POLARIZATION STUDIES IN COLLODION MEMBRANES AND IN SYNTHETIC PROTEIN-LIPOID MEMBRANES*

By MONA SPIEGEL-ADOLF

(From the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia)

(Accepted for publication, October 31, 1936)

While investigating the polarization of brain tissue (1) adequate tissue models became desirable. Some experiments along this line have been published before (2) with the method used for measuring the polarization.

The method consisted briefly in measuring the conductivity of a membrane at various frequencies (560–5000 cycles). For this purpose the membrane was inserted in a three-cell apparatus which was filled with a salt solution. In case of polarization of the membrane there is a difference between the conductivity at high and at low frequency. This difference expressed in percentage of the conductivity at low frequency (Δ) has been used as a measure of polarization. The determinations of Δ require exact minimum positions on the bridge. These can be much more quickly determined than the exact capacity necessary for absolute silence. Therefore when studying experimental changes on living brain the former method seemed preferable. To make direct comparisons possible the same method has been used here. A mica decade condenser was used to determine the parallel capacities necessary to obtain a distinct minimum on the bridge.

The experiments reported here deal mainly with three problems: methods of preparing polarizable collodion membranes of low resistance and some of their properties, relations between polarization and permeability, methods of building a synthetic protein-lipoid membrane which shows polarization, and its reaction to different substances.

* Parts of this paper have been presented before the meeting of the Federation of American Societies for Experimental Biology, at Washington, D. C., March 26, 1936.

I

It has been reported previously (2) that addition of lipoids to collodion causes polarizability in membranes, while membranes made under similar conditions from pure collodion are not polarizable. No explanation has been offered up to this time, but at least three possible interpretations have commanded consideration: specific effects of

TABLE I*

Influence of Holes on the Polarization of Lecithin-Collodion Membranes (I) and Pure Collodion (II) Membranes

No. of holes	Lecithin-collodion					Collodion		
	$K_{560} \cdot 10^{-4}$	C	$K_{5000} \cdot 10^{-4}$	C	Δ	$K_{560} \cdot 10^{-4}$	$K_{5000} \cdot 10^{-4}$	Δ
					per cent			*per cent*
0	4.50	0.066	8.28	0.014	84	3.62	7.94	119.3
1	5.21	0.05	8.31	0.013	59.5	—	—	—
2	5.87	0.036	8.59	0.010	46.4	7.62	11.28	48.5
3	6.85	0.023	8.80	0.008	28.4	—	—	—
4	7.22	0.029	9.18	0.010	27.1	13.80	16.20	17.7
5	7.46	0.027	9.16	0.011	22.8	17.38	19.22	12.9
6	7.70	0.027	9.42	0.009	22.3	21.52	23.04	7.2
7	7.83	0.025	9.44	0.008	20.6	25.24	26.52	5
12	—		—		—	30.76	32.22	4.8
29	—	0.015	—	0.005	12.0	42.12	42.84	1.7
No membrane	—		—		—	90.96	90.96	0

* In all the tables, K = specific conductivity,
Indices 560 and 5000 = frequencies in cycles,
$\Delta = \frac{K_{5000} - K_{560}}{K_{560}} \cdot 100$,
C = capacity in μF.

lipoids, changes in the degree of dispersion, and displacement of the solvent. Therefore, the following experiments were undertaken.

The specific action of lecithin for inducing polarizability can be interpreted as due to a formation of a continuous thin layer with high polarizability, as recently suggested by Bungenberg and Bonner (3). In order to test this assumption, a wire of 0.012 inch diameter was used to punch holes consecutively into a polarizable lipoid-collodion membrane. If the polarizability is due to the intactness of the lecithin

film, an impairment of the film must destroy the polarizability. The results of such a series of experiments are summarized in Table I. The figures indicate that interruptions of the continuity of the membrane only partly destroy the polarization of a lecithin-collodion membrane. The decrease is marked after the first 3 holes. Nevertheless, the decrease does not run parallel to the number of holes. In fact, from the 5 to 7th hole the polarizability remains nearly stable and 22 more holes are required to reduce it again by half. The total area of the holes amounts to about 16 sq. mm., while the surface of

