XC. THE ULTRAFILTRATION OF PROTEINS THROUGH GRADED COLLODION MEMBRANES.

I. THE SERUM-PROTEINS.

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MANY qualitative investigations showing the influence of various physical factors upon the process of ultrafiltration are contained in the literature. Elford [1933] has discussed these effects in a more quantitative manner, based on experimental results with graded collodion membranes [Elford, 1931]. The latter afford a unique advantage in that they provide a wide selection of grades and can be accurately calibrated in terms of average pore diameter; and groups of membranes known to be of the same grade may be used in comparative filtrations with different systems. The present study considers the influence upon the ultrafiltration of proteins of the nature of the solvent medium, its hydrogen ion concentration, the concentration of protein and filtration pressure. It establishes a firmer basis for the standard conditions which have been adopted as being most favourable for ultrafiltration studies on viruses and bacteriophages in this Laboratory (for a partial bibliography see Burnet and Andrewes [1933]). It confirms the values previously presented for the empirical correction factor used in estimating the particle size of a virus or phage from the pore diameter of the limiting membrane which, under those optimum conditions, just completely retains it.

Materials.

The proteins used were highly purified samples of horse serum-albumin and pseudoglobulin, kindly presented to us by Dr H. W. Dudley, to whom we acknowledge our indebtedness. The pseudoglobulin was prepared by precipitation from normal horse serum with half-saturated ammonium sulphate, and subsequent dissolution in water and precipitation with ammonium sulphate three times. The final precipitate was dissolved in water and dialysed under slight pressure. This served to precipitate any remaining euglobulin, which was sedimented by centrifuging. The clear centrifugate, containing the pseudoglobulin, was concentrated *in vacuo* over sulphuric acid to obtain the solid protein. The albumin, contained in the first filtrate from the globulin precipitation, was precipitated three times by the method of Hopkins and Pinkus [1898], the requisite amount of acetic acid for producing a crystalline precipitate being determined in each case by trial. The final precipitate was redissolved in water and dialysed and concentrated *in vacuo* as above.

When the albumin was dissolved in water, or the pseudoglobulin in 0.05 % sodium chloride solution, practically no insoluble residue remained. Protein-free ultrafiltrates of solutions of both proteins in distilled water contained no chloride

detectable by the silver nitrate test and only a trace of sulphate as detected by barium chloride. The ash contents of the solid samples were: albumin, 0.8 %; pseudoglobulin, 0.4 %.

Procedure.

Exclusive use was made of the graded collodion ("gradocol") membranes, prepared and calibrated by the methods of Elford [1931] and Elford and Ferry [1934]. Nickel-plated ultrafilters of the type described by Barnard and Elford [1931] were slightly attacked by the more acidic protein solutions, and accordingly silver-plated models were employed. The components of the apparatus were sterilised separately and assembled under sterile conditions. Filtration was carried out under positive pressure of from 1 to 3 atmospheres of nitrogen. In comparative experiments, six filtrations could be run simultaneously from a pressure distributor attached to one nitrogen cylinder.

In a typical experiment, a solution of protein was made up to the desired concentration in a given solvent medium, and the p_H was adjusted, if necessary, by addition of small quantities of 0.1 *N* or *N* hydrochloric acid or sodium hydroxide. The solution was then filtered through a membrane of grade between 0.5μ and 1.0μ to sterilise it. Filtration through such a membrane affords a very convenient method for removing bacteria and gross aggregates with minimum loss of protein by adsorption. This sterile stock filtrate could be kept for months at 4° without any appreciable change in appearance, p_H (unless originally alkaline, when absorption of CO_2 from the atmosphere soon shifted the reaction), or filtrability. In no case was an antiseptic preservative necessary. When a comparison of different media was required, the stock was made up to twice the final concentration desired, and aliquot parts were diluted with the different media.

