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# BLOOD PROTEINS AND THEIR THERAPEUTIC VALUE[1,2]

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RECOGNITION of the role of the National Research Council in making available to Government Agencies the knowledge and advice of civilian experts in the medical sciences appears to be peculiarly appropriate at this time. I am honored to have been asked to participate in this ceremony, and to discuss blood proteins and their therapeutic value. The Blood Substitutes Sub-Committee of the National Research Council is a small group, the membership of which has changed but little from the time, many months before Pearl Harbor, when it was reorganized and charged with the responsibility of advising the Armed Forces and Government Agencies as to the methods to be employed and the products to be accumulated against military emergency or civilian disaster. Although it has carefully and critically examined the properties of every blood substitute that has been suggested, it

[1] Address delivered on December 11, 1944, at the ceremony of the award to the National Research Council by the American Pharmaceutical Manufacturers Association, New York, N. Y.

[2] The observations on which this appraisal is based involve so many fields and have appeared in such diverse publications that even a partial bibliography would be longer than this address. "A Plan for Collection, Transportation and Administration of Whole Blood and of Plasma in Warfare," by DeGowin and Hardin (*War Medicine,* 1: 326, 1941), reviews the earlier experience on blood transfusions, "The Preparation and Preservation of Human Plasma," by Strumia, McGraw, and Reichel (*Am. Jour. Clin. Path.,* 11: 480, 1941), that on the drying of plasma from the frozen state, and "The Properties and Functions of the Plasma Proteins," (*Chem. Reviews,* 28: 395, 1941), the methods available for their separation and purification. Other references are given in a more recent report on "Blood, Blood Derivatives, and Blood Substitutes" (*Proc. Am. Philos. Soc.,* 88: 159, 1944), in a series of twenty-three communications from different laboratories under the title, "Chemical, Clinical and Immunological Studies on the Products of Human Plasma Fractionation" (*Jour. Clin. Investigation,* 23: 1944), and in four clinical papers that have appeared lately (*Jour. A. M. A.,* 126: 469, 674, 680 and 944, 1944).

has thus far recommended to the Armed Forces only products derived from human blood. Its membership has included the expert who recommended the methods for blood procurement on the basis of which the American Red Cross blood donor program has functioned so successfully; the expert on blood banks, who continuously stressed the place of whole blood in therapy; the expert on dried plasma whose knowledge and experience of many years in its preparation and administration have thus always been available; a professor of medicine; a professor of surgery, and myself, a chemist concerned with the properties of proteins, their separation and purification, and their biological functions.

These experts have met repeatedly with the designated representatives of the Surgeons General of the Army, the Navy, and the Public Health Service, the Red Cross, the Office of Civilian Defense, and the Food and Drug Administration, inviting other experts to meet with them as occasion demanded. Unburdened by an administrative hierarchy or intermediaries they have transmitted their advice directly and supplemented and implemented their recommendations with reports and memoranda on the value and safety of products and of chemical or biological processes. This is the form of civilian advisory function which President Lincoln envisaged in creating the National Academy of Sciences, and it is to be hoped that it will be the form of the agencies which will advise Government regarding the medical sciences in the post-war world.

*Whole Blood.* As a part of the symposium on some fundamental trends in chemotherapy you have asked me to discuss "Progress in Conquest of Knowledge of Blood Proteins and of Their Therapeutic Value." A question which at once arises is how much of the organization for the collection and processing of the human blood, that has been developed during this war, will continue in the post-war world. Whole blood and plasma were well-known therapeutic remedies before this war. Their value has been further proven in military theaters of operation. Whole blood is especially needed when there has been severe blood loss, or when major operations are to be performed in which severe blood loss is anticipated. The red cells which are responsible for the respiratory function of the blood, carrying oxygen to the tissues, are also needed to combat the anemia which frequently occurs in convalescence from wounds. The therapeutic value of the red cells is thus related to their physiological functions. The therapeutic value of other constituents of blood may also be related to their physiological functions.

