315.871

VOLUME 1 • NUMBER 1 • 1967

HAEMATOLOGIA

International Quarterly of Haematology

EDITOR-IN-CHIEF:
S. R. HOLLÁN

EDITOR:
I. BERNÁT

Editorial Board:

G. A. ALEKSEEV (Moscow)
A. ANDRÉ (Liège)
A. ANTONINI (Rome)
J. ARNAUD (Tours)
G. ASTALDI (Tortona)
I. BARTA (Pécs)
G. BAST (Rostock)
H. BEGEMANN (München)
E. BENEDEK (Budapest)
J. BERNARD (Paris)
E. BEUTLER (Duarte, California)
H. BRAUNSTEINER (Innsbruck)
W. DAMESHEK (New York)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Uppsala)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
I. GONZÁLEZ-GUZMÁN (Mexico City)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
G. C. de GRUCHY (Melbourne)
A. HÄSSIG (Bern)
L. HEILMEYER (Freiburg/Br.)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
I. A. KASSIRSKY (Moscow)
E. KELEMEN (Budapest)
A. E. KISELEV (Moscow)
E. KOWALSKI (Warsaw)
P. J. LAH (Belgrade)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Marburg/Lahn)
A. E. MOURANT (London)
C. T. NICOLAU (Bucharest)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Szeged)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM (Liège)
G. RUHENSTROTH-BAUER (München)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Modena)
M. C. VERLOOP (Utrecht)
A. VIDEBAEK (Copenhagen)
S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

HAEMATOLOGIA

is a new 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 some 500 pages.

Subscription price: $ 12 per volume (per year). Orders may be placed with

KULTURA

Trading Company for Books and Newspapers
Budapest 62. P.O.B. 149

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

Contents

Foreword 3

Bernard, J.: Remarques sur la tumeur de Burkitt 5

R. Hollán, S., G. Szelényi, J., Lehmann, H., Beale, D.: A Boston-type haemoglobin M in Hungary: haemoglobin M Kiskunhalas 11

Levin, E., Beutler, E.: Human erythrocyte adenylate kinase 19

Storti, E., Lusvarghi, E., Grignaffini, G. F., Sgandurra, A.: Biological and clinical significance of the adrenalin test in haematology 27

Hořejší, J.: Effect of reduced glutathione on the dissociation curve of haemoglobin. Possible therapeutic use of glutathione in haemorrhagic shock 35

Waubke, R., Gerstmair, G., Trepel, F., Pichlmaier, H., Begemann, H.: Wirkung von Röntgen-Ganzkörper- und -Extrakorporal-Bestrahlung auf die Lymphozyten des peripheren Blutes 45

Чернцова Т. А., Сусоева, В. М.: Опыт лечения миелобромолом больных хроническим миелолейкозом 61

Péter, Á., Szatmári, É., Romhányi, J., Letenyei, K.: Changes in the myelogram in panencephalitis nodosa (Subacute sclerosing leucoencephalitis) 79

Book Reviews 85

Abstracts 88

From the international literature on haematology 96

VOLUME 1

HAEMATOLOGIA

INTERNATIONAL QUARTERLY OF HAEMATOLOGY

EDITOR-IN-CHIEF:

S. R. HOLLÁN

EDITOR:

I. BERNÁT

MEMBERS OF THE EDITORIAL BOARD:

G. A. ALEKSEEV (Moscow) · A. ANDRÉ (Liège) · A. ANTONINI (Roma) · J. ARNAUD (Tours) · G. ASTALDI (Tortona) · I. BARTA (Pécs) · G. BAST (Rostock) · H. BEGEMANN (München) · E. BENEDEK (Budapest) · J. BERNARD (Paris) · E. BEUTLER (Duarte, California) · H. BRAUNSTEINER (Innsbruck) · W. DAMESHEK (New York, N. Y.) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle Washington) · L. GARBY (Uppsala) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest) · I. GONZÁLEZ-GUZMÁN (Mexico City) · F. GRÁF (Budapest) · A. GRAFFI (Berlin) · G. C. de GRUCHY (Melbourne) · A. HÄSSIG (Bern) · (L. HEILMEYER (Freiburg/Br.) · L. P. HOLLÄNDER (Basel) · J. HOŘEJŠÍ (Prague) · M. HRUBIŠKO (Bratislava) · I. A. KASSIRSKY (Moscow) · E. KELEMEN (Budapest) A. E. KISELEV (Moscow) · E. KOWALSKI (Warsaw) · P. J. LAH (Belgrade) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Marburg/Lahn) · A. E. MOURANT (London) · C. T. NICOLAU (Bucharest) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Szeged) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM (Liège) G. RUHENSTROTH-BAUER (München) · V. SERAFIMOV-DIMITROV (Sofia) I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIĆ (Belgrade) E. STORTI (Modena) · M. C. VERLOOP (Utrecht) · A. VIDEBAEK (Copenhagen) S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ

PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES

BUDAPEST 1967

MAGYAR

To the Reader

The lively interest aroused by the periodical *Haematologia Hungarica* – although most of its papers were published in Hungarian – induced us to develop this journal into an international quarterly. Well-known haematologists from 22 countries have kindly contributed to the realization of this object and enabled us to present now the first issue of *Haematologia.*

It was not our intention to add just one more to the number of haematological journals, a number not negligible *in se* but still relatively small considering the rapid development and growing importance of haematology. It is the aim of *Haematologia* to serve as a forum for the achievements of haematologists carrying on research in various countries, thereby paving the way for a closer scientific co-operation.

Beside publishing original papers we also wish to provide our readers with complex and up-to-date information on both research and clinical practice. A General Survey, an Open Forum, Book Reviews, Abstracts of the more important papers from other periodicals and, last but not least, a regular Documentation of the well-known and the less accessible periodicals are to serve this purpose.

Entering the respectable family of haematological journals may we ask the Reader for support in our endeavour to contribute to the advance of our science.

Remarques sur la tumeur de Burkitt

ÉDITORIAL

J. BERNARD

Hôpital Saint-Louis, Paris

(Reçu le 15e juin, 1966)

La tumeur des mâchoires est anciennement connue en Afrique Centrale et Orientale. Des dessins d'enfants noirs aux visages déformés illustrent les carnets de notes des premiers explorateurs, missionnaires ou médecins, qui à la fin du siècle dernier décrivirent le royaume millénaire de Bogenda. Mais l'histoire de ces tumeurs commence avec les travaux de Burkitt et peut être partagée en trois périodes:

La période des safaris de Burkitt: En 1962, Denis Burkitt, chirurgien à Kampala, entreprend plusieurs grands voyages. Il longe les vertes collines d'Afrique, gravit les montagnes du Ruanda-Urundi, parcourt les forêts humides du Tanganyika, les savanes, les déserts. Il cherche à préciser la géographie de la tumeur particulière de la mâchoire dont il a on 1958 en Ouganda reconnu la fréquence et l'originalité.

La période suivante est une période de stagnation, de déception, les progrès qu'on avait espéré rapides se font attendre. Enfin vient une période de mise au point; c'est à cette mise au point que s'est consacré un récent colloque tenu à Kampala.

Ces tumeurs sont en effet très remarquables par l'âge des victimes, par leur allure anatomo-clinique, par la géographie. Les enfants sont seuls ou presque seuls atteints, surtout les enfants de 5, 6 et 7 ans. La maladie est exceptionnelle avant un an, exceptionnelle après 15 ans. La courbe de fréquence absolue ressemble ainsi à celle de la leucémie aiguë de l'enfant avec la même rareté avant un an, le même pic dans le seconde enfance, un peu plus tardif pourtant ici que dans la leucémie. On compte parmi les malades deux fois plus de garçons que de filles. Les tumeurs de Burkitt de l'adulte, dont quelques cas sont connus, ont un double intérêt, elles frappent des immigrants récents, des personnes qui n'ont pas passé leur enfance dans des zônes endémiques; elles sont plus viscérales et moins maxillaires que les formes de l'enfant. Cette autonomie anatomo-clinique de la maladie est assurée. Certes, les discussions des cytologistes et des histologistes persistent, encore que certains caractères, la forte basophilie et l'homogénéité du cytoplasme, l'abondance des vacuoles, la finesse du réseau nucléaire, l'abondance sur les coupes à côté des cellules tumorales, de grands macrophages chargés de lipides, donnent à la tumeur de Burkitt une place assez particulière parmi les hématosarcomes de la série lymphoïde. Mais c'est surtout du côté de la clinique et de l'ana-

tomie macroscopique que l'individualité de l'affection est manifeste. La maladie de Burkitt est un hématosarcome défini par les trois traits suivants: 1. importance et précocité des localisations aux mâchoires, 2. importance de certaines localisations viscérales, surtout ovaires, testicules, système nerveux central, 3. intégrité habituelle de la rate et des ganglions lymphatiques, ce dernier trait étant pour un hématosarcome le plus original.

Le très grand intérêt de la tumeur de Burkitt est de servir de modèle pour les deux problèmes fondamentaux des hématosarcomes humains et particulièrement des leucémies aiguës, celui de l'étiologie, celui de la physiopathologie et des effets du traitement.

Les voyages de Burkitt ont précisé la répartition géographique de la maladie. Il ne semble pas exister de facteur ethnique, à tout le moins la sarcomatose a été observée dans des groupes africains très divers; les enfants indiens vivant en Afrique, les enfants blancs peuvent être atteints. Les constatations sur lesquelles se fonde Burkitt pour imputer la maladie à des facteurs fournis par l'environnement ne permettent pas cependant d'écarter complètement l'intervention de facteurs constitutionnels indépendants de la race.

Le rôle de deux facteurs d'environnement, la température, l'hygrométrie, a été reconnu par les safaris de Burkitt.

La tumeur n'est observée que dans les zônes dont la température moyenne est supérieure à 17° C. Elle est fréquente dans la plus grande partie de l'Ouganda, au Kenya, au Tanganyika autour des grands lacs. Elle manque complètement dans les zônes montagneuses de l'Ouganda et dans toute l'étendue du Ruanda et de l'Urundi, pays montagneux dont l'altitude dépasse 1600 m. Cette altitude limite va s'abaisser avec l'éloignement de l'Equateur. La tumeur connue au Mozambique est inconnue dans le Swaziland tout voisin dont l'altitude n'est que de 600 m. Elle n'est pratiquement jamais observée dans l'extrême Sud du continent.

C'est au Nigeria que le rôle de l'hygrométrie a été le mieux apprécié. Point de tumeur dans le Nord désertique du pays, nombreuses tumeurs dans le Sud, là où les pluies sont abondantes. Le rôle de l'humidité s'explique probablement par l'influence qu'elle exerce sur la végétation.

Les enquêtes faites par les médecins d'Afrique francophone, particulièrement par Camain et son école, enquêtes rassemblées dans la remarquable thèse de Melle Lambert, ont confirmé ces notions. Il est ainsi possible de dessiner autour de l'Afrique une zône particulière, la ceinture de Burkitt. Une zône endémique nouvelle dans des conditions climatiques comparables a été découverte récemment on Nouvelle Guinée. Les cas sporadiques signalés aux Etats-Unis et en Europe ne sont pas tous authentiques et demeurent très peu nombreux. Ainsi le rôle de la température et le rôle de la végétation paraissent établis. L'intervention d'un arthropode vecteur et d'un virus est dès lors sérieusement soupçonnée, soupçonnée mais non prouvée. On doit cependant noter que la répartition générale de la maladie correspond assez bien à celle de certains anophèles, surtout les anophèles Mansoni que les médecins d'Afrique Orientale tiennent actuellement pour un vecteur probable.

Mais les études virologiques piétinent ou paraissent piétiner. Pourtant un progrès important est survenu: dans différents laboratoires des lignées de cellules de Burkitt ont pu en culture être obtenues et perpétuées dans des conditions qui semblent bonnes. Ces lignées facilitent les recherches virologiques dont les résultats pourtant demeurent encore incertains. Tantôt et le plus souvent, point de virus, tantôt trop de virus. Deux sortes de virus ont en effet été isolées: un virus herpétique par plusieurs équipes, des rhéo-virus par d'autres. Il paraît bien s'agir de virus authentiques et non, comme trop souvent dans l'étude des leucémies humaines, de débris de plaquettes ou de mycoplasmes. Il semble bien que les virus trouvés viennent de la tumeur de Burkitt. Il est plus difficile d'affirmer leur rôle étiologique et l'on sait bien que les tissus tumoraux récèlent assez souvent des virus innocents ou apparemment innocents. L'éloignement géographique, l'insuffisance des équipements locaux, d'autres difficultés aussi expliquent peut-être, pour une part, la lenteur des progrès. Des efforts très vigoureux cependant sont envisagés et l'on peut raisonnablement espérer des progrès dans un avenir pas trop éloigné.

Les effets de la chimiothérapie ont apporté d'autres informations importantes. La thérapeutique a en effet été orientée par l'insuffisance des équipements locaux. L'Ouganda ne possède pas de radiothérapie et, fait assez rare en cancérologie, la tumeur de Burkitt a d'emblée et uniquement été traitée par chimiothérapie. Les antimétabolites n'ont pas donné de résultats bien nets. La vincaleucoblastine paraît inefficace, la vincristine n'a pas encore été essayée, la méthylhydrazine n'a été que rarement donnée et sans effet. En fait c'est presque uniquement avec la cyclophosphamide que les essais thérapeutiques ont été poursuivis.

La fréquence des rémissions est certaine. Dans les deux tiers des cas une rémission de bonne qualité est obtenue. Cette rémission est souvent très rapide et s'annonce en quelques jours.

Mais le fait le plus notable est la longue durée de certaines rémissions, durée et qualité si remarquables que la guérison de ces tumeurs a pu être envisagée. La fréquence des très longues rémissions ne peut être évaluée, la surveillance à long terme de ces enfants améliorés est en effet très difficile, mais leur fréquence est assurément élevée, de l'ordre de 10 à 15%, beaucoup plus élevée que celle des longues rémissions des leucémies aiguës. Elles ont généralement été obtenues après une cure assez courte de cyclophosphamide et sans traitement d'entretien.

Dès lors, et c'est là l'orientation actuelle probablement la plus féconde des recherches consacrées à la tumeur de Burkitt, l'accent a été mis sur l'intervention des mécanismes de défense de l'hôte, sur la réaction de l'hôte contre sa tumeur.

Les médecins anglais et africains qui, en Ouganda, au Kenya, au Nigeria, ont, avec beaucoup de vigueur, abordé depuis trois ans l'étude de ces problèmes, ont retrouvé les données établies pour les leucémies aiguës par l'école française dès 1947. Ils ont rencontré les mêmes difficultés. Ils ont étudié, comme l'avaient fait les auteurs français, les effets des transfusions de sang de donneurs sains, les effets des transfusions de sang prélevé à des malades en rémission et noté, dans certains cas, des résultats heureux, fonction peut-être du moment du prélèvement.

Le rôle de la posologie et de la durée de la chimiothérapie est probablement important. Le traitement appliqué par Burkitt est fort mais court, très différent des chimiothérapies multiples et prolongées actuellement appliquées au traitement des hémopathies malignes communes. Ainsi est renouvelé le débat qui oppose partisans de la destruction totale, mathématiques des cellules leucémiques, et partisans d'une destruction moins complète, laissant persister non détruits les mécanismes de défense de l'hôte. Il est possible que Burkitt est presque fortuitement trouvé d'emblée la dose heureuse, celle qui détruit assez de cellules malignes pour permettre aux mécanismes personnels de défense respectés d'intervenir efficacement.

Tout récemment, l'application à la maladie de Burkitt de méthodes nouvelles (immuno-fluorescence, cytotoxicité) ont permis d'ébaucher la mesure de cet état immunologique de l'hôte. Klein a pu, à Stockholm, étudier les effets de sérum prélevé à Nairobi à des malades atteints de maladie de Burkitt à différentes périodes de l'évolution sur des cellules de tumeur de Burkitt. Jean Dausset et F. Kourilksy ont dans notre laboratoire, en collaboration avec Klein et Madame Klein, entrepris des études comparables. Les premiers résultats sont encourageants. Ils doivent certes être interprétés avec prudence. Il paraît cependant hautement probable que le sérum des sujets atteints de maladie de Burkitt contient des anticorps spécifiques de la cellule de Burkitt décelés par immuno-fluorescence et que le taux de ces anticorps varie avec l'évolution, faible au début, augmentant au moment de la rémission, diminuant très tardivement.

Dès lors les questions habituelles se posent: cette réaction immunologique est-elle authentique et importante? Si la réponse est affirmative, quelle est sa cible vraie, cellulaire ou virale? Existe-t-il une relation entre ces changements immunologiques et la rémission complète, sa qualité, sa durée? Si elle est heureuse et utile, comment fortifier, prolonger cette réponse immunologique? Enfin, les méthodes immunologiques peuvent-elles faciliter l'étude épidémiologique de la maladie?

On reconnaît là, presque terme pour terme, les questions qui préoccupent actuellement si fortement les leucémologues. Plusieurs laboratoires se consacrant aux leucémies appliquent depuis plusieurs années des méthodes identiques mais la tâche est difficile, plus difficile semble-t-il que pour la tumeur de Burkitt.

Une troisième leçon a été apportée par ces essais thérapeutique, les aplasies provoquées par la chimiothérapie sont remarquablement rares. Les doses données par Burkitt sont très fortes, de l'ordre de 30 à 40 mg par kilo (par voie veineuse une fois par semaine) soit 600 à 800 mg pour un enfant de 20 kg. La durée du traitement, et nous l'avons dit, est le plus souvent assez courte, mais les aplasies n'ont pratiquement pas été observées et sont en tout cas exceptionnelles. On a proposé d'expliquer cette heureuse tolérance par l'hyperactivité latente de la moelle des enfants noirs soumis depuis longtemps à diverses parasitoses anémiantes et avant tout au paludisme.

On voit ainsi s'ébaucher une nouvelle chimiothérapie, la chimiothérapie géographique qui devra prendre pour thème l'adaptation nécessaire des chimiothérapies, de leur nature, des posologies, aux groupes humains considérés.

Ainsi, en peu d'années, les deux grands problèmes que pose l'étude des leucémies humaines, celui des longues rémissions et des principes mêmes du traitement, celui de l'étiologie et du rôle du virus, se sont trouvés renouvelés et inspirés par l'étude de la tumeur de Burkitt. Il est certes possible que l'hypothèse virale se trouve infirmée, il est possible encore que la tumeur de Burkitt apparaisse comme un désordre très particulier sans signification générale. Mais il est plus raisonnable d'escompter et la démonstration de l'origine virale et la valeur exemplaire de cette démonstration.

A Boston-Type Haemoglobin M in Hungary: Haemoglobin M Kiskunhalas

Susan R. Hollán, Judith G. Szelényi, H. Lehmann and D. Beale

Research Institute of Haematology, National Blood Centre, Budapest and
Abnormal Haemoglobin Research Unit, Department of Biochemistry, Cambridge

(Received August 2, 1966)

An α-chain haemoglobinopathy M was described by us formerly as Hb M Kiskunhalas. This anomaly has been now identified by fingerprint analysis as Hb M Boston. The relative amount of Hb M showed definite fluctuation when examined at different times and there was a change in the Hb M per cent of the patient's blood after a stay under lowered atmospheric pressure.

Congenital cyanosis is the most characteristic symptom of hereditary methaemoglobinaemia. Normally, less than two per cent of the total haemoglobin is present as methaemoglobin in the erythrocytes. The equilibrium between the oxydation of haemoglobin to methaemoglobin and the reduction of the latter to haemoglobin may be disturbed either 1. by an enzymatic defect of the methaemoglobin reductase system, or 2. by an abnormality in the molecular structure of the globin moiety of the blood pigment.

Hörlein and Weber [1] were the first to describe a family suffering from congenital methaemoglobinaemia with unusual spectral characteristics due to an abnormality of the globin. The designation of haemoglobin M (Hb M) was suggested by Singer in 1955. Since then a number of individuals or families with Hb M disease have been described [2–18]. The first report on Hb M Kiskunhalas was presented in 1962, at the 9th Congress of the International Society of Haematology and in 1963, at the 9th Congress of the European Society of Haematology [19, 20]. Detailed data of this case of Hb M disease are first published here.

Case Report

The propositus, M. K., a Hungarian young man, was 19 years old when first seen. He was born cyanotic and was mistakenly thought to have congenital heart disease. There was a noticeable fluctuation in the degree of cyanosis during all his life. He was asymptomatic with the exception of moderate fatigue and dyspnoe on strenuous exercise.

There was no history of drug ingestion, and there were no gastrointestinal symptoms. On physical examination there was a bluish-gray tinge of the skin, lips and nail beds. The lungs were clear. Physical examination, electrocardiograms,

roentgenographic examinations, and the result of heart catheterization failed to disclose congenital heart disease. The liver and spleen were not palpable. Treatment with ascorbic acid had no effect upon the cyanosis. The haemoglobin level was 15 g per 100 ml. The red cells numbered 4.4 million, the leukocytes 6200 per cubic millimeter. The differential formula and the myelogram were within normal limits. The erythrocytes were normochromic and there was no basophilic stippling. Thrombocyte count was 250,000 per cubic millimeter. The number of reticulocytes was not increased.

The patient's blood had a chocolate brown colour which was unaffected by shaking in the presence of air. Since there was no increase in methaemoglobin, haemoglobin M trait was suspected.

Methods

Electrophoresis was carried out in starch-gel and in starch-block at pH 7.1 in phosphate buffer, according to Gerald [2].

Absorption spectra were taken in 1/15 M phosphate buffer pH 6.6 using a Unicam SP 500 type spectrophotometer.

Hybridization experiments

To one volume of the patient's Hb (5–6 g%), one volume of canine Hb (5–6 g%) and one volume of 0.5 M acetate buffer, pH 4.7, was added. The mixture was dialyzed against the above buffer in a carbon monoxide atmosphere at 4° C for 24 hours. The recombination was achieved by a dialysis against Tris-buffer, pH 8.8 for 12 hours, in carbon monoxide atmosphere at 4° C [21]. The hybrids formed were examined by starch-gel electrophoresis.

Fingerprint analysis

Peptide maps were prepared and the abnormal peptide was separated and analyzed by methods previously listed [23].

Results

The oxyhaemoglobin form of the patient's haemolysate did not differ from the normal on paper- and starch-gel electrophoresis at pH 8.6. After oxidizing it to methaemoglobin an abnormal fraction could be detected with starch-block and starch-gel electrophoresis at pH 7.1. This fraction was characterized by a greyish-green colour and decreased cathodic mobility (Fig. 1). Based on its electrophoretic behaviour the abnormal fraction could be classified as a variant of the Hb M-group.

The relative amount of Hb M was determined by the two following electrophoretic methods: 1. the densitometric evaluation of the positive-film made from the starch-gel fractions and 2. on the basis of extinction values of the fractions isolated by starch-block electrophoresis. The results obtained by the two methods were in good agreement (Hb M: 32 and 29 per cent, respectively).

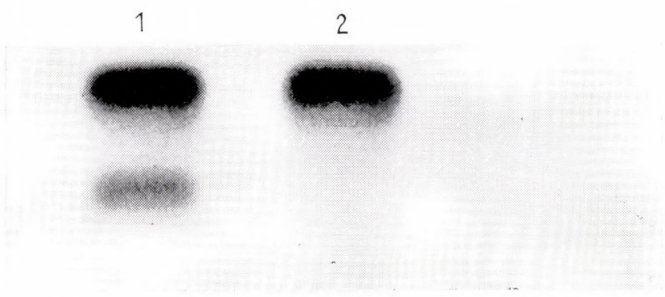


Fig. 1. Starch-gel electrophoretic pattern of oxydized haemolyzates, at pH 7.1 phosphate buffer 7.5 V/cm, three hours pattern 1 : Hb M disease (present patient). Pattern 2: normal adult

According to the spectrophotometric behaviour of the Hbs M the oxyhaemoglobin of the patient's blood differed only slightly from the normal. In the spectral curve of the acid methaemoglobin form of the patient's haemolysate the usual deep absorption minimum at 600 mμ [22] was missing, but the absorption curve attained the peak at 630 mμ through a flat plateau.

The abnormal spectral absorption curve of the isolated methaemoglobin M was even more pronounced. The sharp peak at 630 mμ was completely missing and there was a plateau from 580 – 610 mμ (Fig. 2).

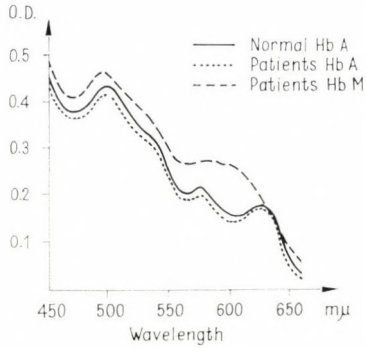


Fig. 2. Absorption curves of fractions isolated from normal (N) and pathological (P) methaemoglobin samples by starch-block electrophoresis

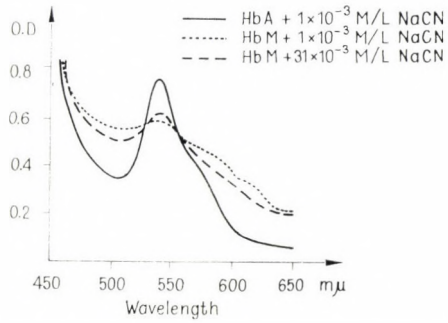


Fig. 3. Changes in absorption of methaemoglobin M as a function of NaCN added

The absorption spectrum of the cyanmethaemoglobin form of the isolated Hb M differed from that of the normal, even after the addition of a large amount of NaCN (Fig. 3).

In our hybridization experiments, in addition to the fractions expected, a new fraction could be detected near the zone $\alpha_2^A\ \beta_2^{Can}$. On the basis of its mobility this fraction corresponds to $\alpha_2^M\ \beta_2^{Can}$ (Fig. 4). This was indicated also by the greyish-green colour of this zone. These results proved that the anomaly resides in the α chain. This was also confirmed by hybridizing the abnormal haemoglobin with Hb S.

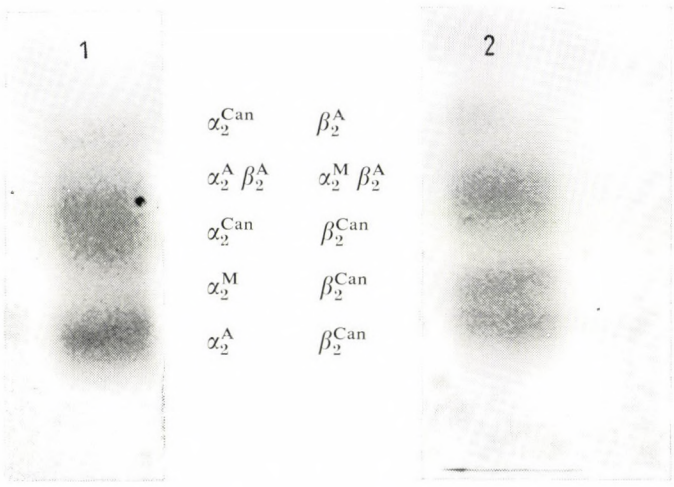


Fig. 4. Starch-gel electrophoretic pattern of fractions obtained by hybridization with canine Hb, at pH 8.8. Tris buffer 9.5 V/cm, 4 ½ hours

The peptide map of the abnormal haemoglobin showed that the histidine containing peptide α Tp VII was missing (Fig. 5). This peptide is known to consist of residues 57 – 60 of the α chain. The faint spot normally representing the peptide α Tp VII – VIII was also missing. A new tyrosine containing peptide was seen near to the position of α Tp III. This new peptide had an electrophoretic mobility of 0.56 (compared with lysine = 1.00) at pH 6.4 and gave a transient yellow colour with ninhydrin. A very faint additional tyrosine positive spot was also found near to the position of β Tp VII. These observations suggested that the residue at α 58 was tyrosine instead of histidine.

Amino acid analysis of the new peptide (electrophoretic mobility = 0.56) and α Tp VII from Hb A confirmed the His-Tyr mutation (Table 1). Hb M Kiskunhalas is, therefore $\alpha_2^{58\ His\text{-}Tyr}\ \beta_2$ and this is the formula reported for Hb M Boston.



Fig. 5. Fingerprint of Hb M Kiskunhalas at pH 6.4

Table 1

Amino acid analysis of α Tp VII from Haemoglobin A and the abnormal peptide from Haemoglobin M Hungary

	$^{\alpha}$TpVII		Abnormal peptide	
	μ moles	molar ratio	μ moles	molar ratio
Gly	0.143	2.1	0.047	2.0
Tyr	0.000	0	0.018	*0.8*
Lys	0.068	1.0	0.023	1.0
His	0.061	*0.9*	0.003	0.1

Most variants of Hb M disease observed so far were dominantly inherited heterozygote anomalies. Since either in the ascending or in the collateral line of the patient's family it was not possible to detect any Hb abnormality, it may be assumed that the occurrence of Hb M in our patient is due to a new mutation. Illegitimacy, however, cannot be ruled out.

As it was mentioned above the degree of cyanosis in the patient varied several times during his life and during our observations as well. The Hb M content of the patient's blood studied at various periods fluctuated between 25 and 32 per cent. However, estimations made simultaneously by various methods corresponded well (Table 2).

Table 2

Haemoglobin M values, as determined by different methods at the same point of time

Method		Starch gel electrophoresis per cent	Starch block electrophoresis per cent
Date	Jan. 23	32	29
	April 10	26	25
Max. deviation from mean		±2.5	±4.5

We have also studied the circulatory and haematologic adjustment of our patient to a moderate degree of hypoxia as produced by ascent to an altitude of 4000 meters in a low pressure chamber. After a stay of 30 minutes under lowered atmospheric pressure (480 Hgmm) there were no significant changes in the blood count, reticulocyte count and oxygen saturation of the patient's blood. Physical examination and electrocardiograms showed an excellent circulatory adjustment. The Hb M content of the patient's blood was 26 per cent before and increased to 31 per cent after the experiment. The normal deviation from mean being ±2.5 per cent.

Discussion

The paramount importance of Hbs M lies in the fact that they have the only known alterations of amino acid sequence that lead directly to an interference with the molecular function. Several types of Hbs M have been decribed so far. Some of these, although originally thought to be different, have been shown by chemical studies to be identical:

Hb M Saskatoon = Hb M Emory = Hb M Kurume = Hb M Radom =
= Hb M Chicago = $\alpha_2^A \beta_2^{63\ Tyr}$
Hb M Boston = Hb Norin = Hb Gothenburg = $\alpha_2^{58\ Tyr} \beta_2^A$
Hb M Iwate = Hb Kankakee = $\alpha_2^{87\ Tyr} \beta_2^A$
Hb M Hyde-Park = $\alpha_2^A \beta_2^{92\ Tyr}$

Our case represents a Boston-type Hb M ($\alpha_2^{58\ His\text{-}Tyr} \beta_2^A$) with a particularly interesting characteristic that the degree of cyanosis often changed in the patient's life, and the relative amount of Hb M showed definite fluctuation when examined at different times. There was a change also in the Hb M per cent of the patient's blood after a stay under lowered atmospheric pressure.

In hereditary methaemoglobinaemia caused by a deficiency of the methaemoglobin-reductase system, the degree of cyanosis, that is the methaemoglobin content of blood, changes periodically according to the season or to nutrition. These fluctuations reflect probably the changes of vitamin C-uptake. In Hb M disease, similar to the other haemoglobinopathies, the changes of the relative amount of the abnormal pigment have not yet been described. It is assumed that not only the structure but also the rate of synthesis, and thus the relative amount of normal Hbs are genetically determined.

The ratio of normal and abnormal Hbs depends obviously not only on their rates of synthesis. All data obtained so far refer to the erythrocytes of the peripheral blood, and do not consider the whole erythron, that is the sum of the erythropoietic cells in the bone marrow and of the extravascular and the circulating erythrocytes.

Mature red blood cells do not synthetize Hb and thus the ratio of normal and abnormal Hbs presumably does not change in the circulating mature erythrocytes. But we have no knowledge yet of the differentiation of the stem cells, of their maturation time or of the release of mature elements into the blood stream in this anomaly. Neither is it known as yet whether Hb A and Hb M are synthetized by the same red cell population. A better insight into the above factors is indispensable for the drawing of definite conclusions from the fluctuation of the relative amount of Hb M in our patient's blood.

Acknowledgements

We are indebted to Dr. S. Lukács and Dr. J. Hídvégi for performing the low pressure chamber experiments and to Miss Zs. Miklós and Miss M. Hasitz for skilful technical assistance.

References

1. Hörlein, H., Weber, G.: Chronische familiäre Methämoglobinämie. *Dtsch. Med. Wschr. 73*, 476 (1948).
2. Gerald, P. S.: The electrophoretic and spectroscopic characterization of Hgb M. *Blood 13*, 936 (1958).
3. Pisciotta, A. V., Ebbe, S., Hinz, J. E.: Clinical and laboratory features of two variants of methemoglobin M disease. *J. Lab. Clin. Med. 54*, 73 (1959).
4. Betke, K., Gröschner, E., Bock, K.: Properties of a further variant of haemoglobin M. *Nature 188*, 864 (1960).
5. Shibata, S., Miyaji, T., Iuchi, I., Ueda, S., Takeda, I., Kimura, N., Kodama, S.: Hemoglobin M Saskatoon. *Acta haemat. Japan 25*, 690 (1962).
6. Miyaji, T., Iuchi, I., Shibata, S., Takeda, I., and Tamura, A.: Possible amino acid substitution in the α chain (α 87 Tyr) of Hb-M Iwate. *Acta haemat. Japan 26*, 538 (1963).
7. Watson, H. C. and Kendrew, J. C.: Comparison between the amino acid sequences of sperm whale myoglobin and of human haemoglobin. *Nature 190*, 670 (1961).
8. Hansen, M. A., Jagenburg, O. R. and Johansson, B. G.: Studies on an abnormal hemoglobin causing hereditary congenital cyanosis. *Acta Paediat. Stockh. 49*, 503 (1960).
9. Gerald, P. S. and Efron, M. L.: Chemical studies of several varieties of Hb M. *Proc. Nat. Acad. Sci. 47*, 1758 (1961).
10. Heck, W. and Wolf, H.: Angeborener Herzfehler mit Cyanose durch pathologischen Blutfarbstoff (Hämoglobin M). *Ann. Ped. 190*, 135 (1958).
11. Kiese, M. and Kurz, H.: Chronische Hämoglobinämie durch pathologischen Blutfarbstoff. *Klin. Wschr. 34*, 957 (1956).
12. Kwon, P. H., Loogen, F. and Mürz, R.: Über zwei differente Formen congenitaler Blutfarbstoffehlbindung. *Dtsch. Arch. Clin. Med. 206*, 503 (1960).
13. Heller, P., Weinstein, H. G., Yakulis, V. J. and Rosenthal, I. J.: Hemoglobin M Kankakee. A new variant of hemoglobin M. *Blood 20*, 287 (1962).
14. Josephson, A. M., Weinstein, H. G., Yakulis, V. J., Singer, L. and Heller, P.: A new variant of hemoglobin M disease: Hemoglobin M Chicago. *J. Lab. Clin. Med. 59*, 918 (1962).
15. Tönz, O., Simon, H. A. and Hassefeld, W.: Untersuchungen einer grossen Hämoglobin M Sippe; Entdeckung eines neuen Blutfarbstoffes: Hb M Oldenburg. *Schweiz. Med. Wschr. 93*, 1311 (1962).
16. Murawsky, K., Szymanska, Z. and Kozlowska, J.: A new variant of abnormal methaemoglobin: Hb M Radom. *Biochim. Biophys. Acta 69*, 442 (1963).
17. Betke, K.: Hämoglobin M.: Typen und ihre Differenzierung (Übersicht). In Hemoglobin Colloquium, Wien, 1961. Stuttgart, G. Thieme Verlag, 1962, p. 39.
18. Farmer, M. B., Lehmann, H. and Raine, D. N.: Two unrelated patients with congenital cyanosis due to haemoglobinopathy M. *Lancet II*, 786 (1964).
19. Hollán, S. R., Szelényi, J. G.: Analysis of a new case of hemoglobin M trait. 9th Congr. of the International Society of Haematology Mexico, 1962.
20. Hollán, S. R. and Szelényi, J. G.: Some new data on hemoglobin M Kiskunhalas. 9th Congr. European Society of Haematology Lisbon, 1963.
21. Betke, K.: Personal communications.
22. Brugsch, J.: Hämoglobin, der rote Blutfarbstoff. Leipzig, G. Thieme Verlag, 1948, p. 17.
23. Watson-Williams. E. J., Beale, D., Irvine, D. Ibadan and Lehmann,H.: A new haemoglobin, D (β—87 Threonine→Lysine) producing no sickle-cell haemoglobin D disease with haemoglobin S. *Nature 205*. 1273 (1965).

Human Erythrocyte Adenylate Kinase *

E. LEVIN and E. BEUTLER

Department of Medicine, City of Hope Medical Center, Duarte, California

(Received October 12, 1966)

A rapid, accurate quantitative method for measuring adenylate kinase activity (ATP + AMP → 2 ADP) in the soluble fraction of erythrocytes is described. The reaction is coupled to the oxidation of DPNH so that it may be followed spectrophotometrically. The conditions and requirements of the reaction, including substrate-velocity relationship, and pH optimum are described. Normal values are recorded and preliminary data bearing on activity in disease states are recorded.

Erythrocyte adenylate kinase catalyzes the reaction ATP + AMP $\rightleftarrows$ ADP. This enzyme is of potential importance in the biosynthesis and degradation of ATP. Its presence in the red blood cell has been demonstrated [1, 2, 3]. The methods used in the past have been chromatographic [1, 2] or have involved the hexokinase reaction to measure the production of ATP [1]. No data are available regarding the activity of this enzyme in the normal individual and in disease states, nor have the kinetic properties of the enzyme and its stability under storage conditions been investigated.

We have developed a method for the determination of adenylate kinase in which the reaction of AMP + ATP $\rightleftarrows$ ADP is coupled to the oxidation of DPNH.

The assay is based upon the method for measurement of ADP as described by Adam [4]. The substrate concentration and quantity of pyruvate kinase and lactic dehydrogenase have been adjusted so that the amount of erythrocyte adenylate kinase is the limiting factor. The reactions used can be summarized in three equations:

1. Adenylic kinase reaction: $\text{AMP} + \text{ATP} \xrightleftarrows{\text{AK, Mg++}} 2\ \text{ADP}$

2. Auxiliary reaction: $2\ \text{ADP} + 2\ \text{Phosphoenolpyruvate} \xrightleftarrows{\text{PK, Mg++}} \text{ATP} + 2\ \text{Pyruvate}$

3. Indicator reaction: $2\ \text{Pyruvate} + 2\ \text{DPNH} \xrightleftarrows{\text{LDH}} 2\ \text{Lactate} + 2\ \text{DPN}.$

* This investigation was supported, in part, by Public Health Service Research Grant HE-07449.

The following abbreviations are used: ATP = adenosine triphosphate, AMP = adenosine monophosphate, ADP = adenosine diphosphate, DPN = diphosphopyridine nucleotide (NAD), DPNH = diphosphopyridine nucleotide, reduced form (NADH), AK = adenylate kinase, PK = pyruvate kinase, LDH = lactic dehydrogenase, SFH = stroma-free hemolysate.

The equilibrium of reactions 2 and 3 is sufficiently far to the right to overcome the ADP dismutation and to reduce virtually all of the pyruvate that is formed.

Materials and Methods

Preparation of stock solutions: The following chemicals were obtained from Sigma Chemical Company. In some assays, DPNH from Boehringer was utilized.

1. Adenosine monophosphate, 22.5 μmoles/ml. The concentration of this solution may be verified both by its optical density at 258 mμ and enzymatically by the method of Adam [4]. This solution is stable at 4° C.
2. Disodium ATP, 22.5 μmoles/ml of 0.04 M Tris borate buffer, pH 9.2. This solution has a final pH of 7.55 and is quite stable when frozen. The concentration of this solution was verified by its optical density at 258 mμ and contamination with AMP and ATP was found to be negligible when the solution was assayed enzymatically for these compounds [4].
3. Disodium DPNH 45 μmoles/ml triethanolamine buffer 0.05 M, pH 7.5.
4. Tricyclohexylammonium phosphoenolpyruvate 43 μmoles/ml of 0.05 M triethanolamine buffer, pH 7.5.
5. Magnesium sulfate 0.245 M.
6. Potassium chloride 2 M.
7. Triethanolamine buffers 0.05 M, pH 7.5 and 6.9.

Substrate mixture: 270 μl of the substrate mixture contained the following: 0.1 ml ATP (2.25 μmole); 0.02 ml DPNH (0.9 μmole); 0.05 ml phosphoenolpyruvate (1.72 μmole); 0.04 ml $MgSO_4$ (9.8 μmole); 0.07 ml KCl (140 μmole). A suitable multiple of the above individual volumes may be mixed, and stored in frozen aliquots suitable for a single day's work. Care must be taken that the final pH of the reaction mixture is between 7.5 and 7.6. The frozen mixture is stable for at least one week.

Auxiliary enzymes:

Lactic dehydrogenase (0.1 mg protein/ml). Dilute 0.01 ml crystalline suspension (Type II, Sigma), (10 mg protein/ml) to 1 ml with 2.2 M ammonium sulfate solution. This dilute preparation of enzyme is unstable and must be prepared each day.

Pyruvate kinase (0.5 mg protein/ml). Dilute 0.1 ml crystalline suspension (crystalline from rabbit muscle, Type II, Sigma) (5 mg protein/ml) to 1 ml with 2.2 M ammonium sulfate solution.

Hemolysate: Unless otherwise stated, blood was collected into ethylene diaminetetraacetic acid (EDTA), 1 mg/ml blood. Hemolysates were prepared within three hours after collection of blood. One cc of blood was washed with 10 volumes of cold isotonic saline and the buffy coat was removed by aspiration. Packed cells were diluted 1 : 30 with triethanolamine buffer, pH 7.5, and the cells were lysed by freezing and thawing the suspension two times. The hemolysate was

then centrifuged at 4° C, 24,000 g for 45 minutes. The stroma-free hemolysate (SFH) was aspirated and used for assay. Assay was carried out 120 minutes after the SFH was removed from the centrifuge.

Procedure: The following were pipetted into each of two cuvettes:

2 ml triethanolamine buffer, pH 6.9
0.27 ml substrate mixture
0.02 ml lactic dehydrogenase suspension
0.02 ml pyruvate kinase suspension

Sufficient SFH (0.015 – 0.02 ml) was added to the test cell so that the optical density at 540 μm was between 0.06 and 0.085. The $O.D._{540}$ is proportional to the hemoglobin concentration and is, therefore, a measure of the quantity of red cells assayed. The reaction was started by adding 0.1 ml AMP solution to the test cuvette. The contents of the cell were mixed rapidly. Spectrophotometric measurements were made at a wave length of 340 mμ with a light path of 1 cm, at a temperature of 30° C. After an initial lag period of about one-half minute, the reaction rate is linear. The enzyme activity is expressed as $\triangle$ $O.D._{340}$/minute/ $O.D._{540}$. One international unit, the quantity of enzyme which will transform 1 μmole of substrate/minute/mg Hb is obtained by multiplying these units by 0.148.

Results and Discussion

I. Conditions affecting enzyme activity of the hemolysate

A) *Anticoagulation and storage stability.* Blood collected in EDTA, ACD. and heparin gave similar results when the assay was carried out with fresh blood, The enzyme activity remained unaltered for at least three months when blood was collected in ACD. Similar results have been reported by Tatibana et al. [5].

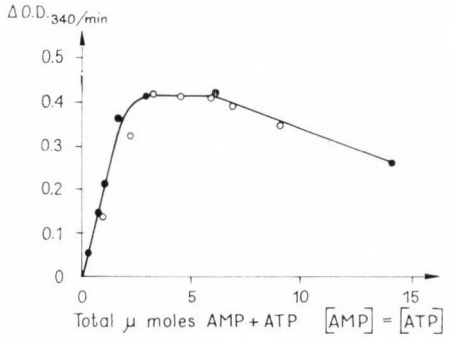


Fig. 1. The relationship between the total concentration of adenine nucleotide and the velocity of the reaction. The open and closed symbols represent data obtained with different SFH on different days. Assay system as given in text except that DPNH content of system was 0.45 μmole

B) *Washing.* SFH from unwashed red cells gave results similar to those from washed cells except in the samples from patients with chronic myelogenous leukemia or myelofibrosis with increased peripheral white blood counts. In the latter instances the activity of the unwashed sample was lower than that of the washed sample. (Cerletti [2] has found no adenylate kinase activity in the white cells.)

C) *The effect of centrifugation of the hemolysate.* Enzyme assays carried out on uncentrifuged red cell hemolysates gave results which were non-linear with dilution. These suggested that an inhibitor was present. High speed centrifugation of the hemolysate, as described under Methods, appeared to remove this inhibitor, and linear results were obtained.

D) *Variation in activity of the SFH as a function of time.* During the study of the reproducibility of the method, it was noted that there were fluctuations in the activity of the SFH for two hours following removal of the SFH from the centrifuge. After this period, enzyme activity was identical to that found immediately after centrifugation, and enzyme activity remained stable for at least 24 hours.

II. Conditions and requirements of the reaction

A) *Substrate-velocity relationship*

1. Nucleotide concentration: Fig. 2 demonstrates the relationship between the initial linear velocity of the reaction and the total nucleotide (AMP + ATP) concentration. For the assay, equimolar concentrations of AMP and ATP were chosen so that the total adenine nucleotide concentration in the reaction mixture would be equivalent to the mid-point of the plateau depicted in Fig. 2. The final nucleotide concentration was 1.9 μmole/ml.

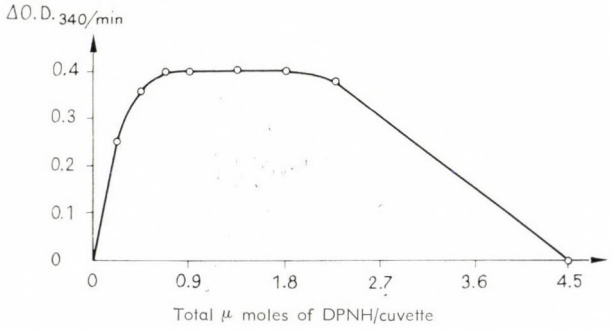


Fig. 2. The relationship between the initial linear velocity and the concentration of DPNH. Assay as given in text

2. DPNH: The effect of DPNH concentration on the initial linear velocity of the reaction is depicted in Fig. 3. The DPNH concentration utilized in the final standard assay was chosen from a point on the plateau of the curve. The final concentration of DPNH was 0.37 μmole/ml.

3. Phosphoenolpyruvate: Varying the final concentration of phosphoenolpyruvate from 0.36 μmole/ml to 1.44 μmole/ml did not affect the initial linear

velocity. The value midway between, 0.70 μmole/ml, was chosen for the standard assay.

4. Magnesium: Magnesium is necessary for the reaction and the final concentration of 4.05 μmole/ml slows the initial velocity by only 2½% in either case.

5. Potassium was found to be inhibitory at very high concentration (118 μmole/ml) and, in fact, in some hemolysates tested, the concentration used (59 μmole/ml) slows the initial linear velocity by 9%. However, since potassium is necessary for the pyruvate kinase reaction and since we were unable to determine if its concentration in some of the hemolysates might be marginal, it was decided to add the ion in a final concentration of 59 μmole/ml.

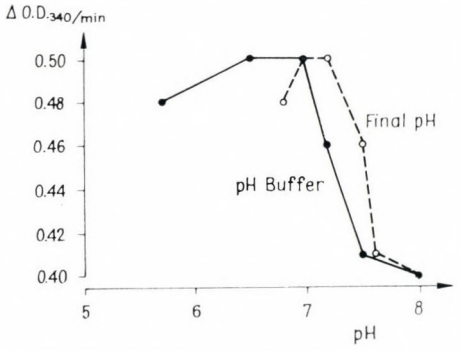


Fig. 3. The relationship between pH and the maximal velocity of reaction. The solid line indicates the pH of the buffer; the dotted line indicates the final measured pH of the reaction mixture. Conditions of the assay as given in the text

6. Auxiliary enzymes: a) Lactic dehydrogenase: the conditions of the assay require an excess of exogenous lactic dehydrogenase. Doubling the amount of lactic dehydrogenase which is used in the standard assay does not affect the initial linear velocity of the reaction. b) Pyruvate kinase: an excess of exogenous pyruvate kinase is also needed in the system, and doubling the amount used in the standard assay does not influence the initial linear rate.

B) *pH*

The reaction has a narrow pH optimum between 7.0 and 7.2. In order to insure the stability of the substrates during their storage, it is necessary to buffer them strongly at pH 7.5 with the Tris borate buffer. In order to adjust the pH of the final reaction mixture to a pH of 7.0 – 7.2, it was necessary to use a triethanolamine buffer, pH 6.9, in the reaction system. This relationship and the relationship between the final pH of the incubation mixture gave the initial linear velocity as shown graphically in Fig. 3.

C) *Linearity of the reaction*

The relationship between the amount of enzyme used and the initial linear velocity is demonstrated in Fig. 4.

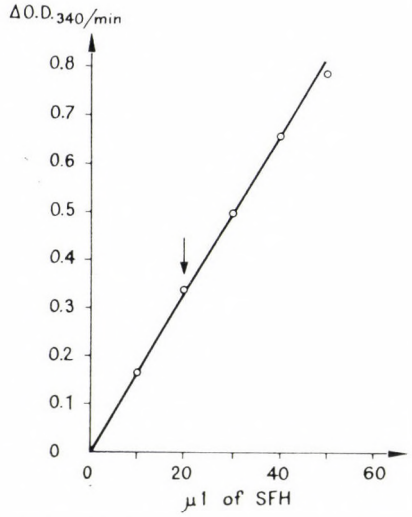


Fig. 4. Relationship between the amount of enzyme solution assayed and the initial linear velocity of the reaction. Assay as given in text

III. Reproducibility

Six replicate determinations were carried out on one normal subject. These gave an average value of 2.483 with a standard deviation of 0.055 units of activity. Eight replicate determinations on the blood of another subject gave an average value of 2.434 with a standard deviation of 0.056 units of activity.

IV. Normal values

Twenty-two normal subjects were found to have an average enzyme activity of 2.526 with a standard deviation of 0.329 units of enzyme activity.

V. Disease states

Two patients with hemolytic disease, one a patient with paroxysmal nocturnal hemiglobinuria with a reticulocyte count of 12.5% and one a patient with autoimmune hemolytic disease with a reticulocyte count of 6%, had values which fell into a normal range. One patient with acute leukemia, one patient with iron deficiency anemia, and one patient with acute intermittent porphyria have been tested. Their values were within the normal range.

References

1. Kashket, S. and Denstedt, O. F.: The metabolism of the erythrocyte. XV. Adenylate kinase of the erythrocyte. *Canad. J. Biochem. Physiol. 36*, 1057 (1958).
2. Cerletti, P. and Bucci, E.: Adenylate kinase of mammalian erythrocyte. *Biochim. et Biophys. Acta 38*, 45 (1960).
3. Overgaard-Hansen, K.: Regeneration of adenosine triphosphate in human erythrocytes by purine nucleotides. *Acta Pharmacol. Toxic. 14*, 67 (1957).
4. Adam, H.: Adenosine-5′-diphosphate and adenosine-5′-monophosphate. In: Methods of Enzymatic Analysis, Edited by H. U. Bergmeyer, 1963, Academic Press, New York, p. 573.
5. Tatibana, M., Nakao, M. and Yoshikawa, H.: Adenylate kinase in human erythrocytes. *J. Biochem. 45*, 1037 (1958).

Biological and Clinical Significance of the Adrenalin Test in Haematology

E. STORTI, E. LUSVARGHI, G. F. GRIGNAFFINI and A. SGANDURRA

Institute of Special Pathology and Clinical Methodology, University of Modena (Italy)

(Received August 13, 1966)

The behaviour of the leukocyte resistance tests, before and after the injection of adrenalin, was studied in normal and splenectomized subjects. It can be concluded that: *a)* the early leukocytosis (after 20′) is particularly due to the "emptying-out" of cellular elements from the storage organs; the tardy leukocytosis (after 3 hours) is exclusively due to the haemodynamic variations of the peripheral circulation; *b)* the adrenalin test is not useful in obtaining information regarding bone marrow function.

The adrenalin test is used in clinical practice both in haematology and in endocrinology.

In the former case, this test is founded upon the immediate effect of the drug (preadrenocortical effect), consisting primarily in pancytosis. In the normal subject, the moment when this pancytosis appears and its degree depend upon the quantity of the drug and the mode of administration. Following a still very common opinion, if the test is carried out with the standard technique (1 mg of adrenalin hydrochloride $1^0/_{00}$, subcutaneously or i. m.) the degree and the quality of the response to the drug (particularly and in practice the leukocytosis, which is more remarkable and controllable than erythrocytosis and thrombocytosis) should supply information about the functional conditions of the spleen and bone marrow.

In the latter case, the test is founded upon the tardy effect of the drug (adrenocortical effect) i.e. upon the decrease of the absolute value of the eosinophils in the blood stream (Recant, Forsham and Thorn's adrenalin test [22]).

Our research is limited to the preadrenocortical effect of the drug, i.e. to the "common adrenalin test", which is expected to shed light on the functional activity of the spleen and bone marrow (Doan et al. [9], Chatterjea et al. [7]). But before setting forth the data obtained from our experiments, which have been carried out by means of the osmotic and mechanical resistance tests in normal subjects and in splenectomized ones (due to trauma or therapy), we will briefly review the latest principal contributions to this subject.

Bierman et al. [5, 6] published the striking results of their researches in man on the effects of adrenalin in the pulmonary and splenic circulations. These authors noted the appearance of leukocytosis and thrombocytosis in the arterial blood $30'' - 60''$ after the beginning of the administration of the drug ($0.1 - 0.3$ mg

intravenously). The leukocytosis in the venous blood appeared 30″ – 120″ after the arterial one. Therefore, it is obvious that the above-mentioned haematological variations originate primarily from the pulmonary circulation which, therefore, must be looked upon as a remarkable storage organ of leukocytes and thrombocytes. Adrenalin (0.2 – 0.3 mg) injected into the human splenic artery gives rise to leukocytosis (which will be primarily granulocytic) in the arterial blood 30″ – 60″ after the beginning of the injection, while in the hepatic veins and in the pulmonary artery it appears only 2′ – 3′ later. It is therefore clear – say Bierman et al. [5] – that this leukocytosis originates immediately from the lung and then from the spleen and other portal organs which are the leukocyte storage depots of the body.

At about the same time, Chatterjea, Dameshek and Stefanini [7] state that the response to adrenalin is essentially alike in the normal and in the splenectomized subjects, and that leukocytosis consists only of mature white blood elements of the myeloid and lymphatic series; furthermore, they maintain that in the cytopenia due to hypersplenism (with blockage of the marrow) or hypoplasia, there is always a close relationship between the response to adrenalin and the functionality of the bone marrow, though they attach no diagnostic value to the test in any clinical state, as it shows only indirectly the functionality of the marrow and the condition of the leukocyte storage organs. Shortly thereafter, Hamilton and Horvath [14, 15] experimentally studied the phenomenon in the dog and they sustained the pulmonary, hepatic and splenic origin of leukocytosis induced by adrenalin, and excluded the mechanism of the intravascular variation in the systemic circulation.

The latest contributions to the significance of the adrenalin test were given by Scaltrini and Confalonieri [23], Grifoni et al. [12], Athens et al. [1] and Fieschi and Sacchetti [10]. But while the former workers (Scaltrini and Grifoni) just studied the problem in its individual stages, stating that after 30′ the leukocytosis appears due to the "emptying-out" of the primary storage organs (lungs, spleen and liver) and that 3 hours later the increase in leukocytes is to be regarded as due to the "emptying-out" of the secondary storage organs (bone marrow), Athens et al. [1], Fieschi and Sacchetti [10] may have definitely solved the problem by studying the leukocyte dynamics by means of DFP^{32}. They have shown that neutrophilic leukocytosis by adrenalin and norepinephrine has no clinical significance since it is secondary to a leukocyte shift from the marginated pool (i.e. the passage into circulation of the white blood cells lining the vascular bed). As mentioned in the above review, though it is incomplete, the biological and clinical significance of the adrenalin test is not yet completely defined. On the contrary, beside some authors supporting its clinical value, and limiting the mechanism of leukocytosis to a mere splenocontraction, there are some workers attaching little interest to the test, since they believe that splenic participation in the increase of white blood cells is insignificant or at most scanty.

As we look upon the theory regarding the spleen "as the single organ" responsible for haematological variations as an old-fashioned concept, there is still to inquire as to whether the leukocytosis following the administration of adrenalin is caused by a mere "shifting" of the white cells from the depots in the body (lungs.

spleen and liver) or by direct stimulation of the bone marrow. In order to solve this problem, we have observed the behaviour of the osmotic and mechanical resistance tests during adrenalin administration both in normal subjects and in subjects splenectomized because of trauma or therapy. We have put together this study to confirm previous researches to observe the particular behaviour of leukocytic resistances in the splenectomized subject.

The osmotic and mechanical resistance tests, which were applied both in biology and in clinical medicine by Storti and Pederzini [29] and by Storti, Bellesia and Lusvarghi [25], are tests of leukocyte functionality showing a constant behaviour under normal conditions, while they may undergo important and characteristic alterations under experimental and clinical conditions [19, 26, 28, 30]. Therefore, in splenectomized subjects, it was noted that even after eight years, the leukocytic resistances, both of poly- and mononucleates, have highly increased as compared with normal values, so that this phenomenon becomes constantly associated with the splenectomized individual [27].

Furthermore, it has been shown that the value of the osmotic resistance of a given leukocyte population under examination is proportional to its age, which means that the leukocytic resistance of the myeloblast is higher than that of the younger granulocyte, and the resistance of the granulocyte with fewer segments is higher than that of the older granulocyte [2, 3, 18]. For instance, the degree of leukocytic resistance in a subject with a leukemoid reaction (Arneth's scheme shifted to the left) is higher as compared with the norme, and much higher than in a patient suffering from Addison's anemia (Arneth's scheme shifted to the right [2]).

It, therefore, follows that by determining the leukocytic resistances (L. R.) before and after the injection of adrenalin, we are able to conclude from the variations in L. R. as to whether the leukocytosis consists mostly of young cells (increased resistance), mostly of older cells (decreased resistance), or simply of mature elements (unchanged resistance). In the first variation, the change could have been caused by a direct stimulation of the bone marrow, in the second one, by an "emptying-out" of the storage organs, and in the third, by a simple mechanical variation of the blood flow.

Materials and Methods

Our research has been carried out in 24 healthy subjects, 20 to 40 years of age, and in 8 subjects splenectomized 5 months to 8 years previously because of traumatic (7 cases) or therapeutic (1 case) reasons. The adrenalin test was carried out in the standard manner: 1 mg of adrenalin hydrochloride ($1‰$) injected s. c. and haematological controls before and after 20′ (that is when the highest leukocytosis appears); ten subjects were also controlled after three hours. The methods of osmotic and mechanical leukocyte resistances performed contemporaneously with the haematological controls, are those of Storti and Pederzini [29], and of Storti, Bellesia and Lusvarghi [25]. The osmotic leukocyte resistance test consists in placing the white blood cells in a hypotonic solution (0.20% NaCl), while the mechan-

ical leukocyte resistance test consists in subjecting the leukocytes to a continuous mechanical trauma in order to exceed their physical resistance threshold. For further details regarding the above two tests, please refer to the original papers.

Results

Figs 1, 2 and 3 show the variations of the leukocyte counts, and the average values of the leukocytic resistance index (L. R. I.) of the polynucleates (neutrophils,

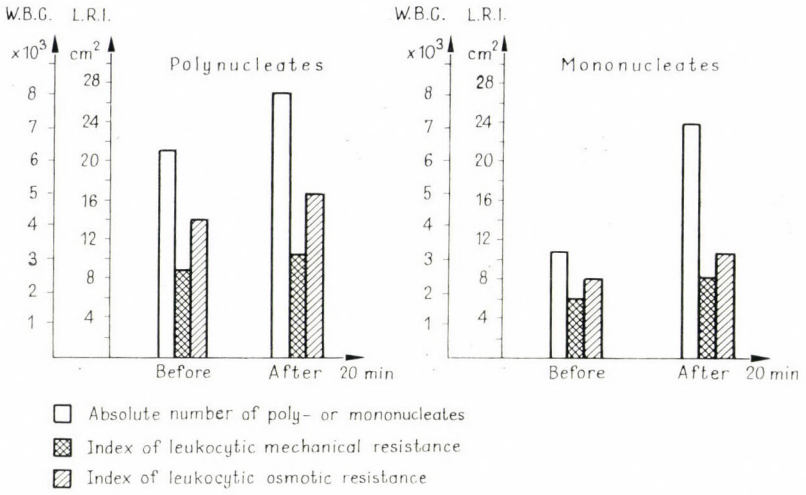


Fig. 1. Absolute numbers and indices of mechanical and osmotic resistances of white blood cells (poly- and mononucleates) before and after 20′ from the injection of adrenalin (1 mg s. c.). Average values on 24 normal subjects

Table 1

Statistical analysis of our results, expressed

		Mechanical polynucleates		
		average	S. D.	P
Normal subjects (24 cases)	Before injection of adrenalin	10.106	1.19	0.02—0.01
	After 20′	11.266	1.02	
Splenectomized subjects (8 cases)	Before injection of adrenalin	8.903	0.68	0.01—0.001
	After 20′	10.441	0.51	
Normal subjects (10 cases)	Before injection of adrenalin	10.232	1.24	0.90—0.80
	After 3 hours	10.556	2.45	

eosinophils and basophils) and of the mononucleates (lymphocytes and monocytes), before and after the injection of adrenalin in the three groups of subjects under examination.

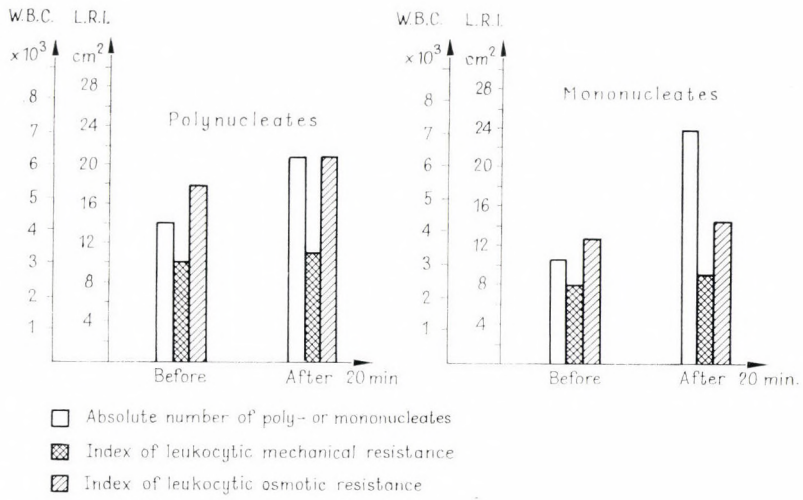


Fig. 2. Absolute numbers and indices of mechanical and osmotic resistances of the white blood cells (poly- and mononucleates) before and after 20′ from the injection of adrenalin (1 mg s. c.). Average values on 8 splenectomized subjects

Table 1 shows the statistical analysis of the collected experimental data.

as leukocyte resistance indices in cm^2

resistance			Osmotic resistance					
mononucleates			polynucleates			mononucleates		
average	S. D.	P	average	S. D.	P	average	S. D.	P
8.007	1.62	0.20—0.10	18.029	1.54	0.01—0.001	12.697	2.10	0.02—0.01
9.097	2.06		21.043	2.08		15.641	2.39	
6.041	1.51	0.10—0.05	14.165	1.25	0.01—0.001	8.183	1.06	0.01—0.001
8.262	1.76		17.068	1.37		10.858	1.84	
8.583	1.48	0.90—0.80	18.474	1.63	0.70—0.60	13.396	1.71	0.40—0.30
8.345	1.31		17.987	2.12		14.630	2.21	

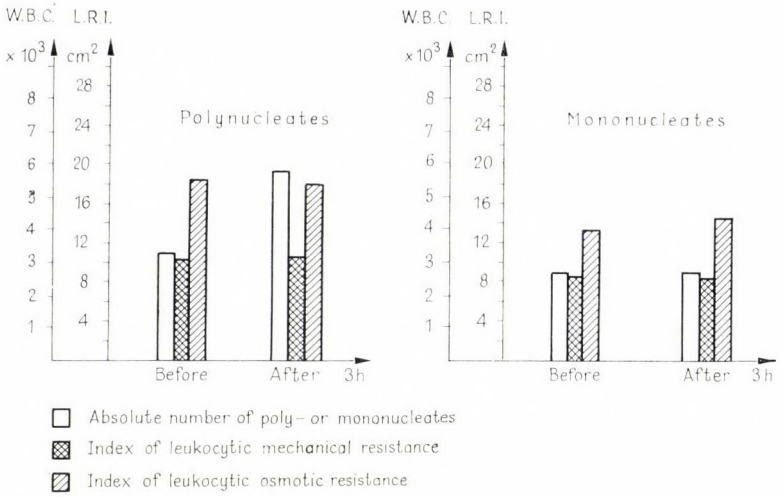


Fig. 3. Absolute numbers and indices of mechanical and osmotic resistances of the white blood cells (poly- and mononucleates) before and after 3 hours from the injection of adrenalin (1 mg s. c.). Average values on 10 normal subjects

Discussion

The following points are brought into focus by our experimental data:

1. A considerable decrease of the osmotic and mechanical resistances of human polynuclear cells in the peripheral blood was observed both in the normal and in the splenectomized subjects 20 minutes after the subcutaneous injection of 1 mg of adrenalin hydrochloride.

2. The mechanical resistance of the mononuclear cells (lymphocytes and monocytes) is not remarkably affected by the adrenalin stimulus, while the osmotic resistance of these elements is significantly decreased.

3. Three hours after the injection of adrenalin, there was a significant variation in the osmotic and mechanical resistances of the mono- and polynuclear cells.

4. The adrenalin induced leukocytosis is identical both in the normal and in the splenectomized subjects.

The conclusions are very clear:

a) The early neutrophil leukocytosis (appearing after 20′) induced by adrenalin may be looked upon as the expression of the "emptying-out" of the W. B. C. from the storage organs (lungs, spleen and liver); the decreasing leukocyte resistance signifies the entrance into circulation of older, "enfeebled" elements, which probably originate from the above-mentioned storage organs [19, 26].

b) The mononuclear components (lymphocytes and monocytes) are probably composed of elements derived from the "emptying-out" of the storage organs and by the entrance into circulation of younger forms from the lymphatic duct, since the osmotic resistance is decreased and the mechanical one remains unchanged.

c) Since the statistical analysis of our data does not show any significant variation of the mechanical and osmotic leukocytic resistances, the modest leukocytosis which appears 3 hours after the injection of adrenalin, may be interpreted as a cellular shift from the marginated into the circulating pool caused by haemodynamic variations of blood flow.

d) If we bear in mind that the early leukocytosis is particularly due to the "emptying-out" of cellular elements from the storage organs, we can easily understand the identical behaviour to the adrenalin stimulus of the normal and of the splenectomized subjects.

We confirm, therefore, the previous researches of Bierman et al. [5, 6], Hamilton and Horvath [14, 15], Athens et al. [1], Fieschi et al. [10] concluding, *from a biological wievpoint*, that the early leukocytosis is due to "demargination" (that is, above all, from the "emptying-out" of the storage organs: lungs, liver and spleen); the tardy leukocytosis is, therefore, exclusively due to the haemodynamic variations of the peripheral circulation, without the participation of any storage organs. *From a clinical viewpoint*, we agree with the past researches of Kreuter [16], Schenk [24], Yang [31, 32], Benhamou [4], Patek and Daland [21], Chatterjea, Dameshek and Stefanini [7], Mřkos et al. [20], Lucia et al. [17], De Franciscis and Polosa [8], Garrey et al. [11], Groisser et al. [13] regarding the identical behaviour of the adrenalin test in the normal and in the splenectomized subjects, and we furthermore agree with Athens et al. [1], Fieschi and Sacchetti [10], that *this test is not useful in obtaining information regarding bone marrow function.* We limit, therefore, the applicability of this test to the semeiological practice of obtaining direct information regarding the contractility of the spleen, and in obtaining indirect general knowledge about the histo-anatomical structure of this organ.

References

1. Athens, J. M., Haab, O. P., Raab, S. O., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M.: Leukokinetic studies. IV. The total blood circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. *J. clin. Invest. 40*, 989 (1961).
2. Bellesia, L., Lusvarghi, E., Mucci, P.: Rapporti fra resistenza osmotica e vitalità dei globuli bianchi. *Haematologica 43*, 97 (1958).
3. Bellesia, L., Lusvarghi, E., Mucci, P.: Wirkung eines Pyrogens (Lipopolysaccharids) aus Salmonella abortus equi auf die osmotische und mechanische Resistenz der weissen Blutkörperchen beim gesunden Menschen. *Schw. med. Wschr. 88*, 929 (1958).
4. Benhamou, E.: L'exploration fonctionnelle de la rate. Masson, Paris 1933.
5. Bierman, H. R., Byron, R. L., Kelly, K. H.: The role of the spleen in the leukocytosis following the intra-arterial administration of epinephrine. *Blood 8*, 153 (1953).
6. Bierman, H. R., Kelly, K. H., Cordes, F. L., Byron, R. L., Polhemus, J. A., Rappoport, S.: The release of leukocytes and platelets from the pulmonary circulation by epinephrine. *Blood 7*, 683 (1952).
7. Chatterjea, J. B., Dameshek, W., Stefanini, M.: The adrenalin (epinephrine) test as applied to hematologic disorders. *Blood 8*, 211 (1953).
8. De Franciscis, P., Polosa, P.: Emocitogramma prima e dopo adrenalina in soggetti splenoprivi per cause traumatiche. *Boll. Soc. ital. Biol. sper. 26*, 782 (1950).

9. Doan, C. A., Wright, C. S.: Primary congenital and secondary acquired splenic panhematopenia. *Blood 1*, 10 (1946).
10. Fieschi, A., Sacchetti, C.: Dinamica dei granulociti: leucocitosi e leucopenie. Atti 62° Congr. Soc. ital. Med. int. Torino 1961, Pozzi, Roma (1961).
11. Garrey, W. E., Bryan, W. R.: Variations in the white blood cell counts. *Physiol. Rev. 15*, 597 (1935).
12. Grifoni, V., Scaltrini, G. C., Confalonieri, C., Conigliaro, C.: La prova dell'adrenalina in ematologia. *Haematologica 44*, 393 (1959).
13. Groisser, V. W., Ruberman, W.: The hematologic response to adrenalin. *J. Lab. Clin. Med. 43*, 386 (1954).
14. Hamilton, L. H., Horvath, S. M.: Immediate blood cell response to epinephrine. *Am. J. Physiol. 176*, 311 (1954).
15. Hamilton, L. H., Horvath, S. M.: Immediate blood cell count response to epinephrine in splenectomized dogs. *Am. J. Physiol. 178*, 58 (1954).
16. Kreuter, H.: Zur Frage der funktionellen Milzdiagnostik, nach Menschen. *Z. ges. exp. Med. 2*, 411 (1913).
17. Lucia, S. P., Leonard, M. E., Falconer, E. H.: The effect of the subcutaneous injection of adrenalin on the leukocyte count of splenectomized patients and of patients with certain diseases of the hematopoietic and lymphatic systems. *Am. J. med. Sci. 194*,35 (1937).
18. Lusvarghi, E., Bellesia, L., Mucci, P., Grignaffini, G. F.: La résistence osmotique de la série granuloblastique dans la phase hématopoïétique du cycle vital leucocytaire. *Sang 30*, 867 (1959).
19. Lusvarghi, E., Bellesia, L., Mucci, P., Grignaffini, G. F.: Le resistenze osmotica e meccanica dei globuli bianchi in un distretto circolatorio periferico nell'uomo. *Progr. med. Napoli 16*, 109 (1960).
20. Mřkos, D., Dostálová, H.: The diagnostic importance of adrenalin test in hypersplenism. *Vnitř. lék. 1*, 99 (1955).
21. Patek, A. J., Daland, G. A.: The effect of adrenalin injection on the blood of patients with and without spleens. *Am. J. med. Sci. 190*, 14 (1935).
22. Recant, L., Forsham, P. H., Thorn, G. W.: Observations on the adrenalpituitary response following epinephrine infusion in man. *J. clin. Endocrin. 8*, 589 (1948).
23. Scaltrini, G. C., Confalonieri, C.: Modificazioni del quadro ematico periferico indotte dall'adrenalina e loro genesi. *Rec. Progr. Med. 23*, 168 (1957).
24. Schenk, P.: Die Adrenalinwirkung auf das Blut des Menschen und ihre Beziehung zur Milzfunktion. *Med. Klin. 16*, 279 (1920).
25. Storti, E., Bellesia, L., Lusvarghi, E.: La resistenza meccanica dei leucociti. Metodi e reperti relativi al normale. *Min. med. 47*, 2202 (1956).
26. Storti, E., Bellesia, L., Lusvarghi, E.: Relationship between leukocatheresis and the behaviour of the osmotic resistance of white blood cells in the principal circulatory areas. *Acta med. scand. 162*, 375 (1958).
27. Storti, E., Bellesia, L., Lusvarghi, E., Mucci, P.: Leukocytic resistances in patients following splenectomy and the relation between the spleen and the viability of white blood cells. *Ann. N. Y. Acad. Sci. 77*, 797 (1959).
28. Storti, E., Bellesia, L., Lusvarghi, E., Mucci, P.: Les résistances des leucocytes. Proc. 7th Congr. europ. Soc. Haemat., London, 1959, p. 336. Karger, Basel 1960.
29. Storti, E., Pederzini, A.: Metodo per la valutazione della resistenza osmotica dei leucociti: reperti relativi al normale. *Progr. med. Napoli 8*, 563 (1952).
30. Storti, E., Pederzini, A.: Clinical significance of the leukocytic resistance. *Acta med. scand. 154*, 417 (1956).
31. Yang, C. S.: Influence of adrenalin on the human spleen and blood picture. *Chin. J. Physiol. 2*, 163 (1928).
32. Yang, C. S., Chang, H. C.: The effect of adrenalin on the circulating blood volume in individuals with normal and enlarged spleens and after splenectomy. *Chin. J. Physiol. 4*, 21 (1930).

Effect of Reduced Glutathione on the Dissociation Curve of Haemoglobin

Possible Therapeutic Use of Glutathione in Haemorrhagic Shock

J. HOŘEJŠÍ

Institute of Haematology and Blood Transfusion, Prague, Czechoslovakia

(1) Glutathione influences both the shape and the situation of the dissociation curve of oxyhaemoglobin. A higher concentration of reduced glutathione in blood causes a shift of the dissociation curve to the right (the Hill's constant is decreased).

(2) Both the shape and the situation of the dissociation curve may be influenced, in vitro, either by the addition of reduced glutathione or by the blockade of sulfhydryl groups (e.g., by mercury compounds).

(3) A shift of the dissociation curve to the right improves the conditions for supplying oxygen to the tissues since, under conditions otherwise identical, a given amount of blood liberates a greater amount of oxygen at a decreased partial pressure of oxygen.

(4) An addition of reduced glutathione to preserved blood or to plasma expanders — of protein or non-protein character — increases the percentage of survival in animals subjected to experimental conditions of a standard haemorrhagic shock.

The presence of reduced glutathione in the erythrocyte is very important primarily for the protection of haemoglobin from an irreversible oxidation to methaemoglobin. This tripeptide, however, appears to play also an important role in the specific function of haemoglobin, i.e. the mediation of the oxygen transport from the atmospheric air into the tissues.

Glutathione in the reduced form (GSH) is capable (similarly as are other compounds containing the -SH radical) of interaction with a great number of proteins, provided that the latter have reactive -SH groups, whereby mixed sulfhydryls are formed. Glutathione takes part also in a variety of enzymatic reactions by influencing the activities of some so-called "sulfhydryl-dependent" enzymes whose activity is conditioned by the preservation of their sulfhydryl radicals. According to Racker [1], GSH protects such enzymes either from oxidation or from toxic effects of heavy metals.

The globin component of haemoglobin contains -SH radicals, too. Normal haemoglobin A (normal adult human haemoglobin, $alpha_2 beta_2$) contains six -SH groups, one in each alpha chain and two in each beta chain. Among these, of course, only two sulfhydryl groups are reactive, viz., those situated in the beta chains in the close vicinity of histidine. These sulfhydryl groups are in a position favouring an effective influence on the oxygen equilibrium (Taylor [2]).

Behlke and Scheller [3] assumed the existence of weak bonding forces between the -SH and vinyl radicals, basing their assumption on the changes

observed in the redox potential of haemoglobin in the presence of N-ethylmaleimide which latter blocks the sulfhydryl. According to Murawski [4], the existence of such bonds may be inferred also from the crystallographic analysis of haemoglobin. Two electrons of the sulfur atom are apparently available for the creation of a labile bond. In the presence of oxygen, these electrons are attracted to the [oxygen-iron] complex by the action of the double-bond system present in the porphyrin ring of the haem. Thus, to the bond between iron and histidine there acceeds another bond, that between the haem and the double-bond system of the polypeptide chain.

To the action of -SH groups the so-called Bohr's effect is ascribed, although not quite univocally. In this interpretation, the said effect would be conditioned by the decreased mutual attraction between a proton and a sulfur atom, the decrease being caused by the shifting of an electron within the assumed bond in the course of oxidation. The (vinyl-sulfhydryl) bond enhances the stability of bivalent iron against oxidation by activating more π electrons. According to Murawski [4], the same mechanism increases the forces bonding together haems and globin.

The present concept assumes that the normal S-shaped dissociation curve of oxyhaemoglobin is conditioned by an interaction between haems depending on the presence of -SH groups. Myoglobins, which do not contain -SH groups, exhibit only a very weak interaction, or possibly none at all, and exist mostly in the monomeric form. Their dissociation curve has also a different shape, being hyperbolic and not S-shaped as is that of haemoglobin. Such a responsibility of the -SH groups both for the interaction of haems and for the Bohr's effect has not, of course, achieved general acceptance. Taylor [2] showed that free sulfhydryl groups may be eliminated with the aid of cystine, whereby mixed disulfides of the type $Hb-S-S-CH_2-CH\ (NH_2)-COOH$ are formed. A change of the behaviour of this haemoglobin product in relation to oxygen could be expected, but in reality its properties are almost identical with those of normal haemoglobin, as far as the course of the dissociation curve is concerned.

A haemoglobin-glutathione complex ($Hb-GSH$) differs in some properties from the original haemoglobin. It was found, e.g., that $Hb-GSH$ complexes have a higher electrophoretic motility, pass more easily through ion exchange columns, but move more slowly on DEAE (diethylaminoethyl) cellulose. The $Hb-GSH$ complex binds also larger amounts of chromium ^{51}Cr. Huishman [5] showed that in blood haemolysates, containing normal haemoglobin as well as some abnormal haemoglobins, marked changes take place on the addition of GSH. Thus, in non-dialyzed haemolysates of adult human blood, in the course of storage at 4 °C lasting about 5 months, a gradual formation of 4 additional fractions *X*, *Y*, *Z*, A_{1d} was observed. An addition of glutathione markedly accelerates the formation of these fractions.

The oxidation of glutathione produces a haemoglobin complex having the structure $alpha_2\ (SH)_2\ beta_2\ (SH_2)\ (SSG)_2$.

The presence of reduced glutathione protects the beta chain of haemoglobin against oxidation. Hill showed that in the presence of oxidants (in vitro) glutathione

is preferentially oxidized by glutathione peroxidase whereby haemoglobin is protected against oxidation.

Our [6] interest in the mechanism of oxygen transport into tissues in anaemia, especially in pernicious anaemia, dates from the year 1933. We studied the dissociation curve and its changes. Both the shape and the situation of the dissociation curve can be essentially expressed in mathematical form with the aid of the so-called Hill's constant, which determines the relationship between x, the partial pressure of oxygen acting upon haemoglobin, and y, the percentage of oxyhaemoglobin formed under the given conditions:

$$k = \frac{y}{x^n\ (100-y)} \qquad (n = 2.5).$$

Normal values of this constant lie within the range from 18×10^{-4} to 39×10^{-4}. In pernicious anaemia we found markedly lower values between 47×10^{-5} and 79×10^{-5}. The curves characterized by this constant are shifted to the right. A similar change of the dissociation curve shape was described at the same time by Litarczek et al. [7]. When trying to find an interpretation of this phenomenon, we turned our attention to the reduced glutathione level in erythrocytes, expressing it in the form of the so-called Gabbe's index:

$$\frac{\text{GSH in mg per 100 g of erythrocytes}}{\text{erythrocytes in millions}}.$$

We found an indirect dependence between the Hill's constant and the Gabbe's index which is not linear but has an approximately hyperbolic shape (Fig. 1).

Later on we tried to prove the influence of glutathione upon the shape and situation of the dissociation curve by experiments in vitro. After the addition of reduced glutathione to the blood we could observe a distinct decrease of the Hill's constant and consequently a shift of the dissociation curve to the right. Cysteine exerts an effect analogous to that exerted by reduced glutathione. The Tables 1 and 2 demonstrate the changes of the Hill's constant caused by the addition of glutathione Table 1) an d of cysteine (Table 2).

Table 1

	Hill's constant	
	Experiment No. 1	Experiment No. 2
Blood, native	0.000250	0.000235
Blood + 25 mg of glutathione per 100 ml	0.000193	0.000182
Blood + 50 mg of glutathione per 100 ml		0.000182
Blood + 75 mg of glutathione per 100 ml	0.000137	0.000156
Blood + 100 mg of glutathione per 100 ml		0.000129

Table 2

	Hill's constant
Blood, native	0.000292
Blood + 25 mg of cysteine per 100 ml	0.000234
Blood + 50 mg of cysteine per 100 ml	0.000198
Blood + 75 mg of cysteine per 100 ml	0.000169
Blood + 100 mg of cysteine per 100 ml	0.000105

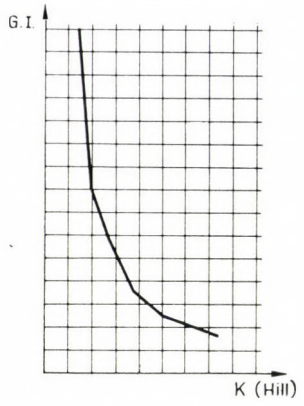


Fig. 1. The relation of Gabbe's index to the value of the Hill's constant

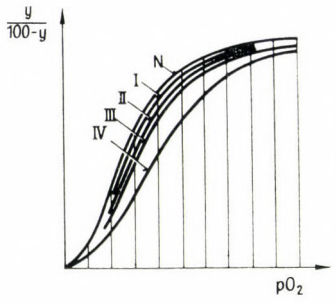


Fig. 2. The dissociation curve of oxihaemoglobin in blood to which rising amounts of reduced glutathione have been added. (25—50—75—100 mg %.) The values of the Hill's constant: native blood 29,2 · 10^{-4}, I. (25 mg %) 23.4 · 10^{-4}, II. (50 mg %) 19.8 · 10^{-4}, III. (75 mg %)16.9 · 10^{-4}, IV. (100 mg %) 10.5 · 10^{-4}

The blockade of -SH groups, e. g., by a mercury compound (we used phenylmercuric borate), has an opposite effect. The Hill's constant increases, and the dissociation curve shifts to the left. This effect of mercury compounds was observed also by Riggs and Wolbach [8] who assumed an influence of glutathione upon the interaction of haem units. The effect of mercury compounds can be cancelled by the adddition of either glutathione or cysteine. The results of such an experiment are presented in the Table 3.

Table 3

	Hill's constant	
	Experiment No. 1	Experiment No. 2
Blood, native	0.000205	0.000173
Blood + phenylmercuric borate	0.000586	0.000275
Blood + glutathione	0.000123	
Blood + cysteine		0.000119
Blood + cysteine + phenylmercuric borate		0.000145

In the course of further studies we used crystalline haemoglobin, prepared by the Drabkin method [9] from haemolyzed human blood. We found that a solution of pure haemoglobin did not exhibit any glutathione effect on the shape of the dissociation curve. Only when the stromata, previously separated by centrifugation from the haemolysate, were added, the effect of glutathione on the shape and situation of the dissociation curve was renewed. We assume, therefore, that the effect of glutathione on the dissociation of haemoglobin is conditioned by the synergism of a component bound on the erythrocyte stroma on whose nature, however, we cannot propose any opinion yet.

The possible significance of a shift to the right, due to an elevated concentration of reduced glutathione in the erythrocyte, for the supply of oxygen to the tissues may be best demonstrated with the aid of the graph (Fig.2) showing the results of one of our experiments. The native blood used in the experiment contained 11.8 per cent w/v of haemoglobin. At the oxygen pressure 100 torr and carbon dioxide pressure 40 torr this blood, having a Hill's constant 29.2×10^{-4}, binds 14.55 ml of oxygen. If the oxygen pressure sinks to 40 torr (which corresponds to the oxygen pressure in mixed venous blood and roughly also to the oxygen pressure in the tissues), then 2.91 ml of bound oxygen per 100 ml of blood are liberated. If the Hill's constant is decreased to 10×10^{-4} by an addition of reduced glutathione, then under conditions otherwise identical as much as 5.96 ml of oxygen are liberated from the same volume of blood, i.e., by 105 per cent more. This effect seems to explain (partly at least) the clinical experience that some anaemias, in particular pernicious anaemias, are not accompanied by more serious dyspnea even if the anaemia has developed to a relatively high degree.

The fact that the shape and situation of the dissociation curve of haemoglobin can be influenced by an addition of reduced glutathione, was an impulse for our experiments aimed at the investigation of a possible enhancement, with the aid of the said mechanism, of the therapeutic efficacy of the transfusion of blood or possibly also of substitutive solutions (plasma expanders) in such clinical states in which a considerable and rapid loss of blood has taken place, viz., in haemorrhagic shock.

The experiments were carried out in dogs exposed to a standard haemorrhagic shock. The conditions of the experiment have been elaborated in our institute so well that the experimental results can be easily compared and statistically evaluated.

The following working scheme has been used for producing the haemorrhagic shock [10].

In dogs weighing 17.5 kg on the average the femoral vein and artery were prepared under aseptic conditions. Into the artery on the one side a canule connected with a manometer was inserted, and from the artery on the other side blood was let out at a rate of 1 ml per 1 kg body weight per 1 minute, until the blood pressure sank to 110 torr. Then the blood-letting was continued so that during further 5 minutes the pressure sank to 84 torr, during another 5 minutes to 60 torr, and during final 5 minutes to 40 torr. At this level, the pressure was maintained

for 60, 70, or 80 minutes by infusion or evacuation of blood, according to the conditions of pressure. When the preset duration of hypotension had been achieved, the infusion of either blood or another therapeutic solution to be tried was started at a rate of 1 ml per 1 kg of body weight per 1 minute. The total volume of the liquid infused depends on the magnitude of the previous loss of blood.

Under the described experimental conditions, about 50 per cent of the experimental animals died when treated by transfusion of blood alone (preserved blood of the experimental animal itself). First of all, the dosage of reduced glutathione, necessary for a manifest therapeutic effect, had to be established. We

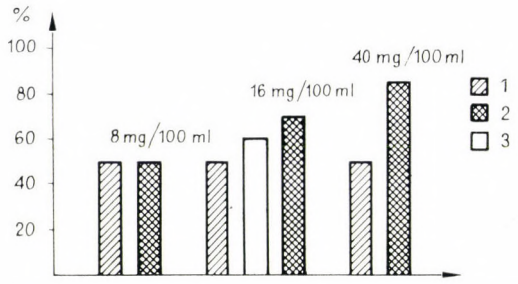


Fig. 3. Therapeutic effect of blood transfusion enriched with rising amounts of reduced glutathione. 1. Controls without fortification. 2. Blood preserves, fortified with rising amounts of GSH. 3. Therapeutic effect of packed cells

used blood preserves fortified with increasing doses of reduced glutathione, 8 – 16 – 40 mg per 100 ml of transfused blood. The graph (Fig. 3) exhibits the results showing that the optimal effect was obtained with the concentration of 40 mg per 100 ml of blood. (In some experiments even higher concentrations were used, but since their use did not produce better results, they have been omitted here.) At this concentration, more than 90 per cent of the experimental animals survived the acute loss of blood, while at the concentration of 8 mg per 100 ml of blood the number of surviving animals was equal to that registered when the blood preserve alone, without glutathione, was used. At the concentration of 16 mg per 100 ml of blood, 70 per cent survived (slightly more than when erythrocyte mass was employed).

The therapeutic effect achieved must be ascribed to glutathione itself, as several circumstances show:

(a) There exists an apparent direct proportionality between the effect achieved and the dose used; the optimal effect was found at 40 mg per 100 ml of blood.

(b) Other compounds containing -SH groups did not exhibit any therapeutic effect. The addition of cysteine to the blood preserve had no favourable effect on the experimental result; the percentage of surviving animals was not increased.

(c) The addition of a mixture of amino acids, constituents of glutathione (viz., glutamic acid, cysteine, and glycine) in respective amounts proportional to a concentration of 40 mg of glutathione per 100 ml of the solution used for the therapy, did not exhibit any favourable therapeutic effect.

In 18 dogs treated with blood containing 40 mg of reduced glutathione per 100 ml, we investigated the changes of the dissociation curves of oxyhaemoglobin. The Hill's constant values were determined, first before the experiment, then immediately after the administration of blood with glutathione, and finally about 1 – 2 hours later.

The blood samples were saturated in tonometers with gas mixtures containing always carbon dioxide at a concentration corresponding to the partial pressure of 40 torr, and oxygen under varied pressures. The saturation of haemoglobin was determined by the usual procedure using Haldane's apparatus; the compositions of gas mixtures in the tonometers were checked with the aid of Haldane's gas analyzer. The results are presented in the Table 4.

Table 4

	Average	Dispersion variance
(1) Hill's constant in native blood, average value from 18 experiments	33.9×10^{-4}	13.9×10^{-4}—8.40×10^{-4}
(2) Hill's constant after the application of blood with GSH	13.4×10^{-4}	30.0×10^{-5}—2.90×10^{-4}
(3) Hill's constant, 1 hour later	14.5×10^{-4}	77.0×10^{-5}—1.95×10^{-4}
(4) Hill's constant, 2 hours later	24.0×10^{-4}	11.0×10^{-4}—7.80×10^{-4}

The administration of blood containing glutathione causes therefore a general decrease of the Hill's constant and a shift of the dissociation curve to the right. This decrease remains at almost the same value during the first hour, and even after 2 hours the values do not return to the initial levels. Such a course we observed in all experimental animals with a single exception in which no change followed the administration. A subsequent analysis of the glutathione solution administered revealed that the greater part of glutathione was present in the oxidized form, the solution having been stored for a relatively long period.

A control experiment in a group of 3 dogs, to which preserved blood containing 50 mg of vitamin C per 100 ml was administered, showed results presented in Table 5.

Table 5

	Average	Dispersion variance
(1) Hill's constant	28.0×10^{-4}	25.6×10^{-4}—3.30×10^{-4}
(2) Hill's constant after the application of blood with vitamin C	22.0×10^{-4}	13.0×10^{-4}—2.80×10^{-4}
(3) Hill's constant, 1 hour later	36.0×10^{-4}	22.0×10^{-4}—5.90×10^{-4}
(4) Hill's constant, 2 hours later	31.0×10^{-4}	27.0×10^{-4}—3.90×10^{-4}

It is evident that here, apart from an insignificant decrease of the Hill's constant immediately after the administration of a therapeutic dose of the blood with vitamin C, we did not observe any shift of the dissociation curve to the right, in contrast to preserves with glutathione added.

Glutathione solution destined for the therapeutic administration was prepared from commercial glutathione Hoffmann – La Roche. The substance was dissolved in sterile physiological solution, and the solution was passed through a Seitz filter. Measured volumes of the solution, necessary for the fortification with

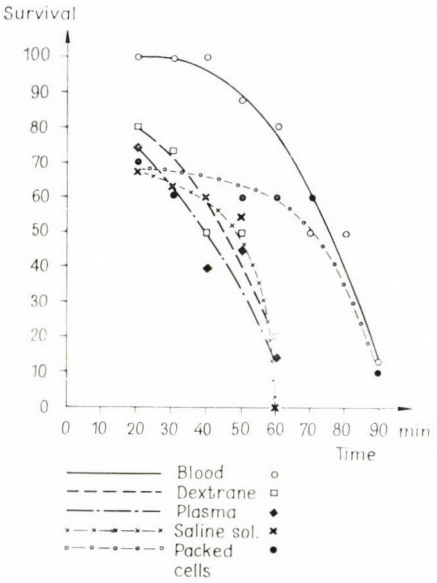


Fig. 4. Therapeutic effect of different preparations in the treatment of haemorhagic shock (standard model)

glutathione of the individual preserves, were filled into ampoules which were flushed with nitrogen and sealed. In this form the solution of glutathione may be stored till the time of use without danger of oxidation. Controls, based on the method of chromatographic separation of oxidized glutathione from the reduced form, proved that the glutathione administered was present in the reduced form. After an ampoule has been opened, the contents must be used up within short time since glutathione exposed to air is relatively rapidly oxidized, thus losing the therapeutic activity. It is best, therefore, to add the necessary amount of glutathione to the therapeutic preparation to be infused immediately before the administration.

Further, we asked the question whether a similarly favourable therapeutic effect of glutathione could be observed if we used in the therapy of acute haemorrhagic shock, instead of whole blood, substitutive solutions of blood plasma where no haemoglobin is present which could act as oxygen carrier.

In further experiments, we employed the most important types of substitutive solutions, viz.: Czechoslovak despecificated plasma, Hémoplasma, BK8 Solution, Subtosan, Dextran (both of medium and high molecular weight), Macrodex, homologous plasma. We compared the results with those obtained previously either with the physiological solution or with isologous blood. It is apparent from the graph (Fig. 4) that favourable therapeutical results fluctuated between 60 and 70 per cent but never exceeded the upper value even if whole blood was employed.

Another graph (Fig. 5) represents the therapeutic effects achieved in a standard haemorrhagic shock model, when certain solutions were administered to

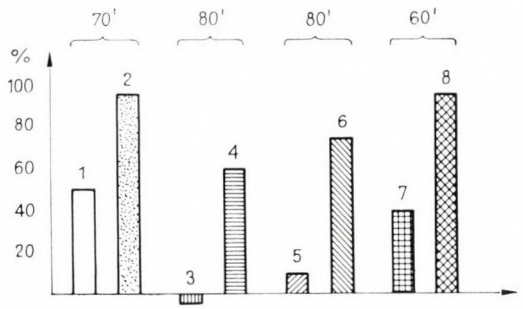


Fig. 5. Survival of experimental animals exposed to the standardized model of haemorrhagic shock, and treated with blood and different blood expander solutions, after fortification with reduced glutathione. 1. Blood preserve, 2. Blood preserve with glutathione, 3. Dextran (mid-molecular), 4. Dextran (mid-molecular) with glutathione, 5. Dextran (high-molecular), 6. Dextran (high-molecular) with glutathione, 7. Modified bovine plasma, 8. Modified bovine plasma with glutathione

which reduced glutathione had been added in doses of 40 mg per 100 ml. There were 4 experimental groups to which the following solutions were administered: (i) blood, (ii) dextran of medium molecular weight, (iii) dextran of high molecular weight, (iv) despecificated bovine plasma. To each group belonged a control group treated with the appropriate solution without glutathione.

In the group treated with blood, 50 per cent of the animals survived; in the group treated with blood containing glutathione, 95 per cent survived. Very marked differences are apparent in both groups treated with dextran. The group receiving dextran of medium molecular weight had a 100 per cent mortality rate; the addition of glutathione raised the percentage of survival to 60 per cent. Still more favourable were the conditions in the group receiving dextran of high molecular weight in which the number of surviving animals was increased by 75 per cent in comparison with the controls. The enrichment with glutathione showed a favourable action also in the administration of despecificated heteroplasma where the number of surviving animals was increased by 55 per cent in comparison with the controls.

All results were evaluated with the aid of the χ^2 test and were highly significant.

In our experiments, glutathione appeared to be a substance favourably influencing the therapy of experimental haemorrhagic shock. A satisfactory interpretation of this effect is naturally not easy. The shift of the dissociation curve to the right, observed at an increased concentration of glutathione in blood, may signify an improvement of the conditions regulating the supply of oxygen to the tissues, as demonstrated by our experiments. In our opinion, however, this is only a partial interpretation of the whole mechanism. Glutathione plays an important role in the protection of a variety of enzymes whose activity is conditioned by the preservation of intact -SH groups. Probably an effect is exerted also on a number of biochemical processes in which such "sulfhydryl-dependent" enzymes play an important role. Further experiments aimed at the solution of this side of the problem may possibly shed more light on some of these questions.

References

1. Racker, E.: Glutathione reductase from bakers'yeast and beef liver. *J. Biol. Chem. 272*, 855 (1955).
2. Taylor, J. F.—Antonini, E.—Wyman, J.: The oxygen equilibrium of cystine-treated human haemoglobin without free sulfhydryl group. *J. Biol. Chem. 238*, 2660 (1963).
3. Behlke, J.—Scheller, W.: Durch SH-Reagenzien bedingte Veränderungen des Redoxpotentials Haemoglobin. *Naturwissenschaften 48*, 717 (1961).
4. Murawski, K.—Zakrzewski, K.: Sulfhydryl groups and haem-globin linkage. *Acta Bioch. Pol. 11*, 379 (1964).
5. Huishman, T. H. J.—Dozy, A. M.—Horton, B. F.—Nechtman, C. M.: Studies on the heterogeneity of hemoglobin. X. The nature of various minor hemoglobin components produced in human red blood cell hemolysates on aging. *J. Lab. clin. Med. 67*, 355 (1966).
6. Hořejší, J.: *a)* Dissociační křivka haemoglobinu u perniciosní anaemie. *b)* Vliv glutathionu na affinitu haemoglobinu ke kyslíku. *Čas. lek. čes. 72*, 227, 263 (1933).
7. Litarczek, G.—Aubert, H.— Cosmulesco, I.—Comanesco, V.—Litarczek, S.: Sur un cas de cyanose auto-toxique par méthémoglobinémie intra-globulaire. *Le Sang 4*, 188 (1930).
8. Riggs, A. F.—Wolbach, R. A.: Sulfhydryl groups and the structure of haemoglobin. *J. Gen. Physiol. 39*, 585 (1956).
9. Drabkin, D. L.: A simplified technique for a large scale crystallization of human oxyhemoglobin. Isomorphous transformations of hemoglobin and myoglobin in the crystalline state. *Arch. Biochem. 21*, 224 (1949).
10. Sterba, O.—Hořejší, J.—Petz, R.: Experimentale Schocktherapie mit den durch Gluthation verstärkten Blutplasmaersatzlösungen. *Blut 12*, 236 (1966).

Wirkung von Röntgen-Ganzkörper- und Extrakorporal-Bestrahlung auf die Lymphozyten des peripheren Blutes *, **

R. Waubke, G. Gerstmair, F. Trepel, H. Pichlmaier und H. Begemann

I. Medizinische Abteilung des Städt. Krankenhauses München-Schwabing und Chirurg. Univ.-Klinik München

(Eingegangen am 16. Oktober 1966)

Bei einer feineren morphologischen Differenzierung der Lymphozyten lassen sich nach Röntgenbestrahlung der Ratte unterschiedliche Reaktionen der verschiedenen Lymphozytentypen nachweisen. Die Lymphozyten mit basophilem Zytoplasma zeigen nach subletaler Ganzkörper-Bestrahlung im peripheren Blut eine raschere Regeneration als die Lymphozyten mit hellem Zytoplasma. Nach extrakorporaler Bestrahlung des Blutes sind die basophilen Lymphozyten nicht nennenswert vermindert, während die hellplasmatischen nachhaltig absinken. Das unterschiedliche Verhalten der einzelnen Lymphozytentypen wird durch differierende Proliferation und Rezirkulation erklärt.

Unter verschiedenen Fragestellungen haben sich bereits zahlreiche Autoren mit der Wirkung von Röntgenstrahlen auf das lymphatische System beschäftigt [2, 3, 7, 13, 16, 18, 37, 38]. Mit dem Fortschritt der Transplantationschirurgie gewinnt die Röntgenbestrahlung des lymphatischen Systems immer größere Bedeutung, weil durch den immunosuppressiven Effekt dieser Maßnahme Transplantat-Rejektionen verzögert werden können. Eine immunologische Fragestellung, über die wir in einer folgenden Veröffentlichung [33] berichten werden, war zunächst der Ausgangspunkt der vorliegenden Untersuchungen.

In dieser Arbeit sollen die Folgen der Ganzkörper- und Extrakorporal-Bestrahlung auf die Lymphozyten des peripheren Blutes beschrieben werden, wobei diese Zellen nach morphologischen Kriterien genauer differenziert wurden. Andere Untersuchungen unseres Arbeitskreises hatten verschiedene Verhaltensweisen morphologisch unterscheidbarer Lymphozyten-Untergruppen erkennen lassen, beispielsweise bei der Phagozytosefähigkeit [32]. Die Lymphozyten wurden in der May – Grünwald – Giemsa-Färbung nach Größe und Zytoplasma-Farbe in sechs Untergruppen eingeteilt. In der Nukleolen-Färbung nach Stockinger – Kellner wurden makro- und mikronukleoläre Lymphozyten unterschieden. Außer Lymphozyten wurden auch andere Zellgruppen – Monozyten, Lymphomonozyten, Granulozyten – berücksichtigt.

* Mit Unterstützung der Deutschen Forschungsgemeinschaft.

** Die Ergebnisse wurden auszugsweise auf der 11. Tagung der Deutschen Gesellschaft für Hämatologie in Innsbruck 1965 vorgetragen.

Die vorliegenden Untersuchungen haben zwei Aspekte: Sie lassen einerseits die Wirkung von Röntgenstrahlen auf die Lymphozyten des peripheren Blutes erkennen und stellen andererseits einen Beitrag zur Funktion morphologisch unterscheidbarer Lymphozyten-Untergruppen dar.

Material und Methoden

Als *Versuchstiere* wurden 260–320 g schwere Sprague–Dawley-Ratten verwendet. Die Ernährung bestand aus Trockenfutter Altromin® und Wasser, dem bei den bestrahlten Tieren sechs Tage lang Tetrazyklin (160 mg/l) zugesetzt wurde. – Als Narkotikum erhielten die Ratten während der Bestrahlung intraperitoneal Chloralhydrat (36 mg/100 g Körpergewicht).

Ganzkörperbestrahlung: 12 Tiere wurden mit 500 r, 5 Tiere mit 550 r bestrahlt (Röntgenstrahlen 250 KV, 20 mA, 0,5 mm Kupfer-Filter, Dosimetrie mit Siemens-Dosimeter).

Extrakorporalbestrahlung: Bei 17 Tieren wurde zwischen A. carotis und V. jugularis durch einen Polyäthylenschlauch von 4 ml Inhalt ein Shunt gelegt. Der Durchfluß des heparinisierten Blutes (500 E/100 g Körpergewicht) betrug in der Minute 12 ml, also ca 75% des Gesamtblutvolumens der Ratte. – Bei 10 Tieren wurde nach Abdeckung des Körpers mit Bleiplatten die Extrakorporal-Bestrahlung mit einer Dosis von 1000 r durchgeführt (genaue Angaben bei Pichlmaier, [23]). Die Bestrahlsdauer lag zwischen 20 und 25 Minuten. – Nach der Bestrahlung wurde das Blut aus dem Shunt-System in die Blutbahn zurückgepreßt. – Mit 7 Kontrolltieren wurde mit Ausnahme der Bestrahlung in gleicher Weise verfahren.

Blutabnahmen: Zu den Zeitpunkten, die aus den Abbildungen ersichtlich sind, wurde Blut aus der Schwanzvene entnommen. Die Blutabnahme am Tage 0 erfolgte zwei Stunden nach Bestrahlung. Um genaue Ausgangswerte zu erhalten, wurde jeweils an drei Tagen vor der Bestrahlung Blut entnommen.

Färbemethoden: May–Grünwald–Giemsa-Färbung 3 Min., Eosin-Methylenblau-Lösung nach May–Grünwald, 2 Min. gepuffertes (pH 7,2) Aquadest, 20 Min. 5%ige Giemsa-Lösung. Modifizierte Nukleolenfärbung nach Stockinger–Kellner [5, 30], 15 Min. wäßrige Methylenblau-Lösung, ca 30 Sek. Zitratpuffer nach Sörensen (pH 4,96). Die genaue Zeit muß empirisch festgestellt werden, die Kerne sollen nur schwach blau angefärbt sein und einen deutlich blau gefärbten Nukleolus erkennen lassen.

Zelldifferenzierung. May–Grünwald–Giemsa-Färbung: Die Lymphozyten wurden nach ihrer Größe in kleine (Kerndurchmesser bis 7,5 μ), mittelgroße (Kerndurchmesser 7,5 bis 10,0 μ) und große (Kerndurchmesser über 10,0 μ) eingeteilt. Diese Größenordnungen sind bei der Ratte recht konstant, in Relativprozent an den Gesamtlymphozyten betragen die kleinen ca 60–70, die mittelgroßen 10–25 und die großen 1–5 rel%. – Außerdem wurden in den einzelnen Größenordnungen Lymphozyten mit tiefbasophilem und Lymphozyten mit wasserhellem Zytoplasma unterschieden, die im Folgenden kurz als basophile bzw. helle

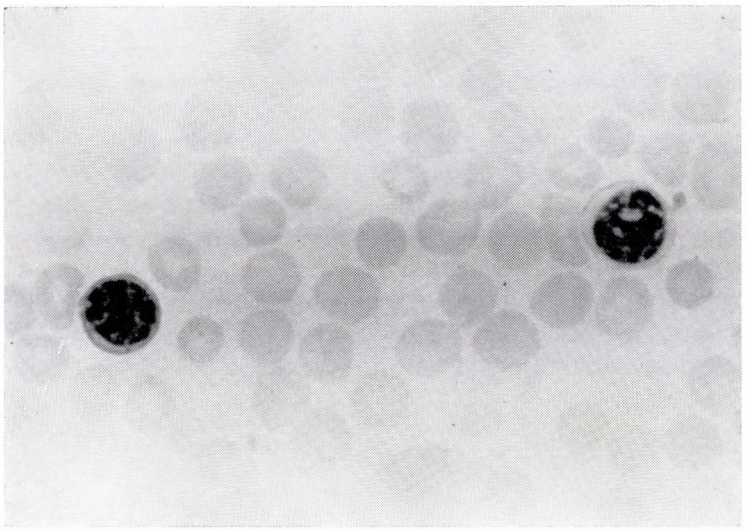


Abb. 1. Heller (rechts) kleiner Lymphozyt, basophiler (links) kleiner Lymphozyt (May—Grünwald—Giemsa-Färbung)

Lymphozyten bezeichnet werden (Abb. 1). In allen Größenordnungen beträgt der Anteil der basophilen Lymphozyten etwa 1/3, der hellen Lymphozyten etwa 2/3. – Außer Lymphozyten (80–90 rel% der Leukozyten) wurden noch Lymphomono-

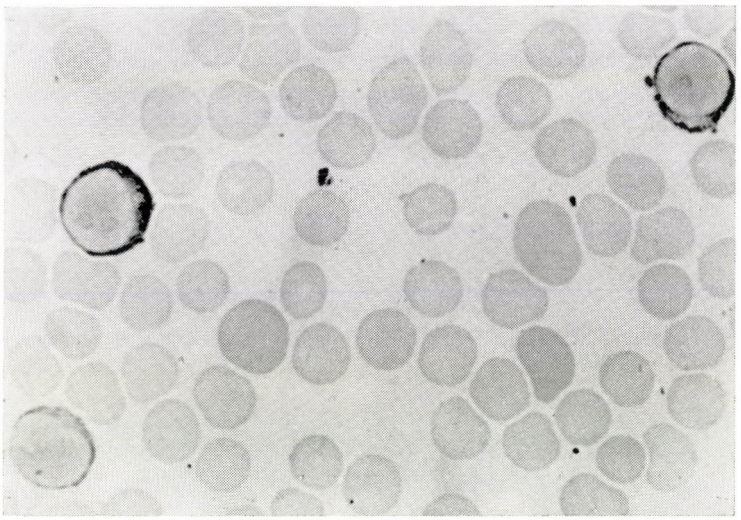


Abb. 2. Kleine Lymphozyten (Nukleolenfärbung mit Methylenblau nach Stockinger—Kellner). Links und rechts oben makronukleoläre Lymphozyten (rechts oben mononukleolär, links oben polynukleolär), links unten mikronukleolärer Lymphozyt

zyten (1 – 3 rel%) unterschieden, die morphologisch zwischen Lymphozyten und Monozyten stehen sowie Monozyten (1 – 5 rel%) und Granulozyten (5 – 10 rel%). In der Nukleonfärbung nach Stockinger – Kellner wurden nur kleine Lymphozyten berücksichtigt und in solche mit deutlich sichtbaren Nukleolen (makronukleoläre, normal ca 90 rel%) und in solche mit nur kleinen oder nicht abzugrenzenden Nukleolen (mikronukleoläre, normal ca 10 rel%) eingeteilt (Abb. 2).

Erfahrungsgemäß schwanken die Leukozytenzahlen bei Ratten von Tier zu Tier. Mittelwerte aus absoluten Zahlen würden daher besonders bei vorliegender Fragestellung das Bild verfälschen. Die absolute Anzahl eines Zelltyps (Mittelwert aus drei Voruntersuchungen) wurde bei jedem Tier als 100% angesetzt, hierzu wurden die weiteren Werte in Beziehung gesetzt. Zur Information wurden jedoch die absoluten Zahlen trotzdem den einzelnen Kurven beigefügt.

Ergebnisse

Die Untersuchungsergebnisse wurden in den Abb. 3 – 22 dargestellt. In den Abbildungen wurden auf der Ordinate die prozentualen Veränderungen angegeben, wobei der Ausgangswert, dessen Schwankungsbreite in absoluten Zahlen beigefügt ist, gleich 100% ist. Auf der Abszisse wurde die Zeit nach Bestrahlung (am Tage 0) in Tagen aufgetragen. Der mittlere Fehler der einzelnen Meßwerte ist durch senkrechte Striche eingezeichnet.

Bei der *Ganzkörperbestrahlung* von 500 r (Abb. 3 – 7) werden die basophilen etwas weniger reduziert als die hellen Lymphozyten. Auffälliger ist der raschere Wiederanstieg bei den basophilen Lymphozyten aller Größenordnungen, der im Vergleich zu den hellen Lymphozyten am dritten Tage nach Bestrahlung und somit ca. 4 Tage eher erfolgt. Während innerhalb der Gruppe der hellen Lymphozyten keine größenabhängigen Unterschiede festzustellen sind, steigen die großen und

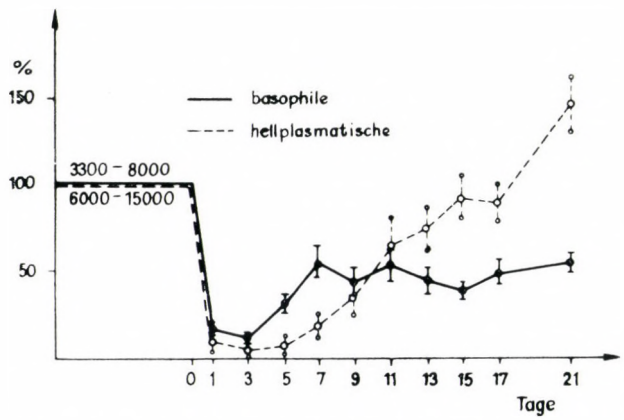


Abb. 3. Ganzkörper-Bestrahlung 500 r, kleine helle und basophile Lymphozyten

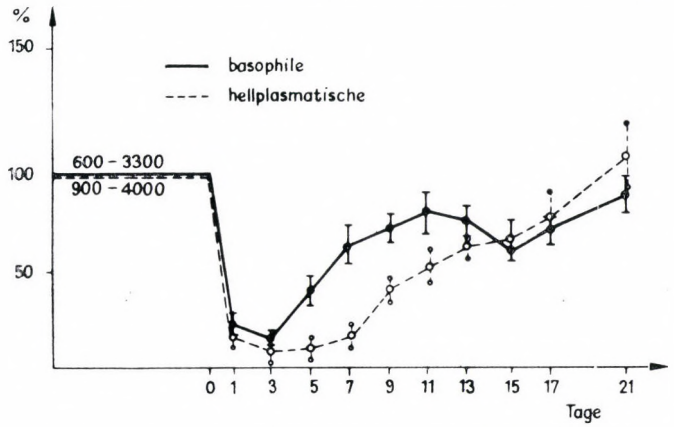


Abb. 4. Ganzkörper-Bestrahlung 500 r, mittelgroße helle und basophile Lymphozyten

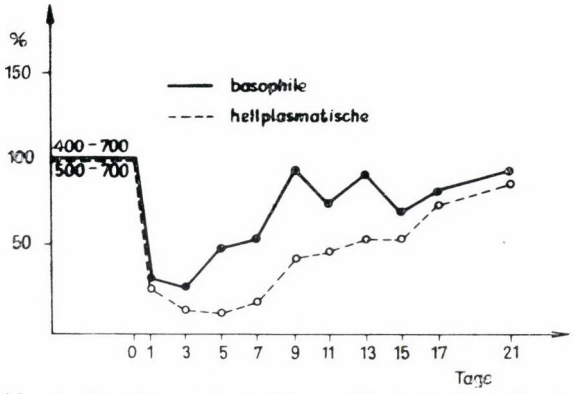


Abb. 5. Ganzkörper-Bestrahlung 500 r, große helle und basophile Lymphozyten

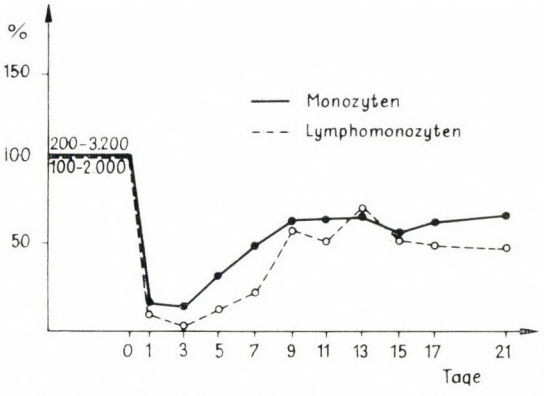


Abb. 6. Ganzkörper-Bestrahlung 500 r, Monozyten und Lymphomonozyten

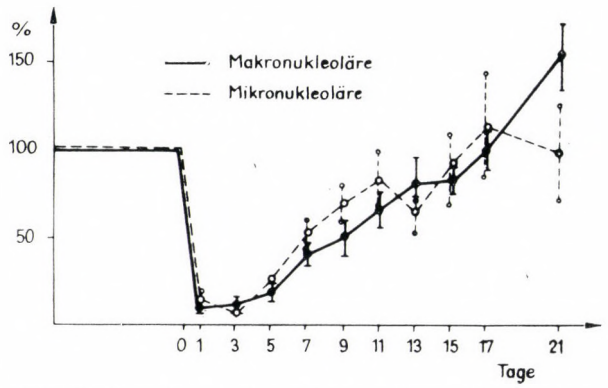


Abb. 7. Ganzkörper-Bestrahlung 500 r, Methylenblau-Färbung. Makro- und mikronukleoläre kleine Lymphozyten

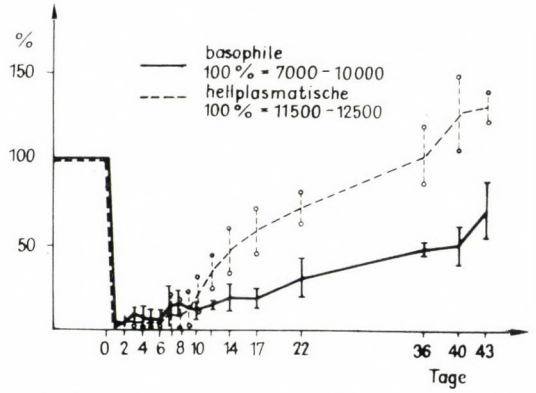


Abb. 8. Ganzkörper-Bestrahlung 550 r, kleine helle und basophile Lymphozyten

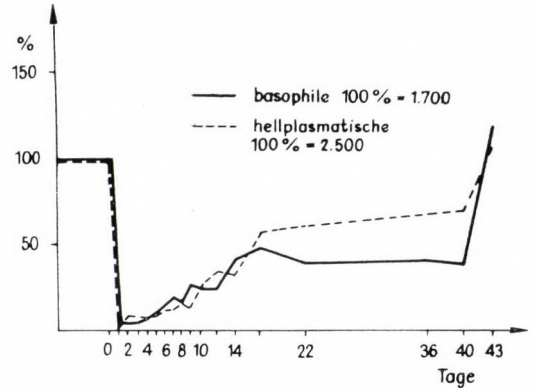


Abb. 9. Ganzkörper-Bestrahlung 550 r, mittelgroße helle und basophile Lymphozyten

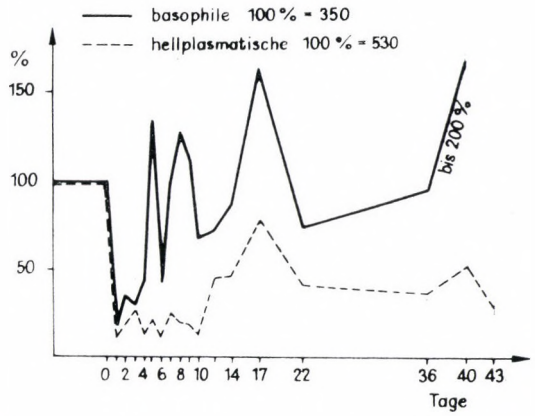


Abb. 10. Ganzkörper-Bestrahlung 550 r, große helle und basophile Lymphozyten

mittelgroßen basophilen Lymphozyten in der Regenerationsphase stärker als die kleinen an. – Die höhere Dosis von 550 r (Abb. 8–11) bewirkt eine ausgeprägtere und länger anhaltende Verminderung der peripheren Lymphozyten. Nur die großen basophilen Lymphozyten zeigen eine ähnliche rasche Regeneration, wie sie nach 500 r zu beobachten war. – Zwischen den übrigen basophilen und hellen Lymphozyten ist in den ersten 10 Tagen nach der Bestrahlung kein Unterschied feststellbar. Nach dieser Zeit steigen die hellen Lymphozyten schneller als die basophilen an, was auch bei Dosis von 500 r gesehen wird. – Ein unterschiedliches Verhalten zeigt sich bei den makro- und mikronukleolären kleinen Lymphozyten nicht.

Durch die *extrakorporale Bestrahlung* (Abb. 13–22) werden die basophilen kleinen Lymphozyten nicht vermindert (Abb. 14), die mittelgroßen (Abb. 16) geringgradig und großen basophilen Lymphozyten (Abb. 18) stärker. Der Abfall

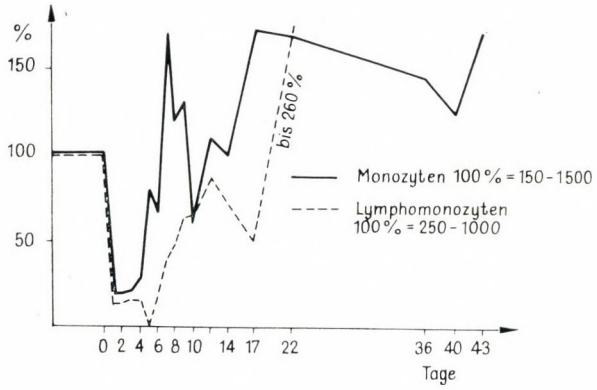


Abb. 11. Ganzkörper-Bestrahlung 550 r, Monozyten und Lymphomonozyten

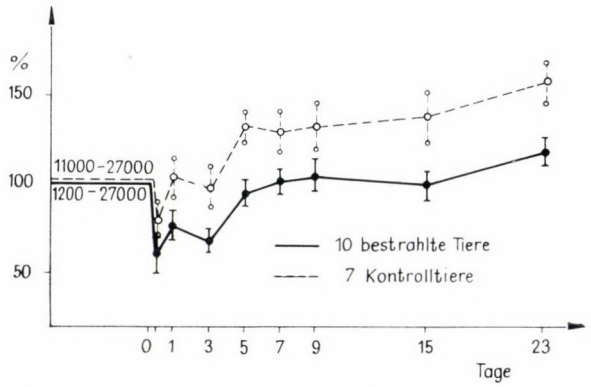


Abb. 12. Extrakorporal-Bestrahlung, kleine Lymphozyten (helle und basophile zusammen)

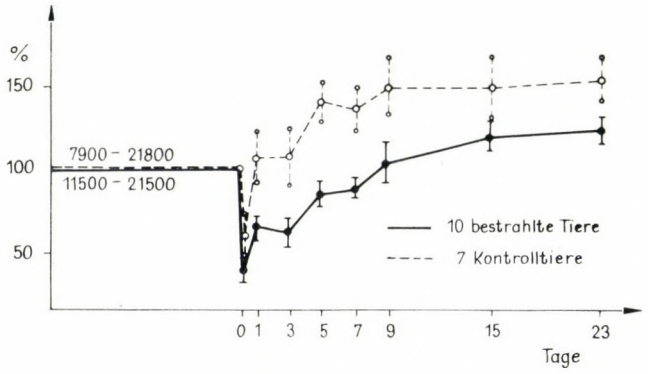


Abb. 13. Extrakorporal-Bestrahlung, kleine helle Lymphozyten

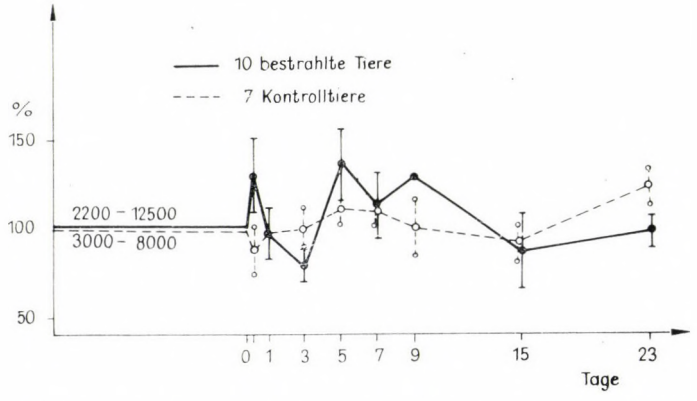


Abb. 14. Extrakorporal-Bestrahlung, kleine basophile Lymphozyten

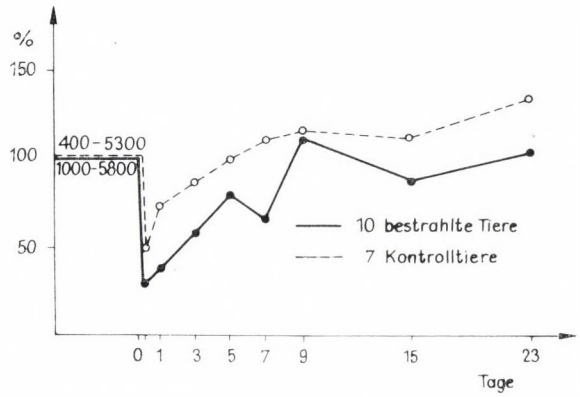


Abb. 15. Extrakorporal-Bestrahlung, mittelgroße helle Lymphozyten

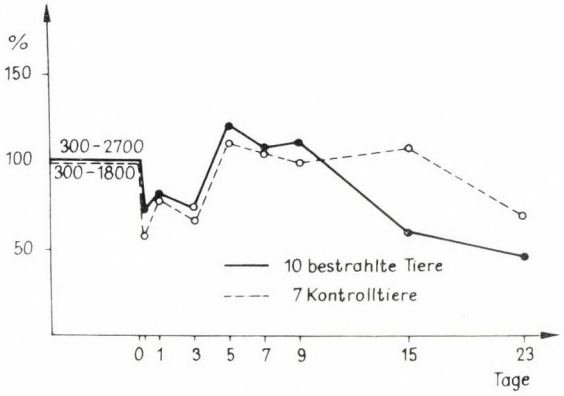


Abb. 16. Extrakorporal-Bestrahlung, mittelgroße basophile Lymphozyten

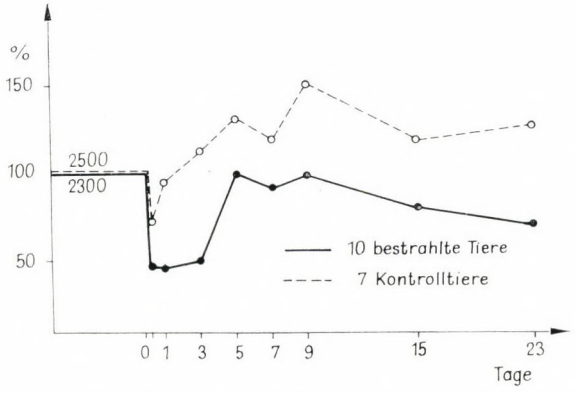


Abb. 17. Extrakorporal-Bestrahlung, große helle Lymphozyten

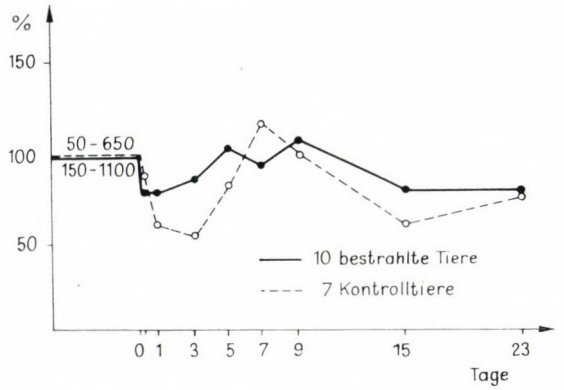


Abb. 18. Extrakorporal-Bestrahlung, große basophile Lymphozyten

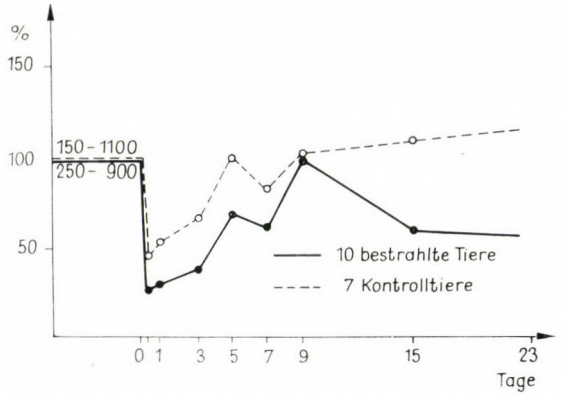


Abb. 19. Extrakorporal-Bestrahlung, Lymphomonozyten

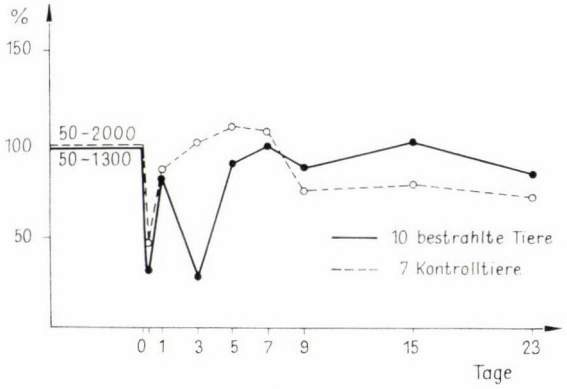


Abb. 20. Extrakorporal-Bestrahlung, Monozyten

der kleinen Lymphozyten (Abb. 12) wird nur durch die kleinen hellen Lymphozyten hervorgerufen (Abb. 13). In der Größenordnung der mittelgroßen und großen Lymphozyten werden die hellen wesentlich stärker und anhaltender als die basophilen vermindert. Die operationsbedingte vorübergehende Lymphopenie bei

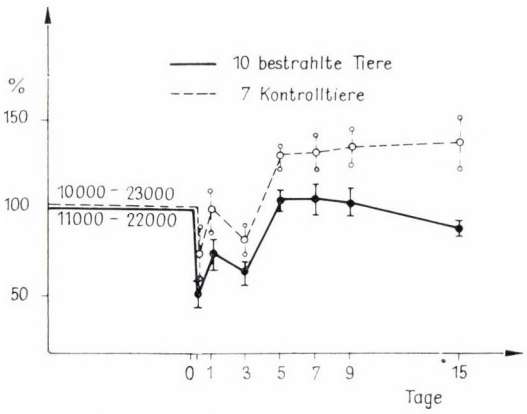


Abb. 21. Extrakorporal-Bestrahlung, Methylenblau-Färbung, makronukleoläre kleine Lymphozyten

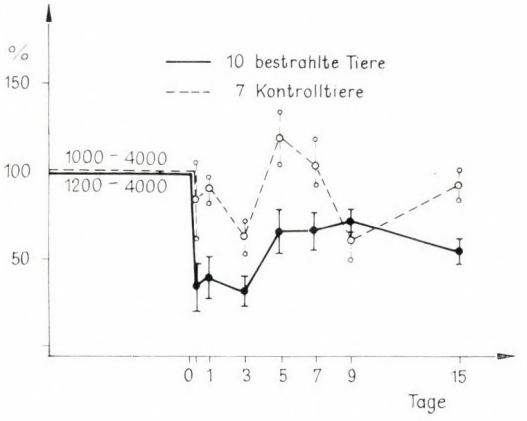


Abb. 22. Extrakorporal-Bestrahlung, Methylenblau-Färbung, mikronukleoläre kleine Lymphozyten

den Kontrolltieren bildet sich viel schneller als die Bestrahlungs-Lymphopenie zurück. – Die Anzahl der mikronukleolären Lymphozyten (Abb. 22) nimmt nach Extrakorporalbestrahlung deutlicher ab als die der makronukleolären Lymphozyten (Abb. 21).

Die Lymphomonozyten und Monozyten reagieren meist verschiedenartig, besonders nach der Ganzkörperbestrahlung von 550 r (Abb. 11) und nach Extra-

korporalbestrahlung (Abb. 19 und 20). Die Monozyten, die in ähnlicher Weise wie die Granulozyten reagieren, gleichen den akuten strahlenbedingten Verlust schneller aus als die Lymphomonozyten. – Das Verhalten der Granulozyten ist uneinheitlich, es kommt nach Extrakorporalbestrahlung und Ganzkörperbestrahlung von 550 r zunächst zu einem raschen Anstieg, dann zu einem steilen Abfall und nach wenigen Tagen zu einer oft sprunghaften Zunahme. Bei der Ganzkörperbestrahlung von 500 r war der anfängliche schnelle Anstieg der Granulozyten nicht nachweisbar.

Diskussion

Die Strahlenempfindlichkeit des lymphatischen Systems beruht auf der Strahlenempfindlichkeit der einzelnen lymphatischen Zelle. Bereits geringe Dosen von 5 r genügen, um morphologische Veränderungen der Lymphozyten in vitro hervorzurufen [37]. Bei höheren Dosen werden Kernpyknosen [11], Kernlappungen und Kernoedem [27, 28], umvollständige Mitosen [19] und Kernzerfall [1] beschrieben. Ähnliche qualitative Veränderungen an Lymphozyten wurden ebenfalls bei den vorliegenden Untersuchungen beobachtet. Die Ursache dieser morphologisch erfaßbaren Veränderungen dürfte vorwiegend auf Störungen des Nukleinsäure-Stoffwechsels beruhen [1, 18, 28, 29 35]. Man macht daher für die besondere Strahlenempfindlichkeit des lymphatischen Systems seinen hohen Nukleinsäuregehalt verantwortlich. Bei einer Ganzkörperbestrahlung gelten 600–800 r als letale Dosis bei der Ratte [24]; im hohen subletalen Bereich zwischen 500 und 550 r nimmt die Strahlenschädigung sprunghaft zu [20]. Hierzu stehen die eigenen Beobachtungen der ausgeprägteren und länger anhaltenden Lymphozytendepression bei Steigerung der Strahlendosis von 500 auf 550 r in guter Übereinstimmung. Etwa 3 Wochen nach einer Ganzkörperbestrahlung von 500 r war im peripheren Blut die Regeneration im Wesentlichen abgeschlossen, bei 550 r nach etwa 6 Wochen. – Um Verhaltensweisen verschiedener Lymphozytengruppen zu studieren, erscheint der Dosisbereich um 500 r besonders geeignet, weil bei dieser Dosierung das lymphatische System vorübergehend massiv geschädigt wird, jedoch die Fähigkeit zur raschen Regeneration behält.

Die Verminderung der peripheren Lymphozyten durch die Ganzkörperbestrahlung wurde von zahlreichen Autoren beobachtet (Zusammenfassungen bei [16, 18, 23]). Bei den eigenen Untersuchungen fiel auf, daß die Lymphozyten verschiedener Größenordnungen ungleich betroffen wurden, und zwar die kleinen Lymphozyten stärker als die mittelgroßen Lymphozyten, und diese wiederum stärker als die großen Lymphozyten. In der Regenerationsphase steigen die Lymphozyten aller Größenordnungen wieder an. Auffällig bei der genaueren morphologischen Differenzierung ist das unterschiedliche Verhalten von Lymphozyten mit hellem bzw. basophilem Zytoplasma. Die hellen Lymphozyten werden durch die Ganzkörperbestrahlung etwas stärker betroffen: Sie sind in den ersten Tagen nach Bestrahlung deutlicher vermindert als die basophilen und regenerieren später, dann jedoch überschießend.

Die extrakorporale Bestrahlung des Blutes wurde in den letzten Jahren besonders als Modell für kinetische Untersuchungen des lymphatischen Systems verwendet [6, 7]. Sie bietet gegenüber der Ganzkörperbestrahlung den Vorteil, die peripheren Lymphozyten isoliert zu treffen, ohne dabei die lymphatischen Bildungs- bzw. Speicherungs-Organe in Mitleidenschaft zu ziehen. – Bei der Extrakorporalbestrahlung wurden in gleicher Weise wie bei der Ganzkörperbestrahlung Lymphozyten mit basophilem bzw. hellem Zytoplasma unterschiedlich betroffen. Die Anzahl kleiner, mittelgroßer und großer hellplasmatischer Lymphozyten sinkt nach extrakorporaler Bestrahlung ab und unterscheidet sich auch beim Wiederanstieg deutlich von den Werten, die bei den Kontrolltieren gefunden wurden. Dagegen zeigten die basophilen Lymphozyten bestrahlter Tiere und unbestrahlter Kontrolltiere keine Unterschiede. Die nachhaltigere Verminderung der peripheren Lymphozytenzahl nach extrakorporaler Bestrahlung wird also durch das Absinken der hellplasmatischen Lymphozyten verursacht.

Die Lymphozytendepression nach extrakorporaler Bestrahlung zeigte in unseren Versuchen ein ähnliches Ausmaß wie bei Cronkite und Mitarb. [7]. Das ist umso bemerkenswerter, als Cronkite beim Kalb eine mehrstündige Bestrahlung durchführte, während wir bei der Ratte nur 20 Minuten bestrahlten. Die Strahlengesamtdosis, die das Blut erhielt, war jedoch in beiden Versuchsanordnungen annähernd leicht. Bei Cronkite wurden pro Minute etwa 5%, bei uns etwa 75% des Gesamtsblutvolumens des jeweiligen Versuchstieres bestrahlt. Cronkite benötigte daher die 15-fache Bestrahlungsdauer (nämlich ca 5 Stunden), um denselben Effekt wie wir in 20 Minuten zu erreichen.

Bei der Deutung der Versuchsergebnisse sind die Operationsfolgen zu berücksichtigen, denn auch die unbestrahlten, nur operierten Tiere zeigten am ersten Tag eine vorübergehende Verminderung der hellplasmatischen Lymphozyten. Diese kann man im Wesentlichen als Stress-Effekt auffassen, der durch die Ausschüttung von Cortison hervorgerufen wird. Die Verminderung peripherer Lymphozyten und die lymphoklastische Wirkung durch Cortison ist experimentell nachgewiesen [4, 8, 12, 15]. Die operationsbedingte Lymphopenie ist jedoch flüchtiger und geringer als der Lymphozytensturz nach Bestrahlung.

In der Literatur finden sich für genauere morphologische Differenzierungen der Lymphozyten nach Röntgenbestrahlung kaum Hinweise. Wiseman [39], der eine ähnliche morphologische Differenzierung wie wir durchführte, berichtete von einem Patienten mit strahlenbedingter Lymphopenie, bei der das Verhältnis zwischen hellen und basophilen Lymphozyten zu Gunsten der basophilen Lymphozyten verschoben war. – Für das unterschiedliche Verhalten von Lymphozytenuntergruppen nach Bestrahlung lassen sich grundsätzlich zwei Erklärungen geben: Entweder sind die basophilen Lymphozyten weniger strahlenempfindlich, oder sie werden im Blut schneller ersetzt als die hellplasmatischen. Ein Unterschied in der Strahlensensibilität dürfte vorhanden sein, ist jedoch gering, wie aus den Untersuchungen mit der Ganzkörperbestrahlung hervorgeht, bei der alle Zellen gleichen Bedingungen unterworfen wurden. Weder der frühere Wiederanstieg der basophilen Lymphozyten nach der Ganzkörperbestrahlung noch die Tatsache, daß diese

Zellgruppe bei der extrakorporalen Bestrahlung kaum betroffen wurde, können mit der geringeren Strahlenempfindlickeit allein erklärt werden.

Der Grund des unterschiedlichen Verhaltens der basophilen und der hellen Lymphozyten dürfte darin zu suchen sein, daß die basophilen Lymphozyten im peripheren Blut rascher ersetzt werden. Nach eigenen Beobachtungen enthält der Ductus thoracicus der Ratte fast ausschließlich basophile Lymphozyten. Bei den großen Mengen von Lymphozyten, die der Ductus thoracicus ständig an das Blut abgibt [14, 25], stellt der Ersatz der geringen Anzahl im peripheren Blut kreisender basophiler Lymphozyten kein Problem dar. Dieses wird besonders dann klar, wenn man den Prozentsatz der im peripheren Blut kreisenden Lymphozyten im Vergleich zur Gesamtlymphozytenzahl mit 1 % annimmt [17] und die Zirkulationszeit eines Lymphozyten im Blut mit 20–40 Minuten ansetzt [7, 35].

Im Gegensatz zu den kleinen basophilen Lymphozyten scheinen die kleinen hellen Lymphozyten nicht oder nur geringgradig zu rezirkulieren, denn ihr strahlenbedingter Verlust im peripheren Blut nach Extrakorporalbestrahlung wird so langsam ausgeglichen. wie es der Neuproduktion kleiner Lymphozyten entspricht.

Das unterschiedliche Verhalten der kleinen hellen und basophilen Lymphozyten nach extrakorporaler Bestrahlung wird ausreichend durch verschiedenartige Rezirkulationen erklärt. Die Unterschiede bei den größeren Lymphozyten scheinen durch Turnover-Differenzen bedingt zu sein. Große und mittelgroße haben einen rascheren Turnover als kleine Lymphozyten [10, 22, 26]. Weitere Untersuchungen unseres Arbeitskreises ergaben, daß die großen und mittelgroßen basophilen Lymphozyten einen wesentlich rascheren Umsatz haben als die großen und mittelgroßen hellplasmatischen Lymphozyten [31].

Die Lymphomonozyten zeigten nach Bestrahlung ein ähnliches Verhalten wie die großen hellplasmatischen Lymphozyten, was für einen lymphatischen Ursprung dieser Zellen spricht. Die Monozyten stehen in ihrem Verhalten zwischen den einen raschen Turnover zeigenden großen basophilen Lymphozyten und den Granulozyten. Das steht in Übereinstimmung mit der neueren Auffassung des Monozyten als einer Zelle mit schnellem Turnover, die aus dem Knochenmark stammt [10, 21, 34].

Ergänzend zur May–Grünwald–Giemsa-Färbung ergaben die Befunde der Nukleolen-Färbung nach Stockinger–Kellner, daß die makronukleolären kleinen Lymphozyten durch die Bestrahlung nicht so stark wie die mikronukleolären kleinen Lymphozyten betroffen wurden. Wenn man die makronukleolären Lymphozyten in polynukleoläre und mononukleoläre aufteilt, ergibt sich, daß sich die polynukleolären wie die basophilen und die mononukleolären (neben den mikronukleolären) wie die hellplasmatischen Lymphozyten verhalten. Auch diese ergänzenden Befunde sprechen für uneinheitliche Reaktionen von morphologisch unterscheidbaren Lymphozyten-Untergruppen.

Die vorgelegten Untersuchungen stellen einen Beitrag zur Funktion morphologisch differenter Lymphozyten dar. Unterschiedliche Verhaltensweisen dieser Zellen nach Bestrahlung, bei der Lebenszeitbestimmung [31] und der Phagozytosefähigkeit [32] ließen eine genauere Differenzierung wünschenswert erscheinen.

Während in diesem Teil der Arbeit die Folgen der Ganzkörper- und Extrakorporal-Bestrahlung erläutert wurden, soll in einer folgenden Arbeit [33] auf immunologische Funktionen morphologisch unterscheidbarer Lymphozytengruppen eingegangen werden.

Literatur

1. Ackermann, G. A., Bellios, N. C., Knouff, R. A. and Frajola, W. I.: Cytochemical changes in lymph nodes and spleen of rats after total body X-radiation. *Blood 9*, 795 (1954).
2. Adelstein, S. J. and Dealy, J. B.: Hematologic response to human whole body irradiation. *Amer. J. Roentgenol., Rad. Ther. and Nuclear Med. 93*, 927 (1965).
3. Barnes, B. A., Brownell, G. L. and Flax, M. H.: Irradiation of the blood: Method for reducing lymphocytes in the blood and spleen. *Science 145*, 1188 (1964).
4. Baserga, A. et Morsiani, A.: La régénération lymphocytaire après prednisone. *Schweiz. Med. Wschr. 91*, 1190 (1961).
5. Begemann, H., Rastetter, J. und Fink, U.: Zur Klassifizierung der Lymphozyten. *Med. Klin. 58*, 706 (1963).
6. Cronkite, E. P., Jansen, C. R., Mather, G. C., Nielson, N. O., Usenik, E. A., Adamik, E. R. and Sipe, C. R.: Studies on lymphocytes. I. Lymphopenia produced by prolonged extracorporeal irradiation of circulating blood. *Blood 20*, 203 (1962).
7. Cronkite, E. P., Jansen, C. R., Cottier, H., Rai, K. and Sipe, C. R.: Lymphocyte production measured by extracorporeal irradiation and labeling techniques. *Ann. N. Y. Acad. Sci. 113*, 566 (1964).
8. Dougherty, T. F. and Frank, J. A.: The quantitative and qualitative responses of blood lymphocytes to stress stimuli. *L. Laborat. 42*, 530 (1953).
9. Everett, N. B., Rieke, W. O., Reinhardt, W. O. and Yoffey, J. M.: Radioisotopes in the study of blood cell formation with special references to lymphocytopoiesis. *Symposium on Haemopoiesis*, p. *43*, London 1960.
10. Fliedner, T. M., Cronkite, E. P. and Bond, V. P.: Potentialities and limitations of H-3-thymidine labeling of haemopoietic cell systems in the study of their dynamics of proliferation. *Proc. VIIIth Congr. Europ. Soc. Haematol.*, Vienna 1961, Part I, *62*, Basel—New York 1962.
11. Forssberg, A.: Effects of small doses of ionizing radiations. *Adv. in Rad. Biol.* 117 (1964).
12. Frank, J. A. and Dougherty, T. F.: Effect of graded amounts of epinephrine on normal and stress lymphocytes of intact and adrenalectomized mice. *J. Lab. Med. 45*, 485 (1955).
13. Gidali, J. and Fehér, J.: Some data on mechanism of leukopenia and leukocytosis following irradiation. *Blood 23*, 27 (1964).
14. Gowans, J. L.: The effect of the continuous reinfusion of lymph and lymphocytes on the output of lymphocytes from the thoracic duct of un-anaesthetized rats. *Brit. J. Exp. Path. 38*, 67 (1957).
15. Grundmann, E.: Experimentelle Untersuchungen über die funktionelle Zytomorphologie der lymphatischen Strukturen bei Entzündung sowie unter Cortison und DOCA. *Beitr. Path. Anat. u. Allg. Path. 119*, 377 (1958).
16. Hagen, U.: Strahlenschädigung der lymphatischen Organe. *Med. Welt. 18*, 1009 (1962).
17. Hamilton, L. D.: Control and functions of the lymphocyte. *Ann. N. Y. Acad. Sci. 73*, 39 (1958).
18. Hug, O.: Die akuten Allgemeinreaktionen bei Ganz- und Teilkörperbestrahlung. *Strahlenbiologie, Strahlentherapie, Nuklearmedizin und Krebsforschung* 581 (1959) Stuttgart.
19. Ingram, M.: Symposium on the immediate and low level effects of ionizing radiation, Venedig 1959.

20. Kohn, H. I. and Kallmann, R. F.: Acute letality studies with the rat: The LD 50, death rate and recovery rate. *Rad. Res. 7*, 85 (1957).
21. Leder, L. D.: Fermentcytochemische Untersuchungen zur Herkunft der Blutmonozyten. *Klin. Wschr. 44*, 25 (1966).
22. Little, J. R., Brecher, G., Bradley, T. R. and Rose, S.: Determination of lymphocyte turnover by continuous infusion of H-3-thymidine. *Blood 19*, 236 (1962).
23. Pichlmaier, H.: Die Bedeutung der Lymphozyten für die Homotransplantation. Habilitationsschrift, München 1965.
24. Rajewsky, B., Aurand, K., Hug, O., Mergler, H., Muth, H., Pauly, H., Schraub, A., Six, E. und Wolf, I.: Strahlendosis und Strahlenwirkung, Stuttgart 1956.
25. Reinhardt, W. O.: Some factors influencing the thoracic duct output of lymphocytes. *Ann. N. Y. Acad. Sci. 113*, 844 (1964).
26. Schooley, J. C., Bryant, B. C. and Kelly, L. S.: Preliminary autoradiographic observations of cellular proliferation in lymphoid tissues, using tritiated thymidine. Stohlmann, F., jr.: The kinetics of cellular proliferation, 208, New York 1959.
27. Shrek, R.: Cinemicrographic observations and theoretical considerations on reactions of lymphocytes to X-rays. *Radiol. 65*, 912 (1955).
28. Shrek, R.: Dual morphologic reactions of rabbit lymphocytes to X-rays. *Am. Arch. Path. 63*, 252 (1957).
29. Sommermeyer, K.: Die Entwicklung der Treffertheorie seit dem Jahre 1946 unter besonderer Berücksichtigung ihrer Anwendung auf die biologische Wirkung energiereicher Strahlen. In: *Strahlenbiologie, Strahlentherapie, Nuklearmedizin und Krebsforschung* 1, Stuttgart 1959.
30. Stockinger, L. und Kellner, G.: Der Lymphozytennukleolus. *Wien. Zschr. Inn. Med. 33*, 135 (1952).
31. Trepel, F. und Rastetter, J.: Untersuchungen zur funktionellen Differenzierung der mononukleären Leukozyten. 11. Tag Dt. Gesellsch. f. Hämatologie, Innsbruck 1965, *Blut*, z. Zt. im Druck
32. Trepel, F., Waubke, R. und Begemann, H.: Phagozytose durch Lymphozyten. *Klin. Wschr. 44*, 256 (1966).
33. Trepel, F., Gerstmair, G., Waubke, R., Pichlmaier, H. und Begemann, H.: Beziehungen zwischen der Abstossung homologer Hauttransplantate und verschiedenen Lymphozytentypen im peripheren Blut. Haematologia 1967.
34. Volkmann, A. and Gowans, J. L.: The origin of macrophages from bone marrow in the rat. *Brit. J. Exp. Path. 46*, 62 (1965).
35. Wahler, B. C. and Widdicombe, J. G.: The blood lifespan of the lymphocyte in rabbits and rats. *J. Physiol. 132*, 41 (1956).
36. Warren, S., Holt, M. W. and Sommers, S. C.: Some cytologic and histochemical studies of radiation reaction. *Am. J. Clin. Path. 22*, 411 (1952).
37. Whitelaw, D. M.: The effect of total body irradiation on the labeling of circulating mouse lymphocytes with tritiated thymidine. *Blood 25*, 749 (1965).
38. Winchell, H. S., Pollycove, M., Andersen, A. C. and Lawrence, J. H.: Relatively selective beta irradiation of lymphatic structures in the dog using Y-90 DTPA. *Blood 23*, 321 (1964).
39. Wiseman, B. K.: Criteria of the age of lymphocytes in the peripheral blood. *J. Exp. Med. 54*, 271 (1931).

Опыт лечения миелобромолом больных хроническим миелолейкозом

Т. А. ЧЕРНЦОВА, В. М. СУСОЕВА

Гематологическая клиника Центрального ордена Ленина института гематологии и переливания крови, Москва.

(Поступила в редакцию 19 ноября 1966 г.)

1. Миелобромол оказывает сильное и быстрое цитостатическое действие, избирательно на клетки гранулопоэза.

Цитостатическое действие миелобромола распространяется и на тромбоцитопоэз. Поэтому при наличии тромбоцитопении лечение миелобромолом должно проводиться в сочетании с гемотрансфузиями и кортико-стероидными препаратами.

2. Миелобромол не оказывает какого-либо тормозящего действия на эритро- и лимфопоэз.

3. При первом обострении хронического миелолейкоза лечение миелобромолом приводит к развитию полноценной ремиссии.

4. Неполная ремиссия достигается при лечении миелобромолом, в сочетании с гемотрансфузиями и кортико-стероидными препаратами, андрогенами у мало курабельных больных, у которых заболевание протекает с вторичным миелофиброзом, или исходом в ретикулез и в бластный криз. Эти больные резистентны к миелосану и к другим цитостатическим средствам.

5. Лечебная доза миелобромола 250—125 мг в сутки.

Для продления ремиссии необходимо систематически применять миелобромол в поддерживающей дозе и тогда ремиссия может длиться до 9 месяцев и возможно более.

6. Поддерживающее лечение миелобромолом проводится в разовой дозе не более 125 мг 1—2 раза в неделю, под строгим контролем числа лейкоцитов и тромбоцитов.

7. Хорошая переносимость миелобромола, отсутствие каких-либо токсических влияний, резистентности в любых стадиях хронического миелолейкоза, заставляет ставить вопрос о внедрении миелобромола в широкую лечебную практику.

Миелобромол — дезоксибромманнит или препарат ДБМ, венгерское средство, изготовленное заводом Хиноин, в состав которого входят: 1,6-дибром, 1,6-дидезокси-Д-маннит. Выпускается в таблетках по 250 мг.

В 1962 году на 8 Международном противораковом конгрессе Шеллеи и Экхардт [1] были доложены экспериментальные данные, которые показали, что 1-6-Дибромманнит тормозит лейкопоэз. Авторами также применялся этот препарат у больных с различными формами лейкоза. Были отмечены положительные результаты при лечении хронического миелолейкоза.

При хроническом миелолейкозе авторы применяли повторные курсы лечения после интервала от 6 недель до 6 месяцев.

Из побочных влияний при лечении миелобромолом, авторы отметили у двух больных — агранулоцитоз, у одного — лейкопению и у 6 — тромбоцитопению. Ни один больной не погиб от побочных явлений.

В отчете о клиническом испытании миелобромола из клиники Дебреценского медицинского института также указывается на эффективность его при хроническом миелолейкозе [2]. Наблюдения проводились на протяжении 10 месяцев. Продолжительность курса лечения варьировала от двух до 10 месяцев. Ремиссия у больных поддерживалась при систематическом контроле и при соответствующей дозировке миелобромола. Резистентности к препарату не отмечалось. Только у двух больных (из 10) снижалось число тромбоцитов. Одному из них ранее проводилась рентгенотерапия, у другого тромбоцитопения носила иммуноаллергический характер.

У всех больных лечение миелобромолом проводилось с хорошим терапевтическим эффектом. Заметного снижения числа эритроцитов под влиянием лечения не отмечалось. У одного больного при развитии лейкопении (до 1200 в 1 мм^3) на протяжении 2—3 недель, наблюдалось выпадение волос. У других больных, несмотря на развивающуюся лейкопению, выпадения волос не было. Нарушения функционального состояния печени за период лечения не обнаруживалось.

Авторы отмечают различие индивидуальной чувствительности к препарату, иногда достаточно было дозы 250 мг в сутки, при общей дозе 2,0 для получения клинической ремиссии. Было указано на кратковременность ремиссии продолжительностью в 3—4 недели. При повторном лечении резистентности к препарату не наступало, была отмечена хорошая терапевтическая активность при выраженной резистентности к ранее применяемому милерану.

Авторы пришли к следующим выводам: начальная суточная доза миелобромола должна быть от 125 до 375 мг на 50 кг веса больного, клиническая и гематологическая ремиссия наступала через 3—6 недель от начала лечения. При снижении числа лейкоцитов до 20 000, следует проводить поддерживающее лечение через день, а позже через два дня по 125—250 мг в сутки. При числе тромбоцитов ниже 100 000 лечение миелобромолом должно проводиться под защитой гемотрансфузий. Миелобромол противопоказан в комбинации с другими цитостатическими средствами.

Mathé и сотр. [4] с соавторами от 1964 года, приводят данные о лечении миелобромолом 13 больных хроническим миелолейкозом, в периоде обострения. Больные получали по 250—750 мг миелобромола в сутки. Суммарная доза была в среднем 5,25 гр. В результате лечения у 8 больных достигнута полная ремиссия, у трех — частичная, и у двух — терапевтического эффекта не наблюдали. Длительность ремиссии варьировала от 3 до 8 месяцев. Препарат хорошо переносился больными.

* * *

Переходим к описанию собственных данных.

Миелобромол нами применялся у 34 больных. Мы сочли возможным выделить у этих больных три группы.

В первую группу мы отнесли 6 больных хроническим миелолейкозом, которым ранее специальное цитостатическое лечение не проводилось Сюда вошли больные в возрасте от 32 до 73 лет. Лечение было начато через 2—4 недели и у одного больного через 4 года от момента установления диагноза хронического миелолейкоза Сроки лечения миелобромолом у этих боль-

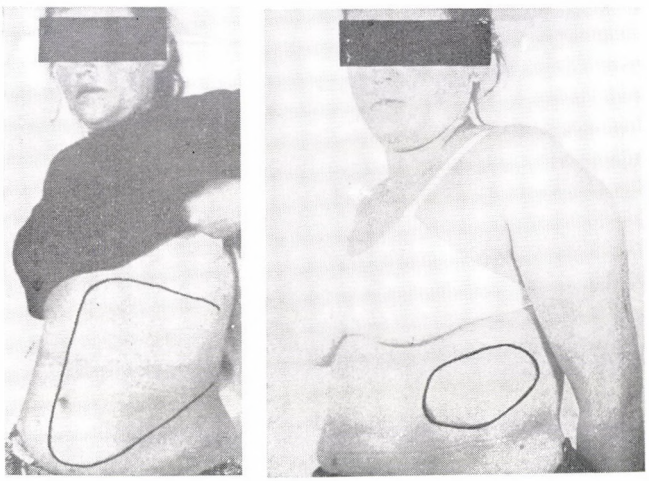


Рис. 1. Больная А-ва. Размер селезенки до и после лечения

ных были от 28 до 180 дней (до 6 месяцев). Общая доза принятого миелобромола варьировала от 3 до 12,5 гр (в среднем 7,5 гр). Наряду с лечением миелобромолом больным назначались витамины B_6 и С, гемостимулин.* У всех больных этой группы к концу лечения развилась полная клиническая и почти полная гематологическая ремиссия. Нормализовались размеры селезенки, которая до лечения достигала следующих размеров по Курлову: от 6 18/9 до 20 30/22 (рис. 1). Показатели гемоглобина и эритроцитов достигали нормальных цифр. Число тромбоцитов снижалось у всех больных, но оставалось к концу лечения на нормальном или субнормальном уровне (140 000—172 000—281 000 в 1 мм^3) (рис. 2).

Во вторую группу мы отнесли 12 больных в возрасте от 17 до 63 лет. Давность заболевания у этой группы больных было от 2 до 6 лет. Все больные 2 группы ранее получали различные цитостатические препараты. У них выявилась резистентность к миелосану** и к другим средствам (допан,

* Дефибринированная сыворотка, ferrum lacticum, cuprum sulfuricum, glucose.
** (=Myleran)

рентгенотерапия). Сроки лечения миелобромолом были от 20 до 169 дней. Общая доза миелобромола варьировала от 5,0 до 17,0 (в среднем 8,9).

У 8 больных (из 12), под влиянием лечения миелобромолом наступило выраженное клиническое улучшение (восстановление сна, аппетита, уменьшение потливости, прибавка в весе до 2—5 кг), заметное уменьшение размеров селезенки. У двух больных этой группы в результате лечения миелобромолом наступило небольшое клиническое улучшение. У осталь-

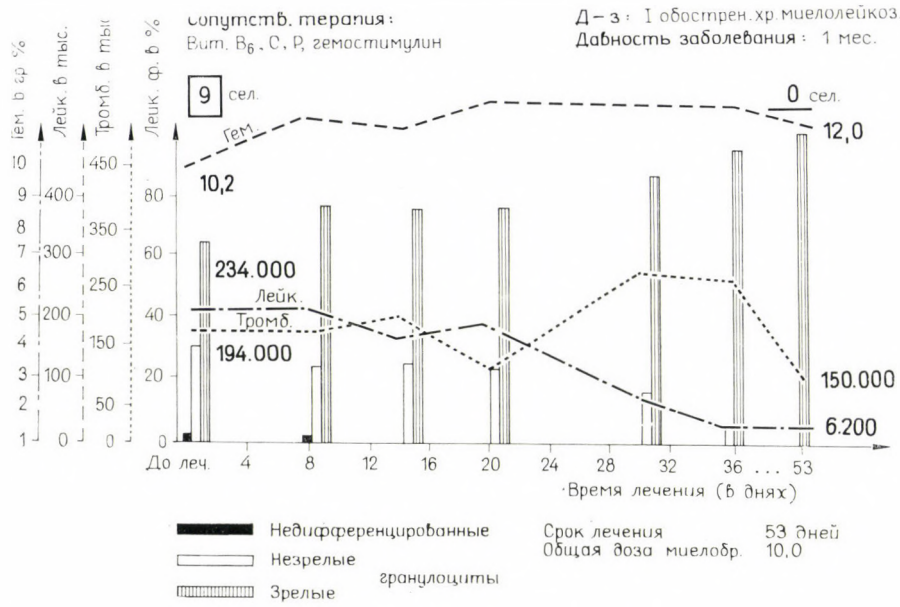


Рис. 2. Периферическая кровь больной К-н Н. Е., 58 лет, 1 группа

ных двух больных почти не наблюдалось никаких сдвигов в сторону улучшения; миелобромол им был отменен в связи с развитием головных болей, тошнот, снижением аппетита.

Трое больных из этой группы, наряду с лечением миелобромолом, получали единичные гемотрансфузии.

У 2 больных к концу лечения наступило снижение числа тромбоцитов. Одному из них за месяц до поступления проводилось лечение допаном в чередовании с миелосаном, но тогда проявлений геморрагического диатеза у этого больного отмечено не было. У другого больного довольно выраженная тромбоцитопения была еще до начала лечения миелобромолом (62 000), которая наступила после почти непрерывного применения миелосана (на протяжении двух с половиной лет). При развитии полной клинической и гематологической ремиссии под влиянием лечения миелобромолом, число тромбоцитов оставалось на том же уровне (64 000—62 000).

Через неделю после окончания лечения миелобромолом число тромбоцитов снизилось и при подсчете обнаружить их не удавалось, развились явления геморрагического диатеза, а через 3 недели больной умер от массивных геморрагий.

На рис. 3 представлено фото-иммерсия стернального пунктата этого больного (М — в 18 лет). Миелокариоциты до лечения — 446 000, после

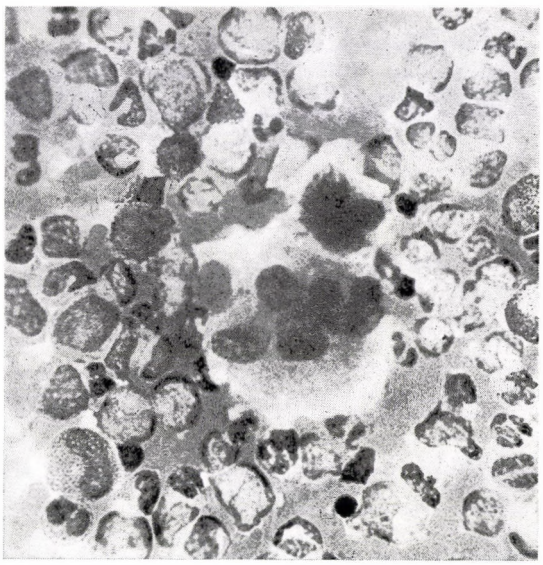


Рис. 3. Фото-имм. стернального пунктата б-го М-ба 28 лет. До лечения

лечения — 114 000, мегакариоциты до лечения 22,2 в мм^3, после лечения – не были найдены. Данные гистологического исследования, произведенные после лечения, свидетельствовали о наличии в костном мозгу очагов гипоплазии, с сохранением мегакариоцитов. Полной аплазии кроветворения отмечано не было (рис. 4).

К 3 группе мы отнесли 16 больных, у 13 больных хронический миелолейкоз протекал с вторичным миелофиброзом, у 3 больных имел место исход в ретикулоз. У одного из них отмечался вторичный миелофиброз, развившийся еще в начальной стадии заболевания. У больных этой группы обострение протекало с гемоцитобластным кризом. Возраст больных был от 18 до 61 года. Давность заболевания — от 2 до 8 лет. Всем этим больным ранее применялось длительное цитостатическое лечение и была выявлена резистентность к миелосану. Срок лечения у этой группы больных был от 1 до 3 месяцев (в среднем 42,5 дня). Общая доза миелобромола на курс лечения была — от 4 до 11,5 г (в среднем 6,79).

Все больные 3 группы получали миелобромол в сочетании с гемотрансфузиями, гормональной терапией (кортикостероидами и андрогенами) и 3 с антибиотиками. В результате проведенного лечения у всех больных, за исключением трех, наступало выраженное улучшение. У одной больной через 6 дней после применения миелобромола полностью нормализовалась температура, у другого — через 10 дней после начала лечения миелобромолом прекратились упорные головные боли, которые расценивались как

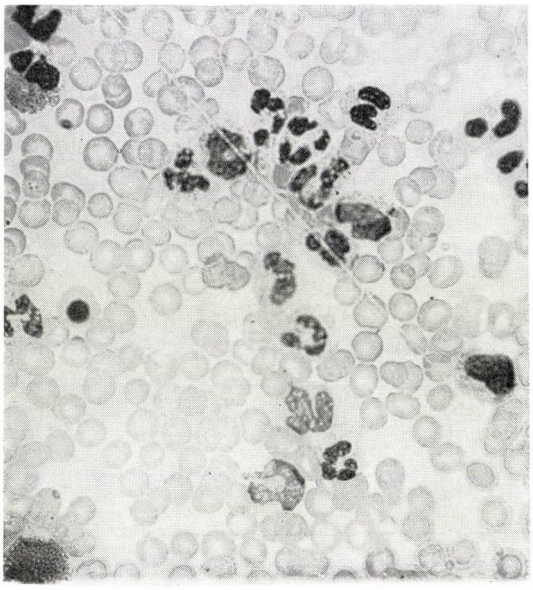


Рис. 4. Фото-имм. стернальнаго пунктата б-го М-ва 18 лет после лечения

результат лейкозной инфильтрации ствола мозга. У всех больных восстановились силы, заметно уменьшились размеры печени и селезенки. Например, размеры селезенки по Курлову уменьшались с 34 42/31 до 14 22/14, с 21 27/13 до 9 25/12, с 12 27/20 до 6 16/15 (рис. 5).

К концу лечения у больных повышались показатели гемоглобина и эритроцитов, хотя полной их нормализации не наступало. Почти у всех больных число лейкоцитов к концу лечения снижалось до нормального уровня и у двух больных с исходом в ретикулоз снизилось до выраженной лейкопении (2500 и 1800). У одной больной (Р-ой 25 лет) с исходом в ретикулоз, под влиянием лечения миелобромолом исчезли лимфатические узлы, селезенка стала пальпироваться у реберного края, в то время как до лечения она выступала на 9 см от края реберной дуги.

Для иллюстрации на рис. 7 представлено фото-иммерсия стернального пунктата этой больной. Лечение миелобромолом ей проводилось на

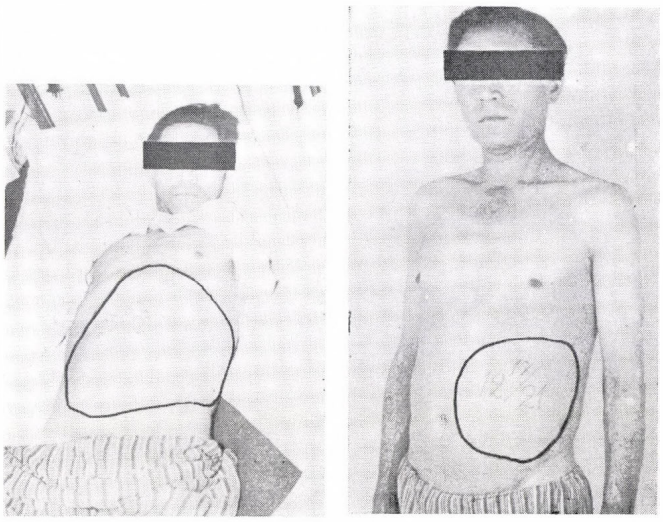


Рис. 5. Больной Л-ов. Размер селезенки до и после лечения. 3 группа

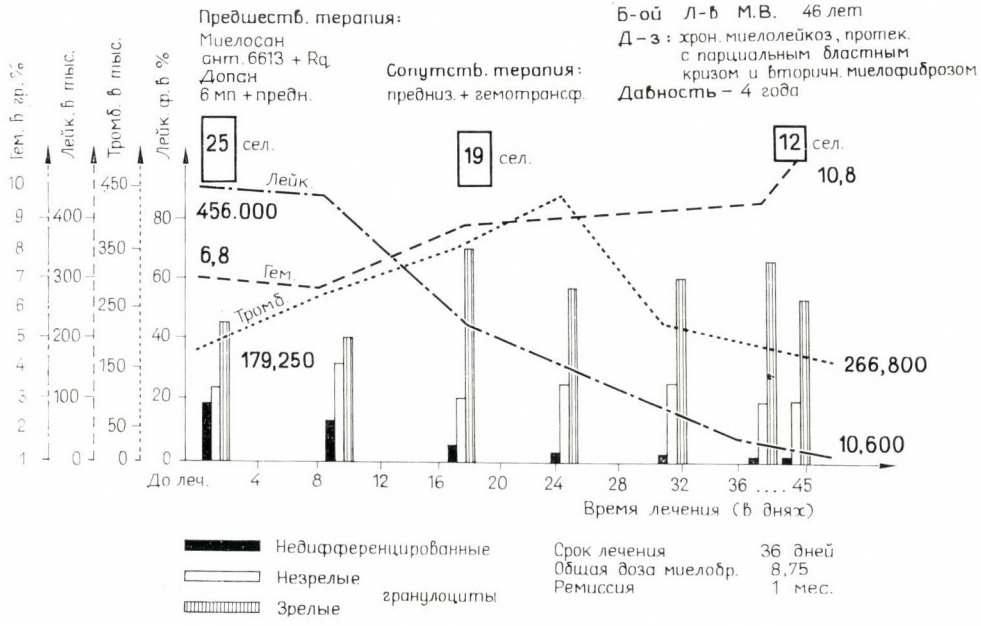


Рис. 6. Периферическая кровь б-го Л-ва 3 гр.

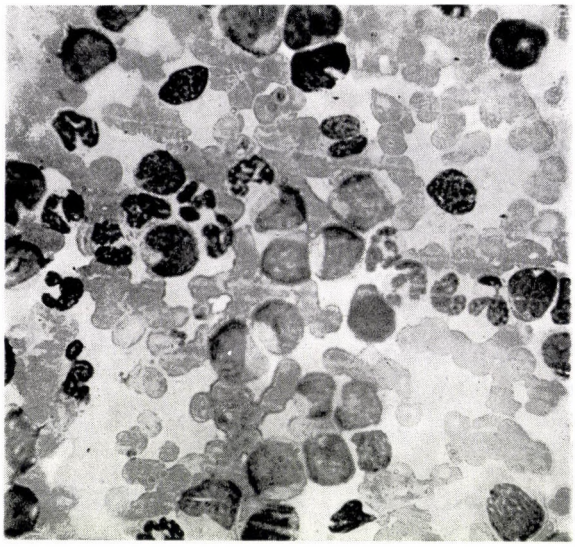


Рис. 7. Фото-имм. стернального пунктата б-ой Р-ой 25 лет. До лечения

протяжении 10 дней в общей дозе 4,0 (в течение 6 дней больная получала по 500 мг pro die). В результате лечения наступило выраженное опустошение костного мозга (рис. 8). На фото-иммерсия показано, что после лечения

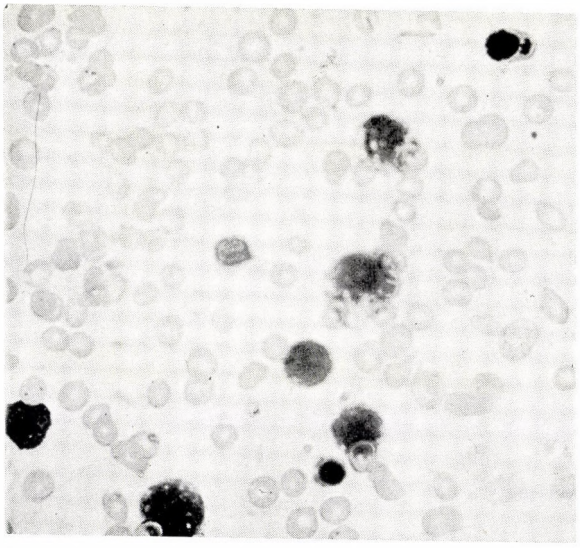


Рис. 8. Фото-имм. стернального пунктата б-ой Р-ой 25 лет. После лечения

имеются лишь клетки лизиса и эритронормобласты, в то время как до лечения, костный мозг был клеточный и был представлен, в основном, недифференцированными клетками и незрелыми гранулоцитами.

Под влиянием лечения у больных 3 группы значительно снизилось число недифференцированных клеток в периферической крови, а у 4 больных полностью исчезли из периферической крови. У одной больной с выражен-



Рис. 9. Фото трепанобиопсии б-ой Ч-ко 18 лет. До лечения

ным гемоцитобластным кризом число гемоцитобластов под влиянием лечения снизилось лишь с 62% до 39%, а через 2 недели снова наступило почти тотальное бластное обострение.

У других больных этой группы наступило значительное созревание клеток гранулоцитарного ряда. Число зрелых гранулоцитов достигало 55—73%.

У двух больных с вторичным миелофиброзом под влиянием лечения развилась умеренная тромбоцитопения и у одной больной — лейкопения. Применение миелобромола проводилось у этих больных с большой осторожностью в дозе не более 125 мг pro die, иногда не каждый день.

На рис. 9, 10 представлены фото трепанобиопсии больной Ч-ко 18 лет, у которой хронический миелолейкоз протекал с вторичным миелофиброзом. На фото, произведенном до лечения, показано, что в костном мозгу содержатся все клетки характерные для обострения хронического миелолейкоза.

