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HAEMATOLOGIA

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Editorial

A message as well as a note of acknowledgement to our contributors, subscribers and readers

A little while ago we examined the past and present state of our Journal. We were proud to note: *Haematologia* is a widely read international periodical. The articles appearing are of high standard and cover broad areas in haematology, immunology and blood transfusion. Just to attest this, the papers published in recent years included reviews of therapeutical trials, reports on diagnostic problems, advances in technology and laboratory tests as well as works dealing with many other complex research subjects. Typographically *Haematologia* is up to par with the best in the field.

At the same time, however, we had to concede that editing and printing problems have sometimes resulted in rather erratic appearances. Well, we are going to fundamentally change this now.

To compensate for preceding arrears of publishing, a slender volume is brought out for this year which will be sent free of charge to all subscribers. The first issue of Volume 17 will reach the subscribers not later than January 1984. This and the succeeding issues will contain the papers already accepted.

The Editors sincerely hope that acquiescing with our efforts, you will keep on contributing to the traditionally high standards as well as to the growing readership of our international Journal with your estimable works and comments.

Another change we wish to announce is that with Volume 15 Elsevier Biomedical discontinues its association with this journal and VNU SCIENCE PRESS BV will join AKADÉMIAI KIADÓ in the distribution of *Haematologia*.

It is also a pleasure to express my sincere thanks to the reviewers for their unfailing courtesy and invaluable advice and help throughout our collaboration.

SUSAN R. HOLLÁN

Editor-in-Chief

Publishers' announcement

Please continue to send your manuscripts to the Editor-in-Chief
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Perspectives in Hematology*

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(Received 6 August 1982; accepted 21 January 1983)

Those of us who have chosen to concentrate our attention on the field of hematology have been very fortunate, indeed. Hematology has proved to be fascinating and exciting. For me, the experience has been especially rewarding because my interest began just at the time that hematology was about to be transformed from a morphologic exercise to a wide-ranging discipline that has contributed enormously to many aspects of biologic science, as well as to the health of man.

The Mystique of the Blood

Our interest is not unique, of course. The blood has always fascinated mankind. Blood caught the imagination of prehistoric man, as cave paintings in northern Spain testify, and it continues to intrigue man today. It has fired the imagination of poets, and puzzled the philosophers; mystics have spun their tales about it and charlatans have used it to embroil their innocent victims. Fortunately, it has also been the subject of study by serious seekers of the truth.

The Bible states that 'the life of the flesh is in the blood' and the Jews considered it to be the seat of the soul; consequently, the drinking of blood was prohibited and the orthodox practice, based on Mosaic Law, of salting meat before its use as food, was designed to rid meat of blood. The Romans, on the other hand, had no such qualms; they drank the blood of fallen heroes so as to imbibe their strength and courage. Before them, the Egyptians employed blood baths for recuperation and rejuvenation. And it was not only in ancient times that such extraordinary properties were attributed to the blood. Even in the fifteenth century, and later, blood was recommended for lunacy, fits, palsy, melancholia and bad disposition. There is the apocryphal story that when Pope Innocent VIII was on his deathbed in 1492, in a last desperate attempt to save his life, he received the blood of three youths, by draught, presumably. Shortly thereafter, he passed on, no doubt to Heaven; the three youths, likewise, one hopes. Nothing is known about the fate of the prescribing physician.

Needless to say, these views were held in the absence of any concept of the composition of blood, its functions or, until William Harvey's discovery, even its circulation. The contrary view, namely that blood could be bad, also had its ardent proponents, as we know, for venisection was practised for many centuries and contributed to the death of many, many victims, including the first president of the United States, George Washington.

The Nature and Functions of the Blood

It is an interesting commentary on the way we often gain insight that allusion to what turned out to be one of the functions of the blood arose as the consequence of the lust for gold in the 16th century. It was Father Acosta, a Spanish priest who accompanied Pizarro on his incursions in the Peruvian Andes who suggested in 1569 that the 'elements of the air' in those lofty heights might be so thin and delicate that it was not suitable for human breathing. He proposed that this might be responsible for the mountain sickness which presented so great a problem for Pizarro. More than another 200 years passed before oxygen was discovered. And even so, the relation of 'thin air' and oxygen to red corpuscles and hemoglobin was not appreciated until after hemoglobin was discovered in 1851 by Funke, and until Hoppe-Seyler in 1865 showed that hemoglobin has the unique property of taking up and discharging oxygen. For untold years, the tiny globules that Leeuwenhoek described in the blood in 1674 were not regarded as being of any importance, even after William Hewson suggested that they surely played some important role since nature had provided them in such abundance.

Thus, for centuries knowledge grew at an infinitely slow pace. The microscopists of the 19th century had an extremely hard time attracting scientific interest and, for the most part, they were scorned by physicians. Goethe wrote in 1829 that, 'Microscope and telescope confuse in reality the pure human judgement.' Madame de Pompadour and George III seemed to have been more interested in microscopy than the scientists of their time. The leukocytes also did not arouse much excitement. Even after they were recognized, their role was long debated. Virchow only acknowledged their relationship to pus after holding a contrary view for many years. Unorthodox ideas find few advocates even today.

That knowledge for a long time grew so slowly is, perhaps, not as surprising as the fact that it grew at all. Roger Bacon, in the 13th century, described the use of single convex lenses and thus, in a sense, was the inventor of the simple microscope. However, he was imprisoned and his writings remained hidden. It was left for the Dutch spectacle maker to describe in the blood what man had never before seen or even dreamed of.

It is worth recognizing how often chance has played a role in scientific progress. Thus, it is largely because Antony van Leeuwenhoek had a friend who had some influence and also because the Secretary of the Royal Society of London was open-minded enough to publish the observations of a man without scientific cre-

dentials that Leeuwenhoek's observations were at least recorded for posterity. On the other hand, one wonders how much sooner understanding would have progressed if William Hewson had not died of an infection sustained during an autopsy at the age of 35. His contributions concerning coagulation of the blood and regarding lymphocytes and the lymphatic vessels were fundamental and very important. Then, again, the story seems to be well founded that Ehrlich might have been expelled from medical school because he was just 'poking around' and not attending the lectures of his professors. His brilliant career might have been interrupted had it not been for the support of Professor Waldeyer. One wonders if George Minot would have carried out his liver therapy trials so successfully if he, as a diabetic, had not believed so strongly in the important role of food in blood formation. One could easily add to these examples of the role of chance in affecting the course of research. Biology is so extraordinarily complex that an unexpected observation, a happy accident, or a new interpretation of some seemingly unrelated finding can result in unforeseen applications and consequences of unpredicted and unpredictable implications.

Hematology in the 1920's

By the 1920's hematology, with a few exceptions, had made no great advances. Of far-reaching importance had been the observation by Landsteiner at the turn of the century that the red cells of some individuals were clumped or agglutinated by the sera of certain other persons. He showed that this was a phenomenon observable in the blood of normal individuals, not a manifestation of illness, as had been thought. As we know, this led to the discovery of the four major blood groups and made blood transfusion possible without the consequences that led in 1668 to the prohibition, by law, of the transfusion of blood. Blood incompatibility between species had been noted before but that not all bloods even of the same species were compatible and that this was a natural phenomenon occurring in normal individuals had not yet been recognized by the beginning of the present century.

Metchnikoff, a zoologist, had discovered phagocytosis and Paul Ehrlich, by means of his blood staining methods had stimulated great interest in blood morphology. A number of the hematologic diseases we recognize today had by the 1920's been described but their pathogenesis was unknown. It is interesting to read the section on diseases of the blood in the first edition of Osler's famous 'Principles and Practice of Medicine' published in 1892. A total of 24 pages was devoted to this subject in a textbook of 1079 pages. In contrast, 39 pages were devoted to typhoid fever and still more to tuberculosis. Elsewhere, almost a page was assigned to paroxysmal hemoglobinuria, but only the form of hemoglobinuria associated with Raynaud's disease and exposure to cold was discussed, not the nocturnal form. Hemolysis was mentioned in relation to pernicious anemia and to 'toxic hemoglobinuria'. There was no entry, however, for acholuric jaundice. Polycythemia vera and hereditary telangiectasia, diseases with which Osler's name is

now associated, were not mentioned. Even in his 10th edition, published in 1925, only 35 out of 1173 pages were devoted to the blood and blood disorders.

Another more modern book, the first edition of Cecil's Textbook of Medicine, published in 1927, devoted only a total of 60 pages to diseases of the bloodforming organs, out of a total of 1500. In Volume II of J. Von Mering's Lehrbuch der Inneren Medizin, edited by L. Krehl and published in 1925, Naegeli discussed all Blutkrankheiten in 29 pages.

On the technical side, the situation was equally bleak. Methods for enumerating red and white cells, developed in the latter half of the 19th century, were difficult to execute with any degree of accuracy and platelet counting amounted to scarcely more than an estimate. The measurement of hemoglobin could only be called crude, even if one ignored the Tallqvist scale – although one could not really ignore the Tallqvist because it was widely used! Hemoglobin was expressed in per cent, supposedly as per cent of normal. To my surprise, however, when I began to develop an interest in hematology, the values that were then being quoted as representing normal were based on a few determinations made in the 19th century by the even cruder methods of that time. This is why I began to develop some data on which normal values could be based, as did a few others, especially E. E. Osgood. I was stimulated to initiate studies in normal male and female students, incidentally, because in 1927 the idea was prevalent in New Orleans, Louisiana, that there existed an 'anaemia of the south'; that is, that normal blood levels in the southern United States were lower than in the North. This, incidentally, proved to be a figment of someone's imagination.

Even though hemoglobin estimation was so crude and no allowance was made for the recognized difference in normal values for men as compared with women, per cent hemoglobin, divided by the percentage of the red cell count, taking 5.0 million RBC's as 100 per cent normal, was used to determine the color index, a procedure introduced by Georges Hayem, the French 'father of hematology'. This index was widely used in Europe and in America, especially to help distinguish pernicious anemia from chlorosis and other 'secondary' anemias.

It is no wonder that the differential diagnosis of anemia was confused. To provide a more accurate measurement of red cells than was then available, I devised a hematocrit which had a sealed bottom, required approximately 1 ml blood and could be easily calibrated, thereby to correct the deficiencies of the microhematocrit which was occasionally being used in the hope of improving hematologic methodology.

Measurement of the size of red cells was of great interest in the 1920's. Cecil Price-Jones had clearly demonstrated the striking differences in the sizes of the red cells in pernicious anemia and in other anemias and the dreaded task of a student or house officer was the work and tedium involved in preparing a Price-Jones curve. The method was not only tedious but very time consuming. However, there was no gainsaying that Price-Jones made his point.

It was in this period that Russell Haden called attention to the Volume Index and introduced the Saturation Index, as complementary to the Color Index. These

procedures troubled me, however, because they were based on the unjustified assumption that a value for 100 per cent could be established for men and women and, it was also assumed, for children. Furthermore, I was impressed that those who used these terms did not really understand what they meant. For this reason, the thought came to me that one could devise a method of calculation that required no assumed normal values. The size and hemoglobin content of red cells could, moreover, be expressed in readily understandable terms. Thus, the calculations of MCV, MCH, and MCHC were introduced. Furthermore, on the basis of improved technical methods and such calculations, I then devised a classification of anemias which offered more than a means for separating pernicious anemia from a miscellany of other forms of anemia.

The Beginning of the Modern Era

I am sure that everyone will agree that the modern era of hematology had its 'official' inception with the publication of a paper entitled, 'Treatment of Pernicious Anemia by a Special Diet,' in the *Journal of the American Medical Association*, *87*, 470 (1926) by G. R. Minot and W. P. Murphy. This was a very significant turning point. It marked the introduction of the methods of experimental medicine in the field of hematology. The paper described a painstaking clinical study, supported by meticulous daily reticulocyte counts, in which test diets were given for periods of 10 days each and the results were observed daily. It might be pointed out, also, that without Ehrlich's 'useless' study of reticulocytes and Minot's finding that their increase heralded remission in pernicious anemia, the length of time required to clearly demonstrate the effect of liver in pernicious anemia would have been so long that the risk for the patients might have prohibited the experiments. Likewise, it would have been very difficult for Castle to show the effect of beef muscle, plain and then predigested, experiments that led to his famous intrinsic factor, extrinsic factor theory.

Minot's and Castle's series of experiments were convincing and had a profound effect on hematology. One began to think about the various steps in erythropoiesis and to ask about mechanisms and substances essential for their production. To me, an intern in 1926, Minot's report was inspiring. I realized how little was known or understood but, like many others, I was profoundly stimulated to begin to enquire.

As one looks back, it is surprising that progress at first was not very rapid. It is interesting to examine the first edition of my own book, *Clinical Hematology*, published in 1942. The words 'stem cell' and 'tissue culture' are only mentioned very briefly. Bone marrow puncture was just beginning to be utilized. Whether or not the red corpuscle possesses a membrane was uncertain. The efficiency of this structure as a carrier of oxygen was appreciated but its anerobic metabolism was suspected by only a few. As to the leukocytes, I stated that our concepts of their functions 'are based largely on inference and are founded to a great extent on the

variations which have been observed to occur in the blood and in the tissues in pathological conditions.' This fact caused me to draw attention to the possibilities for error in this indirect method. The lymphocytes were stated to be still more mysterious than the polymorphonuclear cells and monocytes. Interestingly, on the basis of studies that Rich, Lewis and I conducted, we ventured to suggest that one function of the lymphocyte was concerned in some way with the body's reaction to foreign protein.

Coagulation was a very simple process (don't we wish it were so today!). Oncology, as we know it today, did not exist. 'Mediterranean anemia' was stated to be 'rare, apparently, for less than 100 cases have been reported since Cooley's paper appeared in 1925.' It was stated that, although families have been said to be normal, . . . this claim has not been supported by evidence of thorough examinations of such 'normal adults.' This fact troubled me and I pointed out that my own observations showed that both parents in two families with children having Mediterranean anemia had mild anemia and red cell abnormalities consistent with a 'benign' form of Cooley's anemia. This, as Neel has stated, was the final link between thalassemia and the conditions described by Angelini and by Caminopetros.

Progress was interrupted by World War II, but it is noteworthy that modern chemotherapy had its beginnings in the studies of the biological effects of nitrogen mustard that Goodman, Gilman, Dougherty and others (including myself) conducted. It was also in connection with that war that Peter Medawar began inquiring why skin grafts used in the treatment of burns were rejected – investigations that we now know were so important in illuminating the functions of the lymphocyte. In a later war, incidentally, G6PD deficiency was discovered in the course of a search for more effective antimalarial compounds.

The Present and the Future

All of these investigations, however, were but the prelude. It is in the last three decades that the greatest surge in knowledge and understanding has taken place. There has never before been a comparable growth of knowledge of living things and, among these phenomenal advances in the field of biology, hematology has held center stage. Consider only two examples. It was the investigations of the nature of the sickling phenomenon and, in particular, the observations by Sherman in my laboratory that deoxygenated sickle cells show birefringence in polarized light, that led Pauling and Itano to carry out the studies which resulted in the epochal report, 'Sickle cell anemia, a molecular disease', that initiated the molecular age in biology. And almost more astounding have been the results of the study of the lowly lymphocyte, the cell whose uniformly monotonous appearance hides its astonishing diversity. Who had ever dreamed that these morphologically anonymous cells have such diverse capabilities, possess so long a memory and have played so important a role in the process of evolution.

With all that has gone before, what can we say of the future? Where will cell

culture, the study of cell surface markers, recombinant DNA, monoclonal antibodies, gene splicing, bone marrow transplantation and a host of other advances carry us? Will monospecific antibodies make it possible to distinguish and to target malignant cells? With the better characterization of the leukemias that surface markers provide, will we be able more accurately to destroy the abnormal cells and leave the normal ones unharmed? As Peter Medawar has stated, an important objective of research is the detailed mapping of cell surfaces. When we learn the molecular basis of specificity we may learn why, in development, some cells go one way and others follow another route, why some stick together, others do not. In due course, we are likely to find a way to detect small metastases and perhaps to deliver cytotoxic drugs to malignant cells. Tissue typing will be improved by the use of homogeneous antibodies and this should not only aid organ transplantation but it will further the study of the relationship of human leukocyte antigens (HLA) to the development of various diseases. There are good grounds to hope that one day we will be able to replace genes that are defective.

The visions and possibilities I have mentioned are based on realization of what we know today. It does not require much dreaming to conceive them and even to think of others.

But experience tells us that advances often have come from totally unexpected directions and in unforeseen ways. I find it very humbling when I try to imagine what I might have predicted even 35 years ago. Before we learned about the structure of hemoglobin, how many would have conceived the picture we have today of the abnormal hemoglobins and of thalassemia? Who would have guessed a little more than 30 years ago, before sickle cell anemia was found to be the consequence of an alteration in a single amino acid out of the 146 in the β-globin chain of hemoglobin, that we would find so many hemoglobin abnormalities. Or, even more recently than 30 years ago, who would have dreamed that there exist so many varieties of thalassemia, formed in so many different ways?

Shakespeare wrote:

> Strange it is that our bloods
> of colour, weight, and heat, pour'd all together,
> Would quite confound distinction, yet stand off
> In differences so mighty.
>
> All's Well That Ends Well
> II, iii, 125 – 128

Little did he realize how wisely he was speaking.

A question I was asked when I was beginning my study of the blood and blood diseases was: Why does the blood remain 'normal' in healthy human beings? This, when we stop to reflect, is one of the most difficult questions of all. We are only now beginning to discover ways of investigating some of the regulatory mechanisms and the differences in the regulation of the growth and development of neoplastic cells as compared with normal cells. Hitherto, we have learned mainly by exploring the defects we have encountered, the so-called 'experiments of nature.'

Now more direct approaches are being devised. And, incidentally, when we contemplate the extraordinary number and variety of defects that have been discovered, and consider those that could take place, one must marvel that we ever developed at all! But that is the essence of the evolutionary process.

There is good reason to expect that some day we will know how our complicated and intricate biologic mechanism is regulated, corrected and faulty parts (cells?) destroyed. How soon this will come about and whether or not the answer will contain many surprises we can, each of us, speculate all we will. I suspect that few will dream imaginatively enough. And, no doubt, there also will be answers to questions we have not even thought to ask.

Dangers Ahead

Looking backwards, we have very good reasons to hope for a rosy future. But there are problems. In the field of biologic science we have been blessed with peace and trust in our fellow human beings. There has been more and more free exchange of ideas and support for research has increased, albeit in spurts and starts. Progress has been made because, as time has gone on, more and more ingenious minds have had an opportunity to pursue questions that have excited them. As these were answered, still more questions arose and, so, questions and answers have multiplied at a logarithmic rate.

Hitherto, and for some time now, medical science and hematology in particular, has attracted many young people. Success has bred success. As new discoveries were made, more and more young men and women were stimulated to try their talents. Will this continue? It seems to me that there are fewer now who want to dream and are willing to devote their lives to the search for greater understanding. Is there a general eating away of enthusiasm for research, even some pessimism regarding the future of civilization as a whole?

In certain respects we live in an irrational and even an insane world. Much more energy is spent every day on ways and means to destroy mankind than ways to benefit the human race. These means are becoming more and more widespread and one doesn't know when some madman will set a spark that will destroy all that we have learned. War is not a new experience for man, but now war can be totally devastating. We now are able to destroy all of mankind, not just thousands or a few million. Will sanity ever be restored? Will there be a return to idealism, general good will, a striving for the good of all mankind?

We cannot set these awesome thoughts aside but, nevertheless we must continue to give thought to less overwhelming considerations. One of these is the matter of free communication. An important element in the progress we have witnessed is the fact that communication has been open and ever more rapid. As a result, each investigator has stood on the shoulders of his predecessors and his contemporaries, adding rapidly thereby to our fund of knowledge. This has become the accepted practice and, by and large, credit has been given where it was due. But now a new

aspect is entering biologic science. I refer to the potential exploitation of molecular biology. Discoveries relating to recombinant DNA and monoclonal antibodies have great potential for the manufacture of useful and profitable products. This is exciting and holds much promise. But we hear of scientists who have formed their own companies so as to profit financially from their discoveries and who, as a consequence, have become multimillionaires overnight. We hear of famous universities doing the same or considering steps in this direction. One writer suggested that one such university whose motto includes the word, Veritas, should change it to Cupiditas.

The element of private gain, whether for the scientist or the university or a commercial institution, or for combinations of these, must by its nature stifle the free exchange of ideas. This is extremely disturbing because open communication is the bedrock of science. Hitherto, it has been mainly the developmental aspects of some scientific discoveries that have been in the domain of commercial ventures and the resulting inhibiting effect on free and open scientific research has been fairly limited. The new developments to which I refer bode ill. There is great danger.

One can understand the reasons for the desire of all parties to secure some of the profits that arise out of research. However, while the problem is not altogether new, now for the first time scientists and universities have something extremely valuable to sell and this comes at a time when funds for research support are becoming more scarce and research is becoming more costly. We must find a solution to this problem that is favorable to science. The scientist and the university must be beholden to no interest but the truth. If this ideal is shattered, our dreams for the future will be endangered. The goose has laid a golden egg, but will it be the last?

At the other end of the spectrum is the way in which medicine will be practiced. We are on the threshold of fundamental transformations in health practices as the result of the growth of knowledge and the technologic advances that have been made, such as innovative methods for securing data and computerized systems for integrating and interpreting them. These will have far-reaching social and economic effects. Designed to improve our ability to help our patients, there is nevertheless a danger that the physician will be further and further removed from the patient and may, himself, lose the integrative skills that a well-trained physician develops. When our approach was mainly qualitative, we were forced to pay close attention to the patient. Now that we measure and measure, numbers rather than human qualities tend to dominate. It becomes easier to ignore the fact that each patient has his or her unique features. The need of our patients to communicate with the physician still is there and will never disappear. We must find ways to provide our patients with all the benefits of science but we must do this in a way that is not economically overwhelming. We must also continue to regard the concerns, the fears and the worries of our patients with the same importance as their diseases and the fascinating problems that the latter present.

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Leukemia Antigens and Immunity in Man

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Research on human leukemia antigens and immunity in leukemia has undergone considerable changes within the last few years. Until recently, most of the field was dominated by two of the concepts prevailing overall in tumor immunology, that most tumor cells would be found to have tumor-associated antigens and that most tumors would have demonstrable immunogenicity, which could lead to host resistance against progressive disease. There were many efforts to identify leukemia-associated or specific antigens and indeed several were reported. There were also many studies on specific immune reactivity of leukemia patients against autologous leukemia cells. Upon more careful assessment of these data, however, considerable doubts about the documentation of the specificity were raised. The recent advent of monoclonal antibody technology raised new hopes for the identification of leukemia-specific antigens. However, experience to date has indicated that this approach suffers from most of the same problems of specificity as the previous studies with antibodies in the sera of patients or with antisera produced in heterologous species.

Despite the overall disappointment in the usual failure to identify truly specific leukemia antigens, a quite intriguing and important pattern has emerged. It appears that almost all of the antigens detectable on leukemia cells are differentiation antigens which are appropriate for the stage of development or differentiation of the cells. Normal hematopoietic cells at the same stage of differentiation have been shown to share the same characteristics [1]. In each of the major types of leukemia and lymphoma, the marker information appears to fit well with this overall concept. One example of this relates to chronic myelogenous leukemia. Assessment of the distribution of the marker Philadelphia chromosome has indicated that the target cell is probably the pluripotential stem cell in the bone marrow, with potential for any of its derivatives to be affected by the leukemic process. This could provide a ready explanation why, in contrast to the usual myeloid differentiation in this condition, some cases of blast crisis show lymphoid markers rather than myeloid markers. This concept is of particular relevance to the controversy over the classification of the K562 cell line derived from a patient with chronic myelogenous leukemia in blast crisis. Observations that these cells could be induced to express markers of erythroid differentiation led many investigators to con-

clude that this cell line was inappropriately classified and represented an erythroid leukemia. However, further characterization has revealed that this cell line also has the potential to express markers of myeloid and megakaryocytic differentiation [2]. In further support of this concept, Bonnard et al. [3] presented evidence for accessory functions of this cell line compatible with a macrophage or myeloid differentiation. It is also of note, in view of the widespread use of this cell line as the prototype target cell for studies of human natural killer (NK) activity, that P. Pattengale [personal communication] has found that fresh leukemia cells from patients with chronic myeologenous leukemia in blast crisis are also highly susceptible to attack by NK cells.

It has also been possible to assign most of the lymphocytic leukemias to a particulat phase of differentiation of normal lymphocytes. Although the common acute lymphocytic leukemia antigen was initially thought to be leukemia specific, it was later found to be expressed on a small subpopulation of normal bone marrow cells. It appears that this antigen is an indicator of a very early phase in B cell differentiation, prior to expression of cytoplasmic or surface immunoglobulins. At this Congress*, Jánossy and his colleagues [4] presented evidence for a normal equivalent cell for the usual form of chronic lymphocytic leukemia, which has B cell characteristics. In addition to expressing surface IgM, it paradoxically expresses the T1 antigen which has been mainly associated with T cells, and also has receptors for mouse erythrocytes. They have shown that its normal counterpart, which has the same characteristics, is clearly a peripheral B cell, being found in tonsils and reacting with two new monoclonal antibodies, RFA2 and 3, which appear specific for B cells in the periphery. It is of interest that the normal B cell with these characteristics is quite rare, representing only about 5 per cent of normal B cells and localized at the edges of germinal centers. Jánossy also summarized recent information of Diehl et al. from the FRG regarding the normal cell counterpart of the Reed–Sternberg cell of Hodgkin's lymphoma. This group has developed a monoclonal antibody reactive against the Reed–Sternberg cell and has been able to show that there are small numbers of cells in normal lymph nodes, in clusters in the inner ring of the follicular mantle of lymph nodes, but which is entirely negative with T cells, interdigitating dendritic cells, macrophages, or any cells in the bone marrow or peripheral blood. It still remains to be determined what the cell lineage is of this type of cell. It was pointed out that the ability to accurately classify leukemias and lymphomas has therapeutic implications, since B cell lymphomas respond well to intensive chemotherapy. Further, by identifying antigens that are selectively associated with the particular leukemia or lymphoma, it may be quite useful to devise strategies for autologous bone marrow transplants, in which the bone marrow cells of the patient are pretreated with the appropriate antibodies to eliminate small numbers of leukemia cells, prior to infusion after intensive chemotherapy.

At this Congress,* there was considerable evidence of research activity on characteristics of T cell leukemias and lymphomas, particularly from Japan.

* Int. Congr. ISH–ISBT, Budapest 1982.

In addition to a summary of these diseases by Watanabe in a symposium [5] there were several poster presentations. Nishioka et al. [6] reviewed a large series of lymphoid tumors in Japan and found that almost 80 per cent had T cell characteristics. Other presentations also emphasized the predominance of T cell markers in these diseases, particularly with expression of the T4 antigen, which has been associated with a subpopulation of T cells with helper activity. However, some of the presentations emphasized the hazards of directly equating marker information with functional activity. Yamada [7] reported that some patients with this 'helper' phenotype in fact showed strong suppressor activity for B cell differentiation. Conversely, a case of T cell chronic lymphocytic leukemia from Taiwan was reported by Chen et al. [8] to have the T8 phenotype but helper activity in vitro. One further interesting feature to some of the Japanese studies was the detection of antibodies in most of the patients that reacted with a T cell leukemia-associated antigen by immunofluorescence. It appears that this antigen is related to the human T cell leukemia virus recently described by Gallo and also by some workers in Japan. It was of interest in the presentation by Ichimaru et al. [9] that the majority of spouses and siblings of leukemia patients were also found to have such antibodies. Although such data are intriguing, they need to be interpreted cautiously, since no etiologic link has yet been made between this virus and T cell leukemia or lymphoma. It is also of note that in contrast to the high association of this virus with Japanese T cell tumors, the association is considerably less frequent among cases in the United States.

Another type of leukemia that was emphasized in several of the presentations was hairy cell leukemia. The lineage of the leukemia cells in this disease remains quite controversial, as evidenced by the heterogeneity of information presented at the Congress. Leukemia cells frequently show B cell characteristics, with monoclonal surface immunoglobulins and other B cell features. However, Reyes et al. [10] reported that hairy cell leukemias can display accessory function in T cell colony formation in agar, thus mimicking some features of macrophages. The cases described by this group initially had both monocytic and B cell markers but in culture the monocytic markers predominated. Najfeld et al. [11] reported on an interesting patient who had cells with features of T cells, B cells, and monocytes. The non-T leukemia cells were shown to be phagocytic and expressed monoclonal surface immunoglobulin, and reacted with several anti-hairy cell monoclonal antibodies. It will be of considerable interest to determine if this type of leukemia is derived from one normal cell type and if so, what its features and characteristics are.

A central practical issue, in addition to classification of leukemias and lymphomas, is the possible prognostic information provided by leukemia markers. For real clinical value, it would be important to demonstrate that a given marker would allow better prediction of survival or response to therapy than the currently available criteria. At this Congress* there were a few reports on the possible prognostic value of some markers in leukemia and lymphoma. Kühnl et al. [12] reported on factor B levels in Hodgkin's disease, showing a correlation with length of sur-

vival. Some value of measurements of serum β_2 microglobulin was suggested by Cajozzo [13] et al. as a marker for more aggressive or advanced lymphomas. However, it was not clear whether levels of this marker could discriminate within clinical stages of disease. Of considerable interest was the report by Welte et al. [14] that in childhood acute lymphocytic leukemia, low expression of terminal transferase (TdT) was associated with poor prognosis. Tóth et al. [15] evaluated five acute phase reactants in patients with Hodgkin's disease and non-Hodgkin's lymphoma. They found ceruloplasmin and haptoglobin to be particularly useful, with elevations in untreated patients with advanced disease. Patients with remaining elevations or progressive rises in marker levels after therapy were found to have a poor prognosis. As a general caution about the use of circulating markers, Fertakis et al. [16] reported considerable diurnal fluctuations in levels of carcinoembryonic antigen and β_2 microglobulin in patients with leukemia and lymphoma, as well as in other diseases. They stressed the importance of repeated sampling, to assess more accurately the levels of markers.

