SIZE AND SHAPE OF MACROMOLECULES IN PLASMA EXPANDERS

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ALTHOUGH artificial "plasma expanders" were used during the first world war (gelatin[1]; acacia[2]), it was only during the second world war that their use developed. The main problem has always been, and still is, the selection of the colloidal, macromolecular component, the rest of the solution, the small molecules and ions, presenting no major difficulties. During the last fifteen years many macromolecules of different chemical types have been used in plasma expanders but it is only since 1952 that the importance of molecular weight has been realised in determining the behaviour of a macromolecule in the animal body[3–5]. The shape, structure and electric charge of a macromolecule may modify its passage through a membrane, and since the macromolecules used in plasma expanders vary not only in molecular weight but also in shape and structure care must be taken in comparing the experimental results obtained with different plasma expanders. It is the purpose of this review to examine the present state of our knowledge of the behaviour of macromolecules in the body and draw whatever conclusions are possible.

A plasma expander is used, as an alternative to whole blood or plasma, to restore the circulating blood volume after loss of blood and then maintain it by preventing loss of water into the extravascular space. The basic concept of the control of water balance by the colloidal osmotic pressure exerted across the capillary walls, due to Starling[6], has not been changed by recent work. The suggestion that more than a very small quantity of water is held in circulation by hydration of the plasma proteins is not supported by experiment. Although there is some uncertainty about the degree of hydration of proteins, Alexander and Johnson[7] have concluded that a maximum value is 0·4 g. of water per g. of anhydrous protein, whereas experiments[8] and calculations[9] have shown that 1 g. of serum albumin increases the plasma volume by about 18 ml. The hydration of the macromolecules used in plasma expanders is probably about the same as that of proteins[10] so that the effectiveness of a particular macromolecule in a plasma expander will depend largely upon the value and persistence of the osmotic pressure which it exerts across the capillary wall. This persistence depends upon (i) the rate at which the macromolecule passes through the capillary walls, decreasing the effective colloidal osmotic pressure, and (ii) the rate of its elimination from the body, either by excretion of unchanged polymer or by its degradation to small molecules which do not exert any colloidal osmotic pressure. Also of importance is the interaction of the macromolecules with tissue cells and naturally occurring molecules in the body, possibly resulting in storage of the macromolecule and impairment of normal physiological functions.

No attempt has been made to give a complete bibliography of plasma expanders. Reference will be made only to those publications bearing on the effect of size and shape of the solute particle on the functioning of the macromolecular weight component. There is a considerable "twilight" literature on the subject of plasma expanders in the reports of the U.S. Government Scientists, which are not readily available outside the U.S.A. Since references in the normal scientific literature (e.g., reference 11) to these reports show that they contain valuable information, they are referred to in this review, although the author has not read all these documents.

The macromolecules which have been used in plasma expanders are given in Table I. Most of the work of interest to this review has been carried out with dextran, polyvidone and, to a lesser extent, gelatin preparations. No reference will be made to the use of rutin[12], a low molecular weight flavone glucoside, since its mechanism of action, by decreasing the permeability of the capillary wall, is quite different from all the macromolecules of Table I.

TABLE I

MACROMOLECULES WHICH HAVE BEEN USED IN PLASMA EXPANDERS

Chemical type of macromolecule	Molecular weights used in commercial preparations		
	Average*	Range†	References
Proteins			
Casein digests			
Gelatin preparations:			
Fluid gelatin type (*a*)	M_n = 37,000	4% less than 10,000 M.W.	23
" " (*b*)	M_n = 50,000	—	
Isinglass			
Oxypolygelatin	M_w = 31,200	35% < 10,000; 6% > 100,000	23
Globin			
Hæmoglobin			
Plasma proteins in ascitic fluid			
Polysaccharides			
Acacia			
Dextran: American type	M_n = 42,000	0–160,000	15
British type	—	0–450,000	16
Swedish type	M_n = 34,000	—	11
Methyl cellulose			
Pectin			
Synthetic polymers			
Polyvinylalcohol			
Polyvinylpyrrolidone (Polyvidone):			
American type (*a*)	M_w = 56,000	—	11
" " (*b*)	M_w = 33,000	—	11
British type	—	5% < 10,000; 3% > 100,000	22
German type	M_w = 25,000	—	84

* M_n, number average molecular weight.
M_w, weight average molecular weight.
† The range of molecular weights has been estimated wherever possible from published information.

SIZE AND SHAPE OF MACROMOLECULES IN SOLUTION

Most of the macromolecules in Table I are not homogeneous in molecular weight, and, in order to understand the physiological action of a particular sample, it is necessary to know the distribution of molecular weights as well as the average value. These measurements will also be of value in the quality control of commercially produced plasma expanders.

