NEW EXPERIMENTS ON ANOMALOUS OSMOSIS

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For several years we have been aware of the fact that the well known experiments of Jacques Loeb,[1] Bartell,[2] and others[3] concerning anomalous osmosis[4] through collodion membranes could not be reproduced satisfactorily. The effects obtained were usually insignificant or poor at best.

In connection with new work relating to the general theory[5] of anomalous osmosis it became necessary to clear up this matter, especially since it seemed that any clarification might cast considerable light on some basic membrane properties.

When an electrolyte diffuses through a membrane from a more concentrated to a more dilute solution, the sign and extent of anomalous osmosis are correlated rather clearly[5] with the electrokinetic properties (ζ-potential) of the membrane and with the dynamic membrane potential (ϵ-potential) that is observed. Both of these quantities depend ultimately on the electrical structure of the solid-liquid interphase in the pores of the membrane.

The obvious starting point for any experimental investigation was to test different brands of collodion. The following preparations were tested: Parlodion Mallinckrodt, 5 per cent dissolved in 75 per cent alcohol, 25 per

[1] Loeb, J., *J. Gen. Physiol.*, 1918–19, **1,** 717; 1919–20, **2,** 173, 255, 387, 563, 577, 659, 673; and many other papers in the succeeding volumes of the same Journal.

[2] Bartell, F. E., and Madison, O. E., *J. Physic. Chem.*, 1920, **24,** 444; Bartell, F. E., and Carpenter, D. C., *J. Physic. Chem.*, 1923, **27,** 101, 252, 346; Bartell, F. E., Membrane potentials and their relation to anomalous osmosis, in Mathews, J. H., Colloid symposium monograph, Department of Chemistry, University of Wisconsin, Madison, 1923, **1,** 120; and many other publications.

[3] Preuner, G., and Roder, O., *Z. Elektrochem.*, 1923, **29,** 54; Girard, P., *Compt. rend. Acad. sc.*, 1908, **146,** 927.

[4] By anomalous osmosis we mean those phenomena of liquid mass movement which occur when electrolyte solutions dialyze through membranes but are unexplainable on the basis of the laws of normal osmosis.

[5] Sollner, K., *Z. Elektrochem.*, 1930, **36,** 36, 234; Sollner, K., and Grollman, A., *Z. Elektrochem.*, 1932, **38,** 274; *Tr. Electrochem. Soc.*, 1932, **61,** 477, 487; Sollner, K., *Kolloid-Z.*, 1933, **62,** 31.

cent ether; Collodion Merck, U.S.P.X.; Collodion Baker, U.S.P.; Collodium Schering-Kahlbaum "pro analysi;" Collodium Schering-Kahlbaum "für Membranen;" Collodium Schering-Kahlbaum D.A.B.6.

When tested with uni-univalent electrolytes, *e.g.* KCl, in which we were particularly interested, the first four of these brands of collodion gave no significant positive results. Schering-Kahlbaum collodion "für Membranen" gave doubtful effects. Only Schering-Kahlbaum D.A.B.6. collodion gave decidedly positive results of the order of magnitude described by Loeb.[6] Correspondingly, bi- and trivalent ions gave only very moderate results with membranes of the first four named brands of collodion. Schering-Kahlbaum "für Membranen" gave somewhat higher values. Here too, only membranes prepared from collodium Schering-Kahlbaum D.A.B.6. yielded results approaching in magnitude those reported by Loeb.[6]

This particular brand of collodion, incidentally, was used by Loeb in his experiments nearly twenty years ago. Michaelis[7] likewise in his classical experiments on the dried collodion membrane used Schering-Kahlbaum D.A.B.6, as this brand also gave in his hands the most characteristic and reproducible results. No satisfactory explanation of the cause of this different behavior of the several brands of collodion has been noted in the literature. Thus additional insight into the ultimate cause of the peculiar behavior of Schering-Kahlbaum D.A.B.6 collodion promised to cast additional light on the results of Loeb and Michaelis.

