ULTRAFILTER MEMBRANES AND ULTRAFILTRATION

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I. Introduction

A. TERMINOLOGY

An *ultrafilter* is defined as a filter whose pores or interstices are of colloidal or molecular dimensions; filtration through it, ordinarily with the

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purpose of complete or partial retention of the colloidal or molecular species present in the disperse system filtered, is termed *ultrafiltration.*[2]

1. Diffusion, dialysis, osmosis, and ultrafiltration

The distinction between ultrafiltration and other closely related phenomena is illustrated by the following familiar model. A vessel is divided by a membrane into two compartments, of which one is occupied by an aqueous solution, molecular or colloidal, and the other by pure water. Owing to the concentration gradient across the membrane, the water will tend to diffuse into the solution, and the solutes into the water compartment. If the membrane pores are large compared with the diameters of all solute particles present, so that no specific steric hindrance is offered to the latter, both processes take place at relative rates the same as in *free diffusion.* The only effect of the presence of the membrane (apart from electrokinetic influences) is a reduction of the area through which diffusion can occur, and, in case the pores do not run perpendicular to the membrane surface, a prolongation of the path traversed by the diffusing molecules. If, however, the pore sizes are of the same order of magnitude as the solute particle sizes, the solute encounters a preferential resistance; this may include a simple viscous drag as represented by the Ladenburg correction to Stokes' law (175a), specific molecular interaction, or electroviscous effects. Such diffusion may be termed impeded. *Dialysis* is a differential diffusion, employing a membrane impermeable to the colloidal solutes but permeable to the crystalloidal. The latter diffuse into the water, while the water diffuses into the solution. In dialysis under pressure, the solution is under sufficient pressure so that the hydrodynamical flow of water out of the solution balances the molecular diffusion into it, and the concentration of the colloidal solutes remains unchanged while the diffusible solutes escape. If the membrane is impermeable to all solutes, so that the only diffusion occurring is that of water into the solution, the process is ordinary *osmosis.*[3] At osmotic equilibrium, the solution is under a hydrostatic pressure (the osmotic pressure) which causes sufficient flow out of the solution to balance the molecular diffusion of water into it.[4] Now, if the hydrostatic pressure is increased beyond the osmotic pressure, there is a net flow of water out of the solution compartment, with a concentrating of the solute, and we have *ultrafiltration.* Similarly, in dialysis under pressure, if the pressure is increased so that both water and diffusible solutes flow out of the solution, the experiment becomes one of ultrafiltration.

[2] For brevity, the prefix *ultra* is frequently omitted in this paper.

[3] Dialysis may also be considered as osmosis in the presence of diffusible constituents. This complex process has been discussed by Schreinemakers (252, 253).

[4] Osmotic equilibrium in the presence of diffusible constituents may involve a Donnan membrane equilibrium (50).

Under actual conditions of ultrafiltration, the hydrodynamical flow through the membrane due to the applied pressure is usually so much greater than any diffusion effects that the latter may be considered negligible.

2. Porosity and permeability

The term "permeability" has been frequently used to characterize ultrafilters, as a measure of the size of the pores or interstices in the filter structure. The terminology adopted in this paper, and suggested for general use, designates *porosity* to describe the filter structure, and *permeability* for reference to its behavior in diffusion or filtration of a disperse system. For example, a membrane of given porosity will show varying degrees of permeability to a certain protein depending on whether the experiment is one of diffusion or ultrafiltration, and, in the latter case, on a host of physical factors including the pH, the rate of filtration, and the concentration of the solution.

B. HISTORICAL SURVEY

The study of ultrafiltration has always been closely associated with that of dialysis, and, to a lesser extent, osmosis and the problem of the semipermeable membrane.

Dialysis experiments through artificial membranes of collodion were recorded by Fick (120) in 1855. The first mention of the process now known as ultrafiltration appears to have been by Schmidt (248) in 1856, who found that, when a solution of protein or gum arabic was filtered through an animal membrane, the filtrate was less concentrated than the original solution. Similar observations were made by Hoppe-Seyler (157). Schumacher (254), in 1860, described the collodion sac for dialysis, and Sanarelli (246) introduced it in 1891 for bacteriological work, including ultrafiltration of blood plasma *in vivo*. In 1896, Martin (211, 211a) used a bacteriological candle impregnated with gelatin or silicic acid as an ultrafilter to separate colloids from crystalloids. Borrel and Manea (51) and Malfitano (199) used collodion sacs for ultrafiltration in 1904, and Levy (177), in 1905, ultrafiltered enzymes and showed that dialysis and ultrafiltration did not arrive at the same result.

The classic papers of Bechhold (16, 17, 18), who coined the term "ultrafiltration" in 1906, represented the first systematic study of this subject. By impregnating filter paper with acetic acid collodion, Bechhold prepared the first series of membranes of graded porosities; he was the first to estimate critically the pore sizes in his filters, and first pointed out the rôle of adsorption and other physical factors in the filtration process. In the next twenty years, numerous workers experimented in ultrafiltration and intro-

duced various types of ultrafilters and various methods for grading the porosity (see part II). Among these may be mentioned Bigelow and Gemberling (45b), who, in 1907, prepared flat collodion membranes from ether–alcohol solution, and Zsigmondy and Bachmann (294), who patented in 1918 a graded series of membranes to be manufactured by a similar process. Meanwhile, ultrafiltration technique had been adopted by bacteriologists and physiologists, who employed it in attempts to estimate the particle size of enzymes, toxins, and viruses, and to construct models of vital processes involving membranes.

Ultrafilters, and semipermeable membranes generally, were regarded by some early authors as mechanical sieves, so that permeability was a matter solely of particle dimensions and pore dimensions. The opposite extreme in explaining semipermeability was reached by the capillary attraction theory (45a, 272, 284), which represents the solvent as strongly adsorbed in the pore and transmitted by surface mobility of the adsorbed molecules; and by the theory of partial solubility (179a), which represents the solvent as dissolving into the membrane on one side and out on the other. These theories would, however, predict specific effects dependent on the nature of solute and solvent, whereas the experiments of Duclaux and Errera in 1924 (82, 83) demonstrated the sieve-like behavior of membranes in the flow of various liquids through them, and the work of Collander in 1926 (75) showed that the rate of impeded diffusion of crystalloidal molecules through membranes depended principally on the molecular volume and not on the nature of the diffusing solute. On the whole, there is adequate support for the viewpoint that the fundamental mechanism in ultrafiltration is sieving, modified by adsorption, blocking, and other effects arising from the very large ratio of pore length to pore width and of pore surface to cross-section area in all ultrafilters.

The most significant recent developments in ultrafiltration have been the extensive study by Manegold and collaborators of the structure of collodion membranes, and the introduction by Elford (93) in 1930 of the most satisfactory graded series of collodion membranes yet developed. These filters have been successfully employed by Elford and collaborators to estimate the sizes of particles in a number of biological systems.

C. PRESENT PROBLEMS OF ULTRAFILTRATION

The applications of ultrafiltration to chemical, as well as to biological, problems are twofold: fractionation and study of the composition of disperse systems, and estimation of the particle sizes in disperse systems.

The simplest example of the first type of problem is the preparation of a colloid-free ultrafiltrate from a sol. This is of value in the study of lyophobic colloids (195) and lyophilic colloids (188) alike, and especially in

biological investigations, where much attention, for example, has been given to protein-free ultrafiltrates of blood serum and plasma. Ultrafiltration also permits obtaining the disperse phase in solid form, if desired. An example of the removal of particles of a different order of magnitude is the sterilization of bacteriological systems by ultrafiltration, whose methods are often superior to those involving the use of porcelain candle filters. Water and solutions of inorganic and organic crystalloids may be ultrafiltered to remove stray foreign particles, yielding optically clear filtrates (288a). The applications of ultrafiltration to analytical chemistry, for filtering off colloidal precipitates and the like (as advocated by Zsigmondy and collaborators (296)), have been treated in a monograph by Jander and Zakowski (160). A less simple example of fractionation is the separation of colloidal particles of different sizes. Thus, in a suspension containing bacteria, bacteriophage, and products of bacteriolysis, including a specific soluble substance identifiable by an immunological reaction, suitable filters have served for quantitative separation of the different constituents (66). It has been possible even to effect a partial fractionation of albumin from globulin in serum by ultrafiltration (108). In the case of a polydisperse colloid, successive filtration through membranes of different porosities may fractionate the large particles from the small (16).

The second type of problem—estimation of particle size—is much more difficult and requires a more critical selection of filters and a system of calibration and standardization. It is discussed in part V.

The present paper (a) reviews methods of preparing ultrafilter membranes, of grading porosities, and of calibrating membranes, (b) discusses theoretical and experimental study of the mechanisms involved in ultrafiltration, and (c) reviews experimental application of ultrafiltration to the two types of problems outlined above.

For further discussions of the historical development of ultrafiltration and its applications, reference is made to several reviews by earlier authors (134, 20, 90, 185, 166, 257, 135, 293a, 240, 8); and, in particular, concerning applications to biology, to a recent review by Grabar (141b).

II. Ultrafilter Membranes

A. PREPARATION OF DIFFERENT TYPES OF FILTERS

In any application of ultrafiltration, certain specifications must be set for the filter employed. In the first place, the phenomena which cause the behavior of an ultrafilter to differ from that of an ideal mechanical sieve arise from the high ratio of pore length to pore diameter; and, while it is seldom possible to reduce this ratio below a thousand, it should be limited by choosing the filter as thin as possible. On the other hand, the filter must be mechanically strong enough to withstand the pressure applied in

filtration without distortion or rupture. It must be reasonably isoporous, and free from occasional pores which are much larger than the average. It must not, of course, react with or dissolve in any component of the system which is filtered through it. For a fractionation experiment, rigorous control of filter porosity is unnecessary, so long as the pores are large enough to pass the components desired in the filtrate and small enough to retain those desired in the residue. It is for this reason that so many an early experiment, in which no regulation or calibration of filter porosity was made, succeeded in the desired fractionation. It is desirable, however, to calibrate filters rigorously and to have a wide series of porosities available, in order to attain best efficiency by selecting for a given experiment the most highly porous filter which will yet perform the required separation. And, for estimation of particle sizes, a series of carefully calibrated filters covering a wide range of porosities is an essential requirement. For comparative experiments, groups of filters of exactly comparable porosities must be available.

Most ultrafilter membranes are gelatinous, and in the great majority of cases the gel consists of collodion, i.e., nitrocellulose containing about 11 per cent of nitrogen. This gel is produced from a solution of collodion either in glacial acetic acid (acetic collodion) or in a mixture of volatile solvents including principally ether and ethyl alcohol (ether–alcohol collodion). Preparation of artificial gel membranes of reproducible characteristics requires strict adherence to empirical rules in the minor details of technique; this is particularly true of ether–alcohol collodion membranes, but the latter are the most satisfactory if prepared with the required care.

In the following discussion, gelatinous membranes are classified according to whether the gel is impregnated in a supporting structure, or forms its own support. Mention is also made of non-gelatinous membranes. Particular attention is devoted to the types of filters most frequently mentioned in the literature,—those of Bechhold, Bechhold–König, Zsigmondy–Bachmann, and Elford.

1. Gel membranes impregnated in a support

a. Support of filter paper or cloth. Collodion membranes impregnated in filter paper were introduced by Bechhold (16), and his simple technique still represents the easiest method of preparing a graded series of ultrafilters. A piece of hardened filter paper is soaked in a solution of nitrocellulose in glacial acetic acid. The excess solution is drained from the paper, and the membrane is gelled by immersion in water. The acetic acid is removed by prolonged washing, leaving a film of nitrocellulose (with perhaps some cellulose acetate) imbedded in the filter paper. The higher the concentration of nitrocellulose in the original solution, the lower the

porosity of the membrane (see section B, below). Retention of air by the paper, which might result in microscopic "pinholes" in the final membrane, is diminished by preliminary soaking in pure acetic acid, and practically eliminated by conducting the collodion impregnation in a vacuum. Draining off the excess solution from the paper may leave a layer of irregular and excessive thickness, especially in the case of the more concentrated and hence more viscous solutions; an improved technique involves drawing the soaked paper between rollers of glass or gold-plated nickel before gelation (90, 91).

The chief advantage of the Bechhold membranes lies in the relative simplicity of preparation and the wide range of porosities obtainable (pore diameter from 1 to 5μ down to less than 10 mμ). However, in a given filter, the pore sizes vary over a considerable range (see section C, below), and the limited reproducibility in average pore diameter from one membrane to the next makes comparative experiments difficult, even with rigorous control of experimental technique. Further, the Bechhold filters are rather thicker than self-supporting collodion membranes, and, in contrast to the latter, their thickness increases with decreasing porosity, thus making the ratio of pore length to diameter doubly excessive for the densest membranes.

It is possible also to impregnate ether–alcohol collodion into filter paper, coagulating with water in the same manner (230), but this type of filter has no particular advantages, since ether–alcohol collodion films can be made self-supporting.

Impregnation of collodion in a cloth support has been patented by Duclaux (81), who has also impregnated cloth with cellulose acetate, forming a gel suitable for filtrations with some organic solvents like benzene (80).

b. Refractory support. In some cases, a more rigid support for the ultrafilter gel is employed, such as porcelain, alundum, or metal. Of this type were the earliest impregnated filters, made by Martin (211) by filling the pores of a Chamberland candle of unglazed porcelain with gelatin or silicic acid. The classical "semipermeable" membranes for osmotic experiments, introduced by Pfeffer (233) and developed by Morse and Frazer (217) and Berkeley and Hartley (42), consist of copper ferrocyanide deposited in unglazed porcelain, and have a very low porosity.

The most popular porcelain impregnated filter is that of Bechhold and König, which is a Bechhold membrane with porcelain substituted for filter paper. Crucibles, evaporating dishes, and other vessels, with unglazed bottoms, are impregnated with acetic collodion in the usual way. After use, the nitrocellulose can be burned off. The porosity is varied, as above, by varying the concentration of collodion in the impregnating solution.

Ultrafilters of the Bechhold–König type have been prepared for filtra-

tion of non-aqueous solutions. Bechhold and Szidon (37) studied the gelation of collodion and cellulose acetate in different organic solvents, and found the most satisfactory combination to be impregnation by a solution of collodion in ether, followed by coagulation in toluene.

Alundum thimbles have been employed as support for ether–alcohol collodion (54, 238) in ultrafilters and electro-ultrafilters. Collodion has also been impregnated in wire gauze (127), forming a strong filter for use under pressure.

Ultrafilters impregnated in porcelain and the like have the advantage of mechanical convenience and mechanical strength. They are, however, excessively thick, and can remove large quantities of material from filtrates by adsorption. They should be used only for filtration of large volumes of material, where rigid control of membrane porosity is not required, since accurate calibration is difficult.

c. Support of cellophane or collodion. Cellophane, which in itself acts as a very finely-pored, self-supporting ultrafilter, can be given a still smaller porosity by depositing on it a film of cellulose or collodion (190, 191). By filtering through cellophane a solution of cellulose in Schweitzer's reagent, or of ether–alcohol collodion, membranes are obtained which, in filtration of an aqueous solution of sucrose, retain the sugar in varying degrees, and behave as molecular sieves. Membranes of very low porosity have been prepared also by impregnating copper ferrocyanide in collodion films (60a, 49).

2. Gel membranes with a self-supporting structure

a. Artificial membranes. While acetic collodion membranes must be of the impregnated type, owing to the fragility of the acetic collodion gel, membranes made from ether–alcohol collodion have sufficient strength to be self-supporting. These are made in the form of either sacs or discs.

Collodion sacs were the first artificial membranes to be generally adopted (254, 199, 51), and have been used very extensively, especially in biological research. A test tube is filled with a solution of collodion in ether and alcohol, and is inverted and drained, leaving a film clinging to the interior. After evaporation from this film has proceeded for a given time (sufficient for the collodion to set to a gel), the tube is plunged into water; the sac is loosened, removed, and washed free of the remaining solvents. An alternative is to make the sac on the outside of the test tube, rotating the latter to give an even film. Various authors (255, 214, 279, 133, 127a, 164, 71, 131, 276, 79a) have outlined detailed procedures for preparation of sacs, differing in minor points of technique. In particular, Kallós and Hoffmann (162) coated the glass mold with caramel and formed the collodion film on

this; on immersion in water, the caramel dissolved, facilitating removal of the sac. Huzella (158) used caramel for forms of various shapes; it could be drawn out in threads for molding minute cylindrical capillaries of collodion. Giemsa (137) formed the sac on a moist thimble of filter paper. The latter, before being dipped into the collodion, was slipped over a perforated cylinder of glazed porcelain, and this formed the filtering apparatus, negative pressure being applied to the inside of the cylinder.

The porosity of collodion sacs is varied by adjusting the ratio of alcohol to ether in the solvent, and varying the time of draining and the duration of evaporation; also by adding small quantities of other reagents to the solution. These added substances may also affect the mechanical properties of the membrane (see part II, B).

The collodion sac is particularly popular because of ease of preparation and the large area available for filtration, and because it constitutes its own container and, unlike the disc, does not require a mechanical holder with clamp and gaskets. It is, however, quite unsuited for work requiring uniform and reproducible ultrafilters. In the first place, the porosity of a given sac is different at different points, tending to be greater at the closed end than at the open end, and it is very difficult to make successive sacs of similar porosities, on account of the high viscosity of the collodion and the rapidity with which the solvents evaporate.

These difficulties may be overcome in making collodion disc membranes, as introduced by Bigelow and Gemberling (45b) and developed by Zsigmondy and Bachmann (294), Bartell and Carpenter (11), Bjerrum and Manegold (47), Pierce (234), and Elford (93) (see also Folley (124) and Snell (260)). A thin layer of collodion solution is poured on a carefully levelled glass plate, a surface of mercury, or a glass plate floated on mercury. Regulated evaporation proceeds, either by diffusion of the solvent vapors into a fairly large draft-free enclosure, or the slow passage of known quantities of air of regulated humidity past the glass plate. Convection shields prevent irregular air currents. The temperature is carefully controlled and maintained constant. After sufficient of the solvents has evaporated, the collodion sets to a gel. The evaporation is prolonged a specified time, and is then ended by suddenly covering the collodion film with water. The remaining solvents are washed free (a process requiring as long as two weeks in some cases—depending on diffusion out of the membrane pores), and the film is cut by dies into numerous small discs.

This is the procedure of Elford (93), which has been redescribed in detail by Bauer and Hughes (15). When proper attention is given to consistency of all details in technique, it is possible to prepare from the same sheet forty discs, which differ in porosity by less than 2 per cent from one

another, while successive sheets poured from the same solution of collodion agree in porosity within 10 per cent. The sizes of pores in a given disc vary within comparatively small limits.

The Zsigmondy–Bachmann filters, also, are presumably prepared by a procedure similar to that outlined above. These filters, manufactured commercially by E. de Haen, G.m.b.H., Hannover, and later by the Membranfilter Gesellschaft, m.b.H., Göttingen, are marketed under the names "Membranfilter" and "Ultrafeinfilter." The second firm also prepares membranes of pure cellulose ("Cellafilter"), as introduced by Zsigmondy and Kratz (293b).

It is customary to keep a preservative, such as toluene, thymol, or tryptoflavine, in the water which covers such membranes during washing and storage, since the collodion is particularly favorable to the growth of a mold which enters the pores and completely alters the porosity. The procedure of Elford (93), however, involves a sterile technique throughout, avoiding the presence of preservatives which might have some effect in subsequent filtrations, especially in biological work.

The porosity of collodion disc membranes is varied by the same general methods employed for sacs; these are discussed in section B. Membranes of a very low porosity are prepared by allowing the solvents to evaporate completely from a film of ether–alcohol collodion. Such "dry collodion" films demonstrate even a differential permeability to ions (226, 216a).

Commercial cellophane is a membrane of pure cellulose, with a trace of glycerol. It was formerly possible to obtain grades of cellophane which, when swelled in water, had a porosity of about 4 mμ, and formed very convenient ultrafilters for many purposes (190, 192, 193). McBain and Kistler (190) showed that the water could be replaced, proceeding by way of mutually miscible liquids, by various organic solvents to yield membranes for filtration of non-aqueous solutions. Cellophane of recent manufacture is less porous, and even partially retains sucrose in ultrafiltration. Its porosity may be increased by swelling with concentrated solutions of sodium hydroxide or zinc chloride, but only with difficulty to an extent sufficient to pass sucrose in undiminished concentration (197, 218).

Ettisch and collaborators (116a, 116b) incorporated glycocoll or powdered egg albumin into collodion solutions in order to prepare membranes of mixed ampholyte and collodion. An intimate mixture of protein and cellulose ester was effected by Loiseleur and Velluz (181, 182, 183); a solution of protein in acetic or formic acid was incorporated with an acetic acid solution of cellulose acetate or nitrocellulose, and the mixture employed for impregnation of Bechhold membranes. The porosity, as measured by the

rate of diffusion of potassium chloride through the resulting membranes, increased with the proportion of protein.

b. Natural membranes. Various animal membranes have been employed for purposes of dialysis and ultrafiltration, including goldbeater's skin, Bedicher (a membrane from cow's intestine), pig's bladder, fish bladder, amnion, and chorion (45b, 190, 16). These have the advantage of extreme thinness and a consequent comparatively high rate of filtration, but it is difficult to obtain at will a membrane of any desired porosity.