Standard methods of molecular weight determination have been used and, for details, reference should be made either to some general textbook[7] or to the original papers referred to in Table II, which gives a selected bibliography of molecular weight determinations of the macromolecules used in plasma expanders. Apart from the absolute methods, the experimentally simpler determination of intrinsic viscosity is of value provided the relationship between intrinsic viscosity and molecular weight has been determined for each chemical type of macromolecule.

TABLE II

BIBLIOGRAPHY OF METHODS OF MOLECULAR WEIGHT DETERMINATION

Method	Dextran	Gelatin Preparations	Polyvidone
Absolute Methods			
Light scattering	85, 112	18	13, 25
Osmotic pressure	16	11	13, 25
Sedimentation rate	86–89	18	33
Chemical methods	90	11	
Method Requiring Calibration			
Viscosity	86, 112	91, 92	13, 25, 33

Frank and Levy[13] have, however, found that such a relationship for polyvidone is somewhat dependant upon the molecular weight range of the sample being measured. It may be that the viscosity relationships reported for other macromolecules would, on detailed experimental examination, suffer from the same defect.

Molecular weight distribution determinations appear to have been reported for only dextran, gelatin and polyvidone. Three methods have been used:

(1) The molecular weight distributions of polyvidone samples[10] and of dextran samples[14,15] have been calculated from the concentration variation of sedimentation rate.

(2) Samples of macromolecules have been divided by fractional precipitation into a series of fractions, the molecular weights of which have then been determined. This method has been applied to dextran[16] and to polyvidone[17]. Methods of fractionating gelatin have been described by Gouinlock and others[18] but were not used to determine molecular weight distributions.

(3) A variation of this last method, in which fractions are not actually isolated, is based on the turbidimetric method of Morey and Tamblyn[19]. In this method the weight of the macromolecule precipitated by a particular solvent-precipitant mixture is determined turbidimetrically, the molecular weight of the precipitate being a function of the solvent-precipitant ratio. This method has been applied to dextran[20,21] and to polyvidone[22]. Since very small samples are required this method has been used to determine the molecular weight distribution of dextran[21] and polyvidone[5] isolated from urine.

Fuhlbrigge and others[23] have determined the molecular weight distributions of various preparations of gelatin and oxypolygelatin but the methods used are not reported. The ranges of molecular weights in

some macromolecules used in commercially made plasma expanders is given in Table I.

The macromolecule in solution is hydrated and moves around with its shell of tightly bound water. Very little is known about the structure of this hydration shell for the macromolecules considered in this review but the hydration may be nearly half the dry weight of the macromolecule. The shape of the solute particle, depending very largely on the chemical type of the macromolecule, can be either a compact ellipsoid, a rigid, extended, rod-like molecule or a more or less flexible, randomly-coiled, chain-like molecule. Most of the proteins referred to in Table III are ellipsoids, held together by intra-molecular hydrogen bonding and their shapes are generally indicated by the axial ratios of the ellipsoids. Dextran [24] and polyvidone [25] have now been shown to be more or less flexible molecules, and, although axial ratios can be calculated for them, these can be very misleading since they represent a statistical mean shape of the solute particle and do not take into consideration the variations in orientation of the repeating units of the chain about the mean shape, which are continuously taking place in the solution[26]. Recently, it has been shown that gelatin in solution is a randomly-coiled molecule rather than a compact particle or a rigid rod.[18] This implies that there can be very little intra-molecular hydrogen bonding in the gelatin molecule.

Another property which may be of importance in determining the physiological action of a macromolecule is the charge on it in solution. Dextran has been shown to have no charge[27] and polyvidone is generally stated to have a very small negative charge, equivalent to about one carboxyl group per molecule[28]. Every protein molecule bears a charge depending upon its isoelectric point and the pH of the solution.

The Osmotic Pressure of Solutions of Macromolecules

The colloidal osmotic pressure of human blood is 33 cm. of water (normal range 28 to 48 cm.)[29], about one third[9] of this being due to the Donnan effect as a result of the electric charge on the proteins. Serum albumin, possessing a lower molecular weight than the rest of the plasma proteins, is normally responsible for about three-quarters of the colloidal osmotic pressure.

