestandteilen

Herausgegeben von Prof. Dr. sc. med. Dr. phil. FRITZ MARKWARDT, Erfurt
2., überarbeitete und ergänzte Auflage. 1976. 207 Seiten, 20 Abbildungen, 22 Tabellen
Plasteinband 33,40 M · Bestell-Nr. 793 457 2

Die Erforschung des Blutgerinnungsmechanismus hat die Voraussetzung für eine exakte Diagnostik geschaffen. Die in den letzten Jahren erreichten Fortschritte der Plasma- bzw. Blutfraktionierung bieten Möglichkeiten, bestimmte hämorrhagische Diathesen durch sinnvollen Einsatz der fehlenden Blutstillungsfaktoren zu behandeln. Darüber hinaus wurden Hämostypika entwickelt, die — wie z. B. die natürlichen und synthetischen Antifibrinolytika — teilweise neuartige Arzneistoffklassen darstellen und bessere Möglichkeiten zur Therapie bestimmter Blutungen eröffnen. Den mit der Materie weniger Vertrauten aus den verschiedenen Fachdisziplinen wird mit diesem Fachbuch eine Anleitung zum praktischen Handeln am Krankenbett gegeben.

Bestellungen an den Buchhandel erbeten

JOHANN AMBROSIUS BARTH LEIPZIG

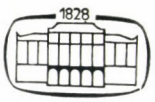
Brain Blood Supply

Proceedings of the 3rd Tbilisi Symposium on Cerebral Circulation
Tbilisi, 5—7 June, 1974

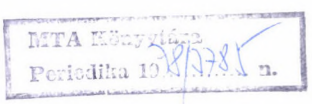
dited by G. I. Mchedlishvili, A. G. B. Kovách, I. Nyáry

The Tbilisi Symposia have won a world-wide reputation because of their unique character. A panel of international experts in physiology, pharmacology, biochemistry, anatomy, neurology, neurosurgery and medical physics, reported on their latest findings. The programme was focused on the most actual problems of cerebral circulation: Physiology, pathophysiology of the vascular smooth muscle cells in the brain; Neural and humoral effects on cerebral vessels; Nervous activity and cerebral circulation; The mechanism of cerebrovascular regulation; General pathology of cerebral circulation. The stimulating discussions that followed each lecture are all included in the proceedings.

In English · 319 pages · several illustrations and tables · 17×25 cm · Cloth
ISBN 963 05 1130 4



AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
Budapest



Card Indexes

H. Ishiyama, K. Okuyama, K. Masuda, J. Ozaki, J. Yasuda

Modifications of the biological activity of anti-D immunoglobulin caused by heat aggregation. Haematologia *11*, 251—258 (1977)

Some of the biological activities known to be confined to certain anti-D sera such as Ripley could be simulated using heat-treated human anti-D Ig preparations. Thus, human red cells sensitized with heat-treated anti-D Ig were found to be capable of causing Fc-receptor on lymphocyte surfaces and of fixing complement. In its other effects such as reactivity to rheumatoid factor and to anti-allotype agglutinators, heat-treated anti-D Ig seemed inferior to such anti-D sera as Ripley.

P. Gergely, T. Bakács

Mitogenicity of fetal calf serum. Haematologia *11*, 259—263 (1977)

The mitogenic effect of fetal calf serum (FCS) has been studied. Human lymphocytes, especially cord blood cells, undergo blastogenesis in media supplemented with FCS. The mitogenic effect is exerted on non-T (B and null) lymphocytes, but not on T cells. Bovine serum albumin (BSA) successfully substitutes for FCS in mitogen-induced lymphocyte blastogenesis. Unlike FCS, it has no mitogenic properties.

G. Simon, A. Lengyel, A. Szücs

Erythrocyte acetylcholinesterase activity is not affected by adenovirus-induced haemagglutination. Haematologia *11*, 265—268 (1977)

No correlation was found between adenovirus-induced haemagglutination and AChE activity of human RBCs as ascertained with three different types of virus. The haemagglutination titre of the adenoviruses did not decrease when RBCs with AChE inhibited by dichlorvos or eserine were used. The receptors involved in active haemagglutination by adenoviruses thus seem to differ from those acting in passive haemagglutination. The AChE inactivating effect of substances such as tannic acid and glutaraldehyde, among others, might be due to a non-specific damage of the RBC membrane.

