The Proteins

CHEMISTRY, BIOLOGICAL ACTIVITY, AND METHODS

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VOLUME II, PART B

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CHAPTER 21

Interstitial Proteins: The Proteins of Blood Plasma and Lymph[1,2]

BY WALTER L. HUGHES

(1) This chapter holds no claims to completeness having been developed around my particular interests. However, it is hoped that the gain in perception and in critical appraisal so achieved will offset the otherwise flagrant omissions. Fortunately, some proteins which might naturally fall in this chapter have been covered in other chapters and so will be mentioned here only in passing. These include hemocyanin (Chap. 14), and fibrin (Chap. 20). It is unfortunate, from my point of view, that omission of fibrin could not justify complete omission of the clotting process since this appears hopelessly complex at the present time. Instead, a brief description has been included centering around fibrinogen and prothrombin with references to more extended treatments. The discussion of immunity has been restricted largely to the nonimmune properties of antibodies, a more complete discussion being found in Chap. 22.

(2) While taking full responsibility for the views here expressed, I would like to express my appreciation to the many friends with whom I have spent hours of stimulating discussion, and whose ideas must naturally appear throughout this chapter. My particular thanks go to David Gitlin, Henry Isliker, John Pappenheimer and the editors.

I. Origin and General Properties of Plasma Proteins

1. Limits of Free Diffusion

The all-important purpose of a circulatory system is obviously the convection of metabolites. Simple calculation will show that diffusion processes cannot supply sufficient metabolite at distances greater than a few millimeters. Thus the diffusion distance for O_2 at normal atmospheric tension at the rate of consumption of typical mammalian tissue has been calculated as 0.2 mm.[3,4]

(3) O. Warburg, *Biochem. Z.* **142**, 317 (1923).
(4) R. W. Gerard, *Am. J. Physiol.* **82**, 381 (1927).

2. Description of Blood, Blood Cells and Plasma

The convecting system evolved in the higher animals developed first through a hemocoele (as in crustacea)—a blood reservoir from which the fluid is pumped through a continuously branching system to the tissues from whence it drains back to the blood cavity, and eventually to a closed vascular system in the higher mollusks (octopus) and the vertebrates.[5,6] Throughout this evolution blood has consisted of a variety of "formed elements" (cells and smaller particles such as the blood platelets) and a large variety of molecules ranging from salts through sugars, amino acids, and other metabolites to the large molecules, the plasma proteins.

The withdrawal of blood, for the purpose of obtaining plasma proteins, requires special precautions if rapid changes following withdrawal are to be avoided. Blood is a self-sealing fluid, and the clotting process accomplishing this is activated by a chemical or physical stimulation of certain components of blood or of the injured tissues. In the case of vertebrate blood such changes may be delayed by preventing contact of the blood with injured cells or with "wettable" surfaces (by insertion of a tubular needle directly into the vascular system and by the use of vessels, tubing, etc., coated with paraffin or other nonwetting material). At the present time this may be most conveniently accomplished by the use of tubing, vessels, and even needles made of plastic[7] or by "siliconing" glassware[8] and coating needles and other metal parts with silicone oil.

Alternatively, blood coagulation may be prevented by stopping one of the chain of events leading to the formation of a clot. This has proven a successful method when study of the blood-clotting process itself was not intended. For this purpose workers have added citrate,[9,10] oxalate, or the sodium salt of ethylenediaminetetraacetic acid[11] as complexing agents for calcium. These interrupt the clotting process by preventing the conversion of prothrombin to thrombin (a process involving calcium ions). Clotting may also be prevented by drawing the blood through a column of cation-exchange resin on the sodium cycle so as to exchange sodium for the blood calcium.[12] Heparin may also be used. This can act at a later stage in the clotting process to prevent the action of thrombin on fibrinogen.[13]

