THE SIZE OF PORES IN COLLODION MEMBRANES.

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In a study of collodion membranes which had been coated with films of gelatin or egg albumin, it was found that the presence of the protein reduced the rate of flow of water through the membranes, as would be expected if the protein film were formed on the walls of the pores.[1] In order to determine the thickness of such films, an attempt has been made to estimate the size of pores in membranes not treated with protein.

The method which was selected as giving the most reasonable and definite results appears to be also the oldest, having been used in 1872 by Guérout.[2] It depends on the assumptions that the membrane acts like a bundle of capillary tubes having a length equal to the thickness of the membrane, and that the law of Poiseuille applies to the flow of water through such short and narrow tubes. The quantities measured are the thickness of the membrane, the rate of flow of water, under a known pressure, through a fixed area of the membrane, and the relative volume occupied by the water in the wet membrane.

Determination of Fractional Volume of Pores.

Membranes of varying permeability were prepared by the method previously described,[1] which is essentially that of Bartell and Carpenter.[3] The permeability was fixed by the time during which the organic solvents were allowed to evaporate before the addition of water. Each large membrane, after at least 1 day in water, was cut into uniform disks with a steel die 3.81 cm. in diameter. The water content of each membrane was determined by weighing several such

[1] Hitchcock, D. I., *J. Gen. Physiol.*, 1925–26, viii, 61.

[2] Guérout, A., *Compt. rend. Acad.*, 1872, lxxv, 1809.

[3] Bartell, F. E., and Carpenter, D.C., *J. Phys. Chem.*, 1923, xxvii, 252.

disks, after blotting between filter papers, in a closed weighing bottle, and then drying to constant weight at 100°C. The thickness was measured, as before, by means of a microscope provided with a micrometer eyepiece, nine readings being made with each membrane.

When the ratio of wet weight to dry weight, w, was plotted against the thickness in cm., l, the points fell close to a straight line. Such a linear relation was previously found by Walpole,[4] who deduced the equation

$$l = m\left(w - 1 + \frac{1}{d}\right). \tag{1}$$

Here l is the thickness of the membrane, m the number of gm. of dry collodion per sq. cm. of wet membrane and d is the density of collodion. The equation follows from the assumptions that the volume of the wet membrane is the sum of the volumes of water and collodion contained in it, and that the density of the collodion itself remains constant in all the membranes. Three determinations were made of the density of the collodion used in the present work, the samples used being a hard lump of air-dried collodion, a membrane of low permeability, and a membrane of high permeability. The values obtained were 1.65, 1.65, and 1.66. The determinations were made at 20°C. with a specific gravity bottle. If Walpole's law of additive volumes did not apply, the figures could not be identical. On testing Walpole's equation with the experimental values for d, m, and w, it was found that the calculated values for l agreed well with those observed.

On the basis of this idea of additive volumes, the fraction of the membrane volume occupied by water, f, is given by the relation

$$f = \frac{m}{l}(w - 1), \tag{2}$$

in which the quantities determined experimentally are the thickness and the wet and dry weights of a known area of membrane.

The assumption involved in this calculation, that all the water in the blotted membranes is in the pores and not combined with the collodion, was roughly confirmed in the following way. Measurements were made of the expansion produced on freezing wet membranes, by

[4] Walpole, G. S., *Biochem. J.*, 1915, ix, 284.

a dilatometer method similar to that described by Foote and Saxton.[5] The results indicated that 85 per cent of the water in a blotted membrane of the most permeable type studied in this work froze above $-6°$C., and that no further freezing took place on cooling to $-18°$C.

Values for the fractional pore volume have been obtained in another way, based on measurements of electrical conductivity. The electrodes were platinized platinum disks forming the ends of a closed cylinder whose sides were glass tubes. The cell was filled with N/50 KCl, and the membrane, after being soaked in the same solution, was supported across the cylindrical space between the electrodes by means of the ground ends of the tubes forming the sides of the cell. The conductivity at 30°C. was measured in the usual way, the source of current being an audio oscillator of 1000 cycles. It was necessary to

TABLE I.

