

## THE STRUCTURE OF THE COLLODION MEMBRANE AND ITS ELECTRICAL BEHAVIOR

### XII. THE PREPARATION AND PROPERTIES OF "MEGAPERMESELECTIVE" PROTAMINE COLLODION MEMBRANES COMBINING HIGH IONIC SELECTIVITY WITH HIGH PERMEABILITY

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#### I

The preceding paper<sup>1</sup> of this series describes the preparation of "megapermselective" collodion membranes which are extremely impermeable to anions, but at the same time are very permeable to univalent cations. The purpose of the present paper is to describe the preparation and the properties of analogous electropositive "megapermselective" membranes which are very impermeable to cations, but at the same time very permeable to univalent anions.

Abrams and Sollner<sup>2</sup> have recently shown that electropositive membranes similar to the activated dried collodion membranes<sup>3</sup> can be prepared by the adsorption of protamine on highly porous collodion membranes and their subsequent drying in air. Such membranes show considerable electropositive activity in solutions ranging from pH 2–10. The "characteristic concentration potential" (0.1 M KCl/0.01 M KCl) of these membranes varied with different specimens from  $-47$  to  $-52$  mv.;<sup>4</sup> the membranes retained their electromotive properties on prolonged contact with water and electrolyte solutions. Their resistance was high and correspondingly their absolute permeability was low, having approximately the same magnitude as that of "activated" collodion membranes.<sup>3</sup> The dried protamine collodion membranes of Abrams and Sollner were of irregular shape and quite wrinkled in appearance; they were fragile and liable to easy destruction. It was also impossible with these membranes to measure accurately the surface area which should be known for many experiments. The present paper describes membranes which are free of these enumerated shortcomings. They are designated "permselective" or "megapermselective" protamine collodion membranes.

<sup>1</sup> Carr, C. W., and Sollner, K., *J. Gen. Physiol.*, 1944, **28**, 119.

<sup>2</sup> Abrams, I., and Sollner, K., *J. Gen. Physiol.*, 1943, **26**, 369.

<sup>3</sup> Sollner, K., Abrams, I., and Carr, C. W., *J. Gen. Physiol.*, 1941, **25**, 7.

<sup>4</sup> To indicate that the concentration potential is opposite in sign to that of the negative membranes, the concentration potential values in the case of the positive membranes are preceded by a minus sign.

## II

In the present investigation we have combined the method used for the preparation of megapermselective collodion membranes described in the preceding paper<sup>1</sup> with the technique of Abrams and Sollner<sup>2</sup> for the preparation of positive membranes. With this new combined technique megapermselective protamine membranes are obtained consistently which combine great absolute permeability with significantly higher "characteristic concentration potentials" than those obtained in the earlier work;<sup>2</sup> these membranes also have a well defined shape, are smooth in appearance, and can stand considerable handling without danger of breakage.

Three-layer porous collodion membranes were cast on the outside of rotating tubes in exactly the same manner as described for the preparation of megapermselective collodion membranes.<sup>1</sup> A 4 per cent solution of Baker Collodion U.S.P. in ether-alcohol (50:50) was used. The porous membranes still on the tubes were immersed in repeatedly changed distilled water for 1 hour to remove the organic solvents. Then the membranes were placed in a 2 per cent solution of protamine sulfate<sup>5</sup> in a 0.025 M sodium borate-sodium hydroxide buffer of pH 11 and placed in an ice box at  $+2^{\circ}$  to  $+5^{\circ}\text{C}$ . After 48 hours they were taken from the solution, washed thoroughly, and dried for 5 hours in air while still on the tubes. Next, they were soaked in distilled water for several hours. Longer soaking in water than with the negative membranes was usually required before the membranes could be taken off the glass tubes. (Occasionally it was necessary to dip the membranes in 98 per cent alcohol for a few minutes to facilitate this removal.<sup>6</sup>) After this had been done, the membranes were fitted with glass rings for easier handling. Only a fraction of the membranes had at this stage the desired degree of ionic selectivity.<sup>7</sup> Further drying in air for at least 3 hours without support regularly results in membranes

<sup>5</sup> The authors are indebted to Eli Lilly and Company, Indianapolis, Indiana, for furnishing samples of this material.

