

CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

VI. THE OSMOTIC PRESSURE OF PLASMA AND OF SERUM ALBUMIN^{1,2}

BY G. SCATCHARD, A. C. BATCHELDER, AND A. BROWN

(From the Research Laboratories of Physical Chemistry, Massachusetts Institute of Technology, Cambridge, and the Department of Physical Chemistry, Harvard Medical School, Boston)

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In order to determine the relative dosage of plasma and of serum albumin in the treatment of shock and to determine their efficiencies in increasing blood volume and other characteristics, we have measured the osmotic pressure of human plasma and serum, of regenerated solutions from dried plasma up to four-fold physiological concentrations, of human serum albumin solutions up to 300 grams per liter in 0.15 molar sodium chloride, and of bovine serum albumin over large ranges of pH, albumin concentration, and salt concentration.³ We have not been able to detect a difference in the osmotic pressures of human and bovine albumin, so we have used the measurements on bovine albumin to extend the range of those on human albumin.

The first function of plasma or serum albumin in shock is to increase the blood volume by holding water in the blood stream. Parts of the water may be injected with the protein, may be shifted from the extravascular fluids, or may be drawn from the intestine. This water must be held in spite of the excess pressure in the capillary bed. Most of this retention of water is not caused by any attraction between the protein and the water, but arises from the fact

that the chemical potential of water is decreased by the presence of dissolved molecules which are not water.⁴

A convenient way of measuring the change in the potential of water produced by the addition of a solute is to measure the decrease in pressure necessary to maintain equilibrium through a membrane permeable to the water but not to the solute, and this pressure is called the osmotic pressure of the solution. If the membrane is permeable to any of the solutes, the pressure is sometimes called the colloid osmotic pressure or

⁴The difference in the potential of any substance at two different places is the least work necessary to bring unit quantity of that substance from the first place to the second if the temperature and total volume of the system are constant. The substance will shift spontaneously from the place of higher potential to that of lower potential if a path is available, and at equilibrium its potential must be the same throughout the system. The rapidity with which equilibrium is attained depends upon the nature of the available paths as well as upon the difference in potential, but the position of equilibrium depends only upon the equality of potential.

There may be a difference in gravitational potential due to different heights in a gravitational field, and this potential is proportional to the difference in height and to the weight of unit quantity. There may be a difference in chemical potential due to different pressures, and this potential is proportional to the pressure difference and to the volume of unit quantity. There may also be a difference in chemical potential due to different chemical compositions. We cannot generalize on the value of this potential, but in very dilute solutions, the difference in chemical potential of each substance is proportional to the difference in the logarithm of its mole fraction. Then the difference in the potential of each solute is proportional to the difference in the logarithm of its concentration, and the difference in potential of the solvent is proportional to the difference in the sum of the concentrations of the solutes, expressed as moles of solute per unit quantity of solvent. These are the three potentials of substances which are physiologically important.

¹ This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

² This paper is Number 16 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.

³ All measurements were made with collodion membranes, which are impermeable to plasma proteins. For technical reasons, the measurements were made at 25° C., where the pressure is about 4 per cent less than at 37° C. All concentrations were calculated using the nitrogen factor 6.25 for both albumin and plasma.

the oncotic pressure. Since this is the only type of membrane across which the pressure is important, we will use the simpler term.

Van't Hoff pressure

About two-thirds of the osmotic pressure at physiological concentrations is explained by the simple theory of Van't Hoff, which says that the osmotic pressure is equal to the pressure difference which would be developed if the solvent were removed and the solutes were gases. If the non-diffusible solute is a non-electrolyte, the osmotic pressure should be proportional to its concentration, and the pressure-concentration ratio should be the same for all substances if the concentration is expressed as moles per unit volume. It is obvious that osmotic pressure per unit mass, however, decreases as the size of the molecule increases. If the concentration is expressed as mass per unit volume, the pressure-concentration ratio is inversely proportional to the molecular weight, and one measurement of the osmotic pressure and of the corresponding mass concentration may be used to determine the molecular weight. The Van't Hoff case is illustrated by curve E in Figures 1 and 2. In Figure 1, the osmotic pressure is

