CHARACTERISTICS OF COLLODION MEMBRANES FOR ULTRAFILTRATION

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Elford's procedure (1, 2) for manufacturing collodion membranes of controlled porosity was modified by Bauer and Hughes (3) to achieve greater ease of use and uniformity of product. In recent years the method has been re-examined, and the evolution of the procedure is presented here.

The later modification in current use is based upon the effect on the ultimate pore size of small amounts of water in the collodion mixture. The basis of the control is therefore the introduction of known amounts of water into the mixture. A further simplification has been the use of propyl alcohol and acetone as the only solvents employed.

The second fundamental improvement lies in the use of a closed chamber for controlled evaporation of the solvents, thus obviating the necessity for temperature and humidity control of the room in which the work is done. Reasonable temperature control, however, is desirable since temperature gradients, with the resulting production of convectional currents in the atmosphere of the container, tend to result in excessive variability in the final membrane.

Stock Solutions

100 gm. of parlodion (Mallinckrodt) are dissolved in 900 gm. (1136 ml.) of anhydrous acetone. This is shaken by machine overnight, or by hand intermittently for 3 or 4 days. For porosity control, propyl alcohol containing 2 per cent of water is used and acetone is added to the mixture in quantities sufficient to maintain a constant volume. The mixture is designed to provide 7 mg. of parlodion per sq. cm. in the finished membrane. The volumes used in preparing the mixture are thus determined by the dimensions of the plate to be used for the evaporation. With the plates in use in our laboratory, a total volume of collodion mixture of 166 ml. is employed. Less propyl solution results in smaller pore diameters. As an example, the following charge yielded a membrane of effective pore diameter of 355 mμ:

	ml.
Parlodion	77
2 per cent water in propyl alcohol	28
Anhydrous acetone	61
Total	166

Apparatus

In the technique of Bauer and Hughes, a leveled cell of plate glass was used. In the present procedure, the base plate is of brass,[1] with the surface lapped to produce a true flat within the ability of the metal to retain its configuration. These plates were prepared by cutting three disks 45 cm. in diameter from a large plate 2 cm. in thickness. The surfaces were trued in a lathe and the disks lapped with fine abrasive in pairs so that ultimately three true flats were obtained. These were then given a thin plating of nickel and brought to a high polish.

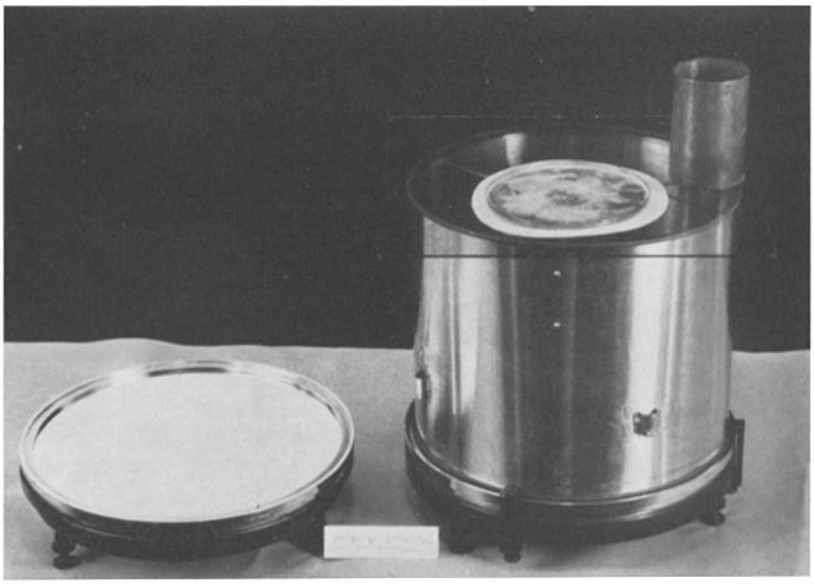

FIG. 1. Equipment for collodion membrane casting. At left is the flat polished metal surface upon which the mixture is poured and at right is a completely assembled unit showing the inclosure which serves to control the drying schedule.

A retaining ring is clamped to the base plate with an intervening gasket of teflon, as shown in Fig. 1. With our plates, the inside diameter of the ring is 35 cm. which is therefore the effective diameter of the pouring surface. On the ring rests a container of stainless steel surmounted by a sheet of plate glass with a central hole. Within the can of stainless steel a moat is provided about one-fourth the distance from the bottom. Within this moat a strip of filter paper 2 inches wide is placed, and, in use, the moat is partially filled with the 2 per cent aqueous propyl alcohol. The filter paper

[1] The original suggestion concerning the use of a metal plate rather than glass, because of the better thermal conductivity, was from Dr. E. G. Pickels. The possibility of using the wet thickness as an index of the pore volume was suggested by Mr. Winfield H. Baker.

insures a constant total evaporation surface for the alcohol. Above the level of the moat four symmetrically placed holes ½ inch in diameter are provided, and hemicylindrical baffles are applied to these apertures either permanently or by the use of Scotch tape. At a somewhat higher level a fifth hole may be added through which a thistle tube

FIG. 2. Elevation view of equipment for collodion membrane casting.

may deliver the mixture to the center of the plate. Near the top, on the brackets shown, there is a small tray 6 inches in diameter resting on a sheet of filter paper about 8 inches in diameter.