N. Freidin, E. M. Mandel, N. Djaldetti

Hypercalcaemia, hyperphosphataemia and cytoplasmic inclusions in a patient with chronic lymphocytic leukaemia. Haematologia *11*, 269–278 (1977)

The history of a patient with chronic lymphocytic anaemia (CLL), known for ten years, is presented. During the terminal stage of the disease, she developed hypercalcaemia and hyperphosphataemia. The peripheral blood and lymph node lymphocytes contained crystalline inclusions in the dilated cisterns of the endoplasmic reticulum. The possible mechanism of hypercalcaemia and hyperphosphataemia and the significance of the crystalline inclusions are discussed.

H. Zywicka-Łopaciuk, A. Janowska-Wieczorek, M. Romejko, K. Swencz-Szczepanik, J. Krzyżanowska-Woźniakowska, R. Michalski, S. Pawelski

Occurrence of malignant neoplasms in patients with chronic lymphatic leukaemia. Haematologia *11*, 279–287 (1977)

In a group of 280 patients with chronic lymphatic leukaemia, a coexistence of malignant tumours diagnosed simultaneously with the leukaemia was found in 14 cases or 5.0%. No significant difference was observed between the development of the second malignant neoplasm in the patients studied and the expected incidence in the general population, taking into account also the sex and age of patients and their place of residence. No statistically significant relation was found between the duration of CLL and the occurrence of second malignancies. No significant correlation was found between the total dose of cytostatic drugs, corticosteroids or radiotherapy and the incidence of cancer.

E. Heilmann, R. Nordiek

Correlations between lymphocyte subpopulation and stage of disease in chronic lymphatic leukaemia of Rai. Haematologia *11*, 289—295 (1977)

T- and B-lymphocytes have been studied in 36 patients with chronic lymphatic leukaemia (CLL). Each twelve cases remained untreated or were treated with chlorambucil or extracorporeal irradiation of the blood (ECIB). The patients were classified according to the staging system proposed by Rai et al. In all groups we found significantly elevated B- and T-cells. There was no correlation between the stage of the disease and the proportion of lymphocytes. B-lymphocytes were reduced significantly by ECIB, whereas T-lymphocytes have not been influenced.

L. Legutko

Relationship between serum copper and bone marrow blasts in acute leukaemia. Haematologia *11*, 297–300 (1977)

Investigation of serum copper level (SCL) and bone marrow obtained on the same day from 45 children with acute lymphoblastic leukaemia (ALL) showed a highly significant correlation between SCL and marrow blasts. The increase of SCL was associated with the progress of ALL and the elevation of the marrow parablast percentage. The highest mean SCL level was observed in cases displaying full marrow involvement with concomitant hyperleukocytosis and/or leukaemic tumours. These observations suggest that SCL can be useful in the management of children with acute leukaemia.

L. Dintenfass

Some aspects of haemorrheology of metastasis in malignant melanoma. Haematologia *11*, 301–307 (1977)

A study of 125 patients with malignant melanoma, of which 30 succumbed to metastases, indicated that plasma viscosity and aggregation of red cells are significantly elevated in patients with metastases, Plots of the intervals between the time of the viscosity test and the time of death against plasma viscosity showed a significant negative correlation; while plots of the same intervals against blood viscosity showed a significant positive correlation.
Viscosity functions (plasma viscosity *vs.* globulin) showed a significant ($P < 0.005$) difference between patients with metastases and patients free from metastases. A significant difference was noted in some functions between patients of A and 0 blood groups.

A. C. Louwagie

I. Increase in bone marrow CFU-S and CFU-C after in vivo phytohaemagglutinin (PHA) administration in mice. Haematologia *11*, 309–320 (1977)

An absolute increase in bone marrow CFU-S was observed within 4 hours after intraperitoneal administration of 3 mg PHA-M or 250 μg PHA-P. At that time, there was no increase in spleen CFU-S, while a pronounced decrease in the number of circulating CFU-S was noted. A two to fourfold increase in bone marrow CFU-C was found within 12 hours after intraperitoneal administration of a similar dose of PHA-M or PHA-P. Addition of PHA-M or PHA-P at small concentrations resulted in a marked inhibition of granulocytic colony formation whereas macrophage colony growth became depressed only at very high concentrations.

A. C. Louwagie

II. Mechanism of increased colony formation of CFU-S and CFU-C after in vivo PHA administration in mice. Haematologia *11*, 321–330 (1977).