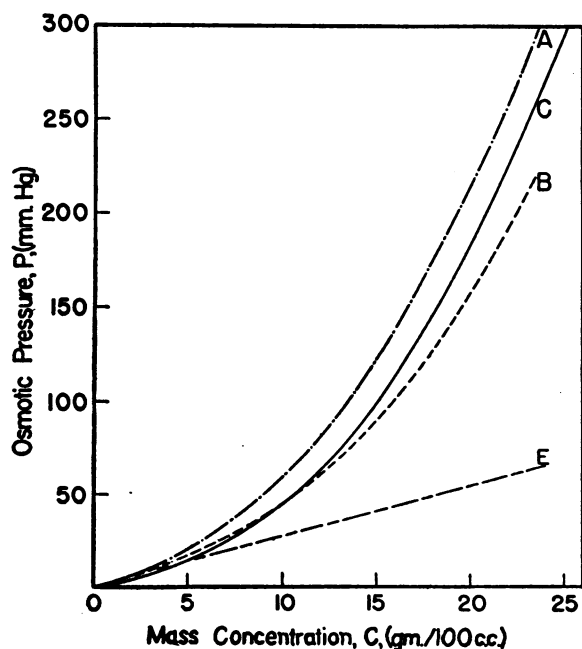


FIG. 1

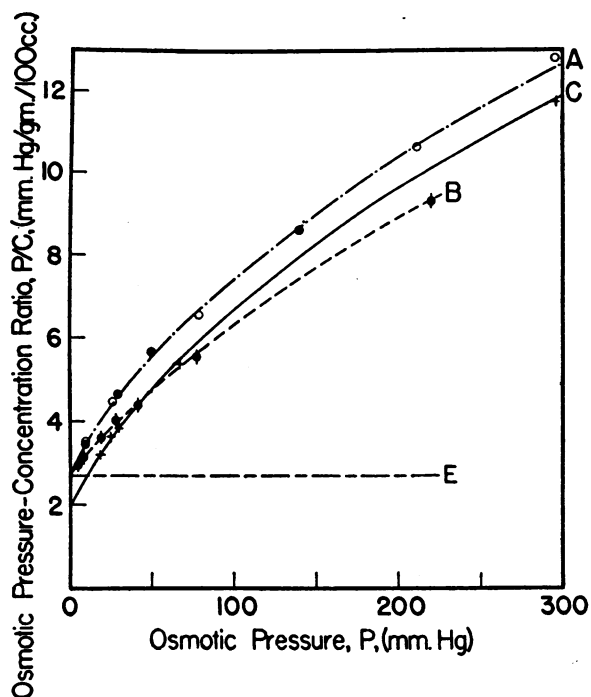


FIG. 2

plotted against the concentration, and Van't Hoff's theory leads to a straight line whose slope is inversely proportional to the molecular weight. In Figure 2, the pressure-concentration ratio is plotted against the pressure, and this curve E is a horizontal line whose ordinate is the slope of E in Figure 1. The curves A in these figures are the measured values for serum albumin at pH 7.4, and the curves B are for albumin at pH 5.4, both in 0.15 M sodium chloride. At pH 5.4, the albumin is iso-ionic; that is, its average net charge is zero.

Donnan pressure

The plasma proteins are not neutral non-electrolytes, and their ionic charges have an important effect on the osmotic pressure. The extension of Van't Hoff's theory to this case was carried out by Donnan. We will limit ourselves to the special case of the sodium salt of a non-diffusible protein anion and sodium chloride, which is a rough approximation of plasma. The solution must be electrically neutral. So, if there is no sodium chloride present, the pressure-concentration ratio corresponds to

the protein ion plus all the sodium ions associated with it.