TABLE II

Influence of Various Substances on the Polarizability of Collodion Membranes

Drying time	Material of membranes	$K_{580} \cdot 10^{-4}$	C	$K_{1000} \cdot 10^{-4}$	C	Δ
min.						*per cent*
60	Pure collodion (Merck)	17.20	0.005	17.20	0.001	0
"	Collodion Merck 2 per cent egg lecithin	11.7	0.025	12.4	0.005	14.2
"	" U.S.P. 2 per cent mastix	23.0	0.005	23.1	<0.001	0
"	" Merck 5 per cent olive oil	8.68	0.012	9.24	0.009	6.2
75	Pure collodion U.S.P.	22.8	<0.001	22.8	<0.001	0
"	" U.S.P., 2 per cent gum benzoin	5.65	0.080	10.90	0.025	92.9
"	" U.S.P., 2 per cent mastix	1.54	0.020	1.58	0.010	2.9
90	Pure collodion U.S.P.	20.4	<0.001	20.4	<0.001	0
"	Collodion U.S.P., 2 per cent camphor	6.27	<0.001	6.27	<0.001	0
"	" U.S.P., 1 per cent cholesterin	23.0	0.010	23.0	0.010	0
"	" U.S.P., 2 per cent mastix	1.39	0.027	1.77	0.010	27.6

the membrane is about 452 sq. mm. In view of these results it does not seem probable that the entire polarization of the lipoid-collodion membrane depends upon the existence of a continuous lecithin film. This opinion gains additional support through the fact that a polarizable membrane of pure collodion shows almost a similar behavior when submitted to the same treatment.

In view of a former observation (2) and of the recent investigations of Fricke and Curtis (4), changes in the degree of dispersion of the membrane colloids had to be considered as possible causes of polariz-

ability. No direct proof to the contrary can be given, but there are some facts that make this assumption improbable, at least, for the range of frequencies used.

It was shown before (2) that it was not possible to produce polarizability by adding phosphatides to gelatin membranes. Neither were attempts successful to induce polarizability in collodion membranes by introducing other colloids, *e.g.* proteins. Finally it could be shown that lecithin sol containing the same amount of lipoid as the polarizable lecithin-collodion membrane, when placed in the middle cell of a three cell apparatus between two parchment membranes, did not show differences in conductivity when measured at different frequencies of the alternating current (Table VIA).

Experiments were begun in order to determine whether the action of the lipoids in rendering collodion polarizable was due to their action on one of the solvents of the collodion. Alcohol and ether are contained in both Merck collodion and U.S.P. collodion. Since cephalin effects the polarizability of membranes quantitatively in the same way as lecithin and since it is insoluble in alcohol, its effect upon the ether was considered. The permeability of collodion membranes depends largely upon the ratio between ether and alcohol (5) and therefore, experiments were started in order to ascertain if additions of ether-soluble, non-volatile substances would act on the polarizability in the same way as lipoids (Table II).

The results summarized in Table II show that substances of different chemical character, such as olive oil, mastix, and gum benzoin when added to collodion are able to induce polarizability. Camphor and cholesterin which separate from solution when the collodion membranes solidify do not have this effect, or only to a very small degree. These results seem to support the view that the influence of the addition of lipoids to collodion is upon the solvent.

Through the information gained by these experiments the preparation of membranes of low resistance and marked polarizability is greatly facilitated. Nevertheless, this introduction of other substances into the collodion complicates the understanding of the mechanism underlying polarizability.

Experiments were therefore started with the purpose of intercepting an intermediate between the wet and the dry stages in the preparation

of collodion membranes. Wilbrandt (6) has given evidence that there is a steady transition from highly permeable wet to completely dried membranes. The dry collodion membranes which have been

TABLE III

Effects of Drying under Pressure on Resistance and Polarizability of Collodion Membranes

(Apparatus filled with 0.02 N KCl, capacitance = 0.4; resistance of apparatus without membranes = 165 ohm = R_0; $C_{560} = 0.015$, $C_{5000} < 0.001$.)