<sup>6</sup> This brief immersion in 98 per cent alcohol swells the protamine covered membranes just enough to facilitate their easy removal from the tubes. Collodion membranes under similar treatment swell strongly and become quite soft and flabby. The absorbed protamine obviously greatly retards the alcohol in reaching the collodion. This interesting effect throws some light on the compactness of such adsorption layers. It warrants further investigation from the point of view of surface chemistry.

<sup>7</sup> The membranes in this state show great differences in selectivity. They give only in rare instances "characteristic concentration potentials" (0.1 M KCl/0.01 M KCl) of  $-52$  mv. or better, though their ionic selectivity is frequently satisfactory with 0.005 N and more dilute solutions. Their resistance is always very low. For work in electrolyte solutions of 0.005 N or lower normality they are therefore in many instances satisfactory, particularly when emphasis must be laid on the lowest possible resistance.

having fully satisfactory electrical and mechanical properties; though perceptibly shrunken they are perfectly smooth and test tube-shaped. These megapermselective protamine membranes are kept either dry or in water to which a crystal of thymol has been added as a preservative. The membranes frequently show a yellowish or brownish discoloration; this however does not affect their other properties or their usefulness. The megapermselective protamine membranes seem to be indefinitely stable when kept in air or water; they also show only very slow deterioration in electrolyte solutions. They can be considered as the electropositive analogues of the (electronegative) megapermselective collodion membranes described in the preceding paper.<sup>1</sup> At present the preparation of the megapermselective protamine membranes requires some skill and patience for optimum results. But there is little doubt that additional experience, as the membranes are used for further work, will gradually eliminate some of the still existing difficulties.

The limits within which changes in the described technique can be made are not nearly so wide as with the negative membranes. The conditions of preparation are apparently critical. The casting of the porous membranes is important. If they are too dense, only low concentration potentials are obtained, probably because many pores are too narrow to admit the protamine molecules. To obtain sufficient porosity with the brand of collodion used the ratio of alcohol to ether in the collodion solutions had to be equal to or somewhat greater than unity. The membranes after drying should have a thickness of 30 to 50  $\mu$ . Membranes thinner than 30  $\mu$  in many instances give low characteristic concentration potentials; they also break more easily on handling. Thicker membranes frequently give low concentration potentials presumably because the protamine had not been able to penetrate sufficiently. The new type positive membrane requires the use of protamine solutions of higher concentration than those recommended by Abrams and Sollner. With 0.2 to 0.5 per cent solutions only occasionally were membranes obtained giving characteristic concentration potentials of  $-52$  to  $-53$  mv. With 2 per cent solutions, however,  $-52$  to  $-53$  mv. was obtained consistently. For optimum results the pH of the protamine solutions should be controlled. In unbuffered solutions, the pH may change by two units, due to the reaction of the alkali solution with the collodion. The use of buffers is therefore indicated. The optimum pH range is rather broad. Above 11.5 the action of the alkali is too severe resulting in very weak membranes; below 10.5 the adsorption becomes too weak to give membranes with the highest selectivity. Membranes prepared in protamine solutions of pH 10.5–11.5 are satisfactory. The buffered protamine solutions can be used repeatedly because only a small amount of the protamine is adsorbed. After repeated use their pH should be adjusted if necessary. The protamine solutions should be kept at ice box temperature, whether in use or not.

In Table I are given the characteristic concentration potentials of some representative megapermselective protamine membranes prepared according to the above given procedure, as well as their resistances when immersed in 0.1 M KCl. The resistances are given as ohms per membrane of about 50 cm.<sup>2</sup>

active area. The potential and resistance measurements were carried out in the manner indicated in the preceding paper.<sup>1</sup> The resistance of these membranes assumes its final, lowest value only slowly, the figures in Table I representing approximately equilibrium values. This time effect warrants further investigation.