Different samples, usually 10 ml. of stock solution, were filtered through various membranes, all of comparable thicknesses and of grades ranging from that which let the protein through in nearly undiminished concentration to that which yielded filtrates giving a negative test with sulphosalicylic acid. For each individual filtration, the filtrate was collected in successive ml. samples and analysed with a Zeiss dipping refractometer. The concentrated residue remaining over the membrane was also analysed. The refractometric analyses were roughly checked by precipitation with sulphosalicylic acid, which was sensitive to a concentration of about 0.005 % protein. The relative concentration, u , of the

Fig. 1. Typical filtration curves.

filtrate, in terms of the concentration of protein in the stock solution (using the refractive index of the protein-free ultrafiltrate as a zero-point) could be expressed with an accuracy of about 2 %. It was plotted against the total volume filtered to give the "filtration curve" (Fig. 1).

The highest point on this curve, $u_{\max}$, was characteristic of the solution and the membrane and was reproducible for comparable conditions to 5–10 %. The value of $u_{\max}$ is largely determined, in curves of type I, by the amount of adsorption of protein by the membrane; in curves of type II, by a process not fully explained, perhaps a true sieving mechanism; in curves of types III and IV, by the stage at which the narrowing and blocking of pores begins. These processes are discussed more fully below. In curves of type V, u is still rising at the end of the experiment, and $u_{\max}$ is significant only in comparing different filtrations which have all been carried to the same point.

Values of $u_{\max}$ for different membranes were plotted against the logarithm of membrane average pore diameter, $\log j$, to give the “end-point curve” (Fig. 2). The end-point, j_e , for the disperse phase in a given system is the A.P.D.¹ of the membrane which just fully retains it.

Fig. 2. Typical end-point curves.

The slopes of the end-point curves vary for different systems, as shown in Fig. 2. Hence the “filtrability” of the disperse phase in a given system cannot well be characterised by the end-point alone; it should be represented by the whole curve. We have selected three points on the curve for a convenient measure of filtrability: the values of average pore diameter, $j_{0.5}$, $j_{0.2}$ and j_e , which correspond with the maximum relative concentrations of filtrates 0.50, 0.20 and 0 respectively. The higher these values are, the less “filtrable” is the system.

The filtrabilities of different systems may be compared among themselves, however, by the values of $u_{\max}$ for one given grade of membrane, represented by the intersections of the end-point curves with the vertical line drawn through the appropriate value of $\log j$, as shown in Fig. 2 by ab .

GENERAL EFFECT OF THE NATURE OF THE SOLVENT MEDIUM ON FILTRABILITY.

The effect of capillary-active substances in decreasing the adsorption zone of the filtration curve and favouring filtration generally has been discussed [Elford, 1933]. The suspending medium uniformly used for filtration studies on viruses and bacteriophages in this Laboratory has been the Hartley's broth which is employed in bacteriological work. The stock broth is prepared from horse-flesh by extraction and digestion with trypsin [Hartley, 1922]. Its effect in facilitating filtration may be due to (1) peptisation of the particles of the disperse phase by adsorption of large residues of broken-down proteins; (2) lowering of surface tension of the solution; (3) peptisation and electrokinetic effects due to the presence of neutral salts.

¹ The abbreviation “A.P.D.” is conveniently used for *average pore diameter*.

The stock broth often contains proteoses; addition of sulphosalicylic acid produces a precipitate which, in contrast with that given by the large serum-proteins, may be redissolved by boiling. Before investigating the influence of broth upon the filtrabilities of the serum-proteins, an effort was made to obtain a proteose-free broth. The standard method of preparation was modified by prolonging the digestion with 5 % Cole and Onslow's pancreatic extract for 2 weeks at 37°. Chloroform was added as a preservative. The resulting "extra-digested" broth gave a negative test with sulphosalicylic acid. Its surface tension at 21° was about 6 % lower than that of water, compared with a 25 % to 30 % lowering in the case of the stock broth. Its frothing power was greatly diminished.

EXPERIMENTAL.