In addition to leukemia markers, another important issue is immunity in leukemia. The shift in emphasis in this field was illustrated by the virtual absence of reports on specific T cell-mediated immunity against human leukemia. The only suggestion that the T cell compartment might be important in resistance against these diseases came from the symposium presentation by Freireich [17] showing that a general marker of T cell competence, lymphoproliferative responses to the mitogen phytohemagglutinin, had some value as a prognostic variable in acute myelogenous leukemia patients before therapy. Low responses were associated with poor prognosis.

Much of the immunologic emphasis at the meeting was devoted to NK cells, which have been shown in animal systems to have a significant role in resistance against tumor growth and particularly against metastasis. Of particular interest at the Congress* was the report by Oshimi et al., [18] who studied the susceptibility of leukemia and lymphoma cells to lysis by NK cells. Of 21 tumors studied, only three were susceptible to normal NK cells, but 11 became detectably susceptible when the effector cells were pretreated with interferon. Some types of tumor cells were particularly susceptible, especially well differentiated lymphocytic leukemia, Hodgkin's lymphoma, and chronic myelogenous leukemia in blast crisis. In contrast, acute myelogenous leukemia cells were quite resistant. Particularly interesting was the observation that autologous lymphocytes from some patients could lyse the leukemia cells, but high doses (about 2500 units) on interferon were required for induction of detectable responses. Several groups reported depressed NK activity in patients with leukemia and lymphoma, and also in patients with other types of cancer. Yagita et al. [19] also found depressed NK activity in patients with aplastic anemia, which appeared to be due, at least in part, to adherent suppressor cells. Some of this suppression appeared to be associated with T cell suppressors for colony forming units. Of particular interest in relation to leukemia were the observations by Yoda et al. [20] that patients with paroxysmal nocturnal hemoglobinuria had depressed NK activity even after interferon pretreatment.

He noted the frequent occurrence of leukemia in patients with this disease, suggesting a possible pathophysiologic link between this tendency and the defective effector mechanism. In addition to attention to the associations between NK activity and disease, there were some reports on the nature of the specificity of recognition by NK cells. Benczúr et al. [21] reported a heterogeneity of specificities, with about seven defined against autologous and allogeneic PHA-stimulated normal cells, in addition to expression on tumor cells. Some activity was depressed by antibodies to β_2 microglobulin, suggesting a role for the major histocompatibility complex. Szabó et al. [22] in a study of autologous cytotoxicity in chronic myelogenous leukemia reported that reactivity was inhibited by the gp70 of two oncogenic viruses, gibbon ape leukemia virus and baboon endogenous virus. The meaning of this intriguing finding remains obscure, however.

It is worthwhile to briefly consider the therapeutic implications of these studies on leukemia antigens and immunity. Although manipulation of potential effector mechanisms might have therapeutic benefits, there is still little evidence to support this approach. In contrast, other approaches are of potential interest. In view of the apparent arrest of leukemia and lymphoma cells at a particular point in differentiation, Sachs [23] emphasized the possibility for stimulating these cells to further differentiation, which in some animal tumor systems resulted in a susbtantial decrease in growth. Also of considerable potential importance is the use of monoclonal antibodies directed against various markers on leukemia cells. A possible major limitation to this approach may be the induction of antigenic modulation by some of these antibodies, as was reported by Ritz et al. [24] in an attempt with a monoclonal antibody against the common acute lymphocytic leukemia antigen. However, some preliminary studies with antibodies to T cell antigens have shown promising results, with at least transient reductions in tumor burden [25]. Particularly intriguing has been the report of a case of B cell lymphoma which was treated by Miller et al. [26] with a monoclonal antibody against the immunoglobulin idiotype. The treatment had a dramatic antitumor effect. It will be of considerable interest to determine how consistently this approach will be successful. More generally, studies over the next year or two will undoubtedly provide more implications about the overall therapeutic value of this approach to the management of leukemia and lymphoma.

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New Trends in Blood Component Therapy

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Red Blood Cells

The blood container

The design of the blood container was the subject of a special symposium chaired by Cash [32]. Most of the closed plastic bag systems for blood collection and preparation of blood components are designed for storage and clinical use of the cellular components and plasma. Lane [33] emphasized the need of a container suited for the large-scale plasma fractionator, making it possible to preserve the excellence of fresh plasma and allowing a rational and hygienic procedure in the plasma fractionation plant. Experience with such a system has been reported.

Although closed systems have been proven to allow the greatest safety in blood component preparation and are recommended for such procedures by many national regulatory agencies, systems which are being opened, e.g. to remove plasma, are also in use. McClelland [35] reported that so-called pig-tail systems are sufficiently safe provided that the procedure is well controlled not to allow reflux [84]. Flexible plastic bags are superior to rigid containers, such as glass bottles, since by their use the separation process is easily done aseptically. As pointed out by Lovric [34] sedimentation can be done by gravity in developing countries where refrigerated centrifuges or freezers are scarce. If glass bottles are used in blood component production, high hygienic requirements should be applied, e.g. work in laminar flow hoods as reported by Strauss [72].

Sometimes it is desirable to enter a container, e.g. in order to pool granulocyte or platelet preparations. Smit Sibinga [36] reviewed the sterile docking devices by which the connection between two containers is sterilized by heat before opening. The principle was reported in 1975 but so far only limited clinical experience is available. Apparently it is difficult to make the procedure sufficiently safe and cost-effective. A new design of a sterile connector was demonstrated at an exhibition by the Travenol Company.

So far most plastic bags used for storage and transfusion of blood components have been made from polyvinylchloride (PVC) using di-ethyl-hexyl-phthalate (DEHP) as plasticizer. The presence of microholes ('pin-holes') in the plastic film and other defects in the bags has been a matter of concern, putting emphasis on proper quality control in the manufacturing process. The fact that DEHP is extracted from the wall of the bag into the plasma has been a matter of discussion for a decade. Still there is controversy if a contamination of blood products with DEHP

is a real risk factor. Recent findings that very large doses of DEHP given orally to rats and mice increase the incidence of liver tumours have not, at least not yet, led to its abandonment in blood bags [1]. No doubt, however, that there is a desire to find materials without these potential disadvantages, but it has apparently been difficult to find other materials with equally good storage properties, at least for the red cells. The gas transport through the wall of the bag is of particular importance in the storage of platelets and will be dealt with below.

Red cell preservation

The ACD and CPD solutions widely used for the collection and storage of blood were originally designed for whole blood. Addition of adenine or adenine + guanosine makes it possible to increase the maximum storage time. Such solutions have been used in Europe since the middle 1960s (for review see [2, 3]) but have not been allowed in other countries like the USA until more recently. The importance of increasing the glucose content of the CPD anticoagulant was emphasized by some authors [63, 64] as well as in the earlier literature [4].

A new trend is to use a separate suspension medium for the red cells, thus separating the function of anticoagulation from that of preservation. Systems such as the SAG and SAGM systems described by ourselves [82] and the Circle Pack System described by Lovric [34] have been used extensively in clinical practice with great success. The suspension media are basically a sodium chloride solution with adenine, glucose and mannitol (SAGM) or adenine, glucose, sodium citrate, citric acid and sodium phosphate (Circle Pack). The initial anticoagulants are normal CPD (SAG and SAGM systems) and CPD with double normal content of glucose (Circle Pack). A closed plastic bag system was used in both procedures. Optimally the system should include four bags by which the following four products are obtained: 1. a platelet poor and white cell depleted red cell suspension (thus containing very little microaggregates), 2. a platelet concentrate, 3. a platelet poor plasma and 4. a buffy-coat preparation. Alternatively, such a system can be used to produce 1. a red cell suspension, 2. a buffy-coat preparation, a maximum yield of plasma which is further processed into 3. cryoprecipitate, and 4. 'cryo-poor' plasma. The buffy coat can be used for interferon production as it is done at a large scale in Finland [24], or as a source of granulocytes in granulocyte transfusion. A three bag system with pig-tail was described by Walker et al. [67]. This may reduce the cost of the system.

When a closed multiple bag system is used, the total content of the respective containers is transfused. This restricts the choice of additives to those which do not cause harmful effects in the recipient. Inosine is an example of an additive which, due to the formation of large amounts of the metabolite hypoxanthine, may raise the serum uric acid level in the recipient after transfusion of a few units, when used in amounts optimal for extended red cell storage. Thus, such a preservation solution is not suitable if the blood unit is expected to be used in massive transfusion

without special treatment. By opening the system toxic additives or metabolites can be removed, e.g. by washing. This means that the unit is not available for immediate use and that the reconstitution procedure may be considered too time consuming in massive transfusions.

Several new resuspension media have been suggested in recent literature and at this Congress. The Adsol™ solution of the Travenol Company is very similar to the SAGM solution but contains about double as much glucose and 25 per cent more mannitol. It is at present under investigation in the USA. Addition of dihydroxyacetone to the SAGM solution improved the oxygen delivering capacity of the red cells as measured by the maintenance of 2,3-diphosphoglycerate (DPG) [71]. Fabre, Leterrier, and Saint-Blancard showed that experimental resuspension solutions containing phosphate, adenine, glucose, guanosine, and sodium chloride in some respects were superior to other tested media including SAGM [61, 62, 70]. In order to reduce spontaneous storage haemolysis when all plasma had been removed they added sorbitol. This apparently had a similar effect as mannitol. Sodium bicarbonate and ammonium bicarbonate had a favourable effect on the maintenance of DPG as shown by Lukasiak et al. [65]. Beutler [5] in 1973 already showed that sodium bicarbonate is very effective in counteracting the acidification during storage and thereby causes maintenance of normal DPG. This is due to the fact that carbon dioxide which is formed through the action of red cell carbanhydrase, escapes from the bag through the plastic wall of the container. In practice, however, bicarbonate solutions cause difficulties because of their instability during sterilization and storage. This is true also for some other substances which have been shown to influence favourably red cell storage, such as ascorbic acid [6] and phospho-enol-pyruvate [7]. As indicated above objections may also be raised when the preservative contains supernormal concentrations of a physiologic substance such as phosphate (20 times the physiologic concentration in some of the experimental preservatives). Obviously, clinical studies are necessary to prove the safety and clinical usefulness of the blood components preserved in several of these experimental solutions.

Another trend in red cell preservation is that other tests are being used more frequently than the traditional biochemical studies in vitro and post-transfusion viability studies in vivo. Evaluation of the morphological changes from discocytes into echinocytes of different grades and echino-spherocytes was used by several research groups. Concerning morphology, CPD-A solution is superior to ACD [61], storage as a concentrate or suspension is superior to whole blood [8], and storage as a suspension in the PAGGS medium is superior to that in SAG [70].

The viscosity at constant haematocrit also changes during storage. Clearcut improvements were found when red cells were stored as red cell suspensions (e.g. in SAG) as compared to traditional storage as red cell concentrate or whole blood [62]. Guanosine and phosphate in the suspension solution ensured further improvements. A more elaborate metabolic investigation than ATP alone is useful in order better to understand the effects of the manipulations. For instance, determination of NAD is useful when substances such as dihydroxyacetone are used [71].

An in vitro model simulating in vivo post-transfusion conditions is also promising [69].

Purification of red cells

Removal of buffy coat as a means of improving the quality of the red cells, which are to be transfused, has been used for many years in some countries [9]. A platelet poor, buffy-coat poor and plasma poor red cell suspension was shown to reduce the incidence of transfusion reactions (febrile, urticarial) in elective surgery patients as compared to whole blood (0.14 per cent and 0.68 per cent, respectively) [82]. This means that even in this respect a red cell suspension which can be stored for 5 weeks is an improved product useful in the majority of patients. In some patients with leukocyte antibodies higher purity from leukocytes must be obtained in order to give a reaction-free transfusion. For practical reasons filtration through cotton wool filters seems to be the method of choice, with an effectiveness of 99 per cent [86], 97 per cent [68], or 92 per cent [85]. Clinical experience of 25 000 units of leukocyte poor blood was reported as very successful both with respect to reduction of febrile reactions and HL-A sensitization [68]. A combination of removal of buffy coat and cotton wool filtration can be expected to be advantageous since the former is effective in removing lymphocytes, and the latter is effective in removing granulocytes [10].

Clinical experience with stored red cell suspensions

Myllylä [66] showed that the post-transfusion survival in vivo of effectively plasma-depleted red cells suspended in SAGM medium was 79.2 per cent (range 87.5–72.3) which was similar to our corresponding figures (83 per cent $\pm$ 5.3 per cent) [82].

We have reported [82] on favourable clinical experience with plasma-poor red cell suspensions (SAGM-system), indicating that plasma or albumin solutions are normally not necessary at blood losses up to 50 per cent of the patient's blood volume. This is in agreement with previous studies of Lundsgaard-Hansen [11]. The new haemotherapy programmes reduced the use of plasma/albumin by 75 per cent and the use of red cells by 25 per cent [82]. Thus, considerable volumes of plasma become available for other purposes, e.g. production of Factor VIII preparations.

Converting B cells to O cells

Goldstein [22] reviewed work to remove the blood group B antigenic determinant by treatment with alpha-galactosidase. After treatment the red cells had 97% 24 h post-transfusional survival in humans whose plasma contained anti-B. The half-time was also normal, 30 days. However, as the procedure includes

several washing and incubation steps it is doubtful whether it will be cost-effective for more general use. In therapeutic use with certain rare blood types the procedure will probably find its first practical application.

Platelets

As early as 1975 Murphy and Gardner [12] showed that prolongation of storage at room temperature can be successfully applied by improving the gas transport through the wall of the container. This can be done by extending the size of the bag or using thinner or more gas-penetrable plastic materials.

A fundamental difference exists between red cells and platelets in that the latter contain mitochondria and can use oxidative phosphorylation for the production of ATP. This way of energy supply to the cell is much more effective than the glycolytic pathway. If oxygen is consumed a switch to anaerobic metabolism occurs and the medium will be acidified rapidly due to the formation of lactic acid. This explains why there is sometimes a sudden drop in pH after one, two or more days of storage. A substantial reduction in viability occurs when the pH decreases below 6.0.

Oxygen consumption depends on the total number of platelets but also on the metabolic activity of the cells, as emphasized by Moroff et al. [74].

The diffusion of oxygen into the bag and of carbon dioxide out of the bag, the latter to reduce the content of carbonic acid, is thus important. The driving force is the difference in partial pressure between outside and inside. When oxygen is consumed by the platelets the pO_2 is lowered which increases the flux into the bag if the conditions permit gas penetration. New plastic materials are available which allow improved gas transport: the PL-732 made of polyolefin material and manufactured by the Travenol Company and the CLX plastic made of PVC with a non-leachable plasticizer and manufactured by the Cutter Company. The usefulness of the latter material is shown in two reports [76, 78] indicating that room temperature storage of platelets may be extended upto 5–7 days in these plastic bags. Similar experience with the PL 732 plastic has been reported earlier [13, 14] and at this congress by Lamy et al. [77].

Other attempts to improve the storage of platelets were done by using different inhibitors of platelet aggregation of which Trapidil and PGE_1 were found the most effective [73]. The stable prostacyclin analogues, 7-oxo PGI_2 and Chinoin M 1296 and M 1308 are also promising [88]. Kotelba-Witkowska et al. [75] used a drug, Craviten, which increased the activity of hexokinase and pyruvate kinase, prevented the fall of the ATP level, increased the cAMP level and inhibited spontaneous aggregation of the stored platelets.

Granulocytes

In his review on granulocyte transfusions Huestis [26] said that the most commonly expressed consensus is that an adequate transfusion should include at least 10^{10} polymorphonuclear neutrophils. To achieve this dose, in most transfusions by centrifugal leukapheresis requires that the mean collection be at least 2×10^{10} PMN because of the unpredictable variability of cell yield from different donors. New antibiotics and an effective regimen to use them have made granulocyte transfusion unnecessary in a large number of patients with granulocytopenia and sepsis, following cytostatic treatment [79]. Although clinical effects even of smaller doses than those mentioned above were claimed by some groups [80] the overall impression is: More granulocytes should be given to fewer patients and more than once daily transfusions may be necessary in some cases.

A special indication may be neonatal neutropenia with depletion of mature marrow neutrophils in infected neonates who are at high risk of death from sepsis. In a recent prospective pilot study all of 7 such infants receiving granulocyte transfusions survived, but 8 of 9 non-transfused patients died [15]. The poor prognosis in this study was correlated with a depletion of the marrow neutrophil storage pool cells, and not with peripheral neutropenia. The given dose of neutrophils was $0.7 \cdot 10^9$/kg of the recipient's body weight, range 0.2 to $1.0 \cdot 10^9$.

No progress in the in vitro storage of granulocytes has been reported; it is still limited to 24 h, preferably at about 20 °C without agitation.

Transfusion in Transplantation

After the discovery by Opelz et al. [16] in 1973 that kidney transplant patients who had received transfusions showed a lower frequency of rejections than non-transfused patients, their observation has been confirmed by many studies. It is, however, unclear which components in the blood are responsible for the effect. It may now be concluded that red cells and plasma do not have this beneficial effect. In his review van Rood [25] showed that protection has been obtained using platelets in an experimental study in monkeys. This is of both theoretical and practical interest since the major histocompatibility antigens are less completely expressed on the surface of platelets than of lymphocytes. Further studies are needed to answer the question whether platelet transfusion regimens prior to kidney transplantation will be superior to the present widespread use of whole blood or red cells with or without partial removal of the buffy coat.

Another practical consequence of the higher immunogenicity of lymphocytes than of platelets was the finding that platelet concentrates depleted of contaminating lymphocytes gave much less refractoriness at repeated platelet transfusions than normal platelet concentrates which are usually at least to some extent contaminated with lymphocytes.

Plasma Components for Therapeutic Use

New fractionation techniques

A new trend in plasma fractionation is the use for separation of plasma proteins of other principles than the alcohol precipitation technique of Cohn. In his review on the subject Curling [23] concluded that the Cohn technique is best suited for large scale fractionation, e.g. in batches of 5000 l plasma/week, while chromatographic methods are useful up to 500 l/week. Affinity chromatography methods for the production of high yield and high purity albumin are available. The most important progress concerns, however, those plasma proteins which are partly denatured during the alcohol fractionation process. By the use of improved methods native preparations are obtained that can be applied intravenously without side effects.

Gamma globulin

Immunoglobulin G preparations obtained by Cohn fractionation are known to cause anaphylactoid reactions if used intravenously particularly at rapid infusion rates. This may be due to the presence of IgG aggregates or Pre-Kallikrein Activator (PKA) and the in vivo generation of vaso-active kinins. Nevertheless, successful intravenous administration of ordinary IgG preparations was observed by McClelland et al. [92] when given slowly (6 per cent complication frequency). The importance of proper testing of IgG preparations was emphasized by Nydegger [37] who showed that large variations exist between IgG preparations available on the market and intended for intravenous use (see also [17]). Some of them do not fulfil the requirement of being safe, potent and efficacious. The usefulness of an animal model in testing the preparations was shown by Bakker et al. [87]; short hypotension in the animals indicated PKA, while prolonged hypotension the presence of IgG aggregates.

Chromatographic IgG prepared by Suomela et al. [81] was native (less than 1 percent non-monomeric IgG), of high purity, and contained potent antibodies to a wide spectrum of bacterial and viral antigens, as shown by Salonen and Suomela [83]. All IgG subclasses were represented.

The clinical usefulness of intravenously administered IgG preparations in immunodeficient patients was shown by Cunningham-Rundles et al. [38]. Serum levels of 4–6 g/l were obtained and the T $^1/_2$ was 2 $^1/_2$–3 weeks. Hospitalization and incidence of febrile reactions decreased considerably.

In addition to antibiotics intravenous IgG was given by von Muralt and Sidiropoulos [39] to infants with neonatal septicaemia. The lethality was reduced from 26 per cent to 10 per cent and the incidence of recurrent infections decreased to one third.

Further experience was reported by Imbach [40] on his recent finding [18] that large doses of intravenous IgG normalized the platelet concentrations in pa-

tients with idiopathic thrombocytopenic purpura (ITP). Excellent results were obtained in acute ITP in children, verified by others in the discussion. In chronic ITP most patients responded, some became refractory [41, 42]. The effect was better in children than in adults. The common dose was 0.4 g/kg/day for 5 days. The initial increment in platelet count was found by Bussel [42] to be predictive of the length of the response. Intravenous IgG was recommended as the method of choice in the initial treatment of ITP. The mechanism is not known but a competitive, protective effect on the platelets or on the megakaryocytes seems possible.

Coagulation factor preparations

Britten [45] pointed out that haemophilia in contrast to many other congenital diseases, is evenly distributed all over the world with an approximative incidence of 50 cases per million inhabitants. The need of Factor VIII to bring bleeding episodes under control was estimated at $4.5 \cdot 10^9$ IU/year. A rough estimate of the world's present production indicated a total of $9 \cdot 10^8$ IU which thus covered about 20 per cent of the total need. Most of the produced Factor VIII is, however, used in the USA and some of the European countries. In the world there is a wide spectrum, from no treatment at all to overtreatment and abuse. It was concluded that a yearly dose of 20 000 IU per haemophiliac, or 1 IU per inhabitant would be a realistic minimum goal of supply in any sufficiently well organized voluntary blood transfusion service. For optimal treatment including prophylaxis at least 1.5–2 IU per inhabitant is probably needed [46].

The instability of Factor VIII causes difficulties obtaining high yields. Rock et al. [90] had made the important observation that the low molecular weight, carrier-free form of Factor VIII:C is lost after 24 h storage of CPD plasma, whereas in heparin plasma or citrated recalcified plasma the small, carrier-free form of Factor VIII:C is stable. By collecting blood directly into heparin or by adding heparin plus calcium to citrated blood or plasma, the total of Factor VIII activity can be maintained. Smit Sibinga et al. [43] had obtained a 63 per cent mean yield and 20–30 IU/ml potency in a routine procedure for production of freeze-dried cryoprecipitate using double precipitation of heparin plasma, which was considerably higher than in cryoprecipitate prepared by the traditional technique from CPD plasma of 44 per cent mean yield and 3–4 IU/ml potency. Also, the so-called thaw-siphon technique gave higher yields [91]. The high purity Factor VIII preparations produced at large scale industrial processes have certain advantages, particularly in home therapy and when very large quantities of Factor VIII have to be administered [44]. The yields are, however, low and the preparations do not contain the von Willebrand factor.

Two major problems in haemophilia treatment were discussed: the difficulties in the 15 per cent of haemophiliacs with inhibitors to Factor VIII, and the occurrence of liver disease. By using non-activated and activated prothrombin complex concentrates, the bleeding episodes had been controlled in 50 per cent and 63 per cent respectively [47]. In a combined American-European study of mainly severe haemo-

philiacs liver disease was investigated. Mild or trivial liver disease was found in 65 per cent of the cases while 7 per cent had severe disease, 7 per cent had cirrhosis, another 10 per cent were suspect of cirrhosis and 7 per cent had acute hepatitis [48].

Attempts to produce non-infective coagulation factor preparations have also been reported. The cold sterilization process using β-propiolactone treatment with ultraviolet irradiation [49, 50] was found to reduce the virus titre about 10 millionfold. Indication was obtained that non-A, non-B hepatitis viruses were also inactivated by these procedures and they were considered promising for practical use. Heat sterilization had also been applied with promising in vitro results [89].

Antithrombin III (AT)

AT is a potent protease inhibitor of activated Factors XII, XI, IX, X, and thrombin when occurring free in solution. Abildgaard [27] chaired a symposium on its biological action and clinical significance. Björk [28] reviewed the biochemistry of AT. After binding to a protease the molecule becomes modified and is cleared from the plasma with T $^1/_2$ 10 min. In the presence of heparin more AT is needed for inactivation of thrombin. The hereditary AT deficiencies were reviewed by Sas [29] and Nagy [30] who described different types of AT deficiency. Nagy surveyed the clinical aspects of the abnormality, and ten Cate [31] reviewed the diagnostic and therapeutic problems including the acquired deficiencies.

The hereditary form of AT deficiency seems to be particularly common in the Gipsy population of Hungary where the over-all incidence was found at 1 : 10 000 by Hungarian studies. The clinical manifestation is deep venous thrombosis which occurs preferentially in the lower extremities and is often accompanied with pulmonary embolism. The first manifestation may occur at the age of 10–16 years. Urinary infections, pregnancy and injuries are precipitating factors. Heparin treatment in AT deficiency may have adverse effects. Hereditary AT deficiency patients with venous thrombosis should be put on a life-long antivitamin K regimen.

Acquired AT deficiency may be due to decreased synthesis (liver cirrhosis), increased loss (proteinuria) or increased consumption (DIC), not infrequently combined in the same patient. Acquired AT deficiency is also a frequent complication of postoperative Gram-negative infections, possibly caused by circulating endotoxins. Very low serum AT levels have a predictive value in the diagnosis of septicaemia.

Concerning the indications for antithrombin concentrates there is general agreement about its value in hereditary deficiency complicated by thrombosis, or as a preventive measure at birth or operation. In acquired deficiency infusion of AT concentrate may normalize the consumption coagulopathy, and apparently be of clinical value. Abildgaard concluded that a common protocol should be elaborated for evaluating the results.

Fibronectin

The biological and clinical significance of plasma fibronectin (FN) was dealt with in a symposium chaired by Lundsgaard-Hansen [51]. Clemmensen [52] reviewed the functional role of plasma FN. Like fibrinogen, FN occurs in soluble form in plasma and in insoluble form in connective tissue. Both proteins are considered to play an important role in tissue repair. In addition FN increases the solubility of fibrin monomers which appears to mitigate the sequels of disseminated intravascular coagulation (DIC). Consumption of plasma FN might thus aggravate organ damage in DIC.

Lundsgaard-Hansen [53] reviewed Saba's concept [19] of plasma FN as a non-antibody, opsonic protein stimulating reticulo-endothelial function. Animal experiments suggest that a depletion of FN reduces the phagocyting capacity. The use of cryoprecipitate, which contains a concentrate of FN together with fibrinogen, Factor VIII and Factor XIII in intensive care patients has given anecdotical evidence that substitution therapy may be important in patients with low levels of plasma FN.

Low levels of FN are often found in severe burns and renal insufficiency after surgical operations [54] and in those patients with malignant haematopoietic disease who are in poor condition [59]. After surgery a rebound occurs with supernormal plasma levels [54].

Rübli et al. [55] investigated 98 patients treated in an intensive care unit. A number of serum proteins were severely decreased in patients with sepsis or DIC. The minimum FN value did not differ between survivors and non-survivors whereas this was the case for antithrombin III: lethality in patients with a minimum AT level ≤ 50 per cent of normal was 66 per cent, with >50 per cent 13 per cent, $p < 0.005$. All septic patients who died after more than two weeks in the intensive care unit had returned to the normal range of FN level prior to death. The study confirmed Saba's concept of an association between low FN levels and life-threatening, septic and/or DIC-associated disease, but a causal relationship has to be proven.

Lundsgaard-Hansen concluded that the question is still open whether fibronectin, recommended as an adjunct to established modes of intensive care, has any beneficial effects. Controlled trials are urgently needed.

Interferon

Nevanlinna [24] reviewed his institute's experience in the large scale production of human interferon-alpha (IF) made from buffy coats. Both the yield of crude and purified interferon (PIF) had increased. Approximately $8 \cdot 10^6$ IU crude IF is produced per buffy coat and the final yield of PIF is around 60 per cent. Production of interferon-gamma from buffy coats has been started using Lens culinaris lectin as inducer. The yield per buffy coat has so far been poor, less than $1 \cdot 10^6$ IU. Clinical trials have been initiated.

The production of IF from buffy coat cells is not an easy procedure, and in spite of large efforts many institutions have failed. The interest in leukocyte IF has decreased in recent years probably reflecting great expectations for the mass production of bacterial IF.

Since, however, the human leukocyte IF products are a mixture of different IFs of which only some are being produced microbiologically in the near future, there may still be room for the human leukocyte derived products. In addition lymphokines other than interferon-gamma such as Interleukin-2 (T cell growth factor) may be of therapeutic interest. After all, human leukocytes is a by-product which can easily be collected in a well organized blood transfusion service.

Therapeutic Apheresis Procedures

Plasmapheresis to remove plasma and replace it with some other fluid normally including albumin and/or donor plasma, has increased tremendously in the last five years. In a workshop on the subject with Shumak as moderator and Rock and Gavrilov as chairmen, Wenz [57] showed that the number of therapeutic plasma exchanges (TPE) in the USA have increased from virtually none in 1977 to an expected number of 80 000 in 1982. The extraordinary thing is that the indications are mainly based on theoretical considerations or anecdotical experience. Only few controlled trials have been performed and few indications have been scientifically established.

The dysglobulinaemic hyperviscosity syndrome where excessive quantities of monoclonal protein are removed, seems to be undisputed [20]. Shumak [56] concluded that in addition to cryoglobulinaemia, Factor VIII antibodies in haemophilia and removal of A/B antibody before bone marrow transplantation may be clearcut indications. Other diseases in which promising results have been obtained are thrombotic thrombocytopenic purpura (TTP) and idiopathic thrombocytopenic purpura (ITP) that have failed to respond to prednisone, and pregnancies at high risk of haemolytic disease of the newborn due to anti-D. Controlled multicentre trials in these diseases have been started in Canada. Another promising indication may be certain cases of aplastic anaemia. A successful case of temporary remission in pure red cell aplasia was also reported [60].

