

THE STRUCTURE OF THE COLLODION MEMBRANE AND
ITS ELECTRICAL BEHAVIOR

IX. WATER UPTAKE AND SWELLING OF COLLODION MEMBRANES IN AQUEOUS
SOLUTIONS OF ORGANIC ELECTROLYTES AND NON-ELECTROLYTES

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I

In a recent publication Carr and Sollner¹ have reported on the water uptake and the swelling of collodion membranes in water and solutions of strong inorganic electrolytes. They determined the weight and volume changes of collodion membranes when placed in water and when transferred from water to solutions of strong electrolytes. It was found that dried collodion membranes swell reversibly to an appreciable extent when placed in water, the swelling varying from 5 to 11 per cent depending on the brand of collodion. The water uptake as determined by the weight increase is larger than could be accounted for by the volume increase, swelling accounting for only 60 to 70 per cent of the water taken up by the membranes. When the "water-wetted dried" collodion membranes were transferred from water to solutions of various strong electrolytes, there was no detectable change in volume. Similarly, when "porous" membranes were transferred from water to solutions of strong electrolytes, there was no significant volume change.

Without giving further details, Carr and Sollner¹ stated that the problem of water uptake and swelling of collodion membranes is more complex in aqueous solutions of many organic compounds. This is of interest in view of the fact that the permeability of collodion membranes to various organic substances in aqueous solutions was found by several investigators to be anomalously high.

Michaelis and Fujita² reported that strychnine, quinine, and other alkaloid cations penetrate dried collodion membranes with approximately the same ease as do sodium ions, which undoubtedly are much smaller.

Collander,³ measuring the relative permeability of about thirty organic non-electrolytes and weakly dissociated compounds, mostly acids, found that with a few exceptions the permeability decreased regularly with increasing molecular

¹ Carr, C. W., and Sollner, K., *J. Gen. Physiol.*, 1943, **27**, 77.

² Michaelis, L., and Fujita, A., *Biochem. Z.*, Berlin, 1925, **161**, 47.

³ Collander, R., *Soc. Scient. Fennica, Commentationes Biol.*, II. 6., Helsingfors, 1926.

size. The compounds which in his experiments deviated most strongly from this regular pattern are propionic acid, valeric acid, monochloroacetic acid, phenol, and *m*-nitrophenol. It is important to note that a membrane kept for some time in a solution of *m*-nitrophenol was found by Collander to contain much more of the solute than would be expected if its concentration in the water contained within the membrane were the same as in the outside solution. Collander links the unusually high permeability of the *m*-nitrophenol with this effect.

Höber⁴ determined the relative permeability through positive membranes of a number of organic and inorganic anions in solutions of their Na salts. For this purpose he used collodion membranes impregnated with a basic dye-stuff. It would be premature to discuss Höber's very interesting results in detail here. It must suffice to state that in many cases the permeability of the organic anions is much higher than the permeability of inorganic anions of smaller molecular size. *E.g.*, the fatty acid ions from acetate to the valerate ion possess permeabilities comparable to that of the much smaller chloride ion; and the fatty acid anions above valeric acid have even greater permeabilities. This behavior Höber attributes to the surface activity of the organic ions which are believed to be concentrated on the pore walls, and thus to move faster across the membrane.

In view of the fact that air-dried collodion membranes swell in water,¹ it seemed advisable to investigate whether the solutions of the substances that were found to exhibit anomalous permeabilities might have a specific swelling effect. The detection of such an effect, of course, would be most important in determining the limits of applicability of collodion membranes to permeability studies; in addition it would cast light on the findings of the above mentioned investigators. We therefore have investigated the swelling of collodion membranes in aqueous solutions of some organic substances and the uptake of solute from these solutions by the membranes.

The organic substances for this investigation were chosen preferentially from three classes of compounds. One group was selected from among those weakly or non-dissociated substances used by Collander which would be most likely to influence the swelling of collodion, such as fatty acids, phenol, and nitrophenol; secondly, some definitely hydrophilic compounds such as glycerine, glucose, and citric acid; and thirdly some strong organic acids, sulfonic acids, were tested, the sodium salts of which were found by Höber to behave very anomalously.

This study has been confined to dried collodion membranes since any specific swelling effect that may be found with dried collodion membranes would also result in a change of structure of "porous" membranes.

⁴ Höber, R., *J. Cell. and Comp. Physiol.*, 1936, **7**, 367.