If it is assumed that the macromolecule has a single molecular weight, M, possesses no electric charge and behaves ideally in solution, the osmotic pressure, in cm. of water, π of a solution (concentration, c g./ml.) at absolute temperature, T, is

$$\pi c = 8{\cdot}48 \times 10^4 \times T/M.$$

The relation between molecular weight and the concentration required to give an osmotic pressure of 33 cm. is given in Figure 1 and indicates that the higher the molecular weight the greater is the concentration of the macromolecules required in a plasma expander. In practice this simple ideal model, in which there are no interactions between molecules, will not hold. In the plasma expander solution, before it is diluted by blood during an infusion, interactions between macromolecules will result in a

higher osmotic pressure, for a given concentration, than is given by Figure 1. The dilution of the plasma expander normally likely to occur during an infusion (e.g., 500 ml. to 2800 ml., an average plasma volume) will reduce the concentration of macromolecule and with it the effect on osmotic pressure of the above type of interaction. After infusion, interactions between the macromolecules and proteins become important but very little experimental data is available on the osmotic pressure of mixed solutions of proteins and macromolecules. Wales and others[30] studying dextran-albumin mixtures and Rowe[31] with dextran-serum mixtures have shown that the osmotic pressure is greater than would be expected from the protein and dextran concentrations, indicating that protein-macromolecule interactions are important.

The macromolecules generally used in plasma expanders are not homogeneous in molecular weight. Apart from the corrections due to intermolecular interaction already discussed, the total osmotic pressure exerted by the macromolecule, as measured by a perfect semi-permeable membrane, will depend upon the number average molecular weight and will be greater than the effective colloidal osmotic pressure exerted in the body across the capillary wall as a semi-permeable membrane, since the low molecular weight molecules will either pass rapidly through the capillary walls, or else be rapidly excreted. Consequently, this effective osmotic pressure, which has never been measured for any plasma substitute, will depend markedly upon the molecular weight distribution of the macromolecule used. It is important, therefore, once the effectiveness of a particular grade of macromolecule has been established, to ensure that all subsequent batches will have the same distribution of molecular weights.

It is the author's opinion, for the reasons already given, that the colloidal osmotic pressure of a plasma expander measured in an osmometer is very nearly meaningless in indicating its effectiveness. Since it is impossible to eliminate all small molecules from commercially prepared samples of macromolecules, careful consideration should be given to the possibility of increasing the effectiveness of a plasma expander by increasing the concentration of the macromolecule to such an extent that, after loss of the small molecules from the blood, the remaining macromolecules would exert an osmotic pressure comparable to that of the normal plasma proteins. Provided the osmotic pressure of the small molecules was small compared with that of isotonic saline, there should be no danger of crenation.

The two macromolecules most widely used in plasma expanders, dextran and polyvidone, do not receive any contribution to their osmotic pressure from the Donnan effect since there is only an extremely small electric charge on them[27,28]. Scatchard[3] has suggested that the ideal macromolecule for a plasma expander would have, under physiological conditions, a similar charge to serum albumin. Such a charge would reduce the concentration of polymer required to produce a given osmotic pressure, and, hence, would result in a solution of lower viscosity, a factor discussed immediately below. The charge on a molecule might also be

important in controlling the rate at which the macromolecule passed through the glomerular membrane or the capillary walls. With the exception of amino-acid polymers, no attempt is being made to develop ionised synthetic macromolecules for use in plasma expanders. The various gelatin preparations have, of course, charged molecules.

The Viscosity of Solutions of Macromolecules

The viscosity of aqueous solutions of macromolecules increases with increasing molecular weight, the exact form of the relationship depending upon the chemical type of the macromolecule. The relative viscosity of solutions of concentration given by the osmotic pressure curve of Figure 1 have been included in that figure and have been calculated assuming that the macromolecule is polyvidone. The viscosity-concentration relationship due to Fikentscher[32] and the viscosity-molecular weight relation due to Scholtan[33] have been used. It is evident, therefore, that increasing the molecular weight of the colloidal component will result in a higher relative viscosity partly due to the increased size of the solute molecule and partly to the increased concentration necessary to give the required osmotic pressure. This conclusion, although qualitatively correct, will be incorrect quantitatively since, as in the discussion of osmotic pressure, no allowance has been made for the range of molecular weights normally present in a macromolecule sample.

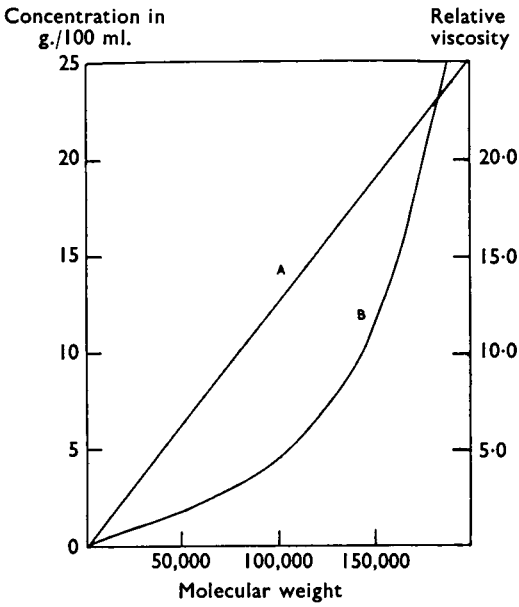

FIG. 1. Curve A. Relation between molecular weight of solute and the concentration required to give an osmotic pressure of 33 cm. The assumptions made are given in the text.

