THE STRUCTURE OF THE COLLODION MEMBRANE AND ITS ELECTRICAL BEHAVIOR

VI. The Protamine-Collodion Membrane, a New Electropositive Membrane

By IRVING ABRAMS and KARL SOLLNER

(*From the Department of Physiology, University of Minnesota, Minneapolis*)

I

Previous publications[1,2] in this series have dealt with the preparation and electrochemical properties of the activated (electronegative) collodion membrane. The activation consisted of an oxidation of the nitrocellulose, a process which caused an increase in the number of dissociable groups in the pores of the membrane. Such membranes show more pronounced effects than similar untreated membranes when tested by electrochemical methods such as the extent of anomalous osmosis, the magnitude of concentration potentials, and the rate of electroosmosis. The purpose of the present communication is to present a method for the preparation and a description of electropositive membranes which are analogous in most respects to the activated collodion membranes.

The literature on electropositive membranes has recently been reviewed by Manegold and Kalauch.[3] Two general methods for the preparation of such membranes are available. First, they have been prepared from inherently electropositive materials, such as the oxides of aluminum, zirconium, or thorium.[4,5] The second method consists of impregnating membranes prepared from collodion or similar materials with some basic organic compound.

One objection to the use of the polyvalent metal oxide membranes is that the electropositive charge is rather easily reversed in the presence of many electrolytes. For this reason they are not suitable for most purposes.

The impregnated membrane, in many instances, has proven more practical. The impregnation of collodion with basic substances may be accomplished either by dissolving such substance in the collodion solution or by adsorbing the material on the surface of a previously cast membrane. Among the

[1] Sollner, K., and Abrams, I., *J. Gen. Physiol.*, 1940, **24**, 1.

[2] Sollner, K., Abrams, I., and Carr, C. W., *J. Gen. Physiol.*, 1941, **25**, 7.

[3] Manegold, E., and Kalauch, K., *Kolloid-Z.*, 1939, **88**, 257.

[4] Prausnitz, P. H., and Reitstötter, J., Electrophorese, Elektroosmose, Elektrodialyse, Dresden, Theodor Steinkopff, 1931.

[5] Sollner, K., and Liapountzeva, T., unpublished.

369

ether-alcohol soluble substances thus far employed are basic dyestuffs[6-8] and alkaloids.[9, 7] *Dried* membranes prepared from such solutions have on occasions given concentration potentials as large as -50 mv.[7] A disadvantage of this method, however, is that in aqueous media the basic substance slowly diffuses from the structure, and in time the membrane again becomes negative. This is the case with the dried membrane, and as might be expected, the loss of the impregnated material is even more rapid with the porous membrane.[10]

The adsorption method which has been used in the production only of *porous* electropositive structures consists of adsorbing a protein on the surface of a previously cast porous collodion membrane. Loeb,[11] for example, employed gelatin, egg albumin, casein, and oxyhemoglobin. Such membranes retain their characteristics practically indefinitely when kept in water. However, the proteins which have been employed are cationic only within restricted limits; *i.e.*, on the acid side of their isoelectric points. Thus membranes prepared with the more common proteins are electropositive only in distinctly acid solution. The preparation of dried membranes by this method has not been reported in the literature.

For the preparation of porous electropositive membranes, we have also employed the adsorption method, but with a protein having an isoelectric point well on the alkaline side of neutrality. Electropositive membranes which correspond in their properties to the dried collodion membrane can be prepared in a similar way by a modification of the adsorption method, as will be shown below.

The protein employed in this investigation was a protamine. Protamines are obtained from ripe fish sperm. They have an isoelectric point of 12.0 to 12.4,[12] and thus are cationic not only in acid, but also in neutral and even in weakly alkaline solution. They have a molecular weight of 2000–4000[13] and are decidedly water-soluble. Because of their basic character, the protamines have a tendency to combine with acidic substances. For these reasons, they seem ideally suited for our purpose.