Procedure

In use, the plate is carefully leveled, using a machinist's level of maximum precision. The can, with its upper and lower edges covered by split gum rubber tubing, is put in place and 100 ml. of propyl alcohol poured into the moat. The tray is par-

tially filled with drierite or silica gel and placed on its brackets. The glass top is put into position and a small plastic cylinder containing drierite over a mesh bottom is placed over the hole. All apertures are closed with Scotch tape. The entire apparatus is then allowed to stand for 2 hours to permit the interior to come to equilibrium with respect to moisture and saturation of the atmosphere with propyl alcohol.

After equilibration, the top is lifted carefully, the tray removed, and the drierite emptied into the plastic cylinder. On replacing the tray, 2 per cent aqueous propyl alcohol sufficient to cover the bottom (70 ml.) is introduced by tube through the aperture in the top plate and the cylinder of drierite repositioned (Fig. 2).

The appropriately shaped thistle tube is introduced through the pouring orifice and the charge delivered to the center of the plate. The Scotch tape is removed from the orifices and the system allowed to remain undisturbed overnight. Under our conditions about one-third of the propyl alcohol in the moat and pan evaporates. The following morning distilled water is introduced by tube in sufficient quantity to cover the membrane completely. The can and other appurtenances may then be removed. The sheet formed on the plate will tend to float after standing for a few minutes. It is carefully removed and placed in a large tray of distilled water where it receives six changes of water in 3 days.

From the large disk, small circular membranes are cut, using a cutter of appropriate dimension for the filters which are to be employed. With our standard size, 40 to 41 individual membranes are prepared from a single sheet. These are placed in a small fruit jar two-thirds filled with distilled water and the whole autoclaved at 10 pounds' pressure for 30 minutes.

Calibration

The equipment for calibration is the same as that previously described and illustrated by Bauer and Hughes (3). With this arrangement, a constant initial head of water is employed and the time of discharge of a predetermined amount is measured. The horizontal position of the calibration cell is to insure constancy of starting pressure irrespective of small variations in the attachment of the cell by the rubber stopper to the burette. We have found it essential to use conductivity water for the calibration because of the presence of particulate material in water which is ordinarily satisfactory for chemical purposes. With conductivity water, no difficulty should be experienced in getting constant values for time of flow in successive trials with the same disk. While a constant pressure head would simplify the mathematical theory, it is more complicated in practice.

Theory of Calibration

For the development which follows, the pores are assumed to be uniformly cylindrical passages normal to the surface of the membrane. By Poiseuille's law of viscous flow through capillary tubes,

$$v = \frac{\pi n r^4 D g h}{8 \lambda \eta} \tag{1}$$

in which v = volume flow per unit area
λ = thickness of membrane
η = coefficient of viscosity
n = number of pores per unit area
r = radius of pore
D = density of fluid
g = acceleration due to gravity
h = height of column

If a is the area of the burette, and A is the exposed area of the membrane,

$$Av = -a\frac{dh}{dt} \tag{2}$$

so that

$$\frac{dh}{h} = -M\,dt, \qquad \text{in which} \qquad M = \frac{A\pi nr^4Dg}{8a\lambda\eta}. \tag{3}$$

Integrating and stipulating the condition that when $t = 0$, $h = H$,

$$h = He^{-Mt}. \tag{4}$$

If the fluid level drops the distance s_0 in t_0 seconds, then the average velocity will be: s_0/t_0. At some time in this period, the instantaneous velocity will equal this average velocity,

$$ds/dt = s_0/t_0. \tag{5}$$

Since $s = H - h = H(1 - e^{-Mt})$,

$$ds/dt = HMe^{-Mt}. \tag{6}$$

So that at some time, t_1,

$$HMe^{-Mt_1} = s_0/t_0; \tag{7}$$

and the column height at time t_1 will be:

$$h_1 = He^{-Mt_1} = \frac{s_0}{Mt_0}.$$

Since M is a quantity needed, it can be obtained directly from (4):

$$M = \frac{1}{t_0}\log_e\frac{aH}{aH - q_0} \tag{8}$$

in which q_0 is the volume flowing in t_0 seconds.