If there is sodium chloride present, its potential must also be the same on the two sides. Since there is more sodium, there will be less chlorine on the side with the sodium proteinate. So the pressure-concentration ratio is smaller. It corresponds to the protein alone when the ratio of protein to chloride is very small, and depends only upon the protein-chloride ratio. The molecular weight cannot be determined from a single osmotic pressure measurement. However, if a series of measurements is made with varying protein concentration but constant salt concentration, the pressure-concentration ratio can be extrapolated to zero protein concentration, and the molecular weight can be determined from this extrapolated value. At pH 5.4, the Donnan curve is the same as the Van't Hoff curve E. The Donnan curve for pH 7.4, where the measured curve is A, lies close to the curve B. It is obvious that the Donnan effect increases rapidly with the net charge of the protein ion. It is, in fact, proportional to the square of the net charge.⁵

⁵ The statement in mathematical equations is more precise and in some ways simpler. We will let C , C_+ and C_- be the concentrations in moles per unit volume of protein, sodium ion, and chloride ion within the membrane, and C'_+ and C'_- the concentrations of sodium and chloride ions outside: c will be the concentration of the protein in grams per unit volume, M its molecular weight, which is c/C , z its valence (a negative number for an anion), T will be the absolute temperature, and R a universal constant.

Van't Hoff's law is

$$P = RT[C + C_+ + C_- - C'_+ - C'_-]$$

$$\frac{P}{c} = \frac{RT}{M} \left[1 + \frac{C_+ + C_- - C'_+ - C'_-}{C} \right]$$

The law of electroneutrality requires that

$$zC - C_- + C_+ = 0$$

and

$$C'_+ - C'_- = 0$$

The law of equilibrium, or equality of the potential of sodium chloride, requires that

$$C'_+C'_- = C_+C_-$$

Combination of the last three equations yields

$$C'_- = C_- \sqrt{1 - \frac{zC}{C_-}}$$

Pressure of real solutions

The Van't Hoff theory assumes that each individual water molecule in a protein solution behaves like a water molecule in pure water and that the decrease in potential of the water which

Combination with Van't Hoff's law yields

$$\frac{P}{c} = \frac{RT}{M} \left[1 + \frac{2C_- - zC - 2C_- \sqrt{1 - \frac{zC}{C_-}}}{C} \right]$$

$$= \frac{RT}{M} \left[1 + \frac{C_-}{C} \left(2 - \frac{zC}{C_-} - 2 \sqrt{1 - \frac{zC}{C_-}} \right) \right]$$

$$= \frac{RT}{M} \left[1 + \frac{C_-}{C} \left(1 - \sqrt{1 - \frac{zC}{C_-}} \right)^2 \right]$$

It is clear from the last equation that the expression in square brackets depends only on zC/C_- for a given value of z , but it is not clear how it approaches unity as zC/C_- approaches zero or even that it does so. To answer these questions, we expand the radical in a Taylor's series to give

$$\sqrt{1 - \frac{zC}{C_-}} = 1 - \frac{zC}{2C_-} - \frac{z^2C^2}{8C_-^2} \dots$$

$$\frac{P}{c} = \frac{RT}{M} \left[1 + \frac{z^2C}{4C_-} + \dots \right]$$

$$= \frac{RT}{M} + \frac{RT(z/M)^2c}{4C_-} = \frac{RT}{M} + \frac{M(z/M)^2P}{4C_-}$$

For a neutral molecule, z is equal to zero, and $P/c = RT/M$ at all concentrations. For an ion $z^2C/4C_-$ is zero only when C is zero, and in dilute solutions P/c increases as a linear function of c when C_- is constant.