The lability of whole blood made it necessary to prepare for this war by the accumulation of reserves of the more stable components of blood. However, work continues in many laboratories[3] in an attempt to increase the span of life of the labile cellular constituents of blood so as to render them available over longer periods of time. Progress in the preservation of red cells both in whole blood and in media in which they can be resuspended may be expected to continue at an accelerated rate because of the methods that are being developed and the knowledge that is being gained.

Many of the conditions for which whole-blood transfusions are used, notably where there is a need for the red blood corpuscles, can probably be successfully provided for by resuspended red cells. Others may be satisfied by the injection of plasma or of a plasma fraction. The longer life of the red cell in whole blood than resuspended in any medium at present known makes it necessary whenever possible to fly whole blood to our Armed Forces. The social economy of using cells and plasma separately in the therapy of different conditions, if either can be demonstrated to be as satisfactory as the whole for the conditions indicated, is, however, so advantageous as to demand consideration in the post-war world.

*Plasma.* The separated plasma can be stored in the liquid state, can be frozen or can be dried from the frozen state. In the liquid state it retains its colloidal properties necessary for the treatment of shock, but the more labile components of plasma, the prothrombin and fibrinogen, concerned with blood coagulation, complement and the antibodies, concerned with immunity, deteriorate with time. For an economical civilian program, therefore, liquid plasma has distinct limitations. In the frozen state, the labile components of plasma are better preserved; here the limitation is the inconvenience for storage and transport and immediate emergency use. In the dried state, most of the labile components of plasma are preserved. Dried plasma has been used by our Armed Forces since, processed to a stable state from the blood collected by the American Red Cross it, as other dried products, can be accumulated as a reserve against need at a future time and place.

Plasma was accumulated in this war largely for the prevention and treatment of shock. Shock as observed in military medicine generally results from a rapid decrease in the volume of circulating blood due to the loss of blood and plasma proteins externally and into damaged tissues. It is most frequently treated, or prevented by injection of plasma proteins. The injected proteins, in so far as they can not readily pass through the kidney, increase the body's reservoir of plasma proteins, and, in so far as they do not readily traverse the capillary walls, increase the

[3] Some under contract with the Committee on Medical Research of the Office of Scientific Research and Development.

plasma volume by drawing water from the tissues into the blood stream. The function of controlling the equilibrium between the water and the electrolytes in the blood and in the tissues is largely performed by the smaller proteins of the blood stream, known as albumins, although all dissolved colloids will, of course, exert some osmotic effect.

*Albumin.* The albumins of plasma represent less than 60 per cent. of the plasma proteins but are responsible for nearly 80 per cent. of the osmotic efficiency of the plasma, upon which its value in the treatment of shock largely depends. Human serum albumin has also been recommended by the Blood Substitutes Sub-Committee to the Armed Forces and, like plasma, it has been processed in large amounts from the blood collected by the American Red Cross. Unlike plasma, albumin is so stable that it can be dispensed in solution; the albumin molecules are so symmetrical that a 25 per cent. solution is iso-viscous with whole blood. The isoelectric, salt-free albumin is prepared as a dry, white powder; it can be redissolved at any concentration and with any diluent. As a concentrate poor in salt, it is proving of value as a diuretic agent. In the treatment of shock it functions as does plasma, largely by virtue of the albumin it contains, by pulling water back into the blood stream, and holding it there. This results in a decrease in viscosity and a restoration of the volume of circulating blood to normal, so that oxygen is transported to the tissues and the other physiological functions of blood are resumed.

Athough traumatic shock occurs frequently enough in civilian life, as a result of automobile, industrial or other accidents, to warrant a peace-time society making provision for its prompt treatment with adequate supplies of plasma or plasma derivatives, the control of certain infectious diseases with plasma derivatives appears to offer at least as great an opportunity for civilian medicine.

