STUDIES ON PERMEABILITY OF MEMBRANES.

VI. Mensuration of the Dried Collodion Membrane (Calculation of Dimensions and of Relations to Certain Biological Membranes).

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In a previous paper of this series a flat form of the dried collodion membrane and its method of preparation was described (1). As this form of membrane presented certain advantages in durability and convenience of manipulation over the previously used bag membranes, it was the type used in most of the subsequent investigations. Electric transfer experiments in which relatively strong electric currents were used (2, 3) and diffusion experiments continued over a period of several months (4) were both made possible because of the resistant and stable properties of these membranes. In experiments to be reported on in the near future the same type of membrane was used for conductivity measurements. In view of the many experiments made with these membranes it has seemed desirable to publish a brief account of measurements of the dimensions and physical properties of some of them. Such measurements are necessary when one wishes to compare diffusion rates, conductivity values, etc., with results obtained with other types of membranes. In the last paper of this series (4), dealing with the diffusion of non-electrolytes, the rates of diffusion of acetone, glycerol, urea and glucose were measured for a number of these membranes but as the result desired was a ratio of the rates at which different substances diffused it was not necessary to reduce the results with any one membrane to terms of unit area and thickness. Nevertheless some points of interest can be brought out by so doing as will be shown presently.

The membranes used in the present study were selected from those

previously used in the non-electrolyte diffusion experiments. The following measurements were made:

1. Area.—The membrane was removed from its bell-shaped glass supporting frame by cutting the attachment around the outside rim with a knife. With scissors the membrane was then trimmed in such a way as to remove as nearly as possible those bits of collodion whose only purpose was to glue the membrane to its frame. There remained a smooth, flat and approximately circular piece of collodion comprising at least most of the previously functioning surface. This was placed in water. For an estimation of the functioning area of the membrane it would have been sufficiently accurate to consider the membrane as a perfect circle and calculate the area from measurements of the diameter. However, as it was desired to use the exact area of the piece removed in calculating the membrane thickness, another method of obtaining the area was employed. A piece of good grade rather heavy white paper was selected and from it a square exactly 15 cm. on each side was cut. The weight of this piece of paper was determined. The membrane was then quickly blotted, placed on a piece of glass with the paper over it and the outline traced with a pencil. The tracing was repeated so that four outlines were made on the one piece of paper. These were now carefully cut out and weighed. The area of the piece of membrane was then calculated from the proportion.

$$\text{Membrane area: } 225 \text{ cm}^2. = \frac{\text{Weight of 4 tracings}}{4} : \text{Weight of } 225 \text{ cm}^2.$$

2. Weight of Wet Membrane.—By the weight of a wet membrane is meant the total weight of a membrane whose pore channels are still filled with water. Following the method of Hitchcock (5) each membrane was quickly blotted between filter papers, wiped dry of surface water, placed in a covered moisture dish and weighed. The average of several such weighings was taken as the correct weight. At first glance it would seem that such weighings must be extremely inaccurate because varying amounts of water might be removed from the pores by the filter paper. It is of interest, then, that the average deviation from the mean rate was never greater than 0.36 per cent and for all the membranes studied averaged 0.12 per cent.

3. Weight of Membrane Immersed in Water.—Several weighings were made of the membrane when it was suspended in water by a fine hair, the average figure being entered as the correct weight. The temperature of the water was recorded.

4. Weight of Dry Membrane.—After all other measurements had been completed the membrane was dried to constant weight in an oven at 60°C. This usually was reached in about 6 hours. With the type of collodion used it was not possible to dry at 100°C. as did Hitchcock. This temperature caused the collodion to become yellow and to lose weight steadily.

From the above measurements the following membrane dimensions were computed:

(a) Thickness: The weight of the wet membrane in air minus the weight when immersed in water equaled the displacement of water. The latter figure when corrected for temperature gave the volume of the membrane. The volume divided by the area gave the thickness. The average thickness of all the membranes studied was 0.0903 mm. This method of obtaining an accurate estimate of the thickness of a thin membrane is essentially the same as that used by Bjerrum and Manegold (6). These authors did not determine the weight of their membranes suspended in water but by using in their calculations a fixed value for the density of collodion reached the same result.

