ELECTROENDOSMOSIS THROUGH MAMMALIAN SEROUS MEMBRANES.

III. The Relation of Current Strength and Specific Resistance to Rate of Liquid Transport. Transport Rate with Serum.

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The present experiments concern the relation of the electric current strength and of the specific electrical resistance of the perfusing liquid to the rate of electroendosmotic flow across mammalian serous membranes. A direct proportionality is found between liquid and electric flow through these membranes, which are complex in structure and heterogeneous in composition. The variables Φ and I are thus connected in the complex case by the same relationship as in the case of simple membranes to which the classical electroendosmotic equation applies. Less simple relations are found when the membranes are bathed in buffers of varying specific resistance. Quantitative determinations are also reported with whole serum and the membranes of living and dead animals. The rate of electroendosmotic flow across dog and cat serosæ bathed in serum has been found to be 0.2 to 0.3 c.mm. per minute per milliampere toward the cathode.[1]

Relation of Current Strength to Rate of Flow.

The experimental set-up is shown in Fig. 1. The membranes were fastened by broad rubber bands over the mouth of the electrode vessel; the inside diameter

[1] The ratio of current strength to liquid flow through any given membrane is independent of the area of membrane through which the current is passing. For if a given constant potential difference is maintained across the membrane, the current strength and the volume of liquid transported in unit time will both be proportional to the area of the membrane through which flow occurs. The dimension of area therefore cancels out and does not appear in the ratio of liquid to electric flow.

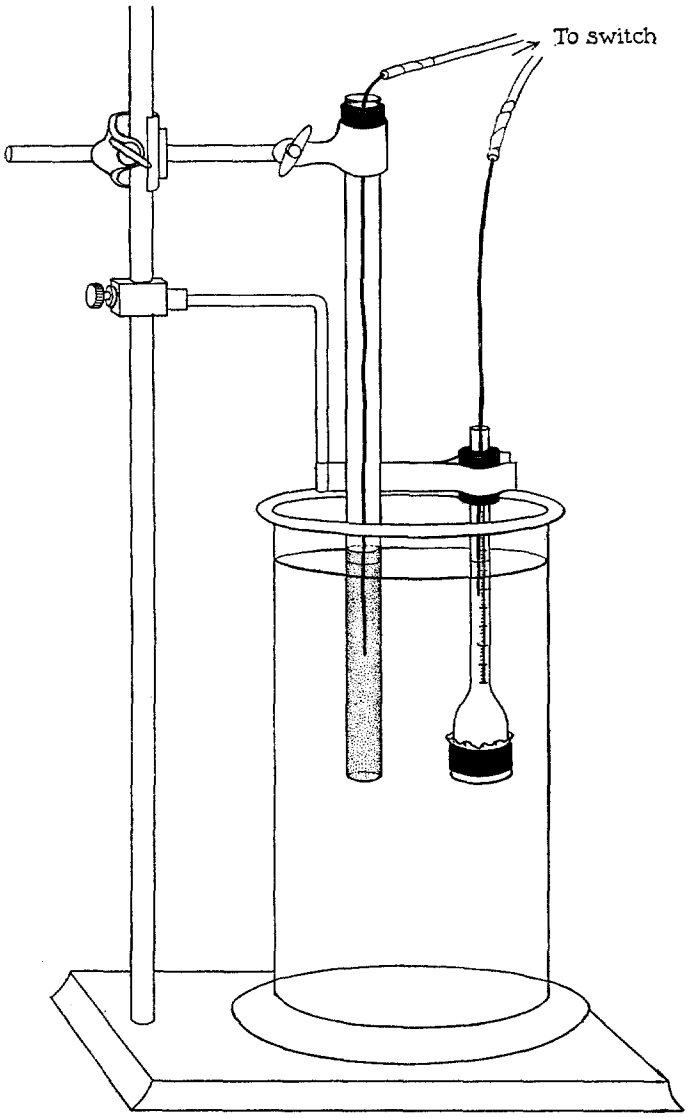

FIG. 1. Arrangement for study of electroendosmotic transport across animal pericardia. See text.

of the mouth was 18 mm. The electrode vessel was filled and emptied with capillary pipettes; it dipped into a large vessel of buffer containing an agar electrode. Current was led into the buffer in the electrode vessel by a platinum wire. The buffer within and without the electrode vessel was the same.