3. Other types of filters

A few types of ultrafilter membranes whose structure is not gelatinous may be cited. Manning (209) plated nickel on 200-mesh wire gauze of nickel or bronze, and thus decreased the sizes of the interstices to give pore diameters of from 50mμ to 300mμ. Warrick and Mack (282) distilled the zinc out of strips of brass, leaving porous copper membranes which showed differential permeability to gases and could serve as semipermeable membranes in the osmosis of aqueous solutions of sucrose. The porosity was evidently very small. Kultashev and Santalov (172a), using a similar procedure, prepared copper membranes permeable to urea and chloride and sulfate ions but not to glucose, and silver membranes permeable to all these crystalloids. Blanc (47a) obtained a porous structure of silica by leaching leucite with strong acid. Prausnitz (236) described ultrafilters of sintered glass, with a mean pore diameter of 1.5 μ. The use of zeolite crystals as molecular sieves has been suggested by Lamb (176), McBain (186), and Pauling (232).

B. METHODS OF VARYING THE POROSITY

An adequate method for grading the porosities of ultrafilter membranes must be capable of varying the porosity continuously, maintaining satisfactory mechanical properties throughout the range. The porosity may be measured in terms of the average pore diameter, as explained in section C.

1. Grading of acetic collodion membranes

The principal method for grading acetic collodion membranes is that originally used by Bechhold,—variation in the concentration of collodion in the impregnating solution. Bechhold (16) found the membrane porosity to be related antibatically with the concentration of collodion, but results were not reproducible from one solution of collodion to the next. In later work, a certain degree of reproducibility has been achieved. The dependence of porosity on concentration, as found by Elford (91), by Krueger and Ritter (170) and Mendelsohn et al. (213), and by Cox and Hyde (76), is

shown in table 1. The average pore diameters are quoted as determined by rate of flow of water (part II, D).

Another method for grading is given by Bechhold and Silbereisen (36). The impregnated paper or porcelain, instead of being gelled in water, is immersed in a weak solution of acetic acid; this results in a membrane of considerably higher porosity. Bechhold and Sierakowski (35) pointed out that solutions of collodion in acetic acid, when stored, undergo an aging effect with a marked decrease in viscosity, and that membranes made from aged solutions are more highly porous than the original. The porosity of a membrane is also increased by heating it to 90–98°C. in a water bath, or by denitration by treatment with ammonium sulfide.

TABLE 1

Grading of acetic collodion membranes

PERCENTAGE OF COLLODION IN IMPREGNATING SOLUTION	AVERAGE PORE DIAMETER IN μ			
	Elford (91)	Mendelsohn et al. (213)	Krueger and Ritter (170)	Cox and Hyde (76)
0.5		0.65	0.94	0.82
1.0	0.51	0.32	0.74	0.64
1.5	0.32		0.52	0.40
2.0	0.25	0.12	0.46	0.34
2.5	0.22		0.38	0.26
3.0	0.18	0.11	0.28	0.24
3.5			0.24	0.18
4.0	0.14	0.10	0.20	0.16
4.5			0.16	
5.0	0.09	0.09	0.16	0.10
5.5			0.14	
6.0	0.07		0.14	0.07

2. Grading of ether–alcohol collodion membranes

In the early experiments on ether–alcohol collodion membranes, where the porosity was graded by varying the evaporation times, no quantitative data on porosity were quoted (45, 45b, 279). The more recent work of Bjerrum and Manegold (47) shows, however, that this method is incapable of producing porosities of greater than about 60 mμ. The shorter the evaporation time, the more highly porous the membrane, but the evaporation must proceed at least long enough to allow the collodion to set to a gel.

Brown (57) prepared a graded series of collodion sacs by allowing them to dry completely and then swelling them in alcohol–water mixtures of varying concentration. The higher the proportion of alcohol in the swelling solution, the higher the porosity of the resulting membrane, but the range of variation was limited, since a concentration of over 96 per cent of alcohol

in the swelling solution would dissolve the collodion. The most highly porous membrane obtained was reported impermeable to filtration of Night blue and Congo red. Bendien and Snapper (40) used a similar procedure, incorporating small amounts of ether into the swelling solution.

Addition of various non-solvents or precipitating agents to a collodion solution was found to increase, to a limited degree, the porosity of membranes prepared from it; among these reagents were glycerol (250), water (224), lactic acid (86), and ethylene glycol (234). The porosity increase was limited by the effect on the strength of the membrane, which became fragile if too much reagent was added, and it was impossible to prepare membranes of high enough porosity for some bacteriological purposes. Asheshov (3), however, prepared membranes of high porosity by adding to the collodion solution a mixture of amyl alcohol and acetone.

Elford (93), as the result of a systematic study of the effect of many reagents on the porosity of membranes prepared from collodion solutions, found that in general addition of good solvents caused a decrease in membrane porosity, and non-solvents or precipitating agents an increase in porosity (cf. Pierce (234)). Amyl alcohol or acetone alone was a good solvent, but in the presence of each other there was an antagonistic effect which resulted in a porosity increase. On this basis, it was possible to compose mixtures of ether, ethyl alcohol, amyl alcohol, and acetone, to which were added small quantities of other reagents, for preparing a graded series of membranes of optimum mechanical properties and with porosities covering a very wide range (2 μ to 2 mμ). Porosities were increased in steps by addition of water or amyl alcohol, and decreased by addition of acetic acid or (107) ethylene glycol monoethyl ether (Cellosolve). Fine adjustments in porosity were made by altering the evaporation time. This permitted grading of porosity on a continuous scale.

The effect of various reagents on the porosity of Elford membranes is shown in figure 1, giving data (103) for membranes made from Necol (from Nobel Chemical Industries, Ltd.). The results of Bauer and Hughes (15) with Parlodion (du Pont) were entirely similar.

The results of adding various reagents to ether–alcohol collodion solutions, as influencing the membranes prepared from the latter, are summarized as follows: Ether–alcohol mixture (4), dilutes the collodion and makes membranes thinner, more porous, and rather brittle; ether (93), dilutes the collodion, making membranes thinner without much alteration in porosity; ethyl alcohol (93), makes membranes thicker and weaker, and decreases the porosity; methyl alcohol (93), decreases porosity; amyl alcohol (4, 93), decreases porosity, but, in the presence of acetone, increases the porosity; water (93, 224), increases porosity; if added in too great amounts, makes membranes brittle and non-uniform; acetic acid (86, 93),

decreases porosity markedly; lactic acid (86), increases porosity; ethyl acetate (4), increases porosity markedly; ethyl formate (4), increases porosity; glycerol (86, 250), increases porosity; castor oil (250), increases porosity and toughness; ethylene glycol (234), increases porosity; Cellosolve (107), decreases porosity markedly.

C. STRUCTURE OF MEMBRANES

Some model of the structure of ultrafilter membranes must be assumed for the quantitative calculation of porosity from calibration data. The limited means for studying membrane structure experimentally must be employed in order to select the most suitable model.

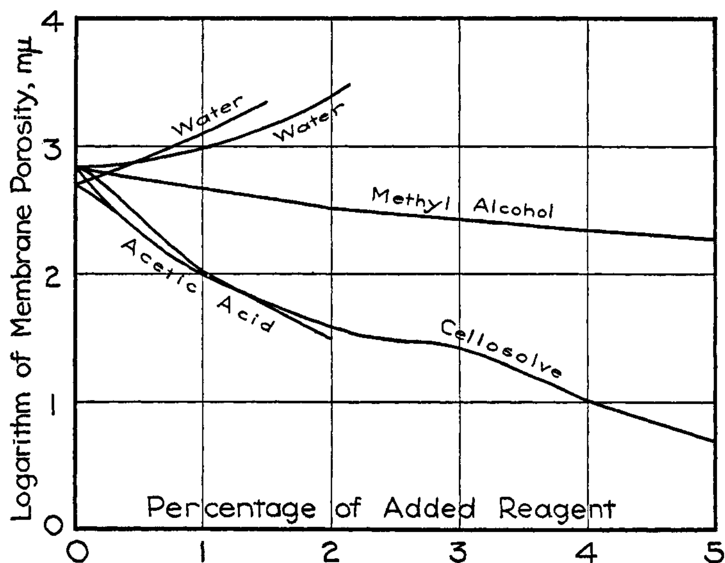

FIG. 1. Influence on membrane porosity of various reagents added to ether–alcohol collodion (Elford's parent amyl alcohol-acetone mixture). The logarithm of the porosity in mμ is plotted against the percentage of added reagent (93, 103).

1. Models of membrane structure

The most simple model of an ultrafilter is a sheet pierced by right circular cylinders, so that the effect in filtration is that of a bundle of cylindrical capillaries. This was the assumption made by Bechhold (18) in calibrating his acetic collodion membranes.

Manegold (201, 202) has discussed possible arrangements of porous structures in some detail, distinguishing between canal structures (pores, cracks) where the solid phase is continuous, and branching structures (packed spheres, packed parallelopipeds, packed rods, etc.) where it is not.

The latter type seems *a priori* the more likely in the case of a gel membrane, but it is much more difficult to treat. As for canal structures, Manegold specifies six arrangements:

(a) Pores (circular cross section), all running perpendicular to the membrane surface.
(b) Pores, a third of the total number running in each of three mutually perpendicular directions, without any intersections.
(c) Pores, oriented in haphazard directions, without any intersections.
(d) Cracks or slits (rectangular cross section), all running perpendicular to the membrane surface.
(e) Slits, a third of the total number running in each of three mutually perpendicular directions, without any intersections.
(f) Slits, oriented in haphazard directions, without any intersections.

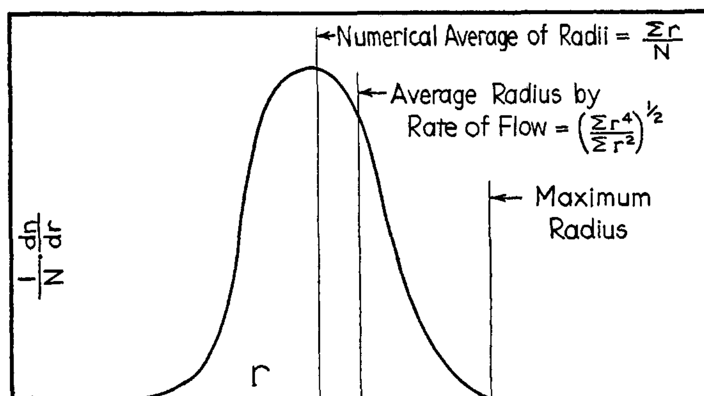

FIG. 2. Schematic distribution curve of pore sizes

As far as rate of flow of water through the membrane is concerned, it is impossible to distinguish between any of these structures. Manegold, after varied experimental studies on ether–alcohol membranes, concluded that structure (f) is the most likely. This was not, however, the only interpretation of his data possible (see below). Most evidence points to structure (a), with slight modifications, as a satisfactory working basis.

As an example of a structure with non-continuous solid medium may be cited the model of closely-packed spheres discussed by Tinker (272). In the case of collodion membranes, this structure is eliminated because it does not provide a large enough proportion of free space (see below; cf. Manegold, Hofmann, and Solf (206)).

Perhaps even more important than the shape and orientation of the interstices of an ultrafilter is the degree of uniformity of their dimensions. This

may be represented by a distribution function; thus, if structure (a) is adopted, the quantity

$$\frac{1}{N} \cdot \frac{dn}{dr}$$

is plotted against r (figure 2), where N is the total number of pores, and dn is the number whose radii lie between r and $r + dr$.

2. Experimental study of structures

a. Microscopical studies. Elford (92) employed films from acetic collodion and ether–alcohol collodion, prepared under various conditions, for studies with the microscope and ultramicroscope. Two types of structure were distinguished. The *microgel* structure has interstices of microscopic dimensions, and is highly irregular, offering pores of different diameters. This type of gel is formed when membranes are prepared from dilute acetic collodion coagulated in water, or ether–alcohol collodion which is coagulated in water before the evaporation has proceeded long enough to set the film to a gel. It results from diffusion of water, a precipitating agent, into the collodion solution while the latter is fluid and the micelles are mobile. On the other hand, the *ultragel* has a very fine, uniform, granular structure, revealed only by the ultramicroscope. This gel is formed when ether–alcohol collodion is allowed to "set" before immersion in water, or when films of acetic collodion of high viscosity or extreme thinness are treated with water. It results from the replacement of solvent by water in a structure which is largely immobile while the replacement proceeds, the collodion micelles being held fast in a previously set gel, or oriented by surface forces in a very thin film, or behaving as if immobile in a solution of high viscosity. In ether–alcohol collodion membranes, which are in practice prepared by immersing in water only after the gel has set, the ultragel structure prevails. In acetic collodion membranes, the structure grades between microgel and ultragel according to the viscosity of the impregnating solution and the thickness of the impregnated film. This explains the lack of uniform porosity in the latter membranes. The ultragel structure is the desirable one; unfortunately the ultramicroscope can give no information concerning its geometrical details.

b. The specific water content. One of the earliest methods of characterizing a membrane was by the proportion of empty space in its structure (279). In practice, the "specific water content" is defined (102) as the relative loss of weight by removal of water from the filter pores, and this is identified with the total volume of all the pores.

The specific water content of ether–alcohol collodion membranes is remarkably high and constant for porosities from 20 mμ to over 1 μ, as

shown by figure 3, which gives data for over two hundred Elford membranes (102). Entirely similar results were given by Manegold and Hofmann (203) for their membranes of pore diameters from 25 mμ to 60 mμ. The proportion of free space averages about 0.87 and is never less than 0.80 for all these membranes. Very close packing of pores in structure (a) would be required to provide this free space; hexagonal close packing, which gives a maximum of 0.905 for the circular cylinders in tangential contact, would barely suffice. Structures (b), (c), (e), and (f) are impossible for lack of room for non-intersecting pores. If pores are to be postulated running in three mutually perpendicular directions, they must be con-

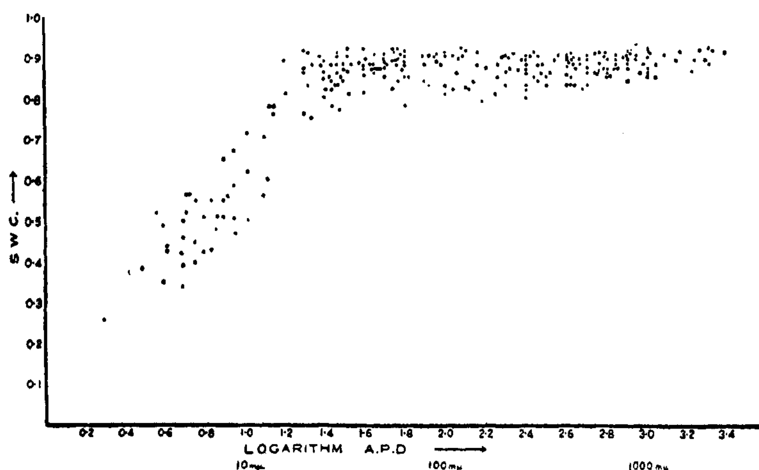

FIG. 3. The specific water content of Elford membranes, plotted against the logarithm of the porosity in mμ (102)

sidered to intersect to an extent dependent on the value of the specific water content (102).

c. Rates of flow and average pore diameter. Estimation of the porosity of a membrane by measurement of the rate of flow of water through it was first suggested by Guérout (146) in 1872. The rate of flow through membranes, as a means of characterizing them, was applied by various workers, who studied its dependence on experimental conditions. It was found to be proportional to the pressure for Pfeffer's copper ferrocyanide membranes (233) and for ether–alcohol collodion membranes (45, 45b, 83, 203), demonstrating that the flow is viscous. However, deviations from the proportionality law have been observed in both directions; the increase in rate of flow with increasing pressure may be greater than linear (203, 176a), which is attributed to distortion of the membrane at high pressures, or to

bringing immobile surface layers of water into motion at high pressures; or it may be less than linear (76), which is attributed to restriction of the area through which flow occurs when the membrane is forced against a perforated support at high pressures. This last effect shows that when rate of flow measurements are made for the purpose of porosity calculations, the membrane must not be supported against a wire gauze or perforated plate, since the calculations require accurate definition of the area through which flow is occurring. In the case of a mechanical support, the area effective in filtration varies from the total area of membrane, at low pressures (76), to the limited area actually opposite the perforations, at high pressures (95). When the effective area is clearly defined, and the pressures employed do not distort the membrane, the rate of flow is almost strictly proportional to the pressure. The dependence of rate of flow on temperature has been found for collodion membranes to be non-linear, and may be entirely attributed to the temperature variation of the viscosity of the flowing liquid (83). Above 75°C., however, this relationship no longer holds, since the membrane structure itself becomes altered and shrinks (76). The reproducibility and independence of time of the rate of flow of water, so long as blocking of pores by foreign particles or molds is eliminated, has been shown by Manegold and Hofmann (203). Cox and Hyde (76) showed that the rate of flow of water through collodion membranes was independent of pH from pH 1 to 12. Solutions of higher alkalinity attacked the nitrocellulose.

Calculation of the absolute porosity of a membrane from rate of flow measurements is made on the basis of three assumptions:

(1) The water flows through parallel cylindrical capillaries of circular cross section (structure (a) of page 387).

(2) The rate of flow of water is governed by Poiseuille's law.

(3) The total volume of pores, as given by the specific water content, represents the total volume effective in filtration; i.e., (*a*) there are no "blind" channels or pores which do not open on the surface; (*b*) there is no appreciable immobilized layer of water lining the pore walls.

In this case, the rate of flow is given by the expression

$$\frac{V}{t} = \sum_{i}^{N_A} \frac{\pi r_i^4 P}{8\eta l} \tag{1}$$

where V is the volume passing in time t, N_A is the number of pores opening on a surface area A, r is the radius of the ith pore, P is the pressure producing flow, η the viscosity of water, and l the capillary length,—set equal to the thickness of the membrane. Now, by assumption 3, the specific water content is given by

$$S = \frac{\sum_{i}^{N_A} \pi r_i^2 l}{Al} = \frac{\sum_{i}^{N_A} \pi r_i^2}{A} \tag{2}$$

and combination of this expression with expression 1 gives

$$\left(\frac{\Sigma r^4}{\Sigma r^2}\right)^{1/2} = 2\left(\frac{2V\eta l}{SAPt}\right)^{1/2} \tag{3}$$

The quantity on the left is obviously an average pore radius ($\bar{r}$) as determined by rate of flow. This type of average weights the larger pores more than does a numerical average (figure 2). The "porosity" of membranes is more frequently referred to by the average pore diameter, j:

$$j = 2\bar{r} = 4\left(\frac{2V\eta l}{SAPt}\right)^{1/2} \tag{4}$$

The extent to which the above assumptions are justified, and the effects of their invalidity on the significance of calculated values of j, are now examined. The consideration is confined to ether–alcohol collodion membranes, and is particularly applicable to those of Elford.

Assumption 1, that of Manegold's structure (a), is the most arbitrary. In support of this assumption, it has been shown that a hexagonal close-packing of cylindrical capillaries will account for the observed water contents of membranes of porosities from 20 mμ to 2 μ; and it is difficult to picture any other structure which would do so and still provide the mechanical strength possessed by these membranes. The hexagonal arrangement is further suggested by the fact that a macroscopic pattern of hexagons and six-pointed stars is formed on the surface of the more porous ether–alcohol collodion membranes during evaporation. It is likely that this pattern is repeated on smaller scales within the visible hexagons, the pores themselves being ultimately formed by such an arrangement. This is supported by Elford's observation (102) that the collodion particles constituting the ultragel tend to link up forming chains and closed rings. The result is probably a structure resembling a honeycomb. Bartell and Van Loo (12a) suggest that, during evaporation, vortices are formed, the elementary unit being a hexagonal cell in which the evaporating solvents stream upward in the center and radiate to the edges. Altogether, a hexagonal arrangement of pore openings in the membrane surface seems probable. It does not follow, however, that the pores run straight and perpendicular to the surface. A honeycomb structure may involve a lateral "staggering" of the constituent nitrocellulose particles, resulting in a certain degree of tortuosity in the pores. This would necessitate, however, an intercommunicating system, and, for the membranes of high water content, Elford and Ferry suggest that the effective pore length or path of flowing water is probably less than twice the membrane thickness (102), this being supported by data for free diffusion through membranes (see below), although Bechhold believes it to be three or four times the membrane thickness (25).

But for membranes of porosity below 20 mμ, where the water content is lower and non-intercommunicating tortuosities are permissible, the channels may be considerably prolonged, so that substitution of the membrane thickness for l in equation 4 makes the calculated value of pore diameter come out too small.

The general validity of assumption 2 is shown by the work of Duclaux and Errera (83), who found rates of flow of water, solutions, and organic liquids through collodion membranes to be inversely proportional to the viscosities of the flowing systems, and proportional to the pressures employed. Poiseuille's law may be invalidated, however, by electrokinetic effects, or by steric effects for pores so small that the water cannot be considered as a continuous medium. These complications, which should be expected for pores less than 10 mμ (94), would make the flow less than that demanded by Poiseuille's law, and make the calculation of average pore diameter by equation 4 come out again too small.