Total time	Dried only		Δ	Dried for 1 hr. and pressed for rest of time		Δ
	K	$R-R_0$		K	$R-R_0$	
			per cent			*per cent*
1 hr. 30 min.	At 560 cycles $1.90 \cdot 10^{-3}$ $C < 0.001$ At 5000 cycles $1.92 \cdot 10^{-3}$ $C < 0.001$	43	0	At 560 cycles $1.28 \cdot 10^{-3}$ $C = 0.014$ At 5000 cycles $1.35 \cdot 10^{-3}$ $C = 0.007$	132	5.8
1 hr. 35 min.	At 560 cycles $1.10 \cdot 10^{-5}$ $C = 0.020$ At 5000 cycles $1.74 \cdot 10^{-5}$ $C = 0.010$	22.89	59	—	—	—
1 hr. 45 min.	Not measurable on account of high resistance			At 560 cycles $6.36 \cdot 10^{-4}$ $C = 0.014$ At 5000 cycles $7.00 \cdot 10^{-4}$ $C = 0.007$	407	10.1
2 hrs.				At 560 cycles $4.04 \cdot 10^{-4}$ $C = 0.100$ At 5000 cycles $6.04 \cdot 10^{-4}$ $C = 0.020$	498	49.6

thoroughly investigated by Michaelis (7) and Northrop (8) are not adapted to the kind of investigations intended. Although it was possible to duplicate the results found by Rein (9) with membranes

made according to Michaelis by directly measuring the membrane potential, the conductivity method which is successful in tissue measurements failed. This failure is probably due to the high resistance of the membrane. Michaelis (10) using the data of Fricke (11) and McClendon (12) came to the conclusion that the membranes of red blood cells have little permeability. However, the aim of this investigation was only to find models comparable to brain tissue in polarizability. After several attempts the following method was used: 10 cc. of collodion were allowed to fill a glass dish of 39.8 sq. cm. After 60 minutes drying at room temperature the adherent membrane

TABLE IV

Influence of Electrolytes on Polarization

Electrolytes isophoretic with 0.02 N KCl	Non-polarizable membrane			Polarizable membrane		
	$\frac{K}{C}\}_{560}$	$\frac{K}{C}\}_{5000}$	Δ	$\frac{K}{C}\}_{560}$	$\frac{K}{C}\}_{5000}$	Δ
			per cent			*per cent*
KCl	$2.82 \cdot 10^{-3}$ $C = 0.001$	$2.82 \cdot 10^{-3}$ $C = 0.001$	0	$1.75 \cdot 10^{-3}$ $C = 0.050$	$2.01 \cdot 10^{-3}$ $C = 0.015$	15.0
HCl	$2.91 \cdot 10^{-3}$ $C = 0.01$	$2.91 \cdot 10^{-3}$ $C = 0.001$	0	$1.74 \cdot 10^{-3}$ $C = 0.014$	$2.02 \cdot 10^{-3}$ $C = 0.006$	16.4
$MgCl_2$	$2.82 \cdot 10^{-3}$ $C < 0.001$	$2.82 \cdot 10^{-3}$ $C < 0.001$	0	$8.24 \cdot 10^{-4}$ $C = 0.054$	$1.08 \cdot 10^{-3}$ $C = 0.013$	29.9
$La(NO_3)_3$	$1.13 \cdot 10^{-3}$ $C < 0.001$	$1.13 \cdot 10^{-3}$ $C < 0.001$	0	$5.54 \cdot 10^{-4}$ $C = 0.04$	$7.48 \cdot 10^{-4}$ $C = 0.008$	35.1

was detached from its glass support and pressed for varying intervals of time between sheets of filter paper under a pressure of 50 gm./sq. cm. The results are summarized in Table III.

U.S.P. collodion and Merck collodion behaved in practically the same way. The results show that the membranes made as described above are of moderate resistance and high polarizability. Furthermore, the superiority of this method over that of complete drying is evident. These "medium" dry collodion membranes show towards electrolytes many of the particular features of dry collodion membranes and living tissue. Their polarizability is increased when in equilibrium with isophoretic salt solutions of bi- and trivalent cations

or with HCl, but is decreased by isophoretic NaOH; collodion membranes containing the same amount of collodion but showing no polarizability are apparently uninfluenced by the higher valency of the ions or by the acidity of the solution (Table IV).