II

The experimental procedure is briefly as follows: flat dried collodion membranes were prepared, their weights determined, and their volumes ascertained with a pycnometer filled with mercury; they then were placed into the solutions of the various organic compounds. After measured duration of immersion in the solutions, the membranes were removed, blotted, and their weights and volumes again determined. Finally, in a number of representative cases the amount of solute contained in the membranes at equilibrium with various solutions was determined.

The technique of the weight and volume measurements was the same as used by Carr and Sollner which was described in another paper.¹

In some cases the weight and volume measurements were made at relatively frequent intervals in order to ascertain the swelling of the membranes as a function of the time. In all cases enough determinations were made to assure that equilibrium values of weight and volume had been reached. The material used throughout this investigation was Baker U.S.P. collodion.

To determine the amount of organic solute contained in the membranes, the following procedure was used: the membrane was removed from the solution and momentarily dipped into distilled water; then it was placed into a 150 × 15 mm. test tube containing *ca.* 10 ml. of distilled water which covered it completely. After a sufficient amount of the organic substance had diffused out of the membrane, in most cases after a few days, the membrane was removed from the solution and the latter was titrated. The membrane was placed again in distilled water and the resulting solution was titrated as before after several days. This procedure was repeated until successive titrations yielded zero values. A membrane which was free of organic solute was used as a control and titrated along with the others. With the acids the titration was carried out with 0.02 N NaOH, using a syringe micro buret which allowed readings to 0.002 ml. The total amount of reagent required for the control was subtracted from the amount required for the titration of the membranes. The phenol solutions were titrated in a similar manner with 0.1 N solution of KBr-KBrO₃. In all cases the analyses were carried out after swelling equilibrium had been established.

The results of the weight and volume measurements are given—as by Carr and Sollner—as milligrams and cubic millimeters weight and volume increase respectively per cubic centimeter of dry membrane, which occur when dry collodion membranes are immersed in the various solutions.

The accumulation of solute by the membranes was calculated as follows: the amount of substance actually present in a membrane was divided by the quantity that would be present if the concentration of the solute in the solution which is contained within the membrane were the same as in the bulk of the

solution. This ratio obviously is a quantitative measure of the relative accumulation of solute within the membrane.

The quantity of solute present in the membrane is found by analysis. The quantity of solute that would have entered the membrane if the measurable weight increase were caused by the uptake of unchanged solution can readily be calculated from the weight increase and the known specific gravity and the concentration of the solution. The former quantity divided by the latter gives the desired ratio which we call the "accumulation factor."

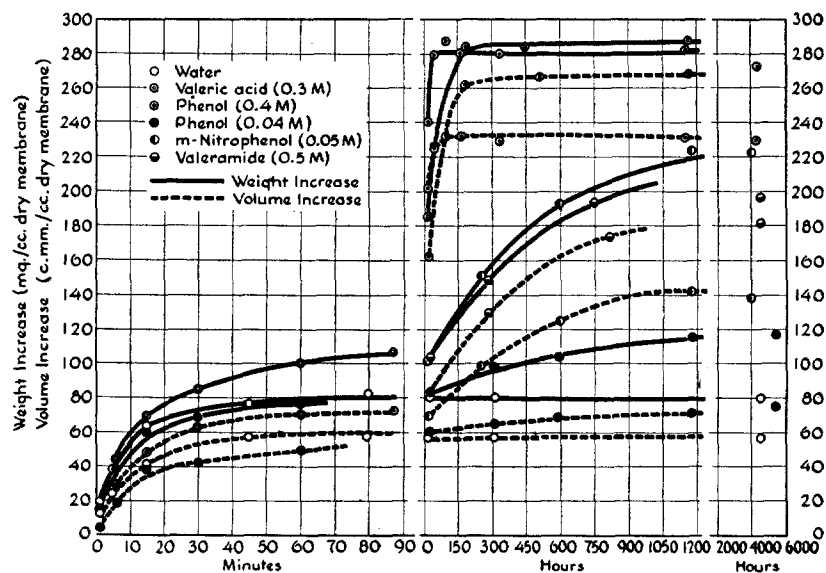


FIG. 1

It is of interest to compare the quantity of solute contained in the membrane with the corresponding weight or volume increases. The clearest picture of this relationship is obtained by comparing the quantity of solute taken up per cubic centimeter of dry membrane with the corresponding "characteristic additional weight increase." This latter quantity we define as the weight increase in solution minus the weight increase in water per cubic centimeter of dry collodion.