(5) C. L. Prosser, Comparative Animal Physiology, Chap. 15, W. B. Saunders, Phila., 1950.
(6) M. Florkin, Biochemical Evolution, Academic Press, New York, 1949.
(7) C. Walter, *Surg. Forum, Proc. 36th Congr. Am. Coll. Surgeons. 1950 (Pub. 1951)*, p. 483.
(8) E. G. Rochow, An Introduction to the Chemistry of the Silicones, Wiley & Sons, New York, 1951.
(9) G. A. Pekelharing, *Beitr. wissensch. Med.* **1**, 433 (1891).
(10) R. Lewisohn, *Med. Record* **87**, 141 (1915).
(11) G. Schwarzenbach, *Helv. Chim. Acta* **30**, 1798 (1947); F. Proescher, *Proc. Soc. Exptl. Biol. Med.* **76**, 619 (1951).
(12) A. Steinberg, *Proc. Soc. Exptl. Biol. Med.* **56**, 124 (1944).
(13) However, *in vivo*, it appears to prevent prothrombin conversion. D. S. Riggs, *New Engl. J. Med.* **242**, 179, 216 (1950); M. Burstein, *Compt. rend. soc. biol.* **144**, 750, 1338 (1952).

If "defibrinated" blood is desired, the blood may be drawn without any precautions and stirred (whipped) while clotting takes place. This prevents gross occlusion of the cells by the clot which contracts to small shreds.[14]

Plasma, the noncellular portion of blood, may be obtained by simple settling (used with horse blood) or by centrifugation of the cellular elements.

The various types of cells in human blood possess quite different densities permitting their separation in a density-gradient column.[15-17] On a larger scale this may be accomplished by differential centrifugation. This process has been made more efficient by the design of special centrifuges[18] and by use of aggregating reagents, such as dextran,[19] for the erythrocytes. The blood platelets have been separated by differential centrifugation and by adsorption on ion-exchange resins.[20]

Blood plasma so obtained from fasting animals is a straw-colored fluid, which visually appears stable, if sterile. However, continuous changes take place on storage, not only among the clotting components, as mentioned above, but also among the lipoproteins, complement, etc.[21] Some changes may be due to enzymes present and some to the natural instability of the protein components. Certainly, these changes may be minimized by storage in the cold, although at 0°C. considerable precipitation of fibrinogen or of cold-insoluble globulin may occur. For this reason the storage temperature of blood has frequently been specified as 4°C. Blood plasma may be stored frozen or dried (by "lyophilization" —sublimation of water under vacuum after freezing the plasma) with marked improvement in stability for many components. However, some of the lipoproteins are badly damaged by this technic (see page 705).

The constancy in composition of blood plasma led to Claude Bernard's concept of the *milieu interieur* which bathed all the cells and buffered them from an adverse external environment. The constancy to which Bernard referred was that of the circulating electrolytes (Table I), which is, in fact, unusually invariant for a "biological constant." Proteins (including lipoproteins) constitute the bulk of the remaining plasma components and these show much greater variation, which can be measured by physicochemical methods against this constant ionic background.

(14) This process of some historical interest was described by Hewson, who first isolated fibrin: W. Hewson, An Experimental Inquiry into the Properties of the Blood, T. Codell, London, 1771.
(15) B. L. Vallee, W. L. Hughes, Jr., and J. G. Gibson, 2d, *Blood* **1**, 82 (1947).
(16) J. W. Ferrebee and Q. M. Geiman, *J. Infectious Diseases* **78**, 173 (1946).
(17) D. W. Fawcett and B. L. Vallee, *J. Lab. Clin. Med.* **39**, 354 (1952).
(18) E. J. Cohn, personal communication.
(19) E. S. Buckley, Jr., and J. G. Gibson, 2d, *Proc. Univ. Lab. Phys. Chem. Related Med. and Public Health* **1**, 45 (1950).
(20) J. L. Tullis, personal communication.
(21) L. E. Krejci, L. Sweeney, and E. B. Sanigar, *J. Biol. Chem.* **158**, 693 (1945).