On the basis of observations to be reported in detail later, it was concluded that certain impurities or groups foreign to the pure ideal nitrocellulose were responsible for the "activity" of the actual collodion. The purer brands show less activity. The high efficiency of Schering-Kahlbaum D.A.B.6 collodion is due to its high content of groups foreign to pure nitrocellulose. More specifically, it was concluded that COOH groups, due to the presence of pectic substances or to oxidation, cause the relatively

[6] Experiments carried out about eight years ago with this brand of collodion gave decidedly less positive results than our present ones carried out with recently acquired Schering-Kahlbaum D.A.B. 6 collodion.

[7] Michaelis, L., and Fujita, A., *Biochem. Z.*, Berlin, 1925, **158,** 28; 1925, **161,** 47; 1925, **164,** 23; Michaelis, L., and Dokan, S., *Biochem. Z.*, Berlin, 1925, **162,** 258; Michaelis, L., and Hayashi, K., *Biochem. Z.*, Berlin, 1926, **173,** 411; Michaelis, L., and Perlzweig, W. A., *J. Gen. Physiol.*, 1926–27, **10,** 575; Michaelis, L., McEllsworth, R., and Weech, A. A., *J. Gen. Physiol.*, 1926–27, **10,** 671; Michaelis, L., Weech, A. A., and Yamatori, A., *J. Gen. Physiol.*, 1926–27, **10,** 685; Michaelis, L., *Bull. Nat. Research Council, No. 69*, 1929, 119; *Kolloid-Z.*, 1933, **62,** 2, and other publications.

great activity characteristic of membranes prepared from this particular brand of collodion.

This conclusion immediately suggested a method of increasing the activity of collodion membranes by oxidation. Following a suggestion of Meyer and Sievers,[8] the membranes were oxidized for several hours with NaOBr solution, prepared by saturating normal NaOH with molecular bromine. The activity of the membranes increases with increasing oxidation time. The better brands of collodion withstand 24 hours oxidation without damage, whereas the poorer grades leak and cannot be oxidized so long. We have not yet made a study of the optimum conditions for maximum activity.

The experimental technique is as follows: Collodion bags are cast in 30 × 110 mm. test tubes and allowed to dry several minutes, the suitable drying time varying considerably with different brands of collodion. Next the bags are filled with water; they loosen from the glass spontaneously and are tied to glass rings with thread while still filled with water. Following this, they are kept in covered glass containers under water to which thymol has been added as a preservative. The membranes so prepared are fitted to rubber stoppers provided with a long capillary tubing (inner diameter about 1½ mm.). Following the suggestion of Loeb,[9] membranes were selected which, when filled with ¼ molar sugar solution and placed in water for 20 minutes, yield an osmotic rise of about 120 mm. of liquid in the capillary manometer. The adjustment of the zero reading is facilitated by a small glass syphon provided with a stopcock, allowing the rapid adjustment of the meniscus in the manometer to the proper level corresponding to the capillary rise over the outside solution.

The bag chosen for actual use is filled with solutions of varying (in our experiments decreasing) concentrations of first sugar, then KCl, K_2SO_4, and K_3-citrate. The rise of the meniscus in the manometer is noted 20 minutes after the bag is placed in water.

In the accompanying figures, the abscissae show molar concentrations in the collodion bag on a logarithmic scale, the ordinates the rise in level of the liquid in the manometer after 20 minutes (with the exception of the values for Loeb's K_3-citrate curve (Fig. 1) which are given after 10 minutes).