Comparative experiments were made to show the effects of various media on filtrability. Solutions of serum-albumin, concentration 0.5 %, were made up in water, 1 % sodium chloride, and 1:1 dilutions in water of extra-digested broth, two different preparations of stock broth, and ox-heart broth (*i.e.* Hartley's broth prepared from ox-heart instead of horse-flesh). Each system, adjusted to approximately the same p_H , was filtered through a membrane of the same A.P.D. A similar experiment was performed with the pseudoglobulin, using 0.05 % sodium chloride solution instead of water. The results are shown in Table I.

Table I. *Filtration of serum-proteins in various solvent media.*

Medium	Albumin. Membrane A.P.D. 14.5 $m\mu$		Pseudoglobulin. Membrane A.P.D. 59 $m\mu$	
	p_H	$u_{max.}$	p_H	$u_{max.}$
Water	7.1	0.14	—	—
0.05 % NaCl	—	—	7.1	0.14
1.0 % NaCl	6.9	0.34	7.3	0.61
Extra-digested broth	7.1	0.36	7.3	0.57
Stock broth, preparation 26-9	7.5	0.46	7.4	0.81
Stock broth, preparation 24-10	7.4	0.33	7.3	0.74
Ox-heart broth	7.4	0.46	7.4	0.73

Results. The data demonstrate the superiority of Hartley's broth as a solvent medium in the filtration of both proteins. Stock and ox-heart broths favour the filtration to about the same extent and are definitely more effective than extra-digested broth, which in turn has about the same effect as the 1 % sodium chloride solution. The superiority of the stock and ox-heart broths over the extra-digested preparation is probably due to the presence in the former of a capillary-active component, which becomes destroyed by prolonged digestion. It is also to be noted that in every instance the improvement in filtration is more marked for pseudoglobulin than for albumin. The mechanism of these effects will be discussed below.

EFFECT OF p_H ON FILTRABILITY.

Serum-albumin.

Experimental. The complete filtration and end-point curves were determined for filtrations of solutions of serum-albumin, concentration 0.4 % to 0.5 % in distilled water, at different values of p_H ranging from 3.0 to 8.8. The characterising

A.P.D. values, $j_{0.5}$ and $j_{0.2}$, were obtained by interpolation, and j_e by extrapolation on each curve. These three quantities are plotted against the p_H in Fig. 3.

Fig. 3.

Fig. 3. p_H -filtrability curves of serum-albumin dissolved in water. *a.* $u_{\max} = 0.50$. *b.* $u_{\max} = 0.20$. *c.* $u_{\max} = 0$.

Fig. 4.

Fig. 4. Comparison of filtrability of serum-albumin dissolved in water and in Hartley's broth. *a.* Solution in Hartley's broth. *b.* Solution in water (interpolated from the data of Fig. 3). (Membrane A.P.D. $20m\mu$.)

Time did not permit a correspondingly complete series of end-point curves to be obtained for filtration of serum-albumin when dissolved in 1 : 1 dilutions in water of stock broth, but comparison was made of systems at different p_H values with one grade of membrane. The values of $u_{\max}$ are plotted against the p_H in Fig. 4, together with the corresponding curve for solutions in distilled water, interpolated from the data of Fig. 3.

Results. The high peak in Fig. 3, at which membranes of A.P.D. $45m\mu$ completely retain serum-albumin in aqueous solution corresponds fairly closely to its isoelectric point, p_H 4.88 [Svedberg, 1930]. It is significant that the adsorption of the protein on collodion particles as a function of the p_H exhibits a high maximum at the same point [Elford, 1933; Ettisch *et al.*, 1930]. Stock broth improves the filtration at all p_H values but most markedly at the isoelectric point and near it on the alkaline side. The minimum filtrability apparently occurs at the same p_H as in distilled water solution. At the optimum p_H values for filtration in aqueous solution, 3.8 and 8, the end-point is about $11m\mu$. The former p_H is just outside the zone within which Svedberg and Sjögren [1930] show serum-albumin to be monodisperse (p_H 4 to 9).