Assumption 3a depends on the absence of cavities in the membrane which are ineffective in transmitting flowing water. It would, therefore, not hold for structures (b) and (c) of Manegold, where the pores are assumed non-intersecting. For either of these structures, the average pore diameter as calculated by equation 4 is too small by a factor of 0.58 (47). However, for a membrane of specific water content of 0.87, intersection is a geometrical necessity, and for pores equally distributed in three mutually perpendicular directions the error introduced by assumption 3a is much less, the factor being 0.75 (102). For the actual membrane, even this is probably an overestimate of the deviation, the factor being more likely still closer to unity, because the membrane, prepared in the form of a thin film with processes like that suggested by Bartell and Van Loo in progress, is not apt to possess an isotropic structure with pores distributed evenly in various directions. Marked orientation should be favored, resulting in preference for channels perpendicular to the surface. On the other hand, for membranes of porosity below 20 mμ, with lower water contents, the possibility of blind channels and even completely isolated sacs arises; the effect of using S in equation 4, instead of a smaller value representing the fraction of volume actually effective in filtration, is again to make j come out too small.

Invalidity of assumption 3b also makes the calculated average pore diameter too small. The magnitude of the effect depends on the ratio of the thickness of the layer of immobilized water to the radius of the pore; it is probably negligible for porosities above 100 mμ (since such membranes can be dried and re-wet reversibly), and below 100 mμ becomes of increasing importance with decreasing porosity.

Elford and Ferry (102) concluded that the effects of all these factors

represent an error in the average pore diameter of not more than 25 per cent, when equation 4 is applied to membranes of porosities greater than 20 mμ.

d. Dialysis, diffusion, and conductivity. When a solute diffuses through a membrane, if there are no specific steric influences (the diffusion is free), the only effect of the membrane is to reduce the area through which the diffusion takes place,[5] and (if the length of the pores is greater than the membrane thickness) to reduce the gradient of concentration. Thus,

$$K' = \frac{K\delta\Sigma q_i}{Al} \tag{5}$$

where δ is the membrane thickness, q_i is the area of the ith pore opening on the top surface, l is the pore length, K the free diffusion constant, and K' the apparent diffusion constant for diffusion through an area of membrane A corresponding to the summation Σq_i. The ratio $\Sigma q_i/l$ may thus be calculated from experimental values of K and K'. The quantity $\delta\Sigma q_i/AlS$ is a pure number which should theoretically be unity for membrane structure (a)—pores all perpendicular to the top surface—and should have the value of $\frac{2}{3}$ for structure (f)—slits haphazardly oriented. Measurements by Manegold (201) permit the calculation of values of $\delta\Sigma q_i/AlS$ for hydrochloric acid, urea, and sucrose. Above an average pore diameter[6] of 35 mμ, these values were independent of the porosity, being 0.64, 0.72, and 0.80 respectively. Below 35 mμ, they decreased with decreasing porosity. Similar results had been noted by Oldenburg (227) for diffusion of sodium and potassium chlorides. The numerical values of $\delta\Sigma q_i/AlS$ for high porosities were interpreted by Manegold as evidence for structure (f) for membranes of porosity over 35 mμ. In view of the preceding discussion, however, structure (f) being actually a geometrical impossibility, an alternative explanation seems more likely. This is that the interstices are pores, not slits, and that there is enough tortuosity in them to decrease the ratio δ/l somewhat below unity, and that a small fraction of them do not open on the top surface, making the ratio $\Sigma q_i/AS$ accordingly less than unity; so that the product, $\delta\Sigma q_i/AlS$, is as low as 0.64 to 0.80. The decrease in the product with decreasing porosity is at first attributable to a change in membrane structure, with increasing tortuosity of pores and an increase in

[5] This reduction of the area through which diffusion occurs is called by Friedman and Kraemer (128) "mechanical blocking," a terminology which should be distinguished from the frequent use of "blocking" to signify progressive clogging of filter pores in the filtration of a disperse phase (part III).

[6] These porosities are in terms of average pore diameter on the basis of structure (a), recalculated from Manegold's data, which are usually expressed in terms of half slit widths on the basis of structure (f).

the proportion of pores which do not open on the top surface,—effects already suggested by the decrease in the water content for porosities below 20 mμ. That the diffusion is at first still "free," so that equation 5 is applicable, is indicated by the fact that hydrochloric acid and sucrose filter in undiminished concentration through membranes of porosity as low as 15 mμ (204), probably as low as 5 mμ (96), and by the fact that a Ladenburg correction for viscous drag, as used by Friedman and Kraemer (128), is capable of accounting for only a small part of the decrease in diffusion with decreasing porosity. At very low porosities, however—perhaps below 5 mμ—the Ladenburg correction, electrostatic and electrokinetic effects (in the case of an electrolyte), and other factors enter to a degree which invalidates equation 5. The ratio K'/K decreases more rapidly and becomes zero at a porosity representing complete impermeability to the diffusing solute.

Very similar considerations hold for conductivity measurements, provided the concentration of electrolyte used is sufficiently high that surface conductivity may be neglected. Manegold and Solf (207), using potassium chloride, showed that the ratio of the specific conductivity through a given membrane to the bulk specific conductivity decreased with increasing concentration, attaining constancy above 0.03 N, where surface conductivity became negligible. Measurements with 0.1 N potassium chloride provided values for $\delta\Sigma q_i/AlS$ of about $\frac{2}{3}$ for a porosity[6] of 40 mμ, decreasing to about $\frac{1}{3}$ for 13 mμ. Measurements by Hitchcock (153) gave somewhat higher values for the quotient, approaching unity for the more porous membranes. Manegold's conclusions of an irregular slit structure (f) to give a value of $\frac{2}{3}$, grading at lower porosities into an irregular pore structure (c), with possible inclusions of isolated sacs, to give a value of $\frac{1}{3}$, may be replaced by the postulate of a structure of pores of a slight tortuosity, the majority of which open on the top surface, the fraction which do not so open, and the degree of tortuosity, increasing with decreasing porosity below 40 mμ.

Michaelis (216) found that two solutions of potassium chloride in different concentrations, between which only a very slight diffusion potential should exist because of the equal mobilities of the two ions, did develop a diffusion potential when separated by a membrane of sufficiently low porosity. Manegold and Viets (208) and Elford and Ferry (103), studying the potential as a function of the membrane porosity, found it to be only very slight above a certain limiting porosity (average pore diameter about 3 mμ (103)), while below this porosity the potential rose sharply to its maximum theoretical value (57 mv. for a tenfold concentration difference). The limiting porosity probably corresponds to the point where the chloride ion is excluded from pores by electrostatic repulsion (cf. part IV, A, 3).

e. Distributions of pore sizes. An indication of the distribution of pores in ultrafilters (cf. figure 2) was obtained by Bechhold (18) by application of Cantor's law (previously suggested by Barus (13) in 1894), as follows. If the opening of a circular capillary is wet with liquid, and a pressure P is required to force through a non-wetting fluid immiscible with the first, then the diameter of the opening is given by

$$d = \frac{4\gamma}{P} \tag{6}$$

where γ is the surface tension of the interface between the two fluids.

If air is forced through a wetted membrane in this way, visual observation of the increase in frequency of bubbling as the air pressure is increased gives a rough measure of the distribution of the largest pores. The pressure at which the bubbling begins determines the maximum pore size, and the ratio of this to the average pore size as determined by rate of flow is an indication of the degree of isoporosity or heteroporosity. For acetic collodion membranes, this ratio is from 5 to 10; for Elford membranes, it is as low as 2, showing these membranes to be relatively isoporous.

The same principle may be applied to forcing a foreign liquid, such as isobutyl alcohol, through a membrane wet with water (33). In this case, much lower pressures are required, since the surface tension is lower. It must be noted, however, that the minute droplets of the foreign liquid which are forced through the pores are invisible until they coalesce to form larger drops, and this takes time (34). For validity of equation 6, the contact angle of the liquid–liquid interface with the pore wall must be 0°. This question has been examined by Erbe (113). The condition for zero contact angle is that a layer of the original liquid remain lining the pores after the second liquid has been forced through, i.e., the original liquid must wet the membrane the better. For cellulose (cellophane and Cella filters) the original liquid should be water, through which isobutyl alcohol is forced. The fact that the pores are still lined with water at this point is indicated by the rate of flow value for the alcohol being too small (after taking into account the difference in viscosity). For nitrocellulose, on the other hand, the isobutyl alcohol wets the better and should serve as the saturating liquid, water being forced through as the second liquid. In this case, the experiment may not be prolonged, or the water will displace from the pore walls the lining of alcohol, giving a contact angle no longer zero. Saturated solutions of the two liquids in each other behave in wetting practically the same as the respective pure liquids.

Combination of the bubble pressure and rate of flow methods gives the Bechhold–Karplus procedure (114) of determining the whole distribution curve schematically illustrated in figure 2. The rate of flow of water

through a nitrocellulose membrane wet with isobutyl alcohol (or *vice versa* for cellulose or an animal membrane) is determined as a function of pressure. At any given pressure, the observed flow represents the total occurring through all pores large enough to pass the non-wetting liquid at that pressure. The curve of flow as a function of pressure is shown in figure 4. From this, by breaking it up into fictitious discontinuities, may be calculated the distribution of pore radii (figure 5a), the distribution of pore areas (figure 5b), and the distribution of flow (figure 5c).

Pisa (235) employed this method to test several types of membranes. Cella filters were far from uniform, the ratio of maximum to minimum pore

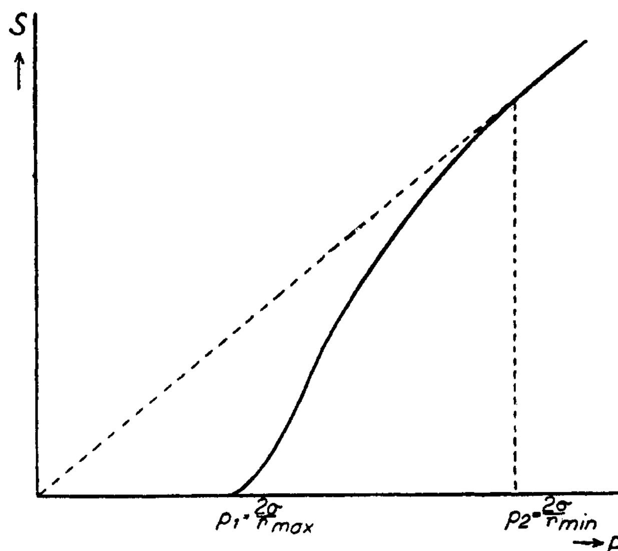

FIG. 4. Flow-pressure curve for the Bechhold-Karplus experiment (114)

diameter ranging from 3 to 5, and two pieces from the same membrane differing considerably in distribution curves and in average pore diameter (also checked by filtration of hemoglobin). Few data were quoted for acetic collodion membranes; in one case there was a sixfold range of pore sizes without a marked maximum in the center of the distribution curve. Among natural membranes, chorion from sheep was found to be quite isoporous, but variable from piece to piece, while amnion was exceptionally isoporous.

A good test for isoporosity is given in filtration experiments with a monodisperse system, especially with a biological material which is detectable in very minute quantities. The porosity ratio of the smallest-pored filter which passes the disperse phase in undiminished concentration to the

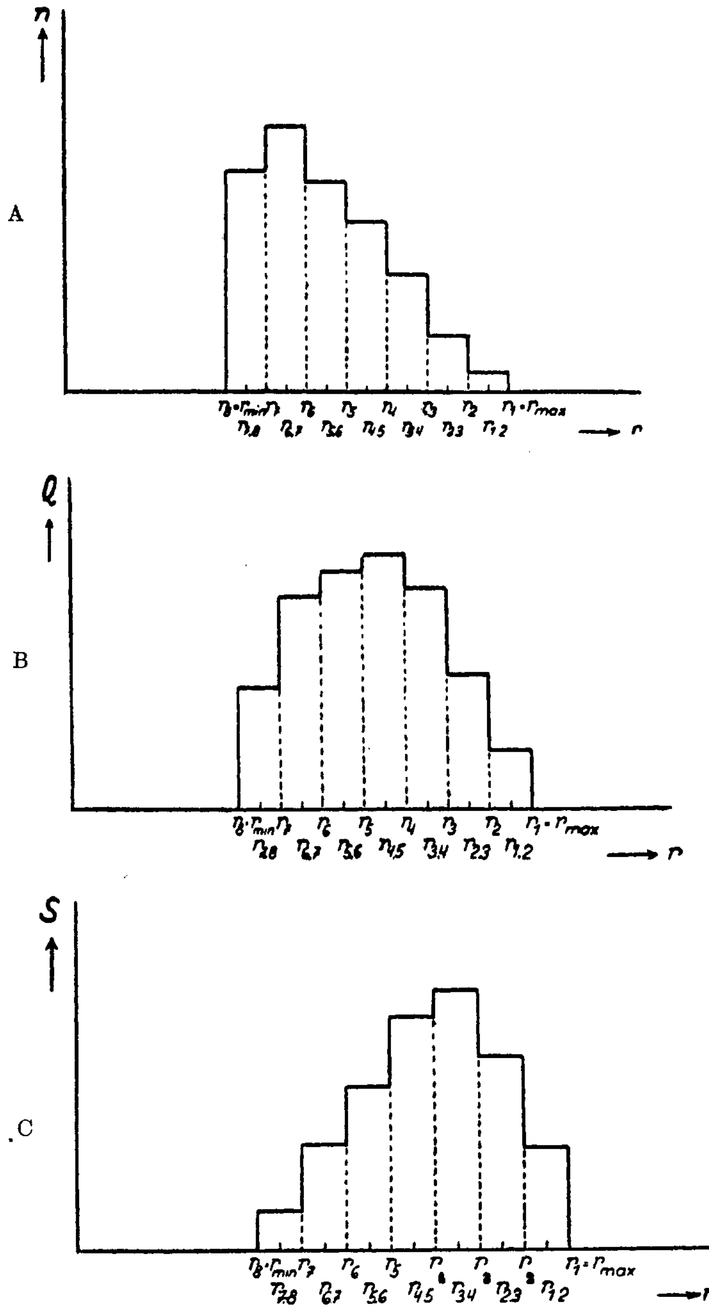

FIG. 5. Distribution curves determined by the Bechhold–Karplus procedure (114). (a) Distribution of pore radii; (b) distribution of pore areas; (c) distribution of flow through individual pores.

largest-pored filter which retains it completely is a measure of the pore uniformity. For filtration of viruses and bacteriophages by Elford membranes, this ratio is seldom greater than 2, a value approximating that anticipated on statistical grounds for a filter of perfect isoporosity (cf. part III, F).

3. Mechanism of variation of porosity

It is remarkable that membranes of collodion may be prepared covering a porosity range of a thousand fold, and particularly that in the greater part of this range ($j = 2\mu$ to $j = 20$ mμ) the total space occupied by the pores is independent of the size of the latter. Increase in porosity is evidently accomplished by redistribution of the nitrocellulose particles so that the pores become larger but also less numerous. This is probably a result of a reversible, gradual aggregation of the nitrocellulose, giving progressively larger particles (102)—particles which are not spherical, but elongated, as shown by streaming double refraction (256). Such gradual aggregation is exceptionally favored by a solvent containing amyl alcohol and acetone, explaining how the method of Elford can give such highly porous membranes with uniformity and adequate tensile strength. Presence of water favors aggregation, but it is not gradual; it produces flocking and coagulation, and the resulting membrane may be fragile and non-uniform. The difference between the action of water and that of amyl alcohol and acetone is clearly shown by titrating collodion solutions with these reagents (107).

The preparation of an ether–alcohol collodion membrane consists essentially in the evaporation of solvents until the film sets to a gel. The degree of aggregation of collodion at any point in the process depends in a highly specific manner on the composition of the solution. The composition of the solution depends in turn on the time elapsed and on the original proportions and volatilities of the various solvents and non-solvents in the solution. Two processes probably occur during the evaporation (107): a gradual aggregation as the proportions of solvents and non-solvents change, and a sudden gelling when the concentration of collodion becomes sufficiently high for the aggregates to lock into a rigid structure. The extent to which the aggregation has proceeded by the time gelation occurs determines, largely, the membrane porosity. Further evaporation from the set gel, accompanied by shrinkage of the gel when it is immersed in water, will decrease the eventual porosity.

D. CALIBRATION OF MEMBRANES

1. Significance of calibration

There are two fundamental questions in characterizing an ultrafilter. One concerns its average pore size and distribution of sizes; the other, its

behavior in filtering particulate systems. The first question is answered by measurements of specific water content, rates of flow, and bubble pressures. The second involves consideration of the complex mechanism of filtration (part III). The second question is the more important in the study of ultrafiltration, as distinguished from that of the membranes themselves, but it is not convenient to characterize ultrafilters in terms of filtration of disperse systems alone. In the first place, the behavior of a filter toward one sol may differ from its behavior toward another of similar particle size. In the second place, characterization by filtration of particles of known size does not provide a continuous scale of grading. Given an ultrafilter, it is hardly possible to command a wide series of sols of different particle sizes so closely spaced as to permit selecting one which the filter retains and another, only slightly finer, which the filter passes. On the other hand, calibration by rate of flow of water assigns a specific average pore diameter to the filter. Values of average pore diameter, as calculated by equation 4, provide a continuous scale, and, for ether–alcohol collodion membranes, are close to representing the true dimensions of the pores (probably within 25 per cent, for porosities above 20 mμ). The relationship between the diameter of a particle and the average pore diameter of the most highly porous filter which retains it is of a specific nature (part III).

2. Procedure of calibration

Calculation of the average pore diameter requires measurement of the membrane thickness, the specific water content, and the rate of flow of water through the membrane. In the convenient procedure of Elford (93) and Elford and Ferry (102), a uniform membrane sheet is cut into about forty discs, of which five or six are employed in measuring the above quantities, the remainder being subsequently available for filtration experiments.

a. Measurement of thickness. The membrane thickness is measured either by a micrometer gauge controlled by a fine spring, with precaution that the membrane is not compressed nor deformed, or by cutting a thin strip of membrane, bending it in the form of a Z to stand on edge, and observing it microscopically with a micrometer ocular. More refined methods may employ an optical lever (161) or even interferometry.

b. Specific water content. The specific water content, S, may be determined in three ways:

(a) By the difference in weights of the membrane with its pores full of water (W_w) and then dried by heating to 60°C. or over sulfuric acid (W_d):

$$S = \frac{W_w - W_d}{\delta A} \tag{7}$$

where δ is the membrane thickness and A the area.

(b) By consideration of the density of solid collodion, ρ; then

$$S = 1 - \frac{W_d}{\delta A \rho} \quad (8)$$

(c) (Acetic collodion membranes only) by assumption that the coagulation does not change the specific volume of the collodion, so that S equals the percentage of acetic acid in the impregnating solution.

c. Rate of flow of water. For a convenient measure of the rate of flow of water, Elford defined an auxiliary quantity, the "R.F.W." (F), in terms which lead to the formulation

$$F = \frac{V\delta}{AtP} \times 60{,}000 \quad (9)$$

where δ is the membrane thickness in millimeters, V is the volume of water which flows through an area A of the membrane in time t under a pressure P (in centimeters of water). Manegold and Hofmann (203) used a similar quantity (expressed in absolute units) for characterizing membranes. For the range of Elford membranes, F varies 10^6-fold, so that apparatus must permit measurement of large and small volumes of water and application of low and high pressures. For the highest porosities a pressure of 10 cm. of water is sufficient; it can be safely increased to 350 cm. of water for the densest membranes. The membrane must be clamped securely to obviate leaks, but damage of the edge must be avoided. The area through which flow occurs must be clearly defined, thus precluding a support of wire gauze or perforated plate behind the membrane. The membrane consequently bulges when pressure is applied, and the resulting displacement of water in the apparatus must be controlled. Apparatus suitable for rate of flow measurements has been described by Brukner (60), Elford (93), Elford and Ferry (102), and Bauer and Hughes (15).

The average pore diameter may be calculated from F (at 20°C.) and S, after introduction of dimensional constants, by the simple expression

$$j \text{ (in microns)} = 0.234 \sqrt{\frac{F}{S}} \quad (10)$$

E. APPARATUS FOR ULTRAFILTRATION

Apparatus for ultrafiltration requires a water-tight clamp for the ultrafilter, with a vessel for the filtering system under pressure and a receiver for the filtrate (often under negative pressure). For specific cases, there are many individual features of the apparatus to be considered.

1. Construction of vessel under pressure

For high pressures the ultrafilter vessel must be constructed of metal. Stainless steel (125) and brass plated with nickel (10, 1) and silver (100) have been employed. For corrosive solutions glass is better, and it has the further advantage of transparency. Grabar (141) has described a filter in which the solution and filtrate need touch nothing but glass and membrane at any point. For filtration of bacteriological systems, the apparatus must be sterilizable and there must be no exposure of metals which have toxic effects. In sacs and filters of the Bechhold-König type, the filter forms its own vessel, which is closed at the top by a rubber stopper sealed with collodion or the like, or left open to the atmosphere for filtration under negative pressure.

2. Support of membrane

The filter membrane may be supported on a perforated metal plate (130, 10) or metal tube (126), a fine wire gauze (61, 190), or a sheet of perforated glazed porcelain (293). Filter paper may be interspersed between the membrane and the rigid support, in an effort to increase the area effective in filtration. The latter is always, however, considerably reduced by a perforated support. The fact that filtration under a substantial pressure (2 atmospheres) takes place only opposite the perforations was shown by experiments of Elford in filtration of hemoglobin (95). A disc of sintered Jena glass (141) forms an admirable support in making use of the entire membrane area, but has the disadvantage of retaining a quantity of filtrate.