If the total pore volume per unit area is defined as:

$$B = \pi r^2\lambda n, \tag{9}$$

then from (3)

$$r = \sqrt{\frac{8Ma\lambda^2\eta}{ABDg}} = C\lambda\sqrt{\frac{M}{B}}. \tag{10}$$

The values of the constant term C must be calculated from the conditions and dimensions of the calibration equipment used. For our equipment, using water as the calibration medium at 25°C. and with a burette area of 0.1773 cm.^{2} and a membrane exposure in the calibration cell of 1.9483 cm.^{2}, the value of C is 2.58×10^{-3}.

Calibration Procedure

The details of calibration are given by Bauer and Hughes. The wet thickness is measured by a paper gauge and is the determination of greatest error. It is felt that better methods may be developed for this purpose.

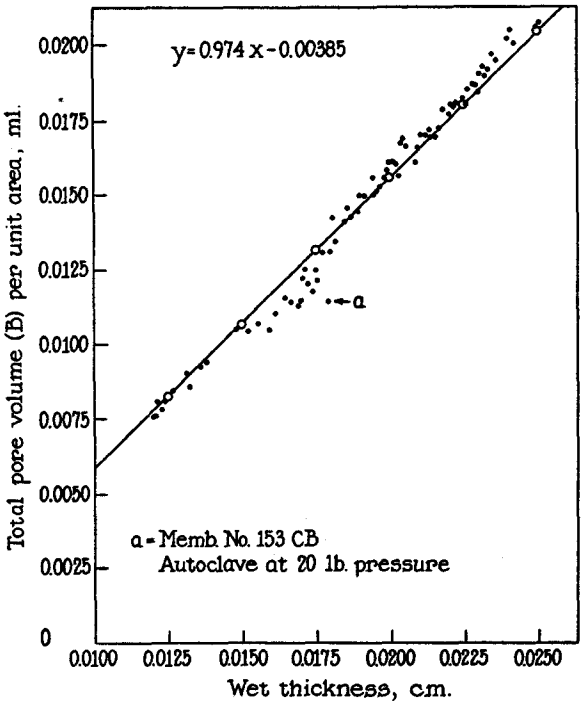

FIG. 3. Wet thickness *vs.* B values.

The total pore volume that has been conventionally determined by the loss of weight on drying in a vacuum is the basis for the further development now in use. Since the amount of collodion per unit area is constant, provided that precise leveling has been obtained, it seemed logical that the total pore volume would be correlated with the wet thickness. Consequently, a long series of calibrations by loss of weight on drying were made and the values of B plotted against the wet thickness (Fig. 3). The straight line fitted to these data by the method of least squares is:

$$y = 0.974x - 0.00385,$$

in which $y = B$ and x is the wet thickness in centimeters.

For the condition $y = 0$ and the mean density of collodion of 1.6712 determined from the calculated collodion volumes and the dry weights, the mass

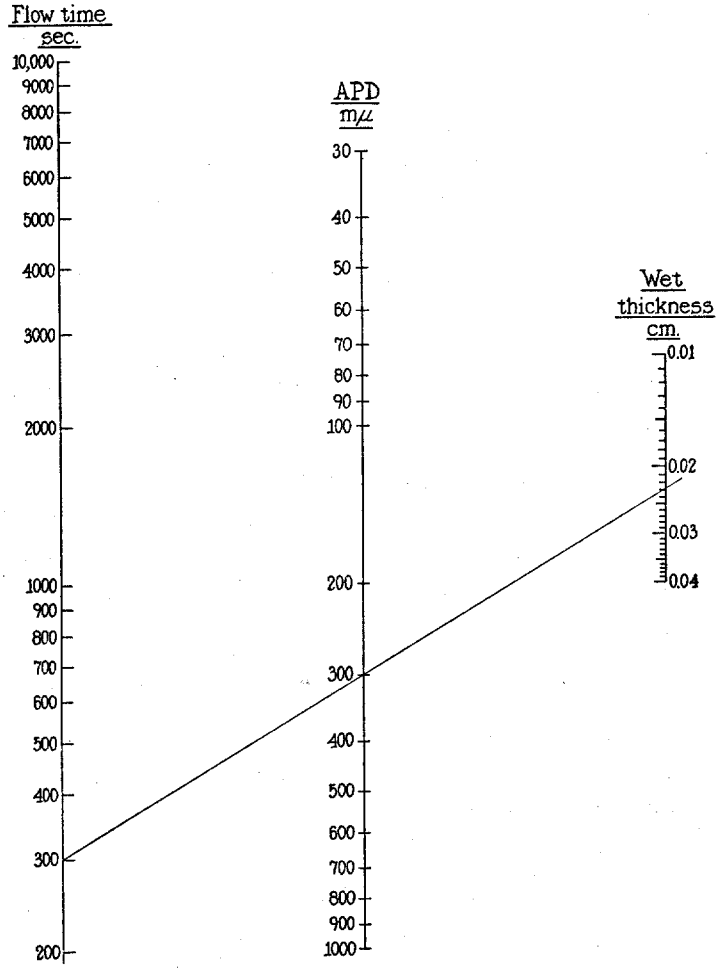

FIG. 4. Nomogram for calibration of collodion membranes. Temperature 25°C. Total flow 5 ml.