Serum pseudoglobulin.

Experimental. A complete set of end-point curves was established for filtrations of pseudoglobulin, concentration 0.4 %–0.5 % in 1 % saline, at different values of p_H ranging from 2.5 to 8. The values of $j_{0.5}$, $j_{0.2}$ and j_e , obtained as for

the albumin, are plotted against the p_H in Fig. 5. A comparison of filtrations in broth at different p_H values, similar to that performed for the albumin, is shown in Fig. 6.

Fig. 5.

Fig. 5. p_H -filtrability curves of serum-pseudoglobulin dissolved in 1 % saline. *a.* $u_{\max}=0.50$. *b.* $u_{\max}=0.20$. *c.* $u_{\max}=0$.

Fig. 6.

Fig. 6. Comparison of filtrability of serum-pseudoglobulin dissolved in 1 % saline and in Hartley's broth. *a.* Solution in Hartley's broth. *b.* Solution in 1 % saline (interpolated from the data of Fig. 5). (Membrane A.P.D. 59 $m\mu$.)

Results. Conditions for the poorest filtrability of pseudoglobulin in 1 % saline obtain at p_H 4.4, where a 90 $m\mu$ membrane completely retains the protein. This reaction is well on the acid side of the isoelectric point (p_H 5.4) in contrast to the close correspondence observed between isoelectric point and point of maximum j_e for the albumin. Adsorption of serum-globulin on collodion particles is greatest over a fairly wide range of p_H in the neighbourhood of the isoelectric point [Ettisch *et al.*, 1930; Elford, unpublished experiments], and the maximum is not sharply defined as in the case of serum-albumin.

Broth is found to improve the filtrability of pseudoglobulin at all p_H values (Fig. 6), but the point of minimum filtrability seems to be displaced still further in the acid direction (p_H 3.7). Since the broth itself exhibits a maximum opalescence at about p_H 3, the minimum filtrability observed at p_H 3.7 may depend upon the resultant stability of the broth colloids and of the pseudoglobulin.

The optimum point for the filtration of pseudoglobulin in 1 % saline, as far as the present investigations extend, is at p_H 8. The corresponding value for the end-point is about 25 $m\mu$.

Effect of neutral salt on filtrability.

Solutions of the albumin containing sodium chloride in concentrations varying from 0.05 % to 2.0 % were filtered at selected p_H values to show how increasing the amount of salt in the medium influenced filtration. The salt concentration producing the maximum effect in each case, together with the actual value of

$u_{\max}$, are shown in Table II. The increase in filtrability due to the salt is least marked with systems of high filtrability (low j_e) in water, and the effect becomes greater as the isoelectric point is approached from the alkaline side.

Table II. *Effect of salt on the filtrability of serum-albumin.*

Concentration of protein 0.5 %.						
p_H	Mem- brane $m\mu$	Concentra- tion of NaCl %	$u_{\max}$ in NaCl solu- tion	$u_{\max}$ in water	Δ $u_{\max}$	j_e in water $m\mu$
7.2	14.5	1.0*	0.34	0.14	0.20	13
6.8	20	0.8	0.90	0.63	0.27	14
4.8	40	2.0	0.95	0	0.95	45

* Only concentration used.

EFFECT OF CONCENTRATION OF PROTEIN ON FILTRABILITY.

Filtration studies on viruses have shown that the filtrability improves with increasing concentration of virus, particularly as the grade of membrane used approaches the end-point. The higher the virus concentration the more quickly is the adsorbing capacity of the membrane satisfied, so that the virus has the greater chance of appearing in the filtrate before blocking sets in. Therefore experiments have always been performed with suspensions of the highest concentrations obtainable, which, however, in the case of virus correspond to molecular concentrations which, for a protein, would be quite undetectable by the sulphosalicylic acid test. In protein solutions, the molecular concentration may be high enough actually to hinder filtration.