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Thus density measurements can be used to estimate total protein, although they will err if the lipide content of the plasma varies. Actually the density method largely ignores the lipides since these have densities close to that of water. Therefore the measurements give estimates for protein agreeing well with nitrogen analysis if one assumes all of the proteins to contain 16% N.[22]

TABLE I
IONIC COMPOSITION OF BLOOD PLASMA

Cations, meq./l.		Anions, meq./l.	
Na^+	142	Cl^-	103
K^+	5	HCO_3^-	27
Ca^{++}	5	HPO_4^-	2
Mg^{++}	3	SO_4^-	1
		Organic acids	6
		Protein	16
	155		155

Alternatively, refractive-index measurements may be used. Since the refractive-index increment of lipide is relatively close to that of protein, this method agrees well with the estimation of protein as total non-dialyzable solids.[22a]

3. REQUIREMENTS FOR CIRCULATORY FUNCTION

The proteins of mammalian plasma show related physicochemical properties, all being negatively charged at physiological pH and having molecular weights varying from 40,000 to 150,000 (a few larger—see Table VI). This relative homogeneity would appear related to function in the blood stream. These molecular sizes are similar to the "pore" sizes of the reticulo-endothelium (these pores being apparently designed so that relatively small variation in membrane structure may permit wide variation in the amount of protein passed). Only traces of protein pass through the membranes of the general capillary bed (including the renal glomerulus) and larger amounts through the membranes of the liver capillaries.[23] Certainly, limited diffusibility would appear desirable

(22) D. D. Van Slyke, A. Hiller, R. A. Phillips, P. B. Hamilton, V. P. Dole, R. M. Archibald and H. A. Eder, *J. Biol. Chem.* **183**, 331 (1950).
(22*a*) S. H. Armstrong, Jr., M. J. E. Budka, K. C. Morrison, and M. Hasson, *J. Am. Chem. Soc.* **69**, 1747 (1947).
(23) E. M. Landis, *Physiol. Revs.* **14**, 404 (1934).

for the maintenance of a high concentration of these substances in the blood. However, perhaps the more interesting question is why the membranes permit any passage at all. Is this an imperfection of nature, a compromise in the interests of high permeability of certain small metabolites, or is such protein permeability necessary as an aid to their function? In the case of some proteins, such as the hormones, the latter interpretation would seem correct. Also, in the case of proteins whose function is transport of metabolites or protection against infection, permeability might seem desirable. It will be interesting in the following pages to look for further functional correlation of the relative permeability for different plasma proteins.

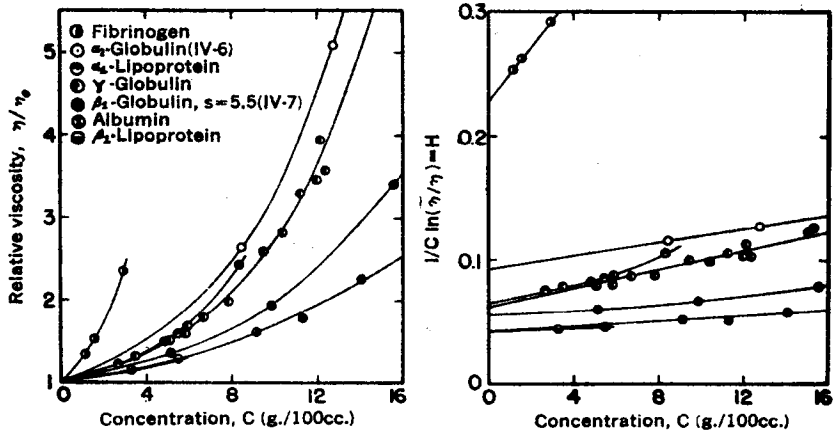

FIG. 1. Viscosity of various plasma proteins at 37°C. (From Oncley *et al.*)[61]

In the interests of osmotic efficiency (see sec. II) all plasma proteins at physiological pH are negatively charged, and also they are all closely related in size (Table VI). These factors may be further related to hydrodynamic efficiency. Certainly, protein–protein interaction, which is accentuated when the proteins bear opposite electric charges, would decrease osmotic pressures and increase blood viscosity. This relative weakness of interaction between plasma proteins has been a boon to the investigator interested in the isolation of individual components.