A number of neurological conditions with known or suspected plasma factors related to the disease have been the subject of treatment in 25 per cent of all TPE procedures in the USA [57]. The conclusion at the workshop was that TPE may be useful in selected cases and to a limited extent in myasthenia gravis, Guillain-Barré syndrome and Refsum's disease, whereas for the moment there is no indication that the procedure is useful in amyotrophic lateral sclerosis. More knowledge has to be collected concerning the mechanism of action of the treatment.

Urbaniak [58] gave a review on the use of TPE in renal diseases. Appropriate indication for the procedure should be considered to be due only when the patho-

genesis is clearly related to a factor predominantly present in plasma. It is also important to prevent resynthesis of this factor. Some centres have experienced good but other centres poor results in Goodpasture's disease. Urbaniak reported that in the experience of his institute none of 19 severe cases had benefited from the treatment while success had been encountered in mild forms of the disease. An obvious difficulty is to initiate treatment sufficiently early during the course of the disease. Immune complex nephritis is a reasonable indication, particularly when there is an exogenous agent. TPE has been used also in allograft rejection after kidney transplantation. In controlled studies the difference was not significant.

There are still many questions in TPE that have to be answered. One of the most important ones concerns the fluid for replacement. Because of the large requirement of albumin or plasma the procedure is very expensive. Practical and unexpensive methods to remove a causative factor more specifically by adsorption are awaited with great expectations. As an example it may be mentioned that preliminary experience with protein-A Sepharose columns for perfusion of blood or plasma to remove IgG 1, 2, and 4 is promising, e.g. in antihaemophilic factor treatment [21]. Great interest is also shown in the possible removal of blocking factors in the treatment of malignancies.

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New Trends in Plasma Fractionation

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Current progress in fractionation concerns not only new fractions but also new techniques and known but considerably improved fractions. The improval of already existing fractions may have different meanings. For instance a higher degree of safety or a fraction with a considerably higher specific activity may be obtained. Both these features may, of course, be observed coincidentally in purer products.

Among these improved fractions we may mention factor VIII concentrates. The most important long-term side reactions associated with substitution therapy of haemophilia A concern the development of hepatitis [1], namely of the non-A, non-B type and the appearance of haemolytic anaemia [2] due to the presence of isoantibodies. Whereas hepatitis B transmission regressed due to better screening of the blood donors, non-A, non-B hepatitis became more frequent. The virus(es) responsible for this type of hepatitis can actually not be evidenced. Consequently, factor VIII concentrates should be submitted to a virus-inactivating treatment. Such a procedure has been in use for many years in the preparation of albumin solutions by heating them to 60 °C in the presence of stabilizer during 10 h. Factor VIII proved to be a very labile protein as heavy losses were observed during fractionation steps performed under rather mild conditions. So it seemed impossible to submit factor VIII preparations to such a rough treatment as heating to 60 °C during several hours. Recently these difficulties have been overcome. It appeared that factor VIII could be protected from heat denaturation by saccharose and glycine [3, 4]. The final product contains only trace amounts of fibrinogen as this undesirable contaminant precipitates during the preparation procedure. The highly purified material has a low protein concentration. It is therefore stabilized before freeze-drying by addition of albumin. It contains much less γ globulins than the non-heated material, but still enough to carry some isoagglutinins. Whereas the product available at present is heated in the liquid state, other laboratories obtained similar results by heating the freeze-dried material. Yet another way has been developed by inactivating the virus by UV irradiation in the presence of β-propriolactone [5]. The efficiency of these treatments is currently checked in chimpanzees. As already mentioned, heating or treatment by β-propriolactone does not allow the removal of isoagglutinins. The best way for obtaining factor VIII

concentrates devoid of anti-A, anti-B activities is obviously the use of isogroup plasma pools as starting material. This has been done by several producers [6].

In summary, factor VIII concentrates have been improved not only as far as the yield and specific activity are concerned, but also with respect to their safety. Unfortunately, the safest products are not those with the best yield, casting aside the availability of the starting material.

More severe quality criteria are now applied to serum albumin preparations. Hypotensive effects have been reported after infusion of plasma protein solutions containing roughly 90 per cent albumin. It soon became clear that the kallikrein-kinin system might be involved [7, 8]. Although bradykinin [9] was detected in some batches, laboratory findings revealed that the causative factor was usually not the end-product of the reaction cascade but the prekallikrein activator which is identical with a Hageman factor fragment (XII f) [7, 10, 11]. Therefore, techniques have been developed to check for the absence of activator, (pre)-kallikrein and kinins [7, 12, 13]. Prekallikrein activator was rarely present in highly purified albumin solutions ($>$ 95 per cent). In addition, oligo- and polymers present in concentrated albumin solutions have been presumed to be the causative agents of side reactions observed occasionally after albumin infusion. Careful analysis of 25 different batches could not, however, reveal any correlation between the amount of di- and trimers in the albumin concentrate and the reported side reactions [14]. Though no objective relationship could be proven between albumin aggregates and the poor tolerance of these preparations, the manufacturers became aware of the eventual harmful effects of these polymers and tests were developed to quantify the amount of di, tri and higher polymers. This is usually done by gel filtration [15] and/or polyacrylamide gradient electrophoresis. It seems, however, that there is no clear difference between oligomers and aggregated material [16]. The latter is eventually more harmful than are the tri- or tetramers. New definitions seem to be oriented towards a tolerance limit of 10 per cent of polymers.

Currently, attention has been drawn to the biological activity of albumin concerning the specific transport role in addition to its main role of maintaining the oncotic pressure in the vascular space. This activity may be checked by a bilirubin fixation test. In contrast to rather generalized ideas, conventional stabilizers influence only slightly the binding characteristics of albumin, whereas the heating process has an additional slight effect [14]. Hence, the bilirubin fixation capacity of albumin in the presence of a conventional stabilizer is well preserved. This feature is important for the paediatrician using this plasma derivative in jaundiced infants.

Much work has been done concerning the improval of i.v. γ-globulin preparations. The relationship between poor clinical tolerance and the presence of aggregates in the γ-globulin preparation inducing complement consumption has been known for a long time and several treatments had been developed to obtain well tolerated γ-globulins. Among these methods we may mention enzymatic degradation, pepsin or plasmin being used for this purpose; chemical modification comprising reduction/alkylation, β-propiolactone treatment and sulphonation

[17–21]; mild acid (pH 4) treatment with small amounts of pepsin; polyethyleneglycol treatment alone or in association with hydroxyethyl starch. Mild acid treatment is one of the oldest known methods [22] which has recently been considerably improved. The product is now presented as a dry powder. Indeed, further aggregation may occur in preparations kept in the liquid form whereas such phenomena are rare in conveniently dried material. During the freeze-drying process protection is obtained by addition of saccharose to the immuno-globulin solution. Very satisfying clinical results obtained with this product have been reported at a symposium held during this Congress [23]. New therapeutic trends with this preparation may also be mentioned as good effects were observed with high dose i.v. γ-globulin in idiopathic thrombocytopenic purpura [24, 25] and autoimmune neutropenia [26].

Among the large variety of i.v. immunoglobulin preparations belonging to the group of modified IgG we may mention the recent improvement [19] of the original Japanese method to sulphitolyse globulins [27]. This modification is of particular interest as it seems reversible in vivo. Other initially promising methods such as precipitation of aggregated material by polyethylene glycol have been questioned in more recent publications [20]. The absence of anticomplementary activity (better expressed as complement consumption), was long the main criterion of the quality control of i.v. γ-globulins. Only recently has it become clear that certain side effects of i.v. γ-globulin preparations might be due to the presence of contact-activated factors with coagulant and vasoactive properties [28]. Certain small molecular weight fragments belonging to the group of kinins or anaphylatoxins [29] have also been found in γ-globulin preparations. This brings us to the quality criteria which should be taken into account.

Intravenous immunoglobulin preparations should have their antigen binding capacity preserved (divalent binding function intact) [21], stimulate macrophage/monocyte phagocytosis [17] and prove to be devoid of polymeric aggregates and antigen/antibody complexes by showing no or little activation of the classical complement pathway. They should reveal no or little inhibition of erythrocyte/antibody rosette formation and little or no non-specific activation of neutrophils [17]. Animal models might be useful to detect short (due to PKA) or long lasting hypotension (due to IgG agregates).

New Techniques

Of the new methods we have to mention first the devices allowing the collection of plasma devoid of cellular components [30–33]. This is a very important feature for future fractionation development. Indeed, most of the plasma is frozen and thawed for the preparation of factor VIII concentrates. Leukocytes and platelets will not withstand these operations and release their enzymes into the surrounding fluid. Many plasma proteins are substrates of leukocyte proteases such as components of the clotting system comprising fibrinogen, factor II, VII, IX, X,

VIII, XIII [34], protease inhibitors such as antithrombin III [35] and α_2-antiplasmin [36], complement components C3 and C5 [37, 38] giving rise to chemotactic breakdown products, and also fibronectin [39]. Leukocyte proteases may thus interfere with the yield and quality of plasma fractions. Furthermore, one has also to take into account the pyrogens of leukocytic origin.

New trends have also been developed concerning the improval of freezing and thawing of plasma to obtain the cryoconcentrates whether as a direct product or as the starting material for intermediate or high purity factor VIII concentrates.

The most impressive new developments, however, concern the separation techniques based on the now widely used chromatographic procedures [40]. The initial method of Curling et al. [41] has been further developed. The results of the first plant using an industrial equipment supplied by Pharmacia for chromatographic processing of albumin were presented by Viljoen et al. [42]. Several posters reported the recovery of albumin by combined anionic and cationic ion exchange chromatography [43–48]. These exchangers comprise different matrix material, Sephadex and Sepharose on the one hand and Trisacryl, for instance, on the other hand [49]. The choice of the matrix might well be of special importance as DEAE-cellulose, for instance, achieves activation of contact factors, whereas DEAE-Sephadex does not [50]. The supporting matrix may also influence the affinity of the proteins for a given adsorbent and thus influence the selectivity of the process [51].

The chromatographic methods have also been used for the purification of hyperimmune γ-globulins [52] and γ-globulin for i.v. use [53, 54]. In this latter process Aerocil, an inorganic adsorbent, is associated with DEAE-Sepharose and SP-Sepharose to give a product devoid of anti-complement activity. These results show that the new chromatographic procedures might overcome the difficulties encountered with the current production of γ-globulins for i.v. use. If this is confirmed, there will be no more necessity for modifying the IgG by chemical treatment, enzymatic digestion or mild acid treatment.

Some authors have shown that a final gel-filtration step might considerably improve the purified fractions, namely the quality of albumin [47].

The efficiency of affinity chromatography for the recovery of albumin from fraction IV using Cibacron blue 3GA-Sepharose C1-6B has been demonstrated [55].

It may further be mentioned that these techniques are sometimes associated with precipitation steps. Engineering applicable to this new type of fractionation is also in progress [56, 57] and the reference data concerning water consumption and the frigorific, calorific and electric energies may already be obtained.

Among the new techniques used in the field of fractionation we may mention the new filtration techniques, namely the use of diafiltration where dialysis for the removal of salts and ethanol is associated with ultrafiltration to obtain the final concentration of the purified fraction [58]. As far as albumin is concerned the procedure seems superior to the usual freeze-drying process.

New Fractions

In the recent past several new fractions have been developed. They may be subdivided in different groups, such as haemostatic components, protease inhibitors and an inactivator of biologically active peptides, immunoglobulin preparations, cholinesterase and fibronectin.

So-called activated prothrombin complex concentrates have been developed for the treatment of haemophiliacs with inhibitor. Such preparations are currently available. Whereas a certain number of clinical observations have already been published, the reaction mechanism of these fractions is still a matter of discussion. Likewise, the laboratory work is comparatively limited [59].

As there was much progress during the last years in the understanding of the molecular properties of factor VIII, there was also a growing interest in concentrates with v. Willebrand factor activity. Two trends may be distinguished. Whereas certain preparations of factor VIII:C have also v. Willebrand factor activity [60], concentrates obtained by the technique of Newman et al. [61] are nearly devoid of v. Willebrand factor. The high molecular weight material exhibiting v. Willebrand activity is removed by the first PEG-precipitation step. This precipitate is used as the starting material in another process which yields v. Willebrand factor concentrate [62]. This new fraction has the advantage of being derived from a by-product of factor VIII:C preparation which from the economical point of view is much more desirable. The different concentrates gave satisfying clinical results.

Prothrombin complex concentrates are now most frequently prepared by adsorption onto DEAE-Sephadex whereas the originally prepared fraction was obtained by adsorption onto $Ca_3(PO_4)_2$ using EDTA plasma or fraction IV_1 as starting material. These earlier preparations contained more factor VII than the newer fractions because a pre-elution step needed for the removal of contaminating, undesirable proteins from DEAE-Sephadex elutes also most of factor VII. To overcome the problems arising from a relative lack of factor VII in these fractions, special concentrates of factor VII were prepared and are currently available.

Concentrates of factor XIII have been known for several years. Similar fractions are now produced by several plants and information concerning clinical indications and effects of this fraction is rapidly increasing. However, several years are usually needed before a new fraction can enter the therapeutic panel.

The same is the case with antithrombin III concentrates. Although they have been put at the disposal of clinicians several years ago, publications concerning its use, posology and clinical effects appeared only recently [63–67].

The high affinity of this antiprotease for heparin became the central pillar of almost all purification techniques based on affinity chromatography on immobilized heparin. The purification of AT III is thus a brilliant example of the usefulness of affinity chromatography [68, 69]. Among the reported indications for the use of this fraction are congenital or acquired AT III deficiency in the presence of acute or moderate DIC, shock, post partum haemolytic syndrome, preeclampsia,

periods of thrombogenic stress, acute liver failure and cerebral thrombosis [63–67, 70, 71].

Substitution therapy with antiproteases must take into account that the inhibitors usually react in a 1:1 molar proportion with enzymes, whereas the enzymes themselves, like the serine-proteases of the clotting system, may catalyse the turnover of several hundred molecules of their substrates. Furthermore, an inhibitor can usually combine with several different enzymes. Consequently, to account for a possible rapid consumption, the aim of substitution therapy with inhibitor concentrates such as AT III must be to bring the plasma concentration to at least 75 per cent of normal. The injection of 1 unit AT III/kg causes a 1 per cent increase in the plasma concentration. An amount of 40 U/kg/day is recommended by several authors, one unit being the amount of activity found in 1 ml of plasma. Also, the efficiency of heparin therapy depends largely on the availability of AT III.

Another important antiprotease of human plasma is α_1-antitrypsin (α_1AT). Although this inhibitor inactivates many proteolytic enzymes, its activity is specially important against the leukocyte proteases. In the view of Laurell [72] the major biological role of α_1AT is self-protection from these enzymes and specially the macrophage elastase. Inherited α_1AT deficiency is well known to be associated with certain α_1AT variants (or Pi-types). The homozygotes PiZZ have only about 15 per cent of the amount of α_1AT found in the most common PiMM type. It could be shown that this deficiency was related to the early development of emphysema. Patients with severe or partial α_1AT deficiency suffering from repeated chest infections may benefit from α_1AT substitution therapy.

The first clinical trial with α_1AT has been published some months ago. A very impure preparation was used needing a large volume (1 l per injection) to be handled but the 5 patients of the PiZZ-type improved considerably and the protease (elastase)-antiprotease imbalance was reversed within the alveolar structures [73]. It is to be hoped that these encouraging results will stimulate the development of suitable techniques for the preparation of concentrates of α_1AT. This protein is most concentrated in fraction IV and since it is very labile it requires a perfect control of technology.

Replacement therapy is also achieved with another antiprotease: the C1-inactivator. Although attacks of hereditary angioedema have been preferentially treated in the past with fresh frozen plasma and considerable progress has been made in the prevention of these episodes by the use of androgens, replacement therapy is obtaining increasing attention. Several publications have reported very satisfying results [74, 75]. Although the exact mechanism of the oedema attack is still a matter of discussion it seems, nevertheless, that activation of C1-esterase with the appearance of complement split products (possibly C2b) is the triggering factor. C1-inactivator is the natural inhibitor of this enzyme but it inhibits also kallikrein and factor XIIa. This explains the good clinical results obtained by v. Starre et al. [76] in correcting the hypotensive effects of 'stable plasma protein' solution, a solution of approximately 90 per cent albumin apparently containing PKA. Control of (plasma) kallikrein activity by this inhibitor will be a helpful tool in kinin-related

hypotension. This inhibitor has, however, no effect on formed kinins. The latter are inactivated by N-carboxypeptidase (or kininase I) which can be isolated from the preluates obtained during prothrombin complex preparation [77].

In conclusion, the kallikrein-kinin system can be controlled by inhibition of the proteolytic enzymes (factor XIIa and kallikrein) and by inactivation of the already formed biologically active peptides. We may notice that anaphylatoxins are inactivated as well [78] underlining that concentrates of N-carboxypeptidase might be a valuable therapeutic enrichment for the treatment of endotoxin shock, severe septicaemia etc.

In the field of immunoglobulins there are also some new preparations. IgM has been isolated from fraction III which has previously been treated by β-propiolactone [79]. IgM preparations have a much higher antibody content against bacterial antigens than has IgG. Preparations containing a mixture of IgG and IgM, or IgG and IgA, or the mixture of all three (called IgGAM) have already been described [80–82] to give good therapeutical results.

Recently IgG preparations with an increased titre of IgG4 have been developed; these preparations contain antibodies against several allergens.

Serum cholinesterase (ChE), another most interesting fraction, has been developed in recent years [83]. Whereas normal serum shows an activity of 3–8 IU/ml, corresponding to a mean specific activity of 0.06 IU/mg protein, the purified product contains 300–320 IU/mg protein [84]. Vials of 5 ml containing an equivalent of 1000 ml of plasma are easily obtained. The enzyme concentrate may be useful in cases of prolonged apnoea after thoracic surgery or electroshock therapy with the use of muscular relaxants. In recent years the relationship between prolonged apnoea after injection of suxamethonium or related drugs and unusual pseudo-cholinesterase phenotypes of these patients has repeatedly been pointed out. As it would be fastidious to screen all patients submitted to thoracic surgery for their ChE phenotype, it would be preferable to have the enzyme concentrates available.

Another even more important indication for ChE therapy is the accidental poisoning of agricultural workers by parathion and related organosphosphates. ChE concentrates may be a helpful emergency treatment. However, the enzyme will be of no help if irreversible tissue damage has been produced.

The purification of transferrin (TF) from AT III supernatant was reported by Jakab et al. [85]. This protein is usually purified from fraction IV obtained during ethanol fractionation of plasma. In association with indium isotopes (^{111}In and ^{113}In) TF is currently used for the evaluation of the plasma volume [86–88].

Haptoglobin (Hp), mostly concentrated in the same fraction IV, has been purified in different laboratories. The haptoglobin-haemoglobin complexes are taken up by the RES thus preventing renal losses of iron under physiological conditions. Renal damage after massive intravascular haemolysis results from a complex mechanism, but part of it is certainly due to Hb.

Hp concentrates gave positive clinical results in patients with massive intra-

vascular haemolysis [89, 90] by avoiding the overload of the kidney by the liberated Hb.

Another promising but clinically not yet investigated fraction is fibronectin. This protein, some years ago a biochemical curiosity called cold insoluble globulin by Mosesson who first described it [91], now encounters increasing interest. On the one hand, the biological activities of fibronectin have become known [92, 93]; on the other hand the deficiency states have also been defined. Fibronectin mediates the cell to cell adhesion and the cell to susbstrate anchoring; it regulates cell locomotion and mediates phagocytosis by tissue macrophages. As its deficiency was observed in severe infections, septicaemia, extensive surgery, burns and other trauma, it is tentative to speculate that these patients would be improved by general or local (burns) treatment with fibronectin concentrates. Some beneficial results have already been reported with cryoconcentrate, a fraction containing fibronectin. Several isolation techniques are already known including affinity chromatography. A comparative study concerning the suitability of certain of these techniques for the preparation of therapeutic concentrates has been presented by Henon et al. [94].

Thus several different aspects of the recent trends in the field of plasma fractionation may be noted. With increasing biological information new fractions are being developed and others, already existing, are being improved. Furthermore, considerable work has been done in the development of new technologies announcing a new era in the scope of fractionation.

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Artificial Blood

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About 30 years ago at a Congress of the International Society of Blood Transfusion, I presented the first report of the transfusion of previously frozen erythrocytes [1]. This work, done in collaboration with P. L. Mollison, led us to believe that we would be able to preserve erythrocytes in large quantities for very long times and that with large reserves, there need never be a shortage. Preservation of erythrocytes in the frozen state has, in some places, contributed to the more efficient use of available blood, but the world-wide need for blood still far exceeds the supply [2]. Since it is likely that the demand will increase faster than the supply, the needs will have to be met by substances other than natural blood or its derivatives.

Blood is a complex fluid with many components and multiple functions. The primary function of blood is to transport oxygen to the tissues and remove carbon dioxide. Several substitutes for blood plasma are available, but they cannot adequately transport oxygen and carbon dioxide. The recent development of preparations which can transport oxygen and carbon dioxide provides the substitutes for the erythrocyte which, in combination with a simulated blood plasma, constitute a long-sought entity, 'artificial blood'. An artificial blood, which is available in large amounts, which is not antigenic and is free of infectious agents, would be an extremely valuable therapeutic agent.

Table 1

Proposed artificial blood preparations

Biological Origin	Synthetic
Solution of Hb	Emulsion of perfluorochemical (PFC)
Solution of modified Hb	Oxygen chelator
Solution of Hb encapsulated in liposomes or synthetic membranes	

The preparations which have been studied in recent years as possible substitutes for the oxygen-transport function of the erythrocyte are listed in Table 1.

Oxygen Chelator

In this category are synthetic organic compounds which combine reversibly with oxygen in the same manner as hemoglobin. Some success has been achieved in the synthesis of compounds which resemble the hemoglobin molecule [3] but the conditions under which they combine with and release oxygen are still quite far from physiological. Although they may ultimately become useful biological oxygen transporters, they are at present still far from clinical usefulness.

Encapsulated Solution of Hb

When blood has been stored for too long in a blood bank, it is not usable because the erythrocyte membrane has lost its viability. The inner contents of the cell, the solution of hemoglobin, can still function as an oxygen transporter. Therefore, it is quite logical to replace the old, non-viable membrane with a new one. Chang et al. [4] and Arakawa and Kondo [5], enclosed droplets of hemoglobin solution in microcapsules and suggested that they might function as substitutes for erythrocytes. However, it has not been shown that these microcapsules will be retained in the circulation for a significant period of time.

Liposomes containing a solution of Hb similar in composition to the contents of the erythrocytes were shown by Djordjevich and Miller [6] to load and unload oxygen in vitro much like the erythrocyte. After infusion in animals, these liposomes persisted in the circulation for about one hour, but it was not shown that they delivered oxygen to the tissues. The major problem with liposome preparations is to prolong their retention and hence their function in the circulation. Another important problem is to prevent the oxidation of the enclosed hemoglobin to methemoglobin and hemichromes. Szebeni et al. [7] have presented results of their studies on the oxidation of Hb in liposomes.

Emulsion of Perfluorochemical (PFC)

Preparations of PFC can serve as transporters of oxygen because of their relatively high solubility for oxygen and other gases. The perfluorochemicals are non-polar liquids which are insoluble in water and do not dissolve salts, glucose or other components of plasma. Emulsions of PFC in a suitable aqueous medium (simulated plasma) have been used as 'artificial blood'. The emulsified PFC contains about the same amount of oxygen as an equal volume of erythrocytes when equilibrated with 100 per cent oxygen. However, unlike the Hb in erythrocytes, the

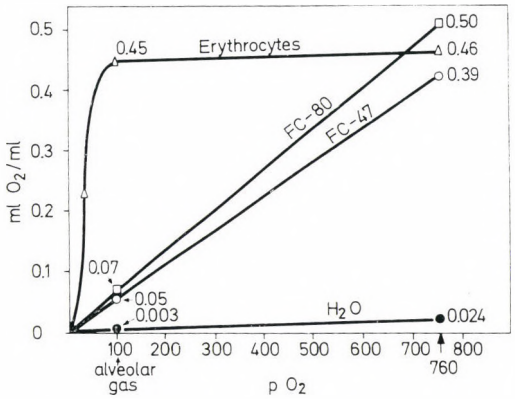

Fig. 1. Oxygen content of packed human erythrocytes, perfluoro compounds (FC-80 and FC-47) and water at 37 °C as a function of oxygen tension. Although PFC carries about the same amount of O_2 as erythrocytes at pO_2 = 760 torr, at pO_2 = 100 torr (alveolar gas) PFC contains only about 15 per cent as much oxygen as an equal volume of erythrocytes (from Ref. [37])

amount of oxygen contained in PFC is a linear function of the oxygen tension (pO_2) to which the PFC is exposed. Therefore, as shown in Figure 1, although the PFC carries about the same amount of oxygen as erythrocytes when exposed to 100 per cent oxygen (pO_2 = 760 torr), when equilibrated with air or alveolar gas (pO_2 = 100 torr) the PFC contains only 15–20 per cent as much oxygen as the same volume of erythrocytes. At present it seems unlikely that new PFC with much higher solubility for oxygen will become available. This means that when an emulsion of PFC is used as a substitute for blood, the recipient animal or man should inspire gas which has a considerably higher oxygen concentration than air.

The ability of an emulsion of PFC to deliver oxygen in adequate amounts to tissues was first demonstrated with an isolated, perfused rat brain preparation [8]. The emulsified PFC maintained the electrical and metabolic activities of this brain as well as erythrocytes did, but in the absence of an oxygen transporter, the electrical activity rapidly disappeared.

The solubility of carbon dioxide in PFC is more than three times greater than that of oxygen. Therefore, the PFC can easily and adequately transport carbon dioxide under physiological conditions. This has been demonstrated with the isolated, perfused rat brain preparation [8]. The mode of transport of carbon dioxide by PFC is different from that of the erythrocyte. The erythrocyte converts most of the carbon dioxide to bicarbonate which is then carried in the plasma; in PFC, little or no bicarbonate is formed and carbon dioxide remains physically dissolved. PFC may be useful experimentally in distinguishing between physiological effects of carbon dioxide, carbonic acid and hydrogen ions.

Use of PFC Emulsions in Animals and Humans

The first demonstration that PFC could provide the major requirement for oxygen in whole animals was the finding [9] that frogs and mice previously infused with PFC emulsion survived much longer than untreated controls in concentrations of carbon monoxide which almost completely blocked the transport of oxygen by hemoglobin. Subsequently, Geyer [10] observed that rats survived after almost complete replacement of blood by PFC emulsion. Clark et al. [11] showed that PFC emulsion provided oxygen in the dog by finding that infusion of PFC emulsion caused an increase in the mixed venous oxygen tension.

The first infusion of PFC emulsion in humans was performed by Makowski et al. [12] in individuals who were legally dead as a result of severe brain trauma. They observed some changes in vital function after PFC infusion which returned to pre-infusion status after several hours. Subsequent studies in human volunteers [13] and in clinical cases [14–16] have indicated that the PFC emulsions are not acutely toxic, although some potentially unfavorable effects were observed. These studies have been done in all cases except that done by the group in China [14], with the PFC emulsion Fluosol-DA, manufactured by The Green Cross Corporation of Osaka, Japan. This preparation contains detergent (Pluronic F-68, BASF Wyandotte) as emulsifying agent. This detergent has been reported to have a variety of actions including effects on blood platelets, blood coagulation and blood viscosity [17]. Therefore, some effects observed after infusion of Fluosol-DA could be due to Pluronic F-68 instead of, or in addition to the delivery of oxygen by PFC.

Lipid-Coated PFC Emulsions

PFC have very unusual physical properties because of their extremely low intermolecular forces, which is due to the presence of the many fluorine atoms. One of the unusual properties is the extremely low surface tension of PFC. Therefore, PFC will affect reactions or phenomena in which surface actions are involved. Although PFC are chemically inert, because of their surface activity, they do affect blood platelets and blood coagulation factors [18, 19]. Therefore, the use of PFC emulsions as artificial blood poses the potential hazards which may result from disorders of platelet function and blood coagulation.

In order to prevent the effects of the surface activity of PFC on platelets and blood coagulation, we attempted to cover the surface of PFC particles with a material which would mask this activity. We chose for this purpose a mixture of cholesterol and lecithin, in the proportion in which they constitute the major components of the erythrocyte membrane. PFC particles coated with this mixture of lipids did not affect aggregation of human blood platelets in vitro and did not cause thrombocytopenia when infused in animals [18]; non-lipid PFC emulsion did affect aggregation of platelets and did cause thrombocytopenia. Subsequently,

we found that cholesterol was not necessary and that emulsions prepared with lecithin alone were just as satisfactory.

The emulsified PFC remains in the circulation for about 3 days after infusion [20]. We made the hypothesis that the lecithin-coated particles first lost the lecithin coating and were then removed from the circulation. If this were true, then slowing the removal of lecithin from the particles should prolong their retention in the circulation. We attempted to do this by maintaining a high concentration of lecithin in the blood by daily injection of lecithin. We have reported the results of this study at this Congress [21]. We found that 4 days after infusion of lecithin-coated PFC emulsion in rats, no PFC remained in the circulation. In rats so infused and then given daily injections of lecithin, 67 per cent of the PFC remained in the circulation after 4 days and complete removal of the PFC did not occur until about 14 days after infusion. Thus, it appears that the lipid-coated emulsion is safer because it does not affect blood platelets and blood coagulation, and that it is more efficacious because its retention in the circulation, and hence its function as transporter of oxygen, can be markedly prolonged.

