EXPERIENTIA

Vol. III - Fasc. 4 Pag. 125-168 15. IV. 1947

Chemical, Physiological, and Immunological Properties and Clinical Uses of Blood Derivatives[1,2]

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The chemistry of any biological system, plant or animal, depends upon the chemistry of the proteins, those nitrogenous molecules called "of the first importance" by MULDER over a century ago. Proteins combine with lipids and with carbohydrates, with vitamins, with fatty acids and with metals, with pigments, with dyes and drugs, with salts and with each other. Proteins may thus be lipoproteins, glycoproteins, mucoproteins, hemeproteins, or they may be enzymes, hormones or antibodies.

The separation and concentration of the various proteins of human plasma was undertaken during this war in order to render as many as possible available as specific therapeutic agents, of value in different conditions, and thus to increase our knowledge and control of the composition of the blood in health and in disease. For precisely as the respiratory function of the blood is performed by the red cells, so the many important physiological and immunological functions of the plasma are performed by its diverse, reactive, protein components.

Discovery of the Protein Components of Plasma

Recognition of the specific chemical functions of the various protein components of the plasma began in the eighteenth century. In his book, "An Experimental Inquiry into the Properties of the Blood", published in 1771, HEWSON described the separation of fibrinogen from plasma: "When fresh blood is received into a bason, and suffered to rest, in a few minutes it jellies, or coagulates, and soon after separates into two parts, distinguished by the names of *crassamentum* and *serum.*"

"It is well known, that the *crassamentum* consists of two parts, of which one gives it solidity, and is by some called the fibrous part of the blood, ... and of another, which gives the red colour to the blood, and is called the *red globules.* These two parts can be separated by washing the *crassamentum* in water, the red particles dissolving in the water, whilst the "fibrous part" remains solid. That it is "the fibrous part" which gives firmness to the *crassamentum,* is proved by agitating fresh blood with a stick, so as to collect this substance on the stick, in which case the rest of the blood remains fluid."[1]

HEWSON thus recognized the water-soluble constituent of the "red globules", the fibrinogen of the "crassamentum" or clot, and the coagulable proteins, largely the albumins, of the serum. Serum proteins, insoluable in water at a slightly acid reaction but dissolved by salt, were recognized by DENIS and SCHERER[2] in 1841. Over a century ago, therefore, the chief protein component of the red cells and at least three protein components of plasma had been recognized.

We now know that the pigment of the "red globules", is the oxygen-combining, iron-containing, prosthetic group of the protein, hemoglobin, and that the red blood cells contain in addition a large number of recently discovered protein components, among them carbonic anhydrase, catalase, phosphatase, choline esterase, hypertensinase and other peptidases. In this communication we shall concern ourselves, however, with the plasma proteins and the specific chemical reactions upon which their physiological functions depend.

The relation of fibrinogen to the clotting of the blood was implicit in HEWSON's discovery. Although HEWSON found ways of blocking fibrin formation it remained for ANDREW BUCHANAN to demonstrate, in 1845, "that fibrin has not the least tendency to deposit itself spontaneously in the form of a coagulum: that like

[1] This paper is Number 62 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross. The large number of colleagues who have collaborated, and the organizational pattern which made these studies possible, are referred to in reference [4] and more fully in reference [5]. Much of this work was carried out under contract recommended by the Committee on Medical Research to the Office of Scientific Research and Development.

[2] An address given, as visiting lecturer of the American-Swiss Foundation for Scientific Exchange, in Bern, February 27; in Zurich, March 3, and in Geneva, March 17, 1947.

[3] Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts.

[4] E. J. COHN, Medicine *24*, 333 (1945).

[5] E. J. COHN, Advances in Military Medicine: The History of the Committee on Medical Research, Chapter 28, Plasma Fractionation, in press.

[1] W. HEWSON, An Experimental Inquiry into the Properties of the Blood, pp. 6–7. T. Cadell, London, 1771.

[2] J. SCHERER, Liebigs Ann. Chem. u. Pharm. 40, 1 (1841).

albumen and casein, fibrin only coagulates under the influence of suitable reagents: and that the blood, and most other liquids of the body which appear to coagulate spontaneously, only do so, in consequence of their containing at once fibrin and substances capable of reacting upon it, and so occasioning coagulation"[1]. The substance which BUCHANAN noted by virtue of its physiological action, was presumably thrombin. Satisfactory studies upon thrombin as a globulin, were, however, not carried out until this century[2].

Physical Properties of Plasma Proteins

Meanwhile albumins and other globulins had been noted in terms of chemical properties, often however, without these properties being associated with specific biological functions. The chemical studies which have continued during the last century have yielded relatively pure preparations of albumins and demonstrated that there were many kinds of globulins.

Molecular Dimensions of Plasma Proteins. Although there is evidence of more than one albumin[3], all serum albumins that have been studied thus far, of human or animal origin, have closely similar molecular properties; isoelectric points near p_H 4·9, molecular weights near 70,000, and molecular dimensions described as ellipsoids 150 Å in length and 38 Å in width. All plasma proteins thus far investigated have diameters of this magnitude or greater. If the smallest dimension is less, the molecule appears not to be retained in the blood stream, but to be lost through the kidney. Among molecules of the same diameter, where loss occurs it appears to be inversely related to the length of the molecule. This should apply not only to rod-shaped molecules of diameters of the order of 20 Å or less[4, 5], but also to the plasma proteins under pathological conditions. Among plasma proteins with roughly the same diameter, of 38 Å, the β_1-globulins, which combine and transport copper and iron, have been estimated to have a length of 190 Å, the γ-globulins, concerned with immunity, of 235 Å, and fibrinogen, concerned with blood coagulation, of 700 Å[6] (Table I).

The viscosity of proteins depends, of course, not upon their size but upon their asymmetry. Thus fibrinogen, the most asymmetrical of the plasma proteins has an intrinsic viscosity six times that of albumin. Were it present in the plasma in large amounts, instead of to but 4 per cent, it would impose considerable burden upon the circulation. Serum albumin is far more symmetrical; a 25 per cent solution being isoviscous with blood[1].

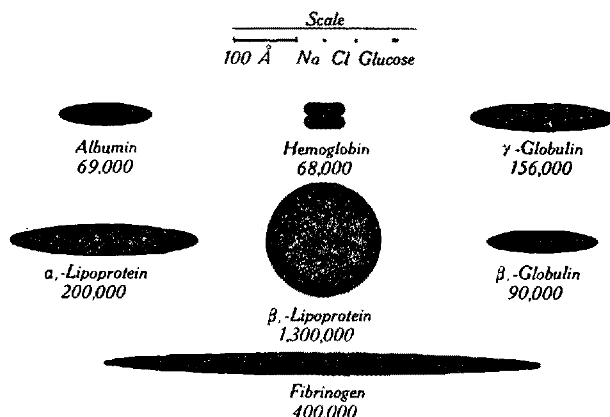


Fig. 1. Relative Dimensions of Various Proteins[2].

The albumins are furthermore at once the most stable, the smallest and the most copious of the plasma proteins. Present in normal plasma to just over 50 per cent they are responsible for nearly 80 per cent of the colloid osmotic pressure which regulates the equilibrium in water and electrolytes between the plasma and the tissues, and thus play the major role in maintaining the volume of the blood upon which normal circulation depends[3].

The molecular size and shape of proteins, once they are separated as homogenous chemical components, may be estimated by measurements of osmotic pressure and of viscosity. The ultracentrifuge renders it possible, however, to distinguish proteins of different molecular dimensions even in so complex a mixture as the plasma. The constant defining the speed of motion of a protein in the field of the ultracentrifuge, for the development of which we are indebted to SVEDBERG[4], has revealed proteins sedimenting in plasma with four very different velocities[5]. Those sedimenting with the smallest velocity include the al-

[1] A. BUCHANAN, London Med. Gaz. *1*, 617 (1845).