В трепанате наблюдаются более массивные балки, чем это имеет место при обычном миелолейкозе. В костно-мозговых полостях наряду с элементами лейкозной инфильтрации, наблюдается развитие фиброретикулярной ткани, вытесняющей на некоторых участках кроветворные элементы костного мозга. После лечения у этой же больной отмечается почти полное уничтожение кроветворных элементов, костно-мозговые полости заполнены



Рис. 10. Фото трепанобиопсии б-ой Ч-ко 18 лет. После лечения

жировыми клетками различной величины и очень грубой ретикулярной стромой.

У двух больных с исходом в ретикулоз, имевшаяся до лечения довольно выраженная тромбоцитопения оставалась на том же уровне. У этих двух больных ремиссия продолжалась 2—3 недели, затем наступило обострение заболевания, протекавшее с почти тотальным бластным кризом. Один из этих больных погиб от присоединившейся двухсторонней пневмонии, другая больная — от кровоизлияния в головной мозг. Третий больной с исходом в ретикулоз, у которого на раннем этапе был выявлен сопутствующий миелофиброз, лечился миелобромолом в течение одного месяца по 250 мг pro die, общей дозой 8 600 мг, оказался резистентным к применяемому лечению и ремиссии не наступило.

Таким образом лечение миелобромолом проводилось нами в дозе не превышающей 250 мг в сутки. Двум больным пытались назначить суточную дозу до 500 мг на протяжении одной недели, но это способствовало

лишь быстрому снижению числа лейкоцитов и не оказывало влияния на скорость развития ремиссии.

При снижении числа лейкоцитов до сублейкемических цифр доза миелобромола снижалась до 250—125 мг через день, а при дальнейшем быстром их снижении больной переводился на лечение миелобромолом в поддерживающей дозе по 125 мг 1—2 раза в неделю.

При уменьшении числа лейкоцитов до нормальных цифр и тенденции к тромбоцитопении, при развитии хотя и неполной ремиссии, лечение миелобромолом заканчивалось.

Ремиссия, полученная при лечении миелобромолом была нестойкой и продолжалась на протяжении 2—3 недель. У больных, которые лечились цитостатическим средством впервые — была 1 1/2 — 4 месяца без поддерживающей терапии. 11 больных после проведенного курса лечения получали миелобромол в поддерживающих дозах по 125 мг 1—2 раза в неделю, при повышении числа лейкоцитов лечение миелобромолом на протяжении 1—2 недель проводилось чаще — ежедневно или через день по 125 мг. У 7 больных несмотря на такую тактику поддерживающего лечения развивалось бурное обострение заболевания через 4—8 месяцев.

У 4 больных при условии проведения поддерживающего лечения миелобромолом ремиссия продолжается от 3 до 9 месяцев.

Мы проводили лечение миелобромолом около двух лет. За этот период больным применялись повторные курсы лечения миелобромолом, который иногда оказывал меньший лечебный эффект или обнаруживалась резистентность к миелобромолу.

При проведении некоторых «функциональных проб» печени до и после лечения (билирубин, белки крови, формоловая и тимоловая пробы, холестерин), нам не удалось выявить какого-либо неблагоприятного влияния миелобромола.

С целью оценки влияния миелобромола на кроветворение были произведены исследования пунктата костного мозга у 20 больных и у 22 больных трепанобиопсия — до и после лечения. В результате лечения наступало заметное уменьшение числа недифференцированных клеток до полной их нормализации, значительное снижение клеток гранулоцитарного ряда, главным образом, за счет незрелых форм, с полной сохранностью клеток красного ряда, и заметным уменьшением числа мегакариоцитов (рис. 11, 12).

Для примера приводим рис. 13, на котором представлены диаграммы больных с давностью заболевания 2 1/2 года — 6 лет. Обострение миелолейкоза протекало у больных с вторичным миелофиброзом. После лечения миелобромолом наряду со снижением числа недифференцированных клеток, незрелых гранулоцитов, метамиелоцитов, значительно увеличилось число клеток красного ряда и лимфоцитов.

Для установления диагноза хронического миелолейкозе трепанобиопсия не имеет преимуществ перед пункцией грудины. Однако данные трепа-

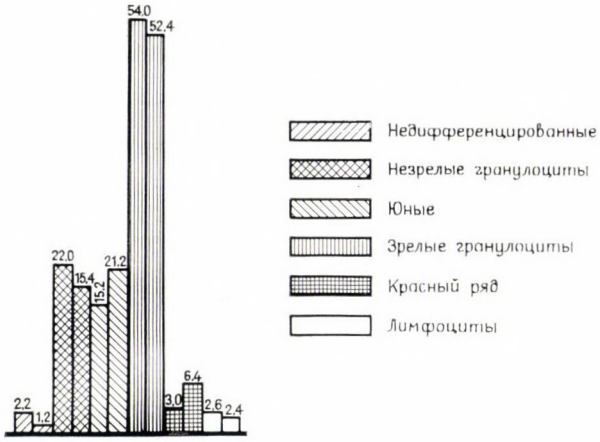


Рис. 11. Диаграмма миелограммы б-го К-ан 1 группы до и после лечения

нобиопсии необходимы для выявления особенностей течения хронического миелолейкоза, развития миелофиброза или трансформации хронического миелолейкоза в ретикулоз и гемоцитобластоз. Кроме того трепанобиопсия позволяет установить возможное развитие аплазии кроветворения.

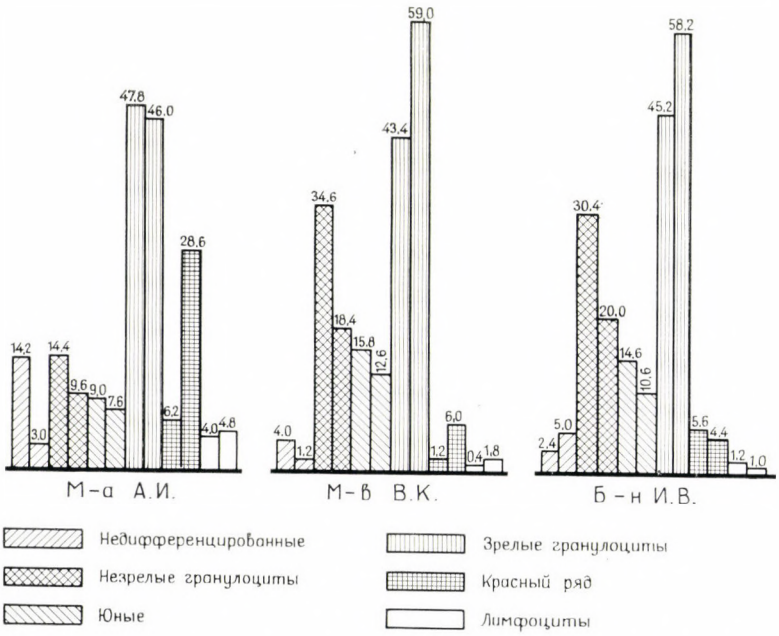


Рис. 12. Диаграммы миелограмм больных 2 группы до и после лечения

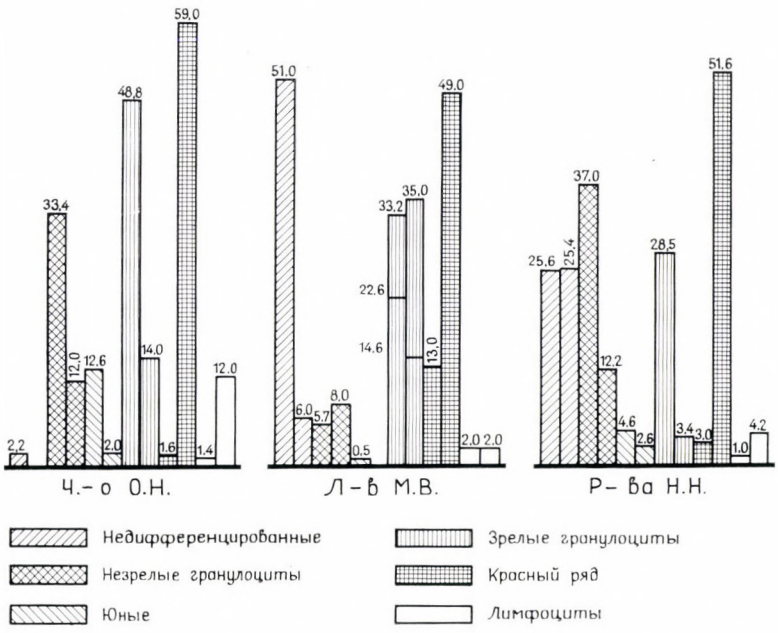


Рис. 13. Диаграммы миелограмм больных 3 группы до и после лечения

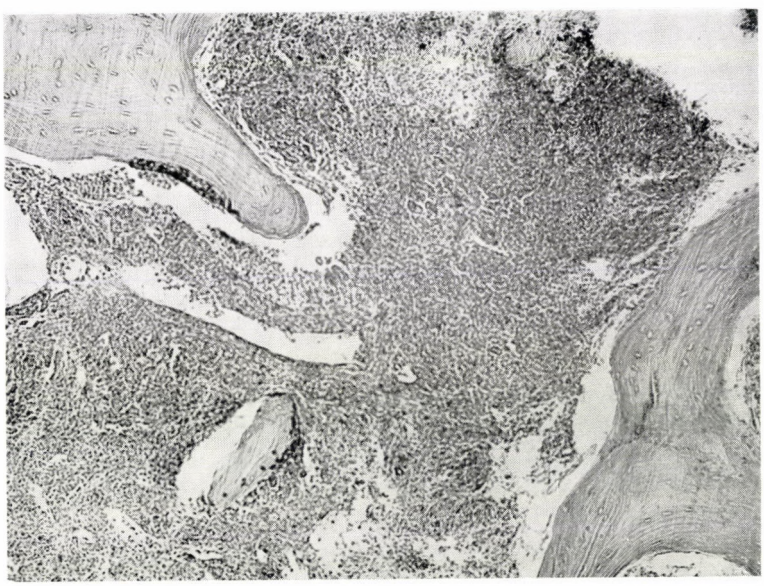


Рис. 14. Фото трепанобиопсии б-го К-ан 1 группы. До лечения

Для примера приводим фото трепанобиопсии больного К-н (рис. 14). До лечения видна масса клеточного состава с преобладанием незрелых гранулоцитов, с полным отсутствием жирового недеятельного костного мозга; после лечения (рис. 15) масса деятельного костного мозга значительно уменьшилась, появилось большое количество жировых клеток и зрелых гранулоцитов. Клиническая ремиссия у этого больного продолжалась 4 месяца без применения поддерживающей терапии.

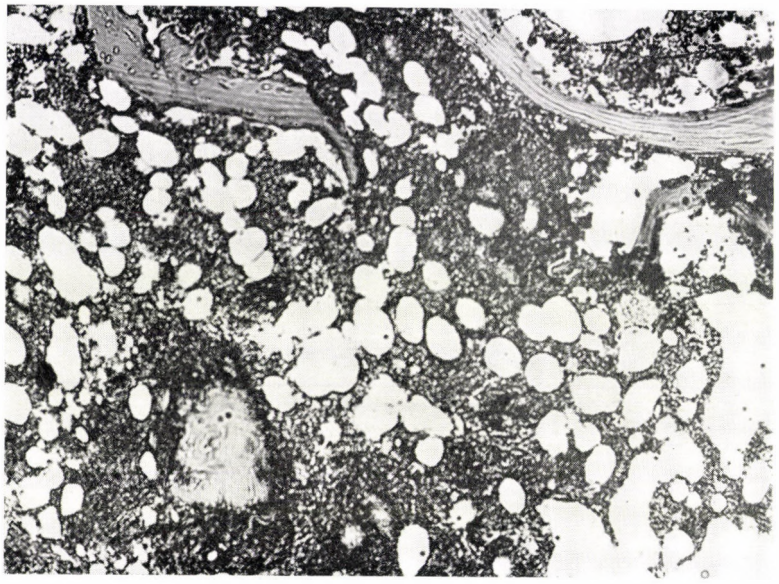


Рис. 15. Фото трепанобиопсии б-го К-ан 1 группы. После лечения

На рис. 16, 17 представлены фото трепанобиопсии больной С, до и после лечения. Костный мозг продолжал оставаться богатым клеточными элементами, однако клеточный состав их изменился в сторону увеличения зрелых форм. Эта больная получила 11,0 миелобромола и ремиссия длилась у нее 2 месяца. Поддерживающее лечение проводилось не систематически.

На рис. 18, 19 представлены фото трепанобипсии костного мозга больного Б. До лечения костно-мозговые полости густо заполнены клеточными элементами; после лечения миелобромолом масса клеточных элементов значительно уменьшена, они располагаются очагово и число жировых клеток ничтожно. Эти последние 2 фото показали, что не у всех больных под влиянием миелобромола проявляется тенденция к развитию гипоплазии кроветворения, костный мозг после лечения продолжал оставаться богатым, но клеточный состав их изменялся в сторону увеличения зрелых форм.

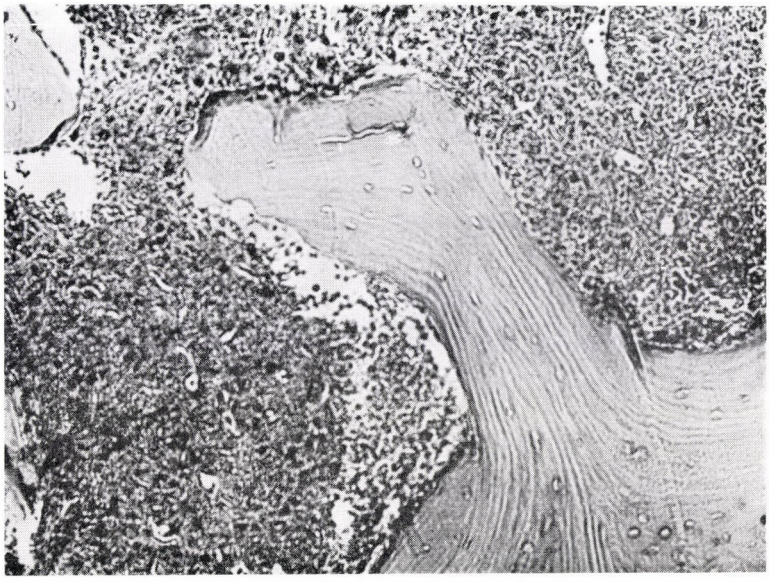


Рис. 16. Фото трепанобиопсии б-ой С. До лечения

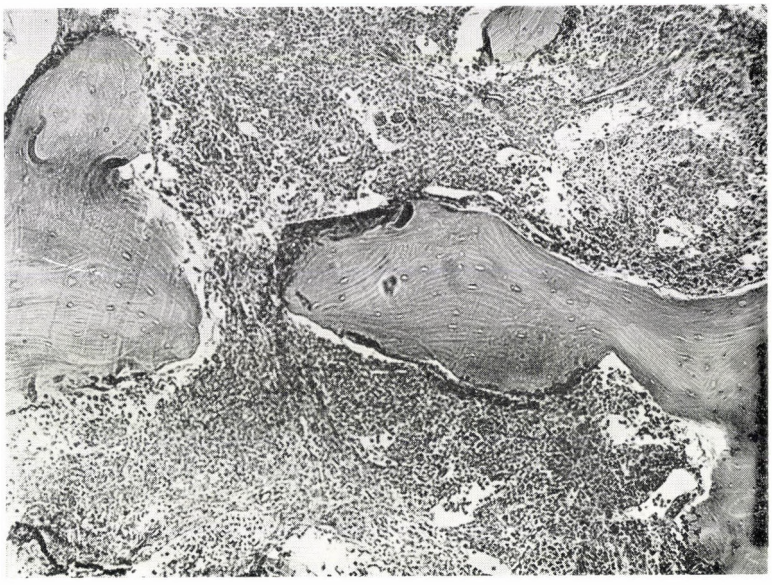


Рис. 17. Фото трепанобиопсии б-ой С. После лечения

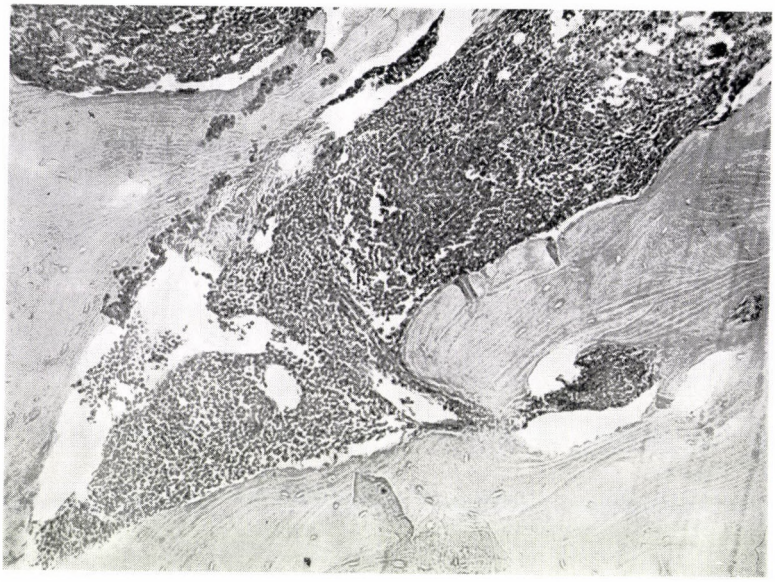


Рис. 18. Фото трепанобиопсии б-го Б-на. До лечения

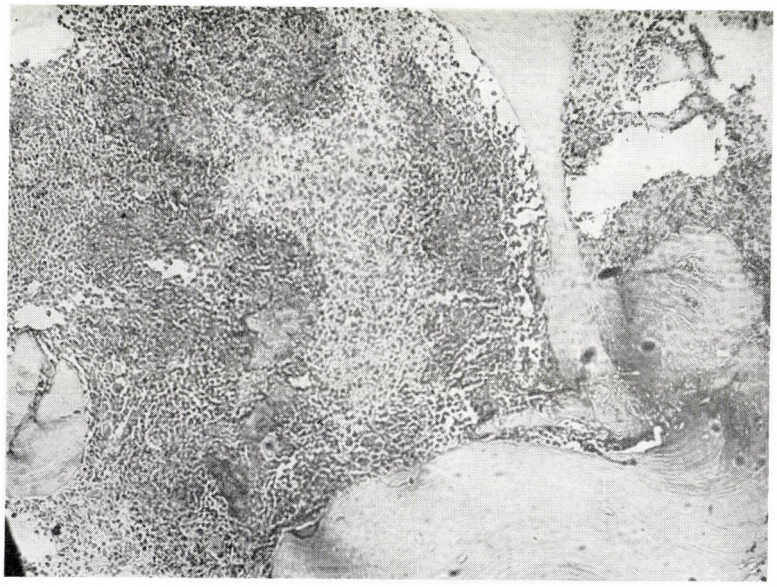


Рис. 19. Фото трепанобиопсии б-го Б-на. После лечения

В результате лечения миелобромолом в костном мозгу наступало заметное уменьшение числа недифференцированных клеток гранулоцитарного ряда, главным образом, за счет незрелых форм, с полной сохранностью клеток красного ряда, и заметным уменьшением числа мегакариоцитов.

У некоторых больных хроническим миелолейкозом, протекающего с очагами миелофиброза, после лечения миелобромолом наблюдалось появление большого количества жировых клеток, значительное обеднение кроветворными элементами.

Литература

1. Шеллеи, К., Экхарт, С.: Клиническое изучение 1,6 дибромманнитола. В кн.: Международный противораковый конгресс, т. 6, стр. 202, 1962.
2. Петраньи, Д.: Отчет о клиническом испытании дидезоксибромманните (ДБМ). Из II терапевтической клиники Дебреценского медицинского института (рукопись).
3. Csomár, G.: Gyógyszereink (1965) 15, 261.
4. Máthé, G., Schneider, M., Cattan., A., Schwarzenberg, L., Amiel, J. L.: Presse méd. *72*, 2135 (1964).

Clinical experience with Myelobromol in the treatment of chronic myeloleukosis

T. A. Cherntsova, V. M. Susoyeva

Thirty four patients were treated with Myelobromol out of whom six had received no previous cytostatic treatment and 12 who had been treated earlier with some cytostatic agent and had become resistant to such treatment. Thirteen patients suffered of myelofibrosis, three of reticulosis. Myelobromol was found to be a potent rapidly acting cytostatic agent with an elective action on granulopoiesis with a slighter action on thrombopoiesis. The drug had no inhibiting effect on erythro- and lymphopoiesis. A complete remission was achieved by the first serial treatment of myeloleukosis, but only partial remissions were observed in cases of myelofibrosis. Patients suffering of the latter disease were resistant to both Myleran and other cytostatic agents. The daily dose of Myeolobromol is 125 to 250 mg. With long term maintenance doses (125 mg once or twice weekly) nine months or longer remissions may be achieved.

Changes in the Myelogram in Panencephalitis nodosa (Subacute Sclerosing Leucoencephalitis)

Ágnes PÉTER, Éva SZATMÁRI, J. ROMHÁNYI, and Klára LETENYEI

Department of Neurology, Department of Medicine No. 1
and Department of Paediatrics No. 1, University Medical School, Budapest

(Received October 24, 1966)

The cytology of the bone marrow has been studied in 18 cases of subacute sclerosing leucoencephalitis (panencephalitis nodosa). In 61 per cent of the cases the lymphoid reticular cells, in 50 per cent the eosinophilic cells and in 33 per cent the plasma cells were increased in number. Four patients had eosinophilia. In one case of cerebral cysticercosis the myelogram showed similarly an increase of reticulum and eosinophilic cells, and in one patient suffering from polymyositis the plasma cell and lymphoid reticular cell counts were increased in the bone marrow smear. From the results it is concluded that the pathologic changes in the myelogram of patients with subacute sclerosing leucoencephalitis (panencephalitis nodosa) confirm the view that the immunologic processes are different in multiple sclerosis and in panencephalitis nodosa. The myelograms found in panencephalitis nodosa indicate that in the pathomechanism of this disease allergic components, too, may play a significant role beside the immune processes. In panencephalitis nodosa the relation between the humoral and cellular defensive activities of the RES is rather peculiar: the humoral antibodies increase in the cerebrospinal fluid, while the cellular reaction is manifest in the nervous tissue and bone marrow, in the first place.

In recent years increased interest was devoted to the analysis of the laboratory changes accompanying panencephalitis nodosa (in the following: PN, which includes also the conditions called subacute sclerosing leucoencephalitis in the French and Anglo-Saxon literature). One of the reasons of this is that PN occupies a peculiar position among the inflammatory diseases of the nervous system as far as the defensive activity of RES is concerned.

An increase of gamma globulins in the cerebrospinal fluid usually indicates intense immunological processes, a presence of antibodies. The highest gamma globulin concentration in the cerebrospinal fluid is found in PN. Gamma globulinorrachia is characteristic also for multiple sclerosis (in the following: MS) and for neurolues.

Now that Bauer and Heitmann [1], Matiar and Schmidt [12], Cervos-Navarro and Matiar [3], Frick [5] and others have published their results, it is accepted that gamma globulins are produced locally intrathecally. It seems likely that gamma globulinorrachia is correlated with the activity of the leptomeningeal plasma cells (Cervos-Navarro and Matiar [3], Schmidt [16], Frick [5], Péter [13]). In MS and neurolues, however, the increase of gamma globulin concentration in the cerebrospinal fluid is accompanied by a lympho-plasmacellular reaction,

and owing to the morphological changes indicative of plasma cell activity Sayk and Schmidt [14], as well as Bischoff [2] go as far as to write about a plasmocytic "Reiz" syndrome in such cases. The cytology of the cerebrospinal fluid in PN is characterized by oligocytosis, by a preponderance of monocytic cell forms, whereas plasma cells occur infrequently and even then without immunoactive properties (Kolár [8], Péter [13]).

Beside the data of the cytogram of the cerebrospinal fluid, a difference in immunological processes is reflected also by an analysis of the protein spectrum of the cerebrospinal fluid. In MS and neurolues it is mainly the fast-migrating fractions of gamma globulins that occur at highest concentrations in the cerebrospinal fluid (Loewenthal et al. [10], Dencker and Kolár [4]), while in PN the slow gamma globulin fractions preponderate [11].

Few attempts have been made to assess the condition of the extraneural RES in these diseases. Schmidt found in 30 cases of MS an average of 13 per cent plasma cells and 16 per cent lymphoid reticulum cells in the bone marrow punctate, and 7 per cent of reticulum cells in the blood count of 50 patients with the same disease [15]. According to Schmidt, the increase of reticulum cells in blood and bone

Table 1

No.	Name	Sex	Diagnosis	Cerebrospinal fluid	
				Gamma globulin rel. %	Number of gamma globulin fractions
1.	Zs. Zs.	F	Panencephalitis nodosa	20.3*	4
2.	H. M.	F	,, ,,	43.4*	5
3.	N. K.	F	,, ,,	46.6*	5
4.	D. L.	M	,, ,,	37.6*	6
5.	F. A.	M	,, ,,	46.4*	5
6.	C. E.	F	,, ,,	34.7*	6
7.	F. T.	M	,, ,,	—	—
8.	B. A.	M	,, ,,	45.2*	5
9.	H. I.	M	,, ,,	29.3*	5
10.	Sz. I.	M	,, ,,	—	—
11.	M. Zs.	F	,, ,,	32.8*	5
12.	K. I.	M	,, ,,	—	—
13.	Sz. M.	M	,, ,,	44.1*	6
14.	K. T.	M	,, ,,	29.0	—
15.	V. J.	M	,, ,,	10.4	—
16.	T. J.	M	,, ,,	22.8	—
17.	H. K.	F	,, ,,	77.6	—
18.	M. P.	M	,, ,,	23.0	—
19.	Sz. A.	M	Cerebral cysticercosis	50.6*	6
20.	B. J.	F	Polymyositis	18.9*	1

* marks the results obtained by agar electrophoresis, otherwise paper electrophoresis was used.

marrow indicates that the RES as a whole participates in the reaction to an organ-specific agent.

There is evidence indicating that extraneural non-specific changes occur also in PN. Kolár [7] has demonstrated an increase of acid phosphatase in the sera from 12 PN patients and that of mucoprotein in a few of them, parallel with the progression of the clinical disease. Remission was accompanied by a decrease. Kolár ascribes these changes to enzymopathy and to a non-specific reaction of connective tissue, respectively.

An increase of extraneural RES activity may also be surmised to occur in PN. By immunoelectrophoresis, Kolár [9] demonstrated pathologic changes in the gamma G globulins. By agar electrophoresis, Loewenthal [11] showed that in 10 out of 11 cases of PN the serum gamma globulins separated into 2 to 6 subfractions.

In order further to analyse the immune processes in PN, we have studied the bone marrow smears and the blood count of our patients. To assess the activity of RES of the NS, the results of the bone marrow studies were analysed in regard to the cytogram of the cerebrospinal fluid, the quantity and number of

Serum Number of gamma globulin fractions	Cerebrospinal fluid		Blood Eosinophils %	Sternum punctate		
	Cell count	Plasma cells %		Plasma cells %	Eosinophils %	Lymphoid reticulum cells %
1	7/3	0	2	1.2	7.0	5.3
2	5/3	2	2	0.5	5.0	2.7
6	16/3	0	2	0.4	8.0	1.0
2	9/3	1	8	0.5	6.5	8.0
—	24/3	2	2	1.2	8.0	1.8
6	1/3	0	14	5.2	17.8	4.1
—	3/3	0	—	0.8	18.8	1.7
—	0/3	0	0	0.0	6.0	0.8
2	13/3	0	10	2.4	22.4	3.6
—	3/3	0	4	5.5	10.2	12.0
4	8/3	0	1	1.6	2.3	0.6
—	5/3	0	0	4.8	3.6	14.0
5	0/3	0	0	3.4	4.0	18.4
—	2/3	0	11	4.4	11.2	1.6
—	15/3	0	—	1.0	17.2	8.8
—	2/3	0	—	5.2	5.2	10.4
—	4/3	0	—	1.8	18.0	2.0
—	1/3	0	5	0.4	5.2	2.8
—	380/3	52% eo. 9%	6	1.0	11.5	5.2
1	740/3	0	2	5.6	7.2	0.4

fractions of serum gamma globulin, the quantity and grade of fractionation of the gamma globulin of the cerebrospinal fluid.

The bone marrow smear and the blood count were analysed in 18 cases of PN, 12 males and 6 females, aged 7 to 15 years. The results are presented in Table 1, together with the data for a male adult patient suffering from cerebral cysticercosis and for one female adult patient with polymyositis, to facilitate comparison.

Two-thirds of our PN patients showed an increase of protein in cerebrospinal fluid (60 to 240 mg per cent).

In the majority of our cases the gamma globulin concentration was significantly increased in the cerebrospinal fluid. Relative per cent values of 20 to 30 were found in 6 cases, 30 to 40 in 3 and over 40 relative per cent in 6. The CSF from 3 patients was not subjected to electrophoresis. In 9 cases out of 10 the CSF showed 5 to 6 gamma globulin subfractions by agar electrophoresis, carried out at the Laboratory of the Bunge Institute, Antwerp, by Dr. Loewenthal.

Sera from 8 patients were studied by agar electrophoresis. With the exception of one case, the gamma globulin fraction was separated into 2 to 6 subfractions. The serum gamma globulin level was not increased in any of our PN cases.

In the majority of our PN cases qualitative analysis of the CSF protein was indicative of an intense neural RES activity, while that of the serum proteins suggested extraneural RES activity.

The cell count in the cerebrospinal fluid was increased insignificantly in only four of our PN cases. Plasma cells could be demonstrated in the CSF in only 3 patients, amounting to 1 to 2 per cent, i.e. they were present in insignificant quantity.

In the blood marrow smears of our PN cases the reticulum cells and eosinophils were increased in number, with a less marked increase of the plasma cell count. On the basis of the data in the literature (Wintrobe [17], Kelemen [6]) in the bone marrow the plasma and lymphoid reticulum cell count is considered to be normal up to 2 per cent, and the eosinophilic granulocyte count up to 7 per cent.

Table 2

	Under 2% (normal)	2 to 5%	5 to 10%	10 to 15%	Over 15%
Plasma cells	11	4	3		
Lymphoid reticulum cells	7	7	3	3	1
Eosinophils:	under 7% (normal)		7 to 10%		
	9		2	2	5

The data for the percentage incidence of various cell types in the sternum punctate in 18 cases of PN are presented in Table 2.

In the bone marrow smear an increase of lymphoid reticulum cells was demonstrated in 11 patients (61 per cent of our cases). The eosinophil count exceeded the normal level in the bone marrow smears from 9 patients (50 per cent). In one case the eosinophilic cells amounted to 22.4 per cent. Eosinophilia was demonstrated in the peripheral blood in 4 cases, including 3, in which the eosinophil count was significantly increased in the bone marrow, too. An increase of plasma cells in the sternum punctate was demonstrated in 6 patients (33 per cent of the cases); values over 5 per cent were found in 3 cases, with the highest 5.5 per cent.

In 3 patients a simultaneous increase of the plasma cell, eosinophilic cell and lymphoid reticulum cell counts could be demonstrated in the bone marrow. In patient C. E. the increases of the three cell forms were rather significant proportionately, with a 14 per cent eosinophilia in the peripheral blood count. In patient H. I. the eosinophilic cells showed the highest increase, with 10 per cent eosinophils in the peripheral blood. On the other hand, in patient Sz. I. the plasma and reticulum cell counts increased more markedly.

As far as the increase of eosinophilic cells is concerned, patient K. T. merits special attention. In this case we found 11.2 per cent eosinophils in the bone marrow and 11 per cent in the blood, together with a 4.4 per cent plasmocytosis in the bone marrow. Many members of the family of K. T. suffered from allergic conditions.

The meningograms of our PN patients have been compared with the bone marrow findings of 4 patients suffering from neurological diseases other than PN. Patient Sz. A. had cerebral cysticercosis, thus, allergic mechanisms had to be taken into consideration in the first place. Patient B. J. had polymyositis, which, as an autoimmune process, evoked the reaction of the RES. The bone marrow changes in our PN cases showed features similar to those found in allergic reactions of the RES.

In patient Sz. A. an increase of eosinophilic and reticulum cells could be demonstrated in the bone marrow, with a significant eosinophilia also in the CSF (9 per cent). In the case of polymyositis we found only plasmocytosis in the bone marrow.

The changes in the myelograms of our PN patients may indicate that *i)* in the genesis of PN allergic mechanisms, too, may play a significant role, alongside the immune processes. Kolár, too, speaks about neuroallergic and immunoalterative properties in PN. *ii)* In PN, there is an extremely peculiar relationship between the humoral and cellular defensive activities of RES. Gamma globulin is significantly increased in the cerebrospinal fluid; the cellular reaction is marked in nervous tissue and bone marrow, but not in the cerebrospinal fluid. 3. The immunopathological processes involved in PN are significantly different from those of MS. *a)* In MS, Schmidt [15] found in the bone marrow plasmocytosis and reticulocytosis, while we found in PN reticulocytosis and eosinophilia in the bone marrow.

b) The gamma globulin subfractions and the immunoglobulins predominating in the CSF in PN are different in properties from those found in MS (Dencker and Kolár [4], Loewenthal [11]). *c)* In MS there is a parallellism between the plasmocytosis and increase of gamma globulin in cerebrospinal fluid (Schmidt [16]), while in PN this correlation is absent (Kolár [8], Péter [13]).

References

1. Bauer, H., Heitmann, R.: Chemische und serologische Untersuchungen bei der multiplen Sklerose. *Dtsch. Zschr. f. Nervenheilk. 178*, 47 (1958).
2. Bischoff, A.: Das Vorkommen von Plasmazellen im Liquor cerebrospinalis bei der multiplen Sklerose. *Dtsch. Zschr. f. Nervenheilk. 185*, 606 (1964).
3. Cervos-Navarro, J., Matiar, H.: Zur Frage der intrathekalen Regulation und Genese der Liquorproteine. *Dtsch. Zschr. f. Nervenheilk. 179*, 614 (1959).
4. Dencker, J. S., Kolár, O.: The cerebrospinal fluid gamma globulin profile in subacute sclerosing leucoencephalitis. *Acta Neurol. Scand. 41*, 135 (1965).
5. Frick, R.: Immunbiologische Untersuchungen über die intrathekale Gammaglobulinbildung bei der allergischen Encephalomyelitiden. *Zschr. f. ges. exp. Med. 138*, 408 (1964).
6. Kelemen, E.: A klinikai haematológia alapvonalai. Medicina, Budapest, 1961.
7. Kolár, O., Obrucnik, M., Farkova, H.: Problematik der extraneuralen biochemischen Äusserungen bei subakuten Encephalitis von Dawson—Pette—Döring—van Boagert. *Der Nervenarzt 36*, 391 (1965).
8. Kolar, O., Behounkova, L., Kotrle, M., Kadlec, A.: Zur Frage der immunbiologischen Bedeutung der Liquorzellen im Verlauf der subakuten Encephalitis von Dawson—Pette—Döring—van Bogaert. *Acta Univ. Palack.* Olomuc *38*, 125 (1965).
9. Kolár, O., Dencker, S. J., Obrucnik, M., Gerna, I., Skatula, Z.: Zur Bedeutung der Gamma G-Globulininfektion im Hirngewebe bei der subakuten sklerotisierenden Leukoencephalitis. *Dtsch. Zschr. f. Nervenheilk. 188*, 222 (1966).
10. Loewenthal, A., van Sande, M., Karcher, D.: The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF gammaglobulins. *J. Neurochem. 6*, 51 (1960).
11. Loewenthal, A.: Results of agar electrophoretic studies of materials obtained from patients treated at the Medical University of Budapest. Details see in 13.
12. Matiar, H., Schmidt, C.: Die Erhöhung der Gamma-Globuline im Liquor. *Dtsch. Zschr. f. Nervenheilk. 178*, 300 (1958).
13. Péter, Á.: The plasma cells of the cerebrospinal fluid. In press.
14. Sayk, J., Schmidt, R. M.: Zur Liquordiagnostik bei der multiplen Sklerose. *Ärztl. Wschr. 11*, 788 (1966).
15. Schmidt, R. M.: Über Veränderungen des peripheren Blutbildes und des Sternalmarkes bei der multiplen Sklerose. *Med. Wschr. 10*, 661 (1955).
16. Schmidt, R. M.: Der Liquor cerebrospinalis während der Behandlung der Neurolues. *Dtsch. Med. Journal 16*, 190 (1965).
17. Wintrobe, M. M.: Clinical hematology. Lea and Febiger, Philadelphia. 1961.

Book Reviews

J. D. Gray and G. Discombe: *Clinical Pathology. Clinical Microbiology and the Process of Disease.* Blackwell Sci. Publ. Oxford 1966.

Clinical pathology perhaps is the largest field of medical sciences whose outlines are hard to draw. The concise character of the book including such a vast material, proves that authors dispose of a large-scale practical knowledge and excellent systematizing capacity coupled with an all-round orientation in literature.

The principal aim of this book is to serve as a guide for general practitioners, clinicians and medical students in making best use of the results gained from clinical laboratory investigations. It also bridges the gap between clinical textbooks and books on laboratory methodology, as well as the no-man's land so often separating the way of thinking and views of clinicians from those of laboratory physicians. This book is of special importance in countries like Hungary where the laboratories of hospitals and clinics are headed by laboratory specialists and not by clinical pathologists in the British sense of the word.

The book with its 857 pages is divided into two parts. In part one 26 chapters deal with the problems of clinical microbiology. Chapter one "The microbiologists, the clinician and the patient" gives a clear definition of clinical pathology and the field of work for clinical pathologist as seen with the eyes of well-versed specialists. A series of excellent examples show that the close collaboration of clinicians and laboratory specialists has an enormous bearing on the future of the patient, on the early and correct diagnosis as well as on the adequate treatment of diseases.

Already in chapter one and furthermore in connection with various problems it is most definitely emphasized that clinical pathologists have to devote considerable time and energy to the prevention of diseases, particularly to the prevention of cross-infection within the hospital.

The next chapter deals with the basic problems of infection, inflammation, immunity and hypersensitivity. Chapters three to eight give an account of definitive bacteriology, virology, protozoology and mycology. These chapters give the clinician an insight into the work done in the laboratory without going into technical details. They also stress the practical value of the methods employed, as well as their limitations. Chapters 9 to 16 are arranged according to regional anatomy. The reactions of various organs to microorganisms, the differential diagnosis of the infections affecting various systems of the body are described. The next chapters are devoted to problems of generalized infections, to pyrexia of unknown origin, to chemotherapy and cross-infection. Chapters 20 to 23 deal with the most important methods of sterilization and disinfection, with the principles of artificial immunization and the prevention of accidental infection among hospital staff. The next three short chapters summarize the notifiable diseases, the techniques for housemen and the microbiological assays of the B-vitamins.

The second part of the book is devoted to the process of disease. Chapters 27 and 28 give some general concept about disease

and its activity. The next chapter is an excellent review of iatrogenic disease, drug toxicity and poisoning. Concise chapters inform the reader about the basic principles of genetics, cytogenetics and autosomal disorders. The chapter on inborn errors of metabolism is one of the most fascinating parts of this excellent book. The tremendous lot of up-to-date information concentrated in these 47 pages is most impressive.

The next chapters deal with the deficiency diseases, with the disorders of function of the gastrointestinal tract and its related glands, with the disorders of the endocrine glands, with the water and electrolytes of the body and with the clinical pathology of bone disease. The last 55 pages comprise excellent reviews on plasma proteins, autoimmunity, disorders of the bleeding and clotting mechanisms. There is an interesting chapter on blood groups, haemolytic disease of the newborn, and blood transfusion. The last chapters are devoted to the anaemias, the leukocytes and the leukaemias. The amount of valuable information packed into these chapters is truly amazing.

The highly enjoyable style of the book gives pleasure to the reader. Excellent systematization and the sequence of extremely interesting topics are a guarantee that this work despite of its concise character is very fascinating.

Susan R. Hollán

A. Kalaidjiew, J. Segal: *Die Struktur biologisch aktiver Eiweisse*. Humboldt Universität, Berlin, 1966.

The correlation between structure and biological function is one of the most thoroughly studied problems at present. The tremendous advance in the methods of structure research in the past ten years led to the clarification of the structure of some biologically active proteins. These methods elucidated not only the primary structure (amino acid sequence), but also the molecular conformation and the structures of active and inactive, native and denatured proteins.

Of the results of protein study this book deals firstly with those supporting the so-called "Faltentrommel" protein model claimed to exist by the authors. Thus, the book does not claim to give a complete survey of the problem — as proved by the relatively small number of references.

This is further reflected in the syllabus: after the first three chapters on protein conformation the following three deal with the problem of denaturation. The rest (about one third) of the book discusses the interaction between proteins and ions, structure and enzyme activity and the electron microscopic study of globular proteins.

The correlation between protein structure and biologic activity is discussed mainly from the point of view of the difference between native and denatured states. It touches briefly on the problem of primary structure, but discusses in detail methods for the detection of the presence of diketopiperazine rings. As the presence of diketopiperazine rings in native proteins is today a subject of discussion, it is quite justified that the authors wish to bring up as many arguments as possible in support of their hypothesis. This question of course cannot be settled by indirect experimental data, though the authors believe that this hypothetical structure plays an important role in the development of the "Faltentrommel" structure which they claim is present in globular proteins.

A separate chapter deals with the "Faltentrommel" protein model which has been constructed in the Institute of Biology of the Humboldt University. This theory is based partly on the X-ray crystallographic and electron microscopic studies of haemoglobin, myoglobin and other globular proteins and partly on certain phenomena of denaturation. Thus they represent a view which to some degree differs from the general opinion concerning the helix content and certain functional correlations (e.g. haem-haem interaction in haemoglobin).

Though it does not claim to give a general survey of the present state of the problem, this book nevertheless offers from many points of view very valuable informations. In this respect the very detailed and thorough methodical part dealing with the processes of denaturation and their irreversible character deserves special attention. This part contains the detailed description of some quite simple methods which may be applied to different proteins. The experimental results and conclusions are grouped from the point of view of the "Faltentrommel" model

the authors wish to prove to exist and which as a model reflecting the protein in its dynamic state is undoubtedly highly interesting.

Judith Szelényi

H. Lehmann and R. G. Huntsman: *Man's Haemoglobin.* North-Holland Publishing Company, Amsterdam, 1966.

This is one of the most successful recently published textbooks. It may well serve as an example of how to summarize one of the most interesting branches of research, one which has yielded so many results, in a way that is fascinating to read, gives an extensive survey of the pertaining literature and at the same time serves as a most up-to-date practical guide. This book which truly reflects the present state of haemoglobin research will be indispensable to all working in this field.

The book is didactically constructed from 25 chapters. It deals first with the function, origin and degradation of the prosthetic group, the haem. Problems concerning the storage and transport of iron, the other haem-containing proteins of the organism, such as the cytochromes, myoglobin, catalase, etc. are discussed in a separate chapter.

Another chapter introduces the reader to the fundamental conceptions of protein chemistry which is necessary for the understanding of the structure of the haemoglobin molecule. The understanding of the section dealing with the structure and evolution of normal human haemoglobins is facilitated by a chapter on the genetical and molecular genetical fundamentals. By this thorough biochemical survey covering many details it is then possible to discuss pathologic haemoglobins and other disturbances of haemoglobin synthesis in the light of the latest results of protein research.

In the treatment of haemoglobinopathies the clinical patterns are traced back to biochemical lesions, and this method, which is highly interesting for both the medical practitioner and the biochemist, is one of the chief merits of the book. Not only drepanocytic anaemia, the Hb C, Hb D and Hb E diseases, which have been known for some time, are described in great detail, but the recently discovered unstable haemoglobins and rarer types are also included. Beside the detection and identification of pathological haemoglobins, therapeutic methods and the prognosis of the illness are also dealt with.

The book also devotes considerable space to the haemoglobin M types which are the cause of the disturbed functions of haemoglobin. Several chapters deal with disorders of the regulation of haemoglobin synthesis. The description of the persisting Hb F, Hb Lepore, the haemoglobins containing only a single type of polypeptide chain, such as Hb Bart's and Hb H, is followed by a detailed essay on the thalassaemia syndrome.

Just as the progress of their research had led the authors to geological haematology and population genetics, in the next chapters the correlation between the distribution of haemoglobinopathies and the spreading of malaria and other epidemics, between the probable paths of the migration of people and other historical and geographic observations unfold themselves before the eyes of the reader.

Like in the preceding ones, in the last five chapters the laboratory tests are dealt with in strict logical order. After a brief theoretical survey of the basic methods (electrophoresis and chromatography), the methods for the study of the primary, secondary and tertiary protein structure are described from the establishing of the amino acid sequence to X-ray crystallography.

The book gives details which may excellently be used for the practical laboratory detection and identification of haemoglobins. The problem of differentiation is presented step by step together with the solutions to the problems, such as electrophoresis at different pH-s and in various media, solubility and thermal denaturation tests, detection of intracellular haemoglobin and the fingerprint method.

Every word in the book reflects the fact that the authors are indeed true pioneers of haemoglobin research, especially because they have extended its scope and at the same time traced it back to the molecular level. Their fascinating style, the interesting examples taken from history, the original photos and ingenious illustrations contribute to the appeal and readability of this scientifically outstanding, highly comprehensive work.

Susan R. Hollán

Abstracts

Studies on iron absorption. V. The effect of ascorbic acid and ethyl alcohol on the absorption of iron in iron-deficient subjects. E. W. Sörensen (Medical Department, School of Medicine, University of Bergen, Bergen, Norway), *Acta med. scand. 180*, 241 (1966).

Iron absorption has been studied by means of oral administration of ferrous iron. The iron has been given together with a standard meal with or without addition of alcohol or ascorbic acid to 60 patients with iron-deficiency. Based upon analyses of serum iron, the results show that both alcohol and ascorbic acid significantly increase iron absorption in about the same measure.

I. Árky

Mechanismen der Bildung von Inosinmonophosphat in menschlichen Erythrozyten. I. Eigenschaften der Adenylsäuredesaminase in menschlichen Erythrozyten (Mechanisms of the formation of inosine monophosphate in human erythrocytes. I. Properties of the adenylic acid deaminase in human erythrocytes). H. Banaschak (Institut für Pharmakologie und Toxikologie der Humboldt Universität, Berlin) *Acta biol. med. germ. 16*, 485 (1966).

The properties of an adenylic acid deaminase in human haemolysates and dialyzed haemolysates were investigated. The ATP content of the haemolysates had been destroyed by iodoacetate pretreatment, the ATPase and phosphatase activities were inhibited by EDTA. The adenylic acid deaminase has an optimal activity at pH 7.8 and 37° C. Due to an allosteric effect the enzyme is activated by its own substrate AMP, as well as by ADP and ATP. The enzyme is inhibited competitively by phosphate, further inhibitors are arsenate, pyrophosphate and p-chloromercuribenzoate. The Michaelis-Menten constant in dialyzed haemolysate at pH 7.8 and 37° C is 2.1×10^{-3} Mol AMP/1. The activity of the enzyme in human erythrocytes is estimated at 2.5×10^{-1} Mol AMP/1 of cells/hour.

I. Szász

Über die Beeinflussung des Blut- und Serumcholesterinspiegels durch Röntgenbestrahlung (About influencing blood and serum cholesterol level by X-ray irradiation). M. Morczek, H.-J. Otto, G. Ebert (Strahleninstitut und die Strahlentherapeutische Klinik der Medizinischen Akademie Magdeburg) *Rad. biol. ther. 4*, 495 (1966).

The introduction of the preparation Colsil for prophylaxis and therapy of nervous irritation following X-ray irradiation is based on the assumption that there is a causal connection between a decrease of cholesterol level in the blood following X-ray irradiation and the development of radiation syndrome. It is remarkable how little justification for using the drug is presented. In a study of patients with abdominal and thoracic area irradiation, authors found changes in the blood and serum cholesterol levels, which did not exceed physiological variation. Colsil proved hardly effective in the therapy of nervous irritation after X-ray irradiation; better drugs are available. *I. Árky*

Zur Bedeutung des 2.3-Diphosphoglycerat Zyklus in Erythrozyten. Beziehungen zwischen Methämoglobin- und 2.6-Dichlorphenolindophenol-Reduktion, Glykolyse und 2.3-Diphosphoglyceratbildung (On the importance of 2.3-diphosphoglycerate cycle in erythrocytes. Connexions between methaemoglobin and 2.6-dichlorophenolindophenol-reduction, glycolysis and 2.3-diphosphoglycerate formation). W. Schröter, P. Winter (Universitäts-Kinderklinik, Hamburg—Eppendorf) *Blut 14*, 16 (1966).

The formation of 2.3-diphosphoglycerate (2.3-DPG) without ADP in haemolysates containing more than 95 per cent methaemoglobin is twice as high as in haemolysates with less than 1 per cent methaemoglobin. The formation of 2.3-DPG can be increased by the dye 2.6-dichlorophenolindophenol to a greater extent than by methaemoglobin. Inversely, the reduction of 2.6-dichlorophenolindophenol in haemolysates with high methaemoglobin content can be diminished by 2.3-DPG, and this inhibition is partly abolished by ADP. 2.3-DPG diminishes the reduction of 2.3-dichlorophenolindophenol in the absence of ADP by inhibition of the formation of NADH in the glyceraldehyde-3-phosphate-dehydrogenase reaction. The nonenzymatic reduction of 2.6-dichlorophenolindophenol by NADH is diminished by 2.3-DPG as well. The results are compatible with the hypothesis that the 2.3-DPG has an importance as a NADH-saving by-way of glycolysis under increased requirements for NADH, e.g. in the case of increased methaemoglobin formation.

I. Szász

The use of a simple whole-body counter for haematological investigations. S. T. Callender, L. J. Witts, G. T. Warner, R. Oliver (The Nuffield Department of Clinical Medicine, Radcliffe Infirmary and Department of Radiotherapy, Churchill Hospital, Oxford) *Brit. J. Haemat. 12*, 276 (1966).

A whole-body counting system is used for studying the absorption processes of haematological importance, using isotope-iron and vitamin B_{12}. The counting system is described: two cm. sodium iodide crystals are mounted, one above the other, below a motorized couch. Collimators restrict the field viewed to some 38 cm. along the couch and further lead protection prevents direct background radiation from reaching the detectors. The total weight is about 4.5 tons, so that installation in normal hospital laboratory is possible. 60 patients have been examined, using either ^{59}Fe or ^{58}Co. In 54 patients faecal samples were also collected while in six patients inconvenience or illness prevented collection. Although every effort had been made, faecal collection was incomplete in 16 cases. In the other cases the average ratio of body retention determined by the whole-body counter and by measurements of faecal samples was 1.01 ± 0.12, so that there is no systematic difference in the results. The avoidance of the inconvenience and uncertainty associated with faecal collection is of great value in clinical practice.

I. Szász

Red cell "pseudomosaicism" in congenital methaemoglobinaemia. A. S. Keitt, T. W. Smith, J. H. Jandl (Thorndike Memorial Laboratory and Second and Fourth Harvard Medical Services, Boston City Hospital and Department of Medicine, Harvard Medical School) *New Engl. J. Med. 275*, 397 (1966).

In a patient with congenital methaemoglobinaemia caused by DPNH-like methaemoglobin reductase (diaphorase) deficiency, there was a heterogeneous distribution of methaemoglobin, mainly in older erythrocytes. This resulted from a decrease in the methaemoglobin reducing activity of ageing erythrocytes, without a simultaneous decrease in diaphorase level. In young cells a secondary pathway, depending on GSH and the hexose monophosphate shunt, but not necessarily on TPNH, that is normally of little importance, is sufficiently active to supplement the deficient diaphorase mechanism. After the decrease in the secondary mechanism, the diaphorase deficiency was limited and methaemoglobin accumulated, producing a "pseudomosaic" pattern of distribution among the erythrocytes. Several curious features of this disease (e.g. its self-limited nature, the tendency to achieve equilibrium, the failure of methaemoglobin to cause an appropriate shift in the oxygen-

dissociation curve and the surprisingly low incidence of polycythaemia) appear to be caused by the segregation of methaemoglobin in a minor population.

I. Szász

The preparation of crystalline human erythrocyte glyceraldehyde-3-phosphate dehydrogenase. M. Oguchi, T. Hashimoto, S. Minakami, H. Yoshikawa (Department of Physiological Chemistry and Nutrition, Faculty of Medicine, The University of Tokyo, Tokyo) *J. Biochem.* (Tokyo) *60*, 99 (1966).

Erythrocyte glyceraldehyde-3-phosphate was purified to study the properties of the enzyme. Human erythrocytes were separated from out-dated ACD-blood, washed with isotonic saline and kept in frozen state. After adding 7 volumes of 4 mM Na_3EDTA, solid ammonium sulfate (430 g/l) was cautiously added to the haemolysate and the resulting mixture was filtered. Solid ammonium sulfate was further added to make 90 per cent saturation. The precipitate was dialyzed against 1 mM Na_3EDTA, then diluted with water to 1500 ml, and adjusted to pH 6.2. Further purification was achieved by adsorption to calcium phosphate gel and subsequent elution by 0.1 M $Na_4P_2O_7$, as well as by the removal of a precipitate produced by 65 per cent ammonium sulfate saturation. The enzyme was then precipitated by adding ammonium sulfate to 90 per cent saturation, desalted on Sephadex G-25 column, applied on a DEAE-cellulose column and eluted with a linear gradient system by increasing the concentration to 50 mM EDTA in 5 mM tris-HCl buffer, pH 8.6. The fractions of the first protein peak contained all of the enzyme activity. After calcium phosphate gel treatment and elution with 0.1 M $Na_4P_2O_7$ ammonium sulfate of 70 per cent saturation was added. The precipitate was centrifuged off. Needle-like crystals appeared in the solution within a few hours. The purified enzyme gave a constant specific activity even after recrystallization, migrated as a single peak in ultracentrifugal analysis, and showed a sedimentation velocity comparable to that of the rabbit muscle enzyme.

I. Szász

Comparison of the concentrations of glutathione and of iodine-reducing substances in human erythrocytes. N. Jesenovec, M. Fišer-Herman (Splošna bolnišnica, Maribor, Zavod za Medicinsku, Biokemiju, Domagojeva 2, Zagreb) *Clin. chim. Acta 14*, 293 (1966).

Concentrations of reduced and oxidized glutathione (GSH, resp. GSSG) were determined by a specific glyoxalase method and by a less specific iodometric method in blood cells from healthy persons (including pregnant women and newborns as well), and from patients suffering from hepatitis, cirrhosis of the liver, uraemia, malignancy, leukaemias, pernicious, sideropenic and posthaemorrhagic anaemia and hyperthyroidism. With the glyoxalase method no significant changes were detected in liver diseases, but in other conditions GSSG was increased and GSH reduced. Total glutathione was found to be much reduced in malignancy and hyperthyroidism. With the iodometric method GSH levels seemed to be higher than with the enzymatic method. The difference was called by the authors: the non-glutathionic oxidizable material. Normal cells contained quite a large quantity of this material, but much more has been found in the cells of patients, particularly in cases of uraemia, pernicious anaemia and malignancy.

I. Szász

Hereditary elliptocytosis and hyperhaemolysis. A comparative study of 6 families with 145 patients. R. A. Geerdink, P. W. Helleman and M. C. Verloop (Medical Department, University Hospital, Utrecht, The Netherlands) *Acta med. scand. 179*, 715 (1966).

In six families with elliptocytosis, about 400 individuals were examined. In 145 members of these families, elliptocytosis was diagnosed. A study of haemolysis in the patients, the normal members of the families serving as controls, disclosed that elliptocytosis caused hyperhaemolysis as a rule. Elliptocytosis did not influence the haemoglobin concentration, haematocrit value and erythrocyte count per volume unit of blood. The patients therefore showed a "compensated haemolysis", regardless of whether or not they belonged to families in which a

genetic linkage between elliptocytosis and rhesus factors exists (i.e. regardless of whether the corresponding genes are or are not localized close together on the same chromosome). A comparative study of the degree of haemolysis in the various families disclosed that the patients in one "non-linked" family showed a more markedly pathological haemolysis than those in one "linked" family. This suggests that, not only genetically but also biochemically, at least two types of elliptocytosis exist.

I. Árky

Haemoglobin Köln (β 98 Valine → Methionine): an unstable protein causing inclusion-body anaemia. R. W. Carrell, H. Lehmann, H. E. Hutchinson (M. R. C. Abnormal Haemoglobin Research Unit, Department of Biochemistry, Cambridge) *Nature 210*, 915 (1966).

Haemoglobin Köln moves more slowly than Hb A on electrophoresis at alkaline pH, and it was confirmed by hybridization that the additional positive charge is situated in the β chain. In spite of that no difference could be detected in the position of any of the tryptic peptides by fingerprinting. On staining for bivalent sulphur β Köln TpXI gave a positive reaction. This suggested that in Hb Köln a neutral amino acid (among the residues 96—104 of the β chain) had been replaced by methionine. Amino acid analysis of this area confirmed that Hb Köln is $\alpha_2 \beta_2^{98\ \mathrm{Val} - \mathrm{Met}}$. Valine in position 98 is in direct contact with the haem and it might be expected that its replacement by a larger residue could interfere with the stability of the tertiary structure of the β chain. The increased positive charge of Hb Köln, which becomes apparent at pH 8.6, could be connected with an increase in the positive charge of a histidine near to the Val in question. The methionine might displace the histidine in position 97, thus bringing it nearer to an aspartic acid residue at 99 or possibly 94. The greater proximity of the negative charge would then produce an increase in positive charge of the 97 histidine and this would depend on the tertiary structure of the molecule maintained. This has been observed in fact, when the increased positive charge of Hb Köln was not demonstrable anymore, after the separation of its α and β chains, or after its digestion with trypsin.

Judith Szelényi

Investigation of erythrocyte turnover with selenium-75-labeled methionine. J. A. Penner (Department of Internal Medicine, Simpson Memorial Institute, The University of Michigan) *J. Lab. clin. Med. 67*, 427 (1966).

Selenomethionine, a gamma-emitting methionine analogue, was employed to study erythrocyte production and survival. In normal individuals, administration of 100 μc of selenomethionine resulted in the concentration of significant amounts of radioactivity in the bone marrow within one hour and in circulating erythrocytes, leukocytes, and platelets within 48 hours. The major portion of erythrocyte activity was found in hemoglobin. Erythrocyte radioactivity in the normal individual increased rapidly during the first 7 days. A relatively sharp decline in activity was observed between 100 and 135 days, indicating that most of the cells initially labeled in the marrow and released into the circulation had survived throughout this period. Patients with acquired hemolytic anemia and sickle cell anemia exhibited increased incorporation of isotope into the red cells initially and a rapid loss of activity during the following weeks as the labeled cells were destroyed. A similar pattern of incorporation was seen in spherocytic anemia; however, splenectomy appeared to reduce the rate of red cell destruction and resulted in a more gradual loss of radioactivity. Little, if any, incorporation of labeled amino acid was observed in erythrocytes of patients with hyporegenerative anemias. It was concluded that selenomethionine is a useful clinical agent in the evaluation of red cell survival and production.

I. Árky

The effect of ε-aminocapronic acid on certain serological reactions. Yu. Musyatovich, Yu. Esuita, M. Belecky (2nd Department of Gynaecology and Obstetrics, Bielostok) *Akusherstvo i Gin.* 3, *28* (1966).

After a brief survey of certain papers on the marked action of ε-aminocapronic acid (EACA) in allergic diseases, the authors describe their own experiments. They studied the change of isohaemagglutination under various conditions both *in vivo* and *in vitro*. Anti-A, anti-B and anti-D sera with a titre of 1 : 128, erythrocytes of groups "A", "B", and "O" (Rh positive) and EACA solutions of 10, 15 and 20 mg%, and isotonic NaCl, respectively, were used. The following results were obtained:

1. At 4° C in systems incubated for 24 hours with 20 mg% EACA solution the isohaemagglutination titre dropped from the 1 : 128 of the controls to 1 : 8.

2. The agglutinate formed was dissolved in EACA; after 18 hours incubation (following 30 minutes shaking) the bulky agglutinate was dispersed, although there was no change in the titre.

3. The agglutinability of erythrocytes incubated 24 hours in EACA solution and then washed, underwent a permanent change; there was a significant decrease in the titre of the sera used.

4. The isohaemagglutination titre of sera treated for 24 hours at 4° C with identical quantities of EACA dropped from 1 : 64 to 1 : 16. Dialysis caused no further reduction in the titre.

5. When the agglutinates were dissolved and centrifuged after 18 hours incubation with 15 mg% EACA solution the supernatant fluid caused no agglutination in dilutions up to 1 : 2, while dilutions of 1 : 28 caused already agglutination in the controls.

P. Tabák

Indophenol oxidase and peroxidase activities in mammalian erythrocytes. I. Siegel (Pathology Department, Albert Einstein College of Medicine, New York) *J. Histochem. Cytochem. 13*, 141 (1965).

Cytochrome oxidase and peroxidase are two of the enzymes not yet demonstrated in mammalian erythrocytes. It was believed that in mammalian erythrocytes cytochrome loses this enzyme as it obtains the major part of its energy by glycolysis. The Nadi reaction was used in the present investigations. Enzyme activity was not determined quantitatively, but the cytochrome oxidase activity found proved the significant respiratory activity of mature mammalian erythrocytes. When oxygen was eliminated, the G-Nadi reaction stopped, but the M-Nadi reaction continued. Potassium cyanide and sodium azide partially inhibited the G- and M-Nadi reactions. Microscopic examinations have shown that the reaction product settles at the periphery of the cell. The Ehrlich ascites tumour cells and haemolyzed erythrocyte stroma showed similar results by the Nadi test. Fixing and heating inhibited the reaction.

Ilona Berzy

Untersuchungen zur Topochemie der eosinophilen Leukozytengranula (Topochemistry of eosinophilic leukocyte granules). B. K. Brzezinski (Anatomisches Institut der Ernst-Moritz-Arndt-Universität, Greifswald) *Acta histochem. 20*, 343 (1965).

Eosinophilic leukocytes were studied cytochemically. The results indicate that the specific granules contain protein components and phospholipids rich in aromatic and basic amino acids. They contain no DNA, but some RNA, which remains unchanged during maturation. The granules of eosinophilic cells contain no polysaccharides, but some neurinic acid, which may explain their PAS activity. The known sudanophilia is not due to lipids; the granules contain fat peroxide, but no cholesterol or choline. Owing to the group high in P, the granules show a marked gamma metachromasia. The lamellar structure of the granules is due to a suitable arrangement of components. The centre of the granules is composed of nucleic acid, protein, and lipid, surrounded by a peripheral enzyme carrier capsule. The experimental results indicate that protein is synthesized in the granules.

Ilona Berzy

Lysozyme in bone marrow and peripheral blood cells. R. S. Briggs, P. E. Perillie, S. C. Finch (Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut) *J. Histochem. Cytochem. 14*, 167 (1966).

The lysozyme activity of cellular elements in the bone marrow and peripheral blood was studied by an indirect histochemical method. Following anticoagulant treatment, blood or bone marrow suspension was mixed in a leucocyte pipette with the dried suspension of Micrococcus lysodeicticus killed by UV irradiation, and after 20 seconds smears were prepared. The smears were incubated for five to ten minutes in a wet chamber, then were air-dried and stained by Wright's method. The lysozyme activity of the cells was manifested in a swelling of the capsule of the surrounding micrococci and the shrinking of the protoplast of the latter. A significant lysozyme activity in the monocytes and the neutrophilic granulocytes as well as in the neutrophilic precursors up to the promyelocytes could be demonstrated by this method. Occasionally, a few mature eosinophilic granulocytes showed traces of lysozyme activity. The basophilic granulocytes, the erythrocytes and their precursors, the megakaryocytes, the blood platelets, the plasma cells, the leukocytes in the tissues and the reticuloendothelial cells of the bone marrow always reacted negatively.

Gy. Lelkes

The ultrastructure of eosinophilic granulocytes in the peritoneal cavity of rats following injection of ferritin. F. Bro-Rasmussen (Department of Anatomy, University of Copenhagen, Copenhagen, Denmark) *Scand. J. Haemat. 3*, 257 (1966).

The function of the granules of eosinophilic cells is not yet quite clear. It has been supposed by several authors that in non-sensitized organisms the granules of the eosinophilic cells possess phagocytotic properties. In the work presented the granules of eosinophilic cells were studied electron microscopically, in non-sensitized organisms after the administration of foreign proteins. The eosinophilic granulocytes were obtained from rats following intraperitoneal injection of ferritin and peritoneal washing at various times with 50 ml saline. Ten minutes after the injection inclusion bodies containing ferritin and similar to phago-lysozomes were visible in the cytoplasm of the eosinophilic granules. Ferritin phagocytosis caused a morphological change in the eosinophilic granules, but the latter did not disappear. In the neutrophilic granulocytes of the peritoneal fluid phagocytosed conglomerates of eosinophilic granules were visible, indicating a degenerative change. The phagocytosis of the eosinophilic granules is regarded as a stage in the degradation of eosinophilic cells.

Ilona Berzy

The steady-state transport of oxygen through hemoglobin solutions. K. H. Keller, S. K. Friedlander (Department of Chemical Engineering, the Johns Hopkins University, Baltimore, Md.), *J. Gen. Physiol. 49*, 663 (1966).

The steady-state transport of oxygen through hemoglobin solutions was studied to identify the mechanism of the diffusion augmentation observed at low oxygen tensions. A novel technique employing a platinum-silver oxygen electrode was developed to measure the effective diffusion coefficient of oxygen in steady-state transport. The measurements were made over a wider range of hemoglobin and oxygen concentrations than previously reported. Values of the Brownian motion diffusion coefficient of oxygen in hemoglobin solution were obtained as well as measurements of facilitated transport at low oxygen tensions. Transport rates up to ten times greater than ordinary diffusion rates were found. Predictions of oxygen flux were made assuming that the oxyhemoglobin transport coefficient was equal to the Brownian motion diffusivity which was measured in a separate set of experiments. The close correlation between prediction and experiment indicates that the diffusion of oxyhemoglobin is the mechanism by which steady-state oxygen transport is facilitated.

G. Gárdos

Formation of a peculiar haem-protein fraction in plasmas containing haemoglobin. E. Bottini, G. Filippi, F. Cozzi, G. Maggioni (Istituto di Genetica, Università, Roma) *Experientia* (Basel) *22*, 19 (1966).

A particular benzidine-positive fraction was observed by means of starch-gel electro-

phoresis, in the Hb containing plasmas of children (378 plasmas examined) in various physiological and pathological conditions (neonatal jaundice, thalassaemia, favism). This new fraction migrates faster towards the positive pole than the free Hb band and is closer to the latter than the MetAlb band. Similar results were obtained by incubating normal adult Hb with umbilical cord plasma. After preserving the plasma for approximately 50 days at 4° C, the results were less apparent. The results of the *in vitro* experiments seem to favour the theory that the new benzidine-positive band is to a large extent a result of the transformation of Hb and that in plasma there are non-ultrafiltrable factors capable of accelerating Hb transformation as compared to haemolysates; these factors appear to be more active in the first few days of life.

Judith Szelényi

Die Wirkung von Triosereduktion auf die Kalium- und Natriumpermeabilität roter Blutkörperchen (Effect of triose reductone on the permeability of K and Na in erythrocytes). H. Passow, E. Vielhaeur (II. Physiologisches Institut der Universität des Saarlandes, Homburg, Saar) *Pflügers Arch. 288*, 1 (1966).

Inhibition of active transport causes potassium leakage accompanied by the uptake of a nearly equivalent amount of Na. A number of agents, such as fluoride, iodoacetate + adenosine, and lead not only inhibit the uphill movements of K and Na, but enhance the downhill movements of K ten- to thirtyfold without appreciably increasing the passive Na influx. Triose reductone and its hydrazine derivative were found to exert similar actions. Triose reductone is readily oxidized by atmospheric oxygen at high pH values, but is fairly stable in the acid range. It reacts spontaneously with hydrazine, although the reaction rate is low. Enzymes present in the interior of the erythrocyte enhance this reaction. The product contains 2 moles of hydrazine per mole of reductone. Neither reductone nor its hydrazine derivatives seem to form chelates with alkaline earth metal ions. Both exert little effect on red cell metabolism as judged from the fact that they induce but little ATP breakdown. They are metabolically converted into unidentified end products. This process is not affected by the presence of ethylenediaminetetraacetic acid (EDTA). The action on cation permeability of triose reductone and of its hydrazine derivative depends on the metabolic state of the cells. In the concentration range studied (0.5—10.0 mmoles/l) fresh erythrocytes are scarcely affected whereas cells incubated in the absence of substrates prior to the addition of the poison lose potassium with little concomitant sodium uptake. The hydrazine derivative is much more effective than reductone itself. In cells preincubated for 16 hours it may cause, within 2 hours, a net loss of about 60 mEq K/kg cells with Na uptake of as little as 5 mEq/kg cells. Potassium loss can be induced only in the presence of Ca. At 0.5 mmoles/l $CaCl_2$ a maximum effect is reached whereas an excess of EDTA prevents the permeability change to occur.

G. Gárdos

Effect of oxidized glutathione on some enzymes of erythrocytes and its relation to erythrocytic enzyme activity and electrophoretic mobility. H. Walter, Juanita F. Caccam (Laboratory of Chemical Biology, Veterans Administrations Hospital, Long Beach, Calif.) *Biochem. J. 100*, 274 (1966).

1. Oxidized glutathione (GSSG) reacts or interacts with some erythrocytic enzymes (glucose 6-phosphate dehydrogenase, aspartate aminotransferase), but not with some others (lactate dehydrogenase).

2. GSSG does not diminish the activity of any of these enzymes and is therefore not responsible for the decreased enzyme activities associated with older erythrocytes.

3. It may be that the reaction of aspartate aminotransferase with GSSG is the cause for the more rapid anodic electrophoretic mobility of this enzyme derived from human erythrocytes as compared with the mobility of the same enzyme from other human tissues.

4. A reinterpretation of some related, previously published, data with regard to the electrophoretic mobility of the abovementioned enzymes from young and old erythrocytes is presented.

G. Gárdos

Chemically induced, selective thrombocytopenic purpura. J. C. Kulis, (Swedish Covenant Hospital, Chicago, Ill.) *Arch. intern. Med. 116*, 559 (1965).

Chemicals can cause selective thrombocytopenia without anemia and neutropenia. In the present study two cases have been presented: one acute and one chronic. In the first case the chemicals involved were 2.2-dichlorovinyl-dimethyl-phosphate and Dieldrin (insecticidal product containing hexachloro-octalhydro-dimethano-naphthalane), and produced acute thrombocytopenia with bleeding into the skin and mucous membranes and gross hematuria. This was a 10-year-old girl. The second case was a 60-year-old man, he had an episode of epistaxis which subsided spontaneously, thereafter petechiae appeared on extremities and in the gastrointestinal tract. The patient had been employed for the last seven years spraying clothes with "Mono-Zone Moth Spray" which contains pyretkins, lethane and petroleum hydrocarbons. The therapeutic use of blood, platelets and steroids in inducing a remission to normal has been demonstrated. The possible pathogenic mechanism in inducing the thrombocytopenic purpura:

1. Immunologic damage to circulating platelets leading to their premature removal from the blood (e.g. chlorothiazide, sulfas, phenylbutazolidin).

2. Direct toxicity to platelets is a dose-related phenomenon (e.g. ristocetin).

3. Direct toxic depression of the megakaryocytes (e.g. chloramphenicol or the gold salts).

The possibility that a drug is the cause must be investigated in each case. A simple test for demonstrating antiplatelet antibodies has been devised by Weintraub, but the results may be negative or inconclusive. The only way of proving the diagnosis would be to administer a test dose of the suspected drug or chemical which is usually too dangerous, and the diagnosis is made by temporal association of the administration of the drug and the reappearance of symptoms and signs.

Elisabeth Margittay

The treatment of autoimmune hemolytic anemia with heparin. A. Ten Pas, R. W. Monto (Division of Hematology, Henry Ford Hospital, Detroit, Mich.) *Amer. J. med. Sci. 251*, 63 (1966).

A patient with disseminated small cell lymphoma complicated by severe autoimmune hemolytic anemia is discussed. The anemia was successfully treated with heparin. The hemolytic process was evidenced by a positive Coombs' test, a low complement titer, and an elevated serum hemoglobin which did not respond previously to splenectomy, adrenocortical steroids or chemotherapeutic agents. Following the administration of heparin subcutaneously, there was a complete remission of the hemolytic process. The Coombs' test became negative: the serum hemoglobin dropped and the complement titer rose to normal levels. Complement apparently is required in some types of red cell destruction in autoimmune hemolytic anemia. Complement titers are depressed in cases with cold agglutinins (50%), but also in the presence of warm antibodies (20%). The utilization of complement, therefore, is a prerequisite in some types of intravascular hemolysis produced by antigen-antibody reactions. Presumably heparin inhibition of this reaction is due to its ability to form loose complexes with the globulin components of complement and in this manner competes with the red cell antibody complex for union with the complement. It is suggested that a trial of heparin, a less harmful agent than corticosteroids or immunosuppressive agents, should be the treatment of choice early in severe autoimmune hemolytic anemia.

Georgina Sik

From the International Literature on Haematology

Acta Haematologica (Basel) **36** (1966) No. 1–2.

Remarques sur l'hématologie géographique. *Bernard, J.* et al. (Hôpital Saint-Louis, 2, Place de Dr. Fournier, Paris X), p. 1.

Comparative haematology of laboratory animals. *Lawkowicz, W.* et al. (ul. Jana Krysta 8/39, Warszawa 42), p. 13.

African macroglobulinaemias. *Trincão, C.* et al. (Inst. de Med. Tropical, Lisbon), p. 26.

Die Atransferrinämien. *Heilmeyer, L.* (Med. Univ.-Klinik, Hugstetterstrasse 55, 78 Freiburg im Br., Germany), p. 40.

Lymphozyten u. Proteine. *Hitzig, W. H.* (Univ. Kinderklinik, Steinwiesstrasse 75, 8032 Zürich), p. 50.

Configuration des macromolécules en solution. Méthodes d'étude. *Sandron, Ch.* (Centre de Recherches sur les Macromolécules, 6, rue Boussingault, Strasbourg), p. 63.

Therapeutische Fibrinolyse. *Deutsch, E.* (I. Med. Univ. Klinik, Spitalgasse 23, Wien IX), p. 89.

Facteurs locaux du premier temps de l'hémostase. *Hugues, J.* (Inst. de Médecine, Univ. de Liège, Liège), p. 109.

Importance of coagulation besides haemostasis. *Koller, F.* (Med. Univ. Klinik, Bürgerspital, 4000 Basel), p. 133.

Acta Haematologica (Basel) **36** (1966) No. 3–4.

The nature of the defects in hemorrhagic disorders. *Owren, P. A.* (Rikshospitalet, Oslo), p. 141.

Chromosomes and leukaemia. *Baikie, A. G.* (Dept. of Med., St. Vincent Hospital, Fitzroy N. 6, Melbourne, Victoria), p. 157.

The viral theory of human leukemia. *Thorell, B.* (Dept. of Pathol., Karolinska Sjukhuset, Stockholm), p. 174.

On the pathogenesis of human leukaemia. *Videbaek, AA.* (Bernstorffsvej 9, Hellerup, Denmark), p. 183.

Structure and heterogeneity of antibodies. *Kabat, E. A.* (College of Physicians and Surgeons, Columbia Univ., 630 W. 168 Street, New York, N. Y.), p. 198.

Les études quantitatives de l'isohémagglutination. *Wurmser-Filitti, Sabine* et al. (Inst. de Biologie Physico-Chimique, 13, rue Pierre Curie, Paris V), p. 239.

Thalassaemia. *Lehmann, H.* (Univ. Dept. of Biochemistry, Cambridge), p. 256.

Die Reifung des Retikulozyten. *Löhr, G. W.* (Med. Univ.-Poliklinik, Robert-Koch-Strasse 7, 355 Marburg/Lahn, Germany), p. 273.

Contribution of cytology to the study of lymphopathies. *Pavlovsky, A.* (Inst. de Investigaciones Hemat., Melo 3081, Buenos Aires, Argentina), p. 296.

Blood (New York) **28** (1966) No. 1.

Quantitative estimation of split products of fibrinogen in human serum, relation to diagnosis and treatment. *Merskey, C.* et al. (Dept. of Med., Albert Einstein College of Med., New York, N. Y.), p. 1.

Studies in the pathogenesis of autoerythrocyte sensitization syndrome. *Groch, G. S.* et al. (Dept. of Intern. Med., Yale Univ. School of Med., New Haven, Conn.), p. 19.

Factor 13 deficiency with severe hemorrhagic diathesis. *Fisher, Sh.* (Dept. of Hemat., Jaffa Government Hosp., Jaffa, Israel), p. 34.

Effect of thymus enclosed in millipore diffusion envelopes on thymectomized hamsters. *Wong, F. M.* et al. (Pratt Clinic, New England Med. Center Hosp., Boston, Mass.), p. 40.

Absence of cis delta chain synthesis in $\delta\beta$ thalassemia. (F-thalassemia). *Comings, D. E.* et al. (Dept. of Med., Univ. of Washington, School of Med., Seattle, Washington), p. 54.

The iron content of jejunal mucosa obtained by Crosby's biopsy in hemochromatosis and hemosiderosis. *Astaldi, G.* et al. (Blood Research Foundation Center, Municipal Hosp., Tortona, Italy), p. 70.

Plasma cells containing iron. An electron micrographic study. *Goodman, J. R.* et al. (Veterans Administration Hosp., San Francisco, Calif.), p. 83.

F^{59} absorption in human subjects using total-body counting technic. *Pollack, S.* et al. (Dept. of Hemat., Walter Reed Inst. of Research, Washington, D. C.), p. 94.

Observations on certain enzyme activities of normal human red cells treated with sulfhydryl compounds. *Sirchia, G.* et al. (Ist. de Semeiotica Medica dell'Univ. de Milano, Italy), p. 98.

Multiple myeloma and normal pregnancy. Report of a case. *Kosova, L. A.* et al. (Hektoen Inst. for Med. Research, Cook County Hosp., Chicago, Ill.), p. 102.

Hemostatic homeostasis. Analytical review. *Spaet, Th. H.* (Dept. of Hemat., Lab. Div., Montefiore Hosp., New York, N. Y.), p. 112.

Blood (New York) **28** (1966) No. 2.

The Chediak-Higashi syndrome: A possible lysosomal disease. *White, J. G.* (Dept. of Pediatrics, Univ. of Minnesota Med. School, Minneapolis, Minn.), p. 143.

Leukocyte response following simultaneous ionizing and microwave (Radar) irradiation. *Thomson, R. A. E.* et al. (Dept. of Rad. Biol. and Biophysics, Univ. of Rochester School of Med. and Dentistry, Rochester, N. Y.), p. 157.

Respiration and glycolysis of normal human lymphocytes. *Hedeskov, C. J.* (Dept. of Med., Marselisborg Hosp., Aarhus Univ. Med. School, Aarhus, Denmark), p. 163.

The effects of phytohemagglutinin on mouse spleen cells in vivo. *Gamble, Ch. N.* (Div. of Exp. Pathol., Scripps Clinic and Research Foundation, La Jolla, Calif.), p. 175.

Ribonucleic acid biosynthesis in human leukocytes. Effects of phagocytosis on RNA metabolism. *Cline, M. J.* (Dept. of Med., Univ. of Calif. School of Med., San Francisco, Calif.), p. 188.

Selective eradication of erythropoiesis by actinomycin D as the result of interference with hormonally controlled effector pathway of cell differentiation. *Reissmann, K. R.* et al. (Dept. of Med., Univ. of Kansas Med. Center, Kansas City, Kansas), p. 201.

Interaction of influenza virus with blood platelets. *Terada, H.* et al. (Blood Research Laboratory, New England Center Hospitals, Boston, Mass.), p. 213.

Erythrocyte mucoids in acquired autoimmune hemolytic anemia. *Tishkoff, G. H.* (Dept. of Med., Univ. of Calif., Center for Health Sciences, Los Angeles, Calif.), p. 229.

Cytogenetic studies in the chronic myeloproliferative syndrome. *Kiossoglou, K. A.* et al. (New England Med. Center Hospitals, Tufts Univ. School of Med., Boston, Mass.), p. 241.

The effect of ellagic acid on the thrombin-induced hemorrhagic syndrome in the rat. *Cliffton, E. E.* et al. (Clotting Mechanism Section, Sloan-Kettering Inst. for Cancer Research, New York, N. Y.), p. 253.

Jordan's anomaly in white blood cells. Report of a case. *Rozenszajn, L.* et al. (Meir Hospital, Kfar-Saba, Israel), p. 258.

Tabular and ultrastructural studies of agranulocytes of the thoracic duct of calves. *Weber, A. F.* et al. (Dept. of Veterinary Anatomy, Univ. of Minnesota, St. Paul, Minn.), p. 266.

Hypocupremia and neutropenia in copper deficiency. *Cordano, Angel* et al. (Grace Dept. of Research, British-American Hospital, Lima, Peru), p. 280.

Blut (München) **14** (1966) No. 1.

Die Genotype-Bestimmung im Kidd-System. *Spielmann, W.* et al. (DRK-Blutspendedienst Hessen, Immunhämat. Abtt., Univ., Frankfurt/Main,) p. 1.

Zur Vererbung der Enzymaktivitäten der Lactat-Dehydrogenase u. Malat-Dehydrogenase im menschlichen Erythrozyten. *Haug, H.* et al. (Med. Univ.-Poliklinik, Würzburg), p. 10.

Zur Bedeutung des 2,3-Diphosphoglycerat-Zyklus in Erythrozyten: Beziehungen zwischen Methämoglobin- u. 2,6-Dichlorphenolindophenol-Reduktion, Glykolyse u. 2,3-Diphosphoglyceratbildung. *Schröter, W.* et al. (Univ.-Kinderklinik, 2000 Hamburg 20, Martinistrasse 52), p. 16.

Präparative Voraussetzungen zur klinischen Histologie des menschlichen Knochenmarkes. *Burkhardt, R.* (8 München 15, Ziemssenstrasse 1), p. 30.

Chronic monocytic leukaemia. *Ryder, R. J. W.* (St. Kevins Hosp., James Street, Dublin, Eire), p. 47.

British Journal of Haematology (Oxford) **12** (1966) No. 4.

The effect of unilateral limb shielding on the haemopoietic response of the guinea-pig to gamma irradiation (150 r). *Osmond, D. G.* et al. (Dept. of Anatomy, McGill Univ., Montreal, Canada), p. 365.

An antithrombin (Maculatin) and a plasminogen activator extractable from the blood-sucking hemipteran, Eutriatoma maculatus. *Hellmann, K.* et al. (Chemotherapy Unit, Cancer Research Fund, Lincoln's Inn Fields, London, W. C. 2), p. 376.

Studies of erythrocyte spicule formation in haemolytic anaemia. *Nathan, D. G.* et al. (Curtis Hemat. Research Lab., Peter Bent Brigham Hosp., Boston, Mass.), p. 385.

Sideruria following a single dose of desferrioxamine-B as a diagnostic test in iron overload. *Ploem, J. E.* et al. (Dept. of Haemat., State Univ. Hosp., Utrecht, The Netherlands), p. 396.

Quantitative assay of Factor VIII (antihaemophilic globulin) using the thrombin generation test. *Pudlák, P.* et al. (Inst. of Haemat., U nemocnice 1, Prague 2), p. 409.

Studies of the leucocyte compartment in guinea-pig bone marrow after acute haemorrhage and severe hypoxia: evidence for a common stem-cell. *Harris, P. F.* et al. (Dept. of Human Biol. and Anat., Univ. of Sheffield, England), p. 419.

A procedure for separating viable lymphocytes from human blood and some studies on their susceptibility to hypotonic shocks. *Thomson, A. E. R.* et al. (Div. of Biochemistry, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W. C. 2.), p. 433.

The cytogenetics and cell metabolism of circulating Ree-Sternberg cells. *Sinks, L. F.* et al. (Dept. of Med., Univ. of Cambridge, Tennis Court Road, Cambridge), p. 447.

The amino acid content of venous blood and marrow aspirate before and after treatment in pernicious anaemia. *Parry, T. E.* (Dept. of Clin. Pathol., Llandough Hosp., Glamorgan, South Wales, Great Britain), p. 454.

Analysis of erythroid homeostatic mechanism in normal and genetically anaemic mice. *Kleighley, G. H.* et al. (Hospital for Sick Children, Toronto, Canada), p. 461.

Platelet function tests in thrombocythaemia and thrombocytosis. *McClure, P. D.* et al. (Hospital for Sick Children, Toronto, Canada), p. 478.

The response of guinea-pig bone marrow to intravenous injection of TAB vaccine. *Adeloye, Adelola* (The Radcliffe Infirmary, Oxford), p. 499.

British Journal of Haematology (Oxford) **12** (1966) No. 5.

The chromosomes in polycythaemia vera. *Kay, H. E. et al.* (Dept. of Clin. Research, The Royal Marsden Hosp., London), p. 507.

The nature of the vitamin B_{12}-binding protein in human serum. *Hardwicke, J.* et all. (Dept. of Exp. Pathol., Medical School Hosp. Centre, Univ. of Birmingham, Birmingham 15), p. 529.

Intracellular location of thromboplastic activity in the cells of human chorion. *Clarke, N.* et al. (School of Pathol., Trinity College, Dublin, Ireland), p. 536.

Haematological studies with methylene dimethanesulphonate (dimethanesulphonoxymethane). *James, R. M. V.* (Paterson Laboratories, Christie Hosp., Manchester, Great Britain), p. 546.

Thalassaemia types among Kurdish Jews in Israel. *Horowitz, A.* et al. (Hadassah Hosp., The Hebrew University, Jerusalem), p. 555.

The binding of vitamin B_{12} by serum proteins in normal and B_{12}-deficient subjects. *Lawrence, Christine* (Albert Einstein College of Med., Bronx Municipal Hospital Centre, New York, N. Y.), p. 569.

In vivo plasma binding capacity for cyanocobalamin. *Schiffer, L. M.* et al. (Med. Research Center, Brookhaven National Lab., Upton, New York), p. 578.

Experiments with Factor VIII separated from fibrinogen by electrophoresis in free buffer film. *Bidwell, Ethel* et al. (Blood Coagulation Research Unit, Churchill Hosp., Oxford), p. 583.

A histochemical method for the demonstration of leucocyte naphthylamidase using two substrates: alanyl- and methionyl-4-methoxy-2-naphthylamide. *Rutenburg, A. M.*et al. (Yamins Lab. for Surgical Research, Beth Israel Hosp., Boston, Mass.), p. 595.

Changes in iron mobilization and utilization induced by acute haemorrhage. *Meital, V.* et al. (Haemat. Research Lab., Hadassah Univ. Hosp., Jerusalem), p. 598.

The effect of acute haemorrhage and acute haemolysis on the intestinal iron absorption in the rat. *Chirasiri, L.* et al. (Korat Hospital, Korat, Thailand), p. 611.

Studies on neutrophil function. I. Physiological and pharmacological aspects. *Penny, R.* et al. (Dept. of Haemat., Postgraduate Med. School of London, Ducane Rd., London, W. 12), p. 623.

Studies on neutrophil function. II. Pathological aspects. *Penny, R.* et al. (Dept. of Haemat., Postgraduate Med. School of London, Ducane Rd., London, W. 12), p. 633.

The diurnal variation of plasma iron turnover and erythropoiesis in healthy subjects and cancer patients. *Lockner, D.* (Isotope Division, Karolinska Inst., Stockholm), p. 646.

Symposia on thalassaemia, and on the complications of leukaemia and of its treatment. *Weatherall, D. J.* (Dept. of Med., Univ. of Liverpool, Ashton Street, Liverpool 3), p. 657

Folia Haematologica (Leipzig) **86** (1966) No. 1.

Vergleichende Hämozytologie niederer Vertebraten. III. Granulozyten. *Fey, F.* (Inst. für Krebsforschung, Lindenberger Weg 70, 1115 Berlin), p. 1.

Observations on adult polynuclear leukocyte nucleus stained with basic dyes. *Costăchel, O.* et al. (Inst. of Oncology, Bulevardul 1, Mai nr. 11, Bucharest), p. 21.

On the ultrastructure of leukemic monocytes. *Smetana, K.* et al. (Laboratory for Electron Microscopy, Alberzova 4, Prague 2), p. 35.

Plasmozyten mit generalisierter Osteosklerose. *Wiedermann, B.* et al. (Polská 46, Olomouc), p. 47.

Megaloblastiche Anämie als Folge einer selektiven Vitamin-B_{12}-Malabsorption. Syndrom Imerslund-Gräsbeck. *Šmrhová, I.* et al. (Kreisinst. für Blutspendewesen des Krankenhauses, Hranice, ČSSR), p. 70.

Sur quelques changements hématologiques accompagnant la tuberculose. *Yordanova, E.* et al. (Inst. d'Hématologie et de Transfusion Sanguine, Sofia), p. 80.

Neue Beiträge zur Pathogenese der Verbrennungsanämie. *Bernát, I.* et al. (Róbert Károly krt. 44, Budapest XIII), p. 85.

Neue Aspekte der Verwendung von Ficin in der Immunhämatologie. *Májský, A.* et al. (Inst. für Hämatologie u. Bluttransf., U nemocnice 1, Prag 2), p. 96.

Folia Haematologica (Leipzig) **86** (1966) No. 2.

Vergleichende Hämozytologie niederer Vertebraten. IV. Monozyten—Plasmozyten—Lymphozyten. *Fey, F.* (Inst. für Krebsforschung, Lindenberger Weg 70, Berlin 1115), p. 133.

Der Einfluß von UV-Bestrahlung auf Leukozytenkernanhänge, weisses Blutbild u. Pigmentierung von Rana ridibunda. *Burmeister, J.* et al. (Gertrudenstrasse 9, 25 Rostock), p. 148.

Beitrag zur Bedeutung der Lymphoidzellen. *Fuchsberger, R.* (Inneren Abtl. im Betriebskrankenhaus, Pov. Bystrica, Leninstrasse 1134-C-1.), p. 153.

Aldrich—Wiskott-syndrome with thrombocytophagy and lipopigment in the reticuloendothelial system. *Kaluš, M.* et al. (Dept. of Clin. Pathol., 1st Clinic of Surgery, U nemocnice 2, Prague 2), p. 164.

Die Beeinflussung des hämopoetischen Systems der weissen Ratte durch einen-N-Lost-Cyclophosphamidester (ENDOXAN-ASTA). *Hambsch, K.* et al. (Med. Poli-

klin., Inst. der Karl-Marx-Univ., Härtelstrasse 16—18, 701 Leipzig), p. 174.

Die Bedeutung der Milz bei der experimentellen autoimmunhämolytischen Anämie. *Sundermann, A.* et al. (Med. Klinik der Med. Akad., Nordhäuser Strasse 74, 50 Erfurt), p. 190.

Die Bestimmung des Erythrozytenabbauortes bei hämolytischen Erkrankungen mit Hilfe von Radioisotopen. *Kuba, J.* et al. (Radioisotopenabteilung, I. Med. Univ., I. P. Pavlova 6, Olomouc, ČSSR), p. 197.

Untersuchungen an vier Jahre gelagertem Trockenplasma. *Tesar, V.* (Bezirksinst. für Blutspende u. Transfusionswesen, Leninallee 203, 402 Halle), p. 209.

Zur Bestimmung des Kalium-, Natrium-, Magnesium- u. Wassergehalts der Erythrozyten. *Elek, S. jr.* et al. (Inst. für Arzneimittelforschung, Szabadságharcosok útja 47—49, Budapest IV.), p. 215.

The use of reduced glutathione for the improvement of the therapeutic effectiveness of blood and plasma substitutes in the treatment of haemorrhagic shock. *Hořejší, J.* et al. (Inst. of Hematology and Blood Transfusion, U nemocnice 1, Prague 2), p. 220.

Über das Verhalten eines modifizierten Rinderserums im Tierversuch. *Knesslová, V.* et al. (Inst. für Hämatologie u. Bluttransf., U nemocnice 1, Prag 2.), p. 226.

Zwei Erythrozytenpopulationen, AB und schwaches A, bei einem Kranken mit chronischer Leukämie. *Májský, A.* (Inst. für Hämatologie u. Bluttransf., U nemocnice 1, Prag 2.), p. 235.

Hémostase (Paris) **6** (1966) No. 1.

Speculations on von Willebrand's disease. *Owen, Ch. A., Jr.* et al. (Mayo Clinic, Rochester, Minn.), p. 1.

La maladie de Moschcowitz. Réflexions cliniques, biologiques et histologiques à propos d'une observation. *Duhamel, G.* et al. (Hôpital Saint-Antoine, 53 Boulevard Diderot, Paris 12.), p. 7.

Synergistic effect of SH-containing compounds and prednisolone in increasing platelets in rats. *Nicola, de P.* (Clinica Med., Univ. of Pavia, Italy), p. 17.

Étude in vitro concernant le mécanisme de neutralisation de l'héparine par ses antagonistes. *Páusesco, E.* et al. (Lab. de Pathologie Exp., Hôpital Fundeni, Bucarest), p. 25.

Les mécanismes de l'action de l'héparine dans l'organisme. *Lomazova, H. D.* et al. (Inst. de Physiol. de l'Age, Pogodinskaja U1. 8, Moscou J-117), p. 41.

Les thrombocytopoïétines. *Markossian, A. A.* et al. (Inst. de Physiol. de l'Age Pogodinskaja U1. 8, Moscou J-117), p. 49.

Effets de l'irradiation à hautes doses sur la fonction plaquettaire in vitro. *Hollard, D.* et al. (Lab. de Recherches Hémat. du Centre d'Études Nucléaires, Grenoble), p. 57.

Treatment of haemophilia with purified factor VIII (FI-O-Ta). *Pinto, de Tezanos M.* (Inst. de Investigaciones Hémat., Acad. Nacional de Med., Melo 3081, Buenos Aires), p. 65.

Étude critique de quatre tests de coagulation dans la surveillance des traitements anticoagulants prolongés (anti-vitamines K). *Chataing, B.* (Lab. de Coagulation de l'Inst. Pasteur, Lyon), p. 73.

Nouvelle Revue Française d'Hématologie (Paris) **6** (1966) No. 3.

Fonctions des plaquettes: Questions sans réponse. *Zucker, Marjorie B.* (American National Red Cross Research Lab., New York Univ. Medical Center, New York, N. Y.), p. 345.

Les lysozymes des leucocytes et du plasma d'origine humaine. *Charlemagne, Danièle* et al. (Lab. de Chimie Biologique, 96, Boulevard Raspail, Paris 6.), p. 355.

Analyses chromosomiques dans l'anémie sidéroblastique idiopathique acquise. *Grouchy, J. de* et al. (Hôpital des Enfants-Malades, 149 rue de Sèvres, Paris XV), p. 367.

Les anticorps anti-gamma-globulines humaines spécifiques de système Gm chez les polytransfusés. *Ropartz, C.* et al. (Centre Départemental de Transfusion Sanguine, Rouen), p. 389.

Diagnostice rapide de l'érythropoïèse extramédullaire par enregistrement continu de l'activité osseuse, hépatique et splénique après injection de radio-fer. *Najean, Y.* et al. (Hôpital Saint-Louis, rue de la Grange-aux-Belles, F. 75-Paris X), p. 399.

Nouvelle Revue Française d'Hématologie (Paris) **6** (1966) No. 4.

Les cellules souches hématopoïétiques. *Mathé, G.* (Hôpital Paul-Brousse, 94-Villejuif, France), p. 473.

L'adhésivité des stromas, des fragments de globules rouges et des fragments de stromas. *Ponder, E.* (Inst. Pasteur, Service de Biophysique, Paris), p. 479.

Étude de la Plasmocytopoïèse. II. Schéma probable du mode de formation des plasmocytes élaboré à partir des résultats de la numération de leurs mitoses. *Sainte-Marie, G.* (Centre National de Transfusion Sanguine, Paris), p. 489.

Nomenclature des facteurs génétiques supportés par les immunoglobulines humaines. *Ceppellini, R.* (Inst. of Med. Genetics, Univ. of Turin, Turin), p. 515.

Проблемы гематологии и переливания крови (Москва) **11** (1966) № 9.

Ближайшие задачи проблемы консервирования крови *Виноград-Финкель, Ф. Р.* с. 3. — Pressing Tasks of the Blood Preservation Problems. *Vinograd-Finkel, F. R.* p. 3.

Новые ограждающие растворы для ультрабыстрого замораживания эритроцитов. *Виноград-Финкель, Ф. Р.* и сотр. (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 7. — New Protective Solutions for Ultra-Rapid Erythrocyte Freezing. *Vinograd-Finkel, F. R.* et al. p. 7.

Рентгенографическое исследование характера затвердевания эритроцитов, консервированных при ультранизких температурах. *Кудряшова, С. Н.* (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 11. — Roentgenographic Examination of the Character of Solidification of Erythrocytes Preserved at Ultra-Low Temperatures. *Kudryashova, S. N.* p. 11.

Современные методы выделения лейкоцитов и кровяных пластинок. *Мопен, Б.* (Париж), с. 15. — Current Methods of Leukocyte Platelet Isolation. *Maupin, B.* p. 15.

Замораживание белых клеток периферической крови для их длительного хранения при ультранизких температурах. *Абезгауз, Н. Н.* и сотр. (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 24. — Freezing of the Peripheral Blood Leukocytes for their Prolonged Preservation at Ultra-Low Temperatures. *Abezgauz, N. N.* et al.p. 24.

Изыскание методов длительного консервирования тромбоцитов в замороженном состоянии (Предварительное сообщение). *Скопина, С. Б.* и сотр. (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 30. — Search of the Methods of Prolonged Thrombocyte Preservation in Frozen Condition. *Skopina, S. B.* et al. p. 30.

О профилактике вирусного гепатита при переливании крови и плазмы. *Филатов, А. Н.* и сотр. (Ленинградский научно-исследовательский институт переливания крови, Ленинград), с. 35. — Prophylaxis of Viral Hepatitis in Blood and Plasma Transfusion. *Filatov, A. N.* et al. p. 35.

Влияние некоторых антикоагулянтов крови на фагоцитарную функцию лейкоцитов. *Шустов, А. И.* (Кафедра военно-морской и радиационной гигиены ВМОЛА им. С. М. Кирова, Ленинград), с. 41. — The Effect of Some Blood Anticoagulants on the Phagocytic Activity of Leukocytes. *Shustov, A. I.* p. 41.

Применение в клинике эритроцитов, отмытых от плазмы простым и доступным методом. *Воробьева, Г. С.* и сотр. (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 43. — The Use in Clinical Conditions of Erythrocytes Washed of Plasma by a Simple and Accessible Method. *Vorobyeva, G. S.* et al.p. 43.

Разработка и экспериментальное изучение нового консервирующего раствора для операции замещения крови. *Насыбуллина, Х. С.* и сотр. (Лаборатория консервирования крови ЦОЛИПК, Москва), с. 48. — Elaboration and Experimental Study of a New Preservative for Blood Substitution. *Nasybullina, Kh. S.* et al. p. 48.

Влияние антибактериальных препаратов, входящих в состав консервирующих растворов, на результаты бактериологического исследования крови. *Сироко, И. А.* (Военно-медицинская ордена Ленина академия им. С. М. Кирова, Ленинград), с. 53. — The Effect of Antibacterial Preparations Included into the Com-

position of Preserving Solutions on the Results of Bacteriological Examination of the Blood. *Siroko, I. A.* p. 53.

Посмертные изменения крови облученных животных. *Рыжков, С. В.* и сотр. (Научно-исследовательская лаборатория крови и тканей ВМОЛА им. С. М. Кирова, Ленинград), с. 57. — Changes in Post-Mortem Blood of Irradiated Animals. *Ryzhkov, S. V.* et al. p. 57.

Проблемы гематологии и переливания крови (Москва) 11 (1966) № 10.

Получение препарата очищенного антигемофильного глобулина и изучение его свойств. *Розенберг, Г. Я.* и сотр. (Центральный ордена Ленина институт гематологии и переливания крови, Москва), с. 3. — Obtaining of a Preparation of Purified Antihemophilic Globulin and a Study of its Properties. *Rozenberg, G.Ya.* et al. p. 3.

Выделение антигемофильного глобулина (VIII фактор) из плазмы и определение его активности. *Пул, Дж. Г.* (Медицинская школа при Стенфордском университете, Калифорния, США), с. 9. — Extraction of Antihemophilic Globulin (VIII factor) from the Plasma and Determination of its Activity. *Pool, J. G.* p. 9.

Гемостатическая фракция плазмы, содержащая четыре фактора (II, VII, IX, X) свертывающей системы кровы. *Штейнбух, М.* и сотр. (Национальный центр переливания крови, Париж), с. 15 — Hemostatic Fraction of the Plasma Containing Four Factors (II, VII, IX, X) of the Blood Coagulation System. *Shteinbukh, M.* et al. p. 15.

Морфологическая характеристика свежезаготовленного костного мозга, применяемого для переливания в клинике. *Ярустовская, Л. Э.* (Гематологическая клиника, радиологическое отделение и лаборатория проблемы донорства ЦОЛИПК, Москва), с. 22. — Morphological Characteristics of Freshly Prepared Bone Marrow Used for Transfusion in Clinical Conditions. *Yarustovskaya, L. E.* p. 22.

Консервирование человеческого костного мозга при низких температурах. *Пегг, Д. Е.* (Радиотерапевтическое отделение Вестминстерского госпиталя, Лондон), с. 26. — Storage of Human Bone Marrow at Low Temperature. *Pegg, D. E.* p. 26.

Изучение трудного костного мозга с целью его использования для лечения лейкозов и апластических состояний. *Мурчакова, А. Ф.* (Клиника факультетской терапии ВМОЛА им. С. М. Кирова, Ленинград), с. 31. — A Study of Cadaver Bone Marrow for Treatment of Leukemia and Aplastic Conditions. *Murchakova, A. F.* p. 31.

Использование пересадок кадаверного костного мозга при некоторых заболеваниях системы крови. *Абдулкадыров, К. М.* (Гематологическая клиника и лаборатория консервирования и пересадки тканей Ленинградского научно-исследовательского института переливания крови, Ленинград), с. 35. — Application of Cadaver Bone Marrow Transplantation in Some Affections of the Blood System. *Abdulkadyrov, K. M.* p. 35.

Трансплантация консервированного костного мозга при лечении острой лучевой болезни в эксперименте. *Кевлишвили, Г. Е.* и сотр. (Научно-исследовательский институт переливания крови им. Г. М. Мухадзе, Тбилиси), с. 37. — The Therapeutic Efficacy of Preserved Bone Marrow Transplantation in the Experimental Therapy of Acute Radiation Sickness. *Kevlishvili, G. E.* et al. p. 37.

Свойства сывороточного фактора Gm (a). *Будяков, О. С.* с. 39. — Properties of the Serum Gm(a)Factor. *Budyakov, O. S.* p. 39.

К вопросу о содержании свободного эритроцитарного протопорфирина у доноров, повторно дающих крови. *Ярлыкова, Е. И.* и сотр. (Кафедра клинической лабораторной диагностики Центрального института усовершенствования врачей, Москва), с. 45. — Concerning the Content of Free Erythrocytic Protoporphirine in Repeat Donors. *Yarlykova, E. I.* et al. p. 45.

Определение количества функционально полноценных клеток в суспензии костного мозга. *Бутомо, Н. В.* и сотр. (Военно-медицинская ордена Ленина академия им. С. М. Кирова, Ленинград), с. 48. — Determination of the Number of Functionally Valuable Cells in the Bone Marrow Suspension. *Butomo, N. V.* et al. p. 48.

К вопросу о получении костного мозга закрытым способом и стабилизация его с помощью катионитной смолы. *Михайлов, В. Г.* и сотр. (Узбекский научно-исследовательский институт гематологии и переливания крови, Ташкент), с. 51. — Concerning the Methods of Obtaining the Bone Marrow by Closed Method and its Stabilization with the Aid of a Cationite Resin. *Mikhailov, V. G.* et al. p. 51.

О консервации костного мозга доноров и больных в растворах. *Михайлов, В. Г.* и сотр. (Узбекский научно-исследовательский институт гематологии и переливания крови, Ташкент), с. 55. — Concerning the Preservation of Donor and Patients' Bone Marrow in Solutions. *Mikhailov, V. G.* et al. p. 55.

Заготовка крови в условиях районной больницы. *Зюзин, Н. Н.* (Отделение переливания крови Даровской Центральной районной больницы Кировской области), с. 59. — Blood Preparation and Storage in Conditions of a District Hospital. *Zyuzin, N. N.* p. 59.

О диспансерном методе обслуживания гематологических больных. *Свядощ, А. В.* и сотр. (Кафедра терапии Казахского института усовершенствования врачей, Алма-Ата), с. 60. — Service Rendered to Hematological Patients on a Dispensary Level. *Svyadishch, A. V.* et al. p. 60.

Рецензия на книгу З. Витте «Переливание костного мозга». *Глузман, Ф. А.* (Киев), с. 61. — A Review on the Book by S. Witte: "Knochenmarktransfusion", München, 1963. *Gluzman, F. A.* p. 218.

Scandinavian Journal of Haematology (Copenhagen) **3** (1966) No. 3.

Assessment of body stores of vitamin B_{12} during maintenance therapy of pernicious anaemia with depot cyanocobalamin preparation Betolvex). *Bastrup-Madsen, P.* (Aarhus Amtssygehus, Aarhus, Denmark), p. 165.

Vitamin E deficiency in the monkey. X. Protoporphyrin and haeme synthesis by peripheral blood and bone marrow. *Stanley Porter, F.* et al. (Duke Univ. Med. Center, POB 2916, Durham, N. C.), p. 175.

Studies on hypoxia. IV. The differential haemopoietic response to moderate and severe hypoxia. *Yoffey, J. M.* et al. (Dept. of Anatomy, The Medical School, Univ. Walk, Bristol 8.), p. 186.

Effect of intermittent hypoxia on regeneration of haemopoiesis in rats treated with cyclophosphamide or total body irradiation. *Høst, H.* (The Norwegian Radium Hospital, Montebello, Norway), p. 193.

Mechanism of action of deferoxaminum on iron absorption. *Balcerzak, S. P.* et al. (Veterans Administration Hosp., Univ. Drive, Pittsburgh, Penn.), p. 205.

Long-term observation of gastrectomized rats with regard to development of vitamin B_{12} deficiency. *Nygaard, K.* et al. (Inst. of Pathol. Anatomy, Rikshospitalet, Oslo), p. 213.

Heredity of combined deficiency of AHG and proaccelerin. *Gobbi, F.* (Istituto Patologia Med. Univ., Policlinico, Modena, Italy), p. 222.

Circulating anticoagulant in a haemophiliac (Evidence for antibody nature). *Svejgård, A.* (Blodbanken, Kommunehospitalet, Århus), p. 227.

Crude or refined intrinsic factor in preparations for the oral treatment of pernicious anaemia. *Berlin, H.* et al. (A. B. Kabi, Stockholm 30.), p. 236.

Scandinavian Journal of Haematology (Copenhagen) **3** (1966) No. 4.

Erythrocyte catalase activity in thalassaemia. *Balcerzak, S. P.* et al. (Veterans Administration Hosp., Univ. Drive, Pittsburgh, Penn.), p. 245.

The ultrastructure of eosinophilic granulocytes in the peritoneal cavity of rats following injection of ferritin. *Bro-Rasmussen, F.* et al. (Dept. of Anatomy, Univ. of Copenhagen, Nørre Allé 63, Copenhagen), p. 257.

Light chain types of transiently occurring cold haemagglutinins. *Harboe, M.* et al. (Inst. for Exp. Med. Research, Ullevål Hospital, Oslo 1), p. 269.

The effect of an enzyme preparation from Aspergillus Oryzae on the aggregation of rabbit platelets in vitro. *Svärd, P. O.* (Research Laboratories of AB Astra, Södertälje, Sweden), p. 277.

Periodic thrombocytopenia or tidal platelet dysgenesis in a man. *Engström, K.* et al. (Med. Dept., County Hospital, Borås, Sweden), p. 290.

Studies on the development of cancer anaemia in humans. *Lockner D.* (Isotope Div. Dept. of Alcohol Research, Karolinska Inst., Stockholm), p. 295.

Reversible agglutination of trypsinized red cells by a γ M-globulin synthesized by the human foetus. *Mellbye, O. J.* (Inst. for Exp. Med. Research, Ullevål Hosp., Oslo), p. 310.

Thrombosis et Diathesis Haemorrhagica (Stuttgart) **16** (1966) No. 1/2.

Determination of plasminogen in human plasma by the lysis time method. *Berg, W.* et al. (Sahlgrenska Hosp., Univ. of Göteborg, Sweden), p. 1.

Activity of plasmin and streptokinase-activator on substituted arginine and lysine esters. *Sherry, S.* et al. (Dept. of Med., Washington School of Med., St. Louis, Mo.), p. 18.

Arterio-venous differences in the components of the fibrinolytic enzyme system. *Ogston, D.* et al. (Dept. of Med., Univ. of Aberdeen, Scotland), p. 32.

Determination of plasminogen in clots and thrombi. *Hedner, U.* et al. (Coagulation Lab., Univ. of Lund, Allmänna Sjukhust, Malmö), p. 38.

Desmofibrin formation and the activity of the fibrin stabilizing factor (FSF) during the cleavage of its SH groups by thrombin. *Buluk, K.* et al. (Dept. of Genetics and Exp. Pathol., School of Med., Bialystok), p. 51.

A comparative study of the solvents commonly used to detect fibrin stabilization. *Tyler, H. M.* (Dept. of Pathol., Inst. of Orthopaedics, Brockley Hill, Stanmore, Middlesex, Great Britain), p. 61.

The influence of human blood cells on the solubility of clot in urea solution and clot lysis. *Kirchmayer, St.* et al. (II. Clinic of Intern. Med., Medical Academy, Cracow), p. 69.

Influence of sex and thyroid principles on the antiproteolytic activity of rat serum. *Wabner, Ch. I.* et al. (Dept. of Biochemistry, Div. of Med., Fort Knox, Kentucky), p. 86.

Platelet adhesiveness: A comparison of the rotating bulb and glass-bead column methods. *Hirsh, J.* et al. (Dept. of Pharmacol., Royal College of Surgeons of England, London), p. 100.

The effect of white cells on platelet aggregation. *Harrison, M. J. G.* et al. (Dept. of the Regius Professor of Med., The Radcliffe Infirmary, Oxford), p. 105.

The effect of sulfhydryl and enzyme inhibitors on platelet aggregation in vitro. *Harrison, M. J. G.* et al. (Dept. of the Regius Professor of Med., The Radcliffe Infirmary, Oxford), p. 122.

A lysosomal concept of the platelet release reaction and viscous metamorphosis. *Kowalski, E.* et al. (Dept. of Radiobiol. and Health Protection, Inst. of Nuclear Research, Warsaw 9), p. 134.

Microtubules and microfibrils in human platelets. *Sixma, J. J.* et al. (Center of Med. Electronmicroscopy, State Univ., Beetsstraat, Utrecht), p. 152.

Le comportement des plaquettes chez le rat. *Sinakos, Z.* (Hôpital Saint-Louis, Paris), p. 163.

A study of coagulation factor levels in women during labour and in their newborn infants. *Nossel, H. L.* et al. (Dept. of Med., Univ. of Cape Town, Cape, South Africa), p. 185.

A simple method for preparing fibrinogen. *Straughn, W.* (Dept. of Pathol. and Biochem., Univ. of North Carolina, Chapel Hill, N. C.), p. 198.

The role of HF (Factor XII) in the pathogenesis of the thrombolytic state induced by venous occlusion. *Iatridis, S. G.* et al. (Dept. of Med., Athena Clinic, Athenai, Greece), p. 207.

Die Permeabilität des Hämostasethrombus gegenüber Plasma. *Witte, S.* (Med. Univ.-Klinik, Erlangen, DBR), p. 218.

Studies on experimentally induced hypercoagulable state in rabbits. *Izak, G.* et al. (Hemat. Research Lab., The Hebrew University-Hadassah Med. School, Jerusalem), p. 228.

Hemorrhagic syndrome in dogs induced by intravenous thrombin. *Girolami, A.* et al. (Sloan-Kettering Inst. for Cancer Research, New York, N. Y.), p. 243.

Pharmacological and chemical characteristics of peanut extracts effective in hemo-

stasis. *Jackson, Blanche* et al. (Dept. of Zoology and Physiology, Louisiana State Univ., Baton Rouge, Louisiana), p. 257.

Influence of peanut extract on bleeding time. *Frampton, V. L.* et al. (Agricultural Research Service, New Orleans, Louisiana), p. 265.

The kinetics of inhibition of the action of thrombin by the fibrinopeptides formed during the clotting of fibrinogen. *Silver, A.* et al. (Dept. of Pathol., Univ. of Louisville School of Med., Louisville, Kentucky), p. 277.

Central nervous system hemorrhages induced by heparin in rats with allergic encephalomyelitis. *Levine, S.* et al. (New York Med. College Center for Chronic Disease, Welfare Island, N. Y.), p. 296.

Distribution, half life time and placental transfer of the protease inhibitor trasylol. *Beller, F. K.* et al. (Dept. of Obstetrics and Gynecology, New York Univ. School of Med., N. Y.), p. 302.

Automatic recording of prothrombin times and other coagulation tests in multiple samples. *Margolis, J.* et al. (Royal Alexandra Hosp. for Children, Camperdown, Sydney), p. 311.

Current blood clotting schemes. *Quick, A. J.* (Marquette Univ. School of Med., Milwaukee, Wisconsin), p. 318.

Transfusion (Philadelphia) **6** (1966) No. 3.

Repeated leukaphoresis of patients with chronic myelocytic leukemia. *Morse, E. E.* et al. (Blood Bank, Johns Hopkins Univ. Hosp., Baltimore, Md.), p. 175.

The transfusion of leukocytes from donors with chronic myelocytic leukemia to patients with leukopenia. *Morse, E. E.* et al. (Blood Bank, Johns Hopkins Univ. Hosp., Baltimore, Md.), p. 183.

Distribution and quantity of leukocyte antigens in the formed elements of the blood. *Bialek, Joan W.* et al. (Dept. of Med., Stanford Univ. School of Med., Palo Alto, Calif.), p. 193.

Preparation of platelet concentrates for transfusion employing adenosine diphosphate. *Flatow, F. A.* et al. (Leukemia Service, National Cancer Institute, Bethesda, Md.), p. 205.

An evaluation of anticoagulant solutions used in the preparation of platelet concentrates. *Kinlough, R. L.* et al. (Dept. of Med., Univ. of Adelaide, Australia), p. 213.

Antihemophilic factor (AHF) stability in fresh frozen blood bank plasma. *Weaver, R. A.* et al. (Univ. of North Carolina, Chapel Hill, N. C.), p. 224.

Stability of Factor VIII in the frozen state. *Britten, A.* et al. (Hemat. Unit, Massachusetts General Hosp., Boston, Mass.), p. 230.

The clinical use of dried fibrinogen (human), and the risk of transmitting hepatitis by its administration. *Anderson, H. D.* et al. (Michigan Dept. of Health Laboratories, Lansing, Mich.), p. 234.

The in vivo survival of Coombs' positive autologous erythrocytes produced by agglomeration. *Valeri, C. R.* et al. (Naval Blood Research Lab., Naval Hosp., Chelsea, Mass.), p. 247.

Observations on the preservation of autologous human erythrocytes using glycerol, slow-freeze technic and agglomeration. *Valeri, C. R.* et al. (Naval Blood Research Lab., Naval Hosp., Chelsea, Mass.), p. 254.

Characteristics of stored human blood kept fluid by urokinase-induced fibrinolysis. *Corn, M.* (The George Washington Univ. Med. Division, General Hosp., Washington, D. C.), p. 263.

Studies on the distribution of the blood group antigen Do^a (Dombrock) and the characteristics of anti-Do^a. *Polesky, H. F.* (Minneapolis War Memorial Blood Bank, Minneapolis, Minn.), p. 268.

A third example of the Rh antigen, E^w. *Winter, Nancy* et al. (Blood Grouping Lab., Boston, Mass.), p. 271.

A portable blood bank refrigerator for temporary storage of blood in surgery. *Darling, Ch. L.* (USAF Hosp. Wright-Patterson, Wright-Patterson Air Force Base, Ohio), p. 273.

Transfusion (Philadelphia) **6** (1966) No. 4.

Assay of clotting factors in outdated blood bank plasma and its potential use for therapy in hemophilia and other hemorrhagic dyscrasias. *Rosenthal, R. L.* et al. (Hospital for Joint Diseases, New York, N. Y.), p. 289.

Plasmapheresis of the patient as a method for achieving effective levels of plasma coagulation factors using fresh frozen plasma. *Perkins, H. A.* (Blood Bank of the San Francisco Med. Society, 270 Masonic Ave., San Francisco, Calif.), p. 293.

Transfusion of platelet concentrates prepared from acidified platelet-rich plasma. *Shively, J. A.* et al. (Dept. of Pathol., The Univ. of Texas M. D. Anderson Hosp., Houston, Texas), p. 302.

Platelet concentrates from acidified plasma: a method of preparation without the use of additives. *Chappell, Wanda S.* (Blood Bank Dept., National Inst. of Health, Bethesda, Md.), p. 308.

The third example of anti-Do^a. *Williams, Catherine H.* et al. (Dept. of Immunohemat., John Elliott Blood Bank of Dade County, Inc., Miami, Florida), p. 310.

Human-type blood factors in gibbons, with special reference to the multiplicity of serological specificities of human type M blood. *Wiener, A. S.* et al. (Dept. of Forensic Med., New York Univ. Med. School, Brooklyn, N. Y.), p. 311.

Blood group, serum genetic factors, and hemoglobins in Cook Islanders. I. Atiu Island. *Douglas, R.* et al. (Auckland Blood Transf. Service, Auckland C. 3, New Zealand), p. 319.

Blood group, serum genetic factors, and hemoglobins in Cook Islanders. II. Rarotonga. *Douglas, R.* et al. (Auckland Blood Transf. Service, Auckland, New Zealand), p. 324.

Anti-M in neoplastic diseases. *Vetter, O.* et al. (Blutspendedienst der Karl-Marx-Univ. Med.-Klinik, 701 Leipzig, Johannisallee, 32), p. 327.

The evaluation of a rapid card technic for ABO and Rh_0 (D) typing. *Cohen, Bernice H.* et al. (Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, Md.), p. 328.

Selection of "safe" group O blood. *Grove-Rasmussen, M.* (Blood Bank and Transfusion Service, Massachusetts General Hosp., Boston, Mass.), p. 331.

Red cell changes as observed by a simple method for continuously recording osmotic fragility. *Shields, Ch. E.* et al. (Army Med. Research Lab., Fort Knox, Kentucky), p. 336.

Heparinized blood versus ACD blood for cardiopulmonary bypass. *Sessler, A. D,* et al. (National Inst. of Health, Bethesda, Md.), p. 360.

Transfusion (Paris) **9** (1966) No. 1.

Les trois problèmes fondamentaux en matière de groupes sanguins et leurs solutions. *Wiener, A. S.* (64 Rutland Rd., Brooklyn. N. Y. 11225), p. 3.

Un cas non leucémique d'antigène H faible. *Blanc, R.* (Blood Transf. Service, Carcassone, France), p. 35.

Groupes sanguins ABO et Rh (D) dans le département de l'Indre. *Kherumian, R.* et al. (Centre Départemental de Transf. Sanguine, Chateauroux/Indre, France), p. 43.

Répartition des groupes Gc dans la population nantaise. *Guihard, D.* et al. (Centre Régional de Transf. Sanguine de Nantes, France), p. 49.

Intermediate rate freezing of human red cells. *Krijnen, H. W.* et al. (Central Lab. of the Netherlands Red Cross Blood Transf. Service, POB 200, Amsterdam), p. 53.

Microscopic observation of the freezing and thawing of suspensions of erythrocytes. *Kuivenhoven, A. C. J.* (Lab. voor Koudetechniek, Mekelweg 2, Delft, The Netherlands), p. 61.

Conservation à basse température de cellules de moelle osseuse chez le Singe. *Muller-Bérat, Christine Nicole* et al. (51—53, rue Belliard, Bruxelles), p. 65.

Études préliminaires sur la conservation du sang à la température de l'azote liquide. *Eygonnet, J. P.* et al. (Centre de Transf. Réanimation de l'Armée "Jean Julliard", Clamart, France), p. 73.

Transfusion (Paris) **9** (1966) No. 2.

Conditionnement de la solution de sérum-albumine humaine sous azote. *Guégan, M.* et al. (Centre de Transf. Réanimation de l'Armée "Jean Julliard", 1, rue Raoul-Batany, 92-Clamart, France), p. 99.

Comparaison des différents tests aux enzymes pour la recherche d'anticorps anti-Rh. *Omar, A.* et al. (Centre de Transfusion de Lyon, France), p. 103.

Groupes sanguins ABO et Rh (D) dans la région de Roanne (Loire). *Kherumian, R.* et al. (École d'Anthropologie, Paris), p. 115.

Le service de transfusion sanguine en Tchécoslovaquie. *Dobry, E.* (Inst. d'Hémat. et de Transf. Sanguine, Prague), p. 123.

Vox Sanguinis (Basel) **11** (1966) No. 5.

Genes and immunoglobulins. *Mårtensson, L.* (Dept. of Med. Microbiology, Paradisgatan 5, Lund, Sweden), p. 521.

Reactions of anti-Gm (f) sera. *Gold, E.* (Reg. Transf. Centre, Southmead Hosp., Bristol), p. 546.

Pa 1, a new inherited alpha-2-globulin of human serum. *MacLaren, J. A.* et al. (221 Longwood Ave., Boston, Mass.), p. 553.

Allotypic differentiation of rabbit gamma-globulins as a cause of posttransfusion shock. *Popielski, B.* et al. (ul. Chalubinskiego 4, Wroclaw), p. 561.

Hematological changes in mice following Freund's adjuvant administration. *Morton Jane I.* et al. (3181 S. W. Sam. Jackson, Park Road, Portland, Oregon), p. 570.

Nonhemolytic febrile transfusion reactions. *Perkins, H. A.* et al. (Irwin Memorial Blood Bank, San Francisco Med. Society, San Francisco, Calif.), p. 578.

Drug-induced haemolytic reactions due to antibodies against the erythrocyte/Dipyrone complex. *Lay, W. H.* (São Paulo Univ. School of Med., Av. Dr. Arnaldo no. 455, São Paulo, Brasil), p. 601.

A second example of anti-Ku in a patient who has the rare Kell phenotype, K^0. *Nunn, H. D.* et al. (Blood Group Reference Lab., Gatliff Road off Ebury Bridge Road, London, S. W. 1.), p. 611.

Linkage study of gamma globulin groups. *Corcoran, Patricia A.* et al. (Blood Grouping Lab., Boston, Mass.), p. 620.

Anti-Gm and anti-globulinic antibodies after multiple pregnancies. *Eyquem A.* et al. (Inst. Pasteur, 28, rue du Dr. Roux, Paris XV), p. 623.

Demonstration of leucocyte iso-antigens on skin fibroblasts by means of the cytotoxic antibody test. *Engelfriet, C. P.* et al. (Central Lab. of the Netherlands Red Cross Blood Transf. Service, POB 200, Amsterdam), p. 625.

Action of plasma from pantethine treated donors on platelet counts and hemorrhagic diathesis in thrombocytopenia. *Nicole, de P.* et al. (Lungoticino Visconti 3, Pavia, Italy), p. 631.

The second example of anti-Do^a. *Webb, A. J.* et al. (Regional Transf. Centre, Southmead Hosp., Bristol), p. 637.

Vox Sanguinis (Basel) **11** (1966) No. 6.

Prevention of Rh haemolytic disease. A method based on the post-delivery injection of the mother with anti-D antibody. *Clarke, C. A.* (Dept. of Med., The Univ. of Liverpool, Ashton, Great Britain), p. 641.

Platelet transfusion. A brief review of practical aspects. *Morrison, F. S.* (Blood Research Lab., U. S. Naval Hosp., Chelsea, Mass.), p. 656.

The purification of human placental plasminogen (profibrinolysin). *Sgouris, J. T.* et al. (Bureau of Laboratories, Michigan Dept. of Public Health, Lansing, Mich.), p. 663.

Antigenic markers on cultured human cells I. Ii, Tj^a, Donath—Landsteiner and "non-specific" autoantigens. *Franks, D.* (Dept. of Pathol., Tennis Court Road, Univ. of Cambridge), p. 674.

Subgroups of A in the South African Bantu. *Brain, P.* (National Blood Transf. Service, POB 2356, Durban, Republic of South Africa), p. 686.

The blood groups of Latvians resident in Australia. *Kariks, J.* et al. (New South Wales Red Cross Blood Transf. Service, 1 York Street, Sydney), p. 699.

Changes of $alpha_1$-lipoproteins in some hemoblastoses. *Loštický, C.* et al. (Inst. of Hemat. and Blood Transf., W. Pieck, Str. 108, Prague 10), p. 705.

The use of crystal violet in the prevention of the transfusional transmission of Chagas-Mazza disease. *Vilaseca, G. C.* et al. (Naval Hospital, J. B. Ambrosetti 699, Buenos Aires, 13), p. 711.

Failure to detect human polymorphism with modification of red cells by Bacillus. *Sloan, Helen* et al. (The New York Blood Center, 310 East 67th St., New York, N. Y.), p. 717.

The incidence of cold auto-haemagglutinins in Melanesian children and adults. *Booth, P. B.* et al. (Red Cross Blood Transf. Service, POB 24, Port Moresby, Papua, New Guinea), p. 720.

Specific binding of gamma globulin to erythrocytes. *Gergely, J.* et al. (Inst. of Haemat., Daróczi út 24, Budapest XI.), p. 724.

A contribution to the blood group frequencies in Tibetans. *Ørjasaeter, H.* et al. (State Inst. of Public Health, Geitmyrsveien 75, Oslo), p. 726.

The frequency of the Lp (a)-factor in Frankfurt/Main. *Seidl, S.* et al. (Dept. of Immunohemat., Univ. Frankfurt, Ludwig-Rehn-Strasse 14, Frankfurt am Main), p. 730.

Simple methods for obtaining purified suspensions of lymphocytes. *Czerski, P.* et al. (Dept. of Histology and Embryology, Warsaw Med. School, 5 Chalubinskiego St., Warsaw), p. 734.

A new sensitive method for studying the polymorphisms of the human low density lipoproteins. *Bütler, R.* et al. (Central Lab. of the Blood Transf. Service of the Swiss Red Cross, Wankdorfstrasse 10, 3000 Berne), p. 738.

I. BERNÁT

OZAENA

EINE MANIFESTATION DER EISENMANGELKRANKHEIT

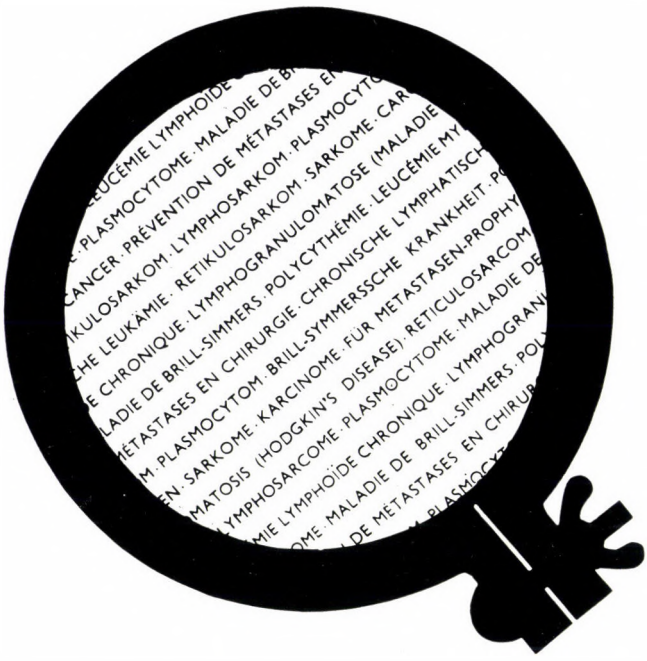
In deutscher Sprache • 152 Seiten • 52, zum Teil mehrfarbige Abbildungen und 10 Tabellen im Text • 15 × 21 cm • Ganzleinen $ 4,90; DM 19,60; £ 1 15s

Nach einer kritischen Übersicht der wichtigeren Ozaena-Theorien weist der Verfasser auf Grund klinischer Untersuchungen und Tierexperimente nach, daß die Ozaena nichts anderes als eine Sonderform der Eisenmangelkrankheit ist. Er befaßt sich eingehend mit den bei der Ozaena auftretenden neuroendokrinen Funktionsstörungen und untersucht den Ursprung der Ossifikationsstörungen und der histopathologischen Veränderungen. Schließlich zeigt er anhand seiner therapeutischen Versuche, daß die Ozaena auf Eisenpräparate gut reagiert und mit diesen in der Mehrzahl der Fälle erfolgreich behandelt werden kann.

AUS DEM INHALT:

I. Kurze Übersicht und Kritik der wichtigeren Ozaenatheorien
II. Material und Methoden
III. Klinik der Ozaena
IV. Ossifikationsstörungen bei der Ozaena. Wachstums- und Entwicklungsstörungen, Entwicklungsanomalien
V. Zusammenhang der Ozaena mit neuroendokrinen Funktionsstörungen
VI. Histopathologie der Ozaena
VII. Die Eigentümlichkeiten beim Vorkommen der Ozaena
VIII. Der Wirkungsmechanismus der Vakzinetherapie bei der Ozaena
IX. Erfahrungen mit der Eisentherapie bei der Ozaena

AKADÉMIAI KIADÓ
VERLAG DER UNGARISCHEN AKADEMIE DER WISSENSCHAFTEN
Budapest V. Alkotmány utca 21



DEGRANOL CHINOIN

Brand of mannomustine dihydrochloride (BCM)

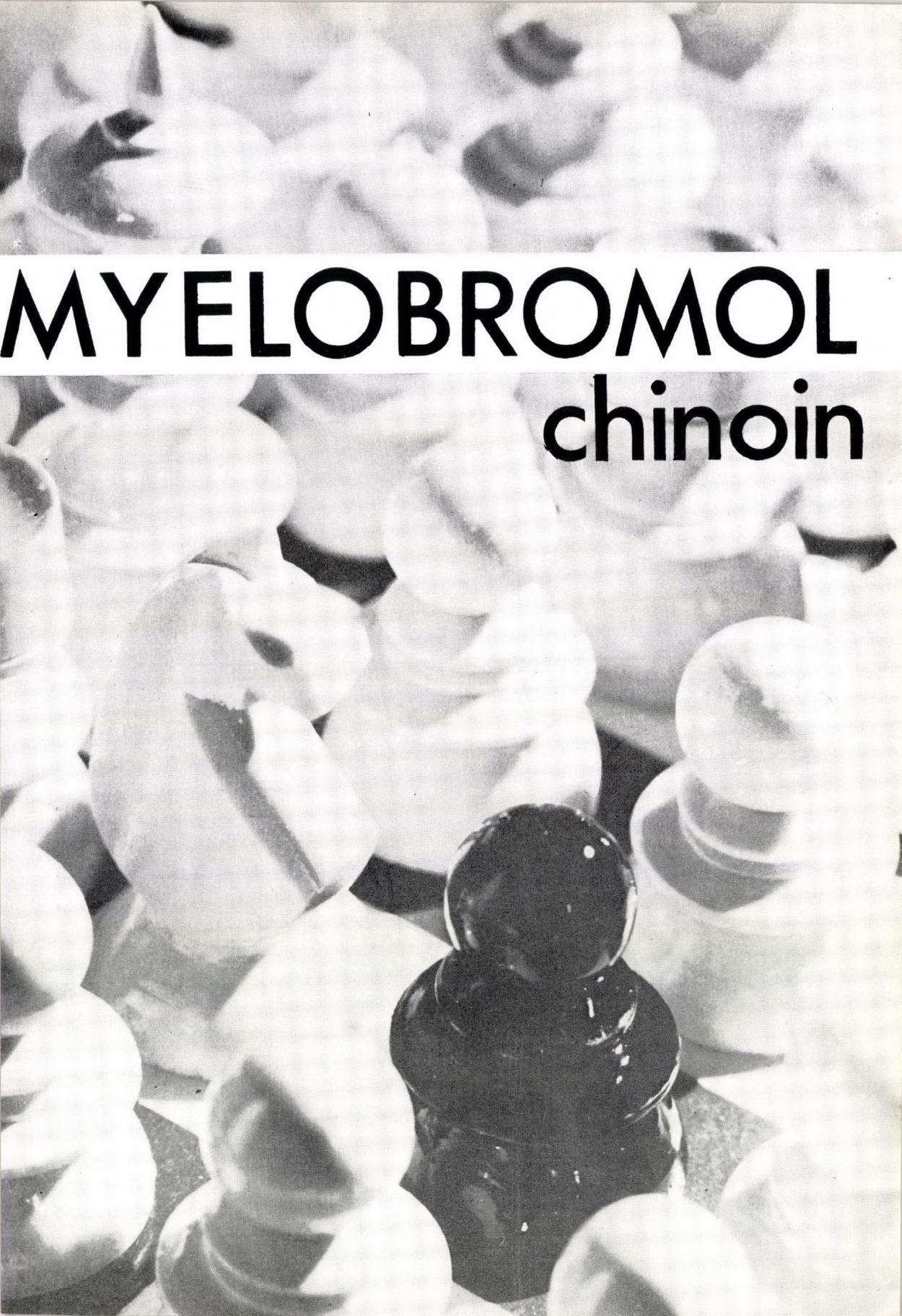
1,6-bis-(β-Chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride

Chronic lymphoid leukaemia
Hodgkin's disease
Lymphosarcoma and other haemoblastoses
In tumor surgery as chemoprophylactic
For direct injection into the cavities with tumorous effusion
DEGRANOL is remarkably safe in adequate dosage

powder ampoules of 50 mg • coated pills of 50 mg

Chemical and Pharmaceutical Works CHINOIN Ltd. • Budapest

Information and literature by MEDIMPEX, • Budapest 5 • P.O.B. 126


MYELOBROMOL
chinoin

MYELOBROMOL
chinoin

1,6-Dibromo-1,6-dideoxy-D-mannitol
tablets of 250 mg.

A specific antigranulopoietic agent

Chronic myelocyetic leukaemia
Polycythaemia vera

Highly selective action
Oral administration
No cross-resistance with other therapies
Response of patients can be controlled by dosage
MYELOBROMOL brings about the prolongation of survival

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126

Publications of
AKADÉMIAI KIADÓ
Budapest

A. BOGSCH and A. LESZLER

Diagnostic Pneumomediastinum

In English · 212 pages · 116 figures · 17 × 24 cm · Cloth · $9.80; DM 39.20; £ 3 10s

The authors discuss the injection of air into the mediastinum as a contrast medium for X-ray examinations. The history and methods of the procedure are dealt with, and its indications and contraindications as well as its special applications for facilitating diagnoses are treated.

M. JULESZ AND K. KOVÁCS

Therapie der endokrinen Krankheiten und ihre theoretischen Grundlagen

In German · 592 pages · 31 figures · 3 tables · 17 × 24 cm · Cloth $16.00; DM 64.–; £5 15s

The present work provides medical practitioners with the necessary fundamentals instead of mere formules for healing. Thus the readers will be able to proceed by themselves to further studies in the field of therapy. The chapters of the work are arranged according to theoretical and practical issues.

I. WITTMANN

Peritoneoscopy I. II.

In English · Vol. I. 187 pages · 50 figures · 21 tables · Vol. II. 500 coloured photos on 171 pages · 21 × 29 cm · Cloth · Together $32.00; DM 128.–; £11 11s

In this work the author discusses the diagnostic methods of peritoneoscopy, its history and his own extensive experience. Volume I describes the methods, instruments, indications and contraindications as well as the dangers of peritoneoscopy. Volume II is a peritoneoscopic atlas containing 500 original coloured photographs and illustrates what has been discussed in Volume I.

Publishing House of the Hungarian Academy of Sciences
Budapest V. Alkotmány utca 21

Publications of
AKADÉMIAI KIADÓ
Budapest

L. BÍRÓ and H. GRABER

Klinische Anwendung der Kortisonderivate

In German · 459 pages · 15 figures · 31 tables · 17 × 24 cm · Cloth $12.00; DM 48.–; £4 4s

The authors deal in detail with the therapeutic employment of corticoids in endocrinology, collagen-fibre diseases, rheumatic fever, cardiology, allergic diseases, haematology, kidney and hepatic diseases and in tumours. The bibliography contains almost 2500 items up to 1965. This book enumerating all Hungarian and foreign ACTH (corticoid) products will be of great value to practising physicians.

B. ISSEKUTZ

Geschichte der Arzneimittelforschung

In German · In preparation · Approx. 450 pages · 17 × 24 cm · Cloth · Approx. $15.00; DM 60.–; £5 5s

An interesting and instructive chapter of the history of civilization is presented to the reader in this volume. The author gives a comprehensive account on the history of drugs, their discovery, recognition of physiological actions, isolation of the active principles from plants, elucidation of their chemical structures, and the synthetic approach based on this information. Systematic improvements of the pharmaceutical value have led to our modern drugs that may be called, not infrequently, practically perfect.

Proceedings of the Fourth Congress of the Hungarian Assocation of Microbiologists Budapest 1964

EDITED by R. BACKHAUSZ

In English · 405 pages · 29 figures · 32 tables · 17 × 24 cm · Cloth $12.00; DM 48.–; £4 4s

This volume contains the entire text of the more important lectures, and gives abstracts of the others. The main topics are: bacteriological and epidemiological problems of enteric infections and the microbiological problems of chemotherapy. Further topics include bacterium genetics, immunology, virology and applied microbiology.

Publishing House of the Hungarian Academy of Sciences
Budapest V. Alkotmány utca 21

Medical Journals of

AKADÉMIAI KIADÓ

Budapest

Acta Chirurgica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor A. BABICS

Subject-matter: surgery, including the disciplines of general surgery, surgical aspects of gynaecology, urology, otorhinolaryngology, orthopaedics, ophthalmology as well as nerve and brain surgery, pulmonary, oral surgery, heart and blood-vessel surgery. Papers in English and German.

Published in four issues, making up a volume of some 400 pages yearly. Size: 17×24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Medica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor I. RUSZNYÁK

Subject-matter: fundamental and applied pathophysiology and its cognate fields. Papers in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17×24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Microbiologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor G. IVANOVICS

Papers in the field of microbiology mainly in English, but also in French, German, or Russian.

Published in four issues, making up a volume of some 400 to 500 pages. Size: 17×24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Morphologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor I. TÖRŐ

Subject-matter: medical morphology, normal and pathological anatomy, forensic medicine and oncology. Papers in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm.
Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Paediatrica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor P. GEGESI KISS

Papers in the field of pediatry in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm.
Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Physiologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor K. LISSÁK

Subject-matter: physiology, including the disciplines of biochemistry, biophysics, and pharmacology. Papers in English, French, German or Russian.

Published in eight issues making up two volumes of some 400 to 500 pages each yearly.
Size: 17 × 24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Bogdán István

A kézirat nyomdába érkezett: 1966 XII 13. Terjedelme: 10 (A/5) iv, 58 db ábra.

66.63222 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to 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.
Central Research Institute of the
National Blood Service
Daróczi út 24
Budapest XI, 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 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) name(s) and initials of the author(s); (2) title of paper; (3) international abbreviation of the perodical; (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 enviromental 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. Coloured 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:

ALBANIA

Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA

A. Keesing
Box 4886, GPO
Sydney

AUSTRIA

Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM

Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA

Raznoiznos
1 Tzar Assen
Sofia

CANADA

Pannonia Books
2 Spadina Road
Toronto 4, Ont.

CHINA

Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Smeckách 30
Praha 2
Postova Novinova Služba
Dovoz tisku
Vinohradska 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Postova Novinova Služba
Dovoz tlace
Leningradska 14
Bratislava

DENMARK

Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough, Northamps.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP.
GPO Post Box 1374
Bombay I

ITALY

Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA

Chulpanmul
Phenjan

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND

RUCH
ul. Wilcza 46
Warszawa

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
Bucuresti

SOVIET UNION

Mezhdunarodnaja Kniga
Moscow G—200

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA

Stechert Hafner Inc.
31 East 10th Street
New York, N. Y. 1003
Walter J. Johnson
111 Fifth Avenue
New York, N. Y. 1003

VIETNAM

Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA

Forum
Vojvode Misica broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

15. III. 1967

Index: 26341

315.871

VOLUME 1 · NUMBER 2 · 1967

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)
J. ARNAUD (Tours)
G. ASTALDI (Tortona)
I. BARTA (Pécs)
G. BAST (Rostock)
H. BEGEMANN (München)
E. BENEDEK (Budapest)
J. BERNARD (Paris)
E. BEUTLER (Duarte, California)
H. BRAUNSTEINER (Innsbruck)
W. DAMESHEK (New York)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Uppsala)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
I. GONZÁLEZ-GUZMÁN (Mexico City)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
G. C. de GRUCHY (Melbourne)
A. HÄSSIG (Bern)
L. HEILMEYER (Freiburg/Br.)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
I. A. KASSIRSKY (Moscow)
E. KELEMEN (Budapest)
A. E. KISELEV (Moscow)
E. KOWALSKI (Warsaw) †
P. J. LAH (Belgrade)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Marburg/Lahn)
A. E. MOURANT (London)
C. T. NICOLAU (Bucharest)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Szeged)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM (Liège)
G. RUHENSTROTH-BAUER (München)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Modena)
M. C. VERLOOP (Utrecht)
A. VIDEBAEK (Copenhagen)
S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

2

HAEMATOLOGIA

is a new 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 some 500 pages.

Subscription price: $12 per volume (per year). Orders may be placed with

KULTURA

Trading Company for Books and Newspapers
Budapest 62. P.O.B. 149

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

Contents

In memory of George Hevesy *(L. Kocsár)* 109
Pietschmann, H., Raab, W., Hartl, O.: Beitrag zur Hämatologie der Porphyria cutanea tarda 113
Trepel, F., Gerstmair, G., Waubke, R., Pichlmaier, H., Begemann, H.: Beziehungen zwischen der Abstoßung homologer Hauttransplantate und verschiedenen Lymphozytentypen im peripheren Blut 119
Danon, D., Shiffmann, A., Efrati, P.: Analysis of osmotic fragility as a routine test in clinical practice 131
Astaldi, G., Airò, R.: Questions and answers on leukemia problems 143
Lennes, G., André, A., Lhoste, C.: Comparaison du taux moyen de complément dans un échantillon de malades et dans un échantillon de personnes normales. — Considérations méthodologiques et application au cas des ulcères duodénaux et gastriques 163
Gergely, J., Árky, I., Medgyesi, G. A.: Iodination of human IgG and its papain-insensitive population 169
Hořejší, J., Svoboda, M., Švorcová, V.: A contribution to the study of calcium and phosphate metabolism in Kahler's disease 171
Péter, Á., Romhányi, J., Letenyei, K.: Cerebrospinal fluid diagnosis of meningeal leukaemia 181
Arnold, R.: Zur Bedeutung der Zellelektrophorese für hämatologische Fragen 193
Szász, I., G. Szelényi, J., Gárdos, G., R. Hollán, S.: Effect of different washing solutions on some characteristics of erythrocytes 207
Open Forum 217
Book Review 231
Abstracts 232
From the international literature on haematology 243

VOLUME 1 · NUMBER 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) · J. ARNAUD (Tours) · G. ASTALDI (Tortona) · I. BARTA (Pécs) · G. BAST (Rostock) · H. BEGEMANN (München) · E. BENEDEK (Budapest) · J. BERNARD (Paris) · E. BEUTLER (Duarte, California) · H. BRAUNSTEINER (Innsbruck) · W. DAMESHEK (New York, N. Y.) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle, Washington) · L. GARBY (Uppsala) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest) · I. GONZÁLEZ-GUZMÁN (Mexico City) · F. GRÁF (Budapest) · A. GRAFFI (Berlin) · G. C. de GRUCHY (Melbourne) · A. HÄSSIG (Bern) · L. HEILMEYER (Freiburg/Br.) · L. P. HOLLÄNDER (Basel) · J. HOŘEJŠÍ (Prague) · M. HRUBIŠKO (Bratislava) · I. A. KASSIRSKY (Moscow) · E. KELEMEN (Budapest) A. E. KISELEV (Moscow) · E. KOWALSKI (Warsaw) † · P. J. LAH (Belgrade) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Marburg/Lahn) · A. E. MOURANT (London) · C. T. NICOLAU (Bucharest) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Szeged) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM (Liège) G. RUHENSTROTH-BAUER (München) · V. SERAFIMOV-DIMITROV (Sofia) I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIĆ (Belgrade) E. STORTI (Modena) · M. C. VERLOOP (Utrecht) · A. VIDEBAEK (Copenhagen) S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES
BUDAPEST 1967

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Bogdán István
A kézirat nyomdába érkezett: 1967. IV. 22. Terjedelme: 10,75 (A/5) ív
67.63780 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

In Memory of George Hevesy

George Hevesy, physicochemist, winner of the Nobel Prize for Chemistry, one of the greatest atomic physicists of all times, deceased July 5th, 1966. George Hevesy was one of the few who used atomic energy for peaceful purposes from the very beginning. As an all-round and inventive scholar, he made revolutionary discoveries in physicochemistry, biochemistry, physiology and also in radiobiology.

Not as an appraisal, since many an official fori have done so while he was still alive, much more as a tribute of respect to him, in the name of Hungarian researchers we feel it our duty to give a brief survey of his life and scientific career. It should all the more be our duty to publish this appraisement in *Haematologia* because in no other branch of medicine or biology have the nuclear methods introduced by Hevesy been applied so widely as in the field of haematology. Investigations based on Hevesy's scientific achievements have brought about revolutionary changes in the aspect and knowledge of haematology based hitherto chiefly on morphological and clinical experience.

George Hevesy was born in Budapest in 1885. He started to study chemistry in Budapest. His studies led him from Budapest via Berlin to Freiburg in Breisgau where he took his doctor's degree in 1908. Afterwards, together with Lorenz, he worked in Zürich at the Institute for Chemistry headed by Willstaetter. While in Zürich, he was an enthusiastic visitor of the lectures delivered by Einstein. In 1911 he went to Manchester to work under Rutherford. While working with Rutherford, he was an eyewitness to the epoch-making discovery of the atomic nucleus. Here, among others, he collaborated with Moseley and Geiger. Also, his life-long close friendship with Niels Bohr dates back to his early Manchester days. Whenever passing through Paris, he never failed to visit Marie Curie, Irene Curie and Frédéric Joliot.

In January 1913 he qualified as a Reader in physico-chemistry at the Budapest University where he lectured for a semester. As an Austrian subject, he served in the Austro-Hungarian Army from 1914 to 1918. On request by Loránd Eötvös, in the autumn of 1918, he undertook to be the director of the II. Institute for Physics at the Budapest University. By the middle of 1919 he left Hungary. Invited by Niels Bohr, he joined Bohr's team at the Institute for Theoretical Physics to be

built in 1920 in Copenhagen. In 1926 Hevesy was appointed professor of physical chemistry at Freiburg University and at the same time the head of its Institute for Physics and Chemistry.

The study of radioactivity constituted Hevesy's main research field. As early as 1913, he discovered that chemical elements might be labelled with such tiny amounts of radioactive substances which could not even be determined by chemical methods earlier. This finding marked the beginning of the first era of using isotope indicators. The introduction of the first biological tracer technique is also one of Hevesy's merits. In 1923 he began to study how labelled lead compounds were taken up by plants and how the seedling labelled this way returned the resulting radioactive salts to its inactive surroundings. These experiments, performed with thorium-B and radium-D as radioactive tracers, were the first to permit some insight into the metabolic dynamism of the living organism.

The second era of using isotopic indicators started by Urey's discovery of deuterium. As early as 1934 Hevesy used deuterium in a biological study of interchange of water molecules between goldfish and the surrounding water.

By making use of the principle of isotope dilution, also introduced by him, he determined the water content and water metabolism of the human body.

The discovery of artificial radioactivity marks the start of the third era. As an ingenious mind, Hevesy immediately realized the possibility of its uses in biology. Using ^{32}P, in 1935 he was the first to demonstrate the dynamics of phosphorus metabolism in rats. This experiment opened up a long series of biological studies performed with radioactive isotopes.

In 1940, by making use of the "isotope dilution principle" again, Hahn and Hevesy were the first to describe the determination of blood volume by the use of ^{32}P. Next, Hevesy elaborated a method for labelling erythrocytes, using ^{42}K (1951) and Th-B (1953) as indicators. In co-operation with Ottesen, in 1945, he determined the life-cycle of the red corpuscles of the hen by isotope techniques.

The use of isotopes as tracers in radiobiological research work is also due to Hevesy's ingenious mind.

In 1938, Hevesy together with Hans v. Euler, studied the radiosensitivity of nucleic acids in normal and tumorous tissues, using ^{32}P as an indicator.

The discovery of activation analysis, a new branch of isotope technique, really unfolding these days, is also one of Hevesy's merits. This, indeed, represents the fourth era of isotope techniques.

In 1936, in co-operation with Hilde Levi, he determined quantitatively the europium content in gadolinium and demonstrated the possibility of measuring rare earth elements by the same method. This method is becoming more and more important not only in experimental but more recently also in clinical medicine. Almost 20 years had to elapse before researchers realized the importance of this method.

In 1943 Professor Hevesy moved to Stockholm to work at the Institute for Organic Chemistry and Biochemistry of the University of Stockholm. From this period spent in Stockholm I should like to focus the attention on his radiobiological

studies connected with nucleic acid metabolism, bicarbonate, glucose and fatty-acid metabolism.

In recent years he studied iron metabolism, cancer anaemia, radiosensitivity of extramedullary haematopoiesis in fish and plasma copper turnover under normal and cancerous conditions.

In search for the missing element 72 Hevesy and Coster studied zirconium minerals. By a very protracted fractionated crystallization of ammonium zirconium hexafluorides they succeeded to prepare "hafnium" in a pure state

In recognition of his scientific merits, in 1943 he was awarded the Nobel Prize for Chemistry, which the war prevented him from receiving until 1944.

In 1957 he became a member of the Peace-Class of the German Order pour la Mérite.

In 1959 he received the "Atoms for Peace" award, Niels Bohr being the only other person in the world to have done so previously.

It would be a hard task indeed, to enumerate his most important scientific decorations, totalling about 30. To mention but some of them, he was elected "doctor honoris causa" by the Universities of London, Cambridge, Uppsala, Copenhagen, Freiburg and Budapest. He was the holder of the Copley-, Faraday-, Bailey-, Sylvanus-, Thomson- and Bohr-medals.

He was a great scholar and a modest man. Everyone who knew him, liked and respected him. We, who had the honour to know him personally, will never forget him. And mankind will respect him for generations for his epoch-making scientific discoveries.

L. KOCSÁR

Beitrag zur Hämatologie der Porphyria cutanea tarda*

H. Pietschmann, W. Raab, O. Hartl

II. Medizinische Universitätsklinik und Medizinisch-chemisches Institut der Universität Wien

(Eingegangen am 30. November 1966)

Es wird über 30 meist schwere bzw. fortgeschrittene Fälle von Porphyria cutanea berichtet. Während die die Leberfunktion betreffenden Laboruntersuchungen zum Teil ein pathologisches Resultat ergaben, wies die Hämopoese in der Mehrzahl weitgehend normale Verhältnisse auf. Da die Leberveränderungen schubweise verlaufen, sollten die Patienten in regelmäßigen Abständen untersucht werden. Wegen ihrer hohen Empfindlichkeit wird auf die Bedeutung vor allem der Transaminasebestimmung im Serum hingewiesen.

Die alte Einteilung von Günther [6] unterscheidet – abgesehen von den symptomatischen bei Intoxikationen – akute, chronische und kongenitale Formen der Porphyrie. Schmid et al. [20] stellten fest, daß bei der kongenitalen Porphyrie die abnorme Porphyrinbildung in den Erythroblasten des Knochenmarkes vor sich geht, während bei den anderen Arten die Porphyrinbildung in der Leber gestört ist. Die häufigste Form der hepatischen Porphyrie stellt die Porphyria cutanea tarda dar. Diese Erkrankung gewinnt zunehmend an Interesse, da einerseits ihre Häufigkeit zunimmt und andererseits sich in der letzten Zeit neue therapeutische Möglichkeiten ergeben haben. Da insbesonders die Diagnose dieser Lebererkrankung allein aus den Hauterscheinungen mit hoher Wahrscheinlichkeit gestellt werden kann, hat sie auch für den praktischen Arzt großes Interesse.

Die Porphyria cutanea tarda (P. c. t.) weist eine eindeutige Bevorzugung des männlichen Geschlechtes auf. Im gemeinsam bearbeiteten Krankengut der II. Medizinischen Klinik und der II. Hautklinik fanden sich unter 30 Fällen nur 3 Frauen. Die erste Manifestation der Erkrankung erfolgte zwischen dem 33. und 75. Lebensjahr, im Durchschnitt um das 50. Lebensjahr. Die Patienten mit Porphyria cutanea tarda waren vorwiegend in Berufen tätig, die mit einem Aufenthalt im Freien verbunden sind, wie Maurer, Landwirte usw. Bei 22 Patienten wurde ein übermäßiger Alkoholkonsum festgestellt, dem somit in erster Linie als auslösender Faktor Bedeutung zukommt. 3 Patienten hatten eine Hepatitis durchgemacht, bei 2 bestand ein Barbituratabusus und ein Patient wurde mehreren Salvarsankuren unterzogen.

Bei allen Patienten fanden sich die dem Dermatologen geläufigen charakteristischen Hauterscheinungen, die den Patienten zum Arzt führen: die Blasenbildun-

* Teilweise vorgetragen am Kongreß für Hämatologie, Budapest 1965.

Tabelle 1

Serumeisenspiegel und Blutbild bei Porphyria cutanea tarda (30 Fälle)

	Path. Werte	Mittelwert	Bereich
Serumeisen	15	176/100 ml	72—313/100 ml
Serumeiweiß	4	7,4 g%	5,5—9,3 g%
Erythrozyten	2	4,3 Mill./cmm	3,4—5,8 Mill./cmm
Hämoglobin	3	14,6 g%	12,8—17,6 g%
Hb_E	0	31,8	27—34
Leukozyten	4	6 300/cmm	3 200—16 000/cmm

gen an licht- und traumaexponierten Körperstellen. Gesicht und Hände waren regelmäßig dunkelbraun pigmentiert. An den Händen und vereinzelt im Gesicht fanden sich neben den frischen Blasen scharf begrenzte Narben als Residuen älterer Veränderungen.

Während die erythropoetischen Formen der Porphyrie durch die Zeichen der hämolytischen Anämie, durch einen Milztumor und durch massive Uroporphyrin I-Ablagerungen in allen Organen charakterisiert sind, weist die Porphyria cutanea tarda prinzipiell andere hämatologische Verhältnisse auf. Die Fälle können auf Grund des Serumeisenspiegels in 2 Gruppen eingeteilt werden (Ippen [10, 11]): mit normalem bzw. erhöhtem Serumeisen. Bei unseren Patienten war der Eisenspiegel in 15 normal, in den übrigen Fällen war er mehr oder weniger stark erhöht. Dagegen unterscheiden sich wie aus der Tabelle 1 zu entnehmen ist, die Mittelwerte der Erythrozytenzahl, der Hämoglobinkonzentration und der Leukozytenzahl nicht signifikant von den Werten Gesunder. Nur bei einzelnen Patienten war die Erythrozyten-, Leukozytenzahl oder die Hämoglobinkonzentration geringgradig erhöht (Heilmeyer [7, 8]). Das gleiche gilt für die Perzentsätze des Differentialblutbildes. Auch die Untersuchung des Knochenmarkes ergab in der Regel normale Verhältnisse, nur vereinzelt war die Erythropoese gesteigert. In mehreren Fällen wurde jedoch eine Vermehrung der Plasmazellen festgestellt. Zytochemische Reaktionen (wie alkalische Phosphatase nach Kaplow, unspezifische Esterase mit Naphthol ASD-Acetat nach Löffler und ATPase nach Wachstein und Meisel) zeigten keine Auffälligkeiten.

Im Gegensatz dazu ergaben die Enzymaktivitätsbestimmungen im Serum häufig ein pathologisches Resultat. Da bisher abgesehen von einzelnen kasuistischen Mitteilungen keine Serumenzymbestimmung bei der Porphyria cutanea tarda bekannt geworden ist, haben wir die Aktivitäten einer Reihe diagnostisch wichtiger Enzyme verfolgt (Bestimmung im optischen Test). Die Aktivität der leberspezifischen Sorbitdehydrogenase war bei 17 von 21 Patienten gesteigert, die der FDP-Aldolase in 13 von 24 Fällen. Hingegen wies die Laktatdehydrogenase mit zwei Ausnahmen eine normale Aktivität auf. Die ausgeprägtesten Aktivitätssteigerungen ließen die Transaminasen erkennen. Die Glutamat-Oxalacetat-Transaminase

war in 23 von 26 Fällen, die Glutamat-Pyruvat-Transaminase 19 von 21 Fällen erhöht (Mittelwert 39,5 mU.; Mittelwert bei Gesunden: 8,2 mU.). Die Aktivität der alkalischen Phosphatase war in allen untersuchten Fällen normal (Tabelle 2).

Tabelle 2

Serumenzyme bei Porphyria cutanea tarda

Enzym	Anzahl	path. Werte	Mittelw. in mU./ml	Mittelw. b. Norm.
Glutam.-Pyruvat-Transaminase	21	19 (90%)	39,5 (6—96)	8,2
Glutam.-Oxalacet.-Transaminase	26	23 (88%)	35,1 (13—77)	9,4
Sorbit-Dehydrogenase	21	17 (81%)	2,6 (0—9,9)	0,3
FDP-Aldolase	24	13 (54%)	4,4 (1—14)	3,2
Lactat-Dehydrogenase	26	2 (8%)	139,0 (48—297)	110,4
alkal. Phosphatase	8	0	31,7 (11,7—49,7)	30,0

Die Bestimmungen der Serumenzyme, insbesondere der Transaminasen, ergaben bei P.c.t. häufiger pathologische Resultate als die übrigen Leberfunktionsproben. Da die Leberveränderungen bei P. c. t. schubweise verlaufen, sind wiederholte Untersuchungen der Transaminasen im Serum für die Verlaufskontrolle von großem Wert. Liegen erhöhte Serumenzymaktivitäten vor, so ist anzunehmen, daß der Krankheitsprozeß progredient ist. Deshalb kommt den Enzymbestimmungen neben der diagnostischen eine wichtige prognostische Bedeutung zu (Neumayr und Pietschmann [15]).

In einigen Fällen fand sich der Bromsulphaleintest im Normalbereich, obwohl die Serumenzymaktivitäten gesteigert waren; ein derartiges Verhalten ist bei Lebererkrankungen recht selten. Bei anderen Patienten fanden sich trotz fortgeschrittener Leberveränderungen nur relativ geringe Zunahmen der Serumenzyme; dies kann damit erklärt werden, daß der Enzymaustritt aus den Leberzellen bei fortgeschrittenem zirrhotischem Umbau parallel mit der Reduktion des Leberparenchyms abnimmt (Amelung [1]). Andererseits werden akute, toxische Einflüsse (Alkohol) bei vorgeschädigter Leber sehr empfindlich durch die Transaminaseaktivität angezeigt (Lachnit und Pietschmann [12]).

Auf die verschiedenen Leberfunktionsproben, über die an anderer Stelle ausführlich berichtet wird (Pietschmann, Raab und Hartl [18]), kann im einzelnen nicht eingegangen werden, die Tabelle 3 gibt einen Überblick. Wegen ihrer prinzipiellen Bedeutung ist jedoch kurz auf die Ergebnisse der Leberbiopsie hinzuweisen. Im Verlauf der Porphyria cutanea tarda fehlt manchmal eine vermehrte Porphyrinausscheidung im Harn (inaktives Stadium), regelmäßig ist jedoch ein vermehrter Porphyringehalt der Leber nachweisbar, der das wesentliche pathogenetische Moment für die Störung der Leberfunktion darstellt (Brugsch [2], Holzner und Niebauer [9]). Entsprechend einer primären Hepatose tritt erst in weiterer Folge eine Aktivierung des Mesenchyms ein, die zum zirrhotischen Umbau führt.

Tabelle 3

Laboratoriumsbefunde bei Porphyria cutanea tarda

	Anzahl	patholog. in
Serumbilirubin	24	11
Gammaglobuline	24	14
Thymolprobe	28	11
Bromsulphaleintest	26	20
Galaktoseprobe	20	16

Auf Grund des familiären Vorkommens der P. c. t. messen Waldenström und Haeger [21] kongenitalen Faktoren beim Zustandekommen der Erkrankung wesentliche Bedeutung bei und bezeichnen die P. c. t. als "inborn error of metabolism". Die echten hereditären Formen scheinen jedoch die Minderheit darzustellen, da trotz gerichteter Anamnese nur selten ein familiäres Vorkommen festzustellen ist; im vorliegenden Krankengut konnte nur bei einem Patienten ein Familienangehöriger eruiert werden, der ebenfalls an einer P. c. t. erkrankt war.

Zur Untermauerung der klinischen Befunde wurden tierexperimentelle Untersuchungen an Ratten durchgeführt, denen Hexachlorbenzol verfüttert wurde (Raab, Pietschmann und Hartl [19]). Es wurde das Blutbild, das Knochenmark sowie die Leber fluoreszenzmikroskopisch untersucht. Nach 6–8 Wochen konnte eine Zunahme der Porphyrinausscheidung im Harn, Serumfermentveränderungen und Leberveränderungen analog der Porphyria cutanea tarda festgestellt werden. Der Hämoglobinwert lag bei den hexachlorbenzolvergifteten Ratten jedoch signifikant niedriger als bei den Kontrolltieren (10,6 $\pm$ 0,8 g% gegenüber 13,2 $\pm$ $\pm$ 1,4%, $p < 0{,}01$). Das Differentialblutbild wies granulozytäre Vorstufen und lymphozytäre Reizformen auf. Das Knochenmark ließ Zeichen einer toxischen Schädigung erkennen. Diese auffälligen hämatologischen Befunde weisen auf die wesentlich höhere Aktivität der experimentellen Intoxikation im Vergleich zur Porphyria cutanea tarda hin. Auch beim Menschen führt die Intoxikation mit Hexachlorbenzol zum Auftreten von Symptomen der P. c. t. (Cam et al. [4], Centingil und Ozen [5], Ockner und Schmid [16]).

Bei allen Patienten wurde neben der diätetischen und medikamentösen Therapie eine Aderlaßbehandlung durchgeführt, auf die erstmals Ippen [10] hingewiesen hat. Wahrscheinlich werden durch den Entzug des Hämoglobins die im Überschuß vorhandenen Porphyrinkörper zur Nachbildung des Hämoglobins herangezogen. Mit der Verminderung des Überschusses an Porphyrinkörpern verschwindet die Licht- und Traumaempfindlichkeit der Haut. Es wurden bis zu 6 Liter Blut, durchschnittlich insgesamt 2–3 Liter entnommen (je 250 bis 500 ml in 1–8 wöchigen Abständen). Wie bei der Hämochromatose tritt auch bei der Porphyria cutanea tarda nie eine Erschöpfung der Blutbildung ein, das Hämoglobin sank unter der Aderlaßtherapie nie unter 12 g%. Bei einigen Fällen wurde eine

Desferrioxamintherapie eingeleitet. Die Aderlaßbehandlung stellt unseres Erachtens zwar nur eine symptomatische Behandlungsform dar, ist aber zweifellos von beträchtlichem therapeutischem Wert.

Literaturverzeichnis

1. Amelung, D.: Fermentdiagnostik interner Erkrankungen. Georg Thieme Verlag, Stuttgart 1964.
2. Brugsch, J.: Die Klinik der Phorphyrie. *Gastroenterologia 97*, 370 (1962)
3. Cam, C.: Intoksikasyona bagli der porfirinleri. *Dirim* (Istambul) *34*, 11 (1959).
4. Cam, C. and Nigogosyan G.: Acquired toxic porphyria cutanea tarda due to hexachlorbenzene. *J. amer. med. Assoc. 183*, 88 (1963).
5. Centingil, A. I. and Ozen, M. A.: Toxique porphyria. *Blood 16*, 1002 (1960).
6. Günther, H.: Die Bedeutung der Hämatoporphyrine in Physiologie und Pathologie. *Erg. allg. Path. 20*, 608 (1922).
7. Heilmeyer, L.: Lehrbuch der inn. Med. Springer. Berlin, Göttingen, Heidelberg 1955.
8. Heilmeyer, L.: *Münch. med. Wschr. 102*, 871 (1960).
9. Holzner J. H. und Niebauer, G.: Fluoreszenzmikroskopischer Nachweis von Porphyrin in der Leber. *Acta hepato-splen.* Stuttgart *10*, 110 (1963).
10. Ippen, H.: Allgemeinsymptome der späteren Hautporphyrie (Porphyria cutanea tarda) als Hinweis für deren Behandlung. *Dtsch. med. Wschr. 86*, 127 (1961).
11. Ippen, H. und Gehrmann, G.: Untersuchungen zur Erythropoese bei der Porphyria cutanea tarda. *Klin. Wschr. 39*, 1960 (1961).
12. Lachnit, V. und Pietschmann, H.: Activity of serum glutamic-oxaloacetic-transaminase and aldolase in workers exposed to halogenated hydrocarbons. *Industr. Med.* Chicago *29*, 523 (1960).
13. Lever, W. F.: Ablagerungskrankheiten körpereigener Stoffwechselprodukte. In: Handbuch der Haut- und Geschlechtskrankheiten, J. Jadasohn, Ergänzungswerk, Bd. III, Teil 1. S. 190—221, Springer-Verlag, Berlin—Göttingen—Heidelberg 1962.
14. Löffler, H.: Zytochemischer Nachweis von unspezifischer Esterase in Ausstrichen. *Klin. Wschr. 39*, 1220 (1961).
15. Neumayr, A. und Pietschmann, H.: Die diagnostische und prognostische Bedeutung des Serumgehaltes an Glutaminsäure-Oxalessigsäure-Transaminase bei Erkrankungen der Leber. *Wien. Zschr. Inn. Med. 40*, 161 (1959).
16. Ockner, R. K. and Schmid, R.: Acquired porphyria in man and rat due to hexachlorobenzene intoxication. *Nature* (London) *189*, 499 (1961)
17. Pietschmann, H.: Unterschiede der Aktivitäten von Serumcholinesterase und Serum-Glutaminsäure-Oxalessigsäure-Transaminase bei Erkrankungen der Leber. *Wien. Zschr. Inn. Med. 41*, 409 (1960).
18. Pietschmann, H., Raab, W. und Hartl, O.: Leberfunktion bei Porphyria cutanea tarda. *Wien. Zschr. Inn. Med. 47*, 349 (1966).
19. Raab, W., Pietschmann, H. und Hartl, O.: Serumenzymaktivitäten bei der experimentellen Porphyrie der Ratte. *Arzneimittelforsch.* Im Druck.
20. Schmid, R., Schwartz, S. und Watson, C. J.: Neuere Ergebnisse auf dem Gebiet der Porphyrien. *Acta haemat.* Basel *10*, 150 (1953).
21. Waldenström, J. and Haeger, B.: The liver in porphyria cutanea tarda. *Ann. Int. Med. 53*, 286 (1960).
22. Wiedmann, A.: Einige Bemerkungen zur Porphyria cutanea tarda. *Wien. med. Wschr. 76*, 426 (1964).

Beziehungen zwischen der Abstoßung homologer Hauttransplantate und verschiedenen Lymphozytentypen im peripheren Blut*

F. Trepel, G. Gerstmair, R. Waubke, H. Pichlmaier, H. Begemann

I. Medizinische Abteilung des Städt. Krankenhauses München-Schwabing, und Chirurgische Universitäts-Klinik, München

(Eingegangen am 2. Dezember 1966)

Bei Ratten bestehen Korrelationen zwischen den Verhalten einiger Lymphozytenuntergruppen und der Abstoßung von homologen Hauttransplantaten. Bei einer Ganzkörperbestrahlung von 500 R 1 Tag vor der Transplantation oder bei einer extrakorporalen Bestrahlung des Blutes mit 1000 R 4 Tage nach der Transplantation wurden die Transplantate durchschnittlich um 10 bzw. um 8 Tage verzögert abgestoßen. Der Beginn des immunologischen Abstoßungsvorganges fiel in beiden Bestrahlungsmodellen mit dem Anstieg der Gruppe der kleinen hellplasmatischen Lymphozyten zusammen. Eine weniger enge Korrelation wurde zwischen der Transplantatabstoßung und großen bzw. mittelgroßen hellplasmatischen Lymphozyten sowie den mikronukleolären kleinen Lymphozyten gefunden. Eine Beziehung zu den kleinen, mittelgroßen und großen basophilen Lymphozyten sowie zu den Monozyten und Lymphomonozyten ergab sich nicht. Die mögliche Rolle des kleinen hellplasmatischen Lymphozyten als immunkompetente Zelle wird diskutiert.

Ein homologes Transplantat (d. h. Gewebe, das von einem Individuum auf ein nicht verwandtes Individuum gleicher Spezies übertragen wird) wird vom Empfänger in der Regel nach mehreren Tagen abgestoßen. Diese Abstoßung ist bekanntlich ein immunologisches Phänomen, das durch lymphatische Zellen (Übersicht bei Billingham und Silvers [5]), genauer: durch kleine Lymphozyten (Übersicht bei Gowans und McGregor [13]) des Empfängers ausgelöst wird.

Die Überlebenszeit von homologen Transplantaten kann durch Senkung der Lymphozytenzahl mit Hilfe einer länger dauernden Lymphableitung aus dem Ductus thoracicus (Pichlmaier und Junginger [20]) oder durch eine Schädigung von lymphatischen Zellen mit Hilfe von Zytostatika (Übersicht bei Schwartz [23]) oder ionisierenden Strahlen (Dempster et al. [9], Übersicht bei Govaerts [11]), erreicht werden. Da vermutet wird, daß nur ein Teil der lymphatischen Zellen immunologisch kompetent, ein anderer aber inaktiv ist (Übersicht bei Gowans und McGregor [13] bzw. Gorman und Chandler [10]), wäre es wichtig zu wissen, ob der immunologisch aktive Zelltyp durch immunosuppressive Maßnahmen selektiv oder nur unspezifisch zusammen mit den anderen lymphatischen Zellen betroffen wird.

* Diese Untersuchungen wurden von der Deutschen Forschungsgemeinschaft unterstützt.

Wir stellten uns die Frage, ob nach verschiedenartiger Anwendung von Röntgenstrahlen die Überlebenszeit homologer Transplantate Beziehungen zur Lymphozytenzahl im allgemeinen oder zu einer morphologisch erfaßbaren Lymphozytenuntergruppe im besonderen erkennen läßt.

Material und Methoden

Wir verwenden in Koloniezucht gehaltene weiße männliche Sprague–Dawley-Ratten (Gewicht 250 bis 300 g) als Transplantatempfänger und männliche Tiere des dunkelhäutigen Inzuchtstammes Berlin-Druckerey IX* als Transplantatspender.