II

As the resistances of the polarizable membranes were in every comparable instance higher than the resistances of non-polarizable membranes, it was natural to assume that they were less permeable for electrolytes than the non-polarizable membranes. Under this assumption polarizability has previously (1–2) been used as a measure

TABLE V

Permeability and Polarization

Time	1 per cent lecithin-collodion membrane Δ = 0	4 per cent lecithin-collodion membrane Δ = 29.5
hrs.		
0	$1.11 \cdot 10^{-5}$ reciprocal ohm	$6.4 \cdot 10^{-6}$ reciprocal ohm
1	$8.40 \cdot 10^{-5}$ " "	$1.33 \cdot 10^{-5}$ " "
2	$1.98 \cdot 10^{-4}$ " "	$2.11 \cdot 10^{-5}$ " "
4	$2.24 \cdot 10^{-4}$ " "	$2.20 \cdot 10^{-5}$ " "
22.5	$8.00 \cdot 10^{-4}$ " "	$4.80 \cdot 10^{-5}$ " "
48	$11.4 \cdot 10^{-4}$ " "	$7.90 \cdot 10^{-5}$ " "

of permeability. In order to ascertain this fact in a quantitative way, the following experiments were started.

Two collodion membranes were made in exactly the same way, both containing the same amount of collodion, but different quantities of egg lecithin, namely the first 1 per cent and the second 4 per cent. The respective resistances were 480Ω and 635Ω when measured in 0.02 N KCl. After a similar exposure to $CaCl_2$ Δ was 0 and 29.5 per cent respectively. Each of the membranes was then inserted into a three cell apparatus. On one side of the membrane there was 0.02 N KCl and the compartment on the other side of the membrane was filled with distilled water. At frequent intervals the electric conductivity of samples of the water were measured. The results of this experiment are summarized in Table V, indicating that the perme-

ability of the membrane showing polarization was approximately only one-fifteenth of that of the non-polarizable membrane.

Another clue as to a relationship between permeability and polarization can be found in the behavior of a single membrane (Table VI), upon ageing.

III

For the studies of some physicochemical problems the collodion membranes described above proved to be adequate tissue models. But their shortcomings became manifest when the influence of biologically active substances was investigated. Therefore, the attempt was made to build up polarizable membranes in which the reactive

TABLE VI

Ageing of a 4 Per Cent Lecithin-Collodion Membrane

Time	Conductivity apparatus filled with 0.02 N KCl				Resistance of membrane	Δ
	$K_{100} \cdot 10^{-3}$	C	$K_{1000} \cdot 10^{-3}$	C		
days					*ohms*	*per cent*
0	4.99	0.06	6.46	0.025	636.6	29.5
3	7.32	0.025	7.95	0.011	381.5	8.6
6	10.91	0.017	11.28	0.008	202.6	3.4

parts consisted solely of substances occurring in animal and human tissues.

No such investigations have so far been successful. Fricke and Curtis (4) could not find polarization in a solution of gelatin, and Abramson and Gray (13) were unable to detect changes in permeability in collodion membranes containing up to 50 per cent lecithin.

Therefore, the problem was attacked from different angles. In the first series of investigations collodion was used as a supporting medium, and different proteins (serum, egg albumin) were introduced into it in the same way as in the membranes used in electrodialysis by Ettisch and Ewig (14). By thoroughly shaking (2 hours on a shaking machine) a certain homogeneity was reached. The membranes were dried in such a way that pure collodion under similar treatment would not yield a polarizable membrane. No trace of polarizability could be

TABLE VI A

Unsuccessful Attempts to Render Membranes Polarizable by Introducing Biological Colloids or to Build Membranes with These Colloids