Fraction of Membrane Volume Occupied by Pores, As Determined by Wet and Dry Weight and by Conductivity.

Designation of membrane......	B 6	B 1	B 8	B 4	B 2	B 7	B 5	B 11	B 3	B 10
f (by weight)............	0.92	0.97	0.89	0.85	0.77	0.62	0.69	0.63	0.41	0.54
f (by conductivity).......	0.90	0.88	0.78	0.79	0.76	0.72	0.64	0.46	0.25	0.11

balance the rather large capacity of the cell, especially when the membrane was in place, by means of variable condensers. The differences in resistance observed with and without the membrane were not large, except in the case of the least permeable membranes, and in the latter case the readings were not well reproducible with different pieces of the same membrane. Accordingly the results of the measurements are given, not as being of much value in themselves, but simply as confirmatory of the order of magnitude of the pore volumes. The latter were calculated on the assumption of cylindrical pores and of additive resistance for the layers of solution inside and outside of the membrane. The relative pore volumes obtained by the two methods are given in Table I.

[5] Foote, H. W., and Saxton, B., *J. Am. Chem. Soc.*, 1916, xxxviii, 588; 1917, xxxix, 627, 1103.

Determination of Pore Size from Poiseuille's Law.

The measurement of the rate of flow of water through the membranes has been described elsewhere.[1] The apparatus was not in a thermostat, but the temperature of the water varied from 18.5 to 24°C. in different experiments. Correction was made for the effect of temperature on the viscosity of water by means of Bingham's data.[6] From four to eight disks of the same membrane were used for successive readings, the average being taken. The highest pressure used was 35 cm. of Hg. The membranes were all visibly stretched to some extent, and the stretching was neglected in the calculation of the rate of flow per sq. cm. The data were reduced to c. g. s. units, the permeability, Q, being defined as the number of cc. of water flowing in 1 second through 1 sq. cm. of membrane under a pressure of 1 dyne per sq. cm. (This differs from the notation of the previous paper.[1]) On the assumption that the membrane behaves like a bundle of capillary tubes of length equal to its thickness, the rate of flow should be given by Poiseuille's law in the form

$$Q = \frac{n \pi r^4}{8 l \eta}. \tag{3}$$

In this equation the unknown quantities are n, the number of capillaries per sq.cm., and r, the pore radius in cm. The quantities Q and l, as defined above, were determined experimentally, while values for η, the viscosity of water at the temperature of the experiment, were taken from the data of Bingham.[6] Thus the equation was solved for values of $n\pi r^4$. The fraction of the membrane volume occupied by pores, f, must on the assumption of cylindrical pores be equal to $n\pi r^2$, and the determination of this quantity has already been discussed. Hence the value of r, the pore radius, should be given by

$$r = \sqrt{\frac{n \pi r^4}{n \pi r^2}} = l \sqrt{\frac{8 \eta Q}{m (w - 1)}}, \tag{4}$$

while that of n, the number of pores per sq. cm., should be

$$n = \frac{n \pi r^2}{\pi r^2} = \frac{m^2 (w - 1)^2}{8 \pi Q l^3 \eta}. \tag{5}$$

[6] Bingham, E. C., Fluidity and plasticity, New York and London, 1922.

The values so obtained are given in Table II, together with the data from which they were calculated.

On plotting the values of r against those for l, the points fell close to a straight line, which may be represented by the empirical equation

$$r = 1.2 \times 10^{-4}\, l. \tag{6}$$

TABLE II.

Calculation of Pore Radii from Measurements of Thickness, Wet and Dry Weight, and Rate of Water Flow.