TABLE I  
Characteristic Concentration Potential and Resistance of "Megapermselective" Protamine Collodion Membranes

Membrane	Characteristic concentration potential 0.1 M KCl/0.01 M KCl $\pm 0.1$ mv.	Resistance in 0.1 M KCl solution $\pm 0.5 \Omega$
	<i>mv.</i>	$\Omega/50$ cm. <sup>2</sup>
a	-52.9	10
b	-53.0	12
c	-52.5	14
d	-53.0	12
e	-52.8	10
f	-53.0	5
g	-53.0	2 $\pm 0.2$
h	-52.8	0.5 $\pm 0.2$
i	-53.0	0.5 $\pm 0.2$

### III

Table I shows that the megapermselective protamine membranes consistently combine desirable electromotive properties with satisfactorily low resistance. It is three to four orders of magnitude lower than the resistance of the dyestuff- or alkaloid-impregnated positive membranes described in the literature. The "characteristic concentration potential" reaches in all cases -52 to -53 mv., the resistance in 0.1 M KCl solution being 0.5 to 15  $\Omega$  per 50 cm.<sup>2</sup> of membrane.

Shape, strength, and stability of these membranes as was indicated above are fully satisfactory.

It must be noted that the characteristic concentration potential of these membranes never reaches the thermodynamically possible maximum of -55.1 mv., the "cation leak" corresponding to -52 and -53 mv. being about 3 and 2 per cent respectively.<sup>1</sup> We shall take up this problem in the next section.

The new megapermselective protamine membranes were also tested for their absolute permeability in a few preliminary experiments on anion exchange across them. A membrane, showing a characteristic concentration potential of -53 mv. and a resistance of 2  $\Omega$  in 0.1 M KCl was filled with 30 ml. of 0.1 M NH<sub>4</sub>Cl solution and placed in 30 ml. of 0.1 M KNO<sub>3</sub>. After 4 hours 0.8 milliequivalent of Cl<sup>-</sup> had exchanged through the membrane, whereas only 0.025 m.eq. of NH<sub>4</sub><sup>+</sup> had entered the KNO<sub>3</sub> solution. This "cation leak" corresponds to

3.2 per cent. Contrary to the situation found with the megapermselective collodion membranes, the positive membranes also show an appreciable leak of univalent cations in more dilute solution. The leak of bivalent cations as  $\text{Ca}^{++}$  or  $\text{Ba}^{++}$  is always much smaller than of the univalent cations.

## IV

The highest characteristic concentration potential that has been obtained with the megapermselective protamine membranes is  $-53$  mv. Since this value was obtained rather consistently, some further experiments were carried out to gain if possible some insight into the causes of this peculiarity of the protamine membranes.

TABLE II  
*Concentration Potentials at Different KCl Concentrations across "Megapermselective" Collodion and Protamine Collodion Membranes*

Concentration of KCl solutions		Concentration potential		
$C_1$	$C_2$	Calculated maximum values mv.	"Megapermselective" collodion membrane $\pm 0.1$ mv.	"Megapermselective" protamine collodion membrane $\pm 0.1$ mv.
<i>mols/l.</i>	<i>mols/l.</i>		<i>mv.</i>	<i>mv.</i>
0.64	0.32	15.9	12.6	-11.5
0.32	0.16	16.0	14.4	-13.8
0.16	0.08	16.2	15.6	-14.3
0.08	0.04	16.3	16.3	-15.8
0.04	0.02	16.6	16.6	-16.0
0.02	0.01	16.9	16.9	-16.2
0.01	0.005	17.1	17.1	-16.2

It is well known that the ionic selectivity of membranes increases rapidly as the absolute concentration of the electrolyte is decreased. Collodion membranes which show relatively low ionic selectivity in 1 M solutions become nearly completely anion-impermeable in 0.01 or 0.005 M solutions. To study this effect with the positive megapermselective membranes a series of concentration potential measurements was made with solutions having a 1:2 concentration ratio. To be able to compare the results with the behavior of negative membranes under similar conditions, parallel measurements were made with megapermselective collodion membranes. The results are given in Table II. The thermodynamically possible maximum values of the concentration potentials at the different concentration levels are also included in Table II. The activity coefficients used for the calculation of these values were taken from standard tables.

Table II indicates that the maximum thermodynamically possible value cannot be reached with protamine membranes even at very low concentrations.

The megapermselective protamine membranes show a significant cation leak<sup>1</sup> under all conditions.