Curve B. The relative viscosity (calculated as described in text) of the solutions defined by curve A.

It is desirable that the relative viscosity of a plasma expander should not be so much greater than that of true plasma (1·8 at 38° C.[29]) as to throw undue strain on the heart. Dilution of the plasma expander during infusion will undoubtedly reduce its relative viscosity but there does not appear to have been any authoritative statement about the maximum permissible relative viscosity. Plasma expanders containing polyvidone have a relative viscosity only slightly above that of true plasma (e.g., Plasmosan; relative viscosity = 2·1) whereas those based

on dextran have a considerably higher viscosity (e.g., the solution accepted by British hospitals must have a relative viscosity between 3·0 and 6·0 at 37° C.[16]).

Elimination of Macromolecules from the Body

The elimination of macromolecules from the circulating blood is of importance in connection with the duration of action of a plasma expander and with the possibility of storage in the body. The more rapid the elimination, the shorter will be the time during which an effective colloidal osmotic pressure is exerted. Absence of a mechanism of elimination must result in storage in the body.

Some macromolecules, such as dextran[34], can be completely metabolised in the body to low molecular weight molecules (in the case of dextran, to sugars and ultimately carbon dioxide), which do not exert an effective colloidal osmotic pressure and can be rapidly excreted by the kidney. It will be shown that, for each chemical type of macromolecule, there is probably a threshold molecular weight above which it is excreted by the kidney only very slowly, and, consequently, only macromolecules which are completely metabolised will be eliminated rapidly when their molecular weight is greater than this threshold value. No information has been published on the effect of molecular weight on the rate of metabolism of dextran. Of the other macromolecules used in plasma expanders, there is no agreement about the metabolism of gelatin[35,36] and the limited degradation of polyvidone, postulated by Campbell and colleagues, can but assist the renal excretion of this macromolecule[5,37].

The main route for elimination of macromolecules from the body is by excretion by the kidney. Most work on the permeability of the normal kidney nephron to large molecules has been concerned with the passage of proteins from the plasma into the urine and is summarised in Table III. There are undoubtedly difficulties in comparing these results. The different animals used may have nephrons of different permeabilities. The results with hæmoglobin may be anomalous since it has been suggested that it alters the nephron permeability[39]. The proteins used may form complexes with the plasma proteins, as has been shown to be the case with lysozyme[40]. It is broadly apparent, however, that protein molecules with a molecular weight greater than about 68,000, the molecular weight of hæmoglobin, do not pass through the normal nephron. A more detailed study indicates that other factors, which may be molecular shape and electrical charge, are significant in determining the renal excretion of macromolecules but no definite correlations are evident. Two exceptions to the simple molecular weight rule are albumin and fetuin. Albumin, the molecular weight of which is now believed to be 65,000 instead of 69,000[41], does not pass through the human nephron, whereas hæmoglobin, of molecular weight 68,000, does. Fetuin of molecular weight 51,000 is not excreted in the urine of rabbits whereas hæmoglobin with a greater molecular weight is[38]. Brant and others in a study of the renal clearance of hæmoglobin in normal and proteinuric

TABLE III

RENAL EXCRETION OF PROTEINS BY NORMAL KIDNEYS (*a*)

Protein injected into plasma	Renal excretion: Animals used	Renal excretion: Present (+) or absent (−) in urine	Molecular weight	Isoelectric (*f*) point	References: Renal excretion	References: Molecular weight	References: Isoelectric point
Lysozyme	Dog	+	15,000	10·5 (0·1)	40	97	104
Myoglobin	Dog	+	17,000	7·0	43	97	101
Ovomucoid	Dog	+	28,000	3·9 (0·1)	40	97	105
Globin	Rat	−	?34,000 or 68,000 (*c*)	7·5 (0·1)	96	103	104
Bence Jones	Dog, cat, rabbit	+	35,000	5·2 (0·02)	94	7	7
Fetuin	Man Rabbit, calf	+ −	35,000		38 38	98	
Gelatin	Dog, cat, rabbit	+	35,000 (*d*)		94	94	
β-lactoglobulin	Dog	+	37,300	5·1 (0·1)	40	97	104
Ovalbumin	Dog, rabbit, calf, rat	+	47,000	4·6 (0·1)	40, 94, 96, 93	97	104
Serum albumin	Man, dog, cat, rabbit, rat	−	65,000	4·7 (0·1)	94, 96, 93	41	104
Hæmoglobin	Man, dog, cat, rabbit	+ (*b*)	68,000	7·0	43, 94, 39, 95	101	101
Conalbumin	Dog	−	87,000	6·8 (0·1)	40	97	105
Casein	Dog, cat, rabbit	−	75,000–100,000	4·0–4·5 (0·1)	94	99	104
Serum globulin	Man, dog, cat, rabbit	−	176,000	γ=5·8 (0·1)	94	7	104
Edestin	Dog, cat, rabbit	−	c.a. 350,000	5·5–6·0	94	100	97
Hæmocyanin helix	Dog, cat, rabbit	−	1,000,000 (*e*)	4·7	94	101	101

NOTES:

(*a*) The work of Bott and Richards[98] on amphibia has not been included in this table.