3. Gaskets

The filter may be sealed by gaskets of rubber (the customary material) or, where this is objectionable, of heavy swollen cellophane (195).

4. Clamping

A circular threaded ring is recommended for clamping, as giving even pressure all around the periphery of the membrane. It should be designed to prevent any shearing of the membrane (10). For higher pressures, where this does not give a tight seal, a series of individual bolts arranged around the periphery is employed (61).

5. Pressures applied

Filtration under negative pressures is not recommended for quantitative work, since the filtrate tends to concentrate by rapid evaporation (7). Further, it is difficult to collect successive samples of filtrate, a procedure necessary for adequate analysis of results. It is convenient, however, for

filtration of large volumes of material through highly porous filters, where the upper vessel may be often replenished. For filtration through membranes of low porosity, very high positive pressures may be desired. The designs of the Göttingen Commercial Filters (188), of Brukner and Overbeck (61, 62), and of Folley and Mattick (125) permit pressures up to 100 atmospheres.

6. Stirring

Bechhold (16) showed that ultrafiltration was often favored and expedited by stirring. He employed a mechanical stirrer with a shaft which emerged from the upper vessel through packing. The apparatus of the Göttingen Filters (188) and of Brukner and Overbeck (61) is equipped with a magnetic stirrer operated from the outside by a rotating electromagnet. Kronsbein (168) devised a reciprocating stirrer for thorough agitation in a filter cell in which the membrane was held vertically.

7. Filtration of small volumes

Apparatus designed especially for filtration of small volumes of material includes the arrangements of Augsberger (7), who employed an inverted Giemsa tube (137) with positive pressure, and de Waard (277) and Tóth (273), who ultrafiltered in a centrifuge, the centrifugal force replacing pressure. The design of Tóth, however, involved a membrane of the Bechhold–König type, which is itself unsuited for filtering small volumes, because of the retention of filtrate.

8. Miscellaneous

Other ultrafiltration apparatus has been described by Walpole (280), Malfitano and Michel (200), Fouard (126), Smith (259), Spiegler (262), Aitken (1), Breedis (52), Zakarias (291), Wilenskii (286), and Thiessen (270).

III. The Mechanism of Ultrafiltration of Disperse Systems

When a disperse system is forced through an ultrafilter, the disperse phase may be less concentrated in the filtrate because it is (a) adsorbed on the surface of the filter and its pores (primary adsorption), (b) retained within the pores or excluded from such blocked pores (blocking), or (c) mechanically retained on top of the filter (sieving). The latter sieve action may result from heterodispersion in the filtered system or heteroporosity in the filter, or from purely statistical effects (compare section F). The principal problem in selecting conditions for carrying out ultrafiltrations is to eliminate effects a and b as completely as possible, so that sieving, the desired effect, is the controlling factor. The operation of these three

mechanisms may change with time as the filtration proceeds, so that, in the first place, examination of the course of filtration throws light upon the problem.

A. EXPERIMENTAL PROCEDURE

The ideal method for following an ultrafiltration experiment would involve continuous measurement of concentration of filtrate and residue during the whole process, without necessitating withdrawal of samples. This is possible in some cases by measurement of refraction or light absorption (208), but imposes serious limitations on the filtration apparatus. It is almost always possible, however, to separate the filtrate into successive small samples for individual analysis, and to withdraw small samples from the residue from time to time if desired. Whenever feasible, a physical analytical method is preferable; refractometry has been successfully used

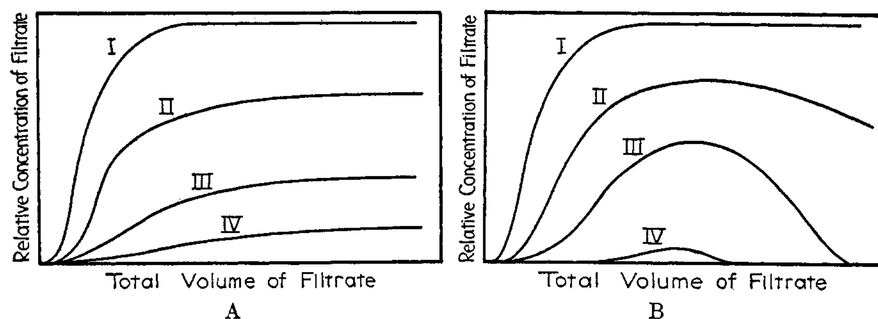

FIG. 6. Typical filtration curves. (a) Normal filtration; (b) abnormal filtration

(188, 94, 193), as well as colorimetry (94) and conductivity (195, 89) and surface tension (89). For biological systems, immunological reactions or animal tests are usually required.

The relative concentration of filtrate is defined as the ratio of the concentration of a momentary small sample of filtrate to that of the original solution. When this is followed through the course of a filtration, a curve of one of the forms shown in figures 6a and 6b results. Curves I arise from filtration through membranes whose pores are far wider than the solute particles. When the pore sizes are of the same order of magnitude as the particle sizes, the curves may take the form of either II-IV in figure 6a (100), or II-IV in figure 6b (100, 235). In every case, the initially low values of filtrate concentration are attributed to a primary adsorption of the solute in the membrane pores; this becomes satisfied as soon as a sufficient volume has been filtered through (depending, of course, on the concentration of the filtering solution). After this, the solute or disperse

phase appears in the filtrate in practically undiminished concentration (curves I); or, if the pores are not large enough to permit this, the filtrate concentration levels off or slowly increases (figure 6a), attributed to sieve action; or reaches a maximum and falls off more or less rapidly (figure 6b), attributed to blocking.

The change with time of the concentration of the residual solution above the filter is also characteristic. When the solute appears in the filtrate in practically undiminished concentration (curves I), the concentration of the residue is also practically unchanged. True sieve action (figure 6a) is accompanied by an increase in the concentration of the residue, which may

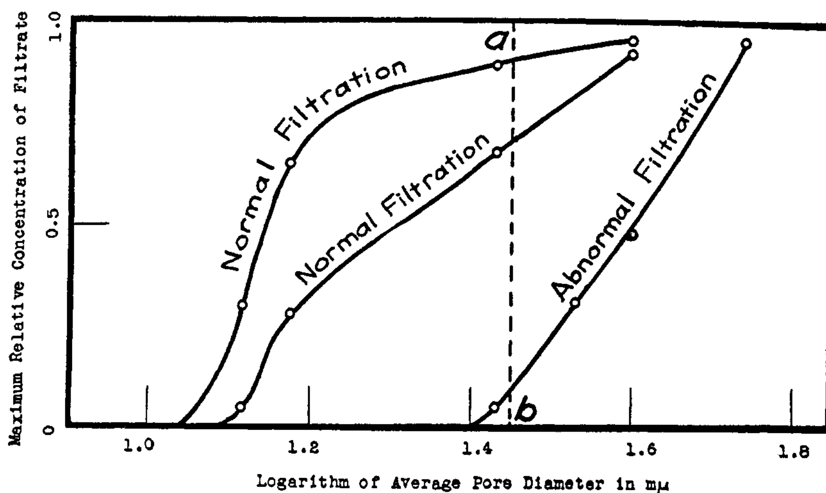

FIG. 7. End-point curves for filtration of serum albumin (100). The maximum relative concentration of filtrate is plotted against the logarithm of average pore diameter in mμ.

be many fold if the proportion of solute passing the filter is small and a large fraction of the original volume is filtered. Blocking (figure 6b) is accompanied by an increase in the concentration of the residue to some extent, but (unless it is occasioned by foreign particles) it always involves colloidal instability of the disperse phase and a tendency to flocculation and precipitation, so that the latter processes often occur in the residual solution, and the disperse phase, instead of concentrating, is precipitated on the membrane. Finally, in the case of marked primary adsorption from a limited quantity of a dilute solution, the concentration of the residue may be considerably diminished.

To obtain a quantitative measure of filterability of a given disperse system, equal volumes of the latter (volumes several times that required

to satisfy primary adsorption) are filtered through membranes of different porosities. The maximum relative concentration of filtrate obtained in each experiment is plotted against the membrane porosity (semilogarithmically, for convenience), and curves of the type shown in figure 7 result. The intersection of such an "end-point curve" with the porosity axis determines the end-point porosity, i.e., the highest porosity which completely retains the disperse phase.

By adjusting experimental conditions, it is possible to isolate effectively the factors of adsorption and blocking, and to study means of their elimination.

B. PRIMARY ADSORPTION

Bechhold (16) pointed out the rôle of adsorption in removing the disperse phase from the ultrafiltrate, and advocated control experiments by shaking bits of membrane with the solution to be filtered, and thus determining the amount of adsorption. This procedure is not adequate, however, for complete interpretation of filtration mechanisms (94).

The effect of primary adsorption in filtration may be studied separately by employing filters with pores very much wider than the solute particles, so that no sieving nor blocking can occur; or by employing extremely dilute suspensions, as is possible in biological systems, so that a large volume is required to satisfy the adsorbing capacity of the membrane.

1. Influence of experimental conditions

a. Concentration of filtering solution. The more dilute the filtering solution, the greater the volume which must pass through the membrane before adsorption is satisfied and the disperse phase begins to appear in the filtrate. Elford's experiments with dyes (94) showed that, over a tenfold variation in initial concentration, the relationship was one of slightly less than inverse proportionality (figure 8). Similar results were found in the filtration of foot and mouth disease virus (129). Infective agents are always employed in very dilute suspension (see table 5); if limited volumes of filtrates from such a system are collected, it is clear that presence or absence of the agent in a filtrate will depend on the initial concentration. This is shown in figure 9 for *B. Prodigiosus* and vaccinia virus. Membranes of high porosity adsorb all the agent from a limited volume of low concentration. For a higher initial concentration, it is necessary to select a membrane of lower porosity—one which will introduce some sieve action—to retain the agent. Finally, for the highest concentrations, only membranes of porosities below the end-point value can retain the agent. This same effect was pointed out as early as 1910 by Steinhardt (263) in the filtration of diphtheria toxin and cobra venom through uncalibrated col-

lodion sacs. It has been studied by Elford and collaborators for foot and mouth disease (129) and six different sizes of bacteriophages (98).

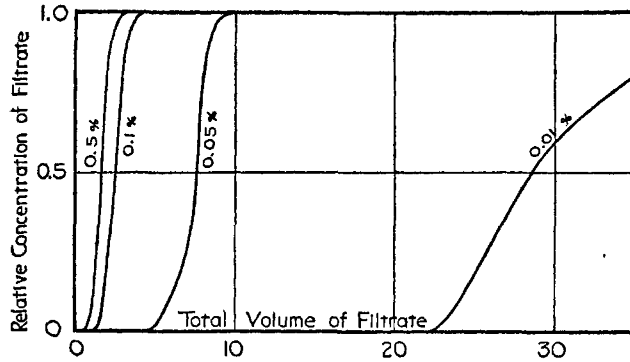

FIG. 8. Dependence of the zone of primary adsorption on concentration, for dilute solutions of dye (94). The numbers opposite the curves refer to initial concentrations of the filtering solutions.

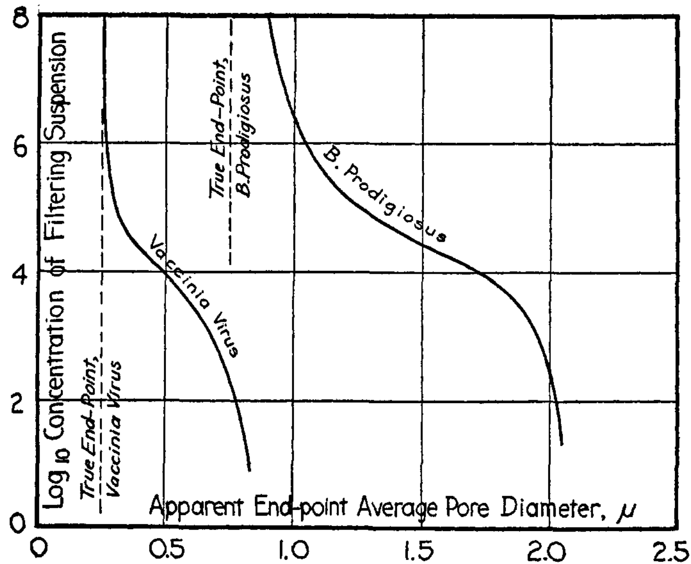

FIG. 9. Dependence of apparent end point on concentration for bacteriological systems, caused by primary adsorption (94)

b. Filtration pressure. Increase of pressure narrows the zone of primary adsorption, presumably by defining more closely against the filter support the areas through which flow can take place and by a tendency to shear

away adsorbed layers in the pores. Figure 10 shows experiments by Elford (94) with the dye Night blue.

c. Thickness of membrane. The thicker the membrane the greater the adsorbing capacity, and accordingly the greater the volume which must be filtered before the disperse phase appears in the filtrate. This was pointed

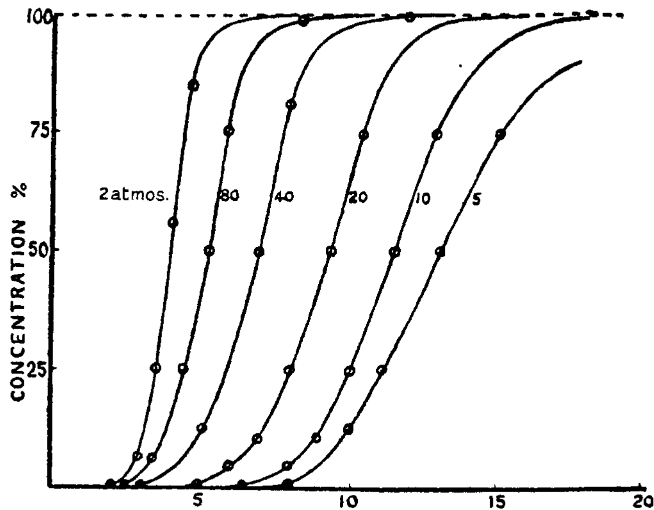

FIG. 10. Dependence of the zone of primary adsorption on pressure, for dilute solutions of Night blue (94). The relative concentration of filtrate is plotted against the total volume of filtrate. The numbers opposite the curves refer to pressures in centimeters of mercury, except the first, which is expressed in atmospheres.

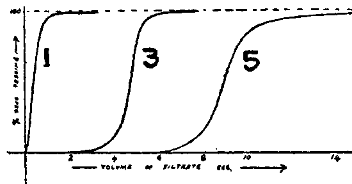

FIG. 11. Dependence of the zone of primary adsorption on membrane thickness, for foot and mouth disease virus (129). The figure opposite each curve indicates the number of membranes through which filtration took place.

out by Steinhardt in experiments with cobra venom (263) and demonstrated by the results of Galloway and Elford with foot and mouth disease virus (figure 11) for membranes piled one on top of another.

d. Capillary-active substances. The presence of a capillary-active substance in the disperse system markedly decreases the zone of primary

adsorption, as shown by the results of Elford on Night blue (figure 12), where saponin and sodium oleate are employed as capillary-active agents.

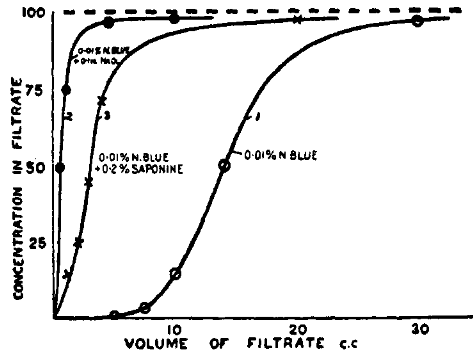

FIG. 12. Dependence of the zone of primary adsorption on the presence of capillary-active substances, for a dilute solution of Night blue (94)

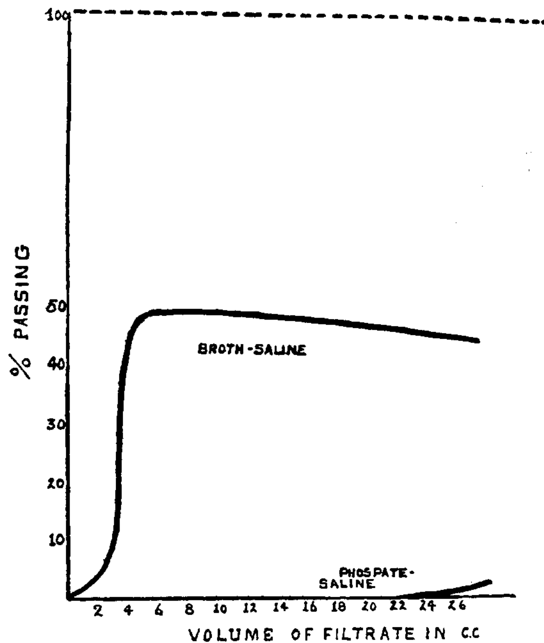

FIG. 13. Dependence of the zone of primary adsorption on the presence of a capillary-active substance, for foot and mouth disease virus (129)

The capillary activity of broth, as contrasted with phosphate-buffered saline, is shown by filtration of foot and mouth disease virus through collodion membranes (figure 13) and through Seitz filter candles (136). The

effect is presumably due to preferential adsorption of the capillary-active agent (see part III, section D).

2. Suppression of primary adsorption

It is clear that the effect of primary adsorption can always be eventually eliminated by filtering a sufficient volume through the filter membrane. The adsorbing capacity is satisfied most quickly for (1) high initial concentrations of the filtering solution, (2) high filtration pressures, (3) thin membranes, and (4) presence of capillary-active substances.

C. BLOCKING

The effect of blocking may be studied separately by employing solutions of concentration sufficiently high to satisfy the primary adsorption at an early stage in the process. Blocking of ultrafilters is closely related to colloidal instability, and may be best studied in filtration of sols of a lyophobic tendency. Proteins near the isoelectric point offer convenient examples.

1. Influence of experimental conditions

a. Concentration of filtering solution. The more concentrated the filtering solution, the sooner blocking sets in, so that, under conditions of severe blocking, it is possible to find the maximum relative filtrate concentration related antibatically to the absolute initial concentration. This is the situation for serum albumin at pH 5.7 (figure 14).

b. Filtration pressure. Increase of pressure favors blocking. In particular, reduction of the applied pressure to zero—i.e., substituting dialysis for ultrafiltration—apparently eliminates the effects of blocking entirely (section G). Bechhold (19) found alternating periods of pressure and no pressure to be effective in filtering solutions of hemoglobin, while Elford (96) has employed an oscillating, reversing pressure to keep the filter pores cleared.

The effect of increased pressure on the filtration of a colloidal sol, favoring blocking and resulting in impaired filterability, is in contrast to the effect on the filtration of an emulsion, as found by Hatschek (148a) for solutions of lecithin. In this case, increase of pressure improves filterability, the droplets of the disperse phase being deformed and elongated and forced through pores too small to admit them in spherical form.

c. Membrane thickness. The thicker the membrane, the greater the ratio of pore length to pore diameter, and the more favorable are conditions for blocking. This has been demonstrated for filtration of serum pseudoglobulin near the isoelectric point (103).

d. Capillary-active substances. Presence of a capillary-active substance suppresses blocking (100), presumably by preferential adsorption with

resultant "lubrication," enabling the particles to slip through the filter pores more readily (part III, D).

e. Foreign particles. Presence of foreign particles, larger than those of the principal disperse phase, may cause serious blocking of the pores by mechanical obstruction, even under conditions where the principal disperse phase itself does not tend to block because of colloidal instability. In fact, filtration of distilled water (rate of flow experiments) has been found to be impeded by blocking from dust particles unless the water is double-distilled or cleared by a preliminary filtration (113). When blocking is occasioned by foreign particles, accompanied by no flocculation of the

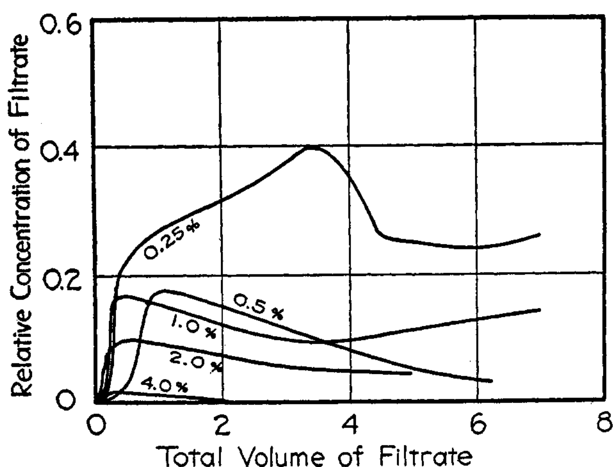

FIG. 14. Dependence of blocking on concentration, for serum albumin at pH 5.7 (100). The numbers opposite the curves refer to the initial concentrations of the filtering solutions.

disperse phase, the concentration of the residue should increase as in true sieve action.

2. Suppression of blocking

It is clear that the effect of blocking can be diminished by (1) low initial concentrations of the filtering solution, (2) low filtration pressure, (3) thin membranes, (4) the presence of capillary-active substances, and (5) absence of gross foreign particles.

D. CAPILLARY-ACTIVE SUBSTANCES

Conditions for suppression of adsorption and blocking agree in demanding the presence of a capillary-active substance, i.e., one which depresses

the surface tension of the solution and is strongly adsorbed in interfaces, in particular, on the pore walls and on the surfaces of colloidal particles.

The effect of a protective colloid in facilitating filtration of lyophobic colloids was pointed out by Bechhold (16).