Supporting material	Introduced material	$K_{560} \cdot 10^{-3}$	C	$K_{5000} \cdot 10^{-3}$	C	Δ
Collodion	Blood albumin, 0.2 gm. shaken in 10 ml. collodion, dried for 60 min.	1.94	<0.001	1.94	<0.001	0
"	As above, treated for 10 min. with 0.02 N $CaCl_2$	2.06	<0.001	2.06	<0.001	0
"	2 per cent blood albumin in collodion, mechanically shaken for 60 min., in 0.02 N KCl	1.46	<0.001	1.46	<0.001	0
"	As above, in 0.01 N HCl	1.78	0.004	1.78	<0.001	0
"	As above, in 0.01 N NaOH	2.69	0.005	2.69	<0.001	0
"	As above, electrodialyzed for 40 min.	2.31	<0.001	2.31	<0.001	0
"	Egg albumin, 2 per cent in collodion, mechanically shaken for 3 hrs., dried for 60 min.	1.71	<0.001	1.71	<0.001	0
30 per cent gelatin	2 per cent heat-denatured egg albumin	2.03	<0.001	2.03	<0.001	0
As above	As above, after electrodialysis	3.55	<0.001	3.55	<0.001	0
Solid gelatin	Treated 24 hrs. with formaldehyde, pressed, 0.02 N KCl + formaldehyde	2.18	<0.001	2.18	<0.001	0
3 gm. gelatin	Treated with 36 per cent formaldehyde, pressed for 2 hrs., 0.02 N KCl + formaldehyde	1.41	<0.001	1.41	<0.001	0
Filter paper	1 gm. casein in 5 ml. 0.1 N NaOH, spread, 3 days in 10 per cent formaldehyde	2.00	0.010	2.00	0.001	0
" "	As above, in 0.012 N HCl	2.56	0.010	2.56	0.001	0
" "	As above, in 0.02 N $CaCl_2$	3.85	0.010	3.85	0.001	0
Parchment paper	Between two membranes 2 per cent lecithin solution, in 0.02 N KCl	1.31	<0.001	1.31	<0.001	0
" "	As above, in $CaCl_2$ of 0.005–0.068 N	No difference in conductivity at low and high frequencies detectable. Capacity at both frequencies <0.001 until N = 0.028. At highest concentration C 560 = 0.02, C 5000 = <0.001.				

detected in these protein-collodion membranes even when treated with $CaCl_2$, HCl, or NaOH or electrodialyzed.

In the second series of investigations gelatin was used for preparing membranes or as a medium for blood proteins, genuine or heat-denatured. It has been mentioned previously that 20 per cent gelatin membranes treated according to Collander (15) with 10 per cent formaldehyde and containing up to 2 per cent lipoids were not polarizable. These results could not be modified by using a thicker gelatin membrane, a membrane containing less water, or one hardened ultimately in 36 per cent formaldehyde. Formaldehyde was added to the fluid used in the conductivity measurements. Furthermore, a membrane consisting of a homogeneous layer formed by a concentrated solution of sodium caseinate on filter paper, did not show any polarizability even when treated with HCl or formaldehyde (Table VIA).

On account of these numerous failures, a fundamental change in the method of preparing the membranes was advisable.

It is well known that certain colloids are adsorbed on surfaces, this process being sometimes followed by reversible or irreversible denaturation as in the case of proteins (16). Experiments were instituted in which protein (egg albumin) was adsorbed on the sintered glass plate of a Jena glass crucible. Sintered glass crucibles No. 3 and No. 4 were used in which the sizes of the pores were, according to the manufacturers, respectively 20–30 μ and 5–10 μ.

In preliminary experiments it was shown that the insertion of a sintered glass plate into the pathway of an alternating current did not lead to polarization after the whole system had been filled with 0.1 N KCl.

With suction a 1 per cent solution of egg albumin was filtered through the glass plate. The filtration rate decreased very soon. In one experiment the interval between two drops was 720/5 seconds after 30 minutes, 1310/5 seconds after 1 hour and 20 minutes, practically nothing for the protein solution as well as for distilled water after 2 more hours.

In order to measure the polarizability of such a prepared filter plate, the following apparatus was devised. A wire gauze platinum electrode (diameter = 20 mm.) was introduced into a funnel in such a way that the electrode plate was tightly fixed in a position parallel to the glass

plate of the crucible. The funnel was then filled to the brim with 0.1 N KCl. The crucible was inserted tightly into the funnel with the help of a rubber band. In order to exclude air bubbles, the bottom of the crucible had been filled previously with a 10 per cent gelatin solution containing 0.1 N KCl. The crucible itself was filled with 0.1 N KCl, and an electrode similar to the one described was immersed in the crucible. A clamp held the electrode fixed so that its plate remained parallel to the first electrode and to the glass plate. The distance between the plates was 30 mm. The conductivity of such a system was about $1.45.10^{-2}$ reciprocal ohm. No trace of polarization could be detected with the usual conductivity measurements.