(*b*) Appears in the urine only when the plasma concentration is greater than 0·1 g.100 ml.[95].

(*c*) The literature (e.g., reference 102) on the use of globin in plasma expanders gives the molecular weight as 34,000. Since it has been shown that globin dimerises very readily, the absence of globin in the urine may indicate that the higher molecular weight of 68,000 is correct.

(*d*) Stated to include breakdown products of about 1,000 molecular weight.

(*e*) There appears to be doubt about this molecular weight since it varies with the concentration of the solution used in the measurement. Hæmocyanin may reversibly dissociate into smaller molecules.

(*f*) The ionic strength of the solution used for the measurements is given in parentheses wherever known.

human subjects concluded that the nephron is more permeable to molecules with large electric charges[42]. Measurements of the actual rates of passage of proteins through the nephron[40,43] indicate that the sharp cut-off in renal excretion at a molecular weight of about 68,000 is not real, there being a gradual decrease in excretion rate with increasing molecular weight until no detectable excretion is observed at molecular weights greater than 68,000.

Many experiments have shown that a limited re-absorption of proteins, whatever their nature, takes place in the kidney tubules, different proteins competing with each other to saturate the tubular re-absorption[44,46]. It is now believed that the absence of higher molecular weight proteins in the urine is not due to the impermeability of the glomerular membrane to these proteins but to the passage of only small quantities of them through the glomerular membrane and their subsequent reabsorption in the tubules. The evidence for this belief, based on the appearance of proteinuria when the homologous plasma protein concentration is raised above normal[47] and on other experiments, has been reviewed by Rather[48]. The evidence from protein studies is, therefore, that the glomerular membrane is permeable to all proteins, the rate of penetration decreasing, but never becoming zero, with increasing molecular weight and changing, to a lesser extent, with molecular shape and electric charge.

The very little evidence available suggests that the renal excretion of macromolecules other than protein involves simple glomerular filtration and that tubular reabsorption does not occur. Although transient deposits of dextran have been found in the tubule cells[49], Wallenius[21] has concluded, from renal clearance experiments with low molecular weight dextran, that there is no tubular re-absorption of dextran, a very small quantity of low molecular weight material merely diffusing by a purely physical mechanism, into the tubule and other cells of the nephron. Campbell and others[5] have shown that polyvidone of high molecular weight is excreted into the urine of human subjects in very low concentrations some 300 hours after an infusion. Such a low concentration would appear to be unlikely if a tubular reabsorption mechanism existed for all molecular weights of polyvidone.

The inverse relation between renal excretion rate and molecular weight has been demonstrated, by measuring the renal excretion of fractions of limited molecular weight range, for dextran[20,21,50–54], polyvidone[55,56], gelatin[57,58,113] and polyethylene glycols[59]. The results of Campbell and others[5] (see Fig. 2), who studied the variation in molecular weight distribution of polyvidone excreted by human subjects, with time after an infusion, support this relationship. Early work on dextran[20,52,53] had suggested that there might be a threshold molecular weight of about 40,000 above which it was not excreted but it seems possible that higher molecular weight dextrans have not been detected in urine owing to lack of a sufficiently sensitive analytical method. The results of Wallenius[21] (see Fig. 3) on dextran clearance rates support this conclusion, the asymptotic approach to the molecular weight axis being similar to the curves obtained with proteins. Campbell and others[5] have shown by

isolating the macromolecule from urine that polyvidone molecules with molecular weights greater than 100,000 are slowly excreted by the kidney, the rate of excretion becoming slow at a molecular weight of about 50,000.