Elimination of PFC

The principal avenue of exit of PFC from the body is via the lungs and to a minor extent via the skin. No measurable amounts are excreted in urine or feces. The PFC is removed from the bloodstream by the reticuloendothelial organs and accumulates mainly in liver and spleen. Many of the PFC are retained in liver and spleen for many months. Clark et al. [22] reported that perfluorotributylamine persisted in liver and spleen for years after a single infusion. After testing a large number of PFC, Clark et al. [23] found that perfluorodecalin was eliminated from liver and spleen relatively rapidly, in a matter of days. However, perfluorodecalin did not form a stable emulsion when treated in the same way as the perfluoroamines. Naito and Yokoyama [24] reported the preparation of a relatively stable emulsion which contained a mixture of 70 per cent perfluorodecalin and 30 per cent perfluorotripropylamine. This preparation is the Fluosol-DA manufactured by The Green Cross Company of Osaka, Japan. This emulsion remains stable only if it is stored in the frozen state and it must be used within one day after thawing. The perfluorotripropylamine contained in the preparation is not eliminated rapidly from the tissues, being retained in the spleen for many months. Heldebrandt et al. [25] have reported at this Congress their evaluation of four new PFC with respect to their retention in tissues and other criteria and concluded that none of them were more desirable than perfluorodecalin.

Solutions of Hemoglobin (Hb)

Since a solution of Hb (outside the erythrocyte) readily combines with oxygen, it is an obvious candidate for use as a transporter of oxygen in vivo. Many early attempts were made to use cell-free solutions of Hb (i.e. hemolysates) as

Table 2

Properties of Hb in plasma
(in comparison with Hb inside erythrocyte)

1. Oxygen loading curve shifted to left:	Oxygen affinity is increased; inadequate release of O_2 to tissues
2. Dissociates into dimers:	Dimers cross glomerulus and are excreted in urine
3. May be oxidized to metHb:	No reductase in plasma; metHb shifts O_2 loading curve of Hb to left and reduces release of O_2 to tissues
4. Cleared by reticuloendothelial system:	Hb removed fairly rapidly from the circulating plasma
5. Cleared Hb catabolized in liver:	Iron accumulates; possible damage to liver

blood substitutes, but in all cases infusion of hemolysate in animals resulted in renal damage. Rabiner [26] demonstrated that stromal components of the erythrocyte were responsible for the renal damage and that infusion of large amounts of a stroma-free hemoglobin solution in animals had no harmful effects and did provide some oxygen to the tissues.

Although solutions of purified Hb can be infused with no resulting harmful effects, the quantity of oxygen delivered is very small compared to Hb in erythrocytes. The changes in properties of Hb when released from the cell into plasma, and their consequences are listed in Table 2. In summary, the Hb in plasma does not adequately release oxygen, it dissociates into dimers which are excreted in the urine, it may be inactivated by oxidation and it is fairly rapidly cleared from the circulation by the liver and catabolized with accumulation of iron in the liver.

Solutions of Modified Hemoglobins

An important advance came when Benesch et al. [27] modified Hb by attaching pyridoxal phosphate to the Hb molecule. The resulting molecule has an affinity for oxygen which is much lower than that of unmodified Hb and thus will more readily release oxygen to the tissues. Greenburg et al. [28] demonstrated the improved delivery of oxygen by this pyridoxalated Hb in experiments in hypotensive animals. However, this modified Hb dissociates into dimers and is excreted in the urine. The concentration of Hb in these solutions is limited by their colloid osmotic pressure to about 7 g/dl, which has an oncotic pressure approximately that of normal blood plasma. This concentration is only about 50 per cent of normal blood Hb concentration, and therefore after infusion of a large volume or total exchange transfusion, the recipient animal will be severely anemic.

In order to prevent the dissociation of Hb and its subsequent excretion in urine, DeVenuto and Zegna [29], Moss [30], and Rozenberg [31] treated pyridoxal phosphate Hb with glutaraldehyde to form an intermolecular bridge cross-linking 2 or more molecules. This 'polymerized' Hb was found to be retained in the circulation for significantly longer times than unmodified Hb. At this Congress, Bonhard [32] has reported the preparation of a similar polymerized pyridoxal phosphate-Hb which is relatively homogeneous and has an oxygen affinity close to that of Hb in the erythrocyte.

Another method of preventing dissociation of Hb was achieved by Benesch et al. [33] by the insertion of a covalently-bound bridge containing a phosphate group between the β chains of Hb. This bridge and its phosphate group prevented dissociation and markedly reduced its affinity for oxygen. Sloviter et al. [34] found that after infusion in rats, none of this intramolecularly cross-linked Hb was excreted in the urine, it was retained in the circulation considerably longer than unmodified Hb and it retained its low affinity for oxygen while in the blood stream. This confirms the notion that the Hb tetramer does not pass through the renal glomerulus and that dissociation into dimers precedes excretion in the urine.

PFC Emulsions vs. Solutions of Hb

In Table 3 are listed the properties and functions of both PFC emulsions and Hb solutions which are important to evaluate the safety and efficacy of a substitute for blood ('artificial blood'). For some of the items, further discussion is warranted:

1. At present, the supply of Hb solution comes mainly from outdated blood-bank blood, much of which is still being discarded. However, if Hb solutions become practical substitutes for blood, the demand for Hb may far exceed the supply available from outdated blood. Then, a unit of Hb solution will require the collection of somewhat more than one unit of blood. In contrast, PFC are commercial, organic chemicals and are available in unlimited amounts.

2. The possible acute toxic effects of the PFC emulsions would result from actions on constituents of blood, both formed elements (especially platelets) and plasma components (coagulation factors and complement). Although the serious damage to kidneys observed after infusion of crude hemolysates are not seen with purified Hb solutions, a transient impairment of renal function has been observed after infusion of purified Hb solution [35]. Further investigation of these effects is needed for both PFC emulsions and Hb solutions.

3. Both PFC emulsions and Hb solutions are cleared from the circulation by the reticuloendothelial system. Clearance of large quantities may cause blockade of the R-E system, with the resulting hazard of loss of resistance to infectious and other foreign agents. Also, repeated infusion of Hb solution would cause iron overload, so that Hb solutions are not likely to be suitable for treatment of chronic anemias such as thalassemias and sickle cell anemia.

Table 3

Comparison of PFC emulsions and solutions of hemoglobin as substitutes for blood

	PFC emulsion	Hb solution
Supply	Unlimited	Not unlimited
Long storage	Possible	Possible
Infectious agents	Absent	Absent
Immunologic effect	Not antigenic	Probably not antigenic
Acute toxic effects, possible targets	Platelets Blood coagulation Complement	Kidney
Chronic toxic effects, possible targets	R-E system	R-E system Iron overload
Respiratory gas required	Elevated oxygen concentration	Air
Duration of action	Days	Hours
Oncotic pressure	Iso-oncotic or variable	Limits concentration of Hb
Optical properties	No color No fluorescence	Colored Fluororescent
Conditions of function	Not blocked by CO, oxidants or low temperature	Inactivated by CO, oxidants and low temperature

4. Hb solutions have the important advantage that they can function adequately in animals breathing air, whereas PFC emulsions will require that the animal breathe gas containing elevated concentrations (perhaps > 50 per cent) of oxygen in order to deliver enough oxygen to the tissues.

5. PFC emulsions have the advantage that they can be retained in the bloodstream, and hence deliver oxygen, for considerably longer times than is presently possible with Hb solutions.

6. PFC emulsions are likely to be useful for treatment of carbon monoxide poisoning and during hypothermia. In these situations Hb solutions cannot deliver significant amounts of oxygen.

7. The absence of color or fluorescence in PFC emulsions permits some measurements which cannot be made in the presence of Hb; e.g. the estimation of the redox status of the brain by fluorescence measurement was possible when PFC replaced blood [36].

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Bone Marrow Biopsy

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After introduction of the bone marrow aspiration technique, during many years the clinical haematologists have relied primarily on cytological examination of bone marrow smears. This was mainly due to technical difficulties in preparing tissue sections and to lack of knowledge in their correct interpretation.

During the last 15 years, however, a histological study of the bone marrow (BM) has been introduced in most haematological centres and a bulk of knowledge has been achieved. Important contributions have been made by Burkhardt [1] in Germany, Duhamel [2] in France, Hernández Nieto [3] in Spain, Rywlin [4], Block [5] and Custer [6] in the United States and by many other authors. The indications of bone marrow biopsy have been greatly expanded.

One of the first and chief indications is the so-called 'drytap'. This indication was very well expressed by Block [5] as follows: 'An acellular marrow smear means that the marrow may be acellular, hypocellular, normocellular, hypercellular, fibrous, osteosclerotic, or replaced by abnormal tissue.'

In Table 1 the indications for BM biopsy are summarized. In the group of BM failure, the examination of marrow specimen can be useful not only in cases of aplasia, but also in myelodysplastic syndromes and in the study of BM grafting. The knowledge of chronic myeloproliferative disorders can be improved by BM biopsy. Among the lymphoproliferative syndromes, biopsy is mandatory for staging in lymphomas and for diagnosis in hairy cell leukaemia. In addition, a useful prognostic information can be drawn from BM biopsy in this disease and in chronic lymphocytic leukaemia. In some acute haemopathies such as acute promyelocytic leukaemia with 'packed marrow' or acute myelofibrosis, BM biopsy may be indispensable.

In the following paper Dr. Seewann from Austria will pinpoint the usefulness of BM biopsy in chronic granulocytic leukaemia. A further paper by Dr. Montserrat from our institution will discuss the prognostic value of BM biopsy in this condition. Recent studies suggest that from histological sections of BM, additional information can be obtained in monoclonal gammopathies. Finally, in an expanding group of miscellaneous disorders, BM biopsy can be useful or even essential.

Some years ago, Dr. Miale ingeniously commented: 'The haematologist

Table 1

Indications for BM biopsy

BM failure	
	Aplasia
	Myelo-dysplastic syndrome
	BM transplantation
Chronic myeloproliferative disorders	
	Chronic myeloid leukaemia
	Polycythaemia vera
	Myelofibrosis/osteomyelosclerosis
	Idiopathic thrombocythaemia
Lymphoproliferative disorders	
	Hodgkin's disease
	Non-Hodgkin's lymphomas
	Chronic lymphocytic leukaemia
	Hairy-cell leukaemia
Acute haemopathies	
	Acute leukaemia with packed marrow (i.e. AML-M_3)
	Acute myelofibrosis (acute megakaryoblastic leukaemia)
Monoclonal gammopathies	
	Multiple myeloma (focal)
	Macroglobulinaemia
	'Benign' monoclonal gammopathies
Miscellaneous	
	Malignant histiocytoses
	Metastatic cancer
	Thesaurismoses
	Granulomatous disorders
	Bone pathology
	Other

who doesn't believe in tissue sections of bone marrow will be limited not only in his diagnostic success but also in his chances of going to heaven.'

I am sure that the number of haematologist addicts to the practice of BM biopsy will increase, and eventually, if Dr Miale was right, the heaven will be crowded by members of our specialty.

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The Value of Bone Marrow Biopsy in Chronic Myeloid Leukaemia

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Seventy-six patients with chronic myeloid leukaemia (CML) could be subdivided by core biopsy into chronic granulocytic leukaemia (CGL, n = 24) and chronic megakaryocytic granulocytic myelosis (CMGM, n = 52). By pure clinical definition 59 patients were grouped as classical CML and 17 showed a course which we termed atypical myelosis. The most reliable criteria for distinguishing between the classical and atypical forms were ALP-Index, peripheral leukocyte and platelet count and the estimated number of megakaryocytes in the bone marrow. The classical myeloses consisted of 40 per cent CGL and 60 per cent CMGM whereas the atypical consisted of CMGM only including all stages of fibrosis. Fibrosis was at the time of bone marrow biopsy found in 20 per cent of classical CML and in about 50 per cent of atypical myeloses. In classical CML Philadelphia chromosome could be detected in all the patients with CGL and in 50 per cent of those with myelofibrosis. Atypical myeloses did not exhibit Philadelphia chromosome. In 70 per cent of the cases it was possible to distinguish between CGL and CMGM by peripheral blood findings and bone marrow cytology.

Keywords: atypical myelosis, bone marrow biopsy, chronic granulocytic leukaemia, chronic megakaryocytic granulocytic myelosis, chronic myeloid leukaemia

*

The clinical diagnosis of chronic myeloid leukaemia (CML) is suggested in an adult patient presenting with high granulocyte counts shifted to the left and splenomegaly. Other typical signs are a low or absent ALP index, a normal or sometimes elevated thrombocyte count [5, 11] and the Philadelphia chromosome. Bone marrow aspiration, although of little diagnostic value, is hypercellular with increased granulocytopoiesis and sometimes also marked megakaryocytopoiesis with many thrombocyte clusters. In cases where a hypocellular bone marrow or a dry tap is found, myelofibrosis (MF) is suggested. MF of bone marrow as the consequence of CML has been found in 20–30 per cent of cases [4]. On the other hand MF was thought to be a separate entity called idiopathic myelofibrosis or agnogenic myeloid metaplasia (AMM) with its own natural history [10].

Signs and symptoms in CML and AMM are sometimes overlapping, and one must suspect at least some relationship between these two disorders.

Termination of polycythaemia vera (PV) in an AMM like syndrome occurs in 15–20 per cent of patients [8] and marrow fibrosis may be present in CML. Whether one should distinguish between AMM, atypical CML and idiopathic thrombocythaemia or consider these syndromes as variants of AMM is debatable [11].

The term atypical myelosis has been used for Philadelphia chromosome negative CML with usually less good prognosis and an atypical course of disease. The absence of Philadelphia chromosome did not exclude a long survival in a few patients although the survival in Philadelphia chromosome negative patients was shorter than that of Philadelphia chromosome positive patients [1].

On the basis of clinical parameters we were able to divide CML into two variants, a classical form and one which we termed atypical myelosis, based on the following criteria [7]:

longer prediagnostic period
higher average age
larger spleen size
lower leukocyte count ($\leq 30 \times 10^9$/l)
slight shift to the left
higher thrombocyte count ($\geq 500 \times 10^9$/l)
elevated ALP-Index
higher number of dacryocytes
lower cellularity in aspired bone marrow
elevated number of magakaryocytes
increased number of thrombocyte clusters in the bone marrow.

None of these findings by itself proved to be typical of atypical myelosis, but taken together a classification was possible and deviating values facilitated the decision.

Karyogenetic investigations were not done routinely but all the patients with atypical myelosis were Philadelphia chromosome negative.

Similar differences between typical and atypical myelosis have been found by Canellos et al. [1] except the platelet counts which tended to be lower in their study.

Histology of bone marrow allows to subdivide CML into two discernible subtypes [3], i.e. chronic granulocytic leukaemia (CGL) and chronic megakaryocytic granulocytic myelosis (CMGM). The histological features of CGL are a neoplastic proliferation of granulocytopoiesis with sometimes a slight reactive increase of megakaryocytes. In contrast, CMGM shows a neoplastic proliferation of two cell lineages, i.e. granulocytopoiesis with uninterrupted maturation starting in the peritrabecular areas and extending continuously towards the venous sinusoids and a neoplastic proliferation of megakaryocytes with atypical cytology (bizarre, oversized cells with hyperlobulated nuclei) and an abnormal site of growth (expansion towards the osseous trabecules). Erythropoiesis may be prominent but does not disclose any abnormal proliferation of erythroblasts.

CMGM initially exhibits no fibres. In the later course an increase in the

amount of fibres in the bone marrow of CMGM patients leads to three other stages [2]:

Stage I: CMGM without any fibres
Stage II: early MF (scattered augmentation of reticulin fibres)
Stage III: MF (overt increase of reticulin and collagen fibres) and
Stage IV: myelosclerosis (partial obliteration of marrow spaces by apposition of newly formed bone).

Abnormal and polymorphous megakaryocytopoiesis is a prominent feature of all stages. Erythropoiesis and granulocytopoiesis are increasingly replaced in the later course of the disease.

Materials and Methods

Bone marrow biopsies were performed in 76 patients with CML. The procedure was usually done at the time of diagnosis but some 10 patients entered the study later on. We used the method described by Jamshidi and Swaim. Bone cylinders were fixed in Schaffer's solution and embedded in methyl acrylate. Semithin sectioning was done without decalcification by the Institute of Pathology, Medical School Hannover, FRG. The histological diagnosis of CGL and CMGM with the different stages of fibrosis were correlated with the two clinical courses, i.e. classical and atypical CML.

Mean age of the 76 patients with CML was 54 and a half years. There were 43 males and 33 females. The histologic classification revealed 24 cases with CGL and 52 cases with CMGM at different stages of fibrosis (I 18, II 14, III 15, IV 5). By pure clinical definition 59 patients were grouped into the classical form of CML and 17 into the atypical variety. The classical CML were histologically inhomogeneous and consisted of 24 (40 per cent) CGL and 35 (60 per cent) CMGM of the following stages: I 12, II 11, III 11, IV 1. All the atypical myeloses belonged to the histologic subtype of CMGM; 6 cases were in stage I, 3 in stage II, 4 in stage III and 4 in stage IV.

The 5 most frequent symptoms were fatigue in 75 per cent, weight loss in 50 per cent, upper abdominal discomfort in 24 per cent, fever in 18 per cent and night sweating in 13 per cent of the patients. The two clinical variants did not show considerable differences in these figures.

Mean enlargement of the spleen below the costal margin was 4.2 cm in CGL, 3.8 cm in CMGM stage I/II and 7.8 cm in MF/MS. In a comparison between classical versus atypical myeloses the differences were 5.1 : 6.5 cm.

The haematological data showed clear differences between atypical CML and the CGL subtype of classical myelosis. The three most reliable criteria in distinguishing the two clinical variants were leukocyte count, platelet count and the ALP-index (Table 1). The lowest mean leukocyte counts and highest ALP-indices as well as the highest mean thrombocyte counts were found in atypical CML. Erythroblasts did not appear to be of diagnostic value whereas tear drop shaped erythrocytes predominated in atypical CML.

Table 1

Haematological data in CML (mean values)

n = 76	Classical CML			Atypical CML
	total 59	CMGM 35	CGL 24	17
L× 10^9/l	107	62	172	16
ALP Index	72	104	26	148
Th× 10^9/l	373	453	256	688
% Patients with				
L > 30× 10^9/l	66	54	83	6
Th > 500× 10^9/l	10	17	0	41
Erythroblasts	51	54	46	59
Dacryocytes	47	65	21	72

The surprisingly high ALP-index in the whole group of classical CML could be explained by the fact that in this group more patients had entered the study later and some of them showed signs of beginning accelerated phase. The ALP-index in this group therefore might not reflect the ALP-index characteristic of the pure chronic phase.

Cellularity of bone marrow aspirates was diminished or absent in 47 per cent of atypical myeloses and in 25 per cent of classical myeloses but in no case of CGL. Megakaryopoiesis was found to be increased in 93 per cent of atypical myeloses, in 54 per cent of classical myeloses and only in 17 per cent of CGL. Thrombocyte clusters predominated with 79 vs. 8 per cent in atypical myeloses as compared to CGL.

In 32 of 76 patients it was possible to perform karyogenetic investigations. There were 13 cases of CGL, all of them showed Philadelphia chromosome. In 7 of the 15 CMGM-I–III of the clinical group classical myelosis (46 per cent) was the Philadelphia chromosome detected. In this group 6 MF were included; three of them showed the Philadelphia chromosome while 4 cases of atypical myelosis were Philadelphia chromosome negative.

The prediagnostic period was 22.9 months in our patients with atypical myelosis vs. 5.6 months in the classical group, whereas no striking differences were found between CGL and CMGM in the classical myeloses.

Conclusion

We have tried to find out whether the histologic subtypes of CML, i.e. CGL and CMGM could be correlated to different clinical courses. The clinical group of atypical myelosis was histologically homogeneous (all cases were CMGM) whereas the classical myeloses consisted of 40 per cent CGL and 60 per cent CMGM. The diagnosis of CMGM or CGL was possible in about 70 per cent

of the cases on the basis of clinical methods, i.e. bone marrow and peripheral blood morphology.

AMM is defined by the trias splenomegaly, extramedullary haematopoiesis and fibrosis or sclerosis of the bone marrow. Thus it was not possible to compare AMM with our group of atypical myelosis since half of these patients did not show fibrosis at the time of bone marrow biopsy.

Courses of CML are found to be very different, with survival times ranging from less than one year to more than ten years with a mean of 44 months [6, 9]. These differences might not depend only on the Philadelphia chromosome defect but also on histological variants.

Thus, bone marrow biopsy in CML revealed that CGL was never accompanied with thrombocythaemia at any time of the disease and neither were bleeding and thrombotic complications ever seen. We have not found transition of CGL into fibrosis, therefore we have to expect a blast crisis to terminate the disease. CMGM on the other hand may result either in blast crisis or fibrosis. Sometimes both conditions, blast crisis and fibrosis, coincide in terminating CMGM. In CMGM we sometimes observed very high thrombocyte counts ($1000–5000 \times 10^9/l$) associated with bleeding complications.

The last stage of CMGM may show a histological architecture indistinguishable from that of AMM. Serial histological investigations in the course of CMGM might show a transition into AMM. The clinical course of atypical myelosis resembles AMM, so it was concluded that AMM was a variant of CML. With regard to therapy, we are not able to anticipate basis therapeutic consequences by a clinical or histological subdivision of CML, but in advanced stages of CMGM or cases of atypical myelosis we are rather reserved in initiating aggressive cytotoxic drug treatment.

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Bone Marrow Biopsy in Chronic Lymphocytic Leukaemia: A Study of 208 Cases

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In 208 cases of chronic lymphocytic leukaemia (CLL) in which a bone marrow biopsy has been performed, the correlation between histopathological bone marrow patterns (interstitial, nodular, mixed and diffuse) and both Rai's clinical stages and the recent International Workshop on CLL proposal (A, B, C stages) has been analyzed. In general, a fairly good correlation has been found between the bone marrow patterns and both Rai's and A, B, C systems. As far as the correlation between the bone marrow patterns and the tumoral load (lymphadenopathy, organomegalies, lymphocytosis, anaemia and thrombocytopenia) is concerned, the correlation was also fairly good.

Bone marrow pattern is an important prognostic factor in CLL and it is suggested to use a combined clinical and pathological (bone marrow biopsy) staging in CLL.

Keywords: bone marrow biopsy, clinical staging, chronic lymphocytic leukaemia

Introduction

In 1977, Hernández-Nieto et al. [1] described four different types of bone marrow involvement in patients with chronic lymphocytic leukaemia (CLL): *interstitial*, *nodular*, *mixed* (interstitial and nodular) and *diffuse*, assuming that in CLL the bone marrow infiltration could start with either interstitial or nodular patterns and then evolve to mixed and diffuse ones. In addition, a significant correlation between these patterns and CLL clinical stages according to Rai et al. [2] was found [1, 3]. Thereafter, other authors have been able to recognize the same types of bone marrow involvement as well as their correlation with Rai's clinical stages [4, 5]. On the other hand, Rozman et al. [6] in 1981 demonstrated that in CLL bone marrow biopsy has a clear prognostic significance, the patients with either interstitial or nodular infiltration patterns have a longer survival than those with mixed or diffuse ones.

In order to explore the prognostic significance of bone marrow biopsy in CLL and its anatomoclinical correlations, in 1981 a Spanish Cooperative Group started a study on a prospective and a retrospective basis. This report is an interim analysis of 208 patients included in the study up to January 1982.

* *Spanish Cooperative Group for CLL Study.*

CLL: Diagnostic Criteria

The diagnostic criteria of CLL were those usually recommended [7]: (i) more than 15×10^9/l lymphocytes in peripheral blood; (ii) bone marrow infiltration by lymphocytes of 50 per cent or more; (iii) less than 10 per cent atypical lymphocytes in either the peripheral blood or the bone marrow. Some cases of otherwise typical CLL with less than 15×10^9/l lymphocytes in peripheral blood were also included. Lymphosarcoma cell leukaemia [8, 9], prolymphocytic leukaemia [10] and leukaemic reticuloendotheliosis [11] were excluded.

Bone Marrow Biopsy

On the basis of preliminary studies four different histological patterns have been analyzed. These patterns were as follows.

Interstitial. Some degree of replacement of normal haemopoietic tissue by mature lymphocytes but with preservation of fat cells and bone marrow structure.

Nodular. Nodules made up of mature lymphocytes appear. These nodules are greater than normal lymphoid follicles and lack clear centres. There is no interstitial infiltration. Fat cells are preserved.

Mixed. A combination of the interstitial and the nodular pattern.

Diffuse. Diffuse lymphoid infiltration with massive replacement of normal haemopoietic tissue as well as fat cells.

The bone marrow biopsies were reviewed independently by two observers without any information of the clinical stage of the patients. When diagnostic discrepancies were found, the two observers reviewed together the biopsy until a final diagnosis was arrived at.

Staging Systems

According to *Rai et al.* [2] Stage 0 is defined by lymphocytosis only. Stage I is lymphocytosis with lymphadenopathy. Stage II is lymphocytosis with enlarged spleen or liver or both. Lymph nodes may or may not be enlarged. Stage III is defined by anaemia (Hb $<$ 11 g/dl or haematocrit $<$33 per cent). Lymph nodes, liver, or spleen may or may not be enlarged. Stage IV is established in the presence of thrombocytopenia (platelets $< 100 \times 10^9$/liter). Anaemia or organomegaly may or may not be present.

International Workshop on CLL [12]. In this staging system, besides anaemia (Hb $<$ 10 g/dl) and thrombocytopenia (platelets $< 100 \times 10^9$/liter), special attention is focussed on the clinical enlargement of spleen, liver, and lymph nodes in the cervical, axillary, and inguinal regions (irrespectively of the lymphadenopathy being unilateral or bilateral), constituting five separate 'lymphoid' areas of involvement. Three stages are considered: Stage A: no anaemia or thrombocytopenia with less

than three areas of lymphoid enlargement. Stage B: No anaemia or thrombocytopenia with three or more involved areas. Clinical stage C: Anaemia or thrombocytopenia regardless of the number of areas of lymphoid enlargement.

Results

The frequency of different bone marrow types, sex distribution and age of 208 cases so far studied are shown in Table 1. The bone marrow type most frequently seen was the diffuse (36 per cent), followed by interstitial (32 per cent), mixed (23 per cent) and nodular (9 per cent). No differences either in sex or age distribution were found among the different bone marrow types.

In Tables 2 and 3 the correlation between Rai's and the International CLL Workshop stages and bone marrow types is shown. In Rai's classification, stages 0 and I (low risk stages) were considered a sole group as well as stages III and IV (high risk stages). Both in Rai's stages and in the A, B and C system, in the less advanced stages (either A or 0 + I) low (nodular, interstitial) infiltration patterns

Table 1

Frequency of different bone marrow patterns, sex and age distribution

	N	I	M	D
Number (%)	19 (9)	66 (32)	47 (23)	76 (36)
M/F	13/6	42/24	34/13	43/33
Age (years, mean ± SD)	62±10	64±10	64±8	62±11

N = nodular; I = interstitial; M = mixed; D = diffuse

Table 2

Correlation between Rai's staging system and bone marrow patterns

Rai's staging system	N	I	M	D
Stages 0 + I				
N (%)	15 (18)	43 (51)	23 (27)	4 (4)
Stage II				
N (%)	3 (5)	18 (30)	16 (27)	23 (38)
Stages III + IV				
N (%)	1 (2)	5 (8)	8 (13)	49 (77)

N = nodular; I = interstitial; M = mixed; D = diffuse

Table 3

Correlation between International CLL Workshop staging system and bone marrow patterns

Workshop staging system	N	I	M	D
Stage A				
N (%)	14 (74)	43 (65)	24 (51)	7 (9)
Stage B				
N (%)	4 (21)	19 (29)	16 (34)	25 (33)
Stage C				
N (%)	1 (5)	4 (6)	7 (15)	44 (58)

N = nodular; I = interstitial; M = mixed; D = diffuse

predominate, whereas in the high risk stages (either C or III + IV) the heavy (diffuse) infiltration type was found. Mixed infiltration type was distributed among the different clinical stages without a clear predominance in any of them. The correlation between bone marrow patterns and clinical stages was not absolute.

In Table 4 the correlation between bone marrow type and different clinical and analytical data is presented. Anaemia and thrombocytopenia were predominantly found in the diffuse type and, in a similar way, lymphocytosis above 50×10^9/l was more common in this particular bone marrow pattern. On the

Table 4

Correlation between bone marrow patterns and some clinical and analytical data

	N (n = 19)	I (n = 66)	M (n = 47)	D (n = 76)
Generalized lymphadenopathy	5 (26)	28 (42)	21 (45)	56 (74)
Splenomegaly	3 (16)	17 (26)	19 (40)	64 (84)
Hepatomegaly	3 (16)	18 (27)	21 (45)	51 (67)
Lymphocytes ($\times 10^9$/l)	28±27	40±33	39±35	112±112
Lymphocytes ($>50\times 10^9$/l)	3 (16)	14 (21)	14 (30)	46 (60)
Haemoglobin (<11 g/dl)	1 (5)	5 (7)	6 (13)	46 (60)
Platelets ($<100\times 110^9$/l)	0 (0)	2 (3)	5 (11)	23 (30)

N = nodular; I = intermediate; M = mixed; D = diffuse

All results are expressed as number (%) of cases presenting a feature or as mean (±SD)

contrary, anaemia and thrombocytopenia were infrequent in the low infiltration types (nodular, interstitial). As a rule, the more advanced the bone marrow infiltration the more often were the lymph nodes, spleen and liver enlarged.