(b) Average cross-section area of pores: On the assumption that the weight of the wet membrane is merely equal to the weight of the dry membrane plus the weight of the water within the channels the latter weight was found and from it by temperature correction (in this case of negligible importance) the volume of the pores was computed. The pore volume divided by the membrane thickness gave an estimate of the average pore area on cross-section. Whether the channels are sufficiently uniform in calibre to allow this figure to be regarded as the area at any cross-section, and more especially the pore area at the two surfaces, it is of course not possible to say.

(c) Part of membrane occupied by pores: The proportion of the membrane occupied by pores was determined by finding the ratio between the pore volume and the volume of the whole membrane. The same percentage result could also be obtained by finding the ratio between the pore area and the area of the whole membrane. For the membranes studied it was found that the pores occupied from 10.5 per cent to 15.4 per cent of the entire membrane (average 13.1 per cent). In studying a series of collodion membranes of varying permeability but of the usual type used for dialysis Hitchcock (5) found that the more permeable membranes of his series were composed of 7 to 8 times as much water as collodion while the less permeable contained half as much water as collodion. In this respect then the figures given here represent a continuation of Hitchcock's series, the dried type of membrane being much less permeable and containing a much smaller proportion of water.

(d) Density of the wet membrane: The density of the wet membrane was determined by dividing the weight of the wet membrane by

its volume (loss of weight in water corrected for temperature). This figure represents the density of the membrane as a whole and in the form that it has been used in our experiments. It is of course a component result depending on the density of dry collodion and of water and of the proportions of the two in the membrane. In our series it varied from 1.567 to 1.602 and averaged 1.583.

(e) Density of the dry membrane: This figure represents merely the density of the dried collodion from which the membranes were made. As the data already collected permitted its estimation for each membrane, it was determined in each case, the result being a check on the accuracy of the original measurements. The weight of the membrane immersed in water subtracted from the weight of the membrane after drying gave (after the usual temperature correction) the volume of the collodion framework of the membrane exclusive of the pore volume. The dry weight divided by this volume gave the density. In the present series the variations were from 1.669 to 1.675, the average density being 1.672. The average deviation from the mean was only 0.11 per cent. The figure is in satisfactory agreement with that of 1.653 given by Hitchcock (5) for the collodion used in his experiments and of 1.72 for that used by Bjerrum and Manegold (6).

DISCUSSION.

The various measurements enumerated in the foregoing paragraphs have been tabulated in Table I. Although we have given these measurements principally to place on record the dimensions of the membranes used in our various experiments, still a few items of interest may be brought out in connection with them.

In the last paper of this series in publishing the results of diffusion experiments the amounts of acetone, urea, glycerol and glucose passing the membranes were compared. The results for each membrane were not expressed in terms of unit thickness and area as only the ratio between the rates holding for two different substances was being studied. In explaining the large differences in the diffusion rates between the substances of larger and smaller molecular size, it was supposed that the rate of diffusion of each substance was proportional to a certain "available pore area." The available pore area differed from the total pore area by the amount of pore area distributed among

holes too small to permit the passage of that substance. Glucose diffused more slowly than glycerol and glycerol many times more

TABLE I.

Membrane	Area	Thickness	Density wet	Density dry	Total area of pores at any cross-section	Part of membrane occupied by pores
	cm.²	*mm.*			*cm.²*	*per cent*
F-1	29.491	0.1056	1.572	1.673	4.401	14.9
F-2	28.930	0.1185	1.584	1.670	3.720	12.9
F-3	31.112	0.0894	1.581	1.670	4.113	13.2
F-5	32.847	0.0950	1.594	1.674	3.880	11.8
F-7	30.043	0.1036	1.589	1.672	3.704	12.3
F-8	27.871	0.0681	1.602	1.674	2.934	10.5
F-9	29.779	0.0886	1.567	1.669	4.486	15.1
F-10	30.275	0.0816	1.593	1.673	3.607	11.9
F-11	26.462	0.1044	1.591	1.671	3.120	11.8
C-6	30.232	0.0766	1.570	1.675	4.655	15.4
C-7	27.514	0.0615	1.571	1.669	4.027	14.6

TABLE II.