Fig. 7. Filtration curves of serum-albumin at different concentrations. *a.* 4.0 %. *b.* 2.0 %. *c.* 1.0 %. *d.* 0.5 %. *e.* 0.25 %. (Membrane A.P.D. 33 $m\mu$; p_H of solution 5.7.)

Experimental. Solutions of serum-albumin at p_H 5.7–5.8 ranging in concentration from 0.25 % to 4 % were filtered through membranes of the same grade. The results are expressed in terms of the relative concentration of filtrate, u , in Fig. 7.

Results. The value of $u_{\max}$ decreases with increasing original concentration of the solution filtered. In the systems of highest protein concentration, the primary adsorption on the membrane is most quickly satisfied, but blocking sets in at such an early stage that the first sample of filtrate collected is the most concentrated. Under such conditions it is almost impossible to obtain reproducible results, as the filtrate concentration is rising so rapidly in the first ml. that a very slight variation of the time at which blocking becomes effective will result in widely conflicting values of $u_{\max}$. Hence the concentrations of 4 %, 2 % and probably 1 % are unsuitable for quantitative comparative experiments. On the other hand, a concentration of 0.25 % is rather low for accurate analyses by the refractometer. The usual concentration used in these investigations, 0.5 %, is apparently the most satisfactory.

EFFECT OF PRESSURE ON FILTRABILITY.

A solution of pseudoglobulin at p_H 5.2 was filtered through membranes of grade 84 $m\mu$ at pressures of 3, 2 and 1 atmospheres and under its own slight hydrostatic pressure in the filter. In the last case, the conditions approximated to those of a diffusion experiment. Fig. 8 demonstrates the effect of increasing

Fig. 8. Filtration curves of serum-pseudoglobulin at different pressures. *a*. No pressure applied. *b*. 1 atm. pressure. *c*. 2 atm. pressure. *d*. 3 atm. pressure. Membrane A.P.D. 84 $m\mu$; p_H of solution 5.2.

pressure in slightly narrowing the adsorption zone [Elford, 1933] and hastening blocking. The range of pressure (1–3 atmospheres) regularly employed in the present studies does not involve much variation in $u_{\max}$.

DISCUSSION.

The p_H -filtrability curves of serum-albumin (Fig. 3) indicate that the p_H range may be divided conveniently into characteristic zones of good and poor filtrability. Between p_H 4.2 and 7, there is a sharp peak of poor filtrability, where the end-point values of average pore diameter lie far above the molecular size (5.4 $m\mu$, disregarding the dissymmetry of the molecule) calculated from ultracentrifugation [Svedberg, 1930; von Mutzenbecher, 1933]. On each side this region grades into a zone of good filtrability, where the curves are relatively flat, and j_e is about twice the molecular size of the protein.

The filtration curves for experiments within the central zone of poor filtrability are invariably of types I, III and IV (Fig. 1). Curves of the other types occur

as this zone merges into the regions of good filtrability; the extreme acid and alkaline systems invariably give curves of types I, II and V. The universality of type I shows a uniform filtration mechanism for membranes which let through the respective systems in nearly undiminished concentration, *i.e.* where $u_{\max}$ lies between 0.9 and 1.0. The distribution of the other types indicates two different mechanisms in those filtrations where $u_{\max}$ lies between 0 and 0.9.

In a case where the p_H lies within the central zone, the relative concentration of the filtrate drops off at an early stage and decreases as the filtration is continued, eventually reaching zero. The pores have been rendered impermeable to protein, presumably blocked by large deposits of protein within them, and the $u_{\max}$ attained is far below the value which might have been reached had this blocking effect not occurred. This process may be termed *abnormal filtration*. On the other hand, when the experiment is made in the extreme acid or alkaline portion of the p_H range, the relative concentration of the filtrate reaches a definite fractional value which is fairly steadily maintained or may even rise gradually as the filtration continues. This is what would be expected for a true sieving mechanism, where the filtrate concentration slowly increases due to the continual gain in concentration of the residue above the membrane [Manegold, 1930] or is maintained constant if the latter effect is offset by a slow blocking. The suggestion of sieving need not necessarily mean that the system filtered is polydisperse. A monodisperse system filtered through a membrane whose pores are not quite uniform in size but have a range of size distribution would exhibit this effect; and our gradocol membranes, while believed to be more uniform than most other types of filters, possess such a range of pore sizes. But regardless of what interpretation is placed upon the filtration curves of types II and V, it is evident that only where these types obtain can the experiments be used for comparing the particle size of the disperse phase with the pore size of the membrane. Hence we propose to term the process which gives rise to these curves *normal filtration*.