[6] Mond, R., and Hoffmann, F., *Arch. ges. Physiol.*, 1928, **220,** 194.

[7] Wilbrandt, W., *J. Gen. Physiol.*, 1935, **18,** 933.

[8] Nakagawa, J., *Jap. J. Med. Sc., III Biophysics*, 1937, **4,** 343.

[9] Harkewitsch, N. K., *Kolloid-Z.*, 1929, **47,** 101.

[10] Several experiments of this nature were carried out and it was found that porous membranes prepared from a collodion solution containing a basic dyestuff or alkaloid retain electropositive characteristics for a period not exceeding 24 hours.

[11] Loeb, J., *J. Gen. Physiol.*, 1920, **2,** 577; and many later publications.

[12] Schmidt, C. L. A., The chemistry of the amino acids and proteins, Springfield and Baltimore, Charles C. Thomas, 1938, 618.

[13] Gortner, R. A., Outlines of biochemistry, New York, John Wiley and Sons, 2nd edition, 1938, 465.

II

The general method, as was indicated above, consists of the adsorption of protamine from an aqueous solution onto the surface of a previously cast porous collodion membrane. Membranes of high activity can be prepared by either of two methods. The one consists of an activation of the collodion membrane (by oxidation) previous to immersion in a solution of salmine sulfate.[14] The other method consists merely of an adsorption of the protein from an isoelectric (pH about 12) protamine solution.

Membranes were cast in test tubes (about 30 × 100 mm.) from a 5 per cent solution in ether-alcohol (75–25) of Baker, U.S.P., collodion. This solution was poured slowly from the tube, the latter being constantly rotated. After the solution ceased to drip from the tube, the solvent was allowed to evaporate for 2 to 3 minutes with the tube in an inverted position,[15] after which time it was immersed in distilled water. The resulting membrane was loosened from the tube and attached by the open end to a glass ring. Following a thorough washing to remove the excess solvent, the membranes in the case of the first method are oxidized with 0.5 M sodium hydroxide.[2] After another washing they are immersed for at least 12 hours in solutions containing 0.5 to 0.8 gm. of salmine sulfate per 100 ml. of solution. Finally, they are removed from the solution, washed, and kept in distilled water.

If the other method is employed, the oxidation step is omitted and the protamine solution used for the adsorption is first brought to its isoelectric point.[16] This may be accomplished either by adding a stoichiometric amount of barium hydroxide to the sulfate solution or by simply adding sufficient sodium hydroxide to bring the pH of the solution up to 12.0.[17] Otherwise, the process is identical.

Actually it is unnecessary to use concentrations of protamine as high as those men-

---

[14] We are indebted to Eli Lilly and Company of Indianapolis who kindly provided us with samples of this material.

[15] This technique differs slightly from that employed previously in that formerly the solvent was allowed to evaporate with the tube in an upright, somewhat inclined position. The resulting membranes were always considerably less dense near the bottom since the solvent evaporation was retarded at the bottom and hastened at the open end. The present technique allows the downward escape of the solvent vapors, and hence the porosity in various parts of the resulting membrane is more nearly the same. In all likelihood, the over-all structure of this membrane is similar to that of the middle section of the membrane used in former work. In addition to being more homogeneous (macroscopically), the present membranes are less likely to tear at the closed end.

[16] This method was employed by Hitchcock (Hitchcock, D., J. Gen. Physiol., 1925, 8, 61) who prepared gelatin- and egg-albumin-collodion membranes.

[17] Whether this second method is basically different from the first is open to some question. The alkalinity of the isoelectric solution undoubtedly brings about some oxidation of the collodion which would result in a strong adsorption of protamine. It is thus possible that the adsorption process in these alkaline solutions is enhanced by a simultaneous oxidation-activation of the collodion.

tioned; *i.e.*, 0.5 to 0.8 per cent solutions. Maximum activation can be obtained with solutions having a concentration of 0.1 gm. per liter. The more concentrated solutions were used to prevent the exhaustion of the protamine since each solution was employed repeatedly.