Membrane No.	Diffusion of glycerol			Diffusion of acetone		
	Rate for entire membrane	Rate for unit portion of membrane	Average deviation from mean unit rate	Rate for entire membrane	Rate for unit portion of membrane	Average deviation from mean unit rate
			per cent			*per cent*
F-1	1.71	0.410		123.0	29.48	
F-2	1.50	0.477		83.2	26.47	
F-3	1.56	0.339	43.6	125.3	27.20	13.3
F-5	0.78	0.191		76.8	18.79	
C-6	7.04	1.157		167.4	27.52	
C-7	2.84	0.433		243.5	37.13	

In the first column for each substance is given the diffusion rate for each of the membranes as previously determined. In the second column this rate has been standardized by multiplying by the membrane thickness (expressed in units of 0.1 mm.) and dividing by the cross-section pore area. It will be seen that the standardized rates are considerably more uniform in the case of acetone than with glycerol.

slowly than acetone because a much larger percentage of the total pore area was available for the substances of smaller molecular size.

In other words it can be inferred from this theory that as the molecular size of the diffusing substance decreases the value of the available pore area will approach that of the total pore area. It would then seem that in studying the diffusion rate of substances of small molecular size one might hope to find some relation to the total pore area whereas with substances of larger molecular size no such relation should exist. The experiments with acetone and glycerol seemed best suited for testing this assumption. Eight membranes were used in these experiments; six were measured by the method described in this paper. In making this test the values for the diffusion rates of acetone and glycerol given in the last paper (4) (Table IV) were reduced to terms of unit thickness (0.1 mm.) and unit pore area (1.0 sq. cm.) by using the dimensions given in Table I of this paper. The results are listed in Table II. It will be seen that according to expectation the standardized rates for acetone are much more uniform than those for glycerol. With glycerol the average deviation from the mean result is 43.6 per cent while with acetone it is only 13.3 per cent. Better agreement than this could scarcely be expected for it must be remembered that according to the theory some part of the total pore area must be taken up by pores too small to permit the passage even of acetone.

It has already been mentioned that one purpose in recording these measurements is to make possible comparisons with other types of membranes and especially with biological membranes. Such comparisons are desirable and even necessary to determine within what limits the dried collodion membrane may be taken as a model for very much more complicated cell membranes. Unfortunately peculiar obstacles stand in the way of him who would determine the dimensions of cell membranes. These membranes are extremely thin and delicate and any kind of direct measurements are impossible. Within recent years several investigators, notably Fricke (7) and McClendon (8), have described a method of approaching the problem indirectly. Both of these investigators have estimated the thickness of the red blood cell membrane from measurements of the electric capacity made with high frequency currents. At best the values given must be regarded as approximations only, for even if the factor of experimental error be eliminated they depend upon assumed values

for the dielectric constant of the membranes. Nevertheless, the method is extremely ingenious and is the best approach to a difficult problem that has been made so far. McClendon has calculated the cell membrane thickness as 3×10^{-8} cm. if he assumes a dielectric constant of 3 and as 3×10^{-7} cm. if he takes the dielectric constant as 10. Fricke, assuming a dielectric constant of 3, but with capacity measurements somewhat different from those of McClendon, calculated the thickness as 3.3×10^{-7} cm. In other words the membrane thickness is almost within the range of molecular dimensions. Recognizing the approximate nature of these figures it is interesting to use them for making some rough comparisons between the dried collodion membrane and the membrane of the red blood cell.

In previous papers there have been enumerated some of the potential effects observable when the dried collodion membrane represents the interphase in concentration chains and in chemical chains. It will be recalled that these potential differences do not depend on the thickness of the interphase but merely on the nature and concentration of the adjacent solutions and on certain properties of the membrane itself. On the other hand the force acting on any charged particle within the membrane depends on the intensity of the electric field at this point and the field intensity does depend on thickness. The electric field intensity (ϵ) is given by the formula

$$\epsilon = \frac{E}{d}$$

where E is the potential difference between the two borders of the membrane and d the membrane thickness. It is thus evident that, for a given potential difference, the thinner the membrane the greater the intensity of the electric field and consequently the greater the force acting upon a charged ion within its borders. If we take the thickness of the average dried collodion membrane of the type used in these experiments as 1×10^{-2} cm. and that of the red blood cell membrane as 3×10^{-7} cm. it is evident that equal potential effects will in the case of the cell membrane give rise to an electric field with an intensity 30,000 times greater than in the case of the collodion membrane. In other words very small electromotive effects may have a large significance with biological membranes.