The first systematic study of the effect of capillary-active substances on membrane permeability was that of Brinkman and Szent-Györgyi in 1923 (53). They employed collodion sacs which were ordinarily impermeable to hemoglobin in ultrafiltration at 3 atmospheres pressure. Such a membrane became permeable to this protein when a dilute solution of sodium oleate was first passed through it. That the hemoglobin was unchanged was shown by filtration of the filtrate from a "soaped" membrane through an "unsoaped" one. The protein was retained by the latter in the usual manner. "Soaped" and "unsoaped" membranes had practically the same porosity, as shown by rate of flow of water, in spite of the difference in permeability to hemoglobin. Treatment of a soaped membrane with calcium chloride rendered it again impermeable. Other capillary-active substances could be substituted for sodium oleate with varying effectiveness,—sodium linolate, sodium glycocholate, digitonium, glycerol monooleinate, and Witte's peptone.

Clausen (72) prepared a capillary-active substance from urine of nephrosis by concentration, dialysis, desiccation, and extraction with alcohol. This substance, a wax, rendered permeable certain collodion membranes which were normally impermeable to protein,—an effect of physiological significance in view of the increased permeability of the kidney tissues to serum proteins in nephrosis.

The conflicting report of Norris (225) that the permeability of collodion membranes is unaltered by the capillary-active agents used by Brinkman and Szent-Györgyi and by Clausen, was really no contradiction to the work of the earlier authors. The results of Brinkman and Szent-Györgyi, who studied permeability to protein in ultrafiltration experiments, are ascribable to the suppression of blocking by the capillary-active agents. The data of Norris refer to permeability to calcium chloride in diffusion experiments; in this case no blocking would be expected in the absence of a capillary-active substance (section G), so that the addition of one would have no effect.

However, Faludi (118) reported that bile salts (of which sodium glycocholate is an example) do not influence the ultrafiltration velocity of serum or plasma when the former are employed at the concentrations occurring in the body. Bedson (39a) found that the permeability of collodion membranes to serum proteins in ultrafiltration was increased by a preliminary soaking in serum, and gave some evidence that the active agent in the serum was cholesterol. Tallerman (266) confirmed the effectiveness of serum,

but not that of cholesterol. Nattan-Larrier et al. (223) found that collodion membranes normally impermeable to complement were made somewhat permeable by sodium oleate, taurocholate, or glycocholate, but not by egg albumin. It should be noted that the profound influence of pH on permeability to protein may overbalance that of a capillary-active agent (cf. part IV, C, 2). Rao (239) found that saponin increased the permeability of collodion, parchment, and various animal membranes to oxalic, lactic, and tartaric acids and sucrose in diffusion. The improvement in diffusion, considered as a function of the concentration of saponin, reached a maximum (rarely greater than 30 per cent) at a very small concentration. In the case of sucrose, a surplus of saponin even decreased the permeability. Amyl alcohol was also effective as a capillary-active agent.

Holman and Krock (156) made the remarkable observation that bacteriological candle filters became readily permeable to bacteria when "oiled" with paraffin wax or liquid paraffin. Oiled portions seemed to have larger pores as shown by the bubble test, but the rate of flow of water was decreased by oiling.

The effectiveness of bacteriological broth as a capillary-active agent was noted by Ward and Tang (281), who showed its influence on the permeability of filter candles to vaccinia and herpes viruses. The effect of Hartley's broth on the permeability of collodion membranes was studied by Galloway and Elford (129) in the filtration of foot and mouth disease virus and by Elford and Ferry (100, 101) in filtration of proteins at various pH values. This broth is ordinarily prepared from horseflesh by extraction and digestion with trypsin (148). The identity of the capillary-active substance in it is not known, except that it is destroyed by prolonged digestion with trypsin (100).

The mechanism of lubrication in filtration with a capillary-active substance is usually interpreted as a coating of the latter on both pore walls and particle surfaces. Evidence of such adsorption in fine-pored membranes has been found in rate of flow experiments by Grollman (145), Cox and Hyde (76), and Elford (96).

E. NORMAL AND ABNORMAL FILTRATION

While the conditions for suppression of primary adsorption and blocking agree in demanding thin membranes and the presence of a capillary-active substance, they disagree in their requirements for concentration and pressure. Optimum values of the latter must accordingly be chosen. For most infective biological systems, the optimum concentration is the highest concentration obtainable; for proteins, 0.5 per cent is satisfactory (100).

When blocking is effectively suppressed, so that the filtration curves

have the forms of figure 6a, the filtration has been termed *normal* by Elford and Ferry (100). Filtration with blocking, giving curves like those of figure 6b, is *abnormal.* The latter is invariably associated with an abnormally high end point, so that filters impermeable to a given disperse phase under conditions of abnormal filtration may under normal filtration become permeable.

Occurrence of abnormal filtration apparently depends on the tendency of the particles of the disperse phase to aggregate and precipitate when they are crowded together, as when they are forced into the membrane pores. If the tendency to aggregation exists, multiple adsorbed layers will be built up within the pores, and pores ten times as wide as the particles may become completely blocked. Without this instability—i.e., when normal filtration obtains—the adsorption may be limited to a single layer, and, by

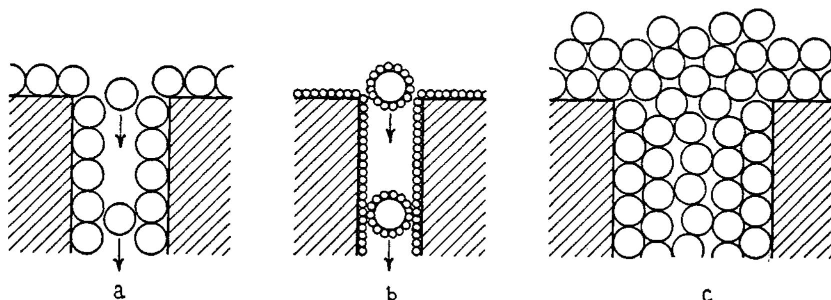

FIG. 15. Schematic representations of normal and abnormal filtration. (a) Normal filtration, in the absence of a capillary-active substance; (b) normal filtration, in the presence of a capillary-active agent; (c) abnormal filtration.

selective adsorption of a capillary-active agent, even that may be suppressed (94). Schematic diagrams of these principles are shown in figure 15.

F. THEORY OF SIEVING

The desired mechanism for retaining the disperse phase in ultrafiltration is a mechanical sieving. The theoretical consequences of an idealized sieving operation are here considered.

1. *Relationship of the sieve constant to the concentrations of filtrate and residue*

Neglecting adsorption and blocking, Manegold and Hofmann (204) assumed the sieving to be expressible by the equation

$$\frac{c_f}{c_s} = \varphi \tag{11}$$

where c_f is the concentration of a momentary small volume of filtrate, c_s is the simultaneous concentration of the filtering solution, and φ is the "sieve constant," independent of c_s. The value of c_s, and the concentration of the total filtrate at any point, are obtainable by integration of appropriate differential equations. The integrals were evaluated for various conditions (a closed system; a system in which the original solution is continuously added to keep the volume of filtering solution constant; a system in which pure solvent is added at intervals to keep up the volume of filtering solution; etc.). For filtration in a closed system, where nothing is added or removed, the concentrations of both residue and total filtrate should increase with time (the latter much more slowly), when the sieve constant is neither 0 nor 1. Such a concentration increase in both residue and filtrate has been reported by Cox and Hyde (76) for filtration of colloidal dyes through Bechhold membranes, and by Duclaux and Hirata (84) for filtration of gelatin through uncalibrated sacs.

2. Statistical evaluation of the sieve constant

The significance of the sieve constant in terms of sizes of pores and solute particles remains to be discussed. For a perfectly monodisperse solute and a perfectly isoporous filter, it has usually been implicitly assumed that the solute would either pass in undiminished concentration ($\varphi = 1$), or be entirely retained ($\varphi = 0$), depending on the relative sizes of pores and particles. This viewpoint must be, however, erroneous. The proportions of solute and solvent which pass the membrane depend on statistical considerations, even when the solute particles are so large that the water can be considered continuous by comparison. The sieve constant should increase gradually from 0 to 1 as the pore size is progressively increased above the end-point value.

On the basis of several simplifying assumptions, it is possible to calculate φ as a function of the ratio of pore size to particle size (119). It is assumed that:

(1) The membrane structure is represented by structure (a) of page 387 and is ideally isoporous.

(2) Adsorption and blocking are absent.

(3) Every solute particle is travelling vertically downwards when its center passes the plane of the surface of the membrane, and, in order to penetrate a pore, it must be wholly within the walls of the latter; i.e., its center must lie within a circle of radius $r - R$, where r is the radius of the pore[7] and R that of the particle.

[7] The pore radius in this case is not that determined by the usual calibration methods, but is the effective radius in filtration, defined on a scale such that $r \leqq R$ for membranes completely impermeable to the solute. This question is more fully discussed in a paper in press in the Journal of General Physiology.

(4) At the mouth of the pore there is no radial component of the hydrodynamical velocity of flow, and the vertical velocity has a parabolic distribution across the capillary in accordance with Poiseuille flow.

(5) The solution above the membrane is homogeneous, thermal motion preventing any accumulation of particles at the mouth of the pore.

In this case, the expression for the sieve constant is evaluated as

$$\varphi = 2\left(1 - \frac{R}{r}\right)^2 - \left(1 - \frac{R}{r}\right)^4 \tag{12}$$

The sieve constant φ is plotted in figure 16 as a function of the logarithm of the ratio r/R. This theoretical curve closely resembles the experimental

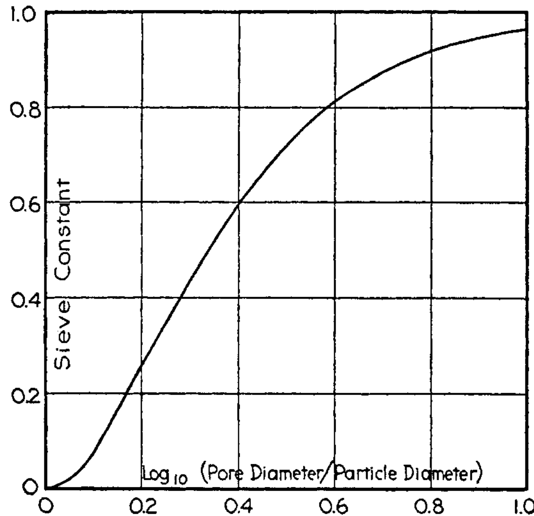

FIG. 16. Theoretical curve for the sieve constant as a function of the logarithm of the ratio of pore diameter to particle diameter

end-point curves for normal filtration shown in figure 7. Several significant values of r/R are noted for reference: (a) For $\varphi = 0.50$ (first indication of fall in filtrate concentration detectable in most viruses), $r/R = 2.18$. (b) For inflection point in curve, $r/R = 1.41$. (c) For end points (i.e., apparent complete retention of disperse phase): in chemical substances, $\varphi = 0.001$, $r/R = 1.022$; in bacteriophages and viruses, $\varphi = 10^{-6}$, $r/R = 1.0007$; theoretical end point, $\varphi = 0$, $r/R = 1.0000$.

A sieving effect—i.e., the partial transmission of the solute, to an extent which varies with the membrane porosity—can thus be anticipated on purely statistical grounds. This indicates as invalid an assumption often implicitly accepted in the literature, that a sieving effect must be due

either to polydispersion in the system filtered or to heteroporosity in the filters employed.

3. Statistical sieving in the absence of isoporosity and monodispersion

In practice ideal isoporosity is never attained, and probably colloidal solutions are never perfectly monodisperse, although some proteins approach this condition closely. When the statistical frequency of pores (or particles) of different sizes is given by a distribution function which is not a sharp peak, the curves of the type of figure 7 or figure 16 are less steep, and sieving occurs over a greater range of filter porosities than is the case for isoporosity and monodispersion, while the apparent end points for chemical and bacteriological systems are much farther apart.

4. Effect of stirring on sieving

The considerations of Manegold and Hofmann (204) hold only in a system where the residue is always homogeneous; i.e., when the rate of filtration is slow compared with diffusion, or when local concentration immediately above the filter is prevented by stirring or agitation. Ershler (115) has discussed the situation where diffusion is negligible, so that the whole of the retained solute remains locally concentrated in a layer of solution just above the surface of the membrane. The concentration of this layer increases until it attains a value c_s/φ, where c_s is the concentration of the body of the filtering solution; then it remains constant throughout the filtration. After the concentration of this layer above the membrane becomes constant, the filtrate has a constant concentration c_s, and the solute apparently passes through the filter unhindered. This effect was demonstrated experimentally for filtration of electrolytes through dense membranes (part IV, A, 3).

G. CONTRAST BETWEEN DIALYSIS AND ULTRAFILTRATION

It has been often pointed out that permeability in dialysis and permeability in ultrafiltration are not comparable. Levy (177) showed that certain collodion sacs were impermeable to ptyalin, rennin, and pepsin in ultrafiltration, but that these enzymes could dialyze through the sacs. Bechhold (16) noted that oxalic acid was separable from colloidal Prussian blue by dialysis, whereas ultrafiltration of the mixture only resulted in a precipitation on top of the membrane. Elford and Ferry (103) found that, although a membrane of porosity 45 mμ was impermeable to isoelectric serum albumin in ultrafiltration at 3 atmospheres pressure, the isoelectric protein could diffuse through a membrane of porosity 14 mμ. It is probable that in diffusion or dialysis, where the penetration of the disperse phase into the membrane takes place by molecular motion only, the effects

of blocking are absent, so that dialysis end-point porosity should be about the same as that found in "normal" filtration.

IV. Experimental Results of Ultrafiltration of Disperse Systems

The previous section has shown the influence of experimental conditions upon the results of ultrafiltration. In applications to the fractionation and study of composition of disperse systems, it is difficult to compare data unless the experimental conditions are completely described,—which has been too rarely the case. Unfortunately, also, much of the earlier work has been done with filters which were characterized imperfectly or not at all. Even in this case, however, some significant comparative results have been obtained.

A. ULTRAFILTRATION OF COLLOIDS AND CRYSTALLOIDS

1. Composition of colloidal sols

Bechhold's original investigations (16, 17) included ultrafiltration of numerous colloidal systems. Heterodispersion of particles in a silver sol was demonstrated by filtration of two fractions which had been prepared by centrifugation; a filter was found which passed the particles of one fraction and retained those of the other. End points of sols investigated were arranged in descending order: Prussian blue, platinum (Bredig), ferric hydroxide, casein, arsenic trisulfide, gold (40 mμ), silver (20 mμ), gold (1 to 4 mμ), 1 per cent gelatin, 1 per cent hemoglobin, serum albumin, diphtheria toxin, protoalbumoses, silicic acid, lysalbumin, deuteroalbumoses A, B, and C, litmus, and dextrin.

Malfitano (199a) studied the composition of colloidal ferric hydroxide, stabilized with hydrochloric acid, by ultrafiltration. The ultrafiltrates (i.e., colloid-free filtrates) contained only hydrochloric acid, and repeated ultrafiltrations (adding water to make up the volume above the filter) resulted in continued hydrolysis, with eventual coagulation of the colloid, the coagulation being to some extent reversible by addition of more hydrochloric acid. Wintgen and Biltz (286b) employed ultrafiltration in conjunction with measurements of conductivity and transference to study the composition of ferric hydroxide micelles. McBain and McClatchie (195), using membranes of cellophane, showed that the composition of ultrafiltrates from a ferric hydroxide sol varied greatly with the rate of filtration; the concentration of simple electrolytes in the filtrate (measured by conductivity) fell off fourfold for a sixfold increase in rate. This is the result to be expected from a blocking effect, but the authors point out that the mutual repulsion of the colloidal particles would prevent blocking (the particles being assumably too large actually to enter the pores), and attribute the results to an internal Donnan effect. The concentration of

the intermicellar fluid varies from point to point, being the least in the immediate neighborhood of the micelles. The ultrafiltrate at zero pressure, or dialyzate of the sol, gives the composition of the dispersing medium far from the influence of the micelles, and is the most concentrated ultrafiltrate obtainable. In filtrations at increasingly high pressures, the micellar domains (ionic atmospheres) are drained to an increasing extent, and the ultrafiltrates correspondingly diluted. Mukherjee et al. (221), on the other hand, reported ferric hydroxide ultrafiltrates of a higher conductivity than that of the original sol.

McBain and Jenkins (188), using Bechhold membranes, ultrafiltered soap solutions, whose colloidal and crystalloidal composition had been previously deduced from osmotic and conductivity measurements. The concentrations of ultrafiltrates through very dense membranes (maximum pore size less than 9 mμ) represented the concentrations of the crystalloidal components, which agreed with the previous data for solutions of sodium laurate below 0.8 N and sodium oleate below 0.5 N. The ultrafiltrate concentration was independent of pressure over a wide range. Very dilute, fresh solutions (0.01 N) of the soaps were completely ultrafilterable, showing absence of colloidal components. In sodium oleate, a fractionation was effected by membranes of maximum pore size from 15 mμ to 75 mμ, the sieving being independent of porosity over this range. This was attributed to separation of ionic micelles from neutral micelles, the former passing the filters and the latter being retained. The concentrations of ionic micelle and neutral colloid thus calculated from ultrafiltration were in agreement with conductivity and freezing-point data. The alkalinity of colloid-free ultrafiltrates was a measure of the degree of hydrolysis of soap solutions. McBain and Lucas (189) filtered 0.6 N sodium palmitate through No. 400 cellophane at 90°C.; the filtrate was 0.24 N in sodium palmitate, which agreed with the concentration of simple crystalloidal soap at this temperature and concentration deduced from conductivity and dew-point lowering.

Zsigmondy and Carius (295), using Zsigmondy–Bachmann membranes, studied ultrafiltration of sols of mercury, arsenic trisulfide, antimony trisulfide, and ferric hydroxide; all but the last appeared to be polydisperse, as evidenced by sieving. The possibility of statistical sieving was not, however, eliminated, in contrast to Bechhold's experiment with colloidal silver.

Wintgen (286a) used collodion membranes to prepare colloid-free ultrafiltrates of stannic acid sols. The proportion of alkali bound to the colloid was found to decrease with increasing alkalinity of the sol.

Wintgen and Löwenthal (286c) Ultrafiltered colloidal chromium hydroxide through Zsigmondy–Bachmann ultrafeinfilters. The greater the

Cl:Cr ratio in the micelles, the more readily filterable were the latter. In a given sol, the colloidal Cl:Cr ratio remained the same even when the suspension medium was ultrafiltered off and the residue re-suspended. Manegold and Hofmann (205) investigated the sieving of two chromium hydroxide sols through ether–alcohol collodion membranes. In the first sol, 25 per cent of the particles passed through membranes of average pore diameter[6] 6.6 mμ; 5 per cent more through 7 mμ; and 13 per cent more through 28 mμ. In the second sol, 30 per cent of the particles passed through a "Kongodicht" Ultrafeinfilter (porosity less than 6 mμ). By comparison of the experimental results with the theoretical filtration curve for a system where the residue is made up at intervals with the pure solvent, it was concluded that the sieve constant was, for some of the suspended particles, neither 0 nor 1.

Bechhold and Szidon (37), using impregnated membranes, ultrafiltered colloidal zinc sulfide, cadmium sulfide, ferric hydroxide, and collargol (silver), dispersed in benzene, toluene, petroleum ether, or linseed oil. The membranes (graded roughly in terms of the concentration of collodion in the impregnating solution) were calibrated by washing out the coagulating liquid (toluene) with alcohol and water and then determining the end points in filtration of hydrosols. The end points of the organosols (org.) and the calibrating hydrosols (aq.) are arranged in descending order: zinc sulfide (org.), cadmium sulfide (org.), Prussian blue (aq.), ferric hydroxide (org.), collargol (aq.), hemoglobin (aq.), collargol (org.), ferric and copper oleates (org.).

Most dyes in organic solvents (37) diffused through the densest Bechhold–Szidon membranes, and were concluded to be molecularly disperse (crystalloidal). This evidence was not, however, conclusive, since colloidal aggregates in reversible equilibrium with crystalloidal components may traverse a membrane by disaggregation, diffusion, and reaggregation. Ultrafiltration, on the other hand, rapidly separates colloidal components from crystalloidal before readjustment of equilibrium can be established. Ultrafiltration of dyes in aqueous solution was studied by Zsigmondy (293), using Zsigmondy–Bachmann membranes. All the dyes filtered more readily than gold sols, but less readily than sucrose. The end points of several are arranged in descending order: Benzopurpurin 4B, Wool black 6B, Congo red, Benzopurpurin 10B. By use of suitable membranes, it was possible to separate one dye from another. The marked difference in filterability between the two Benzopurpurins, whose molecular structures differ only in that the two methyl groups of 4B are replaced by methoxyl groups in 10B, was attributed to the greater hydrophilic character of 10B. Morton (218) studied the filtration of aqueous solutions of dyes at 75°C. through cellulose membranes of average pore diameter 2.1

mμ. Normal filtration obtained. The order of increasing filterability was Chlorazol fast orange AGS, Benzofast blue 8GL, Sky blue FF, Chrysophenine G. In each case, the filterability in the presence of sodium chloride improved markedly with increasing salt concentration up to 0.1 per cent, and for higher concentrations of salt gradually fell off. It was concluded that the degree of dispersion was the least in the absence of salt. An alternative explanation (243a) is that the effect of salt on the dispersion of the dye is always to decrease it, as evidenced by the filterability at higher concentrations, the impaired filterability in the complete absence of salt being due to electrokinetic effects which can be suppressed by small quantities of electrolytes.