For a real solution we may also write

$$\frac{P}{c} = \frac{RT}{M} + bc + dc^2 + \dots$$

or

$$\frac{P}{c} = \frac{RT}{M} + BP + DP^2 + \dots$$

in which b and d or B and D are constants. The latter form is more useful for our purposes because

$$\frac{1}{c} = \frac{RT}{MP} + B + DP + \dots$$

In many cases, D is so small that the term DP may be neglected. Then the volume of solution per gram of protein is equal to the ideal term which is inversely proportional to the pressure plus a term which is independent of the pressure.

B will be composed in general of the Donnan term which is $M(z/M)^2P/4C_-$ plus a term arising from the difference between the force between two protein molecules and that between one of the molecules and the water displaced by the other.

accounts for the osmotic pressure is solely a statistical effect, due to the fact that a certain fraction of the molecules are not water. This is equivalent to assuming that each individual protein molecule in a concentrated solution behaves like a protein molecule in an extremely dilute solution, or that the activity of the protein is proportional to its concentration, and this latter assumption is easier to consider. The Donnan extension considers the macroscopic effect of the charge on the protein ion, but still assumes that the activity of each species is proportional to its concentration.

Real protein solutions are not this simple.⁶ For iso-ionic albumin, the osmotic pressure is much larger than that calculated by the Van't Hoff theory. At other values of pH, however, the increase over the iso-ionic value is much less than predicted by Donnan, so that at pH 7.4, the measured value does not differ greatly from that calculated by his theory, and at still higher values of the pH, the measured pressures are less than those calculated. Real solutions do resemble the Donnan solutions in that the molecular weight can be determined by extrapolating the pressure-concentration ratio to zero concentration or pressure.

Figure 1 shows the curves for osmotic pressure versus mass concentration for serum albumin at pH 7.4 (A) and pH 5.4 (B), for human plasma at pH 7.4 (C), and for an ideal Van't Hoff solute whose molecular weight is 69,000, which is the same as that of albumin (E). The curves all

⁶ The ionic charges on proteins cause deviations from random distribution on the microscopic scale as well as those considered by Donnan, and differences in potential proportional to the square of the net ionic charge. Even in iso-ionic albumin, where the average of the charge is zero, the average of the square of the charge is 6, and at pH 7.4 it is 300. In either solution, the total charge is almost 200, and it is not evenly distributed through the molecule. The resultant electrical field leads to attraction between a protein molecule and a simple ion, or between 2 protein molecules, which is large compared to the effect of the net charges. Moreover, the protein molecule which bears these charges behaves more like an equal volume of a normal organic liquid than an equal volume of water. This molecular framework tends to repel the electrical fields but to attract another framework. The resultant of all these forces leads to effects upon the potential of the water and the resultant osmotic pressure which vary greatly with the ionic charge.

start at zero concentration and pressure, and curves A, B, and E, all have the same initial slope. The value for the molecular weight of serum albumin calculated from these osmotic pressure measurements, 69,000, is in good agreement with that obtained from sedimentation and diffusion measurements reported in the first paper of this series (1). The plasma curve (C) has a smaller initial slope which corresponds to a larger molecular weight, 93,000. All the experimental curves become steeper at higher concentrations. The difference between A and C is proportional to the concentration.

In Figure 2 is shown the pressure-concentration ratio, P/c , versus the pressure.⁷ The ideal Van't Hoff curve, E, is here a horizontal line whose ordinate is the slope of E in Figure 1. A and B intercept E at zero pressure, corresponding to the same initial slopes in Figure 1, but they deviate sharply at higher pressures. The plasma curve, C, has a lower intercept, corresponding to the larger average molecular weight of plasma proteins, and the difference between A and C is here independent of the pressure. If 60 grams per cent of the plasma protein is albumin (1), curve C as drawn would give an average molecular weight of 194,000 for the remaining 40 per cent. However, our measurements do not preclude drawing the intercept at 2.1 rather than 2.0, which would correspond to an average molecular weight of 88,000, and a molecular weight of 150,000 for the 40 grams per cent which are not albumin. The probable value for the average molecular weight of plasma globulins is 170,000.