These experiments were repeated with different proteins, serum albumin, and casein, and with samples of these proteins acidified to increase the adsorption or treated with absolute alcohol or heat coagulated. Still no polarization of the filter plate was obtained.

Furthermore, it was not possible to render the sintered glass plate polarizable by treatment with colloid aqueous lecithin solutions or with alcoholic lecithin solutions, even if it was allowed to remain in the crucible for 12 hours. Even under the coagulating influence of HCl, $CaCl_2$ or $La(NO_3)_3$, it was impossible to cause a noteworthy choking of the glass plate (Table VI B).

Finally experiments were begun in which biological conditions were imitated by using membranes containing both proteins and lipoids. Sintered glass plates prepared with egg albumin in the manner already described were treated with alcoholic egg lecithin solutions. The crucibles were filled with 0.1 N KCl and allowed to stand for 12 hours. After this time, measurements of the conductivity disclosed a decided increase in resistance and capacitance and a definite polarization of the plate, corresponding to a Δ of 5.8 per cent.

The difference in pore size of the original sintered glass plate seemed to be of no importance.

Confirmation of these results was sought by a different method. According to Loeb (17), every impediment to the movement of an ion calls forth a potential difference. To test in this respect the action of the original glass plate and one containing adsorbed protein and lipoid, measurements of potential across those plates were made. Since direct current was to be used, non-polarizable silver-silver

chloride electrodes prepared according to Langelaan (18) replaced the platinum electrodes, and the crucible containing one of the electrodes was fixed with a clamp and dipped into a glass vessel which contained the other electrode. The crucible and the glass vessel were filled with 0.1 N KCl. The air bubble under the crucible was removed by aspiration. A compensation box and a string galvanometer as zero-point instrument were used in measuring the potential necessary to

TABLE VI B

Various Agents Which Did not Render the Sintered Glass Plate Polarizable

Adsorbed material	Conductivity at 560 cycles	C	Conductivity at 5000 cycles	C	Δ
I egg albumin	$4.82 \cdot 10^{-3}$	0.005	$4.82 \cdot 10^{-3}$	0.001	0
II " "	$1.53 \cdot 10^{-3}$		$1.53 \cdot 10^{-3}$		0
I after heating to 100°	$4.42 \cdot 10^{-3}$	0.005	$4.42 \cdot 10^{-3}$	0.001	0
I + HCl	—		—		0
II treated with alcohol	$7.23 \cdot 10^{-3}$	<0.001	$7.23 \cdot 10^{-3}$	<0.001	0
Casein in NaOH neutralized on filter plate	$1.27 \cdot 10^{-3}$	<0.001	$1.27 \cdot 10^{-3}$	<0.001	0
As above, treated with formaldehyde	$5.43 \cdot 10^{-4}$	<0.001	$5.43 \cdot 10^{-4}$	<0.001	0
Casein in HCl	—		—		0
Casein in HCl treated with alcohol	$2.28 \cdot 10^{-3}$	<0.001	$2.28 \cdot 10^{-3}$	<0.001	0
Serum albumin	$1.45 \cdot 10^{-3}$	<0.001	$1.45 \cdot 10^{-3}$	<0.001	0
Lecithin in aqueous solution					
Same as above + 0.1 N $CaCl_2$					
Lecithin solution + 0.02 N $La(NO_3)_3$	No clogging of glass filter				
Lecithin solution + 0.006 N HCl					
Alcoholic lecithin solution	$6.03 \cdot 10^{-4}$	0.002	$6.03 \cdot 10^{-4}$	<0.001	0

compensate that of the system. The results of a series of investigations are summarized in Table VII, indicating that the potential called forth by the protein-lipoid-glass membrane amounts to 4 mv.

These results confirm the results obtained by the measurements of the Δ and point to the usefulness of the latter method.