In respect to the survival probability of patients with CLL according to the sole basis of their bone marrow biopsy pattern, no significant differences were found in the O/E ratio of interstitial type (1.02) as compared to the nodular one (0.93). Therefore, these two patterns were analyzed as a sole group. In Table 5,

Table 5

Statistical analysis (log-rank test) of survival

	Observed/ total	Expected	Observed/ expected	χ^2
Interstitial + nodular	13/85	29.24	0.44	9.02
Mixed	9/47	15.17	0.59	2.51
Diffuse	42/76	19.59	2.14	25.65

χ^2 heterogeneity = 37.18 $p < 0.001$
χ^2 for trend = 31.55 $p < 0.001$

statistical analysis of the three groups (interstitial/nodular, mixed and diffuse) is presented. The estimated median survival probability was greater than 70 months for the interstitial, nodular and mixed groups (non-diffuse patterns) and of 20 months for the diffuse bone marrow type.

Discussion

The prognostic significance of bone marrow histology in CLL has been the subject of several reports. Gray et al. [13] in a study of 115 patients, including cases of lymphosarcoma cell leukaemia, showed that cases with diffuse bone marrow infiltration had a poor prognosis as compared with cases with nodular or mixed (nodular and diffuse) patterns. Although the bone marrow patterns in CLL have usually been described just as nodular (or focal) and diffuse, some authors have distinguished other types of bone marrow involvement in CLL. Thus, Rywlin [14] recognized (i) an interstitial pattern in which the marrow architecture is preserved and lymphocytic infiltrates are found among the haemopoietic cells in between the fat spaces; (ii) a nodular pattern in which there are nodular lymphocytic infiltrates replacing the haematopoietic cell areas as well as fat cells, (iii) a diffuse pattern in which there is a total effacement of marrow architecture and substitution by lymphocytes of normal haemopoietic and fat cells. In addition, there is a mixed pattern

formed by the combination of nodular and diffuse infiltration types. Although in some cases of otherwise diffuse bone marrow involvement, nodular or confluent structures can be seen among the lymphocytic infiltrations, this particular type of mixed pattern (nodular + diffuse) would better be classified as diffuse, since in these cases a great tumoral load is usually present with (lymphadenopathy, organomegalies, lymphocytosis, anaemia and thrombocytopenia). It is important to note that the mixed type described by Rywlin [14] and also recognized by Gray et al. [13], is not the same mixed type that we use in our study (nodular + interstitial).

Recently, Bartl et al. [15] assessing the bone marrow structure in patients with CLL have described two major types of bone marrow involvement: (i) diffuse (non-nodular patterns) which could be subclassified in interstitial and packed marrow types, and (ii) nodular patterns. In this work a certain degree of correlation has also been found between the bone marrow pattern and the clinical stages. It is not clear from the description of these authors whether the interstitial type described by them is the same interstitial type recognized by Hernández-Nieto et al. [1], as the interstitial type described by the latter authors probably represents a lower lymphocytic infiltration than that encountered by Bartl et al. [15] in their so-called diffuse (interstitial) type.

The prognostic significance of bone marrow biopsy has been demonstrated clearly by Rozman et al. [6]. Thus, patients with interstitial and nodular bone marrow involvement had a longer survival than those with mixed and diffuse ones. Since the overall follow-up of the present series is still rather short, no definitive conclusions can be drawn in respect to the eventual differences in the survival probability among patients with interstitial, nodular and mixed bone marrow patterns. The different prognosis of patients with non-diffuse patterns in comparison to those with diffuse bone marrow involvement is clear.

In the present interim analysis we have explored the anatomoclinical correlations of bone marrow patterns in CLL. It has been concluded that the different bone marrow patterns in CLL seem to reflect variable amounts of lymphocytic infiltration along the course of the disease. Therefore, the suggestion seems to be appropriate that in CLL the histological bone marrow patterns could follow the sequence of progression from interstitial and nodular types to the mixed and diffuse ones [6]. Furthermore, in sequential bone marrow biopsies performed in patients with CLL a parallelism exists between the clinical stages and the different bone marrow types. In cases where the clinical stage progresses it is usual that the bone marrow shows a switch to a more heavy infiltration pattern. Conversely, successful treatment of advanced cases of CLL may be accompanied by a regression of bone marrow lymphocytic infiltration or even by a normal biopsy (unpublished observation). Carbone et al. [16] and Charron et al. [17] considering just nodular and diffuse bone marrow patterns have done similar observations.

In this study a certain degree of correlation between the histological types of bone marrow infiltration and the different clinical staging systems in CLL as well as with the tumoral load has been found. On the other hand, it is clear that in CLL the bone marrow patterns have a prognostic significance. Since the correlation

between bone marrow pattern and clinical stage is far to be absolute, it is possible that different subsets of patients, with different prognosis, could be distinguished in the future on the basis of a combined clinical and 'pathological' (bone marrow patterns) staging system [14]. The Spanish Cooperative Group for CLL Study is now testing this possibility on a prospective basis.

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Cytogenetic Factors in Prognosis of the Acute Leukaemias

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Introduction

Chromosome analysis at diagnosis can be used to distinguish poor risk patients from good or better risk patients in both types of acute leukaemia.

With the possibility of bone marrow transplantation in first remission for acute lymphoblastic leukaemia patients, the identification of those who respond badly to current chemotherapeutic regimes is crucial.

Conversely in acute non-lymphocytic patients the fact that there is an important group of long term survivors with conventional therapy makes their identification equally challenging.

First, it will be discussed how chromosomes relate to survival in acute lymphoblastic leukaemia and then consider how this has been demonstrated for acute non-lymphoblastic leukaemia.

Acute Lymphoblastic Leukaemia

The relationship between chromosome findings and prognosis in ALL has been demonstrated in two ways. First, by means of a chromosomal classification based on the chromosome numbers in the cells of the leukaemic tissue at diagnosis [1–4]. Second, by the identification of chromosomal subgroups, some of which have a particularly poor prognosis [3, 5]. The effect of chromosomal classification is different in children and adults and I shall first describe how chromosomes may be used as prognostic indicators in children.

ALL in Children

A relationship between chromosome findings and survival in children was first demonstrated by Secker-Walker et al. in 1978 [1]. We showed at that time that it was important to distinguish patients with different kinds of chromosome abnor-

malities, as well as those with normal chromosomes. Differential prognosis between groups of patients was apparent when the patients were divided into the following categories. Normal – no detectable abnormalities; hyperdiploid – more than 46 chromosomes (the group which did best); pseudodiploid – 46 chromosomes with structural rearrangements (the group which did worst); hypodiploid – less than 46 chromosomes.

Since that time the value of chromosomal classification has been confirmed and the findings extended in other surveys [2–4]. The prognostic effect of chromosomal classification has moreover been shown to be independent of other factors known to influence survival. The findings of our second survey of 93 children with a maximum follow up of 9 years are demonstrated in Figure 1; they confirmed our earlier observation that hyperdiploid groups do better and pseudodiploid groups do worse than those with normal chromosomes. Survival was best in this survey for the 6 hypodiploid patients, none of whom have died; it is difficult to assess the

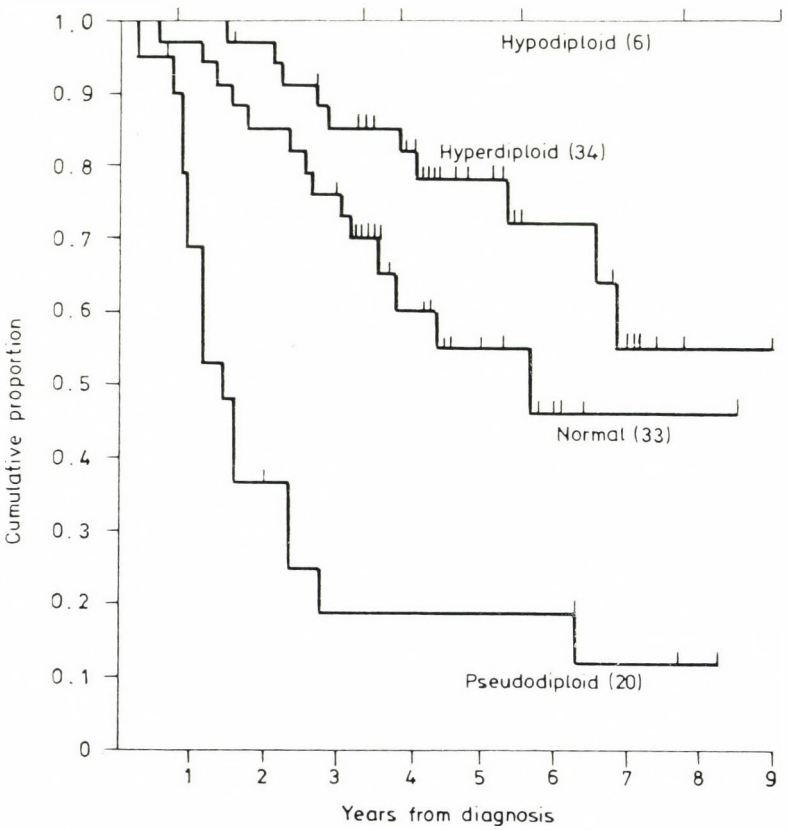

Fig. 1. Survival according to chromosomal category in 93 children with ALL [4]

importance of this in such a small group. When patients with normal chromosomes are compared with those with abnormal chromosomes no difference in survival is found. From Figure 1 it is clear that when the hypo-, hyper- and pseudodiploid groups are combined the opposite effects of the different types of abnormality cancel each other.

A similar classification was used for the 157 children presented at the Workshop at Lund in 1980 [3]. The hyperdiploid patients were divided into two groups, one with 47–50 chromosomes and another with 51–60 chromosomes. Survival was best for the group with more than 50 chromosomes. Those with 47–50 chromosomes did better than those with normal chromosomes and pseudodiploid patients had the worse prognosis.

In three surveys pseudodiploidy has been identified as a poor prognostic indicator. As to the distribution of poor and standard risk children, we know that

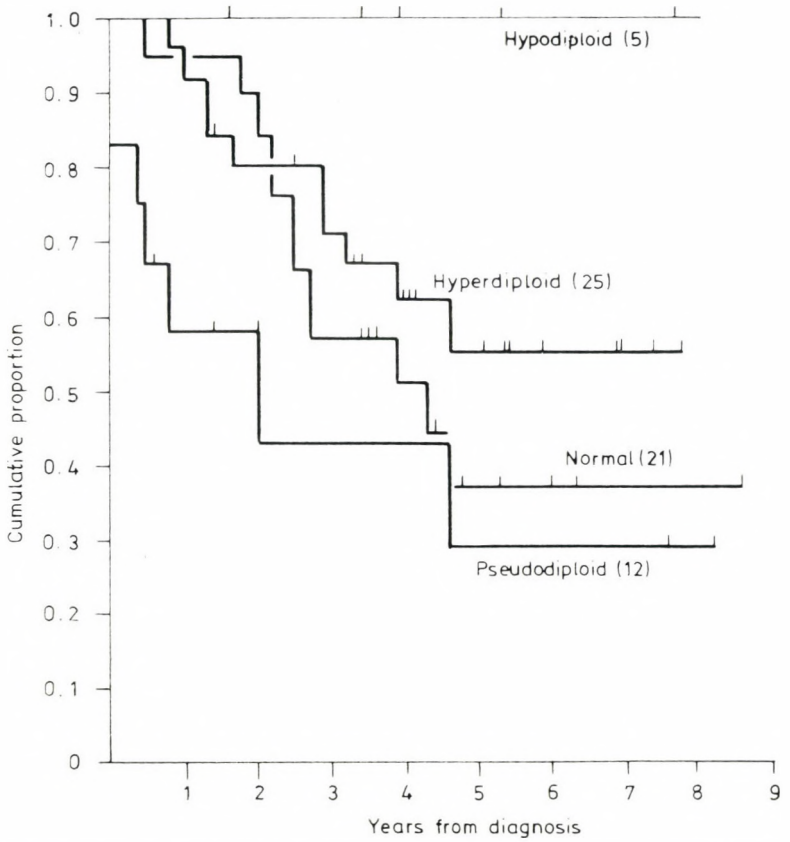

Fig. 2. Remission duration of 93 children with ALL and leukocyte counts below $20 \times 10^9/l$ [4]

pseudodiploidy is associated with male sex [3, 4], high white blood counts above 20×10^9/l [4] or 50×10^9/l [3] and with children aged under 2 or over 11 years [4]. Hyperdiploidy, conversely, is associated with female sex, low white blood count and an age between 2 and 11 years [4]. The unequal distribution of patients by age groups between chromosomal categories was statistically significant in our series ($p < 0.01$). In both surveys patients with a mediastinal mass at diagnosis and those with T cell surface markers were found only in the normal or pseudodiploid categories.

The log-rank analysis was reapplied in our study with adjustment for the effects of the unequal distribution of patients according to sex, age and white blood count. The differences between chromosomal categories remained statistically significant for the duration of the first remission ($p < 0.04$) and for survival ($p < 0.006$).

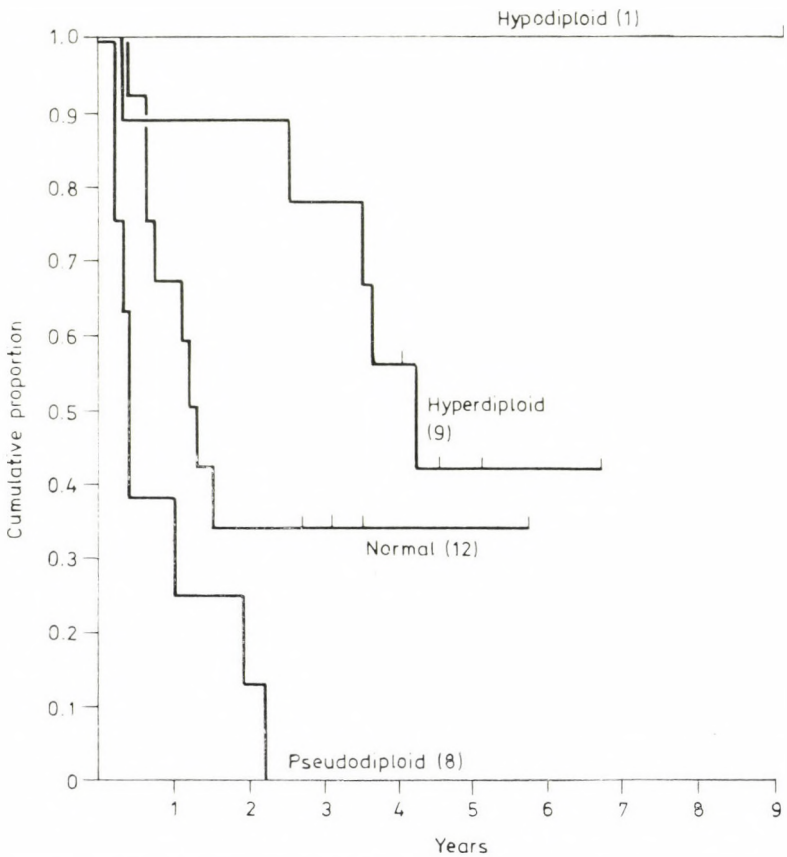

Fig. 3. Duration of remission of 93 children with ALL and leukocyte counts above 20×10^9/l [4]

Figure 2 shows the cumulative proportion of patients surviving by chromosomal category, with low white counts where the hyperdiploids did well and the pseudodiploids badly. Figure 3 shows those with high white counts; here no patient with pseudodiploidy and more than 20×10^9/l white cells was alive after 2½ years.

ALL in Adults

The value of chromosome classification in adults was demonstrated for the first time at the Lund Workshop [3]; 173 patients were considered, and the patients were classified in a number of different ways.

First of all, unlike in children, a comparison of chromosomally normal and abnormal cases was important. The median survival in different chromosomal groups was estimated from life table analysis. Patients with all normal chromosomes had a median survival of 31 months. The presence of an abnormal clone in all or only some of the analyzed cells was a poor prognostic feature with a mean survival of 9 months in each case. This finding was partly a reflection of the statistically significant association between the presence of an abnormal clone with a number of adverse prognostic features (Table 1). AA and AN patients were older and had higher white counts at diagnosis than the NN and NCl groups. Significantly more patients in the normal groups, i.e. 89 per cent achieved complete remission against 55 per cent in the abnormal groups.

When the patients were divided according to modal number it was found that by classifying them simply as normal or abnormal, one small abnormal category with a good prognosis was missed. Patients with more than 50 chromosomes actually did better than the normal category with a projected survival of more than 50 per cent of 'hyperdiploid 51–60' patients alive at 2½ years after diagnosis as compared with 30 per cent of patients in the normal category. Patients with lower numbers in the hyperdiploid range, unlike the children in this group, had an even

Table 1

Association of chromosomal groups with good or bad prognostic features (adults)

	NN	NN NCl	AN	AA	p
Number of patients	21	32	76	44	
Median age, years	24	27	34	35	= 0.02
Median WBC$\times 10^9$/l	13	14	21	26	= 0.05
% Complete remission	95	84	55	54	< 0.001

NN = metaphases all normal; NN NCl = metaphases normal and non-clonally abnormal; AN = metaphases normal and clonally abnormal. AA = metaphases all abnormal [3]

poorer prognosis than those in the pseudodiploid group. So once again the chromosome number was important as a prognostic indicator, but here the only group doing better than that with all normal chromosomes was the group with more than 50 chromosomes.

Five chromosomal sub-groups in ALL characterized by a particular chromosomal translocation or arm deletion have been identified and their survival relative to each other and to those with other chromosome findings was calculated at the Workshop at Lund. Each of these has been found in both adults and children and the effect on prognosis of the particular abnormality appeared to be similar regardless of the age group. The survival relative to each other and to normal cases is shown for adults in Figure 4 and for children in Figure 5.

The Ph′ +ve ALLs, those with a t(8;14) the Burkett-like translocation and those with t(4;11) were found in both children and adults. In adults an additional group was that with other 14q + translocations. All had a worse prognosis than the remaining pseudodiploid patients, and than those classified as normal. In children a fifth group with 6q−, on the other hand, had a good prognosis with a projected survival of nearly 70 per cent at three years.

The incidence of Ph′ +ve ALL is higher in adults than in children, 17 per cent compared with 5.7 per cent at the Workshop. Marker studies were all non-T non-B on the cases tested but recently one T-cell ALL with a Ph′ has been reported [6].

The t(8;14) and half the 14q + cases were B-cell ALL of FAB type L_3. As described by Berger et al. [7] they form a distinct sub-group of ALL with a very poor

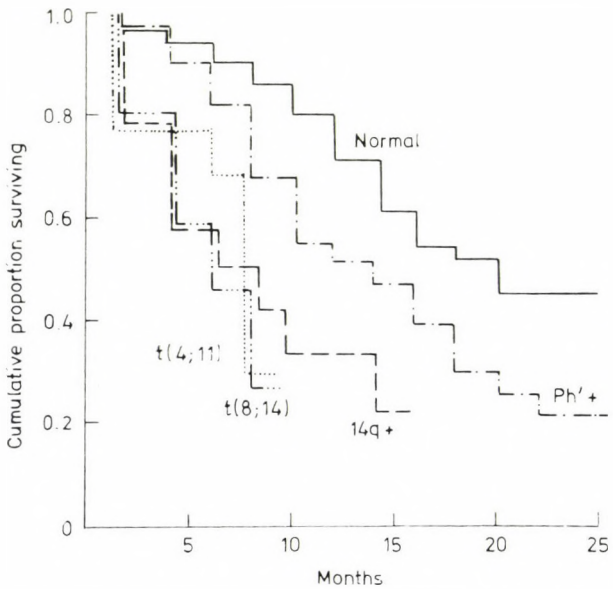

Fig. 4. Survival according to chromosomal subgroups in ALL in adults [3]

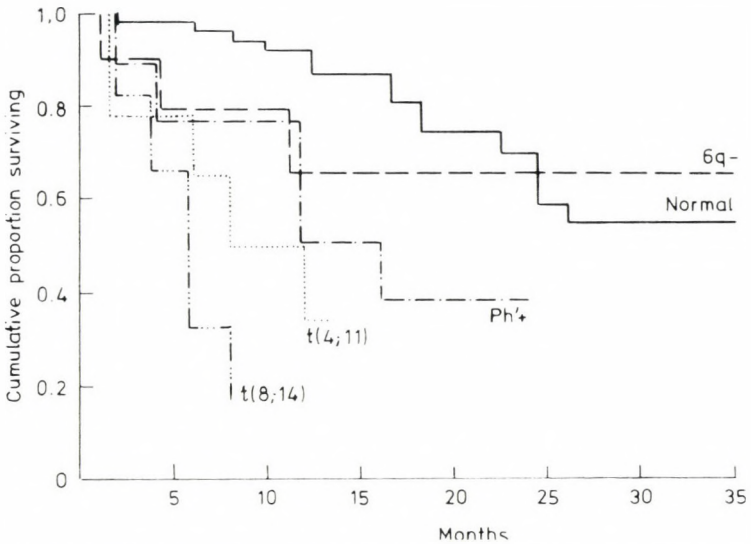

Fig. 5. Survival according to chromosomal subgroups in children with ALL [3]

prognosis. At the Workshop the incidence was 3.8 per cent in children and 5.8 per cent in adults for t(8;14), and for I4q + it was 7 per cent for adults and 2 per cent for children. t4;11 first described by van den Berge [8] was found in about 5 per cent of both children and adults. Most of the children were under 2 years but it occurred in two older children and in 5 adults under the age of 40. All but one patient had very high initial blood counts, and all but one of the cases tested were non-B non-T. The remaining case was a T-cell ALL. In ALL then chromosome analysis has identified good and poor risk groups in both children and adults.

Acute Non-lymphocytic Leukaemia

The importance of chromosome findings in ANLL was first described by Sakurai and Sandberg in 1973 [9] who noted a longer survival of patients with all or some normal chromosomes as compared to those with all abnormal chromosomes. These findings have since been confirmed by Nilsson et al. [10], Golomb et al. [11] and Lawler et al. [12]. In a recent report by Hagemeijer et al. [13], no difference in survival was, however, seen between the chromosome groups NN and AN + AA. Findings at the First International Workshop on Chromosomes [14] suggested that there was a difference in survival between patients with different proportions of normal and abnormal cells, but only in AML FAB types M_1 and M_2.

What was perhaps more interesting was the confirmation at the Workshop of chromosomally abnormal sub-groups with different prognoses. These are shown

in Table 2 with their distribution among the FAB groupings and the relationship of such sub-groups to the attainment of remission and their median survival. Of particular interest is the 8/21 translocation now believed to be associated exclusively with AML M_2, monosomy 7 in AML and the 15/17 translocation found only in APL M_3.

Table 2

Karyotype findings in 139 chromosomally abnormal cases of ANLL

	Ab-normal cases	Percent abnormal cases					Other**
		+8	−7	t(8q−; 21q+)	t(15q; 17q−)	22q−	
AML M_1–M_2	85	20	18	11	—	2.35	48.23
APL M_3	11	—	—	—	81.81	—	18.18
AMMOL M_4	29	6.9	13.79	3.44	—	3.44	72.41
AMOL M_5	2	1*	—	—	—	—	1*
EL M_6	5	1*	1*	—	—	—	3*
Undifferentiated	7	1*	—	—	—	2*	4*
Total	139	22	20	11	9	5	72
CR%	27	32	13	50	33	20	30
Median survival, months	—	6.5	4.0	6.0	1.0	4.0	5.0

* Cases
** Includes −5, −17, −21. Structural change 3, 5, 8, 9, 15, 17, 21 [14]

The 8/21 translocation examined at the 2nd International Workshop [15] was described by Rowley in 1973 [16]. The findings in Table 3 are taken from the 2nd International Workshop in 1979; 46 cases with 8/21 translocation were presented each of which was FAB type M_2. The points to notice here are as follows: the median survival of patients with the translocation, either alone or with other abnormalities, was longer than the 8 months median survival for AML with normal chromosomes shown at the First International Workshop; the very adverse effect on survival of the accompanying loss of a sex chromosome. Unfortunately, the follow-up was not very long but we do know that 11 out of the 46 patients were alive 2 years after diagnosis. Better complete remission rates and longer median survival for 6 t(8; 21) patients compared with 17 normal, and 6 patients with other chromosome abnormalities were reported by Takeuchi et al. [17]. With a maximum follow-up of 18 months there was, however, no significant difference in survival between the t(8; 21) and normal categories. Clearly further follow up of these cases and more studies are needed to find whether t8; 21 is associated with really long-term survival.

Table 3

t(8q−; 21q+) in acute myeloid leukaemia M_2 (46 cases)

Cytogenetics	Cases	Median age, years	CR%	Median survival, months			Alive at 1 year	2
				AN	AA	AN + AA		
t only female	10	52	80	21.5	9.5	14	6	3
male	7	52	71	26	6.5		4	2
t − X female	9	40	56	11	2	5.5	2	0
t − Y male	7	37	71	7	3.5		2	2
t + other	13	31	85	18	14	15.5	9	4
Total	46	45	74	—	—	11.5	23	11

Ref. [15]

Monosomy 7 in adult ANLL was examined by Borgstrom et al. [18] following the 2nd Workshop. Eighteen patients with monosomy 7 were compared with 18 chromosomally normal patients with ANLL in terms of factors which adversely affect prognosis. A very poor remission rate and survival at 18 months of the −7 patients was seen. This could be explained by an increase in fever at diagnosis and afterwards, increased number of infections, and the low granulocyte count. The defective chemotaxis shown by Ruutu et al. [19] to be associated with monosomy 7 may influence the response to infections of these patients.

Acute promyelocytic leukaemia, a rare disease (5 per cent of all acute leukaemias), has a very high early mortality. There is, however, a small but important group of long term survivors in this kind of leukaemia as described by Bernard et al. in 1973 [20]. Of the 80 cases with APL examined at the 2nd International Workshop, half were chromosomally normal, t(15;17) was found in 41 per cent and the other 9 per cent had other chromosome abnormalities (Table 4). Important are the considerably lower median age of the patients with t15;17 alone, coupled with considerably lower complete remission rate and poorer survival, than that in the other groups. The percentage of patients alive at 1 year was greatest in the normal category. In a recent report of 5 APL patients by Fraser et al. [21] the only fairly long term survivor, at 3½ years follow-up, had a normal karyotype at diagnosis. Again in APL more studies and longer follow-up are needed to determine the significance of the karyotype in long term survival, but this seems to be associated with a normal karyotype at diagnosis.

Summary and Conclusions

Chromosome findings at diagnosis can help to distinguish groups of patients with different prognoses. In ANLL there is a small group of patients who achieve long term remissions on conventional therapy. In acute myeloid leukaemia these

Table 4

t(15q+ ; 17q−) in APL M_3 (80 cases)

Cytogenetics	Cases	Median age, years	CR %	Median survival, months	Alive at 1 year, %
Normal	40	43	45	4.0	28
t(15q+ ; 17q−)	23	25	26	1.0	
t(15q+ ; 17q−) +	10	41	30	1.5	18
* Others	7	37	28	5.0	

* 4 = +8, 2 = −7, 1 = ring marker [15]

patients are to be found mainly among those with normal chromosomes at diagnosis. In FAB type M_2, however, patients with the best remission rates are those with 8;21 translocation, providing this is not accompanied by the loss of a sex chromosome. Whether significant numbers of long term survivors will be found among those with t8;21 remains to be seen.

Monosomy 7 carries a poor prognosis.

In acute promyelocytic leukaemia long term survivors may be found among those with normal chromosomes, while those with t15;17 do badly.

In ALL, chromosome investigations have identified groups of patients who do badly on current chemotherapeutic regimes and should perhaps be considered for transplantation during the first remission.

Adults with pseudodiploidy (which includes the Ph′+ves, t8;14s, 14q+s and t4;11s) and hyperdiploidy with less than 50 chromosomes have poor complete remission rates and their projected survival at 20 months is poor. Even those with normal chromosomes do not do too well. In children chromosomal groups with low percentage survival at 8 years are those classified as pseudodiploid, regardless of the white blood count, and those with normal chromosomes and white counts above 20×10^9/l. They are candidates for bone marrow transplantation in the first remission.

Conversely, children with more than 46 chromosomes had a 78 per cent projected survival at 5 years. Among the cases presented at Lund those with a 6q − did well and those with more than 50 chromosomes had a projected survival of more than 90 per cent at three years. In these patients conventional therapy appears to be capable of completely eradicating the leukaemic clone. Bone marrow transplantation at the present state of the art would have nothing to offer.

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The Association of Tn and Leukemia

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Tn polyagglutination or persistent mixed field polyagglutination is a rare acquired erythrocytic state which has been associated with haematologic abnormalities including four reported cases of acute leukaemia. Recent information has proven that the abnormality is clonal and the haematologic findings suggest a mutation of a somatic stem cell. The red cell membrane defect results from a deficiency of B-3-D galactosyltransferase. Tn polyagglutination has been described in patients presenting with acute leukaemia and has preceded acute leukaemia. The Tn syndrome has similarities to other forms of preleukaemic syndromes including paroxysmal nocturnal haemoglobinuria. Patients with Tn should be carefully monitored, although there is little justification for any specific therapy at the present time.