In previously reported experiments in which the transfer number of chlorine in several chloride solutions in different concentrations was determined by means of direct electric transfer experiments it was shown that the electric current itself was capable of altering appreciably the transfer number that existed at the moment of first application of the current. Even with currents of 2 to 4 milliamperes, which were used in most of the experiments, the results of electric transfer experiments were in certain ranges of concentration quite measurably different from transfer numbers estimated for the same membrane from concentration chains. Inasmuch as the rate at which a given electrolyte can diffuse across a membrane depends upon the transfer numbers of its ions (being a maximum when they are each equal to 0.5) it follows that an applied electromotive force can materially change the rate of diffusion of a substance dissociated into ions. For example with one membrane in a medium concentration range with KCl an applied electromotive force of 1 volt was necessary to establish a current of 4 milliamperes across the membrane. Supposing that this membrane was roughly 0.1 mm. thick, this is equivalent to an electric field intensity of 100 volts per cm. To establish the same electric field intensity across the membrane of the red blood cell (thickness taken as 3×10^{-7} cm.) would require a potential difference of only 0.03 millivolt. Potential differences of this magnitude and greater must frequently arise in the animal organism. In fact E. J. Warburg (9) has estimated that a difference in pH of from 7.2 to 7.4 between cells and plasma may give rise to a potential difference of 2 millivolts. It is possible though of course only a surmise that such electromotive effects arising as a result of nerve impulses or because of changes in the concentration of the tissue fluids may be of paramount importance in controlling rates of excretion and secretion by certain cells and be intimately associated with such complex phenomena as the chloride shift.

From the standpoint of the diffusion of non-electrolytes it is interesting to see in how far the dried collodion membrane might serve as a model of a cell membrane if its dimensions were similar. At first glance the recorded rates for the diffusion of glucose with these collodion membranes seem extremely slow and this slowness seems at variance with the rapidity with which the amount of glucose on the

two sides of the red blood cell membrane may become equalized. It will be recalled that with the collodion membranes the fastest rate recorded was only 1.57×10^{-5} millimols per hour for the whole membrane with a diffusion pressure of 2.4 atmospheres. This amounts to 1.56×10^{-6} mg. per minute per sq. cm. of membrane surface at the same pressure. The thickness of this membrane was 7.7×10^{-3} cm. Now it has been estimated that in 1 cc. of blood there are 5,000 sq. cm. of membrane surface (8). If we again take the thickness as 3×10^{-7} cm. and suppose that some physiological event has raised the plasma glucose 72 mg. per 100 cc. above the cell glucose (producing a diffusion pressure of 0.1 atmosphere at 37 C.) we can calculate that if the substance of the red blood cell membrane were dried collodion glucose would diffuse across the membrane at a rate of 8.5 mg. per minute per cc. of blood as long as the pressure was maintained. That is, the entire 72 mg. per 100 cc. originally present as an excess in the plasma could have diffused across the cell membranes in about 5 seconds. Of course the pressure is not maintained for as diffusion continues the pressure falls. But at least we can say that within several minutes the difference in concentration between cells and plasma would no longer be detectable and this is quite in accord with what little is known concerning the rapidity with which glucose may be exchanged between cells and plasma across the normal red blood cell membrane.

SUMMARY.

The flat type of dried collodion membrane used by Michaelis and his associates in numerous investigations has been subjected to mensuration in order that the dimensions of these membranes may be placed on record. The membranes had a functioning area of about 30 cm., were approximately 0.1 mm. in thickness and were composed on the average of 87 per cent by volume of collodion and 13 per cent by volume of pores.

In reviewing some of the previously reported results of diffusion experiments with non-electrolytes in the light of the calculated values for the total pore area for the same membranes additional evidence was presented to show that a smaller molecule (acetone) probably utilizes a much larger percentage of the total pore area for its diffusion than is available for a larger molecule (glycerol).

By using the figures of Fricke and McClendon for the thickness of the membrane of the red blood cell some comparisons were drawn between the dried collodion membrane as a model for certain biological membranes and the red blood cell membrane. In these comparisons emphasis was placed on the exaggerated importance of small electromotive forces and very slight permeabilities when these were associated with membranes of such extreme thinness as the red blood cell membrane.

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