of collodion per square centimeter is 0.0066 gm. From the known amount of collodion used for the entire sheet, the mass would be 0.0070 gm./cm.^{2} In the actual delivery to the plate, a small amount of the charge is lost on the glass-

ware so that the amount actually in the membrane is about 1 per cent lower than this or 0.0069 gm./cm.^{2}

Since, under the conditions of manufacture, the total pore volume per unit area can be obtained directly from the wet thickness, a nomogram was constructed by which the effective pore diameter can be obtained at once from the wet thickness and the time of flow of a standard volume of water. Using a standard procedure (4) for the construction of a nomogram of the type

$$f(u) + f(v) + f(w) = 0, \tag{11}$$

to which equation (10) may be reduced by logarithmic transformation, it is found that the nomogram will consist of three parallel straight lines equally spaced and divided according to the following functional conditions:

$$\begin{aligned} f(u) &= \log t \\ f(v) &= 2 \log r \\ f(w) &= \log f(\lambda) - a \log \lambda - 2 \log C. \end{aligned}$$

Using equal scale factors, the nomogram shown in Fig. 4 results. This illustration applies to a volume of 5 ml. only but in practice we combine with this the scales for 1 ml.

Variance in Effective Pore Diameter

It is evident from the previous discussion that the quantity called "effective" or "average pore diameter" (A.P.D.) is a statistical characteristic of the membrane resulting from the application of certain assumptions concerning the structure and behavior of the material. Apart from the considerations of actual structure, it is reasonable that the porosity of any membrane will not be constant over its entire area. The actual pore diameters will form a frequency distribution at any location on the membrane and the character of this distribution may itself vary significantly from region to region. The individual disks which are used are thus samples of a variable population and there is presented the statistical problem of the degree to which the calibration of a few such disks may characterize the entire lot.

In the usual calibration method, involving the determination of the total pore volume by loss of weight on desiccation, the membranes which are calibrated are destroyed. The greater the variability of the lot, the broader is the zone of uncertainty concerning the remaining membranes which are to be used for actual experiments.

In the technique which we have employed, an arbitrary sample of five membranes is taken at the time of cutting up the sheet, these five being composed of one from the center of the sheet, and four from near the periphery at arcs of 90° from a fixed mark which is placed on the membranes before they are removed from the plate. From the calibrated values of these five membranes a

mean and standard deviation are computed. Since we are concerned with the proportional error in relation to the mean value rather than with the absolute variability, an index of variability, designated by the letter k, is defined as the standard deviation of the five calibrations divided by their mean. From statistical considerations, on the assumption of Gaussian distribution of the calibrated porosities, the probability of a membrane chosen at random from the

TABLE I

Probability of a Membrane Taken at Random Falling within a Fraction u of the Mean A.P.D.

k	$u = 0.10$	$u = 0.05$
0.001	1.000	1.000
0.01	1.000	1.000
0.02	1.000	0.988
0.03	0.999	0.904
0.04	0.988	0.788
0.05	0.954	0.682
0.06	0.904	0.593
0.07	0.847	0.522
0.08	0.788	0.471
0.09	0.733	0.414
0.10	0.682	0.383
0.11	0.637	0.354
0.12	0.593	0.325
0.13	0.558	0.296
0.14	0.522	0.281
0.15	0.497	0.236
0.20	0.383	0.197
0.25	0.311	0.158
0.30	0.236	0.135

lot falling within a certain fraction, u, of the calibrated mean value may be written:

$$p = \sqrt{\frac{2}{\pi}} \int_0^t e^{-\frac{1}{2}t^2} dt,$$

in which $t = \frac{u}{k}$. For values of u of ± 5 per cent and ± 10 per cent, a table of these probability values is obtained (Table I).

Prediction Value of k

The standard calibration sample of five from membrane lot 233 gave an A.P.D. of 990 mμ and k of 0.08. The remaining 36 membranes were then calibrated and the entire lot found to have an A.P.D. of 954 mμ, a k of 0.07, and a coefficient of skewness of -1.0. The frequency distribution is shown in Fig. 5