Bhatia, Ghosh, and Dhar (44) studied the ultrafiltration of colloidal molybdic acid through membranes of ether–alcohol collodion impregnated in filter paper. These membranes retained colloidal ferric hydroxide. About 50 per cent of the molybdic acid in a sol freshly prepared from equivalent amounts of ammonium molybdate and hydrochloric acid was filterable. The fraction filterable decreased with time; it was decreased by addition of acid, increased by addition of base, and increased by dilution. All these effects suggest a partially reversible aggregation, such as would be expected for a lyophobic colloid, but do not demonstrate it conclusively, since the effects of blocking are not evaluated.

Kronsbein (167, 168) studied the ultrafiltration of colloidal silicic acid. He employed Zsigmondy–Bachmann filters, standardizing them by filtration of gold sols (with gum arabic as a protective colloid) whose particle sizes were estimated by ultramicroscopic count. The 40′ Ultrafeinfilters retained completely a gold sol of particle size 4 mμ, and were considered to effect a separation of colloidal from crystalloidal silicic acid. Under conditions where blocking was suppressed as much as possible (by dilution and stirring), the fraction of silica ultrafilterable from a fresh, well-dialyzed sol was 2 to 3 per cent; this decreased with time. A seventeen-year-old sol was 0.5 per cent filterable. Dilution did not increase the fraction filterable. Absence of colloidal silicic acid in the ultrafiltrates and of crystalloidal in the ultimate residue was demonstrated by a colorimetric method. Concentration of an ultrafiltrate by evaporation failed to reaggregate the crystalloidal silicic acid.

Hein and Späte (149), using uncalibrated collodion membranes, found that penta-*p*-bromotriphenylene-chromobromide dissolved in ethylene dibromide was retained in ultrafiltration experiments, and concluded that the solution was colloidal.

McBain and Kistler (193), using membranes of cellophane, ultrafiltered aqueous and non-aqueous solutions of several colloidal electrolytes. The membranes (maximum pore size 4 mμ to 6 mμ as shown by the bubble test) could pass benzene solutions of naphthalene and anthracene in undi-

minished concentration, but were considered to retain any colloidal constituents of a filtering system. Silver bromate dissolved in diethylamine was completely filterable at a concentration of 0.13 N, but at increasingly higher concentrations an increasing fraction of the salt was retained. A trace of water in the solution diminished the fraction retained. Silver nitrate dissolved in piperidine was retained to an extent which increased with the concentration. Similar retention was noted for ammonium iodide in aniline, barium perchlorate and cadmium iodide in amyl alcohol, cadmium iodide in ethyl alcohol, potassium acetate and pyridine in acetic acid, sodium acetate in 50 per cent acetic acid, and cadmium iodide, potassium iodate, and sodium iodate in water. This evidence for existence of colloidal constituents in these solutions supports the indications of anomalies in electrical conductivity and osmotic behavior.

Berczeller (41), using Bechhold membranes, ultrafiltered supersaturated aqueous solutions of menthol, thymol, and naphthol. The concentrations of the filtrates (measured by surface tension lowering) corresponded to saturated solutions, while the residues remained supersaturated; the supersaturated solutions were concluded to be partly colloidal.

2. Separation of colloids from crystalloids

The earliest collodion membranes (254, 246, 244) were of low porosity, and appeared to separate colloids from crystalloids effectively. The fact that collodion membranes do not always perform this separation perfectly was noted as early as 1903 by Gorsline (139), who used membranes which permitted diffusion of peptone, albumose, starch, dextrin, albumin, and certain enzymes. Application of suitable membranes, however, preferably under conditions of normal filtration, will permit the desired fractionation.

Bechhold (16) employed acetic collodion membranes for a practically quantitative separation of gelatin from glycine by ultrafiltration. Boëseken and Meyer (49), not succeeding in preparing collodion membranes sufficiently tight to retain dextrin, used membranes of copper ferrocyanide impregnated in collodion; these permitted reducing sugars to dialyze, but almost completely retained dextrin of molecular weight 5500. McBain (188, 190) has advocated cellophane for separation of colloids from crystalloids, employing it for study of various colloidal systems (see above). For more rapid separation of crystalloidal electrolytes from colloidal sols than can be effected by dialysis, electrodialysis, or ultrafiltration, electro-ultrafiltration may be employed (16, 150).

3. Sieving of crystalloids

Collander (75) studied the impeded diffusion of many organic acids and other compounds through flat collodion membranes, prepared in three porosity grades by the method of Brown. The rate of diffusion was re-

lated antibatically to the molecular size as measured by the molecular refractivities. Exceptions were phenol and *m*-nitrophenol, which diffused abnormally rapidly and were thought to be soluble in the membranes. To each membrane grade corresponded a maximum size of diffusible molecules.

Manegold (201) studied the diffusion of urea, sucrose, and hydrochloric acid through collodion membranes. For membranes whose pores were large enough to permit free diffusion, the results provided information concerning the membrane structure (part II, C, 2); for membranes through which the diffusion was impeded, the order of diffusibility was urea > sucrose > hydrochloric acid, in contrast to the order of free diffusion, hydrochloric acid > urea > sucrose. In general, in impeded diffusion, electrolytes encounter much more retardation than non-electrolytes.

Michaelis (216a) described dry collodion membranes which were permeable (in diffusion) to urea but not to glucose. These were permeable to univalent cations, but not to polyvalent cations nor any anions. This property gives rise to the Michaelis diffusion potential (page 394). Beutner, Caplan, and Loehr (43) suggest that the latter potential is due to chemical reaction between the salt and the collodion; this, however, seems unlikely, in view of its characteristic dependence on membrane porosity (part II, C, 2) and the fact that differential permeability to ions is shown by membranes of copper ferrocyanide as well as of collodion (75). This question is further discussed by Wilbrandt (285a).

Ershler (115), using rather thick ether–alcohol collodion membranes, reported that, under the same conditions of ultrafiltration, crystalloidal electrolytes might be retained to a much greater extent than non-electrolytes. The relative concentration of filtrate from a solution of a non-electrolyte was practically independent of the absolute concentration of the latter; for electrolytes, the relative concentration of filtrate increased markedly with increasing absolute concentration of the original solution. The degree of retention of an electrolyte was the greater, the higher the valence of the ion charged like the membrane, and the lower the valence of the ion charged unlike the membrane. This supported the explanation that the greater retention of electrolytes was due to repulsion of similarly charged ions from the pore walls, resulting in a diminution of the effective pore diameter.

McBain and Kistler (191) were able to separate methyl alcohol from sucrose in aqueous solution by ultrafiltration, using membranes of cellophane impregnated with collodion. Elford and Ferry (103) obtained collodion membranes, of average pore diameter about 2 mμ, which retained 95 per cent of a 1 per cent solution of sucrose in ultrafiltration.

As concerns permeability to crystalloids, three types of membranes can

thus be distinguished, listing the order of decreasing porosity: (1) membranes of porosity greater than 4 to 5 mμ, such as the first cellophanes, through which all crystalloids filter in undiminished concentration; (2) membranes of the Ershler type or the McBain and Kistler impregnated type, which effect a certain sieving of non-electrolytes, and a much greater retention of electrolytes due to electrostatic repulsion of the anions of the latter; (3) membranes of the Michaelis type, which are impermeable to all but the smallest non-electrolyte molecules, and to all electrolytes by virtue of exclusion of their anions.

4. Determination of degree of hydration

The hydration of solute particles may be determined by ultrafiltration by using a reference substance which passes a filter retaining the solute in question. It must be assumed that the reference substance does not affect the solvation equilibrium and is itself not solved, and that the ultrafiltrate represents the interparticulate fluid far from the influence of solvation. Then the filtrate appears to have been concentrated in the reference substance because of retention of solvated solvent.

In this way, McBain and Jenkins (188), using potassium chloride as a reference substance, determined the hydration of potassium laurate to be approximately twelve water molecules per soap molecule. The extensive study of McBain, Kawakami, and Lucas (189) showed that this value was independent of the concentration of soap or salt at high ionic concentrations; internal Donnan effect was shown to be then suppressed. At low ionic concentrations the Donnan effect enters, and causes the apparent hydration to be two to three times as great.

McBain and Kistler (192), using methyl alcohol as the reference substance, obtained a figure for the hydration of sucrose of four molecules of water per molecule of sugar.

Greenberg and Greenberg (144) ultrafiltered solutions of gelatin, casein, starch, glycogen, and serum through uncalibrated sacs. Urea and glucose, as well as salts (in the isoelectric protein solutions), were used as reference substances for estimating the degree of hydration. The fraction of "bound" water was found in every case to be negligible within experimental error. It was concluded that the stability of these lyophilic colloids did not depend on a marked hydration, but more likely on molecular orientation on the particle surfaces.

B. ULTRAFILTRATION OF COLLOIDS OF INDUSTRIAL INTEREST

1. Petroleum

Bechhold and Szidon (37) ultrafiltered Trinidad asphalt in benzene solution through their impregnated membranes. Repeated washing with benzene effected passage of 32 per cent through a membrane which repre-

sented the end point for aqueous hemoglobin. The residue would not redissolve. The retention was shown to be caused by specific adsorption by the collodion. Attempts to ultrafilter petroleum were unsuccessful.

Zaharia and Lucatu (289) ultrafiltered petroleum through membranes of vulcanized rubber at a pressure of 150 atmospheres. All paraffin, and the resins separable by 70 per cent ethyl alcohol, passed the filter; hard and soft asphalts were retained. The asphalt residue redissolved readily in the ultrafiltrate, or in benzene or cyclohexane, but, when solutions in any of these solvents were refiltered, the asphalt was again retained. It was accordingly concluded to be present as a lyophilic colloid.

2. *Nitrocellulose and viscose*

Kumichel (173), using Cella filters and the apparatus of Brukner and Overbeck, studied the ultrafiltration of nitrocellulose. The water in the filter pores was first replaced by the nitrocellulose solvent,—ordinarily acetone. By using a filter of sufficiently low porosity, an ultrafiltrate of practically pure acetone was obtained. Fink, Stahn, and Matthes (122) ultrafiltered viscose through special membranes prepared from an alkylated cellulose thiourethan dissolved in a volatile solvent such as pyridine, with addition of small quantities of a non-volatile solvent such as glycerol chlorohydrin to adjust the porosity. Ultrafiltrates of viscose solutions through such membranes were practically cellulose-free. The degree of esterification of the cellulose xanthate, as determined by ultrafiltration, was in satisfactory agreement with that determined by chemical means. The ultrafiltration data also permitted calculation of the amount of alkali adsorbed by the viscose.

3. *Other systems*

Miscellaneous applications of ultrafiltration to industry include the filtration and concentration of latex by Bechhold–König filters (79); separation of tannins from non-tannins (271, 58); ultrafiltration of fats and oils to reduce the tendency for them to become rancid (287); purification of switch and transformer oils (268); purification of water, removing a large proportion of the ash and silica (155); purification of gelatin and glue (28); ultrafiltration of opium to yield a principle free from fats, resins, wax, and proteins (212); purification of the active principles of belladonna, henbane, and strophanthus (51a); estimation of dextrin in beer (258); analysis of the salt content of soils by removing the electrolytes by electro-ultrafiltration (274, 165); and recovery of cholesterol and higher fatty acids from wool wash-waters (2).

C. ULTRAFILTRATION OF PROTEINS

1. General properties of proteins

Proteins are of particular interest in ultrafiltration, because of the monodisperse character of their solutions and their physical and chemical resemblance to many biological materials of greater complexity. The results of ultrafiltration of proteins may be interpreted in the light of the following characteristic properties.

a. Molecular weights. Svedberg (265) has shown by ultracentrifugation that solutions of most proteins are remarkably monodisperse, and that in most cases the particles do not depart markedly from spherical form. Gelatin and casein are the most common exceptions in being polydisperse. The molecular weights are in many cases approximate integral multiples of 34,000. Certain respiratory proteins have huge molecular weights, that of hemocyanin from *Helix* being 5,000,000. The molecular weight is independent of the pH over a limited range (the Svedberg stability range), usually from pH 4 to 9, except for a slight tendency to reversible aggregation at the isoelectric point. Outside of the stability range, breakdown of the molecules occurs, to varying degrees.

b. Amphoteric properties. A protein particle is usually considered as zwitterionic at the isoelectric point. On the acid or basic side, the protein acquires a net positive or negative charge respectively, due to collection of of hydrogen or hydroxyl ions respectively,—a process which may be considered equally well as chemical combination or Langmuir adsorption (154a).

c. Denaturation. Treatment of proteins by heat, strong acids and alkalis, alcohol, urea, and other reagents causes a reduction of the solubility in water, termed denaturation. Heat denaturation does not affect the molecular weight or cataphoretic mobility, but (in the case of egg albumin) alters the optical activity, refractivity, viscosity, absorption spectrum, and immunological properties of solutions (9). Complete denaturation is characterized by total insolubility at the isoelectric point. Egg albumin—but not serum proteins—is denatured when adsorbed in a surface film (210).

d. Adsorption of proteins. Most of the data on adsorption of proteins refer to saturation values, corresponding to the flat portion of the Langmuir adsorption isotherm. Hitchcock (154), studying the adsorption of gelatin on collodion membranes as a function of pH, found a marked maximum in adsorption at the isoelectric point; on the alkaline side, it fell off; on the acid side, it fell off sharply to a minimum and then increased with increasing acidity. A similar peak at the isoelectric point was found by Palmer (231), who showed further that addition of sodium chloride to

non-isoelectric solutions increased the adsorption to the high isoelectric value. Maxima in adsorption by collodion were found by Ettisch et al. (116) for serum albumin at pH 4.7, serum globulin at 5.3, egg albumin at 4.7, and hemoglobin at 6.7.

Elford (94, 96) studied the adsorption of egg albumin, serum albumin, and serum pseudo-globulin on collodion, quartz, kieselguhr, and kaolin; each protein showed a sharp peak at the isoelectric point (figure 17), the shape of the curve being independent of the nature of the underlying surface. This independence of the underlying surface is also revealed by the cataphoresis experiments of Dummett and Bowden (85) for gelatin adsorbed on particles of quartz, carbon, and copper. This suggests that the peak adsorption at the isoelectric point involves adsorption of protein on protein, i.e., formation of multiple adsorbed layers. In contrast to the

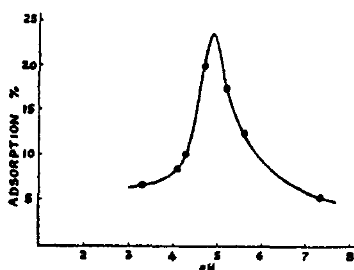

FIG. 17. Adsorption of horse serum albumin by collodion particles, plotted against the pH (94)

egg albumin and the serum proteins, hemoglobin does not have a great tendency to form multiple adsorbed layers, as shown by the specific effect of the underlying surface on cataphoretic mobility of hemoglobin-coated particles (85) and the smaller absolute amount of adsorption (saturated) at the isoelectric point (96). Of the serum proteins, the peak for globulin is much less sharp than for albumin, suggesting a tendency for multiple layer adsorption which extends over a broader pH range. The tendency for excessive adsorption may be identified with colloidal instability, tendency to aggregation, and perhaps with denaturation; and clearly with blocking and abnormal filtration (see below).

2. Filtration of single proteins

Risse (242) showed the effect of pH on the filterability of serum albumin and globulin and hemoglobin, each protein being least filterable at approximately its isoelectric point. Elford and Ferry studied in detail the ultrafiltration of 0.5 per cent solutions of horse serum albumin and pseudo-

globulin (100) and egg albumin, edestin, and hemocyanin (101) through graded collodion membranes, determining the influence of various physical factors, as described below.

a. Influence of solvent medium. In comparative experiments in different solvents at comparable pH values (about 7.2 in each case), filtering through membranes of equal porosity (the value depending on the nature of the protein), the effect of each solvent medium is shown by the form of the filtration curve and the maximum relative concentration of filtrate attained. For egg albumin, serum albumin, serum pseudo-globulin, and hemocyanin, media arranged in the order of increasing favor to filtration are: water (0.05 per cent sodium chloride for globulin); 1.0 per cent sodium chloride, extra-digested Hartley's broth; standard Hartley's broth; Hartley's ox-heart broth. For the albumins, at pH about 7.2, all the solvents permit "normal" filtration; for the pseudo-globulin, normal filtration occurs only with standard or ox-heart broth; and, for the hemocyanin, only broth permits a filtration curve approaching the normal form. In the case of egg albumin and hemocyanin, $M/15$ phosphate buffer favors filtration slightly more than does 1.0 per cent sodium chloride. For edestin, Hartley's broth is much more favorable than 1.5 M sodium chloride.

b. Influence of pH. For egg albumin, serum albumin, and serum pseudo-globulin, the filterabilities have been studied over wide ranges of pH. The variation of the end-point porosities of the three proteins with pH is shown in figure 18. The solvent is water for the albumins, and 1.0 per cent saline for the globulin. For the globulin, the corresponding filtration curves are all abnormal. For the albumins, they are abnormal from pH 4.2 to 7, and normal on the acid or alkaline side of this zone (the boundaries not being sharply defined). The pH zone of abnormal filtration—i.e., blocking—corresponds exactly to the zones in which the end points are excessively high (figure 18), and also to the zone in which excessive adsorption of protein on collodion occurs (figure 17). This suggests that, for proteins, at least, blocking is due to multiple layer adsorption or colloidal instability (100). The dissymmetry in the end-point peaks may be attributed to the charge on the collodion, which is negative throughout, so that on the alkaline side there is a certain repulsion between membrane and protein.

In Hartley's broth as a solvent, the pH zone of abnormal filtration for the albumins is diminished to the narrow range of pH 4.3 to 5.5. For serum pseudo-globulin, it is diminished from extension beyond Svedberg's stability range (outside which the protein is no longer monodisperse) to a limit of about pH 7 on the alkaline side.

It is to be noted that the influence of pH on filterability of proteins does not necessarily imply formation of secondary aggregates in the neighborhood of the isoelectric point. Such aggregates are, in fact, not revealed

in the work of Svedberg; although the latter relates to solutions of lower concentration. That there might be structural aggregates of a type which would not be revealed in ultracentrifugation, however, is suggested by certain properties of jellies (187).

The influence of pH on the filterability of proteins is of importance in the filtration of enzymes, toxins, and viruses, which behave in many ways like proteins, and filter generally more readily in alkaline than in acid solution (243, 5, 219, 228, 229, 97). The acidification of such systems is not carried very far (owing to the destruction, in most cases, of biological activity in acid solution), probably not beyond the isoelectric zone (pH 4.5 to 6); this

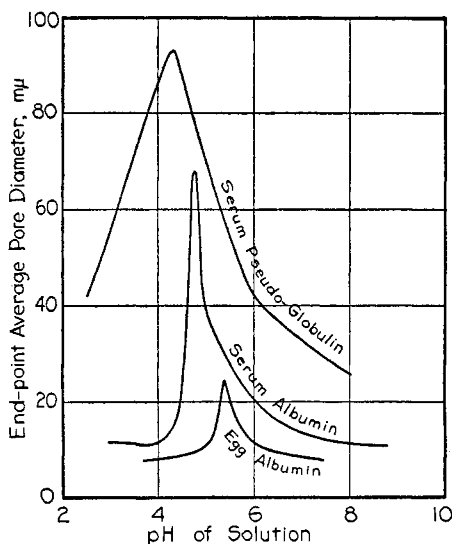

Fig. 18. Dependence of filtration end-point porosity on pH, for proteins (100, 101)

accounts for the lack of observations of ready filterability on the extreme acid side of the pH range, such as noted for proteins.

c. Influence of temperature. Duclaux and Hirata (84) found that the filterability of gelatin increased with temperature from 25° to 70°C.

d. End points. The end-point curves (figure 7) for filtration of proteins are steep, their form being quite similar to that predicted for sieving of a monodisperse system (figure 16), in agreement with the monodisperse character of proteins shown by Svedberg. The end-point porosities for different proteins under conditions where normal filtration obtains, but within Svedberg's pH-stability zone, are listed in table 2. The values are taken from Elford and Ferry (100, 101); in the case of the serum proteins, they are confirmed by Grabar (141)).

3. Filtration of artificial mixtures of the serum proteins

Elford and Ferry (100) noted that, at pH 3.8, membranes of average pore diameter between 40 mμ and 80 mμ completely retain serum pseudo-globulin (in 1 per cent sodium chloride) but pass albumin (in water) in undiminished concentration, when these proteins are filtered singly. In an artificial mixture of the two proteins in 1 per cent sodium chloride at this pH, the filterability of the globulin was increased and that of the albumin greatly decreased, both proteins partially passing a 70 mμ membrane and being retained by one of 40 mμ. This is probably not to be interpreted as due to an albumin-globulin complex (since some fractional sieving of the two proteins can actually be achieved in diluted serum, where the filtration is normal), but rather to the abnormal nature of the filtration of the globulin, which blocks up the pores to itself and to the albumin alike.