*γ-Globulins.* A large scale public health experiment has now supplemented earlier clinical studies in demonstrating the value of the γ-globulins of human plasma in the modification or prevention of measles. These γ-globulins have been separated from the same plasma which yielded the albumin employed in the prevention and therapy of shock.[4] Of the plasma proteins about 58 per cent. is albumin and 11 per cent. γ-globulin. Two fractions of γ-globulin may also be separated, the one richer in euglobulin and the other (Fraction II) in pseudoglobulin. The latter fraction, which has thus far been employed in the study of the control of infectious diseases, represents approximately 5 per cent. of the plasma proteins. None the less γ-globulin may come to be recognized as the most important fraction of the plasma from the point of view of public health.

Pooled normal or convalescent plasma has long been used in the control of certain infectious diseases. Many of the antibodies to the variety of infectious diseases to which the population contributing the blood has been exposed are γ-globulins. Moreover, it has been possible to demonstrate that a population shows heightened immunity following an epidemic of a special disease. The concentration of γ-globulin antibodies over the pooled plasma thus far achieved by fractionation is approximately 25-fold if we compare plasma with the concentrated solution of γ-globulins being made available to the Armed Forces and, through the Red Cross, to public health agencies.[5] As a result titers of certain antibodies, though not necessarily of all, are comparable to those of the corresponding convalescent sera. Therefore, in the prevention or treatment of any disease where the value of convalescent serum has been demonstrated, the effectiveness of a concentrate from pooled normal plasma should be investigated. If convalescent serum or hyper-immune serum is fractionated, still higher antibody titers may be obtained in the concentrated γ-globulins.

The antibodies effective in the modification or in the prevention of measles are present in the more soluble fraction (Fraction II) of the γ-globulins. This is true also of the antibodies for several other virus diseases. Effective use of the γ-globulins in the control of diseases other than measles has been reported. Should these preliminary studies be substantiated by large scale military or public health investigations, the need for γ-globulin may prove even greater than has thus far been anticipated. None the less it should be stressed that in the case of many diseases those bearers of immunity which are the antibodies of the blood stream can not be made available in amounts large enough, or injected soon enough to prevent the onset of disease. In others, the incidence of the disease in susceptibles is so small that it would be

[4] The program of plasma fractionation which has been developed in this laboratory was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. Since August, 1941, it has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

The insight of my many fellow workers in this department has been immeasurably aided by many colleagues—chemical, clinical, immunological and pathological—members of this or other universities, of the Armed Forces, of Committees of the National Research Council, of the American Red Cross or of the commercial laboratories with Navy contracts for plasma fractionation, who labored with us. Indeed, it would be impossible to list here the very large number of individuals and agencies who have collaborated in this work.

[5] See *J. A. M. A.*, 125: 635, 638, 1944.

necessary to immunize passively an entire population in order to offer protection to the one in a thousand who might contract the disease. The blood donors for such an undertaking might not always be available whereas blood donors conscious of the contribution that they were making to the control of other diseases could clearly always be counted on in a society which has responded as has ours in the blood donor program.

Two problems which are chemical, rather than clinical, have thus far limited studies to determine the most effective use of the γ-globulins. The progress that is being made, however, suggests that both are yielding to systematic scientific investigation. The first is concerned with the removal of depressor substances which have heretofore prevented the intravenous use of γ-globulin concentrates. Conditions for the separation of these substances and thus of the preparation of γ-globulins safe for intravenous use appear to have been found, however, and appraisal of the value of γ-globulin intravenously injected in diseases where larger amounts may be needed than can conveniently be administered intramuscularly has been begun. Intravenous injections of 25 cc have been given to small children with acute infections without reaction and 50 cc to children not acutely ill. The injection of a concentrate of γ-globulin equivalent to the amount which could be introduced as plasma or whole blood only by replacing the entire blood volume by transfusion would thus appear to be both possible and practicable. Chemical specifications for the routine production of such preparations are, however, not yet completely satisfactory.