With crucibles prepared in the aforesaid way, some experiments of basic interest for biological problems were made.

The KCl was replaced by other solutions of the same normality.

The Δ was measured after equilibrium had been reached. The results are summarized in Table VIII. According to these figures, the polarization is much increased if potassium is replaced by magnesium. The narcotic effects of magnesium salts are as well known as their precipitating effect upon lecithin sol which is used for analytical

TABLE VII

Polarization of a Protein-Lipoid-Glass Filter Plate

	Ag-AgCl-electrodes		Platinum electrodes		
	Galvanometer readings	mv.	Conductivity at 560 cycles	Conductivity at 5000 cycles	Δ
					per cent
Electrodes alone (= *A*).....	25–15 / 5	2	—	—	—
A + glass filter plate (= *B*)....	30–15 / 5	3	$4.42 \cdot 10^{-3}$ $C = 0.001$	$4.42 \cdot 10^{-3}$ $C = 0.001$	0
B + adsorbed protein-lipoid....	50–15 / 5	7	$2.74 \cdot 10^{-3}$ $C = 0.028$	$2.89 \cdot 10^{-3}$ $C = 0.005$	5.8

TABLE VIII

Action of Various Ions on the Polarizability of Protein-Lipoid-Glass Filter Plate

0.1 N concentration of	At 560 cycles		At 5000 cycles		Δ
	Conductivity	*C*	Conductivity	*C*	
					per cent
KCl	$2.52 \cdot 10^{-3}$	0.028	$2.65 \cdot 10^{-3}$	0.005	5.13
KBr	$2.60 \cdot 10^{-3}$	0.030	$2.77 \cdot 10^{-3}$	0.010	6.50
$MgCl_2$	$1.72 \cdot 10^{-3}$	0.038	$1.88 \cdot 10^{-3}$	0.005	9.25
$AlCl_3$	$6.03 \cdot 10^{-4}$	0.010	$6.46 \cdot 10^{-4}$	0.001	7.05
$La(NO_3)_3$	$6.81 \cdot 10^{-4}$	0.017	$7.42 \cdot 10^{-4}$	0.003	9.0
HCl	$9.94 \cdot 10^{-4}$	0.011	$1.05 \cdot 10^{-3}$	0.003	5.8
NaOH	$1.21 \cdot 10^{-3}$	0.003	$1.23 \cdot 10^{-3}$	0.001	1.6

purposes. Narcotics generally call forth a decrease in permeability of cell surfaces (19). From valency alone the effect of an ion on the polarization of these plates cannot be predicted, lanthanum proving more effective than aluminum. A similar observation has already been made in *in vitro* experiments on brain tissue. Hydrochloric acid

affected the polarizability only slightly more than potassium chloride, while sodium hydroxide decidedly caused a drop in polarization. Analogous observations have been made on animal brain tissue *in vitro* (1). Furthermore, the specific effects of bromides were studied. The effectiveness of bromides in restoring the polarizability of swollen brains in *in vitro* experiments has been ascertained before (1). In another paper (20) a direct action of the bromide on the lecithin molecule was demonstrated. In a series of experiments it was shown that the polarizability of a protein-lipoid-glass plate in equilibrium with potassium chloride was further increased when potassium chloride was replaced by an equinormal solution of potassium bromide.

TABLE IX

Influence of Various Adsorbed Material on the Polarizability of Glass Filter Plate

Material	Conductivity at 560 cycles	C	Conductivity at 5000 cycles	C	Δ
					per cent
Starch	$1.29 \cdot 10^{-2}$	0.030	$1.29 \cdot 10^{-2}$	<0.001	0
Starch + protein	$1.28 \cdot 10^{-2}$	0.022	$1.28 \cdot 10^{-2}$	0.002	0
Starch + lecithin	$6.04 \cdot 10^{-3}$	0.030	$6.29 \cdot 10^{-3}$	0.008	4.2
Protein + alcohol soap solution	$1.14 \cdot 10^{-2}$	0.020	$1.14 \cdot 10^{-2}$	<0.001	0

Finally the influence of a lipoid-soluble agent on polarizability was determined. If ethyl alcohol was replaced by amyl alcohol as a solvent for lecithin, protein-glass filter plates prepared with the amylic solution showed a higher Δ than the ones prepared with the ethylic solution. The narcotic effect of amyl alcohol is twelve times greater than that of ethyl alcohol. In recent experiments (21) it was shown that the salt-binding capacity of lecithin was reduced by ethyl alcohol and practically abolished by amyl alcohol.