III

Fig. 1 gives the relative volume and weight increases of dried collodion membranes in aqueous solutions of several organic compounds as a function of time. The equilibrium values of the weight and volume increases per cubic centimeter

TABLE I
The Interaction of Dried Collodion Membranes with Aqueous Solutions of Various Organic Compounds

1	2	3	4	5	6	7
Solute	Concentration of solution	Weight increase per cc. dry membrane	Volume increase per cc. dry membrane	"Accumulation factor"	Uptake of solute per cc. dry membrane	"Characteristic additional weight increase" per cc. dry membrane
	<i>mols/liter</i>	<i>mg.</i>	<i>c.mm.</i>		<i>mg.</i>	<i>mg.</i>
Water.....	—	80	57	—	—	—
Acetic acid.....	0.54*	97	72	8.7	25.4	17
Propionic acid.....	0.52*	114	90	13.0	52.9	34
Butyric acid.....	0.28*	125	101	22.7	67.8	45
Butyric acid.....	0.49*	161	137	15.6	108	81
Valeric acid.....	0.26*	276	260	32.1	232	196
Monochloroacetic acid.....	0.53*	152	103	9.3	67.8	72
Ethyl alcohol.....	0.5	83	57	§	§	3
Isobutyl alcohol.....	0.5	122	108	§	§	42
Isoamyl alcohol.....	0.5	136	127	§	§	56
Formamide.....	0.5	85	66	§	§	5
Valeramide.....	0.5	196	181	§	§	116
Phenol.....	0.042*	117	76	114	47.4	37
Phenol.....	0.38*	282	232	20.8	234	202
<i>m</i> -Nitrophenol.....	0.05	224	145	§	§	144
Benzene sulfonic acid.....	0.5	75	56	0.00	0.0	-5
β -Naphthalene sulfonic acid.....	0.5	78	57	0.00	0.0	-2
Glycerine.....	0.5	96‡	72‡	§	§	6
Glucose.....	0.5	82‡	70‡	§	§	-8
Citric acid.....	0.5	99‡	75‡	0.00	0.0	9

* Concentration determined analytically after establishment of swelling equilibrium.

‡ These membranes were cast at a later date—the weight and volume increases in water for the membranes in this group are: 90 mg. per cc. dry membrane, and 70 c.mm. per cc. dry membrane respectively.

§ Not analyzed.

of dry membrane for all the substances tested are summarized in columns 3 and 4 of Table I. The "accumulation factors" for several solutes are given in column 5; the solute uptake in milligrams per cubic centimeter of dry mem-

brane and the "characteristic additional weight increase" in milligrams per cubic centimeter of dry membrane are shown in columns 6 and 7.

IV

The data presented in columns 3 and 4 of Table I show that the dried collodion membrane exhibits widely different behavior when placed in solutions of various organic compounds; the weight and volume increases of the membrane differ greatly with the nature and concentration of the solute, being in many cases far larger than in water.

No significant specific swelling effect is observed with the 0.5 M solutions of ethyl alcohol, benzene sulfonic acid, β -naphthalene sulfonic acid, glycerine, glucose, and citric acid. The weight and volume increases are significantly larger than in water alone with acetic acid and the higher fatty acids, monochloroacetic acid, isobutyl and isoamyl alcohol, valeramide, phenol, and *m*-nitrophenol. With formamide the results seem to be ambiguous. In the homologous series of the fatty acids, alcohols, and amides the swelling effect increases with increasing molecular weight. In view of this, one may be inclined to think that a real though small specific swelling effect exists in the case of the formamide solution. The swelling action of a solute is greater, the higher its concentration.

Fig. 1 shows for a few representative cases that the rate at which swelling equilibrium is attained differs markedly for the various solutions. When a dried membrane (prepared from Baker collodion U.S.P.) of about 0.15 mm. thickness is placed in water, swelling equilibrium is reached in approximately 80 minutes; a similar behavior is observed in the case of the solutions of those substances which do not show any specific swelling effect. With solutions which show a pronounced swelling effect this time varies from about 100 hours for 0.26 M valeric acid and 0.38 M phenol to about 1000 hours with 0.042 M phenol and 0.05 M *m*-nitrophenol solutions.

From the data of column 5 it is seen that the relative accumulation of the organic solute within the membrane, expressed by the "accumulation factor," differs very widely with the various solutes. In the two instances in which two concentrations of the same solute were tested the "accumulation factor" is higher with the more dilute solution.