The plasma proteins, excepting fibrinogen, also show similar and relatively small intrinsic viscosities (see Fig. 1). This, too would be a desirable feature in the circulation since it would decrease the work of the heart. Fibrinogen is, of course, necessarily asymmetric (and hence viscous) since it is a precursor to the network of the blood clot.

4. Distribution in Plasma, Extravascular Fluid, and Cells

a. Reservoirs of Plasma Proteins

While the proteins of plasma include a number of readily identifiable components of dimensions such as to favor their confinement to the vascular system, absolute retention is not achieved. Thus it has been shown by tracer technics that approximately 50 per cent of small (relative to plasma content) infusions of serum albumin disappear from the circulation within the first day.[24–26] A considerable portion of this "lost" protein may be explained as being contained in the intercellular spaces due to leakage from the capillaries.[26a] The volume of such spaces is generally considered to be about ⅕ of the total volume of the mammalian organism[27] and hence is approximately 3 × the plasma volume. The protein content of this extravascular fluid must vary widely, the average value lying somewhere between zero and 2–4 per cent—the protein content of lymph.[28] Thus, there would appear sufficient latitude to explain the protein loss following transfusion.

However, lymph represents a concentrated (with respect to protein) extravascular fluid, and the very small permeability of capillaries to plasma proteins coupled with practically complete equivalence between plasma colloid osmotic pressure and average capillary hydrostatic pressure (above tissue pressure)[29] suggests that the extravascular interstitial fluid must contain far less, on the average, than the 1 per cent plasma protein which the above argument would demand. Drinker has pointed out that a quiescent limb does not normally become edematous despite lack of lymph flow.[28] This again suggests that leakage of protein must be extremely small. These arguments apply to the extravascular fluid of skeletal muscle and the appendages. In

(24) A. M. Seligman, *J. Clin. Invest.* **23,** 720 (1944); K. Sterling, *ibid.* **30,** 1228 (1951).

(25) M. P. Deichmiller, F. J. Dixon, and P. H. Maurer [(*Federation Proc.* **12,** 386 (1953)] measured half-lives of several homologous serum albumins (I^{131} tagged): Man 15 days, dog 8 days, rabbit 6 days, and mouse 1.2 days.

(26) This effect was probably first demonstrated in humans by Janeway and Heyl, who followed by Heidelberger's quantitative precipitin technic the disappearance of bovine serum albumin from the blood stream and found 50 per cent disappearance during the first day followed by a much slower rate thereafter.

(26*a*) The dynamic equilibrium between vascular and extra vascular protein has been demonstrated with homologous antibody by D. Gitlin and C. A. Janeway [*Science* **118,** 301 (1953)].

(27) J. L. Gamble, Jr., and J. S. Robertson, *Am. J. Physiol.* **171,** 659 (1952); M. Gaudino, I. L. Schwartz, and M. F. Levitt, *Proc. Soc. Exptl. Biol. Med.* **68,** 507 (1948).

(28) C. K. Drinker and J. M. Yoffey, Lymphatics, Lymph, and Lymphoid Tissue, Harvard Univ. Press, Cambridge, 1941.