In vivo administration of 3 mg PHA-M results in an increase in spleen colonies due to an augmented seeding of CFU-S into the spleen. An increase in the number of CFU-S in the developing nine-day spleen colonies also occurred together with a slight increase in the number of CFU-S in DNA-S phase 24 hours after PHA-M administration. The increased colony formation was due to an increased number of CFU-C from the early culture period onwards but could not be explained by an increase in the number of CFU-C in DNA-S phase. The release of colony-stimulating substances was proved but this only partially accounted for the observed rise in bone marrow CFU-C.

D. Boda, E. Eck

Favourable effect of storage at 0 °C on the O_2 transport function of heparinized blood (Short communication). Haematologia *11*, 331–334 (1977)

The biological value of heparinized blood was found to decrease less on storage at 0 °C than on storage at +4 °C. At 0 °C the P_{50} value decreases substantially later and less, the blood is less acidotic, and the UV absorption of the dialysate is less enhanced than at +4 °C. The findings justify a further search for the conditions suitable for the widespread practical application of storage at 0 °C.

E. Heilmann, M. Vogel

Haemolysis after extracorporeal blood irradiation of man? Haematologia *11*, 335–348 (1977)

Haemolysis was studied before and after extracorporeal irradiation of the blood of 20 chronic lymphadenosis patients. Free haemoglobin, haptoglobin and haemopexin were not affected by irradiation, indicating that no haemolysis was caused by the transit dose accumulated during the applied procedure.

M. Bláha, J. Kačerovský, Z. Hrnčíř, Z. Nožička, J. Špaček, O. Široký

Systemic mastocytosis. Report of a case. Haematologia *11*, 349—357 (1977)

The case of a 20-year-old woman with systemic mastocytosis is reported. The disorder was not preceded by a clinically diagnosed local form of the disease; fibrous changes in organs, bone changes, eosinophilia and increased serotonin, heparin and histamine levels had been observed. Clinical symptomatology was typical and the patient died one year after the first signs of the disease.

J. Nowicka, M. Kotschy, K. Chwistecki, M. Jeleń

Disseminated intravascular coagulation (DIC) during superacute haemolysis in a patient with ovarian dermatoid cyst treated with rifampicin. Haematologia *11*, 359—364 (1977)

A 53-year-old female patient with ovarian dermatoid cyst and lung tuberculosis had been treated with rifampicin. During repeated rifampicin treatment she developed an acute haemolytic syndrome with haemorrhagic diathesis. Laboratory findings showed that it was caused by disseminated intravascular coagulation. Death ensued despite intensive administration of heparin and Trasylol. It is assumed that the repeated rifampicin application had provoked massive haemolysis by an allergic mechanism leading to thrombofibrinolytic haemorrhagic diathesis.

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to the advancement in all fields related to haematology and blood transfusion. Papers in English, French, German and Russian are accepted on the condition that they have not been previously published or accepted for publication.

Manuscripts with a clear carbon copy should be sent to the Editor-in-Chief

Susan R. Hollán, M. D.
National Institute of Haematology
and Blood Transfusion
Daróczi út 24
1113 Budapest, Hungary.

If in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will speed publication. Although every effort will be made to guard against loss, it is advised that authors retain copies of all material which they submit. The editorial board reserves the right to make literary corrections.

Manuscripts should be typed double-spaced on one side of good quality paper with proper margins and bear the title of the paper, name, address and degrees of the author together with the name of the hospital, laboratory or institute where the work has been carried out. The name and full postal address of the author who will be responsible for reading proofs should also be given. An abstract of 50 to 100 words should precede the text of the paper. The paper should not exceed 15 pages including tables and references. The approximate location of tables and figures should be indicated in the margin.

References. Only papers closely related to the author's work should be referred to. The citations should include the name of the author and/or the reference number in parenthesis. A list of numbered references should follow the end of the manuscript.

References to periodicals should mention: (1) Lame(s) and initials of the author(s); (2) title of paper; (3) international abbreviation of the periodical; (4) volume; (5) number of the first page; (6) year of publication in parenthesis. Thus: 14. Bean, W., Mills, A.: Coronary occlusion, heart failure and environmental temperature. *Amer. Heart J. 16*, 701 (1938).

References to books should include: (1) author(s)' name; (2) title; (3) publisher; (4) place and year of publication. Thus: 8. Alsted, G.: The incidence of peptic ulcer in Denmark. Danish Science Press Ltd., Copenhagen 1953.

Illustrations should be selected carefully and only up to a quantity required. Black-and-white photographs should be in the form of glossy prints. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each print. Cloured illustrations should be given only if indispensable. Legends should be brief and attached on a separate sheet. Tables, each bearing a title, should be self-explanatory and numbered consecutively.