Keywords: acute leukaemia, clonal abnormality, preleukaemia, Tn polyagglutination

Introduction

Tn polyagglutination or persistent mixed field polyagglutination is a rare acquired erythrocytic state which has been associated with haemolytic anemia, leukopenia, thrombocytopenia, and acute leukaemia [1, 2].

At the time of this review, four cases of acute leukaemia associated with the Tn red cell state have been reported [2–5]. This paper will review the serology, clonal haematopoietic stem cell origin, biochemical nature, and clinical cases relevant to Tn and leukaemia.

Serology

The red cell abnormalities are well known to immunohaematologists and can be demonstrated by the findings of previously reported patient F. G. [2] (Table 1). The patient's red cells were found to react with anti-A, anti-B, and anti-A,B. Agglutination showed a mixed field appearance. The patient's serum reacted with A cells and B cells but did not react with reagent O cells nor the patient's own cells. The patient's saliva contained H substance but no A or B. It was concluded that the patient's type was O and mixed field polyagglutination had caused the ABO dis-

Table 1

Blood grouping results, F.G.

Red cells	Anti-A	++	(Mixed field)
	Anti-B	±	(Mixed field)
	Anti-A, B	++	(Mixed field)
Serum	A cells	++++	
	B cells	++++	
	O cells	0	
	Auto	0	
Saliva	Secretor of H, not A or B		

crepancy. To determine if T or Tn activation was the cause of the polyagglutination, the patient's red cells were tested with a lectin panel. The cells reacted strongly with *Dolichos biflorus*, *Salvia horminum*, and *Salvia sclarea* but failed to react with *Arachis hypogaea*. As shown in Table 2, the red cells demonstrated the classic pattern of Tn and could be distinguished from other forms of polyagglutination [6].

Table 2

Lectin identification of polyagglutinable red cells

Red cells	Lectin			
	Arachis hypogaea	Dolichos biflorus	Salvia sclarea	Salvia horminum
T-activated	+	0	0	0
Tn-activated	0	+	+	+
Cad-polyagglutination	0	+	0	+
Group O	0	0	0	0
Group A	0	+	0	0
Patient F. G.	0	+	+	+

Further confirmatory tests showed that the red cells failed to react with serum from another case of Tn and that the patient's serum lacked anti-Tn. When the patient's red cells were treated with papain, the Tn activity was eliminated. The patient's red cells were aggregated only weakly by polybrene but were agglutinated strongly by soy bean lectin. The sialic acid level (measured by Dr. Ed Steane) was low, 11.1 $\mu g/10^9$ RBC (normal 13–17 μg) [7]. Beck and co-workers studied this patient's buffy coat and were able to demonstrate greater absorption of lectin activity than by controls, suggesting Tn polyagglutination of buffy coat elements [8].

Development and Biochemistry of Tn

The characteristic features of Tn polyagglutination are thought to result from a mutation of a somatic stem cell since this polyagglutination phenomenon is known to be acquired. A mutation of the pluripotent haematopoietic stem cell occurring at different stages of haematopoietic development may explain the variability in erythrocyte, platelet, or leukocyte polyagglutinability. Recent work by Vainchenker et al. gives strong evidence of the clonal origin of Tn cells [9]. These investigators used cell culture studies to demonstrate that red cells, granulocytes, and platelets which express Tn are derived from a Tn positive clone which undergoes clonal expansion at an unknown stage of development. It was found that the percentage of red cells and platelets in the peripheral blood typing as Tn positive was similar to the percentage of colonies whose cells were uniformly positive for Tn.

The nature of the red cell membrane defect in Tn polyagglutination is now also better understood. Cartron et al. have shown that Tn red cells have a selective deficiency of an enzyme, B-3-D galactosyltransferase, which normally transfers galactose to the Tn structure to then produce T substance [10]. This blockage of galactose transfer due to the deficiency of T transferase results in persistent exposure of Tn antigen with N-acetyl-D-galactosamine as a terminal structure. These facts may also explain the A-like properties of Tn red cells and the lack of M and N antigens on Tn red cells.

Tn and the Leukemic State

A review of 19 published cases of Tn polyagglutination in 1979 included 3 cases associated with myeloid leukaemia [4]. Bird was the first to call attention to this occurrence [3]. A 63 year old male presented with leukemia and the classic features of Tn polyagglutination. Although the paper's title gives the diagnosis of acute myelocytic leukemia (AML), the discussion considers the likelihood that this patient did not have AML. The lymphadenopathy, the inability to aspirate bone marrow, the low leukocyte alkaline phosphatase score, and the elevated vitamin B_{12} level favored the diagnosis of Philadelphia-chromosome-negative chronic myelogenous leukemia (CML). The patient was given cytotoxic chemotherapy and the Tn activation disappeared in 5 months.

Around the time of Bird's report, our patient F. G., previously known to have had Tn polyagglutination for at least 2 years, developed acute myelomonocytic leukaemia [2]. This case was similar to Bird's case with the disappearance of the Tn activity with cytotoxic chemotherapy, giving further support to the description of Tn polyagglutination as persistent, not permanent. There were several differences from Bird's case which expanded the picture of Tn and leukemia. First, our patient's Tn state preceded his leukemia. The leukemic transformation occurred in a patient with pre-existing pancytopenia and hypercellular marrow, a constellation of hematologic findings which commonly precede acute myeloid leukemia and are

called pre-leukemia [11]. The similarities between the pre-leukemic syndrome and Tn polyagglutination suggested a relationship or that perhaps Tn polyagglutination was a specific form of pre-leukemia. The features of what is now called the Tn syndrome are also suggestive of the features of another hematologic disorder, paroxysmal nocturnal hemoglobinuria or PNH [12]. Both disorders are marked by red cell membrane defects resulting from proposed hematopoietic cell mutations, both disorders appear to be clonal, and both feature hemolytic anemia, various cytopenias, and increased likelihood of developing acute leukemia (Table 3).

Table 3

Hematologic syndromes

Features	Tn	Preleukemia	PNH
Acquired nature	+	+	+
Anemia	+	+	+
Hemolysis	+	− − −	+
RBC membrane defects	+	− − −	+
Granulocytopenia	+	+	+
Thrombocytopenia	+	+	+
Leukemic potential	+	+	+

Another important difference in these two cases is morphologic. F. G.'s morphology appeared to be acute myelomonocytic leukemia although his leukemic cells had unusual morphologic features of vacuoles. The problems encountered by Bird in making a firm morphologic diagnosis between AML and CML have already been discussed.

A third case occurring in a 71 year old black male was reported by Baldwin et al. [4]. The patient had had a refractory anemia for two years before presenting with acute myelomonocytic leukemia. His Tn status was identified when leukemia was diagnosed but no immunohematologic investigations had been performed previously. He died before chemotherapy could be instituted.

These three published cases led Dr. Jiji in Baltimore to update his oral presentation at the 1973 American Association of Blood Banks meeting of a patient with pancytopenia and ringed sideroblasts in the marrow along with Tn polyagglutination [5, 13]. The patient was treated with anabolic drugs and pyridoxine with correction of the pancytopenia and almost complete elimination of the clone with Tn polyagglutination. A year later, the patient relapsed with acute myeloid leukemia at which time the Tn polyagglutination returned. The patient died in 1975 at home of bleeding from thrombocytopenia. There are most likely other unpublished cases of Tn and leukemia. A registry of cases of leukemia associated with Tn polyagglutination would be helpful to identify the incidence of this association.

Conclusions

I have reviewed the serologic identification, the biochemical nature, the clonal hematopoietic stem cell origin, and the 4 (or perhaps more) cases of Tn polyagglutination and leukemia. The following conclusions appear to be warranted.

First, among the hematologic abnormalities associated with Tn polyagglutination, we should include hematologic malignancies. We should be careful to avoid limiting this association to cases with acute myelomonocytic leukemia, in view of the difficulties in firmly classifying Bird's case. Perhaps a retrospective morphologic review of these cases by blinded observers could resolve whether this association was limited to AMML or must be broadened.

Second, cytotoxic treatment of several patients with Tn and leukemia has shown that the Tn clone can be ablated, justifying the classification of Tn as persistent but not permanent.

Third, most authors conclude these discussions by stating that any patient or donor with the Tn syndrome merits careful hematologic monitoring. Included in these hematologic observations should be chromosome studies, since certain chromosomal abnormalities are associated with a propensity to develop leukemia [14]. This simplistic conclusion to monitor these patients avoids the real question of what to do with these patients or donors. It could be suggested that a patient with Tn and hematologic findings compatible with a pre-leukemic status could be given cytotoxic therapy. Several pieces of evidence would contradict this suggestion. In 3 of the 4 patients reported, Tn was successfully ablated but leukemia recurred or persisted, once with Tn and twice without a recurrence of Tn. Thus, removal or disappearance of the Tn clone does not mean hematologic cure. Many of the Tn patients have not developed leukemia. Finally, early treatment of pre-leukemia patients has been generally disappointing. The definitive answer of a controlled clinical trial is obviously impossible because of the rarity of these patients. However, the weight of current evidence suggests that cytotoxic chemotherapy would not be indicated.

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Efficacy Control of HB Blood Donor Screening

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If blood donor selection were an efficacious means of preventing posttransfusion hepatitis B, three conditions should be fulfilled: 1. the incidence of the disease should decrease immediately after the exclusion of blood donations that contain presumed infectivity markers; 2. the incidence of the disease should likewise decrease when donor blood is completely replaced by autologous blood; 3. retesting of donors implicated in cases of posttransfusion hepatitis B should regularly detect one or more anti-HBc positive individuals per case.

Since none of these conditions turned out to be fulfilled when tried in the FRG, it remains an open question whether the transfusion-transmitted part of posttransfusion hepatitis B is significant enough to justify laborious donor screening procedures.

Key words: posttransfusion hepatitis, screening for infectivity, transfusion-transmitted hepatitis.

What is the Origin of Posttransfusion Hepatitis?

According to a widely accepted theory, the occurrence of hepatitis in a blood recipient within a certain period after transfusion indicates that at least one of the transfused blood units contained hepatitis agents. This theory was derived from the observation that in blood recipients the incidence of hepatitis is generally higher than in patients without transfusions [1]. However, the elevated hepatitis risk of blood recipients, if analysed in detail, turns out to be an overgeneralization.

In the FRG, a significant statistical association between transfusion and hepatitis could only be established in patients who beside receiving blood transfusions had concomitant surgery [2–4] and in recipients of pooled plasma products [5]. The peculiar problem of pooled plasma products shall not be discussed in this paper.

In recipients with concomitant surgery, two possible pathways of hepatitis agents have to be taken into consideration, namely the administration of blood containing HV and the parenteral exposure to an environment containing HV. The theory of transfusion-transmitted hepatitis stimulated the search for serologic markers which make the infectivity of a donor detectable. In the case of posttransfusion hepatitis B (PTH-B), the hepatitis-Bs-antigen (HBsAg) is regarded as such a marker of infectivity.

What has been Achieved by HBsAg Donor Screening?

If a significant amount of PTH-B had in fact been really transfusion-transmitted, and if HBsAg were indeed a specific infectivity marker and not a mere marker of past HB infection, the elimination of all blood units exhibiting this marker ought to have significantly reduced the incidence of PTH-B.

This appears to have happened when third generation HBsAg donor screening procedures were introduced in the USA. The elimination of Ag positive units was followed by a dramatic reduction of PTH-B [6].

If this reduction of PTH-B following the elimination of all HBsAg positive blood donations were reproducible anywhere, it would give strong empirical support to the assumption that HBsAg testing of blood donors is a useful tool for preventing PTH-B.

Table 1 shows that this reduction by AUSRIA-II-screening of all blood donors could not be reproduced in the FRG, where a similarly significant reduction occurred much later and independently of any special donor selecting procedure.

Table 1

Notified cases of PTH/100 000 blood transfusions

Production period of the blood units involved	Total	HBsAG positive	Positive %
Immediately before RIA-screening	33	27	82
Immediately after RIA-screening	54	46	85
Five years later	27	7	26

Source: Red Cross Blood Centre, Muenster, FRG

Consequently, it remains an open question whether the dramatic reduction of PTH-B in the USA following introduction of the new donor screening was really due to this screening or if it would have occurred anyway.

The observation of hepatitis incidences as such is obviously not an appropriate tool for assessing the efficacy of preventive measures.

In the USA, the assumption of a significant contribution to the prevention of PTH-B by HBsAg donor screening was additionally supported by some previous comparative studies investigating the outcome of HBsAg positive or negative blood transfusions [7, 8]. The conclusiveness of such studies is, however, impaired by the possibility of volatile pseudoassociations, e.g. accidental clustering of HBsAg positive and infective donors.

In the FRG, such comparative studies had already been precluded for ethical reasons when the non-compliance of PTH-B with the HBsAg donor

screening became evident. Therefore we have to envisage the fact that the possible efficacy of this donor screening procedure will remain a mystery forever.

In fact this is the second time that we had encumbered our blood procurement system by an irreversible burden without previous assessment of its clinical efficacy. The first step along this way was the compulsory ALT-screening of all blood donations introduced in 1968.

Undoubtedly, every additional donor screening procedure impaires the general performance of a blood procurement system, which is not completely meaningless because the fear is irrefutable that nowadays 'most bleeding patients who die, die of the blood they did not receive, not from the blood they were given' [9].

Therefore, the experience wilth ALT- and HBsAg-screening, both of them irreversibly established without previous assessment of their clinical efficacy and both without any visible success, should lead to more caution before declaring compulsory all further screening procedures.

What Can be Achieved by Donor Screening Procedures in General?

Basically even the best donor selecting procedure will not prevent hepatitis cases that are not transmitted by transfusion blood. Therefore, we tried to elucidate the maximum transfusion-transmitted part of PTH as a whole. For this purpose four comparative prospective studies were evaluated, two by our European Working Group on PTH [3, 10] and two by independent West German authors [2, 4].

All four studies had in common that blood recipients and control patients of each study were highly comparable and exposed to the same environmental risks of infection at the same theatres within the same periods of time, and were followed for 6 months for biochemical symptoms of hepatitis as well as for HBsAg seroconversion.

Control patients were those, who could be operated upon without blood transfusion or with exclusive administration of autologous blood.

Table 2

Postoperative hepatitis infections in blood recipients and controls

Procedure	No. of patients	Hepatitis or Ag'emia	Per cent
Surgery employing donor blood (> 1000 units)	465	30	6.4
Surgery without donor blood	200	14	7.0

Sources: Copenhagen, Amts Sygehus, 10; Erlangen, University Clinic, 2; Essen, University Clinic, 3; West Berlin, University Clinic 4

Patients who had HB markers or hepatitis symptoms before surgery were not considered eligible for the studies.

As it appears from Table 2, these studies gave no evidence of a nigher specific risk of the blood recipients. This general result applies to each respective study so that they could be summarized without biasing one of them. No other study known to me appearing to demonstrate a hepatitis risk of blood recipients exceeding that of control patients, does disclose how the adequacy of the control patients was established.

But if omission of donor blood transfusion does not visibly reduce the incidence of postoperative hepatitis, neither will any imaginable panel of donor screening procedures do so.

What Can be Achieved by Anti-HBc Donor Screening in Particular?

Nevertheless, a new candidate for further additional donor screening regulations is already advocated, the HB marker anti-HBc. It is said to be the most stable [11] and the most reliable [12] marker of HB infection. Moreover, it is expected to indicate not only HB but also HNANB infectivity [13].

If it is true that HB infection is regularly accompanied by development of anti-HBc, any blood donor who transmits HB to a recipient will carry anti-HBc at least when the hepatitis of the recipient becomes apparent, even if the antibody was not yet demonstrable at the time of the blood donation in question.

And if it is also true that PTH-B is regularly transmitted by the transfused blood, one should regularly find at least one anti-HBc carrying donor per case of PTH-B.

We have tested all implicated donors of 21 notified cases of PTH-B for anti-HBc by CORAB™ (Table 3). The donors of 8 additional cases were tested by the University Transfusion Centre, Tuebingen [14]. In 24 of 29 cases (83 per cent) no donor testing positive for anti-HBc was detected. The frequency of anti-

Table 3

Implication of donors with anti-HBc in cases PTH-B

Country	Cases with anti-HBc positive donor(s)	Cases without anti-HBc positive donor	Total
FRG	5 (17%)	24	29
USA	11 (73%)	4	15

$CH^2 = 13.44$ 1 D. F. $P < 0.0005$

Sources: Red Cross Blood Centre, Muenster, FRG; Tuebingen, University Clinic 14; USA, TTV Study, 16

HBc-carriers among all implicated donors tested was 6 of 132 or 4.5 per cent, thus it did not exceed the rate of anti-HBc carriers in the average West German donor population.

According to recent findings of a French research group, 11 of 13 patients with anti-HBc had no detectable HBV DNA in their liver cells while 3 of 8 chronic HBV carriers had no serologic HB markers [15].

Our retrospective studies do not encourage anti-HBc donor-screening because the PTH-B patients did not receive more anti-HBc containing blood units than recipients in general, and the results of the French group do not even suggest that there exists a statistical correlation between anti-HBc and HBV carriership. Furthermore the correlation between carriership and infectivity may be still another question.

Conclusions

The non-compliance of PTH-B with HBsAg donor screening in the FRG, the identical postoperative hepatitis incidence of recipients and adequately selected controls and the apparent non-correlation of anti-HBc carriership and HBV infectivity neither refute nor corroborate that a certain minority of PTH-B or PTH-NANB might indeed be transmitted by infected donor blood.

The possibly transfusion-transmitted hepatitis cases are, however, regularly concealed by a preponderant number of not transfusion-transmitted hepatitis cases of blood recipients. This makes a conclusive efficacy control of donor selecting procedures practically impossible.

Once the regulation is compulsory, every supposedly preventive measure becomes definitely inaccessible to efficacy control.

This was the case with ALT and HBsAg donor screening in the FRG.

Since the avoidance of donor blood transfusion did not significantly reduce the incidence of postoperative hepatitis, no such reduction by further donor selecting procedures can realistically be expected.

Thus, the introduction at random of one screening test after the other may well prolong the way of blood from the donor to the recipient and perhaps accentuate blood shortages as well as expenses. Whether these drawbacks will be compensated by visible gains in hepatitis prevention can be doubted with good reason.

As far as the third generation donor screening for HBsAg is concerned, we have absolutely no evidence beyond theoretical considerations that it contributed to the obvious reduction of PTH-B in the FRG.

These experiences remind us again that a theory is nothing more than a subjective picture of reality, not reality in itself, and that the strength of our subjective belief in the correctness of a theory is not always a reliable indicator of this correctness.

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New Antisickling Agents

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Sickle cell anemia has variable clinical expression, ranging from benign cases to very severe ones. This situation suggest that, unless a perfect drug is identified, the therapeutic approaches may also vary. Great progress has been made in this last decade in the understanding of the Hb S polymer structure, the kinetics of polymerization and some of the cellular contributions to the disease. This progress has suggested that we could alter the course of the disease by impairing the polymerization of Hb S through three mechanisms: a) interfering in the areas of contacts involved in polymer formation by covalent and non-covalent agents, b) changing the oxygen affinity of Hb S to decrease the changes induced by deoxygenation, c) diluting the Hb S by increasing the MCV of the cells and consequently decreasing its MCHC. This last result is achieved by a modification of the permeability of the red cell membrane.

Keywords: sickle cell anemia, new antisickling agents, Hb S

*

At present, the therapy of sickle cell disease remains a non-specific one. It includes on the one hand a symptomatic treatment of the anemia and the thromboembolic crises, on the other hand a development of hygiene and prophylaxis which by itself may explain the differences of the disease in underdeveloped and industrialized countries. Together with this physiological approach, since more than twenty years the progressive understanding of the molecular pathology of the disease has been an incentive to research of any agent which could specifically inhibit the polymerization of hemoglobin S and the subsequent sickling of the red cell. Most, if not all, of the drugs which have been and still are studied are in fact models of a possible pharmacological action, and the obtained results may be milestones to direct further research.

Among the investigated compounds, some have indeed to be used in vitro at concentrations never reached in vivo. Some are highly toxic, some others do not enter the cell or are active only when not metabolised. They obviously cannot be used clinically as such. In limited cases, as for example cyanate or nitrogen mustards, they have been used for extracorporeal treatment of the red cells which are then reinjected in the patient's circulation. This can even be a temporary treatment when applied to a few selected cases in rich countries. It is clear that it has no practical value for mass therapy, especially in Subsaharan Africa. Nevertheless in these

areas a treatment is badly needed, since the demographic and economic conditions of the major cities increase dramatically the number of homozygotes.

The pathophysiology of sickle cell disease is now well understood, and an efficient therapy could theoretically act at various levels of the vicious circle responsible for the sickling of cells and vasoocclusive crises.

The final goal of any drug remains to inhibit the polymerization of hemoglobin S, acting either on this polymerization itself or on one of the factors responsible for it, namely the concentration of hemoglobin and the degree of deoxygenation. Figure 1 recalls the various natural causes acting on one of these points. It is well known that the contacts of the hemoglobin S polymer are modified by the presence of other hemoglobins, normal hemoglobin F and hemoglobin A_2 or abnormal hemoglobins. The oxygen affinity curve may be shifted by variations in the pH, the temperature, or the anion concentration. And finally, the intraerythrocytic concentration of hemoglobin is partially under the control of the membrane status and the osmolality. The three arrows indicate in a simplified way where the potential therapeutic drugs could act. (For more details see refs [1] and [2]).

1. The drugs disrupting the polymer contacts are all chaotropic agents. They include urea, which was first proposed, and alkylureas which cannot reach in vivo the required concentrations. Aromatic aminoacids and short peptides act well on hemoglobin solutions, but their entrance into the cell is limited. Solvents, detergents, gases are of no therapeutic use.

2. Another large group would involve different products modifying the oxygen affinity of hemoglobin S, and therefore decreasing the proportion of deoxyhemoglobin S. The most thoroughly studied among these drugs was cyanate which has been tried also in vivo. Its toxicity is due to that, despite a preferential binding to hemoglobin, it binds unspecifically to all the proteins.

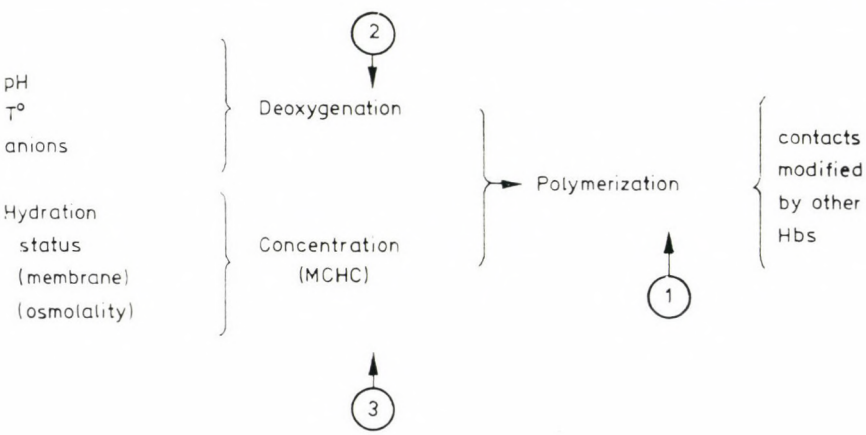

Fig. 1. Possible impact of drugs delaying Hb S polymerization

Among the other compounds, a particular interest is devoted to those which bind to cystein 93, the first model having been cystamine. One has also to mention nitrogen mustards which cross-link with histidine 2 and other bifunctional reagents. The modifying action of the latter compounds is in fact more complex. All the products listed in this second group nevertheless raise the question how much one can increase the oxygen affinity of blood without impairing oxygen delivery to the tissues.

3. Finally, the third group acts only in an indirect way on hemoglobin S polymerization, the primary action being at the membrane level. This group is highly heterogeneous, including drugs as different as zinc, local anesthetics, antiarrhythmic agents, antibiotics, tranquillizers, platelet antiaggregants, etc. Their proposed mechanism of action is by membrane expansion, which has been proved experimentally [3]. Among this wide spectrum of agents, some have pharmacological properties which do not allow any in vivo long-term treatment, or present major side-effects. In contrast, some of them have already been used for other disturbances, and are not obviously contra-indicated in sickle-cell disease. This explains the wide research in this field in the last years.

The fact itself that the proposed drugs are so numerous and have such varying secondary effects proves already that none is outstanding. A practical improvement could perhaps be the combined use of several drugs acting by various pharmacological mechanisms.

A supplementary problem is raised by the difficulty in interpreting the observed results in vivo, this being due to the high clinical polymorphism of the disease, and to the fact that most of the criteria used are not objective such as how to evaluate pain or what is to be called a crisis, etc.

It is also known that the clinical course of the disease may be influenced by a number of factors. Some of these are external to the disease itself, namely those which are related to climatic or ecological conditions, or to the socio-economic status of the patients. The disease is not the same in African Sahelian countries and in the industrialized Western world. In a survey performed recently in villages of Upper Volta, we found 15 per cent heterozygotes, but among more than 1000 subjects there was only one homozygote, and he was less than 5 years old. Due to malnutrition, the lack of medical care, or the very hot and dry climate, the homozygotes do not survive. In African larger cities, where some drugs could be assayed, some improvement of the clinical status may be observed simply by more attention and a better nursing, this being often observed when the efficacy of a new compound is assayed.

It has also been shown that the association of other genetical defects may be beneficial to sickle cell disease. This is now well established for the minor form of α-thalassemia, namely only one α gene expressed per chromosome 16 [4]. It could be true also for the heterocellular form of HPFH. And there are surely other still unknown causes.

This only emphasizes the urgent need for antisickling drugs and the major difficulty in interpreting their potential results.

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Supply and Need of Factor VIII Concentrates

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Demand for factor VIII varies greatly and exceeds supply in many places. Logical analysis of this difficult situation has been undertaken by observing actual usage, calculating potential need, and analysing factors which limit or influence production and distribution. Usage exceeds production potential in some places. It is concluded that factor VIII production/usage in the range of 20 000–60 000 IU per hemophilia A patient per year is both feasible and adequate. Production/use below 10 000 IU is not adequate. Production above 60 000 IU is not practicable using current technology.

Keywords: albumin, blood transfusion, factor VIII, hemophilia, plasma fractionation, supply

Introduction

Hemophilia treatment has made great advances in the last decade. The principal reason for the progress was the increasing availability of the concentrated clotting factor preparations required by hemophiliacs for the control of bleeding.

Clinical advances have created a demand for antihemophilic products which exceeds the supply in many countries. This situation presents interesting challenges to the organizations responsible for the provision of the plasma products required by hemophilic patients. This statement applies equally to those national, regional and local blood transfusion agencies responsible for the preparation of cryoprecipitate and the provision of recovered plasma for fractionation, and to the various organizations who prepare plasma fractions on an industrial scale. The recognition that there are problems in this area has led the International Society of Blood Transfusion (ISBT) [1], League of Red Cross Societies (LoRCS) [2], World Health Organization (WHO) [3], Council of Europe [4], International Federation of Pharmaceutical Manufacturers Associations (IFPMA) [5] and the World Federation of Hemophilia (WFH) [6] to pay special attention to the issues involved in assuring the availability of factor VIII preparations. At the national level, studies of this problem are known to be active in England, Scotland, Sweden, Denmark, the Netherlands, Germany (Federal Republic), France and Canada.

WFH has appointed a Task Force, comprising the five authors of this paper, to attend to the question of worldwide availability of plasma products for hemophilia treatment. Some preliminary thinking was presented in 1981 [7].

Table 1

Factor VIII problems

Users	
	Inadequate supplies in many countries
	Disagreement about dosage need
	High prices in Europe and many developing countries
Providers	
	National blood transfusion services
	difficulty meeting demand
	Industry
	factor VIII need overtaking albumin demand
	low prices in USA

Haemophilia is a generic term encompassing two biochemically different but clinically similar disorders (Factor VIII and Factor IX deficiency). Factor VIII deficiency (hemophilia A) accounts for about 80 per cent of the affected patients and is responsible for all the significant product supply difficulties (Table 1). Factor VIII alone is the focus of this analysis, designed to reduce a conflict, which has become politicized, to a level of scientific logic which will permit rational decision-making.

Methods

The authors have considered available information on production, usage and need of factor VIII and have analysed it in order to identify problems and potential solutions. Issues are considered from the standpoint of both the clinician and the producer of transfusible products. These issues are complex technically, logistically, ethically and geographically; oversimplification has, of necessity, been utilized to aid understanding. Some definitions are offered (Appendix I) to explain the intended meaning of certain key words that might otherwise be ambiguous.

Observations and Discussion

The size of the clinical problem is definable by the number of patients and the amount of factor VIII needed to treat each one. This calculation has been attempted in detail by Cash [8] in his projection of Scottish factor VIII needs.

The number of patients with hemophilia A is remarkably constant around the world, approximately 50 cases per million population. If mildly affected patients are included, the number will be higher; this observation is not consequential when considering product usage since mild cases require little treatment. The assumption of 50 cases per million population is utilized throughout this discussion.

Table 2

Factor VIII usage by country (units/patient/year)

High dosage	
Germany (Fed. Rep.)**	80 000
USA (Treatment centers) [10]	55 000
Belgium*	51 000
the Netherlands*	50 000
USA*	36 000
Moderate dosage	
UK*	22 000
Spain*	19 000
Sweden*	18 000
Austria*	16 000
Finland*	15 000

* See Ref. [5]

** There are no realiable published data for the Federal Republic of Germany. The authors' best estimate, based upon multiple fragments of data, puts total annual factor VIII usage at 200 million units, equivalent to 80 000 units/patient. Henning (personal communication, April, 1981) believes that total usage is 300 million units (Table 12)

The amount of factor VIII needed to treat each hemophilic patient is not constant. There are some remarkable differences from country to country, illustrated in Table 2 and also in the report of the WFH Home Care Committee [9]. Comparable differences have been reported by Levine within the United States [10].