The close resemblance in shape of the curve of j_e as a function of p_H for serum-albumin (Fig. 3) to the curve for adsorption of the protein on collodion particles [Elford, 1933] indicates the important correlation of adsorption with filtrability. However, the primary adsorption occurring at the pore surface, whose equilibration takes place within the first ml. or so of filtrate passing and is responsible for the initial steep rise in the filtration curve, is not the decisive phenomenon in filtration. It determines the value of $u_{\max}$ only in the case of curves of type I. The significance of the similarity of the curves is probably that the high peak in adsorption in the isoelectric region is due to the same process which causes the blocking of pores in abnormal filtration. That process may be a multiple layer adsorption.

The position and shape of the adsorption peak are the same whether the adsorbent is collodion, quartz, kieselguhr or kaolin (Elford, unpublished experiments), suggesting that the peak is due to adsorption not of protein on adsorbent only but of protein on protein, *i.e.* to the formation of secondary adsorbed layers. This corresponds with the tendency to aggregate which prevails in isoelectric protein solutions, as demonstrated by maximum opalescence and a minimum of the "alcohol number" defined by Fenn [1918] at this point. The blocking in the zone of abnormal filtration is then easily explained. When the protein system is forced through the membrane, multiple layers are adsorbed on the sides of the pores; the latter are narrowed and, as the filtration continues, eventually become blocked. The conditions within the pores are exceptionally favourable for formation of protein aggregates and precipitation, owing to the frequency of

collisions in the limited space. On the other hand, in the zones of normal filtration (which may be termed the stable¹ zones), adsorption of the protein is probably unimolecular. The pores are lined with one layer of protein, and other protein molecules slip through without being retained.

The range of p_H values investigated for pseudoglobulin seems to lie entirely within its abnormal filtration zone (Fig. 5), as the end-point at the extreme alkaline side is still more than three times the size of the molecule ($6.9 m\mu$, disregarding molecular dissymmetry). This is confirmed by the filtration curves, which are nearly all of the types I, III and IV. The broad zone of abnormal filtration is quite in agreement with the fact that solutions of pseudoglobulin are generally less stable and more apt to aggregate and precipitate than those of albumin. In solutions whose p_H values lie in the centre of this zone, even the adsorption on the top surface of the membrane apparently suffices to initiate a progressive precipitation. The residue above the membrane after filtration of such a system usually contains a copious precipitate of protein, and the concentration of the supernatant solution, in spite of a decrease in volume of ten- or twenty-fold, is less than that of the stock solution originally introduced into the filter. The adsorption- p_H curve of the pseudoglobulin, again, closely resembles the filtrability curves.

The general effect of salt in improving filtrability, observed at the isoelectric point and on the alkaline side of it, may now be interpreted as a peptising action due perhaps to alteration in charge and hydration of the protein molecule by adsorption of ions. It suppresses aggregation and formation of multiple adsorbed layers and renders filtration less "abnormal." The more abnormal the filtration is in the absence of salt, the more the presence of salt would be expected to affect it; and this is found to be the case (Table II). This is further illustrated by the fact that saline medium improves the filtrability of pseudoglobulin more than that of albumin, solutions of the former protein being characterised by their relative instability and abnormal filtration. The stabilising effect of salt on the globulin is also apparent in decreasing visible opalescence and increasing the "alcohol number." An increase in the electrolyte content of the system will also progressively reduce the Donnan potential at the surface of the protein particle and thus tend to minimise any consequent electro-viscous effects.