A comparison of columns 3 and 4 with column 5 shows a very marked parallelism between the swelling caused by a solute and its tendency to accumulate within the membrane; the compounds which cause the most pronounced swelling are also accumulated most strongly. The increase in the "accumulation factor" with increasing molecular weight in the homologous series of the fatty acids may be specially mentioned. The substances which do not show any specific swelling effect show "accumulation factors" smaller than one; this means that the water in the membrane contains less of the solute than it does

in the surrounding solution. Three of the organic substances tested, benzenesulfonic acid, β -naphthalene sulfonic acid, and citric acid, are actually excluded completely from the membranes.⁵

To use conventional terminology, one may say that typical "hydrophilic" substances have little influence on the swelling of collodion membranes and are not accumulated in them, whereas "carbophilic" substances make the membranes swell and are accumulated.

The characteristic swelling which is observed with some solutes is entirely due to the accumulation of a corresponding quantity of the solute within the membrane, as can be seen from a comparison of columns 6 and 7. The absolute uptake of solute per gram of dry membrane (column 6) is at least as great as the "characteristic additional weight increase" per gram of dry membrane in the same solutions (column 7); with some solutes it seems to be significantly greater. The water content of the membranes in equilibrium with the solutions is therefore scarcely as high, in some cases probably significantly lower, than in pure water.⁶ A collodion membrane which has taken up a significant quantity of organic solute must be considered as a structure substantially different from a similar membrane wetted with water.

A pronounced parallelism is apparent between the anomalously high permeabilities described by Collander with certain compounds and their accumulation and the swelling effect caused by their solutions. All the compounds Collander found to deviate from the expected permeability—phenol, *m*-nitrophenol, propionic acid, monochloroacetic acid, and valeric acid—exhibit a pronounced swelling effect and are accumulated in the membranes. Substances which in our experiments do not show a specific swelling effect and are not accumulated—or which on the basis of their structure must be expected to act so—show normal permeability characteristics according to their molecular size.⁷

⁵ The "accumulation factor" was also determined for two inorganic electrolytes, HCl and HNO₃; both show an accumulation factor smaller than 1; 0.03 in the case of HCl and 0.78 in the case of HNO₃. It is of interest to note that HNO₃, which, as shown by Collander,³ penetrates collodion much faster than does HCl, shows a much higher accumulation factor than the latter. Nitric acid is known to have a strong affinity to cellulose, forming at higher concentrations a definite molecular compound (Knecht's compound).

⁶ The unusually large differences between the figures for the weight and volume increases in the cases of monochloroacetic acid, phenol, and nitrophenol are of course due to the high specific gravities (1.58, 1.07, and 1.48 respectively) of these substances. The converse holds true for the unusually small differences between the values for weight and volume increase found with valeric acid (sp. gr. 0.94) and isoamyl alcohol (sp. gr. 0.81).

⁷ Collander did not test the sulfonic acids. No data seem to be available on their permeability through collodion membranes.

With positive, dyestuff-impregnated collodion membranes the parallelism between anomalous permeability and accumulation is not complete. The unusually high permeability of fatty acid ions in neutral solution is paralleled by the swelling effect and the accumulation of the free acids. The same is not true, however, for the high permeability of aromatic sulfonic acid anions in neutral solution. This discrepancy is hardly surprising in view of the different conditions in Höber's permeability and our swelling and accumulation studies with negative membranes.

On the basis of available information it is impossible to discuss in a satisfactory manner the molecular mechanisms causing the accumulation and the swelling effects and the correlated increase in permeability. The situation is undoubtedly fairly complex. Accumulation can result from adsorption on the micellar surfaces, from filling of intermicellar spaces, and from an absorption of the solute by the micelles. Roughly corresponding to these three degrees of interaction between solute and membrane one would expect little or no specific swelling, moderate specific swelling, and strong specific swelling which in its highest degree approaches dissolution.

These processes obviously are not independent of each other; the observed effects depend upon the nature of the solute and its concentration as well as of the time of interaction between solution and membrane. At low concentrations surface adsorption must be favored over absorption in the interior of the collodion micelles. In a similar manner the alkali salts of organic acids may be strongly adsorbed, but they hardly would dissolve in the mass of the collodion, though the weakly dissociated free acids, which are essentially non-electrolytes, may readily do so.

That the permeability of the accumulated solutes is greater than can be expected on the basis of their molecular size undoubtedly can be due to several different mechanisms. There is first the possibility that a solute which is concentrated in the adsorption layer on the micellar surfaces migrates in the adsorbed state,⁸ as suggested by Sollner⁹ and Höber.⁴ If the adsorption is more pronounced, liquid layers of the solute wetting the membrane may result which, stretching continuously between the two sides of the membrane, could easily account for an increased rate of penetration of the solute. If the micelles absorb and dissolve the solute there is further the possibility that some of the solute diffuses through the swelled collodion micelles themselves. Swelling, furthermore, is bound to result in some change in the pore structure of the membrane which may also lead to an increased rate of permeation of the solute. That the time factor too must be considered is obvious. Adsorption is a fast process and its consequences therefore must be apparent in short time experi-

⁸ Volmer, M., and Estermann, I., *Z. Physik*, 1921, **7**, 13. Volmer, M., and Adhikari, G., *Z. Physik*, 1925, **35**, 170.