Osmotic efficiency

We are particularly interested in the volume of solution per gram of protein, $1/c$, at a given

⁷ The experimental points are shown in Figure 2, whose scale is larger than that of Figure 1. The measurements on plasma are indicated by crosses, those on human albumin by open circles, and those on bovine albumin by filled circles. The measurements at pH 5.4 have a perpendicular line through the circles.

The measurements on albumin at pH 5.4 are carried to dilute enough solutions so that there is little uncertainty about the extrapolation to zero concentration and the resultant molecular weight. The measurements on plasma have not been carried to such dilution, so the curve has been drawn parallel to that for albumin.

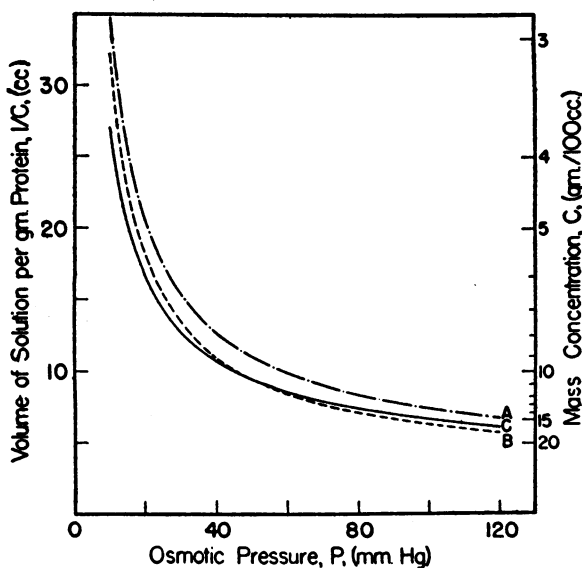


FIG. 3

osmotic pressure. This is shown in Figure 3 for the same measurements.⁸ As in the previous figures, curves A and B refer to albumin at pH 7.4 and 5.4, respectively, and curve C to plasma at pH 7.4. The curve for an ideal Van't Hoff solute corresponding to E of Figures 1 and 2 is omitted. It would lie about 5 cc. below curve B, or about 7 cc. below A at all values of the pressure, as these three curves are nearly parallel. The difference between A and C is almost inversely proportional to the pressure.

Figure 4 shows the volume of solution per gram of protein at 25 mm. pressure as a function of the pH. The curve E represents as before the ideal Van't Hoff solute, with a molecular weight of 69,000, and the curve D represents an ideal Donnan solute with the same molecular weight and the net charge of albumin.⁹ Although the difference between these curves and the experimental values is not very great at the physiological pH, it is evident that this is largely a coincidence, for the experimental and ideal curves cross at large angles.

⁸ Figure 3 is constructed from the same data as Figures 1 and 2 except that the measurements at very high and very low pressures are both omitted to permit a larger scale. The individual points are again omitted.

⁹ The titration curve for human albumin lies somewhat to the left of the curve for bovine albumin, used in estimating net charge for these calculations.

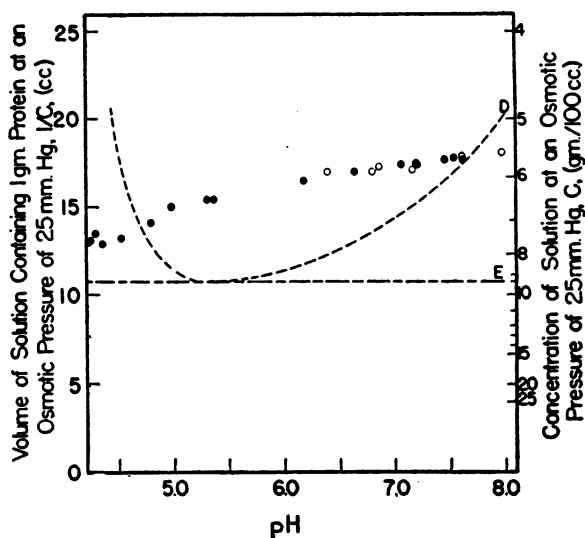


FIG. 4

The experimental values of the volume per gram of protein, $1/c$, between pH 6 and 8 may be expressed by the straight line