[2] J. MELLANBY, Proc. Roy. Soc. London, s. B *107*, 271 (1930); Proc. Roy. Soc. London, s. B *113*, 93 (1933). – W. H. SEEGERS, J. Biol. Chem. *136*, 103 (1940). – W. H. SEEGERS and D. A. MCGINTY, J. Biol. Chem. *146*, 511 (1942). – H. P. SMITH, Ann. Rev. Physiol. *4*, 245 (1942). – W. H. SEEGERS, E. C. LOOMIS and J. M. VANDENBELT, Arch. Biochem. *6*, 85 (1945).

[3] S. P. L. SØRENSEN, C. r. Trav. Lab. Carlsberg *18*, N° 5 (1930). – L. F. HEWITT, Biochem. J. *30*, 2229 (1936); *31*, 360 (1937). – R. M. FERRY and J. L. ONCLEY, J. Amer. Chem. Soc. *60*, 1123 (1938). – J. A. LUETSCHER, Jr., J. Amer. Chem. Soc. *61*, 2888 (1939). – T. L. MCMEEKIN, J. Amer. Chem. Soc. *62*, 3393 (1940).

[4] E. J. COHN, Proc. Amer. Philos. Soc. *88*, 159 (1944).

[5] Characteristic of the various suggested blood substitutes which were examined, but not recommended to the Armed Forces.

[6] J. L. ONCLEY, G. SCATCHARD, and A. BROWN, J. Phys. and Coll. Chem. *51*, 184 (1947).

[1] E. J. COHN, Chem. Rev. *28*, 395 (1941); Trans. and Studies of Coll. of Phys. of Philadelphia *10*, 149 (1942); Medicine *24*, 333 (1945).

[2] This figure is revised from E. J. COHN, Amer. Scientist *33*, 61 (1945) on the basis of the molecular dimensions given in Table I.

[3] E. J. COHN, Proc. Amer. Philos. Soc. *88*, 159 (1944). – G. SCATCHARD, A. C. BATCHELDER, and A. BROWN, J. Clin. Invest. *23*, 458 (1944).

[4] T. SVEDBERG and K. O. PEDERSEN, The Ultracentrifuge. Clarendon Press, Oxford 1940.

[5] P. VON MUTZENBECHER, Biochem. Z. *266*, 226 (1933); *ib. 266*, 250 (1933); *ib. 266*, 259 (1933). – A. S. MCFARLANE, Biochem. J. *29*, 407, 660, 1175, 1202 (1935). – K. O. PEDERSEN, Ultracentrifugal Studies on Serum and Serum Fractions. Almqvist and Wiksells Boktryckeri AB, Uppsala, Sweden, 1945. – J. L. ONCLEY, G. SCATCHARD, and A. BROWN, J. Phys. and Coll. Chem. *51*, 184 (1947).

Table I
Physical Properties of certain separated Plasma Proteins*

	Concentrated in Fraction	Sedimentation Constant	Partial Specific Volume	Intrinsic Viscosity	Molecular Weight	Approximate Dimensions in Å	
		$S_{20,w}$	V	$H_0 \cdot 10^2$	M	Diameter	Length
Serum Albumin	V	4·6	0·733	4·2	69,000	38	150
β_1-Metal-Combining Protein	IV-7	5·5	0·725	5·5	90,000	37	190
γ-Globulin	II	7·2	0·739	6·	156,000	44	235
Fibrinogen	I	9·		25·	400,000	38	700
α_1-Lipoprotein**	IV-1	5·	0·841	6·6	200,000	50	300
β_1-Lipoprotein**	III-0	X	0·950	4·1	1,300,000	185	185

* The values in this table are taken from [1].

** The α-lipoproteins contain 35% lipid, and the β_1-lipoprotein, 75% lipid. The high lipid content explains the high values for partial specific volume. Osmotic pressure studies suggest apparent molecular weights lower than the values recorded here, and considerable leakage through the membranes used, which were tight to most plasma proteins. This observation has been interpreted as indicating an equilibrium between some of the lipid and the lipoprotein.

bumins and certain globulins. The other globulins which have been separated and fibrinogen sediment with other velocities. However, ultracentrifugal analysis does not permit us to distinguish a large number of components in the plasma.

Electrophoretic Mobilities of Plasma Proteins. Solubility studies during the nineteenth century indicated that there were many globulins and classified them as euglobulin or pseudoglobulin, depending upon their insolubility or solubility, in the absence of salt, or in concentrated salt solutions. Electrophoretic analysis, refined by TISELIUS, distinguished serum globulins in terms of their mobility in an electric field and designated them α-, β-, and γ-globulins[2]. Better resolution, by the optical system employed in the analysis, has revealed more than one α-, more than one β-, and more than one γ-globulin. Two α-globulins, α_1- and α_2-, two β-globulins, β_1- and β_2-, and two γ-globulins, γ_1- and γ_2-, the second one, in animals, sometimes termed a T-globulin, are now often designated. If we include the results of electrophoretic analyses the number of protein components of plasma recognized by physico-chemical means has thus increased to nine or more from the three recognized a century ago.

Chemical Interactions of Plasma Proteins

Hormones. A very large number of protein components of the plasma has been postulated on the basis of physiological or immunological properties. Some of these have been concentrated and characterized. Others, like the hormones, are by definition components of the blood, but have rarely been separated from it. Certain of them have been detected in plasma, or in a plasma fraction[1]. Our chemical knowledge of protein hormones, however, has thus far largely been derived from the study of those separated from glandular extracts. Whether their molecular states in the gland are the same as those in which they are released into and transported by the blood stream remains, in most cases, to be determined. In the case of the steroid hormones, the relation of these water-insoluble organic molecules to the lipoproteins of the plasma renders possible the study of the state of steroids in the blood.

Lipoproteins. Recent investigations have demonstrated the presence of different lipoproteins in the plasma, moving in the electric field respectively with the mobilities of α_1- and β_1-globulins. One of these lipoproteins is an asymmetrical molecule with a molecular weight of roughly 200,000. Another is a large spherical lipoprotein with a molecular weight of over a million[2]. These lipoproteins are noteworthy both because of their physical properties and because they render soluble such water-insoluble lipids as cholesterol, carotene and the steroids and because the specificity of their interactions is such that one of the estrogen hormones, estriol, has been found to be combined not with all, but only with one of these lipoproteins, the large spherical β_1-lipoprotein[3].

Immunoproteins. Immunological studies have led to the recognition and the study of complement and

[1] J. L. ONCLEY, G. SCATCHARD, and A. BROWN, J. Phys. and Coll. Chem. *51*, 184 (1947).

[2] A. TISELIUS, Trans. Faraday Soc. *33*, 524 (1937). – L. G. LONGSWORTH, Chem. Rev. *30*, 323 (1942). – S. H. ARMSTRONG, Jr., M. J. E. BUDKA and K. C. MORRISON, J. Amer. Chem. Soc. *69*, 416 (1947).

[1] E. J. COHN, J. L. ONCLEY, L. E. STRONG, W. L. HUGHES, Jr., and S. H. ARMSTRONG, Jr., J. Clin. Invest. *23*, 417 (1944).

[2] J. L. ONCLEY, G. SCATCHARD, and A. BROWN, J. Phys. and Coll. Chem. *51*, 184 (1947).

[3] C. M. SZEGO and S. ROBERTS, Proc. Soc. Exper. Biol. and Med. *61*, 161 (1946). – S. ROBERTS and C. M. SZEGO, Endocrinology *39*, 183, (1946).

its components[1], and of a variety of antibodies[2]. Antibodies have been characterized, as euglobulins and as pseudoglobulins, in terms of their solubilities. Many, but not all, antibodies have been characterized as γ-globulins in terms of their electrophoretic mobilities. Among γ-globulins some have been described of very high molecular weight, others of molecular weights of the order of 156,000. For the most part, however, these immuno-chemical studies have not led to the isolation of pure antibodies of which there could conceivably be as many as the antigens which have led to the production by the body of specific antibodies (Table II).

Enzymes. Prothrombin is presumably present in the body in but small amount and the action of thrombin is now generally regarded as enzymatic. The presence of a large number of other enzymes has since been demonstrated by virtue of their sepcific interactions. Thus there is the proteolytic, fibrinolytic, enzyme now called plasmin, a well-defined serum esterase, two phosphatases, a lipase, an amylase, and a number of peptidases, among them hypertensinase. Most of these substances are far better known in terms of their chemical interactions than of their molecular properties.