Membranes prepared by this method have retained their original electropositive characteristics for a 12-month period, bacterial contamination being prevented by the addition to the water of small quantities of thymol. Indications are that with proper precautions, these properties can be maintained indefinitely.

III

In dealing with the activated collodion membranes, we have employed[1,2,18] three methods for establishing the electrochemical activity of a given porous membrane. These consist of determining (1) the characteristic concentration potential across the membrane, (2) the rate of electroosmosis through the membrane, and (3) the extent of anomalous osmosis. Of these methods, the last is able to distinguish most decisively between specimens having only slightly different electrochemical characteristics. For this reason it has been used extensively in this series of investigations on the structure of the collodion membrane and is used also for the characterization of porous protamine-collodion membranes. For the details of the three methods mentioned, their merits, and their theoretical foundations, the reader is referred to the previous publications. The method used in determining the extent of anomalous osmosis is described below.

Any arbitrarily chosen electrolyte could be used for this purpose. To obtain decisive, characteristic, and easily reproducible effects, a dilute solution of an electrolyte having a divalent ion identical in the sign of its charge with the electrokinetic charge of the membrane is employed. Negative membranes were tested[2] with a salt having a univalent cation and a divalent anion, such as potassium sulfate. Correspondingly, with positive membranes, a di-univalent salt is used, calcium chloride being used in this investigation. With the uni-univalent salts, the effects are not nearly as pronounced. On the other hand, with salts having a trivalent ion, such as aluminum, the latter often adsorbs on the surface of the membrane and so unduly influences its electrochemical behavior.

In testing positive membranes, therefore, the following technique is employed. The membrane bag attached to a glass ring is filled with a solution of 0.01 M calcium chloride and is attached to a rubber stopper holding a capillary manometer tube (inner diameter about 1.6 mm.). The rate of anomalous osmosis is determined by measuring the rise of the meniscus in this tube 20 minutes after the filled membrane had been

---

[18] Sollner, K., Abrams, I., and Carr, C. W., *J. Gen. Physiol.*, 1941, **24**, 467.

immersed in a large volume (about 600 ml.) of distilled water. This rise of the meniscus is taken as an indication of the "activity" of the membrane tested.

The electrochemical behavior of the proteinized membrane varies with the ionic state of the adsorbed protein; therefore, one of the most important points was to determine the dependence of the behavior of protaminized membranes upon pH. Experiments were therefore performed to determine the relationship between anomalous osmosis and pH. A very active protamine-collodion membrane was filled with 0.01 M calcium chloride and tested in the manner described; the pH of the inside and outside media was adjusted to the value desired by the addition of sodium hydroxide or hydrochloric acid. The results
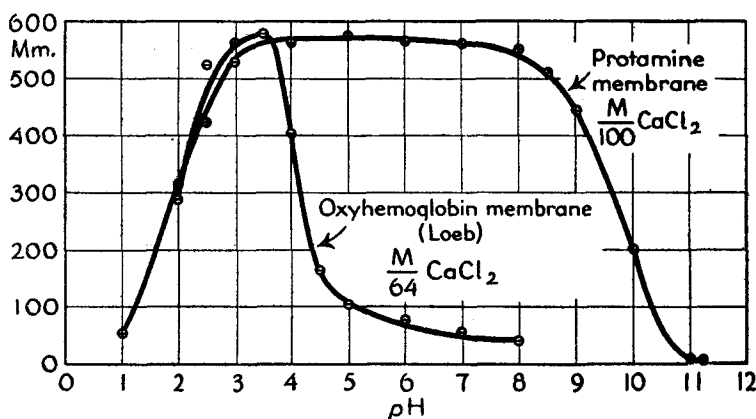

FIG. 1

are shown graphically in Fig. 1, pH being plotted on the abscissa and the values for the manometric rise after 20 minutes on the ordinate.

