ULTRAFILTRATION THROUGH COLLODION MEMBRANES.

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I.

INTRODUCTION.

Since the introduction of the process of ultrafiltration by Martin (1) and the use of collodion membranes (2) as a means of separating dispersed particles from their dispersion medium, much work has been done utilizing collodion sacs in quantitative studies involving the separation of substances in varying degrees of dispersion. Investigators have often contented themselves with merely subjecting the solution under investigation to ultrafiltration through a collodion sac and from the results obtained have drawn conclusions as to the degree of dispersion of the substance under consideration. The effect of the addition of other substances upon the ease with which solutions of colloids pass through a given membrane has been used as evidence of an alteration of the colloid whereas such results may be due to action on the membrane. The passage of a substance through the pores of a membrane is not determined solely by the size of its invariable pores, as is often assumed. The mode of filtration, of pressure applied, of the nature of the filter, and of the solution being filtered, may all affect the concentration of any constituent in the ultrafiltrate. These considerations have been neglected in most of the applications of ultrafiltration described in the literature and this neglect explains the wide variation in results recorded by different observers. The conclusions are often quite erroneous and based on doubtful data. This paper aims to present some observations which may help to test the validity of deductions to be made from ultrafiltration experiments.
II.

The Degree of Uniformity in the Sizes of the Pores of Collodion Filters.

The permeability of collodion filters can be varied by varying the concentration of the collodion solution (3); by adding castor oil or glycerol (4); by varying the time allowed for evaporation of the ether and alcohol; by altering the thickness of the sac; or by immersing in aqueous alcohol solutions of varying concentration (5). Despite the fact that sacs obtained by the same technique will show some degree of constancy as regards permeability towards some specific substance (e.g. hemoglobin or Congo red), it is still to be expected that some variation in the size of the individual pores should persist despite the most careful manipulations. That this is actually the case is seen in the variation in the amount of water filtered through a series of sacs (Table I). These sacs were all prepared simultaneously and appeared alike in all respects. The same surface was exposed to a pressure of 200 mm. of Hg connected to the sacs arranged in parallel.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume filtered in cc. per hour</td>
<td>9.1</td>
<td>9.1</td>
<td>9.9</td>
<td>8.9</td>
<td>9.5</td>
</tr>
</tbody>
</table>

This variation, although not considerable, nevertheless indicates an unavoidable lack of uniformity. Even assuming, moreover, that a series of sacs are prepared so as to appear alike in all respects when tested by their permeability to water, it does not follow that any individual sac will show perfect homogeneity as regards the size of its pores. Indeed, the very nature of their formation seems to delimit this possibility. This fact has usually been overlooked and, as will be seen later, complicates the interpretation of data on the ultrafiltration of many substances. The existence of variation in the size of the pores of a membrane is shown by the ease with which membranes may be prepared which allow a trace of protein or other substance of large molecular size to pass through them. Unless we assume such substances to possess certain particles of a smaller size than the majority, which is not true, then the membrane must possess some pores,
larger than the rest, which allow the passage of the substance in question.

The commonly held belief that collodion holds back only colloidal particles, allowing all crystalloids to pass through, is untrue. No such sharp demarcation exists. The filters ordinarily used in biological work and which are completely impermeable to proteins hold back in part substances of distinctly crystalloidal nature. Thus an eosin solution containing 32 mg. per liter gave an ultrafiltrate containing only 13 mg. per liter, and this, despite the fact that eosin forms a true solution in water (6) as shown by its rapid dialysis (7) and examination under the ultramicroscope (8). Other substances such as sugars (9), non-tans (10), and iso-nitroso-acetyl p-toluol azo-p-toluidin (11) have been described as only partially filterable. And as we shall see later even such simple inorganic salts as sodium chloride and calcium chloride may be partially non-filterable if membranes of sufficiently fine porosity be employed, under certain conditions.

III.

Relation of Pore Size to Dispersion Medium.