Designation of membrane.	Thickness, in cm., $l, \times 10^3$.	Gm. collodion per cm.², $m, \times 10^3$.	Gm. water per gm. collodion, $w - 1$.	Rate of flow in c.g.s. units, $Q, \times 10^9$.	Pore radius in cm., $r, \times 10^6$.	No. of pores per cm.², $n, \times 10^{-10}$.
B 15	21.4	2.45	8.07	2.48	2.08	7
B 6	19.6	2.40	7.54	2.46	2.01	7
B 1	18.6	2.21	8.16	3.03	2.20	6
B 22	17.5	2.39	6.31	2.05	1.80	8
B 8	11.8	2.22	4.72	1.97	1.43	14
B 4	11.1	2.35	4.00	1.98	1.39	14
B 2	11.1	2.28	3.74	1.53	1.31	14
B 17	10.2	2.34	3.29	1.39	1.21	16
B 20	8.6	2.36	2.56	1.16	0.99	26
B 13	8.5	2.38	2.82	1.20	1.02	24
B 7	8.4	2.30	2.25	1.09	1.07	17
B 12	7.1	2.18	2.12	0.673	0.75	37
B 5	6.8	2.24	2.09	1.23	0.97	23
B 21	6.0	2.44	1.03	0.408	0.63	41
B 11	4.6	2.16	1.32	0.585	0.57	61
B 23	3.3	2.55	0.59	0.278	0.40	89
B 14	3.2	2.33	0.59	0.166	0.31	140
B 3	2.9	2.24	0.52	0.247	0.36	100
B 19	2.7	2.39	0.45	0.103	0.24	220
B 10	2.4	2.16	0.60	0.284	0.32	170
B 18	2.2	2.38	0.53	0.236	0.27	270

Likewise an approximately linear relation was found to exist between the observed values of Q and w, according to the empirical equation

$$Q = 4.3 \times 10^{-10}\, (w - 1). \tag{7}$$

It is possible to calculate the pore radii from the values of f obtained from conductivity data. The values so obtained are still of the same order of magnitude, even where the values for f differ widely. Thus, for membrane B 10, where the divergence is greatest, the conductivity method gives $r = 0.69 \times 10^{-6}$ instead of 0.32×10^{-6}. By making use of Walpole's relation, equation (1), the radii may be calculated from the measurements of l and Q, or w and Q. By using the empirical relations given in equations (6) and (7) together with equation (1), the radii may be calculated from a single measurement, either of l, Q, or w. The results do not differ materially from those given in Table II.

The values for n given in Table II are not constant, but show a marked increase as the permeability of the membrane decreased. This is contrary to the conclusion of Bartell and Carpenter,[3] which was adopted by the writer in a previous paper.[1] It happens, however, that the variation of n is such that the relation between the observed amounts of adsorbed gelatin and the calculated relative pore surface is still linear.

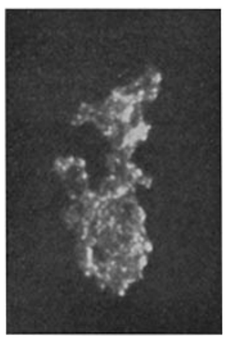

FIG. 1.

Microscopic Structure of Collodion Membranes.

Samples of the membranes used in the present work were examined under the microscope with transmitted light, but showed no definite evidence of structure even at a magnification of 1000 diameters with an oil immersion objective. With dark-field illumination, however, distinct bright granules were visible with much lower magnification. Fig. 1 was made from a photograph of a fragment of collodion which was cut by hand from a wet permeable membrane and mounted in water. The magnification was about 430 diameters, obtained with a 16 mm. objective and a No. 18 Zeiss ocular, the illumination being that from a Zeiss dark-field condenser. All the membranes so examined had a similar appearance; it made no apparent difference whether the membrane was permeable or impermeable, whether the sections were cut with a microtome from membranes imbedded in paraffin, whether they were cut by hand from wet membranes, or whether the microscope was simply focussed on the surface of an ordinary thick membrane. Staining with eosin or methylene blue in alcoholic solution, or with reduced silver (from $AgNO_3$ and pyrogallol developer) caused no apparent difference in the structure. Fig. 1 may be interpreted as showing that the membranes are made up of granules or filaments of collodion, the pores being presumably located in the dark network surrounding the collodion. It has not seemed possible to draw any conclusion concerning the size or number of the pores from these observations, except that the order of their size must be less than 10^{-4} cm. The specimen photographed, like all the others examined, appeared to have about one bright spot per μ, but it is believed that the collodion filaments are much closer together than this would indicate, since the use of an 8 mm. objective did not appear to increase the size of the bright spots but did show a much greater number of hazy spots of less brightness. The whole field, however, was so indistinct that counting was impossible, and with a 4 mm. objective it was not possible to obtain any sharp focus at all.