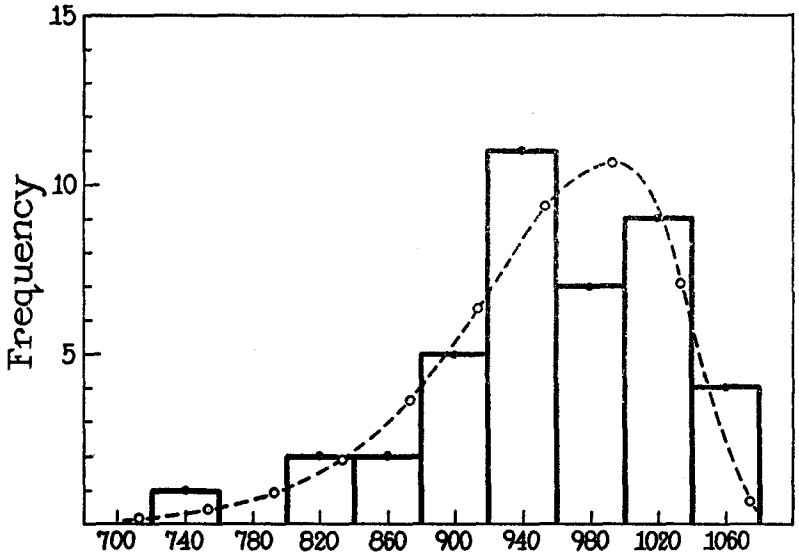

FIG. 5. Membrane lot 233. Observed and theoretical distribution of A.P.D.

TABLE II

Calibration Results of Membrane Lot 233

Values of statistical factors

	Calibration sample	Entire lot
No. membranes	5	41
Mean A.P.D. (in mμ)	990	954
k	0.08	0.07
Distribution of 36 membranes remaining in lot		
	No. membranes falling within 5 per cent of mean	No. membranes falling within 10 per cent of mean
Predicted from sample	17	28
Determined by calibration	19	31
Distribution of entire lot of 41 membranes		
Predicted from true mean and k	21	35
Determined by calibration	24	36

together with a type III curve of the same statistical parameters as the distribution. Neglecting the asymmetry of the distribution and using the probabilities of Table I, with the mean and k of the sample of five, the numbers of

membranes expected to fall within 5 per cent and 10 per cent of the mean were calculated and are shown in Table II. There is good agreement with the actual calibration values of the 36 membranes. The agreement is still closer if the true mean and k for the entire lot are used.

For this particular membrane, it would appear that with samples of five, there is 95 per cent confidence that the mean of the sample is within 6.2 per cent of the true mean of the distribution. For the chosen confidence level, the uncertainty may be reduced either by increasing the size of the calibration sample or by obtaining membranes of smaller k values.

Correlation of Calibration Values with Ultracentrifugation Determination of Particle Size

In another communication (5), the filtration results with these membranes in a series of tropical viruses are given. With one of these viruses (Semliki Forest virus, high mouse passage), it has been possible to prepare a concentrate sufficiently pure to give a good boundary in the ultracentrifuge. From studies with the electron microscope we know that the individual particles of this virus are spherical. The specimens used for ultracentrifugation contained 5 per cent of *rhesus* monkey serum, the components of which sedimented much more slowly than the virus. For the particle density, the value found by Sharp *et al.* (6) for influenza virus (1.104) was used and the viscosity was that of the diluted serum without virus. Assuming that there was no hydration and that the spherical particles were of the above density, the five centrifuge runs employing two different lots of virus gave computed particle diameters of 56.4, 55.5, 54.8, 57.5, and 57.5 mμ. This may be compared with the failure of the virus to pass membranes with a calibrated pore diameter of 61 mμ or less. It readily passed membranes of 69 mμ.

Membrane Structure

In order to visualize the internal structure of the membranes by electron microscopy, replicas of the free surfaces and of microscopic sections of paraffin-embedded membranes were prepared by evaporating silicon monoxide normally to the specimen surface. After dissolving the collodion in acetone to free the replica, the latter was lifted on a specimen screen and lightly shadowed with chromium at an angle of approximately 10°.

Electron micrographs of these replicas at a magnification of 12,600 diameters are shown in Figs. 6 to 13, covering a range of effective pore size from 38 mμ to 739 mμ. From these it appears that the actual structure is one of somewhat irregular, anastomosing passages giving a spongy character to the whole. The orifices are irregular and the passages variable in bore, although, as can be seen by reference to the dimensional standard, they are of the magnitude given by the water calibration.

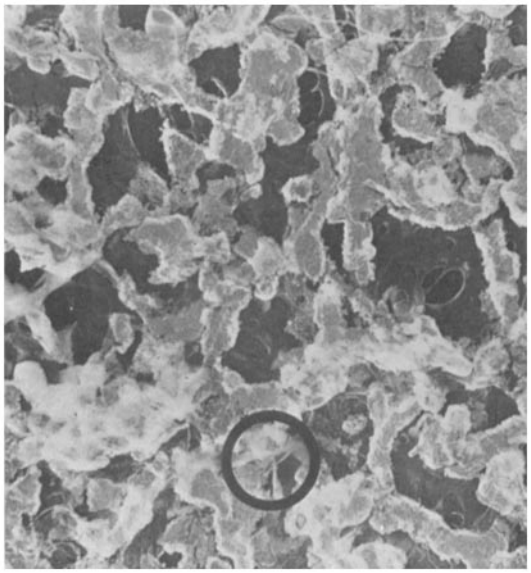

FIG. 6. Surface of a collodion membrane. × 12,600. A.P.D. = 739 millimicrons. k = 0.04. The diameter of the outer circle is 1 micron. The thickness of the black ring represents 0.1 micron.