The second chemical problem concerns the separation of the γ-euglobulins of the plasma (in Fraction III-1) from the isoagglutinins (blood typing globulins), cholesterol and carotenoid bearing β-globulins, so that clinical investigations with this potential source of antibodies may also be begun. The O typhoid agglutinin has been found concentrated in this fraction rather than in Fraction II. The possibility exists, however, that sub-fractionations of the γ-globulins may yield, in time, products derived from the same plasma, some of which are more potent concentrates of certain antibodies; some of others.

*The Fractionation Process* that we have developed yields all the components of human plasma in five major fractions. The number has purposely been made as small as possible so as to facilitate and render economical large scale processes. We shall not stop here to discuss the five-variable physico-chemical system upon which the separations depend except to state that it involves the principles determining electrostatic interactions between proteins and neutral salts, which generally lead to increased solubility, and interactions with less polar organic liquids, which generally lead to precipitating action. The balance between these forces has made possible the definition of conditions leading to far sharper separations than have previously been possible. The theory and general methods, though not the detailed procedures, are equally applicable to plasma and tissue extracts of plant or animal origin; that is, to all systems in which a large number of protein and lipid components are to be separated from each other and without loss of the biological activity upon which their therapeutic value depends.

When the method is applied to plasma, the fibrinogen, which is the structural element of the blood clot, is concentrated in Fraction I. Fraction II + III contains essentially all the prothrombin, which, converted to thrombin, transforms fibrinogen into fibrin; all the immune globulins for which tests have been car-

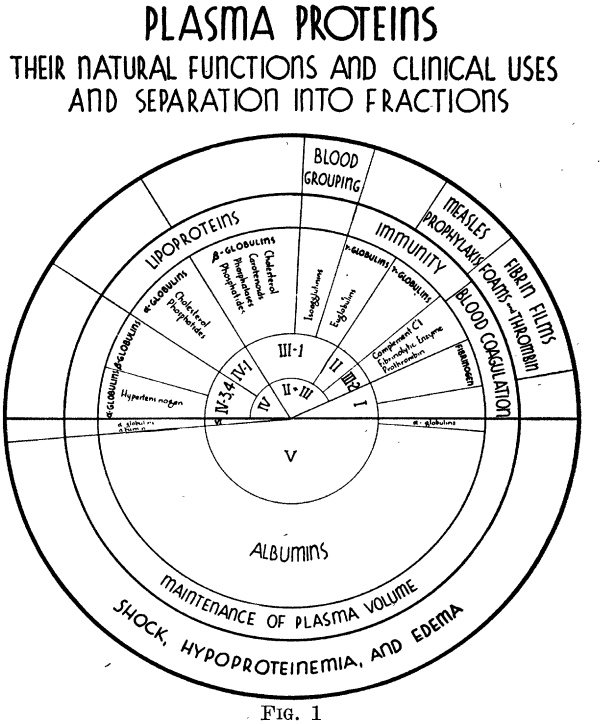


FIG. 1

ried out; the isohemagglutinins and Rh antibodies, of value in blood grouping, as well as all the carotenoids and such part of the cholesterol and phosphatides of plasma as appear to be associated with β- rather than α-globulins; the midpiece of complement; and probably innumerable other components of plasma which are not yet recognized by virtue of their chemical or biological properties. The remaining globulins are concentrated in Fraction IV.

*Isohemagglutinins* have also been prepared under contract with the Navy because of the value of these products of the fractionation of group specific plasma in the typing of whole blood. The products that have thus far been prepared commercially have been frac-

tionated respectively from pools of A or of B plasma. The presence of roughly four times as many A as B donors in our population has heretofore introduced an uneconomical factor in the preparation of blood grouping globulins from human plasma. Recently one of my collaborators has suggested and demonstrated the value of another method of segregating the plasma in pools for the preparation of isohemagglutinins. In this method the small number of B bloods are used to absorb the A anti-B agglutinins from O blood. The resulting fractionated and purified isoagglutinins are not only group specific but are far more avid with respect to the B anti-$A_2$ and $A_2B$ sub-groups than the B anti-A agglutinins prepared from B plasma. And, of course, approximately five times as much of the product is obtained from an equal number of grouped bloods. This process thus makes available an almost equivalent amount of B anti-A and of A anti-B grouping material and greatly increases the economy of the preparation of these highly potent blood-grouping globulins.