Albumins. Until recently the greatest emphasis has been upon the osmotic function of the albumins in maintaining the equilibrium between water and electrolytes in the blood and the tissues. The development of normal human serum albumin for use in military medicine, for the treatment of shock, burns and hyperproteinemia, depended upon the molecular properties of albumin. However, as BENNHOLD[3] and later investigators[4] suggested, albumins interact with a variety of smaller molecules, notably with non-polar anions such as aliphatic fatty acids[5] and are presumably responsible for their transport in the blood stream. Albumins also interact with a variety of dyes[3] including Evan's blue, often used in estimating blood volume[6] with naphthaquinones[7] such as those developed as anti-malarials[8] and with a variety of other dyes[9]. Albumins also combine with a variety of drugs, such

[1] L. PILLEMER, E. E. ECKER, J. L. ONCLEY, and E. J. COHN, J. Exper. Med. *74*, 297 (1941). – L. PILLEMER, Chem. Rev. *33*, 1 (1943). – M. HEIDELBERGER, Amer. Sci. *34*, 597 (1946).

[2] J. F. ENDERS, J. Clin. Invest. *23*, 510 (1944).

[3] H. BENNHOLD, E. KYLIN, and S. RUSZNYAK, Die Eiweißkörper des Blutplasmas. Theodor Steinkopff, Dresden und Leipzig 1938.

[4] R. J. DUBOS and B. D. DAVIS, J. Exper. Med. *83*, 409 (1946).

[5] G. A. BALLOU, P. D. BOYER, J. M. LUCK, and F. G. LUM, J. Clin. Invest. *23*, 454 (1944); J. Biol. Chem. *153*, 589 (1944). – P. D. BOYER, G. A. BALLOU, and J. M. LUCK, J. Biol. Chem. *162*, 199 (1946). – P. D. BOYER, F. G. LUM, G. A. BALLOU, J. M. LUCK, and R. G. RICE, J. Biol. Chem. *162*, 181 (1946).

[6] R. A. RAWSON, Amer. J. Physiol. *138*, 708 (1943).

[7] The specificity of the interaction of albumins with certain dyes and naphthaquinones has led to preliminary studies by L. E. STRONG and D. MITTELMAN on their use in estimating serum albumins by a colorimetric method.

[8] L. FIESER and H. HEYMANN, Publication in press.

[9] I. M. KLOTZ, J. M. LONGFELLOW, and O. H. JOHNSON, Science *104*, 264 (1946). – I. M. KLOTZ, F. M. WALKER, and R. B. PIVAN, J. Amer. Chem. Soc. *68*, 1486 (1946). – I. M. KLOTZ, J. Amer. Chem. Soc. *68*, 2299 (1946).

as atabrin, neosalvarsan[1], and digitoxin[2], mercurials[3] and sulfa drugs[4].

Crystallized Human Serum Albumins. Not all of the porperties that have been ascribed to the albumins are due to these molecules. In order to demonstrate this, it was necessary to prepare highly purified crystallized human serum albumins[5].

Human serum albumins that had been crystallized by earlier methods were demonstrated to contain over two per cent of long chain fatty acid[6]. The albumins that we have crystallized, in very satisfactory yield, from alcohol-water mixtures of defined p_H and ionic strength at low temperatures also contained fatty acid, but the amounts present were far smaller; of the order of one mole stearic acid per mole of albumin. The fatty acid appeared to form an integral part of the crystal structure and, in fact, crystallization has been demonstrated to be greatly aided by the presence of such amounts of fatty acid and by the addition of higher alcohols such as n-decanol. The amounts of the alcohol that have been found useful and with which the albumins combine range from two to ten moles per mole. Crystallized with the aid of such reagents, albumins can be recrystallized under a variety of physical chemical conditions and in a variety of crystal forms.

The resolution of the various albumins that crystallize together required a more specific method of crystallization in order to yield chemical individuals. Conditions for crystallizing horse serum albumin of constant solubility had been determined in our laboratory before the war by MCMEEKIN[7]. This method has thus far not been found effective for crystallizing a fraction of human serum albumin. However, a fraction of the human serum albumins crystallized by the decanol method has been found by W. L. HUGHES, Jr., to form a relatively insoluble crystalline mercury compound (see Figure 2 and Reference [8]). The albumin separated in this way appears to be a chemical individual whose solubility behavior approximates to that of a simple chemical substance.

Although serum albumins combine with a larger number of equivalents of mercury the amount with the albumin in the solid phase is but one-half a mole of mercury per mole albumin. That is to say, each mole of mercury appears to be combined with two albumin molecules in the solid state. In solution,

[1] H. BENNHOLD, E. KYLIN, and S. RUSZNYAK, Die Eiweißkörper des Blutplasmas. Theodor Steinkopff, Dresden und Leipzig 1938.

[2] A. FARAH, J. Pharm. and Exper. Therapeutics *83*, 143 (1945).

[3] L. M. WOODRUFF and S. T. GIBSON, unpublished observations.

[4] B. D. DAVIS, J. Clin. Invest. *22*, 753 (1943). – G. DEROUAUX, Acta Biol. Belgica *3–4*, 170 (1943).

[5] E. J. COHN and W. L. HUGHES, Jr., J. Amer. Chem. Soc., in press.

[6] F. E. KENDALL, J. Biol. Chem. *138*, 97 (1941).

[7] T. L. MCMEEKIN, J. Amer. Chem. Soc. *61*, 2884 (1939).

[8] E. J. COHN, Advances in Military Medicine: The History of the Committee on Medical Research, Chapter 28, Plasma Fractionation, in press.

Table II

Protein Components of Human Plasma separated and concentrated in Diverse Fractions

Protein Component		Estimated Amount in 100 g. Plasma Protein gms	Concentrated in Fraction[4]	Approximate Isoelectric Point	Specific Chemical Interaction
Fibrinogen		4	I-2	5·3	Thrombin
Non-Clottable Protein, insoluble at low temperature		0·15	I-1		
Antihemophilic Globulin[1]			I		
Antibody γ-Globulins	Diphtheria Antibodies[1]	(0·001)			
	Measles Antibodies[1]				
	Mumps Antibodies[1]				
	Streptococci Antitoxin[1]	11	II	7·3	Antigens
	Influenza Antibodies[1]				
	Pertussis Antibodies[1]				
	Typhoid "H" Agglutinins[1]				
Antibody Euglobulins	Typhoid "O" Agglutinins[1]		III-1	6·3	Antigens
Isoagglutinins	Anti-A, Anti-B[1]	(0·03)			
	Anti-Rh Antibodies[1]		III-1		Incompatible Red Blood Cells
Complement Components	C'1	0·4	III-2[2]		Antigen-Antibody Complex
	C'2		IV[2]		
Enzyme Precursors	Prothrombin	0·3	III-2		Thromboplastin
	Plasminogen		III-3		Streptokinase
Serum Enzymes	Thrombin[1]		III-2	4·8	Fibrinogen
	Plasmin[1]		III-3		Proteins
	Amylase[1]				Starch
	Lipase[1]				Lipid
	Peptidase[1]		IV		*l*-Leucylglycylglycine
	Phosphatase[1] (alkaline)		IV[2]		Phosphoric Acid Monoesters
	Esterase[1]	0·02	IV-6	4·5	Acetyl Choline, Ethylbutyrate
Metal-Combining β_1-Pseudoglobulin: crystallized		2·5	IV-7	5·6	Iron and Copper
High Molecular Weight β_1-Globulins (Lipid-poor)	S = 7	2	III-0		
	S = 20	1	III-0		
Iodoprotein[1,3]			IV-6		
Thyrotropic Hormone[1]			IV-4		
Glycoproteins	α_2-Glyco-pseudoglobulin	0.7	IV-6	4·9	
	α_2-Mucoid Globulin	0·5	IV-6	4·9	
Lipoproteins	β_1; 75% Lipid-Containing "X"-Protein	5	III-0	5·6	Estriol, Carotenoids, and other Steroids
	α_1; 35% Lipid-Containing Protein	3	IV-0	5·2	Steroids
Blue-Green Pigment α-Globulin			IV-2		
Bilirubin-Containing α_1-Globulin[3]		0·05	V-1	4·7	Diazo-reaction
Albumin: Crystallized with Mercury			V	4·9	Mercury, Decanol
Albumin: Crystallized with Decanol		50	V	4·9	Fatty Acids, Bile Salts, many Dyes and Drugs

[1] These components represent but small proportions of the fraction and subfraction, and their properties cannot, therefore, be deduced from those of the concentrates in which they have been separated.