(29) E. M. Landis, *Am. J. Physiol.* **82,** 217 (1927); **83,** 528 (1928).

keeping with them, perfusion of the isolated hind limb[30] shows no loss of plasma protein to extravascular space.[31]

Therefore, the large protein losses described above would not appear to be generally distributed, and some extravascular "reservoir" of plasma proteins approaching blood plasma concentrations must exist in some of the internal organs (e.g., the liver) where capillary permeability is known to be very large. (High permeability for the capillaries of the portal system would seem necessary because of the low hydrostatic pressure—see sec. II.)

b. Evidence for Plasma Proteins within Cells

Another possible reservoir of plasma proteins lies within cells. Recent immunochemical studies have demonstrated the rapid appearance of heterologous proteins within cells and particularly within cell nuclei following their injection. Homologous plasma proteins within cells and cell nuclei have also been observed.

An "immunohistochemical method" developed by Coons for this purpose consists in staining alcohol-fixed tissue slices with an antibody to which has been coupled a fluorescent dye. After suitable washing procedures to remove unspecifically adsorbed antibody, the location of the protein against which the antibody is directed may be observed microscopically under ultraviolet light.[32] In this way Coons has been able to observe the rapid penetration (i.e., within 10 minutes) of proteins such as ovalbumin and serum albumin within a variety of cells following their intravenous injection into mice.[33] Haurowitz has also demonstrated the penetration following injection of foreign proteins labeled with radioactive iodine into liver cells of the rabbit. In this way he found radioactivity in the fractions designated: microsomes, the mitochondria, and the nuclei. However, the activity could be separated from the nucleoprotein itself.[34]

Such penetration within cells might be assumed to be the first stage in the immune response of the animal similar to the previously observed

(30) J. R. Pappenheimer, E. M. Renkin, and L. M. Borrero, *Am. J. Physiol.* **167**, 13 (1951).

(31) J. R. Pappenheimer, personal communication. Pappenheimer measures osmotic-pressure changes and therefore might not detect changes in extravascular protein which was not osmotically active. The presence of such "bound" plasma protein is suggested by the occurrence of plasma proteins together with interstitial connective tissue throughout the body in histological sections stained immuno-chemically.[37] If this is not an artifact in the preparation of the histological specimen, further modification of the picture of capillary permeability may be required.

(32) A. H. Coons and M. H. Kaplan, *J. Exptl. Med.* **91**, 1 (1950).

(33) A. H. Coons, E. H. Leduc, and M. H. Kaplan, *J. Exptl. Med.* **93**, 173 (1951).

(34) C. F. Crampton and F. Haurowitz, *Science* **111**, 300 (1950); F. Haurowitz and C. F. Crampton, *J. Immunol.* **68**, 73 (1951).

phagocytosis of particulate antigens.[35] However, the distribution observed by Coons was much more widespread than the cells usually implicated in this mechanism.[36] Using Coons's technic, Gitlin *et al.* have studied the distribution of homologous plasma proteins within cells[37] and have found a similar distribution of serum albumin, γ-globulin, and β-lipoprotein in human tissues. Furthermore, that this represents a true penetration for γ-globulin was indicated by injecting this into a child who genetically was unable to synthesize this protein. The child's tissues, which before injection did not stain for γ-globulin, showed a normal staining pattern after injection.

It is not known whether plasma proteins can reversibly enter and again leave the cell, although heterologous proteins have been shown to disappear from cells at about the same time as disappearance from the circulation should occur.[38] A quantitative approach to these experiments has not been possible, so that the magnitude of the effects is unknown. However, it was noted that only traces of plasma proteins could be detected within muscle cells.[37] Therefore, any large cellular depots must again reside within the body cavity.

The significance of intracellular plasma proteins may lie in their close relation to protein metabolism. Schoenheimer and Rittenberg *et al.* have demonstrated the dynamic equilibrium of plasma proteins[38] with tissue proteins, and Whipple and Madden have shown their importance as amino acid reservoirs in the fasting animal.[39] However, it is not clear whether the plasma proteins are transported intact to the point of their incorporation or are first broken down to the constituent amino acids (see Tarver, Chap. 26). A choice cannot be made from known physical parameters, for while the concentration of most combined amino acids (as proteins) is 10 to 100 times as great as the free concentration in plasma, the capillary permeability to the free amino acid is probably 100-fold that of the protein;[40] and while evidence for cellular uptake of proteins is slowly accumulating, the evidence for uptake of amino acids is much more striking.[41]

(35) A. Bowin and A. Delaunay, Phagocytose et Infections, Herman & Co., Paris, 1947.