Ausgestanzte Hauttransplantate von ca 1,5 cm Durchmesser wurden den dunklen Ratten entnommen und in einem gleich großen Haut-Stanzloch der weißen Ratten mittels eines Gewebeklebstoffes** implantiert. Zur Kontrolle von unspezifischen Veränderungen durch die Operation erhielt jede weiße Ratte neben dem homologen Transplantat ein autologes auf der kontralateralen Seite der Rückenhaut. Die Transplantate wurden täglich auf Nekrosezeichen untersucht. Die Nekrose ist durch Braun-Schwarz-Färbung und Eintrocknung der transplantierten Haut makroskopisch feststellbar. Die zur Nekrose führende zelluläre Infiltration des Transplantats beginnt 4–6 Tage vor der äußerlich sichtbaren Nekrose (Brent [8]), womit sich bei normaler wie bei verzögerter Abstoßung der Beginn der eigentlichen immunologischen Abwehrreaktion aus dem Zeitpunkt der makroskopischen Nekrose durch Substraktion von 4–6 Tagen berechnen läßt. Außerdem stellten wir den Zeitpunkt der vollständigen Abstoßung des nekrotischen Hautmaterials fest, die der Nekrose meist nach 8 bis 10 Tagen folgt.

Zwei Formen der Röntgenbestrahlung wurden angewandt: 1. eine hochdosierte subletale Ganzkörperbestrahlung von 500 R, 2. eine 20minütige extrakorporale Bestrahlung des durch einen Shunt zwischen Arteria Carotis und Vena Jugularis zirkulierenden Blutes mit 1000 R ohne sonstige Strahlenbelastung des Tieres (Einzelheiten der Methode bei Sanguinetti et al. [22]).

Die Ganzkörperbestrahlung erfolgte 1 Tag *vor* der Transplantation. 6 Tiere wurden bestrahlt, 6 nur transplantierte Tiere dienten als Kontrolle. Die extrakorporale Bestrahlung wurde 4 Tage *nach* der Transplantation bei weiteren 6 Tieren vorgenommen. Dabei wurden als Kontrolle 6 transplantierte Tiere verwendet, die statt einer extrakorporalen Bestrahlung nur einen 20 Minuten durchströmten Shunt zwischen Arteria Carotis und Vena Jugularis erhielten. Die Termine der verschiedenen Bestrahlungsarten ergaben sich aus früheren Erfahrungen: Ganzkörperbestrahlung vor Transplantation wirkt immunosuppressiv, nach Transplan-

* Wir danken Herrn Dr. T. v. Kreybig, Toxikolog. Inst. der Universität Tübingen, für die Überlassung dieser Tiere.

** Versuchsklebstoff CHBS 64 Ingelheim, C. H. Boehringer, Ingelheim/Rhein.

tation dagegen eher abstoßungsfördernd (Govaerts [11]). Extrakorporale Bestrahlung am 4. Tag nach Transplantation hatte den besten immunosuppressiven Effekt; am zweiten Tag war der Effekt schwächer und am sechsten Tag nicht mehr nachweisbar (Sanguinetti et al. [22]).

Ausstriche und Leukozytenzahlen vom Schwanzvenenblut der Tiere wurden an mehreren Tagen vor Bestrahlung und 1, 3, 5 usw. bis 21 Tage nach Bestrahlung gewonnen. Die methanol-fixierten Blutausstriche wurden nach May-Grünwald-Giemsa und Stockinger-Kellner (Einzelheiten bei Waubke et al. [26]) gefärbt. Nach der May-Grünwald-Giemsa-Färbung wurden die mononukleären Zellen differenziert in: Monozyten, Lymphomonozyten, große, mittlere und kleine Lymphozyten mit tief-basophilem Zytoplasma und große, mittlere und kleine Lymphozyten mit hellerem Zytoplasma. Einzelheiten dieser Differenzierung wurden an anderer Stelle beschrieben (Trepel et al. [24], [25], Waubke et al. [26]). Nach der Stockinger-Kellner-Färbung wurden die kleinen Lymphozyten eingeteilt in solche mit einem oder mehreren großen Nukleolen (=makronukleoläre Lymphozyten) und in solche mit kleinen oder ohne Nukleolen (= mikronukleoläre Lymphozyten). Einzelheiten der Lymphozytendifferenzierung mit der Nukleolenfärbung wurden von Grundmann [14] und Begemann et al. [3] mitgeteilt.

Ergebnisse

Die homologen Transplantate wurden nach beiden Formen der Bestrahlung um 7 bis 10 Tage verzögert abgestoßen, wobei, wie die Tabelle zeigt, der immunosuppressive Effekt der Ganzkörperbestrahlung etwas stärker war als derjenige der Extrakorporalbestrahlung. Die autologen Kontrolltransplantate heilten bei allen Tieren ein.

Tabelle 1

Die Termine (in Tagen) der verschiedenen Grade der Transplantatabstoßung nach Ganzkörperbestrahlung (GKB) und Extrakorporalbestrahlung (EKB) im Vergleich zu den Kontrollwerten ohne Bestrahlung. Hinter den Bereichsgrenzen sind die arithmetischen Mittelwerte der Abstoßungszeiten in Klammern angegeben

	Beginn der immunolog. Reaktion	Vollständige Nekrose	Vollständige Abstoßung	Verlängerung gegenüber Kontrolle
GKB	13—16*	17—21 (19,5)	23—35 (28)	9—10
Kontrolle	4— 5*	8—10 (9,3)	13—24 (18,9)	
EKB	10—16*	14—21 (18,3)	19—32 (27,1)	
Kontrolle	5— 6*	9—11 (10)	16—25 (20)	7— 8

* Der Termin des Beginns der immunologischen Reaktion ist durch Abzug von 4—5 Tagen vom jeweiligen Zeitpunkt der vollständigen Nekrose errechnet.

Die Veränderungen der verschiedenen Leukozytengruppen nach Ganzkörperbestrahlung und Extrakorporalbestrahlung sind detailliert an anderer Stelle beschrieben worden (Waubke et al. [26]). Während die immunosuppressive Wirkung der Ganzkörperbestrahlung nur geringgradig stärker war als die der extrakorporalen Bestrahlung, hatte die Ganzkörperbestrahlung einen wesentlich intensiveren depressorischen Effekt auf die mononukleären Blutzellen als die Extrakorporalbestrahlung. Durch die Ganzkörperbestrahlung werden alle Zelltypen

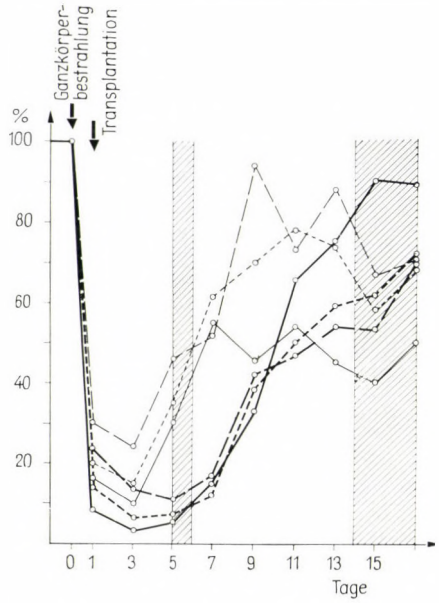


Abb. 1. Das Verhalten verschiedener Lymphozyten-Untergruppen nach Ganzkörperbestrahlung. Die erste Säule gibt die Zeit der Transplantatabstoßung bei den Kontrolltieren mit normalen Lymphozytenzahlen an, die zweite Säule die Zeit der verzögerten Abstoßung bei den bestrahlten Tieren. (Mittelwerte von 6 Tieren)
——— kleine helle Lymphozyten. — — — mittlere helle Lymphozyten.
— — — große helle Lymphozyten. ——— kleine basophile Lymphozyten.
— — — mittlere basophile Lymphozyten. — — — große basophile Lymphozyten

bis zum 21. Tag mehr oder weniger stark vermindert, durch die Extrakorporalbestrahlung nur die Monozyten, Lymphozyten, die hellen sowie mikro- und makronukleolären Lymphozyten bis zum 7. Tag.

Bei einer Synopsis von Transplantatabstoßungszeit und Veränderung der einzelnen mononukleolären Gruppen im Blut nach Bestrahlung ergeben sich einige Korrelationen:

Nach Ganzkörperbestrahlung sind zu dem Zeitpunkt, an dem die immunologische Transplantatreaktion bei den Kontrolltieren mit normalen Leukozytenzahlen beginnt (5–6 Tage nach Bestrahlung, 4–5 Tage nach Transplantation), alle Zellgruppen unter 50% des Ausgangswertes gesenkt (Abb. 1 und 2). Die

basophilen Lymphozyten und die Monozyten wie Lymphomonozyten steigen in den nächsten Tagen auf ihre Ausgangswerte oder ein neues Niveau an, ohne daß die Transplantatreaktion einsetzt. Der Beginn der immunologischen Abstoßung (14–17 Tage nach Bestrahlung, 13–16 Tage nach Transplantation) trifft zusammen mit einem Anstieg der hellen Lymphozyten (große: auf ca 60%, mittlere: auf 60–70% und kleine auf 80–90%) und der makro- sowie mikronukleolären Lymphozyten auf 80% (Abb. 2).

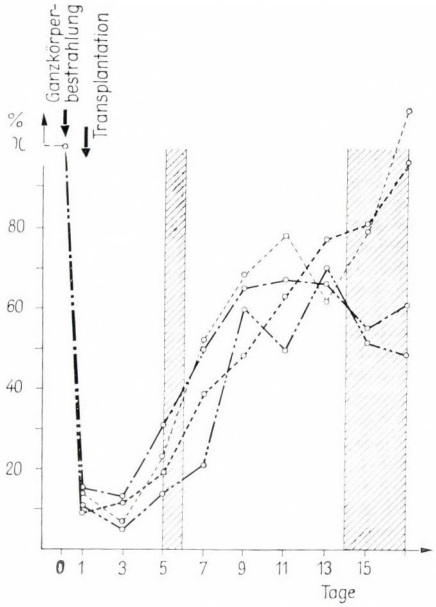


Abb. 2. Das Verhalten von makro- und mikronukleolären kleinen Lymphozyten sowie von Monozyten und Lymphomonozyten nach Ganzkörperbestrahlung (Mittelwerte von 6 Tieren). Erklärung der Säulen: siehe Abb. 1

..... makronukleoläre Lymphozyten. ..... mikronukleoläre Lymphozyten. —·— Monozyten. —··— Lymphomonozyten

Nach Extrakorporalbestrahlung sind zum Zeitpunkt der Transplantatabstoßung bei den Kontrolltieren, die kaum veränderte Leukozytenzahlen aufweisen (1–2 Tage nach Bestrahlung, 5–6 Tage nach Transplantatsetzung) die basophilen Lymphozyten kaum unter das Ausgangsniveau erniedrigt, während die Monozyten auf 60 bis 80%, die kleinen hellen Lymphozyten auf 60%, die großen hellen auf 50%, die mittleren hellen auf 40% und die Lymphomonozyten auf 30% des Ausgangswertes vor Bestrahlung gesenkt sind (Abb. 3 und 4). Die makronukleolären kleinen Lymphozyten liegen gleichzeitig bei 70%, die mikronukleolären bei 50% der Kontrollwerte (Abb. 4). Bei Beginn der durch die Bestrahlung verzögerten Transplantatreaktion (6–12 Tage nach Bestrahlung, 10–16 Tage nach

Transplantation) erreichen oder überschreiten alle vorher erniedrigten Zelltypen die 90%-Grenze ihres Ausgangswertes. Die makronukleolären kleinen Lymphozyten hatten ihren 100%-Wert bereits einen Tag vor Beginn der Abstoßungsphase überschritten.

Zusammenfassend ist eine zeitliche Korrelation zwischen verzögerter Transplantatreaktion und Wiederanstieg der Zellzahl bei folgenden Mononukleären

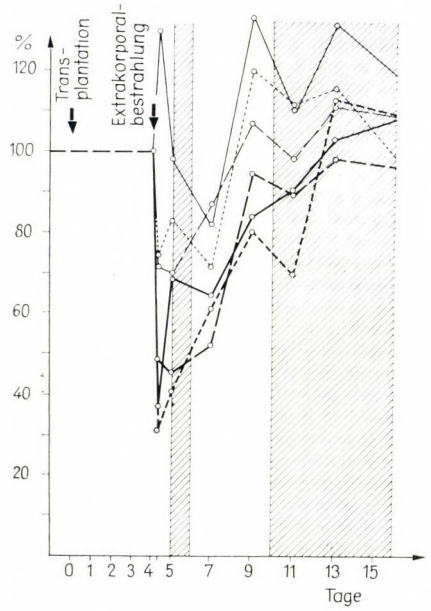


Abb. 3. Das Verhalten verschiedener Lymphozyten-Untergruppen nach Extrakorporalbestrahlung. Bedeutung der graphischen Linien und Erklärung der Säulen: siehe Abb. 1

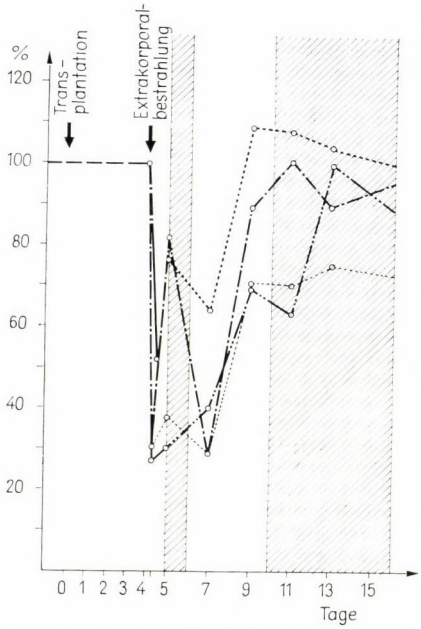


Abb. 4. Das Verhalten von makro- und mikronukleolären kleinen Lymphozyten sowie von Monozyten und Lymphomonozyten nach Extrakorporalbestrahlung. Bedeutung der graphischen Linien; siehe Abb. 2 und Erklärung der Säulen: siehe Abb. 1

festzustellen: Kleine, mittlere und große helle Lymphozyten und mikronukleoläre kleine Lymphozyten bei beiden Bestrahlungsmodellen; makronukleoläre kleine Lymphozyten nur nach Ganzkörperbestrahlung. Niemals fand sich eine zeitliche Korrelation mit den großen, mittleren oder kleinen basophilen Lymphozyten.

Diskussion

Wenn man das Verhalten der einzelnen mononukleären Zellgruppen mit der Abstoßung von homologen Hauttransplantaten nach Röntgenbestrahlung korreliert und daraus Schlüsse auf die Funktion der Zellen ziehen will, so muß

man voraussetzen, daß die einzelnen Zellgruppen in sich homogen sind, zumindest aber, daß innerhalb einer Gruppe alle Zellen annähernd gleiche Reaktionen auf die Strahlen zeigen.

Ehe die immunologische Stellung der Blutzellen aus diesem Vergleich abgeleitet werden könnte, müssen der Eingriff und der Zeitpunkt der Bestrahlung innerhalb des immunologischen Reaktionsablaufes definiert werden:

Bei der Ganzkörperbestrahlung trifft die Schädigung das ganze immunologisch aktivierbare Zellsystem: sowohl im Blut als auch im lymphatischen Gewebe. Der Zeitpunkt der Bestrahlung 1 Tag vor der Transplantation bedeutet, daß die gesamte immunologische Reaktion in der Nachbestrahlungsphase abläuft. Dabei dürfte vor allem der afferente Teil der immunologischen Reaktion, die sogenannte Induktionsphase, während derer das Antigen immunkompetente Rezeptor-Zellen zur Proliferation anregt (Gowans und McGregor [13], Schwartz [23]), betroffen und verlängert sein, weil er in den Zeitraum der schwersten Strahlenschädigung fällt. Der efferente Teil der Immunreaktion, nämlich die Produktion von immunologisch kompetenten Effektorzellen (Gowans und McGregor [13]), setzt vermutlich verspätet aber dann intensiv in der Erholungsphase nach Bestrahlung ein.

Die Extrakorporalbestrahlung trifft naturgemäß nur Blutzellen, während das lymphatische Gewebe unangetastet bleibt. Der Bestrahlungszeitpunkt am 4. Tag nach Transplantation liegt wohl schon nach dem Abschluß der immunologischen Induktionsphase, deren Dauer auf 24 bis 48 Stunden nach Antigenkontakt geschätzt wird (Schwartz [23]). Selbst wenn die Induktionsphase länger dauern würde, so wird sie durch eine Extrakorporalbestrahlung nicht wesentlich gestört, denn eine Extrakorporalbestrahlung 48 Stunden nach Transplantation hatte nur einen geringen immunosuppressiven Effekt (Sanguinetti et al. [22]). Die Extrakorporalbestrahlung greift mit großer Wahrscheinlichkeit nur in die efferente Phase der Immunreaktion ein, indem immunkompetente Effektorzellen, die auf dem (Blut)wege zum Transplantat sind, vernichtet werden. Spezifisch sensibilisierte Effektorzellen müssen bereits 4 Tage nach Transplantation im Blut vorhanden sein, sonst wäre die Extrakorporalbestrahlung zu diesem Zeitpunkt wirkungslos. Vom sechsten Tag an sind sie auch von Billingham et al. ([4], [5]) nachgewiesen worden.

Da wir eine gewisse Immunkompetenz einer Zellgruppe nur aus dem Vergleich zur Transplantatabstoßung erschließen, können wir bei Ganzkörper- wie Extrakorporalbestrahlung nur den efferenten Teil der immunologischen Reaktion erfassen. Es ist jedoch mit einer unspezifischen Methode, wie der hier verwendeten, unmöglich, die wenigen spezifisch gegen ein Hauttransplantat gerichteten immunkompetenten Effektorzellen im Blut (nach Prendergast [21] handelt es sich um weniger als 0,2% der Mononukleären) nachzuweisen. Wir können nur eine Zellgruppe daraufhin prüfen, ob in ihr die spezifischen immunkompetenten Zellen enthalten sein könnten, und ob sie vielleicht die Summe aller auch gegen andere Antigene gerichteten Immunozyten darstellt.

Wie beschrieben, treffen diese Bedingungen nur für die großen, mittleren und kleinen hellen Lymphozyten sowie für die mikronukleolären Lymphozyten zu.

Gowans [12] schloß große und mittelgroße Lymphozyten der Ductus-thoracicus-Lymphe, ohne basophile von hellplasmatischen Zellen zu trennen, von immunologischen Reaktionen aus, während Everett et al. nach immunologischer Reizung das Anschwellen kleiner Lymphozyten bis zum Umfang mittlerer Lymphozyten im Ductus thoracicus beobachteten. Es ist bemerkenswert, daß den Monozyten nach unseren Kriterien keine wesentliche immunologische Effektorrolle zuzubilligen ist. Das steht in scheinbarem Gegensatz zu den Befunden, nach denen immunologische Reaktionen mit monozytenreichen Entzündungsexsudaten (Braunsteiner et al. [7], Billingham et al. [6]) übertragen werden konnten, denn auch diese Exsudate enthalten Lymphozyten (Harris et al. [15]), deckt sich jedoch mit den Vorstellungen nach denen der Monozyt lediglich in der Induktionsphase am immunologischen Geschehen beteiligt ist (Übersicht bei Gowans und McGregor [13]).

Nach diesen Befunden und den eingangs erwähnten Voraussetzungen bleibt nur der kleine und möglicherweise der mittlere helle Lymphozyt bzw. der mikronukleoläre kleine Lymphozyt, der gleichfalls eher ein helles als ein basophiles Zytoplasma hat, als mutmaßlich immunkompetent übrig. Ist die Immunkompetenz hellplasmatischer Lymphozyten nicht ein Widerspruch in sich? Immunkompetenz auf zytologischer Basis schien bisher stets mit Zytoplasmabasophilie verknüpft: 1. Bei Plasmazellen bzw. Plasmoblasten; 2. bei großen pyroninophilen (= basophilen) Zellen, die durch Umwandlung kleiner Lymphozyten aufgrund immunologischer Reizung in vivo (Gowans [12]) oder in vitro (Hurvitz et al. [16], Knight et al. [17] u. a.) entstehen. 3. Darüberhinaus beschrieben Wiener et al. [27] und Prendergast [21] im zellulären Infiltrat von homologen Transplantaten zahlreiche basophile Zellen, darunter auch solche von Lymphozytengröße.

Diese Befunde beweisen jedoch noch nicht, daß immunkompetente kleine Lymphozyten ein basophiles Zytoplasma haben müssen:

Zu 1. Die Plasmazellen erfüllen ihre immunologische Funktion durch Antikörper- d. h., durch Eiweißsynthese. Diese ist bekanntlich von einem hohen RNS-Gehalt im Zytoplasma, der die Basophilie bedingt, abhängig. Für die immunologisch aktiven Lymphozyten ist die Synthese von eiweißhaltigen Antikörpern (z. B. Immunglobulinen) umstritten und wurde bisher in größerem Umfang noch nicht sicher nachgewiesen (Übersicht bei Govaerts [11] sowie Gowans und McGregor [13]). Es wird auch die Ansicht vertreten, daß Lymphozyten nur zytophile Antikörper, die von anderen Zellen synthetisiert worden sind, an der Zelloberfläche tragen (Govaerts [11]). Ein für die immunologische Funktion der Lymphozyten wesentlicher Stoff, der Transfer-Faktor (Lawrence [18]), gehört weder zu den bisher bekannten Immunglobulinen noch zur typischen RNS. Es ist also keine conditio sine qua non, daß die immunkompetenten Lymphozyten ein basophiles (RNS-reiches) Zytoplasma haben müssen.

Zu 2. Die großen basophilen Zellen, die sich aus immunologisch gereizten kleinen Lymphozyten entwickeln, sind nach allen Befunden nicht die eigentlichen immunologischen Effektorzellen, sie stehen vielmehr am Wendepunkt der immunologischen Afferenz zur Efferenz und produzieren die Effektorzellen, nämlich kleine Lymphozyten (Gowans und McGregor [13]). Aus basophilen Stammzellen

müssen nicht unbedingt basophile Endzellen hervorgehen, wie der Vergleich von Myeloblasten und Granulozyten zeigt. Ebenso ist es nicht ausgeschlossen, daß sich hellplasmatische kleine Lymphozyten aus basophilen Vorstufen entwickeln.

Zu 3. Die beobachteten zahlreichen basophilen mononukleären Zellen im Infiltrat bei Transplantatabstoßung müssen nicht die immunkompetenten Zellen sein. Prendergast [21] wies nach, daß nur ein sehr kleiner Teil der infiltrierenden Mononukleären immunkompetent sein kann, während McCluskey et al. [19] zeigten, daß mindestens 80% der Infiltratzellen bei der Allergie vom verzögerten Typ, die der Homotransplantatreaktion ähnelt (Brent [8] sowie Gowans und McGregor [13]), nicht immunkompetent sind. Es ist demnach möglich, daß die neben den zahlreichen basophilen Rundzellen im Infiltrat vorhandenen hellplasmatischen Zellen (Prendergast [21]) die eigentlichen Immunzellen sind.

Für eine Beziehung zwischen hellplasmatischen Lymphozyten und immunologischen Reaktionen vom verzögerten Typ sprechen folgende Befunde:

1. Das Ausbleiben der Transplantatabstoßung während der Depressionsphase der hellplasmatischen Lymphozyten nach Röntgenbestrahlung und das Zusammentreffen der Abstoßung mit dem Wiederanstieg der kleinen hellen Lymphozyten. Im Gegensatz dazu konnte keine Korrelation zu den basophilen Lymphozyten gefunden werden.

2. Ein gleichartiger Befund bei Ratten, die durch mehrtägige Ductus-thoracicus-Drainage lymphopenisch gemacht worden waren: Diese Tiere stießen Transplantate verzögert zu dem Zeitpunkt ab, als die kleinen hellen Lymphozyten wieder anstiegen, während keine zahlenmäßigen Beziehungen zu den kleinen basophilen Lymphozyten bestanden (Pichlmaier und Junginger [20]).

3. Ähnliche Verhältnisse bei zytostatika-behandelten BCG-immunisierten Meerschweinchen: Die Allergie vom verzögerten Typ (Tuberkulintest) war im allgemeinen reduziert, wenn die hellplasmatischen Lymphozyten stark vermindert waren, während die Menge der basophilen Lymphozyten keinen Einfluß zu haben schien (unveröffentlichte Befunde unseres Labors).

4. Gewisse zahlenmäßige Beziehungen bei der antigenbedingten Transformation kleiner Lymphozyten in vitro: Bei der Ratte lassen sich wahrscheinlich wesentlich mehr als die bisher beobachteten 19% der Blutlymphozyten transformieren (Knight et al. [17]). Beim Menschen ließen sich 52–76% der Blutlymphozyten transformieren (Bach und Hirschhorn [1], Hurvitz und Hirschhorn [16]). Im Rattenblut sind 40 bis 75% der Lymphozyten hellplasmatisch. – In der Ductus-thoracicus-Lymphe der Ratte fand Gowans [12] bis zu 5% immunkompetente kleine Lymphozyten. Die Ductus-Lymphe der Ratte enthält über 90% basophile und unter 10% hellplasmatische Lymphozyten.

5. Die beim Menschen beobachtete Vermehrung von azurgranulierten Blutlymphozyten bei immunologischen Reizzuständen (Begemann [2]): Ein Teil der hellplasmatischen Lymphozyten der Ratte weist gleichfalls Azurgranulationen auf, die andererseits in basophilen Lymphozyten nicht gesehen werden.

6. Die besonders lange Lebensdauer der immunologisch kompetenten Lymphozyten (Gowans und McGregor [13]). Die hellplasmatischen Lymphozyten

haben im Durchschnitt einen langsameren Turnover als die basophilen (Trepel et al. [24] und Waubke et al. [26]).

Trotzdem reichen die vorliegenden Befunde nicht aus, die Identität von kleinen hellplasmatischen Lymphozyten und immunkompetenten Zellen des Blutes zu beweisen. Es haben sich lediglich Anhaltspunkte für diese Auffassung ergeben. Technisch schwierigere gezielte Untersuchungen werden zur endgültigen morphologischen Identifizierung des immunkompetenten Lymphozyten nötig sein.

Literaturverzeichnis

1. Bach, F. and Hirschhorn, K.: Lymphocyte interaction: a potential histocompatibility test in vitro. *Science 143*, 813—814 (1964).
2. Begemann, H.: Klinische und experimentelle Beobachtungen am immunisierten Lymphknoten. Freiburg 1953
3. Begemann, H., Rastetter, J. und Fink, U.: Zur Klassifizierung der Lymphozyten. *Med. Klin. 58*, 706—709 (1963).
4. Billingham, R. E., Silvers, W. K. and Wilson, D. B.: Adoptive transfer of transplantation immunity by means of blood borne cells. *Lancet I*, 512 (1962).
5. Billingham, R. E. and S. Silvers, W. K.: Sensitivity to homografts of normal tissues and cells. *Annual Rev. Microbiol. 17*, 531 (1963).
6. Billingham, R. E., Silvers, W. K. and Wilson, D. B.: Further studies on adoptive transfer of sensitivity to skin homografts. *J. Exp. Med. 118*, 397—420 (1963).
7. Braunsteiner, H., Paertan, J. and Thumb, N.: Studies on lymphocytic function. *Blood 13*, 417 (1958).
8. Brent, L.: Tissue transplantation immunity. *Progr. Allergy 5*, 271—348 (1958).
9. Dempster, W. J., Lennox, B. and Boag, J. W.: Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host. *Brit. J. Exp. Path. 31*, 670—679 (1950).
10. Gorman, J. G. and Chandler, J. W.: Is there an immunologically incompetent lymphocyte? *Blood 23*, 17 (1964).
11. Govaerts, A.: Les Anticorps de Transplantation. Brüssel—Paris 1964.
12. Gowans, J. L.: The fate of parental strain small lymphocytes in F_1-hybrid rats. *Ann. N. Y. Acad. Sci. 99*, 432—455 (1962).
13. Gowans, J. L. and McGregor, D. D.: The immunological activities of lymphocytes. *Progr. Allergy 9*, 1—78 (1965).
14. Grundmann, E.: Zur Morphologie der Lymphozyten. *Schweiz. Med. Wschr. 40*, 1186—1197 (1961).
15. Harris, T. N., Harris, S. and Farber, M. B.: Studies on the transfer of lymph node cells. VII. The transfer of cells from blood and peritoneal exudates incubated in vitro with antigenic material derived from Shigella paradysenteriae. *J. Exp. Med. 104*, 663—673 (1956).
16. Hurvitz, D. and Hirschhorn, K.: Suppression of in vitro lymphocyte responses by chloroquine. *New England J. Med. 273*, 23—26 (1965).
17. Knight, S., Ling, N. R., Sell, S. and Oxnard, C. E.: The transformation in vitro of peripheral lymphocytes of some laboratory animals. *Immunology 9*, 565—574 (1965).
18. Lawrence, H. S.: The delayed type of allergic inflammatory response. *Am. J. Med. 20*, 428—447 (1956).
19. McCluskey, R. T., Benacerraf, B. and McCluskey, J. W.: Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions. *J. Immunol. 90*, 466—477 (1963).
20. Pichlmaier, H. und Junginger, T.: Befunde, die 1967 publiziert werden.

21. Prendergast, R. A.: Cellular specificity in the homograft rejection. *J. Exp. Med. 119*, 377—387 (1964).
22. Sanguinetti, F. A., Gerstmair, G., Eisenmann, R., Waubke, R., Rüdenholz, A., Trepel, F. und Pichlmaier, H.: Röntgenbestrahlung des Blutes im extrakorporalen Kreislauf und ihre Bedeutung für die Homotransplantation bei der Ratte. *Arch. Klin. Med.* im Druck.
23. Schwartz, R. S.: Immunosuppressive drugs. *Progr. Allergy 9*, 246—303 (1965).
24. Trepel, F. und Rastetter, J.: Untersuchungen zur funktionellen Differenzierung der mononukleären Leukozyten. Tagung der Deutschen Gesellschaft für Hämatologie, Innsbruck 1965, Blut: im Druck (1967).
25. Trepel, F., Waubke, R. und Begemann, H.: Phagozytose durch Lymphozyten. *Klin. Wschr. 44*, 256—261 (1966).
26. Waubke, R., Gerstenmair, G., Trepel, F., Pichlmaier, H. und Begemann, H.: Die Wirkung von Röntgen-Ganzkörper- und Extrakorporal-Bestrahlung auf die Lymphozyten des peripheren Blutes. *Haemat. 1*, 45—60 (1967).
27. Wiener, J., Spiro, D. and Russell, P. S.: An electron microscopic study of the homograft reaction. *Amer. J. Path. 44*, 319 (1964).

Analysis of Osmotic Fragility as a Routine Test in Clinical Practice*

D. DANON, ANNA SHIFFMANN, P. EFRATI

Section of Biological Ultrastructure, the Weizmann Institute of Science, Rehovoth, Israel and Medical Department B and the Laboratory for Blood Morphology and Cytology, Kaplan Hospital, Rehovoth, Israel

(Received December 20, 1966)

A rapid micro-method for recording red cell osmotic fragility by continuous decrease of salt concentration using the Fragiligraph Model D-2, is described. The blood of 601 cases randomly taken from a department of Internal Medicine was thus tested. About a third of the cases had abnormal osmotic fragility. The results indicate that a more extensive routine use of the test even for cases in which no obvious hematological symptoms are apparent might be of value.

Introduction

Conventional laboratory procedures for determining red cell osmotic fragility are laborious and time-consuming. This is probably one of the reasons why they are seldom employed in clinical practice, unless a pathological state of the red blood cell is actually suspected. Another reason is probably the poor resolution of the conventional test, due to the limited number of points forming the actual curve. As a result of these two limitations little data have been assembled regarding the erythrocyte osmotic fragility picture in pathological conditions which are not of a characteristically hematological nature [1 – 6]. In order to gather such data, and generally allow the performance of the test as routine clinical practice, a method is needed which is rapid, requires neither large volumes of blood nor special preparations, and is sufficiently uncomplicated so that it can be carried out by a laboratory technician without special skills.

Recently, a rapid micro-method has been developed by means of which red cell osmotic fragility curves ("fragiligrams") can be automatically recorded [7].

The time required to carry out a single test is of the order of 5 minutes and only a very small quantity of blood is required (0.04 ml of a 1 : 30 dilution of whole blood). The recorded curve is practically continuous.

The measurement is based on subjecting the cell suspension to a continuous decrease in salt concentration by dialysis, and recording the light transmission

* This work has been partially presented as a preliminary report in the Third Congress of the Asian and Pacific Society of Haematology, and was supported by grant 5/E-2 of the Ford Foundation.

through the blood suspension as hemolysis proceeds. Using this method, a study has been made of the osmotic fragility of red blood cells in patients suffering from various diseases not necessarily of a hematological nature, taken at random from hospitalized cases in a Dept. of Internal Medicine. Cumulative and derivative fragiligrams were recorded, and the results classified on the basis of the clinical diagnosis. No attempt at follow-up was made, i.e. whether the abnormal osmotic fragility determined in some cases later returned to within the normal range was not checked.

Materials and Methods

Blood was withdrawn by means of venipuncture into test-tubes containing heparin or citrate as anticoagulant. Fragiligraph Model D2 (Elron, Electronic Industries Ltd., Haifa, Israel) was used.

The samples were diluted 1 to 30 in isotonic NaCl solution, buffered to pH 7.3 with veronal acetate buffer, and an aliquot of the suspension was introduced into a microcuvette of 0.04 ml capacity two walls of which are made of dialyzing membrane. This microcuvette which is the container cell of the Fragiligraph is held in a distilled water bath which can be maintained at thermostatically controlled temperatures. The cell is positioned so that the membrane window lies in the light path of an electrooptical system consisting of a light source and a photoelectric cell connected to the recording system. Light transmission is initially low, owing to absorption and dispersion due to the presence of the cells. As the medium surrounding the erythrocytes becomes more hypotonic because of dialysis and salt diffusion across the membrane, osmotic hemolysis of the red blood cells takes place. The gradual increase in the number of hemolyzed cells with decreasing ionic strength of the medium results in a progressive increase in the light transmissibility of the suspension, continuous recording of light transmission through the cell suspension during the whole process provides a cumulative osmotic fragility curve.

The ordinate of the graph represents degree of hemolysis and the fragiligram provides a measure of osmotic fragility as a practically continuous curve, rather than in discrete measurements.

A derivative curve, representing incremental hemolysis can be recorded (simultaneously or alternatively) by transmitting the signal from the photoelectric cell through a differentiating amplifier. Since the dialysis causes the salt concentration to decrease exponentially with time, the linear time scale of the recorded graph is essentially identical with an inverse logarithmic scale of salt concentration.

For high accuracy, quantitative measurement of the salt concentration of the suspension is monitored and can be recorded simultaneously with the cumulative or derivative curves. Direct correlation between fragility and salt concentration can thus be obtained, which facilitates comparison of data with that acquired by other methods.

Salt concentration is monitored indirectly by measuring the increasing conductivity of the water surrounding the cell which contains the sample, as dialysi

proceeds. When the blood dilution (1 : 30) is accurately prepared, the base line of the cumulative curve indicates the hematocrit.

Fragiligrams of 601 patients were recorded. On the basis of the final clinical diagnosis on leaving hospital, the patients were divided into 13 groups. The osmotic fragility of the red blood cells was classified as normal (N), increased fragility (I) or decreased fragility (D) and tabulated according to the group divisions.

Results and Discussion

Of the 601 cases examined, 168 abnormal fragiligrams were recorded, 118 showing increased osmotic fragility outside the normal range, and 50 decreased fragility. Tables 1 to 13 present the osmotic fragility findings for the different clinically diagnosed groups. These results are summarized in Table 14. Figs 1 and 2 show typical recordings, Fig. 3 the normal range.

Table 1

1. Blood diseases

Diagnosis	Total	Fragility			
		N.	D.	I.	W. R.*
Hemolytic anemia	8	4	2	1	1
Iron deficiency anemia	9	5	4	—	—
Megaloblastic anemia	2	2	—	—	—
Dimorphic anemia of pregnancy	1	—	—	1	—
Thalassemia minor	1	1	—	—	—
H-Hemoglobinopathy	1	1	—	—	—
Acute leukemia	1	—	—	1	—
Chronic lymphatic leukemia	4	4	—	—	—
Myeloid leukemia	2	2	—	—	—
Lymphosarcoma	2	—	—	2	—
Agranulocytosis	1	1	—	—	—
Myelofibrosis	4	3	1	—	—
Thrombocytopenia	2	—	2	—	—
Thrombotic thrombocytopenic purpura	1	—	—	—	1
Circulating anticoagulants	1	1	—	—	—
Waldenstrom's macroglobulinemia	3	2	—	1	—
Cryoglobulinemia	1	—	1	—	—
Multiple myeloma	3	2	—	1	—
Gaucher's disease	1	—	—	1	—
Hemochromatosis	2	1	1	—	—
	50	29	11	8	2

* Wide range

Table 2

Diseases of the heart

Diagnosis	Total	Fragility		
		N.	D.	I.
Rheumatic heart disease	2	2	—	—
Myocarditis	3	3	—	—
A. S. H. D.	59	47	2	10
Congestive heart failure	20	16	1	3
Cardiomyopathy	2	2	—	—
Benign non-specific pericarditis	4	2	—	2
Hypertensive cardiovascular disease	4	4	—	—
	94	76	3	15

Table 3

Diseases of the lung

Diagnosis	Total	Fragility		
		N.	D.	I.
Tb of lung	2	2	—	—
Pneumonia	19	17	—	2
Pleuropneumonia	4	3	1	—
Bronchopneumonia	9	8	—	1
Infected bronchiectasis	3	3	—	—
Acute bronchitis	4	3	—	1
	41	36	1	4

Table 4

Diseases of the hepato-biliary tract (Pancreas included)

Diagnosis	Total	Fragility		
		N.	D.	I.
Infectious hepatitis	5	2	2	1
Cirrhosis of liver	13	9	3	1
Amebic liver abscess	2	1	—	1
Echinococcus of liver	1	1	—	—
Dubin Johnson's disease	1	—	—	1
Hepatosplenomegaly	3	1	—	2
Acute cholecystitis	2	1	—	1
Cholelithiasis	22	16	3	3
Acute pancreatitis	4	2	2	—
	53	33	10	10

Table 5

Diseases of the digestive tract

Diagnosis	Total	Fragility		
		N.	D.	I.
Stricture of esophagus	1	1	—	—
Peptic ulcer	26	16	2	8
Pyloric stenosis	1	1	—	—
Ulcerative colitis	4	2	—	2
Colitis	4	4	—	—
Gastroenteritis	2	1	1	—
Diverticulosis of large intestine	3	2	—	1
	41	27	3	11

Table 6

Diseases of the urinary system

Diagnosis	Total	Fragility		
		N.	D.	I.
Acute glomerulonephritis	5	4	—	1
Chronic glomerulonephritis	9	4	2	3
Nephrotic syndrome	2	1	—	1
Acute renal failure	4	2	2	—
Chronic renal failure	4	3	—	1
Pyelonephritis	10	8	—	2
Polycystic kidney	1	1	—	—
Nephrolithiasis	1	1	—	—
	36	24	4	8

Table 7

Metabolic diseases

Diagnosis	Total	Fragility		
		N.	D.	I.
Gout	3	3	—	—
Hypothyroidism	5	4	1	—
Hyperthyroidism	5	5	—	—
Diabetes mellitus	33	29	3	1
Congenital dwarfism	1	1	—	—
Sheehan's syndrome	1	—	1	—
Acromegaly	2	2	—	—
Obesity	1	1	—	—
	51	45	5	1

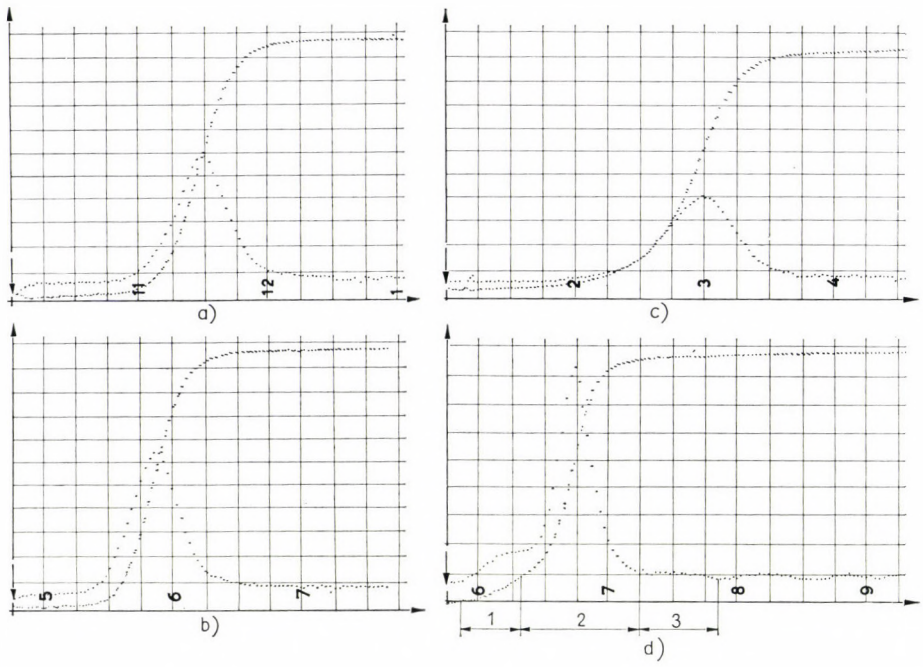


Fig. 1. Photograph of original simultaneously recorded cumulative and derivative curves made with the Fragiligraph Model D-2. *a)* Normal Blood. *b)* Increased fragility in a case of myocardial infarction. *c)* Increased resistance in a case of peptic ulcer with hypochromic anemia. *d)* A case of infectious hepatitis. The derivative curve, recorded with higher amplification than usual, shows that in this case the red cells consist of at least three populations of various osmotic fragility. Ordinate — degree of hemolysis, the plateau indicating complete hemolysis. Abscissa — time in minutes, every vertical line = 30 sec.

Table 8

Diseases of connective tissue and collagen

Diagnosis	Total	Fragility		
		N.	D.	I.
Rheumatic fever	18	13	1	4
Polyarthritis	1	—	—	1
Rheumathoid arthritis	10	8	—	2
Disseminated LE	3	3	—	—
Myositis	1	—	—	1
Myalgia	1	—	—	1
Paget's (bone) disease	1	1	—	—
	35	25	1	9

Table 9

Infectious diseases

Diagnosis	Total	Fragility		
		N.	D.	I.
Malaria	1	1	—	—
Schistosomiasis	1	1	—	—
Brucellosis	2	2	—	—
Colisepticemia	1	1	—	—
Typhoid fever	1	1	—	—
Gluteal abscess	1	1	—	—
Staphylococcal septicemia	3	2	1	—
Acute tonsillitis	1	1	—	—
Sinusitis	4	1	1	2
Upper respiratory infection	3	1	—	2
Prostatitis	2	2	—	—
Dysenteria	1	1	—	—
Mumps orchitis	3	1	1	1
Infectious mononucleosis	4	3	—	1
Meningo-encephalitis	1	1	—	—
Encephalitis	2	1	1	—
F. U. O.	24	20	2	2
Meningococcic meningitis	1	—	—	1
	56	41	6	9

Table 10

Neoplastic diseases

Diagnosis	Total	Fragility		
		N.	D.	I.
Anaplastic Ca of skin	1	1	—	—
Ca of breast	1	1	—	—
Ca of lung	2	2	—	—
Ca of peritoneal cavity	2	1	—	1
Ca of pancreas	12	12	—	—
Ca of stomach	8	7	1	—
Ca of colon	2	—	—	2
Ca of bladder	2	1	—	1
Tumor of brain	2	1	—	1
Tumor of hypophysis	2	2	—	—
Thyroid nodule	1	—	—	1
Generalized carcinomatosis	2	1	—	1
	37	29	1	7

Table 11

Thrombo-embolic diseases

Diagnosis	Total	Fragility		
		N.	D.	I.
Myocardial infarction	52	23	3	26
Cerebrovascular accident	12	9	—	3
Pulmonary embolism	10	8	1	1
Infarction of kidney	1	1	—	—
Thrombophlebitis of leg	1	1	—	—
Endarteritis obliterans	1	1	—	—
	77	43	4	30

Table 12

Poisoning

Diagnosis	Total	Fragility		
		N.	D.	I.
Barbiturate	2	1	—	1
Snake bite	4	2	—	2
Insect bite	1	1	—	—
Largactil	1	1	—	—
	8	5	—	3

Table 13

Miscellaneous

Diagnosis	Total	Fragility		
		N.	D.	I.
Chorea minor	1	1	—	—
Concussion of brain	1	1	—	—
Epilepsy	1	1	—	—
Urticaria	5	5	—	—
Migraine	4	4	—	—
Bronchial asthma	10	8	—	2
	22	20	—	2

Without considering detailed findings, i.e. on the basis of classifying only increases and decreases outside the normal range, the proportion of deviation from the normal seems rather high. There is a considerable proportion of increased fragility in those clinical conditions in which there is a valid suspicion that tissue

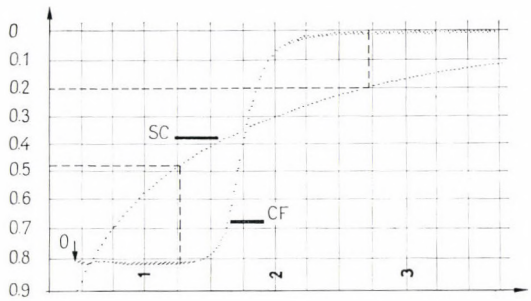


Fig. 2. Photograph of original simultaneous recording of cumulative fragiligram and change of salt concentration as a function of time. Ordinate of the left refers to the exponential salt concentration curve. It can be seen that hemolysis started at a salt concentration of 0.48% NaCl and was completed at 0.20% NaCl. CF — Cumulative fragiligram. SC — Salt concentration

Table 14

Normal, decreased and increased fragility of all the cases examined, classified according to the different clinically diagnosed groups

Groups of diseases	Number of cases	Fragility		
		N.*	D.**	I.***
1. Blood diseases	50	29	12	9
2. Diseases of the heart	94	76	3	15
3. Diseases of the lung	41	36	1	4
4. Diseases of hepato-biliary tract (pancreas included)	53	33	10	10
5. Diseases of the digestive tract	41	27	3	11
6. Diseases of urinary system	36	24	4	8
7. Metabolic diseases	51	45	5	1
8. Diseases of the connective tissue and collagen	35	25	1	9
9. Infectious diseases	56	41	6	9
10. Neoplastic diseases	37	29	1	7
11. Thrombo-embolic diseases	77	43	4	30
12. Poisoning	8	5	—	3
13. Miscellaneous	22	20	—	2
Total	601	433	50	118

*N = Normal D.** = Decreased, I.*** = Increased

injury may have occurred. A strikingly high proportion was found in cases of myocardial infarction, in some of these patients, the osmotic fragility picture was followed for several days, and was found to revert to normality on general clinical recovery.

In view of these findings it is worthwhile noting that certain cases of arteriosclerotic vascular and heart diseases show increased osmotic fragility of the red blood cells before any sign of myocardial lesion is apparent. It is noteworthy in this connection that after experimental soft tissue injury in rabbits there is an increase in osmotic fragility of red blood cells [8].

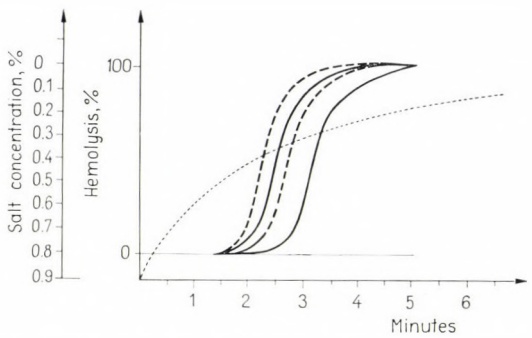


Fig. 3. Range of normal osmotic fragility curves and a salt concentration curve. ..... fresh blood (157 cases) recorded with salt concentration. ——— blood tested after being kept at room temperature for 2 to 12 hours (220 cases). The exponential dotted line rising from left to right describes the change of salt concentration as a function of time. Note the wider range of osmotic fragility among the blood samples left for several hours at room temperature

When a normal red cell population is subjected to increasing osmotic pressure the old cells hemolyse first, followed by the mature cells which form the main population. Hemoglobin is next released by the young cells, until finally reticulocytes are hemolyzed. This sequence of events was described both for drastic [11,12] and gradual hemolysis [13].

The micro-method described for automatically recording the osmotic fragility of erythrocytes is simple, fast and requires negligible amounts of blood. The cumulative osmotic fragility curve, besides providing an overall picture of increased or decreased fragility, also gives information on any alteration in age distribution of the red cells or damage attaining one age group in particular as reflected by modification in the cumulative curve, and even more clearly in the derivative curve [9, 10] (see also Fig. 1).

This detailed information cannot readily be acquired from plots derived from conventional methods consisting of a limited number, say 6—20 points, of which only 3 to 5 actually participate in forming the curve. In the present meth-

od, the curves are practically continuous, consisting of about 250 points. Thus a slight change in modality of the sinusoidal curve is readily apparent in the derivative curve. The micro-method presently described provides automatically recorded information rapidly and simply. The clinical results would indicate that a more extensive routine use of the test for cases in which no obvious hematological symptoms are apparent might be of value.

References

1. Ansley, H. R., Sheldon, W. H., Tenney, A. M., Elderkin, R. D.: Preliminary report of cytopathology of erythrocytes of schizophrenic patients. *Diseases of the Nervous System 18*, 444 (1957).
2. Bohr, D. F.: Erythrocyte fragility in acute infectious hepatitis. *J. Lab. Clin. Med. 31*, 1179 (1946).
3. Havens, W. P.: Infectious hepatitis in the middle east. *J. A. M. A. 126*, 17 (1944).
4. Mc Carty, A. C.: Acute infectious hepatitis. *Kentucky M. J. 44*, 45 (1946).
5. Finks, R. M. and Blumberg, R. W.: Epidemic hepatitis with and without jaundice, some clinical studies on 255 patients among troops in combat zone. *Arch. Int. Med. 76*, 102 (1945).
6. Hoffer, A.: Erythrocytes from schizophrenic patients. *Diseases of the Nervous System 20*, 87 (1959).
7. Danon, D.: A rapid micro-method for recording red cell osmotic fragility by continuous decrease of salt concentration. *J. Clin. Pathol. 16*, 377 (1963).
8. Bergentz, S. E., Danon, D.: Alterations in red blood cells of traumatized rabbits III. Increased osmotic fragility. In preparation.
9. Frei, Y. F., Perk, K.; Danon, D.; Correlation between osmotic resistance and fetal haemoglobin in bovine erythrocytes. *Exp. Cell. Res. 30*, 561 (1963).
10. Danon, D., Frei, Y. F., Rimon, A. and Ben David, A.: Osmotic fragility as a routine test in blood banks. *Transfusion 4*, 339 (1964).
11. Simon, E. R. and Topper, Y. J.: Fractionation of human erythrocytes on the basis of their age. *Nature 180*, 1211 (1957).
12. Marks, P. A. and Johnson, A. B.: Relationship between the age of human erythrocytes and their osmotic resistance: A basis for separating young and old erythrocytes. *J. of Clin. Invest. 37*, 1542 (1958).
13. Danon, D.: Osmotic hemolysis by a gradual decrease in the ionic strength of the surrounding medium. *Bioch. et Bioph. Acta 50*, 102 (1961).

Questions and Answers on Leukemia Problems[1]

G. Astaldi, R. Airò

Institute of Pathology, University of Milan/Italy, and
the Blood Research Foundation Center,[2] Hospital of Tortona/Italy

(Received February 25, 1967)

1. How do you explain the difficulties in showing viruses responsible for human leukemias?

We believe that at present time one can't bring unequivocal proofs in favor or against the viral theory of human leukemias. Very shortly, we would like to answer by saying that one of the hypotheses to explain the difficulties in showing viruses responsible for human leukemias could derive from the possibility that human leukemias are not caused by any viruses. What we have gained so far is only some indirect evidence either in favor or against the viral etiology of human leukemias. Let us discuss some of the most important findings which have been reported in the past few years, and are still waiting for new evidence before any definite conclusion.

The discovery of cell-free transmission of mouse leukemia by Gross in 1951 has led to numerous morphological, biological and biochemical studies of leukemia. At present, we know of the electron-micrographic morphology of the murine leukemic virus. Furthermore, we know that the transmission of this disease is a *vertical transmission*, and that the thymus plays an important role in the development of some of these leukemias. The mouse leukemic viruses are RNA-containing viruses. The so-called *infectious part* of these viruses is the RNA: however, the lipid components also play a considerable role. In fact, the treatment of these pathogenic agents with solvents of lipids, especially ether, causes the loss of their capacity to reproduce the disease.

Until now, several leukemogenic viruses have been described, such as: the Moloney Virus, the Friend Virus, the Graffi Virus, the Rauscher Virus, etc. Moloney thinks that these viruses are different members of the same family, whereas

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

[2] Discussion at the International Symposium on Leukemia: Mendoza/Argentina, Aug. 1966.
(*Participants:* G. Astaldi, Tortona/Italy; J. Bernard, Paris/France; C. Bottura, Sao Paolo/Brasil; L. Braier, Mendoza/Argentina; G. Ducach, Santiago/Chile; A. Dalton, Bethesda/USA; E. Freireich, Houston/USA; J. Furth, New York/USA; H. Hayhoe, Cambridge/United Kingdom; L. Hamilton, Upton/USA; G. Hitching, New York/USA; F. Jimenez De Asua, Buenos Aires/Argentina; J. Maisin, Louvain/Belgium; R. C. Milies, Montevideo/Uruguay; S. Moeschlin, Solothurn/Suisse; J. B. Moloney, Bethesda/USA; G. Oclander, Indianapolis/USA; E. E. Osgood, Portland/USA; A. Pavlovsky, Buenos Aires/Argentina; S. Watanabe, Tokyo/Japan; E. Wiltshaw, London/United Kingdom).

Gross believes that there is only one leukemic virus, i.e., the so called *Mouse Leukemic Virus Type A*. In fact, Gross has recently been able to show an antigen common to his virus and to the virus of Moloney.

As far as the avian leukosis is concerned, this also is a viral disease. Its transmission is not only *vertical*, but *horizontal*, too. Moreover, the avian leukemic viruses also are RNA-containing viruses, and the so-called *infectious part* of these viruses is the RNA. The recent results of Beard et al. show that the metabolism of the virus-RNA in the infected cells is not influenced by actinomycin D, that is to say, the virus-RNA metabolism is independent from the cell-DNA metabolism. Moreover, according to Thorell's investigation, the purine and pyrimidine basis composition of the virus-RNA is very different from the cytoplasmic-RNA. After the virus infiltration, the cytoplasm of these leukemic cells has an elevated RNA content and, in erythroblasts, small amounts of hemoglobin are synthetized. The biochemical data show that the virus specifically changes the cytoplasmic nucleotid metabolism, and that the ATP-ase activity increases, causing disintegration of the cytoplasmic structures and functions.

The avian viruses, as well as those of murine and feline leukemias, are very similar to each other. On the other hand, they are definitely different from the DNA-containing oncogenic viruses, such as polyoma, Shope papilloma, and the adenoviruses. Also, the relationship between the Rous sarcoma virus and the avian leukosis virus – as a helper virus – is a very interesting topic, but the problems dealing with human leukemia are most exciting.

Several facts contradict the role of viruses in causing human leukemias. First of all, human leukemias could not be transmitted to healthy persons by transfusion of leukemic blood, or bone marrow. The *vertical* transmission of leukemias – as can be observed in some strains of mice – has not been shown in humans until now. Mothers of congenital leukemic children are non-leukemic, and leukemic mothers bear non-leukemic children. Up to now we know of more than 150 cases in which leukemic mothers had non-leukemic children, and only 2 cases where a child born by a leukemic mother died from leukemia. Moreover, the genetic characteristics of leukemia – as can be found in some strains of mice – have not been observed in humans. However, investigations on leukemic families have brought some evidence that genetic factors may play a role in the leukemogenesis, as suggested by the incidence of leukemia and cancer in some families, and by the concordance rate with leukemia in identical twins.

During last years, virus-like particles have been observed in leukemic cells in man. Especially the contributions by Dmochowski, Graffi, Magrassi, Murphy, and Negroni are very important from this point. The electronmicrographic appearance of these virus-like particles is very similar to the murine, avian and feline leukemic viruses. However, further investigations have shown that in many instances these virus-like particles were simply mycoplasma, which may or may not be related directly to leukemia. In any case, taking into consideration all the facts known on animal leukemias, especially after the recent observation that also the radiation induced leukemia in rats is due to a virus, one gets the feeling that also

for human leukemias the viral etiology could be possible. Our difficulties in detecting viral agents in human leukemias may be simply due to the inadequacy of our tests, or the low concentration of the leukemogenic viral agent. Regarding this latter possibility, special interest has the observation recently made by Moloney. By inoculating of leukemogenic viruses at low concentration, he was able to obtain leukemia in experimental animals after a definitely prolonged incubation period, and without any possibilities to detect such viruses either in hemic cells, or in hemo-lymphopoietic organs of those animals.

In our laboratory, the purine and pyrimidine basis composition of the RNA of lymphocytic, as well as of granulocytic cell series in both normal and leukemic conditions have been investigated. We have been stimulated in this research project by the recent results of Tamura and Ivanaga, as well as by those of Rottger, who have shown that after infections of RNA-containing virus, the purine and pyrimidine basis composition of the RNA in the infected cells became similar to the virus-RNA. Besides, it is well known that all the leukemogenic viruses isolated until now – from murines, as well as from felines or from avians – are RNA-containing viruses. The results of our investigation taken as a whole show that:

1. Peripheral lymphocytes from normal human blood contain quantities of the four RNA-nucleotides higher than normal granulocytes, but this difference was not significant in the statistical analysis;

2. Peripheral lymphocytes from chronic lymphocytic leukemia contain quantities of the RNA-nucleotides significantly higher than those from normal blood;

3. Peripheral leukocytes from granulocytic leukemia both chronic and acute, contain quantities of the four RNA-nucleotides significantly higher than the peripheral leukocytes from normal blood, and a relationship exists between the RNA-nucleotide contents, and the percentages of blasts of the examined leukocytes;

4. The molar base ratios of the four RNA-mononucleotides – that is to say, the qualitative composition of RNA – resulted always the same, whether the leukocytes were of the granulocytic or lymphocytic series, as well as normal, or leukemic.

In conclusion, in front of some well known facts, we still have many obscure problems in the field of *Virology in Leukemias*, and all of us fervently hope and await that this fundamental problem on the etiology of human leukemia be conquered in our lifetime.

References

1. Astaldi, G.: Introduction to the Symposium on Viral Etiology of Leukaemia. Proc. 10th Congr. Europ. Soc. Haematol., Strasbourg, 1965. In *Haematologica Latina 8*, 161 (1965).
2. Bergolz, V., Dementiera, V.: The application of tissue culture of surviving spleen for the detection of a leucosogenic agent in human leukemic tissue. Proc. Symp. Viral Etiology of Leukemia. 10th Congr. Europ. Soc. Haematol. Strasbourg, 1965. In *Haematologica Latina 8*, 209 (1965).

3. Dmochowski, L.: The search for human tumor viruses. *Tex. Rep. Biol. Med. 21*, 113 (1963).
4. Dmochowski, L.: Present status of viruses as causative agents in cancer. *Southern Med. Bulletin 54*, 65 (1964).
5. Friend, C.: Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exp. Med. 105*, 307 (1957).
6. Graffi, A.: Chloroleukemia of mice. *Ann. N. Y. Acad. Sci. 68*, 540 (1957).
7. Graffi, A. et al.: Sub-microscopic particles in myeloid leukaemia of man. *Deutsch. Med. Forsch. 2*, 12 (1964).
8. Graffi, A.: Zu einigen Fragen der Virusätiologie tierischer und menschlicher Leukämien. Proc. Symp. Viral Etiology of Leukemia. 10th Congr. Europ. Soc. Haematol., Strasbourg, 1965. In *Haematologica Latina 8*, 205 (1965).
9. Gross, L.: Pathogenic properties and "vertical" transmission of the mouse leukemia agent. *Proc. Soc. Exp. Biol. N. Y. 78*, 342 (1951).
10. Kiss, S., Astaldi, G. and Airò, R.: The RNA nucleotide composition in human leukocytes from normal and leukemic cases. *Blood* (in press).
11. Magrassi, F. et al.: Nos derniers apports à l'étude du problème étiopathogénique des leucémies humaines. Proc. Symp. Viral Etiology of Leukemia. 10th Congr. Europ. Soc. Haematol., Strasbourg, 1965. In *Haematologica Latina 8*, 165 (1965).
12. Moloney, J. B.: Biological studies on a lymphoid-leukemia virus extracted from sarcoma 37. I. Origin and introductory investigations. *J. Nat. Cancer Inst. 24*, 933 (1960).
13. Moloney, J. B.: Personal communication.
14. Murphy, W. H., Furtado, D.: Isolation of viruses from children with acute leukaemia. *Univ. Mich. Med. Bull. 29*, 201 (1963).
15. Murphy, W. H. et al.: Virus studies of human leukemia. Proc. Symp. Viral Etiology of Leukemia. 10th Congr. Europ. Soc. Haematol., Strasbourg, 1965. In *Haematologica Latina 8*, 191 (1965).
16. Negroni, G.: Isolation of viruses from leukemic patients. *Brit. Med. J. 1*, 927 (1964).
17. Negroni, G.: Is human leukaemia caused by viruses? Proc. Symp. Viral Etiology of Leukemia. 10th Congr. Europ. Soc. Haematol. Strasbourg, 1965. In *Haematologica Latina 8*, 185 (1965).
18. Rauscher, F. J.: A virus-induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia. *J. Nat. Cancer. Inst. 29*, 515 (1962).
19. Thorell, B.: The viral theory of human leukemia. *Acta Haemat. 36*, 174 (1966).

2. Do you believe that benzol can cause leukemia?

Whether benzol is or is not a leukemogenic agent is still debatable. There are in fact researchers who believe this, while others do not believe that this substance can actually cause leukemic processes.

In the Lombardy region, at the Medical School of the University of Pavia, to which we belong, the Shoe Manufacturing Industries are numerous, and it is known that benzol has been adopted as one of the solvents for adhesives used in the shoe manufacturing. The Government Regulations control and prevent the risk for benzol intoxication in the Industry, but some piece-work was given out to be made at home, and cases of chronic intoxication of benzol unfortunately have risen in the past few years. Similar unfortunate events may represent suitable clinical material for a prospective study on the possible genesis of benzolic leukemia(s). In fact, the so-called human progress unfortunately may bring us to such involuntary medical experiments of human beings, from which man can

only be rehabilitated if he learns to benefit from them by furthering the scope of health and life for humanity.

The larger number of the above-mentioned cases of chronic intoxication of benzol with bone marrow disorders have been admitted to the Worker's Medical Clinic at the Pavia University, Chief of Staff Professor Maugeri, and he checked the progress of some of these cases to leukemic forms, both subacute as well as chronic granulocytic. The incidence of leukemia among the patients with benzolism was such as to suggest a relationship between the benzol intoxication, and the onset of the leukemic process.

It has been reported that the Ph_1-chromosome abnormality appears in the bone marrow cells before the clinical manifestation of leukemia. This would suggest a prospective study of this abnormality among all the people with signs of benzolism. Such a study, together with periodical controls of the blood picture, and the evaluation of the leukocyte alkaline phosphatase scores may help in picking whether a chronic intoxication of benzol really turns to leukemia. When possible, such observations associated with a statistic study on the frequency of leukemia among these patients, as compared with the frequency of leukemia in aplasias from other agents will allow the conclusion whether benzol intoxication may really cause leukemia, either directly, or in concurrence with other etiologic factors.

3. What do you think on the leukokinetic of the leukemic process?

We are particularly pleased to discuss this topic, since our initial research on this subject using the tissue culture technique goes back to 1947 – 50.

In order to evaluate the extent of proliferation of both normal and leukemic blood-forming cells, we first elaborated on the *stathmokinetic test* (Astaldi and Mauri). It developed that this test can provide information of the number of cells which in a given culture, and in a given time enter mitosis. This has been obtained by adding an optimal dose of colchicine to the culture medium. Since all the cells entering mitosis are arrested at the premetaphase by colchicine, the number of mitoses achieved in a given time *(stathmokinetic index)* derives from the number of the cells which have entered mitosis, i.e., from the average duration of interkinesis. In this condition, the stathmokinetic index shows the extent of proliferation of the cells under examination.

In using this method, first of all it was found that the extent of proliferation for a given blood-forming cell series decreases with the progress of maturation. But as far as the leukemic process is concerned, we found from the very beginning of those investigations that the blasts of acute leukemia, as average of all cell population, are characterized with a very low proliferative activity. This showed that the functional defects in acute leukemia cells do not consist only in an absolute incapacity for differentiation or maturation, but also in a low proliferative activity. Furthermore, using the above-mentioned method, i.e., the stathmokinetic test in tissue or cell cultures, it was found that also in chronic granulocytic leukemia the

proliferation activity of the granulocytic series is not increased in respect to the same activity of the corresponding normal cell series. In particular, no significant difference was observed at the promyelocytic stage, whereas at the myelocytic stage the extent of proliferation was lower for the cells from leukemic patients than for those from normal subjects. Also, the lymphoblasts of chronic lymphocytic leukemia, as well as the plasmoblasts of plasmocytoma were endowed with a low proliferative activity.

At that time, our results showing a low proliferation of cells in leukemic processes brought some disorientation not only to us, but also among the hematologists who were convinced of the hyperproliferative condition of the leukemia cells. But in more recent years, our findings have been confirmed by several authors from different countries, mostly after the introduction to the study of cellular kinetics of the radioisotop labeled nucleic acid precursors. For example, Lajtha reported that blast cells in leukemia show a slower rate of DNA-synthesis, and a longer cell cycle than normal myelocytes or normoblasts. Gavosto et al. illustrated that the incorporation of labeled thymidine in acute leukemia cells is very low, compared with that of the blood-forming cells of normal bone-marrow. Using tritiated thymidine, Kilman et al. found labeled blasts only several hrs. after the injection of thymidine, and the peak of the labeled blasts was reached after 36–48 hrs. Thus, Kilman could calculate the generation time of leukemic blasts to be 50–60 hrs., suggesting that these cells do not divide more frequently than the normal granulocytic precursors. Craddock, in studying the proliferation of leukemic cells as reflected by the "in vitro" DNA-synthesis found a lower radioisotopic uptake as compared to the normal precursors of hemic cells, and interpreted these findings as evidence of a definitely prolonged generative time of the acute leukemia cells. Many other reports have confirmed these results. The low rate of proliferative activity in the leukemia cells is associated with a low activity of the enzymes concerned with thymidine phosphorylation, and/or with nucleotide triphosphate polymerization into DNA.

The spectrophotometric method also has shown disorders in the DNA- and RNA-contents in leukemic cells, consisting of an increase of these nucleic acids. This fact is believed to be connected with the delayed nucleic acid metabolism, and therefore with a low proliferative activity. Again, the phase-contrast cinematography of living cells in tissue or cell cultures has shown that the mitotic time in acute leukemia cells is almost double, as compared to that of normal granulocytic cell series (2 hrs. and 20 min. versus 1 hr. and 15 min.).

The low proliferative activity of acute leukemia cells could be connected with the incapacity of these cells to differentiate. In fact, because of their absolute defective maturation, the acute leukemia cells become progressively older from the biochronological viewpoint, and thus less active in proliferation. In other words, the cell population should be very heterogeneous with respect to the age of their single cell components, in spite of the fact that they are more or less similar to each other from the morphologic point of view. We believe that this condition may furnish a possible explanation for understanding the defective capacity in

proliferation shown in acute leukemia by the blast cell population taken as a whole.

This concept, that we had originally presented in order to explain the low cell proliferation in leukemias, has been more recently also emphasized by others. Kilman et al. have suggested that not all the blasts in acute leukemia have the capacity of further division, and that some of those blasts may be *sterile*. Gavosto et al. have reported a more active proliferation of the leukemic blasts during the final blastic crisis of chronic granulocytic leukemia, as well as during remissions of acute leukemia, in which the cell population is more homogeneous with respect to the age of the single cell components. Gavosto has also observed that in acute leukemia the blasts may be divided into two classes, according to the cell diameter. The blasts with higher cell diameter – the younger ones, show a proliferative activity close to normal values. On the contrary, the blasts with smaller cell diameter – the older ones, show a much lower proliferative activity.

From the reported data taken as a whole, it seems logical to conclude that as a result of the action of the leukemogenic agents, the blood-forming cells lose the genetic information involved in the synthesis of specific proteins, or involved in the activity of the enzymes pertaining to differentiation or maturation. In consequence of the fault of these processes, the cells progressively age in the blastic stage, and their proliferative capacity progressively decreases in such a way that the extent of proliferation within the whole blast cell population is definitely lower.

References

1. Astaldi, G.: Differentiation, proliferation and maturation of haemopoietic cells studied in tissue culture. In Ciba Foundation Symposium on Haemopoiesis. London, J. and Churchill Ltd., p. 99, 1960.
2. Astaldi, G.: Opening remarks at the section on tissue culture of hemic cells at the IX Congress of the International Society of Hematology. Proc. IX. Congr. Int. Soc. Hematol. *2*, 1649 (1962).
3. Astaldi, G., Mauri, C.: La valutazione dell'attività proliferativa delle cellule midollari. Studio di un "test statmocinetico". *Haematologica 33*, 321 (1949).
4. Astaldi, G., Mauri, C.: New criteria for the evaluation of the bone marrow cells' mitotic activity. *Le Sang 21*, 378 (1950).
5. Astaldi, G. et al.: Experimental investigations of the proliferative activity of erythroblasts in their different stages of maturation. *Experientia 3*, 1 (1947).
6. Astaldi, G. et al.: Ricerche sperimentali sui processi riproduttivi del midollo normale e patologico. *Boll. Soc. It. Biol. Sper. 23*, 40 (1947).
7. Astaldi, G., Mauri, C.: Recherches sur l'activité proliferative de l'hemocytoblaste de leucémie aiguë. *Rev. Belge Path. Med. Exp. 23*, 69 (1953).
8. Boll, J.: Granulocytopoese unter physiologischen und pathologischen Bedingungen. Springer-Verlag, Berlin, 1966.
9. Boll, J., Gansan, O.: Die Mitosedauer der Paraleukoblasten. *Acta Haematol., 27*, 229 (1962).
10. Craddock, C. G.: Cellular proliferation in normal and leukemic states as reflected by in vitro DNA synthesis. Proc. 9th Congr. Int. Soc. Hemat. Mexico 1962.
11. Craddock, C. G., Nakai, G. S.: Leukemic cell proliferation as determined by in vitro deoxyribonucleic acid synthesis. *J. Clin. Invest. 41*, 360 (1962).

12. Fitzgerald, P. H., Adams, A.: Chromosome studies in chronic lymphocytic leukemia and lymphosarcoma. *Journ. Nat. Cancer Inst. 34*, 827 (1965).
13. Gavosto, F. et al.: Proliferative capacity of acute leukaemia cells. *Nature 187*, 611 (1960).
14. Gavosto, F. et al.: Nucleic acids and protein metabolism in acute leukaemia cells. *Blood 16*, 1555 (1960).
15. Gavosto, F. et al.: Proliferation and maturation defect in acute leukaemia cells. *Nature 203*, 92 (1964).
16. Gavosto, F. et al.: Thymidine incorporation in the chromosomes of human acute leukaemia. In *Current Research in Leukaemia*. Ed. by F. G. Hayhoe, Cambridge, University Press, p. 177 (1965).
17. Gross, R.: Quantitative cytochemical examinations in cases of acute leukaemia. Proc. 9th Congr. Soc. Europ. Haemat. Lisbon *1*, 164. S. Karger, Basel/New York, 1963.
18. Hale, A. J., Cooper, H.: Studies on DNA replication in leukaemic and non-leukaemic leukocytes. In *Current Research in Leukaemia*. Ed. by F. G. Hayhoe, Cambridge, University Press, p. 95 (1965).
19. Kilman, S. A. et al.: Estimation of phases of the life cycle of leukemic cells from labelling in human beings in vivo with tritiated thymidine. *Lab. Invest. 12*, 671 (1963).
20. Lajtha, L. G.: On DNA labeling in the study of the dynamics of bone marrow cell population. In *The Kinetics of Cellular Proliferation*. New York/London, Grune and Stratton, 1959, p. 173.
21. Mauer, A. M., Fisher, V.: Comparison of the proliferative capacity of acute leukaemia cells in bone marrow and blood. *Nature 193*, 1086 (1962).
22. Mauer, A. M. et al.: Kinetics of leukaemic cells. 11th Congr. Int. Soc. Haematol. Plenary Sessions, Sydney, Aug. 1966, p. 42.
23. Muller, D.: The nucleic acid content of normal and leukemic granulocytes and the influence of cytostatic treatment. Proc. 9th Congr. Europ. Soc. Haemat., Lisbon 1963, p. 170. S. Karger, Basel/New York.
24. Pileri, A. et al.: Cytogenetical and proliferative characteristics of acute promyelocytic leukaemia cells. *Europ. J. Cancer 2*, 189 (1966).
25. Salera, U.: Studies on the mitotic growth of haemopoietic cells by the stathmokinetic method. Proc. 9th Congr. Int. Soc. Haemat., Mexico, D. F., 1962.
26. Storti, E., Torelli, E.: RNA and protein metabolism in normal and leukaemic leukocytes as studied by autoradiography. In *Current Research in Leukaemia*. Ed. by F. G. Hayhoe, Cambridge, University Press, 1965, p. 108.
27. Thorell, B.: Studies on the formation of cellular substances during blood cell production. *Acta Med. Scand. Suppl. 200* (1947).

4. What is your opinion of the real development of the leukemic process: hyperplastic, neoplastic, displastic?

At that time our Teacher Professor Ferrata used to go around this question by saying: *Leukemias are leukemias.* In fact, even if he inwardly felt that leukemia is a neoplastic process, his optimistic temperament wanted somehow to rebel against accepting a conclusion that would deprive the leukemic patients of some hope of being cured not only at that time, but following that particular time also, as it was then, and unfortunately is so today. Because of this, we cannot ignore the existence of certain fundamental biological defects common to leukemia and cancer which would suggest a neoplastic, rather than an hyperplastic or displastic nature of the leukemic process, even though our ignorance of the mechanism of

leukemogenesis and cancerogenesis makes if difficult to establish the exact position of leukemia and cancer among all the pathological conditions.

Two main biological abnormalities have been recognized as for neoplastic (leukemic and cancer) cells: 1. *genetical alterations*, as shown by the chromosome alterations present both in animal leukemias, as well as in human leukemias, and in animal and human cancer; 2. *failure of cellular differentiation*, from which derives the low degree of proliferative activity in human leukemias, as we have discussed in the previous paragraph.

These two alterations are consequent to the action of the etiological factor. Here again, we observe that leukemia and cancer share the same etiological agents: radiations, chemical compounds, and viruses are the best known causes of neoplastic diseases. The leukemogenic effect of radiation either in human beings, or in experimental animals and plants has been known long since; but the best evidence has come after the observation of the high incidence of leukemia among the population exposed to the atomic bomb injury, and among people submitted to X-ray radiation for their particular job (radiologists), for therapeutic reasons (such as in irradiated cases of spondylarthritis), or of diagnostic purposes (diagnostic radiography in pregnant women). On the other hand, it is well known that exposure to radiation may induce localized tumors both in experimental animals and in plants.

As far as the chemical compounds are concerned, the leukemogenic effect of benzol has been discussed by several people just in this meeting, too. Also phenylbutazone has been discussed here from the viewpoint of its leukemogenic effect. Other compounds, such as methylcholanthrene, dimethylbenzanthracene, benzopyrene, etc. chiefly cause solid tumors, although some of them have been reported to increase the incidence of leukemia in experimental animals. Well known is the already mentioned role played by viruses in inducing tumors and leukemia in experimental animals. The demonstration by Gross of viral leukemia in mice, transmissible from mother to offspring via milk, or to immuno-incompetent recipients; the long known viral chicken leukemia; the Rous-sarcoma virus capacity of transforming "in vitro" normal cells to malignant cells are certainly unequivocal proofs of the leukemogenic and carcinogenic potentiality of some viruses. As far as human leukemia is concerned, after several reports indicating the existence of virus-like particles in leukemic cells, evident in electronmicrography, it has been found that in many instances those virus-like particles were in effect mycoplasma, and these microorganisms are generally considered to be unrelated to the leukemia etiology. Only a malignant lymphoma in African children, the Burkitt tumor, is proved to be an infectious disease transmitted by insects. This tumor, as well as other malignant lymphomas may end with a leukemic picture thus suggesting a common origin of these diseases.

Whatever the etiological agents of leukemia and cancer may be, the consequence of their action on the cells is a genetic one, and this means that the complex mechanism which regulates the action of genes is involved at first, as a consequence of disturbances in the metabolism of nucleic acids. Because of these genet-

ic alterations, the affected cells keep the potentiality of transmitting the acquired biological disorder to the daughter cells, and this capacity, common to both leukemia and cancer cells, is the most important character which points to the neoplastic nature of the leukemic process.

References

1. Astaldi, G., Mauri, C.: Recherches sur l'activité proliferative de l'emocytoblaste de leucémie aigue. *Rev. Belge Path. Med. Exp. 23*, 69 (1953).
2. Gavosto, F.: Le leucemie acute. *Aggiornamenti clinico-terapeutici.* Ed. Minerva Medica (1966), p. 1.
3. Nowell, P. C., Hungerford, D. A.: The etiology of leukemia: some comments on current studies. *Seminars in Hematology 3*, 114 (1966).

5. What is the diagnostic and prognostic significance of the Philadelphia chromosome?

The discovery by Nowell and Hungerford of a characteristic chromosome abnormality in chronic granulocytic leukemia may be considered as one of the most important findings observed in human leukemia cells. As it is well known, it consists in the loss of a substance in the long arm of one of the small acrocentric chromosomes of the groups 21/22 of the Denver classification. The term "Philadelphia chromosome or Ph1 chromosome" has been suggested by Though in one of the early reports on this topic.

Numerous reports appeared after Nowell's first observation, confirming the existence of this chromosome abnormality, and in all of them, the peculiarity of the chromosome abnormality for chronic granulocytic leukemia has been stressed. We, ourselves, have observed it in Italy for the first time. According to Rudkin et al., the quantity of substance lost in the Ph1-chromosome amounts to 39% of the total DNA present in an analogous normal chromosome, taken as 100. Whether this substance is really lost or only transported to other chromosomes, is not yet established.

It is important to mention that the Ph1-abnormality is present in both granulocyte and erythrocyte precursors, as well as in megakaryocytes, whereas it is absent in lymphoid cells and skin cells of chronic granulocytic leukemia patients. The diagnostic importance of the Ph1-chromosome comes from the fact that it has been found, with rare exceptions, only in chronic granulocytic leukemia. Neither healthy persons, nor patients with other myeloproliferative disorders, including myelosclerosis with myeloid metaplasia, polycythemia vera, etc. show the Ph1-chromosome abnormality. This abnormality, together with the low scores of leukocyte alkaline phosphatase represent the most important aid in clarifying a clinical doubt in the diagnosis of chronic granulocytic leukemia.

It has been sometimes found that the Ph1-chromosome appears in the bone marrow cells before the clinical manifestations of leukemia. This indicates the inquiry of the Ph1-chromosome as a marker, in order to anticipate the development of a leukemic process in the preleukemic stage. This could be important not

only in the case of irreversible diseases, such as myelosclerosis and polycythemia vera, but also, and above all, in acquired bone marrow disorders, such as benzol intoxication, irradiation injuries, etc. The same important value for a prospective inquiry of these pathological conditions would be screened for a drop in the leukocyte alkaline phosphatase activity.

With regard to the prognostic value of the Ph1-chromosome, it has been established that it disappears from the bone marrow cells neither during therapeutic remissions, nor during the blastic crises. On the other hand, since the number of the Ph1-positive cells in peripheral blood drops after therapy according to the decrease of circulating immature cells, the study of the incidence of this chromosome abnormality in peripheral leukocytes may provide some information on the effect of the treatment.

We should like to conclude by mentioning that the hypothesis, that the low phosphatase activity in leukocytes from chronic granulocytic leukemia correlated with the loss of a substance in the small acrocentric 21/22 chromosome, has not been confirmed. Merker has pointed out that the leukocyte alkaline phosphatase activity in chronic granulocytic leukemia may return to normal or above normal ranges during remission, blastic crisis, and marrow insufficiency after treatment, whereas the Ph1-chromosome is constantly present. This would suggest a greater importance of Ph1-chromosome for diagnostic purposes, and of leukocytic alkaline phosphatase activity for testing the efficacy of a treatment.

References

1. Adams, A. et al.: A new chromosome abnormality in chronic granulocytic leukemia. *Brit. Med. J. 2*, 1474 (1961).
2. Astaldi, G., Strosselli, E.: Ricerche citogenetiche nelle emopatie: osservazioni sui cromosomi della leucemia mieloide cronica. *Boll. Soc. It. Biol. Sper. 38*, 109 (1962).
3. Astaldi, G. et al.: Le cellule emiche nella ricerca citogenetica. *Haematologica 47* (suppl.), 1 (1962).
4. Awano, H. et al.: Research in the genetic mechanism of leukemogenesis chromosome in the human leukemia and mouse-leukemia. Proc. 8th Congr. Int. Soc. Haematol., vol. 1, p. 247, 1960 (Pan Pacific Press-Tokyo, 1962).
5. Baikie, A. G.: Chromosomal aspects of leukaemia. 11th Congr. Int. Soc. Hematol. Plenary Sessions, Sydney, Aug. 1966, p. 199.
6. Baserga, A.: Citogenetica e problemi della leucemogenesi. In *Citogenetica delle Leucemie*. Ed. Minerva Medica, Torino, p. 4, 1966.
7. Baserga, A. et al.: Lo studio dei cromosomi nelle emoblastosi. *Haematologica 47* (suppl.), 83 (1962).
8. Bosi, L. et al.: Alterazioni cromosomiche nelle leucemie mieloidi croniche. *Il Progresso Medico 18*, 225 (1962).
9. Brooke, J. H. et al.: Human chromosome uncoiling and dissociation. *Ann. Hum. Genet. 26*, 139 (1962).
10. Eridani, S. et al.: Il cariotipo nelle leucosi: contributo casistico e saggi di analisi statistica. In *Citogenetica delle Leucemie*. Ed. Minerva Medica, Torino, p. 39, 1966.
11. Ford, C. E., Costance, M. C.: Cytogenetic evidence of clonal proliferation in primary reticular neoplasms. *Canadian Cancer Conference 5*, 129 (1963). (Acad. Press Inc., New York, N. Y.)

12. Ford, C. E., Mole, R. H.: Chromosomes and carcinogenesis: observations on radiation induced leukaemias. *Progress in Nuclear Energy*, Series VI, vol. 2, p. 11, 1959.
13. Frei, E. et al.: Studies of the Philadelphia chromosomes in patients with chronic myelogenous leukemia. *Ann. New York Acad. Sci. 113*, 1073 (1964).
14. Gunz, F. W., Fitzgerald, P. H.: Chromosomes and leukemia. *Blood 23*, 394 (1964).
15. Hampel, K. E.: Endoreduplikation bei der chronischen Leukämie. *Die Naturwissenschaften 50*, 619 (1963).
16. Hungerford, D. A.: Chromosome studies in human leukemia. I. Acute leukemia in children? *J. Nat. Cancer Inst. 27*, 985 (1961).
17. Hungerford, D. A.: The Philadelphia chromosome and some others. *Ann. Int. Med. 61*, 789 (1964).
18. Kinlough, M. A., Robson, H. N.: Study of chromosomes in human leukemia by a direct method. *Brit. Med. J. 2*, 1052 (1961).
19. Luers, Th. et al.: Strukturanomalien der Chromosomen bei einer akuten Paramyeloblasten-Leukämie. *Die Medizinische Welt 17*, 948 (1963).
20. Merker, H.: The significance of cytochemical and cytogenetic findings in chronic granulocytic leukemia and related diseases. In *Current Research in Leukemia*. Ed. F. G. J. Hayhoe, Univ. Press, Cambridge, p. 1, 1965.
21. Nowell, P. C., Hungerford, D. A.: Chromosome studies on normal and leukaemic human leukocytes. *J. Nat. Cancer Inst. 25*, 86 (1960).
22. Nowell, P. C., Hungerford, D. A.: Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J. Nat. Cancer Inst. 27*, 1013 (1961).
23. Nowell, P. C., Hungerford, D. A.: Chromosome changes in human leukemia and a tentative assessment of their significance. *Ann. New York Acad. Sci. 113*, 654 (1964).
24. Reisman, L. E. et al.: Chromosome studies in leukemia. 1. Evidence for the origin of leukemic stem lines from aneuploid mutants. *New England Journ. Med. 270*, 591 (1964).
25. Rudkin, G. T. et al.: DNA contents of chromosome Ph1 and chromosome 21 in human chronic granulocytic leukemia. *Science 144*, 1229 (1964).
26. Ruffie, J. et al.: Chromosomes in chronic lymphocytic leukaemia. *Lancet 2*, 227 (1966).
27. Sandberg, A. A. et al.: Comparison of chromosome constitution in chronic myelocytic leukemia and other myelo-proliferative disorders. *Blood 20*, 393 (1962).
28. Sándor, N.: Chromosoma vizsgálatok a haemopoetikus rendszer megbetegedéseiben. *Haemat. hung. 3*, 247 (1963).
29. Speed, D. E., Lawler, S. D.: Chronic granulocytic leukemia; the chromosome and the disease. *Lancet 2*, 403 (1964).
30. Tough, I. M. et al.: Cytogenetic studies in chronic myeloid leukaemia and acute leukaemia associated with mongolism. *Lancet 2*, 411 (1961).

6. Do you believe that a differentiation among acute leukemias may have either theoretical or practical interests?

We believe that a separation among acute leukemias at least in the lymphocytic, granulocytic, and hysto-monocytic types is convenient from both theoretical and practical viewpoints. The subsequent division of the granulocytic leukemia in hemocytoblastic (myeloblastic), and promyelocytic types has mostly a theoretical value, in that it shows that the leukemogenic agent may stop cell differentiation or maturation at different stages. Such separation could help in understanding the

mechanism of leukemogenesis. It will be easier to follow the biochemical and biological modifications (if any) caused by the leukemogenic agent in a single cell line, as well as to compare the altered cells with the normal cells, when we separate one type of leukemia from the other. The different cell lines may differ one from the other in their origin, biochemism, kinetics, etc.

The morphological distinction of the above-mentioned cell lines or cell stages may be substantiated one from the other by means of cytochemical tests. Hemocytoblasts are PAS- and Sudan-negative. Promyelocytes are PAS-positive, as well as peroxidase- and Sudan-positive. Lymphoblasts are slightly PAS-positive, but always peroxidase-negative. Monocytes are PAS-positive and slightly peroxidase-positive. These cytochemical behaviors suggest that the majority of the metabolic systems in leukemic cells are not definitely altered, with respect to the normal cells of the analogous cell line.

Another function connected with blood cells, and not altered in acute leukemia is the immune reactivity in both the humoral and cellular forms. In a group of acute lymphocytic leukemia we have shown that the reactivity of these lymphocytes to phytohemagglutinin (PHA) in cell cultures is well preserved. On the contrary, in chronic lymphocytic leukemia, as well as in other disorders of the lymphoid system the lymphocyte reactivity in cell cultures to antigenic stimulation is defective, as is also the immune mechanism "in vivo". These observations somewhat indirectly substantiate the clonal theory of leukemia, and the different characteristics of the leukemic cells. Furthermore, the capacity to react to the PHA-stimulation in cell culture may help in distinguishing acute lymphocytic leukemia from acute granulocytic leukemia (the hemocytoblastic form).

Finally, therapeutic requirements and prognostic considerations fully justify the separation of lymphocytic acute leukemia from granulocytic acute leukemia. It is a well-known fact since the former is favorably influenced by corticosteroid and antimetabolite treatments, and may last years, sometimes as long as 5 years or even more, while the latter is almost uninfluenced by treatments, and its duration is only a few months long.

References

1. Astaldi, G., Aplicaciones de la histoquimica a la patologia hematica. *Sangre 8*, 349 (1963).
2. Astaldi, G., Sauli, S.: La citochimica nella diagnosi ematologica. *Il Laboratorio nella Diagnosi Medica 9*, 217 (1964).
3. Astaldi, G. et al.: Phytohemagglutinin and lymphocytes from acute lymphatic leukaemia. *Lancet 1*, 1265 (1966).
4. Astaldi, G., Airo, R.: Phytohaemagglutinin and human lymphocytes in short-term cell cultures. In *The Lymphocyte in Immunology and Haemopoiesis*. Ed. by G. M. Yoffey, Bristol Symposium, 1967, p. 73.
5. Hayhoe, F. G. J.: Morphological classification of leukaemias. 11th Congr. Int. Soc. Hematol., Plenary Sessions, Sydney, Aug. 1966, p. 48
6. Hayhoe, F. G. J. et al.: The cytology and cytochemistry of acute leukemias. Her Majesty's Stationary Office, London, 1964.

7. Do you accept the belief that the leukemic cells must be eradicated in order to cure leukemia?

Since our early reports on the low proliferative activity of leukemic cells, and after the observations that no fundamental metabolic differences do exist between normal and leukemic cells, we have occasionally pointed out that the actual therapeutic approaches for treating leukemias are basically inconsistent. The use of antimitotic drugs could be active only on the existence of a higher proliferation in neoplastic cells, as compared with normal ones.

On the other hand, the use of antimetabolites also indicates the necessity of the existence of qualitative differences between the biochemical system of the former as compared to the latter. The ideal situation for the use of antimetabolites would be in the existence in neoplastic cells of such a type of metabolite or metabolic reaction, lacking in normal cells. In this case the antimetabolite would behave strictly selective on the neoplastic cells without damaging the normal ones. Unfortunately, no such peculiar metabolite or metabolic reaction has yet been found in leukemic cells. On the other hand, the lack of certain metabolic factors in neoplastic cells would be of no significance in a specific treatment based on antimetabolites. As a matter of fact, it has been brought even more in evidence that leukemic cells show some metabolic deficiencies. It seems more reasonable to direct our efforts in correcting the biological defects in leukemic cells trying to normalize their maturation process, rather than directing our efforts in inhibiting the growth of leukemic cells.

References

1. Astaldi, G.: Current Treatment of Malignant Blood Disorders. *New Istanbul Contribution to Clinical Science 7*, 40 (1964).
2. Astaldi, G. et al.: Ricerche sperimentali sui processi riproduttivi del midollo osseo normale e patologico. *Boll. Soc. It. Biol. Sper. 23*, 40 (1947).
3. Astaldi, G. et al.: I veleni della mitosi. I fattori che influiscono sulla reattività riproduttiva delle cellule con particolare riguardo ai reperti ottenuti su colture in vitro. *Il Farmaco 2*, 405 (1947).
4. Astaldi, G., Cardinali, G.: The theoretical basis of the use of cytotoxic substances in the chemotherapy of tumors and some aspects of their clinical application. *Gazz. Int. Med. Chir. 42*, 2831 (1957).
5. Astaldi, G., Mauri, C.: New criteria for the evaluation of the bone-marrow cells' mitotic activity. *Le Sang 21*, 378 (1950).
6. Astaldi, G., Mauri, C.: Ricerche sperimentali sui processi riproduttivi del midollo osseo normale e patologico. L'attività proliferativa dell'emocitoblasto della leucosi acuta. *La Settimana Medica 41*, 339 (1953).
7. Astaldi, G., Mauri, C.: Recherches sur l'activité proliférative de l'hémocytoblase de la leucémie aigue. *Rev. Belge de Pathol. et de Méd. Exper. 23*, 69 (1953).
8. Astaldi, G., Meardi, G.: Chemioterapia antimitotica e attività proliferativa delle cellule leucemiche. *Minerva Med.* 1094 (1966).

8. Do you think that some antileukemic vaccines could be realized, and what should be their practical utilization?

The concept that the neoplastic processes including leukemias may be inhibited in their growth by some immunologic mechanisms is an old one, but only recently some experimental evidence in this direction has been brought. Since 1924, J. B. Murphy observed that the resistance to malignant tumors both in experimental animals and in man may have some correlation with the lymphoid tissue, and in these last years the central role played by the lymphatic system on immune reactions has been widely recognized.

Mac Pherson, in a series of brillant experiments was able to show that normal hamster cells cultured in a medium to which the Rous-sarcoma virus was added became malignant, i. e., capable of reproducing the tumor when inoculated in another hamster. In these animals, that received transplants of virus-transformed malignant cells, antibodies appeared which neutralized the Rous-virus. The transformed malignant cells could be reverted to normal cells by transferring them several times in a new culture medium. The transplantation of the latter cells in hamsters would not cause any antibody formation against the Rous-virus: that is to say, the antibodies were specifically formed against the virus-malignant cell.

We would also like to mention that attempts have been made to obtain antibodies against the virus-like particles observed in the leukemic cells and plasma. Rabbits have been inoculated with the portion of leukemic plasma in which the virus-like particles are found after ultracentrifugation. By immunofluorescent techniques it was shown that antibodies against leukemic leukocytes containing the virus-like particles were developed. Such antibodies never reacted with normal leukocytes. Moreover, a cross reaction could be obtained between antibodies prepared in rabbits against mouse leukemia and human leukemia cells.

Anyone can understand the importance of such interesting and stimulating observations, even if some justified criticism has been promoted by these investigations. The results by Alexander, Delorme, and their group, have shown that the growth of the fibrosarcoma induced by 3 : 4-benzopyrene was retarded when the lymphocytes from the thoracic duct of specifically immunized rats were injected i. v. into tumor-bearing rats. Positive results were reached only when tumor fragments of the recipient rats were used to immunize the donor of lymphocytes, thus showing a tumor specific response. In subsequent experiments, the same authors showed that lymphocytes from immunized heterologous animals were also capable to inhibit the specific growth of the tumor. The cells responsible for this reaction probably act by stimulating the immune system of the host, rather than by a direct cytotoxic effect against the cells of the tumor.

A complete inhibition of the growth of the cells of tumors after their transplantation into syngeneic hosts was obtained by Klein and by Mikulska, by mixing the tumor cells "in vitro" with lymphocytes from immunized animals immediately before implantation. Furthermore, specific inhibition of growth of methylcholanthrene-induced sarcomas either "in vivo" or "in vitro" by sensitized

isologous lymphoid cells has been obtained by Rosenau and Morton in mice. Promising results have been obtained by Mathé and his coworkers (Schwarzenberger et al.) in their attempts at treating human leukemia utilizing the "graft-versus-host" immune reaction, consequent to the inoculation of isologous lymphocytes.

All the above-mentioned results clearly suggest that elicitation of the immune defense mechanism may successfully help in the battle against malignant cells. Therefore, attempts at preparing humoral or cellular factors capable to react against malignant cells are well justified, and hold much hope since their target is only the malignant cell in respect to all other cellular components of the body.

References

1. Alexander, P. et al.: The effect of lymphoid cells from lymph of specifically immunized sheep on the growth of primary sarcomata in rats. *Lancet 1*, 1186 (1966).
2. Klein, G. et al.: Demonstration of resistance against methyl-cholanthrene-induced sarcomas in primary autochtonous host. *Cancer Res. 20*, 1961 (1960).
3. MacPherson, I.: Reversion in hamster cells transformed by Rous sarcoma virus. *Science 148*, 1731 (1965).
4. Mikulska, Z. B., Smith, C.: Evidence for an immunological reaction of the host directed against its own actively growing primary tumor. *J. Nat. Cancer. Inst. 36*, 29 (1966).
5. Rosenau, W., Morton, D. L.: Tumor-specific inhibition of growth of methylcholantrene-induced sarcomas in vivo and in vitro by sensitized isologous lymphoid cells. *J. Nat. Cancer Inst. 36*, 825 (1966).
6. Schwarzenberger, L. et al.: Attempted adoptive immunotherapy of acute leukaemia by leucocyte transfusion. *Lancet 2*, 365 (1966).

9. Do you believe that leukemia will be cured?

We have already stressed the necessity that the treatment of leukemias should aim at the correction of defects in the neoplastic cells, rather than at their destruction. Thirty years of experience have confirmed the difficulty to find an agent capable of destroying "in vivo" the malignant cells, without destroying the normal ones. We would like to believe that the leukemogenic agent produces genetical alterations, for which a disorder of the genetical information to the system for protein synthesis in normal cells may occur. This disorder could very well be responsible for the leukemic transformation of these cells, and the removal of this condition could revert the leukemic cells to normalcy.

Biochemistry has clarified the action of different compartments of RNA. We know that the messenger-RNA (m-RNA) is formed on the gene, and that it carries the genetical information to the cytoplasma in order to induce the specific protein synthesis. There are only a few papers dealing with biological activity of this m-RNA. Nevertheless, it is well established that m-RNA – even from other organs and species – is very rapidly resorbed by many kinds of cells. Niu found an induction of enzyme synthesis in tumor cells of liver, by incubating tumor cells

in a solution of liver m-RNA. By the action of m-RNA "in vitro" it is even possible to induce immunity, or the specific behavior in cells of other species, as well as to induce protein production of the species of the donor of m-RNA. Aksenova et al. succeeded in normalizing liver tumor cells by the action of liver m-RNA, in so far as the tumor cells remained alive, but did not grow after transplantation.

These results seem to justify the hope that a normalization of leukemic cells could be arrived at under the influence of an organ-specific m-RNA. Regarding this, Wrba has suggested that the first step of such a research project should be the isolation of a biological active m-RNA from a hematopoietic tissue. The second step should be the study of the possibility of stimulating the activity with this m-RNA in a system of protein synthesis "in vitro" (i. e., ribosomes, labeled amino acids, and ATP). In case of a positive result, the third step should be to investigate the effect in cell cultures of the above-mentioned m-RNA on the maturation process of leukemic cells. The final step of this concept for causal treatment of the leukemic process should be the experiments in patients – checking the application, the dosage, and the frequency of administrations of the compounds, etc. Regarding this, it is interesting to note that Niu has shown that in several cases the activity induced "in vitro" by m-RNA remains stabilized "in vivo" for at least 20 weeks.

These concepts are substantiated by the fact that some experimental and clinical tumor reversibilities have been observed in these past years. A review on this subject has been recently made by Gellhorn, but we should like to confine ourselves only to few examples.

In growing a transplantable mouse lymphosarcoma for several years in cell cultures, Willemina De Bruyn has been able to obtain four cellular strains with different biological behaviors. One strain consists of lymphoblasts and fibroblasts which grow together, and when injected into susceptible mice it reproduces the original tumor. The second strain consists only of fibroblasts, which do not reproduce the tumor. The third strain consists only of lymphoblasts, which are derived from the first strain: these lymphoblasts after 4 months of growth in cell culture without fibroblasts lose their capacity to reproduce the tumor. The fourth strain has been developed using a different medium, and this strain consists of lymphoblasts only. Although both the third and fourth strains consist of lymphoblasts only, only the fourth strain is capable to reproduce tumor.

Similar evidence of neoplastic cell reversibility to normalcy has been seen in the work of MacPherson. He cultured normal cells from hamsters in soft agar, to which Rous-sarcoma virus was added, and those cells became malignant, as seen by their tumor reproducing capacity. MacPherson isolated single cells from these transformed clones, and transplanted them into a new culture medium. These cells grew; but after a weekly transplantation of 8 weeks, 98% of the single cell clones had reverted to normal.

Pierce, injecting cells from an ascites tumor grown for several years to a recipient, could observe after a period of time the formation of tumors histologi-

cally composed either by tumor cells, or by a number of benign tissues, such as cartilage, nerve tissue, and celiated epithelium.

An example of human cancer reversibility has been substantiated by the observations of Kister. He found that a full growing endometrial cancer was converted to normal decidua and normal secretory epithelium, in consequence to the application of progesterone directly into the uterine cavity.

All these facts show that neoplastic cells are not always irreversibly changed, but that they may still have the possibility to become benign again. In view of this, our efforts should be directed to understanding the mechanism of tumor reversibility, and in trying to provoke such reversibility in leukemia. The experiments of MacPherson showed that, when a cell became malignant, it contained the Rous-virus, and when the malignant cell reverted to normal after several transplantations in a new culture medium, it lost the virus. If this model can be used for human leukemia, we really may consider the possibility that the leukemogenic agent produces the genetical alterations whereby a cell becomes leukemic, and that the removal of this agent could revert these leukemic cells to normalcy.

References

1. Aksenova, M. N. et al.: Influence of ribonucleic acids from the liver on implantation and growth of transplantable tumors. *Nature 196*, 443 (1962).
2. Aksenova, M. N. et al.: Effect of ribonuclease on anti-tumour activity of ribonucleic acid from normal thymus. *Nature 207*, 40 (1965).
3. Anos et al.: Evidence for messenger activity of alien ribonucleic acid in chick cells. *Nat. Cancer Inst. Monograph 13*, 155 (1964).
4. Astaldi, G.: Current treatment of malignant blood disorders. *New Istanbul Contribution to Clinical Science 7*, 40 (1964).
5. Cohen, E. P., Parks, J. J.: Antibody production by nonimmunized spleen cells incubated with RNA from immunized mice. *Science 144*, 1012 (1964).
6. De Bruyn, W. M.: Studies of non-tumor and tumor producing cell-lines derived from transplantable mouse lymphosarcoma MBT 86,157. Proc. 9th Congr. Int. Soc. Haemat. Mexico, D. F., 1962.
7. De Carvalho, S.: Effect of RNA from normal human bone marrow on leukemic marrow in vivo. *Nature 197*, 1077 (1963).
8. De Carvalho, S., Rand, R. J.: Comparative effects of liver and tumor ribonucleic acids on the normal liver and the Novikoff hepatoma cells of the rat. *Nature 189*, 815 (1961).
9. Esposito, S.: Ultrastrukturänderungen leukaemischer retikulärer Zellen nach Zusatz von RNS aus normaler Milz zum Kulturmedium. *Experientia 20*, 69 (1964).
10. Esposito, S.: Formation of tryptohanypyriolase in leukemic cells incubated with RNA obtained from spleen of calf embryo. *Acta haemat. 32*, 265 (1964).
11. Esposito, S.: Effect on leukemic cells of ribonucleic acid extracted from calf's spleen. *Nature 203*, 1078 (1964).
12. Esposito, S. et al.: Metodi e prospettive delle ricerche di biologia molecolare con particolare riguardo all'ematologia. *Haematologica 49*, 415 (1964).
13. Esposito, S. et al.: Effect of RNA on leukemia tissue in vivo. *Lancet 1*, 746 (1964).
14. Gellhorn, A.: Editorial on Cancer: Facts and Theories-Clinical Physiology, Chemotherapy, Fundamental Nature and Mechanisms of Gene Control. *Seminars in Hematology 3*, 99 (1966).

15. Hess, M. et al.: The effects of nucleic acids on pituitary ACTH content. *Endocrinology 68*, 549 (1961).
16. Mannick, J. A., Egdahl, R. H.: Ribonucleic acid in "transformation" of lymphoid cells. *Science 137*, 976 (1962).
17. Niu, M. C.: Glucose-6-phosphatase reexamination of the RNA-induced activity in mouse ascites tumor cells. *Science 148*, 513 (1965).
18. Niu, M. C.: Effect of ribonucleic acid on mouse ascites cells. *Science 131*, 1321 (1960).
19. Niu, M. C.: Mode of action of the exogenous ribonucleic acid in cell function. *Nat. Cancer Inst. Monograph 13*, 167 (1964).
20. Niu, M. C.: The stability of the RNS-induced cellular changes. *Fed. Proc. 22*, 354 (1963).
21. Niu, M. C. et al.: Ribonucleic acid induced changes in mammalian cells. *Proc. Nat. Acad. Sci. 47*, 1689 (1961).
22. Niu, M. C. et. al.: RNA-induced biosynthesis of specific enzymes. *Proc. Nat. Acad. Sci. 48*, 1946 (1962).
23. O'Neal, M. A. et al.: Effect of liver ribonucleic acid preparations of Novikoff-Hepatoma cells. *Proc. Amer. Ass. Cancer Res. 3*, 349 (1962).
24. Wrba, H.: Personal communication.
25. Weisberger, A. S.: Induction of altered globin synthesis in human immature erythrocytes incubated with ribonucleoprotein. *Proc. Nat. Acad. Sci. 48*, 68 (1962).
26. Zimmermann, E., Turba, F.: Induktion der Biosynthesestruktur differenter Haemoglobin durch Ribonucleinsäurefraktionen artfremder Reticulocyten. *Biochem. J. 339*, 469 (1964).
27. Zimmermann, E. et al.: Induktion der Synthese artfremden Serumalbumins in Mäuse-Asciteszellen durch nucleolare Ribonucleinsäure aus Leberzellen. *Biochem. J.* 339, 53 (1964).

Comparaison du taux moyen de complément dans un échantillon de malades et dans un échantillon de personnes normales

Considérations méthodologiques et application au cas des ulcères duodénaux et gastriques

(Note préliminaire)

G. Lennes, A. André, C. Lhoste

Université de Liège, Centre de Génétique Humaine, et Laboratoire des Groupes Sanguins

(Reçu le 28 Février 1967)

Considérations méthodologiques

Le dosage du complément contenu dans le sérum est fondé sur la loi de von Krogh, mettant en relation le pourcentage Y d'hémolyse observé dans le système hémolytique et la dose X du sérum étudié introduite dans ce système. On a:

$$X = K \left(\frac{Y}{1 - X} \right)^{\frac{1}{n}}$$

où $\frac{1}{n}$ est une constante bien déterminée, ayant théoriquement une valeur de 0,2 lorsqu'on utilise le système hémolytique de Kabat et Mayer [4].

On voit donc que si $Y = 0{,}5$ (50% d'hémolyse), on a $X_{50} = K$; K nous donne donc la quantité de sérum requise pour produire 50% d'hémolyse. Lorsque $K = 1$, on dit que le sérum contient une unité hémolytique 50%; lorsque $K = 0{,}1$ par exemple, le sérum contiendra 10 unités 50%, soit $1 = 0{,}1$.

D'une façon générale, nous utilisons le sérum humain dilué au 1/10°, de sorte que si X_{50} est la quantité de sérum dilué provoquant l'hémolyse 50%, le taux de complément de ce sérum exprimé en unités hémolytiques 50% sera donné par la relation

$$U_{50} = \frac{10}{X_{50}}$$

En appliquant la transformation logarithmique, la relation de von Krogh peut s'écrire:

$$\log \frac{Y}{1 - Y} = n \log X - n \log K$$

Pour le système hémolytique décrit par Kabat et Mayer, on doit avoir $n = 5 \left(\text{ou } \frac{1}{n} = 0{,}2 \right)$. Cette valeur de $\frac{1}{n} = 0{,}2$ est évidemment toute théorique; de

nombreux auteurs considèrent en pratique que des valeurs comprises entre 0,17 et 0,23 sont encore compatibles avec le système hémolytique de Kabat et Mayer. Beaucoup d'entre eux se bornent en fait à réaliser les conditions expérimentales de Kabat et Mayer et à calculer le taux de complément en unités hémolytiques, tout en supposant $n = 5\left(\text{ou } \frac{1}{n} = 0{,}2\right)$. Une longue pratique des analyses du complément nous a enseigné toutefois l'utilité de calculer chaque fois (ou tout au moins de déterminer graphiquement) la pente de la droite de von Krogh. Cette détermination de la pente permet de vérifier rapidement que les conditions expérimentales n'ont pas varié.

Or, les variations des conditions expérimentales peuvent s'introduire très aisément à l'insu de l'expérimentateur: vieillissement prématuré du lot d'hématies utilisé, variations de la sensibilisation des hématies lors d'un renouvellement de l'ambocepteur, etc . . . Il nous est arrivé par exemple d'obtenir des pentes voisines de 0,27; à l'examen rétrospectif des causes d'erreur, nous nous sommes aperçus pour quelques échantillons que les hématies utilisées avaient été fragilisées par une station trop prolongée dans un réfrigérateur dont la température était tombée au dessous de 0°.

Cependant le calcul de la droite de von Krogh ne sert pas seulement à détecter les variations anormales des conditions expérimentales. Lorsqu'on compare les taux de complément de deux sujets donnés A et B, on compare en fait leur taux de complément en unités 50% ou, en d'autres termes, les concentrations de complément qui chez les deux sujets produisent une hémolyse de 50% des hématies du système. Une telle comparaison doit en fait être indépendante de l'effet choisi et on devrait obtenir un même rapport pour les sujets A et B si on avait choisi une hémolyse de 20%, ou une hémolyse de 80%. Une telle constance du rapport des taux de complément entre les individus A et B est garantie par le parallélisme des droites de von Krogh correspondant aux sujets A et B.

Le choix d'un échantillon

Nous allons maintenant comparer le taux de complément dans un échantillon de personnes souffrant d'un ulcère duodénal d'une part et dans un échantillon de personnes normales sous le rapport de l'ulcère d'autre part. (Nous nous attacherons également au cas de l'ulcère gastrique.)

Pour ce faire, on aurait pu évidemment prendre au hasard un échantillon d'ulcéreux et un échantillon de personnes normales, déterminer les moyennes correspondant à ces deux échantillons indépendants et les comparer ensuite pour juger de la signification statistique du résultat.

Ce procédé nous paraît cependant dangereux. Dans une note précédente, nous avons montré, confirmant par là des résultats de Basevi et Furlan, que le taux de complément a tendance à augmenter en fonction du poids [1]. Lorsqu'on passait de 50 à 110 kg, on observait dans notre échantillon une augmentation du

taux de complément de 2 unités, soit en moyenne une augmentation de 0,33 unités par tranche de 10 kg. Les ulcéreux étant en général caractérisés par un poids légèrement inférieur à celui des personnes normales, la différence que l'on risquerait de mettre en évidence en prenant au hasard un échantillon de contrôle pourrait être imputée à la différence de poids caractérisant les deux échantillons.

C'est la raison pour laquelle à chaque ulcéreux, nous avons associé un témoin ayant le même poids, ou tout au moins un poids très voisin. (La différence de poids est toujours inférieure à 10 kg.) Dans le choix du témoin, nous n'avons pas seulement tenu compte du poids, mais également de l'âge. Basevi et Furlan ont en effet montré également que le taux de complément a tendance à augmenter avec l'âge; nous avons aussi retrouvé cette tendance dans un groupe étudié précédemment. Lorsqu'on passait de la classe correspondant à 25 ans à celle correspondant à 65 ans, on notait une augmentation de 0,8 unités, soit une augmentation de 0,2 unités par tranche de 10 ans, mais nous n'avions pas trouvé d'effet significatif.

Ni les groupes sanguins (AB0, Rhésus), ni le sexe n'ont d'effet marqué sur le taux de complément. Nous nous sommes donc bornés à associer témoin et ulcéreux selon le sexe.

Les prélèvements de sang chez le témoin et chez l'ulcéreux ainsi que les déterminations du complément ont toujours été faits dans des conditions identiques. Nous avons particulièrement veillé à ce que ces opérations soient effectuées à la même date pour un couple de personnes données afin de nous mettre à l'abri des variations systématiques des taux de complément qui pourraient être dues au temps et des légères variations du système hémolytique qui ne sont pas toujours décelées par la méthode de von Krogh.

Les résultats de notre étude sont consignés dans le tableau suivant. Sur une même ligne se trouvent les valeurs correspondant à un couple déterminé, témoin et ulcéreux. La première colonne donne le taux de complément du témoin (T), la seconde le taux de complément de l'ulcéreux (U), la troisième la différence (T − U). Dans la quatrième colonne, nous trouvons l'estimation de la pente de la droite de von Krogh, pente commune à la droite de l'ulcéreux et à celle de son témoin. Cette pente a été déterminée par la méthode des moindres carrés; on notera que les valeurs de la pente correspondant aux différents couples sont très proches les unes des autres et toujours intérieures à l'intervalle de tolérance généralement admis (0,170 à 0,230). Les cinquième et sixième colonnes donnent les poids respectifs du témoin et de l'ulcéreux, les septième et huitième colonnes, leur âge, la neuvième et dernière colonne, le sexe.

La dernière ligne donne les moyennes arithmétiques correspondant aux diverses colonnes de valeurs numériques.

La série des cas d'ulcère duodénal, tout comme celle des cas d'ulcère gastrique semblent caractérisées par un taux de complément inférieur à la normale de l'ordre de 2 unités.

$$28{,}65 < 30{,}94 \text{ et } 30{,}42 < 33{,}02$$

Ulcère duodénal

Taux de complément			Pentes	Poids (Kg)		Age en 1967		Sexe
T	U	T−U		T	U	T	U	
35,1	20,8	+14,3	0,224	59	65	62	51	F
29,4	28,3	+ 1,1	0,204	70	70	42	51	M
29,7	21,7	+ 8,0	0,222	72	70	34	34	M
34,7	31,6	+ 3,1	0,217	78	77	59	52	M
37,0	27,5	+ 9,5	0,218	70	76	46	48	M
32,0	39,4	— 7,4	0,225	61	63	52	63	M
26,5	34,0	— 7,5	0,201	44	43	43	35	F
34,5	34,8	— 0,3	0,215	66	67	51	61	M
29,2	29,2	0	0,217	68	65	63	61	M
29,7	15,9	+13,8	0,198	55	53	55	58	M
23,5	30,7	— 7,2	0,202	69	75	54	65	M
34,2	27,8	+ 6,4	0,225	57	57	37	32	M
26,7	30,7	— 4,0	0,212	63	67	51	47	F
30,94	28,65	+ 2,29	0,2138	64,0	65,2	49,9	50,6	

Ulcère gastrique

Taux de complément			Pentes	Poids (Kg)		Age en 1967		Sexe
T	U	T−U		T	U	T	U	
36,9	30,7	+6,2	0,207	65	65	60	62	M
36,3	28,4	+7,9	0,210	49	49	54	52	F
25,0	28,1	—3,1	0,192	61	63	61	62	M
32,6	27,4	+5,2	0,198	106	105	46	49	M
34,3	37,5	—3,2	0,226	56	62	61	64	M
33,02	30,42	+2,60	0,2066	67,4	68,8	56,4	57,8	

Ces résultats ne sont toutefois pas statistiquement significatifs. Pour l'ulcère duodénal, la moyenne des différences, soit 2,29 ne diffère pas significativement de 0 ($t = 1{,}07$ pour 12 ddl : $P > 0{,}05$). Il en va de même pour l'ulcère gastrique où 2,60 ne diffère pas non plus significativement de 0 ($t = 1{,}09$ pour 4 ddl : $P > 0{,}05$).

Toutefois, le fait que les deux séries de malades donnent des résultats allant dans le même sens, plaide en faveur d'une différence entre les cas d'ulcère digestif et les personnes normales sous ce rapport. Il ne s'agit là pour l'instant que d'une hypothèse de travail, mais elle nous paraît suffisamment plausible que pour nous inciter à continuer notre étude.

Par ailleurs, l'un de nous avait auparavant émis l'hypothèse qu'en cas d'incompatibilité AB0, un foetus ulcéreux en puissance (c'est-à-dire ayant une hérédité

le prédisposant à l'ulcère duodénal) avait plus de chances d'être éliminé par le jeu de cette incompatibilité qu'un foetus n'ayant pas un tel potentiel héréditaire. Cette hypothèse permet d'expliquer notamment l'excès de personnes du groupe 0 que l'on observe chez les personnes souffrant d'un ulcère duodénal sans faire appel à l'hypothèse de la pléiotropie du groupe 0. Elle permet également d'expliquer le très faible pourcentage de couples incompatibles que l'on trouve chez les parents de ces ulcéreux (11 couples: mère A + père 0 contre un couple: mère 0 + père A).

Il nous a paru dès lors intéressant de rechercher chez les ulcéreux une manifestation éventuelle de cette prédisposition héréditaire qui les rendrait plus vulnérables à l'incompatibilité ABO.

Le complément jouant un rôle très important dans l'incompatibilité ABO, et pas de rôle du tout semble-t-il dans l'incompatibilité Rhésus, le taux de complément nous paraissait donc devoir être étudié en relation avec l'ulcère digestif.

La diminution du taux moyen de complément que l'on observe chez ces malades dans notre échantillon pourrait selon nous s'expliquer par un phénomène de consommation, les ulcéreux consommant plus de complément que les normaux dans la mesure où leur prédisposition héréditaire les rend plus sensibles aux phénomènes immunitaires de type ABO.

Cela n'est évidemment qu'une hypothèse à l'heure actuelle. De nombreuses analyses semblables aux nôtres devront encore être effectuées avant de pouvoir être plus affirmatifs. Il nous a paru cependant intéressant de souligner dès maintenant l'aspect méthodologique d'une telle recherche et d'indiquer brièvement le cadre dans lequel elle s'inscrit.

Bibliographie

1. Basevi, A. et Furlan, S.: Il complemento serico nel normale. *Giorn. malat. infett. parass. 9*, 480 (1956).
2. André, A. et Lennes, G.: La valeur moyenne du taux de complément total dans la population adulte normale est-elle en relation avec les groupes sanguins ABO, MN, le sexe, l'âge et le poids? Proc. 10th Congr. int. Soc. Blood Transf. Stockholm 1964 pp. 845—849 (1965).
3. André, A. et Lennes, G.: Choix d'un système hémolytique pour l'étude du complément. *Acta clin. belg. 19*, 220 (1964).
4. Kabat, E. A. et Mayer, M. M.: Experimental immunochemistry, 2nd ed. Ch. C. Thomas Springfield, Illinois 1961.
5. Lennes, G.: Point de vue nouveau sur les relations entre l'ulcère duodénal et les groupes sanguins ABO. *Bull. Acad. Royale belge de Médecine*, VIIe série *I*, 615 à 623 (1961).
6. Lennes, G., André, A., Otto-Servais, M., Dodinval, P. et Heirman-Monseur, F.: L'incompatibilité ABO est-elle en relation avec l'ulcère duodénal? Proc. Second Intern. Congr. of Human Genetics, pp. 855 à 857, 1964.

Iodination of Human IgG and its Papain-Insensitive Population

Preliminary Report

J. GERGELY, I. ÁRKY, G. A. MEDGYESI

Institute of Haematology, National Blood Centre, Budapest

(Received March 5, 1967)

Previous investigations have shown that digestion of IgG with papain in the absence or presence of cysteine produces two different molecular populations. The papain-sensitive IgG is digestible with papain in the absence of cysteine, whereas the papain-insensitive one, only in the presence of, or after pretreatment with, cysteine. The difference between these two types is one of molecular conformation and depends on the Fc part of the molecules [1].

The iodination of the IgG and its papain-insensitive population supplied further evidence of this supposed conformational difference.

Immunochemical pure IgG preparations obtained from individual human sera were trace-labelled with ^{131}I isotope using the chloramine-T method [2]. The amount of radioactivity was measured on a well-type scintillation counter (Frieseke–Hoepfner FH 49 type). The protein content of the IgG solutions was determined by measuring optical density at 280 mμ as well as by estimating nitrogen content by the micro-Kjeldahl method. The specific activity of the "whole" IgG preparations was 200,000–500,000 cpm/1 mg protein. To prepare the papain-insensitive population digestion of the ^{131}I labelled IgG in the absence of cysteine was followed by gel-filtration on Sephadex G.100 [1].

As determined in six individual human IgG preparations and their papain-insensitive populations the specific activity was markedly lower in the latter (Table I).

Table I

Specific activities of IgG preparations and their papain-insensitive populations; cpm/mg protein

Preparation	"Whole"	Papain-insensitive
IgG 4	350,000	210,000
IgG 5	360,000	190,000
IgG 9	100,000	50,500
IgG 10	135,000	80,000
IgG 20	132,000	50,000
IgG 21	228,000	186,000

The pretreatment of IgG with cysteine results in a complete digestibility of protein with papain without further addition of cysteine. Optical rotation measurements revealed an increase in the levorotation of the cysteine-treated IgG indicating that some change in the tertiary structure has occurred [1].

Considering these data we investigated the effect of cysteine treatment on the iodination of the papain-insensitive IgG.

Samples of cysteine-treated and untreated papain-insensitive IgG preparations were labelled with ^{131}I isotope. The specific activities of the cysteine-treated IgG (incubation for 60 min at 37°C with 0.01M cysteine, followed by an exhaustive dialysis) were markedly higher as compared with the specific activity of the same untreated papain-insensitive IgG preparation (Table II).

Table II

Specific activities on untreated and cysteine-treated papain-insensitive IgG; cpm/mg protein

Preparation	Untreated	Cysteine-treated
IgG 4	330,000	510,000
IgG 10	93,000	190,000
IgG B	57,000	81,000
IgG 13	71,000	196,000

The labelling of IgG myeloma globulins showed a higher specific activity of the papain-sensitive types.

The lower specific activity of the papain-insensitive IgG as well as the increase of the specific activity after treatment with cysteine may also indicate a conformational difference between the papain-insensitive and papain-sensitive populations of IgG.

Further details will be published later.

References

1. Gergely, J., Stanworth, D. R., Jefferis, R., Normansell, D. E., Henney, C. S., Pardoe, G. I.: Structural studies of immunoglobulins. I. The role of cysteine in papain hydrolysis. *Immunochemistry 4*, 101 (1967).
2. Bocci, V.: Marcatura di sieroproteine con ^{131}I mediante cloramina T. *Boll. della Soc. Ital. Biol. Sper. 40*, 89 (1964).

A Contribution to the Study of Calcium and Phosphate Metabolism in Kahler's Disease

J. Hořejší, M. Svoboda, V. Švorcová

Institute of Haematology and Blood Transfusion, Prague

(Received March 10, 1967)

1. In Kahler's disease the metabolism of calcium and phosphate has been studied. 14 cases were characterized by electroforesis, immunoelectroforesis, detailed X-ray examination of the skeleton and testing of the renal function. According to X-ray findings all cases were divided in 2 groups: with osteolytic changes and with diffuse osteoporosis.

2. In osteolytic forms we have observed more frequently (in 62%) the gamma G paraproteinaemia. In osteoporotic cases the incidence of gamma A and gamma G paraprotein was of the same proportion (50 : 50%).

3. There were no differences in calcium and phosphate blood levels in both kinds of bone changes.

4. The excretion of calcium in urine after the restricted intake of calcium in the diet was higher in osteoporotic changes than in cases with osteolytic lesions. The excretion of phosphate was similar (equal) in both groups.

5. After the intravenous administration of calcium (10 mg/kg body weight) the blood calcium curve was similar in both groups. But the excretion of calcium in urine was remarkably increased in osteolytic cases (especially in males). In these forms of osseous changes there was also quite distinct elevation of phosphate excretion.

6. The changes observed in myeloma cannot be explained either by activation of hyperparathyreoid secretion or renal regulatory mechanism. They are perhaps due to the specific metabolic activity of the myelomatous tumorous tissue itself and by its influence on bone metabolism.

In Kahler's disease (multiple myeloma) characteristic bone changes are almost regularly present. They can be seen in radiographs either as osteolytic foci in various parts of the skeleton or as diffuse decalcification, osteoporosis. Sometimes combinations of both types of changes can be observed. These changes of the bone structure are almost integral part of the clinical picture of myeloma. Nevertheless not much attention has been paid to the metabolism of bone minerals, namely of calcium and phosphorus.

In some papers published recently higher blood calcium levels have been described (e.g. Bentzel et al. [1], Schmutziger [2], Merival [3]). Schmutziger analysed the metabolism of calcium and phosphorus in myeloma and suggested that in some cases metabolic changes were similar to those seen in hyperparathyroidism. These observations suggested that in myeloma substances similar to parathormone in their effect on Ca and P metabolism might be produced. In some cases the urinary output of phosphate was high, while in others it was low.

Recently we had the opportunity to study in more detail the metabolism of calcium and phosphate in a series of patients with Kahler's disease. We have raised two questions: 1. Might the changes in calcium and phosphorus metabolism be due to a change in parathyroid function? 2. Is the metabolism of P and Ca in the osteolytic form different from that in the osteoporotic one?

Material and Methods

The patients, divided into two groups on the basis of the changes in bone structure, as demonstrated by detailed radiography of the skeleton, were all examined by paper and immuno-electrophoresis, to analyse paraproteinaemia. Bone marrow smears, important in diagnosis, were also studied. Tests were made for NPN and creatinine clearance to assess renal function.

Calcium and phosphorus metabolism was studied before cytostatic treatment. The blood calcium level was determined by manganometric titration,

Table 1

Name	Immunoelectro-foresis	Proteinuria	Electroforesis		Bone marrow smears % of plasmocytes
A) Osteolytic forms					
J. K.	γ g kryoglob +	+++ para γ^2, β_2	Total protein A 40.6 α_1 6,72 α_2 9.88 β 31,3	10,5 g% γ 11.4	37%
J. J.	γ g	+++ para γ_3	Total protein A 29,8 α_1 4,30 α_2 8.1 β 7.9	12,8 γ 49.8	18%
J. S.	γ g	+ —	Total protein A 47.7 α_1 5.8 α_2 9.2 β 14.8	11.1 γ 24.3	—
L. B.	Mikropara	B. J. +	Total protein A 56.20 α_1 6.11 α_2 10.07 β 10.9	8.15 γ 16.6	44%

phosphataemia by the colorimetric method of Fiske–Subbarow. The excretion of both calcium and phosphate was determined in urine collected for 24 hrs. after standard diet with restricted calcium intake which had been given for 3 days before the experiment. Patients were then injected with calcium gluconate intravenously and 10 mg/kg body weight were administered in the course of 3 hrs. interval. The calcaemia was tested repeatedly in the period of 18 hrs. after injection (10′–30′–1 hr.–2–4–6–18 hrs.). And calcium and phosphorus excretion in urine was determined 24 and 48 hrs. after the calcium administration.

All patients thus examined were divided in 2 groups according to skeletal changes (X-ray findings). 24 patients were examined altogether. But not every case was analysed and evaluated. Mixed forms (with both osteolytic and osteoporotic changes) were eliminated and also cases in which osteoporosis might have been caused by another mechanism, as e.g. by osteoporosis of elderly people and of women after menopause. Our study includes 14 cases, 8 with osteolytic and 6 with diffuse osteoporotic skeletal changes. The laboratory findings are presented in Table 1.

NPN Creatinine clearance	Calcaemia	Phosphate in blood serum	Ca excretion in urine 24/hrs	Phosph. excretion in urine 24/hrs
30.4 mg% F 122 ml R 99.3	10.5	2.2	I. 90 mg II. 660 mg III. 140 mg	—
45.4 mg% F 81 ml R 98.1	10.8	2.6	I. 20 mg II. 85 mg III. 36 mg	520.8 mg — 35.1 mg
25.3 mg% — —	10.0	3.8	I. 49.8 mg II. 174.4 mg III. 413.5 mg	29.5 mg 278.3 mg 458.6 mg
28.0 mg% F 102.0 ml R 99.0	10.8	3.3	I. 142.0 mg II. 909.0 mg III. 211.0 mg	1145.0 mg 1208.0 mg 840.0 mg

Table 1

Name	Immunoelectro-foresis	Proteinuria	Electroforesis		Bone marrow smears % of plasmocytes
M. M.	γ A	0	Total protein A α_1 α_2 β	7.85 55.7 5.3 8.6 10.4 γ 19.9	23.6%
M. K.	γ g	+++ para γ_2	Total protein A α_1 α_2 β	7.25 55.4 7.9 14.1 14.4 γ 8.6	92%
B. K.	γ g	+	Total protein A α_1 α_2 β	11.2 35.4 4.4 7.0 6.8 γ 46.2	10.2%
A. H.	since para	+ —	Total protein A α_1 α_2 β	6.6 57.0 8.0 11.2 12.9 γ 10.8	60.5%
B) Osteoporotic forms					
L. Š.	γ A	0	Total protein A α_1 α_2 β	7.6 42.2 3.3 13.3 35.8 γ 5.4	9.6%
A. Šv.	γ g	++ B. J. ++	Total protein A α_1 α_2 β	12.4 47.2 4.2 7.8 7.7 γ 33.0	37.2%
F. B.	γ g	+	Total protein A α_1 α_2 β	11.4 31.5 3.3 4.4 5.6 γ 55.2	16%
J. J.	γ g	+++ γ_2	Total protein A α_1 α_2 β	6.0 49.5 7.1 13.9 10.2 γ 19.3	43%

cont.

	NPN Creatinine clearance	Calcaemia	Phosphate in blood serum	Ca excretion in urine 24/hrs	Phosph. excretion in urine 24/hrs
	30.2 mg%	10.3	3.0	I. 316.0 mg	
F	72 ml			II. 426.0 mg	—
R	99.1			III. 156.0 mg	
	79.6 mg%	10.4	9.1	I. 100.0 mg	378.0 mg
F	18.0 ml			II. 140.0 mg	434.0 mg
R	92			III. 109.0 mg	324.0 mg
	29.3 mg%	9.5	3.2	I. 298 mg	972.0 mg
F	74.3 ml			II. 314.0 mg	720.0 mg
R	99.1			III. 145.0 mg	597.0 mg
	34.0 mg%	9.6	4.2	I. 279.0 mg	—
F	67.7 ml			II. 478.0 mg	801.0 mg
R	99.4			III. 227.0 mg	440.0 mg
	34.6 mg%			I. 384.0 mg	—
F	86.0 ml	10.0	—	II. 665.0 mg	—
R	99.0			III. 212.0 mg	—
	20.6 mg%			I. 154.0 mg	543.0 mg
R	44.5	10.0	3.4	II. 432.0 mg	379.0 mg
				III. 223.0 mg	575.0 mg
	37.8 mg%			I. 324.0 mg	840.0 mg
	—	10.6	4.5	II. 614.0 mg	742.0 mg
	—			III. 226.0 mg	975.0 mg
	45.0 mg%			I. 156.0 mg	—
F	56.0 ml	10.3	3.9	II. 268.0 mg	
R	98.8			III. 110.0 mg	

Table 1

Name	Immunoelectro-foresis	Proteinuria	Electroforesis		Bone marrov smears % of plasmocytes
A. S.	γ A	0	Total protein	10.5	
			A 33.2		
			α_1 4.6	γ 13.9	—
			α_2 5.5		
			β 42.7		
J. V.	γ A	+	Total protein	9.0	
		—	A 38.8		10%
			α_1 7.1	γ 9.7	
			α_2 7.5		
			α_2 36.6		

Results

According to the biochemical findings 57% of the cases had gamma G type of paraproteins in blood serum. In osteolytic cases the incidence of gamma G protein is a little higher (62%), in cases with diffuse osteoporotic changes there is almost equal frequency of gamma A and gamma G type of paraproteinaemia (50 : 50%). In most cases the total protein level is high due to the high proportion of paraprotein. In 8 cases distinct proteinuria was present but only in 2 cases there was Bence–Jones protein present in the urine. Renal functions tested by NPN and creatinine clearance test were within normal limits in all cases but one where renal failure and preuraemic state were present.

The values for calcium and phosphorus metabolism are shown in Table 2. It is evident that in both groups of patients the blood calcium level does not differ and is within normal limits (9.1 – 10.8 mg%). There are no differences between the groups of men and women. The blood phosphate levels are a little more elevated (3.7 mg%) in the group with diffuse osteoporotic changes than in the osteolytic cases (3.2 mg%). The empirical product Ca × P is low in osteolytic and close under the low normal limit in osteoporotic cases, its values fall within normal range.

After intravenous administration the maximum elevation of blood Ca was reached in all cases within 60′. In about one half of cases the hypercalcaemia normalized in 6 hrs. (there was no correlation with the character of the bone changes). The total elevation of Ca in blood is a little higher in the osteolytic forms than in the osteoporotic ones. The elevation of the calcium in blood can be expressed in relation to the original level before the injection, as a quotient, the value of which is 1.45 in osteolytic cases and 1.30 in cases with diffuse osteoporotic changes. In about 50% of the cases examined the blood calcium level returned to the preinjection level within 6 hrs. in both groups of patients.

cont.

NPN Creatinine clearance	Calcaemia	Phosphate in blood serum	Ca excretion in urine 24/hrs	Phosph. excretion in urine 24/hrs
36.0 mg% F 128.0 ml R 99.0	9.1	3.7	I. 406.0 mg II. 699.0 mg III. 191.0 mg	—
30.0 mg% F 96.7 ml R 99.1	10.6	3.2	I. 59.8 mg II. 435.0 mg III. 169.0 mg	390.0 mg 220.0 mg 434.0 mg

After the intravenous injection urinary Ca output increased in both groups of patients. The quantity of calcium excreted in 24 hrs. before the injection of calcium and in 24 hrs. after the injection can be expressed as the relation of the two values (quotient of calcuria). The value of this quotient is higher in the osteolytic form. This has been especially remarkable in men, where very low calcium excretion has been observed after the feeding of low-calcium diet. The higher values of phosphorus excretion in urine after calcium administration has been observed only in osteolytic forms, and never in cases with diffuse osteoporotic changes.

Table 2

Name	Ca in blood	P in blood	Q of calcaemia	Q of Ca excretion in urine	Q of P excretion in urine
A) Osteolytic forms					
J. K.	10.5	2.2	1.34	7.3	—
J. J.	10.8	2.6	1.4	4.0	—
J. S.	10.0	3.8	1.5	3.5	8.5
L. B.	10.8	3.3	1.4	6.5	1.0
M. M	10.3	3.0	1.36	1.3	—
M. K.	10.4	9.1	1.34	1.4	1.1
B. K.	9.5	3.2	1.2	1.0	0.8
A. H.	9.6	4.2	1.9	1.6	—

Table 2 cont.

Name	Ca in blood	P in blood	Q of calcaemia	Q of Ca excretion in urine	Q of P excretion in urine
B) Osteoporotic forms					
L. Š.	10.0	—	1.6	1.7	—
S. Šv.	10.0	3.4	1.3	2.8	0.7
F. B.	10.6	4.5	1.2	1.9	0.9
J. J.	10.3	3.9	1.3	1.7	—
A. S.	9.1	3.7	1.2	1.7	—
J. V.	10.6	3.2	1.24	7.2	0.56

As a control 4 patients were studied without any signs of myeloma and also with normal X-ray picture of the skeleton. In all of these cases both calcium and phosphorus blood levels were normal (8.9 mg% Ca and 3.0 mg% P) and also the excretion of both minerals in urine was within normal limits (308 mg Ca and 717 mg P in 24 hrs.). After the intravenous administration of calcium the excretion of calcium was more than doubled ($Q = 2.6$), the excretion of P was unchanged ($Q = 1.0$).

Discussion

From our findings concerning Ca and P metabolism in Kahler's disease we can conclude that there are no signs of changes which could be attributed to the alteration of parathyroidal hormonal control. In hyperthyreosis with characteristic bone changes there are always characteristic changes in calcium metabolism present, the blood calcium level is high and blood phosphate level low. The normal excretion of phosphate in urine which we have found in myeloma is also very much against the possibility of increased parathyroid secretion. The excretion of phosphate in hyperparathyreosis is elevated because of the impairment of phosphate reabsorption in renal tubuli.

In calcium and phosphorus metabolism an important role is also played by the kidney. That is regularly damaged in myeloma, namely in evolutive period of the disease, when pathological changes involve also the kidney tissue (myelomatous kidney). In our cases no changes in the renal function have been found (of course, only as far as can be concluded from the NPN level and creatinine clearance test). Only in one patient (woman with osteolytic bone changes) there were signs of renal failure and here the phosphate blood level was remarkably

elevated (9.1 mg%). But the excretion of both calcium and phosphate in urine even here was normal and also after the calcium administration we did not observe any characteristic changes.

Renal osteodystrophia can be caused both by glomerular incompetence and tubular impairment. In the first case blood calcium levels are low and there is hyperphosphatasaemia. In tubular lesions there are signs of phosphate diabetes. Nothing of that was observed in our cases.

In both groups of skeletal changes in myeloma we did not see any difference in blood calcium and phosphate level. Differences can be seen only in the values of excretion of calcium which is higher in osteoporotic forms of skeletal changes. After the intravenous calcium administration we have observed quite distinct increase of calcium excretion only in cases with osteolytic changes where also phosphaturia was elevated. This is in contrast with observations in control group.

The increase of calcium and phosphorus excretion in osteolytic forms after the injection of calcium can be perhaps estimated as an evidence of inability of the organism to utilize calcium in a normal way in bone tissue. The administration of calcium in injection seems to stimulate the mobilization of the bone calcium and phosphate. From our present observations we cannot find any feasible explanation for these changes. Only one conclusion can be made: These changes are not due to the elevation of the parathyreoid activity and also the renal mechanism is not involved. The changes we have observed in the metabolism of calcium anf phosphate are perhaps due to the metabolic influence of tumorous, myelomatous tissue itself. Its high metabolic activity is also expressed by high levels of serum alkaline phosphatase. It is of course very remarkable that this high enzymatic activity is not accompanied by washing of calcium and phosphorus out of the bone tissue, where we would expect to find elevated levels of these minerals in the blood plasma.

References

1. Bentzel, C. J., Carbone, P. P., Rosenberg, L. and Bean, M.: The effect of prednisone on calcium metabolism and Ca^{47} kinetics in patients with multiple myeloma and hypercalcaemia. *J. Clin. Invest. 43*, 2132 (1964).
2. Schmutziger, P. and Flury, R.: Störungen des Calcium- und Phosphatstoffwechsels beim multiplem Myelom. *Schweiz. med. Wschr. 92*, 1085 (1962).
3. Merivale, W. H. H.: Mineral metabolism. Fourth chapter from: G. G. Duncan, Diseases of metabolism. W. B. Saunders Co., Philadelphia—London, 1964.
4. Hořejší, J. et al.: Základy chemického vyšetřování v lékařství. 6. vyd. Stát. Zdrav. Naklad. Praha 1964.

Cerebrospinal Fluid Diagnosis of Meningeal Leukaemia

Ágnes PÉTER, J. ROMHÁNYI, Klára LETENYEI

Neurological Department and 1st Department of Paediatrics, Medical University, Budapest

(Received March 12, 1967)

The data of four leukaemic patients are described. Three of the cases suffered from acute lymphoid, one of acute monocytoid leukaemia. No. 3 is an example of neuroleukaemia developing in haematological remission. No. 4 is one of the rare cases in which monocytoid leukaemia is accompanied by neurological involvements.

The authors suggest that on the basis of the CSF cytogram leukaemic infiltration may be primarily responsible for the neurological symptoms of their patients.

In one of the cases hemiplegia, in two symptoms of increased cerebral pressure, in two symptoms of the cranial nerves, and in all four symptoms of meningeal irritation were observed. In all four cases immature cells of the same formation as the pathological cells in the bone marrow were also found in the CSF, together with a number of cells in mitosis.

The authors believe that the cytological examinations of CSF in leukaemia may not only lead to an early diagnosis of neurological involvements but also to an evaluation of the success or failure of the therapy applied.

The neurological symptoms accompanying leukaemia may be produced by leukaemic infiltration, haemorrhage due to thrombopenia or vascular occlusions leading to ischaemia. Vascular occlusion may result from blast cell thrombi or from compression of blood vessels by leukaemic infiltrations. Haemorrhage and neurological changes produced by ischaemia (degeneration of nerve cells, demyelinization, cerebromalacia) may be regarded as secondary consequences of leukaemia. Leukaemic infiltrations of the nervous system belong into the group of systemic leukaemias, and only this second group of changes is called neuroleukaemia.

There is a certain contradiction in the literature as to the incidence of nervous manifestations in leukaemic patients. Leiner [19] demonstrated neurological changes in two out of 27 leukaemic cases, Vivoli [31] in one out of 53, Brandt [4] in 17 out of 206, Schwab and Weiss [27] in 39 out of 334, Shaw et al. [26] in 25 out of 150 cases.

As compared to the microscopic lesions, the neurological symptoms are usually quite negligible [8, 12, 27, 29]. There is a marked difference between the clinical and histological patterns especially in untreated acute leukaemias, because, as compared to other systemic leukaemias, neuroleukaemia develops slowly and the patient may die before the cerebral lesions are so extensive that they cause noticeable symptoms.

A significant change has taken place in the problems related to the neurological manifestations of leukaemia since the use of antimetabolites and steroids (1948–1949). As the modern therapeutic agents tend to prolong the life of leukaemia patients, the incidence of the meningeal form has increased and more than once its occurrence coincides with the period of haematological remission [9, 12, 24, 27].

Up to 1945 (the date of publication of Stodtmeister's great concise work) the histological characteristics and clinical features of neurological lesions of leukaemic origin were essentially clarified. Today an early diagnosis and the evaluation of the therapeutic results are the problems of central importance. On the basis of our experience we believe that CSF cytodiagnosis may offer a significant assistance in finding the correct answer to the latter questions. In the following we wish to point out the importance of CSF cytodiagnosis as illustrated by the tests carried out in four leukaemic patients.

In Hungary neurological lesions accompanying haemopoietic diseases were discussed in the work of Ambrózy et al. [1], and László and Gaál [18].

Case Reports

Case 1, P. H., aged three years

The boy was admitted for the first time at the age of one year after three weeks of high fever and pains in the lower extremities and abdomen. *On admission:* Pale skin, many pinpoint petechiae on the chest, swollen cervical, axillary and inguinal lymphnodes, enlarged liver and spleen were found. *Laboratory findings:* sedimentation rate: 30 mm in one hour; serum electrophoresis: normal; serum calcium, phosphorus, alkaline phosphatase: normal; serum bilirubin: 0.5 mg%; thymol: 2.6 U; gold sol: negative. Blood count: erythrocytes: 2,200,000; leukocytes: 18,000, Se: 1%, Ly: 20%, paraleukoblasts: 79%. Myelogram: paraleukoblasts only. There were hardly any normally mature granulocytes and erythrocytes. X-ray of the chest: negative. X-ray showed reduced calcium content in the long tubular bones.

Course: The patient was given for six weeks gradually decreasing doses of Prednisolone and reached a state of complete clinical and haematological remission. After the first relapse intermittent doses of Prednisolone brought about a few weeks' improvement followed by a deterioration of the patient's condition. On second admission the lymph nodes, liver and spleen were enlarged. Prednisolone and Purinethol therapy resulted in a remission of ten months of duration. The second relapse was preceded by some infectious disease. The peripheral blood count contained 10 per cent paraleukoblasts. Prednisolone and Methotrexate treatment was ineffective, the patient was then given blood transfusion and once a week 0.1 mg per kg vincristine sulphate (Onkovin) intravenously. There was a temporary further deterioration in the differential blood count and meningeal irritation symptoms appeared. At the time of the appearance of meningeal leukaemia the

blood count showed 80% of paraleukoblasts. In the CSF the cell count was 58%, protein 18 mg%, sugar 54 mg%. In the meningogram 38% of paraleukoblasts were found. A partial haematological remission was manifest at the end of the Onkovin treatment. Meningeal leukaemia healed on the administration of 3 times 5 mg of Methotrexate injected intralumbarly, the CSF was normal.

Summing up Case 1: Meningopathy occurred in a three-year-old child in the third attack (second year of the disease) of acute lymphoid leukaemia, during vincristine therapy. Eighty per cent of paraleukoblasts were found in the blood, and 38 per cent in the CSF. After the intralumbar administration of Methotrexate, the neurological symptoms disappeared and the CSF turned normal, but vincristine produced only a partial haematological remission.

Case 2, E. P., aged five and a half years

The girl was admitted with high fever and swollen and painful lower extremities. During the second week in hospital hepatosplenomegaly developed. Her myelogram showed practically only paraleukoblasts, so that she was admitted to the 1st Paediatric Department where after four weeks of Prednisolone and Aminopterin treatment remission set in, the differential blood count showed 24%, the myelogram 12% of paraleukoblasts. Repeated Prednisolone and Aminopterin treatments resulted in a complete remission. On the intermittent administration of Prednisolone for three months headache and vomiting appeared, followed within two months by symptoms of meningeal irritation. The blood count showed 3% paraleukoblasts, cell count in the CSF was 170/3, protein 140 mg%, sugar 20 mg%. The meningogram showed practically only paraleukoblasts with a num-

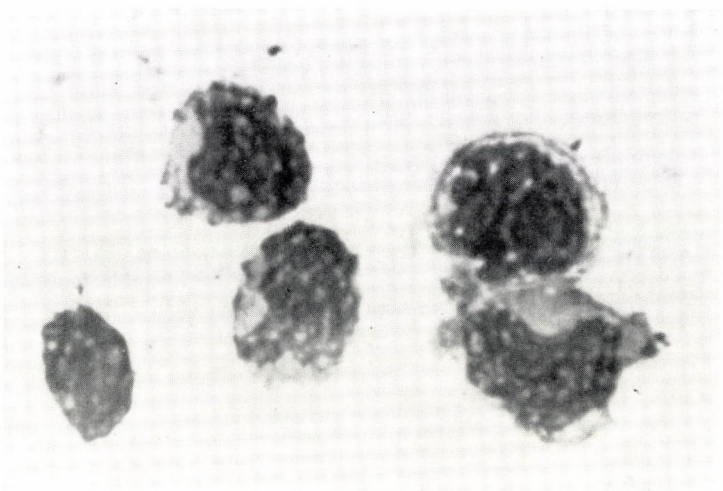


Fig. 1

ber of cells in mitosis (Figs 1 and 2). Therapeutic X-ray irradiation of the skull abolished the clinical symptom and complaints, without a change in the CSF cytogram. On the administration of Prednisolone and Aminopterin the meningeal excitation symptoms reappeared within a short time, followed by bilateral abducent paresis and incipient papilloedema. Parallel with the aggravation of neurological symptoms, changes characteristic of a leukaemic attack were found in the blood count. The blood count was the following at the time of the meningeal symptoms: erythrocytes 4,360,000, leukocytes 13,000, haemoglobin: 82%, Se:

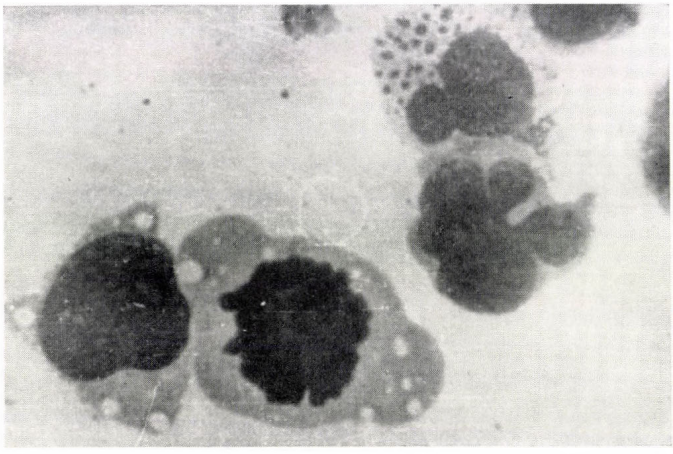


Fig. 2

3%, Ly: 3%, paraleukoblasts 94%. The cell count in the CSF was 124/3, protein 222 mg%, sugar 38 mg%. In response to Prednisolone, blood transfusions and X-ray irradiation of the skull, a certain remission in neuroleukaemia set in, but the general condition of the patient was extremely serious with septic fevers, cutaneous and mucous haemorrhages. In the last stage of the disease the parents insisted on taking the child home.

Summing up Case 2: Clinical and CSF meningitis appeared during the remission following the first attack of acute lymphoid leukaemia of a five and a half-year old little girl. Prednisolone, Aminopterin and X-ray relieved neuroleukaemia, but in the final stage the parents took the child home.

Case 3, S. P., aged eleven years

The boy had periods of high fever before admission. On admission his skin was pale, his body covered with suffusions and petechiae, the peripheral lymph nodes were swollen, the liver and spleen were enlarged. *Laboratory findings:* ESR 85 mm in one hour. Serum electrophoresis: normal. Blood count: erythro-

cytes 2,600,000, leukocytes 6,000, Se 3%, Ly: 9%, paraleukoblasts 88%. In the myelogram practically only paraleukoblasts were found.

Course: During the first week in hospital haematoma and abscess developed at the site of the sternal puncture, accompanied by septic fever and icterus. Therapy consisted of transfusions and the administration of Prednisolone. After temporary serious leukopenia and the lytic disappearance of fever a clinical and haematological improvement set in and maintenance doses of Prednisolone kept the patient in remission for six months. The second leukaemic attack came on

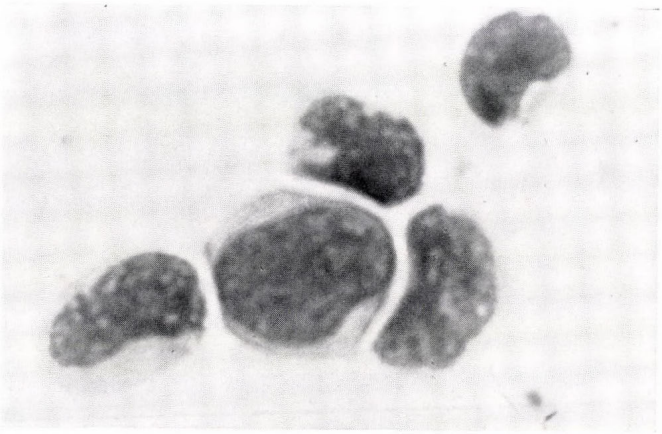


Fig. 3

with nausea, vomiting and vertigo. The myelogram showed more than 50% of the nucleated cells to be paraleukoblasts. CSF cell count: 413/3, protein: 72 mg%, the meningogram showed 92% of paraleukoblasts (Fig. 3 and 4). The CSF meningitis was not accompanied by clinical meningitis. Prednisolone, Leupurine and X-ray irradiation of the skull resulted in a partial remission lasting for two weeks followed by symptoms of meningeal excitation and abducent paresis on the right side. The fundus was intact. CSF: cell count: 90/3, protein and sugar: normal. Leupurine and repeated local X-ray irradiation produced for ten days a complete neurological remission, but after ten days CSF and clinical meningopathy reappeared, which was relieved by intralumbarly injected Methotrexate. Transfusions and Prednisolone therapy led to partial haematological remission, after which the peripheral paraleukoblast count rose to 76%. Death was preceded by fainting, epileptic seizure and the development of hypotonic hemiplegia. CSF findings remained negative even after the development of the cerebral symptoms. Autopsy revealed signs of cerebral oedema. There was no evidence of gross haemorrhage, cerebromalacia or intraparenchymal leukaemic infiltration in the brain slices studied.

Summing up Case3: an 11-year-old boy suffered for 11 months from acute lymphoid leukaemia. Only partial haematological remissions could be achieved during this period. The patient had five leukaemic attacks. In the second active phase symptoms of CSF meningitis, in the third and fourth phases symptoms of CSF and clinical meningitis were manifest. While a complete remission of neuroleukaemia was achieved by repeated X-ray irradiation of the skull and intralumbarly administered Methotrexate, the haematological pattern continued to deteriorate.

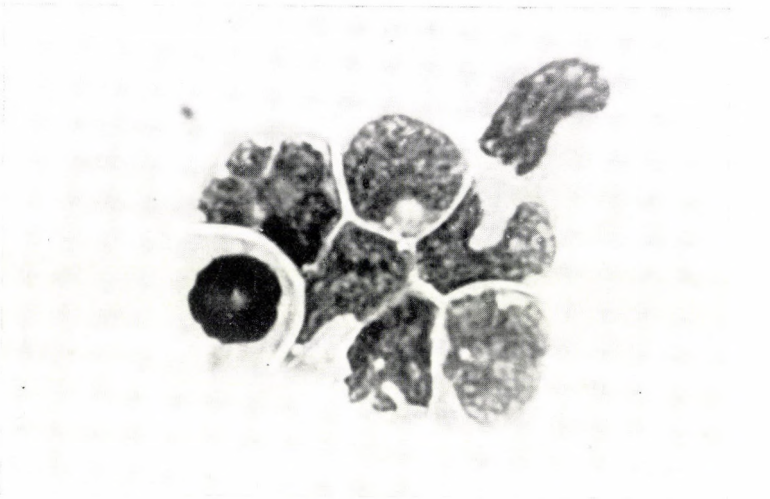


Fig. 4

Case 4, L. Cs., aged 11 months

The baby boy had prior to hospitalization high fever for a week with purpurae on the lower extremities. On admission his skin was pale, with small haemorrhages of the skin and mucosa throughout the body. The lymph glands were swollen, liver and spleen were three fingers' width larger than normal. Laboratory tests gave the following results: ESR 15 mm in one hour, serum calcium, phosphorus, alkaline phosphatase: normal; thymol: 2.5 U; gold sol: negative. Serum electrophoresis: normal. Blood count: erythrocytes 3,000,000; leukocytes 49,000; haemoglobin: 12.2%, promyelocyte: 1%; St: 1%; Se: 10%; Eo: 1%; Ly: 12%; blast: 7%; monoblast: 3%; atypical monocytoid: 12%; atypical lymphocytoid: 23%. In the myelogram half of the nucleated elements were monocyte-shaped cells. There was ample erythrophagocytosis and reduced quantities of elements formed by normal erythro- and granulopoiesis. X-ray of the chest revealed in the lower part of the hilum a bean-sized lymph gland. In the middle part of the parietal bone a hazelnut-sized area was suggestive of osteoporosis.

Course: in spite of the administration of Prednisolone, antibiotics, vincristine and repeated transfusions, the condition of the patient constantly deteriorated. The swelling of the liver and spleen increased and extremely serious anaemia (erythrocyte count: 1,900,000) developed without haemorrhage. In the third week of the illness meningeal excitation symptoms and peripheral facial paresis developed. CSF cell count was 40/3, protein 120 mg%, sugar 40 mg%. In the meningogram 10% of atypical monocytoid cells were found (Fig. 5).

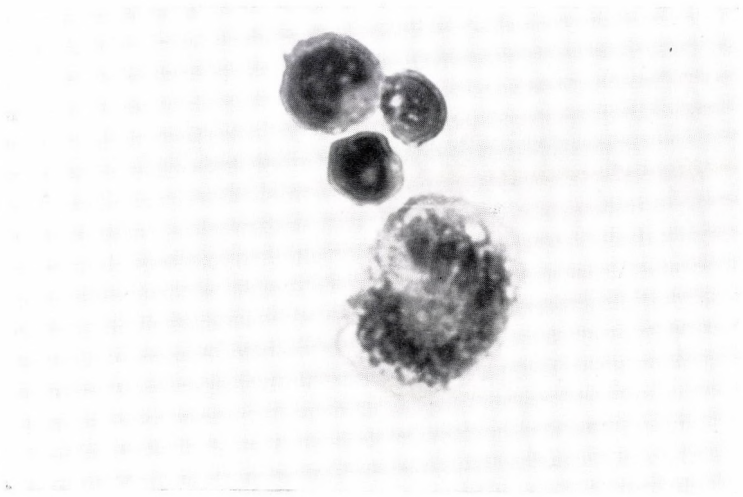


Fig. 5

Summing up Case 4: in the third week of the first attack of acute monocytoid leukaemia of an 11-month-old baby, the clinical and CSF symptoms of meningeal leukaemia were observed during Vincristine and Prednisolone therapy.

Discussion

In four children suffering from acute leukaemia neurological symptoms were observed in various stages of the disease. The youngest of the patients was 11 months, the oldest 11 years old; the duration of the illness varied between four weeks and two years.

Three of the patients had acute lymphoid, one acute monocytoid leukaemia. Reference may be found in the literature to neurological symptoms both in acute and chronic lymphoid and myeloid leukaemia, but only a few authors mention neurological lesions associated with monocytoid leukaemia. Shaw et al. [26] found three cases in Anglo-American literature referring to neurological lesions caused by monocytoid leukaemia. Stodtmeister [28] quotes only the case of Litte-

ral and Malamud [20], the above Case No. 4 is thus a noteworthy pathologic rarity.

The symptoms of meningeal irritation developed in the two acute lymphoid leukaemic patients during combined Prednisolone and antimetabolite therapy, in the case of hyperacute monocytoid leukaemia during Prednisolone and Vincristine therapy in the leukaemic period; before death in the first acute phase (Case No. 4), in the second year of the illness during the third attack (Case No. 1) and in the nine months' period of the disease meningeal irritation accompanied the second, third and fourth attacks (Case No. 3). Case No. 2 is an example of a peculiar form of leukaemic involvement which occurs occasionally since the use of modern therapy, namely the appearance of neurological symptoms during haematological remission.

Sansone and Hauser [24, 15] were the first to mention neuroleukaemia in complete medullary and haematological remission. Since then several authors have called attention to the isolated occurrence of meningeal leukaemia. Gasser [12] observed in five out of ten cases neuroleukaemia. Out of the 17 leukaemic attacks in one of Gasser's patients nine exhibited neuropathological symptoms only. Dost [9] observed in one case, Shaw et al. [26] in eight cases out of 25 meningopathy in the haematological remission period of leukaemia. The isolated leukaemic invasion of the nervous system may be explained by the negligibly small transgression of orally administered cytostatics through the blood-brain barrier [32].

The neurological syndromes accompanying leukaemia may be classified histologically and clinically [6, 29] or only on the basis of the clinical evidence [11, 28]. Most of the authors fail to analyse the correlation between the nature of histopathological lesions and the type of leukaemia, while others deny the existence of any such correlation [8, 29]. Stodtmeister [28], on the other hand, maintains that lymphoid leukaemia is more liable to produce infiltrations, while myeloid leukaemia produces primarily haemorrhages.

The clinical symptoms are determined by the localization of the lesion. Stodtmeister [28] presents a detailed analysis of the nature of the neurological symptoms and the development of the clinical pattern. On the basis of essentially clinical aspects two forms of neuroleukaemia, accompanied namely by 1. cerebral and 2. spinal symptoms may be distinguished. The cerebral form may cause capsular, extrapyramidal or diencephalic lesions, mental confusion, etc. Paraplegy, on the other hand, is one of the spinal symptoms. The leukaemic cells may infiltrate the cerebral or spinal nerves. The neurological manifestation of leukaemia may be localized to the cerebral or spinal meninges and may produce symptoms accompanied by increased intracranial pressure. In all four of the above cases there were clinically meningeal irritation symptoms: rigidity of the nuchal muscles and Kernig's sign positivity. The gradual development of the clinical changes and the morphological nature of the cellular elements in the CSF led us to try to find an explanation for the neurological lesions in the leukaemic infiltration of the meninges. In Case No. 3 death was preceded by an epileptic seizure and dextrolateral

hemiplegia. The epileptic seizure was brought into correlation with the cerebral oedema, and hemiplegia might have been the result of thrombi formed by the blast cells. Autopsy failed to reveal the nature of the gross lesions. The assumed vascular occlusion might have developed just before death, thus there was not enough time for the development of necrosis visible to the naked eye.

On the basis of their 146 cases Schwab and Weiss [27] estimate the incidence of meningeal leukaemia at 17.8%. Up to 1945 Stodtmeister found autopsy evidence of meningeal leukaemic infiltration in 36 cases.

In two out of our four cases symptoms of increased cerebral pressure, i. e. vomiting, abducent paresis and convulsions were observed. Case No. 2 displayed congested papillae. There are many reports on symptoms in leukaemia indicating increased intracranial pressure [12, 24, 26, 28]. Sullivan points out their significantly higher incidence since cytostatic drugs are used.

In Case No. 3 abducent paresis might have been caused by local leukaemic infiltration, as one of the most frequently occurring neurological changes of leukaemia is manifest in the lesion of the cerebral and peripheral nerves due to infiltrations proceeding from the meninges towards the roots. Schwab and Weiss [27] mention in 30% of their cases cerebral nervous symptoms. Shaw et al. [26] found in nine out of 25 leukaemic patients cerebral nervous symptoms. Facial peripheral paresis has a particularly high incidence (see e. g. Case No. 3). According to Stodtmeister [28] up to 1945, 39 leukaemic patients reported in the literature had peripheral facial paresis. Eisenlohr's [10] case is a classic example. Paralysis of the oculomotor nerve has also been observed [6, 8, 23].

In all four cases clinical meningitis was accompanied by an increase in the CSF cell count (40/3 – 418/3), but considerably higher values had also been reported. Schwab and Weiss [27] mention counts as high as 4000/3, Gasser [12] 7000/3, Shaw et al. [26] 11,000/3.

In agreement with published data the protein content of the CSF rose in our cases only slightly or remained normal. According to Schwab and Weiss [27] in meningeal leukaemia an increase in CSF protein has a higher incidence than increase in cell count. They found in 52% of their cases higher protein content and only in 41.4% an increase in the cell count.

In one of our cases CSF sugar showed a significant, in another a slight decrease. This agrees with the observations of Gasser [12], Schwab and Weiss [27] and Shaw et al. [26] who found in some of their cases a lower than normal sugar content of the CSF. The cause for this decrease in CSF sugar might be sought in the high glycolytic activity of the leukaemic cells, or in the high number of blast cells in the CSF [13, 27].

In all four cases paraleukoblasts were found among the cellular elements of the CSF; i. e. the presence of young monocytoid cell forms could be demonstrated. In one case (Case No. 3) neuroleukaemia was diagnosed from the CSF cell pattern prior to the development of the meningeal irritation symptoms. It was not only possible to set up an early diagnosis on the neurological involvements of leukaemia from the examination of the CSF cytogram, but the latter also indicated the

success or failure of local therapy. Thus in Case No. 2 during the period of clinical remission the CSF cytogram indicated the unsatisfactory nature of therapy and after repeated X-ray irradiation of the skull it was again the CSF cytogram which demonstrated the effect of the treatment.

There are but comparatively few reports on the importance of CSF cytodiagnosis. Up to 1945 only in 50% of the neuroleukaemic cases was lumbar puncture carried out; the majority of the authors refers to the cells as lymphocytes. Mierement [22], Barker [2], Litterer [21], Ravenna [23], Heissen [16], Hatta [14], and later Gasser [12], Gilbert and Rice [13], Sansone [24], Shaw et al. [26] describe immature leukoblast type cell forms. Apart from our own material only the paper by Sayk [25], Bischoff [3], Dost [9] and Viala [30] contained of the available literature pictures of paraleukoblasts found in the CSF. The cell formations found in the CSF of our Case Nos 2 and 3 showed close morphological similarity to the cells observed by the above authors. No reference to immature monocytoid cells was found in the literature on CSF cytology.

Our experience suggests the expediency of CSF tests during haematological remission as part of the control tests of leukaemic patients. In the case of symptoms indicating neurological involvement the first task is to disclose the nature of the lesion causing the symptoms of mental confusion. Neurological symptoms due to ischaemic lesions and haemorrhages are less easy to influence. In the case of leukaemic infiltration local treatment (skull X-ray, irradiation, intralumbar antimetabolites) should be applied after which a regression of the meningeal symptoms, i. e. of the CSF syndrome has been observed by a number of authors [12, 26, etc.]. The results obtained by the local treatment of the central nervous system, though it may not have a decisive influence on the fate of the patient, may nevertheless have a significant temporary effect.

References

1. Ambrózy, Gy., Eckhardt, S., Gallai, M.: Rosszindulatú vérképzőszervi megbetegedések idegrendszeri szövődményei (Neurological involvements of malignant diseases of the haemopoietic organs). *Ideggyógy. Szemle 12*, 367 (1959).
2. Barker, L. F.: Neutrophilic myelocytes in the cerebrospinal fluid of a patient suffering from myeloid leukaemia and their significance for the diagnosis. *South. Med. J. 14*, 437 (1921).
3. Bischoff, A.: Liquorcytodiagnostik. *12*, 8 (1962).
4. Brandt, S.: Alterations leucémiques du système nerveux. *Acta psychiat. et neurol. 20*, 127 (1945).
5. Burns, A.: Observations on the surgical anatomy of the head and neck. Wardlaw and Cunningham. 1824. Cit: Critchley.
6. Critchley, M., Greenfield, J. G.: Spinal symptoms in chloroma and leukaemia. *Brain. 53*, 11 (1930).
7. Croizat, P., Revol, L., Bothier, F., Brunat, M., Gauthier, J.: Considération sur le traitment des formes meningées de la leucose aigue par le methotrexate intrarachidien associé à la leucocristine. A propos d'une observation. *Lyon Medical. 213*, 947 (1965).
8. Diamond, J. B.: Leukemic changes in brain. Report of 14 cases. *Arch. Neurol. Psych. 32*, 118 (1934).

9. Dost, F. H.: Leukämische Meningopathie und Iridopathie beim Kind. *Ärztl. Wschr. 11*, 1070 (1956).
10. Eisenlohr, G.: Leukaemia linealis lymphatica et medullaris mit multiplen Gehirnnervenlähmungen. *Virchow's Arch. 73*, 56 (1878).
11. Fried, B. M.: Leukaemia and the central nervous system; with a review of thirty cases from the literature. *Arch. Path. Chicago. 2*, 23 (1926).
12. Gasser, C.: Meningosis leukaemica. *Schweiz. med. Wschr. 90*, 1193 (1960).
13. Gilbert, E. F., Rice, E. C.: Neurologic manifestations of leukaemia. Report of three cases in children simulating acute bacterial meningitis. *Pediatrics 19*, 801 (1957).
14. Hatta, T.: Veränderungen des Rückenmarkes bei der myelogenen Leukämie mit motorischer Lähmung beider unterer Extremitäten. *Zbl. Neurol. 48*, 202 (1928).
15. Hauser, F.: Meningosis leukaemica. *Ann. Paed. 183*, 353 (1954).
16. Heissen, F.: Chlorom und Nervensystem. *Zschr. f. ges. Neurol. Psych. 95*, 248 (1935).
17. Hyman, C. B., Brubaker, C. A., Sturgeon, P.: Intrathecal Methotrexate in the treatment of CNS complications of acute leukaemia. *Clin. Res. Proc. 7*, 90 (1959).
18. László, J., Kalabay, L., Gaál, M.: Alterations cérébrales au cours de la leucémie. *Acta Morph. Acad. Sci. Hung. II/2*, 145 (1952).
19. Leiner, J. H.: Nervous and mental symptoms in diseases of the blood (from study of 141 cases). *New York State J. Med. 28*, 10 (1928).
20. Litteral, E., Malamud, N.: Leukemia with predominant neurologic manifestations. *Neurology. 5*, 740 (1955).
21. Litterer, Diskussion zu dem Artikel Barkers. *South. med. J. 14*, 442 (1921).
22. Mierement, C. W. G.: Ein klinisch unter dem Bilde eines malignen Tumors verlaufender Fall von myeloischem Chlorom. *Virchow's Arch. path. Anat. 215*, 353 (1914).
23. Ravenna, F.: Contributo alla conoscenza dei rapporti fra leucemia e tumori. Chloroma scolorato. *Giorn. clin. med. 2*, 641 (1921).
24. Sansone, G.: Pathomorphosis of acute infantile leukaemia treated with modern therapeutic agents; "meningoleukaemia" and Fröchlich's obesity. *Ann. Paediat. 183*, 33 (1954).
25. Sayk, J.: Cytologie der Cerebrospinalflüssigkeit. Fischer. Jena (1960).
26. Shaw, R. K., Moore, E. W., Freireich, E. J., Thomas, L. B.: Meningeal leukaemia. A syndrome resulting from increased intracranial pressure in patients with acute leukaemia. *Neurology. 10*, 823 (1960).
27. Schwab, R. S., Weiss, S.: The neurologic aspect of leukaemia. *Am. J. Med. Sci. 189*, 766 (1935).
28. Stodtmeister, R., Weicker, H.: V. Leukämie und Nervensystem. *Erg. inn. med. Kinderheilk. 65*, 246 (1945).
29. Trömner, E., Wohlwill, F.: Über Erkrankungen des Nervensystems insbesondere der Hirnnerven bei Leukämie. *Dtsch. Zschr. Nervenheilk. 100*, 233 (1927).
30. Viala, J. J., Gentilhomme, F. M., Chatain, M. B., Moulimer, B., Racle, P., Morel, P.: A propos de 2 cas de meningite leucoblastique traitée par injections intrarachidiennes d'alpha methepterine. *Lyon Med. 123*, 953 (1965).
31. Vivoli, E.: Le complicazioni a carico del sistema nervoso e degli organi di senso nel corso delle leucemie. *Riv. clin. med. 32*, 67 (1931). Ref: *Zbl. Neur. 61*, 792 (1932).
32. Whiteside, J. A., Philips, F. S., Dargeon, H. W., Buchenal, J. H.: Intrathecal aminopterin in neurological manifestations in leukaemia. *Arch. inn. med. 101*, 279 (1958).

Zur Bedeutung der Zellelektrophorese für hämatologische Fragen

R. ARNOLD

Max-Planck-Institut für Biochemie, München

(Eingegangen am 10. April 1967)

Die peripheren Blutzellen des Menschen und verschiedener Versuchstiere lassen sich auf Grund ihrer negativen Oberflächenladung in die 3 Gruppen: Erythrozyten, Lymphozyten und Granulozyten einteilen. Bei der myeloischen und lymphatischen Leukämie, der Lymphogranulomatose und bei akuten entzündlichen Erkrankungen treten charakteristische Abweichungen dieses normalen »Hämozytopherogramms« auf. Um den Mechanismus der im menschlichen pathologischen Hämozytopherogramm auftretenden Veränderungen verstehen zu lernen, wurden die Blutzellen von Ratten- und Mäuseleukämien verschiedener Ätiologie zellelektrophoretisch untersucht. Auch hier traten charakteristische Veränderungen im Hämozytopherogramm auf.

Die Ausschleusung membranunreifer, schnell beweglicher Leukozyten aus den Blutbildungsstätten bei akuten entzündlichen Erkrankungen wird wahrscheinlich durch die Milz kontrolliert.

Die Zellelektrophorese erlaubt die Charakterisierung von Zellen auf Grund ihrer Wanderungsgeschwindigkeit im elektrischen Feld. Alle bisher untersuchten Wirbeltierzellen besitzen auf ihren Oberflächen mehr negative als positive Ladungseinheiten [1, 2]. Werden diese Zellen in einer Elektrolytlösung suspendiert, wandern sie daher beim Anlegen eines elektrischen Feldes zur Anode. Intakte Zellen verschiedenster Herkunft besitzen damit eine dem jeweiligen Oberflächenpotential entsprechende charakteristische elektrophoretische Beweglichkeit [2].

Für die Bestimmung der elektrophoretischen Beweglichkeit von verschiedenen Zellarten hat sich die mikroskopische Beobachtung als besonders vorteilhaft erwiesen. Dadurch können Zellen unterschiedlicher Gestalt und Größe auf Grund ihrer negativen Überschußladung charakterisiert werden. Das gilt in besonderem Maße für die Bestimmung der elektrophoretischen Beweglichkeit von Blutzellen [1, 3]. Die zu messenden Zellen werden in einer möglichst physiologischen Elektrolytlösung aufgeschwemmt und in eine Meßkammer gebracht. Davor befindet sich in einem bestimmten nur wenige mm betragenden Abstand das Objektiv eines Mikroskops.