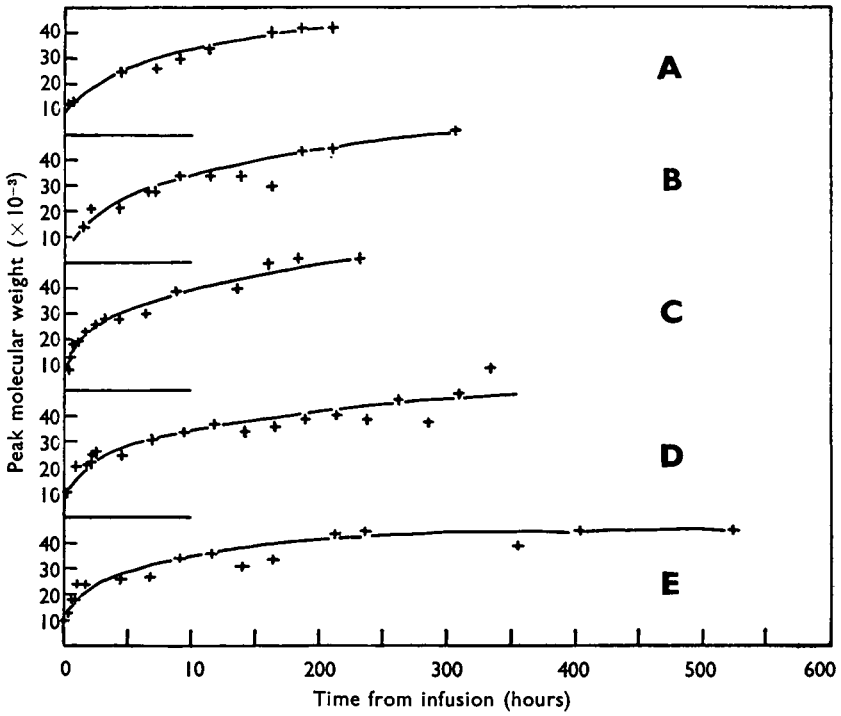

FIG. 2. Increase in peak molecular weight of polyvidone excreted by human subjects with time after an infusion (reproduced with permission from Campbell and colleagues[22]).

This latter molecular weight is supported by Warner's[60] conclusion that there is a sharp cut-off of renal excretion of polyvidone between 35,000 and 40,000 molecular weight. A more sensitive method of analysis would presumably have shown the slow excretion of higher molecular weight polyvidone. Although no actual measurements have been published of the variation of renal clearance rate of polyvidone with molecular weight, it is reasonable to assume a similar relation to that for dextran (Fig. 3).

The renal excretion studies of large molecules reported above are concerned with the normal kidney. It is well established that kidney disease can result in an increase in the glomerular permeability to macromolecules. Starling and Verney[61] have shown that oxygen lack can result in an increase in the permeability of the glomerulus to proteins. Under those clinical conditions of shock necessitating an infusion of a plasma expender, oxygen lack might well be the cause of impairment of the renal circulation so that the glomerulus might pass large molecules

more rapidly than normally. Wallenius[21] has obtained evidence of this with dextran since he detected in the urine molecules of molecular weight 57,000 for two cases of second degree burns whereas normally the maximum molecular weight detected was 46,000. Consequently, the conclusions drawn from the studies of normal renal excretion may be correct qualitatively but may be incorrect quantitatively as a result of increased glomerular permeability in cases of shock. It is, however, evident that for solute particles, size is the most important factor governing the rate of excretion, although shape and electric charge may be important. The glomerular clearance rate becomes very small with increasing molecular weight but there is no experimental evidence suggesting that it actually becomes zero so that, given sufficient time, all macromolecules circulating in the blood could pass through the glomeruli, and, in the absence of tubular reabsorption, appear in the urine. The reviewer believes that the slow excretion of very large chain-like molecules, such as dextran and polyvidone, may be the result of variations in orientation of the repeating units of the chain in solution resulting in changes in the shape of the solute particle from the statistical mean value which is normally measured. Such changes in shape could not, of course, occur with the plasma proteins without rupture of intra-molecular hydrogen bonds and, hence, denaturation.

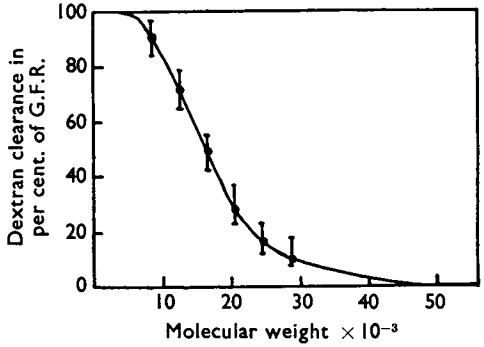

FIG. 3. The renal clearance of dextran (in per cent. of the glomerular filtration rate) and its relation to the molecular weight of the dextran molecules, in normal dogs (after Wallenius[21]).

The bearing of these results on the exact mechanism whereby macromolecules are transported across the glomerular membrane is not discussed here. Wallenius[21] has stated that it is not yet possible to determine which mechanism is correct, restricted diffusion through microscopic pores[62] or diffusion through the gel-like membranes postulated by Chinard[63].