Table 3

Philosophies of hemophilia treatment

	Factor VIII need* units/year/patient
1. Prevent bleeding at all costs	547 500
2. Prevent all severe bleeding	91 250
3. Reduce bleeding by conservative maintenance	51 000
4. Control all bleeding episodes	36 750
5. Treat all bleeding with conservative doses	16 250
6. Control all severe bleeding	13 500
7. Treat all severe bleeding, conserving supplies	7 000
8. Treat only life-threatening bleeding	5 000
9. Minimize treatment, because of risks	2 000
10. No treatment	—

* These numbers differ somewhat from those originally published [7]. The basis for these calculations is lengthy and complex; it is available from Dr. Britten upon request

Analysis of this phenomenon leads to the conclusion that usage is influenced strongly by the philosophy (therapeutic intent) of the clinician (Table 3). Some arithmetical relationships, helpful in interpreting Table 3 and other data, are set out in Appendix II.

Ten different philosophies are considered in Table 3. Each one has a logic which may seem reasonable. Yet the amounts of plasma products required to implement these philosophies vary enormously. Analysis of likely outcomes reveals that philosophies 7–10 will not achieve effective treatment; they will lead to the well-known and severe physical and social disabilities so predictable when treatment is unavailable. These options are therefore not acceptable and must be eliminated from consideration.

Superficial evaluation of philosophies 1–6 could lead to the conclusion that the best clinical results can be expected with philosophy 1. There is, however, a need to consider the factors which limit supplies of factor VIII.

The economic realities of plasma fractionation require producers to make more than one marketable product from each batch of plasma. Production of factor VIII concentrates is limited by the demand/need for albumin. Table 4 illustrates this linear relationship, assuming the production of 25 g albumin and 200 units factor VIII from each liter of fresh frozen plasma fractionated. Factor VIII production is favorably influenced by higher production yields (i.e. > 200 units/liter plasma) and adversely affected by fractionation of source materials depleted of factor VIII.

The critical nature of this factor VIII: albumin ratio becomes clear when considering albumin usage by country (Table 5). Only those few countries which experience a high demand for albumin products are able to cause much factor VIII concentrate to be produced[+] (e.g. Switzerland, Japan, USA, Belgium, Germany

Table 4

Relationship of albumin to factor VIII production*

Liters plasma	Albumin production, kg	Factor VIII production, units $\times 10^6$
4 000	100	0.8
8 000	200	1.6
12 000	300	2.4
16 000	400	3.2

* Assumption: 1 liter fresh frozen plasma generates 25 g albumin and 200 units factor VIII

[+] It is noteworthy that production does not need to be in the same country. Indirectly, high albumin usage in Japan may help sustain enormous factor VIII usage in the FRG, through American production of both products.

Table 5

Albumin usage by country*

Country	Albumin usage (kg/million pop.)
FRG	555
Switzerland	485
USA	296
Sweden	117
UK	45
Brazil	14

* These data were developed from those of IFPMA [5]; this list is incomplete, being selected to demonstrate the wide difference between countries

(Federal Republic)). The difficulty faced by other countries in meeting factor VIII demands becomes apparent.

Factor VIII, in the form of cryoprecipitate, is limited by whole blood needed for transfusion. In most medically developed communities this limitation is based on a need of around 50 000 whole blood donations per million population per year. A further constraint arises in that logistic realities and conflicting service demands decree that cryoprecipitate cannot be prepared from every donated unit (Tables 6 and 7).

Table 6

Factor VIII production limits

Source of product	Factor VIII prod. ($u \times 10^6/10^6$ population/year)				Equivalent u/patient/year
	Fractionation*	Cryoprecipitate	Total	Realistic maximum	
Fractionation	0.8	—	0.8	0.8	16 000
Cryoprecipitate	—	5.0	5.0	3.0	60 000
Combination	0.8	5.0	5.8	3.8	76 000
Realistic Comb.	0.8	2.0	2.8	2.8	56 000

* Assuming albumin use 100 kg/million population/year

Analysis of production potential reveals that a combination of fractionation and cryoprecipitate production leads to optimal factor VIII production only if albumin usage is high; otherwise almost total reliance upon cryoprecipitate (Table 6) must be weighed against dependence upon imported concentrates.

Table 7

Factor VIII production limits

Source of product	Factor VIII Prod. ($u \times 10^6/10^6$ population/year)				Equivalent u/patient/year
	Fractionation*	Cryoprecipitate	Total	Realistic maximum	
Fractionation	3.2	—	3.2	3.2	64 000
Cryoprecipitate	—	5.0	5.0	3.0	60 000
Combination	3.2	5.0	8.2	6.2	124 000
Realistic Comb.	3.2	1.0	4.2	4.2	84 000

* Assuming albumin use 400 kg/million population/year

Plasma that is not fresh frozen is a suitable source of albumin but cannot be effectively utilized for factor VIII production. This relationship is particularly important when the plasma for fractionation is derived from whole blood donation (Table 8); every unit that is not useful for factor VIII reduces the overall production of factor VIII and thus reduces the treatment of hemophilia. Examples of how unsuitable practices may depress factor VIII production are

1. Use of plasma, not fresh frozen, for fractionation
2. Use of placentas as a source of albumin
3. Fractionation for albumin in facilities not producing factor VIII
4. Use of low-yield technology for factor VIII production.

Review of the limitations to factor VIII production demonstrates clearly that philosophies 1, 2 (Table 3) are impractical. There is no way to provide the amounts of plasma products required for general implementation of these philosophies. Reasonable discussion must be limited to philosophies 3–6. Within this range, some countries are clustered in the higher dosage range while others are more conserva-

Table 8

Effect of fractionating 'not fresh' plasma

Fraction of 'not fresh' plasma (units/million population)	Factor VIII production ($u \times 10^6$/year)	
	Albumin production 100 kg/10^6	Albumin production 400 kg/10^6
0	0.8	3.2
10 000	0.4	2.8
20 000	—	2.4
30 000	—	2.0
40 000	—	1.6
50 000	—	1.2

tive (Table 2). The explanation of this difference is that annual production of 10 000–30 000 units per patient is relatively easy in countries with fully developed blood transfusion services. Production in the higher range (> 30 000 units/patient, equivalent to philosophies 3, 4) is possible but difficult, and is being attempted in a few countries only.

Allain [11] approaches the factor VIII usage question from the standpoint of dosage used in the treatment of individual bleeding episodes. His data were used in developing Table 9. Clinical benefit results from higher doses but the gains diminish as dosage is increased, particularly above 15 units/kg. These data can be approximately related to annual usage per patient. Allain's recommendation of at least 26 units per kg, a high-dosage variant of philosophy 4, would imply usage at the rate of more than 60 000 units per patient. With the solitary exception of the commercial fractionation industry in the United States, no country has been able to achieve production at such a level.

Table 9

Effect of dosage upon therapeutic success rate in treatment of hemophilia A

Therapeutic success rate, %	Factor VIII dosage, u/kg	Approximate factor VIII usage, u/patient/year
90	15	36 750
95	20	49 000
99	31	75 950

One must strive for clinically normal patients so a case can be made for using the highest dose possible. The logical conclusion from this discussion is that, while philosophies 5, 6 may be acceptable if supplies are necessarily limited, philosophies 3 or 4 are desirable (Table 10).

If one assumes a worldwide need of 20 000 units per patient, the supply of factor VIII concentrates is inadequate (Table 11). The actual usage pattern is

Table 10

Applicability of different philosophies

Philosophy (from Table 3)	Applicability
1– 2	Impossible
3– 4	Desirable
5– 6	Acceptable
7–10	Not acceptable

uneven, being heavily concentrated in North America and parts of Western Europe (Table 12). Clearly much progress is needed in the development of more general availability. It is necessary to consider ways to increase production and reduce wastage.

There is a growing demand for high purity concentrates because they are convenient and reliable. Unfortunately, as purification increases, the yield is reduced; some factor VIII is lost at each purification step. There is a need to emphasize more economical products, even though their purity is less.

The transfusion of fresh frozen plasma introduces another problem. Plasma transfusion in the United States has increased threefold in 5 years. If this plasma,

Table 11

World supply/need for factor VIII

Population	4 500 000 000
Hemophilia A cases	225 000
Need/case	20 000 u/year
Total factor VIII need	4 500 000 000 u/year
Supply*	±900 000 000 u/year

Supply is ±20% of need

* Calculated from multiple sources of soft data; the assumptions are that 5 million litres of fresh frozen or source plasma were fractionated in 1980 at an average yield of 180 units/litre. These figures do not include single-donor or small pool cryoprecipitate; only fractionation products are considered (Table 12)

Table 12

Factor VIII concentrate usage, 1980 (in millions of units)

USA	400[a]	
FRG	200[b]	(much from USA)
Canada [12]	30	(mostly from USA)
Japan [13]	30	(mostly from USA)
UK [14]	20	(mostly from outside UK)
Rest of world	220[c]	
Total	900[d]	

[a] There are no reliable data. Industry spokesmen estimate usage at 400 million units. The authors's best estimate, based on projections of regional data, is close to that figure. Projection of usage at major treatment centers [10] would put usage at ±720 million units

[b] See Table 2

[c] Calculation is based on subtracting usage for major consuming countries [12–14] from the estimated total production worldwide

[d] See Table 11

now representing nearly 20 per cent of the blood resource, is transfused in the fresh frozen state, factor VIII that could be available for hemophilia treatment is given to patients who do not need it. This wastage is now equivalent, in cryoprecipitate, to 200 million factor VIII units annually in the United States alone.

Table 13 can serve as a basis for considering plasma management in a blood transfusion service. These data are hypothetical, based upon a population of one million from which 50 000 blood donations are collected each year. Table 13 illustrates an undesirable production pattern which is commonly encountered. Each

Table 13

Plasma usage per million population (hypothetical)

Blood donations	50 000			
Plasma available	50 000			
Plasma usage				
Whole blood transfused		20 000*		
Plasma transfused		10 000*		
Plasma fractionated			F. VIII, u	Albumin, kg
Fresh frozen		5 000	200 000	25
Not fresh frozen		5 000	—	25
Cryoprecipitate depleted		10 000	—	50
Cryoprecipitate		10 000	1 000 000	—
Total			1 200 000	100

* Additional cryoprecipitate may be derived (maximum 3 million units)

Table 14

Plasma production scheme in Groningen–Drenthe Region

Blood donations	50 000		
Plasma available	50 000		
Resulting products			
Modifiedwhole blood	15 000		
Plasma for transfusion	10 000		
Plasma for fractionation		F VIII, u	Albumin, kg
Fresh frozen	25 000	1 000 000	125
Not fresh frozen	—	—	—
Cryoprecipitate depleted	—	—	—
Cryoprecipitate	15 000	1 500 000	—
	Total	2 500 000	125

blood donation contains a unit of plasma; each represents one plasma decision. Accounting for this plasma will determine how much hemophilia treatment is possible. The maximum factor VIII yield will result from maximizing cryoprecipitate production and fractionation of fresh frozen plasma. Transfusion of whole blood or plasma, and fractionation of plasma not containing factor VIII, all serve to reduce factor VIII production. Table 14 shows Smit Sibinga's actual production capability in the Groningen–Drenthe region in the Netherlands, much more effective production.

These various observations serve to expose a difficult situation. There is a growing demand and a definable need for factor VIII. Yet there are absolute limitations to the amount that can be produced with current technology. Compromises are necessary in developing a practical policy.

Conclusions

A few specific suggestions are offered to help rationalize the demand for factor VIII and its production. *Clinicians* must be willing to use frozen cryoprecipitate, particularly in a hospital setting. Conserving supplies must always be an important consideration. *Blood transfusion services* must accept the need to maximize factor VIII production including at least some high purity concentrates for home treatment and for patients prone to allergic reactions to cryoprecipitate. Fresh frozen plasma must never be transfused without specific indication, since this wastes factor VIII; factor VIII-depleted plasma is generally a satisfactory substitute. All plasma for fractionation should be fresh frozen since other plasma generates albumin only, thus inhibiting factor VIII production. *Clinicians, hemophilia organizations, blood transfusion services and fractionators* need to work together to coordinate their planning. Only in this way can demand and production potential be rationally linked.

Appendix I

Definitions

Cryoprecipitate	Factor VIII prepared by cryoprecipitation, frozen or lyophilized, single-donor, small-pool, or large-pool.
Concentrates	Lyophilized factor VIII preparations resulting from cryoprecipitation plus further purification steps.
Plasma fractions	Plasma derivatives prepared from large-volume multidonor plasma pools. Examples are albumin, immunoglobulins, factor VIII concentrates, factor IX complex, etc.
Hemophilia A	Congenital deficiency of factor VIII coagulant activity (VIII:C)

Source plasma	Plasma, obtained by *plasmapheresis* and intended for further manufacturing purposes (e.g. fractionation).
Recovered plasma	Plasma, obtained from *whole blood donation* and intended for further manufacturing purposes (e.g. fractionation).
Supply	Equivalent to effective production plus imports minus exports.
Demand	The amount that is *wanted.*
Need	The amount that is needed to achieve a stated clinical objective.
Use (usage)	The amount that is actually used (equal to 'effective demand')

Appendix II

1. *1 blood donation*
   = 450 ml (± 10%) whole blood
   ≡ ± 225 ml plasma
   ≡ 100 units factor VIII as cryoprecipitate (yield 40–50 per cent)
2. *1 liter plasma*
   = 25 g albumin
   ≡ 200 (± 100) units factor VIII as concentrate (yield 10–30 per cent)
3. *Whole blood collection/million population/year*
   = 50 000 donations/year
   ≡ 11 250 liters plasma for fractionation (maximum)
   ≡ 8000 liters plasma – a realistic goal
   ≡ 200 kg albumin
   ≡ 1.6 (± 0.8) million units factor VIII as concentrate
   ≡ 32 000 units factor VIII/patient
4. *Albumin*
   Albumin needs up to 200 kg/million population/year can be met from 'normal' whole blood donations
   Additional albumin can be obtained only by one or more of the following
   1. Plasmapheresis
   2. Overcollection of whole blood
   3. Importation
5. *Albumin and factor VIII*
   4000 liters fresh frozen plasma
   ≡ 100 kg albumin, and 0.8 million units factor VIII
   ≡ 16 000 units factor VIII/patient
   16 000 liters fresh frozen plasma
   ≡ 400 kg albumin, and 3.2 million units factor VIII
   ≡ 64 000 units factor VIII/patient

6. *Hemophilia treatment needs*
   35 hemorrhages/year
   10 severe hemorrhages/year
   15 units factor VIII/kg body weight
   ≡ factor VIII increase of 30 per cent (0.3 u/ml), (controls bleeding ± 90 per cent of the time (Table 9)

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Open Forum on Liability and Reality in Haemotherapy

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Introduction

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Up to present times, transfusional matters had been viewed under clinical, and still more recently, legislative aspects.

Legislation or sets of rules have been designed in numerous countries. The WHO, the League of Red Cross Societies, the European Council and the ISBT have supported that some principles be the basis for this legislation in order to assure transfusions with the greatest possible security, both for the donor and for the receiver.

According to general opinion, everything about transfusion must be kept under the responsibility of Public Health Services. These are responsible for the correct therapeutical use of blood and blood derivatives among the population on both the national and the international scene.

We must not forget, however, that this ethical and clinical responsibility goes along with liability. According to present day life conditions and considering the possibility to process blood derivatives in some countries whereas the raw material comes from another country, we should not consider the liability of Public Health Departments in the restricted frame of one single country, be it producer or user.

This means mutual exchanges between different countries which implies enlarged clinical, technical and juridical controls.

The processing and the use of high quantities of stable derivatives make them a kind of industrial product. Therefore the liability of Public Health Services is great and must take into account not only the criteria previously defined but also economical ones.

There is a kind of supranational responsibility that must aim at distributing among countries with poor productive levels the surplus from other parts of the world.

The responsibility of Public Health Services is assumed in the fields of
research
promotion of routine care
quality control
respect of basic ethical and juridical principles
greatest possible security both for the donor and for the receiver.

This concerns any therapeutical product of human origin in general, and blood in particular: fresh derivatives, on the one hand, and stable derivatives on the other.

Fresh derivatives: the impossible storage except at very high expense and transport difficulties make it hard to process them on a large scale. Obviously all recommendations and ethical rules designed deal particularly with these fresh products that are going to be distributed within a short time. These recommendations must also apply to stable blood derivatives. This requires an industry-like organization with specific controls.

These controls should not overshadow well-known basic ethical principles like volunteering and goodwill.

Experience shows that only under the control of Public Health Services can these ethical principles be taken into account. That is why if private initiative can give good results, it must remain under government control. Governments should therefore choose the most useful way but should also keep it under adequate control.

International relationships are developing considerably and they prompt us to uniform sets of controls and legislation. They are still more necessary, since numerous illnesses are blood transmitted. Moreover, some of them spread in certain countries more than in others. So we have to define an international standard of control.

It is also impossible to equate blood, its fresh and stable derivatives, with drugs, even though their conditioning makes them similar. The supervision must be of a different type. Responsibility rests with Public Health Departments, not only for derivatives produced in their own country but also for those imported from foreign countries.

The responsibility of Public Health Services also covers the choice of analyses to be done in these controls. For economical and practical reasons, we cannot run all the analyses available nowadays. Unfrequent illnesses do not require systematic control of all donors, but liability arises if we do not do so.

A choice must be made in order not to increase too much the expenses of Public Health Departments, which would make it impossible to collect and distribute blood.

All these considerations have led us therefore to reanalyze the responsibility of the Public Health Departments with the following guidelines:

1. Necessity to provide people in each and every country with the fresh and stable blood derivatives they need.

2. Necessity to organize exchanges to distribute surplus production and to organize more efficiently the collection of raw material.

3. Necessity to make it clear that blood and blood derivatives are therapeutical products different from those meant under 'drugs'.

4. To control and encourage industrial processing without rejecting the help provided by non-profit industries.

5. To determine for all countries, respecting their local customs, a uniform

set of criteria with a minimum and maximum, but with the highest possible security for transfusion.

6. These considerations make it clear that Public Health Services must accept the moral responsibility connected with blood and blood derivative delivery.

The need for maximum security in transfusional matters both for the donor and for the receiver, implies juridical liability that will have to be defined in detail and that Public Health Services must be aware of.

This Round Table was conceived in order to give a maximum of information in relation to the evolution in transfusion as it must be conceived on a national and international level, keeping in mind the socio-economical, clinical, juridical and ethical factors.

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International Juridical Aspects of Transfusion

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The Council of Europe has been active in the blood and immunohaematology fields for many years. Since 1962, in fact, the Council of Europe's Committee of Experts on Blood Transfusion and Immunohaematology has contributed much both to the free movement of blood supplies between the Council's member states and to the standardization of blood products [1, 2]. Four important aspects of blood transfusion have received full attention: the organization of blood collection, storage, distribution and transfusion services, staff training, recruitment of donors, and laboratory techniques including quality control. It would be inappropriate to go into great detail concerning over 20 years of work which has resulted in over 15 sets of recommendations to member states and some three agreements (ratifiable instruments). I should prefer on this occasion to describe in some detail our present concern as they affect blood transfusion services in Europe.

Guidelines on the Organization of Blood Transfusion and the Protection of Donors and Recipients

We hope to begin in 1984 the preparation of an innovatory legal instrument to define the responsibilities of national public administrations in the field of blood transfusion. Initially it would be appropriate to set out these guidelines within a Recommendation to be addressed by the Council of Europe's Committee of Ministers to its member states. It is, however, envisaged that this way of suggesting future solutions to current problems could lead to a greater commitment on the part of some at least of the member states, that is, to a ratifiable instrument (an agreement or convention) which would be binding to the Contracting Parties.

The reason for this initiative lies in the existence of developments of which you are all aware in the field of blood transfusion. There are now many new techniques which can expose donors and recipients to greater hazards than has previously been the case. The trend is also towards greater responsibility being taken by the State itself or by the public health administrations. It has sometimes

been observed that where the organization of blood transfusion services has been left to private bodies, there appears to be less efficient supervision of donors and greater incidence of diseases communicable by blood or blood products, diseases which are only detectable by using exceptionally costly techniques. We in the Council of Europe have sought to rationalize the tendency for public health administrations to take greater responsibility for the health of all those within the state's jurisdiction and, to this end, we look very closely at the implications for legislation bound up in new developments in the field of blood transfusion.

To give you an idea of how this general approach is working in practice, I should mention a report drawn up by Professor André in collaboration with Professor Hässig, Dr. Gullbring, Dr. Gunson and Dr. Hantchef for the Committee of Experts' meeting in Ottawa in May of this year, which lists the following recent developments as needing the Committee's close attention:

1. new methods for taking blood, which impose greater constraints and in which the donors are increasingly involved, notably apheresis, plasmapheresis, etc., safety standards for the donor need to be drawn up and enforced;

2. infectious diseases: there may be changes as a result of frequent travel not only by donors but also by recipients;

3. the increasingly frequent exchanges of blood products, particularly stable derivatives;

4. the involvement of the private pharmaceutical industry in the production of stable derivatives and the tendency to consider them medicinal products and forget their human origin;

5. the need for the health authorities to monitor the manufacture of blood derivatives and for controls if they are imported or exported;

6. the need to forecast overall national requirements in blood and its derivatives in order to ensure that optimum use is made of them; this entails stepping up exchanges between countries;

7. the need to involve the doctor who takes the blood and its derivatives and prepares and distributes it in the diagnosis and in the choice of the appropriate blood product;

8. the need to use new or improved diagnostic techniques;

9. the need to monitor laboratory techniques used for taking and preparing blood and its derivatives;

10. the importance of socio-economic factors which necessitate rationalization of monitoring, collection, storage and distribution techniques;

11. the training of specialist staff.

The Council of Europe is an organization whose ethical stance – especially enshrined in the European Human Rights Convention – promotes the dignity of man and gives a special value to everyone's autonomy. It is therefore commonly felt within our member states that the commercialization of blood collection and the subsequent sale of blood and blood products are not compatible with the principle that the human body should not be, whether as a whole or in part, the subject of trade or barter. We therefore are extremely reluctant to

accept that legislations should allow blood or its derivatives collected from voluntary donors to be used for commercial or private gain.

In this context it is especially important to evolve a legal distinction between profits made for private gain and profits (perhaps made by a body set up in the public interest) used to promote blood transfusion.

A second important question has arisen over the basic principle on which blood transfusion systems usually work: we ask for volunteers to give blood and their benevolence is reflected in benefits accruing on a national and international scale. But is it acceptable that the health of the population at large should depend on such a small minority of the population?

Both these questions were dealt with through the efforts of the Committee of Experts on Blood Transfusion and Immunohaematology and the Reykjavík Rules were drawn up in 1975. These Rules have served as a basis for legislation in several European countries. I suspect, too, that they have also inspired the ISBT's own Code of Ethics and that of the Red Cross, and they have been published by the WHO. The Council of Europe's own agreements and protocols in this field concerning the exchange of therapeutic substances of human origin, and the Recommendation on the harmonization of member states on legislations relating to the removal, grafting and transplantation of human substances (No. 29 of 1978) [1] should facilitate positive solutions of these problems. A specific effort in the field of European solidarity is represented by the Council of Europe's continuing support for the European Bank of Frozen Blood of Rare Groups which is based in Amsterdam.

Blood Products and Legal Liability

I should now like to move to the consideration of two other matters which are of interest to us in the Council of Europe. The first issue arises from the fact that some industrialized blood products are being given the status of drugs. This is happening even though such products are of human origin, may have involved a risk for the donor when collected, and may subsequently involve a risk for the recipient, just as ordinary chemical drugs can do. The Council of Europe is convinced that in such a context as this we need to look very closely at the question of liability, whether that of manufacturers or of public health services. The Council of Europe has already drawn up a convention on product liability which has been discussed in relation to the use of products within medical services, but further work on this problem is surely needed. National legislation not only needs to define product liability but also to determine what body should be responsible for the quality control of products and of equipment. In Europe at present governments do not have a uniform approach to these problems. Many of them avoid taking a standpoint on them, and perhaps deliberately maintain a certain degree of indeterminacy in their approach. A few European governments with highly developed public health services to which public accountability is intrinsic have,

however, successfully resolved these problems. Yet others, for the moment, are considering the products as drugs and applying the relevant legislation. One of the Council of Europe's contributions to cooperation in the health field is the European Pharmacopoeia whose specifications are mandatory on Contracting Parties. This organ of the Council of Europe has already noted that certain blood derivatives are included in national monographs as drugs, but clearly governments urgently need to be given precise general guidelines in this field. There can, however, be no intention to impose complete harmonization on European governments in this matter.

The Duties of Health Service Staff

The second general question is that of the duties in law which devolve upon health service staff in their work, especially, of course, doctors. The Council of Europe's Committee of Experts on Legal Problems in the Medical Field (which is at the moment composed of 21 experts from the medical field and 21 legal experts) has already carried out a certain number of studies. These have included the removal, grafting and transplantation of human substances, artificial insemination in human beings and the protection of involuntary patients in mental institutions. Since June of this year, the Committee has begun to prepare an international instrument on the doctor's duties and liabilities. (This notion implies certain rights for the patient, but it is preferred to begin with the traditional notion of the doctor's duties and responsibilities.) This proposed instrument would have an important impact on legal aspects of blood transfusion. For instance, discussions have already taken place on the general definition of the medical intervention: 'All kinds of treatment, intervention or examination having diagnostic, prophylactic, therapeutic or rehabilitative aims, which are administered by a doctor or under his responsibility.'

The instrument would probably contain a clause to the effect that no medical intervention may be administered without the free and informed consent of the patient. This provision would invite us to consider the classification of medical interventions into two groups: routine and second-order. The routine intervention will be one for which the patient's consent may be presumed from the very fact that he has sought the professional services of the doctor. Whilst in this situation the doctor is under no obligation subsequently and expressly to obtain the patient's consent on the basis of information he provides, he would not thereby be excused the general obligation of giving full information to the patient on the latter's state of health unless this is against the patient's interest.

As regards second-order medical interventions these are those which present a certain risk for the patient or affect or are likely to affect the patient's personal privacy. For these second-order interventions the patient's express and specific consent should invariably be required. There would be provisions dealing with the absence of consent where the patient is unable, because of his state of health,

to express his consent and where the patient lacks full legal capacity. These provisions would mean that a patient's legal representative will often be involved in the process of obtaining consent, where relatives are not in existence. Clearly, into this second category, come many diagnostic acts such as X-rays, pathological tests, etc., since these involve the risk of accidental lesion or of patient discomfort. In discussion, the Committee included as a preliminary step blood transfusion in this second category. It must also be noted that conflicts of choice may exist between parents or legal representatives and the patient where the latter is capable of judgment. The patient may, after all desire to benefit from a transfusion refused by other patients. A well-known case of this sort has arisen involving Cooley's anaemia.

It was also recognized by the Committee during these preliminary discussions that the obligation of giving full information to the patient about his health generally lies with the doctor in charge of the case. Any other health staff working, e.g., in a haematological laboratory, a blood bank or in a blood transfusion service, would only have the obligation to inform the patient on the technical aspects of the intervention being carried out, including information on any risks involved. Test results and general prognosis would remain the responsibility of the doctor in charge.

As to the advantages conferred by this international work, the aim of the proposed instrument is not to increase the controls under which doctors work and thereby to obstruct the efficient execution of their duties. Rather it is intended to give doctors the opportunity to carry out their work without the fear that the patient or the patient's relatives will subsequently bring against him a law claiming the doctor's liability. Any contact needs a clear and workable basis which cannot reside solely in the traditional but somewhat nebulous notions of 'trust' and 'entente'.

The field of blood transfusion is traditionally one where international cooperation shows that the principle of solidarity, of sharing the most needed resources between all people, transcends many of the more mundane obstacles to international understanding. I must therefore conclude by expressing the hope that states who wish to enter into cooperation with the Council of Europe in this field should consider doing so for they would be most heartily welcome.

References

1. *Council of Europe Instruments*

Agreement No. 26 on the Exchange of Therapeutic Substances of Human Origin.
Agreement No. 39 on the Exchange of Blood Grouping Reagents.
Agreement No. 84 on the Exchange of Tissue Typing Reagents.
Convention No. 91 on Products Liability in regard to Personal Injury and Death.
Resolution (68) No. 32 on the Establishment in Amsterdam of a European Bank of Rare Groups.

Resolution No. (78) 29 on the Harmonization of Legislations of Member States relating to Removal, Grafting and Transplantation of Human Substances.
Recommendation No. (80) 5 concerning Blood Products for the Treatment of Haemophiliacs.
Recommendation No. (81) 5 concerning Antenatal Administration of Anti-D Immunoglobulin.
Recommendation No. (81) 14 on Preventing the Transmission of Infectious Diseases in the International Transfer of Blood, its Components and Derivatives.