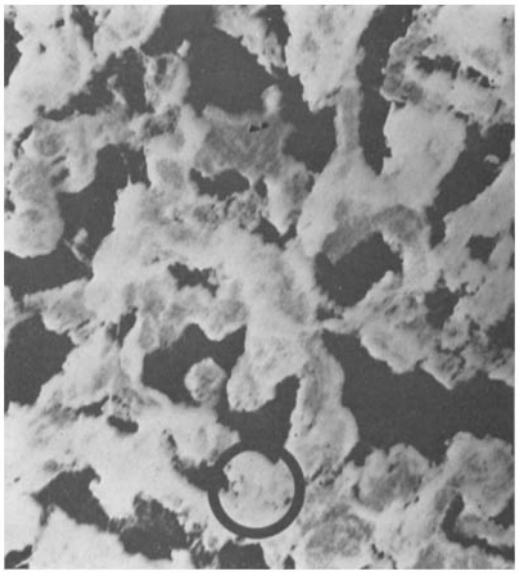

FIG. 7. Oblique section of a collodion membrane. × 12,600. A.P.D. = 739 millimicrons. k = 0.04.

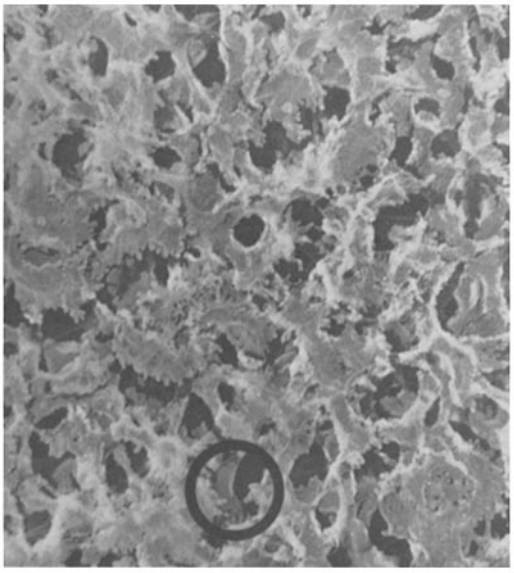

FIG. 8. Surface of a collodion membrane. × 12,600. A.P.D. = 341 millimicrons. $k = 0.04$.

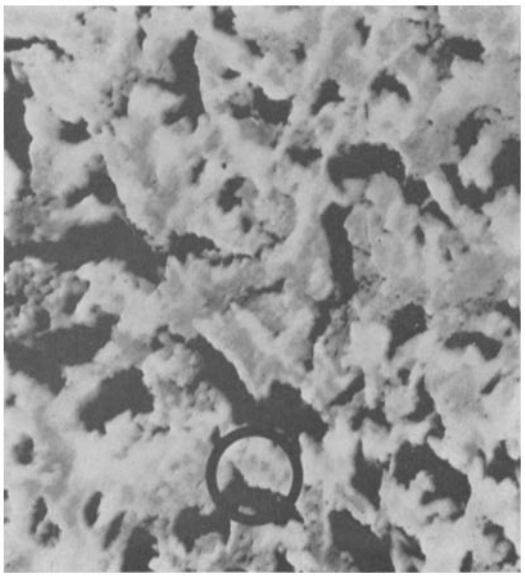

FIG. 9. Oblique section of a collodion membrane. × 12,600. A.P.D. = 341 millimicrons. $k = 0.04$.

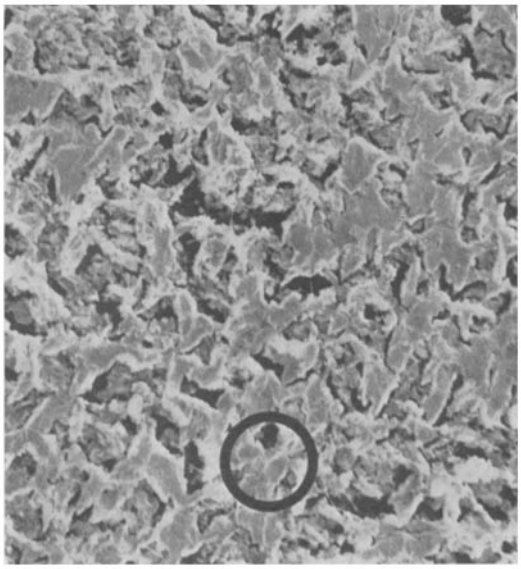

FIG. 10. Cross-section of a collodion membrane. × 12,600. A.P.D. = 162 millimicrons.