Die theoretischen und experimentellen Grundlagen der Zellelektrophorese gehen bis ins Ende des vergangenen Jahrhunderts zurück. Die ersten arbeitsfähigen Apparaturen konstruierten aber erst Northrop und Kunitz (1925), Abramson (1929) sowie Alexander und Sagyers (1948); doch entsprachen sie noch nicht allen Anforderungen. Besonders Ponder (1951), dessen Elektrophoresemodell auf

einer rektangulären, vertikal-waagrechten Anordnung der Meßkammer beruht, trug durch Verbesserung des Elektrodensystems zur weiteren Entwicklung arbeitsfähiger Apparaturen bei. Darauf aufbauend wurde im Arbeitskreis Ruhenstroth-Bauer ein Modell entwickelt, das sich besonders durch die Konstruktion eines Kühlmantels um die Meßkammer zur Ableitung der bei der Messung in störender Weise entstehenden Joule'schen Wärme sowie in der Entwicklung leistungsfähiger Elektroden von den vorhergehenden unterscheidet [4, 5]. Die op-

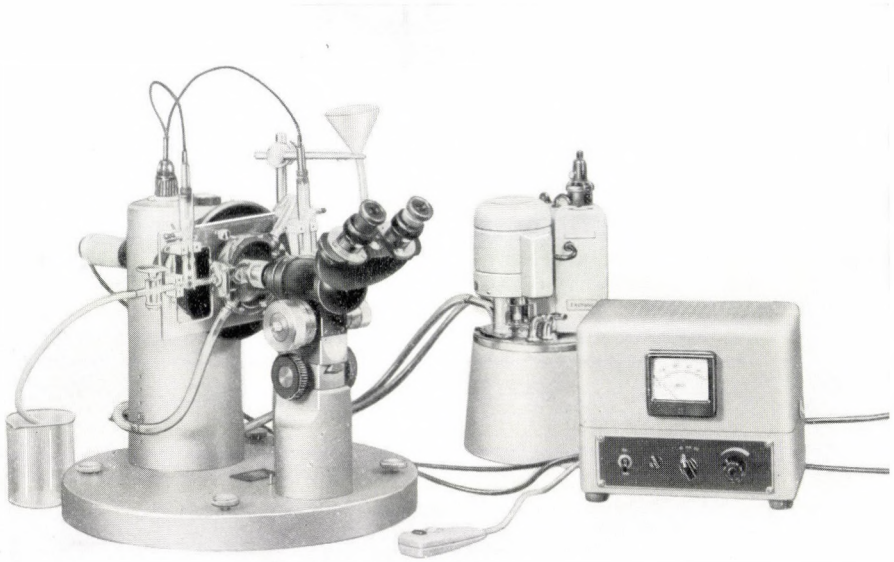


Abb. 1. Das Hämozytopherometer (Zeiss, Oberkochen)

ischen Voraussetzungen dazu wurden von der Firma Zeiss (Oberkochen) geschaffen (Abb. 1).

Kernstück der *Apparatur* ist eine flache Meßkammer aus Glas. Um konstante Temperaturbedingungen zu gewährleisten, ist die Kammer von einem Kühlmantel umgeben. Beidseits der Kammer werden die Elektroden angelegt. Diese Anlage ist in ein Spezialmikroskop eingebaut. Es ermöglicht eine licht- als auch phasenoptische 400 bzw. 800fache Vergrößerung. Das erwies sich besonders bei der Untersuchung der weißen Blutzellen als notwendig. Die Bestimmung der negativen Überschußladung einer Zelle erfolgt innerhalb der Meßkammer in einer der beiden durch die Elektroendosmose bedingten, definierten stationären Ebenen. Mit Hilfe einer Stoppuhr wird die Zeit gemessen, welche eine Zelle für eine bestimmte Strecke eines im Okular befindlichen Eichgitters benötigt. Daraus läßt

sich die elektrophoretische Beweglichkeit berechnen. Sie ist Ausdruck der negativen Überschußladung und wird, auf die Feldstärke 1 bezogen, in [cm^2 sec^{-1} V^{-1}] 10^{-4} angegeben.

I. Elektrophoretische Untersuchungen an Blutzellen des Menschen

Die *Blutzellen des Menschen* lassen sich nach ihrer elektrophoretischen Beweglichkeit in 3 Gruppen einteilen [1, 3]. Das kommt im sogenannten Hämozytopherogramm zum Ausdruck (Abb. 2). Hierbei werden die Ergebnisse der elektro-

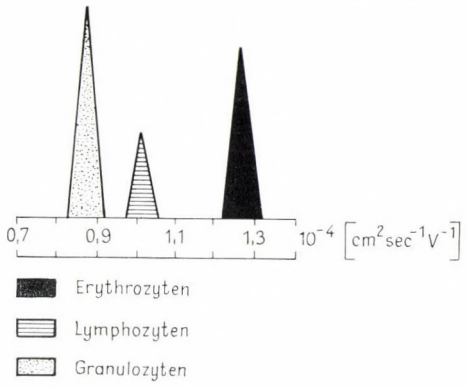


Abb. 2. Das Hämozytopherogramm eines Gesunden. In den Abbildungen 2—8 stellen die Basen der Dreiecke die Streuung der elektrophoretischen Beweglichkeiten der jeweiligen Zellart und die Höhen der Dreiecke die relative Anzahl der gemessenen Zellen dar

phoretischen Messung der einzelnen Zellarten in Form von Dreiecken aufgetragen. Die Basen der Dreiecke geben die Standardabweichung der elektrophoretischen Beweglichkeit und die Höhen das Zahlenverhältnis der gemessenen Zellen zueinander wieder. Die Gruppe mit der größten elektrophoretischen Beweglichkeit besteht aus Erythrozyten. In der nächsten etwas langsamer wandernden Gruppe befinden sich die Lymphozyten. Die geringste elektrophoretische Beweglichkeit besitzen die Granulozyten. Die Streuung der elektrophoretischen Beweglichkeit innerhalb der 3 Zellgruppen beträgt nur wenige Prozente.

Die Relation der gemessenen Zellen im Hämozytopherogramm entspricht nicht dem Differentialblutbild, da für die Bestimmung der elektrophoretischen Beweglichkeit die weißen Blutzellen mittels einer Schrägsenkung angereichert werden müssen. Die Zahl der gemessenen Erythrozyten wird mit 100% angegeben, da die Erythrozyten in beliebiger Zahl vorhanden sind.

Untersucht man das elektrophoretische Verhalten der Zellen in den *Blutbildungsstätten*, so erhält man das in Abb. 3 wiedergegebene Bild [3]. Man findet nur eine einzige Gruppe schnell beweglicher kernhaltiger Zellen. Das Differential-

blutbild zeigt, daß es sich dabei um alle Vorstufen der weißen und roten Blutkörperchenreihe handelt. Offenbar besitzen also die Zellmembranen der myeloischen Reihe eine größere Dichte der negativen Überschußladung als jene der peripheren Granulozyten, während sich die elektrophoretische Beweglichkeit der Erythrozytenvorstufen nicht von derjenigen der ausgereiften Form unterscheidet. Normal bewegliche Lymphozyten oder Granulozyten sind im Knochenmark kaum zu finden. Man muß bei der großen Fülle morphologisch ausgereifter Granulozyten im Knochenmark deshalb annehmen, daß auch diese Zellen eine höhere negative

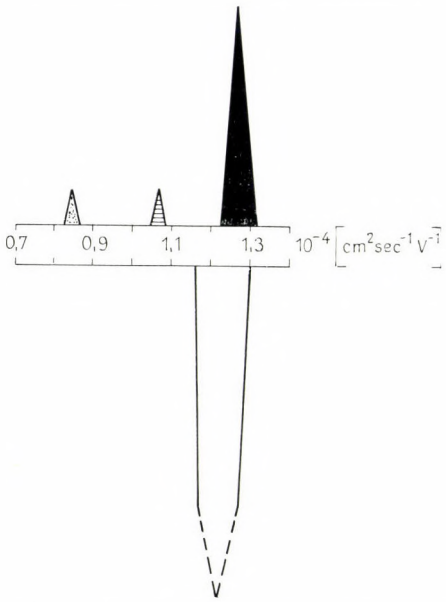


Abb. 3. Das Zytopherogramm des Sternalpunktats eines Gesunden

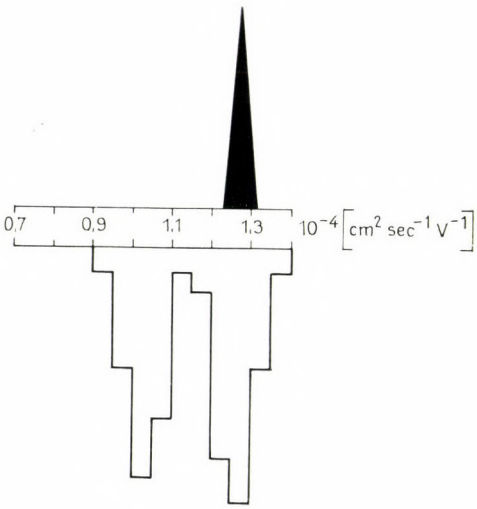


Abb. 4. Das Zytopherogramm eines normalen menschlichen Lymphknotens

Überschußladung besitzen, welche derjenigen der myeloischen Vorstufen entspricht. Wahrscheinlich kommt es erst beim Austritt der reifen Blutzellen aus dem Knochenmark zu einem plötzlichen Verlust der negativen Überschußladung auf den Wert der peripheren Granulozyten [1].

In den Bildungsstätten des lymphatischen Gewebes (Abb. 4) findet man im Zytopherogramm 2 Gruppen von Zellen, die sich hinsichtlich ihrer elektrophoretischen Beweglichkeit unterscheiden [3]. Die eine besitzt die elektrophoretische Beweglichkeit der Lymphozyten im peripheren Blut, die andere Gruppe zeigt ein ähnliches Verhalten wie die im Knochenmark auftretende schnell bewegliche kernhaltige Fraktion. Da sich, wie die Abb. 4 zeigt, die beiden Lymphozytengruppen nicht scharf von einander abgrenzen lassen, kann nicht eindeutig festgestellt werden, ob auch im Lymphknoten, ähnlich wie im Knochenmark, der Membranreifungsschritt von den unreifen Vorstufen zum reifen Lymphozyt sprunghaft erfolgt.

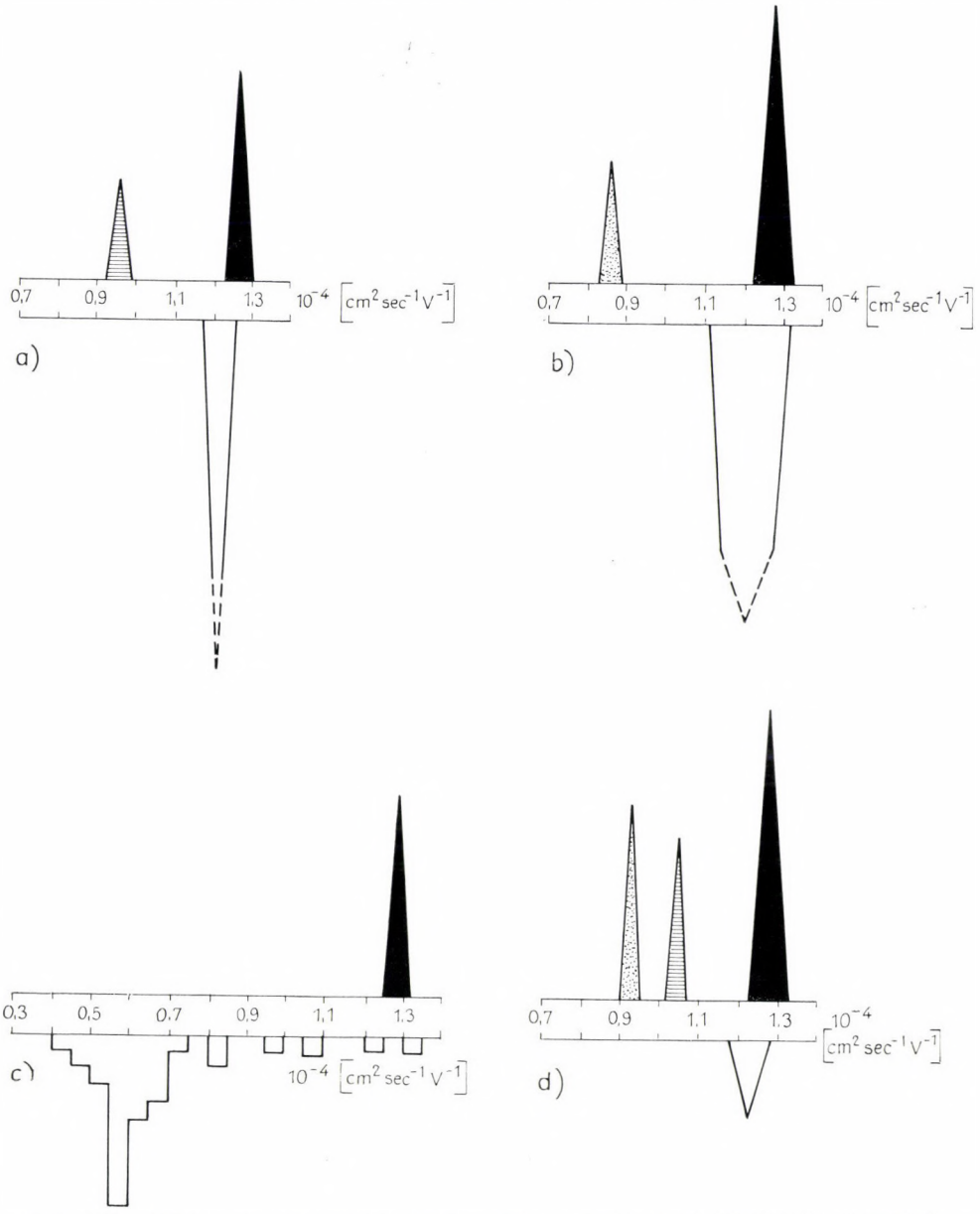


Abb. 5. Das Hämozytopherogramm bei einer myeloischen Leukämie (a), bei einer lymphatischen Leukämie (b), bei einer Lymphogranulomatose (c), bei einer akuten Entzündung (d)

Bei verschiedenen *Erkrankungen* wurden nun Abweichungen vom normalen Hämozytopherogramm gefunden. Dabei werden, wie die Abbildungen 5a–d zeigen, nur die weißen Blutzellen, niemals aber die Erythrozyten betroffen. So tritt

bei der myeloischen und lymphatischen Leukämie (Abb. 5a, b) jeweils eine Gruppe schnell wandernder kernhaltiger Zellen auf [1, 3]. Bei der myeloischen Leukämie fehlt dabei weitgehend die Gruppe der normal beweglichen Granulozyten, bei der lymphatischen Leukämie die der Lymphozyten. Bei den sehr akuten Formen der lymphatischen bzw. myeloischen Leukämie findet man keine normal beweglichen Lymphozyten bzw. Granulozyten mehr. Die Beweglichkeit der bei den besprochenen Leukämieformen auftretenden schnell beweglichen Zellfraktion entspricht der schnell beweglichen Fraktion aus dem Knochenmark und Lymphknoten. Bei der Lymphogranulomatose dagegen findet man eine breite Streuung in der elektrophoretischen Beweglichkeit der weißen Blutzellen an Stelle der im Hämozytopherogramm des Gesunden deutlich von einander abgesetzten Lymphozyten- bzw. Granulozytenblöcke (Abb. 5c). Dabei treten einerseits vereinzelt weiße Blutzellen mit einer geringeren elektrophoretischen Beweglichkeit als die der Granulozyten auf. Andererseits finden sich vereinzelt auch Zellen, die schneller als Lymphozyten wandern. Das elektrophoretische Verhalten der weißen Blutzellen bei der Lymphogranulomatose weist demnach auf Unterschiede in der Membranladung bei sonst scheinbar unveränderten weißen Blutzellen hin. Im Remissionsstadium der Lymphogranulomatose zeigt das Hämozytopherogramm nur ein angedeutetes Streubild. Hier steht im Vordergrund häufig eine zusätzliche schnell bewegliche Fraktion kernhaltiger Zellen, wie man sie regelmäßig u. a. bei entzündlichen Erkrankungen, bei Tumoren mit Knochenmetastasen, beim Lymphosarkom und bei Autoimmunkrankheiten findet (Abb. 5d) [3]. Die elektrophoretische Beweglichkeit dieser Zellfraktion entspricht wiederum der im Knochenmark bzw. Lymphknoten auftretenden kernhaltigen Zellgruppe mit erhöhter negativer Überschußladung. Im Hämozytopherogramm der zuletzt genannten Gruppe finden sich daneben noch die normal beweglichen Lymphozyten und Granulozyten.

In manchen Fällen von sehr akuten *Entzündungen* mit ausgeprägter reaktiver Leukozytose kann im Hämozytopherogramm jedoch anfangs das Bild einer myeloischen Leukämie entstehen, da die Gruppe der normal beweglichen Granulozyten verschwindet. Doch schon nach wenigen Tagen nehmen im Falle der Entzündung die schnell beweglichen Leukozyten ab und es entsteht ein Bild wie in Abb. 5d. Das Auftreten der schnell beweglichen Leukozytenfraktion bei Patienten mit entzündlichen Erkrankungen, Tumoren mit Knochenmetastasen oder Autoimmunkrankheiten deutet darauf hin, daß morphologisch ausgereifte Zellen aus dem Knochenmark und vielleicht auch aus dem Lymphknoten mit einem noch erhöhten Membranpotential ausgeschleust werden. Der normal auftretende, sprunghafte Verlust der negativen Überschußladung beim Austritt der reifen Zellen aus dem Knochenmark bzw. dem Lymphknoten scheint also gestört zu sein. Dabei läßt sich kein Zusammenhang mit dem Ausmaß der Linksverschiebung im Differentialblutbild herstellen.

Das beschriebene Verhalten der Blutzellen ist ähnlich wie bei den anderen Wirbeltierzellen durch die Membranstruktur bedingt, wobei ein Teil der negativen Überschußladung durch Neuraminsäure bedingt ist [6]. Die Faktoren des Blut-

plasmas haben auf die elektrophoretische Beweglichkeit der Blutzellen sowohl im Normalfall als auch in den aufgeführten pathologischen Fällen keinen Einfluß.

Die dargestellten Befunde zeigen u. a., daß die Zellelektrophorese in Zusammenarbeit mit den üblichen klinischen Methoden besonders bei der Sicherung der Diagnosen von Leukämie- und Lymphogranulomatose-Erkrankungen wertvolle Dienste erweisen kann.

II. Elektrophoretische Untersuchungen an den Blutzellen der Ratte und Maus

In einfacher Weise lassen sich mit Hilfe dieser Methode auch die *Blutzellen* von *Laboratoriumstieren* sowie anderer Wirbel- und Säugetiere untersuchen. Das

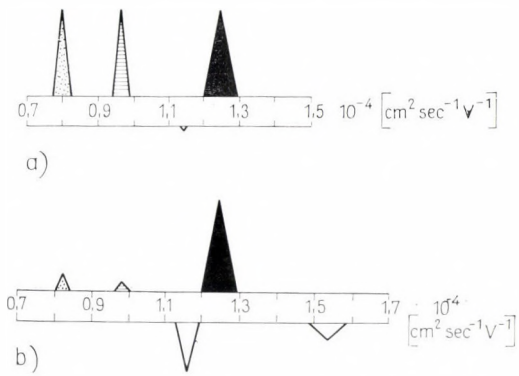


Abb. 6. (a) Hämozytopherogramm einer gesunden Ratte
(b) Das Zytopherogramm des Knochenmarks einer gesunden Ratte

ist deshalb von Bedeutung, da man bei diesen Tieren experimentell in ihrer Ätiologie gesicherte Krankheiten erzeugen kann. So ist die Gegenüberstellung der bei den Tierleukosen zu beobachtenden zellelektrophoretischen Ergebnisse und der beim Menschen gefundenen im Hinblick auf die noch unbekannten Ursachen menschlicher Leukämien wünschenswert. Weiterhin gelingt es im Tierexperiment, Parallelen zu der schnell beweglichen Fraktion kernhaltiger Zellen zu finden, welche beim Menschen im Zuge entzündlicher Prozesse neu auftritt. Dabei läßt sich eine Beteiligung der Milz am Entstehen dieser zusätzlichen Fraktionen nachweisen [7].

Auch die Blutzellen gesunder Ratten und Mäuse lassen sich wie die gesunder Menschen nach ihrer elektrophoretischen Beweglichkeit in 3 Gruppen einteilen (Abb. 6a). Im Gegensatz zum menschlichen Hämozytopherogramm treten allerdings bei Ratte und Maus vereinzelt auch schnelle, kernhaltige Zellen auf, deren elektrophoretische Beweglichkeit etwas geringer als die der Erythrozyten ist [8].

Bei der zellelektrophoretischen Untersuchung von Knochenmarkszellen der Ratte und Maus erhält man das in Abb. 6b wiedergegebene Bild. Neben den nur schwach ausgeprägten Gruppen mit der Beweglichkeit peripherer Lymphozyten und Granulozyten findet man 2 Blöcke schnell beweglicher kernhaltiger Zellen. Die Beweglichkeit des einen dieser Blöcke ist etwas geringer als die der Erythrozyten. Sie entspricht damit den schnellen kernhaltigen Zellen, die vereinzelt im Hämozytopherogramm des peripheren Ratten- und Mäuseblutes gefunden wer-

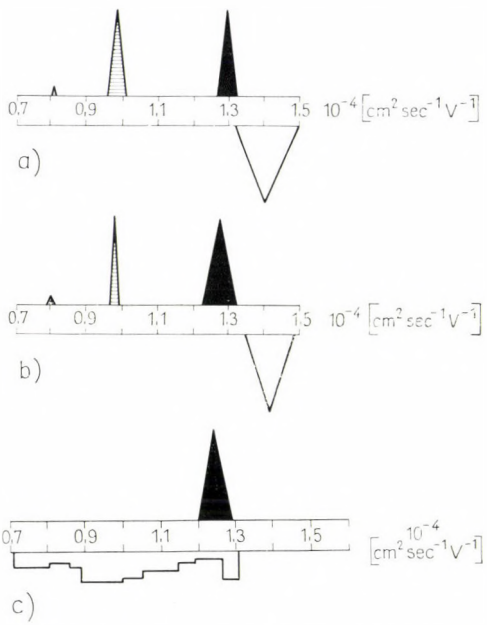


Abb. 7. Das Hämozytopherogramm der Friend-Leukämie (a), der Shay-Leukämie (b), der Graffi-Leukämie (c)

den. Die Beweglichkeit der anderen Gruppe ist etwas größer als die der Erythrozyten [8].

Von den bei Ratten und Mäusen zu beobachtenden Leukämien wurden die Friend-Leukämie der Maus, die Shay-Leukämie der Ratte und die Graffi-Leukämie der Ratte untersucht. Bei der Friend-Leukämie der Maus handelt es sich um eine Virus-bedingte, mit einem Milztumor einhergehende Retikulumzell-Leukämie [9]. 2–3 Wochen nach der intraperitonealen Übertragung von Leukämieblut kommt es zu einer ausgeprägten Leukämie. Zu dieser Zeit verschwindet im Hämozytopherogramm die Gruppe der normal beweglichen Granulozyten fast vollständig (Abb. 7a). Dafür treten schnell bewegliche kernhaltige Zellen auf. Ihr Potential entspricht der Fraktion kernhaltiger Zellen im Zytopherogramm des Knochenmarks der gesunden Ratte, die etwas schneller als die Erythrozyten wandern [8]. Die Shay-Chloroleukämie der Ratte ist im Gegensatz zur zellfrei

übertragbaren Friend-Leukämie nur zellulär überimpfbar [10, 11]. Sie wurde ursprünglich durch Methylcholanthren erzeugt und manifestiert sich je nach Art der Transplantation in 2 verschiedenen Erscheinungsbildern. Einmal entwickelt sich nach subkutaner Injektion von Tumorzellen an der Transplantationsstelle ein lymphoretikuläres Sarkom, welches weiter übertragen werden kann. Injiziert man dagegen neugeborenen Ratten eine Suspension dieser Tumorzellen intraperitoneal, so entwickelt sich innerhalb weniger Tage eine akute periphere Leukämie mit zahlreichen Paramyeloblasten, an der die Tiere nach 2 Wochen sterben. Das Hämozytopherogramm (Abb. 7b) in diesem Stadium gleicht genau dem Bild, wie man es bei der Friend-Leukämie findet [8].

Bei der Virus-bedingten zellulär als auch zellfrei übertragbaren Rattenmyelose nach Graffi entwickelt sich an der Transplantationsstelle ein Tumor mit rundkernigen, plasmareichen, wenig polymorphen Zellen, die Myeloblasten entsprechen könnten, der bei den meisten Tieren nach 4–6 Wochen wieder verschwindet [12]. 5–6 Wochen später treten bei einigen Tieren im Blut vereinzelt unreife Elemente auf. Das Hämozytopherogramm dieser Tiere ist in Abb. 7c wiedergegeben. Man findet eine breite Streuung der elektrophoretischen Beweglichkeit der Leukozyten. Sie erinnert stark an das Hämozytopherogramm, das man bei der menschlichen Lymphogranulomatose beobachtet [8, 13].

Bei der Virus-bedingten Friend-Leukämie und der Shay-Leukämie finden sich, wie gezeigt wurde, zellelektrophoretisch gleichsinnige Veränderungen. Es tritt jeweils eine Gruppe kernhaltiger Zellen auf, deren elektrophoretische Beweglichkeit größer ist als die der Erythrozyten. Sie entspricht der schnelleren Knochenmarksfraktion bei der Ratte. Man kann deshalb vermuten, daß es sich bei dieser Fraktion sowohl im Blut als auch im Knochenmark um morphologisch wie auch hinsichtlich ihrer Membran sehr unreife Vorstufen der Myelopoese handelt. Beim Menschen wurde eine entsprechend schnell bewegliche Gruppe in einigen Fällen von sehr akuten Leukämien gefunden. In diesen Fällen fanden sich ebenso wie bei der Friend- und Shay-Leukämie im peripheren Blut Paramyeloblasten. Diese unreifste Zellart wird jedoch unter normalen Umständen nur im Knochenmark gefunden.

Die Virus-bedingte Graffi-Myelose zeigt dagegen in der breiten Streuung der elektrophoretischen Beweglichkeit der Leukozyten ein von den bisher bei Leukämien gewohnten Hämozytopherogrammen abweichendes Bild. Vergleichbar findet sich eine solche breite Streuung der elektrophoretischen Beweglichkeit der weißen Blutzellen auch bei der menschlichen Lymphogranulomatose. Ein weiterer Fall einer breiten Streuung innerhalb einer morphologisch einheitlichen Zellpopulation wurde von Goldhamsternierenzellen beschrieben, welche mit Polyomavirus infiziert waren [14]. Außerdem kann man bei L- oder HeLa-Zellen eine breite Streuung der elektrophoretischen Beweglichkeit bereits wenige Augenblicke nach Besetzung der Zellen mit Sendai-Virus nachweisen [15].

Aus den Ergebnissen der beschriebenen Tierleukosen geht hervor, daß eine Virusätiologie bei ein und demselben Krankheitstypus zellelektrophoretisch zu ganz verschiedenen Erscheinungsbildern führen kann. Andererseits erhält man

bei der Friend- und Shay-Leukämie trotz verschiedener Ätiologie gleichsinnige Veränderungen im Hämozytopherogramm.

Ähnlich wie bei der Leukämie, gelingt es auch bei Ratten, experimentell eine zusätzliche Fraktion schnell beweglicher Leukozyten zu erzeugen, wie sie beim Menschen vor allem bei akuten entzündlichen Prozessen auftritt.

Um diesen Mechanismus, welcher in den Ausschüttungsmechanismus der Blutbildungsstätten eingreifen muß, besser verstehen zu lernen, wurden 2–3 Monate alte Sprague-Dawley-Ratten einer Röntgenganzkörperbestrahlung unterzogen. Die Einzeldosen betrugen dabei 800, 400 und 150 R. In bestimmten Ab-

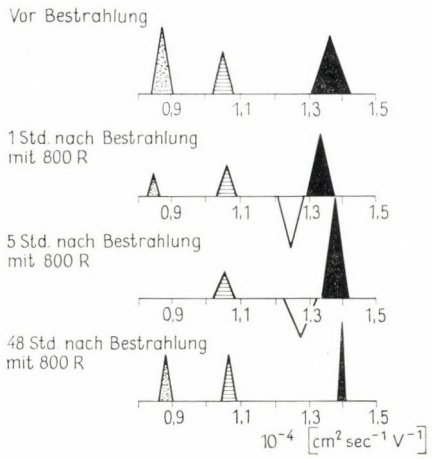


Abb. 8. Hämozytopherogramme von Ratten vor und nach Bestrahlung

ständen nach Bestrahlungsende wurde den Tieren Blut entnommen und zellelektrophoretisch untersucht [8, 16]. Bereits 5–10 Minuten nach einer Bestrahlung mit 800 R treten im Blut vermehrt schnell bewegliche kernhaltige Zellen auf, deren elektrophoretische Beweglichkeit etwas geringer als die der Erythrozyten ist; Abb. 8 zeigt das Hämozytopherogramm solcher Tiere. In der Abb. 9 ist außerdem das ungefähre prozentuale Verhältnis der schnellen Leukozyten zu den normal beweglichen Granulozyten wiedergegeben. Bestimmt man dieses Verhältnis nach einer Ganzkörperbestrahlung mit 800 R als Funktion der Zeit, so ergibt sich folgendes Bild: die normal beweglichen Granulozyten verschwinden in zunehmendem Maße, so daß nach 4 Stunden nur noch schnelle Zellen gefunden werden. Der Block der Lymphozyten bleibt dagegen, wie die Abb. 8 zeigt, erhalten. Nach 10 Stunden treten langsam wieder normal wandernde Granulozyten auf, während die schnell beweglichen Zellen wieder abnehmen, um nach 1–2 Tagen völlig zu verschwinden. Die mitgeteilten quantitative Relationen sind mit der angewandten Methode der Leukozytenanreicherung gut reproduzierbar [17], sie sind jedoch für die genauen quantitativen Verhältnisse der Zellzahlen im peripheren Blut nicht repräsentativ. Daß die Ursache der schnell beweglichen Zellen unter den beschrie-

benen Bedingungen nicht in Denaturierungsvorgängen an den Zellmembranen zu suchen ist, geht aus den Hämozytopherogrammen hervor, die man nach in vitro Bestrahlungen von Rattenbluten mit 800 und 400 R erhält [8].

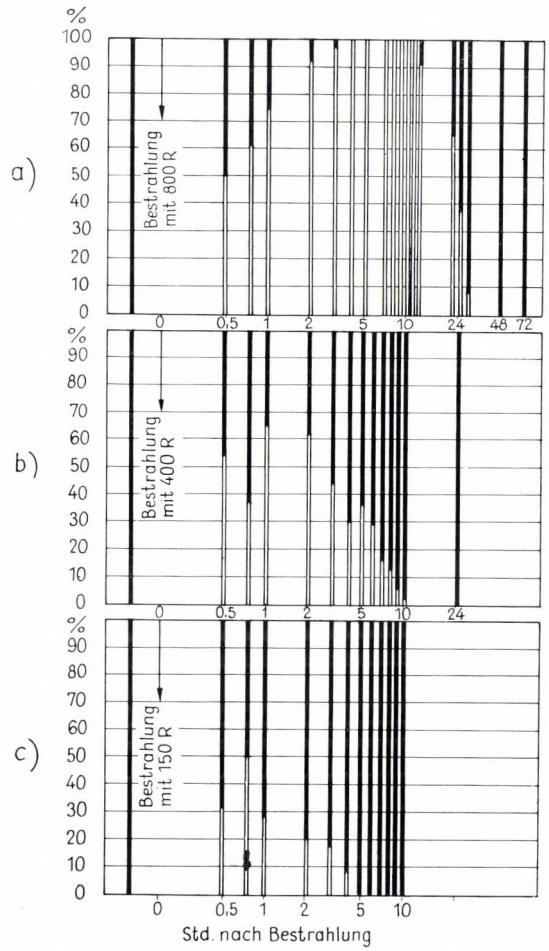


Abb. 9. Prozentuales Verhältnis zwischen elektrophoretisch normal beweglichen Granulozyten (schraffiert) und »schnellen« Leukozyten (nicht schraffiert) von Ratten nach einer Ganzkörperbestrahlung in Abhängigkeit von der Strahlendosis und Zeit

Die beschriebenen Veränderungen im Hämozytopherogramm von Ratten nach einer Ganzkörperbestrahlung sind wahrscheinlich auf eine überstürzte Ausschwemmung von membranunreifen Zellen aus dem lymphatischen Gewebe, vielleicht auch aus dem Knochenmark zurückzuführen [18, 16]. Sie können wohl als organspezifische Antwort der Blutbildungsstätten auf einen den ganzen Or-

ganismus betreffenden Insult angesehen werden. Es kommt also zu einer veränderten Reaktion des Mechanismus, welcher den Nachschub der Leukozyten aus der Reserve der Blutbildungsstätten in die Peripherie reguliert. Unter den vielen organ- und systemspezifischen Reaktionen, welche in erster Linie das Knochenmark, den Intestinaltrakt und das zentrale Nervensystem betreffen, ist bisher keine strahlenspezifische Antwort des Organismus auf einen Strahleninsult bekannt, die in so kurzer Zeit nach Einwirkung der Bestrahlung und mit so geringem Aufwand nachgewiesen werden kann: mit Hilfe der Zellelektrophorese läßt sich eine Ganzkörperbestrahlung bereits nach 5 Minuten nachweisen. Außerdem kann man eine Abhängigkeit von der Strahlendosis sowie dem Zeitpunkt feststellen, zu dem die Bestimmung der elektrophoretischen Beweglichkeit der weißen Blutzellen nach einer Bestrahlung erfolgt (Abb. 9).

Die Ausschleusung membranunreifer Zellen aus den Blutbildungsstätten nach einer Ganzkörperbestrahlung wird wahrscheinlich durch die Milz kontrolliert [7]. Das konnte durch eine Splenektomie gezeigt werden, welcher die Ratten 1–2 Tage vor einer Ganzkörperbestrahlung unterzogen wurden. Das Hämozytopherogramm eines Tieres, dem 2 Tage vor einer Ganzkörperbestrahlung mit 800 R die Milz entfernt wurde, entspricht dem Hämozytopherogramm eines gesunden Tieres. Durch diesen Befund, nach dem der Milz bei der Ausschüttung der schnell beweglichen Zellen aus den Blutbildungsstätten Bedeutung zukommt, konnte gezeigt werden, daß man mit Hilfe der Zellelektrophorese auch in der Frage der Beziehung Milz–Blutbildungsstätten wichtige Anregungen erhalten kann.

Dieser Bericht sollte einen Eindruck von den Anwendungsmöglichkeiten der Zellelektrophorese in der hämatologischen Forschung und Diagnostik vermitteln. Durch diese Art der Charakterisierung von Zellen ist ein neuer Weg eröffnet, auch innerhalb morphologisch scheinbar einheitlicher Zellpopulationen Unterscheidungen vorzunehmen.

Literaturverzeichnis

1. Ruhenstroth-Bauer, G., Straub, E., Sachtleben, P. und Fuhrmann, G. F.: Die elektrophoretische Beweglichkeit von Blutzellen beim Gesunden und bei Kranken. *Münch. med. Wschr. 103*, 794 (1961).
2. Ruhenstroth-Bauer, G., Fuhrmann, G. F., Granzer, G., Kübler, W. und Rueff, F.: Elektrophoretische Untersuchungen an normalen und malignen Zellen. *Naturwiss. 49*, 363 (1962).
3. Rueff, F.: Die Zellelektrophorese in der klinischen Diagnostik. Habilitationsschrift 1962 München.
4. Ruhenstroth-Bauer, G. und Sachtleben, P.: Die Zellelektrophorese. Med. Grundlagenforschung Bd. 2, Georg Thieme Verlag, Stuttgart 1959.
5. Sachtleben, P., Fuhrmann, G. F., Straub, E. und Ruhenstroth-Bauer, G.: Fortschritte in der Methodik der Zellelektrophorese. *Klin. Wschr. 39*, 839 (1961).
6. Ruhenstroth-Bauer, G., Kübler, W., Fuhrmann, G. F. und Rueff, F.: Zur Frage spezifischer Strukturelemente in Tumorzellmembranen: ihr Neuraminsäuregehalt. *Klin. Wschr. 39*, 764 (1961).

7. Gostomzyk, J. G., Arnold, R. und Ruhenstroth-Bauer, G.: Zur Frage der Beziehung zwischen Milz und Knochenmark nach einer Ganzkörperbestrahlung. *Naturwiss. 50*, 704, (1963).
8. Arnold, R.: Die elektrophoretische Beweglichkeit von Blutzellen bei Ratten und Mäusen. Inauguraldissertation 1964, Univ. München.
9. Friend, Ch.: Leukemia of adult mice caused by a transmissible agent. *Ann. N. Y. Acad. Sci. 68*, 522 (1957).
10. Shay, H., Harris, C. and Gruenstein, M.: Effect on male rats of the gastric instillation of methylcholanthrene in "heated" and "unheated" olive oil. *Cancer 4*, 988 (1951).
11. Shay, H., Harris, C. and Gruenstein, M.: Influence of sex hormones on the incidence and form of tumors produced in male or female rats by gastric instillation of methylcholanthrene. *J. Nat. Cancer Inst. 13*, 307 (1952).
12. Graffi, A. and Gimmy, J.: Rattenleukosen durch zellfreie Filtrate aus homologem leukämischem Gewebe. *Z. Naturforschg. 14b*, 747 (1959).
13. Ruhenstroth-Bauer, G., Fuhrmann, G. F. und Arnold, R.: Zur Frage des pathologischen Hämozytopherogramms bei der Lymphogranulomatose. *Dtsch. med. Wschr. 88*, 620 (1963).
14. Forrester, J. A., Ambrose, E. J. and Macpherson, J. A.: Electrophoretic investigations of a clone of hamster fibroblasts and polyoma-transformed cells from the same population. *Nature 196*, 1068 (1962).
15. in Vorbereitung.
16. Ruhenstroth-Bauer, G., Gostomzyk, J. G. und Arnold, R.: Neue hämatologische Befunde an Ratten nach einer Ganzkörperbestrahlung. *Strahlentherapie 122*, 56 (1963).
17. Maupin, B. und Chary, R.: Présentation d'une nouvelle méthode de séparation des leucocytes et des plaquettes sanguines. *Le sang 23*, 336 (1952). Methode modifiziert durch: Ehrhart, H. und Dörfler, E.: Untersuchungen über den Leukozytenstoffwechsel *Blut 10*, 231 (1964).
18. Lücke, C.: in Vorbereitung.

Effect of Different Washing Solutions on some Characteristics of Erythrocytes

Ilma Szász, Judith G. Szelényi, G. Gárdos, Susan R. Holián

Research Institute of the National Blood Centre, Budapest, Hungary

(Received April 15, 1967)

Some characteristics of erythrocytes (maintenance of shape, ion-transport, ATPase and NADPase activities, maintenance of GSH level, permeability of non-electrolytes) are significantly damaged by washing of the cells with physiological NaCl. This damaging effect is even more pronounced when using isotonic washing solutions of low ionic strength (e. g. sucrose). Damages caused by washing in physiological NaCl are partly reversible, partly irreversible. By the use of physiological NaCl supplemented with glucose and Mg the above damages can be prevented to a great extent.

The elimination of plasma or serum by washing is often necessary for the study of erythrocyte chemistry. Most authors use buffered physiological saline as the washing solution. Attention is given to the ionic composition of washing solutions when studying the cation transport processes of reconstituted erythrocyte ghosts [1, 2]. Mollison's studies proved even the fact that the grade of chromium elution from ^{51}Cr-labelled erythrocytes is determined in the first place by the composition of the washing solutions used (physiological saline, ACD, etc.) [3]. In contrast with this, in most of the studies of metabolism, enzyme activities and other characteristics of erythrocytes, this aspect is not respected. It is well known from the literature that magnesium activates many enzymes and is taken up very slowly [4, 5]. Thus, eventual losses occurring during washings cannot be replaced by the cell. It is also known that a great deal of glucose, the main substrate of erythrocytes, is also lost during washings [6]. This may damage the cells secondarily.

With this in mind, we have investigated the changes of some erythrocyte characteristics (maintenance of shape, K-transport, enzyme activities, etc.) caused by washing of the cells. Beside washing solutions of ionic character we have also studied the metabolic effect of non-ionic washing solutions (e. g. sucrose) used by some authors [7] for protecting the protein coat of erythrocytes.

Abbreviations used: ATP: adenosine triphosphate, ATPase: adenosine triphosphatase, G6PD: glucose-6-phosphate dehydrogenase, NADPase: nicotinamide-adenine dinucleotide phosphatase, AchE: acetylcholinesterase, GSH: reduced glutathione, I_m: morphological index, ACD: acid citrate dextrose

Methods

Fresh, defibrinated human blood was used. Erythrocytes were washed three times with four volumes of washing solutions. Centrifugations were performed at room temperature for 10 minutes at 3500 r. p. m. The following washing solutions were used:

1. 166 mM NaCl
2. 6 mM glucose, 163 mM NaCl
3. 5 mM $MgCl_2$, 158 mM NaCl
4. 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl
5. 6 mM glucose, 1 mM $MgCl_2$, 290 mM sucrose

The pH of each washing solution was adjusted to pH 7.4 by Tris.

Iodoacetate and sodium fluoride were used as glycolytic inhibitors. 75% of the experimental system consisted of defibrinated blood or washed erythrocyte suspension. The effective agents were applied in 25% volume, so the haematocrit value was around 30%. The mixtures were incubated at 37°C.

The morphology of erythrocytes was investigated in an isotonic trisodium citrate solution containing 1% formalin described by Dacie [8], between slide and coverslip, with phase-contrast microscope at a 800-fold magnification. The morphological index (I_m) was determined as described in our previous paper [9].

ATP determinations were carried out in two ways: 1. by the spectrophotometric analysis at 260 mμ of the fractions obtained from Dowex-1-Cl ion exchange column [10]; 2. enzymatically with the aid of glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase, according to the Boehringer test [11]. The measured values were expressed as percentages of the original values.

Potassium was determined by flame photometry, magnesium by complexometry [12]. Glucose determinations were carried out with the aid of a specific enzymatic test (Boehringer).

ATP utilizing ("ATPase") enzyme activities were measured in haemolysates prepared by freezing and thawing the erythrocytes twice. In 0.6 ml volume 5 μM ATP, 5 μM $MgCl_2$, 100 μM NaCl, 20 μM KCl and 30 μM Tris-HCl buffer (pH 7.4) were added to a haemolysate of 0.4 ml erythrocyte sediment, and at 0 minute and after 30 minutes of incubation at 37°C the inorganic phosphate was determined in the deproteinized supernatants according to Fiske and Subbarow [13].

Glucose-6-phosphate dehydrogenase activity of erythrocytes was determined by Kornberg and Horecker's method as modified by Marks et al. [14], acetylcholinesterase activity by Michel's [15], reduced glutathione by Mortensen's [16], glutathione stability by Beutler's [17] and the number of superficial SH-groups by Szelényi's [18] method. Erythrocyte permeability for isotonic solutions of nonelectrolytes (glycerol and thiourea) was investigated by the method of Jacobs [19].

Results

When K-free washing solutions were used, the K-loss of erythrocytes did not surpass 1–2%. In Mg-free washing solutions the Mg-loss amounted to 3–5%. In glucose-free washing solutions 65–70% of the glucose content of erythrocytes disappeared. In some blood samples the ATP level decreased by 10 to 15%, in other samples it did not decrease at all. In washing solutions to which 4 mM in-

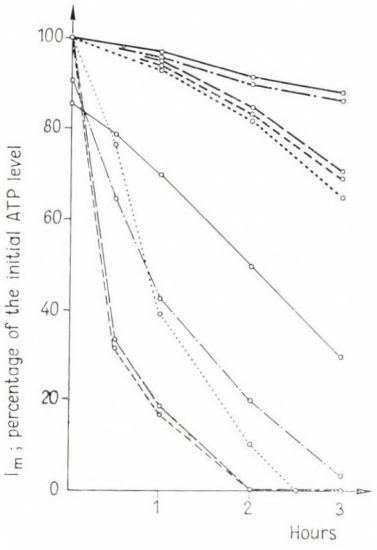


Fig. 1. Kinetics of ATP breakdown (lower lines) and morphological degeneration (upper lines) in the presence of 1 mM iodoacetate, in human erythrocytes washed three times in different washing solutions. ○–○ defibrinated blood, ○—○ washing in 166 mM NaCl ○·····○ washing in 6 mM glucose, 163 mM NaCl, ○–·–○ washing in 5 mM $MgCl_2$, 158 mM NaCl, ○----○ washing in 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl

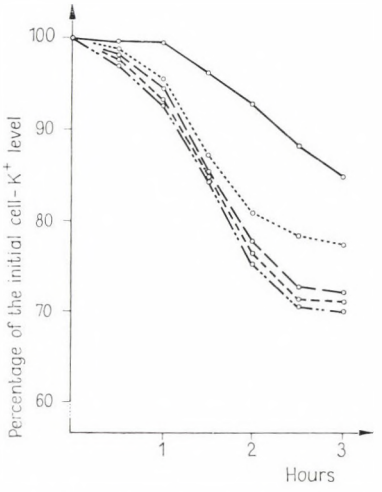


Fig. 2. Effect of washing and resuspension on the kinetics of K-outflow of human erythrocytes caused by 16 mM NaF. ○–○ defibrinated blood, ○—○ washing and resuspension in 166 mM NaCl, ○·····○ washing in 166 mM NaCl, resuspension in fresh serum, ○–·–·–○ washing in 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl, resuspension in Tyrode solution, ○----○ washing in 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl, resuspension in fresh serum

organic phosphate had been added, the ATP breakdown was prevented in every case. On treatment with washing solution *5* of low ionic strength, significant morphological degeneration and decrease in enzyme activities resulted because 70 to 80% of ATP had been broken down.

The rate of ATP breakdown and morphological degeneration in the presence of glycolytic inhibitors was greatly slowed down as a result of the three washings in physiological NaCl. When applying washing solution *2*, there was no decrease in the initial ATP level and the rate of ATP breakdown in the presence of glycolyt-

ic inhibitors slowed down only to a smaller extent. The effect of washing solution *3* on the rate of ATP breakdown was similar to that of washing solution *2*. On washings with washing solution *4* the curve of ATP breakdown in the presence of glycolytic inhibitors was essentially the same as in the case of defibrinated blood. In the I_m values, compared to that of the ATP levels, changes of other type were observed. Erythrocytes washed with physiological NaCl and physiological NaCl containing Mg degenerated at a slower rate.

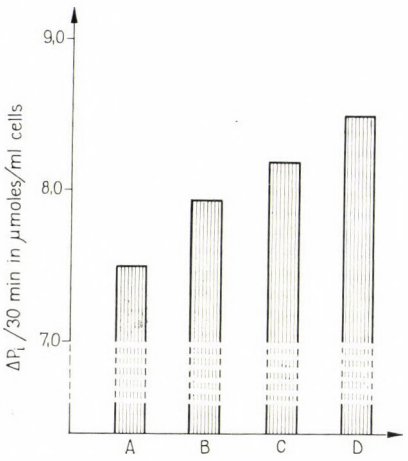


Fig. 3. "ATPase" activities of haemolysates of human erythrocytes washed in different washing solutions. A: washing in 166 mM NaCl, B: washing in 6 mM glucose, 163 mM NaCl, C: washing in 5 mM $MgCl_2$, 158 mM NaCl, D: washing in 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl

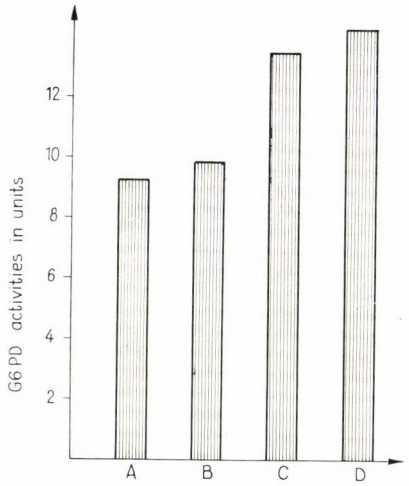


Fig. 4. G6PD activities of human erythrocytes washed in different washing solutions. A: defibrinated blood, B: washing in 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl C: washing in 166 mM NaCl, D: washing in 6 mM glucose, 1 mM $MgCl_2$, 290 mM sucrose

The erythrocytes washed with washing solutions *2* and *4* did not differ significantly from defibrinated blood (Fig. 1). ATP and I_m values were not changed even then, when erythrocytes washed three times were resuspended in fresh serum.

It is interesting, however, that while with erythrocytes washed three times and resuspended in physiological NaCl the rapid K-outflow well known for defibrinated blood in the presence of high concentrations of NaF [20, 21] did not occur, with erythrocytes washed three times in physiological NaCl and resuspended in serum, it partly reappeared. Erythrocytes washed in washing solution *4* and resuspended in Tyrode or serum behaved like defibrinated blood (Fig. 2).

The "ATPase" activities of haemolysates of washed and unwashed erythrocytes were not identical. When to demonstrate Mg-loss, Mg was left out of the activity measuring system, it was found as a rule that the haemolysates of erythrocytes washed with physiological NaCl had the lowest activity. The activity of haemolysates prepared of erythrocytes washed with physiological NaCl containing either

Mg or glucose, was higher and the highest activity was represented by haemolysates prepared of erythrocytes washed with physiological NaCl containing Mg and glucose (Fig. 3). However, the activity of haemolysates prepared of unwashed erythrocytes was mostly lower than that of the haemolysates prepared of washed erythrocytes.

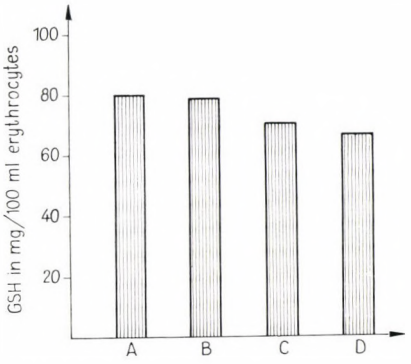


Fig. 5. GSH level of human erythrocytes washed in different washing solutions. For symbols see Fig. 4

Comparing the effect of the various washing solutions on further characteristics of erythrocytes, changes were found in G6PD activity, GSH content and permeability for non-electrolytes, while there was no significant change in AchE activity and in the number of superficial SH-groups. According to our examinations, the results corresponding best to the defibrinated blood can be obtained by the use of washing solution *4*. The increase in G6PD activities after washings with

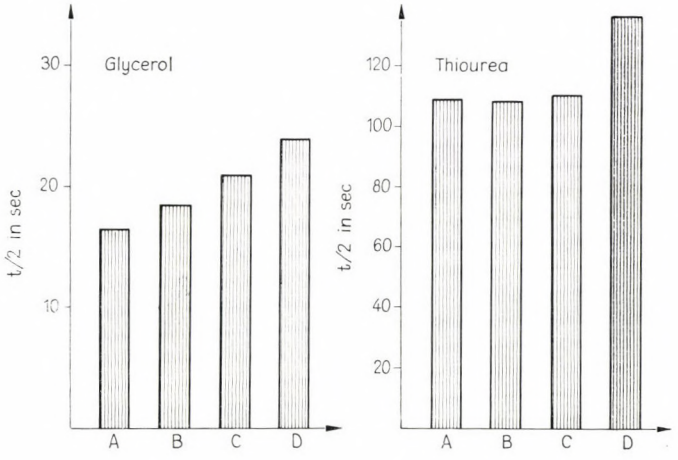


Fig. 6. Half time of glycerol and thiourea penetration of human erythrocytes washed in different washing solutions. For symbols see Fig. 4

physiological NaCl, resp. sucrose is represented in Fig. 4 and suggests damage to the membrane NADPase [22]. Fig. 5 shows the decrease in GSH-level when different washing solutions were used. Fig. 6 demonstrates that after having washed the cells with physiological NaCl, and even more after the washings with sucrose, the rate of penetration of non-electrolytes decreased.

Discussion

It is known from the literature that Na-loss of erythrocytes in Na-Ringer solutions is minimal [6]. According to our measurements K-loss is not considerable either, and though K – Na sensitive enzymes (phosphofructokinase, pyruvate kinase, aldehyddehydrogenase, transport ATPase, etc.) occur in erythrocytes, no functional differences were observed when K-free and K-containing washing solutions had been used. On the other hand, our experiments with Mg-free washing solutions referred to the manifold functional injury of erythrocytes, which can be explained by the role of enzymes activated by magnesium (hexokinase, pyruvate kinase, enolase, ATPase, etc.). With respect to the very slow Mg uptake of human erythrocytes, magnesium loss is to be prevented by magnesium applied in higher concentration than that of the cell (2 – 2.5 mM) during washings.

It is known that the half time of glucose exchange in human erythrocytes is short [23]. On this basis Glynn [6] assumes that during washing glucose disappears from the cells or at least decreases to a minimum. According to our experiments with specific enzyme test, glucose did not disappear from the cells, but diminished essentially, and this was manifest in functional changes, too. When applying glucose-free washing solutions, a small decrease in ATP level may occur. We have found that in these cases, the presence of inorganic phosphate – the regulator of ATP synthesis – is advantageous. But, as it can be supposed that the maintenance of ATP level occurs in these circumstances partly through the breakdown of 2.3 diphosphoglyceric acid, we deem that the physiological equilibrium of the cell can be maintained only by application of washing solutions containing glucose.

It was pointed out by Wilbrandt and Passow [24, 25] that low ionic strength induced a rapid Cl – OH exchange with strong alkalinization of erythrocytes. Indeed, we observed a rapid ATP breakdown – similar to that occurring on alkalinization – when using these solutions. Thus, for the examination of metabolism, cells washed in this way cannot be used at all, at the same time, however, their immunoglobulin coat remains intact. The role of the different ions in this will be described elsewhere [26].

Nakao et al. [27] studied the correlation between ATP content and biconcave shape on erythrocytes washed three times with physiological NaCl. Our present experiments clearly show that this relatively simple intervention influences the phenomenon to a great extent. We have found that the relatively high ATP level cannot be utilized adequately for the maintenance of the biconcave shape. This indicates that in erythrocytes washed with physiological NaCl the so-called "shape maintaining ATPase" was damaged, too. One of the causes of this damage

is the loss in the activator Mg ions during washings. The fact that a greater "ATP-ase" activity can be measured in haemolysates from washed than from unwashed erythrocytes shows that the elimination of platina hexokinase inhibitor plays a role in the extent of "ATPase" activity. The irreversible nature of the damage of the shape maintaining function is proved by the fact that in erythrocytes washed with physiological NaCl, even after resuspension in serum, the original ATP content : morphology relation does not reappear.

Davson [28] described interesting results in connexion with K-outflow from erythrocytes washed three times in physiological NaCl and resuspended in isotonic solutions of different composition. He demonstrated that in the absence of Mg, there is no rapid K-loss caused by NaF in high concentration. We experienced that in erythrocytes washed with a solution containing Mg and glucose and resuspended in Tyrode solution or serum, the rapid K-loss can be fully regenerated, while in erythrocytes washed in physiological NaCl it is only partly attainable.

The fact that the washing solution containing both glucose and Mg causes the least membrane damage is manifested even in that, that the loss in membrane NADPase acting as an inactivator of G6PD is in this case the smallest. This is indicated by the fact that the smallest increases in G6PD activity were observed on treatment with this washing solution.

The damage to erythrocyte membrane is also indicated by the slower penetration of non-electrolytes (isotonic glycerol and thiourea). According to our unpublished investigations, this depends on the extent of membrane damage, and it can be used as an indicator of this. Even in this case the results imply that the smallest damage was caused by washing solution containing glucose and Mg.

The small decrease in GSH level presumably reflects already intracellular changes. According to Carson et al. [29] this can be explained by the role the erythrocyte membrane played in erythrocyte metabolism, or simply by the damage occurring in substrate-free medium. The latter is supported by our experiments with erythrocytes preserved for 48, resp. 96 hours, when the decrease in GSH stability was the most pronounced.

Based on new aspects our experimental results draw attention to the fact emphasized in recognized classical works [30–32] that isotonic NaCl is not a "physiological" washing, resp. suspending medium for erythrocytes. We deem this necessary because a great number of authors do not take sufficient care of the quality of washing solutions when elaborating methods to determine the chemical composition, resp. functions of erythrocytes. Consequently, these procedures offer results, which are reproducible, but which deviate more or less from the characteristics of intact erythrocytes. This fact hinders the comparative evaluation of different erythrocyte functions and characteristics. To obtain comparable results it is of great importance that physiological ion composition and energy supply should be maintained during washing processes of erythrocytes.

*

For the skilful and precise technical assistance thanks are due to Miss Mary Hasitz, Mrs. Eva Irmai, Miss Susan Keresztes and Mrs. Kate Lendvay.

References

1. Hoffman, J. F., Tosteson, D. C., Whittam, R.: Retention of potassium by human erythrocyte ghosts. *Nature 185*, 186 (1960).
2. Sen, A. K., Post, R. L.: Stoichiometry and localization of adenosine triphosphate dependent sodium and potassium transport in the erythrocyte. *J. Biol. Chem. 239*, 345 (1964).
3. Mollison, P. L.: Further observations on the normal survival curve of ^{51}Cr-labelled red cells. *Clin. Sci. 21*, 21 (1961).
4. Rogers, T. A.: Exchange of radioactive magnesium in the rat. *J. cell. comp. Physiol. 57*, 119 (1961).
5. Rogers, T. A., Mahan, P. E.: Exchange of radioactive magnesium in erythrocytes of several species. *Proc. Soc. Exp. Biol. Med. 100*, 235 (1959).
6. Glynn, I. M.: Sodium and potassium movements in human red cells. *J. Physiol. 134*, 278 (1956).
7. Harshman, S., Najjar, V. A.: The binding of autologous γ-globulin with isohemagglutinin activity to human red blood cells. *Biochem. Biophys. Res. Comm. 11*, 411 (1963).
8. Dacie, J. V.: Practical haematology. Chemical Pub. Co., New York (1956).
9. Gárdos, G., Szász, I., Árky, I.: Structure and function of erythrocytes. I. Relation between energy metabolism and the maintenance of biconcave shape of human erythrocytes. *Acta Biochim. Biophys. Hung. 1*, 253 (1966).
10. Deutsch, A., Nilsson, R.: Ion exchange chromatography of inosine phosphates. *Acta Chem. Scand. 7*, 1288 (1953).
11. Bücher, Th.: Über ein Phosphatübertragendes Gärungsferment. *Biochim. Biophys. Acta 1*, 292 (1947).
12. Flaschka, H., Holasek, A.: Eine neue Methode zur Bestimmung des Calciums im Blutserum. *Z. physiol. Chem. 288*, 244 (1951).
13. Fiske, C. R., Subbarow, Y.: The colorimetric determination of phosphorus. *J. Biol. Chem. 66*, 375 (1925).
14. Marks, P. A., Szeinberg, A., Banks, J.: Erythrocyte glucose-6-phosphate dehydrogenase of normal and mutant human subject. *J. Biol. Chem. 236*, 10 (1961).
15. Michel, H. O.: An electrometric method for the determination of red blood cell and plasma acetylcholinesterase activity. *J. Lab. Clin. Med. 34*, 1564 (1949).
16. Mortensen, E.: The nitroprusside method for the determination of reduced glutathione. *Scand. J. Clin. Lab. Inv. 16*, 87 (1964).
17. Beutler, E.: The glutathione instability of drug-sensitive red cells. *J. Lab. Clin. Med. 49*, 84 (1957).
18. Szelényi, J.: Relations between the permeability and the functional state of the erythrocyte membrane. *Haemat. Hung. 6*, 33 (1966).
19. Jacobs, M. H.: The permeability of erythrocytes. *Ergebn. Biol. 7*, 1 (1931).
20. Gárdos, G., Straub, F. B.: Über die Rolle der Adenosinetriphosphorsäure in der K-Permeabilität der menschlichen roten Blutkörperchen. *Acta Physiol. Hung. 12*, 1 (1957).
21. Gárdos, G.: The role of calcium in the potassium permeability of human erythrocytes. *Acta Physiol. Hung. 15*, 121 (1959).
22. Carson, P. E., Schrier, S. L., Kellermeyer, R. W.: Mechanism of inactivation of G-6-PD in human erythrocytes. *Nature 184*, 1292 (1959).
23. Wilbrandt, W.: Die Permeabilität der roten Blutkörperchen für einfache Zucker. *Arch. ges. Physiol. 241*, 302 (1938).
24. Wilbrandt, W.: Die Ionpermeabilität der Erythrozyten in Nichtleiterlösungen. *Arch. ges. Physiol. 243*, 537 (1940).
25. Passow, H.: Passive ion permeability and the concept of fixed charges. *Proc. of the XXIIIrd Internat. Congr. of Physiological Sciences, Tokyo*, 555 (1965).
26. Árky, I., Szász, I., Puskás, E., Gergely, J.: Factors influencing aspecific IgG binding to erythrocytes. *Haematologia 1* (1967). In press.

27. Nakao, M., Nakao, T., Yamazoe, S., Yoshikawa, H.: Adenosine triphosphate and storage of erythrocytes. *J. Biochem. (Tokyo) 49*, 487 (1961).
28. Davson, H.: The effect of some metabolic poisons on the permeability of the rabbit erythrocyte to potassium. *J. cell. comp. Physiol. 18*, 173 (1941).
29. Carson, P. E., Ajmar, F., Hashimoto, F., Bowman, J. E.: Electrophoretic demonstration of stromal effects on haemolysate G-6-PD and GPGD. *Nature 210*, 813 (1966).
30. Rous, P., Turner, J. R.: Preservation of living red blood corpuscles *in vitro*. II. The transfusion of kept cells. *J. Exp. Med. 23*, 219 (1916).
31. Ashby, W.: Study of the mechanism of change in resistance of erythrocytes to hypotonic salt solution. III. A study of the cause of effects produced by cations on the resistance of red corpuscles previously described. *Am. J. Physiol. 68*, 585 (1924).
32. Kerr, S. E.: Studies on the inorganic composition of blood. III. The influence of serum on permeability of erythrocytes to potassium and sodium. *J. Biol. Chem. 85*, 47 (1929).

Open Forum

MODERN TRENDS IN THE TEACHING AND SPECIALIZATION OF HAEMATOLOGY AND BLOOD TRANSFUSION

There has been a dramatic increase in the knowledge of haematology in the last 30 years. Research in this field has expanded both in intensity and in scope. This resulted in further disintegration and a growing number of isolated specialities. Haematology – once limited to morphology and to the clinic of blood diseases – includes today cytochemistry, genetics, immunology, isoserology, biochemistry (especially protein chemistry), blood coagulation and rheology, blood preservation, nuclear science, etc.

It is utterly impossible for the practising physician to keep abreast of this formidable advance. As a consequence there is a great need for experts in haematology and blood transfusion throughout the world. At the same time it is a matter of course that time and again the question arises in what way a working synthesis could be achieved in our era of specialization.

It is highly desirable to re-examine the scope of haematology. In a number of universities there are special courses for haematology. In others it is taught as a part of internal medicine, paediatrics, biochemistry, pathophysiology, etc. In some countries there are chairs for immunohaematology and blood transfusion.

It is believed that many countries would profit from an exchange of experience in this field, and a lively discussion would greatly further the teaching of haematology and blood transfusion.

With this aim in mind prominent scientists from various countries have been invited to participate in the discussion.

GREAT BRITAIN

J. V. Dacie, M. D., F. R. C. P., Professor of Haematology, Postgraduate Medical School of London

Problems of Organization*

New knowledge and expansion in the field of haematology poses new problems for the present and future. The problems I have in mind are three: firstly, the staffing and organization of a department or section of haematology and its

* Part of the inaugural address to the Haematology Society of Australia delivered on August 7, 1962, reprinted by the courtesy of J. V. Dacie, Professor of Haematology, Postgraduate Medical School of London and R. R. Winton, F. R. A. C. P., Editor of The Medical Journal of Australia.

relationships to the clinical-pathological laboratory and the medical department of a hospital; secondly, the staffing and organization of a blood transfusion service in a hospital and the relationship between the blood transfusion laboratory and the department of haematology; thirdly, but not least, organization for research in the blood and its diseases.

Before making specific suggestions on each of these topics, I must stress the very varied nature of the services and organization in haematology which are now available. I refer in this context primarily to hospital diagnostic services rather than to organization for research. The arrangements in fact differ widely in different parts of the world, even across the border between England and Scotland.

In England particularly, haematology has mostly, although not exclusively, been the province of the clinical pathologist and has been carried out in a section of the general clinical-pathological laboratory of the hospital. In England, therefore, a haematologist at the present time is almost always a pathologist who has spent the greater part of his professional life in a laboratory. He may spend all his time on haematological work, running a routine diagnostic service and investigating patients suffering from blood diseases, or he may do this in addition to undertaking another branch of clinical pathology – probably bacteriology or morbid anatomy. However, more and more the trend in the larger laboratories is towards the appointment of a pathologist primarily to do haematological work, to run (probably in addition) the hospital transfusion service, and to help with other branches of clinical pathology only in emergencies or when another member of the staff is on holidays. In large institutions, particularly in the teaching hospitals, the laboratory haematologist, as I have called him (Dacie, 1960), often has his own staff of medically qualified assistants and technicians.

This is the pattern even in teaching hospitals, where one or more members of the professorial medical unit may perhaps specialize in haematology, and where this unit has its own laboratory and staff in which investigations on its own patients are carried out. Even when this is so, the haematological work of the rest of the hospital is usually carried out under the supervision of a laboratory haematologist in the hospital's main clinical-pathological laboratory.

In Scotland, although there have been some recent changes, much of the routine work in the past used to be done by students or technicians in side rooms attached to wards under the supervision of physicians in charge of wards, with occasional consultation or reference to a physician specializing in haematology, who might perhaps be a member of an academic department of medicine. However, within the last few years, central haematological laboratories have been set up in several centres and haematologists have been appointed, with the organization approaching the orthodox English pattern.

In European countries the usual arrangement seems to be for each section of the hospital or ward or group of wards to have its own laboratory, scantily staffed by technicians who usually work under the minimum of supervision, and for one or more members of the medical staff to have a particular interest in haematology. However, it is rare, I believe for a physician to deal solely with patients

suffering from blood diseases; he usually practises general internal medicine in addition to a greater or lesser extent. In America, either system may be found; usually the haematological diagnostic work is under the remote charge of a physician and is done in clinical side rooms or in a single central laboratory which is part of the department of medicine, or less frequently the man in charge may be a clinical pathologist. In America, as indeed in Europe and throughout the world, the great majority of the laboratory work is done by technicians who may occasionally hold a degree in science, but certainly not in medicine.

Although the various systems I have outlined above sometimes work well – depending on the amount of technical help available and the enthusiasm of the medical man in charge – the diagnostic service provided usually falls short of what is desired, and the same is true of the standard of investigation of patients known to be suffering from a blood disease. Although it may be possible to carry out a thorough investigation on a patient suffering from a particular type of disease and to arrive at a precise diagnosis, it is usually next to impossible to cover all areas of haematology effectively. Not only are premises and equipment often inadequate, but there is almost invariably far more work to do than can be properly accomplished. There are usually too few technicians; they are often inadequately trained and insufficiently supervised, and the limited medical staff has too much to do. I have met with few people in Britain, in America, or on the continent of Europe who are even remotely satisfied with the organizations with which they find themselves associated. The reason for this does not seem to me to be in any way obscure. It is because the haematological services – manpower, organization and premises – have been developed in a haphazard fashion from existing facilities, often in a desperate attempt to keep pace with the advancement and application of medical knowledge and the ever-increasing demands for haematological and transfusion services from every section of a hospital. Few haematological departments have been built as such and almost all are under-staffed. The maintenance of a really high standard of work is almost impossible under such circumstances.

I shall start from the premise that every large hospital should have a department or section of haematology, and that even the smallest hospital must provide facilities for the carrying out of basic procedures, such as blood counts and the cross-matching of blood. I visualize the department in the largest institutions as being an autonomous one, with close links with both the clinical-pathological laboratories and the medical department.

Departments of Haematology: their Function and Organization

I visualize the functions of the department of haematology as being five in number. Firstly, it should provide an efficient screening and diagnostic service for the whole hospital, and a service by which the effects of treatment given to patients may be accurately assessed and regulated. This service implies the provision of accurate and expertly interpreted elementary haematological data on in-patients

and out-patients from all sections of the hospital – for example, haemoglobin and packed-cell volume estimations, leucocyte counts, platelet counts, reports on blood films, prothrombin times and so on. Such work more often than not fails to demonstrate any abnormality. It is time-consuming and wearisome, but necessary, and should be carried out by technicians under the supervision of a haematologist, who should look at, or see that a member of his medical staff looks at, the blood film of every new patient. It is important that the haematologist should take a personal interest in this type of work, for in this way he can bolster up the morale of his technicians by making them feel that the work they do, although routine in nature, is very worth while.

All blood films should be covered with a cover-glass, permanently labelled and, ideally, kept. The centralization of this type of service in a single laboratory enables this to be done and high standards to be maintained, and it makes possible the early diagnosis of blood diseases. The volume of such work may be very great and clinicians can help enormously by leaving the decision as to what should be done and what need not be done in a particular case to the haematologist's judgement.

The second function of the department of haematology is the investigation of patients whose blood is abnormal. It goes without saying that all blood and bone-marrow films made in this type of case should be kept and filed away. We should aim to investigate quite deeply every patient suffering from a blood disease so that the diagnosis can be established with accuracy, the response to treatment can be correctly assessed and as much as possible can be learnt from each patient's illness. We can in this way enhance greatly the interest of our work and make the best of the opportunities to advance knowledge that come our way. This is in fact our plain duty, and I will have a little more to say on this later.

The third function of the haematologist and his staff is the care of patients admitted to beds reserved for haematological cases, and the provision of a consultative service for patients elsewhere in the hospital who present with, or who develop, blood disorders.

The question of special beds for patients with haematological disorders is perhaps slightly controversial, but I believe that, where there is a well organized department of haematology, there ought to be a number of beds reserved for patients with blood diseases, who, because they present with particular or long-term haematological problems, or because they were first referred to the haematologist because of his known interest in a particular field, are best looked after by him or his staff. I do not want to suggest that all patients with blood diseases should necessarily be transferred to haematological beds, but, in addition to patients needing complicated haematological investigation, there are others needing repeated hospital admission for, say, blood transfusion or chemotherapy, who would benefit by having this type of bed available for them also.

A further function of the department of haematology would be the provision of out-patient follow-up clinics for patients with certain categories of blood disease – for example, haemophilia and other haemorrhagic disorders, megalo-

blastic anaemia (perhaps these patients should be cared for in a clinic conducted jointly with a gastroenterologist), haemolytic anaemias, haemoglobinopathies, leukaemia and polycythaemia (again, perhaps a radiotherapist should help to conduct the clinic). In this way, patients with different types of haematological disorder can be brought together, and continuity in their treatment or surveillance can be assured.

In this connexion I should like to stress how important it is for patients with blood diseases that each hospital should have on its staff a physician who specializes in chemotherapy, and also a radiotherapist who specializes in, or has a particular interest in, blood diseases. These specialists might well have part-time appointments in the department of haematology. The joint participation of haematologist, chemotherapist and radiotherapist in the management of patients with leukaemia, polycythaemia and allied disorders, can be of great benefit to the patient. Chemotherapy and radiotherapy should be looked upon as complementary (not rival) modes of treatment; by attending clinics managed jointly in this way, the patient can get the treatment best suited to his own particular clinical problem.

I should perhaps mention at this point clinics for the control of anticoagulant therapy. This is a controversial subject and, without going into the merits or otherwise of long-term therapy, I believe that, if anticoagulants are given at all, the regulation of the dosage should be in the hands of a cardiologist, and not a laboratory haematologist. In this way the dosage of the drugs is regulated by a physician, who has a better chance of knowing the patient than has the haematologist, and is in a better position to assess the significance of a patient's symptoms. Whether the tests are actually carried out by a member of the technical staff of the haematology department or of the medical department seems immaterial. A haematological technician might well be lent to the cardiologist for the number of sessions required.

The last function of the department of haematology that I wish to refer to is the running of hospital blood transfusion services. In Britain blood transfusions are usually administered by the haematologist or his designated deputy, and I think this is the best arrangement. Blood diseases and blood transfusion are so inextricably mixed together that it seems to me to be wrong to separate them. Moreover, as examinations for technicians are now organized by the British Institute of Medical Laboratory Technology, students are examined in haematology and blood transfusion technique in the one examination. It is desirable, therefore, to be able to rotate technicians between haematological and blood transfusion work.

Although the technical work of blood grouping and cross-matching can be very well and expertly done by technical staff, it seems to me most desirable that a medical man with, if possible, a genuine special interest in transfusion work should be in charge. Only he or she is in a position to discuss with the clinical staff on equal terms the allocation of blood to particular patients when blood supplies are short, as they so often are.

Staff and Premises of a Department of Haematology

Next I come to staff and premises. For a department of haematology to accomplish all I have just described, it obviously needs a staff of doctors, technicians, secretaries and clerks which is probably considerably larger than that at present allocated to most hospitals, but a large staff is essential if the work is to be done at a high standard. I shall deal with staff first. Should the director of the department be a pathologist or a physician? This may be thought to be an artificial and even unnecessary question, as both can be and are haematologists. Personally, I believe, it is a mistake to differentiate too sharply between pathologists and physicians, or between laboratory workers and clinicians. Both assume important responsibilities in respect of their patients, and in relation to the present problem it does not seem to me to be of great significance whether a particular haematologist has been trained predominantly as a pathologist or as a physician, as long as he is sufficiently experienced and has a deep interest in blood diseases. In any case, local circumstances may well have already decided the issue, an excellent man – pathologist or physician – being already in charge. However, if a new appointment is contemplated, or when the staff of a new hospital is being planned, then there is an opportunity for thought and deliberate selection.

I certainly used to believe that a wide training in laboratory work was essential for a successful haematologist. I still believe this to be desirable, but I now would place less stress on experience in branches of clinical pathology, such as bacteriology, morbid histology and chemical pathology, than I would have done 10 or so years ago. I now in fact rate a wide clinical experience and a good general knowledge of medicine as of more importance to a haematologist than extensive experience in other branches of clinical pathology. I think this change of viewpoint has come about partly as the result of the gradual change in the type of work clinical pathologists now undertake. More and more of the technical work is being undertaken by non-medical technical staff and less and less by the doctors. This applies to all branches of clinical pathology and not only to haematology. One hopes that any lack of skill and breadth of experience in laboratory techniques shown by one's trainees is more than compensated for by their practical and theoretical knowledge of the whole field of medicine and of the mechanisms of disease.

The extent of the haematologist's supporting staff naturally has to be related to the size of his department. If the director is primarily a physician, then it is clearly desirable that his first assistant should be a pathologist and that the technical supervision of the laboratories should be made over to him. On the other hand, if the director is by training a pathologist, then he should have a first assistant, a man or woman who has primarily been trained as a physician. He or she would naturally assume responsibility for the clinical care of the patients occupying the haematological beds and the follow-up clinics. A joint appointment in the departments of haematology and medicine would appear to be appropriate.

Thus my concept of the department of haematology is that it should be an autonomous department closely linked to the other hospital laboratories, because

it shares with them the responsibility for the laboratory investigation of patients, and to the medical department, because of the overlapping clinical responsibilities. As I have indicated above, the director may be pathologist or physician, depending on local circumstances. Junior staff, too, should consist of both pathologists and physicians in training, who could divide the work between them according to which branch of the subject they wished to specialize in.

The premises in which haematological work is carried out have only too seldom been designed for the purpose. Often an old ward, a temporary hut or converted corridor is all that is provided. It goes without saying that the laboratories and ancillary rooms should be properly designed and that they should be in a central site in the hospital area. A laboratory clerk and a proper record organization are most important.

Blood samples for routine tests should be submitted to the laboratory by the clinical staff – if EDTA is used as anticoagulant this is practicable – rather than the laboratory staff being expected to go to the wards to find the patients and to bleed them there. Needless to say we should welcome the introduction of automatic procedures and time-saving gadgets into haematological laboratory work. Electronic counters and automatic staining machines should be widely available. The blood transfusion laboratories should be closely adjacent; again they should be designed for the purpose, with a properly equipped bleeding room if blood is obtained locally, as well as refrigeration facilities close at hand.

The creation of a haematological department on the scale and of the standard I am suggesting will certainly cost a great deal of money. I sincerely hope a larger proportion of the vast cost of running hospital services will be channelled into departments of haematology than in the past. The importance of the subject to my mind clearly merits it.

Research and Haematology, and the Future

I have left consideration of research until last. This is no accident, for the carrying out of research should be our ultimate and highest aim. We cannot be content to let this to be the province of the other man. To make major discoveries needs opportunity, high intelligence, intuition, luck, perseverance, hard work and adequate (but not necessarily generous) facilities. Very few of us are likely to make a major discovery, but something can be done, for instance, by careful observations on patients, the collection of statistical data and the accurate recording of facts. But we can do this type of work – which, if done by many people, ultimately adds up to a great deal – only if the haematologist and his staff have adequate premises and sufficient time to investigate, study and follow-up their patients properly, are not continuously submerged in an avalanche of routine work, and are encouraged to make the best of the opportunities which surround them.

I mentioned earlier the way in which research in blood diseases soon takes the research worker into more basic scientific disciplines. The problem he is pursuing he soon finds, perhaps to be essentially a biochemical one (for example, the exact

nature of the metabolic defect in congenitally defective red cells) – or a physico-chemical one, if he wants to know why certain red cells are the shape they are – or a problem of protein chemistry, if he is trying to isolate and characterize the nature of erythropoietin or intrinsic factor – or even a problem of cytology, if he wants to study chromosome aberrations in leukaemia. Is he likely, whether he be by training primarily a laboratory haematologist or a physician, to be personally sufficiently master of the chemical, physical or cytological techniques which he will have to apply to his problem to make this type of research a practical proposition, even if he has time to apply himself seriously to his problem. The answer is I think that it is most unlikely. As I see it, the only way that fundamental haematological research and the type of problem I have mentioned can be carried out satisfactorily is for the medical research worker who has the problem in front of him, who thinks he knows how to tackle it and who has his patients close at hand, to be able to invite non-medical scientists, chemists, biochemists, biophysicists, biologists and so on, to join his research team, and to be able to offer them the prospects of satisfactory careers in medical research as scientists with the expectancy of permanent posts in the field, if they make a success of their work. At the moment the opportunities for non-medical scientists to work on a long-term basis in the field of medical research are far too restricted, and I would like to see this remedied. By the same token, there should be far more openings than there are at present for permanent appointments in medical research for medical graduates who have shown originality of thought and who wish to make research their career.

I am conscious that I have said little or nothing about the future. The ever-increasing pace of advance in medical knowledge which has taken place in recent years should encourage us to hope that many of the blood diseases which now defeat us will surely eventually be conquered, but it would be foolish to suggest which problem is likely to be settled within the next decade or two and which will be solved much more quickly than this. Some diseases will no doubt be conquered fairly soon but we may be sure that others will remain serious problems throughout our lifetime, and that haematologists here and throughout the world will for many years to come have plenty to do in diagnosing and treating disease and in unravelling further the complexities of the blood and its disorders.

FRANCE

Professeur Jean Bernard, Directeur de l'Institut de Recherches sur les maladies du sang était si gentil de répondre à nos questions concernant l'enseignement et la spécialisation de l'hématologie et de la transfusion sanguine en France.

Comment est organisé l'enseignement de l'hématologie et de la transfusion sanguine dans votre pays?

L'enseignement de l'hématologie est organisé sous la direction de l'Université. L'enseignement préparatoire à la transfusion sanguine est généralement fait

au Centre National et dans les différents Centres Régionaux de Transfusion Sanguine qui dépendent du Ministère de la Santé Publique.

L'enseignement de l'hématologie est obligatoire dans le curriculum des études médicales.

Il est fait à deux périodes:

a — en 3ème années des études médicales, l'hématologie-biologie est enseignée à la même période que la bactériologie et la parasitologie.

b — D'autre part, les étudiants font obligatoirement un stage de trois mois dans un service se consacrant aux maladies du sang, ce stage a généralement lieu en 4ème ou en 5ème année des études.

Quelles sortes de cours spéciaux sont organisées pour les techniciens?

Des cours spéciaux pour les techniciens sont organisés par les chaires d'hématologie des Universités.

Quelle est l'organisation du service de transfusion sanguine dans votre pays?

La transfusion est en France un service national organisé sous la direction du Ministère de la Santé Publique. Il comprend un Centre National de Transfusion Sanguine, une Commission Consultative de la Transfusion Sanguine au Ministère de la Santé, et une série de centres régionaux et départementaux dans chaque département.

Est-ce qu'un Institut d'hématologie existe-t-il dans votre pays? Y a-t-il des chaires d'hématologie à votre Faculté de Médicine?

Il existe plusieurs Instituts d'hématologie en France. Il existe trois chaires universitaires, l'une à l'Université de Paris, la deuxième à l'Université de Strasbourg, la troisième à l'Université de Toulouse. La chaire de Paris a été la première chaire universitaire au monde consacrée à l'hématologie.

Est-ce que les chaires d'hématologie sont en connexion avec des services de transfusion sanguine?

Il existe dans plusieurs villes de France des liaisons étroites entre le service de transfusion et la chaire d'hématologie, par exemple le Professeur titulaire de la chaire d'hématologie de Toulouse, le Professeur titulaire de la chaire d'Hématologie de Strasbourg sont l'un et l'autre Directeur du Centre Régional de Transfusion Sanguine de leur ville.

Y a-t-il une possibilité d'acquérir un diplôme d'hématologie et de transfusion sanguine?

Il existe deux Certificats: L'un, le Certificat d'Études Spéciales d'Hématologie. L'autre, le Certificat d'Études Spéciales d'Hématologie Supérieur. Ils sont destinés aux médecins, étudiants en fin d'études, vétérinaires et éventuellement pharmaciens.

Il existe des diplômes délivrés aux élèves qui ont passé avec succès soit le Certificat Spécial, soit le Certificat Supérieur d'Hématologie. D'autre part comme je l'ai mentionné les Centres de Transfusion organisent un enseignement particulier qui est sanctionné par un diplôme. Ce diplôme n'a pas de valeur gouvernementale, alors que les diplômes d'hématologie sont obtenus après un examen passé sur le plan national et ont une valeur officielle. Il est possible d'obtenir séparément ces différents diplômes.

Quel enseignement théorique et pratique doit passer un médecin qui veut être hématologiste?

Le médecin désirant se consacrer à l'hématologie doit avoir passé au moins un an à suivre l'enseigmenet théorique et pratique préparatoire au Certificat d'Études Spéciales d'Hématologie, le médecin désirant devenir un hématologiste de haut rang doit avoir passé au moins trois ans à préparer le Certificat Supérieur d'Hématologie.

A ces données, il faut ajouter qu'il existe un concours spécial pour obtenir le titre de Professeur Agrégé dans les universités françaises l'un concernant l'hématologie théorique et biologique, l'autre les maladies du sang. Ces concours supposent des épreuves théoriques, leçon faite aux étudiants et épreuve de rédaction sur un sujet déterminé, et des épreuves cliniques et pratiques selon le cas.

THE NETHERLANDS

To inform our readers about the present state of haematology and blood transfusion in the Netherlands Professor Verloop of Utrecht and Professor Van Loghem of Amsterdam have been kindly invited to contribute to this discussion.

How is the teaching of haematology and blood transfusion organized in your country?

Dr. Verloop: The teaching of haematology at the universities in the Netherlands is a part of the teaching in general medicine to undergraduate students. When these students become internists, haematology is usually taught by weekly demonstrations of patients. Important facts about blood transfusion and immunohaematology are also taught.

Dr. Van Loghem: Haematology is taught at the University Medical Schools by professors and readers. Most universities subdivide "haematology" into morphology, clotting, immunohaematology and blood transfusion, and often there are specialists for each of these subjects.

Is it a compulsory subject in medical education?

Dr. Van Loghem: Yes, to a certain extent, as part of the examinations deal with questions on patients suffering from haematologic diseases and a specialist in internal medicine has to follow a stay in haematology, under the guidance of a professor or a reader in haematology.

Dr. Verloop: Haematology is one of the main sub-specialities in the teaching of general medicine to undergraduates.

On what level is it included in the curriculum of medical schools?

Dr. Verloop: Haematology is included in the curriculum of general medicine of the medical faculties of the universities in Holland.

Dr. Van Loghem: It is taught throughout the medical curriculum, e.g. in the third year of the medical study blood morphology and immuno-haematology are treated as part of the lectures on immunology.

Are there postgraduate courses for haematology and blood transfusion?

Dr. Verloop: Postgraduate teaching in haematology and blood transfusion is taken care of at the half-yearly meetings of the Dutch Society of Haematology (more than 200 members). Haematology is also included in postgraduate courses for physicians and for specialists in internal diseases and paediatrics, organized by the universities in The Netherlands.

Dr. Van Loghem: Incidentally postgraduate courses are given, dealing with certain aspects of haematology and blood transfusion.

What sort of training is devoted to haematologic techniques?

Dr. Van Loghem: Medical students are trained in all kind of laboratory techniques, including haematological methods.

Dr. Verloop: Usually the internists are trained to be able to perform simple haematologic estimations, e. g. blood-grouping and cross-matching, determination of haemoglobin, erythrocyte, leucocyte and thrombocyte count. They are also taught blood cell morphology.

How is blood transfusion service organized in your country?

Dr. Van Loghem: Blood Transfusion Service in The Netherlands is under Red Cross control, and comprises 112 blood-transfusion services. Each service has a medical leader, mostly an internal medicine specialist. He controls the medical centre, which is housed in a hospital or municipal medical service, and he is appointed by the Board of the local branch of the Dutch Red Cross. The medical leaders of a province choose from their midst a provincial leader. The eleven provincial leaders are all members of the so-called Central Medical Blood Transfusion Committee. This Committee is directly responsible to the Central Executive Committee of the Dutch Red Cross. Besides the provincial leaders, a few leaders of the Central Executive Committee of the Dutch Red Cross are also members of this Committee as well as representatives of the Dutch Public Health Service, the Head of the Central Laboratory and a few doctors who have made themselves useful in the blood transfusion service.

The Central Medical Blood Transfusion Committee sees to the uniformity of the blood transfusion services in Holland. Rules resulting from consultations ensure the interests of the patients as well as of the donors.

The donor service in Holland is very much centralized under the banner of the Red Cross. Besides, the organization is flexible enough to meet local needs. It leaves freedom for forming blood banks, the need of which varies greatly locally. The demands which the blood to be transferred has to meet are, however, uniform and binding.

The Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, the origin of which dates from 1939, is guided by a foundation in which participate the Dutch Government, The Netherlands Red Cross and the Municipality of Amsterdam.

A staff of qualified physicians, chemists, pharmacists and biologists is charged with the leading of the different departments and laboratories. The Central Laboratory which is operating on a non-profit basis, is the medico-technical centre of the blood transfusion organization in The Netherlands. It provides the majority of Dutch hospitals with blood transfusion equipment and blood derivatives. Besides the large-scale investigations in blood grouping and antibody screening in pregnant women and Red Cross donors, research work is done on various subjects covering the large field of blood transfusion, biochemistry of blood proteins and blood cell preservation, immunohaematology, blood group serology, white cell and platelet serology, immunopathology (autoimmune diseases, transplantation immunology), immunochemistry, biochemistry of blood clotting, blood cell chemistry, blood group genetics and seroanthropology. The Central Laboratory comprises four producing departments and several laboratories.

Is there an Institute for Haematology in your country? Is it a chair of the University Medical School?

Dr. Verloop: Every University Hospital in The Netherlands has a Department for Haematology, usually divided into a Department for Coagulation, a Department for Clinical Haematology and a Haematological Research Laboratory. At the universities of Amsterdam, Leiden, Utrecht and Nijmegen one or more readers (lectors) have been appointed for the teaching of haematology or one of its sub-specialities. In Groningen University there is a chair for haematology occupied by a professor in haematology.

Is there a possibility of obtaining a degree in haematology and blood transfusion? Can these degrees be obtained separately from each other?

Dr. Verloop: In The Netherlands haematology is not an acknowledged speciality. Most doctors working in the field of haematology and blood transfusion are specialized in internal diseases. In the haematological departments of the university hospitals there are always doctors and internists who acquire training in haematology. There is no possibility, however, for obtaining a special degree in clinical haematology or blood transfusion.

Dr. Van Loghem: It is possible to choose a sub-specialism, such as haematology after training in paediatrics or internal medicine. Such specialists are called

"paediatrician-haematologist" or "internal medicine-haematologist". To become a specialist one must be trained for a certain number of years in a recognized university centre, but no examinations are required.

What suggestions would you have for promoting the development of haematology and blood transfusion (education, medical care and research)?

Dr. Verloop: My suggestion for promoting the development of haematology is that care should be taken for haematology to remain a clinical specialism, one of the sub-specialisms of the mother-specialism of internal medicine. Naturally it will be necessary for scientific development of some branches of haematology, such as coagulation, immunohaematology and blood transfusion service, and for the development of haematological laboratory techniques to train haematological pathologists, specially versed in these subjects, and to co-operate with chemists, geneticists, etc. I think, however, that it is of utmost importance that clinical haematology should remain the foster-mother. To bring haematology on a higher level at the universities it should be taught by clinical specialists in haematology; at the same time close co-operation between the sub-departments in haematology (coagulation, immunohaematology and haematological research) should be guaranteed in order to prevent the disintegration of haematology.

Dr. Van Loghem: We do not think that people should be trained in haematology and blood transfusion as a whole. We prefer experts in certain aspects of this very complicated and differentiated field of science. It also depends on whether one wishes to study haematology as a clinical or as a laboratory worker. In our country all kinds of education are possible; haematology is sub-divided into clinical haematology, cytochemistry, genetics, immunology (immunohaematology), blood protein chemistry, immunochemistry, blood coagulation, blood preservation, including cryobiology, etc.

The University of Amsterdam has a chair of immunopathology (since 1959). The University Laboratory of Immunopathology, which is incorporated in the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, deals with studies on immunogenetics, immunohaematology, immunochemistry, autoimmune diseases (other than blood disorders), transplantation immunology, immuno-endocrinology, etc.

ÖSTERREICH

Prof. Dr. E. Deutsch, Vorstand der I. Medizinischen Universitätsklinik, Wien

In Österreich werden Hämatologie und Bluttransfusion nicht in einer eigenen Serie von Vorlesungen vorgetragen, sondern sie sind Teil der Hauptvorlesungen der Inneren Medizin (Hämatologie), der Chirurgie (Bluttransfusion) und der Serologie. Da sie einen Teil des Lehrstoffes dieser Pflichtvorlesungen darstellen, sind sie ein Pflichtgegenstand in der medizinischen Ausbildung, insbesondere in der Inneren Medizin. Diese Vorlesungen werden zwischen dem 6. und 10. Semester gehört. Im Rahmen von Fortbildungskursen, die z.B. von der Wiener

Akademie für Ärztliche Fortbildung abgehalten werden, wird auch Hämatologie und Bluttransfusion vorgetragen. Während der auf das abgeschlossene Studium folgenden praktischen Ausbildung wird sehr grosser Wert auf die Erlernung hämatologischer Techniken und ganz besonders auf die Erlernung der ordnungsgemässen Durchführung von Bluttransfusionen gelegt.

In allen grossen Städten, sowie in den grösseren Krankenhäusern sind Blutbanken organisiert. Diese Blutbanken werden zum Teil vom Roten Kreuz betrieben, zum Teil jedoch von den Krankenhäusern selbst. In den Krankenhäusern ist der Bluttransfusionsdienst meist in den Händen der Chirurgen oder Anaesthesisten, jedoch bestehen beträchtliche örtliche Unterschiede. Es gibt kein eigentliches Institut der Haematologie in Österreich, und es gibt auch keinen Lehrstuhl für Hämatologie. Es besteht aber in neuester Zeit die Möglichkeit, sich für Innere Medizin unter besonderer Berücksichtigung der Hämatologie zu habilitieren. Die Hämatologie ist Schwerpunktsprogramm verschiedener Universitätskliniken (z.B. I. Med. Univ. Klinik in Wien, Med. Univ. Klinik in Innsbruck, sowie der Schwerpunkt verschiedener grosser Krankenhausabteilungen, z. B. Hanuschkrankenhaus in Wien u. a.). Darüber hinaus wurde z.B. an der I. Med. Univ. Klinik in Wien ein Zentrales Gerinnungslaboratorium und ein Thrombosedienst errichtet. Am pathologisch-anatomischen Institut der Universität in Wien besteht eine ausserordentlich aktive Blutgruppenserologie, von der aus die serologische Forschung in ganz Österreich wesentlich befruchtet wurde. Eine besondere Subspezialisierung in Hämatologie ist nicht vorgesehen. Es gibt daher keine Fachärzte für Hämatologie. Dasselbe gilt aber auch für andere Teilgebiete der Inneren Medizin (z.B. Gastroenterologie oder Cardiologie). Wenn durch die strikte Beantwortung der gestellten Fragen der Eindruck erweckt wird, dass in Österreich Hämatologie nicht gepflegt wird, so ist dies grundsätzlich falsch. Die hämatologische Forschung war in Österreich immer ausserordentlich aktiv und ist dies auch heute noch, wenn sie auch keineswegs in starre präformierte Bahnen gelenkt wird. Die bei uns vertretene ganzheitliche Auffassung von der Inneren Medizin lässt eine zu weitgehende Subspezialisierung nicht angezeigt erscheinen, so dass es bisher nicht zur Schaffung von hämatologischen Instituten, Lehrstühlen oder Fachärzten für Hämatologie gekommen ist. Diese Entwicklung hat auch einen wesentlichen historischen Hintergrund. Es hat sich nämlich die morphologische und humorale Hämatologie mehr in der Inneren Medizin, die Transfusionsforschung im Schosse der Chirurgie, und die Serologie im Rahmen der pathologisch-anatomischen Institute selbständig entwickelt. Es besteht zwischen diesen Abteilungen eine sehr intensive und fruchtbringende Zusammenarbeit, wenn es auch noch nie zu einer Vereinigung dieser Sparten zu einem grösseren Ganzen unter Loslösung von den Mutterwissenschaften gekommen ist.

*

In the next issue of *Haematologia* answers from other contributors participating in this discussion will be published.

Further contributions to this discussion or suggestions concerning anyone of the questions would be highly appreciated.

Book Review

F. Gramlich: *Die Rezeptorfunktion der Erythrozyten* (Receptor Function of Erythrocytes). Karger S. Basle, 1966.

Investigations into the processes taking place on the surface of erythrocytes are highly important not only in haematological, but also in general biological research. The erythrocyte is namely one of the objects which are easiest to study, and its surface properties are relatively well known, because of its clinical importance. On the other hand, as a "unit membrane" it may be readily used as a model in investigations concerned with the processes taking place on the surface of cells.

Author discusses the structure and composition of the erythrocyte membrane as the producer and carrier of specific biological functions. This furnishes the basis for the investigation of the development of receptor sites, on the one hand, and of the specific combinative reactions, on the other.

The effect of the environment (medium) on these surface properties is dealt with in detail. Author discusses on an exact physicochemical basis the adsorption of the plasma protein film developing in normal and pathological (e.g. dysproteinaemic) conditions and illuminates its theoretical and clinical importance.

The reactions taking place on the surface of the erythrocyte membrane are analysed from the point of view of the linkage of blood group antibodies (iso-antibodies and autoantibodies) and from the angle of the membrane-modifying effect. In this connection the clinical importance of the surface reactions in vivo is also discussed.

When dealing with heterologous substances (heterohaemolysins, phytohaemagglutinins, heterologous proteins, semi-antigens and microorganisms), author invariably analyses the course of the reactions, their dependence upon the actual condition of the surface (i.e. the erythrocyte), as well as the clinical observations.

It is obvious from the book that author discusses every process on the basis of firm experimental foundations. The techniques necessary to carry out the experiments mentioned in the book are dealt with so thoroughly that these two chapters might be used also as a separate laboratory manual. The reader is told how to carry out preparation of antigens, labelling with isotopes ^{35}S, ^{51}Cr, ^{131}I, sensitization, surface modification, and so forth. This detailed account of the methods is one of the greatest merits of the book.

Beside presenting his own results, author evaluates and surveys comprehensively the pertinent literature, too. It is certain that the publication and wide distribution of this small monograph will contribute to a more thorough examination and study of the as yet unclarified extracellular haemolytic syndromes and through that to a better understanding of their pathogenesis.

Judith Szelényi

Abstracts

Isolated fluorochroming of multiple myeloma- and Waldenstrøm-cells. S. Raptis et al. (II. Medizinische Universitätsklinik, Vienna). *Wien. Z. Inn. Med. 47*, 220 (1966).

Bone marrow smears of patients with various types of paraproteinaemia were treated with gamma-globulin and thiazine red fluorochrome. Isolated fluorescence of the cytoplasm of plasmocytes was observed in cases of β2A-type multiple myeloma and Waldenstrøm's disease. In normal bone marrow smears plasmocytes exhibited only minimum fluorescence, while the fluorescence was entirely absent in reticulosis, acute lymphoid leukaemia, and agammaglobulinaemia. It has been assumed that the observed phenomenon is due to a special affinity of fluorochrome to the paraproteins. Probably the dye enters the cytoplasm through the secretory pores by diffusion.

Ilona Berzy

Haemolysis elicited by Neo-Salvarsan in glucose-6-phosphate dehydrogenase deficiency, combined with hepatic jaundice. F. Michot et al. (I. Med. Abt. Städt. Krankenhauses, München-Schwabing, G. F. R.). *Schweiz. Med. Wschr. 96*, 985 (1966).

A Rhodesian negro treated with Neo-Salvarsan developed viral hepatitis accompanied by haemolytic jaundice. Glucose-6-phosphate dehydrogenase deficiency could be detected in the patient and the clinical picture, the case history, as well as the serological examinations supported the diagnosis of haemolysis elicited by Neo-Salvarsan. It could not be established to what extent hepatitis, as a virus infection, was responsiblt for haemolysis. Till now, Salvarsan has noe been recognized as a drug which elicits haemolysis in glucose-6-phosphate dehydrogenase deficiency.

Ilona Berzy

Cytoplasmic granule formation in myelocytes. An electron microscope radioautographic study on the mechanism of formation of cytoplasmic granules in rabbit heterophilic myelocytes. M. E. Fedorko, J. G. Hirsch (The Rockefeller University, New York, N. Y.). *J. Cell Biol. 29*, 307 (1966).

Immature white cells in the bone marrow actively forming cytoplasmic granules are suitable for a study of lysosome formation. The intracellular flow of tritiated lysine as revealed by electron microscope radioautography was investigated in heterophilic myelocytes of rabbit marrow. Label over the Golgi complex rose to a maximum of 37% of total cytoplasmic grains 30 min after initial exposure to the tracer and fell to 11% after 3 to 4 hr of incubation. Coincident with decrease in label over the Golgi complex, grain counts over granules rose to 32% after 3 to 4 hr. The time sequence of incorporation and flow of tritiated lysine and the percentage distribution of label were similar in bone marrow myelocytes under in vivo and in vitro conditions. The results demonstrate that the Golgi complex incorporates or packs certain basic amino acids or proteins into the cytoplasmic granules of heterophilic myelocytes.

S. Szalontai

Autophagic vacuoles in human red cells. G. Kent, O. T. Minick, F. I. Volini and E. Orfei (Department of Pathology, West Suburban Hospital, Oak Park, Ill.). *Amer. J. Path. 48*, 831 (1966).

Membrane-enclosed bodies containing cytoplasmic organelles, iron particles and segments of erythrocytic cytoplasm were demonstrated in the erythrocytes and circulating reticulocytes of all persons examined. The autophagic vacuoles displaying acid phosphatase activity were increased in number in individuals without spleen or in those with abnormalities of erythropoiesis. They were most abundant in splenectomized persons with concomitant haematologic disorders. Their incidence was high in reticulocytes, even in normal individuals. The bodies appear to play a role in the final stages of red cell differentiation in physiologic and pathologic conditions; their residues are eliminated efficiently in the presence of an intact spleen but accumulate in its absence. In order to attain maximum efficiency, the developing red cell must evidently surrender all superfluous material. According to the study it enlists the aid of the spleen for the effective removal of undigested residues.

S. Szalontai

Investigations into the local blood state of vascular tumours of the skin. I. Pastinszky and M. Kovács (Hungarian Army Medical Corps). *Magyar Onkológia 1*, 38 (1965).

The investigations have been carried out in 23 patients with giant-haemangioma, different in type and extent, but not associated with purpura. On six occasions in every case, invariably in the morning after fasting overnight, upper extremital venous blood was tested for erythrocyte, leucocyte, absolute eosinophil and basophil, as well as thrombocyte counts. Subsequently, blood samples taken from the cavity of the haemangiomas were examined for the same parameters. In every case the thrombocyte count was significantly lower in the blood samples from he angioma than in the circulating blood. The platelet count was 57.8 per cent (mean value) lower in the haemangioma blood than in the peripheral blood. In the blood taken from the cavity of haemangioma the eosinophil count was 40 per cent higher and the basophil count was 60.3 per cent lower. In the intraangiomal blood serum the albumins were decreased by 33.2 per cent and the beta-globulins were increased 3.5-fold. Antithrombocytic antibodies could be detected neither in the peripheral, nor in the haemangiomal blood samples.

Authors attribute the intraangiomal thrombocytopenia to an increased utilization of these elements of blood within the haemangioma. The platelets have not only a mechanical reparative effect, but also influence vasoconstriction. If they decrease in number and if their function is impaired, haemorrhages may occur, but the condition of the vascular wall may also be affected and the symptoms of angiopathy may develop. The decrease of basophil count and the increase of the eosinophil count are also considered to be part of a mechanism protecting the vascular wall. The changes in albumin and globulin values are ascribed to an alteration of the permeability of the vascular wall. It may be assumed that also such a shift in the protein pattern may play a role in the genesis of bleedings from angiomas.

(Autoreferate)

Thrombelastographic studies in physical urticaria. I. Pastinszky and M. Kovács (Hungarian Army Medical Corps). *Allergologia 4*, 243 (1966).

To study the role of blood coagulation relations in physical urticaria, authors have determined the thrombocyte count and carried out thrombelastographic examinations before and after the allergic episodes in cold urticaria (8 patients), heat urticaria (2 patients) and light urticaria (2 patients). It has been found that in patients suffering from physical urticaria there were no changes in the thrombocyte count or in the coagulation pattern as determined by thrombelastography before the application of the stimulus. In every case the induction of urticaria was followed immediately by an increase in the thrombocyte count and by an inhibition of fibrinolysis. Thirty minutes later, the thrombocyte count was 49.71 per cent lower and the rate of fibrinolysis was 164.4 per

cent higher. The other values of the thrombelastogram remained practically unchanged. Authors are of the opinion that the changes of both the thrombocyte count and the fibrinolysis serve to prevent the development of damage to the vascular wall during the allergic episode. The mobilization of thrombocytes and the decreased rate of fibrinolysis prevent an increase of the permeability of the vascular wall. The fall in the thrombocyte count proves that the platelets have been utilized to restore the integrity of the damaged vascular wall. The release of an activator from the vascular wall parallel with the disintegration of thrombocytes results in plasmin production, which is manifested in an increased rate of fibrinolysis.

The results tend to indicate that the intravasal blood clotting relations play an important role in the control of vascular wall permeability in physical urticaria.

(Autoreferate)

Biochemical metamorphosis of hemoglobin in Rana catesbeiana. II. Further studies on the structure and properties of tadpole and frog hemoglobins. K. Hamada, R. Shukuya (Department of Biochemistry, Nippon Medical School, Tokyo). *J. Biochem. (Tokyo) 59*, 397 (1966).

Using *Rana catesbeiana* as source material, tadpole and frog hemoglobins were each separated into two kinds of polypeptide chains by use of carboxymethyl cellulose (referred to as α and β). Tadpole β-chain is responsible for the prominent absorption of tadpole hemoglobin near 290 mμ. Frog β-chain contains two moles of reactive sulfhydryl group as calculated on the basis of the molecular weight being 17,000 for the polypeptide chain. Both tadpole and frog α-chains have the same N-terminal sequence, Gly. Leu and the same C-terminal group, His. On the other hand, the N-terminal sequence and C-terminal group of tadpole β-chain are Val. Ala and Ala, respectively, while those of frog β-chain are Gly. Ser and Glu, respectively. Tryptic and chymotryptic peptide maps prepared from tadpole α-chain are of the same pattern as those obtained from frog α-chain. but those prepared from tadpole and frog β-chains differ from each other in several peptide spots.

G. Gárdos

Electrophoretic demonstration of stromal effects on haemolysate glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. P. E. Carson, F. Ajmar, F. Hashimoto and J. E. Bowman (Genetics Clinic, Department of Pathology, University of Chicago). *Nature 210*, 813 (1966).

Authors previously reported some stromal effects on haemolysate enzymes. The induced changes are 1. inactivation of glucose-6-phosphate dehydrogenase, 2. apparent activation of glutathione reductase, and 3. partial inactivation of 6-phosphogluconic dehydrogenase. The electrophoretic demonstrations of these effects are reported in the present paper. The results show that the loss of G-6-PD activity by heat denaturation or by incubation with stromata can be prevented by added NADP. For 6-P-GD it was shown that the usually distinguishable bands have merged, there was no specific removal of any band. The decrease in 6-P-GD activity of haemolysates induced by incubation with stromata and NADP may be associated with an actual alteration of enzyme protein. Unlike the inactivation of G-6-PD, NADP is also necessary to effect this change. Thus although NADP does not seem to be directly necessary for protection of the catalytic activity of 6-P-GD, NADP may affect its structural state. The erythrocyte membrane does appear to have the capability of altering the metabolism of the erythrocyte by effecting changes in the cytoplasmic enzymes.

Judith G. Szelényi

Rh antibody type in haemolytic disease of the newborn. S. Murray (Regional Transfusion Centre, Newcastle upon Tyne). *Vox Sang. 12*, 81 (1967).

Between 20% and 30% of antenatal sera from Rh negative immunized mothers contain saline agglutinating anti-Rh in addition to the incomplete Rh antibody. Saline antibodies are not merely an early response but recur quite frequently in subsequent

pregnancies and in stillbirths. The presence of saline antibody in the mother is significantly related to increased severity of the disease in the infant both in 1st and later affected cases. This is not affected by the AB0 group, compatibility status or Rh genotype of the infant, but in 1st affected cases only it seems to be related to the mother's AB0 group, and could conceivably be affected by the naturally occurring antibodies of the AB0 system already present. It is with the lower titres of incomplete Rh antibody that the saline antibodies are important, and if cases with saline anti-Rh are excluded, there is then a highly significant relationship between indirect Coombs' titre and severity of disease.

Jolanda Simon

Determination of phosphate: Study of labile organic phosphate interference. E. S. Baginski, P. P. Foa and B. Zak (Detroit General Hospital, Detroit Mich. USA). *Clin. Chim. Acta 15*, 155 (1967).

A newer method for the determination of inorganic phosphate is applicable to the study of phosphate-splitting enzymes. This simple and sensitive procedure for phosphate determination eliminates error due to non-enzymatic hydrolysis of phosphate. A unique feature of the method concerns the fact that the reagents (ammonium molybdate, and citrate-arsenite solution) form a stable colored complex with the inorganic phosphate present, and any additional phosphate liberated after the reagents have been added, cannot react. This procedure appears to be quite suitable for the determination of inorganic phosphate, phospholipids, nucleotides or enzyme hydrolyzed phosphate.

Edith Soós

Effect of ferritin on globin synthesis. J. Van Stone, R. D. Coleman, P. Heller (Veterans Administration West Side Hospital and University of Illinois College of Medicine, Chicago, Ill.). *Nature 210*, 843 (1966).

The effect of ferritin on globin synthesis was investigated in a cell-free system meant to imitate the conditions in sideroblasts. Ribosomes and the enzyme fraction were obtained from reticulocytes and the amount of incorporated valine-1-^{14}C into the nondialysable fraction was considered to be a measure of globin synthesis. Experiments were done with the addition of ferritin, pyridoxal, pyridoxine, pyridoxamine, deoxypyridoxine, or glutathione. The results suggested that ferritin inhibited globin synthesis. This inhibition was counteracted by excess of glutathione in the incubation medium. Pyridoxine and its derivatives neither stimulated globin synthesis nor counteracted the effect of ferritin. It is suggested that in thalassaemia the high ferritin content has some role in the inhibition of globin synthesis but the slightly ameliorating effect of pyridoxine in this disease is indirect through the action of pyridoxine on haem synthesis.

Judith G. Szelényi

Mechanismus der Bildung von Inosinmonophosphat in menschlichen Erythrozyten. II. Synthese des Inosinmonophosphat aus Inosin nach phosphorolytischer Spaltung des Inosins (Mechanism of formation of inosine monophosphate in human erythrocytes. II. Synthesis of inosine monophosphate from inosine upon phosphorolytic decomposition of inosine). H. Banaschak (Institut für Pharmakologie und Toxikologie der Humboldt Universität, Berlin) *Acta biol. med. german. 17*, 261 (1966).

In stroma-free, human haemolysates the IMP synthesis from inosine and ATP is defined by adenylic acid deaminase and inosine kinase reactions. IMP synthesis is dependent on Mg^{++}, K^{+} and phosphate. Phosphate-dependent are both the nucleoside phosphorylase and the IMP pyrophosphorylase reactions, the latter representing the K^{+} dependent stage. The individual stages of the reaction are studied in details. It is concluded that the ribose-5-phosphate pyrophosphokinase reaction is the speed-limiting step in this way of the IMP synthesis.

Ilma Szász

Control of glycolysis in the human red blood cell. I. A. Rose, J. V. B. Warms (Department of Biochemistry, Philadelphia, Penn. 1911). *J. Biol. Chem. 241*, 4848 (1966).

Out of the effects of inorganic phosphate, resulting in the increase of glycolysis rate, the stimulation of hexokinase and phosphofructokinase is recognized, but the cause of the necessary rate increase of pyruvate kinase is not. Therefore kinetic studies with this enzyme from normal and mutant (pyruvate kinase deficient) human red blood cells have been performed. It was established that the increase in the rate of formation of phosphoenolpyruvate is primarily responsible for the increase of the reaction rate in the kinase step. Mutant cells with about 10% enzyme activity reached even saturating levels of phosphoenolpyruvate. According to the authors the explanation for the accumulation of fructose diphosphate and dihydroxyacetone phosphate at high glycolytic rates in normal and deficient cells is that glyceraldehyde phosphate dehydrogenase becomes rate limiting as a result of an increased ratio of reduced to oxidized NAD and 1,3-diphosphoglyceric acid.

Ilma Szász

Studies on erythrocyte glycolysis. II. Free energy changes and rate limiting steps in erythrocyte glycolysis. S. Minakami, H. Yoshikawa (Department of Physiological Chemistry and Nutrition, Faculty of Medicine, University of Tokyo). *J. Biochem. (Tokyo) 59*, 139 (1966).

The mass action ratios and the free energy changes of the individual steps of glycolysis occurring in erythrocytes under physiological conditions are calculated. The thermodynamic data indicate that three reactions catalyzed by hexokinase, phosphofructokinase and pyruvate kinase are rate limiting steps in glycolytic process in erythrocyte. The free energy change for the erythrocyte glycolysis may be calculated to be —52.5 kcal or —53.8 kcal. The efficiency of ATP formation by the steady state glycolysis of erythrocytes is calculated to be about 50%.

G. Gárdos

Plasma volume expanding effect of dextran and plasma. U. F. Gruber, S. E. Bergentz (Kirurgiska kliniken I, Gothenburg, Sweden). *Nord. Med. 76*, 1277 (1966).

The plasma volume expanding effect of an infusion of 500 ml plasma, and a dextran solution was studied in healthy volunteers following withdrawal of 500 ml blood. There was immediate expansion of the plasma volume to almost twice the infused volume. After 2 1/2 hours the plasma volume had decreased to the pre-bleeding value. Autologous plasma infusion immediately restored the plasma volume to the pre-bleeding value and it remained unchanged at the end of 2 1/2 hours. Homologous fresh plasma had the same immediate effect but there was a marked decrease in the plasma volume amounting to the whole volume infused. Two subjects had urticaria and one, chills. Since the blood groups were wholly identical, the reactions probably were due to the presence of immunoglobulins or viable leukocytes, causing an antigen-antibody reaction. It is concluded that the plasma volume expanding effect of homologous plasma infusion is not predictable as that of infusion of dextran or autologous plasma.

E. Benedek

Gm(b) factor in the γ globulin of non-human primates. J. C. Allen (Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Md.). *Nature 209*, 520 (1966).

Inhibitory effect of pooled sera from different species of non-human primates and that from an individual gorilla was compared with the effect on incomplete anti-D coated red cells—anti-Gm(b) agglutinator system. A rheumatoid and a non-rheumatoid human serum and a serum from an immunized Rhesus monkey were used as agglutinators. The non-human primate sera were inhibitory to a different extent when the human agglutinators were used; but only the human serum was an effective inhibitor of the monkey agglutinator. No Vi H-chain sub-group antigenic determinant was found in the non-human primate sera.

G. A. Medgyesi

Biologische Normalwerte bei Wistar-Ratten (Jena) verschiedenen Alters. I. Das normale rote und weisse Blutbild (Normal biological

data of Wistar rats (Jena) at different ages. I. The normal data of erythrocytes and leukocytes). H. Hoffmann, L. Kersten. (Institut für Pharmakologie der Friedrich-Schiller-Universität, Jena). *Z. Versuchstierk. 8*, 227 (1966).

The alterations in the red and white blood-cell counts of Wistar rats (Jena) of different ages are reported. The number of erythrocytes increases to the value of the adult rats after 3 1/2 months (8.52 ± 0.152 Mill./mm^3), while haemoglobin attains these values after about 2 months (16.6 ± 0.15 gr%). There are real differences in the haemoglobin content between male and female rats during the maturation and also in the numbers of erythrocytes of adult rats. The white blood-picture develops more rapidly, for standard values of adults in both sexes were found after 15 days of life (distribution of leukocytes in differential blood picture) and at the end of the 2nd month of life (complete leukocyte-counts).

Esther Cholnoky

Die Normalwerte des Thrombelastogramms bei gesunden Hunden (The normal data of the thrombelastograms of healthy dogs). J. Pospisil, M. Beran (Institut für Biophysik der Karls-Universität, Praha). *Z. Versuchstierk. 8*, 256 (1966).

The thrombelastograms of 60 healthy fasting dogs were evaluated. The average values are as follows: $r = 9.6\text{vt} \pm 1.8$, $k = 5.7\text{vt} \pm 1.3$, $ma = 60.8\text{mm} \pm 6.1$.

Esther Cholnoky

Pyridoxine responsive anaemia determined by an X-linked gene. M. W. Elves, M. S. Bourne, M. C. G. Israels (Department of Clinical Haematology, The Royal Infirmary and University, Manchester). *J. Med. Gen. 3*, 1 (1966).

A family is described of which 83 individuals have been investigated, a pyridoxine responsive anaemia being found in 6 middle-aged men. The salient features of this disease are an anaemia that may vary from mild to severe, and evidence of iron loading. Two of the patients had normal haemoglobin levels at the start of the study, but both showed iron loading which responded to pyridoxine therapy. Osmotic fragilities and haemoglobin studies by electrophoresis revealed no departure from normal in any case. It is suggested that the anaemia in this family is an X-linked recessive condition. Other published familial cases are discussed and all would be consistent with such a mode of inheritance. It was found that the gene responsible was not closely linked to that determining the Xg blood groups.

Esther Cholnoky

Neutrale Plasmalogene und Alkoxydiglyzeride in menschlichem Depotfett (Neutral plasmalogens and alkoxydiglycerides in human fat deposit). H. H. O. Schmid and H. K. Mangold (Hormel Institute, University of Minnesota, Austin, Minn.). *Biochem. Z. 346*, 13 (1966).

Neutral plasmalogens (O-Alken-1-yl-diglycerides) and 1-alkoxydiglycerides are isolated, as pure lipid classes, from human perinephric fat obtained at post mortem from a patient with leukaemia. The aldehydes in the neutral plasmalogens and the glyceryl ethers in the alkoxydiglycerides are mainly saturated and monounsaturated. However, the quantitative compositions of corresponding constituents of the two lipid classes are different. The neutral plasmalogens and alkoxydiglycerides contain the same fatty acids, but in different proportions. Each of these lipid classes exhibits a specific fatty acid pattern.

Elisabeth Török

Die Veränderung der Enzymkapazitäten von Hexokinase, Phosphofruktokinase und Pyruvatekinase roter Blutzellen bei Konservierung in ACD und zitratfreien Stabilisatoren (Changes of hexokinase, phosphofruktokinase and pyruvic kinase activities of red blood cells under preservation in ACD and citrate-free stabilizers). G. Jacobasch, H. J. Raderecht with assistance of S. Scheerer, Ch. Boese, R. Elsner (Physiologisch-chemisches Institut der Humboldt-Universität, Berlin). *Acta biol. med. german. 17*, 275 (1966).

The hexokinase, phosphofructokinase and pyruvic kinase activities of red blood cells were followed in the course of blood preservation, using three stabilizer solutions: ACD, AMD (glucose: 133 mM/l, malonic acid: 114 mM/l, NaOH: 218 mM/l) and ABD (glucose: 150 mM/l, HCl: 30 mM/l, sodium diaethylbarbiturate: 42 mM/l, sodium acetate: 3 H_2O : 44 mM/l). Phosphofructokinase proved to be the most susceptible enzyme, being inactivated by 50 per cent within 6 to 8 hours in ACD medium. After 30 days of preservation, the activity loss exceeded 80 per cent, whereas pyruvic kinase by more than 50 per cent. The initial inactivation of phosphofructokinase in AMD and ABD media was not as high. Since in haemolysate both phosphofructokinase and pyruvic kinase may be inactivated by citrate it is supposed that the inactivation of these two enzymes in ACD medium is accelerated by the action of citrate.

Ilma Szász

Blood-volume changes in severe anaemia in pregnancy. K. A. Harrison, M. B. Lond (Department of Obstetrics and Gynaecology, University of Ibaden, Nigeria). *Lancet 7480* (I), 20 (1967).

The blood-volume changes were estimated by means of chromium labelling methods and by Evans-blue dye technique in anaemic and healthy pregnant women. In severe anaemia in pregnancy, the haemoglobin concentration and the packed cell volume of the venous blood give a very good indication of the total red cell value before the anaemia is treated. In cases of multiple pregnancies, gross splenomegaly, anaemic failure and larger plasma-volume expansion are found. Total blood-volume is reduced in 25% of the patients in whom the haemoglobin concentration is less than 4.4 g per 100 ml, and the packed cell volume of peripheral venous blood is less than 14% and the total red-cell mass is less than 10 ml per kg body weight. In severe anaemia in pregnancy the haematocrit ratio is not constant when the packed cell volume is less than 25%. The total blood-volume can therefore be reliably estimated only by simultaneous measurements of red-cell and plasma-volumes.

E. Benedek

Gleichzeitige Bestimmung von Kalzium- und Chloridionen in derselben Serumprobe (Determination of calcium and chlorid ions in one serum sample). L. Wünsch (Krankenhaus, Usti u/L. Tschechoslowakei). *Clin. Chim, Acta 15*, 127 (1967).

A titration method for the determination of calcium and chlorid ions in one serum sample is described. First calcium is estimated by titration with dinatrium salt of ethylendiamintetraacetic acid, then are the chlorid ions titrated with mercuric nitrate. By the titration of calcium fluorexon, and by the titration of chlorid diphenylcarbazon is the indicator. During the second titration, the rising complex of diphenylcarbazon and mercuric ions is extracted in ethyl acetate to make the reaction more sensitive. The presence of coagulated proteins does not interfere with the titration. The determination can be performed with 0.1—0.5 ml of serum. The satisfactory accuracy, simple equipment and small amount of serum make the method recommendable for diagnostic purposes in children and in other cases where large amounts of blood are difficult to obtain.

Edith Soós

Some differences between primary structures of hemoglobins of man and monkey (Macacus rhesus). M. Teleha and P. Mäsiar (Department of Biochemistry, Safárik University, Kosice). *Collection Czechoslov. Chem. Commun. 31*, 3383 (1966).

Differences between the primary structures of hemoglobins of man and monkey were studied by high-voltage paper electrophoresis and by chromatography. Acidic fragments containing cysteic acid and differing in their chromatographic behaviour were isolated from chymotryptic digests of the two hemoglobins. The fragment which was isolated from monkey hemoglobin did not contain threonine. From a combined tryptic-peptic digest of monkey hemoglobin peptide Ala. Glu. Leu was isolated by chromatography and electrophoresis. This peptide was not found in human hemoglobin submitted to an analogous treatment. From the results of the analyses of the two differing peptides the authors conclude that one

of the differences between the primary structures of human and monkey (M. rhesus) hemoglobin involves position 87 in the β-chains where threonine is replaced by glutamic acid in monkey hemoglobin.

V. Sőtér

Agglutination of tanned sheep erythrocytes by serum from Nigerian adults and children. C. Adeniyi-Jones (University of Ibadan, Nigeria). *Lancet 7483*, 188 (1967).

This investigation shows that the serum of Nigerians commonly contains a factor capable of agglutinating tanned sheep erythrocytes. It has the electrophoretic mobility of gammaglobulin, and is absorbed by tanned cells. Its characterization as a macroglobulin, by the action of the reducing sulphhydryl agent mercaptoethanol suggests its similarity to the heterophil antibodies present in human serum against untanned sheep erythrocytes. It is different from the antibody to the Forssman antigen, since it persists after absorption with normal sheep cells and with guinea pig kidney. It is also different from the heterophil antibody found in patients with infectious mononucleosis since it is not absorbed by ox red blood cells. It creates difficulties in performing haemagglutination tests using tanned cells sensitized with antigen, since it is difficult to determine the agglutination titre with specific antibody. The usefulness of specific haemagglutination tests is thus limited in areas where the factor is common. It was absent from cord blood, but present in 39% of infants, 71% of schoolchildren, 75% of urban adults, and 91% of rural adults. The titre increased with age. The sharing of antigenic determinants among organisms and plant tissues is well known and Olliver-Gonzalez (1952) described the absorption of heterophil antibody against normal sheep erythrocytes by a variety of bacteria and helminth parasites, showing the wide interrelationship of antigens. It is therefore not difficult to envisage existing antibody reacting with the tanned cell surface. The age-distribution of the factor in Nigerian serum suggests that it is an acquired phenomenon, and its macroglobulin nature explains its absence from cord blood. The rise in prevalence and titre with age may indicate increasing exposure to a stimulus which is first applied shortly after birth, and the lower titres and prevalence in urban men may reflect the reaction of population subject to a less intense stimulus. From early life the Nigerian is assailed by viral, bacterial, protozoal, and helminth infections. The early appearance of agglutination at a time when helminth infection is uncommon and malaria not unusual, suggests that a relationship between the agglutination reaction and malaria infection may well exist.

P. Lépes

Lysozyme in bone marrow and peripheral blood cells. R. S. Briggs, P. E. Perillie, S. C. Finch (Department of Internal Medicine, Yale University, New Haven, Conn.). *J. Histochem. Cytochem. 14*, 167 (1966).

By means of an indirect histochemical technique the intracellular lysozyme of the formed elements of the peripheral blood and bone marrow was estimated. Evidence is presented that monocytes as well as mature neutrophils and their precursors extending back to the progranulocyte, contain significant amount of this enzyme. A rare mature eosinophil demonstrated a trace of lysozyme activity. There was no evidence of lysozyme activity in basophils, erythrocytes, megakaryocytes, platelets, plasma cells, tissue mast cells or bone marrow reticuloendothelial cells.

Georgina Sik

The transfusion of leukocytes from donors with chronic myelocytic leukemia to patient with leukopenia. E. M. Morse et al. (National Cancer Institute, Division of Biology Standards, Bethesda, Md.). *Transfusion 6*, 175 (1966).

Leukocytes were collected from donors with chronic myelocytic leukemia by plasmapheresis and transfused into severely leukopenic recipients. The median transfusion of 7×10^{10} granulocytes (range 15 to 35×10^{10}) resulted in a median increase in circulating granulocytes of 1,000 per cu. mm (range 0 to 19,000) one hour after injection. The post-transfusion increment was directly related to

the number of cells injected. Only 4.8 per cent of the injected cells were recovered in the circulating blood volume at one hour (range 0 to 37 per cent). The per cent recovery was directly related to the pretransfusion granulocyte count of the recipient, the more severe the recipient's leukopenia, the lower was the per cent recovery of transfused cells. The transfused granulocytes disappeared from the recipient's circulation with a half time of 24 hours. A number of other factors were found to influence the results of transfusion such as: antileukemic drugs given, the presence of fever, the transfusion vehicle, the AB0 red cell compatibility of donor and recipient and the sequence of transfusion. Clinical responses as measured by disappearance of fever were seen in 54% of the recipients. The fraction of febrile patients responding increased as the dose of leukocytes transfused increased. Severe reactions, manifested by dyspnea, cyanosis, and lung infiltrates were seen in a small percentage, but febrile reactions occurred in 67 per cent of afebrile patients.

Georgina Sik

Intrauterine transfusion. G. C. Allan, W. M. Alpern, E. A. Friedman et al. (Department of Gynecology, Chicago Medical School, Chicago, Ill.). *Obstet. gynec. 28*, 323 (1966).

Intrauterine transfusion is claimed to be effective in preventing intrauterine death from severe erythroblastosis fetalis. The method administering blood to the fetus in utero was first described by Liley in 1963. Twenty-six intrauterine transfusions were performed in 17 patients during a year. Fresh, 0 Rh negativ packed red blood cells, compatible by cross match with the mother's serum were transfused without any antibiotics. These experiments lead the authors to believe that infants born with erythroblastosis fetalis can be kept viable and healthy by this method.

Georgina Sik

A study of conservative and surgical treatment of Werlhof's disease. D. M. Grozdov, A. S. Kukel, L. S. Tsepa (Central Institute of Haematology and Blood Transfusion, Moscow). *Probl. haemat. 12*, 16 (1966).

The paper presents the results of treatment of 343 patients with thrombocytopenic purpura; 213 of these were subjected to surgical intervention. The importance of isolation of the immune forms is underlined; the latter are mostly encountered in acute (subacute) forms and rarely in chronic relapsing Werlhof's disease. Conservative therapy with the use of steroid hormones is sufficiently effective in acute (subacute) forms. In non-immune forms of Werlhof's disease the therapeutic measures, including hormonal therapy, at best provide clinical remission. In this connection indications of splenectomy in this variant of the disease should be widened. Postoperative results are better when the operation is performed at the phase of remission than during the haemorrhagic crisis. The mortality rate is zero in the first case and 5% in the second.

Katalin Natonek

The effect on the plasma proteins of peroral, enteral, intraportal, and intravenous administration of casein hydrolysate. An experimental study in the dog. I. Holm (Department of Surgery, Serafimerlas, Stockholm). *Acta Chir. Scand. 351.* Suppl. (1965).

Author has shown that protein-free diet leads to hypalbuminaemia. The normal plasma protein level can be maintained by the peroral administration of casein hydrolysates. If the same amount of casein hydrolysate is administered enterally, the protein supply is insufficient, due to the evoked diarrhoea or polyuria. As injected intraportally or intravenously daily, in rapid infusions of short duration, appropriate amounts of casein hydrolysate will produce hypalbuminaemia and hyperglobulinaemia. The repeated, rapid infusion of physiological sodium chloride solution evokes the same alterations, in spite of the peroral administration of appropriate amounts of N, probably because the elimination of albumin from the blood is increased by the sodium chloride infusion. If casein hydrolysate is administered in appropriate amounts but in prolonged daily doses, hypalbuminaemia and hyperglobulinaemia can be almost completely prevented and normal plasma protein level can be maintained in many cases. These

results suggest that the plasma protein level can be kept near to normal by the intravenous administration of casein hydrolysate, if the daily doses are introduced in small liquid volumes and in infusions of relatively long duration.

J. Dávid

Separation of contaminating pyrogenic material from commercial bovine serum albumin. B. A. Philip, P. Herbert and J. W. Hollingsworth (Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.). *Proc. Soc. Exp. Biol. Med. 123/2*, 576 (1966).

The authors found a pyrogenic material in commercial bovine serum albumin, which proved to be bacterial endotoxin. From sterile calf serum non-pyrogenic bovine serum albumin was prepared. By Sephadex G-100 column chromatography, the pyrogenic material could be separated from proteins in commercial bovine serum samples. Since bovine serum albumin is widely used in immunological and other studies, the preparations contaminated with endotoxin may modify the results and thus misleading conclusions may be drawn.

J. Dávid

The effect of blood exchange transfusion on the production of immunologic factors. Z. Nejedlá (Institute for Mother and Children Welfare, Prague-Podoli). *Mschr. Kinderheilk. 114*, 107 (1966).

Exchange transfusion removes not only the antibodies causing haemolysis, but also the ones directed against pathogenic microorganisms, that enter the foetus from the mother through the placenta and that ought to protect the foetus in the first months of life. The maternal antibodies protect the newborn up to 2—3 months; after that the infant begins to produce its own antibodies. The half-life of antibodies introduced by exchange transfusion is only 25—30 days. Thus, infants treated by blood exchange are poorly protected from the second month of life on. Author examined 25 infants treated by exchange transfusion and compared the results with those obtained in infants not treated that way. Diphtheria-, tetanus-, and pertussis-antibodies belong to the microglobulins. They are present in smaller amounts in the maternal blood than in donor blood. In the blood of healthy newborns and of those suffering of erythroblastosis these antibodies are present in smaller amounts than in the maternal blood. Newborns with erythroblastosis show immediately before blood exchange unequivocally lower titres than the normal ones. During the first week after blood exchange the titre values of infants are still lower. All infants were immunized with diphtheria-tetanus-pertussis triple vaccine at 3 months of age. In normal infants, aged 6 to 9 months, the antibody titres were higher as a rule. The gamma globulin levels in normal newborns were comparable to the maternal. After the third month of age there was a sudden fall in the values. In the bloods used for exchange transfusion the gamma globulin values were lower than in the maternal blood. In haemolytic disease of the newborn a markedly lower value was found already before the blood exchange than in the normal newborns. The gamma globulin values of this group continued to decrease till the sixth month. The author studied the behaviour of E. coli antibodies as well, which belong to the macroglobulins and cannot pass through the placenta. For this reason the E. coli antibody-level of normal newborns is very low. After exchange transfusion the titre markedly increases, and thus the E. coli antibody-level of infants submitted to exchange transfusion is higher in the first month than in normal infants.

J. Dávid

Antibodies against gamma globulin after repeated blood transfusions in man. J. C. Allen and H. G. Kunkel (Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Md.). *J. Clin. Invest. 15*, 29 (1966).

The authors have examined the sera of 65 children and 85 adults, who had received repeated doses of gamma globulin in the form of transfusion. It became evident that 60 per cent of the children and 30 per cent of the adults developed some kind of antibodies

against gamma globulin. In the children mainly the anti-gamma globulin was (Gm) genetic factor specific, although non-Gm specificity and rheumatoid factor-like activity could also be detected. The assumption that these Gm-specific antibodies were formed by immunization by the transfused and genetically heterologous gamma globulin, is supported by the following: 1) the anti-Gm specificity was directed only against Gm-factors, which were absent from the patient's gamma globulin phenotype; 2) the very high percentage incidence of activity, as compared to control individuals without transfusion; 3) in a 15-year-old individual anti Gm-activity developed during observation, and finally 4) the frequent lack of immunizing Gm-factor in the maternal gamma globulin. In adults haemagglutinating antibodies were found against human incomplete anti-D antibodies. These might be formed *via* immunization by the denatured or aggregated gamma globulin found in the transfused preparation, or the immunization took place with the autologous gamma globulin, as the antibodies against the transfused erythrocytes ("anti-antibodies"). These studies have demonstrated the antigenicity of human isologous gamma globulin and have called attention to the circumstance that Gm-specific antibodies and rheumatoid factor-like activity may be brought about by immunization with isologous gamma globulin. The investigations have shown also the origin of antibodies specific to the Gm-factor of human gamma globulin in clinically healthy persons, and pointed out the antibody character of anti-gamma globulins formed in patients suffering of rheumatoid arthritis.

J. Dávid

On the serological diagnosis of AB0-erythroblastosis. F. W. Bube (Blood Center of Univ. Clin., Clin. Chir., Köln). *Z. Kinderheilk., 95*, 231 (1966).

The serological methods recommended for the examination of AB0-erythroblastosis are often very complicated and fail to give positive results in all cases. A good method should detect complete as well as incomplete antibodies, should be well reproducible, simple, and must not give aspecific positive results. The author replaced the maternal serum by AB-group substance. In this way the isoagglutinins are neutralized. Then A-erythrocytes pretreated with bromelase are added. In addition, fresh AB-serum is also added, since complement is required for the binding of anti-A or anti-B immune antibodies; then the source of agglutination is examined in indirect light. If the test is negative, after washing with physiological sodium chloride solution anti-human globulin is added and the mixture is examined again for agglutination. If the test is now positive, immune-antibodies are present. (For details see the original paper.)

Out of 38 cases of pure AB0-erythroblastosis the test was positive in 37 cases, and similar results were obtained in 26 cases of pure Rh-erythroblastosis. Out of 17 cases of AB0 + Rh-erythroblastosis the test was positive in 16 cases.

J. Dávid

The loss of certain cellular components from human erythrocytes during hypotonic haemolysis in the presence of dextran. H. Magnus, S. G. Ostling and E. G. Persson (University Hospital, Uppsala, Sweden). *Acta Physiol. Scand. 67/1*, 43 (1966).

The loss of certain cellular components (catalase, glucose-6-phosphate dehydrogenase, hexokinase, lactic dehydrogenase, ATP, glutathione) was studied in human erythrocytes treated with haemolytic hypotonic salt solution containing dextran. There was an inverse relationship between the amount of components and the relative disappearance rate from the cell.

J. Dávid

From the International Literature on Haematology

Acta Haematologica (Basel) **36** (1966) No. 5—6

Cell proliferation in leukemia during relapse and remission. I. DNA and RNA synthesis of leukemic cells in the bone marrow in vitro. *Schmid, J. R.* et al. (Mayo Clinic, Rochester, Minn.), p. 313

Aggregation und Desaggregation von Erythrozyten. *Ahrly, A. M.* et al. (I. Med. Klinik und Poliklinik der Univ., Langenbeckstr. 1, 65 Mainz), p. 323

The transformation of human mononuclear leukocytes in vitro. I. Comparison of cells in suspension and attached to coverslips. *Lamvik, J. O.* (Univ. of Bergen, School of Med., Bergen, Norway), p. 335

Studies of lymphocytes and their derivative cells in vitro. I. Biochemical constituents. *Gough, J.* et al. (Dept. of Clinical Haemat., The University, Manchester, England), p. 344

Eosinophil granules and cell maturity: Electron microscopic observations on guinea-pig marrow. *Hudson, G.* (Dept. of Anatomy, Med. School of the Univ., Bristol, England), p. 350

A peculiar benzidine-positive protein fraction in the plasma of newborns and children with various haemolytic conditions. *Bottini, E.* et al. (Istituto di Genetica, Facoltà di Science, Univ. di Roma, Roma), p. 361

Über den Retraktionsvorgang beim Blutplasmagerinnsel. *Fonio, A.* (Poststrasse 19, 7000 Chur, Schweiz), p. 371

On the origin of the skin window macrophages. *Trepel, F.* et al. (I. Med. Abteilung, Städt. Krankenhaus, München—Schwabing), p. 386

Sickle cell disease and glucose-6-phosphate dehydrogenase. *Lewis, R. A.* et al. (National Inst. of Health and Med. Research, P. O. Box 2848, Accra, Ghana), p. 399

A new thalassemic syndrome: Homozygous hemoglobin S disease delta thalassemia. *Thompson, R. B.* et al. (Dept. of Clin. Lab. Sciences, Univ. of Mississippi Med. Center, Jackson, Miss.), p. 412

Blood (New York) **28** (1966) No. 3

Effects of erythroid hyperplasia on patterns of glycine-2-C^{14} uptake in heme and globin of human peripheral blood in vivo. *Nathan, D. G.* et al. (Hemat. Lab., Peter Bent Brigham Hosp., Boston, Mass.), p. 303

The production of hemoglobin C in sheep carrying the gene for hemoglobin A: Hematologic aspects. *Moore, S. L.* et al. (Clemson Univ., Clemson, S. C.), p. 314

Extraction of an erythropoietin-producing factor from a particulate fraction of rat kidney. *Contrera, J. F.* et al. (Dept. of Biol., Graduate School of Arts and Science, New York, N. Y.), p. 330

Urinary excretion of erythropoietin in normal men and women. *Alexanian, R.* (The Univ. of Texas M. D. Anderson Hosp., Houston, Texas), p. 344

Erythropoietin excretion in normal man. *Adamson, J. W.* et al. (Div. of Hemat., Univ. of Washington School of Med., Seattle, Wash.), p. 354

Adverse effect of raised environmental temperature on the expression of hereditary spherocytosis in deer mice. *Anderson, Ruth* et al. (Dept. of Med. and Genetics, Univ. of Washington, Seattle, Washington), p. 365

Fast hemoglobin in lead poisoning. *Charache, S.* et al. (Dept. of Med., The Johns Hopkins Univ. School of Med., Baltimore, Md.), p. 377

Mechanism of the hereditary anemia of Sl^m mutant mice. *Kales, A. N.* et al. (Dept. of Med. and Physiol., Univ. of Chicago, Chicago, Ill.), p. 387

Regeneration of locally irradiated bone marrow. I. Dose dependent, long-term changes in the rat, with particular emphasis upon vascular and stromal reaction. *Knospe, W. H.* et al. (Walter Reed Army Inst. of Research, Washington, D.C.), p. 398

The development of Hodgkin's disease and lymphoma during anticonvulsant therapy. *Hyman, G. A.* (Columbia Univ. College of Physicians and Surgeons, New York, N.Y.), p. 416

Characteristics of cell proliferation in four patients with untreated acute leukemia. *Mauer, A. M.* et al. (The Children's Hosp. Research Foundation, Cincinnati, Ohio), p. 428

Serum anti-γG globulin factors in paroxysmal nocturnal hemoglobinuria. *Kaplan, M. E.* et al. (Dept. of Hemat., The Mount Sinai Hosp., New York, N.Y.), p. 446

The intravascular lifespan of monocytes. *Whitelaw, D. M.* (Univ. of British Columbia, Vancouver, Canada), p. 455

Virus-like particles and cytopathic activity in urine of patients with leukemia. *Ames, R. P.* et al. (National Cancer Inst., National Institutes of Health, Bethesda, Md.), p. 465

Factor VIII (AHF) concentration in pH 6.5 citrate-plasma. *Morrison, F. S.* (St. Mary's Hosp. Med. School, London), p. 479

Blood (New York) **28** (1966) No. 4

Hemoglobin H disease. A family study. *Necheles, Th. F.* et al. (New England Med. Center Hospitals, Boston, Mass.), p. 501

Impairment of red cell viability by exposure to "excess" acid-citrate dextrose. *Mayer, K.* et al. (Memorial Hosp. for Cancer, New York, N. Y.), p. 513

Platelet function in a patient with thrombasthenia. *Zucker, Marjorie B.* et al. (New York Univ. School of Med., New York, N.Y.), p. 524

Erythropoietin in the urine of normal and erythropoietically abnormal human beings. *Van Dyke, D.* et al. (Lawrence Radiation Lab., Univ. of California, Berkeley, Calif.), p. 535

The periodic acid-Schiff reaction in neutrophil leukocytes in untreated and Myleran-treated chronic myelocytic leukemia. A quantitative microspectrophotometric study. *Gahrton, G.* (Inst. for Med. Research, Karolinska Inst., Stockholm), p. 544

A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. *Beutler, E.* (City of Hope Med. Center, Duarte, Calif.), p. 553

The binding of Cr^{51} to hemoglobin. II. In vivo elution rates of Cr^{51} from Hb CC, Hb CS, and placental red cells. *Pearson, H. A.* (Univ. of Florida College of Med., Gainesville, Florida), p. 563

Stilbestrol-induced porphyria: Increase in hepatic δ-amino-levulinic acid synthetase. *Levere, R. D.* (State Univ. of New York, Downstate Med. Center, Brooklyn, N. Y.), p. 569

Detection and quantitation of minor erythrocyte populations in an admixture by means of fluorescent antibody: rhesus monkey. *Bogden, A. E.* et al. (Dept. of Immunol., Mason Research Inst., Worcester, Mass.), p. 573

Cytogenetic and cytochemical studies on marrow cells in B_{12} and folate deficiency. *Menzies, Rosa C.* et al. (Cytogenetics Unit, Christchurch Hosp., Christchurch, New Zealand), p. 581

Failure of methyl palmitate induced reticuloendothelial blockade to affect plasma levels of Factor VIII. *Gaynor, Evelyn* et al. (Dept. of Hemat., Montefiore Hosp. and Med. Center, New York, N.Y.), p. 595

Blood (New York) **28** (1966) No. 5

Hemangioma-thrombocytopenia syndrome associated with microangiopathic hemolytic anemia. *Propp, R. P.* et al. (Albany Med. College of Union Univ., Albany, N.Y.), p. 623

Immunologic aberrations in the Di Guglielmo syndrome. *Finkel, H. E.* et al. (New England Med. Center Hospitals, Boston, Mass.), p. 634

Ribonucleic acid biosynthesis in human leukocytes: The fate of rapidly labeled RNA in normal and abnormal leukocytes. *Cline, M. J.* (Dept. of Med., Univ. of California School of Med., San Francisco, Calif.), p. 650

The detection of iso-antigens on leukemic cells using the cytotoxicity test. *Peacocke, I.* et al. (Veterans Hosp., Durham, N. C.), p. 665

Staining of blood cells with periodic acid/salicyloyl hydrazide (PA-SH). A fluorescent method for demonstrating glycogen. *Burns, J.* et al. (Dept. of Pathol., Radcliffe Infirmary, Oxford, England), p. 674

Human leukocyte metabolism in vitro. II. The effect of 6-mercaptopurine on formate-C^{14} incorporation into the nucleic acid of acute leukemic leukocytes. *Scott, J. L.* et al. (Wadsworth Hosp., Veterans Administration Center, Los Angeles, Calif.), p. 683

Allogeneic marrow engraftment by cross circulation in lethally irradiated dogs. *Epstein, R. B.* et al. (Univ. of Washington School of Med., Seattle, Washington), p. 692

Urinary iron excretion and renal metabolism of hemoglobin in hemolytic diseases. *Sears, D. A.* et al. (Dept. of Hemat., Walter Reed Inst. of Research, Washington, D.C.), p. 708

Iron-deficient diet: Effects in rats and humans. *Kaufman, R. M.* et al. (Dept. of Hemat., Walter Reed Army Inst. of Research, Washington, D.C.), p. 726

The effect of acute inflammation on the utilization and distribution of transferrin-bound and erythrocyte radioiron. *Quastel, M. R.* et al. (Dept. of Med., Univ. of California School of Med., Los Angeles, Calif.), p. 738

Ferritin localization on the erythroblast cell membrane and ropheocytosis in hypersiderotic human bone marrows. *Tanaka, Y,* et al. (Clin. Pathol. Dept., National Institutes of Health, Bethesda, Md.), p. 758

Isolated malabsorption of vitamin B_{12} causing megaloblastic anemia and hyperpigmentation in a Nigerian. *Watson-Williams, E. J.* et al. (Subdepat. of Hemat., Univ. of Ibadan, Nigeria), p. 770

Blut (München) **14** (1966) No. 2

Isolierung und physikalisch-chemische Charakterisierung der Cholinesterase aus Humanserum. *Haupt, H.* et al. (355 Marburg/Lahn, Behringwerke AG.), p. 65

Untersuchungen an länger gelagerten Blutkonserven: Nucleosideffekt und Hexokinaseaktivität. *Blum, K.-U.* et al. (I. Med. Klinik der Freien Univ., 1 Berlin 19, Spandauer Damm 130), p. 76

Untersuchungen mit einem neuen Hämoresistometer. Die mechanische Resistenz der menschlichen Erythrozyten im Blut von klinisch gesunden und kranken Personen sowie von Konservenblut in Abhängigkeit von der Lagerdauer. *Hennig, U.* (Chirurg. Klinik, Städt. Krankenhaus im Friedrichshain, 1018 Berlin, Leninallee), p. 83

Ein Hämagglutinationstest zum Nachweis von Lysozym. *Grossgebauer, K.* (Inst. für Hygiene u. Med. Microbiol., Freie Univ. Berlin, 1 Berlin 65, Föhrer Str. 14), p. 93

Untersuchungen zur Populationsgenetik der Haptoglobin-Untergruppen. *Kehr-Löke, E. R.* et al. (78 Freiburg im Br., Albertstr. 11), p. 97

Blut (München) **14** (1966) No. 3

Die Alius-Grignaschi-Anomalie: Der erblich-konstitutionelle Peroxydasedefekt der Neutrophilen und Monozyten. *Undritz, E.* (CH-4000 Basel, Neubadstr. 83), p. 129

Response of cultured tissue cells to homologous and heterologous blood group substances. *Reřábek, E.* et al. (27 M. Schramma, Prague 6), p. 137

A_1/A_2-Untergruppenbestimmungen in Abstammungsprozessen. *Rex-Kiss, B.* (Med.-Univ., Budapest IX., Üllői út 93), p. 150

Die Bestimmung der Thrombozytenlebenszeit mit Diisopropylfluorophosphat ($DF^{32}P$). *Adam, W.* et al. (Med. Univ. Klinik, 78 Freiburg im Breisgau, Hugstetterstr. 5), p. 155

Das morphologische Blutbild vom europäischen Seehund. *Kraft, H.* (Med. Tierklinik, 8 München 22, Veterinärstr. 13), p. 169

British Journal of Haematology (Oxford) **12** (1966) No. 6

Familial disturbance of fibrin monomer aggregation. *Felten, A. von* et al. (Dept. of Med., Univ. of Zurich), p. 667

Lactate dehydrogenase in the diagnosis and assessment of response to treatment of megaloblastic anaemia. *Emerson, Pauline M.* et al. (Dept. of Clin. Pathol., Guy's Hosp., London, S. E. 1.), p. 678

Observations on the in vitro growth of peripheral leucocytes from patients with myeloproliferative disorders. *Pegrum, G. D.* (Dept. of Haemat., Charing Cross Hosp. Med. School, London), p. 689

Incidence of gastric parietal cell antibody in families of patients with iron deficiency anaemia. *McFadyen, I. J.* et al. (Univ. Dept. of Med. and Pathol., Western Infirmary, Glasgow, W. 1), p. 697

Methylmalonic acid excretion in vitamin B_{12} deficiency. *Bashir, Helen V.* et al. (Dept. of Pathol., Prince Henry Hosp., Little Bay, Sydney), p. 704

Anabolic agents and relative polycythaemia. *Verwilghen, R.* et al. (Dept. of Med., St. Raphael Univ. Clinic, Leuven, Belgium), p. 712

Sites of red cell destruction in the haemolytic anaemia of adoptively immunized NZB mice. *Barnes, R.D.S.* et al. (Dept. of Haemat., Inst. of Child Health, Guilford Street, London, W. C. 1), 717

Gastric acidity and iron absorption. *Jacobs, A.* et al. (Welsh National School of Med., Cardiff, Wales), p. 728

Giant myeloid cells in the bone marrow of protein malnourished infants: Relationship to folate and vitamin B_{12} nutrition. *Spector, I.* et al. (South African Inst. for Med. Research, Johannesburg, S.A.), p. 737

Iron absorption in relation to transferrin saturation and other factors. *Taylor, M. R. H.* et al. (Dept. of Clin. Med., Trinity College, Dublin, Ireland), p. 747

Involvement of the erythroid series in blastic crisis of chronic myeloid leukaemia. Further evidence for the presence of Philadelphia chromosome in erythroblasts. *Clein, G. P.* et al. (Royal Victoria Infirmary, Newcastle upon Tyne 1, Great Britain), p. 754

Kinetics of rapidly labelled RNA in human leukaemia cells. *Sinks, L. F.* et al. (Dept. of Med. Univ. of Cambridge, Tennis Court Rd., Cambridge), p. 759

Platelet aggregation and the availability of platelet Factor 3. *Hardisty, R. M.* et al. (Inst. of Child Health, The Hosp. for Sick Children, Great Ormond St., London), p. 764

Studies on thalassaemia-like anaemia in pregnant immigrants in London. *Beaven, G. H.* et al. (M. R. C. Laboratories, Holly Hill, Hampstead, London), p. 777

Folia Haematologia (Leipzig) **86** (1966) No. 3

Neue Aspekte beim Morbus Osler. *Stinner, Gisela* (I. Med. Klinik, Humboldt-Univ., 104 Berlin, Schumannstrasse 21), p. 249

Über Schädigungen der unreifen Formen der megakaryozytären Zellreihe. *Kissóczy, S.* et al. (Bezirkskrankenhaus, Presov, ČSSR), p. 258

Experimental cholesterine sclerosis as reflected in blood coagulation. *Lusztig, G.* et al. (Dept. of Pathol. Anat. and Histol., County Hosp., Kecskemét, Hungary), p. 272

Examination of the anticoagulant and clearing-like effect of serum in experimental cholesterine sclerosis. *Lusztig, G.* et al. (Dept. of Pathol. Anat. and Histol., County Hosp., Kecskemét, Hungary), p. 283

On the direct effect of cobalt on nucleic acid and protein metabolism in rat's bone marrow cultivated in vitro. *Niebrój, T. K.* (Katowice 6, Warminska str. 18/4), p. 293

Paragammaglobulinämie, charakterisiert durch die Bildung von Gammaglobulin mit defizienter Antigenität, Mangel an Beta-2M-Globulin und ein partielles Antikörpermangelsyndrom. *Backhausz, R.* et al. (Impfstoffproduktions- u. Forschungs-

institut "Human", Budapest X, Szállás u. 5—7), p. 298

Experimentell durch Hexadimethrinbromid ausgelöste Eosinophilie bei Ratten. *Kovács, K.* et al. (I. Med. Univ.-Klinik, Korányi Rkp. 12, Szeged, Hungary), p. 324

Das Verhalten der basophilen Granulozyten bei adipösen Kindern. *Blau, H.-J.* (Univ.-Kinderklinik, 25 Rostock, Rembrandtstr. 16—17), p. 341

Untersuchungen über die gerinnungshemmende Wirkung von Mukopolysacchariden aus der menschlichen Aorta. *Józsa, L.* et al. (Prosektur des Komitatskrankenhauses, Kecskemét, Hungary), p. 345

Hémostase (Paris) **6** (1966) No. 2—3

Mise au point d'une nouvelle méthode de dosage du plasminogène. Application à l'étude des cirrhoses du foie. *Dray, L.* (Clinique des Maladies du Sang de l'Hôpital Broussais, 96, rue Didot, Paris 14), p. 105

A propos de deux cas de purpura thrombopénique idiopathique traités par microdoses de P 32, après échec des thérapeutiques classiques. *Croizat, P.* et al. (Clinique médicale C, Lyon), p. 129

Études sériques et immunologiques au cours des purpuras. *Bachmann, A. E.* et al. (Instituto de Investigaciones Hematologicas de la Académia Nacional de Medicina, Melo 3081, Buenos Aires), p. 139

Étude expérimentale des vitesses de disparition de l'héparine plasmatique après une injection intraveineuse. *Weiss, M.* (Centre Chirurgical Marie Lannelongue, 129, rue de Tolbiac, Paris 13), p. 149

Effets des radiations ionisantes sur la coagulation du sang et la fibrinolyse. Étude expérimentale chez le rat. *Bertrand, H.* (Centre de Transfusion-Réanimation de l'Armée «Jean-Juillard»), Clamart, France, p. 167

The opacity variations of the plasma clot. *Montani, E.* (General Med. Division, Monza Hosp., Monza, Italy), p. 185

Plasmin assay. A new method for the estimation of physiologically active plasmin. *Hunter, D. W.* et al. (Dept. of Pathol., Univ. of Utah Med. Center, Salt Lake City, Utah) p. 207

Pesanteur et rétraction du caillot. *Vergoz, D.* et al. (Centre départ. de Transfusion sanguine de la Seine, Hôpital Saint-Antoine, Paris), p. 207

Nouvelle Revue Française d'Hématologie (Paris) **6** (1966) No. 5

Lymphe et circulation des lymphocytes chez l'homme. *Bernard, J.* et al. (Hôpital Saint-Louis 2, place du Docteur-Fournier, F-75-Paris), p. 585

Applications de la recherche ultrastructurale à l'étude des thrombocythémies. *Jean, G.* et al. (Clinique de Séméiotique Médicale, Univ. de Milan, Italie) p. 591

L'érythrocinétique dans l'hémoglobinurie nocturne paroxystique. *Najean, Y.* et al. (Hôpital-Saint-Louis,F-75-Paris), p. 611

Étude comparative des taux de cofacteur de la staphylocoagulase (C.R.F.) et des taux de facteur II (Prothrombine) dans diverses conditions. *Soulier, J. P.* et al. (Centre National de Transfusion Sanguine, 6, rue A.-Cabanel, F-75-Paris), p. 623

Trois observations de transformation aiguë d'hémopathies lymphocytaires chroniques *Lortholary, P.* et al. (Hôpital Saint-Louis, F-75-Paris), p. 637

Effet de l'incubation à 37°C pendant deux heures sur la capacité de réactivité des cellules immunologiquement compétentes. *Mathé, G.* et al. (Hôpital Paul-Brousse, 14, avenue Paul-Vaillant-Couturier, 94-Villejuif), p. 657

Un nouvel antigène des β-lipoprotéines humaines. *Contu, L.* (Hôpital Saint-François, Nuoro, Italie), p. 671

Nouvelle Revue Française d'Hématologie (Paris) **6** (1966) No. 6

Le rôle de l'érythropoïétine dans la différenciation des globules rouges. *Goldwasser, E.* (Argonne Cancer Research Hosp., University of Chicago, Chicago, Ill.), p. 757

Anomalies des enzymes de la dégradation du glucose érythrocytaire. *Boivin, P.* et al. (Hôpital Beaujon, 100, avenue Général-Leclerc, F-92-Clichy, France), p. 769

Les systèmes d'oxydo-réduction du globule rouge et leurs anomalies. *Kaplan, J. C.*

(Hôpital des Enfants-Malades, 149, rue de Sèvres, F-75-Paris), p. 809

Mécanisme de l'hémolyse dans la microsphérocytose héréditaire. *Najean, Y.* et al. (Hôpital Saint-Louis, 2, place du Dr-Fournier, F-75-Paris), p. 835

Les enzymes de la biosynthèse de l'hème et leurs anomalies au cours de la thalassémie, l'anémie sidéro-achrestique héréditaire et l'anémie sidéroblastique idiopathique acquise. *Gajdos, A.* (Clinique Médicale de l'Hôtel-Dieu, 1, place du Parvis de Notre-Dame, F-75-Paris), p. 845

Anémies hémolytiques congénitales avec déficit isolé en glutathion réduit par déficit en glutathion synthétase. *Boivin, P.* et al. (Hôpital Beaujon, 92-Clichy-France), p. 859

Valeur comparée de la cholinestérase sérique et globulaire au cours de divers troubles hématologiques. *Kissel, P.* et al. (C.H. U., 29, avenue du Général-De-Lattre-de-Tassigny, F-54-Nancy, France), p. 867

Études sur les variations de sept activités enzymatiques et du taux du glutathion réduit érythrocytaires au cours des anémies rénales. *Madec, Y.* et al. (Centre Hospitalo-Universitaire, 1, rue G.-Veil, F-44-Nantes, France), p. 873

Проблемы гематологии и переливания крови (Москва) **11** (1966) № 11

Тканевые факторы сосудистой стенки, пораженной атеросклерозом. *Альфонсов В. В., Кузник Б. И.* (Кафедра нормальной физиологии Читинского медицинского института.) с. 3. — Blood Coagulation Tissue Factors of the Atherosclerosis-Affected Vascular Wall. *Alfonsov, V. V., Kuznik B. I.* p. 3.

О некоторых особенностях свертывания системы крови при гемофилии. *Гутберг Р. А.* (Лаборатория фракционирования белков Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 9. — Some Peculiarities of the Blood Coagulation System in Hemophilia. *Rutberg, R. A.* p. 9

Тромбоцитопоэтическая активность сыворотки крови при остром лейкозе. *Баранов, А. Е.* (3-я кафедра терапии Центрального института усовершенствования врачей.) с. 15. — Thrombocytopoietic Activity in the Blood Serum in Acute Leukemia. *Baranov, A. E.* p. 15

Изучение тромбоцитопоэтических свойств сыворотки крови при экспериментальных тромбоцитопениях. *Намятышева, А. М.* (Патофизиологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 18 — A Study of Thrombocytopenic Blood Serum Properties in Experimental Thrombocytopenia. *Namyatysheva, A. M.* p. 18

Изменение тромбоцитопоэза и содержания тромбоцитов в крови и пунктатах селезенки при сенсибилизации, в анафилактическом шоке и в послешоковом периоде. *Сомова, Е. П.* (Кафедра патологической физиологии Московского ордена Ленина Медицинского института им. И. М. Сеченова.) с. 23. — Changes of Thrombocytopoiesis and of Thrombocyte Content in the Blood and Splenic Puncture Material in Sensitization, Anaphylactic Shock and during the Postshock Period. *Somova, E. P.* p. 23

Об особенностях влияния аминазина на тромбоциты человека. *Безносиков, Б. О., Ильин, И. И.* (Цитологическая лаборатория по изучению лейкозов Ленинградского института переливания крови и лаборатория патологии высшей нервной деятельности человека Института физиологии им. И. П. Павлова.) с. 25. — Peculiarities of Chlorpromazine Effect on Human Thrombocytes. *Beznosikov, B. O., Ilyin, I. I.* p. 25

Изменение фибринолитической активности крови у здоровых лиц в зависимости от возраста. *Зорькина, Н. К.* (Кабинет гериатрии Ставропольского медицинского института.) с. 27. — A Change of Blood Fibrinolytic Activity in Apparently Healthy Persons in Relation to Age. *Zorkina, N. K.* p. 27

Некоторые аспекты изучения функционального состояния тромбоцитов при болезни Гланцмана—Негели (Обзор литературы). *Исаакян, А. И.* (Гематологическая клиника, лаборатория биохимии Центрального ордена Ленина ин-та гематологии и переливания крови Министерства здравоохранения СССР.) с. 28. — Some Aspects of Studying the Function-

al State of Thrombocytes in Glanzman-Naegeli Disease (Literature survey). Isaakyan, A. I. p. 28

Тромбоэластографические изменения при острой почечной недостаточности, вызванной посттрансфузионными осложнениями. *Карасева, Е. В., Кавешникова, Б. Ф.* (Отделение посттрансфузионных осложнений и гемодиализа и клиническая лаборатория Центрального ордена Ленина института гематологии и переливания крови.) с. 34. — Thrombelastographic Changes in Acute Renal Insufficiency Caused by Posttransfusion Complications. *Karaseva, E. V., Kaveshnikova B. F.* p. 34

Тромбогеморрагический синдром при операциях с использованием аппарата экстракорпорального кровообращения в эксперименте. *Лабахуа, Г. Ш.* (Отделение экспериментальной и клинической коагулологии Института экспериментальной и клинической хирургии и гематологии АМН СССР Тбилиси.) с. 38. — Thrombohemorrhagic Syndrome in Operations with the Experimental Use of an Extracorporeal Circulation Apparatus. *Labakhua, G. Sh.* p. 38

Мегакариоцитограмма у доноров. *Ярустовская, Л. Э.* (Гематологическая клиника, радиологическое отделение и лаборатория проблемы донорства Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 41. — Megakaryocytogram in Donors. *Yarustovskaya, L. E.* p. 41

Об изучении ингибитора VIII фактора у больных гемофилией А. *Папаян-Покрышева, Л. П.* (Лаборатория свертывания крови Ленинградского института переливания крови.) с. 46. — A Study of the Inhibitor of Factor VIII in Patients with Hemophilia A. *Papayan-Pokrysheva, L. P.* p. 46

Организация и проведение «дня донора» в Тульской области. *Шкарин, В. В.* (Тульская областная станция переливания крови.) с. 48. — Organization of "Donor Day" in Tula Region. *Shkarin, V. V.* p. 48

К вопросу о «стандартном разводителе» для антирезус-сывороток. *Успенская, О. В.* (Калининская областная станция переливания крови.) с. 49. — "Standard Diluent" for Antirhesus Sera. *Uspenskaya, O. V.* p. 49

Заготовка консервированной крови в посуде малой емкости. *Петров, Д. Г., Винцкевич, А. И.* (Львовский научно-исследовательский институт гематологии и переливания крови.) с. 53. — Preparation of Preserved Blood in Small Containers. *Petrov, D. G., Vintskevich, A. I.* p. 53

Рецензия на книгу А. Маркуш, М. Цукер «Физиология тромбоцитов». *Свиридов, Н. К.* с. 54. — Review on the book by A. Marcus and M. Zucker "Physiology of Thrombocytes". *Sviridov, N. K.* p. 54

Рецензия на книгу Ж. Сальмон «Фибринолиз и сосудистая патология». *Альперин, П. М.* с. 56. — Review on the book by Zh. Salmon "Fibrinolysis and Vascular Pathology". *Alperin, P. M.* p. 56

Замечание к статье Л. И. Идельсона и Э. Г, Радзивальской «Порфириновый обмен при анемиях (№ 1—66 г. стр. 10). *Троицкий, С. А.* с. 59. — A Comment on the Article by L. J. Idelson and E. T. Radzivalskaya: "Porphyrin Metabolism in Anemia". *Troiitsky, S. A.* p. 59

Научная сессия по проблеме фибринолиза. *Климова, К. Н.* с. 59. — Scientific Session on Fibrinolysis Problem. *Klimova, K. N.* p. 59

Протокол заседания гематологической секции Московского общества гематологов и трансфузиологов от 22/2 1966 г. *Ковалева, Л. Г.* с. 61. — Proceedings of a Meeting of a Hematological Section of the Moscow Society of Hematologists and Transfusiologists (Feb. 22, 1966). *Kovaleva, L. G.* p. 61

Отчет о работе Ташкентского общества гематологов и трансфузиологов за 1966 г. *Хакимов, Х. А. Калугина, В. И.* с. 62. — A Report on the Work of Tashkent Society of Hematologists and Transfusiologists for 1966. *Khakimov, A., Kalugina, V. I.* p. 62

Проблемы гематологии и переливания крови (Москва) **11** (1966) 12

Очередные проблемы хирургического лечения заболеваний системы крови. *Гроздов, Д. М.* (Центральный ордена Ленина институт гематологии и переливания крови Министерства здравоохранения СССР,

Москва.) с. 3. — Current Problems of Surgical Treatment of Diseases of the Blood System. *Grozdov, D. M.* p. 3

Клиника и хирургическое лечение болезни Верльгофа. *Шабанов, А. Н.* (Проф. А. Н. Шабанов, Москва.) с. 11. — Clinical Picture and Surgical Treatment of Werlhof's Disease. *Shabanov, A. N.* p. 11

Показания к консервативному и хирургическому лечению болезни Верльгофа. *Гроздов, Д. М., Кукель, А. С., Цепа, Л. С.* (Хирургическая клиника Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 16. — A Study of Conservative and Surgical Treatment of Werlhof's Disease. *Grozdov, D. M., Kukel, A. S., Tsepa, L. S.* p. 16

Ближайшие и отдаленные результаты спленэктомии при болезни Верльгофа. *Шерман, С. И., Сечило, Е. А., Куралева, В. В, Абдулкадыров, К. М.* (Гематологическая клиника и хирургическая клиника Ленинградского института гематологии и переливания крови.) с. 21. — Immediate and Remote Results of Splenectomy in Werlhof's Disease. *Sherman, S. I., Sechilo, E. A., Kuraleva, V. V., Abdulkadyrov, K. M.* p. 21

Обеспечение безопасности спленэктомии при заболеваниях системы крови. *Демидюк, П. Ф., Караванов, А. Г., Пхакадзе, А. Л.* (Отдел клинической хирургии Киевского научно-исследовательского института клинической медицины им. Н. Д. Стражеско и хирургическое отделение Киевского института гематологии и переливания крови.) с. 25. — Provision of Safety of Splenectomy in Diseases of the Blood System. *Demidyuk, P. F., Karavanov, A. G., Pkhakadze, A. L.* p. 25

Хирургическое лечение заболеваний системы крови и операции на их фоне. *Епифанов Н. С., Журавлев, В. А., Корепанов, В. И., Садков, С. А.* (Кировский научно-исследовательский институт переливания крови.) с. 30. — Surgical Treatment of Blood System and Operations against the Background of Hematological Diseases. *Epifanov, N. S., Zhuravlev, V. A., Korepanov, V. I., Sadkov, S. A.* p. 30

Влияние сплнеэктомии при болезни Верльгофа на свертывающую систему крови и свойства тромбоцитов. *Копыстянский, Н. Р.* (Гематологическая клиника Львовского института гематологии и переливания крови.) с. 34. — The Effect of Splenectomy in Werlhof's Disease on the Blood Coagulation System and the Thrombocyte Properties. *Kopystyansky, N. R.* p. 34

Отдаленные результаты спленэктомии при талассемии. *Ахундова, А. М.* (Гематологическая клиника Азербайджанского научно-исследовательского института гематологии и переливания крови, Баку.) с. 37. — Remote Results of Splenectomy in Thalassemia. *Akhundova, A. M.* p. 37

Переломы костей у больных гемофилией. *Абдуллаев, Г. М.* (Хирургическая клиника Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 40. — Fractures of the Bones in Patients with Hemophilia. *Abdullaev, G. M.* p. 40

Лечение гемофилии, осложнившейся анаэробной инфекцией. *Абдуллаев, Г. М., Кузьмина, И. В.* (Хирургическая клиника Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 44. — Treatment of Hemophilia, Complicated by Anaerobic Infection. *Abdullaev, G. M., Kuzmina, I. V.* p. 44

Морфологическая картина крови при болезни Иценко—Кушинга до и после адреналэктомии. *Калинин, А. П., Мардер, О. П.* (Хирургическое отделение Института экспериментальной эндокринологии и химии гормонов АМН СССР, Москва.) с. 48. — Morphological Blood Picture in Itsenko-Cushing's Disease before and after Adrenalectomy. *Kalinin, A. P., Marder, O. P.* p. 48

Протокол заседания секции трансфузиологов Московского научного общества гематологов и трансфузиологов от 18/1 1966 г. *Гроздов, Д. М., Герасимова, Л. И.* с. 52. — Proceedings of a Meeting of the Section of Transfusiologists of the Moscow Scientific Society of Hematologists and Transfusiologists (Jan. 18, 1966). *Grozdov, D. M., Gerasimova, L. I.* p. 52

Протокол заседания секции трансфузиологов Московского научного общества ге-

матологов и трансфузиологов от 15/2 1966 г. *Гроздов, Д. М., Герасимова, Л. И.* с. 53. — Proceedings of a Meeting of the Section of Transfusiologists of the Moscow Scientific Society of Hematologists and Transfusiologists (Febr. 15, 1966) *Grozdov, D. M., Gerasimova, L. I.* p. 53

Протокол заседания секции трансфузиологов Московского научного общества гематологов и трансфузиологов от 22/3 1966 г. *Аграненко, В. А., Герасимова, Л. И.* с. 54. — Proceedings of a Meeting of the Section of Transfusiologists of the Moscow Scientific Society of Hematologists and Transfusiologists (March 22, 1966). *Agranenko, V. A., Gerasimova, L. I.* p. 54

Отчет о работе гематологической секции Ленинградского общества терапевтов им. С. П. Боткина за 1965 г. *Антонов, В. Б.* с. 56. — A Report on the Work of Hematological Section of the Leningrad S. P. Botkin Society of Therapeutists for 1965. *Antonov, V. B.* p. 56

Thrombosis et Diathesis Haemorrhagica (Stuttgart) **16** (1966) No. 3/4

Effects of thorium dioxide upon blood clotting and platelets. I. In vitro studies. *Hjort, P. F.* et al. (Univ. of Southern California School of Med., Los Angeles, Calif.), p. 333

Effects of thorium dioxide upon blood clotting and platelets. II. In vivo studies in rabbits. *McGehee, W. G.* et al. (Univ. of Southern California School of Med., Los Angeles, Calif.), p. 354

The influence of EDTA, trisodiumcitrate and heparin on the ultrastructure of blood platelets. *Libánská, J.* (Pediatric Faculty, Charles Univ., Prague), p. 371

The effect of the contact factors of the coagulation system on platelet aggregation. *Barth, P.* et al. (Med. Univ. Clinic, Heidelberg), p. 378

Suppressive effect of dextran on platelet adhesiveness. *Cronberg, S.* et al. (Univ. of Lund, Allmänna Sjukhuset, Malmö, Sweden), p. 384

Elektronenmikroskopische Untersuchungen an Thrombozyten des Menschen nach Einwirkung verschiedener Gase. *Doenecke, C.* et al. (Pathol. Inst., Univ. Düsseldorf), p. 395

Untersuchungen zum Nachweis antithrombozytärer Heteroantikörper durch Immunfluoreszenz. *Mueller-Eckhardt, Ch.* et al. (Hygiene-Inst., Univ. Kiel), p. 411

Clot-dissolving action of Echis colorata venom in dogs. *Djaldetti, M.* et al. (Rogoff and Welcome Research Laboratories, Tel-Aviv Univ., Tel-Aviv, Israel), p. 420

Action of Newcastle disease virus on human blood platelets. *Cohen, I.* et al. (Rogoff and Welcome Med. Research Laboratories, Tel-Aviv Univ., Tel-Aviv, Israel), p. 430

Thrombozytopenie bei chronischem Cor pulmonale. *Beltrami, G.-M.* et al. (Med. Univ.-Klinik, Bern), p. 443

The purification of human prothrombin. *Shapiro, S. S.* et al. (Dept. of Biol., Massachusetts Inst. of Technol., Cambridge, Mass.), p. 469

Chromatographic differentiation of human prothrombins. *Aronson, D. L.* (National Inst. of Health, Div. of Biol. Standards, Bethesda, Md.), p. 491

Prothrombin and antithrombin: their opposing functions in blood coagulation. *Grannis, G. F.* et al. (The Cardeza Foundation for Hemat. Research, Jefferson Med. College, Philadelphia, Penn.), p. 497

Action of water-insoluble trypsin derivatives on fibrinogen clottability. *Alexander, B.* et al. (Dept. of Biophysics, The Weizmann Inst. of Science, Rehovoth, Israel), p. 507

Studies on the degradation of human fibrinogen by plasmin and trypsin. *Beck, E. A.* et al. (The Johns Hopkins Univ. and Hosp., Baltimore, Md.), p. 526

Kongenitaler Mangel von Faktor V und Faktor VIII (Kasuistische Mitteilung). *Paar, D.* et al. (Zentrallab. der Med. Klinik, Essen, DBR), p. 541

Combined Factor VIII and IX inhibitors in a girl. *Reussi, C.* et al. (Lab. of Coagulation and Fibrinolysis Invest., Rivadavia Hosp., Buenos Aires, Argentine), p. 549

The immunological properties of Factor VIII. I. Studies on the inhibitory activity of specific antisera to human antihemophilic globulin. *Uszyński, L.* (Inst. of Haemat., Warsaw), p. 559

Deficiencies and inhibitors of thromboplastic activity. *Grannis, G. F.* et al. (The Cardeza Foundation for Hemat. Research, Jefferson Med. College, Philadelphia, Penn.), p. 574

The procoagulant in human urine: Purification, assay and some biochemical and physiological properties. *Aoki, N.* et al. (Univ. of Colorado, School of Med., Denver, Colorado), p. 586

Effect of phenobarbital and glutethimide on biological half-life of warfarin. *Corn, M.* (George Washington Univ. Med. Div., District of Columbia Gen. Hosp., Washington, D. C.), p. 606

Einfluss des Alkohols auf die Antikoagulantientherapie. *Riedler, G.* (Gerinnungsphysiol. Labor des Kantonsspitals, Zürich), p. 613

Studies on heparin metabolism in hemostatically different dogs. *Oliveira, M. M.* (Inst. Oswaldo Cruz, Rio de Janeiro, Brasil), p. 636

Experimental thrombosis in rabbits: Evidence that alkaline phosphatase enhances the thrombogenic effect of the injected ellagic acid. *Iatridis, P. G.* et al. (Univ. of North Carolina School of Med., Chapel Hill, N. C.), p. 645

Effects of ellagic acid upon the human fibrinolytic system. *Iatridis, S. G.* et al. (Athena Clinic, Athens), p. 657

Comparative studies of fibrinolytic inhibitors in vitro. *Maki, M.* et al. (New York Univ. School of Med., New York, N. Y.), p. 668

Betalipoproteins and euglobulin lysis time. *Cucuianu, M.* (Inst. for Med. Research, Roumanian Academy of Science, Cluj), p. 687

Effects of Staphylococcal enterotoxin B on the coagulation mechanism and leukocytic response in beagle dogs. A preliminary study. *Gilbert, Ch. F.* (US Army Med. Unit, Walter Reed Army Med. Center, Fort Detrick, Md.), p. 697

Some effects of purified autoprothrombin C in blood clotting. *Müller-Berghaus, G.* et al. (Wayne State Univ. School of Med., Detroit, Mich.), p. 707

A preparation of kininogen for the assay of kallikreins. *Małofiejew, M.* et al. (Dept. of General and Experimental Pathol., Medical School, Bialystok, Poland), p. 723

Studies of the coagulation and prothrombin time in the mouse embryo. *Caruso, R.* et al. (Dept. of Preventive Med., Univ. of California School of Med., San Francisco, Calif.), p. 732

Further purification of the antihaemophilic Factor (AHF). *Veder, H. A.* (Biochem. Lab., Paediatric Clinic, Univ. of Amsterdam, Binnengasthuis, Amsterdam), p. 738

The quantitation of platelet aggregation induced by four compounds: A study in relation to myocardial infarction. *O'Brien, J. R.* et al. (Portsmouth and Isle of Wight Pathol. Service, Portsmouth, England), p. 752

A comparison of platelet stickiness tests during an Atromid-S trial. *O'Brien, J. R.* et al. (Portsmouth and Isle of Wight Pathol. Service, Portsmouth, England), p. 768

Transfusion (Philadelphia) **6** (1966) No. 5

The effect of dipyridamole on the adenosine triphosphate level of stored human blood. *Gibson, J. G.* et al. (Protein Foundation Inc., 110 Francis Street, Boston, Mass.) p. 427

Etiology and pathogenesis of acquired hemolytic anemia. *Zuelzer, W. W.* et al. (Child Research Center of Michigan, Detroit, Mich.), p. 438

Immunochemical studies of the Rh system. IV. Hemagglutination assay of antigenic expression regulated by interaction between paired Rh genes. *Gibbs, Mary B.* et al. (Dept. of Immunochem., Walter Reed Army Inst. of Research, Washington, D. C.), p. 462

The effect of bilirubin on the mammalian erythrocyte. *Cheung, Wan Ho* et al. (The Long Island Jewish Hosp , New Hyde Park, N. Y.), p. 475

Heterophilic antibodies following injection of blood group substances. *Davidsohn, I.* et al. (Mount Sinai Hosp. Medical Center, Chicago, Ill.), p. 487

The effect of filters on the efficiency of platelet transfusion. *Morrison, F. S.* (Wright-Fleming Inst., St. Mary's Hosp. Medical School, London, W. 2). p. 493

Anti-Lu^b and mild hemolytic disease of the newborn: A case report. *Scheffer, Helena*

et al. (Dept. of Pathol., Montgomery Hosp., Norristown, Penn.), p. 497

Absence of anti-Tj^a-like hemolysin in pregnant aborters in Budapest. *Horváth, E.* et al. (Research Inst. of the National Blood Service, Budapest), p. 499

Transfusion (Philadelphia) **6** (1966) No. 6

Studies on blood preservation. The relative capacities of hexoses, hexitols, and ethanol to maintain red cell ATP levels during storage. *Beutler, E.* et al. (City of Hope Med. Center, Duarte, Calif.), p. 537

Relationships between metabolic state and (1) in vivo survival and (2) density distribution of previously frozen human erythrocytes. *Valeri, R. C.* et al. (Naval Blood Research Lab., Naval Hosp., Chelsea, Mass.), p. 543

Relationships between in vivo survival and (1) density distribution, (2) osmotic fragility of previously frozen, autologous, agglomerated, deglycerolized erythrocytes. *Valeri, R. C.* et al. (Naval Blood Research Lab., Naval Hosp., Chelsea, Mass.), p. 554

Experimental studies on the transfusion of blood obtained postmortem. *Grayson, G.* et al. (Kings County Hosp., New York, N. Y.), p. 565

Preparation and in vivo circulation of human platelets preserved with combined dimethylsulfoxide and dextrose. *Djerassi, I.* et al. (Univ. of Pennsylvania School of Med., Philadelphia, Penn.), p. 572

On the possible relation of sialic acid to the Rh factor. *Prager, M. D.* et al. (Wadley Research Inst. and Blood Bank, Dallas, Texas), p. 577

Three examples of anti-Lu_b and related data. *Molthan, Lyndall* et al. (Temple Univ. School of Med., Philadelphia, Penn.). p. 584

Anti-s hemolytic disease: A case report. *Lusher, Jeanne M.* et al. (The Child Research Center of Michigan, Detroit, Mich.) p. 590

Immune anti-N agglutinin in human serum. Report of apparent associated hemolytic reaction. *Yoell, J. H.* (Motion Picture Country Hosp., Woodland Hills, Calif.), p. 592

Concentration of antibodies by freezing serum. *Castro, A.* et al. (Serum Production, Specific Serums, Inc., 20 Hudson Place, Hoboken, N. J.), p. 594

Changes of pH of blood diluted with plasma and plasma substitutes in vitro. *Takaori, M.* (Univ. of Pittsburgh School of Med., Pittsburgh, Penn.), p. 597

Increased haptoglobin serum levels in a group of blood donors. *Castro, de S.* et al. (Lab. de Biol., Facultad de Ciencias, Univ. de Valladolid, Spain), p. 600

Transfusion (Paris) **9** (1966) No. 3

Le pouvoir inhibiteur de la bêta-lipoprotéine sur la streptolysine 0 chez les donneurs de sang de la région parisienne avec et sans antécédents d'ictère. *Badin, J.* et al. (Inst. Prophylactique, Paris 6e), p. 203

Le test au thymol et la transaminase glutamino-pyruvique chez les donneurs de sang de la région parisienne avec ou sans antécédents d'ictère. *Badin, J.* et al. (Inst. Prophylactique, Paris 6e), p. 215

Activité lysozymique de plasma citraté. *Saint-Blancard, J.* et al. (Centre de Transfusion-Réanimation de l'Armée «Jean Julliard», 1, rue Raoul-Batany, 92-Clamart), p. 225

a NEW journal in Excerpta Medica's monthly abstracting series

Hematology

containing 3,600 current abstracts a year

Excerpta Medica's monthly abstracting journals bring to your desk abstracts of current articles and reports of medical and bio-medical literature published throughout the world. These abstracts, prepared, translated and edited by the Foundation's international team of more than 7,000 specialists, provide information which would, due to the language barrier or the unavailability of the original publication, otherwise be inaccessible to you. Monthly author and subject indices enable you to rapidly locate abstracts. The subject index of Hematology is computer-based.