$$1/c = 11.1 + 0.9 \text{ pH}$$

Since the difference between $1/c$ and the Van't Hoff value RT/MP is nearly independent of the pressure, a good approximation for $1/c$ at any small pressure is

$$1/c = 268/P + 0.4 + 0.9 \text{ pH}$$

This corresponds to

$$P/c = 268 + (0.4 + 0.9 \text{ pH})P = \frac{268}{1 + (0.4 + 0.9 \text{ pH})c}$$

COMMENT

The chief uncertainty in determining the efficiency of albumin in increasing blood volume lies in the uncertainty of the osmotic pressure of normal plasma, which goes back to the uncertainty as to the normal concentration of plasma proteins. The composition of the proteins appears to be very constant in physiological plasma (1),¹⁰ but the total concentrations quoted by

¹⁰ Wies and Peters (2) have studied the effect on the osmotic pressure of varying composition of the protein in pathological plasma. Their results cannot be compared with ours directly because they determine proteins by Howe's precipitation method and our compositions are determined by electrophoresis. If we assume with them that

various observers show variations. Gutman and his coworkers (3) quote protein concentrations in serum for normal adults ranging from 6.5 to 7.9 and averaging 7.2 grams per cent. Perera and Berliner (4) quote concentrations from 6.2 to 7.3, averaging 6.8 for normal ambulatory adults, and from 5.4 to 7.0, averaging 6.0 for the same individuals recumbent. The results for plasma should be 0.4 per cent higher than for serum. So we must consider the range from 6 to 8 grams per cent. Since the recipient of a blood substitute will probably be resting, the lower part of the range is probably more important than the higher.

If c is 6 grams per cent, $1/c$ is 16.7 cc. per gram. From curve C, Figure 3, we find the corresponding pressure to be 20 mm., and from curve A we find that at 20 mm. pressure, each gram of albumin retains 20 cc. of solution. If c is 8 grams per cent, $1/c$ is 12.5 cc. per gram. The corresponding pressure is 32 mm., and at that pressure, each gram of albumin retains 15 cc. of solution. If the pressure is 25 mm., each gram of albumin retains 18 cc. of solution, and each gram of average plasma protein retains 15 cc., or the concentration of plasma protein is 6.67 grams per cent. This is the value which we have taken as the norm, with the realization that it may vary more than 10 per cent in normal individuals. This corresponds closely to the average of 17.4 cc. per gram of albumin, given for the increase in blood volume per gram of added albumin by Heyl, Gibson, and Jane-way (5, 6). This average corresponds to 6.9 per cent protein. Their extreme values of 13.2 and 24 cc. per gram correspond to 5.1 and 8.8 per cent protein. Probably part of this variation corresponds to the inevitable errors of measurements of total blood volumes.

If the increase of blood volume on the addi-

the pressure is a linear function of the ratio of globulin to total protein, g , at all small pressures and pH's between 6 and 8, we may determine the effect of globulin from our measurements on normal plasma, which gives

$$P/c = \frac{268(1 - 0.42g)}{1 + (0.4 + 0.9 \text{ pH})c}$$

For normal plasma this equation gives a limiting value at zero concentration which is too large, but it should give a fair representation of the results in undiluted plasma or serum.