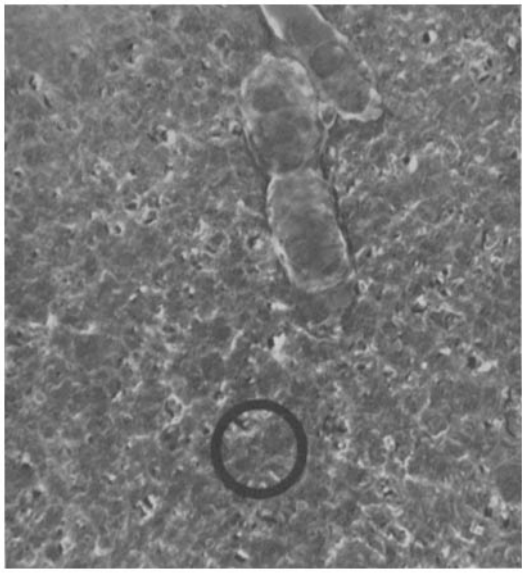

FIG. 11. Surface of a collodion membrane. × 12,600. A.P.D. = 79 millimicrons. k = 0.07. Note replica of microorganisms.

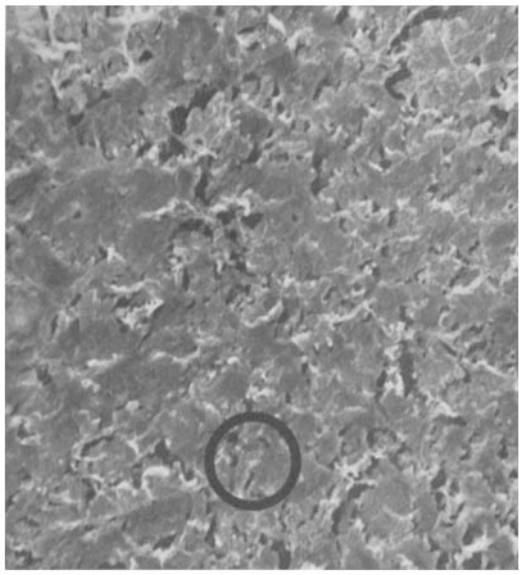

FIG. 12. Oblique section of a collodion membrane. × 12,600. A.P.D. = 79 millimicrons. $k = 0.41$.

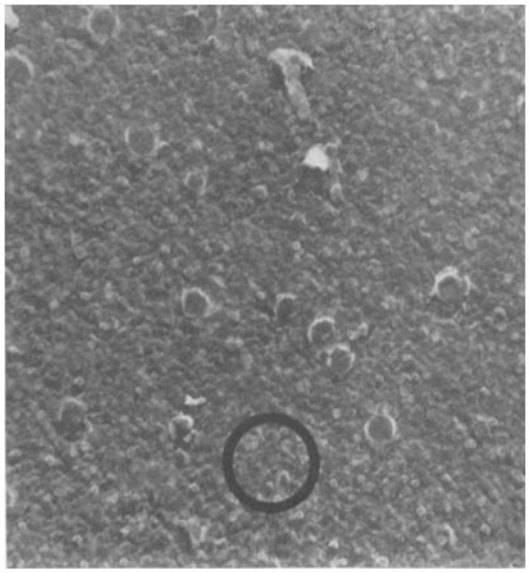

FIG. 13. Surface of a collodion membrane. × 12,600. A.P.D. = 38 millimicrons. $k = 0.04$.

While such a structure as revealed in these preparations will give calibration values related to the general order of magnitude of the passages, it is also clear

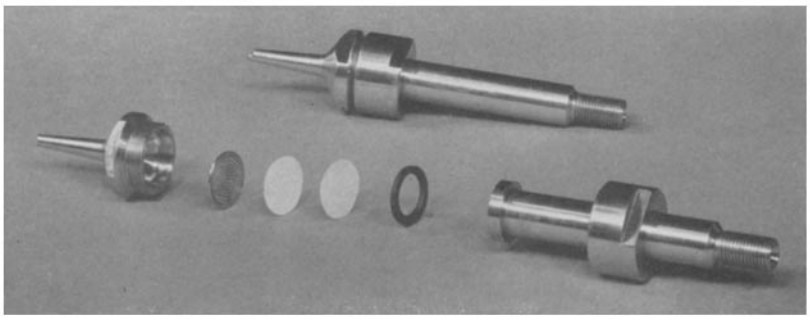

FIG. 14. An exploded view of a filtration unit for the utilization of collodion membranes. In the rear is shown such a unit assembled.