It is probable that the actual size of the pores of a membrane is modified by a layer of fluid adsorbed on the surface of these pores, thus diminishing their effective diameter. Thus Tinker (12) in his microscopic study of artificially prepared copper ferrocyanide membranes showed that the particles must be considered as large micelles around which exists an adsorbed layer of water which diminishes the effective pore diameter of the intermicellar space. Indeed, this view was suggested by Pfeffer (13) as an explanation of the mechanism of semipermeability in his classic experiments on osmotic pressure. The thickness of this adhering layer will naturally be determined by the nature of the medium bathing the pores. The ultrafilterability of a substance will, therefore, be determined in part at least by the nature of the medium in which it is dispersed; and changes in the nature of the medium may alter its ability to pass through a filter.

This view of the nature of the pores of collodion filters seems essential for explaining much that would otherwise seem anomalous. It is in accord, too, with determinations of the actual pore diameters.
Thus the bubble method indicates the size of the pores which allow the passage of collargol to be 200 to 490 \( \mu \text{m} \) whereas filtration through a Chamberland filter would indicate the size of the coarsest particles to be 170 \( \mu \text{m} \) while their actual size is undoubtedly much less (20 \( \mu \text{m} \)) (14). The effective pore size is, therefore, much smaller than the actual pore size as determined by the bubble method. The adsorbed film need not behave like the main body of solvent towards any constituent. Thus, the adsorbed layer of water on the copper ferrocyanide micelle is impermeable to the sugar, giving the membrane its property of semipermeability.

The action of dissolved substances on the thickness of this film may in part at least explain the variations obtained in the rate of passage of various aqueous solutions through collodion membranes. A few typical results are given in Table II. The solutions indicated were ultrafiltered under a constant pressure of 200 mm. of mercury and the amount of ultrafiltrate obtained in a given time noted. The rate of ultrafiltration is compared with the rate with which pure water passed through the same filter under the same conditions.

There is perhaps no single mechanism by which the rate of filtration is affected and hence no individual physical constant can be used in evaluating this effect. Electrolytes might conceivably affect the rate by altering the charge of the membrane double layer (15). Viscosity changes would affect the rate in accord with Poiseuille's law (16). The proteins most probably act by forming an adsorbed layer
on the wall of the pores which diminishes their size. The action of surface active materials such as bile salts is especially marked and seems to depend on their ability to alter the conditions of the layer adsorbed on the walls of the pores. Examples of these three types of substances are given in Table II.

On the basis of the above theory of a variable effective pore size dependent on the medium in contact with it, one can explain many of the changes in permeability on the addition of otherwise inert materials. Thus many dyes, e.g., tetrachlorophenolsulphonphthalein, sodium carminate, etc., appear in the ultrafiltrate in higher concentration from Ringer's solutions than from distilled water. The increase in permeability described by Brinkman and von Szent-Györgyi (17) may be explained in the same way although attempts to render the collodion sacs used in this investigation permeable to hemoglobin by the use of bile salts, sodium oleinate, lanthanum chloride, acid or alkali, etc., failed. They always remained impermeable to this substance.

IV.

The Effect of the Filtering Pressure on the Concentration of the Ultrafiltrate.

If collodion filters, as has been demonstrated above, contain pores of various sizes, the nature of the ultrafiltrate will be influenced by the pressure used in forcing the solution through the filter. This problem has been discussed by McBain and Jenkins (18).

Theoretically, a pressure above the osmotic pressure of those substances which are retained by the filter should be necessary to cause the passage of the remaining substances through the filter. If, e.g., a solution, containing a single solute A, whose osmotic pressure is \( p \), be ultrafiltered through a filter containing a series of graded pores, some of which permit the passage of A while others do not, the concentration of A in the ultrafiltrate will depend on the pressure employed in the ultrafiltration. If a pressure less than \( p \) be employed, separation of the solvent through the pores through which A cannot pass should not occur, and hence the solution should pass unchanged through the larger pores, giving an ultrafiltrate of the same composition as the original solution. As long, therefore, as the filtering pres-
sure is less than the osmotic pressure of the retained constituents, none of these can be separated from the solvent. The use of such pressures should result in the production of an ultrafiltrate in which the diffusible solute is present in the same concentration as in the original solution. To test this hypothesis, solutions were ultrafiltered at pressures less than their calculated osmotic pressures. These substances failed, however, to appear unchanged in concentration in the ultrafiltrate. Instead, with decreasing pressures there was a gradual increase in the concentration of the ultrafiltrate. The explanation of these results seems obvious when we consider the actual conditions prevailing during filtration. Those pores through which a constituent cannot pass are bathed, more or less, by the ultrafiltrate outside of the membrane. The effective pressure necessary to cause separation of pure solvent will, therefore, be the difference in osmotic pressure of this constituent in the solution and ultrafiltrate. Consequently, even at pressures much below the osmotic pressure of a constituent which cannot pass a pore, separation of solvent will occur with consequent dilution of the ultrafiltrate.