In most of these experiments the reaction of the composite membrane appears to be determined by the reactions of the lipoid component. The rôle of proteins seems to be of a more mechanical nature; *e.g.*, that of decreasing the size of the pores in the sintered glass plates. This view is supported by experiments showing that proteins can be replaced by soluble starch. The molecular weight of starch is 100,000

according to Samec (22). Starch alone does not cause polarization of the sintered glass plate.

It has not been possible so far to accomplish these results, if the lipoids were replaced by soluble starch or salts of fatty acids (Table IX). This failure is interesting in view of the many theories stressing the importance of lipoids in biological membranes.

SUMMARY

1. Collodion membranes of high polarizability and low resistance can be obtained either by addition of certain ether-soluble substances such as phosphatides, olive oil, mastix, and gum benzoin, to the collodion or by drying collodion membranes for a limited time under pressure.

2. The permeability of membranes of different polarization has been measured by means of conductivity methods.

3. Sintered glass filter plates of Jena glass crucibles on which proteins and lipoids have been adsorbed show polarization. It could be shown that some narcotics which react with lecithin cause an increase in polarization of the protein-lipoid-glass system. Substitutions of the protein but not of the lipoid were possible, without causing a decrease in the polarizability of the membranes.

REFERENCES

1. Spiegel, E., and Spiegel-Adolf, M., *Am. J. Psychiat.*, 1936, **92,** 1145.
2. Spiegel-Adolf, M., and Spiegel, E., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32,** 139.
3. Bungenberg de Jong, H. G., and Bonner, J., *Proc. Acad. Sc. Amsterdam*, 1935, **38,** 797. *Chem. Abstr.*, 1936, **30,** 354.
4. Fricke, H., and Curtis, H. J., *Phys. Rev.*, 1935, **47,** 974.
5. Thomas, A. W., Colloid chemistry, New York and London, McGraw-Hill Book Co., 1934.
6. Wilbrandt, W., *J. Gen. Physiol.*, 1934–35, **18,** 933.
7. Michaelis, L., *Kolloid-Z.*, 1933, **62,** 2; and coworkers, *J. Gen. Physiol.*, 1926–27, **10,** 575, 671, 685; 1927–28, **11,** 147; 1928–29, **12,** 55, 221, 473, 487.
8. Northrop, J. H., *J. Gen. Physiol.*, 1927–28, **11,** 233; 1928–29, **12,** 435.
9. Rein, H., *Z. Biol.*, 1926, **85,** 217.
10. Weech, A. A., and Michaelis, L., *J. Gen. Physiol.*, 1928–29, **12,** 221.
11. Fricke, H., *J. Gen. Physiol.*, 1925–26, **9,** 137.
12. McClendon, J. F., *J. Biol. Chem.*, 1926, **69,** 733.
13. Abramson, H. A., and Gray, S. H., *J. Biol. Chem.*, 1927, **73,** 459.

14. Ettisch, G., and Ewig, W., *Biochem. Z.*, Berlin, 1928, **195,** 175.
15. Collander, R., *Protoplasma*, 1927, **3,** 213.
16. Spiegel-Adolf, M., *Biochem. Z.*, Berlin, 1932, **252,** 37.
17. Loeb, J., Proteins and the theory of colloidal behavior, New York and London, McGraw-Hill Book Co., 1922.
18. Langelaan, J. W., *Arch. néerl. physiol.*, 1929, **14,** 592.
19. Spiegel, E., and Spiegel-Adolf, M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34,** 799.
20. Spiegel-Adolf, M., *Biochem J.*, London, 1935, **29,** 2413.
21. Spiegel-Adolf, M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35,** 263.
22. Samec, M., Kolloidchemie der Stärke, Dresden-Leipsic, Steinkopff, 1927.