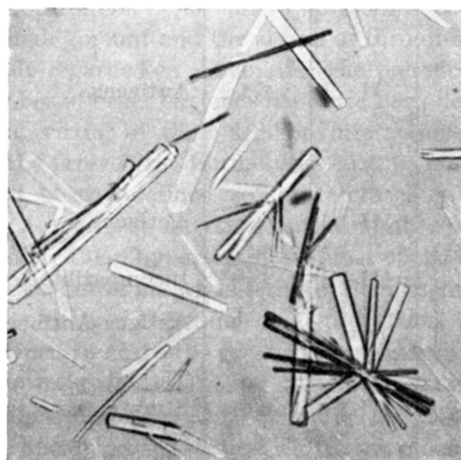
[2] These components have not been tested for since revision of the fractionation process.

[3] Albumin binds more bilirubin than the bilirubin pigment globulin in Fraction V-1 and more iodine than has been found in Fraction IV-6.

[4] When purified chemical components have been separated from fractions they have not been given new fraction numbers. In that case, the fraction number refers to the starting material for the separation of the component.

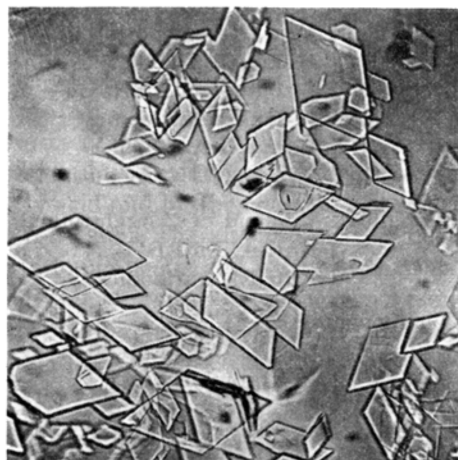
however, this complex dissociates, more near the isoelectric point than in neutral solution where albumin of double molecular weight may be demonstrated in the ultracentrifuge.

Pigment Proteins. Serum albumin had previously been reported to combine with hematin and with bilirubin[1]. Upon adequate recrystallization, the amount of both diminish until they can no longer be readily detected spectrophotometrically. Upon equilibrating such pure serum albumin with these substances, however, combination can be demonstrated and quantitatively estimated. Albumin which we have recrystallized has been studied in equilibrium with hematin[2] and with bilirubin[3]. At alkaline reaction albumin combines with as much as three moles bilirubin per mole albumin. At acid reactions, however, the bilirubin is free and can be removed by dialysis.



a

Ethanol 25 % Γ/2 0·1
Decanol 0·1 % p_H 5·5



b

Ethanol 10 % Γ/2 0·05
$HgCl_2$ 0·01 % p_H 5·5

Fig. 2*a*, *b*. Crystalline Human Serum Albumins

Bilirubin is also a component of a true pigment protein of the blood stream, normally present to less than a tenth of a per cent of the plasma proteins. This bilirubin pigment protein interacts strongly and is, therefore, separated with difficulty from the 50 per cent of albumin in plasma. It has now been separated, however, and alone of adequately purified plasma proteins, gives the indirect van den Bergh reaction[4]. Another pigment protein responsible for a very characteristic blue-green color does not give this reaction and although often found associated with crude albumin preparations is a globulin concentrated in our system of fractionation in Fraction IV-1, whereas the yellow pigment, due to bilirubin, is concentrated in Fraction V-1.

Iodoproteins. Iodine combines with essentially all proteins, entering the phenol ring to form diiodotyrosine and also entering the imidazol ring. Albumin rich in iodine has been prepared and crystallized by SALTER from horse serum[1]. In studying the distribution of iodine in the human plasma fractions that we have separated, some has always been found with the albumin, some, however, has been found in Fraction IV-6. The further study of the iodoprotein in these fractions should reveal more regarding the nature of the plasma molecules of which it is a part.

Metal-Combining Proteins. It has long been known that copper, iron and zinc are combined by plasma protein. The separation of the plasma proteins into fractions in which are concentrated the molecules responsible for specific interactions has yielded, in Fraction IV-7, the β_1-globulin responsible for the combination and transport of copper and iron and perhaps of zinc in the plasma. The close interrelation of the copper and iron in plasma had been noted in clinical studies[2]. The combination of a component of plasma with iron was noted in connection with bacterial studies[3] and has led to its identification in a plasma fraction and further purification and characterization in our laboratory[4]. Physiological studies of the role of the

[1] H. BENNHOLD, E. KYLIN, and S. RUSZNYAK, Die Eiweißkörper des Blutplasmas. Theodor Steinkopff, Dresden und Leipzig 1938.
[2] M. ROSENFELD, personal communication.
[3] N. H. MARTIN, in preparation.
[4] H. T. MALLOY and K. A. EVELYN, J. Biol. Chem. *119*, 481 (1937).

[1] A. M. BASSETT, A. H. COONS, and W. T. SALTER, Amer. J. Med. Sci. *202*, 516 (1941).
[2] L. HEILMEYER and G. STÜWE, Klin. Wschr. *17.2*, 925 (1938). – L. HEILMEYER, W. KEIDERLING, and G. STÜWE, Kupfer und Eisen als körpereigene Wirkstoffe. Gustav Fischer, Jena 1941. – L. HEILMEYER, Handbuch der Inneren Medizin, Vol. II, Blutkrankheiten, Chapter III. Springer-Verlag, Berlin 1942.
[3] A. L. SCHADE and L. CAROLINE, Science *104*, 340 (1946).
[4] After this manuscript was written, but before the address was given, this metal-combining protein was crystallized in our laboratory in collaboration with B. A. KOECHLIN.

separated protein injected into man have begun[1] with a view to determining its function and possible value in therapy.

Clinical Studies upon Plasma Proteins

As each component of plasma has been prepared in a satisfactory state with respect to concentration, stability, and safety for injection in man, clinical studies have begun to determine its uses in therapy. Let us review briefly the knowledge that has been gained thus far with the various products that have been prepared and used in large amounts.

Normal Human Serum Albumin: Shock: The first clinical studies with normal human serum albumin were aimed at defining its physiological action and value in the treatment of shock. Since it was prepared in concentrated solution for convenience in shipping and storage under military conditions, it was first necessary to prove that its injection in large amount was followed by no untoward reactions and resulted in a shift of fluid across the capillary membrane just as occurred across the artificial membrane in an osmometer. After removing a measured amount of blood from human subjects, the increase in plasma volume which followed the injection of a known amount of concentrated serum albumin was determined. In experiments with volunteers, most of whom were Harvard Medical students, the average increase one hour after injection was found to be 17·4 cc. per gm[2], as compared with the 18 cc. per gm[3] predicted from osmotic pressure measurements. With such excellent agreement between results obtained *in vitro* and *in vivo*, clinical trial of this new product was placed on a firm quantitative basis.

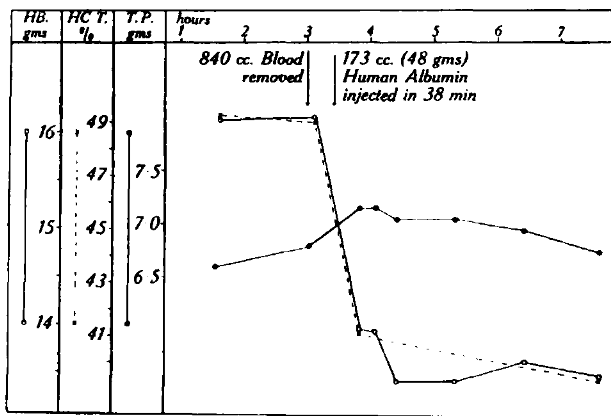


Fig. 3. Changes in the values for the hematocrit, hemoglobin, and plasma protein (falling drop method) in a normal human subject, following a large venesection and the subsequent administration of a concentrated human albumin solution[4].