⁹ Sollner, K., *Kolloid-Z.*, 1933, **62**, 31.

ments; absorption and dissolution are relatively slow processes; their full effect is only felt after prolonged contact between solution and membrane.

From the foregoing discussion it is clear that swelling, accumulation, and anomalous permeability need not necessarily be parallel in all cases. One can readily conceive of instances in which accumulation without swelling may cause an increased permeability.¹⁰

The interaction of a first solute with the membrane must affect the permeability to second solutes. Blocking of some pathways by the first solute,¹¹ changes in the geometrical and electrical structure of the membrane, and interaction between the second solute and the first which is present locally in high concentration, as well as other effects, can occur, depending on the nature and concentration of the solutes. This makes general predictions hazardous. Careful experimental investigation alone can clarify this problem.

Further discussion seems superfluous since the purpose of the present investigation is only to demarcate the approximate limits of the usefulness of the collodion membrane as a general model of the non-swelling, inert, porous membrane.

Collodion membranes seemingly act as inert membranes with the aqueous solutions of the strong, weakly adsorbable¹² electrolytes and those typically hydrophilic organic substances which are reasonably free from carbophilic groups. With all other substances the situation needs careful individual examination.

The instances of a specific interaction between organic solutes and collodion membranes are apparently much more numerous than has previously been assumed. The anomalies of permeability resulting from this interaction which were observed in the past with certain carbophilic compounds are now more clearly understood as a result of the specific interaction of solute and membrane.

In future work it therefore will be necessary to ascertain the extent of a possible interaction between any given solute and the membrane. A sharp distinction must be made between phenomena which are characteristic for porous membranes in general and observations which are due to a specific interaction between solute and membrane.

The use of membranes prepared from other material than collodion, *e.g.*, denitrified collodion (cellulose) or various silicates obviously will be helpful

¹⁰ Membranes of porous character have interstices of a whole spectrum of sizes. If on account of its molecular size the solute cannot enter the smaller pores but may enter the bigger ones and be adsorbed there, then it is possible that in spite of an overall "accumulation factor" smaller than 1, an abnormally high permeability due to adsorption may be found.

¹¹ Weech, A. A., and Michaelis, L., *J. Gen. Physiol.*, 1928, **12**, 55.

¹² Nitric acid according to the results of Collander and ourselves cannot be included in this group.

in clarifying the question of the effects of a specific solute-membrane interaction.

The interaction between solute and membrane is, of course, a phenomenon of considerable interest in its own right, primarily since such interaction undoubtedly plays an important rôle in living membranes. For an investigation of this problem, however, it may be advisable to use membranes more readily comparable to living tissues than collodion.

SUMMARY

1. Dried collodion membranes are known to swell in water and to the same limited extent also in solutions of strong inorganic electrolytes (Carr and Sollner). The present investigation shows that in solutions of organic electrolytes and non-electrolytes, the swelling of dried collodion membranes is not as uniform, but depends on the nature of the solute.

2. The solutions of typically "hydrophilic" substances, *e.g.*, glycerine, glucose, and citric acid, swell collodion membranes only to the same extent as water and solutions of strong electrolytes.

In solutions of typically carbophilic substances (*e.g.*, butyric acid, valeric acid, isobutyl alcohol, valeramide, phenol, and *m*-nitrophenol) the swelling of the membranes is much stronger than in water, according to the concentration used. For the brand of collodion used the swelling in 0.5 M solution was in some cases as high as 26 per cent of the original volume, as compared to 6 to 7 per cent in water. Therefore, in these solutions the "water-wetted dried" collodion membrane is not rigid, inert, and non-swelling, but behaves as a swelling membrane.

3. The solutes which cause an increased swelling of the membranes are accumulated in the latter, the degree of accumulation being markedly parallel with the degree of their specific swelling action.

4. The anomalously high permeabilities of certain carbophilic organic solutes reported by Michaelis, Collander, and Höber find an explanation in the specific interaction of these substances with collodion.

5. The use of the collodion membrane as a model of the ideal porous membrane is restricted to those instances in which no specific interaction occurs between the solute and the collodion.