PASSAGE OF MACROMOLECULES INTO THE LYMPH

The more rapidly do the macromolecules of a plasma expander leave the circulating blood and pass into the lymph, the more rapid is the decrease in colloidal osmotic pressure and, hence, in the effectiveness of the plasma expander. The permeability of the capillary wall to macromolecules has not been studied as fully as has that of the glomerular membrane but qualitatively one would expect similar laws to hold in both cases. Quantitatively, however, there might be very large differences. Forker and others[64], using radiotracer methods, have shown that exchange of plasma proteins between blood and lymph is complete in less

than one hour but no measurements were made on the variation of the rate with molecular weight. Grotte and others[54], using fractions of Swedish dextran of different molecular weights, have shown that molecules with molecular weights as great as 205,000 pass into the lymph, the rate decreasing with increasing molecular weight. More recently Martin[16] has reported experiments (Fig. 4) which show that diffusion into the lymph can be reduced by use of higher molecular weight dextran. Martin[16] and Wasserman and Mayerson[65] have shown that dextran below about 40,000 molecular weight passes rapidly into the lymph. No similar data are available for polyvidone, but, from the work of Fresen and Weese[66] who made a histological examination of lymph nodes and other tissues after infusions of polyvidone, it can be inferred that molecules with molecular weights as great as 400,000 pass into the lymph and that the rate decreases with increasing molecular weight. Little and Wells[67] have shown that molecules of gelatin pass through the capillary wall less readily than do plasma proteins.

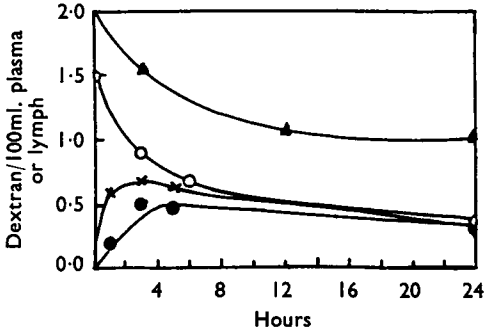

FIG. 4. Rate of decay of dextran fractions A and D from the plasma and their diffusion into the lymph (after Martin[16]). Dose 20 ml./kg. intravenous. Average of normal rabbits.

	Weight average mol. wt.
Fraction A. O—O, plasma, ×—×, lymph	45,000
Fraction D. ▲—▲, plasma, ●—●, lymph	120,000

VARIOUS INTERACTIONS WITH MACROMOLECULES

It is essential that any compound used in a plasma expander should not interfere with the normal functioning of the body. It is well established that macromolecules sometimes form complexes with other macro molecules, such as proteins, and with ions. In most cases no formal chemical bonds are formed, the complex being held together by electrostatic or van der Waals' forces. The present discussion has been limited to those interactions of macromolecules in which the effect of molecular weight is known. For example, the vast literature on the use of polyvidone as a drug delaying agent or as a detoxifying agent will not be referred to since no proper examination of the effect of molecular weight has been made.

It is well established that macromolecules foreign to the blood interact with erythrocytes producing an increase in their sedimentation rate (dextran[68–70,111]; gelatin[69]; oxypolygelatin[71]; polyvidone[69,70,72]) and, in sufficiently high macromolecule concentration, aggregation of the cells which clinically may produce some anæmia[73]. The most detailed work

on the influence of molecular weight has been carried out by Thorsen and Hint[69] who showed that the erythrocyte sedimentation rate increases with increasing molecular weight with dextran and gelatin, and that the minimum molecular weight which will produce aggregation is 59,000 for Swedish dextran and 18,000 for gelatin. These authors also proved that the presence of low molecular weight dextran would prevent, or reduce, the effect of higher molecular weight dextran. Hummel and Hamburger[74] suggest that the minimum molecular weight for aggregation is about 35,000 for polyvidone. In clinical practice, no bad effects have been reported with the grades of dextran, gelatin and polyvidone at present used in plasma expanders.

There is evidence that the antigenicity of dextran depends upon molecular weight. Dextrans of various origin have been shown to be antigenic in man[75] and recently Glynn and others[76] have shown that dextran of high molecular weight has a greater antibody response than has low. Dextran molecules of molecular weight about 5000 were non-antigenic but produced a precipitate with antisera. Smaller molecules acted as non-precipitating haptens. Similar antigenic reactions have not been reported for gelatin preparations or for polyvidone.

Although it has been established that polyvidone and dextran do not interact sufficiently strongly with the plasma proteins to interfere with their electrophoresis[77], it has been proved[78] that polyvidone, acacia and all dextrans except the lowest molecule weights all form insoluble complexes at 4° C. with fibrinogen. At 37° C. no insoluble complexes were formed. No ill effects due to the formation of fibrinogen complexes have been reported in the use of plasma expanders, but a fuller study of this problem seems desirable.