Table III
Effect of concentrated Albumin in Shock
Plasma Volume Increase observed in Patients with Measurements made within 1 ½ Hours of Injection of Albumin

Type of Case	No. Cases	Plasma Volume Increase cc./gram of injected Albumin: Spread	Average
Calculated from Osmotic Pressure Measurements[1]			18
Experimental Hemorrhage in Man[2]	11	13·2–24·1	17·4
Clinical Shock* [3] (No additional saline or small amount)	63	0–31·7	11·7
Clinical Shock* [3] (Additional saline)	20	7–29	17·9

* Newer unpublished, as well as previously published, values[3] are included in this table.

Clinical experience in a number of centers soon demonstrated that albumin could be safely and effectively used also in cases of hemorrhage, trauma, and burns to produce an increase in plasma volume and to bring about clinical improvement. The initial evaluation of albumin in the treatment of shock, led to a report with instruction for its use in May, 1942[4]. In extreme dehydration, as well as in some cases of shock, adequate fluid must be made available, by some route, if maximum hemodilution is to be achieved.

Salt-poor Serum Albumin: Hypoproteinemia. The effect of human serum albumin on hypoproteinemia was next explored, and its safety when administered repeatedly over long periods established[5]. Interest in the use of albumin in the treatment of hypoproteinemic edema, particularly in renal disease, stimulated the development of salt-poor albumin[6], which was made possible by the studies of the stabilization of albumin

[1] C. A. FINCH, personal communication. – C. MOORE, personal communication.

[2] J. T. HEYL, J. G. GIBSON 2nd, and C. A. JANEWAY, J. Clin. Invest. *22*, 763 (1943). – J. T. HEYL and C. A. JANEWAY, U. S. Nav. M. Bull. *40*, 785 (1942).

[3] G. SCATCHARD, A. C. BATCHELDER, and A. BROWN, J. Clin. Invest. *23*, 458 (1944).

[4] Reproduced from "Blood Substitutes and Blood Transfusion", Chapter XXI, C. A. JANEMAY, from data of E. A. STEAD, Jr., and R. V. EBERT, CHARLES C. THOMAS, Publisher, Springfield, 911 (1942).

[1] G. SCATCHARD, A. C. BATCHELDER, and A. BROWN, J. Clin. Invest. *23*, 458 (1944).

[2] J. T. HEYL, J. G. GIBSON, 2nd, and C. A. JANEWAY, J. Clin. Invest. *22*, 763 (1943).

[3] A. COURNAND, R. P. NOBLE, E. S. BREED, H. D. LAUSON, E. DE F. BALDWIN, G. B. PINCHOT, and D. W. RICHARDS, Jr., J. Clin. Invest. *23*, 491, 504 (1944).

[4] L. M. WOODRUFF and S. T. GIBSON, Report to the Subcommittee on Blood Substitutes, Division of Medical Sciences, National Research Council, May 11, 1942; U. S. NAV. M. Bull. *40*, 791 (1942).

[5] C. A. JANEWAY, S. T. GIBSON, L. M. WOODRUFF, J. T. HEYL, O. T. BAILEY, and L. R. NEWHOUSER, J. Clin. Invest. *23*, 465 (1944).

[6] G. SCATCHARD, L. E. STRONG, W. L. HUGHES, Jr., J. N. ASHWORTH, and A. H. SPARROW, J. Clin. Invest. *24*, 671 (1945).

solutions[1]. Albumin is so stable that it alters only slowly even when subjected to high temperatures[2]. By 1945 the specifications for albumin to be used either as a diuretic agent or for the treatment of shock called for a 25 per cent solution of albumin, at p_H 6·8 ± 0·2, stabilized with 0·04 moles per liter of sodium acetyltryptophanate without mercurial preservative, but heated 10 hours at 60° C in the final container. Heating for this period of time has been demonstrated to be sufficient to destroy the virus of homologous serum hepatitis[3]. Proof that salt-poor albumin was as satisfactory for the treatment of shock as the albumin solutions of higher salt content was rapidly obtained with the collaboration of our associates[4]. This very satisfactory product, rich in protein and poor in electrolyte, therefore, replaced the earlier standard albumin solution. The availability of a concentrated solution, low in sodium, of the plasma component most active in maintaining normal osmotic relationships and the one most frequently diminished in protein deficiency with edema provides a solution for parenteral use (Figure 5), which, with solutions of glucose, electrolytes, and amino acids, should make it possible to rectify most disturbances of fluid balance. Its usefulness not only in treatment but in the study of physiological disturbances in diseases such as nephrosis and cirrhosis of the liver is being abundantly demonstrated.

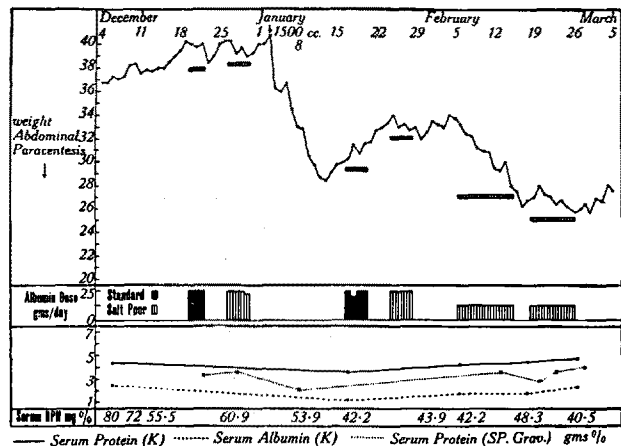


Fig. 4. Changes in body weight of a three-year-old child with nephrosis. Compare response to daily injections of salt-poor albumin (0·3 gm. of Na^+ per 100 cc.) (barred lines) with that to standard albumin (1·0 gm. of Na^+ per 100 cc.) (solid lines)[5].

[1] G. A. BALLOU, P. D. BOYER, J. M. LUCK, and F. G. LUM, J. Clin. Invest. *23*, 454 (1944); J. Biol. Chem. *153*, 598 (1944). – P. D. BOYER, G. A. BALLOU, and J. M. LUCK, J. Biol. Chem. *162*, 199 (1946) – P. D. BOYER, F. G. LUM, G. A. BALLOU, J. M. LUCK, and R. G. RICE, J. Biol. Chem. *162*, 181 (1946).

[2] G. SCATCHARD, S. T. GIBSON, L. M. WOODRUFF, A. C. BATCHELDER, and A. BROWN, J. Clin. Invest. *23*, 445 (1944).

[3] S. S. GELLIS, J. R. NEEFE, J. STOKES, Jr., G. SCATCHARD, L. E. STRONG and C. A. JANEWAY, in preparation.

[4] E. A. STEAD, Jr., and J. V. WARREN and A. COURNAND and A. LOWELL, Reports to the Subcommittee on Blood Substitutes, Division of Medical Sciences, National Research Council, March 14, 1945.

[5] G. HUTCHINS and C. A. JANEWAY, Report to Subcommittee on Blood Substitutes, Division of Medical Sciences, National Research Council, March 14, 1945.