Similar experimental data obtained by Loeb[11] with an oxyhemoglobin-collodion membrane have also been plotted in Fig. 1. As is obvious, the range of pronounced electropositive activity in this case lies between pH 2 and 4, whereas the protamine membrane exhibits optimum effects between pH 3 and 8, the activity being depressed only in extremely acid and alkaline regions; distinct electropositivity remains evident down to a pH of 1 and up to 10. The exact magnitude of the effects at optimum pH obtained with the protamine and the oxyhemoglobin membrane are not strictly comparable since Loeb used M/64 solutions whereas 0.01 M solutions were employed in the other case.

In a previous publication,[1] the typical N shape of the liquid transport curves of Loeb were reproduced with an unoxidized and an oxidized collodion membrane. The electrolytes employed were the chloride, sulfate, and citrate of potassium. For the sake of comparison with regard to both the concentration effect and the general shape of the curves, we carried out analogous experi-

ments with an active protamine-collodion membrane, the salts employed in this case being potassium, sodium, calcium, and cerous chlorides. In every case, the neutral salt solution was used. The results of these experiments are shown in Fig. 2. The osmotic behavior of the membranes with non-electrolytes is
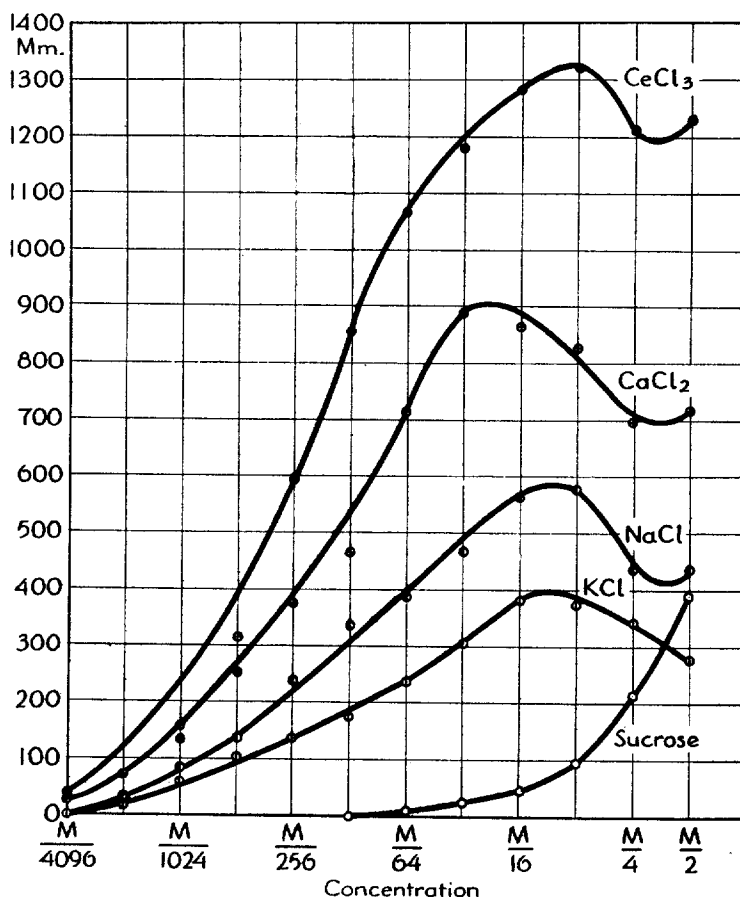
Fig. 2

indicated by the sucrose curve. The manometric rise of the meniscus, representing the rate of transport from the side of the water to the side of the solution, is plotted on the ordinate, with concentration (on a logarithmic scale) on the abscissa.