The obvious and simplest explanation is found in the assumption that some of the critical spots in the heterogeneous<sup>8</sup> pores of the membrane do not carry a positive charge. They may be either uncharged or carry a negative charge. This could be due to several different factors. The protamine molecules having a molecular weight of around 3000 are probably too large to enter many narrow constrictions of the pores even of the porous membranes; thus some critical spots would not carry any positive charge. The alkali and OH<sup>-</sup> ions which are present in the same solution, however, can reach many narrow places and oxidize the pore walls there and thus leave them negatively charged. Or, the protamine carries inherently, as is most likely the case, some carboxyl groups which would occupy the critical spots in some pores; or, the protamine contains some impurity carrying acidic groups which would bring about the same result. At present no decision can be made between these different possibilities. We plan to study this problem further, mainly by the use of different protamine preparations.

## v

The availability of the electropositive megapermselective protamine membrane opens up a still wider field for further investigation than is opened up by the availability of the electronegative megapermselective collodion membrane, for much less is known about the properties of electropositive than of electronegative membranes.

The problems which can successfully be attacked now are quite analogous to those mentioned in connection with the megapermselective collodion membrane.<sup>1</sup> They do not need to be enumerated again.

However, it may be mentioned that megapermselective protamine collodion membranes have been used already with considerable success in the potentiometric determination of anions, such as Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, F<sup>-</sup>, ClO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, BrO<sub>3</sub><sup>-</sup>, IO<sub>3</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and Ac<sup>-</sup>.<sup>9</sup> For the determination of several of these ions no other potentiometric method is known.

The availability of both positive and negative membranes of high ionic selectivity and great permeability now also permits the experimental investigation of various mosaic structures,<sup>10</sup> which may play an important rôle in biological processes. The theory of some mosaic systems was worked out several years ago;<sup>11</sup> for lack of suitable membranes, however, it could not be tested heretofore.

<sup>8</sup> Sollner, K., and Anderman, J., *J. Gen. Physiol.*, 1944, **27**, 433. Sollner, K., and Carr, C. W., *J. Gen. Physiol.*, 1944, **28**, 1.

<sup>9</sup> Sollner, K., *J. Am. Chem. Soc.*, 1943, **65**, 2260.

<sup>10</sup> Höber, R., and Hoffmann, F., *Arch. ges. Physiol.*, 1928, **220**, 558.

<sup>11</sup> Sollner, K., *Biochem. Z.*, Berlin, 1932, **244**, 370.

The successful preparation of the megapermselective protamine membranes suggests the possibility of the preparation of analogous membranes in which various proteins, peptones, and deaminated proteins are used in place of the protamine. Such protein membranes, like the protamine membranes, would contain embedded in a rigid framework substances of particular biological interest which under conditions somewhat analogous to those found in living organisms could thus be investigated conveniently for their electromotive behavior. The use of deaminized proteins should permit the study of strongly electronegative membranes, more comparable to natural membranes than collodion.

#### SUMMARY

The technique of Abrams and Sollner for the preparation of electropositive dried protamine collodion membranes has been improved. Porous collodion membranes cast on the outside of rotating tubes are treated for 48 hours with a solution of 2 per cent protamine sulfate buffered at pH 11. After being washed thoroughly the membranes are dried in air for several hours, soaked in water for several hours, and removed from the tubes. Further drying in air but without support shrinks the membranes slightly. The resulting membranes are designated "permselective" or "megapermselective" protamine collodion membranes. These membranes regularly give characteristic concentration potentials of  $-52$  to  $-53$  mv. and (in  $0.1$  M KCl) resistance of  $0.5$  to  $15$  ohms per membrane of  $50$  cm.<sup>2</sup> area. This resistance is several orders of magnitude smaller than that of the conventional dyestuff- and alkaloid-impregnated positive membranes. The megapermselective protamine collodion membranes can be kept either dry or in water for prolonged periods without detectable deterioration. They are quite smooth, have a regular shape, and stand considerable handling without breakage.

The megapermselective protamine collodion membranes are the electropositive analogues of the electronegative megapermselective collodion membranes described by Carr and Sollner.