Serum γ-Globulin: Measles Antibodies. Very early in the program it was suggested that a globulin fraction might be used for the control of measles and perhaps other infectious diseases. The first of a series of sub-

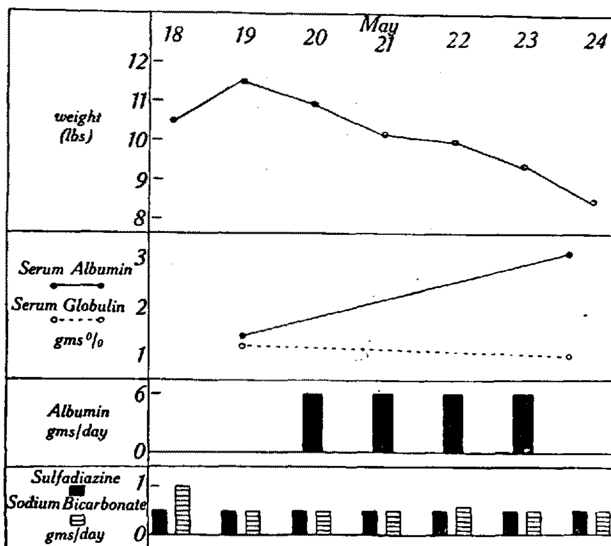


Fig. 5. Clinical course of a baby with severe hypoproteinemia, who was given concentrated human serum albumin 6·25 gm. (25 cc.) per day for four days. On May 19 the baby had severe anasarca and ascites, while on May 24 only slight edema remained about the area of infection on the forehead and scalp[1].

fractionations which yielded a highly purified, stable, concentrated preparation of the antibodies was completed in 1942. This concentrate of γ-globulins (Fraction II) contained all antibodies for which tests were made save the agglutinins against typhoid 0 antigen and the isoagglutinins found in Fraction III-1. By making a solution of Fraction II which contained 16·5 gms of γ-globulin per 100 cc., a preparation having 20–25 times the antibody titer of the original plasma has been achieved.

Table IV

Average Concentration of various Antibodies in Solutions of normal Human Serum γ-Globulins (Fraction II*)

Antibody**	Concentration referred to Plasma
Typhoid Agglutinin, H	19
Mumps, complement fixation	20
Influenza A, complement fixation .	18
Influenza A, Hirst test	10
Influenza A, mouse protection	23
Diphtheria Antitoxin	25
Streptococcus Antitoxin	22
γ-Globulin by electrophoresis	25

* Taken from[2]. ** Titrations of ENDERS[3].

[1] Taken from C. A. JANEWAY, J. A. M. A., *126*, 674 (1944).

[2] E. J. COHN, Amer. Sci. *33*, 61 (1945).

[3] J. F. ENDERS, J. Clin. Invest. *23*, 510 (1944).

The Spring of 1943 was marked by severe measles epidemics in Philadelphia, Baltimore[1] and Boston[2], and as Fraction II with its concentration of γ-globulin was developed, there was ample opportunity to prove its value, in the prevention or modification of measles, when administered in the first half of the incubation period. Large amounts of γ-globulin have since been used in measles prophylaxis. Reactions have been very few, homologous serum jaundice has not occurred, and complications in modified cases of measles have been almost non-existent[3]. These tests have proved that, provided γ-globulin is administered within eight days of exposure, the results will depend on the dose. Thus an intramuscular injection of 0·1 cc./lb. has been established as the protective dose and 0·025 cc./lb. as the modifying dose. The results in children exposed at home, with an expected attack rate of 75–85 per cent, are cited in the following table:

Table V

Relation of Dose of γ-Globulin to Results (1024 Cases: first 8 Days*)

Dose (cc./lb.)	% No Measles	% Mild Measles	% Average Measles	% Attack Rate
0·01 –0·0375	33	63·1	3·9	67
0·0375–0·075	55·2	40·6	4·2	44·8
0·075 –0·2	83·7	15·5	0·8	16·3

* Table taken from [4].

Infectious hepatitis proved to be a very serious problem during this war. No method of controlling this disease was available; hence, the importance of the finding[5,6] that it could either be prevented or modified in exposed individuals by the injection of γ-globulin. Subsequently the usefulness of γ-globulin as a means of passive protection was demonstrated during an epidemic in an institution for children[7].

Though the evidence appears to be satisfactory that γ-globulin has prophylactic value for epidemic infectious hepatitis, a more difficult problem remains in homologous serum jaundice.

[1] J. STOKES, Jr., E. P. MARIS, and S. S. GELLIS, J. Clin. Invest. *23*, 531 (1944).

[2] C. W. ORDMAN, C. G. JENNINGS, and C. A. JANEWAY, J. Clin. Invest. *23*, 541 (1944). – C. A. JANEWAY, Bull. New York Acad. Med. *21*, 202 (1945).

[3] C. A. JANEWAY, J. A. M. A. *126*, 674 (1944).

[4] C. A. JANEWAY, Bull. New York Read. Med. *21*, 202 (1945).

[5] J. STOKES, Jr., and J. R. NEEFE, J. A. M. A. *127*, 144 (1945).

[6] Capt. S. S. GELLIS, J. STOKES, Jr., Maj. G. M. BROTHER, Maj. W. M. HALL, Col. H. R. GILMORE, Maj. E. BEYER, and Capt. R. A. MORRISSEY, J. A. M. A. *128*, 1062 (1945).

[7] Maj. W. P. HAVENS, Jr., and J. R. PAUL, J. A. M. A. *129*, 270 1945).

Table VI

Summary of Results with γ-Globulin in Prevention of Infectious Hepatitis

Study	Given Globulin			Controls		
	Number	Probable Hepatitis	Jaundice	Number	Probable Hepatitis	Jaundice
STOKES and NEEFE[1], U.S.A. (Pennsylvania)	53	21%	6%	278	67%	45%
GELLIS, STOKES, BROTHER, HALL, GILMORE, BEYER and MORRISSEY[2] (Mediterranean Theater of Operations)	406 831 495	— — —	1% 0·4% 0·4%	683 8270 1373	— — —	3·7% 3·4% 2 %
HAVENS and PAUL[3] (Connecticut)	97	8%	2%	155	34%	23%

Mumps. The antibodies against mumps in pooled normal human plasma are concentrated in Fraction II as judged by complement-fixation[4]. Though concentrated 20-fold with respect to the plasma, the use of this concentrate in the prophylaxis or treatment of mumps proved completely ineffective.

In mumps and scarlet fever, unlike measles and poliomyelitis, in which the antibodies are reported to be elevated in convalescence only a few fold, they are elevated 10 to 30-fold. The possibility of preparing far more concentrated antibody preparations against mumps and scarlet fever therefore is far greater if convalescent or hyperimmune, rather than pooled plasma, is fractionated.

Fractionation of mumps convalescent plasma yielded γ-globulin 200 to 300 times as concentrated in the mumps complement-fixing antibodies as normal plasma. Though its prophylactic value has not been proved, its effect in lowering the incidence of orchitis when administered on the first day of disease indicated that it contained adequate amounts of antibody[5].

Other diseases. Trial of γ-globulin in other diseases to which adults are imune has revealed a number in which it appears to be ineffective (see Table VII). These

[1] J. STOKES, Jr., and J. R. NEEFE, J. A. M. A. *127*, 144 (1945).

[2] Capt. S. S. GELLIS, J. STOKES, Jr., Maj. G. M. BROTHER, Maj. W. M. HALL, Col. H. R. GILMORE, Maj. E. BEYER, and Capt. R. A. MORRISSEY, J. A. M. A. *128*, 1062 (1945).

[3] Maj. W. P. HAVENS, Jr., and J. R. PAUL, J. A. M. A. *129*, 270 (1945).

[4] J. F. ENDERS, J. Clin. Invest. *23*, 510 (1944).

[5] S. S. GELLIS, A. C. McGUINNESS, and M. PETERS, Amer. J. Med. Sci. *210*, 661 (1945).

include prevention of chicken pox and infantile diarrhea[1]. Routine administration of globulin to alternate premature babies in a large hospital nursery failed to lower mortality or morbidity in comparison with the controls[2]. On the other hand favorable results have been reported when serum albumin was so administered[3].

Table VII

Summary of Results with Normal Serum γ-Globulin in Common Diseases

Disease	Immunity in Early Infancy	Value of Globulin	
		Prevention	Treatment
Measles	+	+	±
Infectious Hepatitis	–	+	0
Mumps	+	0	0
Scarlet Fever	+	S	S
Poliomyelitis	+	–	0
German Measles	+	S	–
Chicken Pox	0	0	–
Infantile Diarrhea	0	0	0
Homologous Serum Jaundice	–	S	–

Key: + Effective ± Questionable 0 Ineffective – Not known S Under Study.

Blood Typing Globulins: Isoagglutinins. The anti-A and anti-B isoagglutinins of the plasma were concentrated in Fraction III-1. Plasma from donors of the desired groups was fractionated and the isoagglutinins, concentrated in this fraction, proved quite satisfactory, highly concentrated, typing globulins[4].