The curves of Fig. 2 assume the same general shape as those for the electronegative membranes. Even the magnitude of the manometric rises with potassium, calcium, and cerous salts, respectively, are exceedingly similar to those

obtained with the oxidized collodion membrane using potassium chloride, sulfate, and citrate. Thus, we can say that with respect to its anomalous osmotic activity, the porous protamine-collodion membrane is an electropositive counterpart of the porous activated collodion membrane described previously.[1, 2] We might add that the transports with the protamine membranes in neutral solution are considerably greater than those reported by Loeb for gelatin-, egg-albumin-, casein-, or oxyhemoglobin-collodion membranes in acidified salt solutions.

Negative osmosis, a transport of fluid through a membrane from a concentrated to a dilute solution, according to theoretical considerations of Sollner,[19] can be expected only under specific experimental conditions. Generally, with sufficiently porous membranes, if the faster diffusing ion of the electrolyte involved has the same sign of charge as that of the fixed ionic layer of the membrane, negative osmosis can occur. With the porous protamine membrane, then, this phenomenon can be expected to be most pronounced with solutions of strong acids.

In carrying out the experiments, the procedure of Loeb[20] was followed. The membrane filled with distilled water was immersed into acid solutions varying in concentration from $M/1024$ to 2 M. The rise of the meniscus in 10 minutes,[21] representing the flow of liquid from concentrated to dilute solution, is plotted on the ordinate in Fig. 3, with the acid concentration on the abscissa on a logarithmic scale as before. Negative values indicate that the net fluid transport is from solution to solvent.

The striking feature of the curves in Fig. 3 is that negative osmosis is predominant at relatively high concentrations in comparison with those concentrations at which positive anomalous osmosis occurs.

Without entering into the more theoretical aspects of this matter, it might be well to bring out the fact that initial potential differences across such systems as are represented in Fig. 3 are quite high. For example, in the system, 1 M H$_3$PO$_4$ |membrane| water, the initial potential difference was found to be + 180 mv.

Ordinarily, electroosomotic phenomena are absent or very weak in high concentrations of strong electrolyte solutions. Thus the ascription of negative osmosis to electroosmotic forces might raise some question. However, we must consider that in our experimental arrangement, a steep concentration gradient exists within the membrane between the solution on the one side and the water (initially) on the other.

---

[19] Sollner, K., *Z. Elektrochem.*, 1930, **36**, 36; 1930, **36**, 234; Sollner, K., and Grollman, A., *Z. Elektrochem.*, 1932, **38**, 274; Grollman, A., and Sollner, K., *Tr. Electrochem. Soc.*, 1932, **61**, 487.

[20] Loeb, J., *J. Gen. Physiol.*, 1919, **2**, 173.

[21] The duration of these experiments was only 10 minutes (as compared to 20 for the positive anomalous osmosis tests) since the initial rate of rise in this period is much more significant than the average rate over a longer period.

The active electroosmotic process would necessarily occur at such layers of the membrane in which the conditions are optimal for their occurrence.

This deduction is substantiated by the observation that negative osmotic flows persist for relatively short periods, 30 minutes at the most with our membranes, whereas positive anomalous osmotic effects are maintained for an hour or even longer. With the high concentration necessary for negative osmosis, the concentration gradient across the membrane is initially very steep; as diffusion proceeds, the electrolyte concentration in all parts of the membrane is increased and soon becomes high enough to depress electrokinetic effects. At such time, spontaneous electroosmosis (that is, negative osmosis) ceases and true osmotic effects are then observed, the direction of fluid flow being reversed.
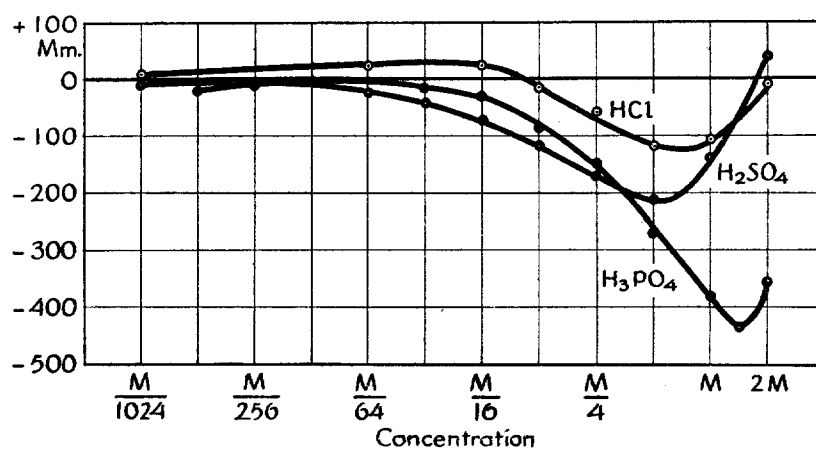