The degree of this dilution of the ultrafiltrate will depend on the pressure employed. The quantity of fluid, $Q$, passing through a tube of length, $L$, and diameter $D$ in time, $T$, under a pressure, $p$, is given by Poiseuille’s formula:

$$Q = \frac{KD^4pT}{L}$$

From this equation in which $K$ is a constant, one would expect that a change in pressure would not influence the concentration of the ultrafiltrate since the relative changes in the amount of solution passing through large and small pores should be equally affected. However, since this simple relationship does not apply to the pores of the magnitude of collodion membranes, this condition will not obtain, and the concentration of ultrafiltrate will vary continuously with pressure as may be seen in the following typical example (Table III).

This fact complicates the process of ultrafiltrations and makes it impossible to take advantage of McBain and Jenkin’s suggestion; i.e., to work below some definite pressure. This pressure will vary continuously and even under pressures much lower than the osmotic
pressure of the substance filtered, dilution of the ultrafiltrate will occur. In general, however, the lower the pressure, the closer does the concentration of ultrafiltrate approximate the true solution and hence results obtained at low pressures are most accurate.

The results of Table III show the great variations in the concentration of the ultrafiltrate which a change in pressure produces. If a large quantity of solution be added to a filter and the filtration continued, without further addition of new solution, there will be a gradual increase in the concentration of the residue left in the filter. This increased concentration of the residue will produce a gradual increase in the concentration of the ultrafiltrate as well. Hence consistent results are only obtainable if the same condition of filtration and the same degree of concentration of the residue are attained in successive experiments.

This problem of the influence of pressure on the concentration of the ultrafiltrate through collodion filters has been usually neglected and reference to the literature shows many instances where such influence, although demonstrated, has been either ignored or misinterpreted. Thus Burian (19) found sodium chloride to appear in lesser concentration (about 10 per cent) in the ultrafiltrate than in the original serum or protein solutions when filtered under a pressure of 10

<table>
<thead>
<tr>
<th>Substance.</th>
<th>Concentration of solution ultrafiltered, mg. per 100 gm. of water.</th>
<th>Pressure, mm. of mercury.</th>
<th>Concentration of ultrafiltrate, mg. per 100 gm. of water.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose bengal.</td>
<td>1000</td>
<td>50</td>
<td>850</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>20</td>
<td>940</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>10</td>
<td>970</td>
</tr>
<tr>
<td>&quot;</td>
<td>5</td>
<td>30</td>
<td>3.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.2</td>
<td>200</td>
<td>1.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.2</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Eosin.</td>
<td>1000</td>
<td>2</td>
<td>1000</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>25</td>
<td>980</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>50</td>
<td>850</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>100</td>
<td>840</td>
</tr>
<tr>
<td>&quot;</td>
<td>1000</td>
<td>700</td>
<td>800</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.2</td>
<td>200</td>
<td>1.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.2</td>
<td>10</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Similar results were likewise obtained by Reid (20). These results are explicable on the assumption that there are pores whose effective size is modified by a layer of adsorbed solvent and which, in these experiments, could not allow the passage of the sodium chloride, but at the high pressures used could transmit the solvent. With increasing pressure these smaller pores with their anomalous filtering power enter into greater consideration.

### TABLE IV.

**Results of the Ultrafiltration of Solution of CaCl₂ Containing 80 Mg. Ca per 100 Gm. H₂O through Protein-Treated Collodion Membranes under 250 Mm. Hg Pressure.**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Concentration of ultrafiltrate, mg. per 100 cc.</th>
<th>Concentration of residue, mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>81</td>
</tr>
</tbody>
</table>

### TABLE V.

**Concentration of the Residue Left after Ultrafiltering 30 Cc. of a CaCl₂ Solution Containing 80 Mg. of Ca per 100 Gm. of H₂O to a Final Volume of 10 Cc.**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Concentration of residue, mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
</tr>
</tbody>
</table>

The production of an ultrafiltrate of too low composition by the use of high pressures is demonstrated by the results of Table IV.