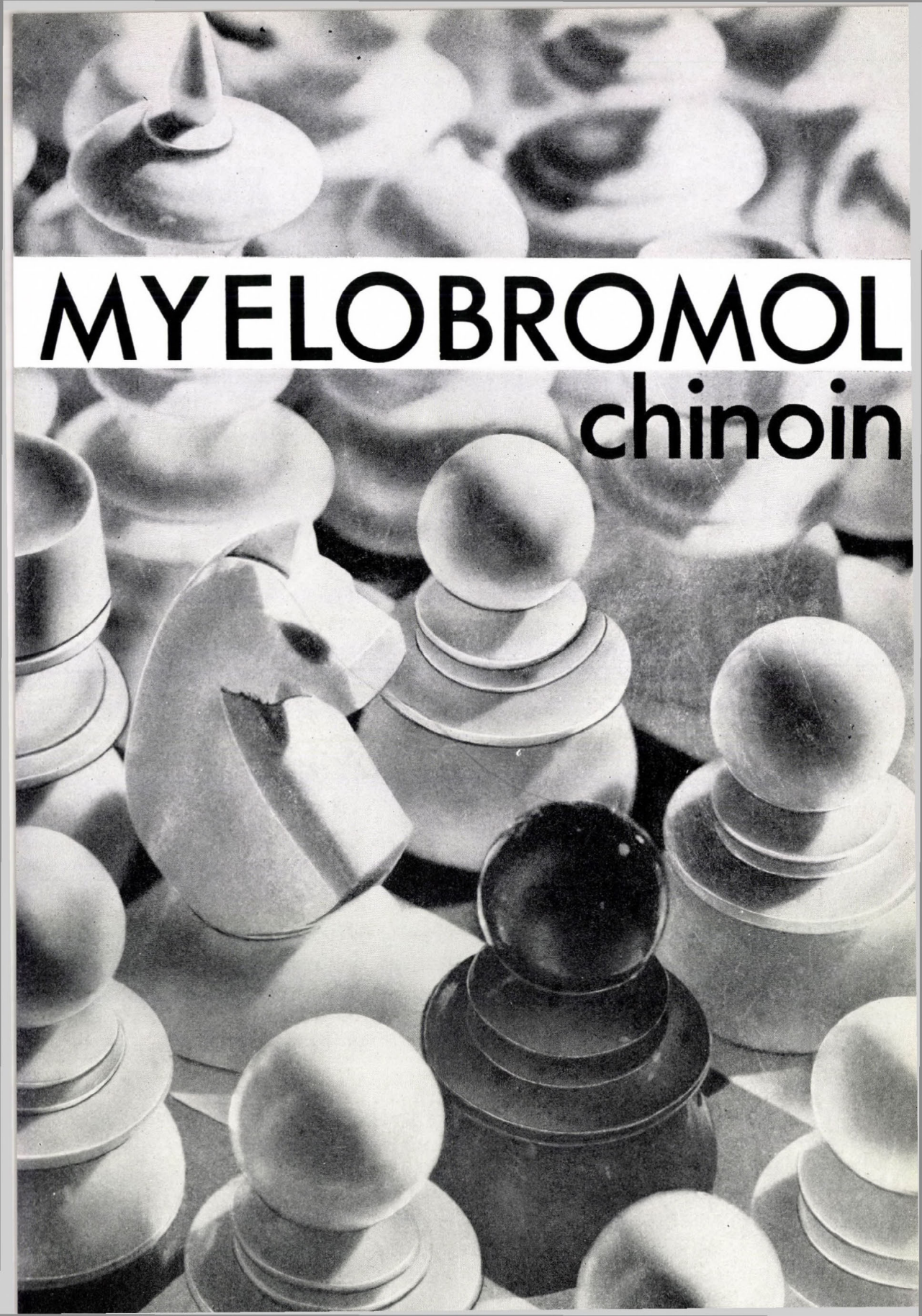
CLASSIFICATION

1. *General aspects*
2. *Erythrocytes, hemoglobin*
3. *Iron metabolism, hemosiderosis, hemochromatosis*
4. *Anemia*
5. *Polycythemia*
6. *Leukocytes*
7. *Leukemia*
8. *Thrombocytes*
9. *Coagulation, fibrinolysis*
10. *Bleeding disorders*
11. *Reticuloendothelial system*
12. *Reticuloendotheliosis*
13. *Immunohematology*
14. *Blood groups, serum groups*
15. *Transfusion*
16. *Transplantation of bloodforming tissues*
17. *Hematological aspects of general disease*
18. *Laboratory techniques*
19. *Miscellaneous*

Annual subscription rate: US $ 30. 00/£ 10. 10. -d. Sterling
For 1967 (April-December): $ 22. 50/£ 7. 17. 6d.

Excerpta medica foundation

119-123 Herengracht, Amsterdam-C, The Netherlands
New York Academy of Med. Bldg., 2 East 103 Street, New York, N. Y. 10029
Chandos House, 2 Queen Anne Street, London, W. 1.


MYELOBROMOL
chinoin

MYELOBROMOL
chinoin

1,6-Dibromo-1,6-dideoxy-D-mannitol
tablets of 250 mg

A specific antigranulopoietic agent

Cronic myelocytic leukaemia
Polycythaemia vera

Highly selective action
Oral administration
No cross-resistance with other therapies
Response of patients can be controlled by dosage
MYELOBROMOL brings about the prolongation of survival

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126

Publications of
AKADÉMIAI KIADÓ
Budapest

A. BOGSCH and A. LESZLER

Diagnostic Pneumomediastinum

In English · 212 pages · 116 figures · 17 × 24 cm · Cloth · $9.80; DM 39.20; £3 10s

The authors discuss the injection of air into the mediastinum as a contrast medium for X-ray examinations. The history and methods of the procedure are dealt with, and its indications and contraindications as well as its special applications for facilitating diagnoses are treated.

M. JULESZ and K. KOVÁCS

Therapie der endokrinen Krankheiten und ihre theoretischen Grundlagen

In German · 592 pages · 31 figures · 3 tables · 17 × 24 cm · Cloth $16.00; DM 64.– ; £5 15s

The present work provides medical practitioners with the necessary fundamentals instead of mere formules for healing. Thus the readers will be able to proceed by themselves to further studies in the field of therapy. The chapters of the work are arranged according to theoretical and practical issues.

I. WITTMANN

Peritoneoscopy I. II.

In English · Vol. I. 187 pages · 50 figures · 21 tables · Vol. II. 500 coloured photos on 171 pages · 21 × 29 cm · Cloth · Together $32.00; DM 128.– ; £11 11s

In this work the author discusses the diagnostic methods of peritoneoscopy, its history and his own extensive experience. Volume I describes the methods, instruments, indications and contraindications as well as the dangers of peritoneoscopy. Volume II is a peritoneoscopic atlas containing 500 original coloured photographs and illustrates what has been discussed in Volume I.

Publishing House of the Hungarian Academy of Sciences
Budapest V. Alkotmány utca 21

Medical Journals of

AKADÉMIAI KIADÓ

Budapest

Acta Chirurgica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor A. BABICS

Subject-matter: surgery, including the disciplines of general surgery, surgical aspects of gynaecology, urology, otorhinolaryngology, orthopaedics, ophthalmology as well as nerve and brain surgery, pulmonary, oral surgery, heart and blood-vessel surgery. Papers in English and German.

Published in four issues, making up a volume of some 400 pages yearly. Size: 17 × 24 cm.
Subscription rate per volume: $ 12.00; DM 48.—; £4 8s

Acta Medica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor I. RUSZNYÁK

Subject-matter: fundamental and applied pathophysiology and its cognate fields. Papers in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm.
Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Microbiologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor G. IVANOVICS

Papers in the field of microbiology mainly in English, but also in French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm.
Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Morphologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor I. TÖRŐ

Subject-matter: medical morphology, normal and pathological anatomy, forensic medicine and oncology. Papers in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Paediatrica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor P. GEGESI KISS

Papers in the field of pediatry in English, French, German or Russian.

Published in four issues, making up a volume of some 400 to 500 pages yearly. Size: 17 × 24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

Acta Physiologica

ACADEMIAE SCIENTIARUM HUNGARICAE

Editor K. LISSÁK

Subject-matter: physiology, including the disciplines of biochemistry, biophysics and pharmacology. Papers in English, French, German or Russian.

Published in eight issues making up two volumes of some 400 to 500 pages each yearly. Size: 17 × 24 cm. Subscription rate per volume: $12.00; DM 48.—; £4 8s

I. BERNÁT

OZAENA

EINE MANIFESTATION DER EISENMANGELKRANKHEIT

In deutscher Sprache · 152 Seiten · 52, zum Teil mehrfarbige Abbildungen und 10 Tabellen im Text · 15×21 cm · Ganzleinen $ 4,90; DM 19,60; £ 1 15s

Nach einer kritischen Übersicht der wichtigeren Ozaena-Theorien weist der Verfasser auf Grund klinischer Untersuchungen und Tierexperimente nach, daß die Ozaena nichts anderes als eine Sonderform der Eisenmangelkrankheit ist. Er befaßt sich eingehend mit den bei der Ozaena auftretenden neuroendokrinen Funktionsstörungen und untersucht den Ursprung der Ossifikationsstörungen und der histopathologischen Veränderungen. Schließlich zeigt er anhand seiner therapeutischen Versuche, daß die Ozaena auf Eisenpräparate gut reagiert und mit diesen in der Mehrzahl der Fälle erfolgreich behandelt werden kann.

AUS DEM INHALT:

I. Kurze Übersicht und Kritik der wichtigeren Ozaenatheorien
II. Material und Methoden
III. Klinik der Ozaena
IV. Ossifikationsstörungen bei der Ozaena. Wachstums- und Entwicklungsstörungen, Entwicklungsanomalien
V. Zusammenhang der Ozaena mit neuroendokrinen Funktionsstörungen
VI. Histopathologie der Ozaena
VII. Die Eigentümlichkeiten beim Vorkommen der Ozaena
VIII. Der Wirkungsmechanismus der Vakzinetherapie bei der Ozaena
IX. Erfahrungen mit der Eisentherapie bei der Ozaena

AKADÉMIAI KIADÓ
VERLAG DER UNGARISCHEN AKADEMIE DER WISSENSCHAFTEN
Budapest V., Alkotmány utca 21



DEGRANOL CHINOIN

Brand of mannomustine dihydrochloride (BCM)

1,6-bis-(β-Chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride

Chronic lymphoid leukaemia
Hodgkin's disease
Lymphosarcoma and other haemoblastoses
In tumor surgery as chemoprophylactic
For direct injection into the cavities with tumorous effusion
DEGRANOL is remarkably safe in adequate dosage

powder ampoules of 50 mg · coated pills of 50 mg

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126

SERIES HÄMATOLOGIE UND BLUTTRANSFUSIONSWESEN

EDITED BY
PROF. DR. F. JUNG · PROF. DR. O. PROKOP ·
PROF. DR. S. M. RAPOPORT · PROF. DR. H. STOBBE

VOLUME 1

DER SIDEROCYT

Physiology and Pathophysiology of Erythropoiesis

By Prof. Dr. Gerhard Brüschke

1962. VIII, 72 pages — 32 figures, incl. 3 unicolour and 4 multicolour illustrations on 4 charts — 12 tables — gr. 8° — half-cloth — MDN 27.50

VOLUME 2

DIE LYMPHOIDZELLEN DES BLUTES

Clinico-haematological Studies on the Structure, Function, and Diagnostic Importance of the Lymphoid Cells of the Blood

By Dr. Dietrich Jorke

1963. IX, 174 pages — 151 figures, incl. 20 coloured — 8 tables — gr. 8° — MDN 50.—

VOLUME 3

DIE PHYTHÄMAGGLUTININE

By Dr. Josef Tobiska

Including Catalogue of all Plants Studied for Phyto-haemagglutinine Content since 1888, Compiled with Botanic Co-operation of Dr. M. Felklová

Scientifically revised by Dr. Wolfgang Reimann

1964. XI, 302 pages — 18 figures — 69 tables — gr. 8° — MDN 58.—

VOLUME 4

AKTUELLE LEUKOZYTENPROBLEME

Selected Contributions from a Symposium

Compiled by Prof. Dr. Wolfgang Plenert

1966. X, 225 pages — 146 figures — 37 tables — gr. 8° — MDN 53.—

IN PREPARATION:

PLASMAZELLE UND PLASMACYTOM

Edited by Prof. Horst Stobbe

The Series will be continued!

AKADEMIE-VERLAG • BERLIN
108 BERLIN, LEIPZIGER STR. 3—4. GDR

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to 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.
Central Research Institute of the
National Blood Service
Daróczi út 24
Budapest XI, 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) name(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. Coloured 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:

ALBANIA

Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA

A. Keesing
Box 4886, GPO
Sydney

AUSTRIA

Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM

Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA

Raznoiznos
1, Tzar Assen
Sofia

CANADA

Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA

Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Smeckách 30
Praha 2
Poštova Novinova Služba
Dovoz Tisku
Vinohradska 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštova Novinova Služba
Dovoz Tlace
Leningradska 14
Bratislava

DENMARK

Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough, Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP.
GPO Post Box 1374
Bombay I

ITALY

Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central

Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA

Chulpanmul
Phenjan

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND

RUCH
ul. Wronia 23
Warszawa

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
Bucuresti

SOVIET UNION

Mezhdunarodnaja Kniga
Moscow G—200

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA

Stechert Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM

Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA

Forum
Vojvode Mišiča broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

27. VII. 1967 Index: 26341

315.874

VOLUME 1 · NUMBER 3 · 1967

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)
J. ARNAUD (Tours)
G. ASTALDI (Milano)
I. BARTA (Pécs)
G. BAST (Rostock)
H. BEGEMANN (Munich)
E. BENEDEK (Budapest)
J. BERNARD (Paris)
E. BEUTLER (Duarte, California)
H. BRAUNSTEINER (Innsbruck)
W DAMESHEK (New York)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Uppsala)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
I. GONZÁLEZ-GUZMÁN (Mexico City)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
G. C. de GRUCHY (Melbourne)
A. HÄSSIG (Bern)
L. HEILMEYER (Freiburg/Br.)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
I. A. KASSIRSKY (Moscow)
E. KELEMEN (Budapest)
A. E. KISELEV (Moscow)
E. KOWALSKI (Warsaw) †
P. J. LAH (Belgrade)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Marburg/Lahn)
A. E. MOURANT (London)
C. T. NICOLAU (Bucharest)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Szeged)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM (Liège)
G. RUHENSTROTH-BAUER (Munich)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Modena)
M. C. VERLOOP (Utrecht)
A. VIDEBAEK (Copenhagen)
S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

HAEMATOLOGIA

is a new 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 some 500 pages.

Subscription price: $ 12 per volume (per year). Orders may be placed with

KULTURA

Trading Company for Books and Newspapers
Budapest 62. P.O.B. 149

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

Contents

In memoriam Edward Kowalski 255

Deutsch, E., Hagenauer, E., Irsigler, K., Köbele, B., Lechner, K., Schmer, G.: Untersuchungen über das exogene Gerinnungssystem 257

Дульцин, М. С., Климова, Н. Ф.: Лечение больных идиопатическим миелофиброзом 275

Szász, I., Árky, I., Gárdos, G.: Structure and function of erythrocytes, III. Effect of membrane modifications on the mechanism maintaining biconcave shape 287

Pawelski, S., Konopka, L., Kotelba-Witkowska, B., Rechowicz, K., Błaszczyk, J.: Observations on the survival of ^{51}Cr-tagged platelets in healthy subjects and in patients with thrombocytopenia 297

Guth, P., Budvári, R.: Inheritable beta-lipoprotein characters (Ag- and Lp-system) 307

Pastinczky, I., Kovács, M.: Study of blood coagulation in familial benign chronic pemphigus of Gougerot-Hailey-Hailey 313

Strausz, I., Barcsák, J., Kékes, E., Szebeni, Á.: Prednisolone-induced acute changes in circulating neutrophil granulocytes, I. In cases of normal granulocyte reserves 319

Strausz, I., Barcsák, J., Kékes, E., Szebeni, Á.: Prednisolone-induced acute changes in circulating neutrophil granulocytes, II. In cases of deficient granulocyte reserves 327

Open Forum 333

Abstracts 337

From the international literature on haematology 347

VOLUME 1 · NUMBER 3

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) · J. ARNAUD (Tours) · G. ASTALDI (Milano) · I. BARTA (Pécs) · G. BAST (Rostock) · H. BEGEMANN (Munich) · E. BENEDEK (Budapest) · J. BERNARD (Paris) · E. BEUTLER (Duarte, California) · H. BRAUNSTEINER (Innsbruck) · W. DAMESHEK (New York, N. Y.) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle Washington) · L. GARBY (Uppsala) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest) · I. GONZÁLEZ-GUZMÁN (Mexico City) · F. GRÁF (Budapest) · A. GRAFFI (Berlin) · G. C. de GRUCHY (Melbourne) · A. HÄSSIG (Bern) · L. HEILMEYER (Freiburg/Br.) · L. P. HOLLÄNDER (Basel) · J. HOŘEJŠÍ (Prague) · M. HRUBIŠKO (Bratislava) · I. A. KASSIRSKY (Moscow) · E. KELEMEN (Budapest) · A. E. KISELEV (Moscow) · E. KOWALSKI (Warsaw) † · P. J. LAH (Belgrade) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Marburg/Lahn) · A. E. MOURANT (London) · C. T. NICOLAU (Bucharest) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Szeged) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM (Liège) · G. RUHENSTROTH-BAUER (Munich) · V. SERAFIMOV-DIMITROV (Sofia) · I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIĆ (Belgrade) · E. STORTI (Modena) · M. C. VERLOOP (Utrecht) · A. VIDEBAEK (Copenhagen) · S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ

PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES

BUDAPEST 1967

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Bogdán István

A kézirat nyomdába érkezett: 1967. VII. 11. Terjedelme: 9,75 (A/5) ív, 31 db ábra

67.64086 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

In memoriam Edward Kowalski
1914 — 1967

Professor Edward Kowalski, Head of the Department of Radiobiology and Health Protection, Institute of Nuclear Research and a member of our Editorial Board, died suddenly in Warsaw on April 16 at the age of 53.

He was born in Sepólno, a small Pomeranian town in Western Poland. Literature, arts, music, philosophy and languages were his favourite pastimes in high school. He wanted to study German philosophy, but financial difficulties did not permit a free choice of the subject of study. A private scholarship enabled him to study pharmacy and he graduated before World War II at the Pharmaceutical Faculty of Poznań University. At the same time he studied also chemistry.

During the war he was educated at the underground Medical University School in Warsaw, where he received his M.D. degree in 1946. During Nazi German Occupation he was an active member of the Polish Resistance movement and he worked as a physician during the Warsaw Uprising in 1944. By his heroism he saved many people, sick and wounded.

From 1946 to 1951 he worked as a research fellow at the Department of Physiological Chemistry of the Medical University School of Warsaw. He received his Ph.D. for a dissertation on alkaline phosphatase. From 1951 to 1960 he headed the Department of Internal Diseases and Laboratory of Clinical Biochemistry, Institute of Haematology, Warsaw. In 1954 he was appointed Research Director and in 1959 Professor of Medicine. From 1955 till his death he was head of the Department of Radiobiology and Health Protection, Institute of Nuclear Research in Warsaw. He was a member of the International Society of Haematology, the New York Academy of Sciences, the Czechoslovak Medical Society of Purkyne, the International Committee for the Nomenclature of Blood Clotting Factors, and the Editorial Boards of the Blood (USA), Blut (FRG), the Haematologia (Hungary), the Atomic Energy Review (IAEA), Medicinskaya Radiologiya (USSR), and several Polish periodicals. He was also an expert of the International Atomic Energy Agency and contributed to solving the problems of waste disposal and radiation hazards.

In the early 'fifties he worked on the activation and inhibition of fibrinolysis and he described the occurrence of plasminogen-like proenzyme in human connective tissues. The most important contribution made by Kowalski and his

associates was the discovery of the anticoagulant properties of fibrinogen proteolysis products (in 1956). Two substances – antithrombin VI (a competitive fibrinogen–fibrin conversion inhibitor) and an inhibitor of thromboplastin production have been isolated from fibrinogen lysate and partially purified (1957–1958). Further work carried out in the early 'sixties demonstrated two types of fibrinogen degradation products: a) "early" or "peak" products, inhibiting mainly thrombin action on fibrinogen, b) "late" products (or "plateau"), inhibiting fibrin monomeric polymerization. Subsequently it was found that fibrinogen breakdown products form complexes with fibrin monomers and that they inhibit clotting. It was further shown that fibrinogen breakdown products inhibit platelet functions in haemostasis. According to Kowalski an inhibition of platelet aggregation in vivo and in vitro by fibrinogen degradation products is a main cause of bleedings in hyperfibrinolytic states. His recent work on fibrinogen degradation products has yielded also evidence concerning fibrinogen structure.

A practical achievement of Kowalski in the field of coagulation and haemostasis was the foundation (in 1956) of an Outpatient Department for Long-term Anticoagulant Therapy and a Dispensary for Haemophiliacs at the Institute of Haematology in Warsaw. In the late 'fifties the first papers describing the results of extensive examination of large haemophiliac population in Poland were published. The other papers published in the same period were on the pathophysiology of fibrinolysis and treatment of thrombotic disease.

Recently, Kowalski and his associates have studied the role of lysosomes in the mechanism of platelet aggregation. He postulated that the activation of platelet lysosomes by thrombin may initiate the release reaction.

Professor Kowalski's achievements in other fields of haematology and radiobiology deserve attention. His studies on the biosynthesis of erythropoietin in kidney and its interaction with plasma proteins, on haem and porphyrine metabolism with particular reference to the influence of ionizing radiation on this process, on the use of radiochromatography in biological research and on bone marrow transplantation into irradiated animals are the most outstanding. Further laboratory research directed by him concerned the mechanism of bone calcification, some aspects of the biochemistry of connective tissues, the influence of ionizing radiation on leukaemic cells in culture and the role of radium D as a contaminant of biosphere and human body.

He published about 130 papers together with his associates.

Professor Kowalski's early death deprived us of a very good friend, an animated scientist and a man of merit.

Untersuchungen über das exogene Gerinnungssystem* **

E. Deutsch, E. Högenauer, K. Irsigler, B. Köbele,
K. Lechner, G. Schmer

(Eingegangen am 25. Februar 1967)

Im exogenen System beteiligen sich an der Aktivierung von Prothrombin die Gewebethrombokinase, die Faktoren VII, X und V sowie Calcium. Ein Vergleich mit dem endogenen System zeigt, daß auch dort die Faktoren V und X wirksam sind, so daß offenbar beide Systeme in eine gemeinsame Endstrecke einmünden. Diese beginnt nach erfolgter Aktivierung von Faktor X. Wir haben uns in unseren Untersuchungen auf jene Faktoren beschränkt, die vor dieser gemeinsamen Endstrecke wirksam werden, und haben uns die Aufgabe gestellt, die in Frage kommenden Faktoren so weit als möglich zu reinigen und festzustellen, ob einer von ihnen Fermenteigenschaften besitzt.

Es wurde zunächst an Aminosäure-β-naphthylamiden geprüft, ob Gewebe- und Plasmathrombokinase, sowie Russell-Vipern-Gift imstande wären, diese zu spalten. Hierbei zeigte sich zu unserer Überraschung, daß alle drei »thromboplastischen« Substanzen synthetische Substrate spalteten. Der naheliegende Verdacht, die Spaltung könnte durch Verunreinigung der beiden erstgenannten Thrombokinasepräparate durch Thrombin bedingt sein, konnte leicht entkräftet werden, da sich die beiden Thrombokinasen in ihrer relativen Aktivität verschiedenen Substraten gegenüber vom Thrombin grundlegend unterschieden: Während Thrombin Arginin-β-naphthylamid und Tosylarginin-β-naphthylamid viel stärker spaltet als Lysin-β-naphthylamid, spalten die beiden Thrombokinasen das Lysin-β-naphthylamid viel besser als TA-β-NA. Russell-Vipern-Gift zeigte wieder ein anderes Spektrum: Leucin-β-naphthylamid wurde etwas besser gespalten als Lysin-β-naphthylamid und dieses wieder viel besser als TA-β-NA. Hiermit war eine durch Thrombin vorgetäuschte Wirkung auszuschließen [4, 15].

Wir gingen nun daran, menschliche Hirnthrombokinase zu reinigen und zu prüfen, ob die Peptidase-Aktivität und die Gerinnungsaktivität tatsächlich miteinander etwas zu tun haben. Es gelang relativ leicht, jeweils eine Aktivität gezielt zu zerstören, wobei die andere weitgehend erhalten blieb: Erhitzen auf

* Vortrag vor der Ungarischen Akademie der Wissenschaften am 9. II. 1967 in Budapest.

** Die Untersuchungen wurden mit Unterstützung des National Institute of Health, Bethesda, Mld, USA, Grant Nr. HE-06425 durchgeführt.

mehr als 60 Grad oder Erhöhen des pH über 11 oder Senken desselben unter 4,5 zerstörte die Peptidase-Aktivität, während die Gerinnungsaktivität erhalten blieb. Extraktion des Präparates mit apolaren Lösungsmitteln zerstörte wieder die Gerinnungsaktivität, während die Peptidaseaktivität erhalten blieb. In schonender Weise konnte die Thrombokinaseaktivität auf der Ultrazentrifuge sedimentiert und von der Peptidaseaktivität frei gewaschen werden. Die Peptidase konnte über Adsorption auf Calciumphosphatgel und Elution mit Phosphatpuffer weiter gereinigt werden. Bei einer Testung an einem breiten Spektrum an Aminosäurenaphthylamiden konnte gezeigt werden, daß unser Ferment eine Exopeptidase ist, da sie die für Endopeptidasen typischen Substrate nicht zu spalten imstande war, und daß es ein Wirkungsoptimum gegen Lysinbindungen aufweist. Da unsere Untersuchungen zeigten, daß dieses Ferment für die Blutgerinnung offenbar keine Bedeutung besitzt, wurde von einer weiteren Reinigung und Charakterisierung abgesehen. Über die physiologische Bedeutung dieses Fermentes ist uns nichts näheres bekannt, es ist aber möglich, daß es mit der von anderen Autoren in Hirngewebe gefundenen Leuzinaminopeptidase identisch sein könnte [2, 5].

Die Träger der Aktivität der Gewebethrombokinase sind große Teilchen. Infolgedessen ließ sich die Thrombokinase nicht mit den üblichen Methoden der Fraktionierung und Säulenchromatographie reinigen und wir mußten zu physikalischen Methoden Zuflucht nehmen. So wurden die Extrakte, die aus acetongetrocknetem menschlichem Hirn hergestellt wurden, zunächst durch langsames Zentrifugieren von groben Partikeln gesäubert, dann wurde auf der Ultrazentrifuge bei 100 000 g zentrifugiert, durch ein besonderes Resuspensionsverfahren nur die kleinen Partikel resuspendiert und diese weiter mit der Ultrazentrifuge gewaschen. Dieses Material ist praktisch frei von Exopeptidase-Aktivität [3, 13].

Da die Annahme gerechtfertigt erschien, daß es sich bei der Gewebethrombokinase um ein Lipoid oder Lipoprotein handeln könnte, wurde der Einfluß verschiedener Lösungsmittel untersucht, indem die gereinigte Thrombokinase zunächst in den Lösungsmitteln suspendiert oder gelöst wurde, dann das Lösungsmittel schonend entfernt und der getrocknete Rückstand wieder in wässeriger Suspension auf seine Gerinnungsaktivität getestet wurde. Es zeigte sich dabei, daß sich die Aktivität durch Lösen in Pyridin und Äther nicht änderte, während die Behandlung mit Methanol, Äthanol, Propanol, Chloroform und verschiedenen chloroformhaltigen Mischungen die Aktivität weitgehend verminderte [3, 13].

Bei Untersuchungen im Mikroskop hatte man den Eindruck, daß in Pyridin eine völlige Lösung erfolgt, während in Äther die Partikel weitgehend erhalten blieben. Es wurde daher für die weiteren Arbeiten Pyridin gewählt. Wurde dieses Material auf Dünnschichtplatten aufgetragen und mit Methanol : Chloroform : Wasser entwickelt, so blieb auf der Auftragungsstelle immer ein in Lipidlösungsmitteln unlösliches Material zurück, welches mit Amidoschwarz angefärbt wurde [13].

Die Lipidfraktionen konnten durch Chromatographie in verschiedenen Lösungsmittelgemischen, durch zweidimensionale Chromatographie und durch Vergleich mit Testsubstanzen als Cholesterin, Cerebroside, Phosphatidyläthanolamin, Lezithin, Sphingomyelin, Phosphatidylinositol und Phosphatidylserin identifiziert werden. Der in den Lipidlösungsmitteln unlösliche Anteil erwies sich auch in höherer Konzentration in Pyridin als unlöslich und konnte in größerer Menge aus einer entsprechenden Menge vorgereinigter Gewebethrombokinase angereichert werden. Nach Hydrolyse ergab die zweidimensionale Chromatographie 15 Aminosäuren, nämlich Arginin, Lysin, Asparaginsäure, Glutaminsäure,

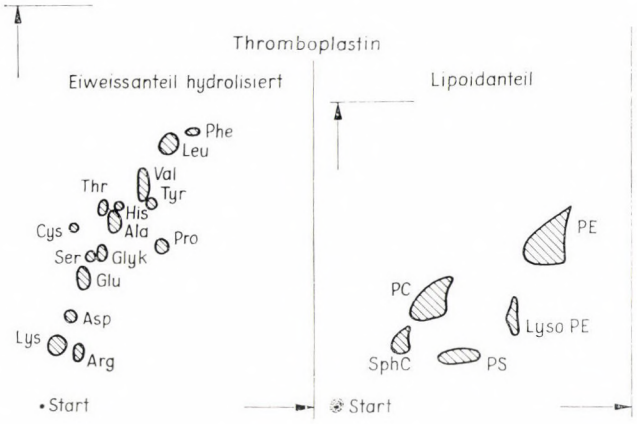


Abb. 1. Zweidimensionale Chromatogramme des Eiweißanteiles (linke Hälfte) und des Lipidanteiles (rechte Hälfte) einer menschlichen Hirnthrombokinase

Cystin, Serin, Glykokoll, Prolin, Alanin, Threonin, Histidin, Tyrosin, Valin, Leucin und Phenylalanin. Es wurde angenommen, daß es sich hierbei um ein Eiweiß oder ein Polypeptid handeln könnte [13] (Abb. 1).

Die beiden Fraktionen verhielten sich in ihrer P : N Relation grundverschieden. Während diese Relation in der vorgereinigten Gewebethrombokinase 1 : 2,24 betrug, war sie im Lipidanteil 1 : 1,42 und im »Proteinanteil« 1 : 41,2 [13].

Die Auftrennung hatte die Charakteristika der Gerinnungsaktivität des Materials grundlegend verändert. Der Lipidanteil zeigte eine viel geringere Aktivität als das Ausgangsmaterial und eine gewisse gerinnungshemmende Wirkung mit zunehmender Konzentration. Er war diesbezüglich ähnlich einem lipiden Aktivator, der durch Ätherextraktion aus dem Hirnpulver gewonnen werden kann, bei dem allerdings die Hemmwirkung viel deutlicher ist. Der »Proteinanteil« erwies sich als vollkommen inaktiv. Wurden nun Lipidanteil und »Proteinanteil« miteinander rekombiniert, so konnten Präparate gewonnen werden, die sogar aktiver als das Ausgangspräparat waren. Allerdings ist für das Rekom-

binat ein größerer Stickstoffgehalt erforderlich als er im Originalpräparat enthalten ist [13].

Es ist schon seit langem bekannt, daß Gewebethrombokinasen die kürzesten Gerinnungszeiten bei Testung am artgleichen Plasma zeigen. Die Unterschiede werden um so größer, je geringer die Verwandtschaft der einzelnen Spezies ist. So ist z. B. die Gerinnungszeit mit einer optimalen Konzentration von Rinderhirnthrombokinase mit menschlichem Plasma wesentlich länger als mit Rinderplasma. Die Charakteristika der Verdünnungskurve sind aber dieselben. Verwendet man jedoch Hühnerthrombokinase mit Menschenplasma, so ist nicht nur die Gerinnungszeit bei optimaler Konzentration noch wesentlich länger, sondern der Kurvenverlauf ist wesentlich verändert und gleicht dem eines lipoiden Aktivators (Abb. 2). Die Speziesspezifität kommt besonders deutlich auch im

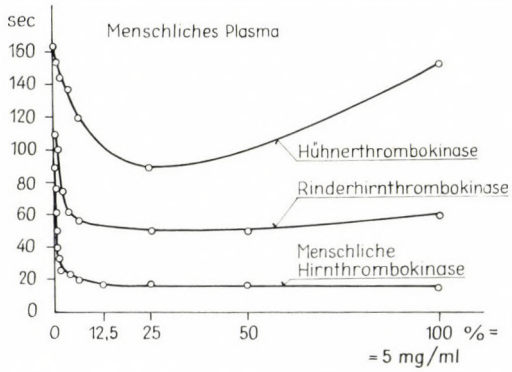


Abb. 2. Speziesspezifität der Thrombokinase. Substratplasma vom Menschen, Thrombokinase von Huhn, Rind und Mensch. Abszisse: Konzentration der Thrombokinasesuspension. Ordinate: Gerinnungszeit in Sekunden

Zweistufenprothrombintest zum Ausdruck. Ein nach Seegers gereinigtes Rinderprothrombin mit Rinderthrombokinase viel besser aktiviert als mit Hühnerthrombokinase [14, 18].

Gewebethrombokinase verschiedener Tierspezies wurde auf der Ultrazentrifuge gereinigt, in Pyridin auf die Dünnschichtplatte aufgetragen und mit dem üblichen Laufmittel zur Chromatographie der Lipoide entwickelt. Es fand sich kein charakteristischer Unterschied in den Lipidfraktionen, was natürlich nicht bedeutet, daß diese wirklich chemisch identisch sind, sondern nur, daß in ihnen dieselben Lipoidklassen enthalten sind.

Die mit Pyridin extrahierten Lipoide gaben mit allen Plasmen Gerinnungszeiten, die wesentlich über 60″ lagen, wobei noch eine geringe Überlegenheit bei Testung am arteigenen Plasma zum Ausdruck kam. Der Lipidanteil der menschlichen Thrombokinase zeigte am Rinderplasma und jener der Rinderthromboki-

nase am menschlichen Plasma die kürzeste Gerinnungszeit erst in entsprechender Verdünnung, so daß die Kurven Ähnlichkeit mit jenen lipoider Aktivatoren haben. Hecht [10] hat mit Ätherextrakten ganz charakteristische Kurven für lipide Aktivatoren erhalten. Diese Extrakte hatten gar keine Speziesspezifität mehr aufzuweisen.

Der »Eiweißanteil« erwies sich im Einstufen- und im Zweistufentestsystem als völlig inaktiv.

Nun wurden Eiweiß- und Lipidanteil verschiedener Spezies in Pyridin kombiniert, das Pyridin verdampft und eine wäßrige Suspension des Rückstandes hergestellt. Bei Testung an verschiedenen Plasmen zeigte sich eindeutig, daß die

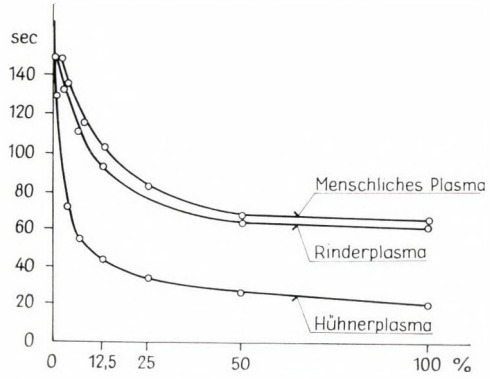


Abb. 3. Rekalzifikationszeit eines thrombozytenfreien Plasmas von Rind, Mensch oder Huhn nach Zusatz eines Thrombokinasere-kombinationspräparates bestehend aus Rinderlipid und Hühnereiweiß. Abszisse: Verdünnung des Kombinates in %. Ordinate: Gerinnungszeit in Sekunden

kürzeste Gerinnungszeit jeweils dann erhalten wurde, wenn der Proteinanteil der Thrombokinase und das Testplasma derselben Spezies angehörten (Abb. 3). Auch im Zweistufentest waren entsprechende Ergebnisse zu erzielen. Es kann aus diesen Versuchen geschlossen werden, daß die Speziesspezifität der Thrombokinasen nicht Ausdruck von Unterschieden im Lipidanteil ist, sondern durch den »Eiweißanteil« bestimmt wird [14, 18].

In der letzten Zeit haben Hecht und van Lit [11] energisch gegen unsere Ansicht, daß es sich bei dem pyridinunlöslichen, mit Amidoschwarz färbbaren Anteil um ein Eiweiß oder Polypeptid handeln könnte, Stellung genommen. Sie konnten zwar dieselben 15 Aminosäuren in diesem Material nachweisen, nehmen aber an, daß es sich entweder um Verunreinigungen handle, die aus dem Rohpräparat in das Reinpräparat mitgeschleppt werden, oder um Aminosäuren, die in Phospholipide eingebaut sind. Es ist hier nicht der Ort, in Einzelheiten

einzugehen, um so mehr, als über die biologische Aktivität der Hechtschen Fraktionen noch nichts bekannt ist. Uns scheint jedoch gerade das Experiment mit der Speziesspezifität sehr beweisend dafür zu sein, daß es sich bei dem pyridinunlöslichen Anteil nicht um ein einfaches Aminosäuregemisch handelt, sondern doch um ein für die Thrombokinasewirkung wesentliches Material.

Als nächstes wurden die plasmatischen Reaktionspartner der Thrombokinase untersucht. Drei von diesen, nämlich Prothrombin, Faktor VII und Faktor X verhalten sich bei Anwendung der einfacheren Adsorptions- und Fraktionierungsmethoden außerordentlich ähnlich. Es war daher zunächst die Frage zu klären, ob es sich hier um unabhängig voneinander im Plasma existierende chemische Einzelindividuen handelt, oder ob diese etwa gemäß der Konzeption von Seegers erst während des Gerinnungsablaufes aus dem Prothrombin entstehen.

Es wurde daher zunächst versucht, die genannten Faktoren voneinander zu trennen. Für diese Untersuchungen wurde wegen der großen erforderlichen Plasmamengen fast ausschließlich Rinderplasma verwendet, aber es wird auch über Einzelversuche an menschlichem Plasma berichtet. Rinderprothrombin wurde genau nach der von Seegers beschriebenen Methode mit einer spezifischen Aktivität von 1200 bis 2500 E/mg Protein hergestellt. Dieses Präparat verhielt sich bezüglich Aktivierung in konzentrierten Salzlösungen und mit biologischen Aktivatoren genau so, wie Seegers dies beschrieben hat. Außerdem war es möglich, in diesem Präparat mit den üblichen Einstufenmethoden zur Bestimmung der Gerinnungsfaktoren des exogenen Systems die Aktivität von Faktor VII und Faktor X nachzuweisen, eine Beobachtung, die Alexander [1] und Streuli [23] bereits bei analogen Präparationen gemacht hatten. Wir waren uns natürlich darüber im Klaren, daß aus diesen Aktivierungsversuchen kein Rückschluß darauf möglich ist, ob die Faktoren VII und X erst während der Gerinnung aus dem Prothrombin entstehen, oder bereits vorher in dem Präparat enthalten waren.

Es wurde daher versucht, die in Frage stehenden Aktivitäten voneinander zu trennen. Rinderprothrombin wurde auf eine DEAE-Zellulose-Säule aufgebracht. Es konnten zwei Eiweißfraktionen eluiert werden, die erste mit 0,175 m Phosphatpuffer pH 7,45, die andere nach Steigerung der Ionenstärke durch Zusatz von 0,2 m NaCl. Bei der ersten Fraktion handelte es sich um ein Prothrombinpräparat, das in Neutralsalzlösungen nicht mehr zu Thrombin aktiviert werden konnte. Es entstand auch nur wenig Thrombin mit Gewebethrombokinase, Faktor V und Calcium, während nach Zusatz von Faktor X viel Thrombin gebildet wurde. Die zweite Fraktion verhielt sich in Einstufentesten wie Faktor X und enthielt auch die ursprünglich nachweisbare Faktor-VII-Aktivität. Wurde die 2. Fraktion der ersteluierten zugesetzt, so verhielt sich die Mischung wie das Prothrombinpräparat vor der Chromatographie [7, 8] (Abb. 4).

Prothrombinpräparate, die in Citrat nicht aktivierbar waren, wurden vor uns bereits von Alexander [1], Soulier [21] und Streuli [23] hergestellt. Mit diesem Experiment glauben wir aber, gezeigt zu haben, daß es möglich ist, Pro-

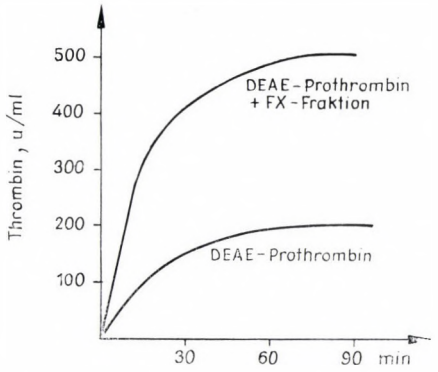


Abb. 4. Aktivierung von DEAE chromatographiertem Prothrombin mit gereinigter Rinderhirnthrombokinase, Faktor V (verdünntes bariumsulfatadsorbiertes Rinderserum) und Calcium mit und ohne Faktor X (zweite Fraktion). Abszisse: Inkubationszeit in Minuten. Ordinate: Einheiten Thrombin per Minute [16]

thrombin einerseits und die Faktoren VII und X andererseits voneinander zu trennen.

Die nächste Frage war, ob sich auch diese beiden Faktoren voneinander trennen lassen. Dies war ebenfalls auf DEAE-Zellulose möglich, jedoch unter Anwendung eines kontinuierlichen Salz-Gradienten, wobei allerdings eine breite Überlappung der Aktivitäten auftrat. Wird jedoch dieser Gradient in zwei kon-

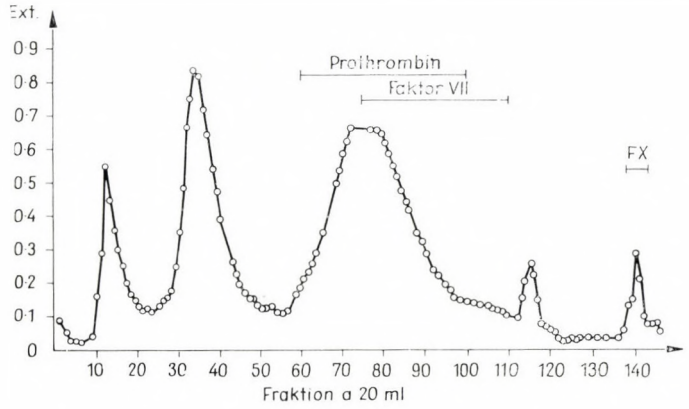


Abb. 5. Trennung von Faktor X von Faktor II und VII mit einer DEAE-Zellulosesäule. Abszisse: Nummer der Fraktion. Ordinate: Extinktion bei 280 mμ

tinuierliche Gradienten aufgelöst, so kann man Faktor VII völlig frei von Faktor X erhalten [5]. Da zu diesen Versuchen Bariumsulfateluat als Ausgangsmaterial verwendet wurde, enthielt das Versuchssystem auch Prothrombin, welches gemeinsam mit Faktor VII eluiert wurde [6]. Dieser Versuch zeigt, daß auch die Faktoren VII und X voneinander getrennt werden können (Abb. 5).

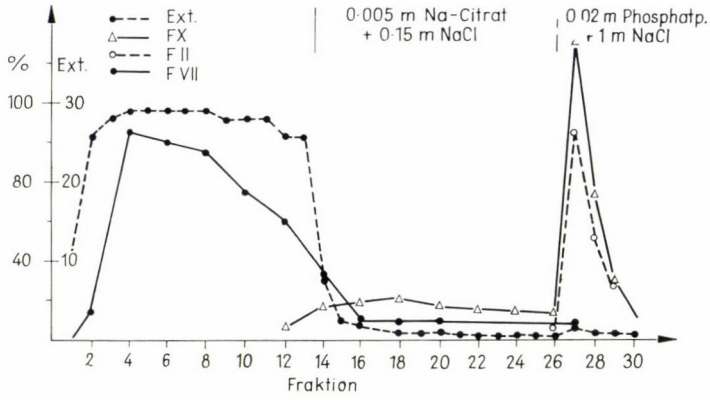


Abb. 6. Trennung von Faktor VII von Prothrombin und Faktor X auf einer DEAE-Zellulosesäule. Menschliches Plasma als Ausgangsmaterial. Abszisse: Nummer der Fraktion. Ordinate: %-Aktivität bzw. Extinktion bei 280 mμ [17]

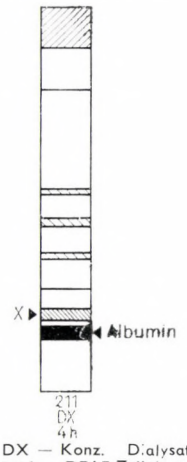
Als nächster Schritt folgte nun die Trennung von Faktor VII und Prothrombin durch Filtration auf Sephadex G 200 [12].

Die Reihenfolge der Trennung der genannten Gerinnungsfaktoren ist offenbar bei verschiedenen Spezies unterschiedlich. Verwendet man menschliches Plasma, für das eine Voradsorption gar nicht erforderlich ist, so wird von der DEAE-Zellulosesäule zunächst Faktor VII eluiert, während Prothrombin und Faktor X erst bei höherer Ionenstärke folgen [17] (Abb. 6).

Aus diesen Untersuchungen kann unseres Erachtens mit großer Wahrscheinlichkeit geschlossen werden, daß die Faktoren II, VII und X auch bei Verwendung von Plasma als Ausgangsmaterial voneinander getrennt werden können, was nicht möglich sein sollte, wenn sie erst während der Gerinnung aus Prothrombin entstehen. Dieser Beweis ist aber hiermit noch nicht erbracht, da nicht mit Sicherheit ausgeschlossen werden kann, daß die Faktoren während der Präparation aus Prothrombin entstehen, da ein partieller Gerinnungsvorgang trotz aller Vorsichtsmaßnahmen nicht verhindert werden kann. Man findet nämlich nach der Elution aus dem Bariumsulfat eine Aktivitätssteigerung von Faktor VII und eine geringe Menge Thrombin, die man auch von der Säule wiedergewinnen kann. Recht beweiskräftig ist jedoch das geschilderte Experiment mit menschlichem Plasma, bei dem Faktor VII von den Faktoren II und X getrennt werden konnte, und in keiner Phase der Präparation Thrombin nachweisbar

war. Es muß daher angenommen werden, daß zumindest Faktor VII als chemisch von Prothrombin unabhängiges Individuum im Plasma enthalten ist.

Die Faktoren VII und X wurden weiter gereinigt und charakterisiert. Faktor X wurde zunächst nach der von Esnouf und Williams [9] beschriebenen Methode hergestellt. Kurz dargestellt bestand die Methode darin, die Faktoren an $BaSO_4$ zu adsorbieren, mit Citrat zu eluieren und an DEAE-Zellulose mit stufenweiser Elution zu chromatographieren. Diese Methode wurde aber später



DX — Konz. Dialysat nach DEAE-Zellulosechromatographie Disk-Elektrophorese. Glycin-Tris-Puffer pH 7,9, Spannung 300 V

Abb. 7. Disk-Elektrophorese von gereinigtem Faktor X

modifiziert, indem das $BaSO_4$ mit 0,2 m Phosphatpuffer gewaschen wurde, wodurch mehr inertes Eiweiß, vor allem Albumin, entfernt wird. Die Elution von der Säule erfolgte mit zwei kontinuierlichen Gradienten, wodurch ebenfalls inertes Eiweiß abgetrennt werden konnte. Als weitere Reinigungsstufe wurde eine Säule mit Hydroxylapatit verwendet [16].

Von diesem Präparat wurden die C- und die N-terminalen Endgruppen mit der Mikromethode von Gray und Hartley mit Fluoreszenzmarkierung der Endgruppen, Hydrolyse bzw. Abspaltung der C-terminalen Endgruppen mit Carboxypeptidase A und Auftrennung mit Hochspannungselektrophorese oder Dünnschichtchromatographie bestimmt [6].

An beiden Seiten wurden Glycin und Serin als Endgruppen gefunden. Auf Grund des Verhaltens bei der Gelfiltration kann angenommen werden, daß die aktive Fraktion ein dem Prothrombin ähnliches Molekulargewicht, also etwa 70 000 hat. Dies entspricht annähernd den Angaben von Esnouf und Williams, die 82 000 fanden. Neueste Untersuchungen mit der Disk-Elektrophorese haben gezeigt, daß auch diese Präparate noch nicht völlig rein sind, sondern noch immer

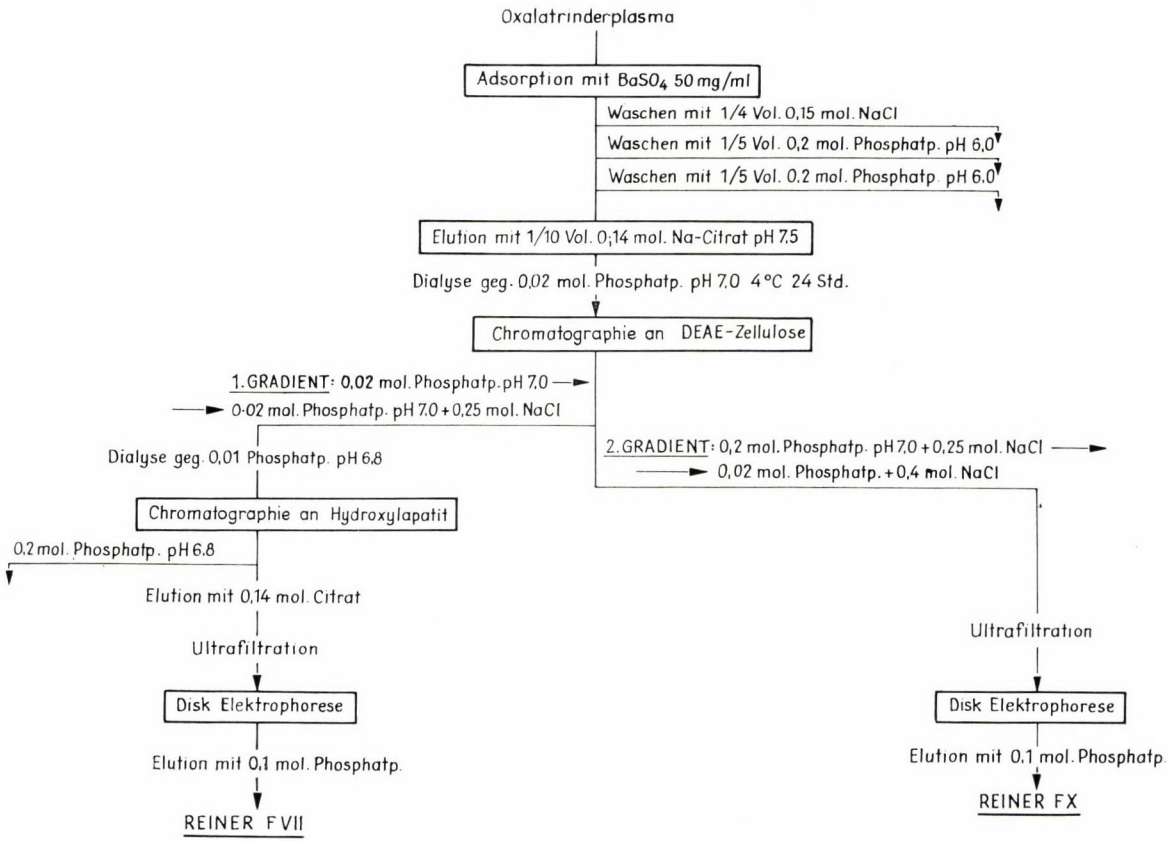


Abb. 8. Diagramm der Trennung von Faktor X und Faktor VII

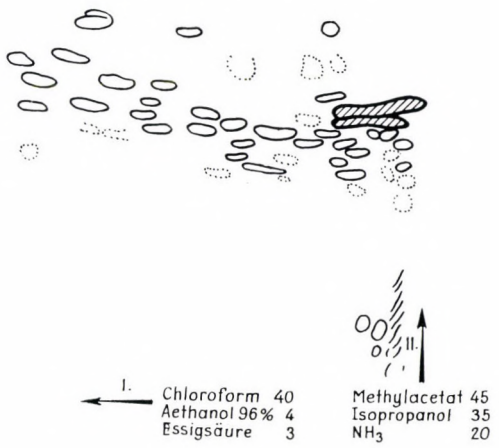


Abb. 9. Fingerprint von Faktor VII. Laufflüssigkeiten und Laufrichtung in der Abb. angegeben

2 stärkere und 4 sehr schwache Eiweißfraktionen enthalten [9]. Die Faktor X enthaltende Fraktion ist uns bekannt und ist noch biologisch aktiv. Die auf diese Weise gewonnenen Mengen haben aber noch nicht ausgereicht, die obigen Untersuchungen zu wiederholen (Abb. 7).

Die Reinigung von Faktor VII stößt auf sehr große Schwierigkeiten. Auch hier sind wir von Rinderplasma ausgegangen, haben an $BaSO_4$ adsorbiert und nach Waschung mit 0,2 m Phosphatpuffer pH 6 mit 0,14 m Natriumcitrat pH 7,4 eluiert. Dann folgte eine Chromatographie auf DEAE-Zellulose mit Elution mit einem kontinuierlichen konvexen Gradienten beginnend mit 0,02 m Phosphat-

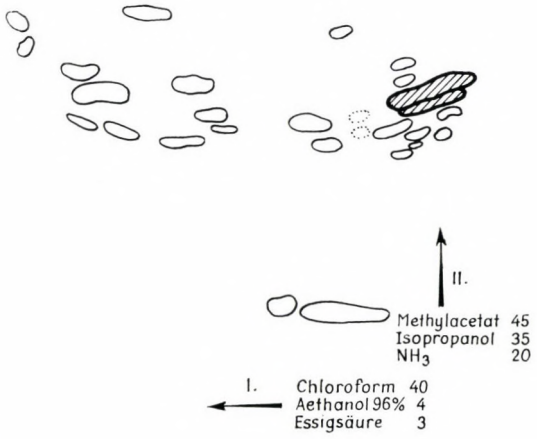


Abb. 10. Fingerprint von Faktor X. Laufflüssigkeit und Laufrichtung in der Abb. angegeben

puffer pH 7 und endend mit Zusatz von 0,25 m NaCl. Hierbei werden Prothrombin und Faktor VII nicht getrennt. Die Fraktion ist jedoch frei von Faktor X. Dann wurde eine Chromatographie an Hydroxylapatit angeschlossen, wobei noch inertes Eiweiß entfernt wurde. Die so erhaltenen Präparationen ent-

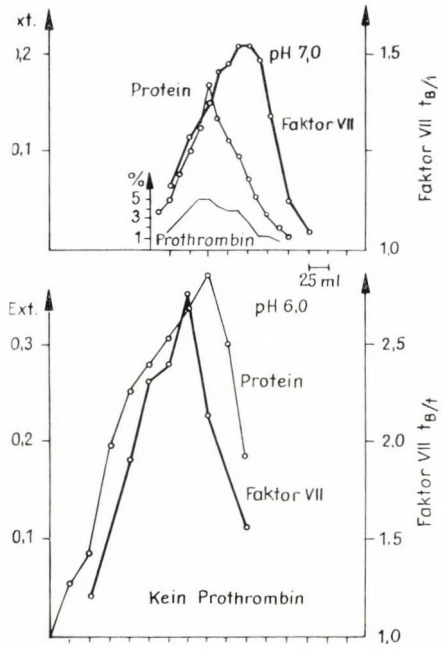


Abb. 11. Chromatographie eines bei pH 7,0 bzw. bei pH 6,0 gewaschenen Bariumsulfateluates aus Rinderplasma. Obere Hälfte: Waschung mit Phosphatpuffer pH 7,0, ausgezogene Linie Prothrombin, ○ — ○: Eiweiß, · — · : Faktor VII. Untere Hälfte der Abb.: Waschung bei pH 6,0. Die Probe enthielt kein Prothrombin, Faktor VII: — · —, Eiweiß: ○ — ○. Abszisse: Nummer der Fraktion. Ordinate: Extinktion bei 280 mμ bzw. Aktivitätsindex

hielten weder Thrombin noch Prothrombinaktivität, wobei wir nicht wissen, ob die Aktivitäten zerstört wurden oder in der Säule verblieben (Abb. 8).

Die Endgruppen dieser Präparation wurden in gleicher Weise wie bei Faktor X bestimmt. Es wurden an beiden Enden Glycin und Serin gefunden. In der Absicht, etwas über den gemeinsamen Aufbau der an sich augenscheinlich ähnlichen Faktoren zu erfahren, wurden von Faktor VII und Faktor X fingerprints angefertigt. Hiezu wurde eine von G. Schmer ausgearbeitete Mikromethode ver-

wendet, die darin besteht, daß das Eiweiß zunächst mit 2 Mercaptoäthanol und Harnstoff reduziert, dann mit Jodacetamid alkyliert und mit Trypsin verdaut wird. Die Peptide wurden mit einem Fluoreszenzfarbstoff markiert und in zweidimensionaler Dünnschichtchromatographie aufgetrennt. Erforderliche Substanzmenge 10^{-3} μ Mol. Hierbei wurde der auffallende Befund erhoben, daß keine grundsätzliche Ähnlichkeit der fingerprints gefunden werden konnte [6] (Abb. 9, 10).

Das Molekulargewicht wurde auf Grund des Verhaltens in der Gelfiltration,

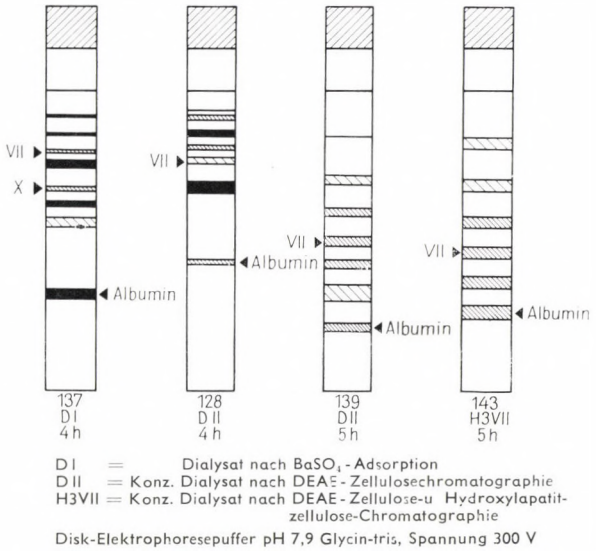


Abb. 12. Disk-Elektrophorese von Serumbariumsulfatadsorbat nach Dialyse (D I), nach DEAE Chromatographie (D II) und nach anschließender Hydroxylapatitchromatographie (H 3 VII)

wo Faktor VII knapp vor dem A-Peak eluiert wird, mit ca 70 000 geschätzt. Dies ist aber offenbar nicht immer so [6].

Im Verlaufe der Ausarbeitung der Methodik war aufgefallen, daß die Trennung auf Sephadex G 200 in Abhängigkeit von der Waschung des an $BaSO_4$ adsorbierten Materials unterschiedlich verläuft. Wird nämlich das $BaSO_4$ mit einem Phosphatpuffer pH 7 gewaschen, so dürfte das Molekül kleiner sein und erscheint erst nach dem A-Peak. Für dieses Molekül wurde ein Molekulargewicht von 59 000 berechnet. Die Aktivität dieses Materials ist deutlich geringer. Es muß daher angenommen werden, daß der Faktor VII in mindestens zwei Molekülgrößen vorkommt (Abb. 11). Diese Präparate weisen in der Disk-Elektrophorese noch immer 3 Banden auf [6] (Abb. 12).

In den letzten beiden Monaten ist es uns gelungen, mit Hilfe der Disk-Elektrophorese präparativ einen nach unserem heutigen Wissen wirklich reinen Faktor VII zu gewinnen. Die Mengen haben noch nicht ausgereicht, um Endgruppenanalysen und fingerprints zu machen.

Es kann kein Zweifel bestehen, daß die im exogenen System wirksamen Gerinnungsfaktoren in Gruppen hintereinander reagieren. Am besten abgeklärt ist die Aktivierung von Faktor X. Dieser aktiviert gemeinsam mit Phospholipid, Faktor V und Calcium das Prothrombin.

Esnouf und Williams [9] haben als erste die Möglichkeit der Aktivierung eines gereinigten Faktor X durch Russell-Vipern-Gift aufgezeigt. Sie haben auch demonstriert, daß der aktivierte Faktor X ein kleineres Molekül hat als seine inaktive Vorstufe. Wir haben fast gleichzeitig mit Spaet und Cintron [22] eine Aktivierung von gereinigtem Faktor X durch Citrat bzw. Ammonsulfat nachgewiesen [16]. Spaet und Cintron sind vom Serum zur Präparation von Faktor X ausgegangen, während wir Plasma als Ausgangsmaterial verwendeten. Die Aktivierung in Citrat erfolgt langsam, wie dies Seegers für sein Prothrombin gezeigt hatte.

Der aktivierte Faktor X verhält sich biologisch wie Autoprothrombin C. Es ist nun von Interesse, daß in den Seegersschen Aktivierungsexperimenten immer gleichzeitig Thrombin und Autoprothrombin C entstehen. Während Seegers annimmt, daß sein Prothrombinmolekül die Muttersubstanz beider Fermente ist, muß man auf Grund der eingangs erwähnten Ergebnisse der Säulenchromatographie eines derartigen Prothrombinpräparates annehmen, daß das Thrombin aus dem Prothrombin, das Autoprothrombin C aber aus dem in dem Präparat enthaltenen Faktor X entsteht. Bei diesem Aktivierungsprozeß greift das Citrat eindeutig am Faktor X an und nicht an dem von Faktor X abgetrennten Prothrombin. Daher ist das von Faktor X getrennte DEAE-Prothrombin auch nicht mit Citrat aktivierbar, sondern erst nach Mischung mit der zweiten von der Säule abgetrennten Fraktion, nämlich dem Faktor X [8].

Faktor X-Präparationen können auch mit Gewebethrombokinase aktiviert werden. Diese Reaktion verläuft wesentlich schneller als die Aktivierung mit Citrat. Es war auffallend, daß verschieden hergestellte Faktor X-Präparate verschieden schnell mit Thrombokinase aktiviert werden konnten. Es zeigte sich dann, daß mit zunehmender Reinheit die Aktivierung langsamer wurde. In einer nächsten Versuchsserie wurde gereinigter Faktor VII neben der Thrombokinase zu dem reinsten verfügbaren Faktor-X-Präparat zugesetzt. Hierbei zeigte es sich, daß die Aktivierung entsprechend beschleunigt wurde, ohne daß die Menge des aktivierten Faktor X wesentlich beeinflußt wurde. Ob eine Aktivierung von Faktor X völlig ohne Faktor VII möglich ist, können wir nicht sagen, da wir kein absolut Faktor-VII-freies System zur Verfügung hatten [16].

Wie aus den Untersuchungen von MacFarlane und Ash [19] hervorgeht, erfolgt die Aktivierung von Faktor X im endogenen System durch aktivierten Faktor VIII. Weiters ist auch eine Aktivierung durch kleine Mengen von Trypsin möglich. Es zeigt sich also, daß Faktor X durch ganz verschiedene Substanzen biologisch und rein experimentell aktiviert werden kann, wobei es sich offenkundig um eine proteolytische Reaktion handelt. Gereinigter Faktor VII allein vermag Faktor X nicht zu aktivieren. Gereinigte Thrombokinase kann Faktor X höchstwahrscheinlich allein nur äußerst langsam, wenn überhaupt, aktivieren.

Bringt man Thrombokinase und Faktor VII zusammen, so ist die volle Aktivität fast augenblicklich vorhanden. Dies steht in einem krassen Gegensatz zu den immerhin recht langen Inkubationszeiten, die für die Aktivierung von Faktor X erforderlich sind [16]. Die Vermutung liegt nahe, daß es sich hier um zwei grundverschiedene Reaktionen handelt. Zu analogen Ergebnissen ist Williams [24] gekommen, der außerdem einmal eine Spontanaktivierung von Faktor VII beobachtet haben will.

Wir können unsere bisherigen Ergebnisse dahingehend zusammenfassen, daß es uns möglich war, wahrscheinlich zu machen, daß die Faktoren VII und X von Prothrombin unabhängige, im Plasma bereits vorhandene Substanzen sind. So gelingt es, durch einen einfachen säulenchromatographischen Arbeitsgang Faktor VII von Prothrombin und Faktor X in menschlichem Plasma zu trennen. Lechner benützte dies zur Ausarbeitung einer einfachen Faktor-VII-Bestimmungs-

Versuchsansatz	TAMe Einheiten
TAMe	0
THROMBOKINASE + TAMe	0
FAKTOR X + VII + TAMe	0,03
FAKTOR X + VII + Ca + TAMe	0,07
FAKTOR X + VII + THROMBOKIN. + Ca + TAMe	2,3

Abb. 13. Entstehung von Esteraseaktivität in Faktor X Präparaten durch Thrombokinase, Faktor VII und Calcium

methode [17]. Prothrombin kann von Faktor X und VII unter anderen Versuchsbedingungen ebenfalls mit DEAE-Zellulose getrennt werden.

In diesem Zusammenhang dürfte es zweckmäßig sein, einige Worte über die unterschiedliche Interpretation der Ergebnisse durch Seegers und durch uns zu sagen. Nach Seegers ist die gemeinsame Muttersubstanz der Autoprothrombine bzw. Faktor VII, IX und X Prothrombin. Dieses zerfällt durch Einwirkung von Thrombin in 3 Teile, nämlich Prothrombin, die eigentliche Vorstufe des Thrombins, einen Hemmstoff und Autoprothrombin III, die Vorstufe von Autoprothrombin C. Wie schon erwähnt, entstehen in den Seegersschen Citratexperimenten immer Thrombin und Autoprothrombin C praktisch gleichzeitig. Wir konnten zeigen, daß DEAE-Prothrombin nicht mehr mit Citrat aktivierbar ist, sondern erst nach Zugabe von Faktor X, an dem das Citrat angreift. Man müßte nun mit Seegers annehmen, daß auf der DEAE-Säule ein analoger Molekülzerfall stattgefunden hat, wie er von Seegers für die Thrombinwirkung postuliert wird. Dagegen sprechen aber die Molekulargewichte. Prothrombin hat nach Seegers ein Molekulargewicht von 68 000. Faktor X nach den Berechnungen von Esnouf und Williams [9] ein solches von 86 000, nach unseren Berechnungen ein solches von etwa 70 000, was bei Verwendung so unterschiedlicher Methoden eine gute

Übereinstimmung darstellt. Außerdem glauben Harmison und Seegers bei Esnouf einen Rechenfehler gefunden zu haben, wodurch sich ihr Wert etwas verringern würde. Auf Grund dieser Berechnungen ist es unmöglich anzunehmen, daß das Prothrombinmolekül Faktor X und die eigentliche Vorstufe des Thrombins enthält. Auch das Molekulargewicht unseres Faktor VII ist so groß, daß man kaum annehmen könnte, daß Faktor VII ein Teil des Prothrombins ist. Es wäre lediglich möglich anzunehmen, daß aus dem Prothrombin durch Absprengung verschiedener sehr kleiner Polypeptide die Faktoren VII oder X entstehen. Dann müßten aber Faktor VII und Faktor X weitgehend denselben Aufbau aufweisen. In diesem Fall müßten sich die fingerprints weitgehend gleichen, was nicht der Fall ist.

Die Aktivierung von Faktor X ist in verschiedenen Systemen möglich, so mit Citrat; Thrombokinase und Faktor VII; Faktor VIIIa; Trypsin oder Russell-Vipern-Gift. Es ist dies eine enzymatische Reaktion, in der Faktor X das Substrat ist, da von seiner Menge die gebildete Menge von Faktor Xa abhängt. Hingegen beeinflussen Thrombokinase + Faktor VII, Russell-Vipern-Gift etc. die Schnelligkeit der Bildung des aktivierten Faktor X. Diese haben daher Fermentwirkung.

Faktor Xa selbst ist ein Ferment, denn während der Aktivierung entsteht Esterase-Aktivität [16] (Abb. 13). Sehr wahrscheinlich gibt es von Faktor X drei verschiedene Zustandsformen, einen Plasma-Faktor X, einen Serum-Faktor X und einen aktivierten, der aus beiden vorgenannten Formen entstehen kann. Faktor VII allein vermag Faktor X nicht zu aktivieren. Hierzu ist außerdem Thrombokinase erforderlich. Die Reaktion zwischen Thrombokinase und Faktor VII ist eine außerordentlich schnelle, über deren Art noch nichts Sicheres bekannt ist. Die Annahme, daß sich Faktor VII in irgendeiner Weise auf dem großen Molekül der Thrombokinase ausbreitet, ist naheliegend, doch fehlen dafür noch sichere experimentelle Unterlagen. Es ist per analogiam sehr wahrscheinlich, daß Thrombokinase + Faktor VII gemeinsam als Ferment zur Aktivierung von Faktor X wirken, wenn es auch noch nicht gelungen ist, ihre fermentative Aktivität an synthetischen Substraten zu erweisen.

Literatur

1. Alexander, B.: Some biochemical, physicochemical and immunochemical studies of prothrombin and proconvertin (Factor VII). Their biopathologic significance. IVth Int. Congr. Biochem., Vienna, 1958, Vol. X, p. 37.
2. Deutsch, E.: Enzymatic and clotting activity of tissue extracts. I. S. Wright, F. Koller and E. Beck: Heparin and Thromboplastin Suppl. 2 ad Vol. IX, *Thromb. Diath. haemorrh.* Stuttgart 1963, p. 189.
3. Deutsch, E., Irsigler, K., Lomoschitz, H.: Studien über Gewebethromboplastin. 1. Reinigung, chemische Charakterisierung und Trennung in einen Eiweiß- und Lipoidanteil. *Thromb. Diath. haemorrh. 12*, 12 (1964).
4. Deutsch, E., Köbele, B., Nesvadba, H.: Untersuchungen über die Spaltung neuer synthetischer Substrate durch Thrombin. *Wien. Z. inn. Med. 43*, 248 (1962).
5. Deutsch, E., Irsigler, K.: An aminopeptidase in human brain tissue thromboplastin preparations. *New Istanbul Contribution to Clin. Sci. 7*, 89 (1964).

6. Deutsch, E., Irsigler, K., Lechner, K., Schmer, G.: Studies on bovine factor VII and factor X. XIth Congr. Int. Soc. Haemat. Sydney, 1966, 281.
7. Deutsch, E., Lechner, K.: Zur Trennung von Prothrombin und Faktor X. Xth Congr. Int. Soc. Haemat. Stockholm, 1964, G 19.
8. Deutsch, E., Lechner, G.: On the Separation of Prothrombin and Factor X. *New Istanbul Contribution to Clin. Sci., Suppl. 1*, 26 (1965).
9. Esnouf, M. P., Williams, W. G.: The isolation and purification of a bovine plasma protein which is a substrate for the coagulant fraction of Russell's Viper venom. *Biochem. J. 84*, 62 (1962).
10. Hecht, E.: Studien über den lipoiden Aktivator der Blutgerinnung. *Thromb. Diath. haemorrh. 1*, 380 (1957).
11. Hecht, E., Oosterbaan van Lit, W. L.: Über die chemische Natur des Thromboplastins aus menschlicher Hirnsubstanz. *Thromb. Diath. haemorrh. 18*, 223 (1967).
12. Deutsch, E., Lechner, K., Schmer, G.: On the Purification of Factor VII from Bovine Plasma. K. M. Brinkhous, I. S. Wright, F. Koller, F. Streuli, F. Duckert: Diffuse intravascular clotting. *Thromb. Diath. haemorrh. Suppl. 20*, 275 (1966).
13. Irsigler, K.: Human brain tissue thromboplastin. In R. B. Hunter, F. Koller, E. Beck: Fibrinogen and fibrin turnover of clotting factors. *Thromb. Diath. haemorrh. Suppl. 13*, 433 (1964).
14. Irsigler, K., Lechner, K., Deutsch, E., Lomoschitz, H.: Studies on tissue thromboplastin. II. Species specificity. *Thromb. Diath. haemorrh. 14*, 18 (1965).
15. Köbele, B.: Untersuchungen über die Wirkung von Thrombin auf neue synthetische Substrate. Dissertation Wien—Tübingen, 1962.
16. Lechner, K., Deutsch, E.: Activation of Factor X. *Thromb. Diath. haemorrh. 13*, 314 (1965).
17. Lechner, K., Deutsch, E.: Eine einfache Methode zur Bestimmung von Faktor VII. *Thromb. Diath. haemorrh. 18*, 252 (1967).
18. Lechner, K., Irsigler, K., Deutsch, E.: Zur Artspezifität von Gewebethromboplastinen. Xth Congr. Int. Soc. Haemat. Stockholm, 1964.
19. MacFarlane, R. G., Ash, B. J.: The Activation and Consumption of Factor X in Recalcified Plasma. *Brit. J. Haemat. 10*, 217 (1964), C 28.
20. Mann, F. D., Hurn, M. M.: Species specificity of thromboplastin. Role of the Cothromboplastin reaction. *Proc. Soc. exp. Biol. Med. 89*, 18 (1952).
21. Soulier, J. P., Weilland, C.: Purification de la prothrombine par la bentonite. II. Propriétés de la prothrombine purifiée. *Path. Biol. 7*, 2541 (1959).
22. Spaet, Th. H., Cintron, J.: Pathways to blood coagulation product I formation. *Blood 21*, 745 (1963).
23. Streuli, F.: On the purification and conversion of human prothrombin. *Thromb. Diath. haemorrh. 3*, 194 (1959).
24. Williams, W. J., Norris, D. G.: Purification of a bovine plasma protein (Factor VII) which is required for the activity of lung microsomes in blood coagulation. *J. Biol. Chem. 241*, 1847 (1966).

Лечение больных идиопатическим миелофиброзом

М. С. ДУЛЬЦИН, Н. Ф. КЛИМОВА

Гематологическая клиника Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва

(Поступила в редакцию в апреле 1967 г.)

1. Миелобромол является эффективным средством для лечения идиопатического миелофиброза. В течение 2—4 недель обычно препарат назначается по 250 мг в день, а в последующие две недели по 125 мг в день.

Миелобромол не вызывает никаких побочных токсических явлений.

2. Миелобромол оказывает медленное цитостатическое действие, не только на миелобластические клетки, но и на фиброзную ткань, в меньшей степени влияет на тромбопоэз и не влияет на эритропоэз.

Как правило, через 2 недели, по окончании курса лечения развивается депрессия лейкопоэза, которая обычно быстро восстанавливается.

3. Ремиссия, достигнутая лечением миелобромолом, продолжается обычно от 4 до 12 месяцев. Поддерживающее лечение миелобромолом в дозе по 125 мг 2—3 раза в неделю удлиняет продолжительность ремиссии.

4. Ценным качеством миелобромола является полное отсутствие резистентности к препарату и это позволяет проводить повторные курсы лечения миелобромолом.

Впервые ассоциацию фиброза костного мозга с лейкемоидной картиной крови описал Wood в 1871 году, и с тех пор это заболевание много раз описывалось под самыми различными названиями. Наиболее часто употреблялись следующие названия: миелоидная метаплазия, лейкоэритробластическая анемия, алейкемический миелоз, хронический не лейкемический миелоз, идиопатический миелофиброз и др.; за самые последние годы это заболевание обозначается как идиопатическая миелоидная метаплазия (Gardner и соавторы, 1961; Pitcock и соавторы, 1962). Мы пользуемся термином идиопатический миелофиброз.

Идиопатический миелофиброз в морфологическом плане может рассматриваться как миелопролиферативное заболевание, характеризующееся фиброзной мезенхимной пролиферацией костного мозга. Если просмотреть естественный ход течения заболевания, то обращает на себя внимание прогрессивное увеличение селезенки. Выраженная спленомегалия часто сопровождается вторичным гиперспленизмом (гемолитической анемией или тромбоцитопенией, либо тем и другим).

Мы полагаем, что спленомегалия у больных миелофиброзом обусловлена не только компенсаторным механизмом, развивающимся в результате фиброретикулярной пролиферации костного мозга, но и такого же

процесса в селезенке. Эти изменения развиваются у больных постепенно на протяжении 5—10 лет.

Наиболее частой причиной для обращения к врачу служит дискомфорт в брюшной полости, наступающий в результате увеличенной селезенки.

На ранних стадиях наличия идиопатического миелофиброза, когда обычно имеет место незначительная анемия (или она отсутствует) и небольшое увеличение селезенки, мы не советуем прибегать к цитостатической терапии. Наша тактика в таких случаях заключается в регулярном наблюдении за больным и применении общеукрепляющей терапии.

При прогрессировании миелофиброза лечение должно быть построено на следующих трех принципах:

1) создание условий стимуляции мезенхимных и ретикулярных клеток к формированию активного гемопоэза. Это достигается, повидимому, лучше всего с помощью препаратов с направленностью анаболического действия — андрогенов,

2) снятие явлений гиперспленизма (применяются кортикостероидные гормональные средства),

3) задержка прогрессирования спленомегалии. С этой целью обычно применяются цитостатики.

В литературе имеются сообщения о благоприятных результатах лечения идиопатического миелофиброза милераном (Dameshek и соавторы, 1958; Videbaek, 1956) с ремиссией от 4 месяцев до 4 лет.

По данным К. А. Хасановой, милеран не способствует уменьшению размеров селезенки и печени у больных с миелофиброзом; он вызывает лишь улучшение общего состояния больных, уменьшение боли в костях и селезенке. Мы не применяли милеран нашим больным, так как на предыдущих этапах лечения он способствовал лишь быстрому снижению числа лейкоцитов и не сокращал размеров селезенки. Поэтому мы решили изучить влияние венгерского препарата миелобромола (Dibrommanit).

Препарат применялся 12 больным с выраженной гепатоспленомегалией, из них было 7 женщин и 5 мужчин, в возрасте от 40 до 59 лет, с давностью заболевания от 2 до 16 лет.

Диагноз идиопатического миелофиброза был установлен на основании наличия гепатоспленомегалии, лейкоэритробластических показателей в периферической крови, стабильных сублейкемических цифр числа лейкоцитов на всем протяжении заболевания, изменения в морфологии эритроцитов (выраженного анизо- и пойкилоцитоза), фиброза костного мозга.

Однако необходимо отметить, что трепанобиопсия не может рассматриваться как единственный метод, пригодный для диагностики идиопатического миелофиброза, так как нередко из-за очагового поражения фиброз костного мозга может быть не обнаружен в определенных полях трепанобиопсии. Как дополнение к диагностике у 8 больных применялись цитохимические и цитогенетические методы. У 7 больных активность щелочной

фосфатазы в лейкоцитах была повышенной и колебалась в пределах от 78 до 236 Ед, у одной больной она была в пределах нормы — 45 Ед. У всех 8 больных филадельфийская хромосома отсутствовала.

Основной жалобой всех больных была общая слабость, чувство дискомфорта в брюшной полости, из-за увеличения селезенки, повышенная утомляемость. Были и другие симптомы: потеря веса у 6 больных, одышка — у 2-х, боли в суставах — у двух.

Увеличение лимфоузлов отмечалось лишь у одной больной.

Гистология костного мозга

При гистологическом изучении костного мозга, полученного методом трепанобиопсии, у всех больных установлен различной степени фиброз. Степень фиброза варьировала от вовлечения небольших участков костного мозга до участков полностью вытесняющих кровотворные клетки фиброзной ткани.

У 5 больных наряду с фиброзом костного мозга отмечались участки новообразования кости.

Гистологическое исследование костного мозга, проведенное у 4-х больных повторно через 1—2 года, показало заметное нарастание фиброза и склероза в костном мозгу.

Миелограмма

При стернальной пункции у 10 больных миелофиброзом игла проходила в кость легко, но аспирация костного мозга была затруднена, по-видимому, из-за фиброзного разрастания в нем. При этом удавалось получить лишь небольшое количество пунктата.

У 2 больных костный мозг получить не удалось: у одного из-за плотности кости и у одной—аспирация не позволила извлечь костно-мозговую ткань.

Содержание миелокариоцитов у 5 больных находилось на нормальном уровне, число их не превышало 51 400 — 77 200. Костный мозг был беден клеточными элементами у 3 больных, и умеренно клеточным — у 2 больных.

При подсчете миелограммы число недифференцированных элементов колебалось от 0,6 до 6,8%; незрелые гранулоциты были в пределах от 10,6% до 23,4%, зрелые гранулоциты составляли 48,4—67,2%. Отмечалось снижение числа клеток красного ряда у всех больных (выраженное у 5 больных от 1,6 до 6%, у 5 — умеренное: от 10,6 до 17,8%).

Гемограмма

Хотя лейкоцитоз был выявлен у всех больных, однако число лейкоцитов в течение заболевания не превышало 50 000 в 1 мм^3. В периферической крови содержание незрелых гранулоцитов колебалось в пределах 3—15—

20%. Непостоянно встречались недифференцированные клетки, число гемоцитобластов не превышало 2—2,5%. Таким образом, наблюдались колебания от относительно нормального дифференцированного подсчета, до приближающегося к развернутой картине, свойственной обострению хронического миелолейкоза.

Эозинофилы, моноциты отмечены у всех больных; они не превышали 3%, и лишь у отдельных больных эозинофилы достигали 4%.

Число тромбоцитов у 9 больных было в пределах нормы. Только у двух больных число тромбоцитов повышалось до 554 000 — 635 000 в 1 мм^3, а у одной больной число тромбоцитов снизилось до 102 000 в 1 мм^3.

Базофилия рассматривается как один из характерных признаков лейкоза.

У наших больных базофилы встречались редко и не превышали 3% — изредка — 4% и у одной больной составляли 5,5%.

Нормохромная анемия отмечалась у 3 больных, выраженная анемия (8,4 г%) у 2 и у одной — умеренная (Нв — 10,8 г%).

В периферической крови постоянно наблюдались ядерные формы красных клеток в большинстве случаев они не превышали — 5—3%, у отдельных больных наблюдалось увеличение их до 8%.

Одним из характерных признаков миелофиброза является выраженный анизоцитоз, пойкилоцитоз и полихроматофилия.

4 из этих больных раньше получали лечение другими цитостатическими средствами; (двое-миелосан, один — допан и одна больная — миелосан и рентгенотерапию на область селезенки) без эффекта.

Миелобромол мы назначали по 250 мг в день в течение 2—4 недель, затем доза уменьшалась до 125 мг pro die. По истечении 5—6 недель, переходили на поддерживающие дозы (обычно по 125 мг 2—3 раза в неделю). Курсовая доза — минимальная 4 г, максимальная 9,6 г. В результате терапии миелобромолом самочувствие больных улучшалось, проходила слабость, улучшался аппетит, увеличивался вес.

У всех больных на 9—12 день приема препарата значительно уменьшались размеры печени и селезенки. Нормализации размеров селезенки не получено ни у одного больного, однако у 2-х больных (В. с давностью заболевания 10 лет и Х. с давностью заболевания — 2 года) после лечения миелобромолом селезенка стала пальпироваться у края реберной дуги; до лечения размеры селезенки были: 6—15/8 см, 5—18/19 см. Размеры печени у этих больных тоже заметно уменьшились.

У всех больных на 3—4 день от начала приема миелобромола отмечалось повышение числа лейкоцитов, затем число лейкоцитов медленно снижалось; курс лечения продолжался в течение 17—47 дней. Препарат обычно отменяли при уменьшении числа лейкоцитов до 6 000.

Миелобромол хорошо переносится больными и не вызывает никаких побочных явлений.

После отмены препарата наблюдалось последующее снижение числа

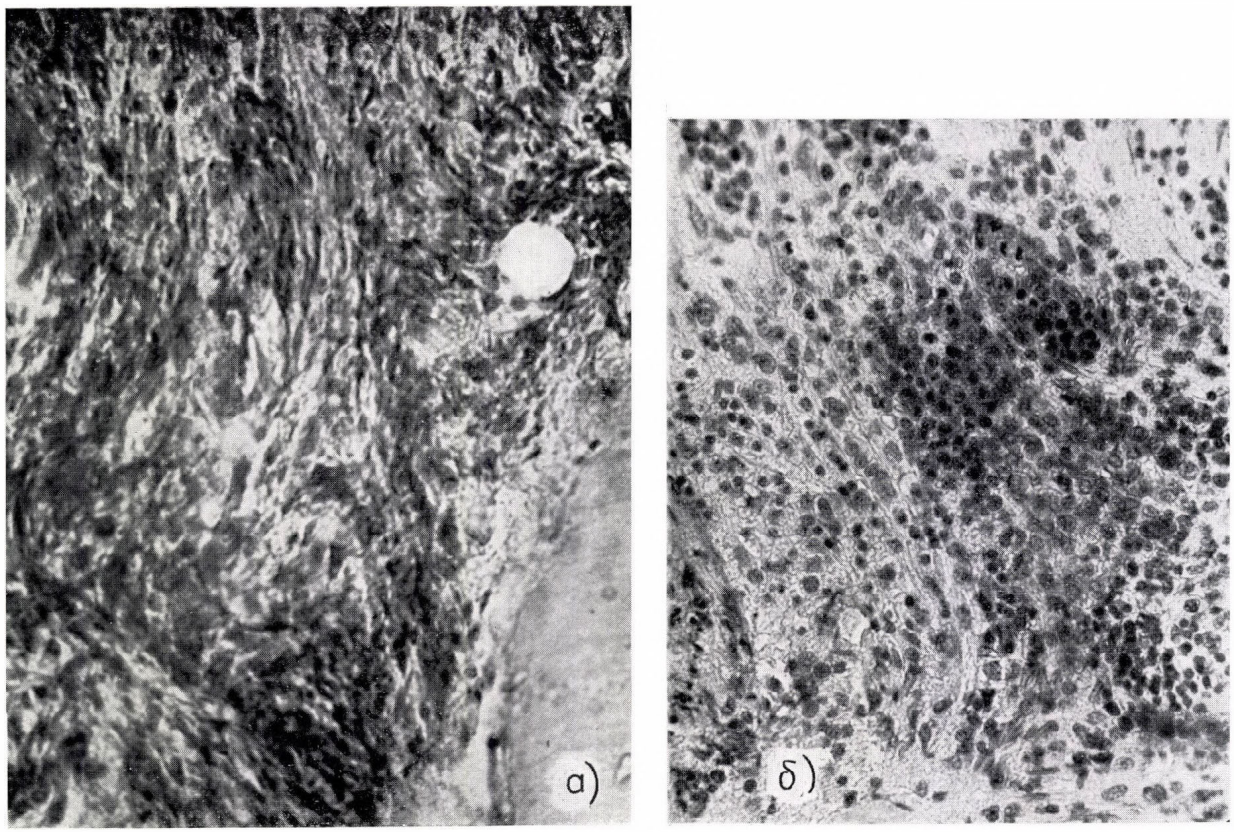


Рис. 1. Трепанобиопсия: а) Диффузный миелофиброз. б) Уменьшение фиброзной ткани и числа миелоидных элементов, сохранение клеток красного ряда после лечения миелофиброзом. Больной Л. Диагноз: идиопатический миелофиброз

лейкоцитов в течение 6—10 дней: у 3 больных — до 2600—3400; у 4-х — до 4000 и только у одной больной В. число лейкоцитов снижалось до 900 (при общей дозе миелобромола 9 250 мг). Обычно через две недели число лейкоцитов повышалось. У 4-х больных число лейкоцитов не снижалось ниже 6000.

У всех больных, за исключением одного, отмечалось улучшение состава лейкоцитарной формулы: исчезали недифференцированные элементы, уменьшалось число незрелых гранулоцитов (с 15—10% до 0—1—2%).

Снижение числа тромбоцитов ниже 100 000 наблюдалось у 3-х больных. У двух — до 50 000 и 37 000 на 10—12 день, а у одной больной лечение было прекращено на 28 день из-за снижения числа тромбоцитов до 59 000. Нужно отметить, что лечение миелобромолом у этой больной было начато при наличии 102 000 тромбоцитов. Через 2 недели число тромбоцитов повысилось до исходных цифр.

Уровень Нв и число эритроцитов за период лечения миелобромолом оставались на исходных показателях.

На 7—10 день после окончания приема препарата отмечалось снижение уровня Нв с 13—12 г% до 10,6 г% у четырех больных.

Назначение нерабола способствовало повышению уровня Нв и числа эритроцитов до исходных показателей. У 8 больных уровень Нв не изменялся.

После проведенного курса лечения миелобромолом при подсчете миелограммы у 6 больных особых изменений, по сравнению с миелограммами до лечения, не отмечалось.

Только у 4-х больных наблюдалось уменьшение числа недифференцированных клеток и зрелых гранулоцитов почти вдвое (с 67,2 до 36,6, с 57% до 20%) и увеличение числа эритро-нормобластов (с 1,6% до 5,4%, с 2,4% до 7,6%; с 10,6% до 16,2%, с 17,8% до 38,4%).

При гистологическом исследовании (трепанобиопсия) костного мозга у этих же больных отмечено уменьшение числа клеточных элементов миелоидного ряда с сохранением клеток красного ряда. В ряде участков волокнистая ткань становилась более нежной и количество ее уменьшалось (рис. 1).

Следовательно миелобромол, в основном, влияет на гранулопоэз. Создается впечатление, что в какой-то мере он способствует рассасыванию фиброретикулярной ткани и возможно подавляет пролиферацию фибробластов. Это предположение подтверждается также резким уменьшением размеров селезенки у этих больных под влиянием лечения миелобромолом.

Мы уже указывали, что спленомегалия у больных миелофиброзом обусловлена не только компенсаторной миелоидной метаплазией, но в селезенке, так же как и в костном мозгу, происходит пролиферация мезенхимных ретикулярных клеток и фибробластов.

Возможно этим же процессом объясняется и неэффективность миелосана у больных с миелофиброзом, так как милеран действует направленно

на миелоидную гиперплазию и не влияет на пролиферацию мезенхимных клеток и фибробластов.

У всех больных под влиянием лечения миелобромолом получены непосредственно хорошие результаты.

Из 12 больных у 5 ремиссия длилась от 4 до 6 месяцев (с поддерживающим лечением миелобромолом). После повторного курса лечения миелобромолом (также при условии поддерживающего лечения) длительность ремиссии увеличивалась до 8—12 месяцев. За остальными 7 больными

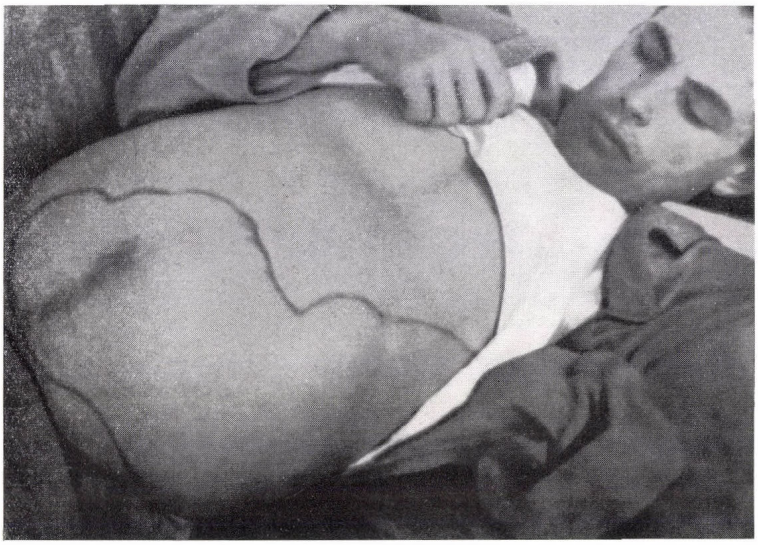


Рис. 2. Гигантская селезенка у больного С. Диагноз: идиопатический миелофиброз

наблюдение продолжается пока в пределах от 1 до 12 месяцев. Больные чувствуют себя хорошо и продолжают получать поддерживающие дозы препарата.

Двое больных поступили в клинику для проведения 3 курса лечения миелобромолом.

Приводим краткую выписку из истории болезни.

Б-ной С. Л. Е., 41 года. Страдает идиопатическим миелосклерозом с 1950 г. До 1963 г. состояние его оставалось удовлетворительным, а затем печень и селезенка стали быстро увеличиваться в размерах, нарастала слабость и потливость. Лечение кортикостероидами особенного эффекта не дало. В марте 1965 г. больной в тяжелом состоянии поступил в гематологическую клинику. Истощен, кожные покровы и видимые слизистые — бледны. Живот резко увеличен в объеме за счет огромной, плотной селезенки, которая занимала почти всю брюшную полость. Размеры селезенки по Курлову — 34—48/32 см (см. рис. 2).

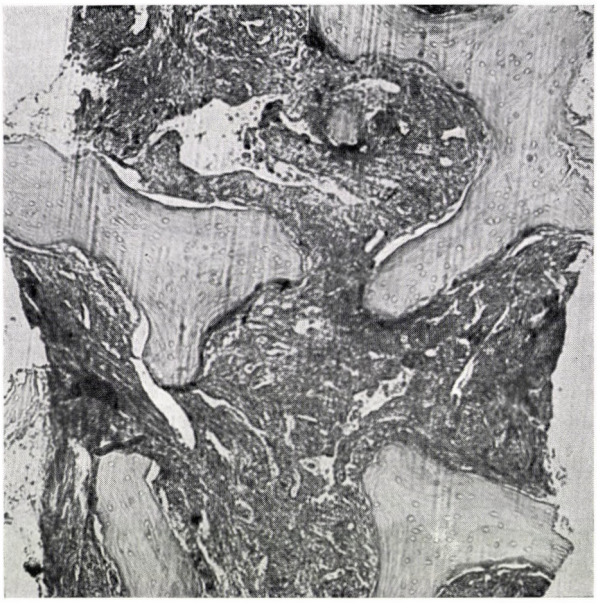


Рис. 3. Трепанобиопсия. Разрастание атипичной коркой и фиброзной ткани в костномозговой полости. Клеточный полиморфизм. Больной С. Диагноз: идиопатический миелофиброз

Печень выступала из-под реберного края на 8 см. Анализ крови от 6/III—65 г. Нв — 15,8 г%, эр. — 5 090 000, л. — 36 000, гемоцитобласты — 0,5%, миелобласты — 0,5%, промиелоциты — 0,5%, миелоциты — 12,5%, метамиелоциты — 4%, п. — 23%, с. — 46,5%, лимф. — 8,5%, баз. — 3%, эоз. — 1%, ретикулоциты — 2‰, тромбоциты — 350 000, РОЭ — 1 мм в час, анизоцитоз, пойкилоцитоз, встречаются ядра мегакариоцитов.

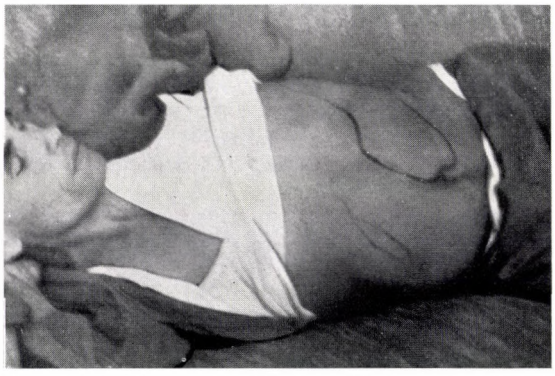


Рис. 4. Резкое уменьшение размеров селезенки у больного С. после лечения миелобромолом

При гистологическом исследовании костного мозга выявлено повышенное костеобразование в виде наслоений незрелой костной ткани и остеоида. Обнаружено разрастание фиброретикулярной ткани; местами она диффузно заполняет костномозговые полости. Клеточный состав полиморфен, однако преобладают эритробласты. Много мегакариоцитов (см. рис. 3).

Во всех костях на рентгенограммах отмечено уплотнение спонгиозного вещества, на фоне которого отмечаются островки склероза.

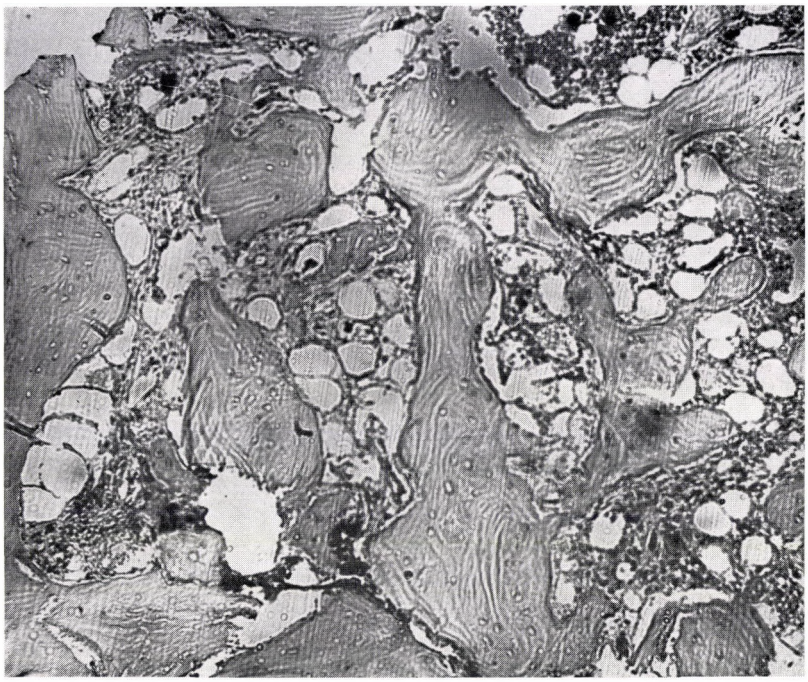


Рис. 5. Трепанобиопсия. Гипоплазия кроветворения с сохранением клеток красного ряда. Уменьшение фиброзной ткани после лечения миелобромолом

При стернальной пункции получить костный мозг не удалось из-за плотности грудины.

В течение 20 дней проводилось лечение миелобромолом в дозе 250 мг в сутки. При снижении числа лейкоцитов до 15 600 доза была уменьшена до 125 мг в сутки, однако через 10 дней препарат пришлось отменить из-за снижения числа тромбоцитов до 50 590; число лейкоцитов было в пределах 10—12 000.

Через 12 дней после отмены миелобромола число тромбоцитов возросло до 78 470, а число лейкоцитов снизилось до 3400.

Всего на курс лечения общая доза миелобромола равнялась 6,5 г.

Размеры селезенки уменьшились до 14–25/18 (см. рис. 4). Заметно сократилась печень, она пальпировалась на 4 см ниже реберной дуги. В результате проведенного лечения улучшилось общее состояние больного, исчезла слабость и потливость. Несмотря на уменьшение размеров селезенки больной прибавлял в весе.

Улучшился также состав периферической крови. Анализ крови от 24/IV—65 г.: Нв — 15,4 г%, эр. — 4 380 000, цветной показатель — 1, л. — 3400. Миелобласты — 1%, миелоциты — 1%, п. — 12,5%, с. — 20%, лимф. — 61,5%, баз. — 2%, эоз. — 1,5%; ретикулоциты — 2‰, тромбоциты — 78 470, РОЭ — 3 мм в час, плазматические клетки — 0,5%.

Гистологическое исследование костного мозга, произведенное после лечения миелобромолом, показало развитие гипоплазии кроветворения, и в ряде участков волокнистая ткань стала более нежной (см. рис. 5). Больной был выписан в удовлетворительном состоянии 27/IV. С 15/V—65 г. начал принимать миелобромол в поддерживающих дозах по 125 мг через день. Число лейкоцитов к этому времени возросло до 9500, а тромбоцитов до 270 000. Всего в амбулаторных условиях он получил 3,25 г миелобромола. Ремиссия длилась 4 месяца.

Повторный курс лечения миелобромолом был начат 1 сентября 1965 г. и продолжался 31 день — всего он получил 6 г. Размеры селезенки уменьшились с 30—49/33 до 13—26/20, печень уменьшилась с 13 до 5 см.

Улучшился состав периферической крови: полностью исчезли недифференцированные клетки, а число незрелых гранулоцитов снизилось с 7 до 2,5%. Число тромбоцитов уменьшилось с 381 000 до 133 000.

Миелобромол был отменен при снижении числе лейкоцитов до 5050.

Через 10 дней после отмены препарата число лейкоцитов снизилось до 2800, число тромбоцитов к этому времени возросло до 190 000, уровень Нв снизился с 13,8 до 10 г%, число эритроцитов уменьшилось с 4 250 000 до 3 170 000.

В связи с развившейся анемией и лейкопенией больному был назначен преднизолон (короткий курс). Через 2 недели количество Нв возросло до 11 г%, эр. — до 3 500 000, лейкоцитов — до 6800.

Ремиссия длилась 10 месяцев. В амбулаторных условиях получал регулярно поддерживающие дозы миелобромола по 125 мг сначала ежедневно, а затем через день.

Последний месяц миелобромол он не принимал, и у него вновь наступило ухудшение, выражавшееся в резком увеличении селезенки.

В третий раз больной поступил в гематологическую клинику для лечения миелобромолом, и вновь были получены хорошие результаты.

Литература

1. Хасанова, К. А.: Лечение миелосаном больных с остеомиелосклерозом и миелофиброзом. *Здравоохранение Таджикистана, 1*, 54 (1961).
2. Dameshek, W. J., Granville, N. B., Rubio, F.: Therapy of the myeloproliferative disorders with mileran. *Ann. New York Acad. Sci., 68*, 1001 (1958).
3. Gardner, F. H., Pringle, J. C.: Androgens and erythropoiesis. II. Treatment of myeloid metaplasia. *New Engl. J. Med. 264*, 103 (1961).
4. Pitcock, J. A., Reinhard, E. H., Justus, B. W., Mendelsohn, R. S.: A clinical and pathological study of seventy cases of myelofibrosis. *Ann. Internal Med. 57*, 73 (1962).
5. Videbaek, A.: Unusual cases of osteomyelosclerosis. *Acta Med. Scand. 153*, 459 (1956).

Treatment of Idiopathic Myelofibrosis

M. S. Dulcin, N. F. Klimova

1. Myelobromol is an effective drug against idiopathic myelofibrosis. Its daily dose is 250 mg for 2 to 4 weeks, then 125 mg for another two weeks. It has no toxic side effects.

2. Myelobromol exerts a slow cytotoxic effect on both the myeloblasts and fibrous tissue. It influences thrombopoiesis to a lesser extent, and erythrocytopoiesis not at all. At the end of the fortnight following discontinuance of administration, leucopoiesis is depressed temporarily.

3. Remission usually lasts 4 to 12 months. Administration of maintenance doses (125 mg two or three times a week) prolongs remission.

4. As no drug resistance develops, myelobromol treatment may be repeated later, if necessary.

Structure and Function of Erythrocytes, III. Effect of Membrane Modifications on the Mechanism Maintaining Biconcave Shape

ILMA SZÁSZ, I. ÁRKY, G. GÁRDOS
Research Institute of the National Blood Centre, Budapest, Hungary

(Received April 25, 1967)

The superficial treatments of the erythrocyte membrane (protein-film desorption, tannin treatment, Al_2O_3 treatment) have no effect on the shape transformation produced by alteration of the ATP content. Contrary to this, the disk-sphere transformation caused by glycolysis inhibitors is slowed down to a great extent by trypsin and papain and to a smaller extent by antibody sensitization. The shape regeneration accompanying fermentative ATP resynthesis is also advantageously influenced by trypsin and papain. To explain these phenomena, the effects of agents mentioned above on glucose transport, glycolysis rate and ATP utilizing reactions were studied. It was established that ATP utilization of haemolysates decreased after antibody-sensitization, papainization and trypsinization; glycolysis of intact erythrocytes was diminished by trypsin treatment, while glucose transport was enhanced by papain treatment. Probably trypsin and papain activate the "shape-maintaining ATPase" and produce excess ATP by inactivating other ATP-utilizing reactions. Antibody sensitization has no direct effect on the shape-maintaining mechanism but, through diminishing hexokinase activity and active K-transport, provides more ATP for the shape-maintaining mechanism. Pancreatic lipase proved to be an effective sphering agent, causing reversible sphering without haemolysis. An attempt was made to give a synthetic explanation of the results.

The maintenance of the biconcave shape of erythrocytes requires ATP [1]. The utilization of the energy of ATP is carried out most probably by a contractile membrane protein with ATPase activity. Recently the extraction of an actomyosin-like protein from erythrocyte membrane was reported [2]. At the same time it is known that through a direct membrane effect, so e.g. with haemolysins attacking both the protein and lipid constituents of the cell membrane, a rapid loss of biconcave shape can be attained [3]. On the basis of this there are still followers of the conception that the maintenance of biconcave shape is secured by the composition and localization of membrane lipids [4, 5].

Abbreviations used in the paper:

ATP:	adenosine triphosphate
ATPase:	adenosine triphosphatase
IA:	iodoacetate (Na-salt)
IgG:	immune gamma globuline
I_m:	morphological index

In our present work we investigate the influence of different membrane modifications on the maintenance of the biconcave shape of erythrocytes. To produce superficial membrane modification, desorption of the protein-film, elimination of the labile lipid fraction, and tannin treatment were carried out. For modification of the membrane structure protein and lipid-splitting enzymes and $Rh_0(D)$ antibodies were used.

Methods

Preparation of the experimental material

Fresh, defibrinated human blood and washed erythrocyte suspensions, resp. were used. The average haematocrit value of the experimental mixtures was 30%. The erythrocytes were washed three times with 4 volumes of washing solutions, and were centrifuged at room temperature for 10 minutes at 3500 r.p.m. Two kinds of washing solutions were used:

A: 5 mM $MgCl_2$, 158 mM NaCl
B: 6 mM glucose, 5 mM $MgCl_2$, 155 mM NaCl

In the experiments involving fermentative ATP resynthesis [6], erythrocytes washed twice in washing solution *B* were incubated with 16 mM NaF, and the suspension was washed once in 10 volumes of washing solution *A* at 4° C. Following this, ATP resynthesis was secured by addition of glucose and incubation at 37° C. The other agents (trypsin, etc.) were added to the system together with glucose.

Desorption of the protein film was done by Furchgott's [7] glass bead method, elimination of the labile lipid fraction by Lovelock's [8] Al_2O_3 method. Tannin effect was investigated on washed erythrocytes in the presence of glycolysis inhibitors at 1 : 10 000 and 1 : 20 000 final concentrations of tannin. The effectivity of the glass bead treatment was controlled with erythrocytes labelled with ^{125}I-albumin, the effectivity of the lipid detachment by the lipid analysis of Al_2O_3, the effectivity of tannin treatment by the Coombs-reaction following protein sensitization.

Erythrocytes were trypsin-treated with the preparation "Lyotryp" (Institute for Serobacteriological Production "Humán", Budapest). One ml of erythrocyte sediment washed previously three times in washing solution *B* was incubated for 30 minutes at 37° C with 0.4 ml of 0.25% trypsin dissolved in a 1 : 1 mixture of 110 mM phosphate buffer (pH 7.4) and 166 mM NaCl. Trypsin was eliminated by washing with solution *B*.

Papain treatment of erythrocytes was performed with crystalline papain (Merck). To 1 ml erythrocyte sediment washed previously three times in solution *B*, 1 ml 0.2% papain activated by 0.25 mg l-cysteine, dissolved in buffered saline was given and the suspension was incubated at 37° C for 30 minutes. Papain was eliminated by washing with solution *B*.

Lipase treatment of erythrocytes was performed with the preparation "Lipáz" (Reanal, Budapest). To the erythrocytes, previously washed three times in

solution *B*, 0.1% lipase in aa. volume, dissolved in 166 mM NaCl was given, and the suspension was incubated for 60 minutes at 37° C. Lipase was removed by using washing solution *B*.

For the antibody sensitization $Rh_0(D)$ positive erythrocytes and immunochemically pure IgG prepared from human sera of high incomplete anti-$Rh_0(D)$ titre were used. To 1 ml erythrocyte sediment 1.5 mg IgG dissolved in 166 mM NaCl was added, and it was incubated for 30 minutes at 37° C. The grade of sensitization was checked by ^{125}I-labelled IgG. Under our experimental conditions 7–12% of the IgG of high antibody content was bound to the surface of erythrocytes.

Determinations

Morphology of erythrocytes was studied in an isotonic trisodium citrate solution containing 1% formalin described by Dacie [9], between slide and coverslip, with phase-contrast microscope, at an 800-fold magnification. The morphological index (I_m) was determined as described in our previous paper [10].

ATP determinations were carried out in two ways: 1. By the spectrophotometric analysis at 260 mμ of the fractions obtained from a Dowex-1-Cl ion exchange column [11]; 2. enzymically with the aid of glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase, according to the Boehringer test [12]. The measured values were expressed as percentages of the original ones.

The activity of enzyme systems utilizing ATP (hexokinase, ATPases) was measured in haemolysates prepared by twofold freezing and thawing of erythrocytes. In 0.6 ml volume 5 μM ATP, 5 μM $MgCl_2$, 100 μM NaCl, 20 μM KCl and 30 μM Tris-HCl buffer (pH 7.4) were added to a haemolysate of 0.4 ml erythrocyte sediment, and at 0 minute and 30 minutes of incubation at 37° C the inorganic phosphate was determined in the deproteinized supernatants according to the method of Fiske and SubbaRow [13]. There was no measurable liberation of inorganic phosphate when ATP was omitted from the system.

Glycolysis rate was determined by measuring glucose consumption iodometrically [14] and lactate production colorimetrically [15].

Glucose transport processes were studied according to the method of LeFèvre and McGinnis [16].

Potassium influx was investigated by the aid of ^{42}KCl. The radioactivities were measured in a well-type scintillation counter.

Results

Effect of superficial membrane treatments

Elimination of the protein-film and the labile lipid fraction, as well as tannin treatment did not influence significantly the changes in shape caused by the decrease in ATP level in experiments with glycolysis inhibitors (1 mM IA, 5–16 mM NaF).

Effect of trypsin treatment

Trypsin treatment produced no detectable changes in the morphology and ATP content of erythrocytes. But when ATP was broken down by glycolysis inhibitors (1 mM IA, 5–16 mM NaF) in erythrocytes pretreated with trypsin, or in the presence of trypsin, we found that while the rate of ATP breakdown

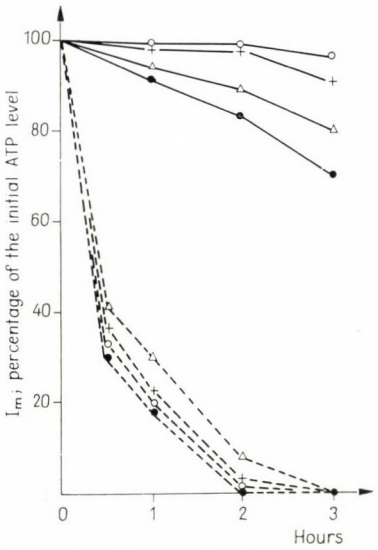


Fig. 1. Kinetics of ATP breakdown (broken lines) and morphological degeneration (solid lines) in the presence of 8 mM NaF with human erythrocytes pretreated by trypsin, papain and anti-Rh_0(D).
● —— ● Control; ○ —— ○ trypsin;
+ —— + papain;
△ —— △ anti-Rh_0(D)

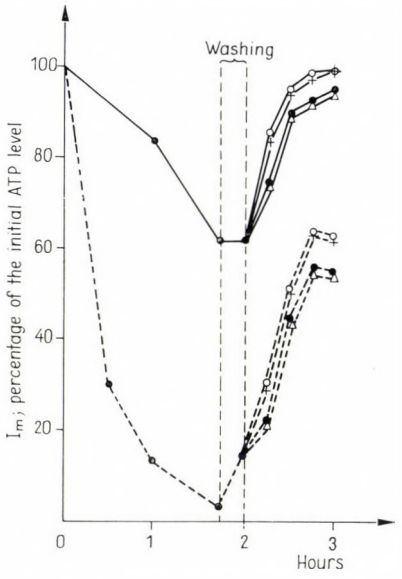


Fig. 2. Effect of trypsin, papain and anti-Rh_0(D) on fermentative ATP resynthesis (broken lines) and morphological regeneration (solid lines). For symbols see Fig. 1, for details of experiment see text

Table 1

Glucose consumption and lactate production in human erythrocytes pretreated with trypsin and papain

Time min.	Control		Trypsin		Papain	
	Glucose	Lactate	Glucose	Lactate	Glucose	Lactate
	in μmoles/ml erythrocyte suspension					
0	15.6	0.28	16.2	0.11	15.9	0.29
60	15.0	1.44	15.7	1.10	15.3	1.45
120	14.4	2.64	15.4	1.70	14.6	2.84

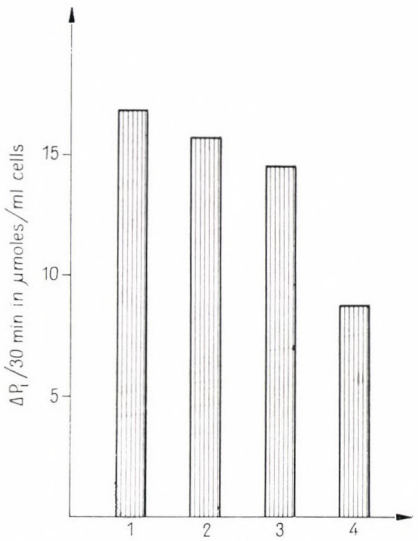


Fig. 3. "ATPase activity" of haemolysates of human erythrocytes pretreated with trypsin, papain and anti-Rh_0(D). 1: control; 2: trypsin; 3: anti-Rh_0(D); 4: papain

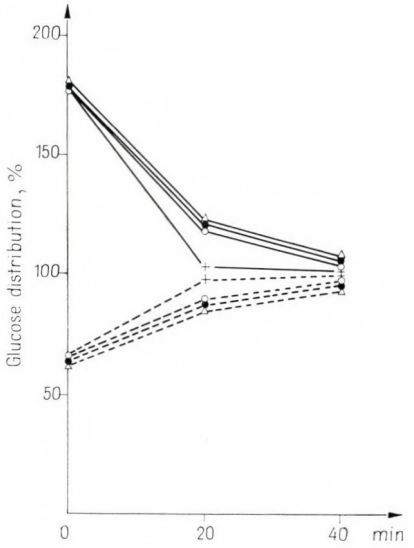


Fig. 4. Kinetics of glucose transport of human erythrocytes pretreated with trypsin, papain and anti-Rh_0(D). Glucose content of erythrocytes is represented by broken lines, glucose content of the medium by solid lines. For symbols see Fig. 1

decreased only to a very small extent in trypsin-treated erythrocytes, morphological degeneration slowed down significantly (Fig. 1). On fermentative regeneration of erythrocytes in the presence of trypsin the rates of both the ATP resynthesis and morphological regeneration increased (Fig. 2). ATP utilization by trypsin-treated erythrocytes decreased a little (Fig. 3), both glucose consumption and lactate production slowed down (Table 1), but the rate of glucose uptake did not change (Fig. 4). Neither did the ^{42}K influx differ from that of the untreated erythrocytes.

Effect of papain treatment

Pretreatment or treatment with papain produced the same changes in morphology and ATP content as trypsin: the rate of ATP breakdown in the presence of glycolysis inhibitors slowed down to a small, that of the morphological degeneration to a significant extent (Fig. 1), the fermentative resynthesis and morphological regeneration accelerated (Fig. 2). Unlike trypsin, papain caused a pronounced decrease of ATP utilization by erythrocytes (Fig. 3). The rate of glycolysis did not change significantly (Table 1), the rate of glucose transport, however, increased significantly (Fig. 4). There was no change in the rate of ^{42}K influx as compared with that of untreated erythrocytes.

Effect of sensitization with anti-$Rh_0(D)$

The rate of ATP breakdown in the presence of glycolysis inhibitors decreased to a well measurable extent in $Rh_0(D)$ positive erythrocytes sensitized with anti-$Rh_0(D)$, and the rate of morphological degeneration slowed down in accordance with this (Fig. 1). In the fermentative resynthesis experiments the sensitization with anti-$Rh_0(D)$ caused no significant changes as compared with the controls (Fig. 2). ATP utilization was measurably diminished by this treatment (Fig. 3), but glucose transport was practically unaffected (Fig. 4). There was a small decrease in the rate of ^{42}K influx.

Effect of lipase treatment

On lipase treatment washed erythrocytes went through a rather rapid sphere transformation (almost 100% within 1 hour) without a significant loss in ATP content. But this phenomenon proved to be reversible, in response to serum or serum protein fractions the cells regained their original disk form.

Discussion

Ponder [3] demonstrated that the shape-transformation effect of haemolysins is strongly influenced by the proteins of the medium and erythrocyte membrane. Contrary to this, we have found that the change in shape caused by internal changes in metabolism is not influenced by the presence, absence or modification of the superficial protein film. As it is known from the works of Schulman [17, 18] the drugs with phenolic groups, e.g. tannin and p,p'-dihydroxystilbene get adsorbed to protein monolayers, and so modify their structure, but do not disperse them. Thus, they cannot penetrate, and haemolyze the cells, and they have, even at high concentration, an agglutinating effect only. Our experiments show as well that the modification (tanning) of the superficial protein-film of erythrocytes and the accessible protein components of the membrane surface, resp. does not influence the changes in shape regulated by ATP.

The situation is different in the case of agents reacting with the deeper structures of membrane. As it is known, trypsin decomposes the sialic acid layer on the outer surface of the erythrocyte membrane [19, 20] and, penetrating the membrane structure, it destroys some blood group antigens (M, N) [21] and increases the reactivity of some others [$Rh_0(D)$] [22]. Furthermore, there is Whittam's [23] conception, according to which both the blood group antigens and erythrocyte membrane enzymes are localized in the so-called elinin plaques. Among the membrane proteins isolated the elinin rods resemble most closely the structure of the myofibrils [24], so it may be supposed that this fraction plays a role in the maintenance of the shape of erythrocytes. Furthermore, trypsin, on the basis of its known effects, seems to act at the site of the elinin plaques. Unfortunately, it cannot be proved directly whether it is the elinin that

represents the "shape-maintaining ATPase" activity, as Wheeler and Whittam [25] demonstrated that the preparative methods used in the isolation of elinin destroy ATPase activities.

The most probable explanation for the effect of trypsin on morphology is that it activates the "shape-maintaining ATPase", while diminishes some other ATP-utilizing reactions (so e.g. hexokinase activity), providing thus excess ATP for the activated shape-maintaining mechanism. In this respect, "shape-maintaining ATPase" is similar to the smooth muscle myosin-ATPase, which can be activated by trypsin as well [26]. The effect of papain on the shape-maintaining mechanism resembles that of trypsin. We cannot tell, however, which ATP-utilizing reactions are damaged by papain, how is the excess ATP rendered available for the activated shape-maintaining mechanism. The data of the experiments on ^{42}K accumulation indicate that transport-ATPase is affected neither by trypsin, nor by papain. From the point of view of the site of action of papain, theoretically it is interesting that the rate of glucose uptake is significantly enhanced by this enzyme. This can be well made consistent with the idea that the glucose carrier is localized in the elinin-plaques as well [27].

The decrease in glycolysis rate of $Rh_0(D)$ positive erythrocytes sensitized *in vitro* by specific antibodies has already been demonstrated [28]. In our experiments we could prove the decrease of ATP utilization of haemolysates prepared from erythrocytes coated by anti-$Rh_0(D)$. We experienced also the partial inhibition of ^{42}K uptake in antibody-sensitized erythrocytes. Concordant results have been obtained in experiments with glycolysis inhibitors, too: there was a well measurable decrease in the rate of ATP breakdown which sufficiently explained the decrease in the rate of morphological degeneration. This suggests that the probable loss in the hexokinase and transport-ATPase activities results in a higher ATP level in the presence of IA, and this is utilized by the unaffected shape-maintaining mechanism. Naturally, the antibody sensitization resulting in a decreased glycolysis rate is not advantageous for the morphology during fermentative ATP resynthesis.

We could bring about an effective sphering, similar to the effect of snake venoms with haemolytic and phospholipase activity, with a lipid splitting enzyme preparation of pancreatic origin. As it is known, the effect of snake-venom phospholipase *A* on erythrocyte membrane lipids comes forth even in the presence of serum proteins, but sphering effect does not occur under these circumstances [29, 30]. In our experiments the disk shape could be protected or regenerated by serum and serum protein fractions as well. The fact that the elimination of the labile 30% of the erythrocyte lipids by Lovelock's method did not result in a similar effect shows that the site of action of lipase effect that produces sphering must be searched in the stronger bound membrane lipids.

We do not deem contradictory the connection between membrane convexity and lipid distribution demonstrated by autoradiographic examinations on the one hand, and the data about the dependence of shape on ATP content and membrane proteins on the other. In our opinion it seems most probable that

the contractile protein rods, which have different lengths and which possess ATPase activity, are connected by lipids. When ATP utilization is possible, they are in contracted state, and maintain biconcave shape, but if the fixation of the end points at lipoids ceases, contraction is no more possible, sphere transformation occurs. Thus in the maintainance of biconcave shape the contractile protein rods play an active functional role, while the lipids are of structural importance.

*

Authors are indebted to Dr. J. Gergely for the preparation of immunochemically pure IgG of high anti-Rh_0(D) titre. For the skilful and precise technical assistance thanks are due to Mrs. Eva Irmai and Miss Susan Keresztes.

References

1. Nakao, M., Nakao, T., Yamazoe, S., Yoshikawa, H.: Adenosine triphosphate and shape of erythrocytes. *J. Biochem. (Tokyo) 49*, 487 (1961).
2. Ohnishi, T.: Extraction of actin and myosin-like proteins from erythrocyte membrane. *J. Biochem. (Tokyo) 52*, 307 (1962).
3. Ponder, E.: Hemolysis and Related Phenomena. Grune and Stratton, New York (1948).
4. Kates, M., Allison, A. C., James, A. T.: Phosphatides of human blood cells and their role in spherocytosis. *Biochim. Biophys. Acta 48*, 571 (1961).
5. Murphy, J. R.: Erythrocyte metabolism. VI. Cell shape and the location of cholesterol in the erythrocyte membrane. *J. Lab. Clin. Med. 65*, 756 (1965).
6. Gárdos, G., Straub, F. B.: Über die Rolle der Adenosintriphosphorsäure in der K-Permeabilität der menschlichen roten Blutkörperchen. *Acta Physiol. Hung. 12*, 1 (1957).
7. Furchgott, R. F., Ponder, E.: Disk-sphere transformation in mammalian red cells. *J. Exp. Biol. 17*, 30 (1940).
8. Lovelock, J.: The physical instability of human red blood cells. *Biochem. J. 60*, 692 (1955).
9. Dacie, J. V.: Practical Haematology. Chemical Pub. Co., New York (1956).
10. Gárdos, G., Szász, I., Árky, I.: Structure and function of erythrocytes. I. Relation between the energy metabolism and the maintenance of biconcave shape of human erythrocytes. *Acta Biochim. Biophys. Hung. 1*, 253 (1966).
11. Deutsch, A., Nilsson, R.: Ion exchange chromatography of inosine phosphates. *Acta Chem. Scand. 7*, 1288 (1953).
12. Bücher, Th.: Über ein Phosphatübertragendes Gärungsferment. *Biochim. Biophys. Acta 1*, 292 (1947).
13. Fiske, C. R., SubbaRow, Y.: The colorimetric determination of phosphorus. *J. Biol. Chem. 66*, 375 (1925).
14. Hagedorn, H. C., Jensen, B. N.: Die Ferricyanidmethode zur Blutzuckerbestimmung. *Biochem. Z. 137*, 92 (1923).
15. Barker, S. B., Summerson, W. H.: The colorimetric determination of lactic acid in biological material. *J. Biol. Chem. 138*, 535 (1941).
16. LeFèvre, P. G., McGinniss, G. F.: Tracer uptake vs. net uptake of glucose through human red cell surface. New evidence for carrier mediated diffusion. *J. Gen. Physiol. 44*, 87 (1960).
17. Rideal, E. K., Schulman, J. H.: Reactions with monolayers and their biological analogies. *Nature 144*, 100 (1939).
18. Schulman, J. H.: In "Cytology and Cell Physiology", Bourne, G. H. (Editor) 2nd ed. Oxford Univ. Press, London (1951).

19. Ponder, E.: Effects produced by trypsin on certain properties of the human red cell. *Blood 6*, 350 (1951).
20. Cook, G. M. W., Heard, D. H., Seaman, G. V. F.: Sialic acids and the electrokinetic charge of the human erythrocyte. *Nature 188*, 1011 (1960).
21. Cook, G. M. W.: Linkage of sialic acid in the human erythrocyte ultrastructure. *Nature 195*, 159 (1962).
22. Morton, J. A., Pickles, M.: Use of trypsin in the detection of incomplete anti-Rh antibodies. *Nature 159*, 779 (1947).
23. Whittam, R.: Transport and Diffusion in Red Blood Cells. Arnold, London (1964).
24. Moskowitz, M., Calvin, M.: On the components and structure of the human red cell membrane. *Exp. Cell Res. 3*, 33 (1952).
25. Wheeler, K. P., Whittam, R.: Structural and enzymic aspects of the hydrolysis of adenosine triphosphate by membranes of kidney cortex and erythrocytes. *Biochem. J. 93*, 349 (1964).
26. Bárány, M., Bárány, K., Gaetjens, E., Bailin, G.: Chicken gizzard myosin. *Arch. Biochem. Biophys. 113*, 205 (1966).
27. Aleksandrowitz, J., Gaertner, H., Urbanczyk, J.: In "Nuclear Hematology", Szirmai, E. (Editor). Academic Press, New York (1965).
28. Storti, E., Vaccari, F., Baldini, E.: Changes in red cell metabolism in presence of incomplete antibodies. *Experientia 12*, 108 (1956).
29. Ibrahim, S. A., Thompson, R. H. S.: Action of phospholipase A on human red cell ghosts and intact erythrocytes. *Biochim. Biophys. Acta 99*, 331 (1965).
30. Kurup, P. A.: Action of phospholipase A of the venom of Heterometrus Scaber on human red cell ghosts and intact erythrocytes. *Naturwissenschaften 53*, 84 (1966).

Observations on the Survival of ^{51}Cr-tagged Platelets in Healthy Subjects and in Patients with Thrombocytopenia

S. Pawelski, L. Konopka, Barbara Kotelba-Witkowska, K. Rechowicz, J. Błaszczyk

Department of Clinical Medicine, Department of Blood Conservation and Preparation, Department of Haematology, Institute of Haematology, Warsaw, Poland

(Received May 15, 1967)

Survival time of ^{51}Cr-tagged platelets was determined and the surface radioactivity was measured over spleen and liver in a group of 10 healthy subjects and 16 patients with various types of thrombocytopenia. The normal values obtained in the control patients approached those reported by other authors. On the other hand, in all 9 patients with idiopathic thrombocytopenia platelet survival was reduced. In one case of the group the platelet life span was normalized after splenectomy. At the same time the haemorrhagic manifestations disappeared and the platelet count increased. The platelet survival was either normal or shortened in the group of patients with symptomatic thrombocytopenia associated with impaired formation of platelets. Determination of platelet life span and morphological examination of the thrombopoietic system are valuable in the diagnosis of the mechanism of thrombocytopenia. The determination of the spleen–liver index is helpful in setting up the indication of splenectomy.

Determination of the life span of platelets tagged with radioactive isotopes is of essential importance in the diagnosis and classification of thrombocytopenias. These functional tests can complete the as yet not quite satisfactory morphological diagnostic criteria of thrombocytopenia based on the investigations of the thrombopoietic system (megakaryocytic and amegakaryocytic thrombocytopenias). Determination of the survival of labelled platelets permits us to gain a better insight into the mechanism of thrombocytopenia, which may be due to [3, 5, 9]:

1. Increased breakdown of platelets in the peripheral blood associated with increased or normal thrombopoiesis (e.g. idiopathic thrombocytopenia, immunothrombocytopenia).

2. Normal survival time and impaired production of platelets in the bone marrow (e.g. postirradiation thrombocytopenia, or thrombocytopenia due to a toxic influence of various chemical substances).

3. Impaired production associated with decreased platelet life span (e.g. thrombocytopenia in hypersplenism).

The methods of determination of the survival time of tagged platelets are also important when it is necessary to assess the biological value of preparations of platelet concentrates for clinical use [2, 6, 7]. It has been shown that the autologous, as well as the isologous platelets survive in the blood of a healthy recipient for 8–11 days [4, 5, 8, 9, 10]. This fact has made it possible to use isologous

platelets for the determination of platelet survival in patients with pronounced thrombocytopenia, in whom it is difficult to obtain satisfactory concentrates of autologous platelets [9].

Of the radioactive isotopes (^{32}P, ^{51}Cr, ^{35}S, ^{14}C), radioactive chromium is most widely used in clinical investigations for the tagging of platelets [2, 3, 4, 5, 8, 9, 10]. The method allows not only the determination of the platelet survival, but also the identification of the site of greatest platelet breakdown by measurements of surface radioactivity. The demonstration of increased radioactivity over the spleen and simultaneous shortening of the platelet life span might be useful for establishing the indications for splenectomy in idiopathic thrombocytopenia [3, 5, 9, 10].

Consistent results could be obtained by the use of fresh and active platelet concentrates. The isoimmunization phenomena appearing in patients after repeated blood transfusions restrict somewhat the determination of platelet survival time. The frequency of development of anti-platelet isoantibodies which shorten the survival time of transfused platelets increases with the number of transfusions [5, 9, 13].

This work has been undertaken in order to find the normal value of platelet survival and at the same time to evaluate the biological properties of the platelet concentrate which is being produced at this Institute. Isologous platelets were tagged with ^{51}Cr and the site of their breakdown in various types of thrombocytopenia was determined.

Material and Methods

Blood from healthy donors was collected into siliconized bottles containing the ACD solution. It was centrifuged at 4° C at 800 r.p.m. for 30 min., then the platelet-containing supernatant was decanted and centrifuged again at 1,700 r.p.m. for 30 min. The residue, which was free of erythrocytes and leucocytes, was suspended in 10 ml of a solution made of 1 vol. 0.9% saline and 9 vols. of 3.3% $^{51}CrO_4$ of neutral sodium citrate. $Na_2{}^{51}CrO_4$ (300 μc) was added and the suspension incubated for 30 min. Thereafter, 1 vol. of platelet-free plasma was added, mixed and 2 ml aliquot was withdrawn for the determination of total radioactivity. The suspension was centrifuged and the sediment resuspended in 30 ml of a saline-sodium citrate mixture which contained 100 mg of vitamin C. An aliquot (2 ml) was withdrawn for the determination of radioactivity and the platelet suspension was transfused into recipients, using a plastic set for blood transfusions. Samples of blood (20 ml) were taken from the subjects each day for 8 consecutive days into siliconized tubes. The platelets were isolated by the differential centrifugation method and resuspended in 2 ml of a sodium citrate solution in 0.9% saline. The following values were calculated from determinations of the radioactivity of samples in a scintillation counter:

1. the effectiveness of tagging: i.e. the ratio of platelet-bound radioactivity to total radioactivity applied.

2. the percentage of platelets recovered in 24 hours after transfusion, using the formula given by Baldini et al. [2].

3. the survival time of ^{51}Cr-tagged platelets, which was determined by extrapolation to the radioactivity of samples on consecutive days, accepting as 100% the radioactivity found 24 hours after the transfusions.

4. the spleen–liver index: measurements of radioactivity over spleen, liver and heart, respectively, were performed daily for 8 days by the method of Najean et al. [9].

The investigations included:

1. a control group of 10 patients from our Department showing no abnormalities in thrombopoiesis and with no transfusion of blood or platelet concentrate in the history. The data obtained in this group were accepted as normal values.

2. a group of 16 patients with thrombocytopenia. In 9 cases of this group idiopathic thrombocytopenia had been recognized on the ground of the clinical and haematological findings. The duration of the disease varied from several months to several years. Splenomegaly was present in only one patient (case 2). With the exception of one case (case 4), the remaining 8 patients were treated with corticosteroids and in 7 of them this treatment was followed by a temporary disappearance of the haemorrhagic manifestations and an increase of the platelet count. In 7 other cases there was a symptomatic thrombocytopenia associated with a proliferative syndrome (cases 10, 11, 13, 14, 15), gastric carcinoma (case 16) and toxic damage to the bone marrow (case 12).

None of these patients had haemorrhage and none of them was transfused when these investigations were being performed. It is known that both haemorrhage and blood transfusions can affect the life span of platelets. In 12 patients investigations were begun at least 4 weeks after the end of treatment with corticosteroids and in only 5 cases (cases 1, 7, 10, 12, 16) these drugs were administered as a vital indication during these investigations. Cases No. 10 and 11 with the proliferative syndromes were given cytostatic agents. One case was reinvestigated (case 1) at 9 months following splenectomy, while cases 3 and 4 were reinvestigated after 2 and 10 months of corticosteroid treatment, respectively.

Results and Discussion

The effectiveness of platelet tagging was $25.08 \pm 6.71\%$, as measured by the present method.

After platelet transfusion to 10 recipients from the control group no reactions were observed and after 24 hours $29.4 \pm 12.31\%$ of the transfused platelets were demonstrated in the circulation. Their survival time was 9.00 ± 0.26 days (with the range from 8 to 10.5 days). Thus the recovery as well as the survival time of the platelets agreed with the results obtained by other authors [2, 3, 4, 5, 8, 9, 10]. This shows that the method of preparation of platelet concentrates used at this Institute of Haematology is satisfactory and such concentrates may be used in therapy as well as for the determination of thrombocyte survival.

Table I

Results of studies in idiopathic thrombocytopenia

No.	Initials Age Sex	Platelet count, 1 cu. mm	Megakaryocytes in bone marrow			24-hour platelets recovery %	^{51}Cr-platelet survival in days	Spleen–liver index	Remarks
			Approximate evaluation	Quantitative evaluation in 1 cu. mm	Thrombopoietic function				
1.	S. M.	38,600	present	50	inhibited	0.56	1	3.0 : 1.0	before splenectomy
	38, M	148,600	numerous	45	normal	19.20	8	—	9 months after splenectomy
2.	K. H. 18, F	18,900	very numerous	—	inhibited	4.30	1	2.1 : 1.0	
3.	B. I.	38,640	present	35	partly inhibited	4.30	1	1.4 : 1.0	before treatment
	17, F	68,200	very numerous	333	normal	7.50	3.5	1.0 : 1.0	after 10 months of prednisone treatment, 20 mg daily
4.	Sz. P.	single	present	20	inhibited	0.84	1	0.5 : 1.0	before treatment
	31, M	15,250	present	20	normal	9.12	1	1.2 : 1.0	after 2 months of prednisone treatment, 40 mg daily
5.	P. A. 19, M	single	present	20	normal	0.12	1	2.0 : 1.0	
6.	G. H. 14, F	single	very numerous	73	normal	0.43	2	1.2 : 1.0	
7.	U. T. 15, F	52,380	present	25	normal	0.56	3	3.0 : 1.0	during prednisone treatment
8.	K. J. 26, F	18,000	very numerous	—	inhibited	0.77	1	0.4 : 1.0	
9.	G. R. 47, M	26,000	present	58	normal	8.90	3	0.4 : 1.0	

Measurements of surface radioactivity in the control group showed the mean spleen–liver index to be 1.2 : 1.0 (range: 0.8 to 1.5 – 1.0). This was accepted as normal.

The results of isotope and haematological studies in the group of idiopathic thrombocytopenia are compared in Table I and Figure 1. In all patients in this group the survival time of ^{51}Cr-tagged platelets was reduced, associated with the presence of numerous megakaryocytes (usually not platelet-forming) in the bone marrow. The megakaryocyte count in the bone marrow ranged from 20 to 78

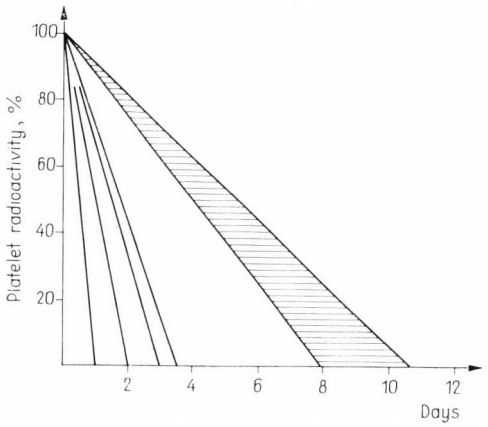


Fig. 1. Curves of platelet survival in idiopathic thrombocytopenia (the first curve is that of cases 1, 2, 3, 4, 5, 8). The hatched area corresponds to the range of normal values

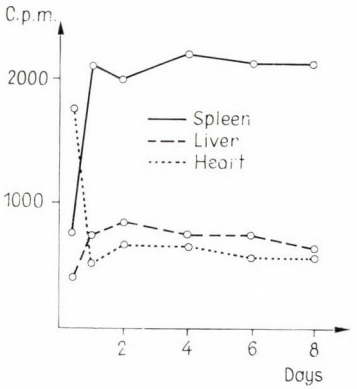


Fig. 2. Measurements of surface radioactivity from ^{51}Cr platelets in a case of idiopathic thrombocytopenia with good effect of splenectomy

(per 1 cu.mm.) with a marked prevalence of immature forms (Types I and II).* No close correlation could be found between decreased survival and the platelet count.

In 4 cases the spleen–liver index was increased (from 2 : 1 to 3 : 1), while in the remaining cases this index was normal or even decreased.

The data obtained permit us to recognize the shortening of the survival time of the platelets as the main cause of thrombocytopenia in this group. In 4 cases (cases 2, 3, 6, 7) decreased survival of ^{51}Cr-tagged platelets could be attributed to previous blood transfusions. The absence of detectable anti-platelet isoantibodies in these cases does not permit one to rule out completely their presence, since the methods for the detection of these antibodies are far from being precise.

In case 1 splenectomy was performed because of marked shortening of the survival time of ^{51}Cr-tagged platelets, their increased sequestration in the spleen (Fig. 2) and failure of prednisone therapy. Immediately after operation haemor-

* Thanks are due to E. Wichrzycka, M. D., who prepared the megakaryocytograms and furnished certain data included in this work.

Table II

Results of investigations in symptomatic thrombocytopenia

No.	Initials Age Sex	Clinical diagnosis	Platelet count in 1 cu. mm	Megakaryocytes in bone marrow			24-hour platelet recovery %	^{51}Cr-platelet survival in days	Spleen—liver index
				Approximate evaluation	Quantitative evaluation in 1 cu. mm	Thrombo-poietic function			
10.	D. E. 27, M	Chronic myelo-cytic leukaemia	55,500	single	—	normal	26.5	8.5	0.7 : 1.0
11.	E. W. 23, M	Chronic myelo-cytic leukaemia	24,960	single	1	normal	64.7	8.5	3.0 : 1.0
12.	S. A. 25, F	Drug-induced thrombocytopenia	51,600	single	—	normal	21.9	9.0	1.0 : 1.0
13.	Ch. F. 36, M	Acute myelocytic leukaemia	32,040	single	7	normal	5.4	2.5	1.7 : 1.0
14.	B. S. 25, M	Hodgkin's disease	63,800	single	—	normal	12.5	4.0	1.8 : 1.0
15.	Ch. W. 52, M	Chronic lympho-cytic leukaemia	single	present	13	normal	10.0	5.0	0.5 : 1.0
16.	K. A. 72, F	Gastric cancer	40,000	present	16	normal	8.1	4.5	1.0 : 1.0

rhagic manifestations disappeared and the platelet count was normalized. Nine months after splenectomy these investigations were repeated showing that the survival time of ^{51}Cr-tagged platelets and the thrombopoietic activity of the bone marrow (increased percentage of megakaryocytes type III and IV) have been normalized.

In two other patients (cases 3 and 4) prednisone treatment in daily doses of 20 and 40 mg for 10 months and 2 months, respectively, was followed in case 3 by a disappearance of overt signs of haemorrhagic diathesis, a twofold increase of platelet count and a slight increase of platelet survival, with a simultaneous increase of the megakaryocyte count to 333 per 1 cu.mm. and return of the normal thrombopoietic activity. In the second patient only a slight improvement was noted, with a decrease of the intensity of haemorrhagic manifestations. The platelet count in the peripheral blood remained, however, low and there was a striking shortening of the platelet survival.

Among the secondary thrombocytopenias (Table II) in 3 cases (cases 10, 11, 12), with the platelet count in the peripheral blood from 24,960 to 55,500 per 1 cu.mm., the 24-hour platelet recovery and survival were normal. In all these cases only single megakaryocytes were found in the bone marrow. This was true also for case 12, in which thrombocytopenia was supposed to be due to the toxic action of previously taken drugs (pyramidon, Gardan, phenacetin).

In the remaining 4 cases (cases 13, 14, 15, 16) decreased platelet recovery after 24 hours and shortening of the survival time were observed. These manifestations were, however, less pronounced than in the group of patients with idiopathic thrombocytopenia. The bone marrow of these patients was hypoplastic and it contained fewer megakaryocytes, with mature forms (types III and IV) prevailing.

The results for the patients with secondary thrombocytopenia show that in the first 3 cases thrombocytopenia was due exclusively to a decreased thrombopoiesis. In the remaining 4 cases decreased platelet survival might suggest that thrombocytopenia was due not only to a decreased thrombopoiesis, but also to thrombocytolytic factors present in the peripheral blood. Because of numerous blood transfusions performed in the 4 latter patients the possibility of isoimmunization should be taken into account, even though no thromboagglutinins were found. The reduction of platelet survival time indicates that, irrespective of the mechanism of this reduction, the possibility of treating these patients with thrombocyte concentrates is limited.

The results of the platelet survival determinations, as presented by us, are in agreement with the data reported by other authors concerning idiopathic, as well as secondary thrombocytopenia [3, 4, 5, 6, 9, 10]. In case of idiopathic thrombocytopenia the platelet survival is always reduced, apart from the periods of complete remission [5, 9, 10]. In certain cases in this group the platelet survival time may be normalized by treatment with corticosteroids or by splenectomy [5, 9, 10]. The treatment is followed by a disappearance of haemorrhagic manifestations and an increase of the count in the circulation. The latter effect, as

brought about by splenectomy, is mainly due to the removal of the primary destruction site of thrombocytes. The spleen is also known to delay the release of mature platelets from the bone marrow [5].

In most cases of secondary thrombocytopenia due to hypoplasia or aplasia of the thrombopoietic system the platelet survival is normal [3, 7, 9, 10] and the only cause of thrombocytopenia in these conditions is an impairment of platelet formation in the bone marrow. The investigations of Cohen et al. [5], just like ours, have shown, however, that hypoplasia of the thrombopoietic system may be associated with a shortened platelet survival. This phenomenon is commonly caused by immunological processes: autoimmunization due to the main disease (e.g. proliferative and neoplastic diseases) or isoimmunization due to blood transfusions. The unreliability of the present serological methods of detection of autoantibodies and isoantibodies does not permit to demonstrate a close correlation between their presence in the blood of patients with thrombocytopenia and shortening of the platelet survival. There is also no correlation between the frequency of detection of isoantibodies and the number of transfusions, or the volume of transfused blood [5, 9, 10, 13]. Shortening of the platelet survival, particularly in malignancy, may also be associated with thrombotic phenomena, which cause an increased breakdown of autologous, as well as of transfused platelets [12, 14]. Marked splenomegaly represents an additional factor which affects the platelet survival, due to an increase of the splenic pool of platelets. It has been shown that the spleen can accumulate up to 90% of all platelets causing an apparent shortening of their survival [1, 11].

The use of the spleen–liver index as a criterion to indicate splenectomy in idiopathic thrombocytopenia has increased the percentage of favourable results of this treatment. In 19 cases out of 22 described by Najean et al. [9], in which increased breakdown of ^{51}Cr-tagged platelets in the spleen was observed, very good late results of splenectomy have been obtained. Of 17 other patients with a low index, or even with an increased breakdown of ^{51}Cr-tagged platelets in the liver, only 6 benefited from splenectomy. Favourable late results of splenectomy, as in case 1, allow one to conclude that a more widespread use of the isotope criteria in indicating splenectomy in thrombocytopenias will be helpful in prognosis.

Conclusions

1. Normal survival time of ^{51}Cr-tagged platelets is 8 to 10.5 days, and the normal index of the spleen–liver surface radioactivity ranges from 0.8 to 1.5 : 1.0.

2. In the group of 9 patients with idiopathic thrombocytopenia shortening of the survival time of ^{51}Cr–tagged platelets was identified as the basic cause of thrombocytopenia.

3. In 3 patients out of 7 with secondary thrombocytopenia the survival time of ^{51}Cr-tagged platelets was normal and the only cause of thrombocytopenia

was a decreased production of platelets by the bone marrow. In the remaining 4 cases thrombocytopenia was due to a decreased platelet production and to thrombolysis.

4. Determination of the survival time of platelets and the morphological examination of the thrombopoietic system help to clarify the mechanism of thrombocytopenia.

5. The use of the spleen–liver index may be useful for establishing the indications for splenectomy in thrombocytopenia, as the prognosis of splenectomy can be predicted more accurately.

References

1. Aster, H. H.: Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. *J. Clin. Invest. 45*, 645 (1966).
2. Baldini, N., Costea, N., Dameshek, W.: The viability of stored human platelets. *Blood 16*, 1669 (1960).
3. Bleifeld, W., Gehrmann, G.: Über die Bedeutung der radioaktiven Markierung von Thrombozyten mit Cr^{51} in der Diagnostik von Thrombozytopenien. *Dtsch. med. Wschrft. 91*, 1594 (1966).
4. Burger, T.: Problems of determining thrombocyte life span. *Acta med. Acad. Sci. hung. 23*, 109 (1967).
5. Cohen, P., Gardner, F. H., Barnett, G. O.: Reclassification of thrombocytopenias by the Cr^{51}-labeling method for measuring platelet life span. *New Engl. J. Med. 264*, 1294 and 1350 (1961).
6. Cronkite, N. P.: Measurement of the effectiveness of platelet transfusion. *Transfusion 6*, 18 (1966).
7. Freireich, E. J.: Effectiveness of platelet transfusion in leukemia and aplastic anemia. *Transfusion 6*, 50 (1966).
8. Gradner, F. H., Cohen, P.: Platelet life span. *Transfusion 6*, 23 (1966).
9. Najean, Y., Ardailleu, N., Caen, J., Larrieu, M. J., Bernard, J.: Survival of radiochromium-labeled platelets in thrombocytopenias. *Blood 22*, 718 (1963).
10. Najean, Y., Larrieu, M. J., Bernard, J.: Survie de plaquettes marques au radiochrome. Étude de 104 observations. *Nouv. Rev. franç. Hémat. 1*, 36 (1961).
11. Penny, R., Rozenberg, M. C., Firkin, B. G.: The splenic platelet pool. *Blood 1*, 27 (1966).
12. Rapaport, S., Chapman, Ch. G.: Coexistent hypercoagulability and acute hypofibrinogenemia in a patient with prostatic carcinoma. *Amer. J. Med. 27*, 144 (1959).
13. Shulman, R. N.: Immunological consideration attending platelet transfusion. *Transfusion 6*, 39 (1966).
14. Verstraete, M., Vermylen, J., Vanderbroucke, J.: Excessive consumption of blood coagulation components as cause of hemorrhagic diathesis. *Amer. J. Med., 38*, 899 (1963).

Inherited Beta-Lipoprotein Characters (Ag- and Lp-System)

P. GUTH, R. BUDVÁRI
Institute of Forensic Medicine, University Medical School, Pécs

(Received May 26, 1967)

The human isoprecipitins of the beta-lipoprotein type, discovered by Allison and Blumberg in 1961, offer proof that human blood serum proteins have antigenic activity within the species ("allotypia"). They have clinical significance in the mechanism of development of complications following transfusion, in the compatibility of transplanted tissues and organs, and in the possibility of foetal-maternal immunization. As inherited serum protein systems, beta-lipoproteins play a significant role also in cases of disputed paternity. Authors have tested 532 blood sera for Lp(a, x) character. The Lp(a) character occurred in 39.47 per cent, the Lp(x) one in 17.29 per cent of the sera tested. By studying these properties, in paternity proceedings, 4.8 per cent exclusion could be achieved.

The theory of "horror autotoxicus", i.e., that the human or animal organism does not produce antibodies against its own substances (cells, proteins, erythrocytes) prevailed for a long time in classical immunology. The discovery of blood groups pierced the first hole into this theory, because it has meant the existence of "isoantibodies" within the species. Later, also "organospecific" antigens have been discovered, but until 1959 there had been no evidence to indicate that antibodies against proteins of normal blood serum would exist in man, although it had already been suspected that such an "allotypia" might exist. This hypothesis was supported, on the one hand, by the fact that, not infrequently, complications best explainable by a "protein incompatibility" developed, despite a strict adherence to transfusion regulations, following the transfusion of bloods compatible from apparently all respects. On the other hand, since 1956 various hereditary variants of the proteins of human blood serum, the so-called serum groups (haptoglobin groups, transferrin-, gamma globulin groups, Gc-, InV-system, etc.) have been discovered and shown to be capable of producing by virtue of their antigenic properties antibodies within species.

The first antibody of isoprecipitin nature was discovered in 1961 by Allison and Blumberg in the serum of a patient (C. de B.) who had received repeated transfusions [1]. This serum precipitated 57 per cent of the normal human serum samples in the Ouchterlony agar-gel diffusion test. The antigen, designated by the symbol Ag(a), proved to be a beta-lipoprotein. Within a relatively short time, several antigens belonging to this system have been discovered and designated Ag(a), Ag(b) and Ag(x), in analogy to the designations used in the Gm

system. The Ag(b) character was discovered in 1963 by Blumberg and Riddel [5], and the Ag(x) by Hirschfeld [15]. According to Bundschuh [8], in Berlin the incidence of the Ag(a) property was 65 per cent, of the Ag(x) 35 and of the Ag(b) 97 per cent. In Hungary, Horváth and Simon [16] demonstrated the Ag(a) character in 58.8 per cent, while according to Kobiela it occurred in 40.7 per cent in Poland.

Attempts have been made by a few researchers to produce by artificial immunization anti-lipoprotein antibodies, which were obtained earlier from a natural source: the blood serum of patients who had received repeated transfusions. (Strictly speaking, in Hungary Gergely et al. [12], studying the immunopathological mechanism of arteriosclerosis, had prepared rabbit serum containing anti-lipoprotein antibodies.)

In 1963, Berg produced anti-lipoprotein serum by injecting purified human beta-lipoprotein into rabbits. This serum, however, precipitated only 34.08 per cent of the sera, i.e. in a lower percentage than the anti-Ag(a) serum. The newly-discovered lipoprotein antigen was designated by Berg with the symbol Lp(a) [2].

In 1964, Bundschuh immunized horses with various human sera, in an effort to prepare anti-Gc serum. Following absorption with Lp(a)-negative human sera, the precipitating anti-human horse serum proved to be suitable for use also for the demonstration of the Lp(a) character. The blood samples examined by this serum showed in about 20 per cent double precipitation band, invariably associated with the Lp(a) property. Bundschuh [8, 10] designated the new antigenic character, closely conjugated with the Lp(a) one, with the symbol Lp(x). According to Bundschuh and Vogt the Lp(a) character had almost the same incidence rate, 35 per cent, as that reported by Berg. Vámosi and Prokop report on a 35.1 per cent [19], Jarosch [17] for Upper Austria a 35.3 per cent incidence of the Lp(a) character in the sera tested. Recently, Bundschuh (personal communication) [7] has probably discovered by means of his anti-Lp rabbit serum a new Lp-character, called for the time being Lp(e). It seems to occur in 50 per cent.

In 1965, Berg reported to have found in the serum of a polytransfused patient such an isoprecipitin which reacted in 42 per cent with normal human sera [3]. In view of the fact that, like the characters Ag(a), Ag(b), Ag(x), Bp(a) and Lp(x), the newly-detected antigen proved to be a low-density lipoprotein, it was designated Ld(a). It was also called cold precipitin, being active from 4 to 20° C, but not at 37° C.

Thus, today we know 6 different, inheritable antigens of the beta-lipoprotein of blood serum, each of which can be demonstrated exclusively with homologous anti-serum: Ag-system: Ag(a), Ag(b), Ag(x); Lp-system: Lp(a), Lp(x), Lp(e) ?; Ld-system: Ld(a).

In view of the fact that all the different isoprecipitins have shown beta-lipoprotein specificity and the antigens are all low-density beta-lipoproteins, Blumberg [5] suggested that they should be uniformly termed Lp-system. By immunological and statistical methods Rittner [18] (1966) showed, however, that the Ag-system and the Lp-system are independent systems in the human

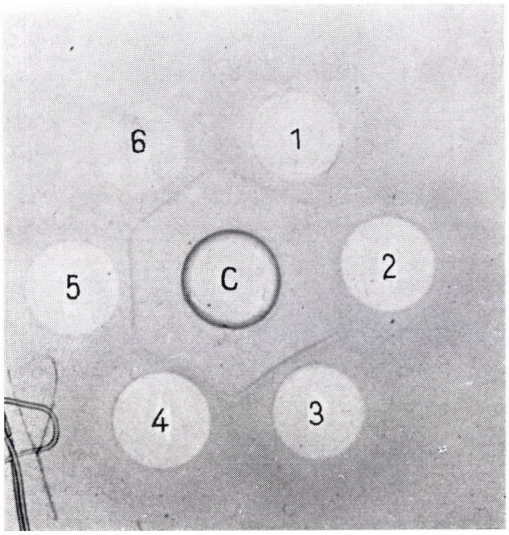


Fig. 1. Agar-gel diffusion preparation (stained) for the determination of Lp(a) and Lp(x) characters

blood serum, therefore it has remained justified to designate them with different symbols. At the same time, he suggested that the Ag- and Lp-antigens might be linked to the same beta-lipoprotein molecule as partial antigens.

In 1966, at the beta-lipoprotein symposium in Marburg, Berg [2] pointed out the technical difficulties of demonstrating the Ag- and Lp-properties. According to this author evaluation may be falsified or unreliable in case anti-Lp or anti-Ag sera are over- or under-absorbed or the agar-gel is contaminated. Circular lipoprotein precipitates around the wells and sera from haemolyzed blood present further sources of error in the Ouchterlony techniques. Therefore the participants

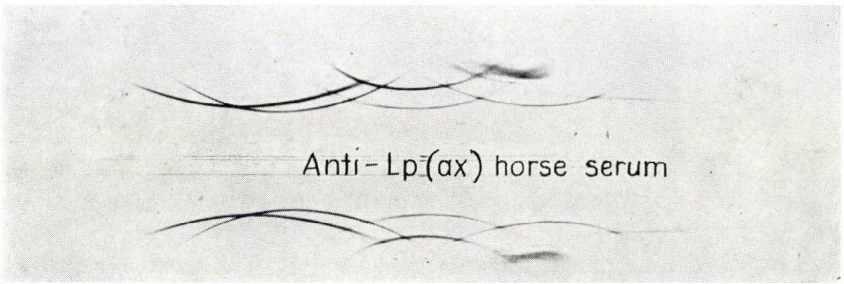


Fig. 2. Precipitations of normal human sera after electrophoresis with unabsorbed anti-Lp(a, x) horse serum. Note thick beta-lipoprotein band

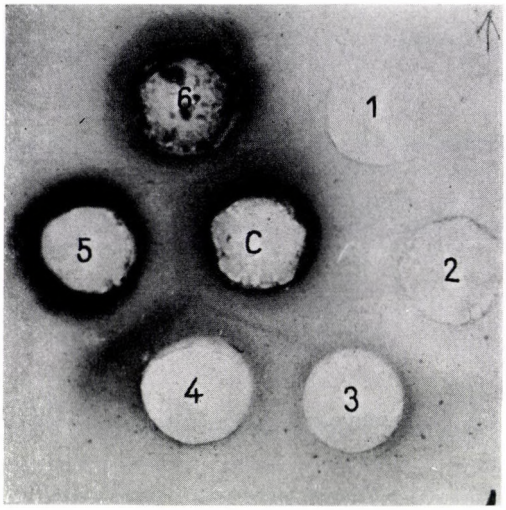


Fig. 3. In central well absorbed anti-Lp(a, x) horse serum, in well 4 maternal serum, in well 5 serum from V. E., aged 4 months

of the symposium agreed in that the reproducibility of the results with the same serum under standard circumstances (distance of diffusion, temperature, time of reading the result, staining, etc.) is an essential requirement of the reliability of testing.

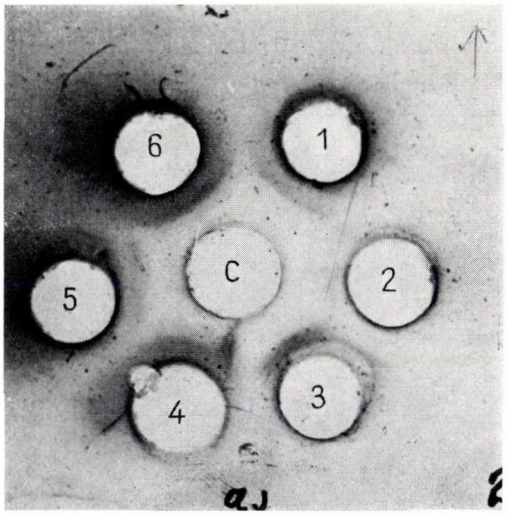


Fig. 4. In central well V. E.'s serum, in wells 1 to 6 adult normal human sera. Only adult serum 2 was not precipitated

It is necessary to check the results for instance in paternity cases, at other Institutes.

Since 1965, routine tests are being carried out using anti-Lp(a, x) horse serum received from Dr. Bundschuh at the Institute of Forensic Medicine, University Medical School, Pécs. The immune precipitation technique is used. On suitably defatted slides 1.5 per cent veronal or phosphate buffer agar-gel is poured to a layer thickness of about 2 mm. After the gel has solidified, 7 wells 2 to 2.5 mm in diameter each are made in it, and the gel is sucked off from the wells. In the central well (C) horse serum absorbed with Lp(a, x) human serum is placed, and clock-wise the serum samples to be tested (Fig. 1). Diffusion lasts 24 hours at room temperature, in a wet chamber. After washing and soaking in saline and distilled water, and drying, the preparations are stained with Sudan black or Amido-black.

The anti-Lp antibody content of the anti-Lp(a, x) horse serum had been demonstrated by running in agar-gel normal human sera, which were precipitated with our unabsorbed anti-Lp(a, x) horse serum, in an immunoelectrophoretic system. In stained preparations thick beta-lipoprotein band(s), staining selectively with Sudan black, were obtained from the start hole toward the anode, behind the alpha$_2$-macroglobulin arc and between the transferrin and the start hole (Fig. 2).

During the past 2 years we tested 532 serum samples, received partly from blood-typing material, partly from the Blood Service, Pécs; 39.47 per cent of the sera were Lp(a) positive and 17.29 per cent were Lp(x) positive [in association with Lp(a) character].

Not much is known as yet about the biological significance of the Lp-system. Berg, on the basis of his experiments, assumes that the survival time of transplanted human skin is correlated with the Lp-compatibility of the donor and recipient. It is another important observation that isoprecipitins may be produced not only in response to repeated transfusions, but also as a result of foetal-maternal incompatibility. In 1965, Dürwald demonstrated anti-beta-lipoprotein antibodies in sera from women pregnant the sixth or the seventh time [11]. We, too, have regularly tested sera from infants 3 to 6 months of age against maternal sera, to demonstrate whether they contained antibodies precipitating maternal proteins. Sera from two children proved to do that. In one case, that of an infant four months of age, precipitating antibodies were produced following the transfusion of 60 ml of blood of the same blood group after antrotomy [6, 14] (Fig. 3). In the other case, that of a 6-month-old child, the mother had been treated against syphilis during pregnancy, the infant was given no transfusion. In both cases the infants' sera strongly precipitated the maternal serum, as well as about 90 per cent of the normal adult human sera tested (Fig. 4).

Geserick et al. [13] have shown that the mechanism of inheritance for the antigens of beta-lipoprotein character is identical with that of the Gm-characters and thus the Lp-system may be used in paternity tests. Theoretically paternity may be excluded in 18 per cent of doubtful cases. In our material of 131 cases

we could rule out 7 men out of 144, i.e. 4.8 per cent. The exclusion of two of the seven men was confirmed on the basis of AB0 and Rh characters. Owing to the technical difficulties outlined above, the routine use of the beta-lipoprotein system is not yet practicable in the cases of questionable affiliation.

References

1. Allison, A., Blumberg, B. S.: An isoprecipitation reaction distinguishing human serum protein types. *Lancet I*, 634 (1961).
2. Berg, K.: A new serum type system in man — the Lp system. *Acta Path. microbiol. scand. 59*, 369 (1963).
3. Berg, K.: A new serum type system in man — the Ld system. *Vox Sang. 10*, 513 (1965).
4. Blumberg, B. S., Alter, H. J., Riddel, N. M., Erlandson, M.: Multiple antigenic specificities of serum lipoproteins detected with sera of transfused patients. *Vox Sang. 9*, 128 (1964).
5. Blumberg, B. S., Riddel, N. M.: Inherited antigenic differences in human serum beta-lipoproteins. A second antiserum. *J. clin. Invest. 42*, 867 (1963).
6. Budvári, R.: A szérumfehérjék öröklődő csoporttulajdonságainak jelentősége. *Orv. Hetil. 37*, 1729 (1965).
7. Bundschuh, G.: Personal communication.
8. Bundschuh, G.: Anti-Lp(a, x) vom Pferd. *Ärztl. Lab. 10*, 309 (1964).
9. Bundschuh, G., Geserick, G., Marek, Z., Fünfhausen, G.: Anti-Ag nach 16 Transfusionen—Frequenz in der Berliner Bevölkerung. *Dtsch. Gesundheitswesen 18*, 819 (1963).
10. Bundschuh, G., Vogt, A.: Die Häufigkeit des Merkmals Lp(x) in der Berliner Bevölkerung. *Humangenetik 1*, 379 (1965).
11. Dürwald, W., Leopold, D., Krämer, K.-H.: The formation of precipitating antibodies after multiple pregnancies. *Vox Sang. 10*, 94 (1965).
12. Gergely, J., Gerő, S., Jakab, L., Székely, J., Virág, S., Czuppon, A.: Vizsgálatok beta-lipoproteid antigénnel. *Orv. Hetil. 102*, 1450 (1961).
13. Geserick, G., Bundschuh, G., Ringk, H.: Über die Verwertbarkeit der menschlichen Serumeigenschaft Ag in der forensischen Serologie. *Dtsch. Gesundheitswesen 18*, 1668 (1963).
14. Guth, P., Budvári, R.: Vorkommen eines anti-Lp(a) ähnlichen Isopräzipitins im Säuglingsserum. In press.
15. Hirschfeld, J.: Investigations of a new anti-Ag antiserum with particular reference to the reliability of Ag typing by micro-immunodiffusion tests in agar gel. *Science Tools 10*, 45 (1963).
16. Horváth, E., Simon, J.: Anti-Ag ellenanyag előfordulása politranszfundáltak savóiban. *Haemat. hung. 6*, 209 (1966).
17. Jarosch, K.: Ein »neues« Erbmerkmal des menschlichen Serums Lp(a) und seine Phaenotypenfrequenz in Oberösterreich. *Wien. klin. Wschr. 115*, 839 (1965).
18. Rittner, Ch.: Bestehen Beziehungen zwischen dem Ag- und dem Lp-System? *Z. Imm. Allerg. Klin. Imm. 130*, 229 (1966).
19. Vámosi, M., Prokop, O.: Über die Frequenz von Lp im Raum Halle. *Z. ärztl. Fortbild. 60*, 827 (1966).

Study of Blood Coagulation in Familial Benign Chronic Pemphigus of Gougerot-Hailey-Hailey

I. Pastinszky, M. Kovács
Hungarian Army Medical Corps

(Received May 28, 1967)

Detailed coagulation studies have been made in 15 cases of chronic familial pemphigus, originating from 10 families.