Authors will receive page proofs which must be sent back by return mail.

Authors are entitled to 50 reprints free of charge.

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

AUSTRALIA

C.B.D. Library and Subscription Service
Box 4886, G.P.O.
Sydney, N.S.W. 200zff

Cosmos Book and Record Shop
145 Acland Street
St. Kilda 3182, Vic.

Globe and Co.
694—696 George Street
Sydney 2000 N.S.W.

AUSTRIA

Globus
Vertrieb Ausländischer Zeitschriften
Höchstädtplatz 3
A-1200 Wien XX

BELGIUM

"Du Monde Entier" S.A.
Rue du Midi 162
B-1000 Bruxelles

Office International de Librairie S.A.
Avenue Marnix 30
B-1050 Bruxelles

BULGARIA

Direkzia R.E.P.
11 pl. Slaveikov
Sofia

CANADA

Pannonia Books
P. Box 1017
Postal Station "B"
Toronto, Ont. M5T 2T8

CHINA

CNPICOR
Periodical Division
P.O.B. 88
Peking

CZECHOSLOVAKIA

Mad'arská Kultura
Narodní Trida 22
115 66 Praha I

Postova Novinova Sluzba — dovoz tisku
Vinohradská 46
Praha 2

Postova Novinova Sluzba — dovoz tlace
Leningrádská 14
Bratislava

DENMARK

Munksgaard's Boghandel
Norregade 6
dK-1165 Kobenhavn K

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
SF-00100 Helsinki 10

FRANCE

Office International de Documentation et Librairie
48, rue Gay-Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Zeitungsvertriebsamt
Strasse der Pariser Kommune 3—4
1004 Berlin

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
7000 Stuttgart I
Postfach 46

GREAT BRITAIN

Blackwell's Periodicals Department
P.O. Box 40
Hythe Bridge Street
Oxford OX1 -EU

Bumpus Haldane and Maxwell Ltd.
Cowper House
Olney, Bucks MK46 4BN
Collet's Holdings Limited
Denington Estate
Wellinborough, Northants NN8 2QT

W. M. Dawson and Sons Ltd.
Cannon House
Folkestone, Kent

H. K. Lewis and Co. Ltd.
136 Gower Str., P.O.B. 66
London WC1E 6BS

HOLLAND

Martinus Nijhoff
P.O. Box 269
Den Haag
Swets and Zeitlinger
Keizersgracht 487
Amsterdam C

ITALY

Licosa
Via Lamarmora 45
Casella Postale 552
50121 Firenze

JAPAN

Igaku Shoin Ltd.
Foreign Department
Tokyo International
P.O. Box 5063
Tokyo

Maruzen Co. Ltd.
P.O. Box 5050
Tokyo International 100-31
Nauka Ltd .
Yasutomi Bldg. 5F
2—12,
Kanda Jinbocho, Chiyoda-ku
Tokyo 101

NORWAY

Tanum-Cammermeyer
Karl Johansgt. 41—43
Oslo I

POLAND

B.K.W.Z. Ruch
ul. Wronia 23
00840 Warszawa

ROMANIA

D.E.P.
Bucuresti
D.E.P.
Oradea

SOVIET UNION

Mezhdunarodny pochtamt
tseh-4
Moscow
Pochtamt-Import
Leningrad

SWEDEN

Almqvist and Wiksell Förlag A.B.
Box 2120
S-103 13 Stockholm 2

Wennegren-Williams AB
Subscription Department
Nordenflychtsvägen 70
Fack
S-104 25 Stockholm 30

SWITZERLAND

Karger Libri A.G.
Petersgraben 31
CH-4011 Basel

USA

Ebsco Industries, Inc.
1st Ave. North AT 13th Street
Birmingham, Alabama 35201
FAM Book Service
69th Fifth Avenue
New York, N.Y. 10003

Intercontinental Medical Book Corporation
381 Park Avenue South
New York, N.Y. 10016

J. B. Lippincott Company
East Washington Square
Philadelphia, Pa. 19105
Stechert-Macmillan, Inc.
7520 Westfield Avenue
Pennsauken, N.J. 08110

YUGOSLAVIA

Forum
Vojvode Misica broj 1
21000 Novi Sad

Jugoslovenska Knjiga
Terazije 27
11000 Beograd

Prosveta Export and Import
Teraizje 16l1
11001 Beograd

ndex: 26861

HU ISSN 0017—6559