TABLE 2

Filtration end points of proteins

PROTEIN	SOLVENT MEDIUM	pH	END-POINT POROSITY
Egg albumin	Water	7.5	7–8 mμ
Egg albumin	Hartley's broth	7.6	6 mμ
Serum albumin	Water	8.0	11 mμ
Serum albumin	Hartley's broth	7.8	9–10 mμ
Serum pseudo-globulin	Hartley's broth	7.8	11–12 mμ
Edestin	Hartley's broth	7.6	18 mμ
Hemocyanin (*Helix*)	Hartley's broth	7.3	55 mμ

4. Composition of protein-free ultrafiltrates

Greenberg and Greenberg (143) prepared protein-free ultrafiltrates of alkaline solutions of casein by filtration through uncalibrated collodion sacs. The concentrations of added salts (sodium chloride, potassium chloride, sodium sulfate, potassium acetate) were always greater in the ultrafiltrate than in the original solution. The relative excess of salt in the filtrate (10 per cent to 80 per cent) was independent of the casein concentration, but decreased with increase in the absolute concentration of the salt. The concentration of urea in an ultrafiltrate was the same as in the corresponding original solution. These results indicate the influence of a Donnan effect.

Polanyi (235a) pointed out that the concentration of salt in the protein-free diffusate or ultrafiltrate of a protein solution does not exactly represent the concentration of free or unbound salt in the original solution, owing to retention of water of hydration by the protein, causing the proportion of

unbound or diffusible crystalloid to appear too large. Augsberger (6) applied this principle to the recalculation of data on ultrafiltrates of serum (table 3). Eversole, Ford, and Thomas (117) compared the proportion of "bound" calcium in mixtures of gelatin and calcium acetate as determined by dialysis and by calcium electrodes. The former method, taking into account the Donnan distribution, gave considerably smaller figures for the bound calcium in the protein solution, as predicted on the basis of retention of water of hydration. The magnitude of hydration required, however, was far greater than that indicated by ultrafiltration (page 423).

D. ULTRAFILTRATION OF SERA

1. *Filtrations in which all protein is completely retained*

Ultrafiltration has been applied by many workers to the study of the composition of protein-free ultrafiltrates of body fluids, particularly blood serum. The work here quoted refers to mammalian sera, chiefly of the horse, ox, and man. The degree to which the various electrolytes present in serum and plasma are retained with the proteins has received the most attention. Results are usually expressed in terms of the percentage filterable, i.e., the relative concentration of the particular constituent in the filtrate as referred to the original serum.

a. Normal blood serum. The data of eleven papers on ultrafiltrates of normal blood serum and plasma are summarized in table 3.

With few exceptions, the results are in good agreement, considering the wide variations in techniques and procedures. The data for the difference in the filterabilities of calcium in serum and in plasma are, however, contradictory. In two cases, evidence was cited that the non-filterable portions of different constituents were actually bound to the protein and not retained in any other way. Thus, the nitrogen content of serum ultrafiltrates (286, 108) checked with the non-protein nitrogen of the original serum, i.e., the nitrogen content after the proteins were precipitated by trichloroacetic acid or the method of Folin and Wu. Also, the non-filterable fractions of sodium and potassium are equal to the fractions carried down with the proteins when the latter are precipitated by alcohol (178). However, the apparent binding of calcium to the serum proteins is about twice as great when they are precipitated by alcohol as when they are ultrafiltered off.

von Deseö and Lamoth (78) and Eissner (88) studied the variation in serum ultrafiltrate concentration with the volume filtered. In the former investigation, Zsigmondy–Bachmann Ultrafeinfilters were employed, and physiological saline, followed by Ringer's solution, was first passed through these to saturate them with the salts present in serum. Both reports showed a slow increase in the total concentration of solutes in the ultra-

filtrate as the filtration proceeded. The ratio of sodium to chloride remained unaltered (88). The effect was attributed to progressive increase in the retention of water of hydration in the residue, as the concentration of protein increased there. In view of the fact that the rate of filtration decreased with time (78), an alternative explanation may be an inner Donnan effect, which corresponds to the suggestion of Ambard and Deviller

TABLE 3

Composition of protein-free ultrafiltrates of serum and plasma (expressed in relative concentrations referred to the filtering solution)

AUTHOR	TYPE OF MEMBRANES	PERCENTAGES FILTERABLE								
		Cl	Na	K	Ca	Mg	P	N	Urea	Sugar
Serum										
Rona and Takahashi* (245a)					63–75					
Cushny (77)	Walpole sacs	100	100	100	62–70				100	
Tschimber and Tschimber (275)	Giemsa sacs		100		50–60	70	30			
Richter-Quittner (241)	Giemsa sacs			0						100
Blum and Delaville (48)			100	100	Part	Part	Part			
Augsberger (6)†			90		57–69	70–80				<100
Wilenskii (286)‡	Special sacs							3		
Lévy and Pacu (179)		100	90	95	64	60–70				
Schmidt-Hebbel (249)					70–73					
Bendien and Snapper (40)	Special sacs				50					
Watchorn and McCance (283)					57	75				
Plasma										
Wilenskii (286)‡	Special sacs							3		
Lévy and Pacu (179)		100	90	95	72	60–70				
Schmidt-Hebbel (249)					50–55					

* Diffusion, not ultrafiltration.
† Recalculations of various data.
‡ Serum or plasma diluted 1:10.

(1a). Wilenskii (286) found that the nitrogen content of the ultrafiltrate was independent of the volume filtered, giving a flat filtration curve.

Eissner (88) reported that when the pH of serum (normally about 8.0) is altered to 5.8, the calcium becomes almost completely filterable. The influence of added salts on the filterability of calcium at constant pH was studied with acetic collodion membranes by Brull et al. (64), who found

that different ions increased the filterability in the order of a Hofmeister series: citrate > tartrate > chloride > iodide > thiocyanate. The calcium became completely filterable at a salt concentration of about 0.05 *N*, except in the case of citrate, where 0.02 *N* was sufficient. The citrate ion was supposed to act directly on the calcium, rather than on the proteins.

The effect of pH and neutral salts on the velocity of ultrafiltration of diluted serum was studied by Ellinger and Neuschloss (112). Progressive increase in pH from 7.7 to 8.9 caused first an increase, then a decrease in the velocity. Salts decreased the velocity in the order of a Hofmeister series: citrate > sulfate > acetate > chloride > bromide > iodide > thiocyanate. Caffein in small amounts decreased the velocity at pH 8.4, but increased it at pH 7.1.

Brühl (59) determined organic acids in ultrafiltrates of normal and pathological sera. The normal concentration lay between 7.7 and 10.2 millimolar; in uremia, after tetanic cramps, and under a ketogenic diet, the concentration of acid was increased to a varying extent up to 29.5 millimolar.

b. Pathological blood sera. Blum and Delaville (48) noted that for certain pathological sera the relative concentration of sodium in ultrafiltrates is less, and that of calcium more, than for normal serum. Scholtz (251) stated that, in cases of shrunken kidney, the filterability of calcium was decreased. Watchorn and McCance (283) studied the filterabilities of calcium and magnesium in various pathological cases, and found, in general, little correlation of the results with diagnosis. In pregnancy, however, the non-filterable fractions were decreased.

c. Milk. Rona and Michaelis (245) found 40 to 50 per cent of the calcium in milk to be diffusible. Wha (285) studied both dialysis and ultrafiltration of milk. Most of the calcium was able to pass through collodion membranes in dialysis. In ultrafiltration, the relative concentration of calcium in ultrafiltrates of fresh milk was 50 per cent; of sour milk, 100 per cent. Potassium and chlorides passed the filter in undiminished concentration for both fresh and sour milk; phosphorus was 40 per cent filterable.

2. Filtrations in which sieving of proteins occurs

a. Filtration end points of proteins. Bendien and Snapper (40) ultrafiltered normal blood serum through collodion sacs of graded porosity. The albumin–globulin ratio was always higher in the ultrafiltrate than in the original serum, indicating that the albumin particles are smaller than the globulin and not bound to the latter in any complex. These observations are confirmed by the quantitative work of Elford, Grabar, and

Fischer (108). The end-point porosities of Elford membranes for the proteins in native serum are given in table 4 (100). These values are in agreement with the end points of the purified proteins dissolved in corresponding media (table 2).

The fact that the end point of albumin is lower than that of globulin, and that membranes of porosity somewhat greater than the end points of both proteins are more permeable to the albumin, accounts for the increased albumin-globulin ratio in the transudate of edema and in the urine of albuminuria.

b. Association of other constituents with protein fractions. Bendien and Snapper (40), by comparing the relative concentration of a non-protein constituent in a serum filtrate with the relative concentrations of albumin, of globulin, and of total protein in the same filtrate, were able to conclude to which protein fraction that constituent was bound. In this way, they found that the non-ultrafilterable or protein-bound calcium is probably

TABLE 4

End points of proteins in native serum

SERUM	pH	END POINT OF ALBUMIN	END POINT OF GLOBULIN
Undiluted serum	8.2	9–10 mμ	11 mμ
Serum:broth, 1:9	7.6-7.8	9–10 mμ	11 mμ
Serum:saline, 1:9	7.8-8.0	11 mμ	13 mμ

bound by albumin alone. Bilirubin is also bound by albumin; lipochrome, not by the albumin nor by all the globulin; cholesterin and lecithin, not by the albumin nor by all the globulin, but perhaps partly bound by the euglobulin.

In a similar way, deKruif and Eggerth (172) used ultrafiltration through uncalibrated sacs to show that anaphylatoxins were associated with the globulin fraction.

3. Ultrafiltration in vivo

It is possible to lead the blood stream of an animal through an ultrafilter, after injection of heparin to prevent clotting, and thus to obtain an ultrafiltrate of the blood *in vivo*. A convenient apparatus is described by Geiger (132). Brull (63) performed this experiment, finding that chlorides, dextrose, and non-protein nitrogen passed the filter in undiminished concentration, while phosphorus and calcium were partially retained, i.e., the results were similar to those of ultrafiltration *in vitro* (table 3).

E. ULTRAFILTRATION OF CHEMICAL SUBSTANCES OF BIOLOGICAL ACTIVITY

1. Enzymes

Strada (264) filtered various enzymes (cleared by preliminary passage through filter candles) through uncalibrated collodion sacs. The pepsin of gastric juice was retained, but the hydrochloric acid was passed in undiminished concentration, showing that it was not associated with the enzyme. The trypsinogen of pancreatic juice and kinase passed the filter separately, but, when mixed, their proteolytic activity was retained. Bechhold and Keiner (26) found that enterokinase was retained by a 4 per cent Bechhold membrane, while trypsin passed a 10 per cent membrane. In weak alkaline solution, however, the kinase passed membranes which retained all protein. Lagrange and Suarez (175) filtered several enzymes through Bechhold–König filters; the order of increasing filterability was trypsin, lipase, vitellase, amylase. Jacoby (159) filtered urease through Zsigmondy–Bachmann membranes; the wide range of porosities over which apparent sieving occurred seemed to indicate that the enzyme was polydisperse. Grabar and Riegert (142a) employed Elford membranes in the ultrafiltration of urease; the activity was found to be associated with polydisperse particles of a protein nature. The end point of the most nearly homogeneous preparation was similar to that of serum globulin. In ultrafiltration of urease partially digested by tryptase, it was shown (142b) that the filterable breakdown products possessed none of the activity of the urease. Grabar (141a) filtered invertase through Elford membranes; the enzyme passed a membrane of porosity 13 mμ with little loss in concentration, while the end point was placed at 10 mμ. The particles were concluded to be similar in size to those of serum albumin (end point 9 to 10 mμ). Snell (260) employed membranes impermeable to amylase but permeable to more highly disperse substances for concentration and purification of malt extract.

2. Toxins and antitoxins

Bechhold–König filters have been used by Sierakowski and collaborators (180, 237) to concentrate and purify scarlatinous and diphtheria toxins. The latter has been purified by Wadsworth and Quigley (278) by concentration over impregnated ether–alcohol collodion membranes which retain all protein but pass 94 per cent of the nitrogen content of the unfiltered system. The residue, when diluted to the original volume, has undiminished activity, showing negligible adsorption. Zajdel (290) found that a 4 per cent Bechhold–König filter retained proteins, but passed diphtheria antitoxin; when the active, protein-free ultrafiltrate was filtered through a 10 per cent filter, the antitoxin was retained and concentrated, while salts

and amino acids passed. Le Guyon (147) found that diphtheria toxin and antitoxin filtered about equally well, both passing at high concentrations collodion sacs which were impermeable to hemoglobin.

Bechhold (16) found it impossible to obtain ultrafiltration data for arachnolysin (poison from *Epeira diadema* spider) or stapholysin, due to strong adsorption by the acetic collodion membrane.

3. Other substances

Le Guyon (147), using collodion sacs which passed Congo red in undiminished concentration, found that pyocyanin (extracted from *B. Pyocyaneus* with chloroform) was completely ultrafilterable; so was tuberculin. Gough (140), in an extensive study of tuberculin, isolated two fractions, consisting of proteins and proteose, respectively. In filtration through Elford membranes, the protein fraction was retained at a porosity of 9 mμ; the proteose passed a membrane of porosity 4 mμ. W. Smith (259a), in a study of the "precipitating substance" from vaccinia virus, reported the end point of the former, determined by Elford, as 6 mμ; the particles of the precipitating substance were concluded to be similar in size to those of egg albumin. Krueger (169) prepared bacterial antigens by grinding bacteria and filtering through an acetic collodion membrane to remove debris. Burnet (66) separated a specific soluble substance from bacteriophage-lysed cultures of bacteria by ultrafiltering off the phage. Taylor, Braun, and Scott (267) found that insulin passed collodion membranes of various grades in undiminished concentration, once the primary adsorption was satisfied. Spain and Newell (261) ultrafiltered ragweed pollen extracts through cellophane. The hay fever allergen was present in the filtrate from No. 300 cellophane, but was retained by No. 1200. Grabar and Koutseff (142), in a study of ricin extracts, isolated two fractions, one toxic and the other allergic. In filtration through Elford membranes, the toxin was retained at a porosity of 7 mμ; the allergen passed membranes of porosity 4 mμ.

F. ULTRAFILTRATION OF BACTERIOLOGICAL SYSTEMS

Interpretation of ultrafiltration experiments with bacteriological systems, such as bacteriophages and viruses, on the basis of comparisons with chemical systems, such as colloids and crystalloids, frequently leads to erroneous conclusions. The marked differences between these two types of dispersions which must be taken into account in comparative studies are summarized in table 5.

The high dilution of bacteriological suspensions makes the effect of primary adsorption particularly marked; blocking probably enters only in the presence of gross foreign particles, which however are often present (tissue debris, etc.). The effect of heteroporosity is illustrated by com-

parison of a chemical and a bacteriological disperse system in filtration through a membrane whose *average* pore diameter is below the end-point porosity. The distribution of pore sizes may be spread to include a fair proportion of pores larger than the end point, and yet these large pores may not let through enough of the chemical system to be identified in the filtrate (i.e., 10^{13} particles per cubic centimeter). On the other hand, even a few large pores may let through enough bacteriological infective units for identification, since these can be detected in so much smaller amounts (24). For an ideally isoporous filter, however, on the basis of statistical sieving, the apparent end points of chemical and bacteriological

TABLE 5

Contrasts between chemical and bacteriological systems

	CHEMICAL SYSTEMS	BACTERIOLOGICAL SYSTEMS*
Concentration of filtering solution employed	10^{16} to 10^{19} particles per cc.	10^{3} to 10^{8} particles or infective units per cc.
Minimum concentration detectable	10^{13} to 10^{14} particles per cc.	1 to 10 particles or infective units per cc.
Minimum relative concentration detectable	$1/10^{3}$ to $1/10^{5}$	$1/10^{3}$ to $1/10^{8}$
Accuracy in determination of relative concentration	Two significant figures	Virus: a power of ten bacteria or phage: 1 significant figure
Effect of heteroporosity in filter	Spreading distribution of pore sizes may have little effect	Spreading distribution of pore sizes makes end point appear too small

* In the case of bacteria and bacteriophage, the infective unit is directly identifiable with the particle. For viruses, where analysis is possible only by animal inoculation, the infective unit is the minimal infective dose for the animal concerned.

systems are close together (within about 2 per cent); so, for comparing results of filtrations of the two types of systems, the filters employed should be as nearly isoporous as possible.

Early work on the ultrafiltration of bacteriological systems is not reviewed completely here, attention being devoted principally to investigations in which the considerations of the preceding paragraph have been taken into account; and, in particular, the work of Elford, since it represents a series of studies carried out under uniform experimental conditions with a view to estimation of particle sizes. Estimations of the actual sizes of infective particles are discussed in part V.

1. Bacteriophages

A bacteriophage is a principle which, introduced into a culture of certain bacteria, multiplies within the cells, eventually killing and disintegrating or lysing them. d'Herelle, who discovered the phenomenon, showed that bacteriophage passed bacteria-tight filter candles, and that the concentration of a suspension of it could be estimated by plating successive dilutions on agar plates sown with the susceptible bacteria, the clear, sterile plaques appearing after incubation being ascribed to individual phage particles (cf. reference 24).

Early reports on ultrafiltration of bacteriophages represent an example of the untrustworthy conclusions arising from failure to consider the significance of table 5. Thus, it was deduced that the particles of phage were smaller than various proteins, or than strychnine nitrate molecules (cf. reference 98). This would have placed the particle diameter at less than 6 mμ. The more reliable work of Bechhold, Leitner, and Ornstein (27), taking into consideration the maximal pore sizes of the filters employed, placed the size of the smallest pores through which their phage could pass as about 40 mμ, while application of correction factors estimated the particle size as 55 mμ (24). The extensive investigation of Elford and Andrewes (98), employing highly isoporous membranes, showed that different strains of bacteriophage had different sizes, but all considerably larger than protein molecules—the end points ranging from 25 mμ to 110 mμ.

The end-point curve of Elford and Andrewes for each different phage strain was very steep, the first falling off in filtrate concentration being detected at a porosity about twice the end point. This resembles the result predicted for sieving of an ideally monodisperse system (part III, F), and indicates the high uniformity of particle size in each phage strain. The contrary statement, that bacteriophage is polydisperse, was made by Wollman and Suarez (288), reporting that Bechhold membranes of different porosities let through different concentrations of phage, but the range of porosities over which this sieving occurred was not great, and the effect may have been a simple statistical sieving. The experiments of Bronfenbrenner and Hetler (55), indicating that bacteriophage is not autonomous, but adsorbed on inactive particles, were not confirmed by Elford and Andrewes.

The filtration end point of a given phase was found to be independent of the organism on which it was cultivated. Different phages were adsorbed to about the same extent by collodion particles, showing that the differences in end points were not due to adsorption phenomena. Two phages of different end points could be separated by fractional ultrafiltration. By repeated washing of a phage suspension over a membrane which retained

it, keeping up the volume of residue by addition of broth, it was possible to free the suspension from bacterial protein, with very little loss in the concentration of phage. Filtration of such a purified phage, when suspended in broth, gave the same results as that of the unwashed preparation, but in saline the purified phage filtered less readily, and the filtration curves were of the abnormal form, suggesting that purification had deprived the phage of a stabilizing agent. The actual end points of the different bacteriophage strains, following the serological classification of Burnet (65), are listed in table 9.

2. Viruses

The infective agents known as viruses have been commonly distinguished from bacteria by (1) their ability to pass bacteria-retaining filters, (2) their invisibility in the microscope, and (3) their failure to multiply in the absence of living cells (68). The early definition of these agents in terms of their ready filterability (as exemplified by the term "filterable virus"), dating from the first reports on tobacco mosaic in 1896 and foot and mouth disease in 1898, soon led to ultrafiltration experiments.