Fig. 1 gives for comparison a characteristic set of curves published by Loeb.[10]

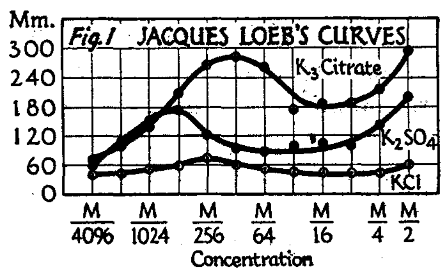
Fig. 2 shows the behavior of a membrane cast from one of the less active brands of collodion (Merck U.S.P.). This behavior is very characteristic and membranes cast from one particular lot of collodion under the same conditions give astonishingly reproducible results when properly selected

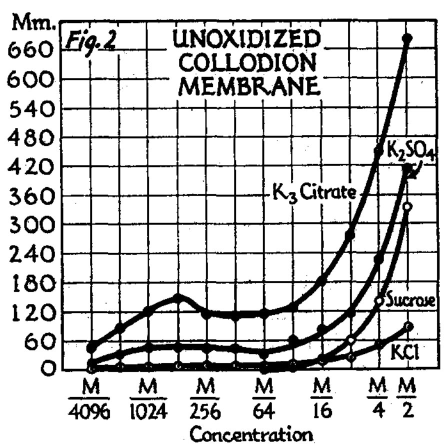
[8] Meyer, K. H., and Sievers, J.-F., *Helv. Chim. Acta*, 1936, **19**, 665.

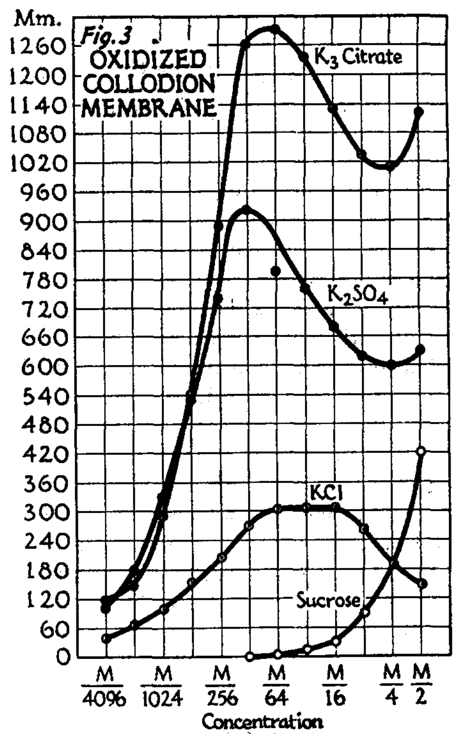
[9] Loeb, J., *J. Gen. Physiol.*, 1919–20, **2**, 93.

[10] Loeb, J., *J. Gen. Physiol.*, 1919–20, **2**, 564.

after the test with sugar (120 mm. rise after 20 minutes). The curves given in Fig. 2 were actually obtained with two membranes, as the first one was damaged in the middle of the K_3-citrate curve. Another one was substituted giving the same sugar value and satisfactory agreement within a few millimeters at several test points of the KCl, K_2SO_4, and the unfinished K_3-citrate curves.







This latter membrane was then oxidized for 24 hours, washed thoroughly, and again tested with sugar, KCl, K_2SO_4, and K_3-citrate in the order indicated. The values so obtained with the "activated" membrane are given in Fig. 3.

The difference between the unoxidized and the oxidized membrane is quite striking. The activity of the oxidized membrane surpasses by far the activity of those used by Loeb.

The differences would be still larger if one measured volumes transferred against a zero pressure and not pressure rises with a progressively increasing back filtration.

The oxidation method affords a simple and rational means of "activating" membranes of otherwise inactive collodion in order to reproduce the Loeb experiments on anomalous osmosis. In addition it seems to open up a rather promising field of membrane research, for example, the comparison of membranes with nearly equal permeability for non-electrolytes but showing remarkable differences in their behavior towards electrolytes. The cause for this difference obviously has to be looked for in the great difference of the charge densities in the pores of the unoxidized and the oxidized membranes.

SUMMARY

1. It is impossible to reproduce Loeb's observations on anomalous osmosis with membranes prepared from relatively pure brands of collodion, whereas positive effects can be obtained using collodion containing acidic impurities.

2. The inactive (purer) collodion membranes may be activated by oxidation with NaOBr solution.

3. Properly oxidized membranes give much greater anomalous osmotic effects than those described by Loeb.