The Rumpel—Leede sign was always negative. In two cases pinhead-sized haemorrhages were seen in the skin of the neck, where it was in touch with the collar. In these patients pinching the neck produced bleedings of pinhead-size (Jürgens' test). In some cases the cutaneous changes appeared at times as bloody-serous crusts, obviously vascular in origin, as shown by the normal thrombocyte count and coagulogram. The thrombelastogram showed an increased coagulability in one case and an increased rate of fibrinolysis in another. Ultimately, no constant defect in the coagulation system could be demonstrated in chronic benign familial pemphigus, just like it is the case with the various types of epidermolysis hereditaria bullosa, the other commonest type of vesicular genodermatosis.

Blood clotting studies in cases belonging to the pemphigus group of disease were carried out for the first time by Soulier [22]. This author demonstrated an increased antithrombokinase activity in three cases accompanied by manifest haemorrhagic diathesis.

In a previous paper (Pastinszky et al. [17]) we described 12 cases of pemphigus and demonstrated true hypoprothrombinaemia in 9 patients, an increased antithrombokinase activity in 1 case. The three cases of true hypoprothrombinaemia that were given vitamin K gave but a transient response. A moderate hypoprothrombinaemia was observed in only one of our six cases of dermatitis herpetiformis Duhring.

Especially interesting are the reports describing in various kinds of bullous dermatitis (pemphigus vulgaris, pemphigus erythematosus, dermatitis herpetiformis), conditions similar to haemophilia, due to the presence in circulating blood of antithrombokinase-like anticoagulant substances ("Hemmkörper Haemophilia", Taylor's disease) (Soulier [22]; Klingmüller et al. [10, 11]). It might be suggested that the pemphigus disease itself might evoke the process of autosensitization against the patient's own thrombokinase.

Among the genodermatoses accompanied by bullous efflorescence the two commonest are chronic benign familial pemphigus (in the following: CBFP) and epidermolysis hereditaria bullosa (EHB), while the bullous types of Darier's disease, ichthyosis bullosa and epidermodysplasia verruciformis bullosa (Herzberg [5, 8]) are less common.

Blood clotting has been studied by numerous authors (Dorn [5], Tiong Hoo Tio et al. [23], Plenert [18], Sardinsky [19], Baumann [2], Cox [4], Lewis [13], Zum Winkel [24], Langhof [12]) in the various forms of EHB, viz., (1) EHB simplex Köbner, (2) EHB dystrophica or polydysplastica Hallopeau-Siemens (including Herlitz's lethal epidermolysis bullosa), (3) EHB dystrophica Cockayne-Touraine (including Pasini's EHB albopapuloides). Most of the authors described hypercoagulopathy, a tendency to thrombosis, in EHB [4, 7, 13, 24]. A reduction of clotting time was found by Dorn [5], as well as Tiong Hoo Tio [23]. Alexander et al. [1] have observed an association of EHB and parahaemophilia (hereditary deficiency of factor V) in three generations, without the two diseases having influenced each other. In the Köbner type of EHB Plenert [18] found increased thrombocyte counts at intervals. Linser [14] demonstrated, with normal coagulation time, an increased heparin-like antithrombin level. The coagulogram was often normal (Sardinsky [19], Tiong Hoo Tio [23]). Recently, Schnyder et al. [20], who thoroughly studied EHB cases collected in Switzerland, found no abnormality in the coagulation.

In the past few years we examined clinically and subjected to detailed laboratory studies 15 individuals from 10 families, suffering from chronic benign familial pemphigus.

There is variance of opinion in the literature as to the nosological place of this rare bullous genodermatosis.

(1) Some authors believe it to be a bullous variant of Darier's disease;

(2) others claim that it is a variant of EHB;

(3) most authors, including us, consider it to be an independent nosological entity.

As it is known, in this irregularly inherited pemphigoid genodermatosis there is a tendency to acantholytic bullation, a congenital weakness of the epidermis to exogenous (mechanical, thermal, actinic, chemical, etc.) microtraumas. In the cases observed by us pea-sized confluent vesicles and bullae and erosions, on dime- to palm-sized erythemic bases were visible marginally, then polycyclic, mycosiform erythemato-squamous-ulcero-crusted plaques were seen in the areas of predilection: on the nape, collar-like on the side of the neck, in the ocular and oral angles, also in the face (after shaving), most often in the contact fold areas (in the armpits, under the breast, in the perianal-genital-pubic-crural regions), less frequently in the umbilical region and on the limbs. Fetid papillary vegetations were common in the folds. The changes heal with slight pigmentation. The Nikolsky sign is positive around the large bullae. After removal of adhesive tape vesiculous-erosive, punctate haemorrhages are visible in the skin (Sonck [21]).

In a smaller number of our cases some lesions were covered by bloody, dried crusts. In two cases in the area where the collar rubbed against the skin of the neck fine pinhead-sized bleedings were visible and the Jürgens pinching test was positive. The Rumpel–Leede sign was negative. Similar punctate haemorrhages of the skin at sites exposed to tugging and rubbing have been described

by Forman and Calnan [6]. Crust-coated haemorrhagic lesions have also been described by several workers (Casala and Abulafia [3], Orbaneja Morales, Robledo y Casto [15]).

We have been inspired to undertake coagulation studies in our CBFP cases by the above observations, by the literary data suggesting that in the pemphigus group disorders of the physiological coagulation often occur and, finally, by the inconsistent data published as regards the other common bullous genodermatosis, EHB.

In the literature available for us we have found no publication dealing with coagulographic studies in CBFP.

Methods

The Rumpel–Leede and the Jürgens tests were carried out by the usual techniques.

In analysing the coagulogram (involving fresh citrated and native blood samples), coagulation time was determined by Duke's method, clotting time of recalcified plasma was determined by the fibre-pulling technique.

The Quick index for the prothrombin level, being a relative value, was not calculated.

Percentage prothrombin level was estimated by comparison with diluted control plasma.

Acceleration of clotting by serum (per cent) was determined according to Horn et al.

Percentage prothrombin consumption was determined by a modification of the Biggs and Macfarlane method. The value of consumption reflects the rate of autocatalytic thrombin production, i.e. the functions of factors VIII, IX, X and XI, as well as of the thrombocyte factor 3.

Clotting time in the presence of thrombin (sec.) was determined according to Horn et al. From the thrombin time we wished to draw conclusions as to the presence of antithrombin.

Clotting time in the presence of thrombin and toluidine blue (sec.) was determined also according to Horn et al. The test demonstrates the quantity of free heparin.

The rate of thrombin inactivation was determined by Gerendás' method. This test indicates the capacity of serum to eliminate thrombin activity; this is calculated graphically, and the k value given in dm represents the speed coefficient of the reaction.

Fibrinogen was measured gravimetrically.

Percentage fibrinolysis was calculated by comparison of the 24-hour gravimetric values.

In the evaluation of the thrombelastogram we relied upon the normal values described by Hartert.

r – 8 min. 40 sec.
k – 3 min. 20 sec.
a_{max} – 55 mm.

Deviations not exceeding 3.5 per cent, 10 per cent and 2.3 per cent from the respective normal values were not considered pathological.

Thrombocyte count was determined by the direct counting method of Feissly-Lüdin, taking into consideration the tendency of thrombocytes to agglutinate.

For lack of an exact standardization method, we omitted the heparin tolerance test.

Other examinations (blood grouping, Rh, cryoglobulin, liver function, direct and indirect Coombs tests) were done as usual at our Hospital.

Results

The results are presented in Table I.

Table I

Case	Rumpel–Leede sign	Jürgens test	Thrombocyte count, 1000/cu. mm	Erythrocyte count, million/cu. mm	Leucocyte count, 1000/cu. mm	Haemoglobin, per cent	Bleeding time, sec.	Clotting time of recalcified plasma, sec.	Prothrombin level, per cent	Percentage acceleration of clotting by serum
Normal value	negative	negative	200 to 400	4.5 to 5	6 to 9	80 to 100	100 to 180	180 to 250	80 to 110	15 to 20
1. We. F.	neg.	neg.	280	5.4	13.5	98	150	180	90	15
2. B. Gy.	neg.	neg.	300	4.8	7.7	92	160	220	85	17
3. Sz. F.	neg.	neg.	280	4.46	6.1	82	140	180	95	20
4. W. J.	neg.	neg.	220	4.82	4.9	94	120	190	80	17
5. L. F.	neg.	neg.	320	4.6	5.4	84	130	200	90	18
6. Z. Gy.	neg.	neg.	260	4.32	11.0	89	130	170	90	29
7. We. M.	neg.	neg.	290	3.9	10.5	83	100	185	100	19
8. Sz. K.	neg.	neg.	360	4.9	9.9	91	130	275	100	22
9. B. F.	neg.	neg.	270	5.0	8.7	93	115	160	70	18
10. Wo. M.	neg.	neg.	260	4.5	10.0	88	115	170	100	21
11. Wo. F.	neg.	neg.	360	4.76	9.1	91	150	190	100	18
12. K. A.	neg.	neg.	310	4.20	8.7	87	170	240	80	20
13. S. F.	neg.	neg.	290	4.3	9.6	85	190	210	85	19
14. F. K.	neg.	neg.	260	4.7	8.4	90	180	200	90	17
15. B. T.	neg.	neg.	270	4.2	7.0	86	190	210	110	18

Discussion

The number of our cases is relatively low, yet it would be rather difficult to collect more cases of such a rare genodermatosis.

Evaluation of the results has shown no constant defect in the mechanism of blood clotting in CBFP. Except in two cases, the results obtained were normal.

In Case No. 3 (Sz. F.) the thrombelastogram showed increased coagulability, the r and k values were shorter, the elasticity of the thrombus was increased. In Case No. 4 (W. J.) the r, k and a_{max} values were normal, but fibrinolysis was increased.

The punctate haemorrhages observed in the skin of the neck exposed to rubbing as well as the bloody-crusty changes visible at times may be explained by a local mechanical lesion of vascular origin and consequent haemorrhage.

The coagulographic results obtained by us are in harmony with those published by Schnyder et al. [20] as found in EHB; thus, no constant defect or disorder in the mechanism of blood coagulation exists in these two commonest forms of bullous dermatosis.

Prothrombin consumption, per cent	Thrombin time, sec.	Thrombin inactivation, k value	Thrombin time with toluidine blue	Fibrinogen, mg per 100 ml	Fibrinolysis, per cent	Thrombelastogram	Blood group	Rh (D)	Cryoglobulin	Coombs test, direct and indirect	Liver function test
40 to 70	23 to 26	k = 0.25 to 0.35	15 to 18	200 to 400	10 to 20	negative			negative	negative	negative
60	26	0.30	17	260	20	neg.	A	+	neg.	neg.	neg.
70	24	0.30	17	350	18	neg.	A	+	neg.	neg.	neg.
60	26	0.30	18	300	17	*	0	+	neg.	neg.	neg.
70	19	0.35	18	250	30	neg.	AB	—	neg.	neg.	neg.
66	25	0.28	18	320	20	neg.	0	—	neg.	neg.	neg.
60	25	0.28	19	320	15	neg.	0	+	neg.	neg.	neg.
58	18	0.27	18	280	21	neg.	A	+	neg.	neg.	neg.
57	19	0.32	16	200	23	neg.	0	+	neg.	neg.	neg.
62	20	0.29	17	380	19	neg.	0	+	neg.	neg.	neg.
64	20	0.31	19	210	18	neg.	AB	—	neg.	neg.	neg.
49	24	0.32	17	330	19	neg.	AB	+	neg.	neg.	neg.
66	25	0.29	16	290	20	neg.	0	+	neg.	neg.	neg.
68	26	0.32	17	300	18	neg.	B	+	neg.	neg.	neg.
72	25	0.30	18	260	17	neg.	A	+	neg.	neg.	neg.
56	24	0.28	17	310	19	neg.	A	+	neg.	neg.	neg.

* increased coagulability

References

1. Alexander, B., Goldstein, R.: Parahaemophilia in three siblings. *Amer. J. Med. 13*, 255 (1952).
2. Baumann, R., Krause, S.: Kasuistischer Beitrag zur Epidermolysis bullosa hereditaria dystrophica. *Dermat. Wschr. 130*, 1003 (1954).
3. Casalá, A. M., Abulafia, J.: Pemphigus familiaris benignus Hailey-Hailey. *Argent. Derm. 7*, 77 (1957).
4. Cox, J. N.: Epidermolysis bullosa hereditaria letalis. *La Presse Méd. 69*, 883 (1961).
5. Dorn, H.: Beobachtungen an zwei Familien mit Epidermolysis bullosa hereditaria simplex. *Ztschr. Haut- u. Geschl. Krh. 23*, 40 (1957).
6. Forman, L., Calnan, L. D.: Chronic familial benign pemphigus (Hailey and Hailey). *Proc. 10th Internat. Congress of Dermatology (London) 1952;* 526 (1953).
7. Fuchs, F.: Epidermolysis bullosa hereditaria dystrophica. *Dermat. Wschr. 125*, 303 (1952).
8. Herzberg, I. J.: Vesiculo-bullöse Erkrankungen. In: Gottron—Schönfeld: Dermatologie und Venerologie. Vol. 2./1. G. Thieme, Stuttgart, p. 676 (1958).
9. Jung, E. G.: Gerinnungsphysiologische Untersuchungen und Therapie der hereditären Epidermolysen. Ref. *Derm. Wschr. 151*, 1006 (1965).
10. Klingmüller, G.: Pemphigus vulgaris mit Hemmkörperhämophilie. *Arch. klin. exp. Dermat. 206*, 614 (1957).
11. Klingmüller, G., Leinbrock, A.: Pemphigus vulgaris mit Hemmkörperhämophilie. *Der Hautarzt 7*, 200 (1956).
12. Langhof, H.: Zur Epidermolysis bullosa. *Derm. Wschr. 126*, 764 (1952).
13. Lewis, I. C., Steven, E. M., Farquhar, J. W.: Epidermolysis bullosa in the newborn. *Arch. Dis. Child. 30*, 277 (1955).
14. Linser, P.: Über die Epidermolysis bullosa hereditaria und ihren Zusammenhang mit der Raynaudschen Krankheit. *Arch. Derm. Syph.* (Berlin) *84*, 369 (1907).
15. Orbaneja, G., Morales, Robledo y Castro: Pénfigo cronico benigno familiar (enfermedad de Hailey-Hailey). *Actas dermo-sif.* (Madrid) *54*, 43 (1963).
16. Pastinszky, I.: Beiträge zur Pathogenese, Nosologie und Genetik des Pemphigus familiaris benignus (Gougerot-Hailey-Hailey). *Congr. nationalis dermatol.* Budapest. 21—24, 4 (1965); Pemphigus. Occupational dermatoses due to chemical sensitization. Budapest, Akadémiai Kiadó, 1967, pp. 415–428.
17. Pastinszky, I., Rácz, I., Kovács, E., Geszti, O.: Coagulogrammuntersuchungen bei in die Pemphigusgruppe gehörenden Krankheiten. (In Hungarian, with German Summary.) *Bőrgyógy. Vener. Szemle 28*, 85 (1952). Ref. in *Zbl. Haut- u. Geschl. Krh. 83*, 54 (1953).
18. Plenert, W.: Studie zur Blasenbildung bei der Epidermolysis bullosa. *Z. Kinderh. 78*, 329 (1956).
19. Sardinsky, T.: Epidermolysis bullosa in the newborn. A Dissertation. Basel, 1961.
20. Schnyder, U. W., Jung, E. G., Salamon, T.: Zur Klassifizierung, Histogenetik, Gerinnungsphysiologie und Therapie der hereditären Epidermolysen. *Arch. klin. exp. Derm. 220*, 38 (1964).
21. Sonck, E.: Personal communication. *Congr. nationalis dermatol.* Budapest *21*, 4 (1965); Pemphigus. Occupational dermatoses due to chemical sensitization. Budapest, Akadémiai Kiadó, 1967, pp. 407–415.
22. Soulier, A.: Cit. Schmied: Die Blutgerinnung. Maudrich, Wien, 1951.
23. Tiong Hoo Tio, Waardenburg, P. J., Vermeulen, H. J.: Blood coagulation in epidermolysis bullosa hereditaria. *Arch. Derm. Syph.* (Chicago) *88*, 24 (1963).
24. Zum Winkel: Epidermolysis bullosa dystrophica. *Zbl. Haut- u. Geschl. Krh. 104*, 346 (1959).

Prednisolone-Induced Acute Changes in Circulating Neutrophil Granulocytes

I. In Cases of Normal Granulocyte Reserves

I. Strausz, J. Barcsák, E. Kékes, Ágnes Szebeni
Third Department of Medicine, Postgraduate Medical School, Budapest

(Received June 5, 1967)

Prednisolone has the immediate effect of inducing neutrophil granulocytosis without a shift to the left, releasing cells from granulocyte reserves; it is accompanied by multiphasic changes in the alkaline phosphatase activity of granulocytes. This complex action of prednisolone may be utilized for functional test since the extent of quantitative changes is characteristic, under conditions of normal granulocytopoiesis and sufficient granulocyte reserves.

Neutrophil granulocytosis induced by the administration of ACTH, cortisone or metisteroids is accompanied by changes in granulocytic alkaline phosphatase activity (GAPA). The extent and course of these reactions depend on the intensity of granulocytopoiesis and the size of granulocyte reserves, as well as on the amount of steroids and the duration of administration. This study is concerned with the acute effect of prednisolone on the number of circulating granulocytes and their alkaline phosphatase activity in individuals with normal granulocytopoiesis.

Material and Methods

Twenty-four patients, 12 males and 12 females, between 20 and 60 years of age and with normal hemopoiesis were studied.

The patients received one or two doses of prednisolone within a day, the second following the first by 8 to 12 hours. Administered orally, the single or first dose was 5 to 50 mg, the second dose 5 to 25 mg. The intravenously injected dose did not exceed 12.5 mg.

The following examinations were made usually before, and 1/2, 1, 2, 4, 6, 12, 24, 48 and 72 hours after the first dose: leucocyte count and differential blood count; number of lobes of the neutrophilic granulocyte nucleus (Hynek's index); GAPA-score according to Kaplow [6]; absolute number and percentage of cells belonging to each group of various grades of GAPA.

Results

Administration of prednisolone was followed in some cases by a slight drop of the granulocyte count after 15 to 30 min., but the number of granulocytes began to rise in most cases after the first 30 min., reached a peak at very

different times (between the 3rd and 24th hours), and returned to the pretreatment level mostly after 24 to 72 hrs. Both the intensity of the reaction and its duration depended on the quantity of the single or double dose: smaller single doses induced moderate and shorter reactions than the single or repeated administration of larger ones.

The diagrams illustrate the results of the peroral administration of two daily doses of 25 mg each. Such dosage – regarded as "standard" – had been

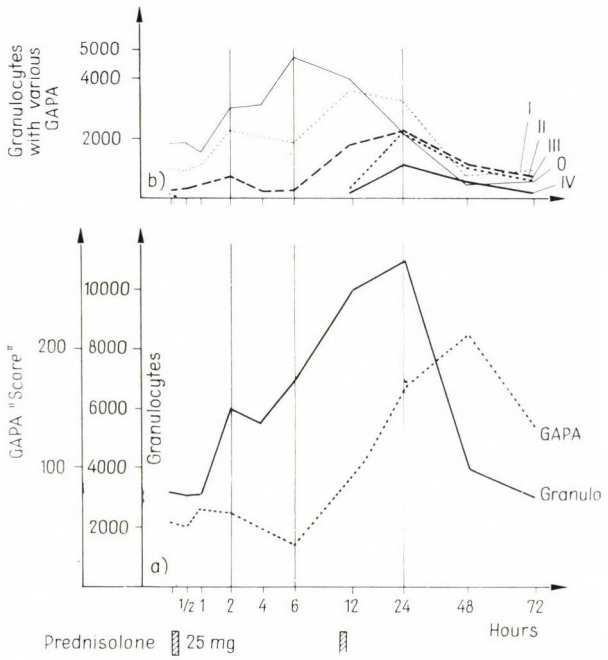


Fig. 1. E. N., female aged 21, with normal hemopoiesis; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

found suitable for the study of the granulocytosis induced by prednisolone under both physiological and pathological conditions of hemopoiesis. The granulocyte count rose two- or threefold, but we regarded an absolute numerical rise of 3000 to 10,000 as characteristic for normal reaction.

Neutrophil granulocytosis was not accompanied by a shift to the left, and changes in the number of lobes of neutrophilic granulocyte nucleus were not regular. It occurred only in less than one third of the cases that an initial diminution in the number of lobes of granulocyte nucleus was followed by a rise which coincided with a decrease or cessation of granulocytosis. In the majority of cases,

however, the number of lobes of granulocyte nucleus remained unchanged or, its changes were rhapsodic.

The GAPA-score usually decreased after the first dose of prednisolone, and the duration of this phenomenon covered a fairly wide range (1 to 6 hrs) also when using the same doses. This was followed by a gradual rise with a peak between the 24th and 48th hours, mostly at a time when the granulocyte count

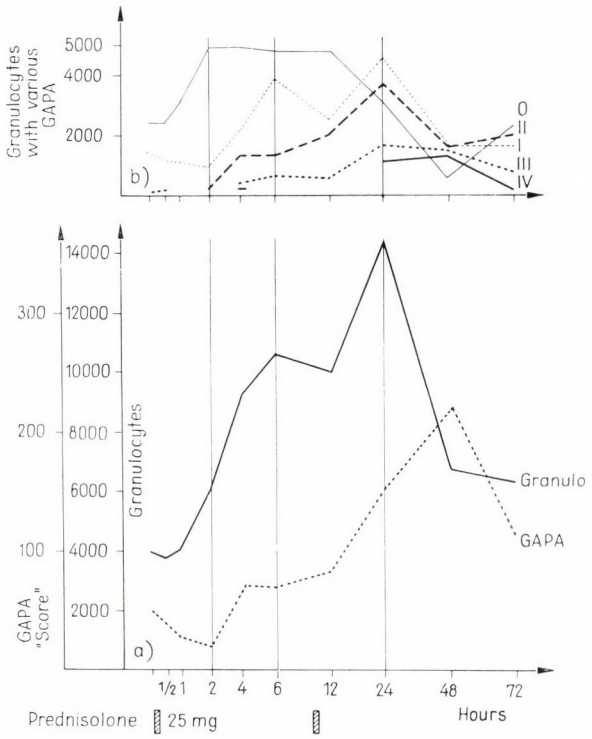


Fig. 2. I. L., hematologically normal female, aged 38; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

was descending or approached (and even returned to) the base-line value. Single intravenous injection of 5 to 12.5 mg prednisolone induced a decrease of GAPA-score, and a subsequent rise was absent or very moderate.

Observations on the changes in the ratio of granulocytes of different GAP activity, determining the GAPA-score, result that at the time when the GAPA-score went down, the number of cells with no (grade zero) or slight alkaline-phosphatase activity (grade I) showed notable increase, determining also the measure of granulocytosis. At this initial period, cells of grades III and IV (if

present) disappeared and the number of those belonging to grade II decreased. It was 1 to 6 hrs after the administration of prednisolone that cells of grade III, and after 3 to 12 hrs that ones of grade IV appeared or reappeared. The peak of granulocytosis usually coincided with the highest number of cells belonging to grades zero and I; there were, at this time, also granulocytes of the higher grades present; they were few but widely different in number so that, at this

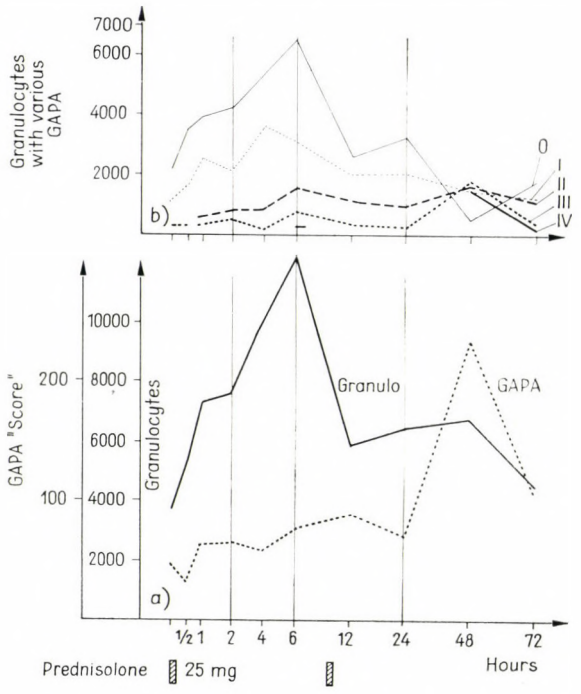


Fig. 3. F. G., 22 years old male, with normal hemopoiesis; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

time, as compared to the initial level, the GAPA-score was higher. Thereafter, the number of cells of grades zero and I diminished and that of the GAP-negative ones usually dropped below the initial value after 48 to 72 hours. There occurred a simultaneous increase in the number of cells with higher activity, which then became predominant. The ratio of the values regained its original level after 72 hours, in most cases still later. The percentage distribution of cells in Kaplow's various groups at the onset and at the time of maximum GAPA-score is shown in Table I.

Table I

Percentage distribution of GAP-negative granulocytes and ones of various grades of GAPA before the administration of prednisolone and at the time of maximal GAPA-score

Grades of GAP activity (after Kaplow)	Neutrophil granulocytes per cent	
	Before treatment	At time of maximal GAPA-score
0	40—70	7—20
I	25—50	18—40
II	5—20	21—25
III	0— 5	10—30
IV		8—21

Discussion

The effect of prednisolone on the number of circulating granulocytes and on the GAPA-score has been studied by several authors (Valentine et al. [15, 16]; Trubowitz et al. [14]; Plenert [9]; Strausz et al. [12]). Our previous experiments showed that granulocytosis induced by prednisolone consists initially of granulocyte release from the reserves (Strausz et al. [12]). The acute effect of one or two daily doses of prednisolone on neutrophil granulocytes was studied in the present investigations. It was found that no shift to the left in the differential blood count accompanied the increase in granulocyte count. The changes observed were similar to those caused by the injection of pyrogenic substances (Pyripher, Pyrexal). It was demonstrated by Craddock et al. [4], Athens et al. [1] and Sacchetti [10] that the increase in leucocyte count following the administration of Pyrexal was due to a mobilization from the marrow granulocyte pool. The so-called "Pyrexal test" measuring the quantity of granulocytes mobilizable from the marrow reserve is based on this assumption. The granulocytosis elicited by prednisolone, like that due to pyrogens, is not accompanied by a shift to the left in cases of normal hemopoiesis. Notwithstanding the similarity, the effect of pyrogenic substances on the release of granulocytes seems not to be due to adrenocortical hormones (Soylemezoglu and Wells [11]).

Changes in GAPA-score, as elicited by prednisolone, can also be induced by pyrogenic substances (Merker [8]): decreasing first, the "score" begins to increase and usually reaches a peak 24 hours after the maximum of granulocytosis at a time when the granulocyte count is already diminishing, or has even returned to the normal value. It has already been noted that after a single intravenous injection of 12.5 mg prednisolone the rise following the diminution of the GAPA-score was either slight or absent, whereas a significant rise of GAPA-score was invariably registered after the peroral administration of a single dose of 50 mg,

or two daily doses of 25 mg each. The fact that a significant elevation of the GAPA-score requires prolonged treatment with prednisolone has been observed also by Valentine et al. [16].

It is clear from the diagrams, and was found also after the peroral administration of a single prednisolone dose of 25 to 50 mg that the rise in the number of GAP-negative cells, and cells belonging to the various grades of GAPA, began successively, and reached peak values at different times; likewise it turned to, or below the initial levels also at different times. Thereafter the number of GAP-negative (i.e. enzymatically inactive) cells dropped usually below the initial level, while – simultaneously – the number of grade III and IV cells rose to the maximum. Pretreatment values were restored after 72 hours and even later. If initially present, cells of grades III and IV temporarily disappeared and the number of grade II cells decreased before they began to appear in large numbers. These phenomena are illustrated in the diagrams in 5 diphasic curves, and it can be seen that the ascending and descending phases of the various curves are displaced in time: where, for instance, the curve representing the number of GAP-negative cells is ascending, that representing the number of the most active cells is descending.

Changes in the absolute number and in the relative value of GAP-negative and differently GAP-active cells determine changes in GAPA-score. It is evident that the first or only effect of prednisolone is a rise in the number of GAP-negative and slightly positive cells, and a reduction in that of the more active cells, an effect invariably observed, irrespective of the dose or route of administration. Increase in number of highly GAP-active cells is not the rule: it may fail to occur after a single intravenous dose of prednisolone. It was observed by Plenert [9] in cases of rheumatic carditis of children that leucocytosis was accompanied by a diminution of GAPA-score during prolonged treatment with prednisolone.

Like Plenert [9], we regard changes in the alkaline phosphatase activity of granulocytes as a multiphasic reaction. The first sign of the prednisolone effect is a disappearance of highly active granulocytes from circulation, presumably due to selective sequestration (Trubowitz et al. [14]). Simultaneous increase in the number of GAP-negative cells is so sudden that they must be supposed to originate from depots. It is most probable that cells of grade I come from the same depot as the GAP-negative cells; this depot is presumably in the bone marrow, because medullary granulocytes contain less alkaline phosphatase than peripheral ones (Trubowitz et al. [13]). Some authors state that also cells of high phosphatase activity derive from the bone marrow, and ascribe their appearance and their increasing number to the cell-maturating effect of prednisolone (Valentine et al. [15]; Plenert [9]). Short-term treatment resulted in the elevation of Hynek's index after 24 hours in the studies of Jährig and Brieger [5], a phenomenon, which they regarded as a sign of accelerated maturation. We did not find this regularly, as it was only in a third of the cases that Hynek's index and the GAPA-score changed parallelly.

Considering that Weissel and Lohmann [18] demonstrated the presence of "activator" substances in the blood, which are capable of promoting the alkaline phosphatase activity of granulocytes, it is conceivable that prednisolone increases the phosphatase activity of granulocytes by means of this factor. Certain authors (Trubowitz et al. [14]; Wiltshaw and Moloney [19]) observed no direct effect of prednisolone and hydrocortisone on the enzymes of the granulocytes, while Knudtson and Evanger [7] found that hydrocortisone elevated the GAPA-score in vitro; again, Cox and McLeod [3] demonstrated that the presence of prednisolone in the culture medium considerably increased alkaline phosphatase activity in cultures of epithelial cells. Athens et al. [2], as well as Sacchetti [10] showed that following long-term administration of prednisolone granulocytes remained in the blood stream longer than normally. Elevated GAPA is, according to Wachstein [17], a sign of physiological maturity. If the rise of GAPA takes place only in the peripheral blood, the effect of prednisolone would mean that the number of mature or "overmature" cells became higher in the circulating blood.

Considering that the granulocytosis caused by adrenaline is due to cells mobilized from marginal depots (Athens et al. [1]; Sacchetti [10]) and is accompanied by an elevation of the GAPA-score, the possibility cannot be excluded that cells with high alkaline phosphatase activity – appearing in increasing numbers after prednisolone administration – are also of marginal origin (Trubowitz et al. [14]).

The mechanism through which prednisolone produces the described effects is still not fully elucidated. Our investigations have shown that in cases of normal granulocytopoiesis and of sufficient granulocyte reserves changes in the number of granulocytes and in the GAPA-score following administration of prednisolone should be regarded as characteristic.

References

1. Athens, J. W., Haab, O. P., Raab, S. O., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M.: Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover in normal subjects. *J. Clin. Invest. 40*, 989 (1961).
2. Athens, J. W., Haab, O. P., Raab, S. O., Boggs, D. R., Cartwright, G. E., Wintrobe, M. M.: Mechanism of steroid granulocytosis. *J. Clin. Invest. 41*, 1342 (1962).
3. Cox, R. P., McLeod, C. M.: Hormonal induction of alkaline phosphatase in human cells in tissue culture. *Nature 190*, 85 (1961).
4. Craddock, C. G., Perry, S., Wentzke, L. E., Lawrence, J. S.: Evaluation of marrow granulocytic reserves in normal and disease states. *Blood 15*, 840 (1960).
5. Jährig, K., Brieger, H.: Veränderungen des peripheren Leukozytogramms unter Prednison im Kindesalter. *Folia Haemat. 80*, 352 (1963).
6. Kaplow, L. S.: A histochemical procedure for localizing and evaluating leucocyte alkaline phosphatase activity in smears of blood and marrow. *Blood 10*, 1023 (1955).
7. Knudtson, K. P., Evanger, A. E.: Standardization of the leukocyte alkaline phosphatase test. *Am. J. Clin. Path. 38*, 264 (1962).

8. Merker, H.: Die alkalische Leukozytenphosphatase; ihre hämatologische und allgemeinklinische Bedeutung. *Dtsch. Med. Wschr. 90*, 484 (1965).
9. Plenert, W.: Studien zur Zytochemie der Leukozytenphosphatasen im Kindesalter. III. Mitteilung: Die Beeinflussung der AGP-Aktivität durch Steroidpräparate. *Folia Haemat. 80*, 52 (1963).
10. Sacchetti, C.: La Granulocitopoiesi. Fisiopatologia e Clinica. Cappelli, Bologna, 1963.
11. Soylemezoglu, B., Wells, J. A.: Comparison of leukocyte response to ACTH and bacterial pyrogen. *Proc. Soc. Exper. Biol. and Med. 77*, 43 (1951).
12. Strausz, I., Kékes, E., Szebeni, Á.: Mechanism of prednisolone-induced leucocytosis in man. *Acta haemat. 33*, 40 (1965).
13. Trubowitz, S., Feldman, D., Benante, C., Hunt, W. M.: The alkaline phosphatase content of the human polymorphonuclear leukocyte in blood and marrow. *Am. J. Clin. Path. 31*, 483 (1959).
14. Trubowitz, S., Moschides, E., Feldman, D.: Alkaline phosphatase activity of the polymorphonuclear leukocyte in rapidly induced leukopenia and leukocytosis. *J. Lab. Clin. Med. 57*, 747 (1961).
15. Valentine, W. N., Follette, J. H., Hardin, E. B., Beck, W. S., Lawrence, J. S.: Studies on leukocyte alkaline phosphatase activity: relation to "stress" and pituitary-adrenal activity. *J. Lab. Clin. Med. 44*, 219 (1954).
16. Valentine, W. N., Follette, J. H., Solomon, D. H., Reynolds, J.: The relationship of leukocyte alkaline phosphatase to "stress", to ACTH, and to adrenal 17-OH corticosteroids. *J. Lab. Clin. Med. 49*, 723 (1957).
17. Wachstein, M.: Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. Clin. Med. 31*, 1 (1946).
18. Weissel, M., Lohman, D.: Alkalische Leukozytenphosphatase und Blutplasma. In Plenert, W.: Aktuelle Leukozytenprobleme. *Hämatologie und Bluttransfusionswesen*, Band 4. Akademie Verlag, Berlin, 1965.
19. Wiltshaw, E., Moloney, W. C.: Studies on various factors influencing leukocyte alkaline phosphatase activity. *J. Lab. Clin. Med. 47*, 691 (1957).

Prednisolone-Induced Acute Changes in Circulating Neutrophil Granulocytes

II. In Cases of Deficient Granulocyte Reserves

I. Strausz, J. Barcsák, E. Kékes, Ágnes Szebeni
Third Department of Medicine, Postgraduate Medical School, Budapest

(Received June 5, 1967)

In cases of deficient granulocyte reserves, prednisolone increased the circulating neutrophil granulocyte count less than normally. This increase amounted mostly to a few hundred cells, and never reached 3000. A slight shift to the left occurred in half of the cases. Changes in GAPA-score are usually multiphasic similar to, but smaller in extent and shorter in duration, than normally. Whether the granulocytopoiesis and granulocyte reserves are normal or deficient, increase in number of granulocytes is of characteristic extent: it indicates the amount of mobilizable granulocyte reserves. Therefore, prednisolone can be employed for the purposes of functional tests and has in this respect many advantages over Pyrexal.

The increase in the number of neutrophil granulocytes and changes in their quality, as induced by certain agents (bacterial lipopolysaccharides; adrenaline), serve to measure the size of granulocyte reserves and the intensity of granulocytopoiesis [3, 2, 1, 5]. Administration of prednisolone elicits a characteristic acute reaction, such as granulocytosis and changes in the GAPA-score, if granulocyte reserves and granulocytopoiesis are sufficient, but the response is quantitatively different if they are deficient [7].

Material and Methods

The material of the present study consisted of 10 female and 6 male patients aged from 45 to 62 years, whose granulocyte count was less than 3000. The following factors were responsible for granulocytopenia:

cytostatics (malignant disease of haemopoietic organs)	in 6 cases
amidazophen	„ 2 „
hypersplenia (cirrhosis of the liver)	„ 4 „
unknown aetiology	„ 4 „

The patients received two peroral prednisolone doses of 25 mg, each with a ten-hour interval between them. The following determinations were made before, and 1/2, 1, 2, 4, 6, 12, 24, 48 and 72 hours after the first dose: the number of leucocytes and differential count; number of lobes of the neutrophil granulocyte nucleus (Hynek's index); GAPA-score according to Kaplow [4]; absolute

number and percentage of cells belonging to each group of various grades of GAPA. The bone marrow was examined in every case prior to the experiment. In six cases investigations were repeated after the cessation of granulocytopenia.

Results

The acute changes caused by prednisolone in granulocyte count and in granulocytic alkaline phosphatase activity (GAPA) are illustrated in 4 cases. The

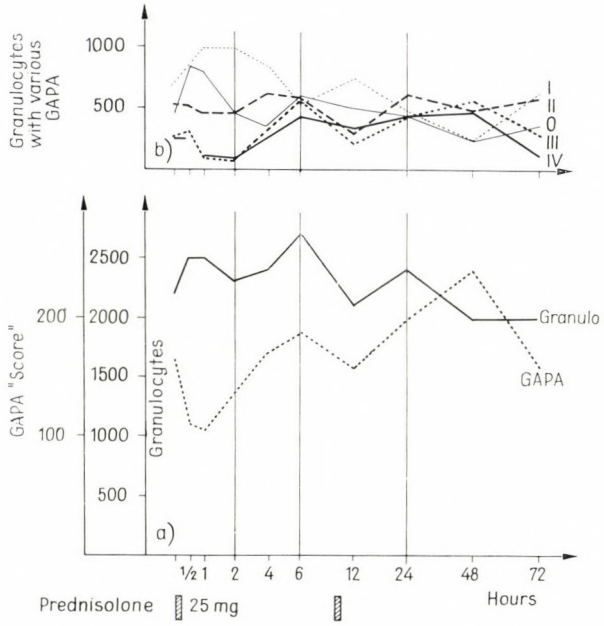


Fig. 1. P. S., 61 years old female, with cirrhosis of the liver and granulocytopenia caused by hypersplenia; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

pretreatment value of GAPA-score was normal in the first three cases. Administration of prednisolone resulted in a slightly increased granulocyte count; the maximum increase did not exceed a few hundred cells. The differential blood count remained unchanged in one case, while in the other two the number of neutrophil bands has doubled. The Hynek's index did not change. After a temporary decrease, the GAPA-score began to rise, reached a peak after 24 to 48 hours, and then sank back to the initial value. As it can be seen from the diagrams, alterations of the GAPA-score were due to changes in the ratio of GAP-negative and GAP-positive cells of various enzymatic activity: increase of GAP-negative cells was

accompanied by a decrease in the number of GAP-positive cells, while the subsequent reduction in the number of GAP-negative cells occurred at the same time as that of the highly GAP-positive cells increased.

In one of the cases the value of GAPA-score was high before the treatment (Fig. 4). The administration of prednisolone provoked no significant rise of the granulocyte count. The GAPA-score decreased because of the appearance of

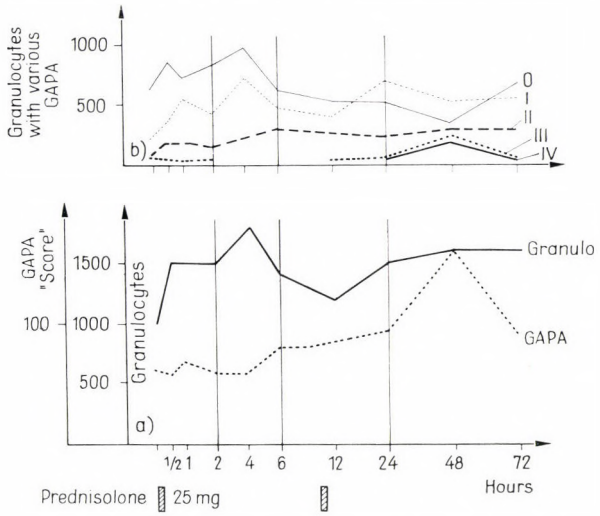


Fig. 2. L. M., 56 years old male, with granulocytopenia of unknown etiology; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

GAP-negative cells, while the ratio of cells of high activity slightly diminished. Increase in GAPA-score was not observed even at a later period.

Changes in the other cases were similar to those shown in the diagrams: the numerical increase of granulocytes rarely amounted to more than one thousand cells. In half of the cases we observed a slight shift to the left, that is an average duplication of neutrophil bands only. The number of lobes of granulocyte nucleus remained unchanged; changes in the GAPA-score, i.e. changes in the proportion of cells of various GAPA depended on whether the GAPA-score was normal or elevated before the treatment. The phenomena elicited by the administration of prednisolone were as in the first three cases, or like those in case 4, accordingly.

Prednisolone, administered after the cessation of granulocytopenia elicited a normal reaction: without a shift to the left, the number of granulocytes rose; the maximum increase was always higher than 3000 cells [8].

Discussion

Administration of prednisolone to patients with deficient granulocyte reserves of various aetiology elicited abnormal reactions [7]. Increase in the granulocyte count amounted to a few hundred cells only, while in cases of normal reaction, it always amounts to more than 3000. Experience has shown that in case the granulocyte count does not increase by 3000 cells, the extent of the reaction

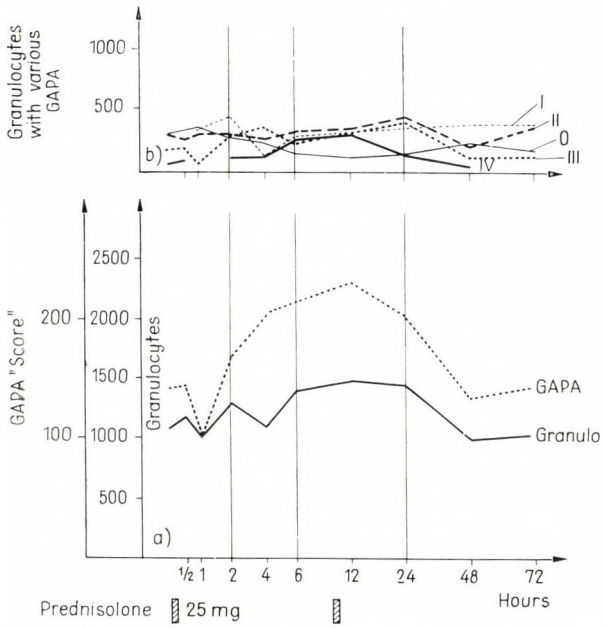


Fig. 3. S. G., 75 years old male, suffering from lymphosarcoma with granulocytopenia during treatment with Endoxan; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

is smaller than the normal, where a decrease in the number of mobilizable granulocytes can be inferred. This is usually a sign of insufficient granulocytopoiesis. As regards alkaline phosphatase activity, the changes in the proportion of "mobilizable" granulocytes were similar to the normal cases, although in some instances the extent and duration of the rise of GAPA-score were lower than the average. The reaction produced by prednisolone became normal after the cessation of granulocytopenia.

Granulocytosis, usually accompanied by pyrexia, can be induced with bacterial lipopolysaccharides. This reaction serves as a functional test for the measurement of medullary granulocyte reserves (Pyrexal test). If the elevation

of the granulocyte count is slight, or absent, it indicates deficiency of reserves: in other words, the number of mobilizable granulocytes is reduced. In such cases the differential blood count shows a shift to the left. In clinical practice, Heilmeyer [3], Craddock et al. [2], as well as Sacchetti [5] have found the Pyrexal test useful.

Granulocytopenia may occur without damage to the bone marrow, and so without a deficiency of medullary granulocyte reserves. In such cases the reaction

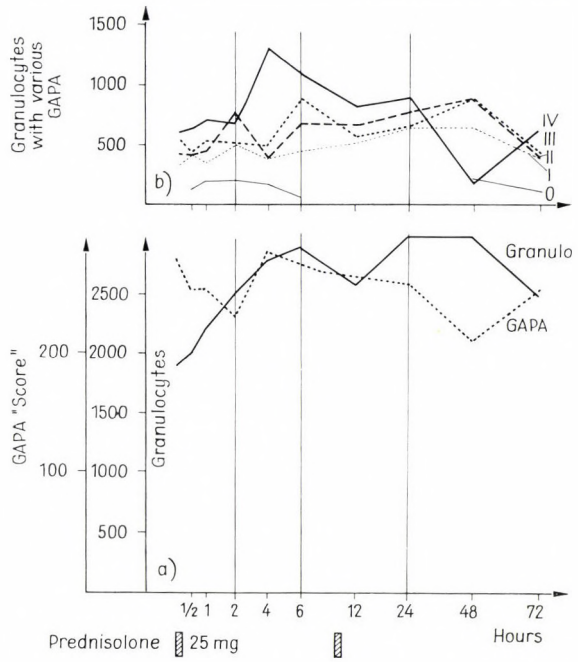


Fig. 4. A. Sch., 69 years old female, suffering from lymphosarcoma with granulocytopenia during treatment with Endoxan; a) acute effect of prednisolone on granulocyte count and GAPA-score, b) on the absolute number of granulocytes at the various grades according to Kaplow's scoring of phosphatase activity

to the administration of prednisolone or Pyrexal is normal. Examination of the bone marrow points in certain cases, especially in lymphocytic leukaemia, to the possibility of deficient granulocyte reserves; in cases like this, we have observed a normal reaction to prednisolone.

Discussing the usefulness of the Pyrexal test, Craddock et al. [2] suggested the possibility that an insufficient reaction need not indicate, as a rule, a primary depletion of granulocyte reserves. It should be taken into account that the "consumption" of granulocytes is accelerated (as in experimental leucopheresis), and that a mechanism of this kind may, for instance, play a role in hypersplenia.

As far as the changes in granulocyte count and in GAPA-score are concerned, the effect of prednisolone is similar to that of Pyrexal; there is, however, no evidence to prove that the action of Pyrexal requires mediation of adrenocortical hormones [6]. Like Pyrexal, prednisolone can be used for functional test. Following its administration the extent of the increase in granulocyte count indicates the amount of mobilizable granulocytes. Prednisolone has many advantages over Pyrexal: 1) a single dose of 25 mg, or a daily dose of 2×25 mg may be administered to any patient without risk; 2) prednisolone causes no undesirable side effects; 3) it is readily available.

After the administration of prednisolone the GAPA-score underwent the same multiphasic changes as in normal hematopoiesis, but, in general, they were numerically smaller and shorter in duration. This was due to the fact that the proportion of GAP-negative cells and cells of various GAP-activities was like the normal, independently of their absolute number.

References

1. Athens, J. W., Haab, O. P., Raab, S. O., Mauer, A. M., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M.: Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover in normal subjects. *J. Clin. Invest. 40*, 989 (1961).
2. Craddock, C. G., Perry, S., Wentzke, L. E., Lawrence, J. S.: Evaluation of marrow granulocytic reserves in normal and disease states. *Blood 15*, 840 (1960).
3. Heilmeyer, I.: Funktionsprüfung der Leukopoese des Knochenmarks. *Dtsch. Med. Wschr. 82*, 644 (1957).
4. Kaplow, L. S.: A histochemical procedure for localising and evaluating leucocyte alkaline phosphatase activity in smears of blood and marrow. *Blood 10*, 1023 (1955).
5. Sacchetti, C.: La Granulocitopoiesi. Fisiopatologia e Clinica. Cappelli, Bologna, 1963.
6. Soylemezoglu, B., Wells, J. A.: Comparison of leukocyte response to ACTH and bacterial pyrogen. *Proc. Soc. Exper. Biol. and Med. 77*, 43 (1951).
7. Strausz, I., Kékes, E., Szebeni, Á.: Mechanism of prednisolone induced leucocytosis in man. *Acta haemat. 33*, 40 (1965).
8. Strausz, I., Barcsák, J., Kékes, E., Szebeni, Á.: Prednisolone-induced acute changes in circulating neutrophil granulocytes. I. In cases of normal granulocyte reserves. *Haematologia 1*, 319 (1967).

Open Forum

MODERN TRENDS IN THE TEACHING AND SPECIALIZATION OF HAEMATOLOGY AND BLOOD TRANSFUSION

Haematology – once limited to morphology and to the clinic of blood diseases – includes today cytochemistry, genetics, immunology, isoserology, biochemistry (especially protein chemistry), blood coagulation and rheology, blood preservation, nuclear science, etc. It is utterly impossible for the practising physician to keep abreast of this formidable advance. As a consequence there is a great need for experts in haematology and blood transfusion throughout the world. At the same time it is a matter of course that time and again the question arises in what way a working synthesis could be achieved in our era of specialization. It is believed that many countries would profit from an exchange of experience in the teaching of haematology and blood transfusion. With this aim in mind prominent scientists from various countries have been invited to participate in the discussion.

In our previous issue the first contributions to this discussion were published.

J. V. Dacie, Professor of Haematology, Postgraduate Medical School of London, acquainted our readers with the organization of haematology in England and Scotland. In his paper, which is a part of his inaugural address to the Haematology Society of Australia delivered in 1962, he dwells at length on problems concerning the organization and function of haematological departments, the providing of high standard diagnostic service and treatment of patients with haematological disorders, the running of hospital blood transfusion services, the training and research in haematology and the future of this branch of medicine.

Professor Jean Bernard, Director of the Research Institute for Blood Diseases in Paris described the organization and function of haematology, the organization of the blood transfusion service, the system of teaching and postgraduate training and the possibility of specialization in France.

Professor J. J. Van Loghem of Amsterdam, Professor M. C. Verloop of Utrecht and Professor E. Deutsch of Vienna made us acquainted with the state of haematology and blood transfusion in their respective countries.

As the comments made hitherto have revealed, the organization and function of this new and rapidly developing field of science show great variety according to countries, especially in the relationship between haematology and blood transfusion and with other branches of medicine, respectively. The aspects of

teaching and postgraduate training of haematology and blood transfusion in the above countries are essentially different, too.

Further contributions to the discussion are given in this issue.

NORWAY

Dr. P. F. Hjort, Head of the Department for Haematology and Blood Transfusion, University Clinic, Oslo

The teaching of haematology and blood transfusion is organized within the scope of the universities. It is a compulsory subject in medical education during clinical training, i.e. in the 4th – 6th years. Postgraduate courses are organized for attaining knowledge in the application of haematologic techniques during their training. Doctors specialized in internal medicine and paediatrics during the course of medical training are taught the simple haematologic techniques (scanning of blood smears and bone marrow).

Blood Banks generally are attached to larger hospitals. The University Clinic in Oslo has a Department for Haematology whose head is assistant professor of clinical haematology. Blood Banks function independently of the Department of Haematology.

Haematology so far is not recognized as an independent specialty. Degrees cannot be obtained in this subject and examinations are not compulsory.

I share the opinion that university clinics should have departments for haematology with inpatient and out-patient services and research laboratories. In general hospitals no special sections are required for haematology but a staff of well-trained internists and paediatricians specialized in haematology.

POLAND

Professor W. Rudowski, Director of the Institute of Haematology, Warsaw

The teaching of haematology in Poland is included in the curriculum of internal diseases while the teaching of blood transfusion is attached to general surgery. As a matter of course neither of these subjects require a separate examination. On what level the teaching of haematology and blood transfusion is included in the curriculum, depends on the individual interest of the lecturer in the subject. Postgraduate courses in haematology and blood transfusion are organized regularly. Training in haematologic techniques is mainly devoted to morphology.

The Institute of Haematology in Warsaw consults in all transfusion problems and supervises the Blood Transfusion Centres of the country. The network of Blood Banks attached to hospitals are controlled by the Blood Transfusion Centres. There are no chairs for haematology at the University Medical School. There is only one at the Postgraduate Medical School.

To qualify in blood transfusion the first degree of specialization in one of the basic branches of medicine (surgery, internal medicine, gynaecology and

obstetrics, paediatrics, chemical pathology) is compulsory, after which the candidate is obliged to acquire a three-year training in serology, microbiology, blood preservation and storage, etc. in a Blood Transfusion Centre or a Blood Bank. This is followed by a final examination, a prerequisite of the second degree in blood transfusion.

For promoting the development of haematology and blood transfusion (education, medical care and research) the following suggestions are made:

Haematology

The modernization of laboratory equipment and diagnostic methods in the university hospitals as well as in the larger hospitals, and providing the Institute of Haematology with modern laboratory equipment and technical facilities are indispensable.

Blood transfusion

Postgraduate teaching should be extended with a possibility for specialization in serology for doctors and biologists. Units of immunopathology for pregnants should be organized. International collaboration for standardizing the production of blood derivatives and the equipment used in blood processing and blood transfusion should also be promoted.

FRANCE

Docteur G. Bilski-Pasquier, Professeur à la Faculté de Médecine,
Médecin des Hôpitaux de Paris

L'enseignement de l'hématologie se fait de deux façons en France:

a) l'hématologie proprement dite est enseignée à la Faculté de Médecine,

b) la transfusion sanguine est enseignée dans le cadre même du Centre National de Transfusion, au cours d'un enseignement de trois mois destiné aux futurs Chefs de Centre de transfusion, l'enseignement comportant des exposés théoriques et des travaux pratiques à l'issue desquels des épreuves écrites et orales donnent droit, si l'on est admis, à une attestation d'Ancien Élève du Centre National de Transfusion Sanguine.

L'enseignement de l'hématologie est obligatoire dans le courant des études médicales.

L'enseignement de l'hématologie est fait en 3ème année de médecine, pendant 30 heures, comportant un enseignement théorique et, surtout, enseignement dirigé par, petits groupes et travaux pratiques. L'enseignement est sanctionné, d'une part, par un examen théorique en fin d'année.

Il existe des cours d'hématologie et de transfusion sanguine destinés aux médecins et pharmaciens. Il s'agit d'une part du Certificat d'Études Spéciales

d'Hématologie et, d'autre part, du Certificat d'Études Supérieures d'Hématologie, le premier comportant des cours, des travaux pratiques, un stage, un examen final, le deuxième nécessitant en plus la rédaction d'un mémoire, un stage dans un service d'hématologie et un examen final.

De plus, il existe un certain nombre de cours complémentaires de cytologie, d'immunologie, d'hémostase, aussi bien à Paris que dans les facultés de province. Tous ces cours comportent évidemment des manipulations pratiques nombreuses.

Le service de la transfusion sanguine en France dépend essentiellement des Centres de Transfusion Sanguine, coiffés par le Centre National de Transfusion Sanguine à Paris, avec de nombreuses directions régionales correspondant dans l'ensemble à des villes de Faculté ou d'École de Médecine.

De plus, quelques groupes privés se chargent de transfusions sanguines en ville mais représentent quelque chose d'assez négligeable par comparaison avec l'importance des centres officiels.

Il existe un Institut d'Hématologie dirigé par Professeur Jean Bernard, à l'Hôpital Saint-Louis, institut de recherches et de traitement groupant de nombreux laboratoires et un matériel d'enseignement considérable.

Il existe une Chaire de Clinique des Maladies du Sang dont le titulaire, à Paris, est le Professeur Jean Bernard, une deuxième chaire à Strasbourg, titulaire Professeur Waitz, une troisième chaire à Toulouse dont le titulaire est le Professeur Ruffie. De plus, un certain nombre de professeurs ont une vocation hématologique, trois à Paris, deux à Lyon, un à Marseille.

L'Institut d'Hématologie de Paris est en liaison assez souple avec la Transfusion Sanguine par l'intermédiaire du Professeur Agrégé Dausset qui est immunologiste et par les liens qu'il a avec le Centre National de Transfusion Sanguine.

Abstracts

Идиопатический миелофиброз и вторичный миелофиброз при хроническом миелолейкозе

(Idiopathic myelofibrosis and secondary myelofibrosis in chronic myeloid leukaemia). M. S. Dultsin, N. F. Klimova, T. A. Cherntsova, A. V. Zacharova (Central Institute of Haematology and Transfusion, Moscow) *Probl. Gemat. 12*, 1 (1967).

Idiopathic myelofibrosis is distinguished as an individual disease, differing from chronic myeloid leukaemia, and characterized by fibrous mesenchymal bone marrow proliferation. There is always a slow, progressive enlargement of the spleen and liver. Marked splenomegaly is often accompanied by secondary hypersplenism, haemolytic anaemia or thrombocytopenia, or by both. Observations carried out in patients with secondary myelofibrosis in chronic myeloid leukaemia indicated that the former was capable of developing at various stages of the disease. Secondary myelofibrosis in chronic myeloleukaemia failed to improve the course of leukaemic process, and evidently promoted the development of early resistance to cytostatics.

Katalin Natonek

Intravenous pyelography in multiple myeloma. V. A. Vix (Veterans Administration Hospital, Minneapolis, Minn.) *Radiology 87*, 896 (1966).

Bartels et al. reported the case of a patient with multiple myeloma (MM) in 1954, who died in acute renal failure following intravenous pyelography (IVP). Since then more such cases have been reported in the literature. As a result of these observations, several investigators have claimed that IVP is absolutely contraindicated in MM. Based upon the chart-review of his own patients, the author has attempted to determine the hazards of IVP in cases of MM. The material examined, dating back to 1949, contained 99 histologically confirmed cases of MM. First of all it has been clarified whether IVP was performed or not, but particular attention has been paid naturally also to blood urea nitrogen, serum and urine electrophoretic data. Out of the 88 patients, IVP was performed in 40 on 52 occasions, mostly in a search for malignant tumors before establishing the diagnosis. Normal excretion was found in 33 cases, while pathologically decreased excretion was found in 7 cases. In the latter 7 patients pronounced Bence—Jones proteinuria or amyloidosis (on postmortem examination) was detected. As to azotemia, 48 patients out of 85 had it. It is noteworthy that 21 patients had azotemia out of the 23 who showed Bence—Jones proteinuria. Out of the 23 patients 13 were submitted to IVP. Comparison of the renal status and IVP, yielded the following evidence. Although blood-urea-nitrogen determinations were not carried out in every case immediately after IVP, its elevation, however, has never been observed and no rapid change has taken place in the clinical condition of any patients after the examination. Rapidly progressing azotemia was observed in 14 cases. Out of these, in 8 cases IVP had not been performed at all, while in the remaining cases there

was no correlation in time. The paper mentions also some pathologically clarified renal disorders accompanied by MM and in this connection some pathogenetic problems, partly unsolved. Summing up, author claims that the origin of renal damage plays no role in the contraindication to IVP; the same precautions should be taken in cases of MM as in any other disease with poor renal function. If IVP is performed in the presence of moderately impaired renal function, on the basis of an appropriate indication, dehydration is to be avoided by all means and a higher amount of contrast medium should be administered in high dilution. Finally, on the basis of clinical experience, author definitely denies that the contrast medium (diatriozate) would by itself have an adverse effect in MM.

J. Forrai

Effect of strenuous and graded exercise on fibrinolytic activity. I. Sudhakaran Menon, F. Burke, H. A. Dewar (Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne, G. B.) *Lancet 7492* (I), 700 (1967).

Notably increased fibrinolytic activity after strenuous exercise has been observed in 58 trained athletes and to a lesser but still significant degree in 10 volunteers after moderate graded exercise. The maximum increase in fibrinolytic activity in the untrained volunteers was reached within 1 1/2 min. of their exercise and no greater effect was achieved by prolonged further exercise. The increase in activity was, however, found to persist for at least 1 hour after the exercise. To obtain the beneficial effects of exercise and prevent thromboembolism it is not necessary to involve the actual endangered part of the body — exercise of the arms, for instance, will benefit a leg encased in plaster.

Cecilia Zsdánszky

The indication for transfusion of freshly drawn blood. H. A. Oberman (Department of Pathology, University of Michigan, Chicago, Ill.) *JAMA 93*, 199 (1967).

The use of freshly drawn blood for transfusion should be justified by the clinical and laboratory findings manifested by the patients in relation to the known effects of storage of blood. Empirical administration of this product is wasteful, for it depletes the available donor pool and interferes with blood-bank inventory. This review of a variety of clinical special situations (chronic anaemia, thrombocytopenia, extracorporeal circulation, leukopenia, haemolytic disease of the newborn, some coagulation disorders, severe liver diseases, haemolydialysis) that frequently occasion request for freshly drawn blood, indicates that most can be treated optimally with specific blood components, in relatively fresh blood.

E. Benedek

Chromosome studies in acute leukaemia. M. K. Jensen, S. Killmann (Haematological Section, Medical Department A, Rikshospitalet, Copenhagen) *Acta. Med. Scand. 181*, 47 (1967).

The cytogenesis of five patients with acute leukaemia was studied. The patients were selected according to some criteria: the bone marrow metaphases show aneuploid modes and at the same time at least ten per cent of the mitotic figures of the bone marrow aspirates occurred in the erythroid series. In four of the patients the percentage of mitoses which occurred in the erythroid series clearly exceeded the percentage of normal metaphases. The data suggested that in acute leukaemia abnormal karyotypes are present not only in the leukaemic blast cells but also in the red cell population. In contrast, lymphocytes cultured from the peripheral blood showed normal karyotypes. The presence of abnormal karyotypes in both the red cell and white cell series implies that the erythroid precursors in acute leukaemia are not remnants of normal erythropoiesis but are directly involved by the leukaemic process concurrently with the white cells.

E. Benedek

Standardization of the one-stage prothrombin time for the control of anticoagulant therapy. R. Biggs, K. W. E. Denson (Medical Research Council, Churchill Hospital, Oxford, G. B.) *Brit. med. J. 1*, 84 (1967).

The control of anticoagulant therapy by the one-stage prothrombin time suffers from the fact that the results of this test are reported differently at different centres. Therefore it seems advisable to improve the general standard control. The calibration scale of commercial preparations could be adjusted to the scale, preparations made individually in clinical laboratories could be similarly calibrated. Moreover, attention to sensitivity to the coumarin defect would undoubtedly improve the reliability of control. A method of standardizing the test has been devised so that all may use the same scale of measurement adjusted in each case to fit the preferred thromboplastin. The method is based on the preparation of the standard thromboplastin with which other preparations can be compared.

Edith Soós

A rapid method for the isolation of coeruloplasmin. R. P. Stokes (Department of Experimental Pathology, Medical School, University of Birmingham, G. B.) *Clin. Chim. Acta 15*, 517 (1967).

A simple method for the isolation of coeruloplasmin is described. The method obviates precipitation procedures and employs two-stage ion exchange chromatography using both diethylaminoethyl (DEAE) Sephadex, an anion exchanger, and carboxymethyl (CM)-Sephadex, a cation exchanger, which are capable of dealing with volumes of plasma, or equivalent amounts of Cohn fraction IV-1 of the order of 1000 ml/g dry weight of the powdered exchanger. Using this technique, coeruloplasmin has been recovered from plasma, serum or Cohn fraction IV-1 at a recovery rate of approximately 70% and in an acceptable state of purity as judged by immunochemical, electrophoretic and ultracentrifugal criteria.

Edith Soós

Untersuchung der verschiedenen Wirkung von Heparin und Streptokinase auf die Eiweißstoffe des menschlichen Blutplasmas mit Hilfe der Diffusion und Elektrophorese in Fibrin-Agar-Gel (Investigation of the various effects of heparin and streptokinase on human blood plasma proteins by diffusion and fibrin-agar-gel electrophoresis). I. Stepánek, J. Andrasina, M. Mattová et al. (Institut für Seren und Impfstoffe, Prag) *Coll. Czech. Chem. Comm. 32*, 466 (1967).

Die neuesten Erkenntnisse über die Wirkung des Heparins auf die Fibrinolyse sprechen für einen komplizierten Reaktionsmechanismus, wobei die Ansicht überwiegt, daß die Anwesenheit des Heparins eine fördernde Wirkung auf die Fibrinolyse hat. In dieser Mitteilung ist die Untersuchung der Wirkung des Heparins und der Streptokinase auf die Eiweißstoffe des menschlichen Blutplasmas mit Hilfe der elektrophoretischen Analyse und des Verfahrens der doppelten Diffusion in Fibrin-Agar-Gel gegeben. Es ist sehr wahrscheinlich, daß das Plasminogen, das durch Einwirkung von Streptokinase in die fibrinolytisch aktive Form übergeht, der profibrinolytische Faktor, der durch Einwirkung von Heparin in die fibrinolytisch aktive Form übergeht, und der autoaktivierende fibrinolytische Faktor durch verschiedene Mechanismen in den Zustand fibrinolytisch aktiver Eiweißstoffe umgewandelt werden. Die fibrinolytisch aktiven Formen dieser Eiweißstoffe sind wahrscheinlich ein Bestandteil des komplexen fibrinolytischen Mechanismus, der für die Lyse des Fibrinkoagulums verantwortlich ist. Die von uns vorgelegten Ergebnisse stehen im Einklang mit den Arbeiten, wonach Plasminogen kein homogener Eiweißstoff ist.

Elisabeth Török

Dihydroxyazetonstoffwechsel in Bluthämolysaten bei Lebererkrankungen (Dihydroxyacetone metabolism in haemolysates with liver diseases). E. Bielik (Central Laboratory of the District Hospital, Banská Bystrica) *Z. ges. Inn. Med. 22*, 93 (1967).

Haemolysates of sixty healthy persons, resp. patients without liver diseases, thirty hepatitic and fifteen cirrhotic patients were investigated. In the haemolysates of erythrocytes from patients with cirrhosis, dihydroxyacetone was utilized half as fast as in the normal controls. The dihydroxyacetone con-

sumption could be normalized by the addition of ATP, adenosine, inosine and adenine. The limiting factor of the consumption of dihydroxyacetone seems to be the ATP, present in a lower concentration in the erythrocytes of patients with parenchymal liver lesion.

Ilma Szász

Adenosinetriphosphatase activity of erythrocyte membrane in hereditary spherocytosis. K. Nakao, Sh. Kurashina, M. Nakao (Third Department of Internal Medicine, University of Tokyo, Tokyo) *Life Sciences 6*, 595 (1967).

Five patients with hereditary spherocytosis and ten control persons were investigated. The erythrocyte membranes were prepared from a hypotonic haemolysate of washed erythrocytes, and the ouabain-sensitive, Na—K—ATPase activity and the ouabain-insensitive ATPase activity of these preparations were determined. The quotient of the ouabain-sensitive and ouabain-insensitive ATPase activities were found to be lower than 1.0 (range: 0.08—0.67) in normal persons and higher than 1.0 (range: 1.4—11.3) in patients with hereditary spherocytosis. The decrease in ouabain-insensitive ATPase activity in hereditary spherocytosis was more significant than the increase in ouabain-sensitive ATPase activity. These results elucidate the aethiopathogenesis of hereditary spherocytosis better than the data about the increased turnover of Na known before.

Ilma Szász

Radiophosphate "closed" system efflux rate constant and ATP metabolism in intact human red cells. J. J. Imarisio, T. R. Jamison (Radioisotope Service, Veterans Administration Hospital, Cincinnati, Ohio) *J. Lab. Clin. Med. 69*, 23 (1967).

The ^{32}P efflux rate constant of red cells, previously incubated with isotope phosphate was accurately determined using perfusion dialysis technique in "closed" conditions, in which only efflux of phosphate following uptake of isotope phosphate was allowed. The ATP/ADP quotient was determined chromatographically. Phosphate flux was shown to be dependent almost exclusively on the synthesis and degradation of ATP. Experiments with glucose deprived fresh red cells proved that the method was suitable for studying the ATP losing state of cells. The ^{32}P efflux rate constant was closely reproducible on repeated determinations on the same cells, but showed great variation from patient to patient. In spite of this, selected untreated thyreotoxic patients showed a statistically significant difference in ^{32}P efflux rate constant from a group of euthyreoid controls. The method seems suitable for the comparative study of endocrine and other influences on the ATP metabolism of intact cells.

Ilma Szász

New anticoagulant from lysed fibrinogen. E. Triantaphyllopoulos, D. C. Triantaphyllopoulos (Department of Physiology, University of Alberta, Edmonton, Alberta) *Brit. J. Haem. 13*, 28 (1967).

Once fibrinogenolysis had been initiated, few, if any fibrinogen molecules remained unaffected. Even the fraction which could still be precipitated by 0—25 per cent ammonium sulphate saturation showed differences from intact fibrinogen. Its solubility decreased very early, its coagulability gradually disappeared and the fraction itself acquired anticoagulant properties. The ability of the 0—25 per cent fraction to prolong the thrombin clotting time of intact fibrinogen varied with incubation in a way similar to that of the 25—50 per cent fraction (AFIF). It was, however, less potent and it disappeared after heating at 60°C for 15 minutes. The two anticoagulant fractions could be differentiated by the electrophoretic and thermostable protein patterns, the degree of precipitability by ether, the effect of alkaline pH buffers and the effect of fraction concentration on the inhibitory activity. The main electrophoretic component of the 0—25 per cent fraction, responsible for the bulk of the inhibitory effect at 100 per cent coagulable period (CP), had a slightly slower mobility and lower specific activity than the main component of

the 25—50 per cent fraction. Purified thrombin liberated acid soluble protein fragments from the 0—25 per cent fraction equal to about 1.5 per cent of the total protein.

Cecilia Zsdánszky

Further studies on the effect of adenine in blood preservation. O. Akerblom, C. H. De Verdier, M. Finnson, L. Garby, C. F. Högman, S. G. O. Johansson (Department of Clinical Chemistry, Blood Transfusion Service, Uppsala) *Transfusion 7*, 1 (1967).

Clinical and experimental studies on effect of adenine supplementation to ACD blood are reported. The frequency and type of transfusion reactions in 5,595 transfusions of ACD-adenine blood (storage time 0—35 days) was the same as in 4,519 transfusions of ACD blood (storage time 0—21 days). There was no change in the plasma haptoglobin level in 10 recipients during and after transfusion of 29 to 35 days old ACD-adenine blood. The relation between posttransfusion viability in vitro hemolysis, erythrocyte ATP content and duration of storage were studied. Supplementation with adenine increased the posttransfusion viability and the erythrocyte ATP content, these effects were noticeable after two weeks of storage. A negative correlation was found with respect to posttransfusion viability and the concentration of total lipids in the medium. A significant covariation between the concentrations of the three nucleotides ATP, ADP and AMP was found. This indicates that erythrocyte adenylate kinase dominates in the regulation of relative concentrations of these nucleotides.

Cecilia Zsdánszky

A revised staining procedure for haptoglobins. Priscilla J. Singh (The New York Blood Center, New York) *Vox Sang. 12*, 78 (1967).

The most often used method of staining for the demonstration of haptoglobin is the benzidine-hydrogenperoxide method following starch gel electrophoresis, suitable for staining haeme-containing complexes. The disadvantages of the method are that the boundaries of the haptoglobin bands are not sharp enough, the stained gel must be read immediately, therefore attempts have been made to prevent diffusion of the dye. The procedure described by Singh is a variant of the Giblett—Steinberg method; the gel is stained with benzidine HCl according to Smithies, the excess dye is removed by washing and contrast staining is employed with amido black 10 B. In the preparations so stained the boundaries of the haptoglobin bands have very sharp outlines. It is suggested that at the site of the haptoglobin bands insoluble benzidine-amido black complexes are fixed.

Maria Ambrus

Lack of linkage between the Lp and Ag serum systems. K. Berg (University Institute of Forensic Medicine, Rikshospitalet, Oslo) *Vox Sang. 12*, 71 (1967).

By precise analysis of the pedigree of 8 Norwegian families, author has studied the genetical relationship of two beta-lipoprotein antigens, Lp(a) and Ag(a). Anti-Lp(a) serum from rabbit, homologous anti-Ag(a) and C. de B. serum obtained from Blumberg have been used.

Mathematical analysis has shown that the Lp^a and Ag^a antigens occupy two different loci, which are not correlated either closely or distantly.

The free or the 0.5 recombination fraction is apparently the most likely. From Berg's family studies we have to conclude that the Lp and the Ag loci are not correlated with each other, we deal with two separate loci or systems within the structure of beta-lipoproteins.

Maria Ambrus

Polymorphism of the human low-density lipoproteins. R. Bütler (Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Berne) *Vox Sang. 12*, 2 (1967) An Editorial.

The most complex group of human serum proteins is that of the lipoproteins. They are classified by ultracentrifugal sedi-

mentation analysis, on the basis of which distinction is made between low-density and high-density variants. The heterogenous class of lipoproteins is represented by the low-density lipoproteins (LDL), having a density of 1.063 to 0.90 per g/ml, corresponding flotation constants of from St 0 to 400, mol weight from 1 to 100 million, diameter of spherical particle 200 to 400 Å. In electrophoretic analysis they migrate as beta-lipoproteins.

Their lipid content is 75 to 90 per cent, in the form of triglycerides, phosphatides, esterified and non-esterified cholesterol and free fatty acids. Their chemical properties are determined by the protein component. In their antigenicity an important role is played by their carbohydrate component, representing 3.2 per cent of the total substance, in the form of hexose.

In 1961, Allison and Blumberg described the presence of isoprecipitin in the serum of a polytransfused patient. Later, it was shown to be an anti-LDL antibody and authors proved that LDL was a heterogenous serum protein fraction not only physicochemically, but also immunologically, and that it showed genetically determined antigenic specificity. [The anti-LDL isoprecipitin found in the patient's serum was called anti-Ag(a), the inductor antigen Ag(a) (1961).]

In his detailed referate, Bütler deals with the problem of terminology, which truly reflects the polymorphism of the LDL system.

At present, two groups of LDL group variants are known:

1. Ag-groups
2. Lp-groups

Standardization of the Lp-nomenclature is in progress. In 1965, Berg suggested the introduction of a new symbol, the "Ld-system", which did not seem justified because he had demonstrated the LDL factor discovered by him by means of human isoprecipitin, consequently, we had on hand a formerly unknown Ag factor.

A recently discovered genetical variant of the LDL system is represented by the "double beta-lipoprotein" described by Seegers (1965).

By two-dimensional (paper/starch) electrophoresis Seegers demonstrated the presence of a double LDL band in the serum from a female, aged 42 and suffering from post-gastrectomy anaemia, as well as in the sera from 6 members of her family. It was stated that this was a new group variant of the LDL system, and the gene responsible for it had a low frequency. In the paper the mode of development of the group-specific anti-LDL antibodies is discussed, attributing significance in the first place to the number of transfusions and to the basic disease. In addition, Dürwald's results for sera from multipara are described. The results of the family studies by Berg and Mohr are dealt with in detail, in which the mode of heredity of Lp groups has been clarified and it has been shown that the LDL antigens are not related to other serum groups. Author discusses also the investigations of Hirschfeld, who elucidated precisely the genetical process of the Ag groups, as well as the exact antibody specificities of the C. de B. serum.

Finally, Bütler emphasizes that the Lp and Ag groups raise a number of interesting population-genetical problems. The LDL polymorphism is also of immunobiological interest, because beside the Gm groups the Ag ones stand alone as verified human anti-plasmic isoimmunization factors. Although to this date there are no reports on clinical manifestations, plasma incompatibility, the discovery is of great interest.

Maria Ambrus

Absorption of unwanted anti-A-B agglutinins from anti-Rh D grouping sera using time-expired formalized heat-treated A_1B Rh D positive red cells. J. V. Lockyer (Regional Transfusion Centre, Bristol, G.B.) *Vox Sang. 12*, 75 (1967).

A simple and reliable method is recommended for the elimination of anti-A and anti-B agglutinins from specific antibody-containing 0 sera. The method is based on Milgrom's experiment, who used stroma material obtained by lysis of AB Rh_0 (D) positive erythrocytes for the absorption of 0 anti-D test serum with success, without observing a change in the reactivity or titre of anti-D. Later, Gold, Lockyer and Tovey demonstrated that on treatment with

formalin the A antigen carrier erythrocytes lost their agglutinability, but continued to absorb unchangedly anti-A agglutinin, whereas the Rhesus antigens lost both properties. The resistance difference of the antigens of the two blood group systems may be utilized in the absorption procedures, when we may use instead of AB Rh negative test cells Rh positive ones.

Author describes his own method, by means of which it has been proved that it is only the antigens of the AB0 system, and to some extent also those of the MNSs system that resist treatment with formalin, while the antigens of other blood group systems have the same resistance as the Rh antigens. To avoid interference by denatured Hb, the test cells were previously fixed by heat (autoclave, at 120° C for 30 minutes), then the heat-treated cells were incubated in formalin solution diluted 1 in 30 for 24 hours at 37° C. The formalin-treated test suspension was incubated with equal volume of test serum at +4°C for 2 hours, then the absorbed serum was tested for presence of anti-A and anti-B agglutinin in salty medium at +4° C and was subjected to enzyme test. Comparison of the pre-absorption specific antibody titre with the postabsorption one, made in every case, never showed a decrease in titre.

After use, the absorbed agglutinins were removed by washing from the surface of the heat-fixed, formalin-treated cells and the test cells were stored in a bacteriostatic solution at +4°C till next used.

The procedure has been employed for the absorption of a wide variety of test sera (anti-C, D, E, c, e, M, N, S, P_1, Fy^a, Kp^b), with full success.

Maria Ambrus

Studies of 6-N-hydroxylamino-9-β-D-ribofuranosylpurine in mouse leukaemia. I. H. Burchenal, M. Dollinger, I. Butterbaugh, D. Stoll, A. Ginor-Sorella (Divisions of Experimental Chemotherapy and Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York, N. Y.) *Biochem. Pharmacol. 16*, 423 (1967).

An adenosine analog, 6-N-hydroxylamino-9-β-D-ribofuranosylpurine (HAPR) has shown activity against transplanted mouse leukaemias L 1210 and P 815 and against sublines of these resistant to 6-mercaptopurine, methotrexate, vinicristine, and 1-β-D-arabinofuranosyl cytosine (Ara C). Conversely, a line of L 1210 resistant to HAPR was still sensitive to 6-mercaptopurine, methotrexate, 5-fluorouracil and Ara C but was cross-resistant to 6-methylmercaptopurineriboside (6 MeMPR). Adenosine given simultaneously, blocked the antileukaemic effects of HAPR, but adenine and guanine did not. It is postulated by analogy with 6 MeMPR that HAPR may be phosphorylated to an active nucleotide by adenosine kinase activity. The antileukaemic effects of HAPR were potentiated by 6-mercaptopurine.

Elisabeth Török

Degradation of human fibrinopeptides in serum. A. Teger-Nilsson, B. Blombach (Blood Coagulation Research Department, Karolinska Institutet, Stockholm) *Acta Chem. Scand. 21*, 307 (1967).

Fibrin formation is preceded by the release of fibrinopeptides from fibrinogen. Appreciable amounts of fibrinopeptides were thus expected to be present in blood serum. They may, however, rapidly degrade after their release from fibrinogen. Fibrinopeptide B, lacking its C-terminal arginine, was recently isolated from clot supernatants of thrombin-treated purified fibrinogen from several species. It was suggested that the peptide derivative was produced through enzymatic degradation by a contaminating exopeptidase of plasmatic origin. It is known from the work on blood kininases that blood contains carboxypeptidase B-like enzymes. The present investigation was undertaken in order to study the fate of fibrinopeptides in serum.

Elisabeth Török

Immune anti-N agglutinin in human serum. J. H. Yoell (Motion Picture Country Hospital, Woodland Hills, Calif.) *Transfusion 6*, 592 (1966).

A case of apparent hemolytic transfusion reaction due to presence in serum of

anti-N is reported. The reaction was characterized by demonstration of anti-N in the patient's serum and MN cells in the offending unit. The patient's phenotype proved to be M. Reasons for reporting this unusual case are: 1) It is believed to be the second instance of hemolytic transfusion reaction due to anti-N yet reported, and 2) Cases of this type again underscore the ease with which saline agglutinating antibodies can be missed in routine crossmatch practice, unless meticulous attention is paid to examination of microscopic agglutinates after the "immediate spin" technic.

Jolán Simon

Inhibition of the anti-globulin reaction by nonspecifically cell-bound human gammaG-immunoglobulin. P. J. Grob, A. Vaucher, H. Isliker (Institute of Biochemistry, University of Lausanne, Lausanne) *Vox Sang. 12*, 132 (1967).

The positive direct anti-globulin (Coombs) reaction displayed by a number of patients with agammaglobulinemia can be suppressed by intravenous administration of human gammaG-immunoglobulin (IgG). In an attempt to explain this in vivo phenomenon the authors have studied the in vitro reaction between anti-D sensitized human red cells and anti-globulin serum in the presence and absence of cell-bound nonspecific human IgG. The following conclusions can be drawn: in vitro a positive anti-globulin reaction of anti-D sensitized human red cells may be suppressed by reacting these cells with non-specific IgG. The inhibitory effect is proportional to the amount of IgG fixed per cell. No significant difference was observed when the red cells were incubated with non-specific IgG before or after sensitization with anti-D. Evidence is presented that the anti-globulin elutes cell-bound IgG, which is thus removed from the cells by the formation of specific complexes. It is open to question whether the suppression of the positive anti-globulin reaction in patients with agammaglobulinemia by intravenous administration of human gammaG-immunoglobulins can be explained by a similar mechanism.

Jolán Simon

Knochenmarksveränderungen bei Typhus abdominalis (Changes in bone marrow in Typhus abdominalis). R. J. Oechslin (Medizinische Klinik des Bürgerspitals, Solothurn) *Acta haemat. 37*, 11 (1967).

Bone marrow studies in 7 patients with acute typhoid fever revealed hyperplastic and left shifted granulocytopoiesis. This finding was considered to correspond with the pronounced toxic neutrophilic reaction in the peripheral blood observed. Erythropoiesis was decreased in the marrow, immature forms predominating. The most striking and unusual findings were diffusely scattered large reticular cells with characteristic features of phagocytosis. These phagocytic marrow cells apparently correspond to proper typhoid cells as they may be recognized in microscopic section materials.

Susan Kőszeghy

Delayed haemolytic transfusion reactions simulating auto-immune haemolytic anaemia. B. E. E. Croucher, M. C. Crookston, J. H. Crookston (Blood Transfusion Laboratory, Toronto, Ont.) *Vox Sang. 12*, 32 (1967).

Seven cases, in which the presence of a blood group iso-antibody was unsuspected until several days after transfusion, are described. In one case the rapid appearance of multiple antibodies produced signs and symptoms simulating auto-immune haemolytic anaemia. In two of the seven cases, antibodies were detectable in the pre-transfusion serum only when cells containing a double dose of the corresponding antigen were used.

Susan Kőszeghy

Treatment of haemophilia (Factor-VIII deficiency) with human antihaemophilic factor prepared by the cryoprecipitate process. C. R. M. Prentice, R. T. Breckenridge, W. B. Forman, O. D. Ratnoff (Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio) *Lancet 1*, 457 (1967).

A plasma fraction rich in antihaemophilic factor (A.H.F.) made by the cryopre-

cipitate process has been evaluated in the treatment of 11 bleeding episodes in eight haemophilic patients. In vitro assay of the material as diluted for infusion showed the average bag of cryoprecipitate contained 113 units of A.H.F. but there was a great deal of variability. The activity of cryoprecipitate after infusion into patients was, on average, 81% of the in vitro activity. 393 precipitates were used for the treatment of the 11 bleeding episodes. This material could be made easily by the hospital blood bank, and no other supplies of A.H.F. concentrate were needed once a stock of precipitates had been built up. After the extraction of A.H.F. the whole blood was used for most routine blood-transfusion purposes. No excessive blood-loss or complications due to haemorrhage were noted in any patient. One patient developed jaundice 2 months after therapy, but otherwise no serious complications arose from the use of the material. In particular, there has been no resistance to therapy or development of a circulating anticoagulant, although the latter complication has been observed elsewhere. A provisional scheme for the treatment of haemophilic patients is given.

Judith Pintér

The Factor-VIII activity in fresh and stored plasma. A. E. Preston (Oxford Regional Blood Transfusion Centre, Churchill Hospital, Headington, Oxford) *Brit. J. Haemat. 13*, 42 (1967).

An investigation into the Factor-VIII activity in fresh and stored plasma is reported and the present methods of production at the Oxford Regional Blood Transfusion Centre are described. Simple methods for increasing the Factor-VIII activity of therapeutic plasma are suggested. Special equipment did not prevent the loss of activity which occurs following the freezing, storage and thawing of fresh frozen plasma. A comparison between plasma stored at 4°C for less than 18 hours is made with fresh frozen plasma and the use of the former is suggested for routine treatment of minor injuries when fresh frozen plasma is not available.

Judith Pintér

Hypofibrinogenaemia without increased fibrinolysis in leukaemia. J. Hirsh, J. G. Buchanan, G. C. Gruchy, A. G. Baike (University of Melbourne, Department of Medicine, St. Vincent's Hospital, Melbourne) *Lancet 1*, 418 (1967).

Abnormally low levels of plasma-fibrinogen are described in 4 cases of leukaemia. Measurements of coagulation and related tests revealed no evidence of increased fibrinolysis, but in each case there was indirect evidence of excessive utilization of fibrinogen. In hypofibrinogenaemia the amount of fibrin in a blood clot is diminished, so methods for demonstrating increased fibrinolysis which depend on the patient's blood to provide substrate may give false results. Hypofibrinogenaemia in acute leukaemia has been thought to be associated mainly with promyelotic leukaemia, but the findings in these 4 cases do not support this. Haemorrhagic effects were severe in these cases, possibly because the hypofibrinogenaemia was associated with thrombocytopenia and other coagulation defects.

Georgina Sik

Analysis of pleiotropic effects of W and f genic substitutions in the mouse. Elizabeth S. Russell, Eleanor C. McFarland (The Jackson Laboratory, Bar Harbor, Maine) *Genetics 53*, 949 (1966).

An inbred mouse strain, FL/Re-f/f, has been developed along with congenic stocks differing at the W and f genetic loci. This makes possible quantitative evaluation of effects of these genic substitutions on postnatal blood values and body growth (0—60 days) and distribution of hair pigmentation. — At birth f/f mice have fewer and smaller red cells, and lower mean hematocrit percentages than congenic normal mice; all evidence of anemia has disappeared by 7 days postnatal. — Intralitter paired comparisons have demonstrated a highly significant increase in number and decrease in mean cell volume in W/+ mice over the observed congenic +/+ mice, without evidence of anemia, since these opposing effects balance each other. (This unequivocal microcytosis in W/+ mice is surprising,

since W/W mice suffer from very severe macrocytic anemia.) Substitution of f/f for f/+ results in significantly impaired total body growth, already apparent at birth and persisting at least to 60 days postnatal. W/+ versus +/+ genic substitution has no significant effect on body growth. — Both f/f versus f/+ genic substitution and W/+ versus +/+ genic substitution, lead to a moderate degree of ventral white-spotting; combination of these two gene substitutions (W/+ f/f mice) produces a hyperadditive spotting effect. The pigment restriction and restricted growth associated with f/f versus f/+ genic substitution may be secondary results of fetal anemia, or may result from direct action of f genes in primarily affected tissue. The pigment restriction associated with W/+ versus +/+ genic substitution, and the hyperadditive portion of pigment restriction in W/+ f/f mice, may be attributed to primary gene action in primarily affected tissues rather than to secondary of W gene action in hematopoietic tissue.

Esther Cholnoky

From the International Literature on Haematology

Acta Haematologica (Basel) **37** (1967) No. 1.

Studies of Heinz-body formation. *Borges, A.* et al. (Hosp. das Clínicas, Pediatría, São Paulo, Brazil), p. 1.

Knochenmarksveränderungen bei Typhus abdominalis. *Oechslin R. J.* (Med. Klinik, Bürgerspital, 4500 Solothurn, Schweiz), p. 11.

Cell proliferation in leukemia during relapse and remission. II. DNA synthesis of leukemic cells in the peripheral blood in vitro. *Schmid, J. R.* et al. (Dept. of Med., University, Kantonsspital, 8006 Zürich), p. 16.

The transformation of human mononuclear leukocytes. II. Precursors of large mononuclear cells on coverslips. *Lamvik, J. O.* (Univ. of Bergen, School of Med., Bergen, Norway), p. 32.

Studies of lymphocytes and their derivative cells in vitro. *Gough, J.* et al. (Dept. of Clin. Haemat., The University, Manchester, England), p. 42.

Aconitase activity in iron deficiency. *Swarup, Sushiela* et. al. (Indian Council of Med. Research, School of Tropical Med., Calcutta, India), p. 53.

Agglutinin synthesis regulation independent of antigen. *Rewald, E.* et al. (Corrientes 3550, Mar del Plata, Argentina), p. 62.

Acta Haematologica (Basel) **37** (1967) Nos. 2—3.

Erythrokinetic studies in thalassemia with simultaneous radioactive tracers (Fe^{59} and Cr^{51}). *Schettini, F.* et al. (Clinica Pediatrica, Univ. di Bari, Bari, Italia), p. 65.

Relationship of paroxysmal nocturnal hemoglobinuria to other pH-dependent hemolytic systems: Role of acetylcholinesterase. *Laforet, M. T.* et al. (Children's Hosp., Med. Center, Boston, Mass.), p. 88.

Survey for erythrocyte glucose-6-phosphate dehydrogenase deficiency in Hawaii. *Jim, R. T. S.* (Professional Center, 1481 S. King St., Honolulu, Hawaii), p. 94.

The potential of lymphocytes from patients with leukaemia and reticuloses to transform under the influence of phytohaemagglutinin. *Elves, M. W.* et al. (The Robert Jones and Agnes Hunt Orthopaedic Hosp., Oswestry, Shropshire, England), p. 100.

Failure of shielding the thymus to induce recovery of bone marrow after radiation. *Adams, D.* (Dental School, Heath, Cardiff, Wales), p. 109.

The nuclear segmentation of eosinophils under normal and pathological conditions. *Sparrevohn, S.* et al. (Ewaldsvej 15, Rungsted Kyst, Denmark), p. 120.

Hämolytische Anämie bei Sarkoidose der Milz. *Wyss, S.* et al. (Med. Abtl., Kreisspital, 8708 Männedorf, Schweiz), p. 126.

A case of idiopathic aplastic anaemia associated with trisomy-21 and partial endoreduplication. *Erdogan, G.* et al. (Capa Internal Clinic of Istanbul Med. School, Vakif Guraba Hosp., Capa, Istanbul), p. 137.

Kasuistischer Beitrag zum Krankheitsbild der akuten Eosinophilen-Leukämie. *Hauswaldt, Ch.* et al. (Med. Univ.-Klinik, Göttingen), p. 143.

Multiple antigenic changes in a case of acute leukaemia. *Ayres, M.* et al. (Departa-

mento de Puericultura e Pediatría, Univ. Federal do Pará, Belém, Pará, Brazil), p. 150.

Acta Haematologica (Basel) **37** (1967) No. 4.

Effet de l'insuline et de quelques autres facteurs sur la glycolyse des leucocytes humains mesurée in vitro. *Antonioli, J. A.* et al. (Clin. méd. de l'Univ., Lausanne, Suisse), p. 161.

Haemoglobin Koelliker: A new acquired haemoglobin appearing after severe haemolysis: α_2 minus 141 Arg β_2. *Marti, H. R.* et al. (Med. Univ. Poliklinik, Hebelstrasse 1, Basel), p. 174.

The thalassaemia syndromes. VI. Two subtypes of sickle cell-beta thalassaemia disease: (a) Normocytic type of sickle cell-beta thalassaemia disease. (b) Microcytic type of sickle cell-beta thalassaemia disease. *Aksoy, M.* et al. (Istanbul Med. School, Capa Internal Clinic, Vakif Guraba Hosp., Istanbul), p. 181.

Identification of ferritin within Gaucher cells. An electron microscopic and immunofluorescent study. *Lorber, M.* et al. (Dept. of Physiol. and Biophy., Georgetown Univ. Schools of Med. and Dentistry, Washington, D.C.), p. 189.

Characterization of glucose-6-phosphate dehydrogenase in Sardinian children with congenital nonspherocytic haemolytic anaemia. *Schettini, F.* et al. (Clinica Pediatrica dell'Univ., Policlinico 171, Bari, Italia), p. 198.

Übergang einer arzneimittelempfindlichen Erythropathie bei Glukose-6-Phosphatdehydrogenase-Mangel in eine chronische hämolytische Anämie. *Busch, D.* et al. (Med. Univ.-Klinik, Hugstetter Strasse 55, 78 Freiburg im Breisgau), p. 206.

Christmas disease in a girl with female karyotype. *Rozman, C.* et al. (Intern. Clinic, Dept. of Haemat., Univ. of Barcelona), p. 217.

Bilten transfuzije (Beograd) **20** (1966).

Patogeneza idiopatske trombocitopenijske purpure. *Stefanović, S.*. et al. (No address given), p. 7.

Klinicka slika idiopatske trombocitopenijske purpure. *Ruvidić, R.* et al. (No address given), p. 13.

Laboratorijski nalazi u nasih bolesnika sa ITP. *Ristić, M.* et al. (No address given), p. 21.

Tok i prognoza ITP. *Radojičić, B.* et al. (No address given), p. 29.

Rezultati lečenja idiopatske trombocitopenijske purpure na internoj klinici a u Beogradu. *Konečni, J.* et al. (No address given), p. 33.

Rezultati lečenja idiopatske trombocitopenijske purpure. *Berović, R.* et al. (Interne Klinike B Med. Fakulteta, Beograd), p. 39.

Iskustva u lečenju hronicne idiopatske trombocitopenijske purpure na klinici za unutrašnje bolesti VMA. *Radojičić, B.* et al. (Klinika za unutrašnje bolesti VMA), p. 45.

Terapija primarnih trombocitopenija (Akutnih i hronicnih). *Stojimirović, E.* et al. (Pedijatrijska klinika Med. Fakulteta, Beograd), p. 55.

Nasa iskustva u lečenju idiopatskih trombocitopenija (ITP) kod odraslih. *Nedelkovski, J.* et al. (Interna klinika Med. Fakulteta, Skopje), p. 63.

Nase iskustvo u tretiranju trombopeničnih purpura kod dece. *Sadikario, A.* et al. (Dečja klinika Med. Fakulteta, Skopje), p. 71.

Osvrt na liječenje esencijalne trombopenične purpure na internim klinikama medicinskog fakulteta u Sarajevu. *Ibrahimbegović, F.* et al. (Interne klinike Klinicke bolnice, Sarajevo), p. 83.

Završni osvrt. *Berović, R.* (Interne Klinike B. Med. Fakulteta, Beograd), p. 87.

Neka ispitivanja u vezi krvnih grupa sistema Lewis. *Latal-Duančić, M.* et al. (Zavod za transfuziju krvi SRH, Zagreb), p. 95.

Blood (New York) **28** (1966) No. 6.

A study of histamine in myeloproliferative disease. *Gilbert, H. S.* et al. (Dept. of Hemat., Mount Sinai Hosp., New York, N.Y.), p. 795.

Classification and evolution of patterns of erythropoiesis in polycythemia vera as studied by iron kinetics. *Pollycove, M.* et al. (Donner Lab., Univ. of California, Berkeley, Calif.), p. 807.

Further cases of Hb Q-H disease (Hb Q-α thalassemia). *Lie-Injo Luan Eng* et al.

(G. W. Hooper Foundation, Hemat. Unit, Univ. of Calif. Med. Center, San Francisco, Calif.), p. 830.

Treatment of autoimmune hemolytic anemia in children with azathioprine (Imuran). *Hitzig, W. H.* et al. (Dept. of Pediatrics, Univ. of Zurich, Zurich, Switzerland), p. 840.

Tritiated thymidine as a cytocidal agent in human leukemia. *Greenberg, M. L.* et al. (Med. Research Center, Brookhaven National Lab., Upton, N.Y.), p. 851.

The lactate dehydrogenase of hemopoietic cells. *Starkweather, W. H.* et al. (United States Veterans Administration Hosp., Ann Arbor, Mich.), p. 860.

A radioautographic study of hemopoietic repopulation using irradiated parabiotic rats. Relation to the stem cell problem. *Caffrey Tyler, Ruth W.* et al. (Dept. of Biol. Structure, Univ. of Washington School of Med., Seattle, Wash.), p. 873.

DNA replication patterns of normal human leukocyte cultures. Time sequence of DNA synthesis in relation to the H^3-thymidine incorporation over the nucleolus. *Ribas-Mundó, M.* (Dept. of Hemat., Clinic for Intern. Med., Barcelona, Spain), p. 891.

The ultrastructure of negatively stained platelets. Some physiologic implications. *Bull, B. S.* (Clin. Pathol. Dept., National Inst. of Health, Bethesda, Md.), p. 901.

Observations on the presence of pteroyl polyglutamates in human serum. *Banerjee, D. K.* et al. (Dept. of Hemat., School of Tropical Med., Calcutta, India), p. 913.

Plasma infusions in immunologic deficiency states: Metabolic and therapeutic studies. *Stiehm, E. R.* et al. (Dept. of Pediatrics, Univ. of California School of Med., San Francisco, Calif.), p. 918.

Blood (New York) **29** (1967) No. 1.

The effects of two alkaloids derived from Vinca rosea on the malignant cells of Hodgkin's disease, lymphosarcoma and acute leukemia in vivo. *Marmont, A. M.* et al. (Hematology Center, Ospedali-Civili, Genova-Sampierdarena, Italy), p. 1.

Studies of lymphocyte kinetics in man. *Perry, S.* et al. (National Cancer Inst., National Institutes of Health, Bethesda, Md.), p. 22.

The effects of allografts of thymic epithelial reticular cells on the lymphoid tissues of neonatally thymectomized mice. *Hays, Esther F.* (Lab. of Nuclear Med. and Rad. Biology, Dept. of Biophysics-Nuclear Med., Univ. of California, Los Angeles), p. 29.

Evidence for the origin of plasma cells from adventitial cells. I. Sensitized subcutaneous connective tissue examined by light microscopy. *Hirata-Hibi, M.* (Langley Porter Inst., Univ. of California Med. Center, San Francisco, Calif.), p. 41.

RNA metabolism of normal and leukemic leukocytes. II. Ribonuclease. *Silber, R.* et al. (Dept. of Med., New York Univ. School of Med., New York, N.Y.), p. 57.

The size and transit time of nondividing subpool of precursor cells in acute leukemia. *Shiuming Lin, M.* et al. (Dept. of Med., The Ohio State Univ., Columbus, Ohio), p. 63.

Composition and structure of cytoplasmic globules in leukemic lymphocytes. *László, J.* et al. (Durham VA Hospital, Durham, N. C.), p. 77.

Detection of glucose-6-phosphate dehydrogenase deficient heterozygotes. *Stamatoyannopoulos, G.* et al. (Dept. of Med., Univ. of Washington, Seattle, Washington), p. 87.

The effect of plethora on growth and differentiation of normal hemopoietic colony-forming cells transplanted in mice of genotype W/W^v. *Till, J. E.* et al. (Dept. of Med. Biophysics, Univ. of Toronto, Ontario, Canada), p. 102.

The influence of hypoxemia and cobalt on erythropoietin production in the isolated perfused dog kidney. *Fisher, J. W.* et al. (Univ. of Tennessee Med. Units, Memphis, Tenn.), p. 114.

Regulation of fetal and adult hemoglobin formation in patients with sickle cell disease transfused to normal hematocrits. *Gabuzda, Th. G.* et al. (Jefferson Med. College, Philadelphia, Pa.), p. 126.

Protease content and fibrinolytic activity of human leukocytes. *Astrup, T.* et al. (James F. Mitchell Foundation, Washington, D.C.), p. 134.

Coated charcoal assay of erythrocyte vitamin B_{12} levels. *Kelly, A.* et al. (The Mount Sinai Hosp., New York, N.Y.), p. 139.

Leukemia or lymphoma occurring subsequent to an autoimmune disease. *Oleinick, A.* (National Cancer Institute, National Institutes of Health, Bethesda, Md.), p. 144.

Blood (New York) **29** (1967) No. 2.

An electron microscopic study of thrombin-induced disseminated intravascular coagulation. *Margaretten, W.* et al. (Dept. of Pathol., Columbia Univ. Col'ege of Physicians and Surgeons, New York, N.Y.), p. 169.

Malic and lactic dehydrogenase isozymes of normal and leukemic leukocytes separated on glass bead columns. *Rabinovitz, Y.* et al. (Vet. Adm. Hosp., Research Service, Hines, Ill.), p. 182.

Effects in vivo hyperoxia on erythrocytes. V. Changes of RBC glycolytic intermediates in mice after in vivo oxygen under high pressure. *O'Malley, W.* et al. (Dept. of Med., Ohio State Univ., Columbus, Ohio), p. 196.

Studies on the macroglobulins of human serum. III. Quantitative aspects related to cold agglutinin. *Wollheim, F. A.* et al. (Dept. of Med., Univ. Hosp., Minneapolis, Minn.), p. 203.

Chromosomal aberrations common to three types of monoclonal gammopathies. *Houston, Elsie W.* et al. (Univ. of Texas, Med. Branch, Galveston, Texas), p. 214.

A marrow chromosomal abnormality preceding clinical leukemia in Down's syndrome. *Demayo, A. P.* et al. (Cornell Univ. Med. College, Dept. of Pediat., New York, N.Y.), p. 233.

The effect of splenic X-irradiation on the ferrokinetics of chronic leukemia with a clinical study. *Awwad, H. K.* et al. (Faculty of Med., Univ. of Alexandria, United Arab Republic), p. 242.

Neutrophile alkaline phosphatase changes in tularemia, sandfly fever, Q fever and noninfectious fevers. *Beisel, W. R.* (US Army Med. Unit, Fort Detrick, Frederick, Md.), p. 257.

Nuclear bodies of normal and pathological human lymph node cells: an electron microscopic study. *Brooks, R. E.* et al. (Dept. of Pathol., Univ. of Oregon Med. School, Portland, Oregon), p. 269.

Blood (New York) **29** (1967) No. 3.

The influence of hemoglobin concentration on the distribution pattern of the volumes of human erythrocytes. *Weed, R. I.* et al. (Univ. of Rochester, School of Med., Dept. of Radiation and Biology, Rochester. N.Y.), p. 297.

The regeneration of reduced glutathione in normal and glucose-6-phosphate dehydrogenase deficient human red blood cells. *Kosower, N. S.* et al. (Albert Einstein College of Med., Bronx, N.Y.), p. 313.

Treatment of hereditary spherocytosis in peromyscus by radiation and allogenic bone marrow transplantation. *Steinmuller, D.* et al. (Inst. of Cancer Research, Div. of Pathol., Fox Chase, Philadelphia, Pa.), p. 320.

Differential consumption of coagulation factors resulting from activation of the extrinsic (tissue thromboplastin) or the intrinsic (foreign surface contact) pathways. *Nossel, H. L.* (Columbia Univ., College of Physiol. and Surg., New York, N.Y.), p. 331.

The relation of blood platelet survival and distribution to ^{14}C-serotonin distribution and excretion. *Heyssel, R. M.* et al. (Vanderbilt Univ., School of Med., Nashville, Tenn.), p. 341.

Radioactive diisopropyl fluorophosphate as a platelet label: an evaluation of in vitro and in vivo technics. *Bithell, T. C.* et al. (Univ. Utah, College of Med., Salt Lake City, Utah), p. 354.

Hematologic effects of splenic implants. *Tarnuzi, A.* et al. (Univ. Gadjah Mada, Dept. of Pathol., Jog Jakarta, Indonesia), p. 373.

Ultrastructural observations on platelet adhesion reactions. I. Platelet-fibrin interaction. *Erichson, R. B.* et al. (Montefiore Hosp. and Med. Center, Dept. of Hematol., New York, N.Y.), p. 385.

Studies of the changes in leukocyte alkaline phosphatase following pyrogen stimulation in chronic granulocytic leukemia. *Perillie, P. E.* (Yale Univ., School of Med.: Dept. of Internal Med., New Haven, Conn.), p. 401.

Analytical review: Multiple differentiation in megakaryocytes and platelets. *Paulus, J.-M.* (Univ. Liège, Dept. Clin. Pathol. Med., Liège, Belgium), p. 407.

Blut (München) **14** (1967) No. 4.

Hämoglobingehalt und Hämoglobinkonzentration in Erythrozyten nach Schichtungszentrifugation. *Kleihauer, E.* et al. (Univ.-Kinderklinik, 74 Tübingen, Rümelinstr. 23.), p. 193.

Karyometric investigations of the erythroblastic system. *Ławkowicz, W.* et al. (Dept. of Haemat., Inst. of Haemat., Chocimska 5, Warsaw), p. 199.

Die Anwendung des Alkohols zur Konservierung der Testerythrozyten in der immunohämatologischen Praxis. *Kout, M.* et al. (Inst. für Hämat. u. Bluttransf., U nemocnice 1, Prag 2.), p. 202.

Vergleich der Proteinasen-Inhibitoren aus Lunge und Leber vom Rind mit besonderer Berücksichtigung ihres Einflusses auf Fibrinolyse und Blutgerinnung. *Werle, E.* et al. (Klin. chem. Inst. der Univ., Nussbaumstrasse 20, 8 München 15.), p. 206.

Zytochemische Untersuchungen an Zellen eines extramedullären Plasmozytoms des Magens. *Castrup, H. J.* (Pathol. Inst. der Univ., Josef-Stelzmannstr. 9, 5 Köln-Lindenthal), p. 211.

Zur Lebenserwartung unbehandelter und behandelter Plasmozytomkranken. *Ramsbott, C.* et al. (I. Med. Klinik der Freien Univ., Spandauer Damm 130, 1 Berlin 19.), p. 217.

Blut (München) **14** (1967) No. 5.

The platelet resistance in idiopathic thrombocytopenic purpura. *Storti, E.* (Inst. di Patol. Med. dell'Università, Via del Pozzo, Modena, Italy), p. 257.

Die Wirkung von ^{60}Co-Gammastrahlen auf die Lebensdauer und Feinstruktur peripherer Thrombozyten. *Sebestik, V.* et al. (Inst. für Hämat. u. Bluttransf., Prag 2, U nemocnice 1), p. 261.

Über die Blutkonzentrationen der Leukozyten u. Thrombozyten bei Eisenmangel. *Voigt, D.* et al. (I. Med. Univ.-Klinik der Charité, Schumannstr. 20/21, X 104 Berlin), p. 267.

Zur Frage der endokrinen Beeinflussung der alkalischen Leukozytenphosphatase. *Müller, D.* (Med. Univ. 74 Tübingen), p. 273.

Stimulation in vitro of cells from patients with myeloproliferative disorders. *Pegrum, G. D.* (Dept. of Haemat., Charing Cross Hosp. Med. School, London, W. C. 2), p. 277.

Über einen im Rhesus-Blutgruppensystem wirksamen Phenolwasserextrakt aus menschlichen Erythrozyten. *Thiele, O. W.* et al. (Physiol.-chem. Inst. der Univ., 34 Göttingen, Humboldtallee 7), p. 284.

Blut (München) **14** (1967) No. 6.

Die Milzszintigraphie. *Pfisterer, H.* (Inst. für Hämatol., Gesellschaft für Strahlenforschung mbH, Ass. mit EURATOM, Ziemssenstr. 1, München), p. 321.

The mechanical resistance of platelets: a description of the method, results in normal cases, and preliminary results in pathologic cases. *Storti, E.* et al. (Istituto di Patol. Med. dell'Univ., Via del Pozzo, Modena, Italy), p. 325.

Gc-Darstellung mittels Hydroxylapatit-Säulen-Chromatographie. Immunelektrophoretische Untersuchungen unter besonderer Berücksichtigung des alpha$_2$-Bereiches. *Roelcke, D.* et al. (Serol. Inst. der Univ., 69 Heidelberg), p. 331.

Doppelbrechung in Blutzellen nach Supravitalfärbung mit Toluidinblau. *Missmahl, H. P.* (Med. Univ.-Klinik, Otfried-Müller-Strasse, 74 Tübingen), p. 341.

Stellungnahme zum indirekten Vaterschaftsausschluss über das Blutgruppensystem MNSs. *Fiedler, H.* et al. (Robert Koch-Inst., Nordufer 20, 1 Berlin 65.), p. 346.

Zur elektronenmikroskopischen Darstellung der ATP-spaltenden Fermentaktivität in Knochenmarkszellen. *Pakesch, F.* et al. (Lazarettgasse 14, II. Med. Univ. Klinik, A 1000 Wien), p. 348.

Morphologische Veränderungen einer Plasmozytomzelle während 4 Tagen in vitro. *Boll, I.* (Städt. Krankenhaus Neukölln, Rudower Strasse 56, 1 Berlin 47.), p. 351

Untersuchungen über Generationszeiten in der Erythropoese unter verschiedenen Funktionszuständen. *Müller, D.* (Med.

Univ.-Klinik, Otfried-Müller-Strasse, 74 Tübingen), p. 356.

British Journal of Haematology (Oxford) **13** (1967) No. 1.

Platelets in haemostasis and thrombosis. *Lüscher, E. F.* (Theodor Kocher Inst., Univ. of Berne), p. 1.

Effects of human plasma on thrombelastographic patterns produced by fibrinolytic agents in fibrin clots. *Egeblad, K.* et al. (Surg. Dept. H, Copenhagen Hosp., Gentofte, Denmark), p. 10.

Factor-VIII (antihaemophilic factor) activity in sickle-cell anaemia. *Abildgaard, C. G.* et al. (Univ. of Illinois College of Med., Chicago, Ill.), p. 19.

New anticoagulant from lysed fibrinogen. *Triantaphyllopoulos, E.* et al. (Univ. of Alberta, Edmonton, Alberta, Canada), p. 28.

The Factor-VIII activity in fresh and stored plasma. *Preston, A. E.* (Oxford Regional Blood Transf. Centre, Headington, Oxford), p. 42.

The anaemia of erythropoietic porphyria. II. Studies of some red-cell intermediates. *Zail, S. S.* et al. (South African Inst. for Med. Research, Johannesburg, S.A.), p. 60.

Spontaneous lymphocyte transformation in vitro: Negative evidence from a study of thymidine uptake. *Caron, G. A.* (Univ. of Oregon Med. School, Portland, Oregon), p. 68.

Vitamin B_{12} absorption by inhalation. *Shinton, N. K.* et al. (Coventry and Warwickshire Hosp., Coventry, England), p. 75.

The inhibition of anaerobic glycolysis in red cells by leukaemic leucocytes. *Sabine, Jean C.* (Univ. of California School of Med., San Francisco, Calif.), p. 80.

Red-cell metabolism in hereditary spherocytosis. *Bronwen Loder, P.* et al. (Dept. of Med., St. Vincent's Hosp., Fitzroy, Victoria, Australia), p. 95.

Developmental and acquired variations in erythrocyte carbonic anhydrase isozymes. *Weatherall, D. J.* et al. (Dept. of Med., Univ. of Liverpool, England), p. 106.

Alterations in cellular sodium, potassium, calcium, magnesium, copper and zinc levels during the development and maturation of erythrocytes in the rabbit. *Valberg, L. S.* et al. (Dept. of Med., Queen's Univ., Kingston, Ontario, Canada), p. 115.

Hereditary elliptocytosis: A report of two families from New Guinea. *Pryor, D. S.* et al. (Angau Memorial Hospital, Lae, New Guinea), p. 126.

Microangiopathic haemolytic anaemia: The occurrence of haemolysis in experimentally produced vascular disease. *Brain, M. C.* et al. (Dept. of Haemat., Royal Postgraduate Med. School, London), p. 135.

Folia Haematologica (Leipzig) **86** (1966) No. 4.

Der Monozyt, seine Genese und seine Rolle bei der Reaktivität des Organismus. *Grigorowa, O. P.* (Inst. für Pädiatrie u. kindliche Chirurgie RSFSR Moskau G-34, Krapotkinskaja 37), p. 377.

Zur Rolle der basophilen Leukozyten und Mastzellen in Fibrinolyse und Allergie. I. Wirkung von Fibrinolytika und antifibrinolytika auf den Degranulierungsgrad von basophilen Leukozyten. *Frick, G.* et al. (Med. Univ.-Klinik, 25 Rostock, Ernst-Heydemann-Strasse), p. 389.

Beitrag zur Frage der extramedullären Hämopoese. *Szász, Gy.* et al. (Gorkijfasor 11/a, Budapest VII), p. 404.

Über den Nachweis fetalen Hämoglobins bei erworbenen Blutkrankheiten mit der Elutionsmethode. *Scholz, R.* (12 Frankfurt/Oder, Heinrich-Hildebrand-Strasse 24), p. 419.

Astrozytose, eine Sonderform der Acanthozytose. Beitrag zum Studium der Erythrozytenanomalien. *Gingold, N.* et al. (Hämat. Abtl. des Bezirksspitals Griviţa Roşie, Bukarest, Bd. 1 Mai, 37—39), p. 436.

Bericht über eine Vitamin B_6-Mangelanämie. *Wohllebe, W.* et al. (Med. Univ.-Klinik u. Poliklin., 22 Greifswald, Friedrich-Loeffler-Strasse 23/a), p. 445.

Antithrombin activity of serum from the native or recalcified blood. *Gaertner, H. A.* et al. (Kraków, Dzierzýnskiego 19a/4), p. 454.

Beitrag zum familiären Vorkommen eines P-Antikörpers. *Stangel, W.* et al. (Bratislava, Ulica Febr., vitazstva 17), p. 459.

Hémostase (Paris) **6** (1966) No. 4.

La staphylocoagulase. *Soulier, J. P.* (Centre National de Transf. Sanguine, 6, rue Alexandre-Cabanel, Paris XVe), p. 213.

The platelet aggregation defect in the von Willebrand-Jürgens-syndrom on the Åland Islands. The possible existence of an "anti-Willebrand-factor". *Jürgens, J.* et al. (Dept. of Med., Univ. of Frankfurt/Main), p. 225.

Some investigations into the reserve fibrinolytic activity in patients with febrile and thrombo-embolic diseases. *Van Walree, R. N. E.* et al. (Dept. of Intern. Med. B, Municipal Hosp., Dijkzigt, Rotterdam), p. 233.

Major surgery in the presence of a primary capillary haemorrhagic disorder. *Hobbs, K. E. F.* et al. (Dept. of Cardio-thoracic Surgery, The Royal Infirmary, Sheffield, Great Britain), p. 241.

Investigations on the blood coagulation and the euglobulin clot lysis time in superficial thrombophlebitis. *Szczepanski, M.* (Dept. of Surgery, Inst. of Haemat., Warsaw), p. 247.

Aspects biologiques et résultats expérimentaux de l'utilisation de la streptokinase en circulation régionale isolée. *Baille, Yves* et al. (Chaire d'Anat. Appliqué, Faculté de Médecine de Marseille, France), p. 255.

Purpura thrombopénique aigu, secondaire à l'absorption de quinine. *Vergoz, D.* et al, (Centre Départ. de Transf. Sanguine, 53, boulevard Diderot, 75-Paris-XIIme), p. 273.

Fibrinolytic activity and reduction of platelet adhesiveness following prolonged administration of ethyl-chlorophenoxy-isobutyrate (Atromid-S). *Gibelli, A.* et al. (Dept. of Med., Univ. of Pavia, Italy), p. 285.

Evaluation du pro-activateur du système fibrinolytique par thromboélastographie. *Migne, J.* et al. (Centre de Recherches du Service de Santé des Armées, Div. d'Hémobiol., Paris), p. 291.

Il Sangue (Milano) **39** (1966) No. 6.

Trasfusioni di sangue nel feto in utero. *Liley, A. W.* (Postgraduate School of Obstetrics and Gynaecology, Univ. of Auckland, New Zealand), p. 244.

Valore antropologico dei gruppi sanguigni. *Bartolo, M. De* (Istituto di Antropologia, Univ. di Roma, Italia), p. 251.

Trasfusioni ed eritroenzimopatie. *Salvidio, E.* et al. (Istituto di Patologia Speciale Medica, Univ. di Genova, Italia), p. 255.

L'attivita medico sociale dei servizi trasfusionali. *Santi, L.* et al. (Istituto di Clinica Medica Generale, Univ. di Genova, Italia), p. 261.

Il Sangue (Milano) **40** (1967) No. 1.

Contributo all'interpretazione patogenetica delle emorragie precoci del neonato. *Santangelo, G.* et al. (Univ. degli Studi di Messina, Clin. Pediatrica, Messina, Italia), p. 4.

Frequenza e aspetti della "malattia emorragica del neonato". *Chiappe, F.* et al. (Univ. di Cagliari, Istituto di Puericultura, Cagliari, Italia), p. 10.

Timo e linfociti. *Baserga, A.* (Istituto di Patologia Medica, Univ. di Ferrara, Italia), p. 15.

Nouvelle Revue Française d'Hématologie (Paris) **7** (1967) No. 1.

Études familiales portant sur les alloantigènes leuco-plaquettaires. *Dausset, J.* et al. (Hôpital Saint Louis, 2, place du Dr. A.-Fournier, F.-75-Paris X^{e}), p. 5.

Surproduction des porphyrines et variations du taux hépatique et érythrocytaire de l'atp au cours de l'intoxication alcoolique aiguë du rat. *Gajdos, A.* et al. (Lab. de Recherches biochimiques, 1, place du Parvis-Notre-Dame, F.-75-Paris IVe), p. 15.

Étude de la cinétique des polynucléaires après marquage «in vitro» par le radiochrome. I. Étude critique de la méthode et résultats obtenus chez les sujets normaux. *Dresch, Catherine* et al. (Lab. des Isotopes, Hôpital Saint-Louis, 2, place du Dr.-Fournier, F.-75-Paris X^{e}), p. 27.

Étude de la cinétique des polynucléaires après marquage «in vitro» par le radiochrome. II. Résultats obtenus dans les granulopénies. *Dresch, Catherine* et al. (Lab. des Isotopes, Hôpital Saint-Louis, 2, place du Dr.-Fournier, F.-75-Paris X^{e}), p. 49.

Formation de cils rudimentaires dans les cellules sanguine primitives du sac vitellin d'embryons de rat et de poulet. *Breton-Gorius, Janine* et al. (Inst. de Pathol., Hôpital Bicêtre, F.-94-Le Kremlin-Bicêtre, Paris), p. 79.

Leucocytes et fibrinolyse. II. Étude des leucocytes pathologiques. *Tatarsky, I.* et al. (Ramban Government Hosp., Haifa, Israel), p. 95.

Les isozymes de la lactico-déshydrogénase du globule rouge. *Schapira, Fanny* et al. (Hôpital des Enfants Malades, 147, rue de Sèvres, F.-75-Paris XVe), p. 109.

Scandinavian Journal of Haematology (Copenhagen) **3** (1966) No. 5.

The reactivity of human cord lymphocytes in mixed cell cultures. *Lamvik, J. O.* (The Gade Inst., Dept. of Pathol., Bergen, Norway), p. 325.

Screening for irregular blood group antibodies. Selectogen procedure compared with other methods. *Dybkjaer, E.* (Blood Bank and Grouping Lab., Århus Kommunehosp., Århus C, Denmark), p. 332.

Gelation of soluble fibrin in plasma by ethanol. *Godal, H. C.* et al. (Haemat. Research Lab., Dept. IX, Ullevål Hosp., Oslo, Norway), p. 342.

Synthesis of donor type γ-Globulin following thymus transplantation in hypo-γ-globulinaemia with severe lymphocytopenia. *Harboe, M.* et al. (Inst. for Exp. Med. Research, Ullevål Hosp., Oslo 1, Norway), p. 351.

Lymphocyte number and response to phytohaemagglutinin in chronic lymphocytic leukaemia. *Sharman, Christine* et al. (Cytogenetics Unit, Christchurch Hosp., Christchurch, New Zealand), p. 375.

Inhibition of red cell aggregation by TAME. *Godal, H. C.* et al. (Haemat. Research Lab., Dept. IX, Ullevål Hosp., Oslo, Norway), p. 383.

A group of patients with impaired plasma fibrinolytic system and selective inhibition of tissue activator-induced fibrinolysis. *Brakman, P.* et al. (The James F. Mitchell Foundation, 5401 Western Ave., N.W., Washington, D.C.), p. 389.

Methylmalonic acid excretion and serum vitamin B_{12}. *Holmberg, C. G.* et al. (Dept. of Clin. Chem., Univ. Hosp., Lund, Sweden), p. 399.

Thrombosis et Diathesis Haemorrhagica (Stuttgart) **17** (1967) No. 1/2.

Fibrinolytic system in patients with hypertensive cardiovascular disease. *Prokopowicz, J.* et al. (Dept. of Physiol. Chemistry, Medical School, Bialystok, Poland), p. 1.

Rapid determination of profibrinolysin (plasminogen) in plasma. *Nanninga, L. B.* (Dept. of Physiol., Univ. of Texas Med. Branch, Galveston, Texas), p. 8.

A method for the quantitative assay of inhibitor of plasminogen activation in human serum. *Bennett, N. B.* (Dept. of Med., Univ. of Aberdeen, Scotland), p. 12.

The inhibitory effect of sialic acid on fibrinolysis. *Rubin, H.* et al. (Dept. of Med., Maimonides Hosp., Brooklyn, N.Y.), p. 23.

Effects on fibrin clot lysis of a trypsin inhibitor in peanuts (Arachis hypogaea). *Egeblad, K.* (James F. Mitchell Foundation, Inst. for Med. Research, Washington, D.C.), p. 31.

Spontaneous fibrinolysis demonstrated by immunological technique. *Laursen, B.* et al. (Dept. of Med., Sundby Hosp., Copenhagen, Denmark), p. 42.

The effect of trasylol on blood coagulation after intravenous injection. *Gormsen, J.* et al. (Med. Dept., Sundby Hosp., Copenhagen, Denmark), p. 51.

Vergleichende Untersuchungen zur Wirkung von Urokinase und Streptokinase im Tierversuch. *Hiemeyer, V.* et al. (Med. Univ.-Klinik, Freiburg i. Br., Germany), p. 58.

Soluble unclottable complexes formed in the presence of fibrinogen degradation products (FDP) during the fibrinogen-fibrin conversion and their potential significance in pathology. *Lipinski, K.* et al. (Dept. of Radiobiol. and Health Protection, Inst. of Nuclear Research, Warsaw 9, Poland), p. 65.

Influence of fibrinogen split products on platelets. *Barnhart, M. I.* (Wayne State Univ. School of Med., Dept. of Physiol. and Pharmacol., Detroit, Mich.), p. 78.

Platelet aggregation in mammalians (human, rat, rabbit, guinea-pig, horse, dog). A comparative study. *Sinakos, Z.* et al. (Clinique

Médicale B. Hosp. Hippocration, Univ. de Thessalonique, la Grèce), p. 99.

Effect of temperature, velocity gradient and i.v. heparin on in vitro blood coagulation and platelet aggregation. *Dintenfass, L.* et al. (Dept. of Med. Univ. of Sydney, Sydney), p. 112.

Defibrination syndrome followed by hypercoagulable state. *Rachmilewitz, E. A.* et al. (Dept. of Med. B, Hadassah Univ. Hosp., Jerusalem, Israel), p. 120.

Experimentelle Verbrauchskoagulopathie nach intravenöser Zufuhr von Fett und Endotoxin. *Huth, K.* et al. (Med. Klin. u. Poliklinik, Justus-Liebig-Univ., Giessen), p. 129.

Verbrauchskoagulopathie und Lysekoagulopathie bei menschlichen Äquivalenten des Sanarelli-Shwartzman-Phänomens (generalisiertes Shwartzman-Phänomen). *Graeff, H.* et al. (Univ.-Frauenklinik, Heidelberg), p. 144.

Studies on the pathogenesis of the generalized Shwartzman reaction IV. Production of a Shwartzman-like phenomenon with autoprothrombin-C. *Rodríguez-Erdmann, F.* et al. (New England Med. Center Hospitals, Boston, Mass.), p. 156.

Hypercoagulable state induced in humans by the intravenous administration of purified ellagic acid. *Girolami, A.* et al. ("Clotting Mechanisms" Section, Memorial Sloan-Kettering Cancer Center, New York, N.Y.), p. 165.

Inherited fibrinogen abnormality causing thrombophilia. *Egeberg, O.* (Inst. for Thrombos. Research Med. Dept. A, Rikshospitalet, Oslo, Norway), p. 176.

Studies on the enzymatic activity of the antihemophilic factor (Factor VIII). *Creveld, S. van* et al. (Hemophilia Clinic, Huizen, The Netherlands), p. 188.

Congenital combined deficiency of Factor VIII (antihaemophilic globulin) and Factor V (proaccelerin) in two siblings. *Gobbi, F.* et al. (Dept. of Med. Pathol., Univ. of Modena School of Med., Modena, Italy), p. 194.

The conversion of prothrombin to thrombin. *Baker, W. J.* et al. (Dept. of Physiol. and Pharmacol., Wayne State Univ. School of Med., Detroit, Mich.), p. 205.

The prothrombin time in the newborn: Effect of contact activation. *Shanberge, J. N.* et al. (Dept. of Pathol., Evanston Hosp., Evanston, Ill.), p. 214.

The blood clotting properties of rabbit peritoneal leukocytes in vitro. *Rapaport, S. I.* et al. (Dept. of Med., Univ. of Southern California School of Med., Los Angeles, Calif.), p. 222.

Effects of clotting on the label in iodinated fibrinogen in different species. *Regoeczi, E.* et al. (National Inst. for Med. Research, Mill Hill, London, N.W. 7.), p. 235.

Removal of thrombokinase from commercial thrombin. *Kerwin, D. M.* et al. (Dept. of Pathol., Yale Univ. School of Med., New Haven, Conn.), p. 247.

The effect of hirudin, a potent antithrombin, on the thromboplastin generation test. *Caldwell, M. J.* (Dept. of Laboratories, Henry Ford Hosp., Detroit, Mich.), p. 256.

Anticoagulant properties of an acid mucopolysaccharide preparation isolated from rat kidney. *Allalouf, D.* et al. (Rogoff and Wellcome Med. Research Inst., Beilinson Hosp., Petah, Tikva, Israel), p. 264.

Mechanism of interaction between a phosphoinositide anticoagulant and a phospholipid activator in the Stypven type conversion of prothrombin to thrombin. *Kahn, M. J. P.* et al. (Univ. of Brussels, Belgium), p. 273.

The anticoagulant activity of 3.3'-(p-alkylthio-benzylidene)-bis-4-hydroxycoumarins and their oxidation products. *Guminska, Maria* et al. (Dept. of Physiol. Chemistry, Medical Academy, Cracow, Poland), p. 277.

L'action du facteur VIII (globuline antihemophilique) sur la consommation du facteur X pendant la coagulation. *Mayer, G.* et al. (Inst. d'Hémat. de la Faculté de Médecine, Strasbourg, France), p. 287.

Transfusion (Paris) **9** (1966) No. 4.

Fréquence du facteur Gm (4) parmi quelques populations blanches, noires et jaunes. *Ropartz, C.* et al. (Centre Départemental de Transfusion Sanguine, 76 F. Bois-Guillaume, France), p. 293.

Préparation de facteur anti-hémophilique VIII concentré à usage thérapeutique: la cryoprotéine VIII. *Combrisson, A.* et al. (Centre Départemental de Transfusion Sanguine, Seine, France), p. 303.

Groupes sanguins AB0 et Rh (D) dans le département de la Charente. *Khérumian, R.* et al. (Centre Départemental de Transfusin Sanguine, Charente, France), p. 321.

Accident transfusionnel mortel chez un sujet A_2 dû à des anticorps anti-A_1. *Doitchinova, N.* et al. (Institut d'Hématologie et de Transfusion Sanguine, Sofia), p. 327.

Transfusion (Philadelphia) **7** (1967) No.1.

Further studies on the effect of adenine in blood preservation. *Åkerblom, O.* et al. (Dept. of Clin. Chem., Univ. Hosp., Uppsala, Sweden), p. 1.

Relationship between lipid fractions of erythrocytes and their *in vivo* survival following preservation with glycerol and the slow freeze technic. *Almond, D. V.* et al. (Naval Hosp., Naval Blood Research Lab., Chelsea, Mass.), p. 10.

The effect of cooling regimens on the transplantation potential of marrow. *Lewis, J. P.* et al. (Dept. of Med., Presbyterian St. Luke Hosp., Chicago, Ill.), p. 17.

Aberrant U blood accompanying Rh_{null}. *Schmidt, P. J.* et al. (Blood Bank Dept., National Inst. of Health, Bethesda, Md.), p. 33.

Hypocalcemia and defective hemostasis after massive blood transfusion. Report of a case. *Aggeler, P. M.* et al. (Med. Service, Univ. of California School of Med., San Francisco, Calif.), p. 35.

A study related to the significance of hemolysis observed among aborters, non-aborters and infertility patients. *Vos, G. H.* (Dept. of Pathol., King Edward Memorial Hosp. for Women, Subiaco, Australia), p. 40.

Autoimmune hemolytic anemia associated with anti-f: Report of a case. *Meara, J. F.* et al. (Dept. of Clin. Pathol., Cleveland Clin. Foundation, Cleveland, Ohio), p. 48.

Auto anti-hr' in the absence of anemia. *Bell, C. A.* et al. (Cedars Med. Center, Los Angeles, Calif.), p. 51.

Serological and immunochemical studies of snail (Otala lactea) anti-a: A simple purification method. *Bhatia, H. M.* et al. (Dept. of Immunochem., Boston Univ. School of Med., Boston, Mass.), p. 53.

The blood inventory control system — helping blood bank management through computerized inventory control. *Catassi, C. A.* et al. (Alameda Contra Costa Med. Assoc., Oakland, Calif.), p. 60.

Vox Sanguinis (Basel) **12** (1967) No. 1.

Polymorphisms of the human low-density lipoproteins. *Bütler, R.* (Central Lab. of Blood Transf. Service, Wankdorfstr. 10, 3000 Berne), p. 2.

Three examples of a new red cell antibody, anti Co^a. *Heistö, H.* (Blood Bank, Ullevål Hosp., Oslo, Norway), p. 18.

Irregular transmission of a blood-group complex in one family of pigs following irradiation. *Andresen, E.* (The Royal Veterinary and Agricultural College, Copenhagen), p. 25.

Delayed haemolytic transfusion reactions simulating auto-immune haemolytic anaemia. *Croucher, Betty E. E.* et al. (Blood Transf. Lab., Toronto Gen. Hosp., Toronto, Canada), p. 32.

Studies on the nature of the immune adherence receptor. I. The inhibition of immune adherence by soluble mucoids and mucopeptides and by human erythrocyte ghosts. *Nelson, D. S.* et al. (Dept. of Bacteriol., Univ. of Sydney, Sydney, N.S.W.), p. 43.

Vox Sanguinis (Basel) **12** (1967) No. 2.

Rh antibody type in haemolytic disease of the newborn. *Murray, Sh.* (Regional Transf. Centre, Newcastle upon Tyne, England), p. 81.

The favorable effect of ACD on the viability of fresh and stored human platelets. *Morrison, F. S.* et al. (Blood Preservation Hosp., Chelsea, Mass.), p. 90.

A simple concentration osmometer and its application to the determination of the oncotic efficiency of plasma substitutes. *Nitschmann, Hs.* (Inst. for Organic Chemistry, Freiestrasse 3, 3012 Berne), p. 106.

The effect of exchange transfusion on the development of immunological factors. *Nejedlá, Z.* (Inst. for the Care of Mother and Child, Prague-Podoli), p. 118.

Vox Sanguinis (Basel) **12** (1967) No. 3.

Blood group chimerism. *Hart, Mia van der* et al. (Central Lab. of the Netherlands Red Cross Blood Transfusion Service, Amsterdam), p. 161.

Monospecific lymphocytotoxic antisera, an absorption study. *Walford, R. L.* et al. (Dept. of Pathol., Univ. of California School of Med., Los Angeles, Calif.), p. 173.

Leukocyte antigens and the mixed lymphocyte culture reaction. *Iványi, D.* et al. (Inst. of Dent. Res., Acad. of Sciences, Prague), p. 186.

Blocking effect of rheumatoid factor on complement fixation by thrombocyte isoantibodies. *Kissmeyer-Nielsen, F.* et al. (Blood Bank, Århus Kommunehosp., Århus, Denmark), p. 199.

Hemmaglutinins (lectins) extracted from Maclura pomifera. *Jones, J. M.* et al. (Dept. Biol., Wichita State Univ., Wichita, Kansas), p. 211.

Two new antibodies, anti-N_b and anti-N_c in the N blood group system in pigs. *Saison, Ruth* (Univ. Guelph, Ontario Vet. College, Guelph, Ont., Canada), p. 215.

Sul, a new group antigen in the MN system. *Konugres, Angelyn* et al. (Boston Lying-In Hosp., Blood Bank, Boston, Mass.), p. 221.

Further family data to the inheritance of Ag(x). *Rittner, Ch.* (Inst. of Forensic Med., Univ. of Bonn, Germany), p. 225.

Cold agglutinins of anti-specificity in alcoholic cirrhosis. *Rubin, H.* et al. (Dept. Hematol., Mt. Sinai Hosp., New York, N.Y.), p. 227.

The precipitin in the anti-Ld(a)-serum. *Berg, K.* et al. (Rockefeller Univ., New York, N.Y.), p. 231.

A new method for the cytological differentiation of foetal and adult erythrocytes. *Szelényi, Judith G.* et al. (Research Inst. of National Blood Service., Budapest), p. 234.

Vox Sanguinis (Basel) **12** (1967) No. 4.

Studies on agglutination by macromolecular antibodies. III. Agglutination by immune antibodies. *Tönder, O.* (The Broegelmann Research Lab. for Microbiol., Univ. of Bergen, Norway), p. 241.

Nonspecific interaction of the papain-insensitive and papain-sensitive populations of human IgG with erythrocytes. *Gergely, J.* et al. (Inst. of Hemat., National Blood Center, Budapest), p. 252.

Thrombocytopenia due to quinidine or quinine. Report on a series of 28 patients. *Weerdt, Ch.M. van der* (Central Lab. of the Netherlands Red Cross Blood Transf. Service, Plesmanlaan 125, Amsterdam-W, Netherlands), p. 265.

The direct antiglobulin (Coombs') test in hypertensive patients. *Feizi, T.* et al. (The Royal Free Hospital, London, W.C. 1.), p. 273.

The amount of anti-D on red cells in haemolytic disease of the newborn. *Hughes-Jones, N. C.* et al. (Exp. Haemat. Research Unit, Wright-Fleming Inst., London, W. 2.), p. 279.

A new reagent, anti-K_e, in the K blood group system of pigs. *Saison, Ruth* (Dept. of Veterinary Bacteriol., Ontario Veterinary College, Univ. of Guelph, Ontario, Canada), p. 287.

Caryogrammes from stored blood. *Govaerts, A.E.* et al. (The Blood Bank, Univ. Hospitals, Brussels, Belgium), p. 293.

Cytotoxic effect of human alloimmune antibodies on epidermal cells. *Ivašková, Eva* (Inst. of Exp. Biol. and Genetics, Czechoslovak Acad. of Science, Prague 4.), p. 295.

In vitro effects of dipyridamole on platelet adhesiveness to glass. A preliminary communication. *Rosner, P.* et al. (Lab. of Haemostasis, Blood Transf. Center, Geneva 1200, Switzerland), p. 300.

Another example of a "naturally-occurring" anti-K. *Tegoli, J.* et al. (Blood Bank, Memorial Hosp. Med. Center, 444 East 68th Street, New York, N.Y. 10021), p. 305.

The turnover of alpha$_2$-macroglobulins in the nephrotic syndrome. *Kluthe, R.* et al. (Med. Univ. Policlinic, Freiburg i. Br., Germany), p. 308.

A modified method of determining the AB0 group of blood stains on textiles. *Halvorsen, K.* et al. (Blood Group Lab., Haukeland Hosp., Bergen, Norway), p. 312.

Possible sources of error in the blood grouping of dried blood stains on textiles. *Nordhagen, R.* (Blood Group Ref. Laboratory, National Inst. of Public Health, Geitmyrsveien 75, Oslo, Norway), p. 317.

Gm typing with microtiter plates. *Borel, Halina* et al. (The New York Blood Center, 310 East 67th Street, New York, N.Y. 10021), p. 319.

News items

XIIth INTERNATIONAL CONGRESS OF BLOOD TRANSFUSION

The XIIth International Congress of Blood Transfusion will be held in Moscow, Soviet Union, in August, 1969.

Scientific Programme: The following subjects have been chosen for the plenary sessions: transfusion of blood and plasma volume expanders, problems of immunology, preservation of blood and blood cells, transplantation of haemopoietic tissue and other organs.
A session will be devoted to the discussion of leucosis.
Documentary films on blood donation will be projected.

Languages: Papers may be given in Russian, English and French. Application to present a paper should reach the Secretariat before December, 1968.

Registration: Application for participation will be accepted not later than January, 1968.
All correspondence and inquiries concerning the Congress should be addressed to:
The Secretariat of the Soviet National Organization Committee
Central Institute of Haematology and Blood Transfusion
Novozykovski proezd 4
Moscow A–167, USSR

Professor Dr. A. E. KISSELEV

Zeitschrift für die gesamte

INNERE MEDIZIN

und ihre Grenzgebiete

KLINIK — PATHOLOGIE — EXPERIMENT

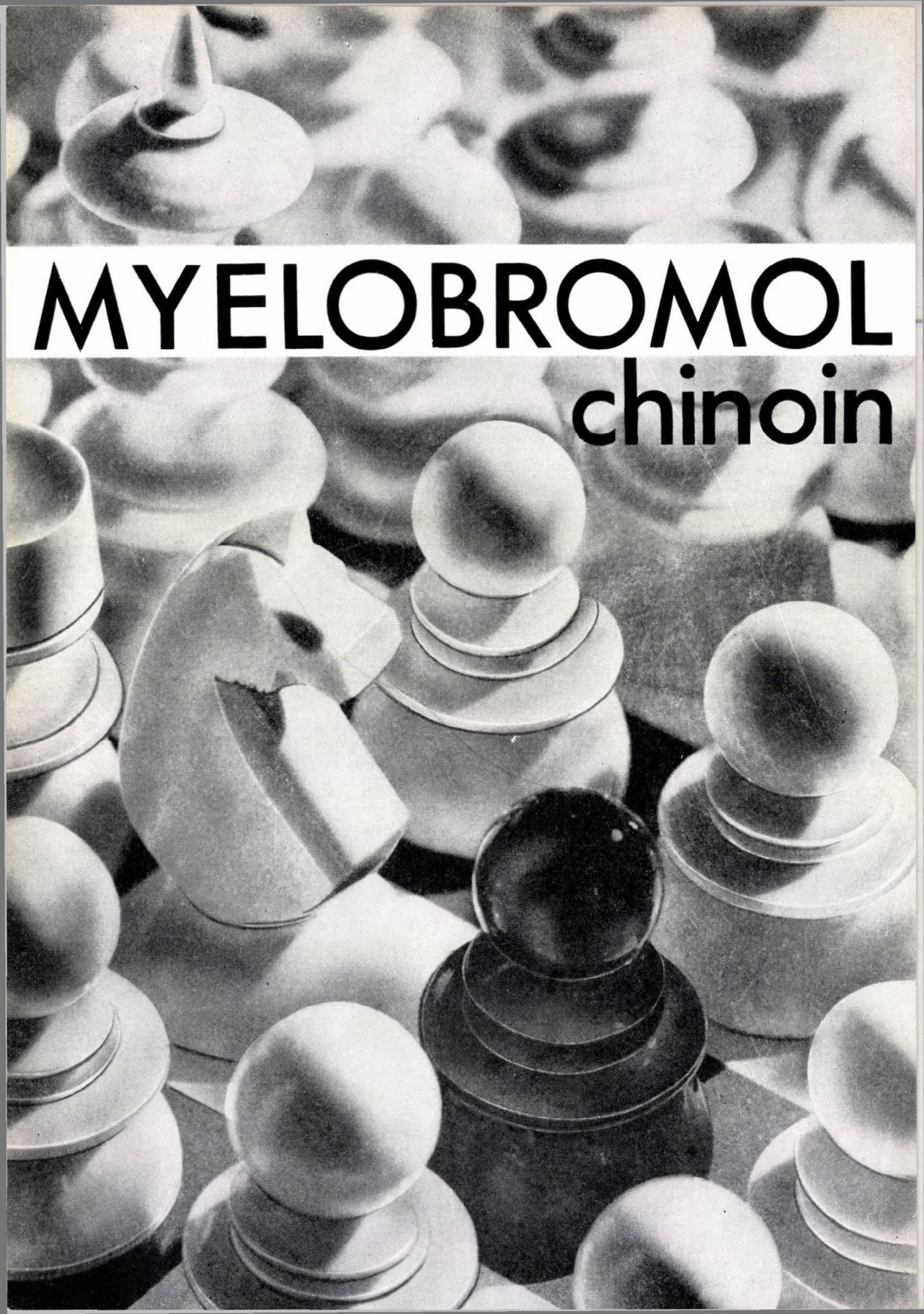
Organ der Sektion Innere Medizin der Deutschen Gesellschaft für Klinische Medizin. Begründet von Theodor BRUGSCH. Herausgegeben von R. EMMRICH, Leipzig, und M. GÜLZOW, Rostock. Redaktionskollegium: H. DUTZ, Berlin; F. MÜLLER, Greifswald; E. PERLICK, Leipzig; F. H. SCHULZ, Berlin; A. SUNDERMANN, Erfurt. Schriftleitung: H. TRENCKMANN, Leipzig.

Die 1946 gegründete »Zeitschrift für die gesamte Innere Medizin und ihre Grenzgebiete« hat sich zur Aufgabe gestellt, aus dem großen Gebiet der Inneren Medizin einschließlich ihrer zahlreichen Spezialfächer und vielen Grenzfächer die neuesten wissenschaftlichen Erkenntnisse und Fortschritte zu vermitteln und damit zur Weiterbildung der interessierten Ärzteschaft beizutragen. Dies geschieht in Form von Übersichten, Originalarbeiten, wissenschaftlichen Kurzmitteilungen und Schilderungen besonderer Krankheitsfälle. Die Verbreitung der Zeitschrift umfaßt nicht nur den deutschen Sprachraum, sondern auch Ost- und Westeuropa, wozu die in englische und russische Sprache übersetzten Zusammenfassungen der Arbeiten wesentlich beitragen. Zugleich ist die Zeitschrift das Publikationsorgan der Sektion Innere Medizin der Deutschen Gesellschaft für klinische Medizin.

Jährlich 24 Hefte in 14täglichen Abständen. Bandumfang 1296 Seiten. Format 21 × 29,7 cm. Zur Zeit Jahrgang 22. Einbanddecken nach Rückfrage beim Verlag.

Den Bezugspreis in Ihrer Landeswährung nennt Ihnen Ihr Zeitschriftenhändler, in den sozialistischen Ländern das Außenhandelsunternehmen bzw. der maßgebende Importbuchhandel. In Westdeutschland und im gesamten Ausland besitzt jede einschlägige Buchhandlung bzw. Zeitschriften-Vertriebsstelle die Möglichkeit der Beschaffung.

VEB GEORG THIEME LEIPZIG


MYELOBROMOL
chinoin

MYELOBROMOL
chinoin

1,6-Dibromo-1,6-dideoxy-D-mannitol
tablets of 250 mg

A specific antigranulopoietic agent

Cronic myelocytic leukaemia
Polycythaemia vera

Highly selective action
Oral administration
No cross-resistance with other therapies
Response of patients can be controlled by dosage
MYELOBROMOL brings about the prolongation of survival

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126

Thrombosis et Diathesis haemorrhagica

Editores: K. M. BRINKHOUS, Chapel Hill · F. BÜCHNER, Freiburg i. Br., E. DEUTSCH, Wien · J. E. JORPES, Stockholm · F. KOLLER, Basel, J. ROSKAM, Liège · J.-P. SOULIER, Paris · I. S. WRIGHT, New York

Redactor: P. MATIS, Tübingen

Co-Editores: P. M. Aggeler, San Francisco · H. Alessandri, Santiago de Chile · B. Alexander, Boston · N. Alkjaersig, St. Louis · S. Amano, Kyoto · T. Astrup, Washington · J. L. Beaumont, Paris · F. K. Beller, New York · J. Bernard, Paris · R. Biggs, Oxford · Y. Bounameaux, Elisabethville · Ch. E. Brambel, Baltimore · C. L. Conley, Baltimore · F. Duckert, Basel · G. Fanconi, Zürich · P. Fantl, Melbourne · J. Favre-Gilly, Lyon · J. H. Ferguson, Chapel Hill · E. L. Gomes, Rio de Janeiro. · R. Gross, Köln. · H. Hartert, Kaiserslautern. · L. Heilmeyer, Freiburg i. Br. · R. B. Hunter, Dundee. · L. B. Jaques, Saskatoon. · F. L. J. Jordan, Utrecht. · J. Jürgens, Frankfurt. · Th. Koller, Basel. · H. G. Lasch, Gießen. · W. Lehmann, Kiel. · E. A. Loeliger, Leiden. · E. F. Lüscher, Bern · R. G. Macfarlane, Oxford. · R. Marx, München. · G. Meyer, Buenos Aires. · Th. Naegeli, Tübingen. · P. de Nicola, Pavia. · P. A. Owren, Oslo. · A. Pavlovsky, Buenos Aires. · A. J. Quick, Milwaukee. · F. E. de Salamanca, Madrid. · W. Sandritter, Gießen. · W. H. Seegers, Detroit. · R. Schoen, Göttingen. · H. E. Schultze, Marburg/Lahn. · F. H. Schulz, Berlin. · Sol Sherry, St. Louis. · H. A. Thies, Hamburg. · M. Verstraete, Louvain · S. I. de Vries, Amsterdam. · J. Waldenström, Malmö. · S. Watanabe, Hiroshima. · S. Witte, Erlangen ·

1967. Band XVII und XVIII, (pro Band 4 Hefte bzw. 2 Doppelhefte) Bandpreis DM 74,—, zuzüglich Postgebühren. [Bände I bis VI vergriffen, als Reprints erschienen bei Swets & Zeitlinger, Amsterdam/Holland, zum Preise von je DM 120,—]

F. K. SCHATTAUER-VERLAG · STUTTGART



DEGRANOL CHINOIN

Brand of mannomustine dihydrochloride (BCM)

1,6-bis-(β-Chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride

Chronic lymphoid leukaemia
Hodgkin's disease
Lymphosarcoma and other haemoblastoses
In tumor surgery as chemoprophylactic
For direct injection into the cavities with tumorous effusion
DEGRANOL is remarkably safe in adequate dosage

powder ampoules of 50 mg · coated pills of 50 mg

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126

Methods of Information in Medicine

Methodik der Information in der Medizin

Journal of Methodology in Medical Research,
Information und Documentation

Zeitschrift für die Methodenlehre der medizinischen Forschung,
Information und Dokumentation

Editores: H. K. Beecher, Boston, M. Eden, Cambridge/Mass.; M. Fishbein, Chicago; C. M. Fletcher, London; E. D. Freis, Washington; P. Hall, Stockholm; R. Huron, Toulouse; A. Kent, Pittsburgh; S. Koller, Wiesbaden; L. Lasagna, Baltimore; L. Martin, Bruxelles; W. Modell, New York; O. Nacke, Bielefeld; R. H. Orr, Philadelphia; A. Proppe, Kiel; D. D. Rutstein, Boston; J. G. Scadding, London; V. N. Slee, Ann Arbor; W. A. Southern, North Chicago; J. W. Sweeney, New Orleans; E. Weigelin, Bonn; I. D. Welt, Washington; E. Welte, Mönchengladbach

Redactores: G. WAGNER, Heidelberg · G. OBERHOFFER, Bonn
H. V. PIPBERGER, Washington

1967. Band VI (4 Hefte), pro Band DM 36,—, zuzüglich Postgebühren



F. K. SCHATTAUER-VERLAG · STUTTGART

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to 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.
Central Research Institute of the
National Blood Service
Daróczi út 24
Budapest XI, 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 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) name(s) and initials of the author(s); (2) title of paper; (3) international abbreviation of the perodical; (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. Coloured 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.

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

Reviews of the Hungarian Academy of Sciences are obtainable at the following addresses:

ALBANIA

Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA

A. Keesing
Box 4886, GPO
Sydney

AUSTRIA

Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM

Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA

Raznoiznos
1, Tzar Assen
Sofia

CANADA

Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA

Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Smeckách 30
Praha 2
Poštova Novinova Služba
Dovoz Tisku
Vinohradska 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštova Novinova Služba
Dovoz Tlace
Leningradska 14
Bratislava

DENMARK

Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und- Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough, Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP.
GPO Post Box 1374
Bombay I

ITALY

Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central
Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA

Chulpanmul
Phenjan

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND

RUCH
ul. Wronia 23
Warszawa

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION

Mezhdunarodnaja Kniga
Moscow G—200

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA

Stecheri Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM

Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA

Forum
Vojvode Mišiča broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

16. XI. 1967 Index: 26341

315.871

VOLUME 1 · NUMBER 4 · 1967

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)
J. 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)
W. DAMESHEK (New York)
E. DEUTSCH (Vienna)
G. DISCOMBE (London)
C. A. FINCH (Seattle, Washington)
L. GARBY (Uppsala)
G. GÁRDOS (Budapest)
J. GERGELY (Budapest)
I. GONZÁLEZ-GUZMÁN (Mexico City)
F. GRÁF (Budapest)
A. GRAFFI (Berlin)
G. C. de GRUCHY (Melbourne)
A. HÄSSIG (Bern)
L. HEILMEYER (Freiburg/Br.)
L. P. HOLLÄNDER (Basel)
J. HOŘEJŠÍ (Prague)
M. HRUBIŠKO (Bratislava)
I. A. KASSIRSKY (Moscow)
E. KELEMEN (Budapest)
A. E. KISELEV (Moscow)
E. KOWALSKI † (Warsaw)
P. J. LAH (Belgrade)
H. LEHMANN (Cambridge)
J. J. van LOGHEM (Amsterdam)
G. W. LÖHR (Marburg/Lahn)
A. E. MOURANT (London)
C. T. NICOLAU (Bucharest)
E. NOVÁK (Budapest)
P. A. OWREN (Oslo)
L. A. PÁLOS (Budapest)
K. RÁK (Szeged)
S. RAPOPORT (Berlin)
F. REIMANN (Istanbul)
J. ROSKAM (Liège)
G. RUHENSTROTH-BAUER (Munich)
V. SERAFIMOV-DIMITROV (Sofia)
I. SIMONOVITS (Budapest)
J. P. SOULIER (Paris)
S. STEFANOVIĆ (Belgrade)
E. STORTI (Modena)
M. C. VERLOOP (Utrecht)
A. VIDEBAEK (Copenhagen)
S. I. de VRIES (Amsterdam)

AKADÉMIAI KIADÓ
PUBLISHING HOUSE
OF THE
HUNGARIAN ACADEMY
OF SCIENCES
BUDAPEST

HAEMATOLOGIA

is a new 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 some 500 pages.

Subscription price: $ 12 per volume (per year). Orders may be placed with

KULTURA

Trading Company for Books and Newspapers

Budapest 62. P.O.B. 149

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

Contents

Dausset, J.: Le système Hu-1. 359

Pasternak, G., Pasternak, L.: Immunologische Charakterisierung Graffi-Virus-infizierter Zellen der Maus 369

Bütler, R., Brunner, E.: Investigations on common antigenic structures of some human and animal serum proteins 375

Dintenfass, L.: Dynamics of blood coagulation introducing a new coagulation factor: "velocity gradient" 387

Solymoss, B., Gabbiani, G., Selye, H.: Blood coagulation changes in the thrombohaemorrhagic phenomenon (THP) 401

Hollán, S. R., Szelényi, J. G., Breuer, J. H., Medgyesi, G. A, Sőtér, V. N.: Structural and functional differences between human foetal and adult erythrocytes 409

Rechowicz, K., Szymanowska, Z., Konopka, L., Murawski, K., Pawelski, S.: Methaemoglobinaemia due to insufficiency of the reducing systems of erythrocytes . . . 421

Gidáli, J., Fehér, I.: Differentiation and maturation of haemopoietic stem cells in spleen colonies 429

Barta, I., Patakfalvi, A.: Tuberculosis of peripheral lymph nodes 437

Open Forum 445

Book Review 455

Abstracts. 456

From the international literature on haematology 461

Contents of Volume 1 481

Author Index 483

Subject Index 485

VOLUME 1 · NUMBER 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) · J. 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) · W. DAMESHEK (New York, N. Y.) · E. DEUTSCH (Vienna) · G. DISCOMBE (London) · C. A. FINCH (Seattle, Washington) · L. GARBY (Uppsala) · G. GÁRDOS (Budapest) · J. GERGELY (Budapest) · I. GONZÁLEZ-GUZMÁN (Mexico City) · F. GRÁF (Budapest) · A. GRAFFI (Berlin) · G. C. de GRUCHY (Melbourne) · A. HÄSSIG (Bern) · L. HEILMEYER (Freiburg/Br.) · L. P. HOLLÄNDER (Basel) · J. HOŘEJŠÍ (Prague) · M. HRUBIŠKO (Bratislava) · I. A. KASSIRSKY (Moscow) · E. KELEMEN (Budapest) · A. E. KISELEV (Moscow) · E. KOWALSKI † (Warsaw) · P. J. LAH (Belgrade) · H. LEHMANN (Cambridge) · J. J. van LOGHEM (Amsterdam) · G. W. LÖHR (Marburg/Lahn) · A. E. MOURANT (London) · C. T. NICOLAU (Bucharest) · E. NOVÁK (Budapest) · P. A. OWREN (Oslo) · L. A. PÁLOS (Budapest) · K. RÁK (Szeged) · S. RAPOPORT (Berlin) · F. REIMANN (Istanbul) · J. ROSKAM (Liège) · G. RUHENSTROTH-BAUER (Munich) · V. SERAFIMOV-DIMITROV (Sofia) · I. SIMONOVITS (Budapest) · J. P. SOULIER (Paris) · S. STEFANOVIĆ (Belgrade) · E. STORTI (Modena) · M. C. VERLOOP (Utrecht) · A. VIDEBAEK (Copenhagen) · S. I. de VRIES (Amsterdam)

MAGYAR TUDOMÁNYOS AKADÉMIA KÖNYVTÁRA

AKADÉMIAI KIADÓ

PUBLISHING HOUSE OF THE HUNGARIAN ACADEMY OF SCIENCES

BUDAPEST 1967

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Bogdán István
A kézirat nyomdába érkezett: 1967. XI. 8. Terjedelme: 11,4 (A/5) ív
67.63780 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

Le système Hu-1

J. DAUSSET

Institut de Recherche sur les Maladies du Sang, Hôpital Saint-Louis, Paris, France

(Reçu le 28e juillet 1967)

Le système Hu-1 est le système principal d'histocompatibilité de l'homme analogue au système H-2 de la souris. Il est composé de plusieurs antigènes qui sont rassemblés en trois séries d'antigènes fréquemment associés. Les séries «3» et «7» montrent une distribution contrastée. L'existence d'un système principal est montrée par les études familiales. Les allèles du système Hu-1 ont été calculés à partir des phénotypes et déduits à partir des familles. L'importance de ce système principal en oncogenèse et en transplantation est soulignée.

Depuis la dernière décade un travail considérable a été fait pour définir les antigènes leucocytaires mais c'est seulement maintenant que le concept d'un système principal de groupe leucocytaire auquel apparemment la majorité des antigènes connus a été complètement accepté.

Nos connaissances sur ce système principal sont dues aux recherches de plusieurs équipes travaillant indépendemment.

En 1958 Dausset a décrit le premier antigène leucocytaire, l'antigène Mac et a émis l'hypothèse que ces antigènes pourraient jouer un rôle essentiel en transplantation [4]. En 1960 il a observé que les reins greffés fonctionnels étaient ceux pour lesquels n'existait pas d'incompatibilité décelable à l'aide d'une batterie de sérums de spécificité inconnue [5]. Van Rood et al. décrivent deux autres antigènes (4^a 4^b) et par une analyse statistique ils montrent qu'ils se comportent comme des allèles et décrivent ensuite d'autres antigènes [23–25]. Payne et al. [18] et Bodmer et al. [1] définissent les antigènes LA 1 et LA 2 (analogue à l'antigène Mac) et LA 3 et montrent qu'ils se comportent également comme des allèles. Pour un certain temps on a cru que ces derniers antigènes appartenaient à un système différent de ceux décrits par Van Rood. Cependant Dausset, Ivanyi et Ivanyi [7] ont décrit 10 antigènes (dont cinq n'avaient pas encore été décrits). Ils observent leurs associations dans la population caucasienne et postulent qu'ils appartiennent à un seul système immunogénétique très complexe qu'ils proposent, en suivant Snell et al. [22] d'appeler le système Hu-1 (Histocompatibilité Humaine n° 1). Des greffes de peau en collaboration avec F. T Rapaport ont montré clairement que la survie des greffes pouvait être prédite des rapports existant entre les groupes leucocytaires des donneurs et des receveurs. Il a ainsi été démontré que les antigènes 1 (Mac), 3 (4^a) et 7 (4^b) étaient des antigènes de transplantation. Une telle démonstration a également été faite par Van Rood et al. [25] pour l'antigène 10 (7^c).

Tableau 1

Études familiales

Étude par double back cross des relations entre les antigènes leucocytaires

Antigènes étudiés	Combinaisons chez les enfants				
	+ +	− −	+ −	− +	
1 et 3	2 1	2 2			$p < 0{,}03$
1 et 4	1	2	2	3	$p < 0{,}02$
1 et 10	3	1	2 7	2 4	$p < 12 \cdot 10^{-5}$
1 et 11			2	0	
1 et 12			7 3	4 1	$p < 12 \cdot 10^{-5}$
11 et 3			2 2	1 4	$p < 0{,}008$
11 et 4			2	4	$p < 0{,}03$
11 et 10			3	5	$p < 0{,}008$
12 et 3	3	3			$p < 0{,}03$
12 et 4	3	3			$p < 0{,}03$
12 et 5	3	3			$p < 0{,}03$
12 et 10	4 3 4 2	4 0 7 1	3 3	2 2	$p < 1 \cdot 10^{-7}$
3 et 4	2 4 1 3	4 4 2 3			$p < 8 \cdot 10^{-6}$
3 et 5	1 3 5	2 3 5			$p < 2 \cdot 10^{-6}$
4 et 5	3	3			$p < 0{,}03$

L'hypothèse de l'existence d'un système principal d'histocompatibilité chez l'homme (comme c'est le cas pour toutes les espèces étudiées jusqu'ici) a été fermement établie par des études familiales. De telles études ont été faites en utilisant la méthode du «double back-cross» [10–12]. Il n'a été trouvé aucune ségrégation indépendante entre les antigènes leucocytaires au cours de notre première étude sur 18 familles et de notre seconde étude sur 31 familles. La probabilité d'un «linkage» par exemple entre les antigènes 1 (Mac) et 10 (7^c) est de $p < 12 \cdot 10^5$ (tableau 1). Au contraire il a été démontré que le système Hu-1 était indépendant des systèmes érythrocytaires ABO, Rh, MNSs, Kell, P, Jk, Duffy et Secréteur, des systèmes sériques Hp, Tf, Gm et Gc et du système plaquettaire Ko. D'autres familles étudiées en commun au cours de la Workshop de Turin (1967) ont également souligné l'absence de ségrégation indépendante entre les antigènes appartenant au système principal.

Il est ainsi possible de faire maintenant une decription plus détaillée des caractères du système Hu-1. C'est le système le plus complexe connu chez l'homme, plus complexe que le système Rhésus.

Tableau 2

Exemple de déduction des allèles Hu-1

	Père	Mère
Antigènes	1, 7, 8, 12, 10	11, 3, 13, 4
Allèles	1 7 8	3, 13, 4
Supposés	12 7 10	11, 3, 13, 4

	Type I 3 Enfants	Type II 4 Enfants
Antigènes	1, 3, 13, 4, 7, 8	11, 1, 3, 13, 4, 7, 8
Allèles	3, 13, 4	11 3, 13, 4,
Supposés	1 7 8	1 7 8

	Type III 2 Enfants	Type IV 2 Enfants
Antigènes	11, 12, 3, 13, 4, 7, 10	12, 3, 13, 4, 7, 10
Allèles	11 3, 13, 4,	3, 13, 4
Supposés	12 7 10	12 7 10

Chaque antigène est défini par plusieurs anticorps réagissant presque identiquement. Mais il est probable que certains de ces antigènes sont définis artificiellement car tous les anticorps utilisés à cette définition ne sont pas dirigés contre la même spécificité mais contre des spécificités qui sont fréquemment associées dans une population donnée [14]. On peut prendre comme comparaison l'antigène D du système Rhésus: Il a été montré être composé de plusieurs facteurs antigéniques Rh^A, Rh^B, Rh^C et Rh^D lorsque des anticorps anti-D apparemment monospécifiques ont été testés et absorbés sur des hématies de populations noires. La même situation existe déjà dans les groupes leucocytaires et être même pour eux, la situation la plus habituelle.

Tableau 3

Liste des antigènes leuco-plaquettaires fréquence et synonymes

Correspondance approximative des nomenclatures					Fréquence dans la population
Dausset	Payne	Van Rood	Shulman	Térasaki	
1 (Mac)	LA2*	8^{a}*	PlGrLyB 1*	2*	56
2		6^{a}			85
3		4^{a}*		3	69
13					42
4					44
14			PlGrLyF 1		43
5	4^{c}*			6	14
6					33
7		4^{b}*		7	67
8		7^{d}			20
9		6^{b}*			46
10	4^{d}	7^{c}* (6^{c})		5	29
11	LA1*			1*	23
12	LA3				33
		7^{a}	PlGrLyC 1		26 à 30
		7^{b}			76
		9^{a}			65
		5^{a}			33
		5^{b}			97

* Correspondance vérifiée par échange de sérums avec les antigènes de Dausset.

Ces antigènes ainsi définis du système Hu-1 forment des séries ou mosaïques d'antigènes fréquemment associés.

Il est remarquable qu'il existe ainsi trois grandes séries d'antigènes:

– La série «1» ou Mac est actuellement constituée seulement par les antigènes 1 et 2 mais il a été récemment montré [12] que cette série possède d'autres antigènes qui n'ont pas encore reçu de nombre. Ces antigènes ont été détectés soit par des études familiales, soit en absorbant des sérums anti-1 sur des cellules d'individus.

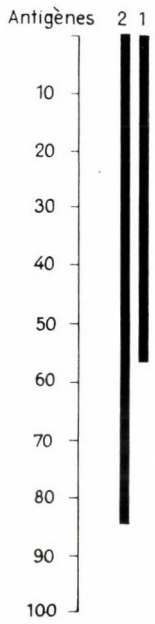


Fig. 1. Distribution des antigènes de la série «1» (Mac) chez 100 individus. Elle est composée des antigènes 1 et 2. L'antigène 1 est «inclus» dans l'antigène 2

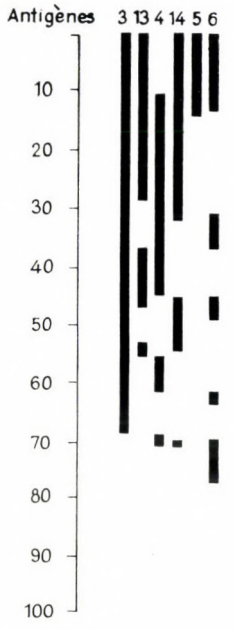


Fig. 2. Distribution antigènes de la série «3» chez 100 individus. Elle est composée de 6 antigènes fréquemment associés dans la population française. Les antigènes 13 et 5 sont inclus dans l'antigène 3. L'antigène 5 est inclus dans l'antigène 14. Les antigènes 4 et 5 ont une relation négative

– La série «3» ou 4^a est composée de 6 antigènes fortement associés avec l'antigène 3 qui est le plus fréquent de cette série. L'antigène 3 peut être considéré comme le «marqueur» de cette série. On peut remarquer que les antigènes 13 et 5 sont complètement «inclus» dans l'antigène 3 (fig. 1) c'est à dire que par exemple il n'existe aucun individu 13+3 –. L'antigène 5 est complétement «inclus» dans l'antigène 14 (qui est l'antigène $PlGrLy^{F1}$ de Shulman et al. [21]. Les antigènes 4 et 5 présentent une association négative quoiqu'ils soient, tous deux, pratiquement «inclus» dans l'antigène 3 (fig. 2).

– Dans la série «7», l'antigène 7 est le plus fréquent des 6 antigènes de la série. L'antigène 10 est complétement «inclus» dans l'antigène 9. L'antigène 8 est «inclus» dans l'antigène 7.

Cette série possède encore les antigènes 11 (LA 1) et 12 (LA 3) qui se comportent comme des allèles (fig. 3).

Les séries «3» et «7» présentent une distribution contrastée dans la population caucasienne comme le montre la fig. 4. Le fait que les antigènes 11 et 12 sont

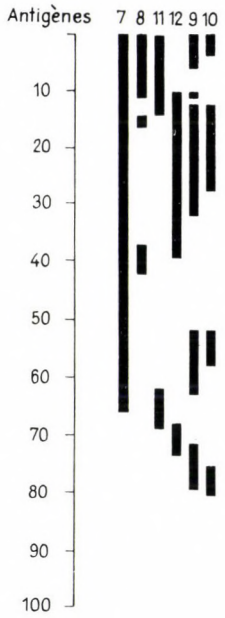


Fig. 3. Distribution des antigènes de la série «7» chez 100 individus. Elle est composée de 6 antigènes. L'antigène 8 est «inclus» dans l'antigène 7. Les antigènes 11 et 12 ont une relation négative

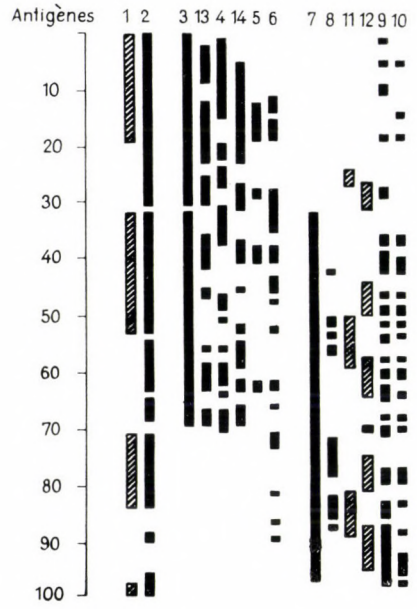


Fig. 4. Distribution des 14 antigènes du système Hu-1 chez 100 individus non apparentés de la population française. Les séries «3» et «7» sont classées de façon à faire apparaître leur distribution contrastée. La série «3» est groupé vers le haut. La série «7» vers le bas. Les antigènes hachurés 1, 11 et 12 se comportent comme des allèles

également allèles de l'antigène 1 rend vraisemblable que ces antigènes appartiennent à un seul système quoiqu'il n'y ait pas de corrélation significative entre l'antigène 1 et les antigènes des deux autres séries.

Cette hypothèse a été largement vérifiée par les études familiales dont il a déjà été fait mention. Ces études ont également montré que les antigènes de chaque parent sont transmis en deux groupes d'antigènes qui constituent les allèles du système. Un exemple est donné dans le tableau 2. Pas plus de quatre types d'enfants n'ont été observés comme cela était attendu si tous les antigènes étudiés appartenaient bien à un seul système.

Le système Hu-1 est composé à l'heure actuelle d'au moins 14 antigènes dont on trouvera les synonymes et les fréquences dans le tableau 3. Parmi eux les plus immunogéniques, – comme par exemple l'antigène 1 ou Mac – ont été déterminés par différents auteurs et de ce fait ont reçu plusieurs noms.

Il est certain que d'autres antigènes déjà décrits comme les antigènes 6^a, 7^a, 7^b [25] et d'autres antigènes non encore décrits, appartiennent également à ce même système.

Au contraire on sait déjà que les antigènes 5^a 5^b [16] et probablement 9^a [25] appartiennent à d'autres systèmes.

Il doit aussi être souligné que les antigènes spécifiques des granulocytes comme NA1 et NB1 [15] ou des lymphocytes comme Ly^{D1} [20] n'appartiennent pas au système Hu-1. Il en va de même naturellement pour les antigènes spécifiques des plaquettes comme ceux des systèmes Zw^a (Pl^{A1}) – Zw^b [19], [26] ou Ko^a Ko^b [27], [6] Les antigènes du système Hu-1 sont présents sur les plaquettes mais également sur tous les tissus de l'organisme.

Les fréquences des différents allèles du système ont pu être grossièrement estimées à partir des fréquences des phénotypes en se basant sur les calculs de Bernstein. Les plus fréquents allèles sont présentés sur le tableau 4. La fréquence des allèles a aussi été déduite de ceux trouvés chez les 50 parents de 25 familles. Ces fréquences ainsi calculées et déduites sont relativement proches (tableau 5).

De nombreuses études sont encore nécessaires pour connaître les détails du système Hu-1 dont la complexité est extrême. C'est dès maintenant un bon instrument pour les études anthropologiques. Des études préliminaires ont été faites chez les japonais, les noirs et les asiatiques [7–8] qui ont montré des différences parfois franches.

Tableau 4

Les 35 allèles les plus fréquents du système Hu-1

1, 2, 3, 13, 4	1,2	2, 1, 7	4,0	2, 1, 9	1,6
1, 2, 3, 13, 4, 14	1,7	2, 1, 7, 10	1,7	2, 1, 9, 10	3,0
1, 2, 3, 13, 6, 5, 14	3,0	2, 1, 7, 8	3,1	12, 9, 6	1,2
1, 2, 3, 4	5,3	2, 1, 7, 8, 9, 10	1,2	12, 6	3,3
1, 2, 3, 6	1,3	11, 7	2,5		
1, 2, 3, 6, 14	3,6	11, 7, 10	1,2		
11, 2, 3	1,1	11, 7, 8	3,9		
11, 2, 3, 4	1,5	11, 7, 8, 9	2,1		
2, 3, 13, 4	4,2	12, 7	3,0		
2, 3, 13, 4, 14	7,7	12, 7, 10	1,8		
2, 3, 13, 6, 5, 14	2,9	12, 7, 9	3,8		
2, 3, 13, 14	1,0	12, 7, 9, 10	5,1		
2, 3, 4	1,5	7, 9	5,3		
2, 3, 14	2,2	7, 9, 10	1,1		
2, 3	2,9	7	1,5		
2, 4	1,1				

Tableau 5

Allèles Hu-1 pour les antigènes 1, 3, 7

1	3	7	
—	—	—	0,081
+	—	—	0,051
—	+	—	0,270
—	—	+	0,313
+	+	—	0,176
+	—	+	0,109
			1,000

Tableau 6

Comparaison des fréquences des allèles calculés à partir des phénotypes avec celles des allèles déduits à partir de 25 familles

	Allèles calculés %	Allèles déduits %
1—3	17,6	13
11—3	2,6	4
12—3	1,2	2
1—7	10,9	10
11—7	9,7	7
12—7	13,7	12
3	23,9	24
7	7,9	18
nul	13,7	9

L'importance en biologie de l'existence d'un système principal d'histocompatibilité chez l'homme est un fait heureux. Il a d'ores et déjà permis des succès relatifs à l'homotransplantation entre individus apparentés. Il peut être espéré qu'à côté des antigènes A et B du système ABO qui ont été montré être de forts antigènes de transplantation [3, 8 – 10], un nombre limité d'antigènes du système Hu-1 seront pris en considération au cours des futures transplantations d'organes. Il n'a pas encore été déterminé avec certitude quels antigènes jouent le plus grand rôle en transplantation mais il peut être supposé que, comme chez la souris, les antigènes qui sont sérologiquement les plus immunogènes sont aussi les plus importants en transplantation.