Suspensions of virus are difficult to obtain free from foreign matter which hinders filtration. In the case of plant-attacking viruses, the presence of sticky material from the plant tissues, which readily blocks ultrafilters, has long delayed the application of filtration methods. Viruses which attack animals are occasionally obtainable suspended in high concentration in body fluids, as foot and mouth disease in vesicular lymph from guinea pigs, and fowl plague in chicken serum. More frequently, however, the virus is found concentrated in some organ of the animal attacked, and often present in intracellular inclusions. The virus must be freed by grinding the tissues and breaking up the cells by autolysis and plasmoptysis. The resulting tissue debris and fatty materials must be removed before the suspension can be used for ultrafiltration experiments, on account of the blocking effects which arise from the presence of foreign particles. Centrifuging, filtration through coarse sand filters, and successive filtrations through collodion membranes of selected porosities well above the end point may serve to remove particles larger than those of the virus, leaving the latter in bacteria-free suspension, together with proteins and salts (10). Even the protein may be sometimes removed by washing the suspension over a membrane which retains the virus. Purification by adsorption and elution has been tried with varied success. Any drastic treatment is apt to inactivate the virus. The elimination of blocking effects by progressive purification of virus probably accounts for observations that the more highly a virus is purified, the smaller its particles seem to become. Too complete purification, however, may conceivably impair

the filterability of a virus by depriving it of the capillary-active substances which aid filtration. It may also diminish the viability of the virus. Alteration of the pH, in particular, markedly affects the viability of most viruses, which rapidly become inactivated on the acid side of neutrality.

a. Foot and mouth disease. A very complete study of the ultrafiltration of foot and mouth disease was made by Galloway and Elford (129), using Elford membranes. The availability of small experimental animals of uniform susceptibility permitted a greater variety of experiments and more accurate analysis of filtrates than in most cases. There was no blocking, the filtration curves being normal in form. For filtrations through membranes of progressively lower porosity, the first marked diminution in filtrate concentration occurred at a porosity of 60 mμ, while the end point was 25 mμ. A mixture of the virus and coli bacteriophage (end point 65 mμ) could be quantitatively separated by a 65 mμ filter, while a 25 mμ filter quantitatively resolved a mixture of the virus and hemoglobin (end point 10 mμ). Variation of the pH between 6.4 and 8.7 for suspension in broth or phosphate saline was without effect on the filterability, in contrast to the marked superiority of alkaline reactions found by Busch (70) for the much more highly adsorbing Bechhold–König ultrafilters. The effect of medium, concentration, and membrane thickness on primary adsorption has been previously mentioned (part III).

b. Poliomyelitis. The particle size of the virus of poliomyelitis was estimated by Krueger and Schultz (171) from filtration through acetic collodion membranes as less than 300 mμ, and by Clifton, Schultz, and Gebhardt (73), who applied the same technique to a purified preparation, as less than 50 mμ. Elford, Galloway, and Perdrau (106) found the end-point porosity for filtration through graded collodion membranes to be 25 mμ, taking into account the low concentrations of the suspensions obtainable. Theiler and Bauer (269), using the technique of Elford, found an end point of 35 mμ; the filtration was, however, abnormal, with marked blocking, and the concentration of the virus suspension used was lower than that of Elford, Galloway, and Perdrau, so that a higher figure would be expected.

c. Louping ill. The virus of the louping ill of sheep was filtered by Elford and Galloway (105) through graded collodion membranes, the end-point porosity being determined as 40 mμ. It was possible to separate partially this virus from bacteriophage C36 (end point 60 mμ) and from bacteriophage S13 (end point 25 mμ) by suitable membranes.

d. St. Louis encephalitis. Bauer, Fite, and Webster (14a) ultrafiltered the virus of St. Louis epidemic encephalitis through Elford membranes, and found the end-point porosity to be below 66 mμ. Elford and Perdrau (109), using the same technique, placed the end point at a porosity of 60 mμ, in good agreement.

e. Yellow fever. Findlay and Broom (121) filtered yellow fever virus through Elford membranes, and found the end-point porosity to be 54 mμ. It was possible to concentrate the virus a thousandfold over membranes of porosity 50 mμ. Bauer and Hughes (15), employing the same technique, placed the end point at a porosity of 55 mμ, in excellent agreement.

f. Rift Valley fever. Broom and Findlay (56) applied the Elford technique to the ultrafiltration of Rift Valley fever virus, and placed the end point at a porosity of 70 mμ.

g. Fowl plague. Bechhold (22), using acetic collodion membranes, estimated the sizes of the largest pores which retained fowl plague virus to be 1.4 to 1.9 μ, and, by applying correction factors, judged the particle size to be 200 mμ. Elford and Todd (111), using Elford membranes, placed the end point at an average pore diameter of 125 mμ. When a mixture of the virus and Staph. K bacteriophage (end point 110 mμ) was filtered through a 125 mμ membrane, the virus was retained, and a trace of phage passed into the filtrate.

h. Vesicular stomatitis. Galloway and Elford (129a) filtered the virus of vesicular stomatitis, and placed its end point at a porosity of 0.13 μ, thus differentiating it markedly from foot and mouth disease virus. This result was confirmed by Bauer and Cox (14).

i. Borna Disease. Elford and Galloway (104) determined the end-point porosity in filtration of Borna Disease virus to be 0.175 μ. The end-point curve was not so steep as for most viruses, indicating possibly greater departure from uniformity in the particle sizes. The virus could be concentrated fivefold by filtering off the suspension medium through a 0.15 μ membrane. The virus could be easily separated from that of louping ill (end point 40 mμ) in a mixture of the two.

j. Newcastle Disease. Burnet and Ferry (69) ultrafiltered the virus of Newcastle Disease through Elford membranes, and placed the end point at a porosity of 0.16 μ.

k. Herpes. Zinsser and Tang (292) reported that herpes virus passed ultrafilters which retained colloidal arsenic trisulfide, but was retained by others which passed casein and fowl plague virus. Bedson (39a) filtered herpes through ether–alcohol and acetic collodion membranes, and found the end point to be sufficiently higher than those of the serum proteins to permit separation from the latter. That the end point of herpes is higher than that of fowl plague was confirmed by Elford, Perdrau, and Smith (110), who found the end point of the former to be 0.20 μ by the Elford technique.

l. Infectious ectromelia. Barnard and Elford (10) filtered the virus of infectious ectromelia through Elford membranes and placed the end point at 0.20 μ. It was possible to purify and concentrate the virus over a membrane of average pore diameter 0.11 μ.

m. Canary virus. Burnet (67) reported the end-point porosity of canary virus, as determined by Burnet and Elford, at 0.25 μ.

n. Vaccinia virus. Bechhold and Schlesinger (30) filtered vaccinia virus through Zsigmondy–Bachmann filters and deduced a particle size of 0.2 μ. Elford and Andrewes (97) studied the ultrafiltration through graded collodion membranes. The end point was placed at a porosity of 0.25 μ.

o. Rous sarcoma. Ultrafiltration of Rous sarcoma virus, and tumor extracts generally, is particularly difficult because of the presence of sticky foreign material which is very effective in blocking filters. Zinsser and Tang (292) found its behavior similar to that of herpes (see above). Mendelsohn, Clifton, and Lewis (213) quoted the end point for Bechhold membranes as 110 mμ. Elford and Andrewes (99) were able to rid the virus suspensions of unwanted constituents by washing above a membrane which retained the virus, and by filtration of such purified material placed the end point for Elford membranes at 0.15 μ to 0.20 μ.

3. Spirochetes

Hindle and Elford (152) found that, when suspensions of spirochetes were filtered at 37°C., the organisms passed filters of such low porosity as to assure that they were penetrating the pores "end-on," their length being at least 50 times their thickness. It was considered that coils and bends were perhaps straightened out while the spirochetes were traversing the pores. The filtrates were of low concentration, as would be expected from statistical considerations, since an organism must be suitably aligned with a pore in order to enter it, and this imposes a serious restriction. The end point of *Treponema pallidum* was judged to be 0.4 μ; of *Leptospira biflexa* and *Leptospira icterohaemorrhagiae*, 0.2 to 0.25 μ.

4. Bacteria

a. Filtrations in which sieving of bacteria occurs. Most early experiments on sieving of bacteria were made with filter candles of unglazed porcelain, which have a high adsorbing capacity. Mudd noted the influence of pH (cf. part IV, C, 2) and of motility (220); when the motility of *Vibrio percolans* was suppressed by anesthetics or cold, it was retained by filters normally permeable to it. Heymans (151) reported that the filterabilities of different bacteria parallelled the tendencies of their respective infections to spread in the organism. Elford (91, 94) studied the filterability of various bacteria through Bechhold and Elford membranes. The cells were somewhat deformable, so that the end point decreased with increasing filtration pressure. Values for the end points extrapolated to zero pressure, corresponding to absence of deformation of the bacteria, are given in table 6. The fact that the end points (in terms of average pore diameter) for

the acetic collodion membranes lie below the true particle sizes indicates the heteroporosity of such membranes, considerable numbers of pores having diameters greater than the average value. The Elford membranes are evidently more nearly isoporous.

b. Filtrations in which all bacteria are retained. The advantages of large-pored collodion membranes over filter candles for preparation of sterile filtrates were pointed out by Eichhoff (87) and Meyeringh (215) for Zsigmondy–Bachmann membranes, and by Elford for his graded collodion membranes (93). Bacteria cannot grow through the membranes, which are, further, quite free from gross holes such as may occur in candles. The rate of filtration is substantially higher than through candles. The membranes may be used to obtain sterile suspensions of spirochetes (152), viruses, enzymes, proteins, and any thermally labile principles; while, even

TABLE 6

Filtration end points of bacteria

BACTERIUM	END POINT AT ZERO PRESSURE (AVERAGE PORE DIAMETER)		SIZE (MICROSCOPICALLY)
	Acetic collodion membrane	Elford membrane	
B. Coli	0.47μ		0.5–1μ by 1–2μ
B. Prodigiosus	0.43μ	0.75μ	0.75–1.0μ
B. Bronchisepticus	0.40μ		0.4μ by 1μ
Bovine pleuropneumonia (spheres)	0.35μ	0.35μ	0.2–0.25μ

for thermally stable substances, they offer means of sterilization sometimes more convenient than heat. They also serve to concentrate highly dilute bacterial suspensions, so that the organisms can be identified visually (87).

V. Quantitative Estimation of Particle Sizes

In the preceding section, remarks have been confined to the behavior of ultrafilters, characterized in various ways, in the filtration of different disperse systems; filtration end points have been quoted, expressed in terms of average pore diameter, without any attempt to deduce from these figures the sizes of the particles retained.

There are more direct methods than ultrafiltration for determining particle sizes. Of these, perhaps the most powerful at present is ultracentrifugation, applicable to both monodisperse and polydisperse systems. Accurate application of that method, however, as so far developed (265, 196) demands elaborate apparatus and a means of analyzing the system

while in the process of centrifuging. The optical methods employed for analysis require a substantial concentration of the disperse phase and the absence of foreign constituents of similar optical properties. These conditions are in some cases difficult to fulfill. The technique of measurement of diffusion has not been developed to a point which permits quantitative estimation of particle sizes, although qualitative comparisons are possible. The most direct method of measuring the sizes of particles is by optical observation, which is applicable down to diameters of 0.2 μ, while, by application of ultra-violet photomicrography, particles of diameters as low as 0.10 μ may be resolved.

Ultrafiltration has been applied to estimation of particle sizes in cases where other methods are unsuited. The procedure consists in determining the filtration end-point porosity and applying to that figure an empirical correction factor representing the ratio, *diameter of particle/end-point average pore diameter.* The correction factor, which is itself a function of the end-point porosity, is determined by filtration of systems of known particle size.

A. EXPERIMENTAL REQUIREMENTS

In order that the correction factor (for a given end-point porosity) be the same for the system whose particle size is to be determined as for that whose particle size is known, experiments must be carried out under comparable conditions. In particular, normal filtration must obtain. For example, a comparison of the end points of serum albumin (figure 18), with the known particle size (5.4 mμ) shows that, in the center of the pH zone of abnormal filtration, the correction factor has the very low value of 0.12; while on either side, with normal filtration, it is as high as 0.5. Abnormal filtration is invariably associated with an abnormally low correction factor, and one which is more sensitive to slight modifications in experimental conditions, and unsuited for quantitative comparisons. The criterion of normal filtration, in terms of filtration curves (figure 6a, 6b), must be established. In selecting uniform experimental conditions, it is convenient to employ a capillary-active substance in the suspensions. For proteins and all protein-like systems, Hartley's broth at pH 7.4 to 7.6 has proved to be a suitable standard suspension medium. For lyophobic colloids, inorganic stabilizing agents are perhaps better.

The membranes used for quantitative comparative work must have high reproducibility and be highly isoporous. The most successful in this respect are those of Elford. The superiority of his technique is evidenced by the quantitative agreement in ultrafiltration results recently obtained by different laboratories employing it (part IV, F), in contrast to the complete lack of agreement in ultrafiltration studies which existed in the literature previously.

B. DETERMINATION OF THE CORRECTION FACTOR

Various authors have outlined tables of correction factors, notably Krueger and Ritter (170), Bechhold (23), and Elford (94), the values depending on the types of membrane used, the terms in which they were calibrated, and the experimental conditions adopted. However, owing to

TABLE 7

Determination of the empirical correction factor

PARTICLE	SIZE DETERMINED BY	PARTICLE SIZE, $2R$	END-POINT A.P.D. BY FILTRATION j_e	REFERENCE	RATIO $2R/j_e$
Bacillus prodigiosus	Microscopy (94)	0.5 -1.0μ	0.75μ	(94)	1.0 (average)
Bovine pleuropneumonia (spheres)	Microscopy (94)	0.25–0.3μ	0.35μ	(94)	0.8 (average)
Infectious ectromelia	Ultra-violet photomicrography (94)	0.13–0.14μ	0.20μ	(94)	0.67 (average)
Gold sols*	Ultramicroscopic count (94)	50–60 mμ 35 mμ 20 mμ	80 mμ 60 mμ 40 mμ	(94)	0.7 0.6 0.5
Hemocyanin (*Helix*)	Ultracentrifugation (265)	24 mμ	55 mμ	(101)	0.44
Edestin	Ultracentrifugation (265)	8 mμ	18 mμ	(101)	0.44
Serum pseudoglobulin	Ultracentrifugation (222)	6.9 mμ	11–12 mμ	(100)	0.6
Serum albumin	Ultracentrifugation (265)	5.4 mμ	9–10 mμ	(100)	0.57
Oxyhemoglobin	Ultracentrifugation (265)	5.4 mμ	10 mμ	(94)	0.54
Egg albumin	Ultracentrifugation (265)	4.3 mμ	6 mμ	(101)	0.72

* The capillary-active substance present in this case was not broth but sodium oleate.

the superiority of Elford's membranes, and the greater volume of data to which his factors are applicable, his system is the only one here discussed.

The empirical correction factor for converting the end-point porosity (average pore diameter), for filtration in a standard solvent, to the particle

diameter of the disperse phase was determined by Elford for various suspensions of known particle size, in Hartley's broth at pH 7.6 to 7.8. The results are summarized in table 7.

The empirical factor, $2R/j_e$, when plotted against the end-point porosity, is seen to pass through a minimum at about 25 mμ average pore diameter (figure 19, curve I). This is not surprising. It is to be expected that the ratio *particle size/true size of pores retaining particles* would decrease uniformly with decreasing pore size, as shown schematically in curve III. On the other hand, the ratio of the average pore size, as determined by the calibration methods of part II, D (page 398), to the true pore size, may be expected to fall off only slightly down to about 20 mμ, and then rapidly;

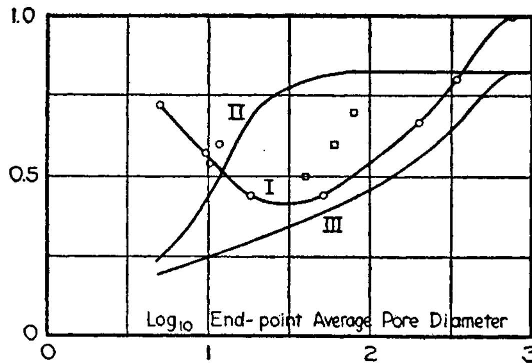

FIG. 19. Correction factors, plotted against end-point average pore diameter in mμ. Curve I, *particle size/end-point average pore diameter* (experimental). The circles refer to the determinations with proteins, viruses, and bacteria; the squares, to those with gold sols. Curve II, *average pore diameter as calibrated/true pore diameter* (schematic). Curve III, *particle size/true pore diameter at end point* (schematic).

this is shown schematically in curve II. The ratio of these two quantities thus estimated gives values for the ratio *particle size/end-point average pore diameter* which fall on the experimental curve, showing a minimum. The average pore diameter of calibration is always smaller than the true pore diameter, and the size of particles retained is always smaller still. Since the correction factor includes any discrepancy between the calibration porosity and the true dimensions of the pores, such discrepancy introduces no error in the estimation of particle sizes by application of the factor.

Elford (94), in applying the correction factor, expressed it as a range between two values, as shown in table 8; this procedure has been followed by most other authors employing his technique.

C. APPLICATION TO BACTERIOLOGICAL SYSTEMS: EXPERIMENTAL RESULTS

Table 9 shows the particle sizes of various viruses, bacteriophages, and spirochetes, as quoted by Elford and other authors by application of the factors given in table 8. The last column gives the particle sizes as calculated from values of the factor obtained directly from figure 19; it is practically equivalent to the fourth column except for end points near 100 mμ, where Elford's factor changes abruptly.

The sizes of particles of bacteriological systems are thus estimated by correction factors which are determined, at the top of the porosity range, by filtration of other bacteriological systems, and, at the bottom of the range, by filtration of chemical systems. The error introduced by this last comparison (cf. table 5) is lessened by the isoporous qualities of the filters employed, but it certainly tends to make the figures quoted at the bottom of the list too small. Opposing this tendency are the effects of blocking by foreign bodies, which cannot always be eliminated, and the difficulty of saturating the primary adsorptive powers of even a thin

TABLE 8

Elford's use of the correction factor (94)

MEMBRANE AVERAGE PORE DIAMETER	FACTOR EMPLOYED
10 to 100 mμ	0.33 to 0.5
100 to 500 mμ	0.5 to 0.75
500 to 1000 mμ	0.75 to 1.0

membrane by dilute virus suspensions. These effects tend to make the estimated particle sizes too large, and it is on their account that Elford's factors (table 8) are displaced somewhat toward low values.

The dependability of the particle sizes at the top of the list (where the factors, having been determined by filtration of bacteria, should be perfectly applicable) is borne out by ultra-violet photomicrography of virus particles (table 10). Another technique whose data can be compared with the filtration results is that of the bucket ultracentrifuge, a method developed by Bechhold and Schlesinger for ultracentrifugation of systems which must be sampled for analysis (29). This technique does not permit so thorough or accurate an analysis of a disperse system as that of Svedberg, but it has been successfully applied to different viruses and bacteriophages, and the results give remarkable confirmation of the relative sizes shown by filtration. The centrifuge places the absolute values higher (table 11). Again, diffusion experiments with different phages place them in the same order of increasing size as does filtration (98), although the significance of

TABLE 9

Estimation of particle sizes in bacteriological systems

PARTICLE	END POINT	ELFORD'S FACTOR (TABLE 8)	PARTICLE SIZE QUOTED BY AUTHORS	EXPERIMENTAL FACTOR (FIGURE 19)	PARTICLE SIZE FROM EXPERIMENTAL FACTOR
	mμ		mμ		mμ
Treponema pallidum spirochete* (152)	400	0.5–0.75	200		
Vaccinia virus (97)	250	0.5–0.75	125–175	0.7	175
Canary virus (67)	250	0.5–0.75	125–175	0.7	175
Leptospira spirochetes* (152)	200–250	0.5–0.75	100		
Herpes virus (110)	200	0.5–0.75	100–150	0.67	130
Infectious ectromelia virus (10)	200	0.5–0.75	100–150	0.67	130
Pseudo-rabies virus (105a)	200	0.5–0.75	100–150	0.67	130
Rous sarcoma No. 1 (99)	200	0.5–0.75	100		
Borna disease virus (104)	175	0.5–0.75	85–125	0.65	110
Newcastle disease virus (69)	160	0.5–0.75	80–120	0.63	100
Vesicular stomatitis virus (129a)	130	0.5–0.75	70–100	0.60	78
Fowl plague virus (111)	120	0.5–0.75	60–90	0.60	72
Bacteriophages Staph. K, D4, D12 (98)	110	0.5–0.75	50–75	0.59	65
Bacteriophages D54, S41 (98)	90	0.33–0.5	30–45	0.54	49
Rift Valley fever virus (56)	70	0.33–0.5	23–35	0.49	34
St. Louis encephalitis virus (14a)	66	0.33–0.5	22–33		
St. Louis encephalitis virus (109)	60	0.33–0.5	20–30	0.47	28
Bacteriophages C36, D13, D20, D48 (98)	60	0.33–0.5	20–30	0.47	28
Yellow fever virus (121)	54	0.33–0.5	18–27	0.45	24
Yellow fever virus (15)	55	0.33–0.5	18–27	0.45	25
Bacteriophage C13 (98)	45	0.33–0.5	15–20	0.43	19
Louping ill virus (105)	40	0.33–0.5	15–20	0.43	17
Bacteriophage S13 (98)	25	0.33–0.5	8–12	0.41	10
Foot and mouth disease (129)	25	0.33–0.5	8–12	0.41	10
Poliomyelitis virus (106)	25	0.33–0.5	8–12	0.41	10
Poliomyelitis virus (269)	35†	0.33–0.5	12–17		

* Diameter of spirochete quoted.

† Titer of virus lower than in experiments of reference 106.

TABLE 10

Sizes of virus particles: comparison between results of filtration and ultra-violet photomicrography (68)

VIRUS	SIZE OF INFECTIVE UNITS BY ULTRAFILTRATION	SIZE OF PARTICLES BY PHOTOMICROGRAPHY
Vaccina virus	0.125–0.175μ	0.15μ
Canary virus	0.125–0.175μ	0.16–0.17μ

calculation of absolute values of the sizes from diffusion is not established (24).

In the future, it is likely that these other physical methods will be developed to the point where they will yield more reliable determinations of particle sizes than ultrafiltration. Ultrafiltration will, however, when conducted under suitable experimental conditions, probably remain the most valuable technique for preparing homogeneous and homodisperse systems to which the more elaborate methods can be applied.

TABLE 11

Sizes of virus and bacteriophage particles: comparison between results of filtration and the bucket ultracentrifuge

PARTICLE	SIZE BY ULTRAFILTRATION		SIZE BY ULTRACENTRIFUGE
	Column 4, table 9	Column 6, table 9	
Bacteriophages: C16	50–75 mμ	65 mμ	90 mμ (247)
C21	30–45 mμ	49 mμ	75 mμ (247)
L	30–45 mμ	49 mμ	75 mμ (247)
D20	20–30 mμ	28 mμ	50 mμ (247)
S13	8–12 mμ	10 mμ	20 mμ (247)
Viruses: Vaccinia	125–175 mμ	175 mμ	200 mμ (30)
Canary	125–175 mμ	175 mμ	120 mμ (32)
Herpes	100–150 mμ	130 mμ	200 mμ (31)
Fowl plague	60–90 mμ	72 mμ	110 mμ (30)

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