By using a higher pressure—1 atmosphere—more striking results were obtained as shown in Table V. These results may in part explain the low and discordant values obtained in ultrafiltration studies of the Ca in blood (21). The use of membranes differing as regards the size and uniformity of their pores and the use of varying pressures must result in such discrepancies.
An insidious error to be guarded against in the use of collodion membranes to obtain quantitative measurements of the state of a substance in solution, is a failure to obtain a true ultrafiltrate due to adsorption of the substance under investigation by the filter (22). One must ensure that a real filtration is taking place and that the results are not vitiated by adsorption.

The adsorption by the filter of substances to which it is impermeable is likewise of great importance in modifying the nature of the ultrafiltrate. This is especially true of solutions containing proteins such as blood plasma or other tissue fluids which have been widely used in biological work. The adsorption of a film of protein on collodion filters was described by Loeb (23) and has been quantitatively studied by Hitchcock (24). A filter on whose pores a layer of protein has been adsorbed will obviously be less permeable than before treatment. This would be especially marked if we consider the more hydrophilic nature of the protein as compared to collodion, and the consequently increased thickness of the layer of adsorbed solvent.

The manner in which this phenomenon may lead to misinterpretation of results is exemplified by the recent work of Rosenthal (25). As a result of ultrafiltration studies of rose bengal in the presence of proteins, he concluded that this substance is completely bound since none of the dye appears in the ultrafiltrate under such conditions. The impossibility of such a conclusion is, of course, obvious when one considers the nature of the adsorption of dye on protein which necessitates the existence of a considerable concentration of the former in the free condition at equilibrium. As a matter of fact, dye enters the collodion sac and is found in the ultrafiltrate after an initial adsorption of some dye by the filter. This ultrafiltrate cannot be considered as the true concentration of free dye since only a fraction of the dye comes through from an aqueous solution, which, we have already seen, is due to impermeability of some of the pores to this substance. This fact would preclude the possibility of using collodion as a means of determining the binding of this dye. Furthermore, clogging of the pores by the protein helps to still further diminish the actual amount
obtained in the filtrate. The protein test suggested by Rosenthal, and based on a supposedly complete binding of rose bengal, is due to the buffering action of the protein and would be given by any buffering solution.

The use of collodion for determining the degree of peptization of colloids is likewise open to objection on the same grounds. The fact that a fixed and constant percentage appears in the ultrafiltrate does not necessarily indicate that this fraction is in a state of greater dispersion than that contained in the residuum. Nor would an alteration of this filterable quantity on the addition of acid necessarily indicate the true peptization since the addition of the latter in itself might affect the composition of the ultrafiltrate. The same criticism is applicable to other studies based on the partial permeability of the filter (26).

The effect of adding surface-active materials such as sodium lysalbinate, the bile salts, etc., and the increase in permeability consequent on such additions should be noted. Their action may result from an effect on the adsorption by the membrane and not on the degree of dispersion of the colloid under investigation as has been assumed.

v.

Comparison of Ultrafiltration and Dialysis as a Means of Determining the Binding of Phenol Red by Blood Albumin.

In a previous paper (27) the adsorption of phenol red (phenolsulfonphthalein) by proteins has been studied using ultrafiltration as a means of obtaining the intermicellar fluid; and the results thus obtained were applied in studying the excretion of this substance by the kidney (28). The nature of phenol red is such as to permit its study in this way. Its highly crystalloidal character which manifests itself in the extreme rapidity with which it diffuses through membranes such as parchment, enables it to pass through collodion in unchanged concentration even when the pores of the latter are at their minimum size. Moreover, the small extent of its adsorption by collodion makes practical its use for such studies.