2. *Reports*

Blood Transfusion Problems in Europe. 1960.
Essential Aspects of Tissue Typing. 1975.
The Production and Use of Cellular Elements in Blood. 1976.
The Indications for the Use of Albumin, Plasma Protein Solutions and Plasma Substitutes. 1977.
Preparation and Use of Coagulation Factors VIII and IX for Transfusion. 1979.
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Industrial Processing

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Initially, transfusion of blood from a donor to a recipient was a medical act performed at the bedside. The modern possibilities for the preparation of cellular components and plasma derivatives from donor blood have led to the development of out-of-hospital blood services with mass production of biologicals of human origin. These products must fulfil quality requirements which are just as strict as those applied to pharmaceuticals. In-hospital donations and transfusions of blood remain medical acts performed under the responsibility of the staff of the institution. The preparation of blood products by regional or national blood services should induce public health services to supplement their pharmaceutical control institutions with a unit supervising good manufacturing and laboratory practices related to these products. Another important task of the unit would be the surveillance of imported biologicals of human origin.

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Blood Transmitted Illnesses

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The prevention of transfusion reactions calls for stringent selection of blood donors and sufficiently sensible cross matches. In most countries these prerequisites have been laid down in guidelines.

Despite of proper precautions, infectious agents happen to be transmitted by blood and blood products. That is why I confine myself to discuss only infectious diseases.

A very large number of infective agents pathogenic to man are potentially transmissible by parenteral exposure to blood or blood products. The requirements for such transfer of infection include three major points:

1. Presence of infectious agents for a certain period in individuals not severely ill, not limited to narrow age groups and not confined to small geographical areas.
2. Stability of these agents in blood or blood products.
3. Susceptibility to infection in a significant proportion of the recipient population.

Blood transmitted infectious diseases are caused by

I. Virus diseases of
   major importance (e.g. hepatitis types B and non-A, non-B;
   occasional importance (e.g. EB and CMV) and
   suspected importance (e.g. slow viruses).
II. Parasitic and bacterial diseases.

I. Virus Diseases

Hepatitis

Transmission of hepatitis by blood or blood products is one of the most serious complications of blood transfusion, constituting a hazard to the life and health of blood recipients as well as a legal hazard to physicians and blood bank services. Blood transmissible virus hepatitis occurs in two major types:

hepatitis type B and
hepatitis type non-A, non-B.

Hepatitis type B

The introduction of HBsAg screening in the early seventies has reduced the proportion of infectious donors, so that more recent surveys of the occurrence of post-transfusion HBV infection cover missed cases of new or recent infections so that much lower rates of viraemia will now be encountered. Instead of post-transfusion hepatitis the new term 'transfusion associated hepatitis' becomes more and more popular.

Socio-economic background is the most important determinant, but also sex and ethnic origin are relevant factors as well as environmental factors such as illicit drug addiction or male homosexuality.

In combination, risk figures will range from 0.1 per 1000 for Scandinavian volunteer donors to perhaps 50 per 1000 in paid donors or in Southern Europe.

In principle, the methods for prevention HBV transmission with blood and blood products contain three strategies:

1. Blood donor selection
2. Treatment of the products
3. Immunisation of the recipients.

ad 1. The screening of blood donors for HBsAg has been most successful, especially in areas with high prevalence of carrier donors.

After general application of third-generation tests for HBsAg in all blood donations, the occurrence of hepatitis B in recipients decreased to less than 0.2 per 1000 units in most areas.

However, in some studies the prevalence of HBsAg carriers is too low and/or the occurrence of non-A, non-B hepatitis too high to yield impressive results.

In many situations, another form of donor selection has been performed simultaneously: avoidance of paid donors who carry an increased risk of B as well as non-A, non-B hepatitis. The impact of this selection alone is probably as effective as donor screening for HBsAg although few exact figures are available.

Much debated is the possible benefit from anti-HBc testing. It should be realized that the presence of anti-HBc alone in a donor serum might be obtained in approximately 10 per cent of all HBV infected individuals, and in most cases it will reflect past infection with no infectivity left. However, a few cases of HBV transmission from such blood have been reported, but no figures exist to establish the value of this additional testing.

ad 2. The fractionation of pooled plasma, by one of several methods yields therapeutically useful material, which has some risk of transmitting viral hepatitis. Terminal inactivation of albumin and plasma protein fraction (PPF) has been mentioned and is generally used in all areas. Immunoglobulin preparation is also a safe procedure.

Among the high risk products are fibrinogen, antihaemophilic factor VIII and factor IX concentrate.

In the last two years special techniques have been developed to produce a safe factor VIII and IX concentrate without a significant loss of activity.

Attempts to dilute viruses by erythrocyte washing have not proved sufficiently effective.

ad 3. Addition of hyperimmune serum globulin with a high titre of anti-HBs seems to be a possible solution, but the costs of this immuno globulin severely limit its application.

A final approach to the control of HBV infection in recipients of blood products is active immunization of susceptible recipients. This is now a practical possibility after the development of a safe and effective HBsAg vaccine. The efficiency of the vaccine in relation to the large dose of virus that may be provided by blood transfusion has not yet been established, nor has the possibility of protection by vaccination after the time of transfusion. Such cost/benefit studies have to be carried out before reasonable benefits can be expected from the selection of recipients.

Immunoglobulin post-exposure prophylaxis with standard immunoglobulin has failed to be of any value in preventing blood transfusion infection, while the role of anti-HBs-hyperimmunoglobulin has not yet been fully evaluated.

Hepatitis type non-A, non-B

Non-A, non-B hepatitis is a diagnosis of exclusion. Acute viral hepatitis due to the hepatitis A or B virus can usually be diagnosed by serologic tests. Hepatitis occurring in the absence of these serologic markers is, however, not necessarily non-A, non-B viral hepatitis. Other infectious diseases such as mononucleoses, CMV infection or syphilis as well as other diseases of the liver, viz. drug-induced liver disease, cholecystitis, etc. must also be excluded. The diagnosis of non-A, non-B hepatitis should be made with caution and it must rest on the combination of typical epidemiological features, history, clinical findings, and serum biochemical laboratory tests. No serological tests are so far known to detect past or present infection with non-A, non-B virus.

Circumstantial evidence suggests the existence of two or three types or strains of non-A, non-B virus. In clinical trials some patients had several bouts of acute hepatitis not associated with hepatitis A virus or hepatitis B virus infections. In PTH, differences in the incubation period ranging from 7 to 150 days have been observed. Also, different patterns are seen in the course of diseases. Some cases are of short duration, others are biphasic or present prolonged liver enzyme, eg ALT, elevation. Finally, the histological appearance of changes in the liver cells suggests two separate types of non-A, non-B hepatitis. These findings, however, have not been sufficiently reproduced in the chimpanzee model.

Virus distribution in the course of infection and the duration of viraemia are only sporadically known. Viraemia may be present for 1–2 weeks prior to the onset of symptoms in acute infections, and may last for some weeks after the onset. Prolonged viraemia, a virus carrier state lasting more than one year has been documented with and without signs of liver damage. As regards the distribution or excretion of non-A, non-B virus transmission, studies have demonstrated the presence of the agent in whole blood, plasma, serum and fractionated factor VIII and IX products as well as in liver homogenate. The presence of virus in other body fluids or excretions has not been observed.

As regards the potential routes of infection, the above mentioned inocula have successfully been administered subcutaneously, i.m. and i.v. No oral infection has been reported. The infectious dose used in experimental infections has been higher than that used in hepatitis B studies. Needle-stick exposures have also resulted in infection. In cases not related to blood transfusion, a parenteral exposure is not regularly found but, as stated, other viruses may be responsible for these cases.

No systematic studies have been published on the stability of the virus(es) or methods for its inactivation. There are no reports on the transmission of non-A, non-B virus by albumin or some other heated material, or by immunoglobulin products.

Epidemiological studies of implicated blood donors revealed a series of potentially useful associations all of which, however, await serological confirmation:

1. The risk of non-A, non-B post-transfusion hepatitis increases with the number of units transfused and the number of donors involved.

2. HBV markers (anti-HBs, anti-HBc) are more prevalent in implicated donors than in controls. These markers are, however, found in less than half of the cases.

3. Liver enzyme elevation in some implicated donors is estimated at 20 per cent.

4. Race, sex, age or history of jaundice have no correlation with the risk of non-A, non-B infection; however, paid donors carry a 2–4 fold higher risk than do volunteers.

II. Parasitic and Bacterial Diseases

Malaria

At present, malaria is the second major cause of non-immunological transfusion accidents after viral hepatitis.

The parasite develops and multiplies in the parasitized red cells, and, by an asexual (erythrocytic) cycle, it produces either merozoites or gametocytes (sexual cycle).

Today there is no easily administered serological routine test for malaria. Therefore the failure of detection of malaria in blood donors seems to be due to insufficient questioning of the donors, lack of knowledge about endemic zones, and to the existence of asymptomatic forms.

Serology may solve many of these problems in the near future. Its cost should not be excessive in non-endemic zones and the supply of specific antigens should be ensured through international cooperation.

At this time the following suggestion would be acceptable:

A potential donor from a malaria area, whatever his malaria case history and whatever the length of his stay, should not be accepted within six months after having left the endemic zone. Immigrants from a malaria area should not be accepted from six months up to three years after having left this area. This quarantine period can be shortened, if suitable immunological tests for malaria are performed and found to be negative.

There are many other human parasitoses which are transmissible by blood transfusion, e.g. Chagas' disease in the north of South America, filariasis or leishmaniasis. Recently, a case of babesiosis, transmitted by blood has also been described.

Syphilis

The frequency of this disease has declined significantly in the great majority of countries ($\simeq$ 1/10 000), although there are signs of an increase at present.

While the serodiagnosis of syphilis is simple and many of these tests can easily be automated, tests should be performed with all donated blood.

It should not be forgotten that there is a long serological negative phase and also that blood storage at 4 °C inactivates the treponema in about 72 h.

A blood product containing syphilitic antibodies may induce positive seroconversion in the recipient; this condition disappears in approximately three weeks.

In conclusion, blood services and commercial companies should be obliged to declare the quality of the original material, the size of the pool, the source of the plasma, the geographical origin of the donors and the number and quality of the screening tests used for prevention of transmission of infectious diseases.

International Cooperation in Blood Transfusion

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Since many years, the World Health Organization (WHO), the League of Red Cross Societies (LRCS) and the International Society of Blood Transfusion (ISBT) have tried to contribute to the development of national blood transfusion services in developing countries by several approaches.

Briefly summarized, the following activities were undertaken:

1. Engagement of consultants or temporary advisers who are sent to developing countries, mainly under the auspices of the WHO or other international organizations.

2. Organization of courses for fellows from developing countries, either in the industrialized countries or in the developing countries themselves.

3. Training of fellows in blood transfusion laboratories in various parts of the world.

Another way of developmental cooperation is the sending of a surplus of blood components, as has, e.g., been done in the past by the Netherlands Red Cross which has distributed large amounts of dried plasma before outdating. In later years, red cell concentrates and some protein components, such as gammaglobulin, have been provided by other countries. However, the transport of red cell concentrates is a very expensive procedure, which needs an organization enabling quick distribution from the airport to blood banks and hospitals under optimal conditions, particularly in tropical and subtropical areas. In countries with a poor organizational infrastructure this has encountered great difficulties and the result is that the surplus of red cell concentrates are sent to communities that in principle should not need them, since the willingness to spend blood is not sufficient. As far as protein components are concerned, most non-profit blood transfusion organizations are unable to help developing countries to cover their needs of albumin, PPS and fraction VIII concentrates. The surplus available is mainly pooled i.m. gammaglobulin, a product which so far has had limited applications.

It is quite clear that the only way to solve the problem of blood cell and protein components in developing countries is the organization of national blood transfusion services able to cover their own needs. The difficulties encountered in setting up such self-supporting national organizations have been so far:

a) A poor socio-economic situation which hampers the willingness to donate blood on a non-remunerated basis.

b) Poor health of the population caused by malnutrition and endemic (tropical) diseases.

c) Religious principles which sometimes are an obstacle against blood donation.

d) Large-scale collection of plasma by pharmaceutical companies, against payment, which of course is a welcome source of income for the poorest people of the world.

Fortunately in many countries precautions are taken nowadays by the government to protect the population against these harmful procedures, referred to by Dr. David-West, regarding the situation in Africa.

In spite of the above mentioned difficulties it is clear that national organizations with a non-remunerated donor population should be created in the developing countries and the initiatives taken in the past and the present should be further expanded in the future.

A prerequisite for such a set up, however, is that the governments of the countries involved are willing to stimulate the principle of self-support, based on a non-profit system, and this should be ratified by law or other official rules.

The organizational structure should preferably consist of a central institute for blood transfusion with a network of regional blood banks.

The main tasks of the central institute should be:

1. The recruitment of voluntary, non-remunerated blood donors, in close collaboration with the regional blood banks.

2. The training of laboratory, technical and administrative staff.

3. The preparation of the necessary protein components from plasma, preferably by cheap methods (chromatography).

4. Quality control of blood cell and protein components, including the prevention of transmissible diseases.

5. Furthermore, the central institute should have the disposal of a blood group reference laboratory and a department for the preparation of blood grouping reagents.

In the first stage of development such a country needs support for setting up an adequate organization, which implies financial, scientific and technical aid from one or more industrialized countries. In general the financial support will be the largest problem. However, all industrial countries have funds for developmental cooperation, and multi- or bilateral agreements with developing countries. It would therefore be of great importance if definite commitments could be made under the auspices of WHO and in collaboration with the LRCS and ISBT, in such a way that each industrial country should earmark a certain percentage of its funds for developmental cooperation, to assist in the building up a national blood transfusion organization in one or more countries in need of it.

I realize that such assistance exists already, but only incidentally and on a limited scale, without the framework of an international body which should

promote the collaboration in this field. If WHO would be able to set up an international cooperation, the subsequent activities are not so difficult to realize. Transmission of knowledge and experience can be achieved by courses and fellowships in donor countries, with a follow-up in the receiving country itself. The highest priority should be given to the recruitment of voluntary, non-remunerated donors in the countries involved, and this can be achieved with the aid of the public health authorities, assisted by well-trained public relation officers who should explain to the population why their blood is needed. All other items, such as laboratory equipment, training of laboratory, technical and administrative staff, and transport facilities, can be procured without too many difficulties.

As I mentioned before, a lot of activities have already been developed, but this is still insufficient, and it is therefore highly desirable that WHO, in collaboration with the LRCS and the ISBT, and in close consultation with the governments of the donor and of the recipient countries, creates a constructive project for financing the development of national blood transfusion organizations wherever they are needed.

ANNOUNCEMENT — ISBT

Jean Julliard Prize

The 9th Jean Julliard Prize, which was established by the Internation Society of Blood Transfusion in memory of its first Secretary General, will be awarded during the XVIII International Congress of Blood Transfusion, to be held in Munich (FRG) from 22 to 28 July 1984.

This prize is reserved for scientists under 40 years of age in recognition of recently completed scientific work on Blood Transfusion and related subjects.

In order to qualify, candidates must forward six (6) copies of an unpublished manuscript or recently published papers including a curriculum vitae to the Secretary General, Professor Ch. Salmon, 53 boulevard Diderot, 75571 PARIS CEDEX 12, France

before the 15th January 1984

This prize will be awarded during the Congress. The value of the prize is 3.000 Swiss francs.

Further information may be obtained from the Secretary General. The regulations for the Jean Julliard Prize are included in the Statutes of the Society.

ABSTRACTS

Relative reducibilities of complexes of Fe(III), Co(III), Mn(III) and Cu(II) with apotransferrin using e_{aq}^- and CO_2^-. J. H. Sommer, P. B. O'Hara, C. D. Jonah, R. Bersohn (Department of Chemistry, Columbia University, New York, N.Y. and Argonne National Labs, Argonne, IL, USA). *Biochim. Biophys. Acta 703*, 62 (1982).

Metal complexes of transferrin with iron (III), cobalt(III), manganese(III) and copper(II) were exposed to the short-lived, extremely powerful reductants e_{aq}^- and CO_2^-. The amount of reduction of the metal ions was measured by the bleaching of the visible charge-transfer absorbance. All of the complexes show less than 7 per cent metal reduction. A kinetic model was formulated and reductive rate constants derived for each complex. The rate constants were of the order 10^8 M^{-1} s^{-1} for e_{aq}^- and 10^6 M^{-1} s^{-1} for CO_2^-. Although these rate constants are almost 10 orders of magnitude faster than those determined for transferrin with conventional chemical reductants, they are 2–4 orders of magnitude slower than those for aquo- and other protein complexes of the same metal ions. Iron transferrin, the biologically important species, is 2–3 times less reducible than any of the other complexes, which is explained by a small structural difference. Direct reduction as a mechanism for iron release in the reticulocyte seems unlikely in view of the results mentioned above.

A. Egyed

The exchange of Fe^{3+} between acetohydroxamic acid and transferrin. Spectrophotometric evidence for a mixed ligand complex. R. E. Cowart, N. Kojima, G. W. Bates (Department of Biochemistry and Biophysics and the Texas Agricultural Experiment Station, Texas A. M. University, College Station, Texas, USA). *J. Biol. Chem. 257*, 7560 (1982).

Transferrin, the serum iron transport protein, provides an excellent model for studying biological metal ion exchange reactions. A curious problem is that while a mixed ligand species of chelate-Fe^{3+}-protein is anticipated from theoretical considerations and supported by kinetic results, no clear spectrophotometric evidence for such an intermediate has heretofore been obtained. In this study of the exchange of Fe^{3+} between acetohydroxamic acid and transferrin such evidence has been found. The reaction of Fe^{3+}-acetohydroxamic acid with apotransferrin-CO_3^{2-} is distinctly biphasic when examined by stopped flow spectrophotometry. The first phase is complete within $\sim$ 4 s and results in the formation of a transient species with a distinct spectral maximum at 432 nm. The second phase requires $\sim$ 2 min and results in the formation of Fe^{3+}-transferrin-CO_3^{2-}. It is suggested that the transient species is a mixed ligand complex. The reaction rate-concentration relationship for the formation of the intermediate is linear for Fe^{3+}-acetohydroxamic acid and hyperbolic for apotransferrin-CO_3^{2-}. This suggests a rate-limiting labilization of Fe^{3+}-(acetohydroxamic acid)$_3$ preceding attack by the apotransferrin-CO_3^{2-}. The reverse reaction, the removal of

Fe^{3+} from the Fe^{3+}-transferrin-CO_3^{2-} by acetohydroxamic acid, does not provide spectral evidence for the intermediate. The velocity-concentration relationship shows a hyperbolic dependence on acetohydroxamic concentration and a linear dependence on Fe^{3+} of Fe^{3+}-transferrin-CO^{2-} resulting from a conformational change.

A. Egyed

Failure of metallothionein to bind iron or act as an iron mobilizing agent. N. Kojima, C. R. Young, G. W. Bates (Department of Biochemistry and Biophysics, Texas Agricultural Experiment Station, Texas A. M. University, College Station, TX, USA). *Biochim. Biophys. Acta 716*, 273 (1982).

There are reasons to suggest metallothionein might act as a transient intracellular iron transport agent. Rabbit kidney metallothionein was isolated from animals treated with Cd in order to induce metallothionein synthesis. Attempts were made to form the iron-protein complex via several reaction routes. There was no evidence for complex formation. Metallothionein also failed to mobilize iron from ghosts of rabbit reticulocytes specifically labelled with ^{59}Fe.

A. Egyed

Isolation from haemolysate of a proteinaceous inhibitor of the red cell Ca^{2+}-pump ATPase. Its action on the kinetics of the enzyme. A. Wüthrich (Department of Veterinary Pharmacology, University of Bern, Bern, Switzerland). *Cell Calcium 3*, 201 (1982).

The purification to apparent homogeneity of a small protein from the cytosol of human red cells is described. The procedure consists of a combination of anion-exchange-chromatography, ultrafiltration, $(NH_4)_2SO_4$- and heat-precipitation. The resulting protein is a potent inhibitor of $(Ca^{2+} + Mg^{2+})$-ATPase of erythrocyte membranes and of Ca^{2+}-uptake into inside-out vesicles. Membrane $(Na^+ + K^+)$-ATPase is not affected by the inhibitor. The peptide migrates as a single band in SDS gels. Its apparent molecular weight is 19 000. It causes inhibition of the Ca^{2+}-pump by decreasing Ca^{2+}-affinity at all calmodulin concentrations.

G. Gárdos

The reconstitution of the human erythrocyte sugar transporter in planar bilayer membranes. J. K. Nickson, M. N. Jones (Department of Biochemistry, University of Manchester, Manchester, UK). *Biochim. Biophys. Acta 690*, 31 (1982).

The degradation of human erythrocyte membrane proteins in relation to the identification of the monosaccharide transporter has been investigated in whole membrane preparations and membrane protein extracts by polyacrylamide gel electrophoresis in sodium n-dodecyl sulphate and iodine-125 labelling. Evidence is presented for the degradation of band 3 polypeptide to lower molecular weight material some of which appears in region 4.5 of the polyacrylamide gel electrophoresis profile. It is found that the degradation process is inhibited by phenylmethylsulphonyl fluoride and is only significant in membrane extracts in the absence of detergent (Triton X-100) and on prolonged incubation at 37 °C, conditions which do not prevail during the isolation of membrane protein extracts for reconstitution studies. Extracts of band 3 and band 4.5 have been prepared and reconstituted in bilayer lipid membranes. The permeabilities of the reconstituted systems to d-glucose have been investigated and it is found that only bilayers incorporating band 4.5 exhibited enhanced monosaccharide transport. A linear relationship between d-glucose transport and the concentration of protein in the aqueous phase bathing the bilayers suggests a partitioning of the protein into the bilayer. Reconstitution is stereospecific and inhibited by cytochalasin B.

G. Gárdos

Modulation of ATPase activities of human erythrocyte membranes by free fatty acids or phospholipase A. G. Schmalzing, P. Kutschera (Department of Toxicology, Uni-

versity of Tübingen, FRG). *J. Membrane Biol. 79*, 65 (1982).

The artificial insertion of increasing amounts of unsaturated fatty acids into human erythrocyte membranes modulated ATPase activities in a biphasic manner, depending on the number and position of double bonds, their configuration, and the chain length. Uncharged long-chain fatty acid derivatives with double bonds and short-chain fatty acids were ineffective. Stearic acid stimulated Na^+K^+-ATPase only. Anionic and con-ionic detergents and -lysophosphatidylcholine failed to stimulate ATPase activities at low, and inhibited them at high concentrations. Mg^{2+}-ATPase activity was maximally enhanced by a factor of 2 in the presence of monoenoic fatty acids; half-maximal stimulation was achieved at a molar ratio of cis(trans)-configurated C18 acids/membrane phospholipid of 0.16 (0.26). Na^+K^+-ATPase activity was maximally augmented by 20% in the presence of monoenoic C18 fatty acids at 37 °C. Half-maximal effects were attained at a molar ratio oleic (elaidic) acid/phospholipid of 0.032 (0.075). Concentrations of free fatty acids which inhibited ATPase activities at 37 °C were most stimulatory at reduced temperatures. At 10 °C oleic acid increased Na^+K^+-ATPase activity fivefold (molar ratio 0.22). Unsaturated fatty acids stimulated the effect of calmodulin on Ca^{2+}-ATPase of native erythrocyte membranes (i.e. increase of V_{max} from 1.6 to 5 μmol PO_4^{3-} phospholipid^{-1} h^{-1}, decrease of K_{Ca} from 6 μM to 1.4–1.8 μM). Stearic acid decreased K'_{Ca} (2 μM) only, probably due to an increase of negative surface charges. A stimulation of Mg^{2+}-ATPase, Na^+K^+-ATPase, and Ca^{2+}-ATPase could be achieved by incubation of the membranes with phospholipase A_2. An electrostatic segregation of free fatty acids by ATPases with ensuing alterations of surface charge densities and disordering of the hydrophobic environment of the enzymes provides an explanation of the results.

G. Gárdos

The stoichiometry of the Ca^{2+} pump in human erythrocyte vesicles: modulation by Ca^{2+}, Mg^{2+} and calmodulin. C. K. Akyempon, B. D. Roufogalis (Laboratory of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada). *Cell Calcium 3*, 1 (1982).

Active Ca^{2+} uptake and the associated (Ca^{2+} + Mg^{2+})-ATPase activity were studied under the same conditions in an inside-out vesicle preparation of human red blood cells made essentially by the procedure of Quist and Roufogalis (*J. Supramolec. Structure 6*, 375–381, 1977). Some preparations were treated with 1 mM EDTA at 30 °C to further deplete them of endogenous levels of calmodulin. As the Ca^{2+} taken up by the EDTA-treated inside-out vesicles, as well as the non-EDTA treated vesicles, was maintained after addition of 4.1 mM EDTA, the vesicles were shown to be impermeable to the passive leak of Ca^{2+} over the time course of the experiments. In the absence of added calmodulin, both active Ca^{2+} uptake and (Ca^{2+} + Mg^{2+})-ATPase were sensitive to free Ca^{2+} over a four log unit concentration range (0.7 μM to 300 μM Ca^{2+}) at 6.4 mM $MgCl_2$. Below 24 μM Ca^{2+} the stoichiometry of calcium transported per phosphate liberated was close to 2 : 1, both in EDTA and non-EDTA-treated vesicles. Above 50 μM Ca^{2+} the stoichiometry approached 1 : 1. When $MgCl_2$ was reduced from 6.4 mM to 1.0 mM, the stoichiometry remained close to 2 : 1 over the whole range of Ca^{2+} concentrations examined. In contrast to the results at 6.4 mM $MgCl_2$, the Ca^{2+} pump was maximally activated at about 2 μM free Ca^{2+} and significantly inhibited above this concentration at 1 mM $MgCl_2$. Calmodulin (0.5–2.0 μg/ml) had little effect on the stoichiometry in any of the conditions examined. The possible significance of a variable stoichiometry of the Ca^{2+} pump in the red blood cell is discussed.

Ágnes Enyedi

Effects of trifluoperazine and mitogenic lectins on calcium ATPase activity and calcium transport by human lymphocyte plasma membrane vesicles. A. H. Lichtman, G. B. Segel, M. A. Lichtman (Departments of Radia-

tion Biology and Biophysics, Medicine and Pediatrics at the University of Rochester School of Medicine, Rochester, N.Y., USA). *J. Cell. Physiol. 111*, 213 (1982).

The phenothiazine, trifluoperazine, and the mitogenic lectins, phytohemagglutinin (PHA) and Concanavalin A (Con A), were tested for their effects on human lymphocyte plasma membrane Ca-activated Mg-ATPase and ATP-dependent calcium uptake. Trifluoperazine completely inhibited Ca-uptake when present from the start of the assay at concentrations of 100 μM or more. When added during measurement of calcium uptake, trifluoperazine reduced the rate of vesicular calcium accumulation but was unlike the calcium ionophore, A 23 187, which caused a rapid release of accumulated calcium from the vesicles. Trifluoperazine also inhibited membrane vesicle Ca-ATPase activity, but this inhibition was non-specific since the Mg-ATPase and Na, K-ATPase activities were inhibited to similar extents at the same concentration of the phenothiazine. In contrast, concentrations of PHA and Con A, which are mitogenic for lymphocytes, did not cause any change in Ca-uptake when added to suspensions of membrane vesicles. Con A had no effect and PHA had a weak inhibitory effect on Ca-ATPase activity.

Ágnes Enyedi

The effect of insulin on glucose transport in rabbit erythrocytes and reticulocytes. S. G. Albert (Division of Endocrinology, Department of Internal Medicine, Saint Louis University School of Medicine, Saint Louis, Missouri, USA). *Life Sci. 31*, 265 (1982).

Insulin binding and 3-O-Methylglucose transport have been studied in erythrocyte- and reticulocyte-enriched fractions of blood cells is order to determine if the increased number of insulin binding sites in reticulocytes is associated with a glucose transport response to insulin. In these experiments rabbit reticulocytes demonstrate an eight-fold increase in total insulin receptors when compared to erythrocytes. Glucose transport activity in the erythrocyte has a K_m of 3.2 mM. Reticulocytes demonstrate a saturable glucose transport activity of lower affinity, K_m 18.9 mM. Neither the erythrocyte, nor the reticulocyte glucose transport activity was capable of an increased response to insulin. The low affinity glucose transport activity in reticulocytes could allow a fourfold increase in facilitated glucose transport at supraphysiological glucose concentrations that might occur in poorly controlled diabetes mellitus.

Ágnes Enyedi

OBITUARY

Distinguished French Professor and long-time member of our Editorial Board, ROBERT ARNAUD had died on the 22nd December, 1982.

Born in Abilly, France, he had graduated from Tours Medical University. His first tutor was professor Antoine Vialle. It was in his laboratory that the young doctor took a liking to bacteriology, haematology and pathology. Then came Paris and the Pasteur Institute. Later he headed a haematological and clinical laboratory established by him. Subsequently he has developed the Tours *Laboratoire de Recherches Biologiques* into an internationally renown scientific centre.

Holder and knight of the French Order of Merit, Professor Arnaud had been a highly respected member of both the French medical society and the International Society of Blood Transfusion (ISBT). An amiable and captivating personality, a good organizer and a fine researcher – he was the classical physician.

Our friendship began with a chance acquaintance. During on ISBT meeting someone had suggested the grounding of "coordinating faculties" in each of the member countries to facilitate international cooperation between nations. This is how the Tours Blood Transfusion Centre and the NIHBT from Budapest became twins. The collaboration had among others also resulted in numerous exchange visits of and friendships between workers of the two institutes.

Professor Robert Arnaud. An eminent figure in both the theory and practice of blood transfusion. A true friend whose memory will be enshrined by us.

Susan R. Hollán

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Manuscripts should be sent to the Editor-in-Chief:

Prof. Susan R. Hollán
National Institute of Haematology and Blood Transfusion
Daróczi út 24
H-1113 Budapest, Hungary

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