The extra-digested broth probably peptises the protein in much the same way as does the sodium chloride, owing to its high content of salts and amino-acids (Table I). The other broths, however, have an additional effect, and this is probably "lubrication" due to preferential adsorption of a capillary-active constituent on the surface of the membrane pores [Elford, 1933], and consequent suppression, not only of multiple-layer adsorption, but even of unimolecular adsorption of the protein, thereby making conditions the most favourable possible for filtration.

Stock broth can thus improve the filtrability of albumin even at a p_H at which filtration in distilled water solution is normal. Fig. 4 demonstrates that the filtrability is, in fact, increased by broth over the entire p_H range, and the zone of abnormal filtration is much narrower (p_H 4.3–5.5) than for aqueous solutions of the protein (p_H 4.2–7).

¹ Our use of this term should not be confused with that of Svedberg, whose " p_H -stability ranges" refer to stability of monodispersion, with respect to breakdown (occasionally aggregation) of protein molecules. "Stability" is used in this paper with respect to potential aggregation and precipitation.

Optimum conditions for filtration.

It is essential in determining the filtration "end-point" for estimating the particle size of a disperse system to ascertain and utilise the optimum conditions for filtration. These obtain when the filtration is "normal," and when the end-point value is as close to the actual particle size as possible. The fact that a protein in solution, as found by Svedberg in his ultracentrifugation studies, is uniformly monodisperse only within certain definite characteristic ranges of p_H , makes it necessary to confine filtration within these prescribed limits. In the studies with virus suspensions also, a restriction upon the permissible working conditions is imposed by the p_H range of viability in each instance. The evidence presented in the foregoing sections clearly indicates that for the serum-proteins the requirements for optimum filtration conditions would be fulfilled by adopting Hartley's broth, at p_H 8, as the standard suspension medium. This medium at p_H 7.4–7.6 has been consistently used in ultrafiltration analyses of virus and bacteriophage suspensions in this laboratory. Experience has shown that both virus and phages filter under these conditions much more readily than when contained in saline or Ringer solution [Galloway and Elford, 1931; Elford and Andrewes, 1932]. Further, their filtrability in broth is not appreciably affected by changes of p_H within the range of viability whereas in saline and Ringer the filtrability of the viruses and phages decreases as the p_H is changed in the acid direction. It must be remembered in this connection, however, that when working with viruses it is seldom possible to detect with certainty variations in concentration less than tenfold.

The ratio, particle size/end-point A.P.D., for the serum-proteins.

An empirical correction factor, derived from filtration studies with suspensions of known particle size, has been used in calculating the particle sizes of viruses and bacteriophages from the values of their experimentally determined end-points [Elford, 1933]. The ratio, particle size/end-point A.P.D., was evaluated individually for certain bacteria, gold sols, haemoglobin and egg-albumin. The correction factor was given as ranging from this ratio to a figure about 30 % below it. It is of interest to derive the values of the ratio for the serum-proteins (Table III). The sizes of the protein particles, denoted by $2r$, are calculated from

Table III. *Particle sizes and end-points of the serum-proteins.*

	Medium	Particle size, $2r$ $m\mu$	End-point j_e ($m\mu$)	Ratio $\frac{2r}{j_e}$
Serum-albumin	Water, p_H 8.0	5.4	11	0.49
Serum-albumin	Broth, p_H 7.8	5.4	9–10	0.54–0.60
Serum-pseudoglobulin	Broth, p_H 7.8	6.9	11–12	0.57–0.63

Svedberg's and von Mutzenbecher's values for the molecular weights, neglecting their slight dissymmetry and regarding them as spherical. The end-points under the optimum conditions, *i.e.* solution in Hartley's broth at p_H 7.8, were obtained by extrapolation from the lower portions of the end-point curves of the respective systems. The end-point for serum-albumin in water at p_H 8.0 is from the data of Fig. 3.