Presently it was found, however, that random pools of group 0 plasma yield a better source of anti-A agglutinin than group B plasma, and that the anti-B activity of group 0 plasma could be removed by mixing group 0 and group B blood. Since 0 donors are encountered much more frequently than B donors, this method also makes far greater amounts of blood grouping globulins available[5].

Anti-Rh antibodies. Besides the naturally occurring isoagglutinins, other isoagglutinins may on some occasions arise through the immunization of human subjects. These antibodies include the isoimmune anti-A and anti-B agglutinins, produced in response to injection, and the class known as the anti-Rh antibodies, sometimes produced after injection, or sometimes arising during pregnancy, and directed against the "Rh factors", which are present in most human red cells, independent of the A and B factors. The concentration and processing of anti-Rh reagents was particularly necessary because of the increasing importance of Rh typing in the prevention of obstetrical accidents as well as hemolytic transfusion reactions.

[1] R. H. HIGH, N. A. ANDERSON, and W. E. NELSON, in preparation.

[2] L. K. SWEET, M. J. HOWELL, L. G. MCMURRAY, Lt. W. A. MCMAIN, Jr., and Lt. G. J. ANTELL, in preparation.

[3] L. K. SWEET, personal communication.

[4] L. PILLEMER, J. L. ONCLEY, M. MELIN, J. ELLIOTT, and M. C. HUTCHINSON, J. Clin. Invest. *23*, 550 (1944). – E. L. DEGOWIN, J. Clin. Invest. *23*, 554 (1944).

[5] M. MELIN, J. Clin. Invest. *24*, 662 (1945).

The method of concentrating the anti-Rh antibodies in Fraction III-1 was in most respects similar to that used for the anti-A and anti-B isoagglutinins[1]. However, there were additional difficulties resulting from the specificities of the serums, the greater chemical and physical instability of the anti-Rh agglutinins, the neutralization of the naturally-occurring anti-A and anti-B isoagglutinins, and finally the production of a stable, bacteria-free, specific anti-Rh reagent. These difficulties were overcome by serum albumin as a diluent[2], and it is now possible to produce useful anti-Rh reagents in larger quantity and of much better quality than were heretofore available.

Substances concerned in Blood Clotting. The great potential value for clinical use, in many conditions, of prothrombin[3], thrombin[4], fibrinogen and other components of the clotting mechanism was recognized at the very beginning of our fractionation of human plasma[5].

Fibrinogen, present in human plasma at a concentration of approximately 2·5 gms/l, clots readily and is much more susceptible to denaturation than most blood proteins. Most of the fibrinogen of plasma was concentrated in Fraction I which was preserved in the dried state and readily dissolved on addition of water to form stable solutions. Fibrinogen can readily be further purified, preparations having been obtained in which over 98 per cent of the protein was clottable. The degree of purification and the concentration at which it is dissolved and made available may thus be determined by the uses to which it is to be put.

Prothrombin and Thrombin. Prothrombin is far more difficult to separate in pure form. It is present in but small amount[6]. Its activity rapidly declined in the presence of whole fresh plasma and of certain plasma fractions. Antithrombic activity appeared to be concentrated most strongly in the lipoprotein in Fractions III-0 and IV-1. Fraction III-2, though largely β-glob-

[1] J. L. ONCLEY, M. MELIN, J. W. CAMERON, D. A. RICHERT, and L. K. DIAMOND, Ann. N. Y. Acad. Sci. *46*, 899 (1946).

[2] JAMES W. CAMERON and LOUIS K. DIAMOND, J. Clin. Invest. *24*, 793 (1945). – L. K. DIAMOND and R. L. DENTON, J. Lab. and Clin. Med. *30*, 821 (1945).

[3] J. MELLANBY, Proc. Roy. Soc. London, s. B *107*, 271 (1930). – W. H. SEEGERS, H. P. SMITH, E. D. WARNER, and K. M. BRINKHOUS, J. Biol. Chem. *123*, 751 (1938).

[4] W. H. SEEGERS and D. A. MCGINTY, J. Biol. Chem. *146*, 511 (1942).

[5] J. T. EDSALL, R. M. FERRY, and S. H. ARMSTRONG, Jr., J. Clin. Invest. *23*, 557 (1944).

[6] Estimated to be 0·2 gm/l in beef blood[7].

[7] W. H. SEEGERS, E. C. LOOMIS, and J. M. VANDENBELT, Arch. Biochem. *6*, 85 (1945).

ulin[1], was relatively free of β-lipoproteins, and rich in prothrombin. This prothrombin was promptly converted to thrombin by the addition of calcium ion and tissue thromboplastin. It was considered of importance that no protein of non-human origin be introduced into our preparations. As a human thromboplastin, the tissue globulin from placental extract was found to be highly satisfactory[2]. Thrombin so prepared could be further subfractionated, or could be filtered and dried directly from the frozen state, to yield a human thrombin preparation of adequate potency and purity for clinical purposes. Although such preparations lost most of their thrombic activity within two or three days in the dissolved state at room temperature, they have remained essentially unchanged in the dry state even after heating at 50° C for two years.

Structure of the Fibrin Clot. Conditions controlling the mechanical and chemical properties of fibrin clots have been determined. These properties are profoundly modified by variations in p_H, ionic strength, temperature, thrombin and fibrinogen concentration[3]. One extreme type of clot formed at neutral or slightly alkaline p_H was transparent, gelatinous, friable, and crumbly, with very low tensile strength and elongation. Another type of clot formed near p_H 6·3 was opaque, doughy, non-friable, and synerized easily with extrusion of water and enormous decrease of volume; yielding a clot of high tensile strength.

Both types of clot consist of networks of fibers. In the clear clot the fibers consist of individual chain molecules, whereas in the opaque type they are bundles of molecules aggregated side by side. These studies of the properties of fibrin clots determined the optimum conditions for preparing fibrin film[4].

Fibrin Film as a Dural Substitute. Fibrin film has been successfully used to replace dura which had been removed, whether or not the underlying tissue had suffered injury[5]. Adhesions between the dura and the underlying tissue were not observed. The film slowly disappeared in the course of several months and was replaced by a neomembrane of fibrous tissue. "Fibrin film has proved more satisfactory than any other material tested as a dural substitute. In addition to its safety, fibrin film is well adapted for use in the repair of dural defects because of its translucence, flexibility, ease of handling, and adaptability to any contour". (See p. 684 in reference [6].)

[1] E. J. COHN, J. L. ONCLEY, L. E. STRONG, W. L. HUGHES, Jr., and S. H. ARMSTRONG, Jr., J. Clin. Invest. *23*, 417 (1944).

[2] R. C. ELEY, A. A. GREEN, and C. F. MCKHANN, J. Pediat. *8*, 135 (1936).

[3] J. D. FERRY and P. R. MORRISON, J. Clin. Invest. *23*, 566 (1944); J. Amer. Chem. Soc. *69*, 388 (1947).

[4] J. D. FERRY and P. R. MORRISON, J. Amer. Chem. Soc., *69*, 400 (1947). – J. D. FERRY, M. SINGER, P. R. MORRISON, J. D. PORSCHE, and R. L. KUTZ, J. Amer. Chem. Soc., *69*, 409 (1947).

[5] O. T. BAILEY and F. D. INGRAHAM, J. Clin. Invest. *23*, 597 (1944).

[6] F. D. INGRAHAM and O. T. BAILEY, J. A. M. A. *126*, 680 (1944).

Fibrin Foam and Thrombin in Hemostasis. When active and sterile preparations of human thrombin became available in February, 1942, clinical studies demonstrated that it was inadequate for the control of bleeding from larger vessels, since the clot formed by the thrombin was rapidly swept away by the further flow of blood.

The use of oxidized cellulose, as a solid matrix in conjunction with thrombin for the control of bleeding, was reported in 1943[1]. The possibility of making a similar solid matrix from one of the constituents of human plasma occurred to us. This would have the advantage that the product would not be in any sense a foreign body, but actually a protein of human origin[2]. HARVEY CUSHING over 30 years ago suggested the use of fibrin for hemostasis, and preliminary experiments were made in this direction during the first world war. CUSHING was, of course, responsible for the wide-spread use of small pieces of muscle as hemostatic agents, which proved by far the most effective general technique for hemostasis in neurosurgery up to the beginning of 1943.