*The α- and β-globulins* concentrated in Fraction IV have recently been subdivided into two fractions, both sufficiently stable to be dried from the frozen state and reconstituted as clear soluble protein concentrates. One of these sub-fractions (IV-1) contains the remaining lipids of the plasma which, in this preparation, appear to be associated with α-globulins, largely with $\alpha_1$-globulins. The remainder of Fraction IV, tentatively designated IV-3, 4, is almost free of lipids, though rich in α- and β-globulins and is subject to further sub-fractionation in the interest of concentrating and purifying the diverse components which it contains.

In this process, all the proteins in plasma concerned with the coagulation process are concentrated in Fractions I and III-2; the immune globulins in II and III–1; the lipo-proteins in III–1 and IV–1 and the albumin in Fraction V which represents approximately 50 per cent. of the plasma proteins. Since the albumin in this fraction makes by far the largest contribution to the colloid osmotic pressure of plasma, fractionation yields albumin to perform this function and other fractions to be employed in therapy in connection with more specific physiological functions.

Not only has Fraction II been prepared in a state safe for intravenous use in the last months, but so also has Fraction IV-3, 4. What are the physiological functions and what are the therapeutic uses of this large fraction rich in α- and β-globulins, poor in lipids and antibodies and in components of the coagulation mechanism? The chemist is fulfilling his function when he makes these α- and β-globulins available in soluble, stable and safe form for intravenous use. We can depend on our clinical colleagues, armed with these tools, to determine the functions of these substances in the economy of the body and to discover their uses in therapy.

*Fibrinogen, Thrombin and the Uses of Fibrin Products.* In the subfractionation of Fraction II + III to yield the γ-globulin antibodies in Fraction II, prothrombin is of necessity first separated in Fraction III–2. Prothrombin may be converted to thrombin with human thromboplastin and large amounts accumulated and made available as a sterile dry powder ready for reconstitution and immediate emergency use. For stopping the flow of blood, in surgical procedures, thrombin is the only component of the clotting mechanism which must be supplied. For the most effective use of thrombin in hemostasis, however, it must be applied with a matrix which can hold the thrombin in the bleeding area until clotting is completed. A porous matrix soaked in thrombin solution can achieve this mechanical result. Fibrin foam is such a matrix formed from human fibrinogen and thrombin. It effectively controls bleeding from oozing surfaces and from veins, even very large ones. Though not recommended for brisk arterial hemorrhage, it has proved very effective in neurosurgery in controlling hemorrhage from the dura, from tumor beds, from dural sinuses and from large cerebral veins. In general surgery it has been reported effective, in a small number of patients, in controlling hemorrhage from the cut surface of the liver and kidney, in jaundice patients, in thorocic operations, and in stopping bleeding in hemophilia.

In the dry state fibrin foam is a porous material composed of strands of fibers separated by air spaces of macroscopic size. It readily absorbs water, saline or thrombin solution, and can be used in conjunction with a sulfa drug or with penicillin. The physical properties of the foam can be varied by controlling the conditions of manufacture. Since fibrin foam is prepared completely from human proteins and is rapidly absorbed with minimal tissue reaction, it may be left in place, thus preventing recurrence of bleeding. It is easily handled in the operating room, and, like serum albumin, is immediately available for use in emergencies.

Just as the asymmetry of certain synthetic polymers is responsible for the remarkable mechanical properties of industrial plastics, the fibrinogen molecule endows products of this human protein with remarkable mechanical properties. Among these products is fibrin film. Fibrin film may be prepared from fibrinogen and thrombin as a strong, rubbery sheet which can be stretched reversibly from two to three times its original length. This material can be made in various shapes and thicknesses, and in the form of seamless tubing. Its mechanical properties can also be varied

from a soft, rubberlike elasticity to a parchmentlike consistency.