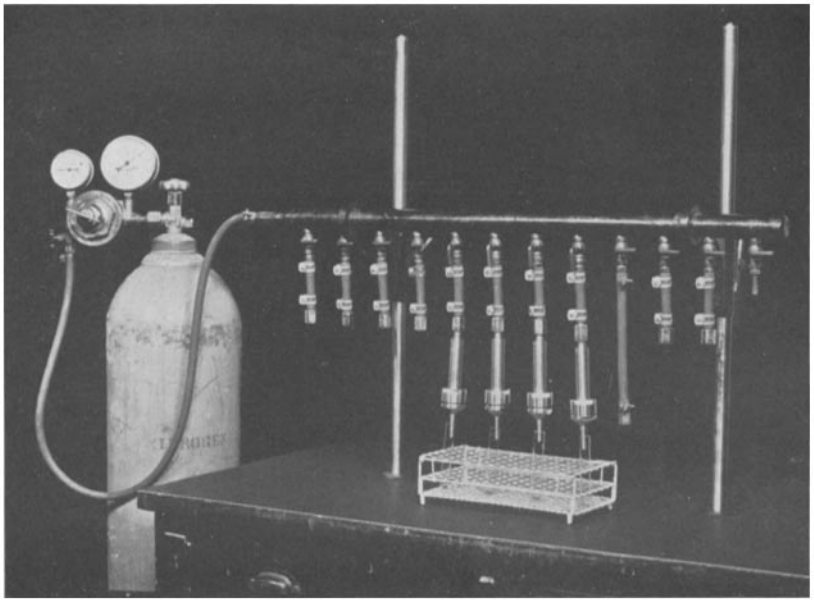

FIG. 15. A manifold for the simultaneous filtration of a biological fluid through collodion membranes of various pore diameters. This process is accelerated by the application of a positive pressure of the order of 10 pounds per square inch (obtained from a tank of nitrogen) to the fluid in the individual filtration units.

that the conditions of the idealized membrane used for the mathematical development of the calibration theory do not, in fact, exist. The intercommunicating passages have a total length appreciably longer than the thickness of the

membrane and the maximum diameter of the particle which will pass will be partially dependent upon the minimum diameter of the passage.

The microscopic studies have been limited to membranes prepared by the method described in this paper and have not been comparative. It is evident, however, that considerable variability of structure can exist even in membranes prepared by presumably identical techniques.

Method of Employing Membranes in Ultrafiltration

The detail of the technique has been completely described by Bauer and Hughes (3). Essentially, the membrane is separated from an underlying perforated plate by a disk of filter paper which permits the entire permeable surface of the membrane to be utilized. Since the publication of the paper cited, we have developed a filter of smaller dimensions which is especially suitable for the filtration of small volumes. This filter is machined from stainless steel. The exploded assembly is shown in Fig. 14 and the method of use in Fig. 15. The filter disk is approximately 2.5 cm. in diameter.

DISCUSSION

From the foregoing, it is clear that membranes prepared by the method described differ materially in their structure from that assumed in the development of the theory of ultrafiltration. Not only is there considerable variability in the diameters and lengths of the pores, but inevitably there is also variation over the entire membrane. The figure for pore diameter obtained by calibration is thus a number more or less closely related to the behavior of the membrane in use. The variability of individual membranes cut from the larger sheet is dependent upon many factors operating at the time of manufacture. Since the permeability behavior of a lot of membranes cut from the same sheet exhibits a frequency distribution, it would appear that the precise definition of an "end-point" might well be that calibrated porosity of a lot of membranes which will pass the virus being studied 50 per cent of the time. With a distribution near Gaussian this definition would apply irrespective of the value of k.

It would be most desirable to calibrate the membrane actually used in a filtration, but usually this is impractical and a small sample is ordinarily calibrated as in the method described. The uncertainty with respect to a membrane chosen at random from the container can only be reduced by diminishing the value of k. Careful temperature control and elimination of air currents in the room will greatly assist in obtaining low k values.

Water calibration appears to be a sound and useful method, but there is always an area of uncertainty concerning the actual porosity due to the character of the structure and the possible interaction of the material being filtered with the substance of the membrane. It would seem advisable to confirm endpoints given by a particular set of membranes by filtering additional biological

material of known size and shape. This procedure would give due recognition to the empirical element in the use of such membranes.

Because of the lack of concordance between the idealized structure and that actually found, it is evident that there will be more or less discrepancy between the water calibration results and the dimensions of particles which will pass the pores irrespective of such factors as the surface charge of the particles and the adsorption of protein material to the walls of the passages. The factors relating theoretical calibration and practical experience may vary considerably dependent upon the mode of preparing the membrane and the type of material filtered. In the instance cited, the calibrated average pore diameter of the membranes seems to be closely related to the size of a virus as determined by centrifugation. It is apparent that further studies of this type are in order, together with careful measurements of the densities of the viruses being studied in their completely hydrated state.

SUMMARY

An apparatus for the production of graded collodion membranes is described. The theoretical considerations of calibration are discussed in relation to the demonstrable structure and statistical characteristics of the membranes. A new definition of an "end-point" is suggested.

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