The use of fibrin foam and thrombin produced prompt and effective hemostasis in many conditions. "Its most obvious sphere of usefulness is that of the control of oozing from small vessels. The use of foam has materially shortened many neurosurgical operations because the bleeding from the dura, from beneath the edges of the bone flap, and from the spinal veins can be quickly brought under control. More important, however, is the fact that fibrin foam adequately controls the oozing from the beds of tumors."

"Another field in which the fibrin foams have proved of great value is in the control of bleeding from large venous channels. When there is oozing from large superficial cerebral veins, ... such oozing may be controlled by the use of foam. It is also possible to stop bleeding from ... dural sinuses. ... In dealing with blood vessel malformations of the cerebrum, ... fibrin foam is not merely an agent to shorten neurosurgical procedures, but has succeeded where other agents have failed. Fibrin foam can hardly be expected to control bleeding from large arteries[3]."

Further studies indicated that fibrin foam and thrombin might be of use in general surgery[4]. It has been reported of value in controlling hemorrhage from the cut surface of the liver and kidney, in prostatectomies[5], gynecological and thoracic operations, tooth extractions[6], thyroidectomies, and in stopping the bleeding in hemophilia.

[1] T. J. PUTNAM, Ann. Surg. *118*, 127 (1943). – V. K. FRANTZ, Ann. Surg. *118*, 116 (1943).

[2] O. T. BAILEY and F. D. INGRAHAM, J. Clin. Invest. *23*, 597 (1944).

[3] F. D. INGRAHAM, O. T. BAILEY, and F. E. NULSEN, J. Neurosurg. *1*, 171 (1944).

[4] O. T. BAILEY, F. D. INGRAHAM, O. SWENSON, J. J. LOWREY, and E. A. BERING, Jr., Surgery *18*, 347 (1945).

[5] W. C. QUINBY and E. K. LANDSTEINER, N. E. J. M. *233*, 267 (1945).

[6] P. K. LOSCH, J. Oral Surg. *3*, 177 (1945).

Fraction I in the Treatment of Hemophilia. Several years before the war, injection of a euglobulin fraction of normal plasma had been shown to be effective in shortening the clotting time of hemophilic blood[1]. This component has been found in high concentration in Fraction I. The usual dose found effective was approximately 200 mg. of protein, most of which, of course, was fibrinogen which had been demonstrated not to be the active component in this condition.

In most hemophilic patients, the injection of 200 to 400 mg. of Fraction I from an active preparation caused the clotting time to drop nearly to normal within the first half hour and to remain lowered for a period of 4 to 6 hours or more. The clotting time rose again slowly but reinjection caused it to fall once more[2]. The results have been most striking in children.

Several hemophilic patients who required tooth extractions received injections shortly before this operation, which was then carried out without excessive bleeding. Where oozing was noted after the extraction, and it was found that the clotting time had increased, reinjection of Fraction I immediately lowered the clotting time and stopped the oozing.

In several cases where the patient was suffering from internal hemorrhage, a marked arrest of bleeding was reported as a result of the injection of Fraction I and a reduction in clotting time from the usual hemophilic level of over half an hour to a much more nearly normal level.

In addition to these favorable results, however, there have been several cases of well-established hemophilia in which the results of injection of Fraction I were negative, even when the material used had been active in other patients. Also, in some patients who had previously responded either to transfusion of blood or to the injection of Fraction I, there were periods during which the clinical response was unsatisfactory to intravenous injection of blood or the Fraction. Variability in response from patient to patient and in the same patient from time to time suggested that conditions diagnosed as hemophilia may not always be due to deficiency of the same chemical component.

Fraction I, like most of the other fractions and subfractions that have been developed, must still be considered to consist of a number of components, one of which has a very high temperature coefficient of solubility, whereas the antihemophilic globulin has a far higher thermal stability than the fibrinogen. The chemical separation of the diverse components of this fraction, however, remains to be accomplished.

[1] A. J. PATEK, Jr., and F. H. L. TAYLOR, J. Clin. Invest. *16*, 113 (1937). – E. L. LOZNER and F. H. L. TAYLOR, J. Clin. Invest. *18*, 821 (1939).

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Zusammenfassung

Die chemischen Methoden, die wir für die Trennung, Konzentrierung und Reinigung von Proteinen, Glykoproteiden und Lipoproteiden irgendeines biologischen Systems durch Fraktionierung in Alkohol–Wasser-Mischungen bei jeweils festgelegten p_H, Salz- und Eiweißkonzentrationen und bei Temperaturen unterhalb des Gefrierpunkts, die für die Verhinderung der Denaturierung notwendig sind, entwickelt hatten, führten bis jetzt zur Entdeckung und Konzentrierung von über 25 verschiedenen Eiweißkomponenten des menschlichen Plasmas. Zu diesen gehören mehrere voneinander verschiedene Albumine; Immunglobuline, welche sich in ihren physikalischen Eigenschaften und durch ihre Wechselwirkung mit Antigenen unterscheiden; Lipoproteide, welche sich durch ihre physikalischen Eigenschaften und durch ihre Wechselwirkung mit Steroiden unterscheiden; Enzyme mit Protease-, Peptidase-, Lipase-, Phosphatase und Esterasewirkung; Thrombin, Fibrinogen und das antihämophile Globulin, das an der Blutgerinnung beteiligt ist; Glykoproteide, Iodoproteide und das metallbindende Protein, welches sowohl mit Eisen als auch mit Kupfer reagiert und wahrscheinlich Transportfunktionen im Plasma auszuüben hat.

Die Zahl dieser Plasmaproteine ist viel größer als die, die durch die Elektrophorese oder durch die Ultrazentrifuge entdeckt werden können. Die chemische Fraktionierung ergab mindestens vier β_1-Globuline und mindestens zwei α_1- und zwei α_2-Globuline. Zu den α_2-Globulinen gehören mindestens zwei Arten von Glykoproteiden, zu den α_1-Globulinen das Globulin der Fraktion V-1, welches Bilirubin enthält, und das Lipoproteid in Fraktion IV-1. Zu den β_1-Globulinen gehört sowohl das carotinreiche Euglobulin, welches mit der dreifachen Menge seines eigenen Gewichts sich mit Lipoiden verbinden kann, als auch ein hochmolekulares, lipoidfreies β_1-Globulin. Diese beiden Globuline werden in der Fraktion III-0 angereichert. Auch die Fraktion III enthält β_1-Globuline mit verschiedenen molekularen Eigenschaften. Eine andere Fraktion, IV-7, welche die eisenbindende Komponente des Plasmas enthält, besteht hauptsächlich aus einem lipoidfreien β_1-Globulin und steht den Albuminen hinsichtlich seines osmotischen Verhaltens näher als irgendein anderes Globulin. Diese β_1-Globuline haben außer der Eigenschaft, elektrophoretisch nicht unterscheidbar zu sein, keine andere gemeinsam. Die lipoidbindende Plasmakomponente ist ein β_1-Euglobulin, die eisenbindende β_1-Komponente ein Pseudoglobulin. Sie unterscheiden sich durch ihre Größe, Form, Löslichkeit, chemische Zusammensetzung und Reaktionsfähigkeit und durch ihre physiologische Funktion.

Ausgehend von der Annahme, die vorderhand tatsächlich nur eine Annahme ist, daß jeder Bestandteil des menschlichen Blutes eine wichtige physiologische Funktion ausübt, und daß die Menge irgendeiner Plasmakomponente, die für die Ersatztherapie verwendet wird, so groß zu bemessen ist, daß es zu einer Wiederherstellung und nicht zu einer Störung der Konzentrationen der verschiedenen physiologisch wichtigen Plasmakomponenten kommt, versuchten wir so viele als möglich der einzelnen Eiweißkomponenten zu trennen und zu konzentrieren, um sie als spezifische, bei manchen Krankheitszuständen sich als wertvoll erweisende therapeutische Agenzien der menschlichen Gesellschaft, die das Blut zur Verfügung stellte, zum Zwecke einer möglichst wirkungsvollen und ökonomischen Verwendung zurückzugeben.