Fig. 3

To further extend the analogy between the porous protamine and the porous oxidized membranes, comparative electroosmosis experiments were performed with protamine, oxidized, and unoxidized membranes.

Several collodion membranes were prepared from Baker, u.s.p., collodion according to the method described above. One third of this group was used as such, a second third was oxidized for 3 hours with molar sodium hypobromite, and the remaining third was impregnated with isoelectric protamine. Following a thorough washing, all of these membranes were subjected to electroosmosis experiments for which a current of 10 milliamperes was passed through the membrane, the latter being attached to a rubber stopper holding a graduated manometer tube of 1 cm. diameter. The electrodes consisted of a platinum wire spiral inside the membrane and a platinum wire cage outside.[2] In each experiment, a current of 20 milliamperes was applied for 10 minutes. Then the current was then reduced to 10 milliamperes and after some time the volume transported in 10 minutes was recorded. The polarization before the actual measurements was necessary (especially with the dilute solutions) in order to have a constant condition of polarization during the actual measuring

period. The electrolytic solutions used were 0.1, 0.01, and 0.001 M potassium chloride. The results of these experiments with a membrane representative of each group are given in Table I, the volume changes being expressed as milliliters per hour.

The similarities in magnitude of the electroosmotic transports with protamine and oxidized membranes are obvious; those of the unoxidized membranes fall considerably below this level.[22]

A few words concerning the physical properties of the porous protamine-collodion membranes may be added here. The pore space of our membranes is about 80 per cent of the actual membrane volume; the concentration potentials obtained with various electrolytes across such membranes are only a few millivolts different from those obtained on free diffusion. This situation is quite analogous to the observations with the oxidized porous collodion membranes.[2]

TABLE I

Electroosmosis through Protamine-Collodion, Oxidized, and Unoxidized Collodion Membranes
(Current 10 M Amps., Membrane Area about 100 Cm.²)

Concentration of KCl solution	Electroosmotic flow per hr.*		
	Protamine-collodion membrane	Oxidized collodion membrane	Unoxidized collodion membrane
moles per liter	*ml.*	*ml.*	*ml.*
0.001	−4.38	+4.64	+2.84
0.01	−1.68	+1.80	+0.36
0.1	−0.08	+0.21	+0.00

* The plus sign indicates transport towards the cathode, a minus sign transport towards the anode.

The ohmic resistance of such membranes (thickness of 0.03 to 0.04 mm.) when immersed in 0.05 M potassium chloride solution, is very low, about 1 ohm—on the average—per 100 cm.² of membrane; the ohmic resistance is not significantly different from the original membranes which are not coated with protamine.

The rate of filtration of water or electrolyte solutions through such membranes seems to be somewhat lower than that observed with the ordinary collodion membranes. For example, the filtration rates through three of our typical membranes previous to the adsorption of protamine were 1.83, 2.35, and 1.55 ml. per hour under an average hydrostatic head of 15 cm. of water, the filtering area being roughly 100 cm.² After the adsorption of the protamine, these values were 1.61, 1.77, and 1.18 ml. per hour. To minimize disturbances which could be caused by electrical forces, molar potassium chloride solution

[22] Electroosmotic transport values given previously (Sollner, K., Abrams, I., and Carr, C. W., *J. Gen. Physiol.*, 1941, 25, 16) were erroneously expressed as transport values per 20 minutes; they are actually transport rates per hour.

was used in these filtration experiments. This observation agrees qualitatively with the observation of Hitchcock[23] that the filtration rate of collodion membranes after the adsorption of gelatin was considerably reduced, the stronger effect with gelatin being due most likely to its greater molecular size.