The mechanical properties of the soft, rubbery films (as shown by their stress-strain curves) bear a marked similarity to those of the elastic ligament of the neck, the *ligamentum nuchae*. The tough films with delayed elasticity resemble rather wool or hair in their mechanical properties. It may be possible to imitate, with different types of fibrin products, still other natural structures of the body.

The fine structure of fibrin film has been shown to involve pores which in one type are of the order of 60 Ångstrom units in diameter. Hemoglobin molecules in solution pass through these pores readily, but plasma globulins are partially and fibrinogen molecules completely retained.

The mechanical properties of certain types of fibrin films make them suitable for use as dural substitutes and in the prevention of meningocerebral adhesions. The duration of their persistence in the body can be adjusted by suitable treatments. They have been used in neurosurgical operations and appear to be excellent materials for these purposes, patients having been followed for as long as fifteen months without the appearance of unfavorable sequelae. Other types of fibrin film and of fibrinogen plastics are being tested for other surgical applications.

Albumin and the immune globulins, fibrin foam and thrombin, have been licensed under the National Institute of Health, and have been prepared in large amounts under contract with the Navy. Fibrin films, which were developed in our laboratory several years ago, presented a considerable problem as a satisfactory sterilization procedure adaptable to their large scale preparation remained to be developed. I am happy to be able to report that a satisfactory procedure in which the films are sterilized by steam in the final container has been developed and is making large numbers of films prepared in this way available for final appraisal.

*In Summary,* the knowledge of blood proteins and their therapeutic value that has been gained in this war enables us to approach the future with a very different view than was possible a decade ago to even the most farsighted physician who was taking advantage of all scientific knowledge then available, especially that contributed by Landsteiner on blood grouping, in arranging for safe blood transfusion from donor to patient.

In the past, repeated blood transfusions had, of course, often been administered but not to the extent that our British colleagues practised, using blood and serum banks, during the first years of this war. Their experience from such multiple transfusions indicated on the one hand the value, in certain conditions, of injecting large amounts of plasma proteins, and on the other the need for red cells when such large amounts of serum proteins were administered.

The program of the American Red Cross has demonstrated the extent to which carefully controlled centralized collection and processing centers can contribute both to the safety of the donor and to that of the recipient of one or another of the products prepared from the blood contributed. The record that has been achieved is a fine one and redounds to the credit of the American Red Cross and the laboratories of the pharmaceutical industry that have loyally collaborated in this nationwide undertaking.

The need for multiple transfusions makes it necessary to use the blood contributed by a number of donors in treating a single individual. Indeed, whether we are dealing with the colloidal properties of the plasma proteins in shock or with the immune properties of the γ-globulins—which contribute but little to the osmotic efficiency of the plasma—the therapeutic value of massive doses, under certain conditions, has become apparent.

The control of infectious diseases by passive immunization with γ-globulins may well be the largest need of a civilian population for a blood derivative and one to which a civilian population can be expected to contribute in the interests of the modification and control of a children's disease, such as measles, until such time as the immunity of each growing generation is achieved.

In the fractionation of blood to obtain the γ-globulins, the red cells and the proteins which they contain, as well as the other plasma proteins, become available for the therapeutic uses for which they are needed; the albumin for the treatment of shock, hypoproteinemia, edema and—prepared in the salt-poor condition—as a diuretic agent; fibrin foam and thrombin as a hemostatic agent; fibrin film as a substitute for a natural body membrane and the large number of other cellular, protein and lipid components, whose physiological function and chemical nature are only beginning to be explored, for whatever therapeutic purposes may prove the most important.

Starting with the assumption, which must for the present remain an assumption, that every part of human blood performs an important natural function, we must continue, as we have begun, to make available as many as possible of its diverse cellular, protein and lipid components, separated and concentrated as specific therapeutic agents, of value in different conditions, in the interests of the most effective and economical use by a society of the blood which it contributes.