The end-point for the albumin in water is naturally higher than in broth, even though the filtration is quite normal in both cases, because of the additional "lubrication" afforded by the latter medium. The ratios $\frac{2r}{j_e}$ for the two proteins

in broth agree satisfactorily with the value (0.54) for haemoglobin in the same medium [Elford, 1933].

It is hoped to present later similar data for proteins of still larger molecules, when a general tabulation of the values of the ratios will be made.

Filtration of artificial mixtures.

Examination of the respective end-point curves showed that at p_H 3.8 membranes of A.P.D. 40 $m\mu$ to 80 $m\mu$ retain pseudoglobulin (in saline) completely, whereas albumin (in distilled water) passes them in nearly undiminished concentration. To determine the behaviour of a mixture of the two, a solution at p_H 3.8 was made up to 0.5 % in respect to each protein in 1 % saline and filtered through several grades of membranes in this range. The results are shown in Table IV.

Table IV. *Filtration of artificial mixtures.*

0.5 % albumin + 0.5 % pseudoglobulin in 1 % NaCl p_H 3.8.

Membrane A.P.D. $m\mu$	Pseudoglobulin (1/2 sat. $(NH_4)_2SO_4$)	Albumin (sulphosalicylic acid)
70	Positive	Positive
55	Trace	Trace
40	Negative	Negative

Under these conditions, the filtrability of the pseudoglobulin is increased, and that of the albumin is greatly decreased. The improvement in the case of the former protein is probably the result of a stabilising influence exerted by the albumin. On the other hand, the drop in filtrability of the albumin no doubt means that the pores, clogged by multiple adsorbed layers of pseudoglobulin (here undergoing abnormal filtration), are blocked to both proteins equally. Hence the data do not necessarily indicate formation of an albumin-globulin complex. (The mixture is not strictly comparable with the pure solution of albumin, owing to the presence of the sodium chloride in the former; but any effect due to the salt would be small compared with the great decrease in filtrability observed).

Filtration of horse serum.

In order to compare the filtration end-points of serum-albumin and globulin as determined in pure solutions and in their native state in serum, reference is made to the work of Elford, Grabar and Fischer. Their experiments, details of which will be published later, yield the end-point values for the proteins in undiluted serum and in dilutions in broth and in saline (Table V).

Table V. *End-points of the serum-proteins in native serum.*

	Pressure 1-3 atm.		End-point of globulin $m\mu$
	p_H	End-point of albumin $m\mu$	
Undiluted serum	8.2	9-10	11
Serum: broth, 1 : 9	7.6-7.8	9-10	11
Serum: saline, 1 : 9	7.8-8.0	11	13

It is seen by inspection of Tables III and V that the filtrability of each serum-protein is characterised by the same end-point whether the system filtered is normal serum or the pure solution of the protein in Hartley's broth at p_H 7.8.

This indicates that serum-albumin and globulin exist largely as individual molecular species in their native state in serum, a conclusion also reached by Svedberg and von Mutzenbecher in their ultracentrifugation studies.

SUMMARY.

1. The filtration curve, end-point curve and a quantitative measure of filtrability are defined.
2. The influence of the nature of solvent medium, p_H , neutral salts, concentration of protein and filtration pressure on the ultrafiltration of serum-albumin and pseudoglobulin through graded collodion membranes is described.
3. These effects demonstrate two types of filtration, termed normal and abnormal, and are interpreted in terms of adsorption of protein within the membrane pores.
4. Hartley's broth at p_H 7.6–7.8 is found to be the best solvent medium for filtration experiments with the proteins, as previously noted for bacteriophages and animal viruses.
5. The ratios, particle size/end-point A.P.D., are given for the two proteins for optimum filtration conditions.
6. The filtration end-point of each protein is the same in pure solution in Hartley's broth at p_H 7.8 as in native horse serum.

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