Validity of the Assumptions Used.

In the preceding calculations use was made of Poiseuille's law, which has been derived only for straight capillary tubes of uniform diameter. Experimentally, the rate of flow of water through these membranes has been found to be proportional to the pressure,[1, 2, 7, 8] and inversely proportional to the thickness; that is, the use of two or three membranes in place of one reduces the flow to one-half or one-third its original value. Duclaux and Errera[8] found that the rate of flow of different liquids through similar membranes varied inversely as the viscosity of the liquids. So far the data are in agreement with Poiseuille's law. It might be assumed, however, that the fourth power relation would not apply to interstices between granules or filaments. Guérout[2] tested this point by measuring the rate of flow of water through sand whose grains had a mean diameter of 0.1 mm. He calculated that the capillary spaces between the grains must be about 0.0004 sq. mm. in cross-section, while his experiments on the rate of flow gave a value, based on Poiseuille's law, of 0.0002 sq. mm. This deviation may be partially accounted for by assuming that in the case of spherical grains the length of the capillaries ought to be greater than

[7] Bigelow, S. L., *J. Am. Chem. Soc.*, 1907, xxix, 1675.

[8] Duclaux, J., and Errera, J., *Rev. gén. colloïdes*, 1924, ii, 130; 1925, iii, 97. In the latter paper these authors used Guérout's method to obtain figures for cellulose acetate membranes; they found $r = 1.3 \times 10^{-6}$ cm. and $n = 10^{11}$ pores per sq. cm.

the thickness of the layer of sand in the ratio of half the circumference to the diameter, or of π to 2. In any case these considerations render it probable that the values given for the mean pore radii in Table II are at least of the right order of magnitude.

The work of Bartell and Carpenter,[3] however, led them to calculate much larger pore radii for similar membranes. Their figures for the pore diameters in three membranes are 0.701, 0.934, and 1.681 μ, or, for the radii, 35, 47, and 84 $\times 10^{-6}$ cm. These figures are from 40 to 130 times as large as those in Table II. They were obtained by measurement of the pressure required to force air through a wet membrane, the relation involved being essentially the same as that used in determining surface tension by the capillary rise method. Their figures are based on the assumptions that the pores behave like capillary tubes, that they retain their original size under the pressures used (from 1.76 to 4.23 kg. per sq. cm.), that the angle of contact between water and collodion is zero, and that the surface tension of water is the same in such capillaries as it is in larger volumes. Attempts were made to apply their method to some of the membranes used in this work, but no definite flow of bubbles was obtained even at much greater pressures. This fact, together with the admitted stretching of the membranes under such pressures, renders it likely that the calculations based on Poiseuille's law are more nearly correct.

SUMMARY.

By the application of Poiseuille's law to the rate of flow of water through collodion membranes, it is calculated that the membranes used had pore radii of the order of 0.3 to 2 $\times 10^{-6}$ cm. On the same basis the number of pores per sq. cm. appears to vary from 270 $\times 10^{10}$ to 7 $\times 10^{10}$, decreasing with increase in pore size. Reasons are given for preferring these figures for the radii to figures, 100 times as large, which were calculated by others. Microscopic examination of the membranes, with dark-field illumination, indicates that they are made up of solid granules or filaments of collodion much less than 1 $\times 10^{-4}$ cm. in thickness.

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