(36) For the effect of altered physicochemical properties on protein distribution within cells (phagocytosis?) compare: D. Gitlin, *Proc. Soc. Exptl. Biol. Med.* **74,** 138 (1950).

(37) D. Gitlin, B. H. Landing, and A. Whipple, *J. Exptl. Med.* **97,** 163 (1953).

(38) R. Schoenheimer, S. Ratner, D. Rittenberg, and M. Heidelberger, *J. Biol. Chem.* **144,** 545 (1942).

(39) G. H. Whipple and S. C. Madden, *Medicine* **23,** 215 (1944).

(40) J. R. Pappenheimer, *Physiol. Revs.* **33,** 387 (1953).

(41) H. N. Christensen, T. R. Riggs, and N. E. Ray, *J. Biol. Chem.* **194,** 41 (1952)

c. *Evidence for Cellular Proteins in Plasma*

The presence of stray proteins in plasma as leakage from tissues might be expected either from dying cells or as a result of a slight permeability of the cell membranes. Substances whose presence in plasma might be so explained are the hydrolytic enzymes such as amylase, phosphatases, and esterases. The presence of catalase, occasionally considered to be a plasma enzyme, has been traced to hemolysis during blood collection, since its concentration is proportional to that of hemoglobin in the plasma.[42] The presence of peptidases in serum has been similarly ascribed to erythrocyte destruction.[42a] Recently, ovalbumin has been detected in chicken plasma, highest in laying hens. However, the concentration never exceeded 0.1 per cent of the total protein.[43]

In pathological conditions, changes in the plasma proteins are frequently observed which are proving increasingly useful in diagnosis. These changes can either involve an altered level of a normal plasma constituent (e.g., decreased albumin concentration in nephrosis) or the appearance of one or more new components, normally not detectable in plasma. An interesting example of the latter case is McCarty's crystalline "C reactive component"[44] (a protein occurring during the acute phase of certain infections). Further discussion of pathological changes is considered outside the scope of this chapter, and the reader is referred to the excellent review by Gutman.[44a]

5. Metabolism of Plasma Proteins

Metabolism of the plasma proteins is considered at greater length in Chap. 26 (Tarver), but it is interesting to point out in passing that the major component of plasma, serum albumin, appears to act as a protein reserve in the fasting animal. The turnover rate for human serum albumin is nevertheless quite slow (7 per cent/day),[25] indicating the relative stability of this protein toward catabolism or exchange phenomena. Nevertheless, the stability *in vitro* of albumin under physiological conditions is so great[45,46] that disappearance cannot be a function of "wearing out" in the circulation. Some sites of formation of certain

(42) G. E. Perlmann and F. Lipmann, *Arch. Biochem.* **7**, 159 (1945).
(42*a*) E. L. Smith, G. E. Cartwright, F. H. Tyler, and M. M. Wintrobe, *J. Biol. Chem.* **185**, 59 (1950).
(43) M. E. Marshall and H. F. Deutsch, *J. Biol. Chem.* **189**, 1 (1951).
(44) M. McCarty, *J. Exptl. Med.* **85**, 491 (1947).
(44*a*) A. B. Gutman, *Advances in Protein Chem.* **4**, 156 (1948).
(45) G. Scatchard, S. T. Gibson, L. M. Woodruff, A. C. Batchelder, and A. Brown, *J. Clin. Invest.* **23**, 445 (1944).
(46) P. D. Boyer, G. A. Ballou, and J. M. Luck, *J. Biol. Chem.* **162**, 199 (1946).

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