That ultrafiltration can be used to study the binding of certain substances is shown by the following experiments in which the results of
The binding of a 4 per cent albumin solution was determined in the following manner. The solutions containing phenol red were placed in diffusion shells (Schleicher and Schüll No. 579) which were closed by a rubber stopper and placed in a glass tube but slightly larger than the shells, in order to permit the use of a minimal quantity of pure water in the outer tube. The dye quickly diffuses into the outer fluid and equilibrium was insured by shaking the tubes until the concentration of dye in the outside fluid became constant (50 hours). None or negligible amounts of protein were found in the outer fluid. From such determinations the amount of dye bound was determined and could be compared with that previously found by ultrafiltration. The agreement which is within experimental error is shown in Fig. 1.

This agreement indicates that filtration of albumin solutions through the filters under the pressures and conditions used in the investigations described for phenol red (27) are valid as indicating a true separation of intermicellar fluid. At higher pressures or using denser membranes high values would undoubtedly be obtained. The application of the
same method to other dyes or crystalloids towards which collodion shows a lesser degree of permeability is, however, subject to considerable error.

VI.

The Effect of Variations in the Method of Preparation of the Membranes.

It will be clear from the above consideration of the effect of the size of the pores, pressure employed, etc., that the nature of the ultrafiltrate may be altered by variations in the structure of the membranes employed. In some cases such variations will be more pronounced than others depending on the relation of the size of the constituents in the ultrafiltrate to the size of the pores of the membrane. Standardization of the method of preparation is difficult since slight variations in the concentrations of the collodion used, the thickness of the membranes, duration of the time allowed for drying, etc., all affect the physical properties of a membrane, including the pore size. Each experimenter has used his own technique in the preparation of his membranes and in the method of filtration and consequently the wide divergence of the results recorded in the literature is what would be expected from the conditions outlined above. Even using the same technique, one obtains slight variations as shown in Tables I and IV. In Table V is shown the divergence possible as a result of varying the thickness of the membrane. Tube 2 of this table was the same as those employed in obtaining the data of Table IV. In preparing Tube 1, attempts were made to obtain an exceedingly fine porosity and the results of this variation in the method of preparation manifests itself in the divergence of its value from those obtained with the tubes used in the other experiments.

Unless the same details of procedure are followed as were used in making the collodion sacs used in this investigation, the results cannot be duplicated. Thus filtration of calcium chloride solutions through more porous membranes would show the same concentrations in the ultrafiltrate and solution ultrafiltered. This would be no safe criterion, however, for the use of blood serum or protein solutions as the latter might cause a sufficient decrease in the size of the pores to invalidate their use in determining the state of calcium in the solution.
studied. Use of membranes of a finer structure, on the other hand, might give results for the binding of phenol red much higher than the true values as obtained by dialysis experiments.

The membranes used in this laboratory are made in the following manner. An 8 per cent solution of dry negative cotton in a mixture of equal parts of absolute alcohol and ether serves as the source of the collodion. This solution is poured into seamless test-tubes (18 mm. in diameter), rotated for several minutes to give a uniform coating to the glass and allowed to dry (mouth of tube projecting downwards) for 30 minutes. A second coating is then similarly applied and after the excess ether has evaporated (about 30 minutes) the membrane is removed and preserved in distilled water in the ice chest. These membranes are perhaps the most suitable for filtrations of biological material at low pressures. The use of high pressures on comparatively thick and dense membranes is likely to involve greater errors.

VII.

CONCLUSIONS.

It is obvious that the factors considered in this paper render data obtained by ultrafiltration open to criticism unless they are checked by other methods and precautions are taken for the elimination of the vitiating effects which have been described.

As regards the mechanism of ultrafiltration, the view of a sieve-like action as most experimental evidence indicates, is adequate, if all the factors are considered which might modify the effective pore size. The behaviors of collodion membranes which seem contrary to a mechanism of ultrafiltration based on the existence of a system of pores, can be explained on the basis of a variable layer of adsorbed fluid on the walls of the pores. It is, therefore, unsound to make any deductions about living tissues from the demonstration of changes produced in the behavior of collodion membranes. Thus, the increase in the rate of filtration of water through collodion by diuretics (29) or the change of permeability due to the presence of surface-active materials, gives us no information about their action in the living organism. The effect of these substances on a sieve-like membrane of the type of collodion would not necessarily bear any analogy to that
exerted on the emulsion type of membrane of living cells. The mechanisms of the reactions necessary to produce the same effects in such widely differing systems may be entirely unrelated.

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