tion of protein is to equal the volume of solution retained by the protein, the osmotic pressure of the plasma must be the same after the addition of the protein as before. It is probable that this condition is nearly fulfilled in hemorrhagic shock and also in traumatic shock. Any error in this assumption will be in the direction of increasing the pressure during the infusion and thus giving a smaller increase in blood volume. It is possible, however, that there are cases in which the assumption is so greatly in error that it is more accurate to assume that the blood volume is constant, and to determine the increase in osmotic pressure per gram of protein at constant plasma volume. Thus, an increase in osmotic pressure per gram of protein at constant volume becomes of interest. This will, of course, depend upon the total plasma volume, of which we have no measure here. Our measurements do show the change in pressure for unit change in concentration, and most clearly in Figure 1. This change, dP/dc , for albumin is 2.7 at zero concentration, 3.9 when c is 2, 5.1 when c is 4, 6.3 when c is 6, and 8.4 when c is 8 grams per cent. The values of dP/dc for average plasma protein at the same concentrations are 2.0, 2.8, 3.8, 4.0, and 6.4. For albumin or for plasma, the change in pressure with changing concentration increases rapidly as the concentration increases.

The comparison of the relative efficiencies of serum albumin and of plasma is more certain. Within the accuracy with which we read our curves, the relative efficiencies are independent of the pressure. There is a slight increase in the relative efficiency of albumin as the pressure decreases, but we may take, for all physiological pressures, our result that each gram of albumin retains as much fluid as 1.2 grams of average plasma protein. The pooling of plasma from several donors and the relative constancy of conditions of collecting blood reduce the fluctuations in the concentration of plasma. At present, the Red Cross pooled citrated plasma contains 6 grams per cent of protein (1). So each gram of albumin corresponds to 20 cc. of citrated plasma and 25 grams of albumin corresponds to 500 cc. of citrated plasma. This relationship is the basis for the present containers used by our armed forces,—500 cc. of citrated plasma for the

dried plasma container or 100 cc. of 25 per cent albumin.

SUMMARY

The osmotic pressures of plasma and of serum albumin at 25° have been measured over ranges of concentration and pH, much wider than the physiological ranges. The extrapolation of the osmotic pressure-concentration ratios to zero concentration yields a molecular weight of 69,000 for albumin. A similar extrapolation for plasma yields an *average* molecular weight of about 90,000. This corresponds to an *average* weight of about 170,000 for the 40 per cent of the protein which is not albumin. The osmotic pressure-concentration ratios increase rapidly with increasing concentration. At pH 7.4, this increase corresponds to the Donnan effect of the ionic charges, but measurements over a pH range show this to be a coincidence.

The volume of fluid held in the blood stream by each gram of albumin should be about 18 cc. but should vary with the protein concentration of the plasma. Each gram of albumin is equivalent to 1.2 grams of plasma protein or 20 cc. of the current Red Cross citrated, pooled plasma.

BIBLIOGRAPHY

1. Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., Jr., and Armstrong, S. H., Jr., Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J. Clin. Invest.*, 1944, **23**, 417.
2. Wies, C. H., and Peters, J. P., Osmotic pressure of proteins in whole serum. *J. Clin. Invest.*, 1937, **16**, 93.
3. Gutman, A. B., Moore, D. H., Gutman, E. B., McClellan, V., and Kabat, E. A., Fractionation of serum proteins in hyperproteinemia, with special reference to multiple myeloma. *J. Clin. Invest.*, 1941, **20**, 765.
4. Perera, G. A., and Berliner, R. W., The relation of postural hemodilution to paroxysmal dyspnea. *J. Clin. Invest.*, 1943, **22**, 25.
5. Heyl, J. T., Gibson, J. G., 2nd, and Janeway, C. A., Studies on the plasma proteins. V. The effect of concentrated solutions of human and bovine serum albumin on blood volume after acute blood loss in man. *J. Clin. Invest.*, 1943, **22**, 763.
6. Janeway, C. A., Gibson, S. T., Woodruff, L. M., Heyl, J. T., Bailey, O. T., and Newhouser, L. R., Chemical, clinical, and immunological studies on the products of human plasma fractionation. VII. Concentrated human serum albumin. *J. Clin. Invest.*, 1944, **23**, 465.