Il est aussi très vraisemblable que les antigènes de transplantation jouent un rôle important dans la défense de l'organisme contre les mutations [2]. Toutes les cellules mutantes et les cellules néoplasiques peuvent être considérées comme des homogreffes et sont peut être rejetées par le même mécanisme.

Dausset et Dupuy [13] ont aussi suggéré que les antigènes viraux ou les néo-antigènes induits par les virus pourraient être aussi reconnus par les systèmes d'histocompatibilité. Seuls les individus possédant des antigènes proches des antigènes viraux ou des néo-antigènes viraux seraient susceptibles à ces virus. La découverte de Lilly et al. [17] que la susceptibilité de la souris au virus leucémique de Gross était liée à H-2^K est en faveur de cette hypothèse.

Remerciements

Nous tenons à remercier tout spécialement le Docteur P. Ivanyi et P. Demant qui ont largement participé à ce travail et qui ont bien voulu se charger du calcul des allèles.

Bibliographie

1. Bodmer, W. P., Bodmer, J., Adler, S., Payne, R., Bialek, J.: Genetics of "4" and "LA" human leucocyte groups. *Ann. N. Y. Acad. Sci. 129*, 473 (1966).
2. Burnet, M.: Role of the thymus and related organs in immunity. *Brit. med. J. 2*, 807 (1962).
3. Ceppellini, R., Curtoni, E. S., Leigheb, G., Mattius, P. L., Miggiano, V. C., Visetti, M.: An experimental approach to genetic analysis of histocompatibility in man. In: Conference and Workshop in Histocompatibility Testing, Munksgaard, Copenhagen, 1965, p. 13.
4. Dausset, J.: Iso leuco anticorps. *Acta haemat. (Basel) 20*, 156 (1958).
5. Dausset, J., Colombani, J.: Isoanticorps anti-leucocytaires et anti-plaquettaires. Sérologie et importance pratique, en particulier pour les greffes. Proc. VIII Congr. Int. Soc. Blood Transfusion, Tokyo, 1960, Karger, Basel, 1962, p. 324.
6. Dausset, J., Berg, P.: Un nouvel exemple d'anticorps anti-plaquettaire Ko. *Vox Sang.* (Basel) *8*, 341 (1963).
7. Dausset, J., Ivanyi, P., Ivanyi, D.: Tissue alloantigens in humans. Identification of a complex system (Hu-1). In: Histocompatibility Testing, Munksgaard, Copenhagen, 1965, p. 51.
8. Dausset, J., Rapaport, F. T., Colombani, J., Feingold, N., Legrand, L., Reviron, J.: Rôle du système érythrocytaire AB0 dans l'histocompatibilité humaine. *Presse méd. 73*, 2237 (1965).
9. Dausset, J.: Discussion on histocompatibility antigens in Immunopathology. IV Int. Symposium, Monte-Carlo, 1965. Schwabe, Basel, 1966, p. 211.
10. Dausset, J., Ivanyi, P., Feingold, N.: Tissue allo-antigens present in human leucocytes. *Ann. N. Y. Acad. Sci. 129*, 386 (1966).
11. Dausset, J., Benoit, P., Legrand, L., Berroche, L. Y., Moullec, J.: Études familiales portant sur les allo-antigènes leuco-plaquettaires. *Nouv. Rev. franç. Hémat. 7*, 5 (1967).
12. Dausset, J., Ivanyi, P., Colombani, J., Feingold, N., Legrand, L.: The Hu-1 system. In: Histocompatibility Testing, 1967, Munksgaard, Copenhagen, 1967.
13. Dausset, J., Dupuy, J. M.: Coïncidences ou relations fondamentales entre le leucémogenèse et les antigènes de transplantation. *Nouv. Rev. franç. Hémat. 7*, 161 (1967).
14. Ivanyi, P., Dausset, J.: Allo-antigens and antigenic factors of human leucocytes. An Hypothesis. Symposium of Tissue typing 1965. *Vox Sang.* (Basel) *11*, 326 (1966).
15. Lalezari, P.: A new neutrophile specific antigen. Its role in the pathogenic of neonatal neutropenia. *Fed. Proc. 25*, 371 (1966).

16. Leeuwen, A. Van, Eernisse, J. G., Rood, J. J. Van: A new leucocyte group with two alleles: leucocyte group five. *Vox Sang.* (Basel) *9*, 431 (1964).
17. Lilly, F., Boyse, E. A., Old, L. J.: Genetic basis of susceptibility to viral leukaemogenesis. *Lancet 2*, 1207 (1964).
18. Payne, R., Tripp, M., Weigle, J., Bodmer, W. F., Bodmer, J.: A new leukocyte iso-antigen system in man. Cold Spring Harbor Symposium. *Quant. Biol. 29*, 285 (1964).
19. Shulman, N. R., Aster, R. H., Leitner, A., Hiller, M. C.: Immuno reactions involving platelets. V. Post-transfusion purpura due to a complement fixing antibody against a genetically controlled platelet antigen. A proposed mechanism for thrombocytopenia and its relevance in "auto-immunity". *J. Clin. Invest. 40*, 1597 (1961).
20. Shulman, N. R., Marder, V. J., Hiller, N. C., Collier, E. M.: Platelet and leukocyte iso-antigenes and their antibodies: Serologic, physiologic and clinical studies. *Progr. Hemat. 4*, 222 (1964).
21. Shulman, N. R., Moor-Jankowski, J., Hiller, M. C.: Platelet and leukocyte isoantigens common to man and other animals. In: Histocompatibility Testing, Munksgaard, Copenhagen, 1965, p. 113.
22. Snell, G. D., Hoecker, G., Amos, D. B., Stimpfling, J. H.: A revised nomenclature for the histocompatibility-2 locus of the mouse. *Transplantation 2*, 777 (1964).
23. Van Rood, J. J.: Leucocyte grouping. A method and its application. Pasmans, Den Haage, 1962.
24. Van Rood, J. J., Van Leeuwen, A.: Defined leukocyte antigenic groups in men. In: Histocompatibility Testing. Nat. Acad. Sci. Publ. No. 1229, Washington, D. C., 1965, p. 21.
25. Van Rood, J. J., Van Leeuwen, A., Schippers, A. M. J., Vooys, W. H., Frederiks, E., Balner, H., Eernisse, J. G.: Leucocyte groups, the normal lymphocyte transfer test, and homograft sensitivity. In: Histocompatibility Testing, Munksgaard, Copenhagen, 1965, p. 37.
26. Van Loghem, J. J., Dorfmeijer, H., Van der Hart, M., Schreuder, F.: Serological and genetical studies on a platelet antigen (Zw). *Vox Sang.* (Basel) *4*, 161 (1959).
27. Van der Weerdt, Ch. M., Van der Wiel-Dorfmeijer, H., Engelfriet, C. P., Van Loghem, J. J.: A new platelet antigen. Proc. 8th Congr. European Society of Haematology, Vienna 1961, Karger, Basel, 1962, p. 379.

Immunologische Charakterisierung Graffi-Virus-infizierter Zellen der Maus*

G. PASTERNAK, L. PASTERNAK

Institut für Krebsforschung der Deutschen Akademie der Wissenschaften zu Berlin, Experimenteller Bereich, Berlin-Buch

(Eingegangen am 21. August 1967)

Graffi-Virus-induzierte Leukämien der Maus enthalten spezifische Antigene, die in vivo durch Erzeugung von Transplantationsresistenz und in vitro mit der Fluoreszenz-Antikörper-Technik, dem Zytotoxizitätstest und durch Absorptionsversuche nachgewiesen werden können. Die an der Membran der Leukämiezellen lokalisierten spezifischen Antigene entstehen unter dem Einfluß der Virusinfektion. Diese Antigene scheinen jedoch nicht nur in leukämischen Zellen produziert zu werden. Bereits 14 Tage nach Infektion neugeborener Mäuse mit dem Graffi-Virus besitzen Zellen der Milz die charakteristischen virusinduzierten Oberflächenantigene, obgleich die Tiere zu dieser Zeit noch keine Leukämien entwickelt haben. Weitere Untersuchungen über Virus-Zell-Wechselwirkungen mit immunologischen Methoden wurden an »natürlich« und »künstlich« mit dem Graffi-Virus infizierten neoplastischen Geweben durchgeführt. In diese Versuche wurden einbezogen: 1. das Landschütz Sarkom I, 2. Gross-Leukämien des AKR-Stammes, 3. chemisch induzierte Leukämien und 4. ein UV-induziertes Sarkom. Probleme der antigenen Konversion durch das Graffi-Virus sowie der Differenzierung zwischen viralen und neuen zellulären Antigenen in der Membran infizierter Zellen werden diskutiert.

Verschiedene Probleme der viralen Leukämogenese der Maus lassen sich mit immunologischen Methoden untersuchen. Es konnte nachgewiesen werden, daß infizierte Zellen charakteristische Antigene enthalten, mit denen der Organismus in bestimmter Wechselwirkung steht. Während immunologisch inkompetente Mäuse gegen diese Antigene tolerant werden und Leukämien entwickeln, zeigen normale erwachsene Tiere dagegen Abwehrreaktionen, die die Entstehung einer Leukämie nahezu vollständig verhindern. Übereinstimmende Befunde wurden für die Leukämieviren von Graffi, Gross (Passage A) und Moloney berichtet [1, 2; 3–5].

Die Anwesenheit spezifischer Antigene wurde zuerst in Leukämiezellen festgestellt [6–10]. Durch Behandlung von Mäusen mit virusenthaltenden Geweben oder mit Viruspräparationen konnte Resistenz gegen syngen transplantierte Leukämiezellen induziert werden. Leukämien gleichen viralen Ursprungs zeigten unabhängig vom histologischen Typ Kreuzresistenz [11, 12]. Damit war angedeutet, daß sie identische tumorspezifische Transplantationsantigene besitzen und daß die Antigene durch das Virus kodiert werden. In den Sera resistenter Tiere

*) Nach einem Vortrag auf dem »European Cancer Meeting« in Wien, 3. Juli 1967.

waren außerdem Antikörper vorhanden, die mit Leukämiezellen in vitro reagierten und das Leukämievirus neutralisierten [13]. Wie die indirekte Immunofluoreszenztechnik und der Zytotoxizitätstest zeigten, waren spezifische Antigene an der Oberfläche der Zellen lokalisiert. Die Antikörper konnten durch Leukämiezellen vollständig aus den Immunsera absorbiert werden. Für die erwähnten Techniken ist jedoch noch nicht geklärt, ob sie identische oder auch differente antigene Spezifitäten messen.

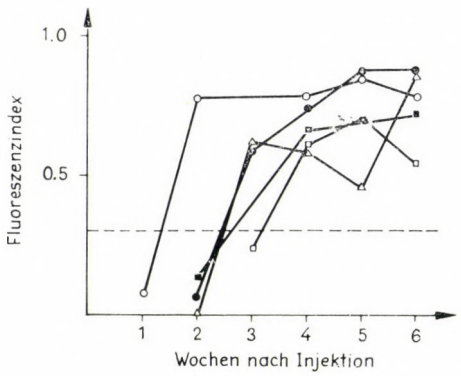


Abb. 1. Nachweis der Produktion von Graffi-Virus in »natürlich« und »künstlich« infizierten neoplastischen Geweben der Maus

● Chemisch induzierte Leukämie, infiziert mit dem Graffi-Virus
■ Graffi-Virus-infiziertes UV-Sarkom
○ Primäre Graffi-Leukämie
□ Landschütz-Sarkom I
△ Graffi-Virus-infizierte Gross-Leukämie 664967 b

In unseren Untersuchungen mit dem Graffi-Leukämievirus der Maus interessierte uns, wann diese Antigene nach Infektion von Mäusen auftreten und welche Zellen überhaupt in der Lage sind, virusinduzierte Antigene zu bilden. Es konnte mit Hilfe der Absorptionstechnik festgestellt werden, daß bereits 14 Tage nach Infektion neugeborener Mäuse mit dem Graffi-Virus die charakteristischen virusinduzierten Oberflächenantigene der Leukämiezellen in den Zellen der Milz vorhanden waren [14]. Zu diesem Zeitpunkt besteht noch keine Leukämie. Zellen aus Lymphknoten und Thymus absorbierten nicht die spezifischen Antikörper. Werden erwachsene Mäuse mit dem Virus infiziert, so kann zwar das Virus im Knochenmark, in der Milz und in den Lymphknoten nachgewiesen werden, die charakteristischen Oberflächenantigene fehlen jedoch anscheinend in den Zellen der genannten Organe. Möglicherweise werden sie sofort nach ihrer Entstehung zusammen mit den Zellen durch Immunprozesse eliminiert. Das Virus selbst scheint aber, wenn es intrazellulär in bestimmten Zellen lokalisiert ist, vor der Immunreaktion geschützt zu sein. Verschiedene »natürlich« und »künstlich« mit

dem Graffi-Virus infizierte neoplastische Gewebe wurden in die Versuche zur Virus-Zell-Wechselwirkung einbezogen und auf die Anwesenheit von Virus und virusinduzierten Antigenen untersucht. Als »natürlich« mit dem Graffi-Virus infiziert wurde das Landschütz-Sarkom I bezeichnet, aus dem 1954 das Graffi-Virus isoliert worden war [15]. »Künstliche« Infektionen mit dem Graffi-Virus wurden durchgeführt, indem die vorher als nicht infiziert nachgewiesenen neoplastischen Gewebe bis zu 5mal auf Graffi-virämischen Tieren passagiert wurden. Anschließend wurden die Tumoren auf normale Rezipienten transplantiert. Folgende maligne Gewebe wurden untersucht: Gross-Leukämien des AKR-Stammes, Methylnitrosoharnstoff-induzierte Leukämien und ein UV-induziertes Sarkom. Die Anwesenheit des Graffi-Virus in den Geweben wurde mit dem MAP-Test bestimmt [16]. Hierbei wird die Fähigkeit des Virus, Antikörper gegen Leukämiezellen zu induzieren, für einen quantitativen Virusnachweis verwendet. Die Latenz der Antikörperbildung ist umgekehrt proportional zur Virusdosis. Antikörper wurden mit der Immunofluoreszenztechnik gemessen, und es wurde ein Index errechnet, bei dem Werte $\geqq 0{,}3$ als signifikant positiv bewertet wurden.

In Abb. 1. ist gezeigt, daß alle »künstlich« oder »natürlich« mit dem Graffi-Virus infizierten Gewebe das Graffi-Virus ständig produzieren. Spätestens 4 Wochen nach Beginn des MAP-Tests enthalten alle Sera Antikörper. Vergleichsweise ist der MAP-Test mit einer primären Graffi-Leukämie dargestellt; hier sind bereits 2 Wochen nach Injektion Antikörper nachweisbar.

Eine Übersicht über die Anwesenheit Graffi-Virus-induzierter Antigene in primären Virusleukämien ist in Tabelle 1 dargestellt. Während die Graffi-Virus-induzierten Leukämien aller untersuchten Mäusestämme die mit verschiedenen Techniken nachgewiesenen Antigene enthielten, fehlten sie in den spontanen Gross-Leukämien. Beide Leukämieformen gehören unterschiedlichen Antigensystemen an. Tabelle 2 enthält eine Übersicht über die Antigene in »künstlich« und »natürlich« mit dem Graffi-Virus infizierten Geweben. Insgesamt wurden 10 verschiedene Gross-Leukämien mit dem Graffi-Virus infiziert [17]. Nur eine einzige zeigte das von Stück und Mitarb. [18] beschriebene Phänomen der antigenen

Tabelle 1

Nachweis von Graffi-Virus und -Virus-induzierten Antigenen in primären Virusleukämien

	Tumorspezifische Transplantationsantigene	Antigene, nachgewiesen durch			Graffi-Virus
		Immunofluoreszenz	Zytotoxtechnik	Absorption	
Graffi-Leukämien (CBA, XVII, C57 BL)	+	+	+	+	+
Graffi-Leukämien (AKR)	+	+	+	+	+
Spontane Gross-Leukämien (AKR)	—	—	—	—	—

Tabelle 2

Nachweis von Graffi-Virus und -Virus-induzierten Antigenen in »künstlich« und »natürlich« mit dem Graffi-Virus infizierten neoplastischen Geweben

	Tumorspezifische Transplantationsantigene	Antigene nachgewiesen durch			Graffi-Virus
		Immunofluoreszenz	Zytotoxtechnik	Absorption	
Gross-Leukämien					
a) antigen konvertiert	+	+	+	+	+
b) nicht konvertiert	—	(±)	—	(±)	+
Chemisch induzierte Leukämien					
a)	+	+	n. g.	n. g.	+
b)	—	(±)	n. g.	n. g.	+
UV-induziertes Sarkom	(+)	(±)	n. g.	n. g.	+
Landschütz-Sarkom I (natürl. inf.)	—	(±)	—	(±)	+

n. g. = nicht getestet

Konversion. Ihre Zellen besaßen alle Graffi-Virus-induzierten Oberflächenantigene. Die anderen 9 Leukämien waren im Immunofluoreszenztest nur schwach positiv. In gleicher Weise konnte auch nur ein schwacher Absorptionseffekt der Zellen mit Graffi-Immunsera festgestellt werden. Alle anderen Techniken ergaben sogar

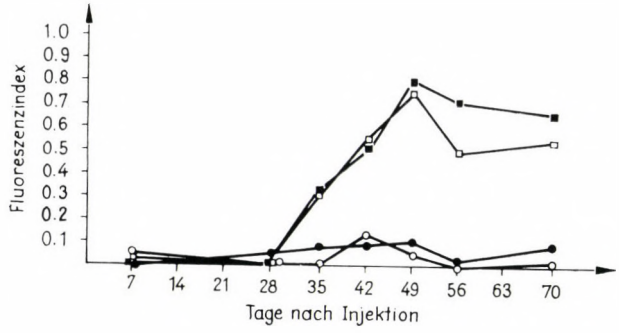


Abb. 2. Verlust virusneutralisierender Aktivität von Graffi-Immunsera, die mit Sarkom I-Zellen absorbiert waren

○ = zellfreies Homogenisat + nicht absorbiertes Graffi-Immunserum
□ = ,, ,, + Sarkom I-absorbiertes Graffi- ,,
● = ,, ,, + mit normalen Zellen absorb. ,, ,,
■ = ,, ,, + Normalserum

negative Resultate. Chemisch induzierte Leukämien zeigten ein ähnliches Verhalten. Wieder waren antigen konvertierte Leukämien festzustellen und Leukämien, die zwar das Graffi-Virus produzierten, jedoch die induzierten Membranantigene quantitativ vermindert enthielten oder nur einen Teil qualitativ differenter Antigene zu produzieren schienen. Sowohl das UV-induzierte Sarkom als auch das Landschütz-Sarkom I verhielten sich im wesentlichen wie die nicht konvertierten Leukämien. Ursachen für das unterschiedliche Verhalten von Geweben hinsichtlich der Expression virusinduzierter Antigene sind noch unklar.

Zur Natur der Antigene in Graffi-Virus-infizierten Zellen wird die Hypothese aufgestellt, daß die ständig Virus freisetzenden Graffi-Leukämiezellen sowie alle durch das Virus antigen konvertierten Leukämien virale und neue zelluläre Antigene in der Membran enthalten. Die Graffi-Virus-infizierten Sarkome und die nicht konvertierten Leukämien enthalten dagegen nur virales Antigen [19].

Das abschließend auf Abb. 2. dargestellte Versuchsergebnis stützt diese Hypothese. Landschütz-Sarkomzellen, die das Graffi-Virus enthalten, eliminieren virusneutralisierende Antikörper aus einem Graffi-Immunserum, ohne die Antikörper zu absorbieren, die mit der Oberfläche von Leukämiezellen reagieren. Weitere Untersuchungen zu dieser Hypothese werden durchgeführt.

Literatur

1. Old, L. J., Boyse, E. A.: Antigens of tumors and leukemias induced by viruses. *Fed. Proc. 24*, 1009 (1965).
2. Sjögren, H. O.: Transplantation methods as a tool for detection of tumor-specific antigens. *Progr. exp. Tumor Res. 6*, 289 (1965).
3. Klein, E., Klein, G.: Immunological tolerance of neonatally infected mice to the Moloney leukaemia virus. *Nature* (Lond.) *209*, 163 (1966).
4. Axelrad, A.: Antigenic behaviour of lymphoma cell populations in mice as revealed by the spleen colony method. *Progr. exp. Tumor Res. 6*, 30 (1965).
5. Pasternak, G.: Virusinduzierte Antigene im präleukämischen Stadium der myeloischen Leukämie der Maus. *Folia haemat.* (im Druck).
6. Pasternak, G., Horn, K.-H., Graffi, A.: Untersuchungen zur Frage der Isoimmunität gegen virusinduzierte Leukosen der Maus. *Acta biol. med. germ. 9*, 314 (1962).
7. Klein, G., Sjögren, H. O., Klein, E.: Demonstration of host resistance against isotransplantation of lymphomas induced by the Gross agent. *Cancer Res. 22*, 955 (1962).
8. Sachs, L.: Transplantability of an X-ray-induced and a virus-induced leukemia in isologous mice inoculated with a leukemia virus. *J. Nat. Cancer Inst. 29*, 759 (1962).
9. Pasternak, G., Graffi, A.: Induction of resistance against isotransplantation of virus-induced myeloid leukaemias. *Brit. J. Cancer 17*, 532 (1963).
10. Klein, E., Klein, G.: Antigenic properties of lymphomas induced by the Moloney agent. *J. Nat. Cancer Inst. 32*, 547 (1964).
11. Horn, K.-H., Pasternak, G., Graffi, A.: Antigencharakteristika virusinduzierter hämatologisch differenter Mäuseleukämien. *Acta biol. med. germ. 12*, 626 (1964).
12. Pasternak, G.: Antigenic properties of Graffi virus-induced leukemia in mice. Comparative Leukaemia Research. In: Proc. Int. Wenner-Gren Symp., Pergamon, London, 1966, pp. 157—160.

13. Pasternak, G.: Serologic studies on cells of Graffi virus-induced myeloid leukaemia in mice. *J. Nat. Cancer Inst. 31*, 71 (1965).
14. Pasternak, G., Pasternak, L.: Demonstration of Graffi leukemia virus and virus-induced antigens in leukemic and nonleukemic tissues of mice. *J. Nat. Cancer Inst. 38*, 157 (1967).
15. Graffi, A., Bielka, H., Fey, F., Scharsach, F., Weiss, R.: Gehäuftes Auftreten von Leukämien nach Injektion von Sarkomfiltraten. *Naturwissenschaften 41*, 503 (1954).
16. Klein, E., Klein, G.: Mouse antibody production test for the assay of the Moloney virus. *Nature* (Lond.) *204*, 339 (1964).
17. Pasternak, G., Micheel, B.: in Vorbereitung.
18. Stück, B., Old, L. J., Boyse, E. A.: Antigenic conversion of established leukaemias by an unrelated leukaemogenic virus. *Nature* (Lond.) *202*, 1016 (1964).
19. Pasternak, G.: Differentiation between viral and new cellular antigens in Graffi leukaemia of mice. *Nature* (Lond.) *214*, 1364 (1967).

Investigations on Common Antigenic Structures of some Human and Animal Serum Proteins

R. BÜTLER, E. BRUNNER

Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross,
Berne, Switzerland
(Received September 5, 1967)

Antisera were prepared by absorption of rabbit immunesera against different human serum proteins with sera of various mammals. The cross-precipitation patterns obtained on animal sera using these absorbed antisera are discussed. The group- and/or species-specificity of antigenic structures shared by human and animal serum proteins was investigated by means of comparative immunodiffusion tests using non-absorbed and absorbed antisera.

Due to the antigenic relationship of animal (including human) serum proteins, an antiserum prepared against a serum protein of one animal species will cross-react with respective serum proteins of other species. Based on this cross-reactivity, various serum proteins of mammalian origin were compared with the use of the immunodiffusion test of Ouchterlony in this institute by Roulet et al. [15*]. The results obtained led the authors to speculate on the minimum number of antigenic determinants in human serum proteins and on the number of different antibodies contained in the immune sera. A number of investigators have used comparative immunodiffusion methods with animal sera for the study of antigenic structures of human (and animal) serum proteins [1, 6, 8, 9, 10, 12, 13, 14, 16, 17]. Our own experiments were initiated by the question of whether some of the antigenic structures of human serum proteins which can be demonstrated in this way are the expression of group specificity or whether they merely reflect species specific differences.

Materials and Methods

1. Human sera

A pool of 200 normal sera, as well as individual sera from healthy blood donors of Berne were used, the latter representing the standard serum panel used in our studies of serum protein polymorphisms [4].

2. Animal sera

Fresh sera of the following nine mammalian species were used: rhesus monkey, cat, dog, cow, sheep, pig, horse, guinea pig and mouse.

* Because of repeated quotation of this reference the number is only indicated once.

3. Antisera

The following eight immune sera against human albumin, transferrin, α_2-macroglobulin and LDL (low density lipoprotein) were at our disposition: anti-albumin Nr. 929 F (Behringwerke, Marburg/Lahn), anti-transferrin Nr. 682 c (Behringwerke, Marburg/Lahn), anti-α_2-macroglobulin Nr. 777 b (Behringwerke, Marburg/Lahn), anti-betalipoprotein (anti-LDL) Nr. 1002 (Behringwerke, Marburg/Lahn), anti-α_2-macroglobulin 929 (Berne), anti-LDL K2 (Berne), anti-LDL K3 (Berne) and anti-LDL K4 (Berne). The four sera mentioned last are rabbit antisera which we had prepared for another investigation: the anti-α_2-macroglobulin serum 929 was obtained by immunization of a rabbit with an antigen/antibody complex (α_2-macroglobulin/anti-α_2-macroglobulin) using α_2-macroglobulin of a single donor [5]; the three anti-LDL sera were obtained by immunization of rabbits with single donor LDL (prepared according to Burstein).

4. Comparative immunodiffusion experiments

The rosette pattern was used in all experiments (6 wells arranged around a central well; well diameter 5 mm; distance between wells 5 mm). Absorbed or non-absorbed antiserum was placed in the central well, human or animal sera to be compared were placed in the peripheral wells. Experimental conditions of the immunodiffusion experiments have been described in detail in a previous publication [4].

5. Absorption experiments

In a preliminary step, the antiserum was mixed with increasing amounts of an animal serum and incubated at 37°C for 1 hr and at 4°C for 12 hrs. Precipitates were removed by centrifugation. Supernatants were placed in the peripheral wells of a rosette with the central well containing either pooled human serum (positive control) or the animal serum used for absorption (negative control). Absorption of the antiserum was considered to be complete when no precipitation lines were obtained with the animal serum used for absorption; the corresponding ratio of antiserum to animal serum was used for absorption of larger quantities.

General conditions of immunoprecipitation remained unchanged.

Results and Discussion

In preliminary studies some of the experiments performed by Roulet et al. were repeated. As could be expected, the results obtained differed from those of Roulet et al. because different antisera were used. If the principles as outlined by Roulet et al. were applied to the interpretation of our results, a smaller number of "antigenic determinants" had to be postulated for the different serum proteins under investigation. This behaviour may be explained on the basis of the assumption that our immunesera contained less antibodies against common antigenic

structures of human and animal serum proteins. In addition, differences in general experimental conditions cannot be excluded. Results of these preliminary investigations will not be detailed in this paper.

If the immunesera indeed contained a mixture of multiple antibodies directed against different antigenic structures of the respective human and animal serum proteins, it should be possible to reduce the number of these antibodies by absorption with different animal sera; the absorbed sera would then give "more specific" reactions with certain antigenic structures of the respective serum proteins than non-absorbed sera. Thus, in the first series of experiments, absorption studies were carried out.

1. Reaction patterns of antisera against human serum proteins after absorption with animal sera

Two anti-α_2-macroglobulin immunesera (serum 929 Berne and serum 777 b Behring) as well as four anti-LDL immunesera (serum 1002 Behring and sera K2, K3 and K4, Berne) were absorbed with all animal sera cross-reacting in precipitation tests (see Table 1); absorption ratios were varied until no precipitin reaction occurred with the animal serum used for absorption. The absorbed sera were set up both against human sera and against all animal sera giving cross-reactions. The results are summarized in Tables 2 to 7; the different animal species are listed according to the decreasing number of positive reactions obtained with absorbed antisera.

The following conclusions may be drawn from these experiments:

i) Antisera against human serum proteins when absorbed with cross-reacting animal sera give different and distinct reaction patterns in tests against human

Table 1

Cross-precipitation reactions of antisera against human serum proteins with animal sera

Animal serum \ Immune serum	anti-albumin 929 F (Behring)	anti-transf. 682 c (Behring)	anti-α_2m 777 b (Behring)	anti-α_2m 929 (Berne)	anti-LDL 1002 (Behring)	anti-LDL K2 (Berne)	anti-LDL K3 (Berne)	anti-LDL K4 (Berne)
rhesus monkey	+	+	+	+	+	+	+	+
cat	+	—	+	+	+	+	+	+
dog	+	—	+	+	+	+	+	+
horse	+	—	+	+	+	+	+	+
sheep	+	—	+	+	+	+	+	+
cow	+	—	+	+	+	+	+	+
pig	+	—	+	+	+	+	+	+
guinea pig	—	+	—	—	+	+	+	+
mouse	—	—	—	0	0	0	0	0

+ cross-precipitation — no cross-precipitation 0 not done

Table 2

Cross-precipitation reactions of anti-α_2-macroglobulin 777 b (Behring) absorbed with different animal sera

Reaction with	Immuneserum absorbed with							
	rhesus monkey	cat	horse	sheep	cow	pig	dog	not abs.
man	+	+	+	+	+	+	+	+
rhesus monkey	—	+	+	+	+	+	+	+
cat	—	—	+	+	+	+	+	+
horse	—	+	—	+	+	+	+	+
sheep	—	+	+	—	+	+	+	+
cow	—	+	+	—	—	+	+	+
pig	—	—	—	—	—	—	+	+
dog	—	—	—	—	—	+	—	+

Table 3

Cross-precipitation reactions of anti-α_2-macroglobulin 929 (Berne) absorbed with different animal sera

Reaction with	Immuneserum absorbed with							
	rhesus monkey	cat	horse	pig	sheep	cow	dog	not abs.
man	+	+	+	+	+	+	+	+
rhesus monkey	—	+	+	+	+	+	+	+
cat	—	—	+	+	—	+	—	+
horse	—	—	—	+	+	+	—	+
pig	—	—	—	—	—	—	+	+
sheep	—	—	—	—	—	—	—	+
cow	—	—	—	—	—	—	—	+
dog	—	—	—	—	—	—	—	+

Table 4

Cross-precipitation reactions of anti-LDL 1002 (Behring) absorbed with different animal sera

Reaction with	Immuneserum absorbed with								
	rhesus monkey	cat	dog	pig	guinea pig	sheep	horse	cow	not abs.
man	+	+	+	+	+	+	+	+	+
rhesus m.	—	+	+	+	+	+	+	+	+
cat	—	—	+	+	+	+	+	+	+
dog	—	—	—	+	+	+	+	+	+
pig	—	—	+	—	+	+	+	+	+
guinea pig	—	—	+	+	—	+	+	+	+
sheep	—	—	+	+	+	—	+	—	+
horse	—	—	+	+	+	+	—	—	+
cow	—	—	+	—	—	—	—	—	+

Table 5

Cross-precipitation reactions of anti-LDL K2 (Berne) absorbed with different animal sera

Reaction with	Immuneserum absorbed with								
	rhesus monkey	cat	horse	pig	guinea pig	dog	sheep	cow	not abs.
man	+	+	+	+	+	+	+	+	+
rhesus m.	—	+	+	+	+	+	+	+	+
cat	—	—	+	+	+	+	+	+	+
horse	—	+	—	+	+	+	+	+	+
pig	—	+	+	—	+	+	+	+	+
guinea pig	—	+	+	+	—	+	+	+	+
dog	—	+	—	+	+	—	+	+	+
sheep	—	+	—	+	+	+	—	+	+
cow	—	+	—	—	+	+	+	—	+

Table 6

Cross-precipitation reactions of anti-LDL K3 (Berne) absorbed with different animal sera

Reaction with	Immuneserum absorbed with								
	rhesus monkey	cat	dog	horse	pig	guinea pig	sheep	cow	not abs.
man	+	+	+	+	+	+	+	+	+
rhesus m.	—	+	+	+	+	+	+	+	+
cat	—	—	+	—	+	+	+	+	+
dog	—	—	—	—	+	+	+	+	+
horse	—	—	—	—	+	+	—	+	+
pig	—	—	—	+	—	—	+	—	+
guinea pig	—	—	—	—	—	—	+	+	+
sheep	—	—	—	—	—	+	—	—	+
cow	—	—	—	—	—	—	—	—	+

Table 7

Cross-precipitation reactions of anti-LDL K4 (Berne) absorbed with different animal sera

Reaction with	Immuneserum absorbed with								
	rhesus monkey	cat	horse	sheep	dog	guinea pig	pig	cow	not abs.
man	+	+	+	+	+	+	+	+	+
rhesus m.	—	+	+	+	+	+	+	+	+
cat	—	—	+	+	+	+	+	+	+
horse	—	+	—	+	+	+	+	+	+
sheep	—	+	+	—	+	+	+	+	+
dog	—	+	+	+	—	+	+	+	+
guinea pig	—	+	+	+	+	—	+	+	+
pig	—	+	—	+	+	—	—	+	+
cow	—	+	—	—	+	—	—	—	+

or animal sera. The most likely explanation for this phenomenon may be that different antigenic structures are shared by human and the corresponding animal serum proteins. Those animal serum proteins which react positively with the greatest number of different absorbed antisera would thus possess the greatest number of such common antigenic structures. Furthermore, it could be shown that practically with each of the animal sera used for absorption, a specific reaction pattern was obtained; it can be expected, therefore, that a great number of additional reaction patterns may be obtained by the use of immunesera absorbed with additional animal sera.

ii) The different antisera against human serum proteins obviously contain a series of antibodies directed against several of these common antigenic structures.

2. Problems in connection with the relative contribution of comparative immunodiffusion and absorption experiments in study of antigenic structures of human serum proteins

From results obtained in absorption experiments as described above, and from results of comparative studies by Roulet et al. and other authors it may appear that human serum proteins possess a complex antigenic make-up part of which is also present in corresponding serum proteins of certain lower mammals. This interpretation of course prompts reflections as to the minimum number of "antigenic determinants" contained in different serum proteins; estimates of this kind were presented by Roulet et al. However, some facts should be emphasized which, in our opinion, have not been sufficiently taken into consideration.

a) Some methodological sources of error have to be considered. It is well known that patterns obtained in comparative immunodiffusion may be divided into three main categories: the reactions of "non-identity", of "partial identity" and of "identity". It is generally accepted that spur formation is the expression of partial identity or antigenic relationship. This interpretation is, however, subject to some restriction: when the concentrations of the two compared antigens are very unequal (non-balanced system), sometimes a spur formation may be observed in spite of complete antigenic identity of the two antigens [11]. On the other hand, the difference in the antigenic structures of closely related antigens may be so small that spur formation is either absent or hardly recognizable. Thus, comparative immunodiffusion experiments may lead to erroneous results, too many reactions of partial identity being found in the first and too few in the second case. It may well be that some of the contradictory results obtained by Roulet et al. are attributable to errors of this kind.

b) Errors cannot be excluded in absorption experiments. Very small quantities of antibody not detectable by visible precipitation may still be present in "absorbed" antisera. In this case it would be expected that the number of positive reactions observed will be too small.

c) Of more importance, in our opinion, than such methodological shortcomings is the interpretation of the results. Roulet et al. interpreted their results on the basis of an immunological model in which the reaction of simple "specific"

antibodies, i.e. antisera containing mixtures of many such antibodies, with complex antigens composed of a number of different antigenic determinants, is postulated. Hirschfeld [7] has recently pointed out that immunological reactions may also be interpreted in a different fashion. In his terminology the model mentioned above would be a "simple-complex" system; in addition to this model, "complex-simple" systems (= reactions of complex "polyspecific" antibodies with simple antigens) and "complex-complex" systems are employed for the explanation of immunological reactions. The "complex-complex" system very likely represents the true serological condition. Assuming methodological reliability of our absorption experiments, closer inspection of Tables 2 to 7 appears to indicate that the results cannot be interpreted without contradiction on the basis of a "simple-complex" model. Since absorption experiments revealed the presence of multiple antibodies in the immunesera used, a pure "complex-simple" model may not apply. We have to assume, therefore, that human and also animal serum proteins are complex antigens bearing a multitude of antigenic determinants; furthermore, it appears that the corresponding antisera against human serum proteins contain mixtures of both simple and complex antibodies; these antisera obviously do not contain only simple "monospecific" antibodies directed each against a single antigenic determinant, as stated by Roulet et al. For the interpretation of our absorption experiments a complicated "complex-complex" model would have been required which, on its part, would have represented only a tentative approach to the real serological situation. Since the main subject of the present investigation was the question of whether or not species- and/or group-specificity of common antigenic structures of human and animal serum proteins could be demonstrated, no attempt was made to create adequate immunological models and to speculate on the number of antigenic determinants of human serum proteins.

3. Studies on group- and species-specificity of antigenic structures shared by human and animal serum proteins

This problem has been approached by three different lines of experiments.

3.1. Comparison of individual human sera with animal sera in immunodiffusion test against non-absorbed immunesera. When pooled human serum is compared with animal sera in immunodiffusion against antihuman serum protein antisera, reactions of partial identity are always obtained. Human serum proteins, therefore, possess at least one antigenic structure which is different from those contained in the corresponding animal serum proteins (cf. [15]). In a first series of experiments we replaced the pooled human serum by individual human sera in the following arrangement.

A pair-wise comparative test was performed with a cross-reacting animal serum and individual human sera against the total of 8 antisera at our disposal (see Materials and Methods). A total of 24 individual human sera was compared with each of the cross-reacting animal sera (according to Table 1). Reactions of partial identity were obtained in each of the 1320 combinations (24×55); not a single indisputable reaction of identity was observed.

The following conclusions may be drawn from these results. Antibodies present in the immunesera on a random basis revealed among others, antigenic structures which human albumin, transferrin, α_2-macroglobulin, and LDL, respectively, possess in addition to those contained in the corresponding animal serum proteins used for comparison. Since these "human specific" antigenic structures could be demonstrated in all the 24 individual human sera tested, they very likely are species-specific.

3.2. Comparison of individual human sera in immunodiffusion tests against absorbed immunesera. The demonstration of reactions of partial identity in a pair-wise comparison of individual human sera against an unabsorbed immuneserum would be indicative of the presence of group-specific antigenic differences of human serum proteins. Reactions of identity are, however, regularly obtained with individual human sera in such experiments, as we could confirm in a series of preliminary investigations. Considering the possibility that in a given immuneserum a weak antibody supposedly directed against a group-specific antigenic structure may be present among a number of additional strong antibodies directed against species-specific configurations, then it appears entirely possible that the theoretically expected spur-formation fails to occur in spite of the immunologic non-identity of the respective serum proteins in the two individual human sera compared. As outlined above, the number of antibodies present may be reduced by absorption of the immunesera with different animal sera, thus increasing the specificity. It may be possible, therefore, that a suitable antiserum can be prepared by absorption, containing the supposed antibody directed against a group-specific antigenic determinant either alone or together with a reduced number of additional antibodies. It may be expected that group-specific differences of serum proteins are more easily revealed by means of absorbed antisera.

In a first series of experiments, a total of 6 immunesera (anti-α_2-macroglobulin 929 Berne, anti-α_2-macroglobulin 777b Behring, anti-LDL 1002 Behring, anti-LDL K2 Berne, anti-LDL K3 Berne and anti-LDL K4 Berne) was exhaustively absorbed with rhesus monkey serum. In a pair-wise comparison of 8 individual human sera against all absorbed immunesera, reactions of identity were obtained in all the 168 combinations (6×28); in no instance was a definite spur-formation observed. Rhesus monkey serum was chosen for absorption in these experiments since absorption experiments (see Tables 2 to 7) revealed that antisera obtained in this fashion contain only antibodies which react exclusively with human serum proteins, while no reaction occurs with corresponding animal serum proteins. The results of these experiments demonstrate, however, that these antibodies obviously are directed against species-specific antigenic structures.

In a second series of experiments, one immuneserum (anti-α_2-macroglobulin 777 b Behring) was absorbed exhaustively with cross-reacting animal sera of four different species (cat, sheep, horse, cow). Again, only reactions of identity were observed in a total of 112 combinations (4×28) when 8 individual human sera were compaired pair-wise against each of the absorbed antisera. It is, therefore, very unlikely that an antibody against a group-specific antigenic structure of the

human α_2-macroglobulin should be present in immuneserum anti-α_2-macroglobulin 777 b Behring, provided that this antigenic configuration was present in one of the 8 individual human sera used in this experiment. It may indeed be assumed that an antibody directed against a group-specific antigenic structure should have been present in a sufficiently pure form in one of the absorbed antisera to give a reaction of partial identity.

Another experimental arrangement would have been, of course, the comparison of individual human sera against antisera absorbed with different individual human sera instead of animal sera. It is well known that experiments of this kind have led to the discovery of the Lp-serum group system [2, 3]. A series of such experiments using the immunesera employed in this investigation failed to reveal group-specific factors of the respective serum proteins. Details are not given in this report as we confined ourselves to the study of supposed group-specific antigenic structures shared by human and animal serum proteins.

3.3. Comparison of individual human sera with animal sera in immunodiffusion test against absorbed immune sera. Again, it is assumed that an antibody against a group-specific antigenic structure A of the cross-reacting serum protein is present in the immuneserum used. It is further assumed that by absorption of an immuneserum with a suitable animal serum, we succeed in obtaining an antiserum which, in a given animal X, reacts exclusively with the common antigenic structure A (being group-specific for the human serum protein). In comparison of human serum from an A(+) individual with serum of animal X against the absorbed antiserum a reaction of partial identity may be expected to occur (spur-formation because of the additional species-specific antigenic determinants in the human protein); on the other hand, a reaction of non-identity (crossing of the precipitation lines) should be obtained when serum of an A(−) individual is being compared with the serum of animal X.

These theoretical considerations have been experimentally tested with anti-LDL immuneserum K2. This antiserum, absorbed exhaustively with each cross-reacting animal serum, was set up against 24 individual human sera and each of them was compared pair-wise with an animal serum; of course, only animal sera still cross-reacting with the absorbed antiserum were used (see Table 3). In all instances a reaction of partial identity was observed; not a single reaction of complete non-identity (pure cross-precipitation) was obtained. Therefore, immuneserum K2 obviously contains no antibody against a group-specific antigenic structure of human LDL; this confirms the results of the experiments described above.

4. Final considerations

In summary, our results show that the 8 rabbit immunesera against human albumin, α_2-macroglobulin and LDL, respectively, contain mixtures of antibodies – probably simple and complex – directed against serum protein structures shared by man and some other mammals. The common antigenic structures which were detectable with immunesera at our disposal seemed to be species-

specific throughout; these immunesera appeared unsuitable for the demonstration of group-specific structures. Nevertheless, the experimental arrangements used in this study may still be regarded as promising for the detection of new group-specific iso-antigenic structures of human serum proteins. Success of course will mainly depend on the particular antibody content of the immunesera available for studies of this kind. Theoretically, one should expect that differences in iso-antigenic structures may be revealed with the highest probability when immunesera are being used which were obtained by immunization with single donor serum proteins (e.g. immunesera 929, K2, K3 and K4). However, since Bundschuh [3] was successful in demonstrating group-specific iso-precipitins against human LDL in a polyvalent horse immuneserum, the examination of immunesera prepared by immunization with pooled human serum proteins should also be promising.

In the interpretation of our results it has to be taken into consideration that only a relatively small number (24) of individual human sera was used in comparative studies. Group-specific iso-antigenic structures of a low incidence could, therefore, have been missed.

More experimentation will be necessary to answer the question of whether or not group-specific antigenic structures shared by human and animal serum protein may be detected. It is difficult to predict which of the experimental arrangements used in this study will be the most suitable for this purpose.

Acknowledgement

We wish to thank Dr. M. Hess, Theodor Kocher Institute of the University of Berne, for reading and correcting the manuscript.

References

1. Augustin, R.: Species differences in serum proteins. Protides of Biological Fluids; Proc. 9th Coll. Bruges 1961, p. 207 (1962).
2. Berg, K.: A new serum type system in man — the Lp-system. *Acta path. microbiol. scand. 59*, 369 (1963).
3. Bundschuh, G.: Anti-Lp(a, x) vom Pferd. *Ärztl. Lab. 10*, 309 (1964).
4. Bütler, R., Brunner, E., Vierucci, A., Morganti, G.: Comparative studies on anti-Ag sera in immunodiffusion and passive hemagglutination methods. *Vox Sang. (Basel) 13*, 327 (1967).
5. Bütler, R., Elsener, R.: Specific serum-protein antibodies. *Lancet 1*, 387 (1967).
6. Goodman, M.: The specificity of proteins and the process of primate evolution. Protides of Biological Fluids, Proc. 12th Coll. Bruges 1964, p. 70 (1965).
7. Hirschfeld, J.: Serological codes: Interpretation of immunogenetic systems. *Science 148*, 968 (1965).
8. James, K.: A study of the alpha$_2$-macroglobulin homologues of various species. *Immunology 8*, 55 (1965).
9. Kaminski, M.: Relations immunologiques entre les protéines sériques de quelques mammifères. *Bull. Soc. Chim. biol. (Paris) 39*, Suppl. 1, 85 (1957).

10. Moor-Jankowski, J., Brown, K. S.: Serum Antigens in Mammals: Cross-reacting iso-immune and hetero-immune systems. *Exp. Med. Surg. 21*, 147 (1963).
11. Ouchterlony, Oe.: Gel diffusion techniques. "Immunchemie", 15th Colloquium of the Gesellschaft für Physiologische Chemie, Mosbach/Baden 1964. Springer, Berlin—Heidelberg—New York 1965, p. 13.
12. Picard, J. J., Heremans, J. F., Vandenbroek, G.: Serum proteins found in primates. I. Proteins of man, "Macacus irus" and "Lemur mongoz". *Vox Sang. (Basel) 7*, 190 (1962).
13. Picard, J. J., Heremans, J. F., Vandenbroek, G.: Serum proteins found in primates. II. Serum proteins in some other primate species. *Vox Sang. (Basel) 7*, 425 (1962).
14. Picard, J. J., Heremans, J. F., Vandenbroek, G.: Serum proteins found in primates. III. Comparative analysis of the antigenic structure of several proteins. *Mammalian 27*, 285 (1963).
15. Roulet, D. L. A., Gugler, E., Rosin, S., Renaud, N. M., Hässig, A.: Untersuchungen über die Antigenstruktur menschlicher Serumproteine. *Vox Sang. (Basel) 5*, 479 (1960).
16. Scheiffarth, F., Götz, H., Soergel, K.: Immunoelektrophoretische Studien über heterologe Präzipitinreaktionen. *Int. Arch. Allergy 10*, 82 (1957).
17. Williams, C. A., Wemyss, C. T. Jr.: Experimental and evolutionary significance of similarities among serum protein antigens in man and the lower species. *Ann. N. Y. Acad. Sci. 94*, 77 (1961).

Dynamics of Blood Coagulation
Introducing a New Coagulation Factor: "Velocity Gradient"

L. DINTENFASS

Laboratory of Haemorheology, Department of Medicine, University of Sydney, Sydney, Australia

(Received July 24, 1967)

This paper presents a review of work carried out in my laboratory in the field of rheology of blood coagulation. Coagulation of human blood is studied by means of the rotational cone-in-cone viscometer and the variable-frequency thromboviscometer (VFTV). Coagula formed in these instruments represent a spectrum of clots, red thrombi, white thrombi and "suspension coagula" (suspension of microthrombi), the particular type of the morphology depending on the flow velocity (velocity gradient) at which the coagulation process proceeds. In a parallel manner, the latent clotting times, the thrombus formation times, and the consistency of clots or thrombi are affected by the velocity gradient at which the process of coagulation proceeds. An increase of the velocity gradient leads to the decrease of clotting times and decrease of clot (thrombus) consistency. These phenomena are mirrored by the colloidal and molecular changes in the structure of coagula. The basic effect of the velocity gradient is due to an increase in the diffusion rate, increase in the frequency of collision of coagulation factors, and in orientation of the polymerization process of fibrinogen-fibrin transition. The coagulation phenomena are influenced by the velocity gradient-induced mechanochemical transformations, although the details are still unclear. It is suggested that the velocity gradient represents a basic coagulation factor, and that a study of coagulation under dynamic (as against static) conditions is required in order to appreciate physiology and pathology of coagulation in vivo.

Introduction

It is rather amazing to review the field of blood coagulation and note that, with few exceptions, the bulk of the studies is carried out in test tubes under static conditions. It is amazing, as the obvious point, that blood flows and that coagulation processes must take place under the conditions of flow (even if only of slow flow), is somehow lost or disregarded.

Surely the question could be asked whether the coagulation processes proceed in a similar manner under conditions of flow as under static conditions? or is the role of coagulation factors or profactors affected by the flow of blood and by the velocity and nature of this flow? or is the significance of the levels of factors or profactors found in the test tube (that is, in a static test) similar or even relevant to the levels of these factors in the flowing blood? These questions should be pondered about in the future, but my aim is a less ambitious one: to show that blood flow velocity (or more strictly, the velocity gradient) is one of the basic coagulation parameters.

My studies lead to a conclusion that the velocity gradient affects the triad of clotting times, consistency and morphology of blood coagula, in a predetermined manner; changes in the numerical values of the velocity gradient resulting in a spectrum of coagulation processes, spectra of clotting times, consistencies and morphologies.

Instrumentation and Techniques

Two basic instruments were developed and used in these studies. The first one was a cone-in-cone rotational viscometer [1, 2, 3, 4] equipped with a copper beryllium torsion strip of 0.025×0.002 inch cross-section. The rotational viscometer permits measurement of the viscosity of blood over a large range of rates of shear; it is also a very sensitive tool for following changes in the blood viscosity during the initial stages of coagulation. The viscometer (Fig. 1) operates at the temperature of 37°C, the temperature being controlled by a "JUMO" mercury-contact thermometer. Deflections of the internal cone, suspended on the torsion strip, are observed as movements of a lightspot on a semicircular scale.

The second instrument, the variable-frequency thromboviscometer, named VFTV [5, 6], is an oscillating version of the cone-in-cone viscometer, and is fitted with a much stiffer torsion strip of 0.075×0.008 inch cross-section (Fig. 2) The thromboviscometer allows to follow changes in the viscosity of coagulating blood, and permits determinations of consistency of final clots or thrombi, and determination of the clotting times. Plots of experimental data resemble the tuning fork-like curves of the thrombelastograph. However, in contradistinction to the latter, the VFTV operates over a frequency range from 1 to 300 cycles per minute, and over a range of amplitude from 1 to 120 degrees. Consequently, the lower range of shear rates in VFTV is about hundred-fold higher than that existing in the thrombelastograph.

Under experimental conditions, in which oscillation of large amplitude is used, the deflection of the torsion strip has enough time to come to equilibrium within each oscillation; consequently, the deflection of the torsion strip is a measure of the viscosity of the fluid (or gel) tested. This viscosity can be expressed in the fundamental units of viscosity, poises.

The coefficient of proportionality between the rate of shear and the number of revolutions per minute equals 0.732 for the cone-in-cone rotational viscometer. The mean shear rate in the VFTV is equal to frequency of oscillations (double amplitude/360°) $\times$ 0.732

In tests run at the fixed amplitude of 110 degrees (a complete arc swept during one cycle being 220 degrees), the deflection of the torsion strip corresponding to a standard oil of 1 poise is 0.138 scale units at 10 cycles per minute, 0.83 scale units at 60 cycles per minute, etc.

Samples of blood were obtained from healthy donors and from patients, using either glass or plastic syringes. In the case of plastic syringes, timing started from the moment when blood was poured into the gap between the two cones.

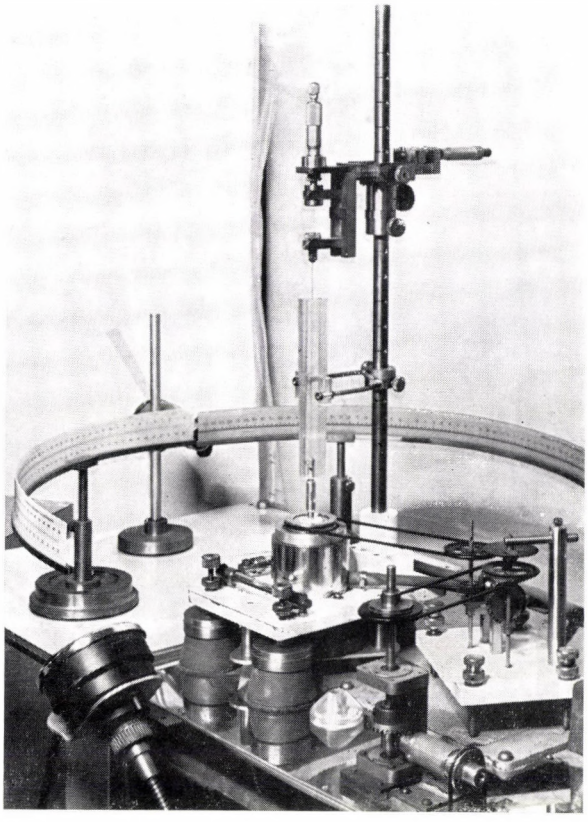


Fig. 1. The cone-in-cone rotational viscometer. The photograph shows the top of the trolley viscometer. The centre is occupied by the coaxial cone-in-cone unit enclosed in a thermostated jacket. The copper beryllium torsion strip is supported by a suspension controlled by two (a horizontal and a vertical) micrometers. A deflection of the torsion strip is measured by means of a lightspot reflected from a galvanometer mirror onto a semi-circular plastic scale. Blood sample is poured into the space between the cones

In the case of glass syringes, the stop-watch was started as soon as the first drop of blood appeared in the syringe. There is no fundamental difference between results obtained both ways; clotting times obtained from blood samples collected by means of plastic syringes are usually 20 to 30 seconds shorter than when glass syringes are used.

Coagulation times are defined as follows. The latent clotting time is measured till the moment when blood viscosity starts to increase, the maximum consistency

of thrombus (clotting) time is measured till the moment when the maximum consistency of thrombus (or clot) is observed; the actual thrombus formation time is the difference of the two clotting times described.

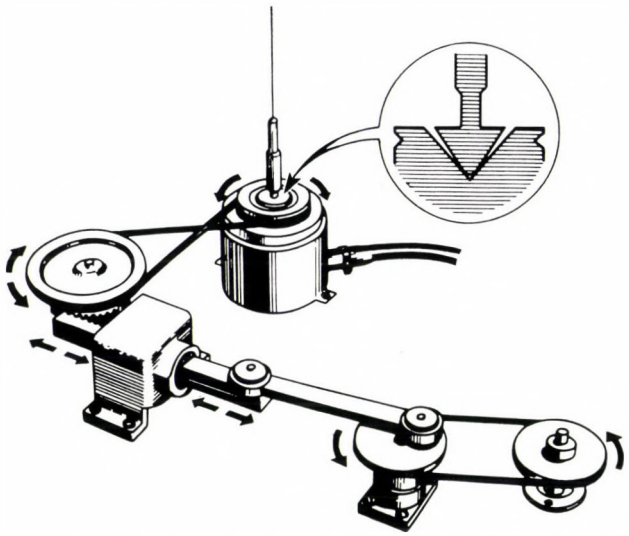


Fig. 2. Diagrammatic representation of the reciprocal drive of the variable-frequency thromboviscometer (VFTV). The instrument is resembling the one showed in Fig. 1. The sample to be tested is poured into the gap between the two cones

Rheological nomenclature:

The "rate of shear" or the "velocity gradient" is a function of the velocity of the fluid laminae or of the surfaces surrounding the fluid, and of the geometry of these surfaces. In the simple case of two parallel surfaces, spaced apart by a gap d, with one surface remaining stationary and the other moving at a velocity V, the velocity gradient between these two surfaces is equal to V/d; the units of velocity (cm/sec) are divided by the units of distance (cm) in order to yield the units of the rate of shear (sec^{-1}).

Discussion and Review of Results

The key role of the velocity gradient on the clotting time of blood was recognized first by Dintenfass [1, 3], although a vague and diffuse knowledge that shaking affects clotting time has been prevalent for a long time. Most investigators of coagulation and thrombosis employ tests based on timing of the onset of clotting in a tilted test tube, or in thrombelastographs, although such tests produce a blood clot. Yet the morphology of a thrombus is known from the year 1882 to be different

from that of a clot [7, 8]. The Chandler's apparatus [9] helps to bridge the gap between clotting in a tube and thrombosis in the body, but does not permit an investigation of the influence of the various levels of the velocity gradient on the clotting time, consistency or morphology of coagula, and does not permit a quantitative approach to these phenomena.

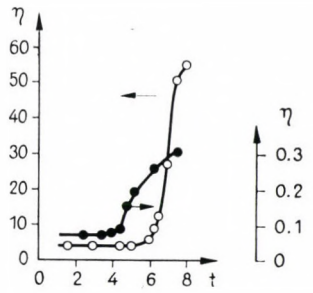


Fig. 3. Changes of blood viscosity, with time, during the initial stages of the coagulation process (after Ref. 1). Curves were obtained at low shear rate (circles, left-hand viscosity scale) and at medium shear rates (dots, right-hand viscosity scale). Viscosity in poises is plotted against time in minutes

The techniques for production of white and red thrombi by means of the cone-in-cone viscometer were established by Rozenberg and Dintenfass [10, 11] in 1963. The influence of the velocity gradient on the consistency and molecular rheology of clots and thrombi formed in the variable-frequency thromboviscometer was reported [5] first during the annual meeting of the Haematology Society of Australia (Adelaide, April 9, 1965).

Subsequent studies crystallized our thoughts on the role of the velocity gradient in blood coagulation in healthy donors, and our attention was then directed to the velocity gradient-affected pathology of coagulation [12, 13, 14, 15, 16].

The first observed variation in the coagulation process was that of the latent clotting times. Figure 3 illustrates one of the first experiments; viscosity of blood is plotted against time at two, low and high, shear rates (0.18 and 56 sec^{-1}). Figure 4 illustrates the effect of velocity gradient on the latent clotting time in blood of six donors; in this figure the relative increase in viscosity is plotted against time. A more informative summary of the data obtained by means of the cone-in-cone viscometer is contained in Figure 5 in which clotting time is plotted against a function of the velocity gradient. It is of particular interest that the dotted line (Fig. 5), which was originally suggested and plotted on purely theoretical grounds, was confirmed in its validity by more recent experiments in which siliconized needles and syringes were employed for venipuncture.

The numerical values of the latent clotting times, obtained by the cone-in-cone viscometer and the VFTV, differ at lower range of the velocity gradients. This is due to the fact that a stiffer torsion strip of the VFTV reacts later, as it responds to a certain elevation of the viscosity of the coagulating blood. However, it appears, at this stage, to be more convenient to standardize on the results obtained by means of the VFTV (Table 1).

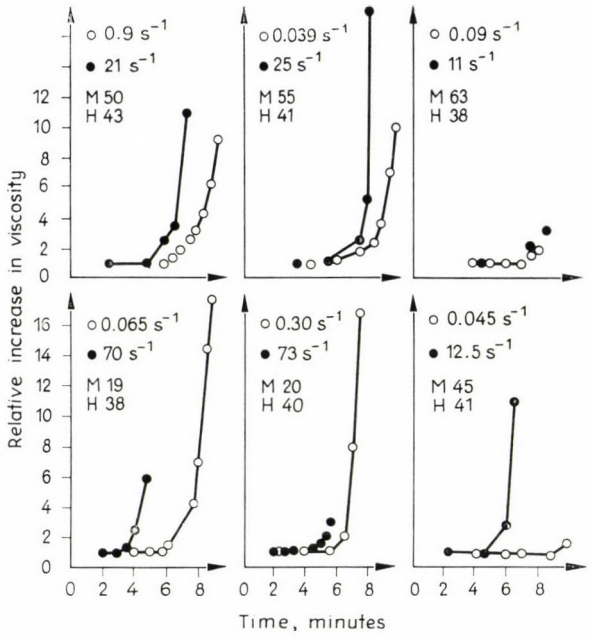


Fig. 4. Changes of blood viscosity during the initial stages of coagulation. Data obtained by means of the cone-in-cone viscometer and plotted as the relative viscosity increase (that is, the ratio of viscosity measured during coagulation to the viscosity of uncoagulated blood). Six male donors were tested at two shear rates each (s^{-1} = shear rate in reciprocal seconds; H = haematocrit; M 45 = male aged 45 years)

An increase in the velocity gradient leads to a pronounced decrease in the clotting times. The clotting times of some minutes duration at near-zero shear rates are giving way to the clotting times of a minute or two duration at higher shear rates, or a few seconds duration at shear rates above 400 sec^{-1}.

The VFTV supplies information not only on the duration of latent clotting time, and on the duration of time required for the formation of the maximum consistency-thrombus, but supplies information on the consistency and rheology of the coagula forming and formed during the coagulation process. Figure 6

Table 1

Effect of the velocity gradient on clotting times in VFTV (after [16])

Coagulation system	Mean shear rate, sec^{-1}	Latent clotting time	Maximum thrombus consistency clotting time
I	0.55	5 min 4 sec	14 min 24 sec
IV	26.5	3 min 11 sec	7 min 33 sec
VI	79	2 min 14 sec	3 min 57 sec
VII	400	0 min 10 sec	below 30 sec

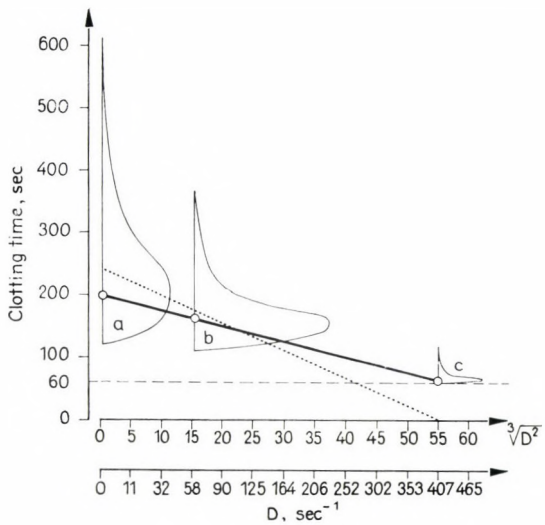


Fig. 5. Latent clotting times as a function of the velocity gradient (after Ref. 5). Results obtained by means of the rotational cone-in-cone viscometer. Clotting time in seconds is plotted against the velocity gradient in reciprocal seconds. Zero time is the moment of venipuncture, as glass syringes were used. The broken horizontal line (60 seconds line) indicates the beginning of the test proper. Areas described as *a*, *b* and *c* represent schematically the frequency distribution of latent clotting times at three shear rates. Function $D^{2/3}$ was employed in order to obtain a straight line. The inclined dotted line represents an approximate function of the latent clotting time and velocity gradient when no time is lost due to the required preparatory work and, thus, coagulation tests can proceed immediately after venipuncture. This approximation has been confirmed recently by tests in which siliconized needles and syringes have been used

illustrates a complete test on a single blood sample. The results, obtained at 10, 60 and 180 cycles per minute, are plotted as viscosity in poises against the coagulation time in minutes. Figure 7 supplies the actual tuning-fork like curves obtained in the VFTV and plotted in the scale units against time.

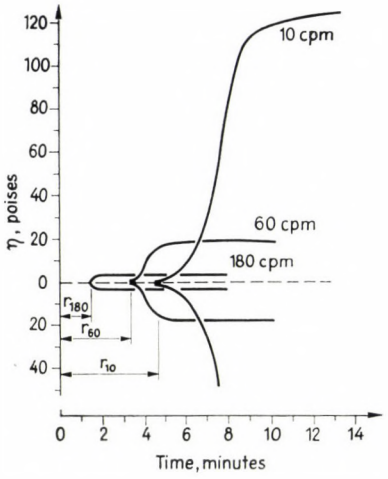


Fig. 6. A typical VFTV pattern seen in a single donor when coagula are cast at different velocity gradients (different frequencies of oscillations). *r* with a subscript 180, 60 or 10 refers to the latent clotting time measured at 180, 60 or 10 cycles per minute. The maximum thrombus consistency clotting time is measured till the moment when a stable (maximum) consistency is obtained

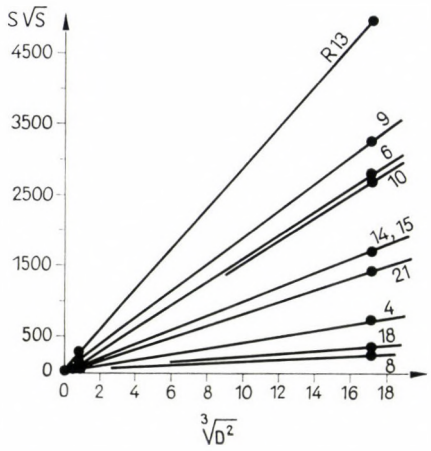


Fig. 7. Typical VFTV patterns seen in healthy donors. Viscosity of coagulum is plotted in the scale units against time in minutes. The maximum thrombus consistency is marked in poises; the frequency of oscillations employed during the particular test is marked in cycles per minute (cpm) (Ref. 16)

The velocity gradient may affect the clotting times through at least three mechanisms:

i. by a directly facilitated diffusion and collision frequency of the coagulation factors and profactors;

ii. by a decrease in blood viscosity, which follows an increased shear rate in a thixotropic medium, and thus by facilitated diffusion and collision of coagulation factors;

iii. by an influx of the mechanical energy which might affect the chemical integrity of the red cells and platelets, with a possible release of some additional coagulation factors.

Of these three mechanisms, the first two are already adequate to trigger off an effect akin to an explosive polymerization. In the process of coagulation the first stages, as exemplified by Macfarlane's cascade [17], are the slowest stages

of coagulation. These mechanisms enable to accelerate the processes in these first stages.

Microscopic examination reveals [10, 11, 12] that an increase in the velocity gradient influences the morphological structure of the blood coagula. When the

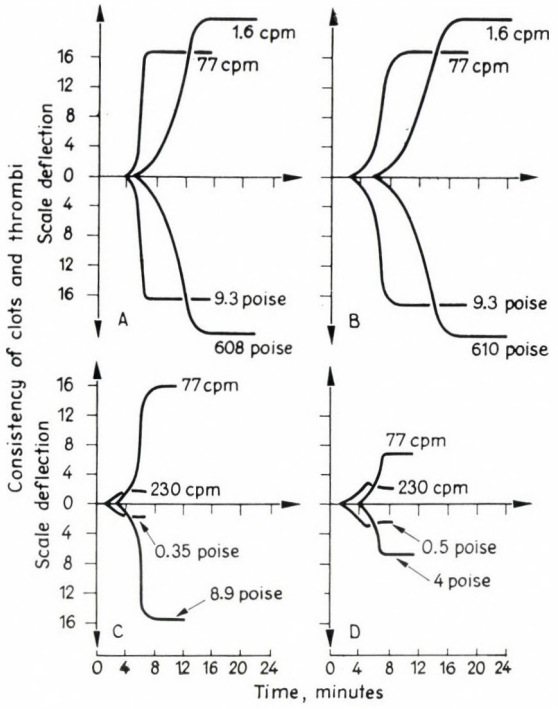


Fig. 8. Aggregation of platelets as a function of the velocity gradient (after Ref. 11). The area of amorphous masses, composed of platelet aggregates, S, is determined from all sections of each of the blood coagula (clots or thrombi) formed in the cone-in-cone viscometer and is plotted as $S\sqrt{S}$ against $D^{2/3}$, where D is the velocity gradient at which the respective clot or thrombus was formed. The slope of each curve depends on the intrinsic adhesiveness of platelets and is specific to each individual donor

coagulation of blood takes place at near-zero rates of shear, a red clot is formed in which the red cells are embedded in a random mesh of fibrin. When coagulation proceeds at higher rates of shear ($30-100\ sec^{-1}$), an artificial white thrombus is formed, containing large masses of platelet aggregates. Blood coagula formed at intermediate velocity gradients show characteristics intermediate between the red clots and white thrombi. The degree of platelet aggregation increases as the velocity gradient, employed in the particular coagulation process, increases (Fig. 8). As the

velocity gradient increases, more and more blood remains fluid and less and less volume is occupied by the coagulation products. When blood coagulation proceeds at velocity gradients above 400 sec^{-1}, the coagulated blood remains quite fluid, the coagulation products being composed of discrete globular or irregular particles of fibrin and platelet aggregates. It is, in fact, a suspension of such discrete particles in fluid blood; hence, the term "suspension coagulum".

The changes in the morphology are mirrored in the changes in the consistency of coagula. The consistency and rheology of blood coagula depend on the velocity gradient at which such coagula are formed, as well as on the velocity gradient at which these coagula are tested. The "casting" velocity gradient determines the final rheological (as also morphological) properties of the clots and thrombi. Coagula formed at very low rates of shear exhibit gel-like and highly viscous and thixotropic properties, and show viscosities of the order of 100 to 1000 poises (if tested at low shear rates). Coagula (red thrombi and white thrombi), formed at medium shear rates (appr. 20 to 60 sec^{-1}) exhibit viscous and thixotropic properties but of only few, or less, poises. Suspension coagula formed at high velocity gradients show viscosities analogous to the viscosities of the original, uncoagulated, blood (Table 2).

The rheology of blood coagula and their morphology are intimately intertwined. In clots formed at zero rate of shear, the dominant feature is the three-dimensional network of fibrin, the meshes of this network retaining the aggregates of red cells. A slight increase in the rate of shear leads to certain orientation of the network structure, the holes in the network permitting some of the disaggregated

Table 2

Effect of velocity gradient on consistency and morphology of coagula formed in VFTV (in parts after [5] and [16])

Coagulation system	Mean shear rate*, sec^{-1}	Viscosity of uncoagulated blood** poises	Mean maximum viscosity+ of the thrombus, $\bar{x}$, poises	Morphology
I	0.55	1.1	910	red thrombus
II	1.8	0.74	125	red thrombus
III	9.4	0.2	20	red/white
IV	26	0.1	17	white thrombus
V	44	0.08	3	white thrombus
VI	79	0.06	1.7	white thrombus
VII	400	0.04	0.04	"suspension coagulum"

* used during the coagulation process;

** a decrease of blood viscosity with increasing shear rate is due to thixotropy;

+ although thrombi are slightly thixotropic, the levels of thixotropy and viscosity are reduced over the whole range of shear rates when coagulation proceeds at increasingly higher rate of shear (Ref. 5).

red cells to escape. Simultaneously, platelets, which formerly took part in the network structure while retaining their individuality, now become aggregated (agglutinated), and platelet masses become prominent. Coagulation proceeding at still higher shear rates leads to highly orientated fibrin polymerization, to a complete disaggregation and escape of the red cells, and to formation of large masses of platelet aggregates. At very high shear rates, fibrin tends to polymerize in a globular form. The changes in the fibrin strands on a molecular level (changes in the organization, sequence, orientation, etc.) might be affected by the stresses and strains acting during the polymerization and induced by the velocity gradient. If fibrin formation proceeds through a stage of reversible fibrinogen-fibrinogen polymerization, breakage and reformation of bonds may be greatly influenced by the state of flow.

Behaviour of platelets under the velocity gradient is not yet completely understood, although it clearly leads to their aggregation. The rate of platelet aggregation increases as the shear rate employed during the coagulation increases. Usually, in suspensions of the reversibly aggregated cells or colloidal particles, an increase in the rate of shear leads to a disaggregation of these cells or particles. A progressive process of platelet aggregation, at the simultaneously increasing shear rates, suggests an irreversible phenomenon; i.e., that the surface properties of the platelets undergo a profound change. Such a change will cause such a system to become, from the colloidal point of view, a new entity in each of the subsequent short periods of time. It may be speculated that the process of aggregation of platelets under velocity gradient is due to some irreversible change in the platelet membrane. It might be a purely biochemical sequence of events (involving, perhaps, a release of some chemical agents) or, alternatively, it might be due to some mechano-chemical transformation.

Consequently, mechano-chemical transformation might play a role in the coagulation proceeding under a velocity gradient, *via* aggregation of platelets, orientation of the fibrin network, and orientation of the fibrin polymerization.

A principal point emerges that the blood flow velocity, or the velocity gradient, exerts a parallel effect on the clotting time of blood, on the rheology of the coagula, and on the colloidal and morphological structure of these coagula. The possible spectrum (Table 3) of the various types of clots, red thrombi, white

Table 3

Effect of velocity gradient on blood coagulation in vitro

Velocity gradient	Low: near zero	Medium	High (appr. 400 sec^{-1})
Clotting times	Some minutes	Few min	Few seconds
Consistency	High: a gel	Medium	Low (nearly that of blood)
Morphology	Red clot	White thrombus	"Suspension coagulum"

thrombi and suspension coagula depends on the progressive variation in the following factors, which – in their turn – depend on the velocity gradient:

(a) on the progressively increasing diffusion and collision frequency of coagulation factors and profactors, especially in the first stages of coagulation, due to the presence of a velocity gradient as such, and due to the lowered blood viscosity;

(b) on the progressive orientation of the fibrin network, an orientation of the molecular structure of the fibrin fibres, a progressive fracture of the network, and finally on the "balling in" of fibrin;

(c) on the progressive disaggregation of the red cell aggregates, and a progressive escape of the single red cells from the fibrin network;

(d) on the progressive aggregation of platelets, the degree of aggregation resulting from the presence of the velocity gradient being modified by the intrinsic adhesiveness of platelets peculiar to the individual donors.

It must be made clear that, in this outline, a progressive variation in the diffusion and collision of the clotting factors, orientation of fibrin, disaggregation of red cells, and aggregation of platelets, indicates a functional dependence on the velocity gradient. A new equilibrium is obtained at each step of the progressive increase in the value of the velocity gradient. The actual levels of these equilibria, and thus the final details of the blood coagula, can be expected to be affected also by the genetic, metabolic, dietary, etc. etc., background of the individual donor.

But not only triad of clotting times, consistency and morphology of clots and thrombi is affected and, indeed, directed by the velocity gradient. Thrombus contraction [16] takes place within few minutes at high or medium velocity gradient. It is worth noting that the usual time, under static conditions, is of the order of one hour [18]. Hartert already recognized [19] that retraction in a glass tube does not reflect the conditions in vivo; he also realized the importance of the viscosity of the fluid obstructing the pathways of the contracting fibrin network. We observed [16] not only that the presence of high and medium velocity gradients (ca. $20-100$ sec^{-1}) decreases the viscosity of the fluid in the pathway of fibrin network, but also that fibrin network formed at such velocity gradients might offer, perhaps, less resistance to movement. As already described, the velocity gradient causes aggregation of platelets and might affect their function through a mechano-chemical transformation. Fonio suggested recently [20] that clot retraction under static conditions is already motivated by a mechano-chemical process triggered off by the lactic acid; were this valid, how many times greater an effect could be expected under flow conditions.

The question studied by us now is if the mechanism of fibrinolysis is affected by the velocity gradient. A related work on perfusion lysis was reported recently by Bouvier et al [21].

Finally, in order to illustrate the relevance of the ranges of shear rates used in our studies to the conditions in vivo, a few words on the numerical values of shear rates in vessels. The rates of shear (velocity gradients) existing in circulation vary greatly not only from one vessel to another, but also within any single vessel.

Due to the presence of a "profile" of the velocity gradient, blood travelling along the axis of any vessel is under conditions of near-zero or zero shear rate. The maximum rate of shear is in the layer adjacent to the vessel wall. The flow pattern, and thus the rate of shear, varies with time, not only as a result of physiological loading on the cardiovascular system, but also due to the pulsatile nature of flow. Consequently, the shear rate varies from zero to a certain maximum value at the wall, but even the latter value may vary from zero to a maximum with each pulse. Approximate mean values for shear rates in arteries will be from 20 to 100 sec^{-1}, with maxima up to 500 sec^{-1} possible under certain conditions: in veins, the mean shear rates will be from 10 to 30 sec^{-1}, with maxima of 50 sec^{-1} quite possible; in capillaries, the mean shear rates would be between 10 to 20 sec^{-1}. It should be remembered that the mean shear rates given here will correspond to the maximum value in each of the velocity gradient profiles, and that zero shear rate is a part and parcel of each of such profiles.

Conclusions

It is my belief that the velocity gradient plays a key role in the fields of thrombosis and coagulation, and that the velocity gradient should be considered one of the basic factors or parameters.

The role of the velocity gradient might be of paramount importance in the dynamic balance existing between thrombosis and fibrinolysis in blood circulation. A delicately balanced equilibrium between coagulation and fibrinolysis, suggested by Astrup [22], can perhaps be shifted into one or other direction by the velocity of flow or velocity gradient existing in a particular vessel. This balance might be affected by the molecular rheology of the blood and the blood cells.

It appears reasonable that coagulation should be studied as a dynamic process, a process affected by the velocity of blood flow. As the conditions of flow, and thus the levels of velocity gradients, vary from vessel to vessel, as well as they vary with time, the rate and direction (reversibility) of the coagulation process should vary from vessel to vessel and should vary in time.

Extraordinary importance could be ascribed to the existence of suspension coagula. These might play a key role in some disorders of microcirculation.

It might appear significant that the general pattern of response of coagulation processes to the increasing velocity gradient is as true for normals as for thrombotics or haemophiliacs, although the rates of response are different.

On the basis of our experience, it is possible to suggest that it is quite feasible that blood coagulation tests, restricted to static conditions, might tend, *eo ipso*, to misrepresent coagulation processes as they take place under physiological and pathological conditions. If it is our aim to understand and control processes of coagulation, thrombosis or fibrinolysis, we might have perhaps to accept that experimental conditions more alike those in vivo, that is dynamic rather than static, should be employed.

Acknowledgements

These studies were supported by Senior Fellowship grants and grants-in-aid of the National Heart Foundation of Australia.

I am indebted to my collaborators, and especially to Dr. M. Rozenberg for his collaboration on the morphology of thrombi, to Dr. P. Castaldi for collaboration on clotting times in haemophiliacs, and to Drs. D. Julian, G. Miller, J. Yu and C. Grace for their occasional collaboration on various aspects of coagulation.

References

1. Dintenfass, L.: Thixotropy of blood and proneness to thrombus formation. *Circulat. Res. 11*, 233 (1962).
2. Dintenfass, L.: An application of a cone-in-cone viscometer to the study of viscosity, thixotropy and clotting of blood. *Biorheology 1*, 91 (1963).
3. Dintenfass, L.: Viscosity and clotting of blood in venous thrombosis and coronary occlusions. *Circulat. Res. 14*, 1 (1964).
4. Dintenfass, L.: A trolley viscometer for estimating viscosity and clotting of blood in hospital wards. *Lancet 2*, 567 (1964).
5. Dintenfass, L.: Effect of velocity gradient on the clotting time of blood and on the consistency of clots formed in vitro. *Circulat. Res. 18*, 349 (1966).
6. Rosenberg, M., Dintenfass, L.: Platelet aggregation in the variable-frequency thromboviscometer. *Nature (Lond.) 211*, 525 (1966).
7. Bizzozero, J.: Über einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. *Virchows Arch. path. Anat. 90*, 261 (1882).
8. Mason, R. G.: Pathogenesis of thrombosis: platelet contribution. *Science 138*, 833 (1962).
9. Poole, J. C. F.: A study of artificial thrombi produced by a modification of Chandler's method. *Quart. J. exp. Physiol. 44*, 377 (1959).
10. Rozenberg, M., Dintenfass, L.: Thrombus formation in vitro: a rheological and morphological study. *Aust. J. exp. Biol. med. Sci. 42*, 109 (1964).
11. Dintenfass, L., Rozenberg, M.: The influence of the velocity gradient on in vitro blood coagulation and artificial thrombosis. *J. Atheroscler. Res. 5*, 276 (1965).
12. Rozenberg, M., Dintenfass, L.: Platelet aggregation in Waldenström's macroglobulinaemia. *Thrombos. Diathes. haemorrh. (Stuttg.) 14*, 202 (1965).
13. Dintenfass, L., Rozenberg, M.: Effect of temperature, velocity gradient and i. v. heparin on in vitro blood coagulation and platelet aggregation. *Thrombos. Diathes. haemorrh. (Stuttg.) 17*, 112 (1967).
14. Dintenfass, L., Julian, D. G., Miller, G.: Viscosity of blood in normal subjects and in patients suffering from coronary occlusion and arterial thrombosis. *Amer. Heart J. 71*, 587 (1966).
15. Dintenfass, L., Castaldi, P.: A study in viscosity and clotting of blood in haemophiliacs. *Thrombos. Diathes. haemorrh. (Stuttg.) 11*, 267 (1964).
16. Dintenfass, L., Yu, J., Grace, C.: to be published.
17. Macfarlane, R. G.: The basis of the cascade hypothesis of blood clotting. *Thrombos. Diathes. haemorrh. (Stuttg.) 15*, 591 (1966).
18. Macfarlane, R. G.: A simple method for measuring clot retraction. *Lancet 1*, 1199 (1939).
19. Hartert, H.: Clot retraction. Kinetics and correlation with the clotting process. *In* Proc. IV. Int. Congress on Rheology, Wiley, New York 1965, Vol. 4, p. 571.
20. Fonio, A.: Über den Retraktionsvorgang beim Blutplasmagerinnsel. *Acta haemat. (Basel) 36*, 371 (1966).
21. Bouvier, C. A., Gruendlinger, J., Berthoud, S.: Clot lysis in a perfusion system. *Thrombos. Diathes. haemorrh. (Stuttg.) 15*, 205 (1966).
22. Astrup, T.: Biological significance of fibrinolysis. *Lancet 2*, 565 (1956).

Blood Coagulation Changes in the Thrombohaemorrhagic Phenomenon (THP)

B. SOLYMOSS, G. GABBIANI, H. SELYE

Institut de Médecine et de Chirurgie experimentales, Université de Montréal, Montreal, Canada

(Received August 8, 1967)

A thrombohaemorrhagic phenomenon (THP) can be elicited in rats by certain polysaccharides or metals when given in conjunction with catecholamines. The coagulation defect observed is characterized by simultaneous decrease of fibrinogen level and thrombocyte count, low prothrombin content, prolonged recalcification, partial thromboplastin and thrombin times and decreased thromboplastin generation. Some of these changes occur within one hour and all of them are obvious four hours after treatment. The blood coagulation changes in THP are due to extensive intravascular clotting followed by depletion of some coagulation components.

A thrombohaemorrhagic phenomenon (THP) can be elicited regularly in the rat and other species by several agents (mostly polysaccharides and metals), especially if these are given in conjunction with catecholamines and other vasoconstrictors or severe systemic stressors. The syndrome is characterized by multiple haemorrhages and microthromboses which are located mainly in the kidney, duodenum, heart and lung, depending upon the eliciting agents used. In addition, very severe and sometimes necrotizing thrombohaemorrhagic lesions are observed at the site of catecholamine injection. In its morphologic manifestations the THP resembles the generalized and local forms of the Sanarelli – Shwartzman phenomenon and, although elicited by different means, like the latter it can be prevented by heparin [1, 4, 6].

Since intravascular clotting plays an important role during the production of THP, we have studied the blood coagulation changes in two forms of the phenomenon elicited by different means. The first form is characterized by intestinal lesions [5]; the second affects the small and large veins of certain muscles [6, 7].

Materials and Methods

One hundred and twenty female Sprague-Dawley rats with an initial body weight of 204 g (range 195–210 g) were divided into 12 equal groups for two experiments. The animals of Group 1 received 1 ml of distilled water intravenously and served as controls for both experiments.

In the *first experiment* (Table 1) the animals of Groups 2, 3 and 4 received 8 mg of agar (Agar, U.S.P., Brickman and Co., Montreal, Canada) in 1 ml of

warm (40° C) distilled water intravenously and were killed 1, 4 and 24 hours later, respectively.

In the *second experiment* (Table 2) Groups 2 and 3 received 1 ml of distilled water intravenously and four intramuscular injections of 5 μg epinephrine in 1 ml distilled water in each leg (in the lower and upper calves and thighs). Groups 4 and 5 received 4 mg scandium chloride ($ScCl_3$, K and K Laboratory, Plainview, N.Y., U.S.A.) in 1 ml distilled water intravenously and immediately thereafter four injections of 1 ml distilled water intramuscularly, in each of the hind legs, as the rats of Groups 2 and 3. Groups 6 and 7 were given both epinephrine and $ScCl_3$ as described above. Groups 8 and 9 received $ScCl_3$ as Groups 6 and 7 and in addition a single subcutaneous injection of 40 μg of epinephrine in 0.5 ml distilled water on the scalp. Groups 2, 4, 6 and 8 were exsanguinated one hour and Groups 3, 5, 7 and 9 four hours after the treatment.

All the manipulations were performed under light ether anesthesia. We used the exposed left jugular vein for intravenous injections. 4.5 ml of blood were taken by aorta puncture and mixed in a ratio of 9 : 1 with 1.34% sodium oxalate solution in a siliconized tube. Immediately thereafter, we examined the thromboplastin generation [8]; recalcification time [9]; partial thromboplastin time [10] with Platelin® (Warner-Chilcott, Toronto, Ont., Canada); prothrombin content [11] with Calsoplastin® (Baltimore Bacteriological Laboratory, Baltimore, Md., U.S.A.); thrombin time [12]; fibrinogen content [12]; thrombocyte and leucocyte counts [12].

The organs were inspected with a binocular magnifier and the intensity of the thrombohaemorrhagic lesions was estimated in terms of an arbitrary scale in which 0 = no lesion, 1 = just detectable, 2 = moderate and 3 = most pronounced lesions, as described previously [5].

Results

THP lesions and blood coagulation changes induced by agar. As seen from Table 1, one hour after the intravenous injection of agar there was a significant decrease in recalcification time and thrombocyte count ($P < 0.001$) and an increase in thrombin time ($P < 0.005$). At this time mild thrombohaemorrhagic duodenal lesions were already present.

More pronounced changes were observed at four hours. There was an increase in recalcification ($P < 0.001$) partial thromboplastin ($P < 0.001$) and thrombin times ($P < 0.001$); and a decrease in thromboplastin generation ($P < 0.005$), prothrombin content ($P < 0.005$), fibrinogen concentration ($P < 0.001$), thrombocyte ($P < 0.001$) and leucocyte counts ($P < 0.005$). The severity of duodenal lesions was increased.

Twenty-four hours after the treatment, there was still a significant increase in recalcification time ($P < 0.001$) and a decrease in thrombocyte count ($P < 0.001$). The intensity of the duodenal lesions was less marked than in the groups killed four hours after the agar administration.

THP lesions and blood coagulation changes induced by $ScCl_3$ and epinephrine. The findings in the animals treated with epinephrine and $ScCl_3$ and killed after 1 hour are shown in Table 2. Epinephrine caused a significant increase in partial thromboplastin time ($P < 0.001$), thrombin time ($P < 0.001$) and thrombocyte count ($P < 0.001$).

$ScCl_3$ elicited a decrease in thromboplastin generation ($P < 0.001$), prothrombin content ($P < 0.001$) and fibrinogen concentration ($P < 0.005$) with a parallel increase in recalcification time ($P < 0.001$), partial thromboplastin time ($P < 0.001$) and leucocyte count ($P < 0.001$). In 20% of the animals small thrombi were observed in the femoral and iliac veins, in the neighbourhood of muscles injected with distilled water.

Table 1

Duodenal lesions and changes in blood coagulability produced by agar

Groups	1	2	3	4
Tests \ Time of autopsy	Controls	1 hour	4 hours	24 hours
Thromboplastin generation (sec) at:				
4 min	9.7 ±0.5	10.6 ±0.2	14.2 ±1.4	11.0 ±0.5
8 min	10.3 ±0.4	10.9 ±0.4	14.5 ±1.2	12.1 ±0.5
12 min	11.3 ±0.6	11.6 ±0.2	15.2 ±1.1	12.9 ±0.4
16 min	11.4 ±0.3	12.3 ±0.3	16.6 ±1.1	13.2 ±0.6
Recalcification time (sec)	66.8 ±6.5	51.8 ±1.7	89.7 ±5.0	84.8 ±4.8
Partial thromboplastin time (sec)	40.6 ±1.8	44.1 ±2.3	79.1 ±4.1	36.8 ±2.3
Thrombin time (sec)	18.7 ±0.3	21.5 ±0.7	21.8 ±1.4	18.8 ±0.8
Prothrombin (%)	95.8 ±2.1	86 ±3.0	60 ±5.0	100 ±5.0
Fibrinogen (mg%)	463.5 ±14.3	408.0 ±12.8	383.0 ±25.7	545.5 ±18.4
Thrombocyte count (per mm^3)	626,000 ±20,500	355,000 ±20,400	198,000 ±27,000	288,000 ±30,600
Leucocyte count (per mm^3)	8,100 ±460	7,600 ±665	6,100 ±420	9,900 ±760
Duodenal lesions:				
% of incidence	0	70	90	760
degree (scale 0—3)	0	1.5	2.3	1.0

The most marked changes were found in the Group treated with epinephrine and $ScCl_3$. Compared with the animals treated with $ScCl_3$ only (Group 4), there was a further increase in recalcification time ($P < 0.001$), partial thromboplastin time ($P < 0.001$) and a decrease in prothrombin content ($P < 0.001$), fibrinogen concentration ($P < 0.001$), thrombocyte and leucocyte counts ($P < 0.001$). In all animals large thrombi were found in the femoral and iliac veins.

Group 8 received the same amounts of $ScCl_3$ and epinephrine as Group 6 but epinephrine was administered in a different region. Here, thrombi did not

Table 2

Thrombosis and changes in blood coagulability after

Groups	1	2	3
Treatment / Tests	Controls	Epinephrine i. m.	
		1 hour	4 hours
Thromboplastin generation (sec) at:			
4 min	9.7 ±0.5	10.0 ±0.3	11.2 ±0.5
8 min	10.3 ±0.4	10.7 ±0.4	11.9 ±0.4
12 min	11.3 ±0.6	11.8 ±0.4	12.5 ±0.3
16 min	11.4 ±0.3	12.4 ±0.5	13.2 ±0.2
Recalcification time (sec)	66.8 ±6.5	78.0 ±4.8	68.1 ±2.9
Partial thromboplastin time (sec)	40.6 ±1.8	73.2 ±3.1	49.8 ±2.6
Prothrombin (%)	95.8 ±2.1	86 ±6.2	86 ±4.0
Thrombin time (sec)	18.7 ±0.3	25.1 ±0.9	20.6 ±1.0
Fibrinogen (mg%)	463 ±14.3	445.5 ± 8.8	560.5 ±12.5
Thrombocyte count (per mm^3)	626,000 ±20,000	822,000 ±31,000	843,000 ±32,000
Leucocyte count (per mm^3)	8,100 ±460	8,900 ±680	8,500 ±390
Venous thrombosis: % of incidence	0	0	0
Venous thrombosis: degree (scale 0—3)	0	0	0

develop; there was a significant difference between Groups 6 and 8 in thromboplastin generation ($P < 0.005$), recalcification time ($P < 0.005$), partial thromboplastin time ($P < 0.001$), prothrombin content ($P < 0.001$), fibrinogen concentration ($P < 0.001$), thrombocyte count ($P < 0.001$) and leucocyte count ($P < 0.005$).

The blood coagulation changes and thrombotic lesions of the animals killed 4 hours after treatment are summarized in Table 2. The results of Group 3 show that epinephrine did not have a lasting effect on the different parameters. Partial thromboplastin and thrombin times were normal and the fibrinogen concentration

epinephrine and scandium chloride administration

4	5	6	7	8	9
$ScCl_3$ i.v.		Epinephrine i.m. + $ScCl_3$ i.v.		Epinephrine s.c. + $ScCl_3$ i.v.	
1 hour	4 hours	1 hour	4 hours	1 hour	4 hours
14.1 ±0.5	12.3 ±0.7	16.4 ±0.9	22.7 ±1.2	11.0 ±0.5	11.0 ±0.6
14.8 ±0.6	12.1 ±0.7	16.1 ±0.7	19.4 ±0.7	12.9 ±0.8	11.2 ±0.3
15.4 ±0.7	13.5 ±0.5	17.5 ±0.6	19.6 ±0.3	12.7 ±0.7	13.3 ±0.6
17.3 ±0.6	14.9 ±0.4	17.5 ±0.6	20.8 ±0.7	14.7 ±0.6	14.4 ±0.3
208.3 ±16.2	203.7 ±20.8	275.4 ±13.3	>300	184.3 ±21.0	167.7 ±29.6
148.4 ±10.3	107.9 ±17.1	333.5 ±43.0	389.4 ±38.9	106.3 ±5.2	69.3 ±6.8
30 ±4.0	27 ±2.0	10 ±2.1	4 ±2.1	55 ±10.0	82 ±4.0
>100	>100	>100	>100	97.2 ±1.5	97.3 ±2.7
414.5 ±16.7	374.0 ±15.5	264.5 ±20.0	184.1 ±41.4	423 ±10.9	446.5 ±23.1
620,000 ±20,900	507,000 ±27,000	345,000 ±32,400	235,000 ±16,500	832,000 ±37,700	705,000 ±19,000
12,700 ±720	9,200 ±720	9,700 ±750	11,600 ±410	12,500 ±800	12,000 ±590
20	20	100	100	0	0
0.5	0.7	2.6	2.8	0	0

was increased ($P < 0.001$) in comparison with Group 2. On the other hand, the changes induced by $ScCl_3$ after 1 and 4 hours were similar.

In Group 7 the changes of blood coagulation were still more marked than in Group 6. The thromboplastin generation ($P < 0.005$), prothrombin content ($P < 0.005$) and thrombocyte count ($P < 0.005$) were further decreased.

In Group 9 partial thromboplastin time ($P < 0.001$) and prothrombin content ($P < 0.005$) returned to normal as compared with Group 8.

Discussion

Agar is a mixture of at least two polysaccharides, agarose and agaropectin. After careful purification its sulphate content is below 1%, hence, agar does not possess the anticoagulant properties of sulphated polysaccharides [13, 14]. On the contrary, it might enhance thrombocyte aggregation and intravascular clotting as several colloidal solutions do [15]. It is possible also that endotoxin contamination or shock, produced by agar, play a role in the activation of intravascular clotting. The THP elicited in rats by agar is characterized by duodenal lesions. The peculiar localization of these lesions might be partly due to the sensitivity to anoxia of the gastro-intestinal mucosa and its particular susceptibility to injury produced by intravascular clotting [16].

$ScCl_3$ delays blood coagulation in vitro and in vivo [22]. Its effect on prothrombin content is similar to that caused by other rare earth metals [23]. In spite of this anticoagulant action, the administration of $ScCl_3$ together with epinephrine produced thrombosis in vivo [7, 22]. However, the mechanism of this reaction is not yet clear.

Epinephrine has a dual effect on blood coagulation and thrombus formation in that small doses promote whereas large doses inhibit these processes [17, 18]. The effect of small doses might be partly due to the release of a thromboplastic substance from the arterial wall [19]. In addition, epinephrine is known to increase thrombocyte count and aggregation [20, 21]. Accordingly, our rats showed an increase in thrombocyte count already one hour after the treatment.

It has been found that in the vessel wall epinephrine induces an oedematous reaction consisting of an enlargement of amorphous extracellular spaces in the subendothelial and medial layers accompanied by a fractionation of elastic and a dissociation of collagen fibres [24]. Recent observations reveal that anticoagulants might enhance platelet deposition at the site of endothelial injury [25]; hence, a similar mechanism may also play a role in our experiments.

Increased intravascular coagulation may be suspected when low fibrinogen concentration and thrombocyte count, decreased prothrombin content and thromboplastin generation are found simultaneously. All these changes were demonstrated in our experiments. In both experiments, the thrombocyte count was markedly affected, suggesting that thrombocytes might play an important role in the THP. We cannot exclude that other factors (e.g., stasis and vascular lesions) also participate in the development of THP.

In both experiments thrombocyte count, fibrinogen and prothrombin content were decreased while recalcification and partial thromboplastin times increased as soon as one hour after the treatment. All these changes became more marked four hours later.

At the present time there are no satisfactory techniques to correlate predisposition to thrombosis with blood coagulation changes. Our results confirm this view, but the above-mentioned signs (low fibrinogen concentration and thrombocyte count, decreased prothrombin content and thromboplastin generation) may have a definite value in the early recognition of widespread thrombosis.

Acknowledgement

This paper was supported by grants from the USA Army Medical Research and Development Command (Grant No. DA-49-193-MD-2039); the USPHS National Heart Institute (Grant No. 5-R01-HF-06182) and the Fondation du Maladie du Coeur.

The authors wish to thank Madame C. Jubert and Mademoiselle D. Bélanger for their technical assistance.

References

1. Selye, H.: The Thrombohemorrhagic phenomena. Thomas, Springfield 1966.
2. Selye, H., Tuchweber, B.: Ein durch verschiedene Agenzien erzeugbares thrombohaemorrhagisches Syndrom. *Allergie u. Asthma 10*, 335 (1964).
3. Selye, H., Tuchweber, B., Gabbiani, G.: Effect of heparin upon various forms of the thrombohemorrhagic phenomenon. *Blood 26*, 533 (1965).
4. Selye, H.: The thrombohemorrhagic phenomenon as a pluricausal disease. *Perspect. Biol. Med. 9*, 226 (1966).
5. Selye, H., Baic, D., Tuchweber, B.: Choledochus ligature in the prevention of experimental thrombohemorrhagic duodenal necrosis. *Amer. J. dig. Dis. 11*, 45 (1966).
6. Selye, H., Tuchweber, B., Rohan, P.: Thromboses in large veins and pulmonary embolisms induced by catecholamines or serotonin. *Nature (Lond.) 208*, 900 (1966).
7. Gabbiani, G., Jacqmin, M. L., Selye, H.: Thrombohemorrhagic lesions induced by combined treatment with rare earth metals and epinephrine. *J. Pharmacol. exp. Ther. 152*, 275 (1966).
8. Sirridge, M. S., Bowman, K. S., Allwin, J. F.: A simple whole blood screening test for disorders of thromboplastin generation. *Amer. J. clin. Path. 37*, 551 (1962).
9. Owen, C. A., Mann, F. D., Hurn, M. M., Stickney, J. M.: Evaluation of disorders of blood coagulation in the clinical laboratory. *Amer. J. clin. Path. 25*, 1417 (1955).
10. Langdell, R. D., Wagner, R. H., Brinkhous, K. M.: Effect of antihemophilic factor on one-stage clotting tests; presumptive test for hemophilia and a simple one-stage antihemophilic factor assay procedure. *J. Lab. clin. Med. 41*, 637 (1953).
11. Quick, A. J.: The prothrombin in hemophilia and in obstructive jaundice. *J. biol. Chem. 109*, 73 (1935).
12. Perlick, E.: Gerinnungslaboratorium in Klinik und Praxis. Thieme, Leipzig 1960, p. 272.
13. Araki, C.: Seaweed polysaccharides. In: Carbohydrate chemistry of substances of biological interest (ed. M. L. Wolfrom), Proc. IV. Int Congr. Biochemistry Vol. *1*, Symp. *1*, 15 Pergamon Press, London 1958.

14. Young, E. G.: Carrageenin and related polysaccharide sulphates. Coll. int. Centre nat. Rech. Sci. (Paris) No *103*, 173 (1961).
15. Hoigné, R.: Über die Veränderungen von Blutgerinnungsfaktoren, Thrombocyten und Leukocyten im anaphylaktischen Schock, beim Arthus Phänomen und beim Sanarelli—Shwartzman Phänomen. Thesis, Zürich 1951.
16. Hardaway, R. M., Husni, E. A., Geever, E. F., Noyes, H. E., Burns, J. W.: Endotoxin shock. A manifestation of intravascular coagulation. *Ann. Surg. 154*, 791 (1961).
17. Cannon, W. B., Gray, H.: Factors affecting the coagulation time of blood. II. The hastening or retarding of coagulation by adrenalin injections. *Amer. J. Physiol. 34*, 232 (1914).
18. Rowsell, H. C., Hegardt, B., Downie, H. G., Mustard, J. F., Murphy, E. A.: Adrenaline and experimental thrombosis. *Brit. J. Haemat. 12*, 66 (1966).
19. Shimamoto, T., Ishioka, T.: Release of a thromboplastic substance from arterial walls by epinephrine. *Circulat. Res. 12*, 138 (1963).
20. Adelson, E., Rheingold, J. J., Parker, O., Buenaventura, A., Crosby, W. H.: Platelet and fibrinogen survival in normal and abnormal states of coagulation. *Blood 17*, 267 (1961).
21. O'Brien, J. R.: Some effects of adrenaline and anti-adrenaline compounds on platelets in vitro and in vivo. *Nature (Lond.) 200*, 763 (1963).
22. Solymoss, B., Selye, H., Gabbiani, G.: Predisposition to thrombosis not reflected by blood coagulogram. *J. clin. Path. 19*, 331 (1966).
23. Knoop, F., Thomas, K.: Die blutgerinnungshemmende Wirkung der seltenen Erden. *Hoppe-Seylers Z. physiol. Chem. 272*, 65 (1942).
24. Shimamoto, T., Sunaga, T.: Edematous arterial reaction by adrenaline and cholesterol and its prevention by MAO inhibitor observed by electron microscopic technique. *Jap. Heart J. 3*, 581 (1962).
25. Fulton, G. P., Akers, R. P., Lutz, B. R.: White thromboembolism and vascular fragility in the hamster cheek pouch after anticoagulants. *Blood 8*, 140 (1953).

Structural and Functional Differences between Human Foetal and Adult Erythrocytes

Susan R. HOLLÁN, Judith G. SZELÉNYI, Judith H. BREUER, G. A. MEDGYESI, Vera N. SŐTÉR

Central Research Institute of the National Blood Service, and Research Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

(Received July 1, 1967)

Lipid structure, non-electrolyte permeability, electrophoretic mobility, AchE and G6PD activity of human foetal and adult erythrocytes were studied. Between the lipid composition of the two cell types the most consistent difference was found in fatty acids esterified to total phospholipids. The concentration of linoleic acid ($C_{18:2}$) was significantly lower, the oleic acid ($C_{18:1}$) content slightly lower and the palmitic acid ($C_{16:0}$) level higher in foetal red cells. Eicosotrienoic acid ($C_{20:3}$) was detected in nearly 50 per cent of the cord blood samples while in none of the adult red cells. Permeability of the foetal cells to non-electrolytes was decreased. Both isotonic glycerol and thiourea penetrate slower into foetal erythrocytes than into adult ones. Electrophoretic mobility of adult red cells was found to be slower than that of foetal cells. AchE activity was lower and G6PD activity higher in foetal red cells. Based on differences in the membrane structure of the two cell types a new method was developed for their cytological differentiation. Differences of the structural organization and permeability of foetal and adult erythrocytes are discussed from the point of view of cell differentiation, red cell life-span and the intricate interaction between haemoglobin and its normal environment, the red cell.

It is well known that foetal erythrocytes contain predominantly Hb-F ($\alpha_2 \gamma_2$), while Hb-A ($\alpha_2 \beta_2$) is the major component of the blood pigment in adult red cells. In the switch from Hb-F to Hb-A production the synthesis of one type of polypeptide chain, that of the γ-chain, is repressed and the synthesis of the β-chain is initiated. Since the chemical structure of both haemoglobins involved is well defined, this simple biochemical system proved to be useful in the study of the genetic control of differentiation. We are still far from understanding the mechanism by which the switch from foetal to adult Hb synthesis takes place. As foetal erythrocytes have significantly higher oxygen affinity than their adult counterparts, it has been suggested that the change in oxygen tension from intrauterine life to extrauterine environment might be the activating factor which is repressing γ-chain synthesis and derepressing or activating β-chain synthesis [1, 2]. But neither this hypothesis, nor the assumption that gonadotropic hormones might play this role [3] can give an explanation for the occurrence of Hb-F synthesis in some haematological diseases during adult life. On the basis of a definite correlation between increased erythropoietic activity and the reappearance of Hb-F synthesis it has been assumed by Baglioni [4, 5] that red cells produced

after few cell divisions from stem cells may possibly contain more Hb-F than red cells produced after several divisions of the erythroblasts. The theoretical consideration that the differentiation of protein synthesis is linked to a definite cellular differentiation is confirmed by the fact that foetal red cells differ not only in their haemoglobin content from adult erythrocytes. However, the three types of Hb-F containing cells: the foetal red cells, the erythrocytes in "high F gene" anomaly, and the postulated pathologically differentiated Hb-F containing erythroid clones in thalassaemia and other severe anaemias, differ in their cellular structure and function in many respects. In spite of this Hb-F shows the same chemical structure in all these conditions. A comparison of the cellular membranes is much more difficult, because our knowledge of the chemistry and physics of the normal foetal red cell membrane is inadequate and often contradictory. It is the major purpose of this paper to investigate and correlate some structural and functional characteristics of normal human foetal and adult red cell membranes.

Materials and Methods

Thirteen cord blood samples were obtained at the end of term pregnancies. Delivery was uncomplicated and the infant appeared normal at birth in each case. Blood was drawn from the umbilical vein immediately after the delivery of the placenta using sodium citrate as anticoagulant (1 vol. to 4 vol. blood). Adult blood samples were taken from 20 healthy blood donors. The blood was centrifuged at $+4^\circ$ C and the erythrocytes were washed with physiological saline at the same temperature until the supernatant was free of protein.

Red blood cell lipids were extracted by the III method of Ways and Hanahan [6]. The only modification was the addition of BHT* as antioxidant to the solvents at a final concentration of 0.005 per cent. The extracts were stored in sealed glass vials under N_2 at $+4^\circ$ C.

Cholesterol was determined by the Lieberman-Burchard method, total esterified fatty acids (TEFA) as described by Hack [7], and lipid phosphorus as described by Allen [8].

Thin layer chromatography of the main lipid classes was performed on 0.25 mm Kieselgel G layers, activated at 110° C for 25 minutes. Petroleum ether–diethyl ether–acetic acid (90 : 10 : 1, v : v : v) mixture was used as solvent. The spots were revealed by spraying with phosphomolybdenic acid solution and heating at 110° C for 20–25 minutes. Phospholipids were separated on Kieselgel H (Merck) layers. The adsorbent was suspended in 0.001 mol Na_2CO_3. The plates were activated at 110° C for one hour. Before activation a mixture of chloroform, methanol, acetic acid and water, 75 : 45 : 12 : 6 (v : v : v : v) was run through the layer for cleaning. The same solvent was used for the development of the chromatogram [9]. The spots were revealed by exposure to iodine vapour or by spraying with 50 per cent H_2SO_4 and heating at 180° C for 30 minutes.

* 4-methyl-2,6-ditertiary butyl phenol.

The fatty acid distribution of red cell total phospholipids was studied by gas-liquid chromatography of the corresponding fatty acid methylesters. Phospholipids were converted into methyl esters using methanolic sulphuric acid. Gas-liquid chromatography was performed on a 5 feet column using an Aerograph A-600-C type gas chromatograph (H_2 flame detector) with diethylene glycol succinate as the stationary phase. The column temperature was 188° C.

Non-electrolyte permeability of foetal and adult red cells was studied in fresh blood drawn into citrate solution (v/v 9 : 1). The permeability studies were carried out according to the method of Jacobs [10] using isotonic (0.3 M) glycerol or thiourea solutions previously adjusted to pH 7.5; 0.02 ml blood was mixed with 3 ml of the penetrating agent. The change in optical density was read at 600 mμ every 15 seconds. The half-time of haemolysis was determined graphically from the plotted curves.

Acetylcholinesterase (AchE) activity of erythrocytes was determined by Michel's [11], *glucose-6-phosphate dehydrogenase* (G6PD) by Marks' [12] and *reduced glutathion* (GSH) by Mortensen's [13] method.

Electrophoretic mobility of foetal and adult red cells was measured with an Opton Cytopherometer at 6 mA by the method of Pollack et al. [14]. All electrophoretic measurements were made on erythrocytes washed thrice in physiologic saline solution and brought to a final 0.2 per cent suspension with M/15 phosphate buffer of pH 7.45, the osmolarity of which was adjusted to 0.3 by the addition of NaCl. All mobility determinations were carried out at +25° C. At least 30 determinations were made in both directions by changing the polarity of the electrodes and the average commuting time determined. Differentiation of foetal and adult cells in blood smears was carried out with the *acid elution* [15] and the *urea methods* [16].

Results

Main lipid class levels. Quantitative determination of cholesterol, tota esterified fatty acids and lipid phosphorus revealed considerable individual differences (Table 1). Most of the values were, however, within or near thel normal adultrange.

Thin layer chromatography of the main lipid classes using the petroleum ether–diethyl ether–acetic acid solvent system did not reveal any difference between the cord blood and normal adult erythrocyte extracts. The presence of free cholesterol and free fatty acids was detected in both, while only traces of triglycerid and cholesterol esters were found. *Phospholipids* were resolved into sphingomyelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl inositide fractions. Sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine have been identified by comparison with the chromatogram of hen egg yolk phospholipids. Comparing the phospholipids of cord blood erythrocytes with those of normal adult blood we have found in some of the cord blood samples a markedly stronger spot of phosphatidyl inositides (Figs. 1 and 2).

Table 1

Main lipid class levels of foetal erythrocytes

Cord blood sample No.	Cholesterol 10^{-12}	TEFA	Lipid P
		mg/cell	
135	88	128	11.3
187	83	250	16.8
225	170	237	15.1
245	236	208	17.2
252	328	332	8.8
253	182	172	12.3
17	306	415	18.8
42	170	178	5.8
49	129	113	3.6
62	126	133	4.2
67	124	165	10.9
83	83	125	5.6
90	180	192	13.5
Adult RBC normal range (30 blood donors)	100—145	146—214	9.5—13.5

The fatty acid distribution of the total phospholipids of cord blood erythrocytes was found to differ from that of adult red blood cells in its significantly lower level of linoleic acid ($C_{18:2}$), in the higher concentration of palmitic acid ($C_{16:0}$),

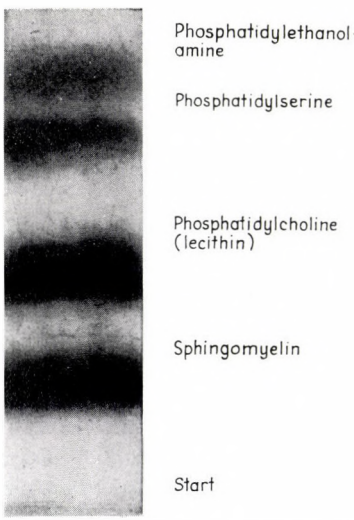


Fig. 1. Phospholipid fractions of adult erythrocytes separated by TLC

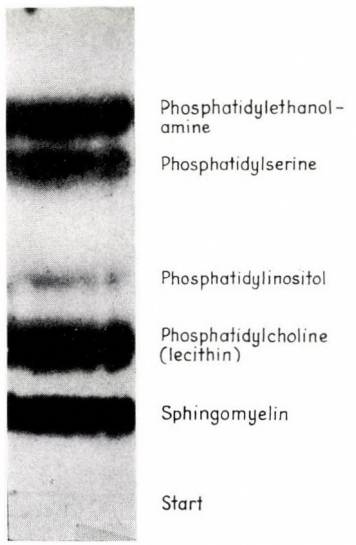


Fig. 2. Phospholipid fractions of foetal erythrocytes separated by TLC

and a lower oleic acid ($C_{18:1}$) content. Eicosotrienoic acid ($C_{20:3}$) could be detected in 4 of the 9 cord blood samples (Table 2).

Permeability to non-electrolytes of foetal and adult red cells was studied by following the rate of haemolysis in isotonic solutions of glycerol and thiourea.

Table 2

Fatty acid distribution of the total phospholipid of adult and foetal erythrocytes (major components in relative percentage)

Fatty acids as methyl esters	Adult +	Foetal + +
$C_{16:0}$	23.3 (19.9—25.9)	28.9 (25.8—31.0)
$C_{16:1}$	2.0 (0.7— 3.6)	2.2 (1.6— 3.4)
$C_{18:0}$	20.8 (18.7—23.4)	20.7 (18.2—22.7)
$C_{18:1}$	20.2 (17.5—23.6)	17.1 (16.6—17.6)
$C_{18:2}$	12.6 (10.9—14.5)	5.5 (4.3— 6.6)
$C_{20:3}$ + + +		2.7 (2.3— 3.9)
$C_{20:4}$	21.0 (17.0—24.7)	22.5 (18.9—27.5)

+ Average of 30 experiments.
++ Average of 9 experiments.
+++ $C_{20:3}$ acid was found in 4 out of 9 cases.

The curves in Fig. 3 represent the clearing of the cell suspension due to haemolysis of the cells which resulted from the penetration of the non-electrolyte into the cell. The rate of haemolysis is inversely proportional to the permeability of the cell. The permeation of both glycerol and thiourea is slower into the foetal red cell than into the adult. This is well reflected in the differences of the values for the half-time of haemolysis (Table 4).

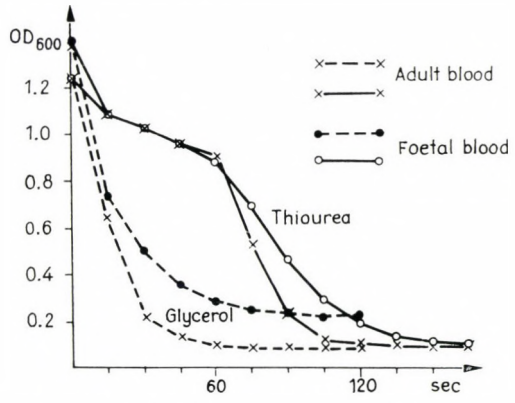


Fig. 3. Rate of haemolysis of adult and foetal erythrocytes in isosmotic glycerol and thiourea

There were striking differences in *AchE* and *G6PD activity* between foetal and adult erythrocytes. Foetal red cells had significantly lower AchE activity and significantly higher G6PD activity than adult erythrocytes. The GSH level showed more marked individual variations in foetal than in adult erythrocytes. There was, however, no significant difference in the average GSH level between the two cell types (Table 3).

Microelectrophoretic measurements showed that foetal erythrocytes have a much higher *electrophoretic* mobility than adult red cells (Table 4).

Based on the differences in the organization and permeability of foetal and adult erythrocytes a new method was developed for the detection of foetal erythrocytes in fixed blood smears [16, 17]. Fig. 4 represents an artificial mixture of equal volumes of adult and cord blood after urea treatment. (Ethanol fixed blood smears were incubated for 5 min at 37° C in a 4 molar urea solution at pH 7.6.) The method allows a clear-cut differentiation of foetal from adult cells. The membrane of the foetal cells is affected only slightly and the Hb remains in the cell, in contrast to the adult cells which are freed most of their Hb. In parallel studies with Kleihauer-Betke's acid elution method and the urea method there was perfect correlation in the results of cord blood samples, and in those

Table 3

AchE and G6PD activities and GSH levels of adult and foetal erythrocytes

	Adult RBC	Foetal RBC
AchE (ΔpH/h)	0.75 (0.60—0.90)	0.42 (0.25—0.58)
G6PD (ΔOD/min/g Hb)	10.3 (8.0—12.0)	15.2 (12.7—22.6)
GSH (mg/100 ml RBC)	69 (60—100)	66.1 (37—81)

Table 4

Non-electrolyte permeabilities and electrophoretic mobilities of adult and foetal erythrocytes

		Adult RBC	Foetal RBC
Non-electrolyte permeability	t/2 glyc+	22 (19— 26)	53 (37— 63)
	t/2 thio++	123 (115—132)	140 (116—175)
Electrophoretic mobility (10^{-4} cm^2/sec/volt)		1.28 (1.20—1.38)	1.43 (1.34—1.50)

\+ Half-time of haemolysis (in sec) in isotonic glycerol.
++ Half-time of haemolysis (in sec) in isotonic thiourea.

of the blood of newborns and infants of different ages. The two methods gave similar results when applied for the detection of foetal cells in maternal circulation. In haemolytic anaemias and in other conditions associated with reticulocytosis the urea method revealed less false-positive foetal cells [17, 18].

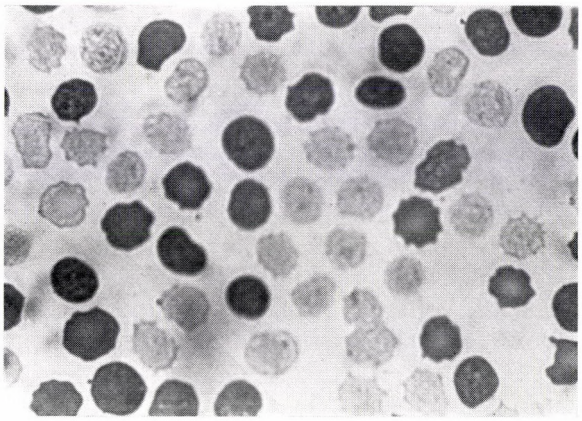


Fig. 4. Urea treatment of the smear of an artificial mixture of equal volumes of normal adult and cord blood

Discussion

Membrane biochemistry occupies a central position in modern biology. Erythrocyte ghosts, being readily isolated, present a unique opportunity for investigating the chemical composition and molecular structure of biointerfaces. The abundance of lipids in the red cell ghost raises the question of how these compounds are arranged molecularly. Based on results of electron microscopy and X-ray analysis, the cell membrane is proposed to be a bimolecular lipid layer sandwiched between proteins. There is, however, considerable evidence for the globular micellar orientations of the lipid molecules in the membrane lipoprotein complex [19, 20]. There is good reason to believe that transitions occur between the different lipid phases being in dynamic equilibrium [21]. Predominantly globular proteins are absorbed to the cytoplasmic envelope [22]. The external envelope of the plasma membrane possesses polysaccharide compounds [23].

In spite of accumulating data we still lack information concerning the detailed structure and metabolism even of the normal adult red cell membrane. The suggested membrane models are extremely tentative. We know still less about the differentiation of the cell membrane, about the structure and function of the foetal cell.

From the membrane components the lipids have thoroughly been analyzed [6, 24, 25, 26, 27] whereas the data of foetal erythrocytes are few and contradictory [28, 29, 30]. Detailed data have been published recently by Crowley et al. [31].

Minor differences in the methods of extraction and the oxidation of unsaturated fatty acids during the lipid analysis may result in different experimental data. To avoid this risk a powerful antioxidant was used and extreme care was taken in the extraction procedures. In spite of these we have found great individual differences in the results of the quantitative determination of the main lipid class and the phospholipid class levels. This may originate from the varying relative amount (60–80 per cent) of foetal cells in different cord blood samples.

The analyses of the fatty acid composition of foetal erythrocyte phospholipids gave more unequivocal results. In good agreement with the data of Crowley et al. [31], the linoleic acid ($C_{18:2}$) content of foetal red cells was found to be significantly lower, and their oleic acid ($C_{18:1}$) content slightly lower than those of the adult erythrocytes. Besides these we have found higher concentration of palmitic acid ($C_{16:0}$) in foetal red cells. Eicosotrienoic acid ($C_{20:3}$) could be detected in nearly 50 per cent of the cord blood samples, while in none of the adult erythrocyte extracts.

The lower linoleic acid ($C_{18:2}$) content and the appearance of eicosotrienoic acid ($C_{20:3}$) were found to be characteristic of red blood cells produced during a fat-free diet [32].

A resemblance can be found between the decreased permeability to non-electrolytes of foetal red cells and that of red cells in certain haematological diseases. Changes in non-electrolyte permeability could be observed in the same group of diseases where alterations in the lipid structure of the red cell membrane and the shortening of the life span of the erythrocytes could be demonstrated as well [33, 34]. Alterations in lipid structure, non-electrolyte permeability and decreased red cell survival characterize the red cells of animals kept on fat free diet [32, 35]. These data correspond well with the shorter life-span of foetal erythrocytes [36] differing in their lipid structure and non-electrolyte permeability from their adult counterparts.

The membrane component responsible for the slower penetration of glycerol and thiourea in these cases is not yet known. On the basis of changes in the non-electrolyte permeability of red blood cells exposed to lipase [37] and considering the species differences in erythrocyte permeability connected with the lipid composition [38, 39], it was concluded that membrane lipids might be important determinants of permeability. In spite of these, a close correlation could not be detected between fatty acid distribution, phospholipid composition, and non-electrolyte permeability [35, 40].

To obtain a many-sided approach to the problem, further functional and structural characteristics were studied. The activity of AchE, which is known to be a membrane-bound enzyme, was determined. The significantly lower AchE activity of foetal cells was in good agreement with the results of Kaplan et al. [41] and referred again to their shorter life-span. The higher G6PD activity observed is considered to be characteristic of young red blood cell population [42], although it may have also resulted from an irregularity of the stromal NADPase-inhibitor [43].

There are a few data concerning the other membrane components. Since the first description of isolated structural proteins of biomembranes a great deal of work has been done to characterize their structure and function [44–49]. Membranes can maintain their integrity after the removal of a major portion of their lipid. The structural proteins appear to be key elements in the regulation of enzyme activities and protein synthesis. Actually only a few methods for preparation and electrophoretic characterization of red cell membrane proteins have been published [50, 51, 52] and a new model of the protein conformation in cellular membranes has been proposed [53].

The mucoid components determining the surface charge of erythrocytes were studied by microelectrophoresis. In agreement with literary data [54] differences in mobility between adult and foetal erythrocytes were observed. This argues again for the immaturity of the membrane of foetal red blood cells, because it is well known that cells lose the negative excess charge of the surface during maturation. Since the charge distribution of the surface is an important factor of the membrane, it might influence certain functional behaviour of the cell.

The above characteristics of foetal erythrocyte membranes permit their cytological differentiation from adult ones, based on their different resistance to urea [16]. AchE is an integral part of the red cell membrane. Decrease of its original activity can serve as a convenient parameter for investigations of the effects of chemical treatment on intact red blood cells and their respective ghosts. A gradual decrease of AchE activity was observed by Lauf and Poulik [55] when erythrocytes or ghosts were treated with urea in concentration less than 4 M. A complete inactivation of AchE occurred when the urea molarity was raised from 4 to 8 M. The 4 M urea concentration proved to be very suitable for the differentiation of the structurally differing foetal and adult erythrocytes. The results obtained by the urea treatment were in good agreement with the results of the acid elution method in cases of erythrocytes produced during foetal life. The overestimation of the number of cells containing foetal haemoglobin in cases of haemolytic anaemia, determined by the acid elution method [15], might have been due to the discussed similarities of foetal membranes to those produced under increased erythropoietic activity. Owing to the intensive action of urea on membranes, the number of overestimated cells is less if this agent is used for the cytological differentiation of adult and foetal erythrocytes.

By comparing normal adult erythrocytes with foetal ones it can be shown that most of the investigated characteristics are divergent. Apart from those discussed above, there are differences in their ultrastructure [56], size [57], mechanical [58] and osmotic fragility [59]; heat [60] and acid-resistance [61] as well.

The basic functional characteristic of foetal erythrocytes, their greater affinity to O_2, cannot be explained by the properties of Hb-F. It does not exist in solutions of Hb-F, or in erythrocytes of adults with hereditary persistence of Hb-F [62]. This fact draws attention to differences in the cellular organization of erythrocytes containing Hb-F. The structure of Hb-F is the same in erythrocytes of different origin, while their metabolic activities and presumably their membranes

are different. Since in connection with the affinity to O_2 the metabolic state of the cell is negligible [62], it might be the membrane structure which influences this behaviour.

Effects of the membrane on the affinity to O_2 of Hb-A was demonstrated by Hořejši and Komarková [63]. On the other hand, Benesch and Benesch suggested that certain organic phosphate compounds might induce allosteric modifications in the haemoglobin structure which result in changes of its affinity to O_2 [64]. As discussed previously [17], the haemoglobin and the membrane might mould each other's structure. Results presented in this paper support this assumption. On the basis of the revealed differences a specific phospholipid-protein interaction is suggested which may have a considerable influence on the allosteric properties of haemoglobin. This may even explain the higher affinity to O_2 of erythrocytes produced during foetal life.

Acknowledgements

We are indebted to Dr. S. Ferkó for the supply of cord blood samples and to Miss M. Hasitz, Mrs. E. Hetényi and Mr. I. Sugár for skilful technical assistance.

References

1. Allen, D. W., Jandl, J. H.: Factors influencing relative rates of synthesis of adult and fetal hemoglobin in vitro. *J. clin. Invest. 39*, 1107 (1960).
2. Thomas, E. D., Lochte, H. L. Jr., Greenough, W. B., Wales, M.: In vitro synthesis of foetal and adult haemoglobin by foetal haematopoietic tissues. *Nature (Lond.) 185*, 396 (1960).
3. Rucknagel, D. L., Chernoff, A. I.: Immunologic studies of hemoglobin; fetal hemoglobin changes in circulation of pregnant women. *Blood 10*, 1092 (1955).
4. Baglioni, C.: Correlations between genetics and chemistry of human hemoglobins. In: Taylor, J. H.: Molecular genetics. Academic Press, New York 1963, pp. 467—468.
5. Baglioni, C.: Chromosomal and cytoplasmic regulation of haemoglobin synthesis. Proc. XIth Congr. Int. Society of Blood Transfusion. Sydney 1966, pp. 170—176.
6. Ways, P., Hanahan, D. J.: Characterization and quantification of red cell lipids in normal man. *J. Lipid Res. 5*, 318 (1964).
7. Hack, M. H.: A method for the estimation of fatty acid esters. *Arch. Biochem. 58*, 19 (1955).
8. Allen, R. J. L.: cit. Ivor Smith (ed): Chromatographic and Electrophoretic Techniques. William Heinemann Ltd. London I. 370 (1960).
9. Skipski, V. P., Peterson, R. F., Barclay, M.: Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J. 90*, 374 (1964).
10. Jacobs, M. H.: The permeability of the erythrocyte. *Ergebn. Biol. 7*, 1 (1931).
11. Michel, H. O.: An electrometric method for the determination of red blood cell and plasma acetylcholinesterase activity. *J. Lab. clin. Med. 34*, 1564 (1949).
12. Marks, P. A., Gross, R. T.: Erythrocyte glucose-6-phosphate dehydrogenase deficiency: evidence of differences between negroes and Caucasians with respect to this genetically determined trait. *J. clin. Invest. 38*, 2253 (1959).
13. Mortensen, E.: The nitroprusside method for the determination of reduced glutathione. *Scand. J. clin. Lab. Invest. 16*, 84 (1964).
14. Pollack, W., Hager, H. J.: A study of the forces involved in the second stage of hemagglutination. *Transfusion (Philad.) 5*, 158 (1965).

15. Kleihauer, E., Braun, H., Betke, K.: Demonstration von fetalem Hämoglobin in den Erythrozyten eines Blutausstrichs. *Klin. Wschr. 35*, 637 (1957).
16. Szelényi, J. G., Hollán, S. R.: A new method for the cytological differentiation of foetal and adult erythrocytes. *Vox Sang. (Basel) 12*, 234 (1967).
17. Hollán, S. R., Szelényi, J. G.: On the methods of differentiating intracellular haemoglobins. Int. Symp. Comp. Haemoglobin Structure. Thessalonica, 1966.
18. Hollán, S. R., Szelényi, J. G.: Foetal cells in maternal circulation after delivery and interruption. *Acta med. Acad. Sci. hung.* (In press.)
19. Luzzati, V., Husson, F.: The structure of the liquid-crystalline phasis of lipid-water systems. *J. Cell Biol. 12*, 207 (1962).
20. Lucy, A.: Globular lipid micelles and cell membranes. *J. theor. Biol. 7*, 360 (1964).
21. Kavanau, J. L.: Structure and functions of biological membranes. *Nature* (Lond.) *198*, 525 (1963).
22. Robertson, J. D.: Ultrastructure of excitable membranes and the crayfish median-giant synapse. *Ann. N. Y. Acad. Sci. 94*, 339 (1961).
23. Finean, J. B.: The nature and stability of the plasma membrane. *Circulation 26*, 1151 (1962).
24. Reed, C. F., Swisher, S. N., Marinetti, G. V., Eden, E. G.: Studies of the lipids of the erythrocyte. I. Quantitative analysis of the lipids of normal human red blood cells. *J. Lab. clin. Med. 56*, 281 (1960).
25. Hanahan, D. J., Watts, R. M., Pappajohn, D.: Some chemical characteristics of the lipids of human and bovine erythrocytes and plasma. *J. Lipid Res. 1*, 421 (1960).
26. Farquhar, J. W.: Human erythrocyte phosphoglycerids. I. Quantitation of plasmalogous, fatty acids and fatty aldehydes. *Biochim. biophys. Acta 60*, 80 (1962).
27. Nelson, G. J.: Composition of neutral lipids from erythrocytes of common mammals. *J. Lipid Res. 8*, 374 (1967).
28. Helmy, F. M., Hack, M. H.: Comparison of the lipids in maternal and cord blood and of human amniotic fluid. *Proc. Soc. exp. Biol. (N. Y.) 111*, 591 (1962).
29. Boyd, E. M.: Lipid composition of blood in newborn infants. *Amer. J. Dis. Child. 52*, 1319 (1936).
30. Bentley, H. P. Jr.: Erythrocyte phospholipids in the newborn infant. *Proc. Soc. exp. Biol. (N. Y.) 111*, 591 (1962).
31. Crowley, J., Ways, P., Jones, J. W.: Human fetal erythrocyte and plasma lipids. *J. clin. Invest. 44*, 989 (1965).
32. Farnsworth, P., Danon, D., Gellhorn, A.: The effect of fat-free diet on the rat erythrocyte membrane. *Brit. J. Haemat. 11*, 200 (1965).
33. Hollán, S. R., Szelényi, J. G.: Glycerol and thiourea permeability of normal and abnormal human erythrocytes. XVth Coll. Protides of Biological Fluids. Brugge 1967.
34. Hollán, S. R., Breuer, H. J., Medgyesi, Gy.: Study of the lipid composition of red blood cells. *Haemat. hung. 5*, 17 (1965).
35. Walker, B. L., Kummerow, F. A.: Erythrocyte fatty acid composition and apparent permeability to non electrolytes. *Proc. Soc. exp. Biol. (N. Y.) 115*, 1099 (1964).
36. Garby, L., Sjölin, S., Vuille, J. C.: Studies on erythrokinetics in infancy. V. Estimations of the life-span of erythrocytes in the newborn. *Acta pediat. (Uppsala) 53*, 165 (1964).
37. Ballantine, R., Parpart, A. K.: The action of lipase on the red cell surface. *J. cell comp. Physiol. 16*, 49 (1940).
38. De Gier, J. J., Van Deenen, L. L. M.: Some lipid characteristics of red cell membranes of various animal species. *Biochim. biophys. Acta (Amst.) 49*, 286 (1961).
39. Kögl, F. J., De Gier, J. J., Mulder, I., Van Deenen, L. L. M.: Metabolism and function of phosphatides. Specific fatty acid composition of the red cell membranes. *Biochim. biophys. Acta (Amst.) 43*, 95 (1960).
40. De Gier, J. J., Van Deenen, L. L. M., Van Senden, K. G.: Glycerol permeability of erythrocytes. *Experientia (Basel) 22*, 20 (1966).

41. Kaplan, E., Tyson-Tildon, J.: Changes in red cell enzyme activities in relation to red cell survival in infancy. *Pediatrics 32*, 371 (1963).
42. Sailer, S., Sandhofer, F., Braunsteiner, H.: Bestimmung der Glucose-6-Phosphatdehydrogenase-Aktivität in den Erythrozyten zur Erkennung von hypo- u. aplastischer Erythropoese. *Acta haemat. (Basel) 30*, 193 (1963).
43. Carson, P. E., Schrier, S. L., Kellermeyer, R. W.: Mechanism of inactivation of G6PD in human erythrocytes. *Nature (Lond.) 184*, 1292 (1959).
44. Green, D. E., Tindale, H. D., Criddle, R. S., Chen, P. V., Bock, R. M.: Isolation and properties of the structural protein of mitochondria. *Biochem. biophys. Res. Commun. 5*, 109 (1961).
45. Richardson, S. H., Hultin, H. O., Green, D. E.: Structural proteins of membrane system. *Proc. nat. Acad. Sci. (Wash.) 50*, 821 (1963).
46. Hendler, R. W.: Protein synthesis as a membrane-oriented cellular activity. XVth Coll. Protides of Biological Fluids. Brugge 1967.
47. Criddle, R. S.: The proteins of membrane structures. Ibid.
48. Warren, L., Glick, M. C.: The turnover of proteins and other components of animal cell surface membrane. Ibid.
49. Firkin, B. G., Wiley, J. S.: The red cell membrane and its disorders. In: Browne and Moore: Progress in Hematology. Vol. 5. Grune and Stratton, New York 1966, pp. 26—60.
50. Azen, E. A., Orr, S., Smithies, O.: Starch gel electrophoresis of erythrocyte stroma. *J. Lab. clin. Med. 65*, 440 (1965).
51. Poulik, M. D., Lauf, P. K.: Heterogeneity of water soluble structural components of human red cell membranes. *Nature (Lond.) 208*, 874 (1965).
52. Zahler, P. H., Wallach, D. F. H.: Isolation of lipid-free plasma membrane proteins by gel filtration on Sephadex LH-20 using 2-chloroethanol-water as solvent. *Biochim. biophys. Acta (Amst.) 135*, 371 (1967).
53. Wallach, D. F. H.: Protein conformation in cellular membranes. XVth Coll. Protides of Biological Fluids. Brugge 1967.
54. Rottino, A., Angers, J. W.: Electrophoretic mobility of the red blood cell during human pregnancy. *Amer. J. Obstet. Gynec. 82*, 1302 (1961).
55. Lauf, P. K., Poulik, M. D.: Structural integrity of acetylcholinesterase of human red cell. XVth Coll. Protides of Biological Fluids. Brugge 1967.
56. Perk, K., Danon, D.: Comparative structure analysis of foetal and adult type red cells in newborn and adult cattle. *Res. Vet. Sci. 6*, 442 (1965).
57. Horan, M.: Studies in anaemia of infancy and childhood: the haemoglobin, red cell count, and packed cell volume of normal English infants during the first year of life. *Arch. Dis. Childh. 25*, 110 (1950).
58. Bos, S. E.: Mechanical fragility of erythrocytes. *Maandschr. Kindergeneesk. 23*, 335 (1955).
59. Reimann, F., Yenen, E.: Der Einfluss von fetalem Hämoglobin auf die Bestimmung der osmotischen Resistenz im Blute des Neugeborenen. *Acta haemat. (Basel) 27*, 359 (1962).
60. Betke, K., Kleihauer, E.: Fetaler und bleibender Blutfarbstoff in Erythrozyten und Erythroblasten von menschlichen Feten und Neugeborenen. *Blut 4*, 241 (1958).
61. Kleihauer, E.: Struktur der fetalen Erythrozyten. *Wien. Z. inn. Med. 44*, 17 (1963).
62. Nechtman, C. M. J., Huizman, T. H. J.: Comparative studies of oxygen equilibria of human adult and cord blood red cell hemolysates and suspension. *Clin. chim. Acta 10*, 165 (1964).
63. Hořejši, J., Komarková, A.: The effect of factors contained in the membranes of red blood cells on the shape of the dissociation of hemoglobin. *Clin. chim. Acta 4*, 391 (1959).
64. Benesch, R., Benesch, R. E.: The effect of organic phosphates from the human erythrocytes on the allosteric properties of haemoglobin. *Biochem. biophys. Res. Commun. 26*, 162 (1967).

Methaemoglobinaemia Due to Insufficiency of the Reducing Systems of Erythrocytes

K. Rechowicz, Z. Szymanowska, L. Konopka, K. Murawski, S. Pawelski

Department of Clinical Medicine and Department of Biochemistry, Institute of Haematology, Warsaw, Poland

(Received June 12, 1967)

Seven cases of methaemoglobinaemia due to failure of the reducing systems of erythrocytes have been observed; six of them belonged to the same family. The erythrocytes of three siblings failed to utilize glucose as well as lactate for reduction of methaemoglobin. This suggested, in addition to cytochemical findings, a simultaneous deficiency or inactivation of both methaemoglobin reducing specific enzymatic systems. Other members of the family showed a deficiency of one of these systems. The presence of non-oxidized haemoglobin in cases of probable failure of both reducing systems is a proof of the important role of the structure of the haemoglobin molecule in the inactivation of oxidizing factors. On the other hand, failure of the methaemoglobin reducing systems seems to lead to grave functional disturbances, as shown by its frequent association with mental deficiency. In one case psychological testing revealed a long-standing mental retardation.

Gibson [3] in 1948 demonstrated the presence of two independent methaemoglobin reducing enzymatic systems in the human erythrocyte. The first of these methaemoglobin reductases (called formerly diaphorase) linked to $NADH_2$ and utilizing directly lactate as hydrogen donor was isolated and obtained in pure form in 1962 [13]. The second reductase linked to $NADPH_2$ and utilizing glucose (or rather its 6-phosphate ester) as hydrogen donor was purified a year later [14].

Under physiological conditions reduction of methaemoglobin in the erythrocyte takes place mainly through the action of the $NADH_2$-dependent reductase, while the $NADPH_2$-dependent one remains a reserve system, although recently it has been shown [6] that glutathione can also be the reserve system in cases of failure of the $NADH_2$-dependent reductase. Deficiency of one of these methaemoglobin reducing systems is congenital and it is inherited as a recessive autosomal character. The result of this deficiency is methaemoglobinaemia with cyanosis as the presenting symptom. Glutathione deficiency led to methaemoglobinaemia in only some of the reported cases [12, 18].

The close association of the methaemoglobin reducing systems with the metabolic pathway of glucose is the cause why haemoglobinaemia may develop in response to non-specific causes; the nosological syndromes observed then may be partly different. Because of this we thought it worthwhile to report several cases observed by us. Three of them seem to have been caused by a simultaneous failure of both methaemoglobin reductase systems.

Methods

Blood methaemoglobin level was determined by the method of Evelyn and Malloy [3]. Blood haemoglobin was estimated as cyanmethaemoglobin [2]. Activity of the methaemoglobin reductase systems was measured by the modified method of Pisciotta et al. [11] and Betke et al. [1], according to the method described in a previous paper [10] except for using 50 mM sodium nitrite instead of 5 mM. The presence of methaemoglobin in erythrocytes was detected by the method of Kleihauer [7]. Foetal haemoglobin was determined by one-minute alkaline denaturation [15]. Glucose-6-phosphate dehydrogenase activity in the erythrocytes was measured by the method of Zinkham [19] and glutathione stability was tested according to the method of Szeinberg et al. [17] in the presence of glucose.

Case Reports and Results of Investigations

Case 1. A male patient aged 38 years was admitted because of easy fatigability and dyspnoea of effort, thought previously to be due to stenosis of the pulmonary artery. The cyanotic hue was not limited to the face but involved also the ear lobes, the oral mucosa and the distal parts of the extremities. A systolic murmur was heard over the cardiac area, loudest over the pulmonary artery and at the point of Erb. X-rays of the lungs and heart, ECG, phonocardiography, spirometry, an examination involving the use of Cardiogreen to exclude interatrial or interventricular shunt, were within normal limits. Erythrocytosis was found in peripheral blood (haemoglobin values reaching 18.3 g per 100 ml); there were 35.2% erythroblasts in the bone marrow. The methaemoglobin level was 5 g per 100 ml at admission and 10 g per 100 ml later (probably as a result of the diet low in vitamin C). Electrophoresis of the haemoglobin [16] and the absorption spectrum of acid methaemoglobin showed no abnormal haemoglobins. The content of foetal haemoglobin was 1.4%. Glutathione stability and glucose-6-phosphate dehydrogenase activity of the erythrocytes were normal. The erythrocytes repeat-

Table 1

Reduction of methaemoglobin by erythrocytes after 5 hours incubation with lactate or glucose, expressed in per cents of the initial amount of methaemoglobin

Erythrocytes	Per cent of methaemoglobin after 5 hours	
	in glucose	in lactate
Control	46	47
Case 1	86	87
Case 2	64	65

edly failed to reduce methaemoglobin (produced by sodium nitrite) in the presence of lactate or glucose (Table 1). Methylene blue (1 mg per 1 kg of body weight with and without vitamin C) administered twice intravenously lowered the methaemoglobin level (Fig. 1).

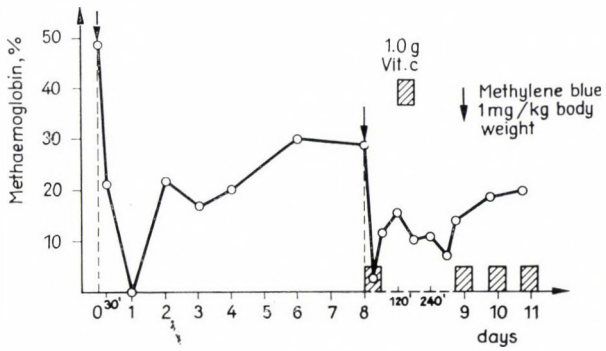


Fig. 1. Case 1. Effect of methylene blue and ascorbic acid on methaemoglobin content in blood of Case 1

Examination of the patient's family revealed similar disturbances of methaemoglobin reduction in two of the patient's siblings. The erythrocytes of three other members of this family showed no ability to utilize lactate as a source of hydrogen for the reduction of methaemoglobin, although in the presence of glucose they reduced methaemoglobin normally (Fig. 2).

Case 2. A girl aged 15 years was admitted to the Second Department of Clinical Medicine, Pomeranian Medical School, with the diagnosis of methaemoglobinaemia. Her complaints included easy fatigability, periodical headaches and

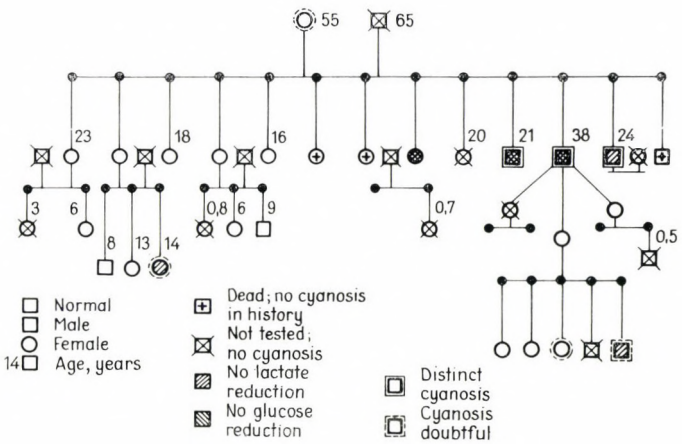


Fig. 2. Case 1. Family tree

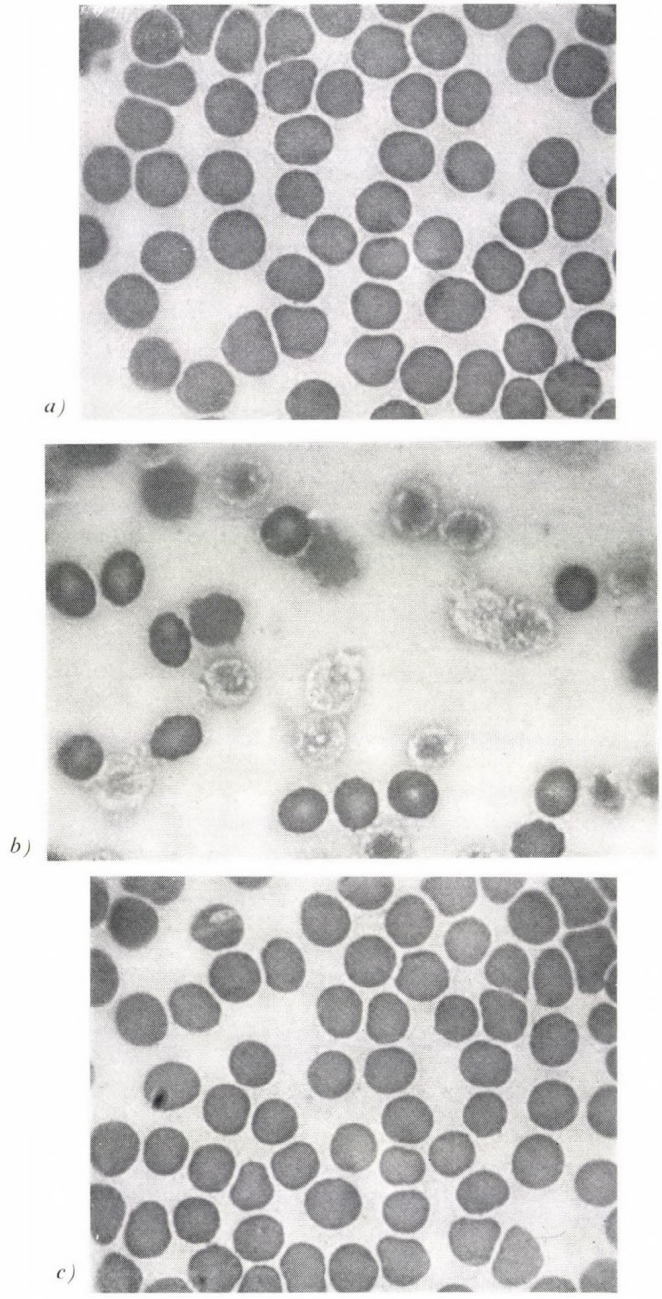


Fig. 3. Blood smears stained by Kleihauer's method. (a) erythrocytes in Case 1; (b) 1 : 1 mixture of normal erythrocytes and erythrocytes containing 100% methaemoglobin; (c) 1 : 1 mixture of normal and patients' (Case 1) erythrocytes

episodes of palpitation and cardiac pain. The lips, cheeks, ear lobes and fingertips were moderately cyanosed. Chest X-rays, ECG, phonocardiogram, spirometry yielded normal findings. The blood methaemoglobin level ranged from 2.1 to 3.0 g per 100 ml. The blood haemoglobin level was 13–14 g per 100 ml. Erythroblastic activity in the bone marrow was normal. Starch gel electrophoresis of haemoglobin did not reveal abnormal components. Foetal haemoglobin amounted to 0.4%. The absorption spectrum of acid methaemoglobin was normal. The erythrocytes showed a decreased methaemoglobin reduction in the presence of lactate or glucose (Table 1), but the results obtained in three consecutive determinations varied over a wide range. No cyanosis was observed in the mother, the father and two brothers of the patient.

In both cases blood films were examined in order to determine the distribution of methaemoglobin in the erythrocyte population. The method of Kleihauer [7] was not sufficiently sensitive to demonstrate the presence of methaemoglobin in any of the patients, even when the blood methaemoglobin level (Case 1) amounted to 18% of all haemoglobin (Fig. 3a). An artificial mixture of 50% normal erythrocytes with 50% erythrocytes exposed previously to sodium nitrite showed the presence of two types of cells, one type containing oxyhaemoglobin, the other methaemoglobin (Fig. 3b). The content of methaemoglobin in the erythrocytes of the patients was too small to be demonstrated by this method even when we stained a 1 : 1 mixture of normal erythrocytes and of the patient's erythrocytes (Fig. 3c).

Discussion

The presence in the human erythrocytes of independent enzymatic systems capable of changing methaemoglobin into its functionally active derivative provides a considerable protection of haemoglobin against the action of oxidative factors. More or less pronounced methaemoglobinaemia results from failure of one of these reducing systems. The greater part of methaemoglobin remains, however, in the active form, which depends on the undisturbed function of the remaining methaemoglobin reducing systems. Numerous examples of congenital methaemoglobinaemia resulting from failure of one of the methaemoglobin reductase systems have been described. The blood methaemoglobin level in cases of $NADH_2$-dependent reductase deficiency reached 40% and in cases of $NADPH_2$-dependent reductase deficiency [9, 10, 12] it approached 10% of all haemoglobin. Determinations of the activity of methaemoglobin reducing systems showed in the cases of $NADH_2$-dependent reductase deficiency an inability of the erythrocytes to utilize as direct hydrogen donors lactate – and in some cases also glucose. In the case reported by Müller et al. [10] reduction of methaemoglobin occurred only in the presence of lactate and the authors explained this phenomenon as a selective deficiency of $NADPH_2$-dependent reductase. In our patient (Case 1) and in his two siblings the erythrocytes failed to utilize glucose as well as lactate as hydrogen donors for methaemoglobin reduction. This suggested a simultaneous

deficiency of both reducing enzymes and may explain the high methaemoglobin level (54%) in this case.

Cytochemical investigations failed to show non-stained erythrocyte populations in isolated $NADH_2$-dependent reductase deficiency. The activity of the remaining methaemoglobin reducing systems then depends on glucose-6-phosphate dehydrogenase, activity of which enzyme decreases with the ageing of erythrocytes [6]. In these cases the population of "old" erythrocytes contained considerable amounts of methaemoglobin and these erythrocytes underwent elution in the course of Kleihauer's staining method. The lack of non-stained erythrocytes may suggest the following enzymatic disturbances in these types of methaemoglobinaemia: a disturbance of the enzymatic system depending on the activity of glucose-6-phosphate dehydrogenase, an enzymatic block in the metabolic pathway of glucose, and a failure of both methaemoglobin reductase systems. The first possibility can be excluded on the basis of the results of other investigations. As to the second possibility there are no experimental data available to support it. The cytochemical results thus are suggestive of a failure of both methaemoglobin reductase systems. The cytochemical abnormalities in these cases of methaemoglobinaemia showed at the same time a uniform distribution of methaemoglobin in the whole erythrocyte population.

In the literature concerning congenital methaemoglobinaemia depending on a failure of the enzymatic reducing systems, cases have been reported displaying an absence of one of the two enzymatic systems, and other cases with an absence of the $NADH_2$-dependent reductase with simultaneous decrease of glutathione [12]. Only Lamy et al. [8] reported a case with a metabolic defect resembling the one observed in our Case 1. However, despite a seemingly profound metabolic disturbance almost half of the haemoglobin remained in the reduced from. This shows that the specific structure of the haemoglobin molecule is one of the most important factors keeping the iron of the haemoglobin in the bivalent state. The non-enzymatic substances present in the erythrocyte reduced glutathione, in the first place and to some extent ascorbic acid and sulphhydryl compounds like cysteine and ergothioneine, may also prevent oxidation of certain amounts of haemoglobin. On the other hand, a methaemoglobin reductase deficiency causes graver functional disturbances than an isolated methaemoglobinaemia. This is indicated by the frequent occurrence of mental retardation in cases of methaemoglobinaemia associated with failure of the reducing systems, and normal mental development in cases of methaemoglobinaemia due to presence of haemoglobin M. Since 1960, eleven patients belonging to 7 families have been described of congenital methaemoglobinaemia associated with mental retardation and with non-characteristic neurological disturbances in some cases.

Our patients displayed no neurological abnormality. In Case 1, psychological examination showed mental retardation with an I.Q of 75 according to Wechsler. However, this test failed to show any evidence of intellectual degradation, which suggests that the mental defect was a long-standing one, probably beginning in early childhood.

As to the cause of the association of mental retardation with methaemoglobinaemia due to methaemoglobin reductase deficiency hypothetical speculations have only been offered [4]. It seems, however, to be certain that the main cause of the mental defect is not a chronic hypoxia of the central nervous system. It is evidently a contributing factor and should be treated early with methylene blue, vitamin C, and similar drugs.

References

1. Betke, K., Stein, H., Tönz, O.: Untersuchung einer Familie mit kongenitaler Methämoglobinämie durch Reduktasinsuffizienz. *Dtsch. med. Wschr. 87*, 65 (1962).
2. Crosby, W. H., Munn, J. I., Furth, F. W.: Standardizing a method for clinical hemoglobinometry. *U. S. Armed Forces med. J. 5*, 693 (1954).
3. Evelyn, K. A., Malloy, H. T.: Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *J. biol. Chem. 126*, 655 (1938).
4. Fialkow, P. J., Browder, J. A., Sparkes, R. S., Motulsky, A. G.: New mental retardation in methemoglobinemia due to diaphorase deficiency. *New Engl. J. Med. 273*, 840 (1965).
5. Gibson, O. T.: The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia. *Biochem. J. 42*, 13 (1948).
6. Keitt, A. S., Smith, T. W., Jandl, J. H.: Red-cell "Pseudomosaicism" in congenital methemoglobinemia. *New Engl. J. Med. 275*, 398 (1966).
7. Kleihauer, E.: Methode und Mechanismus des Nachweises von Methämoglobin in Erythrocyten eines Blutausstriches. *Klin. Wschr. 42*, 853 (1964).
8. Lamy, M., Frezal, J., Jammet, M. L., Josso, N.: La methémoglobinémie congénitale récessive. *Nouv. Rev. franç. Hemat. 3*, 105 (1963).
9. Müller, J., Murawski, K., Koziorowski, A., Wilińska, Ł.: Badania nad methemoglobinemia wrodzoną wywołaną brakiem reduktazy methemoglobinowej krwinek czerwonych. *Pol. Arch. Med. Wewnęt.* (in press).
10. Müller, J., Murawski, K., Szymanowska, Z., Koziorowski, A., Radwan, L.: Hereditary deficiency of $NADPH_2$-methaemoglobin reductase. *Acta med. scand. 173*, 243 (1963).
11. Pisciotta, A. V., Ebbe, S. N., Hinz, J. E.: Clinical and laboratory features of two variants of methemoglobin M-disease. *J. Lab. clin. Med. 54*, 73 (1959).
12. Bishop, Ch.: The Red Blood Cell. Acad. Press, New York 1964.
13. Scott, E. M., McGraw, J. C.: Purification and properties of diphosphopyridine nucleotide diaphorase of human erythrocytes. *J. biol. Chem. 237*, 249 (1962).
14. Shargo, E., Falcone, A. B.: Human-erythrocyte-reduced triphosphopyridine nucleotide oxidase. *Biochim. biophys. Acta (Amst.) 67*, 147 (1963).
15. Singer, K., Chernoff, A. I., Singer L.: Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. *Blood 6*, 413 (1951).
16. Smithies, O.: An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. *Biochem J. 71*, 585 (1959).
17. Szeinberg, A., Asher, Y., Sheba, C.: Studies on glutathione stability in erythrocytes of cases with past history of favism or sulfa-drug-induced hemolysis. *Blood 13*, 348 (1958).
18. Townes, P., Morrison, M.: Investigation of the defect in a variant of hereditary methemoglobinemia. *Blood 19*, 60 (1962).
19. Zinkham, W. H.: An in vitro abnormality of glutathione metabolism in erythrocytes from normal newborns: mechanism and clinical significance. *Pediatrics 23*, 18 (1959).

Differentiation and Maturation of Haemopoietic Stem Cells in Spleen Colonies

J. GIDÁLI, I. FEHÉR

Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

(Received August 10, 1967)

Quantitative and qualitative composition of colonies arising in the spleen of BALB/c mice irradiated with 750 R ^{60}Co and given 10^5 bone marrow cells were studied. Colonies dissected from the spleen were studied in smears. This method allowed a finer differentiation than the conventional histological processing, and yielded values for the qualitative composition of colonies which were different from those reported in the literature in that 63% erythroid, 10.1% granuloid and 26.9% mixed colonies were found. The maturation degree of colonies arising from the same bone marrow pool and studied at the same date ranged between wide limits.

Several methods have been developed for the quantitative determination of haemopoietic stem cells. The methods of Gurney [5], Hodgson [6] and Blackett [2] measuring the total amount of the stem cell compartment by the incorporated ^{59}Fe are suitable to determine only stem cells differentiated into erythron.

Provided the cells forming colonies in the spleen are identical with haemopoietic stem cells, the method developed by Till and McCulloch [12] makes it possible to count stem cells differentiated into any direction.

As a rule, this method is used for the quantitative determination of stem cells. However, the recognition that each colony originates from a single lodged stem cell and is thus a clone, renders the method most suitable for the study of factors acting upon the lodging and differentiation of stem cells. Most authors using the method have contented themselves with counting the colonies only; only a few have studied the qualitative distribution of the colonies, probably owing to the fact that according to the original description [12], though the colonies are easily distinguished from splenic tissue in any histological section, the different cell types, especially the immature forms, are difficult to recognize.

Our experiments were designed to study the action of humoral factors on the lodging and differentiation of stem cells. We, therefore, needed a method that, in addition to counting the colonies, would allow a detailed analysis of their qualitative distribution. To satisfy these requirements, we have developed an alternative of Till–McCulloch's method.

Materials and Methods

A total of 121 male and female inbred mice 8–12 weeks old, weighing 18–25 g, of the strain BALB/c was used. The animals were irradiated with 750 R ^{60}Co gamma rays (dose rate, 106 R/min). Five hours after irradiation the animals were given 1×10^5 isologous bone marrow cells in 0.3 ml volume, intravenously. Bone marrow cells were obtained from the thighs of isologous donors and suspended in saline. Three parallels were used to determine in a haemocytometer the nucleated cell count in the suspension. The required cell concentration was adjusted by adding saline and 200 IU/ml penicillin were added to the suspension. This was handled sterile, kept in thawing ice and injected to the recipients within 30 min. after preparing the suspension.

The animals were kept on normal laboratory diet with 1–2 μg/ml of oxytetracycline in their drinking-water.

The recipients were always processed on the 10th day after the injection of bone marrow cells. Up to this time about 40 to 60% of the irradiated experimental animals died spontaneously.

On the 10th day the surviving animals were killed by cervical dislocation. Their spleen was removed and weighed. Colonies on both sides of the spleen were counted under a binocular lupe, freed from splenic tissue, suspended in a drop of serum and smeared on slides covered with chromium gelatin. After fixing in methanol and staining with Giemsa-solution buffered to pH 6, the slides were studied under high power.

Experimental

In the first experiment the mean spleen colony count for 8 groups on the 10th day amounted to 7.44 ± 0.5 colony/spleen. The standard error of the mean was rather high within the single experimental groups while the difference between the means was slight. After irradiation with the same dose, 0.75 so-called endogenous colony per spleen has formed in animals not given bone marrow cells. The average weight of the spleen was 52.6 ± 8 mg (Table 1).

Some authors [9] found a strict correlation between spleen weight and colony-count. Accordingly, they suggested that instead of the laborious colony-counting, weighing of the spleen would also suffice. We studied this correlation under our experimental conditions. Indeed, a strict correlation was found between average colony-count and average spleen weight. However, when plotting individual values it appeared that though spleen weight is suitable for informative studies, owing to the high standard error it cannot replace colony-counting in individual cases (Fig. 1). The correlation between colony-count and spleen weight has become even more slack if, subsequent to bone marrow transplantation, the recipient mice were given some factor acting on colony formation. Fig. 2 displays the correlation between spleen weight and colony-count in recipients given 2 Co U erythropoietin or 0.06 μg *S. typhi* endotoxin daily. Upon the effect of this treatment the

Table 1

Colony-count in the spleen of mice irradiated with 750 R and injected with 10^5 bone marrow cells

Serial No of experiment	Number of animals	Mean colony-count	S. E.*	Mean spleen weight	S. E.**
1	15	7.00	±5.35	50.1	±16.8
2	17	7.53	±1.52	51.3	±19.8
3	16	7.32	±5.85	50.7	±20.6
4	14	7.57	±1.40	52.7	±24.4
5	14	7.96	±1.62	58.4	±18.2
6	4	7.75	±7.45	43.8	±18.9
7	13	8.00	±3.78	58.7	±23.9
8	8	6.43	±5.90	55.0	±28.0
Mean		7.44	0.53**	52.58	4.84**

* S. E.: quadratic deviation of individual results
** S. E.: quadratic deviation of means for groups

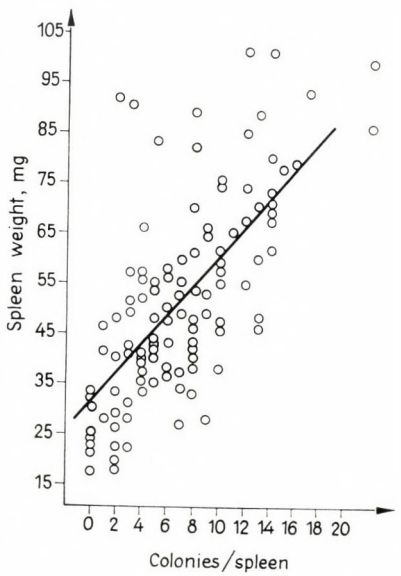


Fig. 1. Correlation between spleen weight and colony-count in control BALB/c mice

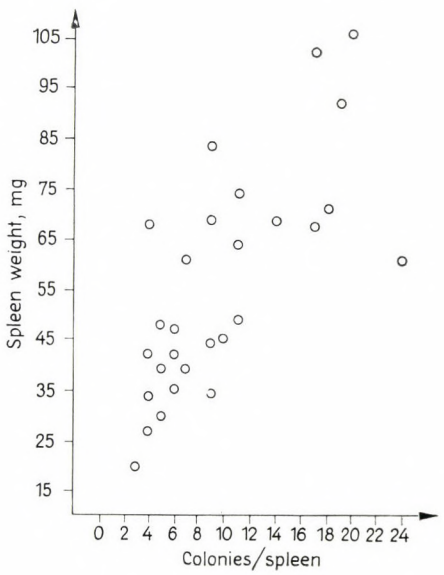


Fig. 2. Correlation between spleen weight and colony-count in BALB/c mice treated with erythropoietin or endotoxin

colony-count increased. Accordingly, spleen weight correlated with the colony-count even less than in the untreated group shown in Fig. 1.

Qualitative classification of colonies was done on the basis of smears prepared from dissected colonies. By this method the colony type cannot be recognized in more than 5 to 6% of the cases. The smears that cannot be classified contain mostly cellular fragments and some cells resembling lymphocytes. Such preparations derive probably from the smearing of splenic nodules appearing like colonies under the lupe. Naturally, these undistinguishable preparations were disregarded when evaluating the experiment and were deducted also from the macroscopic colony-count.

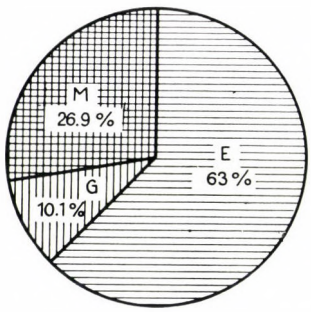


Fig. 3. Qualitative distribution of colonies in recipient mice injected with 10^5 bone marrow cells
(Mean of 121 mice)
E: erythroid colony, G: granuloid colony, M: mixed colony

Under our experimental conditions, 63% of the colonies contained erythroid, and 10.1% of them, granuloid elements, while 26.9% were mixed colonies containing both erythroid and granuloid elements (Fig. 3). Only those colonies were specified as mixed which, in addition to the predominant cell type, contained at least 5% mature and immature elements of the other cell series.

Next, the ratio between immature and nature elements in colonies formed from the same bone marrow suspension was studied. The animals were treated as above. The colonies were dissected on the 10th day following bone marrow transplantation. In every smear 1000 cells were counted. Cells still capable of dividing were specified as immature, non-dividing cells as mature ones.

Although all the colonies had originated from the same suspension and grew for the same period and under identical conditions, the ratio of immature to mature cells within the single colonies varied between wide limits.

In granuloid colonies the ratio of mature elements ranged from 10 to 50%. Forms more mature than metamyelocytes occurred in 1 to 2%. However, since polymorphonuclear granulocytes in a like proportion were found in the erythroid

colonies, the formed cells may have come into the colonies from the circulation (Table 2).

The erythroid colonies also displayed different degrees of maturation (Table 3).

As expected, in mixed colonies the degree of maturation, and the ratio of the two components varied between wide limits (Table 4).

The mitotic index in the above colonies was also studied. Mitoses per 1000 cells counted in each colony and the mitotic index was expressed in per mil (Tables

Table 2

Granuloid colonies originating from the same bone marrow pool

Serial No.	Ratio immature: mature cells*	Mitotic index per thousand
1	0.65	22
2	0.93	21
3	1.25	24
4	1.38	22
5	4.00	32
6	4.06	27

* Immature cells: Myeloblast, promyelocyte, myelocyte.
Mature cells: Metamyelocyte, band cell, polymorphonuclear granulocyte.

Table 3

Erythroid colonies originating from the same bone marrow pool

Serial No.	Ratio immature: mature cells*	Mitotic index per thousand
1	1.03	24
2	1.78	63
3	2.56	18
4	59.00	6
5	14.80	19
6	4.15	11
7	18.10	12
8	1.12	10
9	21.00	7
10	2.12	12

* Immature cells: normoblast, basophilic normoblast.
Mature cells: polychromatic normoblast, orthochromatic normoblast.

2, 3, 4). No correlation was found between maturation degree and mitotic index of the colonies. Especially for erythroid colonies, the mitotic index showed a marked fluctuation from 6 to 63 per mil.

Table 4

Mixed colonies originating from the same bone marrow pool

Serial No.	Ratio granuloid : erythroid colonies	Ratio immature : mature cells*		Mitotic index per thousand
		erythroid series	granuloid series	
1	0.26	37.0	2.26	6
2	0.34	8.0	0.47	17
3	0.36	3.0	4.55	30
4	0.45	3.3	0.74	18
5	0.54	21.6	0.70	42
6	0.70	17.8	1.28	27

* See Footnotes to Tables 2 and 3.

Discussion

Among the usual methods, that of Till and McCulloch [12] is the one best suited for the study of the differentiation of stem cells. In this original form, however, the method does not allow a detailed analysis of the colonies, and in the case of younger colonies, not even the recognition of the colony type. On the other hand, count and location of colonies in splenic tissue can easily be studied in histological sections fixed according to Bouin or Helly–Maximow and stained with haematoxylin-eosin. In such sections, however, only mature cells of a characteristic structure such as metamyelocytes or normoblasts, can reliably be recognized. Accordingly, if the quality distribution or maturation degree of the colonies must also be studied the colonies should be dissected and their smears be analyzed.

There is but one team [3] who reported on the number of unidentifiable or undifferentiated colonies in histological sections. The frequency given by this team agrees well with our 5% incidence.

As already mentioned, most authors neglected to observe the qualitative composition of the colonies, even in experiments designed to study the stimuli acting on the differentiation of stem cells [4]. Independently of each other, three research teams have specified the erythroid-colony count with 42, 43 and 45%, respectively [8, 3, 7]. In our experiments this value was found to be 63%, in good agreement with the 63% found in smears by Juraškova et al. [7]. In contrast to the 21, 35 and 14% granuloid colonies found by the above authors, we found

such colonies in 10.1 % only. However, the ratio of mixed colonies (26.9 %) exceeded the values 16, 13 and 23 % observed by the mentioned three teams [8, 3, 7]. In all probability, the differences were due to the higher sensitivity of the smear method rather than to the specific reaction of the mouse strain used by us. In stained smears the single cells are easily distinguished and in addition to the predominant type, representatives of other series can also be recognized.

In our experiments, about 30 % of the colonies were mixed ones. The ratio of the two kinds of cell series varied per colony (Table 4). If they occurred in about the same number, classification of the colony was easy. However, when besides cells constituting the major part of the colony the other cell series was represented by only a few characteristic precursors and mature cells, the colony type was difficult to determine. According to the clonal origin, irrespective of the numerical ratio of the components, all the colonies that contained elements of more than a single cell series should be considered as mixed colonies, but in our experiments 10 days after transplantation there was hardly any colony which would not have contained sporadic representatives of the cell type other than the predominating one. The presence of mature elements was easy to explain by assuming that they had originated from circulating blood. This explanation was hardly acceptable for the presence of immature forms. Accordingly, considered mixed ones when those colonies were both the mature and immature elements of the non-dominant cell series occurred in at least 5 %. No such numerical definition of mixed colonies has been used by any of the authors, probably because the conventional histological evaluation method does not yield such exact values. The high number of mixed colonies found by us was probably also due to the applied technique. A similarly high number of mixed colonies was found by Juraškova et al. [7] who were working with a method resembling ours.

Spleen colonies are undoubtedly of clonal origin [1], but how do mixed colonies arise? It is improbable that they should originate from two stem cells lodged near each other but differentiated into different directions. Both the high ratio of mixed colonies and the occurrence of elements belonging to the other cell type in almost every colony contradict the assumption.

The hypothesis that the mixed colony also originates from a single stem cell is much more consistent with experimental facts. It may be supposed that during the first divisions the pluripotent stem cell differentiates into both directions, but then some inhibiting factor stops stem cell differentiation in one of the directions. If this inhibition occurs early, the colony will contain besides the predominant cell series a few cells belonging to the other cell series. If the inhibition occurs later, the number of the latter elements will be closer to the predominant ones.

Arisal of mixed colonies might be imagined also in the way that right at the beginning the stem cell differentiates but in one direction and its differentiation into the other direction starts but later.

Our observation that colonies arising from stem cells of the same bone marrow pool display different maturity degrees at the same date (Tables 2, 3, 4)

is analogous with those of Siminovitch et al. [10]. These authors demonstrated that the stem cell count ranged between wide limits in colonies originating from the same bone marrow pool. They assumed that this phenomenon was based on the random distribution of "birth" and "death" processes in a colony.

The assumption that mitoses in the colonies occur in a random fashion, furnishes an explanation for the observation that often no correlation can be demonstrated between the maturity degree of the colony and its mitotic index. This hypothesis is confirmed by the fact that colonies hardly contain or do not contain mature elements during the first 5 days after transplantation while the number of mature elements increases abruptly at later dates.

References

1. Becker, A. J., McCulloch, E. A., Till, J. E.: Cytological demonstration of the nature of spleen colonies derived from transplanted mouse marrow cells. *Nature (Lond.) 197*, 452 (1963).
2. Blackett, N. M., Roylance, P. J., Adams, K.: Studies of the capacity of bone marrow cells to restore erythropoiesis in heavily irradiated rats. *Brit. J. Haemat. 10*, 453 (1964).
3. Bleiberg, I., Liron, M., Feldman, M.: Reversion by erythropoietin on the suppression of erythroid clones caused by transfusion-induced polycythaemia. *Transplantation 3*. 706 (1965).
4. Bruce, W. R., McCulloch, E. A.: The effect of erythropoietic stimulation on the hemopoietic colony-forming cells of mice. *Blood 23*, 216 (1964).
5. Gurney, C. W., Lajtha, L. G., Oliver, R.: A method for investigation of stem-cell kinetics. *Brit. J. Haemat. 8*, 461 (1962).
6. Hodgson, G. S.: Erythrocyte Fe-59 uptake as a function of bone marrow dose injected in lethally irradiated mice. *Blood 19*, 460 (1962).
7. Juraškova, V., Tkadlecek, L., Drasil, V.: Note on the differentiation of colonies of haemopoietic tissue cells in the spleen of irradiated mice. *Folia biol. (Praha) 10*, 381 (1964).
8. Lewis, J. P., Trobaugh F. E. Jr.: Haematopoietic stem cells. *Nature (Lond.) 204*, 589 (1964).
9. Popp, R. A., Congdon, C. G., Goodman, J. W.: Spleen weight as a measure of marrow cell growth in irradiated mice. *Proc. Soc. exp. Biol. (N. Y.) 120*, 395 (1965).
10. Siminovitch, L., McCulloch, E. A., Till, J. E.: The distribution of colony forming cells among spleen colonies. *J. cell. comp. Physiol. 62*, 327 (1963).
11. Smith, W. W., Brecher, G., Budd, R. A., Fred, S.: Effect of bacterial endotoxin on the occurrence of spleen colonies in irradiated mice. *Radiat. Res. 27*, 369 (1966).
12. Till, J. E., McCulloch, E. A.: A direct measurement of the radiation sensitivity of normal mouse marrow cells. *Radiat. Res. 14*, 213 (1961).
13. Till, J. E., McCulloch, E. A., Siminovitch, L.: A stochastic model of stem cell proliferation based on the growth of spleen colony-forming cells. *Proc. nat. Acad. Sci. (Wash.) 51*, 29 (1964).