The storage in the body of the macromolecular component of a plasma expander is of importance as a possible source of interference with normal physiological functions although such interference has never been found in cases where storage of macromolecules has been reported. Experiments involving massive doses of the macromolecule can be disregarded since these conditions of high concentration are never likely to be met in normal clinical use of plasma expanders. Stenger and Müller[79] have concluded that the ability of the body to store macromolecules is mainly of academic interest. Storage in the tissue of a macromolecule might well depend upon molecular weight since the deposition of a water soluble molecule in tissue will require the formation of some kind of complex with the tissue molecules. It has been shown by many authors[53,80] that only temporary storage of dextran occurs, mainly in the liver, spleen, kidney and lymph nodes. It would appear that no strong complex formation is associated with the storage of dextran and that its temporary presence in various cells may be due to a mere physical diffusion into them[21]. If this is correct it would appear that increase in the molecular weight of the dextran would reduce the quantity temporarily stored in the cells. The evidence about the storage of polyvidone is conflicting[37,72,79,81,72] and may partly be due to the use of different grades of the polymer since it has been demonstrated[64,83] that increase in molecule

weight increases the possibility of storage. The reviewer believes that a careful, critical examination of the methods used for the detection of polyvidone in tissue cells is necessary before this problem can be finally solved. No information has been published on the question of storage of gelatin preparations.

The Ideal Macromolecule for a Plasma Expander

In this review an attempt has been made to show the importance of solute particle size and shape in governing the behaviour of macromolecules in the body. Although many more experimental studies would be necessary to complete our knowledge of this subject, it should be possible, at least in principle, to determine the essential properties of the ideal macromolecule for a plasma expander, and, hence, to select both the chemical type and molecular weight range most suitable. When the ideal macromolecule has been finally designed, economic manufacture of it might well prove impossible so that some compromise would be necessary.

The major difficulty in the selection of the macromolecule is the determination of the duration of effectiveness required for a plasma expander. It would appear ideal for the macromolecule to remain in the circulating blood and not to pass into lymph, thereby reducing the osmotic pressure. It would then be eliminated at such a rate that the colloidal osmotic pressure decreased as the osmotic pressure due to the plasma proteins returned to normal. Although it is obvious that an exact balance between the osmotic pressures of the added macromolecules and of the plasma proteins would be an impossibility, an approximation might be arrived at from a knowledge of the time taken for the regeneration of plasma proteins. Unfortunately, most published information on regeneration times in human subjects is for cases of malnutrition. Wallace and Sharpey-Schafer[106] have shown that in human subjects not suffering from shock, regeneration of the plasma proteins is virtually complete in 3 to 90 hours, but no similar studies have been made on human subjects who are in a severely shocked condition through loss of blood. Ebert and colleagues[107] have shown that in dogs, severely shocked after considerable loss of blood which was replaced by an equal volume of isotonic saline, regeneration of proteins was extremely slow. More recently Semple[108], also working with dogs, replaced 45 per cent. of the blood with a dextran solution, and found that protein regeneration was fairly rapid. This last author concluded that dextran did not retard protein regeneration but there does not seem to be any evidence whether the addition of a macromolecule actually assists regeneration. Kozoll and others[109] (pectin), Feigan and others[110] (oxypolygelatin) and Gropper[11] (dextran) have obtained evidence that these macromolecules may actually stimulate the appearance of proteins from some store in the body, thereby producing a greater effect in maintaining plasma volume than can be explained simply by the osmotic pressure produced by the infusion. If this should be proved correct, the fundamental approach to the development of a plasma expander becomes even more difficult and experimental studies

would be required on the effect of molecular weight of different macromolecules on protein regeneration. It must be admitted that, at the present time, our basic knowledge is quite inadequate for an attempt to be made to develop a plasma expander by the suggested *a priori* method.

An alternative approach, and the one actually used in practice, is to determine the clinical effectiveness of different plasma expanders containing macromolecules of different chemical type and molecular weight range. Another difficulty arises, the differences between the requirements of various users which result in differences of medical opinion. Some require a fairly short acting plasma expander for use in cases of hæmorrhagic shock where there has been a single loss of blood[81] whereas others require a longer acting plasma expander which can be used in cases of severe burns, where there is a prolonged loss of fluid from the burn surface[50]. This review is not the place to discuss this question but it appears to the reviewer that the different plasma expanders available to-day may each be of value in the particular application most suited to it.

Our knowledge of the behaviour of macromolecules in the body is very incomplete, and, even when further experimental studies have completed this knowledge, the ideal macromolecule for use in a plasma expander would probably have properties very similar to those of serum albumin, normally responsible for the greater part of the control of fluid balance. This would suggest that the use of serum albumin itself might simplify the development of plasma expanders, provided that it could be obtained in large quantities in a form suitable for long-term storage. The present developments in large-scale fractionation of plasma proteins and in the preparation of despecified sera may be an indication that, in the future, artificial molecules such as dextran, oxypolygelatin and polyvidone, may be replaced by serum albumin in plasma expanders.

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