We may add that membranes exposed to an isoelectric protamine solution assume a slightly yellowish tinge. This effect is probably not due to the protamine, but rather to the alkalinity of the solution. Membranes immersed in a slightly alkaline solution without protamine for relatively long periods also assume a yellow coloration. Whatever the exact nature of this effect may be, it seems to be of no significance for the structure of the membrane.

IV

As was mentioned previously, stable electropositive membranes which correspond in their properties to the activated dried collodion membrane are not described in the literature.

Protamine and its salts are practically insoluble in ether-alcohol. Therefore, dried positive membranes cannot be prepared from collodion solutions containing protamine in the dissolved state.

The adsorption of protamine on dried collodion membranes also does not yield satisfactory results; it was found that the negative character of the membranes is only slightly reduced; in no case was the electrokinetic charge reversed. This is easily understood if we consider the fact that the pores of dried collodion membranes are too small to allow the entry of molecules as large as protamine. Thus, the protamine is excluded from the very spots which determine the electrochemical characteristics of the membrane.

A successful approach to the problem consists of adsorbing protamine on porous collodion membranes followed by drying. The technique is quite simple. Porous protamine membranes are prepared as described in a preceding section and dried in air for at least 6 hours. After this time, they assume a shrunken, irregularly wrinkled appearance and are ready for use.

Their most characteristic property is, of course, their electromotive behavior. The characteristic concentration potential—KCl 0.1 M/KCl 0.01 M— closely approaches the thermodynamically possible maximum. Some typical values are given in Table II.

In another series of six such membranes, the concentration potentials at the end of 3 months were found to vary between -47 and -52 mv. Such membranes offer relatively little resistance to the passage of current just as do the dried collodion membranes which are activated by oxidation or prepared from activated collodion.

[23] Hitchcock, D., *J. Gen. Physiol.*, 1925, **8**, 61.

TABLE II

Characteristic Concentration Potentials across Dried Protamine-Collodion Membranes. +KCl
0.1M/KCl 0.01M—

Membrane	Concentration potential after storage in water			
	1 day	1 wk.	2 wks.	3 wks.
	mv.	*mv.*	*mv.*	*mv.*
a	−52.2	−47.5	−49.5	−48.7
b	−50.7	−49.2	−48.0	−48.2
c	−51.4	−51.0	−50.5	−49.5
d	−50.1	−48.7	−48.9	−49.0
e	−48.6	−49.7	−48.0	−46.2
f	−51.0	−50.3	−49.4	−47.6

Thus, the experimental evidence indicates that the dried protamine-collodion membrane can be considered as an electropositive analogue of the dried activated collodion membrane—just as the porous protamine-collodion membrane is an electropositive analogue of the porous activated collodion membrane.

SUMMARY

1. Strongly electropositive porous membranes were prepared by the adsorption of protamine (salmine) on porous collodion membranes. These membranes retain their electrochemical chracteristics for at least 12 months without change.

2. They are distinctly electropositive between pH 1 and 10, the range of most pronounced electropositive behavior occurring in solutions between pH 3 and pH 8. The filtration rates and ohmic resistance of these membranes do not differ significantly from similar uncoated membranes.

3. The porous protamine-collodion membranes show very pronounced positive anomalous osmosis, the observed effects with proper electrolytes being similar to those obtained with oxidized collodion membranes. They also show very conspicuous negative osmosis with strong acids.

4. Protamine-collodion membranes which correspond in their properties to the activated dried collodion membranes were prepared by the adsorption of protamine on porous collodion membranes followed by drying in air. The concentration potentials across such dried protamine-collodion membranes closely approach the thermodynamically possible maximum.