Tuberculosis of Peripheral Lymph Nodes

I. Barta, A. Patakfalvi

First Department of Medicine, University Medical School, Pécs, Hungary

(Received July 26, 1967)

Among the patients admitted with lymphadenopathy to our Department during the year 1966, 12 suffered from isolated cervical lymph node tuberculosis. Enlargement of the cervical, supraclavicular, axillary and inguinal lymph nodes varied from case to case. It is often possible to differentiate lymphogenic genesis from the haematogenic one. In generalized lymphadenopathy the lymph nodes are seldom larger than a nut, are dense, painless, show no tendency to breaking through. The clinical symptoms are not characteristic, the routine laboratory tests are non-contributory. The highly increased sensitivity to tuberculin is conspicuous. Biopsy plays the decisive role on establishing the diagnosis. After an analysis of differential diagnostic problems it is emphasized that the clinical pattern of tuberculosis of the lymph nodes has changed, its development has shifted in the direction of adulthood, coalescence does not occur and scrofulosis has practically disappeared. This may be attributed to a modification of the immune activity of lymphoreticular tissue as a result of BCG inoculation, chemotherapy and chemoprophylaxis.

The role of lymph nodes in the genesis and course of tuberculosis is clearly shown by the fact that there is practically no tuberculosis without some form of lymph node involvement. Mostly there are only local foci showing a tendency to demarcation, encapsulation, but sometimes tuberculosis is restricted to the lymph nodes. Since it has become possible to study *in vivo* some of the hidden lymph nodes (the cervical and supraclavicular ones by prescalenus biopsy, the mediastinal ones by mediastinoscopy, the retroperitoneal ones by lymphadenography), it could be proved that the incidence of non-localized tuberculous lymphadenopathy is higher than it had been assumed. In the large patient material of Seth, even when isolated tuberculosis had been diagnosed, several regions were found to have been involved in 30 per cent of the cases.

The same applies to the affection of peripheral lymph nodes. Even when it is in a single region that the change appears, later a considerable part of, or sometimes the entire, peripheral lymph node system may become involved. The patient then does not report at a tuberculosis dispensary, but will be admitted to a medical department and this accounts for the seeming decrease in the incidence of lymph node tuberculosis in the specialized institutes. Recently, we have been impressed by the fact that as related to lymphadenopathies of other aetiology the incidence

of generalized lymph node tuberculosis is remarkably high. Table 1 shows the number and percentage of such patients admitted to our Department in 1966.

The 12 patients with peripheral lymph node tuberculosis represent 17.4%. The various regions were differently involved, in most cases bilateral enlargement of the cervical lymph nodes was in the foreground, but in some cases involvement of the cervical, supraclavicular, axillary and inguinal ones could be noted. To illustrate the variability of the clinical pattern of lymph node tuberculosis depending on the region or regions involved, we shall report some pertinent cases.

Table 1

Tuberculous lymphadenitis	12
Lymphogranulomatosis	26
Chronic lymphadenosis	23
Lymphosarcoma	4
Reticulosis	3
Reticulosarcoma	1
Total	69

Case 1. Mrs. K. J., aged 41. In 1960 a lymph node had been excised from the right side of the neck. Histologic examination showed no sign of tuberculosis or malignancy. After half a year of increasing anorexia, weakness, and a loss of weight of about 10 kg, she was admitted on October 1, 1966, with subfebrility and enlarged cervical lymph nodes.

On both sides of the neck the lymph nodes were pea to bean-sized, painless, mobile. Physical examination of the thorax and abdomen revealed no changes. The nervous system was normal. Laboratory tests: urine analysis normal. Blood counts: erythrocytes 3,700,000 per cu. mm, Hb 13.2 g per 100 ml, leucocytes 4,800 per cu. mm. Differential count: eosinophil 1, polymorphonuclei 71, lymphocytes 23, monocytes 5%. ESR: 13 mm in 1 hour. Serum electrophoresis: albumin 50%, alpha$_1$ globulin 4, alpha$_2$ 13, beta 12, gamma globulin 24%. Mantoux test: strongly positive at 1 in 100,000 dilution. On chest X-rays a horizontal narrow band was visible extending from the right hilum laterally. Histologic examination of the excised cervical lymph node (Institute of Pathology, Pécs) revealed tubercle-like foci bounded by epitheloid cells and fibrosis. The patient was treated with streptomycin and isoniazid. Four months later she was afebrile, gained weight, the lymph nodes decreased in size.

This case shows that cervical lymph node tuberculosis may occur also in older age. The process may be bilateral and the lymph nodes do not merge. This was formerly a surgical disease, now it is a problem for the physician.

Case 2. V. Gy., male, aged 31 years, was admitted on January 2, 1966, with fever of 38° C of about one week's duration, anorexia and swelling in the right

armpit. On the left side supraclavicularly and in the armpit pea to bean-sized lymph nodes, on the right side in the armpit one nut-sized and several bean-sized lymph nodes were palpated. Physical examination showed normal conditions.

Laboratory tests. The urine was normal. Blood counts: erythrocytes 4,200,000 per cu. mm, Hb 15.5 g per 100 ml, leucocytes 4,200 per cu. mm. Differential count: eosinophils 10, polymorphonuclei 44, lymphocytes 41, monocytes 3%. ESR: 20 mm in 1 hour. Serum electrophoresis: albumin 58%, globulins $alpha_1$ 7, $alpha_2$ 7, beta 17, gamma 11%. Mantoux test strongly positive at 1 in 100,000 dilution. Chest X-rays showed pathologic change. Histologic examination of the lymph node excised from he rightt armpit revealed under the capsule delimited small necrosed areas containing many Koch bacilli and some epitheloid cells. The patient was treated with35 g of streptomycin and daily 10 tablets of isoniazid. One year later he had no complaints and only a few lymph nodes could be palpated.

In this case the disease presented itself as an acute lymphadenopathy extending to a number of regions and the diagnosis could be established only on the basis of biopsy. The acute affection was cured by antituberculotic treatment.

Case 3. Mrs. B. A.. 46 years of age, had noticed enlargement of the cervical lymph nodes, subfebrility, fatigue and a loss of weight of 10 kg 4 to 5 months before admission. On admission she was pale, asthenic; bean to nut-sized lymph nodes could be palpated behind the sternocleidomastoid muscle on both sides, in the armpit on the left side and in the inguinal region on both sides. Physical examinations yielded normal conditions except enlargement of spleen and liver.

Laboratory tests. The urine was normal. Blood count: erythrocytes 4,200,000 per cu. mm, Hb 14.0 g per 100 ml, leucocytes 9,200 per cu. mm. Differential count: eosinophils 4, polymorphonuclei 72, lymphocytes 21, monocytes 3%. ESR: 35 mm in 1 hour. Serum electrophoresis: albumin 65%, $alpha_1$ 8, $alpha_2$ 2, beta 13, gamma globulins 12%. Mantoux test strongly positive at 1 in 100,000 dilution. Chest X-rays showed no change. Cervical lymph node biopsy (Institute of Pathology, Pécs) revealed confluent epitheloid foci with several Langhans-type giant cells in them; in the centre of a few tubercles caseation was found. After 6 months treatment with streptomycin and isoniazid the condition greatly improved, temperature was normal, body weight increased, the ESR returned to normal. Small lymph nodes could still be palpated in the cervical regions.

This case is illustrative of the generalized form of lymph node affection. The peripheral lymph nodes were enlarged in almost every region.

Diagnosis and Differential Diagnosis

The clinical symptoms of peripheral lymph node tuberculosis are not characteristic. The patient usually seeks medical advice after a period of weakness, fatigue and loss of weight, complaining of enlarged lymph nodes in the cervical area or in the armpit. The acute disease is accompanied by remittent fever, the chronic form by subfebrility, but febrile and afebrile periods may alternate. The

laboratory tests are non-contributory. In the blood counts, moderate hypochromic anaemia, at the onset lymphocytosis, in the generalized form leucopenia and lymphopenia are found. Excessive leucocytosis with leukaemoid reaction is exceptionally observed. The ESR is increased, in the electropherogram sometimes an increase of alpha$_2$ and gamma globulins may be demonstrated. The Mantoux test is strongly positive, and this is what mostly calls attention to the tuberculous aetiology. The decisive evidence is, of course, supplied by lymph node biopsy.

In the smears the cytologic pattern is variable, though certain common changes may always be detected. In the initial phase (Fig. 1) no characteristic changes are demonstrable; lymphocytic and reticulocytic hyperplasia may occur in other inflammatory conditions, too, but the low neutrophilic leucocyte count is conspicuous. The cells typical of tuberculous involvement (Fig. 2) appear only later. The epitheloid cell count varies over a wide range. Degeneration of the nucleus and cytoplasm marks the onset of caseation. Thus, the cytological preparation allows conclusions as to whether exudation, proliferation or caseation dominates in the process. The nuclei of the Langhans cells (Fig. 3) are not located so regularly as in the sections, where in addition to the presence of specific elements the arrangement of cells is also characteristic, although in the early stage specific changes are infrequent. Detection of Mycobacterium tuberculosis of course decides the diagnosis.

In differential diagnosis, various conditions should be ruled out, thus in the early stage acute benign generalized lymphadenopathy [1] and infectious mononucleosis, especially its atypical forms with uncharacteristic haematological and serological changes. It is more difficult to rule out non-specific chronic lymphadenopathy, in the early stage of which there is proliferation of reticulum cells just like in tuberculosis. The epitheloid granuloma of sarcoidosis is easily mistaken for a tubercle. Tuberculous lymphadenitis is often surprisingly similar to lymphogranulomatosis. Differentiation from macrofollicular lymphoblastoma is made possible by needle biopsy and from a lympho- or reticulosarcoma by the histological changes.

Discussion

Generalized tuberculous lymphadenopathy may develop by the lymphogenic or haematogenic route. Lymphogenic infection begins in one region; in the beginning one single lymph node is only affected, then the process spreads to adjacent ones and through the lymphatics the tubercle bacillus reaches the next region where it affects other lymph nodes. The affection may begin in the cervical, supraclavicular, axillary or inguinal lymph nodes. A selective enlargement is, however, mostly transitory and even if the process remains localized for a long time, later other regions become affected. Thus, every change extending to more than one region, so also bilateral cervical tuberculous lymphadenopathy, should be considered a sign of a generalized process. If the primary focus escapes detection, it is difficult to reconstruct the route of infection.

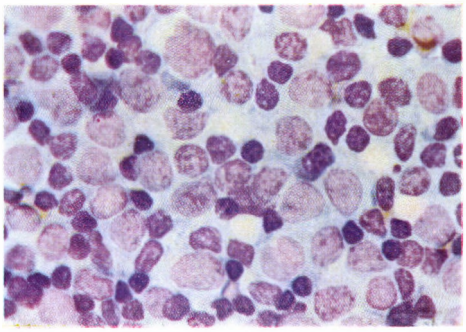


Fig. 1. Large number of reticulum cells with light blue plasma and big nuclei among the lymphocytes

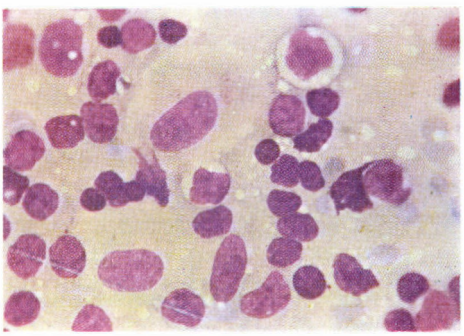


Fig. 2. Lymphocytes, epitheloid and reticulum cells

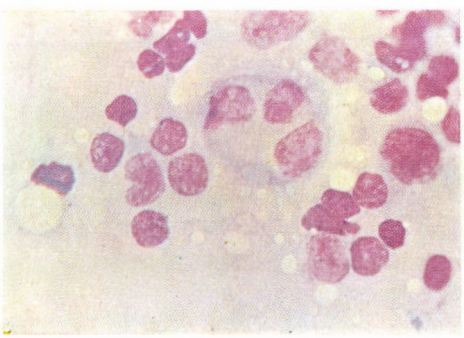


Fig. 3. Langhans-type cells with an irregular nuclear arrangement

Another type of tuberculous lymphadenopathy is due to haematogenic infection. This is suggested by a simultaneous bilateral symmetrical enlargement of the nodes, by the absence of coalescence, the presence of calcified foci or inactive changes in the lungs or pleura, or eventually by a typical history. Active pulmonary tuberculosis seldom occurs together with generalized specific lymphadenopathy. Baumgartner showed more than 50 years ago that generalized lymphadenopathy may be also haematogenic. He injected a homogeneous suspension of tuberculosis bacilli into the carotid of animals, and observed the development of extensive isolated lymph node tuberculosis.

In generalized tuberculous lymphadenopathy the lymph nodes seldom exceed nut size; they are dense, painless, do not adhere to the environment and do not show the slightest sign of a tendency to break through. Apart from the relatively higher incidence of this form the other fact disclosed by our investigations is that the clinical pattern of cervical tuberculous lymphadenopathy has undergone remarkable changes. Scrofulosis, a classical entity a few decades ago, has disappeared. Coalescence and fistula formation do not occur. The condition has become a disease of adults, though formerly it was observed mainly in children and was attributed to bovine-type infection [3, 4, 5, 6, etc.].

What accounts for the delay in the development of lymph node tuberculosis and for the alteration of the nature of the process? It is easier to explain why the disease now occurs in adults. BCG vaccination, chemotherapy and chemoprophylaxis are delaying the primary infection till early or late adulthood, as a result of which tuberculosis of the lymph nodes also develops later. It is more difficult to explain why the nature of the pathological process has changed. It is most plausible to seek the cause of this in the immunological relations. A defective function of lymphatic tissue rich in immunocytes is difficult to demonstrate, as in tuberculosis the organism does not produce such specific antibodies by the estimation of which the function of cells producing immunoglobulins could be appraised. One of us, co-operating with Gyenei, has attempted to gain insight by spleen biopsy into the immune activity of the organism in various forms of pulmonary tuberculosis. The reticulum and plasma cells and the transfer forms showed the most colourful quantitative and qualitative changes which were interpreted as a sign of defensive activity. It appears likely that not only the modification of the local process, but also its generalization may be ascribed to an insufficient function of immune cells.

Treatment

The diagnosis of tuberculosis must always be established as early as possible and specific treatment must be begun as early as possible. As several regions are affected, medical treatment should be instituted in the first place. If combined isoniazid, PAS and streptomycin treatment fails to cause the lymph nodes to disappear, or decrease in size, other combinations should be used eventually with

cycloserine, ethioniamide, pyrazinamide or viomycin. Conditions associated with a hyperergic reaction are best influenced by cortisone. Surgical treatment should be contemplated exclusively when the process is restricted to one single region.

References

1. Barta, I.: Über die generalisierte gutartige Lymphadenopathie. *Z. ges. inn. Med. 21*, 149 (1966).
2. Baumgartner, P.: Über das Verhältnis der Lymphogranulomatose zur Tuberkulose. *Münch. med. Wschr. 61*, 1545 (1914).
3. Brügger, H.: Handbuch der Tuberkulose. Vol. IV. Thieme, Stuttgart 1963.
4. Dietrich, P. J.: *Präv. Med. 7*, 198 (1962).
5. Schmid, P. Ch.: Zervikale tuberkulose Lymphdrüsen an Kindern. Ätiologie, Diagnose und Therapie. *Arch. Kinderheilk. 42*, Suppl. 1960.
6. Schulze, W.: Über die Pathogenese und Therapie der zervikalen Lymphdrüsentuberkulose in den Jahren 1955—1961. *Tuberk.-Arzt. 17*, 226 (1963).
7. Seth, P. P.: Surgical treatment of tuberculous lymphadenitis. *J. Indian med. Ass. 40*, 1966 (1963).

Open Forum

In the last two issues of Haematologia, J. V. Dacie, Professor of Haematology, Postgraduate Medical School of London; Professor Jean Bernard, Director of the Research Institute for Blood Diseases, Paris; Professor J. J. Van Loghem, Director of the Laboratory for Blood Transfusion, Amsterdam; Professor M. C. Verloop, University Medical School, Utrecht; Professor E. Deutsch, University Medical School, Vienna; Professor P. F. Hjort, University Medical School, Oslo, Professor G. Bilski-Pasquier, University Medical School, Paris and Professor W. Rudowski, Director of the Institute of Haematology, Warsaw, have contributed to the discussion of "Modern trends in the teaching and specialization of haematology and blood transfusion".

In this series of discussions further contributions are presented:

SOVIET UNION

Professor A. E. Kiselev, Director of the Central Institute for Haematology and Blood Transfusion, Moscow, has been invited to acquaint our readers with the teaching and specialization of haematology and blood transfusion in the Soviet Union.

How is the teaching of haematology and blood transfusion organized in your country?

Research work and practical training in haematology and blood transfusion on a high level are performed in the institutes of haematology and blood transfusion and in the large regional centres of every Republic of the Soviet Union.

Is it a compulsory subject in medical education and on what level is it included in the curriculum of medical schools?

The curriculum of the medical universities stipulates for medical students the study of haematology and blood transfusion. However, due to the overcrowded schedule, the time devoted to these subjects is not sufficient for achieving the level that would meet present scientific requirements.

Are there postgraduate courses for haematology and blood transfusion?

Postgraduate training courses in haematology and blood transfusion are conducted in the postgraduate medical schools. In addition, training courses for

doctors working in preventive-curative medical services are also held regularly in the institutes of haematology and blood transfusion and in the large regional centres.

New methods for the production of preserved blood and blood derivatives as well as technical methods used in haematology are worked out in the institutes and regional centres of the Blood Transfusion Service.

Is there a possibility of obtaining a degree in haematology and blood transfusion?

Scientific degrees for researchers and specialization for technical assistance of the Soviet Blood Transfusion Service are obtainable both in haematology and blood transfusion. Apart from the university medical schools and the Soviet Medical Academy, the Central Institute for Haematology and Blood Transfusion is also authorized to accept theses. Many candidates for doctorate and doctors of medical sciences are on the staff of these institutes.

What technical training and examinations are required for specialization?

The training of doctors and secondary personnel (nurses) is governed by an organized program which stipulates 245 hours of study for doctors and 22 hours for nurses.

What suggestion would you have for promoting the development of haematology and blood transfusion (education, medical care and research)?

To conduct successful work in haematology and blood transfusion which will meet the standards of up-to-date scientific requirements calls for extensive and complex investigations carried out by researchers from various fields of science. In addition, an exchange of researchers and information by way of international meetings and symposia are of great importance in maintaining advanced research in haematology and blood transfusion.

UNITED STATES

Dr. Tibor J. Greenwalt, Medical Director, Blood Program, American National Red Cross

The horizons of hematology have expanded into a vast multidisciplinary complex. This diversification seems to be a major factor in maintaining an adequate input of qualified and motivated trainees so that no general shortage of trained persons in this field exists. Unfortunately blood transfusion and isoimmunohematology have not done so well as we shall see.

There is no fixed pattern for teaching hematology in our medical schools. In some institutions the related laboratory training is given in the department of pathology and the hematologists have contact with the medical students only during the clinical years. In other medical schools the entire program of teaching is in the hands of hematologists who may also be responsible for the operation of the clinical hematology laboratories and the blood transfusion service. The extent

of training in hematology given to students varies greatly and depends to some extent on the fame and personality of the Chief of the Hematology Section and the support given by the Chairman of the Department of Medicine. Alas, training in blood banking and hemotherapy can be stated in general to be almost nonexistent in medical schools.

Postgraduate training in hematology can be obtained in several ways. Most often it is in the form of postdoctoral fellowships following a medical internship and residency. Financial support is most frequently through training grants or fellowships supported by the National Institutes of Health. Occasionally training can be obtained as part of the residency program of a hospital. A few are trained by working in practice with a qualified hematologist. Courses sponsored by the American College of Physicians or independently by universities are available but these are short term reviews which do not qualify a person as a specialist. Some internists have become qualified hematologists because of a well directed interest, attendance at meetings and taking of special courses.

There is no national institute for hematology or blood transfusion in the United States and in the medical schools hematology is a section of the Department of Medicine headed by a faculty rank of Associate Professor or Professor. There is no board for certifying or special degrees awarded to specialists in Hematology or blood transfusion at the doctoral level. This possibility has been discussed by committees involved in certifying internists and certifying pathologists. For the present perhaps it is best to let the hematologist and transfusionist gain recognition on the basis of his achievements.

Blood banking and blood transfusion therapy enjoy a much less advantageous position. Medical students usually receive practically no training in this subject. Training programs, fellowships and residencies in this field are practically nonexistent. To my knowledge there are only three institutions offering formal training in blood banking and related disciplines. Those actively engaged as blood bank directors generally have strayed into the field from hematology, pathology, anaesthesiology, surgery, etc. and picked up training from experience and association.

Relatively few blood banks are directed by physicians with special training and many positions are open. This deplorable situation has multiple origins. Hematologists are themselves to blame because they have for the most part treated blood banking as a field of little interest to them and not worthy of much attention. This is probably the case with other physicians, many of whom seem to feel that it is silly for a qualified physician to devote his full time to the operation of blood banking and transfusion service. These attitudes result from the lack of realization of what a broad and complex multidisciplinary area the field of blood banking and hemotherapy has become and the possibilities for research and development which exist. The lack of organization of transfusion facilities in this country is also to blame. The Blood Program of the American National Red Cross has 56 Regional Blood Centers which collect almost three million pints of blood annually from volunteer blood donors. The members of the American Association of Blood

Banks collect most of the rest of the blood. The majority of the member banks of the American Association of Blood Banks are small hospital blood banks. The Red Cross and the AABB have established standards which they maintain by regular inspections of facilities. Blood banks are also regulated by the Division of Biologics Standards of the Department of Health, Education and Welfare when they ship products over state lines or voluntarily seek licensure by this agency.

The major deficiencies of blood banking could be remedied if the problems of training qualified blood bank directors could be solved. This is a two-pronged problem: training programs must be established and suitable candidates for training must be recruited.

BULGARIA

Professor V. Serafimov-Dimitrov, Director of the Research Institute of Haematology and Blood Transfusion, Sofia

Today's scientific efforts have led to a so far unexperienced advance of the analytical studies in all fields of medicine. Medical disciplines have increased in number and new specialized fields of knowledge have developed within the limits of the classical specialities. They require a considerably narrower profile and specialization of physicians employed in scientific and public health institutions. Haematological science has also been affected by this process. For instance, haemocytology ceased to be a part of clinical haematology. Isoserology developed into a separate discipline and became a new science by itself. Almost immediately the basis was laid for the delimitation of immunohaematology. Chemistry and physicochemistry provided a number of new methods of investigation of the protein contents of blood plasma and fractionation of plasma was introduced in medical practice. A broad field of haematology, blood clotting, has become a science *per se*. Preservation of blood and haematopoietic tissues developed into a separate branch. The use of ionizing radiations in basic research, industry and medicine leads to the development of nuclear haematology. The list of haematological disciplines has become quite a long one and this necessitates the creation of specialized branches of contemporary haematological science. In my opinion, the differentiation of the specialized haematological disciplines implies the creation of a generalized science which should unify the fundamental problems of haematology. General haematology could become the basis of each specialized haematological discipline. It should also treat the principal problems of normal and pathological morphology, physiology, biochemistry and immunology of the haemopoietic system.

Up to now, scientific and practical work has made it necessary to create eight paraclinical and two clinical disciplines. The eight paraclinical ones are: haemocytology, haemohistology, haemocytobiochemistry, nuclear and functional haematology, blood clotting, preservation of blood, isoserology and immunohaematology. The two clinical disciplines are clinical haematology and transfusional haematology.

The problems of haematology should occupy adequate space in the training program of medical students and physicians. Knowledge in haematology is needed by all physicians, independently of their speciality. What should be the limits of haematological training in undergraduate and postgraduate education? No doubt, modern haematological diagnosis and therapy could be provided only by specially trained physicians. The general course of undergraduate university training should therefore cover knowledge in general haematology. This does not imply that physicians who graduate from higher medical institutes are prepared to provide qualified haematological assistance. Nontheless undergraduate training should provide a minimum of knowledge which would render the young physician adequate training in the field of haematopoietic disorders and ensure a minimum experience in laboratory techniques employed in general therapy. In Bulgaria, medical students acquire basic knowledge in clinical haematology at the departments of medicine. Stress should be laid on the fact that at least for the present their training in haematology could not pretend for thoroughness, since none of the university departments of medicine are specially devoted to haematology.

Training in transfusional haematology is acquired at the institutes of biology, pathophysiology, and forensic medicine and at the department of surgery. Teaching of isoserology, immunohaematology, and of pathology, clinical symptomatology and treatment of hypovolemic disorders is partial and nonsystematized. In its present form it therefore annihilates the chance for the acquisition of solid knowledge and practice in haematology needed by each physician. Attempts are now made to provide a more concentrated training of medical students in transfusional haematology and a more fundamental knowledge in clinical haematology. In my opinion the responsibility for training in clinical and transfusional haematology in medical faculties should be entrusted to the departments of medicine which should be equipped with a proper cytological and coagulational laboratory and a well-organized centre for blood transfusion with isoserological and immunohaematological laboratory.

On the basis of the present stage of development of haematological disciplines it is assumed that specialization in both haematological branches – clinical haematology and transfusional haematology – should be accomplished in postgraduate training. Specialization in clinical haematology now exists worldover and implies no more unresolved questions. Somewhat different is the situation with transfusional haematology. Almost everywhere, it is called transfusiology. Independently of whether or not an official examination is required for the acquisition of this speciality this is already imposed by everyday practice. Nevertheless in many countries training in transfusiology is quite limited, not systematic, and covers only a narrow profile of the field. For this reason we have changed not only the designation of the speciality but also the program for specialization. In my view transfusional haematology represents a medical discipline with a broad spectrum, based on isoserology, immunohaematology, blood clotting, preservation of blood (also the problems of blood donation) and plasma fractionation. This is why the transfusional haematologist should master these disciplines. It is therefore

clear that transfusional haematology is a clinico-laboratory discipline with a therapeutic aspect.

For the present only one haematological speciality exists in this country – clinical haematology. The adoption of the speciality of transfusional haematology will soon follow. The two haematological specialities could be mastered by physicians with a previous basic training such as internal medicine or paediatrics for clinical haematology and internal medicine, surgery or gynaecology and obstetrics for transfusional haematology. Specialization in transfusional haematology should be allowed after one year preliminary apprenticeship in the basic speciality, while that in clinical haematology should follow specialization in internal medicine or paediatrics. Specialization in transfusional haematology should continue for four years and in clinical haematology for two years (after three years of specialization in the basic speciality). Postgraduate teaching in clinical and transfusional haematology is directed by the department of haematology at the Postgraduate Medical Institute (ISUL), which is arranging annual courses of four months in clinical haematology and of three months in transfusional haematology. Every specializing physician should complete the course. Specialization itself is acquired in certain institutions of the Haematology and Blood Transfusion Service according to a program devised by the Chair in Haematology and approved by the Ministry of Public Health and Social Welfare, and is bound to passing an examination in the theoretical and practical aspects of the field. During the training period the physician specializing in clinical haematology should master 1. the fundamentals and theories of general haematology; 2. pathogenesis, clinical symptomatology, diagnosis and therapy of systemic disorders of the blood; 3. interpretation of haemograms, myelograms and haemopoietic tissue biopsy; 4. the necessary minimum in immunological, isoserological and coagulational tests; 5. the necessary minimum in the problems of transfusional therapy. During the training period the physician specializing in transfusional haematology should master 1. the theoretical basis of general and transfusional haematology; 2. the clinical symptomatology and therapy of hypovolemic disorders, of transfusional reactions and complications, of systemic disorders, of the blood (the latter within the range of a necessary minimum); 3. sufficient theoretical and laboratory experience in isoserological and immunohaematological tests; 4. theoretical and practical minimum on coagulational and cytological methods of examination; 5. definite theoretical and practical knowledge of the scientific, practical and organizational problems of obtaining, preservation and transfusion of blood and the preservation and transfusion of bone marrow.

Blood transfusion is a widespread procedure. Still no official rules exist in this country as regards specialization of transfusiologist physicians. Training is unilateral, mainly in technical and industrial problems. This has deprived the work of transfusiologists of its character and has not assisted the improvement of the scientific level of their practice. We think that the introduction of a speciality in transfusional haematology would change matters favourably. The designation of the specialist itself witnesses a clinico-therapeutic tendency. Simultaneously, in

order that no drawback should occur in the technical, productional and laboratory activities, at the transfusion centres, special training of nurses and laboratory technicians will be organized. They will also specialize in courses organized by the Section for the specialization of nurses, medical and laboratory technicians at the Ministry of Public Health and Social Welfare. This will soon place the technical and laboratory activities of the Transfusion Service predominantly in the hands of highly qualified and specialized nurses and laboratory technicians.

It is clear that specialization in clinical and transfusional haematology could not alone satisfy the demands of everyday practice. Clinical haematology requires specialists qualified in cytology, blood clotting and immunohaematology, whereas transfusional haematology requires specialists qualified in isoserology, immunohaematology, blood clotting and preservation of blood. Specialists in these fields are mainly needed at the big haematological and transfusion centres. In fact, the problem concerns the qualification of specialists in haematology who would devote themselves to these more specialized fields. One thing needed in this country and which we are aiming at is that specialization and perfection should follow the training and specialization in clinical and transfusional haematology. Besides, every haematologist should master the basis of haematological science, general haematology. This would allow to avoid the harmful tendency to specialize in a definite haematological branch without previous broad training in general haematology. Stress should be laid upon the fact that specialization has a number of advantages, but also serious drawbacks. One of these drawbacks is the lack of an outlook on the general problems in medicine, on the condition of the patient. Man is an integral and indivisible phenomenon. The pathological changes in a biological system never remain isolated. For that reason, therapeutic assistance administered by a narrow specialist should always be combined with a broad clinical outlook which could not be cultivated without solid knowledge in a basic medical discipline. This is why we claim that haematologists need training in general haematology.

Specialization in haematology cannot be accomplished without an adequately qualified teaching staff. Well equipped clinical departments and laboratories where specialization could take place are also needed. Teachers in haematology should also possess a definite profile. The theoretical and practical courses in clinical and transfusional haematology should be conducted by haematologists – clinicians, cytologists, coagulationists, experienced specialists in blood taking and preservation. Therefore we insist on training physicians in haematology by specialized teaching teams. This provides a high theoretical level and a good practical training for the specializing haematologist.

For the present in Bulgaria the main centre for specialization in haematology is the Scientific Research Institute in Haematology and Blood Transfusion. At the same time it is the basis for the Department of Haematology of the Postgraduate Medical Institute in Sofia. The haematological clinic and the laboratories of the immunological, pathophysiological, morphological, biochemical, organizational and methodological sections of this Institute provide conditions for a completely

modern training in haematology. The same Institute produces test sera and assists the laboratory for fractionation and the lyophilization laboratory and other departments of the Blood Transfusion Centre in Sofia. District Centres of Haematology are now being organized. Each of them will provide highly specialized haematological assistance to the population of an area with 1,500,000 inhabitants. These centres will also train specialists in clinical and transfusional haematology. The country's Haematological Service which is already almost completely organized will rely upon these centres. Together with the Institute and the Department of Haematology they will train specialists not only in these two but also in other fields of haematology.

In our country there exists a tendency to create such a structure of the Haematological Service which would help the specialization and integration of modern haematology. I hope that the above-mentioned notes illustrate the tendency of our Ministry of Public Health and Social Welfare and its Haematological Institute to provide specialized haematological assistance by means of creating narrow specialities and specialists.

A few words are also needed on integration. Integration is a prerequisite of the advance of haematology, the mutual coverage of the spheres of the haematological disciplines. In this respect, highest integration results from the interrelationship between clinical and transfusional haematology. For example, immunohaematology is an important section of clinical haematology which developed on the basis of isoserology, a basic discipline of transfusional haematology. Preservation of blood was a specialized field with a biochemical profile serving the aims of blood transfusion only. Now the problems of bone marrow preservation comprise a considerable part of this discipline which thus became a necessity in clinical haematology. Considerable integration occurs also in blood clotting where the problems of clinical and transfusional haematology intermingle. Presently, haemostasiology is a necessary component of both haematological disciplines. For that reason we insist on their unification for the purposes of a more monistic approach in organizing the Haematological Service, the training of specialists and the therapeutic activities in haematology.

The recent advance of the haematological sciences has created a considerable number of specialized haematological disciplines. At the same time the significance of haematology within medical science has greatly increased. This leads to the necessity for specialization with integration within the scientific studies, everyday practice and training in haematology. In our opinion the various problems of training and specialization in haematology could be solved in the following manner:

1. Medical faculties should provide a concentrated fundamental training in general haematology; the young physician should start his practice with some knowledge and personal experience in clinical and transfusional haematology.

2. Only specialized physicians should be allowed to haematological practice for which two specialization profiles are needed: clinical and transfusional haematology. On the basis of these specialities new, more specialized branches

should be created in cytology, isoserology, immunohaematology, haemostasiology, etc.

3. Integration of the specialized haematologists is needed. This integration demands that training specialization, therapeutic activities and scientific studies should be performed at complex haematological institutions.

4. The principle of specialization and integration has been applied in the organization of the educational, research and therapeutic institutions in Bulgaria.

POLAND

Professors A. Aleksandrowicz and H. Gaertner, University Medical School, Warsaw

An "Open Forum" discussion becomes today necessary at the parting of the ways leading either to the highest specialization or to the sensible integration of sciences. It is not necessary to explain how difficult it is for us haematologists to follow at the same time both trends, specialization and integration. The growing intensity of research, progressing differentiation of science and expanded medical and social needs of populations outride the means and trends in the teaching of and specialization in haematology and transfusiology. Therefore, the training of experts in haematology and transfusiology at special faculties or courses will not resolve any of the arising problems if the medical and social problems of mankind and each country or nation are ignored.

From that point of view we regard the answer to the questionnaire as more informative than helpful. The fundamentals of haematology and transfusiology are taught in Poland as a part of internal medicine, and other subjects of the medical studies and postgraduate specialization. To this extent haematology and transfusiology are compulsory subjects in every year of medical training, especially in the fourth and fifth years, in the scope of internal medicine and paediatrics. For those who want to specialize in haematology or transfusiology, the Institute of Postgraduate Medical Education in Warsaw organizes different courses devoted to haematological problems. In the teaching of haematology and transfusiology only the routine methods and techniques are taught.

The organization of the Polish transfusion service is very efficient. The Institute of Haematology in Warsaw, although not a chair of the University Medical School, is operated by well-known academic workers and supervises the whole blood transfusion service which consists of large regional centres and smaller blood transfusion services in hospitals.

In Poland we have no possibility to obtain a scientific degree in haematology or transfusiology. Even professors or associate professors specialized in haematology receive their scientific degree in internal medicine or other medical branches. After the highest (second) degree of specialization in any fundamental medical speciality (e.g. internal medicine, paediatrics) the physician may obtain a specialization in haematological fields. This is separated from other professional specializations.

With the present necessarily narrow way of haematological specialization and the more or less successful integration of haematology with other (medical and non-medical: classical, social and technical) sciences and even arts – our ideas and deeds aim at improving the prevention of proliferative diseases. In view of the leukaemogenic effect of many an industrial product or by-product, especially in the war industry, we postulate an appropriate sanitary control of industry and a limitation of armaments or even better – a total disarmament. Therefore, our Department participates in the Peace Research program of the Polish Academy of Sciences concerning the prophylaxis of proliferative diseases by means of military and psychological disarmament. The problems of prophylaxis are closely related to those of epidemiological, geographical, genetic and social haematology. We study these intricate and interesting problems together with other scientific institutes in order not only to solve some of these questions but also to emphasize their importance to medical students and workers. With anthropometric, dactylographic and enzymatic methods we are attempting to select the predisposed people who should be kept away from the environments invaded by leukaemogens. At the same time we are stressing the leukaemogenic danger of radiation (e.g. abuse of X-rays), of chemical detergents, insecticides and weed-killers. This is why our Department seeks co-operation with the technical and classical sciences in the fight against the diseases of civilization, especially those manifesting themselves with proliferative conditions.

We see the progress of haematology in the solution of the entangled problems of the interactions between biophysical and social environment and the population and its members, especially those with genetic predisposition, between viruses, genes and chromosomes, between ribonucleases and ribonucleins (which also form the subject of our studies). To achieve such a progress it is necessary to include the problems of social haematology not only in medical training but also in medical practice and research and to arouse for these problems the interest of all nations throughout the world.

Book Review

Die Störungen der Bluthämsynthese mit besonderer Berücksichtigung der sideroachrestischen Anämien und erythropoetischen Porphyrien. (Disturbances of blood haeme synthesis, with special reference to sideroachrestic anaemias and erythropoietic porphyrias.) By L. Heilmeyer, Freiburg/Br. Co-authors, R. Clotten and L. Heilmeyer, Jr. August, 1964. 182 pages, 88 illustrations, 35 tables, size 15.5 × 23 cm. Price 56 DM (Georg Thieme Verlag, Stuttgart)

In this excellent monograph the disturbances of haeme synthesis in various pathological conditions, and especially in those specified in the title, are discussed systematically. At the Freiburg Department of Medicine, methods have been developed for the determination of haeme precursors in erythrocytes, urine and faeces. In addition, in incubated mature human erythrocytes the process of porphyrin synthesis from delta aminolaevulinic acid to coproporphyrin has been studied under normal and pathological conditions. It has been demonstrated that in the course of that synthesis the mature erythrocytes of healthy man possess the same enzymes as the nucleated red blood cells of birds, usually involved in experiments of this kind. These methods yielded several interesting results allowing a better insight into the pathophysiology of haeme synthesis.

In the Foreword the hope is expressed that the work will open up new fields for clinical research.

In the first chapters the symptoms and signs of the disturbances of haeme synthesis are dealt with. In the next chapter L. Heilmeyer, Jr. is discussing the problems of biosynthesis of haeme. A special part is devoted to the methods of examination and their critical evaluation, and one, written by R. Clotten, to the determination of porphyrins and their precursors in urine and blood.

This is followed by a chapter on the disturbances of haeme synthesis in iron deficiency, regenerative haemolytic anaemia, sideroachrestic anaemias including pyridoxin-sensitive and other symptomatic forms (lead poisoning, INH, etc.), erythropoietic porphyrias, various haematological conditions (anaemias, panmyelopathies, etc.), and infectious diseases. Haeme synthesis by leucocytes and the disorders of haeme synthesis in diseases of the liver are also discussed.

There is every reason to hope that this excellent book will stimulate further research into haeme synthesis.

The presentation of the book is a praise to the publisher's excellent standard of production.

I. Bernát

Abstracts

Steigerung der Glykolyse durch den Einfluss von Ammoniumionen auf die Phosphofruktokinaseaktivität. (Enhancement of the glycolytic rate by the effect of ammonium ions on phosphofructokinase activity.) F. Kloppick, G. Jacobasch, S. Rapoport (Institut für Physiologische und Biologische Chemie der Humboldt-Universität, Berlin) *Acta biol. med. germ. 18*, 37 (1967).

Ammonium ions at a final concentration of 12—29 mM are increasing glucose breakdown in human erythrocytes, first of all by increasing its utilization through the Embden—Meyerhof-pathway. This effect of the ammonium ions is apparent between pH 7.0 and pH 7.4; it is not detectable at pH 8.0. Glucose-6-phosphate determinations proved that the increase in the glycolytic rate was caused by a disinhibition of phosphofructokinase. The connexion between the stationary hexose phosphate level and the glycolytic rate is discussed with regard to pH dependence and ammonium effect.

Ilma Szász

Die Konzentration von Nukleotiden und anderen säurelöslichen P-Verbindungen im Blut der Ratte während der Erythrozyten-Reifung und Alterung. (Concentration of nucleotides and other acid-soluble phosphorus compounds in maturing and aging erythrocytes of the rat.) B. Oswald, G. Dassler (Institut für Pathologische Physiologie der Friedrich-Schiller-Universität, Jena) *Acta biol. med. germ. 18*, 163 (1967).

In the blood of embryonic (19th and 22nd day of pregnancy), pregnant adult (19th and 22nd day of pregnancy), young (5, 10, 20 and 30 days old) as well as healthy anaemic and artificially polycythaemic adult rats, nucleotides and inorganic phosphorus, hexose monophosphate (HMP) and 2,3 diphosphoglyceric acid (2,3DPG) from erythrocytes were separated by ion exchange chromatography, and analysed qualitatively and quantitatively. Inorganic phosphorus, HMP and all nucleotides are present in rat reticulocytes at high concentration, only the 2,3DPG level is low. About 50% of the total nucleotides consists of adenine nucleotide. The reticulocytes of adult anaemic rats differ from those of embryos and young animals by a strikingly high guanosine-5'-triphosphate content.

Ilma Szász

Über den Einfluss des anorganischen Phosphats auf die Glykolyse; seine Unwirksamkeit auf die Hexokinase des Menschenerythrozyten. (Effect of inorganic phosphate on glycolysis; its uneffectiveness on the hexokinase of human erythrocytes.) G. Gerber, E. Kloppick, S. Rapoport (Institut für Physiologische und Biologische Chemie der Humboldt-Universität, Berlin) *Acta biol. med. germ. 18*, 305 (1967).

The effect of inorganic phosphate at a final concentration of 10 mM on the hexokinase activity of human erythrocytes in the presence of glucose-6-phosphate as inhibitor has been studied. Hexokinase was separated from phosphohexoisomerase by ion exchange chromatography. Inorganic phosphate had no effect on this purified hexokinase, though in stroma-free haemolysates it caused the disinhibition of hexokinase. The explana-

tion of this is that in haemolysates inorganic phosphate accelerates the degradation of glucose-6-phosphate by activating phosphofructokinase. This is important from the point of view of glycolysis regulation, because it means that the hexokinase reaction can be excluded as a direct site of action of inorganic phosphate and only phosphofructokinase and glyceraldehydephosphate dehydrogenase can be regarded as the possible sites of P_i action.

Ilma Szász

Bestimmung des Blutzell-und Blutplasmagehaltes von Leber, Niere und Milz der Ratte. (Determination of the blood cell and blood plasma content of liver, kidney and spleen of the rat.) J. G. Reich, U. Till, H. Frunder (Physiologisch-Chemisches Institut der Friedrich-Schiller-Universität, Jena) *Acta biol. med. germ. 18*, 383 (1967).

The erythrocyte and blood plasma content of the rat liver, kidney and spleen has been investigated by the ^{32}P, the haemoglobin and the Evans blue methods. The procedure with ^{32}P labelled erythrocytes proved to be the most sensitive and reliable. For routine studies it is not suitable because its sources of error cannot be controlled. The determination of the organ erythrocyte content by haemoglobin measurements yields reliable results if extraction of haemoglobin is made with digitonin containing isotonic saline and haemoglobin estimation in the turbid organ extracts is performed by a 3 wavelength method. The cyanhaemiglobin procedure is correct but involves higher standard errors. The Evans blue method is suitable for the determination of the plasma content for organs. However, the haematocrit value in the blood of the organs differs considerably from that in peripheral blood. In liver and kidney it is lower, in spleen it is significantly higher than in venous blood. The blood plasma content of the investigated organs amounts to 18% of the wet weight.

Ilma Szász

Studies on the preservation of human blood. II. The relationship of the erythrocyte adenosine triphosphate levels and other in vitro measures to red cell storageability. R. J. Dern, G. J. Brewer, J. J. Wiorkowski (Department of Medicine, Stritch School of Medicine, Ann Arbor, Mich.) *J. Lab. clin. Med. 69*, 968 (1967).

Blood samples of 37 donors were stored at 4°C for 21 and 28 days in either ACD or CPD anticoagulant. Subsequently, ATP, osmotic fragility, mean corpuscular volume, free plasma haemoglobin, NH_3, dextrose, inorganic phosphate, plasma chloride, potassium, sodium and pH, were estimated and viability of red cells *in vivo* was determined by a 24 hour postautotransfusion survival method. The highest degree of correlation between determinations *in vitro* and *in vivo* was found in the case of the poststorage ATP level. This correlation was great enough to raise the possibility of the use of ATP determination in stored blood to predict *in vivo* RBC viability. Beside ATP only osmotic fragility was moderately correlated with poststorage viability. The correlation between prestorage ATP levels and poststorage RBC viability was not of predictive value.

Ilma Szász

Sodium and potassium permeability of red blood cells in dependence of the pH. K. Pfleger, W. Rummel, E. Seifen (Department of Pharmacology and Toxicology, University of Saarland, Homburg/Saar) *Pflügers Arch. ges. Physiologie 295*, 255 (1967).

Permeability of rabbit red cells to sodium and potassium ions was found to depend on pH. Sodium net flux showed a maximum at pH 6.6, potassium at pH 7.0. Isotope studies revealed that the pH-dependent maxima of the net flux were due to an increase of the downhill movements, while uphill movements were slightly influenced. Both uphill and downhill movements have Q_{10} values of more than 2.0. Structural elements of the membrane with ampholytic character such as proteins and phosphatides are assumed to be responsible for the change in permeability induced by variations in pH.

Ilma Szász

Synthesis and use of O-stearoyl polysaccharides in passive hemagglutination and hemolysis. V. Hämmerling and O. Westphal (Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen) *J. Biochem. 1*, 46 (1967).

Lipid-free polysaccharides generally are not attached to the surface of erythrocytes.

In contrast O-stearoyl derivatives of polysaccharides show a high affinity for red blood cells. By reaction of polysaccharides with stearoyl chloride in dimethyl formamide as a solvent in the presence of pyridine, derivatives are obtained which exhibit marked erythrocyte-sensitizing activity. The chemical conditions for optimal sensitization of erythrocytes were investigated for a number of polysaccharides, e.g. starch, dextran and several lipid-free bacterial cell wall polysaccharides. A content of about 5 % by weight of O-stearoyl groups has been found most suitable for producing erythrocyte-sensitizing properties without modifications of the serological specificity of the polysaccharide. Sheep erythrocytes coated with O-stearoyl polysaccharide are specifically agglutinated by anti-polysaccharide antibody (passive hemagglutination) or lyzed when guinea pig complement is present at the same time (passive hemolysis). Further applications of the method by using derivatives of O-stearoyl polysaccharides are mentioned.

Elisabeth Török

Иммуногематологические исследования костного мозга при анемиях. (Immunohaematological bone marrow changes in anaemia.) N. I. Syubaeva (Military Medical Academy, Leningrad) *Probl. Gemat. 12*, 16 (1967).

Employment of the antiglobulin reaction with bone marrow cells in 36 patients with various types of anaemia has offered a possibility of detecting antibodies against bone marrow in hypo- and aplastic anaemias. Such antibodies were also revealed in cases of macrocytic haemolytic anaemia with a negative direct Coombs test. No antibodies against bone marrow were found in patients with iron-deficiency and B_{12}-deficiency anaemia.

Natalia Tomka

The first example of auto-anti-Xg^a. M. Yokoyama, D. T. Eith, M. Bowman (The Kuakini Research Foundation and St. Francis Hospital, Honolulu, Hawaii) *Vox Sang.* (Basel) *12*, 138 (1967).

The sex-linked antigen, Xg^a, controlled by a gene on the X chromosome, was first described by Mann et al. in 1962. A patient, who had been transfused many times, was found at crossmatching to have an antibody to a "new" antigen which was named Xg^a. Two further examples were reported subsequently. The present authors observed the same antibody in a 6 para 4 pregnant patient who thus represented a case of haemolytic anaemia with an autoimmune antibody produced against her own red cells. The antibody, demonstrated in her serum and in eluates from her red cells, was found to be anti-Xg^a and is the first case of auto-anti-Xg^a and the first example of the antibody to be found in a woman.

Jolán Simon

Prenatal T-Transformation? J. R. Jørgensen (Blood Bank, University Hospital, Copenhagen) *Vox Sang.* (Basel) *13*, 225 (1967.

Polyagglutinability means a change in the antigenic structure of red cells so that they are agglutinated by the majority of normal human adult sera. The *in vivo* phenomenon is found both as a permanent and as a transient condition. Permanent polyagglutinability is very rare. Transient polyagglutinability has a somewhat greater incidence, and is seen especially in patients with severe infections, but may also occur in apparently healthy subjects. It is generally considered a so-called "T-transformation". Transient polyagglutinability occurs in all age groups, but the incidence is greatest among young subjects. It has not yet been observed in children under the age of 4 weeks. An account is given of the first case of transient polyagglutinability in a newborn infant who also had icterus gravis. Polyagglutinability is interpreted as a prenatally occurring T-transformation of the foetal erythrocytes. No infection which might have caused such a T-transformation could be demonstrated. A causal relationship between polyagglutinability and neonatal jaundice is considered unlikely.

Jolán Simon

Seltene Doppelimmunisierung als Folge dreier Schwangerschaften und einer Bluttransfusion. (Rare double-immunization following three pregnancies and one blood transfusion.)

W. Schneider (DRK-Blutspendedienst Niedersachsen, Institut Rotenburg) *Ärztl. Lab. 13*, 288 (1967).

Homospecific immunization by pregnancies and/or transfusions of blood are an indirect danger not only for the child, but also for the mother if she urgently requires blood transfusion, since frequently the procuring of suitable donor blood may present difficulties. This applies particularly to cases with antibodies outside the Rh-system and Rh-antibodies not directed against the factor D. This observation of immunization against factor C, with haemolytic disease of the newborn and subsequent additional immunization against the Kell-factor by transfusion of only one Kell-positive preserve in childbed, calls for preventive serological examination of pregnant women and explains the strong antigenicity of the Kell-factor.

Jolán Simon

Successfully operated giant liver haemangioma in a newborn infant. (An unusual case of Kasabach—Merritt's syndrome.) Gy. Korányi, A. Rajk, J. Róbert (Péterfy Sándor Municipal Hospital, Budapest) *Helv. paediat. Acta 22*, 201 (1967).

A newborn with 3400 g birthweight was admitted on the second day of life with the suspicion of intestinal obstruction — then was marked jaundice, with an indirect serum bilirubin value of 19.7 mg. In the abdomen a tumour of apple size was palpable, X-rays showed on the left side under the diphragm a homogeneous shadow with convex lower edge. The tumour increased rapidly in size. Surgery on the next day revealed a firm bleeding tumour in the left hepatic lobe which then was resected and on account of a transversal fissure, the spleen was also removed. The cavernous haemangioma weighed 190 g and measured $10 \times 10 \times 5$ cm. The operation was tolerated well, recovery was complete in three weeks. Another interesting feature of the case was the massive haemolysis with jaundice, without incompatibility. After the intervention, the jaundice vanished rapidly. The case was at the same time a special instance of Kasabach—Merrit's syndrome. Neonatal thrombopenia prevented the development of thrombocytosis likely to occur after splenectomy. The authors believe that the cause of both haemolysis and thrombocytopenia was the congestion in the tumour which acted as an arterio-venous shunt. In the literature 4 cases of surgically treated neonatal liver haemangioma have been reported but all the patients with a bleeding haemangioma died before or during the operation; the present case was the first to recover.

Autoreferate

Vergleichende Untersuchungen zwischen ACD-Vollblutkonserven, Erythrozytenkonzentrat und Erythrozytenresuspensionen. (Comparative study of ACD-whole-blood preserves, erythrocyte concentrates and resuspended erythrocytes.) D. Hoffmann, H. J. Altenbrunn, H. J. Raderecht (Bezirksinstitut für Blutspende- und Transfusionswesen, Berlin) *Z. ges. inn. Med. 22*, 51 (1967).

1. Erythrocytes stored at 4°C in glucose-saccharose-citrate solution were studied (pH, inorganic phosphate, ATP, ADP, potassium, free haemoglobin, osmotic resistance and kinetics of the haemolysis) and compared with ACD-whole-blood preserves. A change of the biochemical criteria was found which was nearly of the same kind. The phenomena of haemolysis appeared earlier in the erythrocyte concentrates. 2. The initial loss determined with ^{51}Cr (second day after infusion) was the same in 20 days old suspensions of erythrocytes and of ACD-whole-blood preserves. In erythrocyte concentrates, however, it was four to five times as much. 3. The half survival time of the erythrocytes determined with ^{51}Cr resisting after the initial loss apparently increases during storage. This is due to an increased reception of ^{51}Cr in erythrocytes which artificially grew old. 4. Apart from the determination of ATP and the kinetics of haemolysis above all the determination of the initial loss of erythrocytes marked by ^{51}Cr is useful for the comparison of the efficiency of conservation ingredients. 5. The erythrocytes in the ACD-whole-blood preserves and the suspension of erythrocytes are of the same value concerning their durability. Erythrocyte concentrates stored for more than two weeks should not be used for cell substitution.

Judith Pintér

Metabolic changes in human red cells during incubation of whole blood in vitro. B. D. Gomperts (University College Hospital, Medical School, London) *Biochem J. 102*, 782 (1967).

Whole blood was incubated at 37° C while being dialyzed against a large volume of iso-osmotic bicarbonate-buffer pH 7.4. The buffer contained glucose and the essential inorganic components of blood plasma in proportion. After 3 hours incubation there was a loss of red cell 2,3 diphosphoglycerate. Isotope experiments have shown this to have been due to an accelerated rate of destruction of the compound. Simultaneously, there was an increase in red cell osmotic fragility. After prolonged incubation there was a decrease in metabolic rate and in the ratio lactate production to glucose consumption. There is a continuous loss of total adenine nucleotide and after the first 12 hours of incubation, a tendency for intracellular and extracellular Na^+ and K^+ to become equilibrated. The standard deviation of osmotic fragility of the red cells increased exponentially with the period of incubation.

Georgina Sik

Cytologic studies of enzymes in malignant tissues. A. Elizalde, P. Miller (Division of Oncology, Department of Medicine, Maimonides Hospital, Brooklyn, N. Y.) *Cancer 20*, 423 (1967).

In the aspirated and smeared malignant cells and bone marrow cells of the patients acid and alkaline phosphatase, aminopeptidase and nonspecific esterase activity have been studied by cytochemical techniques. Certain patterns have been determined for a number of tumours and these might allow to establish their primary site. Distinction between certain anaplastic carcinomas and lymphoreticular sarcoma is possible on the basis of enzyme studies. Similar distinctions appear to be true for pulmonary and genitourinary tract carcinomas but it was not feasible to determine a general method for distinguishing anaplastic carcinoma from sarcoma. Further study of the diagnostic value of the technique appears to be warranted.

Georgina Sik

CO_2 formation from formate in red blood cells. S. Rapoport, M. Müller (Institut für Physiologische und Biologische Chemie der Medizinischen Fakultät der Humboldt-Universität, Berlin) *Acta biochim. pol. 14*, 143 (1967).

The mechanism of CO_2 formation from formate by rabbit red blood cells was studied using aminotriazole as specific inhibitor of catalase. Inhibition occurs only at low H_2O_2 and formate concentrations. Both erythrocytes and reticulocytes produce CO_2 from formate under endogenous conditions, which indicates the existence of stationary concentrations of both formate and H_2O_2. The more immature the cell, the lower the concentration of H_2O_2 and the higher that of formate. Inhibition studies with aminotriazole, cyanide and azide indicated that CO_2 formation proceeds via catalase. Data on duck erythrocytes which contain about 0.1% of the catalase activity of rabbit red blood cells, have confirmed the assumption.

Elisabeth Török

From the International Literature on Haematology

Acta Haematologica (Basel) **37** (1967) No. 5—6.

PAS-positive erythroblasts in kidney diseases. *Klein, H. O.* et al. (Univ. of Cologne, Inst. of Pathol., Köln), p. 225.

Die Lokalisation der unspezifischen Esterase in foetalen Erythrozyten und deren Vorstufen. *Peters, H.* (Pathol. Inst., Univ. of Kiel, Kiel), p. 240.

Studies on the metabolism of iron sorbitol. *Nakao, K.* et al. (Univ. of Tokyo, 3rd Dept. of Intern. Med., Tokyo, Japan), p. 253.

Study on nine families with haemoglobin Lepore in Campania. Hb Lepore trait, heterozygosity for Hb Lepore and β-thalassaemia, homozygosity for Hb Lepore. *Quattrin, N.* et al.(Ospedale Cardarelli Italia), p. 266

Hereditary methemoglobinemic cyanosis due to diaphorase deficiency in three successive generations. *Özsoylu, S.* (Ankara Univ., Hacettepe Faculty of Med., Ankara, Turkey), p. 276,

Hereditary methemoglobinemia due to DPNH-methemoglobin reductase deficiency. Report of a family. *Angelopoulos, B.* et al. (Dept. of Pathol. and Physiol., Univ. of Athens, Greece), p. 284.

Metabolism of leucocytes in a case of acute leukemia. *Salen, G.* et al. (Dept. of Gastroenterol., Jefferson Med. College, Philadelphia, Penn.), p. 294.

Early appearance of blast-like cells in the thoracic duct lymph of rats given bacterial endotoxins. *Haemmerli, Gisela* et al. (Pathol. Inst., Univ. of Zurich, Switzerland), p. 301.

Hematological and serological behaviour of chicks inoculated with myeloblastosis virus. *Gullbring, B.* et al. (Karolinska Sjukhuset, Stockholm, Sweden), p. 311.

Blood (New York) **29** (1967) No. 4.

The Chediak-Higashi syndrome: Cytoplasmic sequestration in circulating leukocytes. *White, J. G.* (Univ. of Minnesota, School of Med., Dept. of Pediat., Minneapolis, Minn.), p. 435.

Cyclic neutropenia in grey collie dogs. *Lund, J. E.* et al. (Washington State Univ., Dept. of Veterinary Pathol., Pullman, Washington), p. 452.

In vitro development of bone marrows from patients with neutropenia. *Lau, P.* et al. (Univ. of Pennsylvania, School of Med., Dept. of Med., Philadelphia, Pa.), p. 462.

Studies on the regulation of hemopoietic spleen colonies. *Bleiberg, I.* et al. (Weizmann Inst., Scientific Section of Cell Biology, Rehovoth, Israel), p. 469.

Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. *Gross, T. Ruth* et al. (Albert Einstein College of Med., Dept. of Pediat., New York, N. Y.), p. 481.

Conversion of cyanocobalamin to a physiologically occurring form. *Reizenstein, P.* (Karolinska Hosp., Dept. of Internal Med., Stockholm, Sweden), p. 494.

Separation of vitamin B_{12}-binding proteins of serum, gastric juice and saliva by rapid DEAE cellulose chromatography. *Retief, F. P.* et al. (Mt. Sinai Hosp. and School of Med., Dept. of Hematol., New York, N. Y.), p. 501.

A new technic for the production of an in vivo labeled fibrinogen. *Gans, H.* et al. (Univ. of Minnesota, School of Med., Dept. of Surg., Minneapolis, Minn.), p. 517.

The uptake of fibrin and fibrin-degradation products by the isolated perfused rat liver. *Gans, H.* et al. (Univ. of Minnesota, School of Med., Dept. of Surg., Minneapolis, Minn.), p. 526.

Disseminated eosinophilic collagen disease. *Pierce L. E.* et al. (Mercy Hospital, Buffalo, N. Y.), p. 540.

Brief report: The Di Guglielmo syndrome: studies in hemoglobin synthesis. *Necheles, Th. F.* et al. (New England Med. Center Hosp., Blood Res. Lab., Boston, Mass.), p. 550.

Chronic lymphocytic leukemia — an accumulative disease of immunologically incompetent lymphocytes. *Dameshek, W.* (Mt. Sinai Hosp., Dept. of Hematol., New York., N. Y.), p. 566.

Influence of immunologic hyperstimulation on murine viral leukemogenesis. *Siegel, B. V.* et al. (Univ. of Oregon Med. School, Dept. of Pathol., Portland, Oregon), p. 585.

Comparative response of normal human thymus and lymph node cells to phytohemagglutinin in culture. *Winkelstein, A.* et al. (Univ. of California, Dept. of Med., Los Angeles, Calif.), p. 594.

Cytoplasmic fibrils in mixed lymphocyte cultures. *Parker, J. W.* et al. (Univ. of South California, School of Med., Dept. of Pathol., Los Angeles, Calif.), p. 608.

Reutilization of DNA breakdown products from lymphocytes in lumen of intestine. *Kotani, M.* et al. (Kyoto Univ. Med. School, Kyoto, Japan), p. 616.

A method for counting phytohemagglutinin-stimulated lymphocytes. *Stewart, C. C.* et al. (Univ. of Pennsylvania, School of Med., Dept. of Pathol., Philadelphia, Pa.), p. 628.

The effect of phytohemagglutinin upon glucose catabolism in lymphocytes, *MacHaffie R. A.* et al. (Oregon State Univ., Student Health Service, Corvallis, Oregon), p. 640.

Purification without loss of viability of neoplastic plasma-cells from the ascites passaged mouse myeloma, X5563. *Gordon, B. L.* et al. (Univ. of California, School of Public Health, Berkeley, Calif.), p. 647.

Chronic myelocytic leukemia with two Philadelphia chromosomes and prominent peripheral lymphadenopathy. *Duvall, Ch. P.* et al. (National Cancer Inst., Med. Branch, Bethesda, Md.), p. 652.

Fine structural demonstration of acid phosphatase activity in Auer bodies. *White, J. G.* (Univ. of Minnesota School of Med., Dept. of Pediat., Minneapolis, Minn.), p. 667.

Leukocytosis, bone marrow hyperplasia and leukemia in chronic magnesium deficiency in the rat. *McCreary, Patricia A.* et al. (Presbyterian St. Lukes Hosp., Dept. of Med., Chicago, Ill.), p. 683.

A plasma factor in chronic lymphocytic leukemia which stimulates DNA synthesis and blastogenesis in leukocyte cultures. *Kasakura, Sh.* et al. (McGill Univ., Royal Victoria Hosp., Dept. of Med., Montreal, Quebec, Canada), p. 691.

Blut (München) **15** (1967) No. 1.

Die RHS-Clearance wärmeveränderter Erythrozyten, ein neues Kriterium bei Erkrankungen des Knochenmarks. *Fischer, J.* et al. (Med. Univ.-Klinik. Langenbeckstrasse 1, 65 Mainz), p. 1.

Erythroblasten mit elliptischen Kernen. *Löffler, H.* et al. (Med. Univ.-Klinik, Klinikstrasse 32b, 62 Giessen), p. 7.

Vergleich zweier Bestimmungsmethoden der alkalischen Leukozytenphosphatase. *Überla, K.* et al. (Inst. f. Med. Statistik u. Dokumentation, Langenbeckstrasse 1, 65 Mainz), p. 13.

Zur Anwendung enzymhistochemischer Methoden an bioptisch gewonnenem Knochenmarkgewebe. *Lorbacher, P.* (New England Center Hosp., Pratt Clinic, Boston, Mass.), p. 19.

Zytochemische Untersuchungen bei unreifzelligen Leukosen des Kindesalters sowie Pleura- u. Peritonealergüssen des Erwachsenen. *Neth, R.* et al. (Univ. Kinderklinik, Martinistrasse 52, Hamburg-E), p. 29.

Verhinderung schwerer Meno- u. Metrorrhagien bei hämatologischen Erkrankungen durch Ovulationshemmer. *Braunsteiner, H.* et al. (Med. Univ.-Klinik, Innsbruck, Austria), p. 31.

Therapieergebnisse bei Behandlung primärer u. sekundärer Hämochromatosen mit Desferal. *Wöhler, F.* (Med. Univ.-Klinik, Hugstetterstrasse 55, 78 Freiburg i. Br.), p. 33.

Neuere Gesichtspunkte zur Methodik u. Anwendung der Autoradiographie. *Dörmer, P.* (Inst. f. Hämatologie, Landwehrstrasse 61, 8 München 15), p. 42.

Blut (München) **15** (1967) No. 2.

Hämolytisch-urämisches Syndrom im Erwachsenenalter. *Schubothe, H.* et al. (Med. Univ.-Klinik, Hugstetterstrasse 55, 78 Freiburg i. Br.), p. 65.

Lebensdauer und Abbauort menschlicher Thrombozyten. *Bleifeld, W.* et al. (Abtlg. Innere Med. I. der Klinischen Anstalten, 51 Aachen, Goethestrasse), p. 70.

Untersuchungen zur funktionellen Differenzierung der mononukleären Leukozyten. *Trepel, F.* et al. (Städt. Krankenhaus München-Schwabing, I. Med. Abtlg., Kölner Platz 1, 8 München 23), p. 76.

Die Proliferationskinetik des Plasmocytoms in vitro und ihre Beeinflussung durch Stilbamidin. *Boll, Irene* (Rudower Strasse 56, 1 Berlin), p. 82.

Die Myelotomie bei unklaren Säuglingsanämien, dargestellt an zwei Krankheitsfällen von Myelofibrose. *Ruhrmann, G.* (St. Hedwig-Klinik, 84 Regensburg, p. 89.

Quantitative Hämagglutination. *Allert, U.* et al. (Radiol. Inst. der Univ., 78 Freiburg i. Br., Albertstrasse 23), p. 96.

Gen-Defekte im Haptoglobin-System. *Schwerd, W.* et al. (Inst. f. Gerichtliche u. Soziale Med. der Univ., 87 Würzburg, Versbacher Landstrasse), p. 99.

Die immunhämatologische Laboratoriumsdiagnostik autoantikörperbedingter Anämien. *Mueller-Eckhardt, Ch.* (Med. Klinik u. Poliklinik, Justus-Liebig-Univ., 63 Giessen), p. 101.

Blut (München) **15** (1967) No. 3.

Ergebnisse cytologischer und cytochemischer Untersuchungen an Lymphozyten in vitro. *Fischer, R* et al. (Univ. Bonn, Pathol. Inst., Bonn), p. 125.

Über den Mechanismus der Lactatbildung in Schweineerythrozyten bei Einsatz verschiedener Substrate. *Reinauer, H.* et al. (Univ. Düsseldorf, Physiol. Chem. Inst., Düsseldorf), p. 133.

Das Verhalten des Knochenmarkes bei der tierexperimentellen Eiweissmangelanämie unter Behandlung mit anabolen Steroiden. *Hunstein, W.* et al. (Med. Univ. Göttingen, Göttingen), p. 142.

Adenosintriphosphatase activity in hereditary spherocytosis. *Mircevová, L.* et al. (Inst. Hematol. and Blood Transf, U nemocnice 1, Prague 2), p. 149.

Iron deficiency as a possible contributory factor in the development of dislocation of the hip. *Aksoy, M.* et al. (Capa Intern. Clinic, Istanbul Med. School, Istanbul, Turkey), p. 153.

Autoradiographische Untersuchungen lymphoproliferativer Erkrankungen nach in-vitro-Markierung. *Rastetter, J.* et al. (Med. Abtl. des Städtischen Krankenhauses, München), p. 157.

Blut (München) **15** (1967) No. 4.

Kristallisation von reinem fetalem Hämoglobin aus menschlichem Nabelblut. *Stöffler, G.* et al. (Kinderklin. der Univ., 74 Tübingen, Rümelinstrasse 23), p. 193.

Lysosomen: Untersuchungen zur Feinstruktur und einfacher polarisationsoptischer Nachweis. *Missmahl, H. P.* et al. (Med. Univ. Klinik, 74 Tübingen, Otfried-Müller-Strasse), p. 198.

Verlust der spezifischen Thrombozytenantigene bei bösartigen Geschwülsten. *Májsky, A.* et al. (Inst. f. Hämatol. und Bluttransf., U nemocnice 1, Prag 2), p. 201.

Untersuchungen über die Einwirkung verschiedener Hämodiluentien auf das Blut bei der extrakorporalen Perfusion. *Leutschaft, R.* (Chirurgische Univ.-Klinik, 852 Erlangen), p. 206.

Das Verhalten der Faktoren des "Prothrombin-Komplexes" bei der Säulenchromatographie an Anionenaustauschern. *Voss, D.*

(Med. Univ.-Poliklin., 355 Marburg/Lahn, Robert-Koch-Strasse 7/a), p. 214.

Elektronenspinresonanz-Untersuchungen an Hämoglobinkomplexen. *Rein, H.* et al. (Berlin-Buch, Inst. f. Pharmakologie der Deutschen Akad. der Wissenschaften), p. 221.

Die Wirkung von heterologem Antilymphozytenserum auf die homologe und heterologe Knochenmarksübertragung bei supraletal bestrahlten Mäusen. *Thierfelder, S.* et al. (Inst. f. Hämatol., 8 München 15, Landwehrstrasse 61), p. 225.

British Journal of Haematology (Oxford) **13** (1967) No. 2.

Familial thrombocytopenic thrombocytopathy. *Cullum, C.* et al. (Stanford Univ. School of Med., Dept. of Med., Palo Alto, Calif.), p. 147.

Erythropoietic cell proliferation during recovery from acute haemorrhage. *Lord B. I.* (Christie Hosp., Holt Radium Inst., Manchester, Lancash., Great Britain), p. 160.

Diffusion chamber studies of rat peripheral leucocytes in the presence of phytohaemagglutinin. *Johnson, L. I.* et al. (New York Univ., Dept. of Biol., New York, N.Y.) p. 168.

The effect of age on the creatine in red cells. *Griffiths, W. J.* et al. (St. Thomas Hosp., Med. School., Dept. of Haemat., London), p. 175.

Platelets in the carcinoid syndrome: A chemical and ultrastructural investigation. *Crawford, N.* et al. (Univ. of Birmingham, Dept. of Physiol. Chemistry, Birmingham, Warwick, Great Britain), p. 181.

Glandulae fever and specific viral infections: uptake of tritiated thymidine by circulating leucocytes. *Besser, G. M.* et al. (St. Bartholomew Hosp. Med. College, London), p. 189.

An electron microscope study of bone marrow in rheumatoid disease. *Wardle, E. N.* et al. (Univ. of Manchester, Dept. of Clinical Haematol., Manchester, Lancash., Great Britain), p. 194.

Physiology of the regulation of platelet production. *Gabriele, G. de* et al. (London Hosp., London E. 1), p. 202.

Regulation of platelet production: "Thrombopoietin". *Gabriele, G. D. de* et al. (London Hosp., London E. 1), p. 210.

Patient variation in pernicious anaemia, as shown in a clinical trial of cyanocobalamin, hydroxocobalamin and cyanocobalamin-zinc tannate. *Tudhope, G. R.* et al. (Queens College, Dept. of Pharmacol., Dundee, Scotland), p. 216.

Separation of lymphocytes of rat bone marrow by combined glass-wool filtration and dextran-gradient centrifugation. *Morrison, J. H.* (Kent State Univ., Dept. of Biol. Science, Kent, Ohio), p. 229.

The aplastic anaemia-paroxysmal nocturnal haemoglobinuria syndrome. *Lewis, S. M.* et al. (Royal Postgraduate Med. School, Dept. of Haematol., London W. 12), p. 236.

Haemoglobin F Texas I ($alpha_2gamma_2$ 5 Glu → Lys): A variant of haemoglobin F. *Jenkins, G. C.* (London Hosp., London E. 1), p. 252.

The cellular basis for the defect in haemopoiesis in flexed-tailed mice. II. The specificity of the defect for erythropoiesis. *Fowler, H. J.* et al. (Univ. of Toronto, Dept. of Biophysics and Med., Toronto, Ont., Canada), p. 256.

British Journal of Haematology (Oxford) **13** (1967) No. 3.

The nature and significance of platelet products in human plasma. *Wolf, P.* (Dept. of Exp. Pathol., Univ. of Birmingham, Great Britain), p. 269.

Histochemical studies on leucocyte alkaline phosphatase in non-leukaemic granulocytosis: effects of anticoagulants and role of zinc and magnesium. *Perillie, P. E.* et al. (Yale Univ. School of Med., Dept. of Intern. Med., New Haven, Conn.), p. 289.

Red cell life span in iron deficiency anaemia. *Loría, A.* et al. (Inst. Nacional de la Nutrición, Calle Dr. Jiménez 261, México 7, D. F.), p. 294.

Haemoglobin J-Bangkok: A clinical, haematological and genetical study. *Pootrakul, S.* et al. (Div. of Haemat., Univ. of Med. Sci., Bangkok, Thailand), p. 303.

The effect of cortisol on the rate of autohaemolysis of red cells incubated in vitro.

Morley, A. A. (Haemat. Dept., Royal Postgraduate Med. School, Ducane Road, London, W. 12), p. 310.

Potentiation of purified erythropoietin with serum proteins. *Gardner, E., Jr.* et al. (Dept. of Med., Med. College of Georgia, Augusta, Ga.), p. 317.

Atypical hereditary spherocytosis: Biochemical studies and sites of erythrocyte destruction. *Zail, S. S.* et al. (Dept. of Haemat., South African Inst. for Med. Research, Johannesburg, S. A.), p. 323.

Accumulation of iron in mitochondria of erythroblasts. *Goodman, J. R.* et al. (Research Laboratories, Veterans Administration Hosp., San Francisco, Calif.), p. 335.

The fine structure of fibrin. *Kay, D.* et al. (Sir William Dunn School of Pathol., Univ. of Oxford, Oxford), p. 341.

Haematological effects of the idiopathic splenomegaly seen in Uganda. *Richmond, J.* et al. (Dept. of Med., Univ. of Edinburgh, Scotland), p. 348.

A diffraction method for measuring the average volumes and shapes of red blood cells. *Child, J. A.* et al. (Dept. of Haemat., Guy's Hosp., London), p. 364.

The fibrinolytic response to moderate exercise and intravenous adrenaline in the same subjects. *Cash, J. D.* et al. (Southeast Scotland Reg. Blood Transf. Research Laboratories, Royal Infirmary, Edinburgh, Scotland), p. 376.

Regulation of platelet production: "Hypersplenism" in the experimental animal. *Gabriele, G. de* et al. (Commonwealth Serum Laboratories, Melbourne, Australia), p. 384.

Köln haemoglobinopathy. Further data and a comparison with other hereditary Heinz body anaemias. *Jones, R. V.* et al. (North West Surrey Group Lab., St. Peter's Hosp., Chertsey, Surrey, Great Britain), p. 394.

The platelet destruction site in thrombocytopenic purpuras. *Najean, Y.* et al. (Laboratoire de Isotopes, Centre de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, Paris), p. 409.

Folia Haematologica (Leipzig) **87** (1967) No. 1/2.

Die Entwicklung von Fibrinolysehemmstoffen. *Markwardt, F.* (Pharmakologisches Inst. der Med. Akademie, 50 Erfurt), p. 5.

Diagnose u. Differentialdiagnose der hyperfibrinolytischen Blutungen. *Vogel, G.* (Med. Klinik der Med. Akademie, 50 Erfurt), p. 15.

Klinische Erfahrungen mit p-Aminomethylbenzoesäure (PAMBA) bei schweren akuten Fibrinolyseblutungen in der inneren Medizin. *Sundermann, A.* et al. (Med. Klinik der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 22.

Fibrinolyseblutungen in der Chirurgie u. ihre Behandlung mit p-Aminomethylbenzoesäure. *Hellinger, J.* (50 Erfurt, Reichartstrasse 19), p. 32.

Das Bild der fibrinolytischen Störung im uterinen Blut u. die Beeinflussbarkeit durch PAMBA. *Flähmig, M.* et al. (Frauenklinik der Med. Akademie, 50 Erfurt, Walkmühlstrasse 7), p. 41.

Die Bedeutung der Fibrinolysesteigerung bei Nachgeburtsblutungen u. ihre Behandlung mit PAMBA. *Sieg, U.* et al. (Frauenklinik der Med. Akademie, 50 Erfurt, Walkmühlstrasse 7), p. 51.

Untersuchungen über die fibrinolytische Aktivität des Liquor cerebrospinalis u. ihre Beeinflussbarkeit durch PAMBA. *Hellinger, J.* et al. (50 Erfurt, Reichartstrasse 19), p. 61.

Anwendung von PAMBA in der Zahn- u. Kieferheilkunde. *Rothe, G.* 50 Erfurt, Augustinerstrasse 38), p. 67.

Hyperfibrinolytische Blutungen in der HNO-Heilkunde u. ihre Behandlung mit PAMBA. *Nuernbergk, W.* (Hals-Nasen-Ohren-Klinik der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 72.

Einfluss von p-Aminomethylbenzoesäure (PAMBA) auf den fibrinstabilisierenden Faktor. *Vogel, G.* et al. (Med. Klinik der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 77.

Begleitfibrinolysen bei hämatologischen Erkrankungen u. ihre Beeinflussung durch PAMBA. *Mey, U.* et al. (Med. Klinik der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 80.

Fibrinolyseblutungen im Kindesalter u. ihre Behandlung mit PAMBA. *Hoffmann, W.* et al. (Kinderklinik der Med. Akademie 50 Erfurt, Am Schwemmbach 32/a), p. 88.

Zur Wirkung der Para-Aminomethylbenzoesäure (PAMBA) bei Polyarthritis acuta u. progressiva. (Vorläufige Mitteilung.) *Wessel G.* (Med. Klinik der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 94.

Zur Dosierung der Antifibrinolytika im Neugeborenenalter. *Rogner, G.* (Kinderklinik u. Poliklinik der Univ., 69 Jena, Kochstrasse 2), p. 100.

Vergleichende Untersuchungen über Antifibrinolytika. *Landmann, H.* (50 Erfurt, Schillerstrasse 58), p. 106.

Über die Kombinationswirkung von PAMBA u. Contrykal. *Klöcking, H.-P.* (Pharmakologisches Inst. der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 113.

Die antifibrinolytische Wirkung von p--Aminomethylbenzoesäure in Bakterien- u. Gewebekulturen. *Perlewitz, J.* (Pharmakologisches Inst. der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 118.

Bestimmungsmethoden für Fibrinolysehemmstoffe im biologischen Material. *Richter, M.* (Pharmakologisches Inst. der Med. Akademie, 50 Erfurt, Nordhäuserstrasse 74), p. 123.

Untersuchungen über den Mechanismus der Strahlenschutzwirkung der proteolytischen Enzyme. *Danysz, A.* et al. (Pharmakologisches Inst. der Med. Akademie, Bialystok, Polen), p. 128.

Postoperative Komplikationen durch erworbenen Faktor-XIII-Mangel. *Thies, H. A.* et al. (Chirurgische Klinik, 71 Heilbronn/Neckar, DBR), p. 135.

Folia Haematologica (Leipzig) **87** (1967) No. 3.

Ein Beitrag zur Nachweismethodik zirkulierender Tumorzellen im peripheren Blut. *Baudach, H.* et al. (Robert-Rössle-Klinik, 1115 Berlin, Lindenberger Weg 80), p. 153.

Zur Rolle der basophilen Leukozyten und Mastzellen in Fibrinolyse u. Allergie. II. Wirkung eines Antihistaminikums und einer allergischen Sofortreaktion auf Zahl und Degranulierung basophiler Leukozyten. *Frick, G.* et al. (Med.-Univ.-Klinik, 25 Rostock, Ernst Heydemann Strasse), p. 178.

In vitro-Untersuchungen über die Wirkung des Zigarettenrauches auf die Vitalität der Leukozyten. *Schröder, H.* et al. (Inst. f. gerichtliche Med. u. Kriminalistik, Univ. Hamburg, 2 Hamburg-Sollstedt, Butenfeld 34), p. 190.

Beitrag zur Zählung und Differenzierung der Leukozyten aus beiden Körperhälften. *Fuchsberger, R.* (Werkkrankenhaus, Povazanska Bystrica, Leninstrasse 1134-C-1), p. 199.

Das Knochenmarkbild bei Kryoglobulinämiekranken. *Biró, I.* et al. (Postgraduate Med. School, Szabolcs u. 33, Budapest, XIII, Hungary), p. 209.

Untersuchungen des menschlichen Knochenmarkes unter Vinblastinsulfatwirkung. *Kühböck, J.* et al. (2. Med. Univ.-Klinik, 1090 Wien, Garnisongasse 13), p. 220.

Methodischer Beitrag zum Nachweis thrombozytärer Autoantikörper. *Grimm, J.* et al. (Städt. Kinderklinik, 90 Karl-Marx-Stadt, Dresdner Strasse), p. 230.

Nachweis einer diaplazentar übertragenen Immunothrombopenie mit Hilfe der Immunofluoreszenztechnik. *Bast, G.* et al. Bezirkskrankenhaus, Innere Klinik, 25 Rostock-Südstadt), p. 237.

Modifikationen des H-Erythrozytenantigens bei Kranken mit Leukämien. *Májský, A.* (Inst. f. Hämatol. u. Bluttransf., U nemocnice 1, Prag 2), p. 249.

Hp-, Gm-, Inv- und Tf-Typen in Santiago de Cuba (Cuba). *Herzog, P.* et al. (Inst. f. Hämatol. u. Bluttransf., U nemocnice 1, Prag 2), p. 260.

Hémostase (Paris) **6** (1966) No. 5.

Plaquettes et virus. *Maupin, B.* (Centre de Transfusion-Réanimation de l'Armée «Jean-Julliard», 92 Clamart, Paris), p. 301.

Two cases of haemorrhagic disease arising from fibrin stabilizing factor deficiency. *Ottaviani, P.* et al. (Inst. of Med. Semeiotics, Rome Univ., Rome), 317.

Studies on the evaluation of factor XIII (fibrin stabilizing factor) by means of the MIA-tolerance (visual and thrombelastographic technique). *Nicola, P. de* et al. (Med. Clin., Univ. Pavia), p. 325.

De l'adhésivité et de l'agrégation plaquettaires chez les diabétiques traités par l'acide

linolénique. *Doumenc, J.* et al. (Lab. d'Hémostase, Service d'Hématologie de l'Hôtel-Dieu, Parvis Notre-Dame, Paris IVe), p. 333.

The initiation of clotting in lipaemic blood: Contact phase and platelet participation. *Coccheri, S.* et al. (Inst. for Occupational and Preventive Med., Bologna University Med. School, Bologna), p. 339.

Mesure de l'agrégation des plaquettes. Activité antiagrégante de l'héparine. *Aznar, J.* (Départ. de Biochimie, Faculté de Médecine, Univ. Navarra, Pampelune, Espagne), p. 351.

Ellagic acid-induced acceleration of intravascular clotting and thrombolysis. *Iatridis, S. G.* (Dept. of Med. Athena Clinic, Univ. of Athens), p. 363.

Conception nouvelle des troubles de la coagulation sanguine dans l'athérosclérose. *Matchabelli, M.* (Avenue Vaja Pchavela, Quartier 3, bâtiment 20, Tbilissi 49, URSS), p. 373.

A study on hypercoagulability. *Akman, N.* et al. (Div. of Hemat., Cerrahpasa Intern Clinic, Istanbul Univ., Aksaray, Istanbul, Turkey), p. 381.

Il Sangue (Milano) **40** (1967) No. 2.

Considerazioni sulla conservazione degli eritrociti mediante congelamento. *Molla, W.* et al. (Ist. di Transfusione Avis, Milano, Italia), p. 52.

Porpora trombopenica neonatale e malattia emolitica da incompatibilità nel sistema Rh. *Pecorella, F.* et al. (Clinica Pediatrica, Ospedale dei Bambini "G. di Cristina", Univ. Palermo, Italia), p. 65.

Nouvelle Revue Française d'Hématologie (Paris) **7** (1967) No. 2.

Érythropoïétine et érythropoïese. *Adamson, J. W.* et al. (Univ. of Washington, School of Med., Seattle, Washington), p. 157.

Coïncidences ou relations fondamentales entre la leucémogenèse et les antigènes de transplantation. *Dausset, J.* et al. (Hôpital Saint-Louis, 2, place du Dr.-Fournier, F.-75-Paris X), p. 161.

Mécanisme de l'action thérapeutique de la restauration retardée chez les souris irradiées. *Duplan, J. F.* et al. (Inst. du Radium, Lab. Pasteur et Fondation Curie, Paris V), p. 167.

Protoporphyrie érythropoïétique secondaire. *Albahary, C.* et al. (Centre Hosp., Serv. Med. Interne, St. Denis, France), p. 177.

Mesure de l'adhésivité plaquettaire au verre. *Rosner, P.* et al. (Centre de Transf. sanguine, Hôpital univ. Genève), p. 185.

Le test de la filtrabilité érythrocytaire (TFE). Une méthode simple d'étude de certaines propriétés microrhéologiques des globules rouges. *Teitel, P.* (Centre d'Hématologie, Str. dr. Felix 89, Bucarest), p. 195.

Mycoplasmes et hémopathies humaines. *Leclerc, J. C.* et al. (Hôpital Saint-Louis 2, place du Dr.-Fournier, F.-75-Paris X), p. 215.

Nouvelle Revue Française d'Hématologie (Paris) **6** (1967) No. 3.

La rubidomycine. *Bernard, J.* et al. (Hôpital Saint-Louis, 2, place du Dr.-Fournier, F.-75-Paris X), p. 317.

Corrélations entre les caractéristiques microrhéologiques (filtrabilité) des globules rouges et leur séquestration splénique et hépatique. *Teitel, P.* (Centre d'Hématologie, Str. dr. Felix 89, Bucarest), p. 321.

Le fer médullaire dans la mucoviscidose. *Mouriquand, C.* et al. (Clinique Médicale Infantile, Faculté de Médecine de Grenoble, 21, rue Lesdiguières, F. 38-Grenoble, France), p. 339.

Anomalie de la formation du caillot dans un cas de IgG myélome. *Praga, C.* et al. (Istituto di Semeiotica Medica dell'Univ., Milano), p. 353.

La maladie de Burkitt hors d'Afrique. *Willemin-Clog, L.* et al. (Clinique Médicale Infantile, C.H.U., F. 63-Clermont-Ferrand, France), p. 367.

L'association de la radiothérapie endolymphatique et des agents alkylants dans le traitement des hématosarcomes rétropéritonéaux. *Bonadonna, G.* et al. (Istituto Nazionale per lo Studio e la Cura dei Tumori, Univ. di Milano, Italia), p. 381.

Hémoglobinopathies en Tunisie. A propos d'une observation familiale avec étude de l'arbre généalogique. *Ben Rachid, M.S.* et al. (Inst. Pasteur, Tunis), p. 393.

Проблемы гематологиии и переливания крови (Москва) **12** (1967) № 1

К проблеме ремиссий при лейкозах. *Кассирский, И. А., Флейшман, Е. В., Рынская Л. М.* (Клиническая база — Центральная клиническая больница им. Семашко Н. А. Министерства путей сообщения.) с. 3. — Remissions in Leukaemia. *Kassirsky, I. A., Fleishman, E. W., Rynskaya, L. M.* p. 3.

Идиопатический миелофиброз и вторичный миелофиброз при хроническом миелолейкозе. *Дульцин, М. С., Климова, Н. Ф., Черницова, Т. А., Захарова, А. В.* (Гематологическая клиника и цитологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 12. — Idiopathic and Secondary Myelofibrosis in Chronic Myeloleukaemia. *Dultsin, M. S., Klimova, N. F., Cherntsova, T. A., Zakharova, A. V.* p. 12.

К вопросу о патогенезе и перспективах лечения лейкоза. *Александрович Ю., Наскальский, Г., Шнайд, Я.* (3-я кафедра и клиника внутренних болезней Краковской медицинской академии, Польша) с. 24. — Pathogenesis of Leukaemia and Prospects of its Treatment. *Alexandrovich, Yu., Naskalsky, G., Schnaid, Ya.* p. 24.

Клинические варианты желтух при некоторых заболеваниях системы крови. *Митерев, Ю. Г., Хазанов, А. И.* (Главный военный госпиталь им. Н. Н. Бурденко, Москва). с. 27. — Clinical Variants of Icterus in some Diseases of the Blood System. *Miterev, Yu. G., Khazanov, A. I.* p. 27.

Электрофорез белков крови у больных эритремией, леченных радиоактивным фосфором. *Николаева, Н. В., Папуш, Н. Д.* (Гематологическая клиника и лаборатория фракционирования белков Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва). с. 32. — Electrophoresis of Blood Protein in Patients with Erythraemia Treated with Radiophosphorus. *Nikolaeva, N. V., Papush, N. D.* p. 32.

Метапластические процессы в желудке и слепой кишке при лейкозах (рентгенологические наблюдения). *Мартышкин, Г. П.* (Рентгенологический отдел Ленинградского научно-исследовательского института гематологии и переливания крови.) с. 34. — Metaplastic Processes in the Stomach and the Caecum in Leukaemia (X-ray observations). *Martyshkin, G. P.* p. 34.

Применение ауто- и аллотрансфузии костного мозга в сочетании с повышенными дозами химиопрепаратов при лечении больных лимфогранулематозом и опухолевым ретикулозом. *Хуцишвили, Г. Э.* (Гематологическая клиника Центрального ордена Ленина института гематологии и переливания крови Министерства СССР, Москва.) с. 37. — Application of Bone Marrow Auto- and Allotransfusion Connected with Increased Doses of Chemopreparations in the Treatment of Patients with Lymphogranulomatosis and Tumour Reticulosis. *Khutsishvili, G. E.* p. 37.

Клинико-морфологические особенности лейкозов у новорожденных. *Юдин, Ю. Г.* (Патоморфологический отдел Московского областного научно-исследовательского института им. М. Ф. Владимирского.) с. 41. — Clinico-Morphological Characteristics of Leukaemia in the Newborn. *Yudin, Yu. G.* p. 41.

К вопросу об этиологии спонтанного лейкоза у мышей линии C_{57}. *Настак, Е. Лунгу, М., Исайя, Г., Балмуш, Г., Попеску, Гр., Дона, Г., Руттер, Г., Чуфеку, Е.* (Бухарест) с. 43. — Some Data on the Aetiology of Spontaneous Leukaemia in Mice of the C_{57} strain. *Nastak, E., Lungu, M., Isaya, G., Balmush, G., Popescu, Gr., Dona, G., Rutter, G., Chufecu, E.* p. 43.

Ассоциация хромосом в кроветворных клетках у мышей. *Жарова, Е. И., Раушенбах, М. О.* (Лаборатория патологии крови Института экспериментальной и клинической онкологии АМН СССР, Москва). с. 47. — Association of Chromosomes in the Haemopoietic Cells of Mice. *Zharova, E. I., Raushenbakh, M. O.* p. 47.

О трактовке изменений количества «барабанных палочек» в зрелых нейтрофилах при хроническом миелозе у женщин. *Шац, В. Я.* (Клинико-диагностическая лаборатория Института онкологии АМН СССР, Ленинград.) с. 51. — Assessment of the Changes in the Number of Drum-

sticks in Mature Neutrophils in Women with Chronic Myelosis. *Shatz, V. Ya.* p. 51.

Заболеваемость лейкозом в Сочи и Туапсе. *Лебедев, В. Н., Беляева, З. Н., Костомарова, В. Г., Юничева, Р. Х.* (Гематологическое отделение Сочинского онкологического диспансера.) с. 52. — Some Data Concerning the Incidence of Leukaemia in Sochi and Tuapse. *Lebedev, V. N., Belyaeva, Z. N., Kostomarova, V. G., Yunicheva, R. Kh.* p. 52.

Ведущие проблемы современной французской гематологии. *Алексеев Г. А.* с. 54. — Main Problems of Current French Haematology. *Alexeev G. A.* p. 54.

Проблемы гематологии и переливания крови (Москва) **12** (1967) № 2.

Гематологическая служба СССР и ближайшие задачи ее развития. *Папко, Г. Ф.* (Клинический отдел института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 3. — Haematological Service in the USSR and Immediate Tasks of Development. *Papko, G. F.* p. 3.

Клиника и лечение серповидноклеточной анемии и ее вариантов. По данным наблюдений в Гане. *Токарев, Ю. Н.* с. 7. — Clinical Picture and Treatment of Sickle-Cell Anaemia and of its Variants (according to Observations in Ghana). *Tokarev, Yu. N.* p. 7.

Иммуногематологические исследования костного мозга при анемиях. *Сюбаева, Н. И.* (Клиника факультетской терапии Военно-медицинской академии им. С. М. Кирова, Ленинград.) с. 16. — Immunohaematological Studies of the Bone Marrow in Anaemias. *Syubaeva, N. I.* p. 16.

К вопросу об обмене железа при болезни Маркиафава—Микели. *Корчмарк, И. Ф., Ливандовский, Ю. А., Лорие, Ю. И.* (Гематологическая клиника Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 22. — Some data on Iron Metabolism in Marchiafava-Micheli Disease. *Korchmark, I. F., Livandovsky, Yu. A., Lorie, Yu. I.* p. 22.

Содержание глюкозо-6-фосфат дегидрогеназы в эритроцитах и их ультраструктура при болезни Маркиафава—Микели. *Дульцина, С. М.* (Цитологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 28. — The Content of Glucoso-6-Phosphate Dehydrogenase in Erythrocytes and their Ultrastructure in Marchiafava-Micheli Disease. *Dultsina, S. M.* p. 28.

Гаптоглобин при гемолитических анемиях у детей. *Алимова, М. М., Евдокимова, А. И., Юрков, Ю. А., Королева, Т. С.* (Институт педиатрии АМН СССР, Москва.) с. 31. — Haptoglobin in Children with Haemolytic Anaemias. *Alimova, M. M., Evdokimova, A. I., Yurkov, Yu. A., Koroleva, G. S.* p. 31.

К вопросу о кобальтосвязывающей способности белков сыворотки крови. *Идельсон, Л. И., Жуковская, Е. Д.* (Группа действ. члена АМН СССР проф. И. А. Кассирского на базе Центральной клинической больницы МПС.) с. 35. — Some Data on the Cobalt-Bindig Capacity of Blood Serum Proteins. *Idelson, L. I., Zhukovskaya, E. D.*, p. 35.

Современные представления об эритропоэзе в норме и патологии. *Липац, А. А.* (Центральный институт гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 38. — Modern Views on Erythropoiesis in Normal and Pathological Conditions (Survey). *Lipatz, A. A.* p. 38.

Современное состояние вопроса об изменении системы крови при хронической бензольной интоксикации. *Магагина, Г. Е.* (Кафедра терапии санитарно-гигиенического факультета I Московского медицинского иснститута им. И. М. Сеченова.) с. 45. — Changes Observed in the Blood System in Chronic Benzene Intoxication (Survey). *Magagina, G. E.* p. 45.

Ошибки и достоверность «количественной» визуальной цитофотометрии. *Гурбанов, В. П.* (Группа действ. члена АМН СССР проф. И. А. Кассирского, Москва.) с. 49. — Errors and Veritability of "Quantitative" Visual Cytophotometry. *Gurbanov, V. P.* p. 49.

Аутоиммунная гемолитическая анемия больного хроническим лимфолейкозом с гипогаммаглобулинемией. *Вайдзиня, В. П., Яворский, Л. И.* (Кафедра терапии факультета усовершенствования врачей

Рижского медицинского института.) с. 57. — Autoimmune Haemolytic Anaemia in a Patient Suffering from Chronic Lympholeukaemia with Hypogammaglobulinaemia. *Vaidzinya, V. P., Yavorkovsky, L. I.* p. 57.

Влияние различных типов ионизирующей радиации из различных источников на гемопоэтическую ткань. (Совещание экспертов в Вене.) *Чертков, И. Л., Козинец, Г. И.* с. 60. — Effect of Various Types of Ionizing Radiation from Various Sources on Haemopoietic Tissue (A Meeting of Experts in Vienna). *Chertkov, I. L., Kozinets, G. I.* p. 60.

Проблемы гематологии и переливания крови Москва **12** (1967) № 3.

О необходимости определения полных резус-антител. *Умнова, М. А.* (Лаборатория по изучению и стандартизации групп крови Центрального института гематологии и переливания крови Москва.) с. 3. — The Necessity of Determining Complete Rhesus-Antibodies. *Umnova, M. A.* p. 3.

О возможности использования эритроцитов, длительно консервированных раствором Цолипк 7^{b}, в качестве стандартов. *Ичаловская, Т. А., Бейлина, В. Б., Самусева, Г. С.* (Лаборатория по изучению и стандартизации групп крови Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 7. — Some data on the Possibility of Using Erythrocytes Preserved for a Long Term with TSOLIPK 7^{b} Solution. *Ichalovskaya, T. A., Beilina, V. B., Samuseva, G. S.* p. 7.

Метод тройного определения эритроцитных подгрупп A_1—A_2 при помощи фитаглютининов. *Потапов, М. И.* (Научно-исследовательский институт судебной медицины Министерства здравоохранения СССР, Москва.) с. 13. — A Method for Triple Determination of Erythrocyte Subgroups A_1—A_2 with Phytagglutinins. *Potapov, M. I.* p. 13.

Иммунологическое значение плазмоклеточных островков. *Сас, Д., Ковач, М.* (Будапешт, Венгрия.) с. 17. — Immunological Significance of Plasmacellular Islands. *Szász, G., Kovács, M.* p. 17.

Функциональная активность коры надпочечников у доноров. *Касимов, Г. И., Гусейнов, Г. А.* (Азербайджанский научно-исследовательский институт гематологии и переливания крови.) с. 20. — Functional Activity of Adrenal Cortex in Donors. *Kasimov, G. I., Guseinov, G. A.* p. 20.

Гемопоэтины при острой почечной недостаточности гемотрансфузионного происхождения. *Кахетелидзе, М. Г., Комаров, Л. С.* (Отделение посттрансфузионных осложнений и гемодиализа и патофизиологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР.) с. 23. — Haemopoietins in Acute Renal Insufficiency Induced by Blood Transfusion. *Kakhetelidze, M. G., Komarov, L. S.* p. 23.

Влияние повторного взятия костного мозга на кроветворение у доноров. *Мамедов, А. И.* (Азербайджанский начно-исследовательский институт гематологии и переливания крови.) с. 29. — The Effect of Repeated Bone Marrow Puncture on Haemopoiesis in Donors. *Mamedov, A. I.* p. 29.

Влияние переливания консервированной крови на содержание электролитов сыворотки и кислотно-щелочное разновесие. *Павлова, З. В., Суджян, А. В.* (Лаборатория анестезиологии Института экспериментальной клинической онкологии АМН СССР, Москва.) с. 32. — The Effect of Transfusion of Preserved Blood in the Content of Serum Electrolyte and the Acid-Base Balance. *Pavlova, Z. V., Sudzhyan, A. V.* p. 32.

Порфирия. *Идельсон, Л. И.* (Группа дейст. члена АМН СССР проф. И. А. Кассирского на базе Центральной клинической больницы МПС.) с. 36. — Porphyria (Literature survey). *Idelson, L. I.* p. 36.

По поводу статьи Ю. Н. Кремера и А. А. Шмидта «Некоторые аспекты проблемы парентерального питания». (Письмо в редакцию.) *Чаплыгина, З. А., Богомолов, Л. Г.* с. 46. — Concerning the Paper of Yu. N. Kremer and A. A. Schmidt: "Some Aspects of the Parenteral Nutrition Problem" (A letter to the Editor). *Chaplygina, Z. A., Bogomolov, L. G.* p. 46.

Опыт получения в больших количествах универсальных сывороток антирезус методом адсорбции. *Кронберг, А. Я.* (Изосерологическая лаборатория Приморской краевой станции переливания крови.) с. 48. — Obtaining of Universal Antirhesus Sera in High Quantities by Absorption. *Kronberg, A. Ya.* p. 48.

Лиофильная сушка антирезусной сыворотки. *Ашкенази, А. Я.* (Мурманская областная станция переливания крови.) с. 49. — Freeze-dried Antirhesus Serum. *Ashkenazi, A. Ya.* p. 49.

Из опыта работы резус-лаборатории Молдавской республиканской станции переливания крови. *Вайсблит, Р. И.* и *Лободенко, Р. Л.* с. 51. — The Work of the Rhesus-Laboratory of the Moldavian Blood Transfusion Centre. *Vaisblit, R. I., Lobodenko, R. L.* p. 51.

К вопросу о предупреждении осложнений, обусловленных переливанием крови, несовместимой по системе АВО. *Максимов, П. М.* (Госпитальная хирургическая клиника Ивановского медицинского института и Ивановская областная станция переливания крови.) с. 53. — Prevention of Complications Caused by Transfusion of Incompatible Blood in the ABO System. *Maximov, P. M.* p. 53.

Некоторые новые формы агитации безвозмездных доноров. *Волобоев, Н. А.* (Чимкентская областная станция переливания крови.) с. 56. — Some New Means of Propaganda for Recruiting Voluntary Donors. *Voloboev, N. A.* p. 56.

Гематологические показатели у здоровых людей в условиях климата Белого моря. *Любимова, В. Т.* с. 58. — Haematological Indices of Healthy Individuals Influenced by Climatic Conditions of the White Sea. *Lyubimova V. T.* p. 58.

Научно-практическая конференция по гематологии и переливанию крови в Челябинске. *Умнова, М. А.* с. 59. — Conference on Haematology and Blood Transfusion in Chelyabinsk. *Umnova, M. A.* p. 59.

Проблемы гематологии и переливания крови (Москва) **12** (1967) № 4.

Усовершенствование растворов для консервирования эритроцитной взвеси при положительной температуре. *Гинзбург, Ф. Г., Сухова, А. Г., Данцер, Н. А.* (Биохимическая лаборатория и лаборатория консервирования крови Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 3. — New Methods of Improving Solutions for Preserving Erythrocyte Suspension at Positive Temperature: *Ginzburg, F. G., Sukhova, A. G., Dantser, N. A.* p. 3.

Стабильные пастеризуемые белки плазмы и источники их получения. *Киселев, А. Е., Фром, А. А., Никитенко, А. А., Русанов, В. М.* (Центральный институт гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 9 — Stable Pasteurized Plasma Proteins and their Sources: *Kiselev, A. E., From, A. A., Nikitenko, A. A., Rusanov, V. M.* p. 9.

Усовершенствованные консерванты с лимонной кислотой и их клиническое испытание. *Олдурова, С. В.* (Лаборатория консервирования крови Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 14. — Preservatives Improved by Citric Acid and their Clinical Trial. *Oldurova, S. V.* p. 14.

Посттрансфузионная выживаемость эритроцитной взвеси, консервированной на растворе Цолипк-8, обогащенной инозином, аденином и желатиной. *Тальская И. Н., Бурдыга Ф. А., Сухова А. Г.* (Лаборатория консервирования крови Центрального ордена Ленин аинститута гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 17. — Posttransfusion Survival Time of Erythrocyte Suspension Preserved in TSOLIPK No. 8 Solution, Enriched with Inosine, Adenine and Gelatine. *Talskaya, I. N., Burdyga, F. A., Sukhova, A. G.* p. 17.

Сравнительная оценка различных питательных сред для бактериологического контроля на стерильность консервированной крови. *Болотников Ф. И., Мартынова В. А., Сельцовская Г. С., Марголина А. Н.* (Бактериологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 20. — Comparative Assessment of Various Nu-

tritive Media for Preserved Blood sterility Test. *Bolotnikova, F. I., Martynova, V. A., Seltsovskaya, G. S., Margolina, A. N.* p. 20.

Изучение режимов центрифугирования крови для увеличения выходов плазмы и интактных эритроцитов. *Федорова, Л. И., Чернышева, Н. Н., Альперович, В. В.* (Экспериментально-производственная лаборатория получения компонентов и препаратов крови Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 23. — A Study of the Blood Centrifugation System for Increasing Plasma and Intact Erythrocyte Output. *Fedorova, L. I., Chernysheva, N. N., Alperovich, V. V.*, p. 23.

Содержание 17-оксикортикостероидов в плазме фибринолизной крови. *Кулешов, Ю. Я., Мурашов, Б. Ф., Стельмах, В. И.* (Клиника инфекционных болезней и Научно-исследовательская лаборатория центра крови и тканей Военно-Медицинской ордена Ленина академии им. С. М. Кирова, Ленинград.) с. 25. — 17-Oxycorticosteroid Content in Fibrinolytic Blood Plasma. *Kuleshov, Yu. Ya., Murashov, B. F., Stelmakh, V. I.* p. 25.

Усовершенствованный метод лиофильной сушки плазмы, стабилизированной глюкозой. *Подольский, М. В., Карлова, Н. Г., Розенберг, Г. Я.* (Лаборатория лиофилизации биопрепаратов и лаборатория фракционирования белков Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 27. — Improved Method of Freeze-Drying of Plasma Stabilized with Glucose. *Podolsky, M. V., Karlova, N. G., Rozenberg, G. Ya.* p. 27.

Приготовление сухой кадаверной плазмы методом лиофилизации и ее биохимическая характеристика. *Пафомов, Г. А., Зыкова, К. И., Артамонова, С. А., Гарина, М. М.* (Лаборатория переливания крови Московского городского начное-исследовательского института скорой помощи им. Н. В. Склифосовского.) с. 32. — Preparation of Dry Cadaveric Plasma by Lyophilization and its Biochemical Characteristics. *Pafomov, G. A., Zykova, K. I., Artamonova, S. A., Garina, M. M.* p. 32.

Об участии лимфатической системы в биосинтезе продуктов белковых гидролизатов. *Рзаев, Н. А., Гусейнов, Г. А.* (Биохимическая лаборатория Азербайджанского начно-исследовательского института гематологии и переливания крови.) с. 37. — The Role of the Lymphatic System in the Biosynthesis of Protein Hydrolysate Products. *Rzaev, N. A., Guseinov, G. A.* p. 37.

Методы получения жировой эмульсии и ее свойства. *Суздалева, В. В., Малахова, Г. М., Казакова, Г. И.* (Лаборатория физико-химии крови и кровезаменителей Центрального ордена Ленина института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 40. — Obtaining of Fat Emulsion and its Properties. *Suzdaleva, V. V., Malakhova, G. M., Kazakova, G. I.* p. 40.

Кровезаменители в экстракорпоральном кровообращении. *Шлоссер, В.* (Хирургическая университетская клиника, Марбург-на-Лане, ФРГ.) с. 43. — Blood Substitutes in Extracorporeal Circulation. *Schlosser, V.* p. 43.

Состояние костномозгового кроветворения у собак после замещающих переливаний посмертной крови облученных животных. *Рыжков, С. В., Чесноков, П. Т., Крылова, Г. П.* (Научно-исследовательская лаборатория, центр крови и тканей Военно-медицинской академии им. С. М. Кирова. с. 48. — Haemopoiesis of Dog Bone Marrow Following Replacement Transfusions of Postmortem Blood of Irradiated Animals. *Ryzhkov, S. V., Chesnokov, P. T., Krylova, G. P.* p. 48.

Плазмаферез при острых отравлениях атропином. *Кузьмин Д. С., Веденский А.* (Рентгенологический отдел и лаборатория консервирования и пересадки тканей и органов Ленинградского научно-исследовательского института переливания крови.) с. 51. — Plasmapheresis in Acute Atropine Poisoning. *Kuzmin, D. S., Vedensky, A. N.* p. 51.

К вопросу консервирования трупного костного мозга. *Асаенок, И. С., Данилов, И. П.* (Лаборатория консервирования тканей Белорусского начно-исследовательского института гематологии и переливания крови.) с. 54. — Preservation

of Cadaver Bone Marrow. *Asaenok, I. S., Danilov, I. P.* p. 54.

Опыт работы Днепропетровской областной станции переливания крови по заготовке и клиническому использованию фибриногена. *Трунова, Л. И., Шитова, В. И.* (Днепропетровск.) с. 58. — The Work of Dniepropietrovsk Regional Blood Bank and Transfusion Centre connected with the Preparation and Clinical Use of Fibrinogen. *Trunova, L. I., Shitova, V. I.* p. 58.

Стерилизация пластикатных мешков с консервирующим раствором методом двукратного автоклавирования. *Блинкин, С. А., Гутник, Р. Б., Болтанова, З. М.* (Киевский научно-исследовательский институт гематологии и переливания крови.) с. 59. — Sterilization by Double Autoclaving of Plastic Bags with Preserving Solution. *Blinkin, S. A., Gutnik, R. B. Boltanova, Z. M.* p. 59.

2-ой симпозиум по методам стерилизации во врачебной и фармацевтической практике. *Виноград-Финкель, Ф. Р., Федорова, Л. И.* с. 61. — Second Symposium on Methods of Sterilization in Medical and Pharmaceutical Practice. *Vinograd-Finkel, F. R., Fedorova, L. I.* p. 61.

Проблемы гематологии и переливания крови (Москва) **12** (1967) № 5

О лимфоците периферической крови. *Терентьева, Э. И.* (Центральный институт гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 3. — Peripheral Blood Lymphocytes. *Terentieva, E. I.* p. 3.

Гистохимическое исследование селезенки при болезни Верльгофа. *Маринов, Х. Г.* (Лаборатория патологической анатомии опухолей человека Института экспериментальной и клинической онкологии АМН СССР и патологоанатомическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 7. — Histochemical Examination of the Spleen in Werlhof's Disease. *Marinov, Kh. G.* p. 7.

Цитохимические особенности элементов костномозгового кроветворения при различных вариантах острого лейкоза. *Ленская, Р. В., Терентьева, Э. И.* (Цитологическая лаборатория и гематологическая клиника Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 12. — Cytochemical Characteristics of Bone Marrow Haemopoiesis in Different Variants of Acute Leukaemia. *Lenskaya, R. V., Terentieva, E. I.* p. 12.

Гистоэнзиматические исследования в гематологии. *Лавкович, В., Кшеминска-Лавкович, И., Шмигольский, С., Литвин, И.* (Варшава) с. 18. — Histoenzymatic Examinations in Haematology. *Lavkovich, V., Ksheminska-Lavkovich, I., Shmigolsky, S., Litvin, I.* p. 18.

К вопросу о цитологической картине пунктатов лимфатических узлов при хроническом лимфолейкозе. *Свядищ, А. В.* (2-ая кафедра терапии Алма-атинского института усовершенствования врачей.) с. 21. — Some data on the Cytological Picture of the Lymph Node Puncture Materials in Chronic Lympholeukemia. *Svyadishch, A. V.* p. 21.

Цитохимические исследования хронического миело- и лимфолейкоза на ранних этапах развития заболевания. *Терентьева, Э. И., Казанова, Л. И., Чернцова, Т. А.* (Центральный институт гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 25. — Cytochemical Investigations of Chronic Myeloid- and Lymphoid Leukaemia in Early Stages of Development of the Disease. *Terentieva, E. I., Kazanova, L. I., Cherntsova, T. A.* p. 25.

Некоторые аспекты морфологической дифференциации хронического лимфолейкоза. *Миансарян И. Т.* (Академическая группа члена АМН СССР И. А. Кассирского.) с. 28. — Some Aspects of Morphological Differentiation of Chronic Lymphoid Leukaemia. *Miansaryan, I. T.* p. 28.

Цитохимическое исследование фермента глюкозо-6-фосфат дегидрогеназы при фавизме. *Дульцина, С. М.* (Цитологическая лаборатория Центрального института гематологии и переливания крови Министерства здравоохраненя СССР, Москва.) с. 33. — Cytochemical Examination of the Glucoso-6-Phosphate De-

hydrogenase Enzyme in Favism. *Dultsina, S. M.* p. 33

Об одном своеобразном варианте ядерной асегментации гранулоцитов имитирующем гомозиготную форму пельгеровской аномалии лейкоцитов. Проф. *Алексеев, Г. А.* (3-я кафедра терапии Центрального института усовершенствования врачей.) с. 37. — A Characteristic Variant of Nuclear Asegmentation of Granulocytes (Stodtmeister type) Imitating Homozygus Form of Pelger Anomaly of Leukocytes. *Alexeev, G. A.* p. 37.

О сегментации ядер нейтрофилов крови у нормальных пробандов из семей с аномалией. *Шац, В. Я.* (Клинико-диагностическая лаборатория института онкологии АМН СССР.) с. 45. — Nuclear Segmentation of Blood Neutrophils in Normal Probands with Pelger Anomaly. *Shatz, V. Ya.* p. 45.

Зависимость эффективности переливок лейкоза мышей от содержания анеуплодных клеток в перевивочном материале. *Хрусталев, С. А.* (Лаборатория патологии крови Института экспериментальной и клинической онкологии, Москва.) с. 47. — Relationship of the Effect of Mouse Leukaemia Transplantation on the Content of Aneuploid Cells in the Transplanted Material. *Khrustalev, S. A.* p. 47.

К вопросу о поступлении лейкоцитов из периферической крови в костный мозг. *Соколов, А. Б.* (Кафедра фармакологии 2-го Московского медицинского института им. Н. И. Пирогова.) с. 49. — Passage of Leukocytes from the Peripheral Blood into the Bone Marrow. *Sokolov, A. B.* p. 49.

Эритропоэтические свойства плазмы при экспериментальном нефрите. *Моисеева, О. И., Рощина, Г. М.* (Лаборатория экспериментальной и клинической гематологии Института физиологии им. И. П. Павлова АН СССР Ленинград.) с. 52. — Erythropoietic Properties of Plasma in Experimental Nephritis. *Moiseeva, O. I., Roshchina, G. M.* p. 52.

Клиническое значение определения трансферрина. *Ливандовский, Ю. А.* (Гемотерапевтическая клиника Центрального института гематологии и переливания крови Министерства здравоохранения СССР, Москва.) с. 57. — Clinical Significance of the Determination of Transferrin. *Livandovsky, Yu. A.* p. 57.

К казуистике острой гемолитической анемии у лиц с недостатком глюкозо-6-фосфатдегидрогеназы в эритроцитах. *Яворковский, Л. И.* (Кафедра терапии факультета усовершенствования врачей Рижского медицинского института.) с. 60. — Casuistics of Acute Haemolytic Anaemia in Persons with Glucoso-6-Phosphate Dehydrogenase Deficiency in Erythrocytes. *Yavorkovsky, L. I.* p. 60.

Scandinavian Journal of Haematology (Copenhagen) Suppl. **1**, 1967.

Studies on methyl-B_{12} in man. *Stahlberg, K.-G.* (Univ. Hosp., Dept. of Intern. Med., Lund, Sweden), p. 1.

Scandinavian Journal of Haematology (Copenhagen) Suppl. **2**, 1967.

Studies on the phagocytic activity of neutrophilic leukocytes. With special reference to chronic myeloproliferative conditions and megaloblastic anemia. *Brandt, L.* (Univ. Hosp., Dept. of Intern. Med., Lund, Sweden), p. 1.

Scandinavian Journal of Haematology (Copenhagen) **4** (1967) No. 1.

Inhibition of thromboplastic activity by Trasylol. In vitro investigations concerning the mechanism of inhibition. *Amris, C. J.* et al. (Dept. of Clin. Chem., Rikshospitalet, Blegdamsvej 9, Copenhagen Ø), p. 3.

Desferrioxamine-induced urinary iron excretion in normal and iron-deficient subjects. *Hallberg, L.* et al. (Dept. of Inter. Med. II, Sahlgren Hosp., Göteborg, Sweden), p. 11.

Fibrinolysis and coagulation. I. Effect of calcium on lysis of euglobulin. clots. *Helle, I.* (Haemat. Research Lab., Dept. IX, Ulleval Hosp., Oslo, Norway), p. 21.

Ferrokinetics in patients suffering from chronic renal disease and anaemia. *Magid, E.* et al. (Med. Dept. C, Diakonissestiftelsen, Peter Bangsvej 1, Copenhagen F), p. 33.

The frequency of Kappa and Lambda chains in pathologic serum gammaG, gammaA, gammaD and gammaμ immunoglobulins. *Laurell, C.-B.* et al. (Dept. of Clin. Chem., Allmänna Sjukhuset, Malmö, Sweden), p. 46.

A case of haemolytic anaemia due to pyruvate kinase deficiency. *Hanel, H. K.* et al. (Dept. of Clin. Chem., Bispebjerg Hosp., Copenhagen NV), p. 53.

Factors influencing the lysis of artificial thrombi by urokinase. *Ogston, D.* et al. (Dept. of Med., Univ. of Aberdeen, Foresthill, Aberdeen, Scotland), p. 61.

"Radial segmentation" of the nuclei in lymphocytes and other blood cells induced by some anticoagulants. *Norberg, B.* et al. (Dept. of Intern. Med., Lasarettet, Lund, Sweden), p. 68.

Separation of lymphocyte-stimulating and agglutinating activities in phytohaemagglutinin (PHA) from Phaseolus vulgaris. *Weber, T.* et al. (Minerva Found. Inst., Tölögatan 12 A, Helsingfors 10, Finland), p. 77.

Scandinavian Journal of Haematology (Copenhagen) **4** (1967) No. 2.

Tne kinetics of eosinophil granulocytes in rats. Autoradiographic studies. *Bro-Rasmussen, F.* et al.(Inst. B of Anatomy, Univ. of Copenhagen, Nørre Allé 63, Copenhagen Ø), p. 81.

The influence of acute and long-term exercise on adenosine diphosphate-induced platelet thrombosis and plasma lipids in rats fed on a 40 per cent butter diet. *Nordöy, A.* et al. (Med. Dept. A, Univ. Hosp, Oslo, Norway), p. 88.

Acquired selective vitamin B_{12} malabsorption. *Hippe, E.* (Herlufsvaenge 18, Naestved, Denmark), p. 97.

Gastric factors influencing iron absorption in anaemic patients. *Jacobs, A.* et al. (The Cardiff Royal Infirmary, Cardiff, G. B.), p. 105.

Studies on the macroglobulins of human serum. IV. The frequency of light chain types K and L in polyclonal and monoclonal gamma M. *Wollheim, F. A.* et al. (Dept. of Clin. Chem., Allmänna Sjukhuset, Malmö, Sweden), p. 111.

Iso-immune neonatal purpura caused by anti-PlGrLy^{B1}. *Svejgaard, A.* et al. (Blood Bank and Blood Grouping Lab., Kommunehosp., Århus C, Denmark), p. 118.

The effect of antigens on DNA-synthesis in cultured lymphocytes. *Bouveng, R.* et al. (Dept. of Med., Lasarettet, Lund, Sweden), p. 125.

Reversible agglutination of trypsinized red cells by normal human sera. Identification of the reversing factor as an amino acid. *Mellbye, O. J.* (Inst. for Exp. Med. Research, Univ. of Oslo, Ullevål Hosp., Oslo, Norway), p. 135.

Studies on hypoxia V. Changes in the bone marrow during hypoxia at 10,000 and 20,000 feet. *Yoffey, J. M.* et al. (Dept. of Anat., Med. School, University Walk, Bristol 8), p. 145.

A new bone marrow biopsy needle. *Stavem, P.* (Med. Dept. A, Rikshosp., Oslo, Norway), p. 158.

Scandinavian Journal of Haematology (Copenhagen) **4** (1967) No. 3.

The effect of demecolcine on the oxalate--induced formation of radial-segmented nuclei (Rieder cells). *Norberg, B.* et al. (Dept. of Intern. Med., Lasarettet, Lund, Sweden), p. 161.

Gastric secretion of acid and intrinsic factor during histamine infusion in 14 cases of duodenal ulcer. *Dotevall, G.* et al. (Dept. of Int. Med. I, Sahlgrenska, Göteborg, Sweden), p. 169.

Substitution of plasma in culture medium with dextran, glucose or albumin for in vitro studies of lymphocytes. *Bergman, B.* et al. (Haemat. Unit, Med. Dept., Lasarettet, Lund, Sweden), p. 176.

The effect of dietary folate deficiency on the synthesis of methylcobalamin in the chick. *Lindstrand, K.* et al. (Univ. of Lund, Inst. of Biochem., und Sweden), p. 181.

Determination of platelet factor-3 by a modified prothrombin consumption test. *Farská, Irena* et al. (Inst. of Haematol., U nemocnice 1, Prague 2), p. 187.

Immunogenetic and immunological problems of allogenic haemopoietic radiochimaeras in man. *Mathé, G.* et al. (Inst. of Cancer Immunogen., Assoc. of C.-Bernaud, Villejuif, France), p. 193.

The relationship between duodenal mucosal iron concentration and iron absorption in human subjects. *Allgood, J. W.* et al. (Washington Univ. School of Med., Div. Haematol., St. Louis, Mo.), p. 217.

Lack of cytotoxicity by human thymocytes in vitro. *Holm, G.* (Wenner Gren Inst., Nortullsgt 16, Stockholm), p. 230.

Thrombosis et Diathesis Haemorrhagica (Stuttgart) **17** (1967) No. 3/4.

"Inhibitor like" effects of haematoporphyrin on Factor VIII (antihaemophilic globulin). *Davis, R. B.* et al. (Blood Coagulation Research Unit, Churchill Hosp., Headington, Oxford, G. B.), p. 299.

Contact activation of Hageman factor and the interaction of Hageman factor and plasma thromboplastin antecedent. *Haanen, C.* et al. (Dept. of Med. and Biochem., Univ. of Nijmegen, The Netherlands), p. 307.

Study of thrombincoagulase. *Soulier, J. P.* (Centre National de Transfusion Sanguine, Paris), p. 321.

Method of purification and properties of anti-XIa (inhibitor of the contact product). *Niemetz, J.* et al. (Dept. of Hemat., The Mount Sinai Hosp., New York, N. Y.), p. 335.

Kinetic aspects of the interaction of blood-clotting enzymes. II. The repletion between time and plasma contraction in prothrombin-time estimations. *Hemker, H. C.* et al. (State Univ. of New York at Buffalo, Med. School, N. Y.), p. 349

The role of thrombin in fibrinogen biosynthesis. *Pickart, L. R.* et al. (Gerontology Res. Inst., Sansum Clinic Res. Found., Santa Barbara, Calif.), p. 358.

Further studies on the "Dynia" clotting abnormality. *Pechet, L.* et al. (Dept. of Pathol., Univ. of Colorado Med. Center, Denver, Colorado), p. 365.

Effects of thrombin on factor VIII activity: Stabilization of factor VIII by glycine ethyl ester. *Kisker, C. T.* (National Inst. of Health, Div. of Biol. Standards, Bethesda, Md.), p. 381.

Activation of chymotrypsinogen by preparations of thrombokinase derived from bovine plasma. *Milstone, J. H.* et al. (Dept. of Pathol., Yale Univ. School of Med., New Haven, Conn.), p. 388.

Determination of fibrinogen (and fibrinolysis) in small quantities of plasma. *Lázár, G.* (Inst. of Pathophysiol., Med. Univ., Szeged, Hungary), p. 401.

Urokinase evaluated with the experimental radioactive embolus. *Hume, M.* (Dept. of Surgery, Yale Univ. School of Med., New Haven, Conn.), p. 405.

Fibrinolysis: mathematical and experimental studies. *Liniger, W.* et al. (IBM Watson Res. Center, Yorktowo Heights, New York, N. Y.), p. 412.

The thrombolytic effect of heparin and a heparin-like substance, SP 54. *Fitz Gerald, D. E.* et al. (Dept. of Med., Univ. of Pittsburgh, Pittsburgh, Pa.), p. 418.

Purification of human plasminogen. *Roberts, P. S.* et al. (Dept. of Med., Med. College of Virginia, Richmond, Va.), p. 423.

Fibrin deposition in the rabbit kidney produced by protease inhibitors. *Beller, F. K.* et al. (Depts. of Obstetrics and Gynecology, New York Univ. School of Med., N. Y.), p. 427.

Preparation and properties of anticoagulant split product of fibrinogen and its determination in plasma. *Nanninga, L. B.* et al. (Dept. of Physiol., Univ. of Texas, Med. Branch, Galveston, Texas), p. 440.

Une méthode photométrique rapide pour le dosage du plasminogène activable et de la plasmine. *Bouvier, C. A.* et al. (Centre de Transfusion Sanguine, Hôpital Univ., Genève), p. 452.

Fibrinogen break-down products and clotting parameters. *Gormsen, J.* et al. (Dept. of Med., Sundby Hosp., Copenhagen), p. 467.

Influence of plasma lipoproteins on the fibrinolytic activity. *Skrzydlewski, Z.* et al. (Dept. of Physiol. Chem., Med. School, Bialystok, Poland), p. 482.

The interaction of different phospholipids on the blood clotting mechanism. *Bourgain, R. H.* et al. (Univ. Libre de Bruxelles, Belgium), P. 491.

Physical factors in the thromboplastic activity of phospholipids. *Gobbi, F.* et al. (Dept. of Med. Pathol., Univ. of Modena, School of Med., Modena), p. 495

Studies on aggregation and adhesion of platelets. *Shively, J. A.* et al. (Univ. of Texas M. D. Anderson Hosp., Houston, Texas), p. 508.

Platelet aggregation by magnesium ion. *Des Prez, R. M.* et al. (Dept. of Med., Vanderbilt Univ., Nashville, Tenn.), p. 516.

Platelet numbers and life span in acute and chronic renal failure. *Stewart, J. H.* (Kanematsu Memorial Inst., Sydney Hosp., Sydney, Australia), p. 532.

Defekte Thrombusstruktur und Hemmung der Thrombusbildung bei Defibrinierungsblutungen infolge ungerinnbarer Fibrinogenderivate. *Steichele, D. F.* (Univ.-Frauenklinik, Tübingen), p. 543.

Studies on clot tensile strength. *Rubenstein, E.* (Dept. of Med., Stanford Univ. School of Med., San Mateo., Calif.), p. 552.

Spontaneous hemorrhage in anticoagulant-treated rats when subjected to excitant or depressant drugs. *Lucas, O. N.* et al. (Dept. of Physiol. and Pharmacol., Univ. of Saskatchewan, Saskatoon, Canada), p. 561.

Die Auslösung einer massiven Thrombosierung durch Epsilon-Aminokapronsäure nach vorangehender Aktivierung des Gerinnungssystems. *Herschlein, H. J.* et al. (Univ.-Frauenklinik, Tübingen,

The clot promoting effect of adenosine-5'-diphosphate (ADP). II. Site of action. *Pizzuto, J.* et al. (Dept. of Med., Univ. of Utah College of Med., Salt Lake City, Utah), p. 578.

Coagulation factors in pregnant women and premature infants with and without the respiratory distress syndrome. *Markarian, Marguerite* et al. (Dept. of Ped., Univ. of Colorado Med. Center, Denver, Colorado), p. 585.

Thrombosis et Diathesis Haemorrhagica (Stuttgart) Suppl. **22**, 1967.

Biologie des Plasmins. *Astrup, T.* (Inst. of Med. Res., Mitchell Found., Washington, D. C.), p. 5.

Biochemie der Fibrinolyse. *Schwick, G.* (Behringwerke AG, Marburg-L.), p. 27.

Methodische Ergänzungen zur Thrombusbildung und Thrombolyse in vivo. *Martin, M.* (Anat. Inst., Univ. Freiburg i. Br., Freiburg), p. 47.

Klinik der anaphylaktoiden Purpura. *Landbeck, G.* (Univ. Kinderklinik, Hamburg), p. 73.

Zur Morphologie der Purpura Schönlein-Henoch, licht- u. elektronenmikroskopische Befunde in Beziehung zur Ätiopathogenese. *Macher, E.* (Univ. Hautklinik, Freiburg i. Br.), p. 87.

Hämostaseologische Bedeutung der Erythrozyten. *Marx, R.* (Med. Univ. Kinderklinik, München), p. 131.

Erythrozytäre Gerinnunsaktivität. *Lechler, E.* (Theodor Kocher Inst., Bern), p. 139.

Transfusion (Philadelphia) 7 (1967) No. 2.

Japanese with no detectable Rh blood group antigens due to silent Rh alleles or deleted chromosomes. *Ishimori, T.* et al. (Tokyo Med. and Dental Univ., Dept. of Kegal Med., Bunkyo, Tokyo, Japan), p. 84.

Gonzales (Go^a), a new blood group character. *Alter, A. A.* et al. (Maimonides Hosp., Brooklyn, N. Y.), p. 88.

On the blood group antigens Bu^a and Sm. *Lewis, M.* et al. (Univ. of Manitoba, Dept. of Pediatrics, Rh Lab., Winnipeg, Manitoba, Canada), p. 92.

The in vivo effects of deglycerolized agglomerated erythrocytes transfused in multiple units to stable anemic patients. *Almond, D. V.* et al. (Naval Hosp., Naval Blood Res. Lab., Chelsea, Mass.), p. 95.

Observations on autologous, previously frozen, deglycerolized, agglomerated, resuspended red cells. I. Effect of storage temperatures. II. Effect of adenine supplementation of glycerolized red cells prior to freezing. *Valeri, C. R.* et al. (Naval Hosp., Naval Blood Res. Lab., Chelsea, Mass.), p. 105.

A new Rh allele, r^{yn} ($R^{-1, 2}$ w^{3} w^{4}). *Morton, N. E.* et al. (Univ. of Hawaii, Dept. of Genet., Honolulu, Hawaii), p. 117.

The effect of the addition of adenine and nucleosides at the beginning of storage on the concentrations of phosphates of human erythrocytes during storage in acid-citrate-dextrose and citrate-phosphate dextrose. *Chanutin, A.* (Univ. of Virginia, School of Med., Biochem. Lab., Charlottesville, Va.), p. 120.

The rapid infusion of large quantities of ACD adenine solution into humans. *Shields, Ch. E.* et al. (US Army Med. Res. Lab., Blood Transf., Res. Div., Fort Knox, Ky.), p. 133.

Comparison of immune and naturally occurring antibodies as test reagents for blood grouping diabetic patients. *Guy, L. Ruth* et al. (Univ. of Texas, SW Med. School, Dept. of Pathol., Dallas, Texas), p. 138.

Enzymatic activities, ADP-induced aggregation and survival in vivo as criteria of

platelet viability during storage. *Caen, J.* et al. (Hosp. St. Louis, Paris), p. 143.

Organization of serologic work in the USA *Umnova, M. A.* (Inst. of Hematol., Moscow), p. 148.

The incidence of isosensitization following blood transfusion. *Grobbelaar, B. G.* et al. (National Blood Transf. Service, Durban, South Africa), p. 152.

Transfusion (Philadelphia) **7** (1967) No. 3.

The effect of several variables on cryoprecipitated factor VIII (AHG) concentrates. *Pool, Judith G.* (Stanford Hosp., School of Med., Palo Alto, Calif.), p. 165.

Concentrated antihemophilic factor (AHF) from outdated blood. *Weaver R. A.* et al. (Univ. of North Carolina, Dept. of Pathol., Chapel Hill, N. C.), p. 168.

Disappearance rates of coagulation factors: Transfusion studies in factor-deficient patients. *Bowie, E. J. W.* et al. (Mayo Clinic and Found., Sect. of Med. and Clin. Pathol., Rochester, Minn.), p. 174.

Coagulation parameters following extracorporal circulation using acid-citrate-dextrose blood buffered with tris (hydroxymethyl) aminomethane (THAM). *Philips, Louise L.* et al. (Columbia Univ., College of Physiol. and Surg., Dept. of Obstetrics, New York, N. Y.), p. 185.

The Lutheran blood groups: A progress report with observations on the development of the antigens and characteristics of the antibodies. *Greenwalt, T. J.* et al. (American National Red Cross, Washington, D. C.), p. 189.

Comparative studies of the viability of human platelets stored at 4 C in plastic and glass containers. *Rolovic, Z.* et al. (Tufts New England Med. Center, Blood Res. Lab., Boston, Mass.), p. 204.

Blood transfusion in the USSR: Notes on a short visit. *Vaughn, J.* (Charlotte Memorial Hosp., Charlotte, N. C.), p. 212.

A simple quality control for the anti-globulin phase of the compatibility test. *Allen, T. E.* et al. (Walter Reed General Hosp., Med. Center, Blood Bank. Sect., Washington, D. C.), p. 230.

A simplified centrifuge for the separation and processing of blood cells. *Tullis, J. L.* et al. (Cytol. Lab., Protein Found. Inc., Boston, Mass.), p. 232.

Vox Sanguinis (Basel) **12** (1967) No. 5.

Quantitative measurements concerning A and B antigen sites. *Economidou, Joanna* et al. (Exp. Haematol. Res. Unit., Wright-Fleming Inst. of Microbiol., London, W. 2.), p. 321.

On the presence of γG and β1C globulins in renal glomeruli of aging and neonatally X-irradiated mice. *Guttman, P. H.* et al. (Cancer Res. Inst., Univ. of California School of Med., San Francisco, Calif.), p. 329.

Crossreacting antibody and saliva agglutinins. *Jakobowicz, Rachel* et al. (Red Cross Blood Transf. Service, Melbourne, Australia), p. 340.

An apparently new B_h-like human blood type. *Kitahama, M.* et al. (Medico-Legal Section, National Research Inst. of Police Science, 6, Sanban-cho, Chiyoda-ku, Tokyo, Japan), p. 354.

Immune adherence and blood cell destruction. *Van Loghem, J. J.* et al. (Central Lab. of the Netherlands Red Cross Blood Transf. Service, Amsterdam), p. 361.

Immune adherence of leucocytes and fibroblasts derived from skin, sensitized by cytotoxic leucocyte isoantibodies and complement, to the surface of indicator cells. *Melief, C. J. M.* et al. (Central Lab. of the Netherlands Red Cross Blood Transf. Service, Amsterdam), p. 374.

The effect of external version on antenatal immunization by the Rh factor. *Vos, G. H.* (Dept. of Pathol., King Edward Memorial Hosp. for Women, Subiaco, Australia), p. 390.

A new allele, Hp^1, in the hemopexin system in pigs. *Baker, L. N.* (Antigenic Lab., Iowa State Univ., Ames, Iowa), p. 397.

Vox Sanguinis (Basel) **12** (1967) No. 6.

Relationship of the anticomplement Coombs test to the classes of immunoglobulins. *Hudgins, W. R.* et al. (Univ. of Tennessee Hosp., Memphis, Tenn.), p. 401.

Factors influencing the agglutinability of red cells. III. Physico-chemical studies on ox red cells of different classes of agglutinability. *Uhlenbruck, G.* et al. (Max-Planck-Inst. for Hirnforsch., Goldenfelsstrasse 21, 5 Köln-Lindenthal), p. 420.

Enzyme reacting specific autoantibodies in individuals showing no signs of autoimmune haemolytic anaemia. *Dybkjaer, E.* et al. (Blood Bank and Blood Grouping Lab., Kommunehosp., Århus, Denmark), p. 429.

A two-component system of human serum agglutinating gelatine-coated erythrocytes. *Wolff, I.* et al. (Dept. of Clin. Pathol., Hanusch-Krankenhaus, Vienna), p. 443.

Further observations on A_m^h and O_m^h phenotypes. *Bhatia, H. M.* et al. (Blood Group Ref. Centre, Indian Cancer Res. Centre, Bombay-12, India), p. 457.

Studies on a mollusc extract using inhibiting and non-inhibiting salivas. *Gold, E. R.* et al. (Reg. Transf. Centre, Southmead Hosp., Bristol, G. B.), p. 461.

An incomplete antibody for red cells in Salmon caviar. *Uhlenbruck, G.* et al. (Max-Planck-Inst. für Hirnforsch., Goldenfelsstrasse 21, 5-Köln-Lindenthal), p. 465.

Vox Sanguinis (Basel) **13** (1967) No. 1.

Distribution of Ag(x) and Ag(y) antigens in some populations. *Hirschfeld, J.* et al. (Statens Rättskemiska Lab., Stockholm 60), p. 1.

Anti-i: Identification of the "non-specific" cold-agglutinin. *Bell, C. A.* et al. (Cedars of Lebanon Div., Cedars-Sinai Med. Center, Los Angeles, Calif.), p. 4.

The mixed leucocyte reaction in inbred strains of rats. *Elves, M. W.* (Charles Salt Res. Centre, Robert Jones and Agnes Hunt Orthopaedic Hosp., Oswestry, Shropshire, G. B.), p. 7.

The distribution of the red cell acid phosphatase variants in Vienna. *Speiser, P.* et al. (Pathol.-Anat. Inst., Univ. Wien, Spitalgasse 4, Wien IX), p. 12.

Further studies on auto-anti-Xg^a antibody. *Yokoyama, M.* et al. (Kuakini Research Found., Honolulu, Hawaii), p. 15.

Multiple phenotypic abnormalities associated with Rh_{null} (/——/——/). *Schmidt, P. J.* et al. (Blood Bank Dept., Clinical Centre, National Inst. of Health, Bethesda, Md.), p. 18.

Relation between anti-Ag (b) and anti-Ag(t) reagents. *Hirschfeld, J.* et al. (Statens Rättskemiska Lab., Tomtebodavägen 30, Stockholm 60), p. 21.

Anti-Ge, as a transfusion problem. *Nunn, Hilary D.* et al. (Blood Group Ref. Lab., Gatliff Rd. off Ebury Bridge Rd., London, S. W. 1.), p. 21.

The preservation of dog agglutinogens in liquid nitrogen. *Strott, Mary C.* et al. (National Inst. of Health, Bethesda, Md.), p. 27.

Inter- and intra-species variations within the Xh antigen. *Rittner, Ch.* (Inst. of Forensic Med., Univ. of Bonn, 53 Bonn), p. 29.

Vox Sanguinis (Basel) **13** (1967) No. 2.

Effect of thymectomy on the golden hamster (Mesocricetus auratus). IV. Comparative studies on two hamster strains. *Sherman, J. D.* (Framingham Union Hosp., Framingham, Mass.), p. 129.

Anti-IB, an expected "new" antibody detecting a joint product of the I and B genes. *Tegoli, J.* et al. (Blood Bank, Memorial Hosp. Center, 444 East 68th Street, New York 21, N. Y.), p. 144.

Demonstration of the Rh gene complex D(c) (E). *Heiken, A.* (Blodgruppsserologiska avdelningen, Statens Rättskemiska Lab., Tomtebodavägen 30, Stockholm 60), p. 158.

A rare blood group antigen, Jn^a. *Kornstad, L.* et al. (State Inst. of Public Health, Geitmyrsveien 75, Oslo 1, Norway), p. 165.

Studies on the Yt blood group system. *Giles, Carolyn M.* et al. (Blood Group Ref. Lab., Gatliff Rd. off Ebury Bridge Rd., London, S. W. 1.), p. 171.

Reactions of anti-J, anti-R and pig anti-A with human group A cells. A pilot study. *Gold, E. R.* (South Western Reg. Transf. Centre, Bristol, G. B.), p. 181.

A study of anti-5b leuco-agglutinins. *Lawler, Sylvia D.* et al. (Royal Marsden Hosp., London, S. W. 3.), p. 187.

Cell specific and common antigens on human granulocytes and lymphocytes demonstrated with cytotoxic hetero-antibodies. *Thorsby, E.* (Inst. for Exp. Med. Research, Ullevål Hosp., Oslo 1, Norway), p. 194.

Vox Sanguinis (Basel) **13** (1967) No. 3.

Immunochemical studies of lectins. I. Partial purification of the hemagglutinating protein of Sophora japonica. *Miller, J. T.* et al. (Found. for Research on the Nervous System, 36 The Fenway, Boston, Mass.), p. 209.

Two incomplete agglutinins associated with anti-Le^a in rabbit antisera against gum arabic. *Matsuzawa, Shigetaka* (Juntendo Univ. School of Med., Dept. of Forensic Med., Tokyo, Japan), p. 218.

Prenatal T-transformation? A case of polyagglutinable cord blood erythrocytes. *Jørgensen, J. R.* (Univ. Hosp., Blood Bank, Rikshosp., Copenhagen, Denmark), p. 225.

Serum agglutinators of erythrocytes sensitized with enzyme digested anti-Rh antibodies. *Waller, M.* (Med. College of Virginia, Dept. of Med., Richmond, Va.), p. 233.

Three new phenotypic systems in the blood of sheep. *Tucker, E. M.* et al. (Inst. of Animal Physiol., Babraham, Cambridge, G. B.), p. 246.

An examination of bovine blood typing antibodies by gel-filtration analysis. *Hines, H. C.* (735 Stadium Drive, Columbus, Ohio), p. 263.

A biological test for injury to the human red cell. *Stuart, A. E.* et al. (Univ. of Edinburgh, Dept. of Pathol., Edinburgh, Scotland), p. 270.

A method for the isolation of white cells from the blood of sheep by differential lysis with hypotonic saline solutions. *Dain, A. R.* et al. (A. R. C. Inst. of Animal Physiol., Dept. of Exp. Pathol, Cambridge, G. B.), p. 281.

Unusual suppression of secretion of blood group substance A. *Tegoli, J.* et al. (Memorial Hosp., Med. Center, Blood Bank, 444 E 68th Street, New York, N. Y.), p. 285.

Vox Sanguinis (Basel) **13** (1967) No. 4.

Genetically determined variants of serum alkaline phosphatase: A review. *Robinson, J. C.* et al. (National Inst. of Health, Bethesda, Md.), p. 289.

Membrane destruction in paroxysmal cold hemoglobinuria. *Scott, J. G.* et al. (Univ. of Rochester, School of Med., Rochester, N. Y.), p. 308.

Anti-Dib-first and second examples. *Thompson, P. R.* et al. (Los Angeles, County Gen. Hosp., 1200 State St., Los Angeles, Calif.), p. 314.

Serum lipoprotein allotypes Ag(x) and Ag(y) in Japanese. *Okochi, K.* (Tokyo Univ. Hosp., Blood Transf. Service, Tokyo, Japan), p. 319.

Comparative studies on anti-Ag sera in immunodiffusion and in passive hemagglutination methods. *Bütler, R.* et al. (Central Lab. and Blood Transf. Service, Berne) p. 327.

The first example of anti-Henshaw in human-serum. *MacDonald, K. A.* et al. (Reg. Blood Transf., Brentwood, Essex, G. B.), p. 346.

Radioimmunoassay of human urinary immunoglobulin G. *Saravis, C. A.* et al. (Harvard Med. School, Peter Bent Brigham Hosp., Dept. of Med., Boston, Mass.), p. 349.

The Rh gene complex cD-segregating in a negro family. *Leyshon, W. C.* (National Inst. of Dental Res., Human Genet. Branch, Bethesda, Md.), p. 354.

Comparison of lectin anti-H reagents. *Flory, L. L.* (Univ. of Cambridge, Dept. of Pathol., Cambridge, G. B.), p. 357.

A and H antigens on leucocytes. *Flory, L. L.* (Univ. of Cambridge, Dept. of Pathol., Cambridge, G. B.), p. 362.

Contents

Volume 1

Foreword 3

Bernard, J.: Remarques sur la tumeur de Burkitt 5

R. Hollán, S., G. Szelényi, J., Lehmann, H., Beale, D.: A Boston-type haemoglobin M in Hungary: haemoglobin M Kiskunhalas 11

Levin, E., Beutler, E.: Human erythrocyte adenylate kinase 19

Storti, E., Lusvarghi, E., Grignaffini, G. F., Sgandurra, A.: Biological and clinical significance of the adrenalin test in haematology 27

Hořejší, J.: Effect of reduced glutathione on the dissociation curve of haemoglobin. Possible therapeutic use of glutathione in haemorrhagic shock 35

Waubke, R., Gerstmair, G., Trepel, F., Pichlmaier, H., Begemann, H.: Wirkung von Röntgen-Ganzkörper- und -Extrakorporal-Bestrahlung auf die Lymphozyten des peripheren Blutes 45

Черницова, Т. А., Сусоева, В. М.: Опыт лечения миелобромолом больных хроническим миелолейкозом 61

Péter, Á., Szatmári, É., Romhányi, J., Letenyei, K.: Changes in the myelogram in panencephalitis nodosa (Subacute sclerosing leucoencephalitis) 79

Book Reviews 85

Abstracts 88

From the international literature on haematology 96

In memory of George Hevesy *(L. Kocsár)* 109

Pietschmann, H., Raab, W., Hartl, O.: Beitrag zur Hämatologie der Porphyria cutanea tarda 113

Trepel, F., Gerstmair, G., Waubke, R., Pichlmaier, H., Begemann, H.: Beziehungen zwischen der Abstoßung homologer Hauttransplantate und verschiedenen Lymphozytentypen im peripheren Blut 119

Danon, D., Shiffmann, A., Efrati, P.: Analysis of osmotic fragility as a routine test in clinical practice 131

Astaldi, G., Airò, R.: Questions and answers on leukemia problems 143

Lennes, G., André, A., Lhoste, C.: Comparaison du taux moyen de complément dans un échantillon de malades et dans un échantillon de personnes normales. — Considérations méthodologiques et application au cas des ulcères duodénaux et gastriques 163

Gergely, J., Árky, I., Medgyesi, G. A.: Iodination of human IgG and its papain-insensitive population 169

Hořejší, J., Svoboda, M., Švorcová, V.: A contribution to the study of calcium and phosphate metabolism in Kahler's disease 171

Péter, Á., Romhányi, J., Letenyei, K.: Cerebrospinal fluid diagnosis of meningeal leukaemia 181

Arnold, R.: Zur Bedeutung der Zellelektrophorese für hämatologische Fragen 193

Szász, I., G. Szelényi, J., Gárdos, G., R. Hollán, S.: Effect of different washing solutions on some characteristics of erythrocytes 207
Open Forum 217
Book Review 231
Abstracts 232
From the international literature on haematology 243
In memoriam Edward Kowalski 255
Deutsch, E., Hagenauer, E., Irsigler, K., Köbele, B., Lechner, K., Schmer, G.: Untersuchungen über das exogene Gerinnungssystem 257
Дульцин, М. С., Климова, Н. Ф.: Лечение больных идиопатическим миелофиброзом 275
Szász, I., Árky, I., Gárdos G.: Structure and function of erythrocytes, III. Effect of membrane modifications on the mechanism maintaining biconcave shape 287
Pawelski, S., Konopka, L., Kotelba-Witkowska, B., Rechowicz, K., Blaszczyk, J.: Observations on the survival of ^{51}Cr-tagged platelets in healthy subjects and in patients with thrombocytopenia 297
Guth, P., Budvári, R.: Inheritable beta-lipoprotein characters (Ag- and Lp-system) 307
Pastinczky, I., Kovács, M.: Study of blood coagulation in familial benign chronic pemphigus of Gougerot-Hailey-Hailey 313
Strausz, I., Barcsák, J., Kékes, E., Szebeni, Á.: Prednisolone-induced acute changes in circulating neutrophil granulocytes, I. In cases of normal granulocyte reserves 319
Strausz, I., Barcsák, J., Kékes, E., Szebeni, Á.: Prednisolone-induced acute changes in circulating neutrophil granulocytes, II. In cases of deficient granulocyte reserves 327
Open Forum 333
Abstracts 337
From the international literature on haematology 347
Dausset, J.: Le systeme Hu-1 359
Pasternak, G., Pasternak, L.: Immunologische Charakterisierung Graffi-Virus-infizierter Zellen der Maus 369
Bütler, R., Brunner, E.: Investigations on common antigenic structures of some human and animal serum proteins 375
Dintenfass, L.: Dynamics of blood coagulation introducing a new coagulation factor: "velocity gradient" 387
Solymoss, B., Gabbiani, G., Selye, H.: Blood coagulation changes in the thrombohaemorrhagic phenomenon (THP) 401
R. Hollán, S., G. Szelényi, J., H. Breuer, J., Medgyesi, G. A., N. Sőtér, V.: Structural and functional differences between human foetal and adult erythrocytes 409
Rechowicz, K., Szymanowska, Z., Konopka, L., Murawski, K., Pawelski, S.: Methaemoglobinaemia due to insufficiency of the reducing systems of erythrocytes 421
Gidáli, J., Fehér, I.: Differentiation and maturation of haemopoietic stem cells in spleen colonies 429
Barta, I., Patakfalvi, A.: Tuberculosis of peripheral lymph nodes 437
Open Forum 445
Book Review 455
Abstracts 456
From the international literature on haematology 461

Author Index

A

Airò, R. 143
Aleksandrowicz, A. 453
André, A. 163
Árky, I. 169, 287
Arnold, R. 193
Astaldi, G. 143

B

Barcsák, J. 319, 327
Barta, I. 437
Beale, D. 11
Begemann, H. 45, 119
Bernard, J. 5, 224
Beutler, E. 19
Bilski-Pasquier, G. 335
Blaszczyk, J. 297
Breuer, J., H. 409
Brunner, E. 375
Budvári, R. 307
Bütler, R. 375

C

Chernstova, T. A. 61

D

Dacie, J. V. 217
Danon, D. 131
Dausset, J. 359
Deutsch, E. 229, 257
Dintenfass, L. 387
Dulcin, M. S. 275

E

Efrati, P. 131

F

Fehér, I. 429

G

Gabbiani, G. 401
Gaertner, H. 453
Gárdos, G. 207, 287
Gergely, J. 169
Gerstmair, G. 45, 119
Gidáli, J. 429
Greenwalt, T. J. 446
Grignaffini, G. F. 27
Guth, P. 307

H

Hagenauer, E. 257
Hartl, O. 113
Hjort, P. F. 334
Hollán, S., R. 11, 207, 409
Hořejší, J. 35, 171

I

Irsigler, K. 257

K

Kékes, E. 319, 327
Kiselev, A. E. 445
Klimova, N. F. 275
Köbele, B. 257
Konopka, L. 297, 421
Kotelba-Witkowska, B. 297
Kovács, M. 313

L

Lechner, K. 257
Lehmann, H. 11
Lennes, G. 163
Letenyei, K. 79, 181
Levin, E. 19
Lhoste, C. 163
Loghem, J. J., van 226
Lusvarghi, E. 27

M

Medgyesi, G. A. 169, 409
Murawski, K. 421

P

Pasternak, G. 369
Pasternak, L. 369
Pastinczky, I. 313
Patakfalvi, A. 437
Pawelski, S. 297, 421
Péter, Á. 79, 181
Pichlmaier, H. 45, 119
Pietschmann, H. 113

R

Raab, W. 113
Rechowicz, K. 297, 421
Romhányi, J. 79, 181
Rudowski, W. 334

S

Schmer, G. 257
Selye, H. 401
Serafimov-Dimitrov, V. 448
Sgandurra, A. 27
Shiffmann, A. 131
Solymoss, B. 401
Sőtér, V., N. 409
Storti, E. 27
Strausz, I. 319, 327
Susoyeva, V. M. 61
Svoboda, M. 171
Švorcová, V. 171
Szász, I. 207, 287
Szatmári, É. 79
Szebeni, Á. 319, 327
Szelényi, J., G. 11, 207, 409
Szymanowska, Z. 421

T

Trepel, F. 45, 119

V

Verloop, M. C. 226

W

Waubke, R. 45, 119

Subject Index

Acetylcholinesterase activities in foetal and adult erythrocytes 409
— — in washed erythrocytes 207
Adenosine triphosphatase activities in erythrocytes 207, 287
Adenosine triphosphate (ATP), breakdown in erythrocytes 207, 287
Adenylate kinase activity in erythrocytes 19
Adrenaline, induction of thrombohaemorrhagic phenomenon by 401
— test 27
Albumin 375
Alkaline phosphatase activity in granulocytes 319, 327
Antigenes, leucocyte 359
—, specific for Graffi-virus leukaemias 369
Antileukaemic vaccines 143
Anti-Rh_0, effect on erythrocyte metabolism 287
Antisera, against serum proteins 375

Benzol, leukaemogenic effect of 143
Blood transfusion, teaching and specialization in 217, 333, 445

Calcium metabolism in Kahler's disease 171
—, role in prothrombin activation 257
Cerebrospinal fluid, antibodies in 79
— — cytogram in leukaemias 181
Chromatography, of the components of tissue thrombokinase 257
Coagulation changes in thrombohaemorrhagic phenomenon 401
— factor, in exogeneous coagulation system 257
— —, velocity gradient as 387
— studies in familial chronic pemphigus 313
Complement, in sera of healthy and ill persons 163
Cytotoxicity test 369

Electrophoresis, of foetal and adult erythrocytes 409
—, of peripheral blood cells 193
Erythrocytes, antibody coating of 287
—, differentiation of foetal and adult 409
—, effect of different washing solutions on 207
—, electrophoretic mobility of 193, 409
—, enzyme activities in 19, 207, 287, 409, 421
—, glutathione level in 207, 409
—, ion transport of 207, 287
—, lipase treatment of 287
—, lipid composition of 409
—, lipid structure of 409
—, osmotic fragility of 131
—, papain treatment of 287
—, penetration of non-electrolytes into 207, 409
—, shape maintenance of 207, 287
—, trypsin treatment of 287

Factor VII, fingerprint analysis of 257
— —, isolation on DEAE-cellulose column 257
— —, prothrombin activation and 257
Factor X, activation of 257
— —, disk-electrophoresis of 257
— —, end-groups of 257
— —, fingerprint analysis of 257
— —, isolation on DEAE-cellulose column 257
— —, prothrombin activation and 257
— —, purification of 257
— —, TAMe activity of 257
Fatty acids, in foetal and adult erythrocytes 409

Fibrinogen, in thrombohaemorrhagic phenomenon 401
Fingerprint analysis of Factor VII 257
— — of Factor X 257
— — of Haemoglobin M Kiskunhalas 11
Fluorescens-antibody technique 369
Fragiligrams, erythrocyte osmotic fragility curves 131
Fragiligraph 131
Fragility, osmotic, of erythrocytes 131

Genetics, of congenital methaemoglobinaemia 421
—, of leucocyte antigenes 359
Glucose-6-phosphate dehydrogenase activities in foetal and adult erythrocytes 409
— — — in methaemoglobinaemias 421
— — — in washed erythrocytes 207
Glutathione, effect in haemorrhagic shock 35
—, effect on the dissociation curve of oxyhaemoglobin 35
—, in foetal and adult erythrocytes 409
—, in washed erythrocytes 207
— stability 421
Granulocytes, alkaline phosphatase activity in 319, 327
—, electrophoresis of 193
—, ribonucleic acids of 143
Granulocytosis, induced by prednisolone 319, 327

Haematology, teaching and specialization in 217, 333, 445
Haemocytopherograms, normal and pathological 193
Haemoglobin M Boston 11
— — Kiskunhalas, fingerprint analysis of 11
— — —, fluctuation of its relative amount in the patient 11
Haemorrhagic shock, effect of glutathione in 35
Histocompatibility, antigenes and 359

Immunodiffusion 375
Immunoglobulins, gamma (IgG), iodination of different populations of 169
Immunprecipitation, cross reactions in 375
Ion transport of erythrocytes 207
Irradiation, bone marrow transplantation after 429
—, effect on lymphocytes 45
—, effect on skin transplantation 119

Kahler's disease, calcium and phosphate metabolism in 171

Leucocytes, H-2 antigene system of 359
—, Hu-1 antigene system of 359
—, resistance test, before and after adrenaline injection 27

Leucocytosis, induced by adrenaline 27
Leucoencephalitis, subacute sclerosing 79
Leucokinetics, in leukaemias 143
Leukaemias, chemically induced 369
—, diagnosis of meningeal 181
—, differentiation among acute 143
—, Gross 143, 369
—, leucokinetics in 143
—, neoplastic character of 143
—, neurological symptoms in 181
—, ribonucleic acids in 143
—, role of messenger ribonucleic acid (m-RNA) in 143
—, viral etiology of 143
Lipase, effect on erythrocyte morphology 287
Lipids, composition of erythrocytes 409
Lipoproteins, beta, inherited antigenic characters of 307
—, low density 375
Liver function tests, in Porphyria cutanea tarda 113
Lp-system, in paternity proceedings 307
Lymph nodes, tuberculosis of 437
Lymphocytes, differences in basophilia of 45
—, electrophoresis of 193
—, graft extrusion and changes in sub-groups of 119
—, ribonucleic acids of 143
—, X-ray sensitivity of sub-groups of 45

Macroglobulin, α_2 375
Methaemoglobin reductase activities in erythrocytes 421
Methaemoglobinaemia, congenital 421
Myelobromol, cytostatic effect in chronic myeloleukosis 61
—, effect in idiopathic myelofibrosis 275
Myelofibrosis, Myelobromol treatment of 275
Myelogram, in panencephalitis nodosa 79
Myeloleukosis, Myelobromol treatment in chronic 61
Myeloma, multiple, calcium and phosphate metabolism in 171

Non-electrolytes, penetration into erythrocytes 207, 409

Osmotic fragility of erythrocytes 131
Osteoporosis, in Kahler's disease 171
Oxyhaemoglobin, effect of glutathione and mercury compounds on its dissociation curve 35

Panencephalitis nodosa, cytology of bone marrow in 79
Papain, effect on erythrocyte structure and functions 287
—, sensitivity of IgG populations towards 169
Paraproteinaemia, in Kahler's disease 171
Partial thromboplastin time, in thrombohaemorrhagic phenomenon 401
Paternity proceeding, Lp-system in 307
Pemphigus, chronic familial 313
—, coagulation studies in 313
Philadelphia chromosome, diagnostic and prognostic significance of 143
Phosphate metabolism in Kahler's disease 171
Phospholipids, composition of foetal and adult erythrocytes 409
Platelet, aggregation as a function of velocity gradient 387
—, count in thrombohaemorrhagic phenomenon 401
—, sequestration 297
—, survival in healthy and thrombocytopenic persons 297
Porphyria cutanea tarda 113
Prednisolone, induction of granulocytosis by 319, 327
Prothrombin, activation of 257
— in thrombohaemorrhagic phenomenon 401

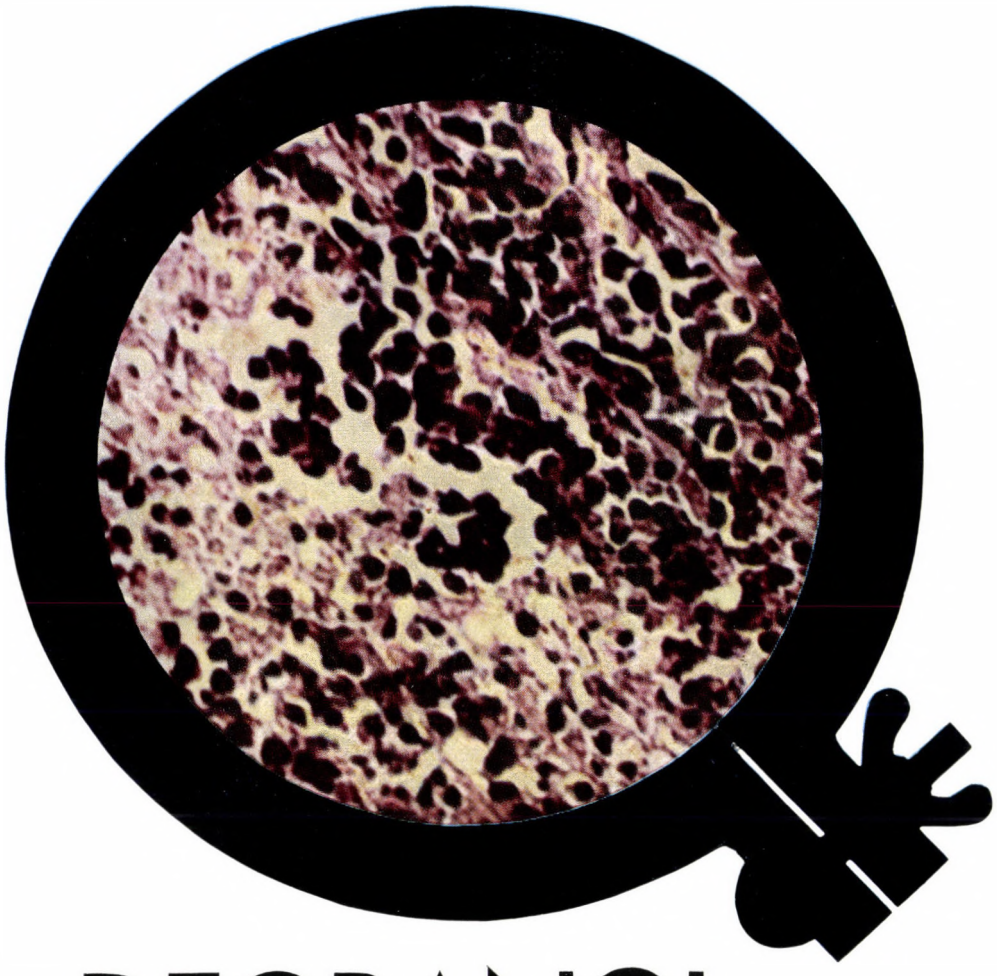
Recalcification time, in thrombohaemorrhagic phenomenon 401
Rheology, of blood coagulation 387
Ribonucleic acids, in leukaemias 143
— —, messenger, role in leukaemias 143

Sarcomas, haematosarcomas 5
—, induced by UV irradiation 369
—, Landschütz 369
Serum enzymes, in Porphyria cutanea tarda 113
— proteins, antigenic structure of 375
Species specificity of serum proteins 375
— — of tissue thrombokinase 257
Spleen, stem cell colonies in 429
Splenectomy, indication in thrombocytopenias 297
Stathmokinetic index 143
— test 143
Stem cells, in spleen 429

TAMe activity, in activated Factor X preparations 257
Thrombin time, in thrombohaemorrhagic phenomenon 401
Thrombocytopenias, platelet survival in 297
—, splenectomy in 297
Thrombohaemorrhagic phenomenon, in rats 401
Thromboplastic substances, cleaving effect on synthetic substances 257
Thromboplastin generation in thrombohaemorrhagic phenomenon 401
Tissue thrombokinase, amino acid chromatography in hydrolysates of 257
— —, clotting activity of the protein and lipid components of 257
— —, lipid chromatography of 257
— —, prothrombin activation and 257
— —, purification of 257
— —, species specificity of 257
Transferrin 375
Transplantation, of homologous skin 119
Trypsin, effect on erythrocyte structure and functions 287
Tuberculosis, of peripheral lymph nodes 437
Tumour, Burkitt's, its geographical distribution and chemotherapy 5

Ulcer, serum complement in doudenal and gastric 163

Velocity gradient, blood coagulation and 387
Viruses, Graffi 143, 369
—, leukaemic 143, 369
Viscosity, changes in blood during coagulation 387



DEGRANOL CHINOIN

Brand of mannomustine dihydrochloride (BCM)

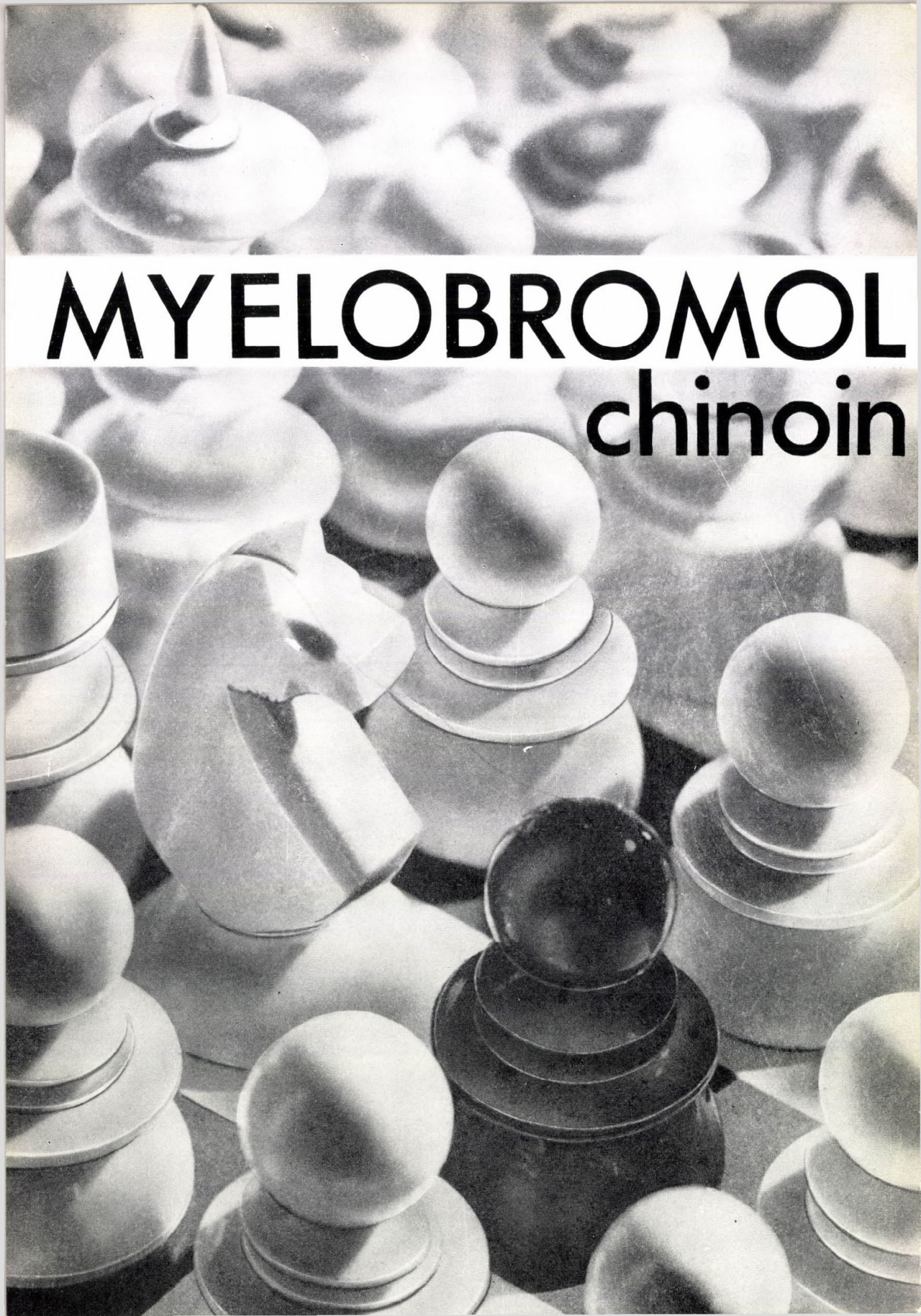
1,6-bis-(β-Chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride

powder ampoules of 50 mg · coated tablets of 50 mg

Chronic lymphoid leukaemia
Hodgkin's disease
Lymphosarcoma and other haemodlastoses
In tumor surgery as chemoprophylactic
For direct injection into the cavities with tumorous effusion
DEGRANOL is remarkably safe in adequate dosage

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O.B. 126


MYELOBROMOL
chinoin

MYELOBROMOL
chinoin

1,6-Dibromo-1,6-dideoxy-D-mannitol
tablets of 250 mg

A specific antigranulopoietic agent

Chronic myelocytic leukaemia
Polycythaemia vera

Highly selective action
Oral administration
No cross-resistance with other therapies
Response of patients can be controlled by dosage
MYELOBROMOL brings about the prolongation of survival

Chemical and Pharmaceutical Works CHINOIN Ltd. · Budapest

Information and literature by MEDIMPEX · Budapest, 5 · P.O B. 126 · Hungary

FOLIA MEDICA NEERLANDICA

Netherlands Journal of Internal Medicine

As a special issue nr. 4/5, 1967 just appeared:

SYMPOSIUM ON BLOOD COAGULATION

Hfl. 15,–

CONTENTS

Introduction
The pathways of blood coagulation
Treatment of Haemophilia B with purified Factor IX (PPSB)
Fats, blood coagulation, fibrinolysis and atherosclerosis
Thrombolytic therapy
The effect of some sedatives (phenobarbital, gluthertimide, chlordiazepoxide, chloralhydrate) on the rate of disappearance of ethyl biscoumacetate from the plasma
Blood platelets and haemostasis
Current concepts in thrombocytopenia
Diagnosis and treatment of disseminated intravascular coagulation

Folia Medica Neerlandica 6 nrs. each volume. Subscription Hfl. 35,–.

Obtainable from your bookseller or the publisher

DE ERVEN F. BOHN N. V.

Haarlem-Holland.

News items

XIIth INTERNATIONAL CONGRESS OF BLOOD TRANSFUSION

The XIIth International Congress of Blood Transfusion will be held in Moscow, Soviet Union, in August, 1969.

Scientific Programme: The following subjects have been chosen for the plenary sessions: transfusion of blood and plasma volume expanders, problems of immunology, preservation of blood and blood cells, transplantation of haemopoietic tissue and other organs.
A session will be devoted to the discussion of leucosis.
Documentary films on blood donation will be projected.

Languages: Papers may be given in Russian, English and French. Application to present a paper should reach the Secretariat before December, 1968.

Registration: Application for participation will be accepted not later than January, 1968.
All correspondence and inquiries concerning the Congress should be addressed to:
The Secretariat of the Soviet National Organization Committee
Central Institute of Haematology and Blood Transfusion
Novozykovski proezd 4
Moscow A—167, USSR

Professor Dr. A. E. KISSELEV

MAGYAR TUDOMÁNYOS AKADÉMIA

NOTICE TO CONTRIBUTORS

HAEMATOLOGIA is designed for the publication of original papers, preliminary reports and reviews which contribute to 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.
Central Research Institute of the
National Blood Service
Daróczi út 24
Budapest XI, 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 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) name(s) and initials of the author(s); (2) title of paper; (3) international abbreviation of the perodical; (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. Coloured 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:

ALBANIA

Ndermarja Shtetnore e Botimeve
Tirana

AUSTRALIA

A. Keesing
Box 4886, GPO
Sydney

AUSTRIA

Globus Buchvertrieb
Salzgries 16
Wien I

BELGIUM

Office International de Librairie
30, Avenue Marnix
Bruxelles 5
Du Monde Entier
5, Place St. Jean
Bruxelles

BULGARIA

Raznoiznos
1, Tzar Assen
Sofia

CANADA

Pannonia Books
2, Spadina Road
Toronto 4, Ont.

CHINA

Waiwen Shudian
Peking
P. O. B. 88

CZECHOSLOVAKIA

Artia
Ve Směčkách 30
Praha 2
Poštová Novinová Služba
Dovoz Tisku
Vinohradská 46
Praha 2
Mad'arská Kultura
Václavské nám. 2
Praha I
Poštová Novinová Služba
Dovoz Tlace
Leningradská 14
Bratislava

DENMARK

Ejnar Munksgaard
Nörregade 6
Copenhagen

FINLAND

Akateeminen Kirjakauppa
Keskuskatu 2
Helsinki

FRANCE

Office International de Documentation et Librairie
48, rue Gay Lussac
Paris 5

GERMAN DEMOCRATIC REPUBLIC

Deutscher Buch-Export und -Import
Leninstraße 16
Leipzig 701
Zeitungsvertriebsamt
Clara Zetkin Straße 62
Berlin N. W.

GERMAN FEDERAL REPUBLIC

Kunst und Wissen
Erich Bieber
Postfach 46
7 Stuttgart S.

GREAT BRITAIN

Collet's Holdings Ltd.
Dennington Estate
London Rd.
Wellingborough, Northants.
Robert Maxwell and Co. Ltd.
Waynflete Bldg. The Plain
Oxford

HOLLAND

Swetz and Zeitlinger
Keizersgracht 471—487
Amsterdam C
Martinus Nijhof
Lange Voorhout 9
The Hague

INDIA

Current Technical Literature
Co. Private Ltd.
India House OPP.
GPO Post Box 1374
Bombay I

ITALY

Santo Vanasia
Via M. Macchi 71
Milano
Libreria Commissionaria Sansoni
Via La Marmora 45
Firenze

JAPAN

Nauka Ltd.
92, Ikebukur O-Higashi 1-chome
Toshima-ku
Tokyo
Maruzen and Co. Ltd.
P. O. Box 605
Tokyo-Central

Far Eastern Booksellers
Kanda P. O. Box 72
Tokyo

KOREA

Chulpanmul
Phenjan

NORWAY

Johan Grundt Tanum
Karl Johansgatan 43
Oslo

POLAND

RUCH
ul. Wronia 23
Warszawa

ROUMANIA

Cartimex
Str. Aristide Briand 14—18
Bucureşti

SOVIET UNION

Mezhdunarodnaja Kniga
Moscow G—200

SWEDEN

Almquist and Wiksell
Gamla Brogatan 26
Stockholm

USA

Stecheri Hafner Inc.
31, East 10th Street
New York, N. Y. 10003
Walter J. Johnson
111, Fifth Avenue
New York, N. Y. 10003

VIETNAM

Xunhasaba
19, Tran Quoc Toan
Hanoi

YUGOSLAVIA

Forum
Vojvode Mišiča broj 1
Novi Sad
Jugoslovenska Knjiga
Terazije 27
Beograd

25. II. 1968. Index: 26341