The source of E.M.F. was connected to the two ends of a 666 ohm slide-wire rheostat. The experimental lines were led off, one from one end of the rheostat and the other from the rheostat slider. By moving the slider, therefore, the

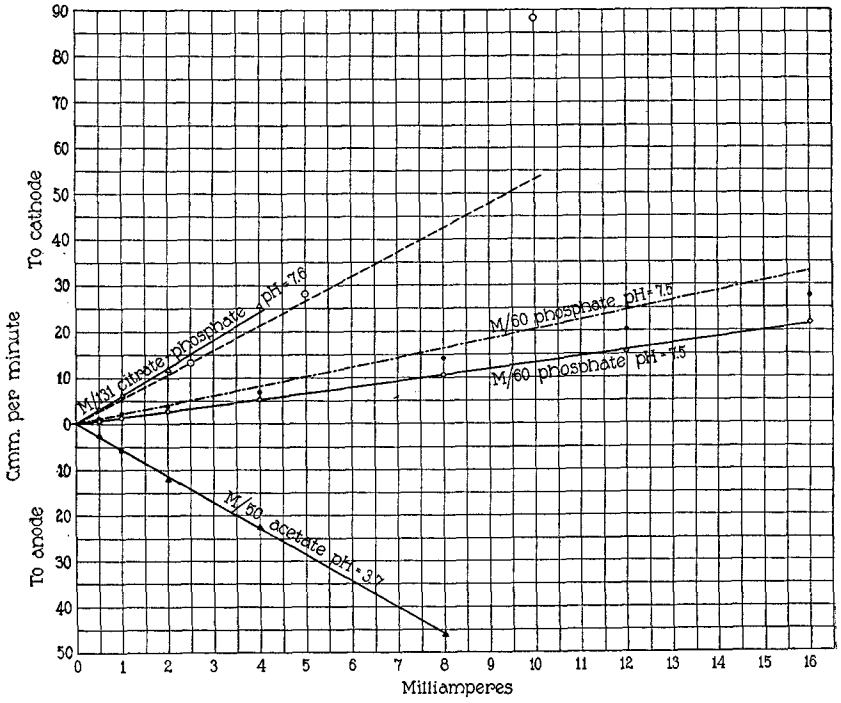

FIG. 2. The relation between volume of liquid transported in unit time (ordinates) and electric current strength (abscissæ) for several systems.

P.D. between the experimental lines could be varied continuously from zero to the total applied E.M.F. A pole-charging switch and a Weston standard milliammeter were included in the circuit. The experimental procedure finally adopted gave satisfactorily reproducible results with a minimum of error due to temperature changes, leakage, bulging of the membrane, polarization, etc.

The data obtained are presented in Fig. 2. The points fall with considerable accuracy along the solid straight lines, and these pass

through the origin; the proportionality of liquid and electric flow even with minimal current strengths is thus indicated.

The points do not fall satisfactorily along the broken lines. In the case of the dash line (M/131 citrate-phosphate data) the erratic position of the points above 4.0 milliamperes is almost certainly due to the effect upon the membranes of chemical changes about the platinum electrode. In these experiments the electrode was brought near the membrane in order to obtain the desired current strengths. Later the electrode was kept well up in the narrow vertical tube.

In the case of the broken line with M/60 phosphate mixture, a disturbing factor seems to have been that the membranes were applied very laxly over the mouth of the electrode vessel.

The slopes of the straight lines indicate the rate of liquid transport per milliampere characteristic for each system. These are:

Buffer mixture of Na_2HPO_4 and citric acid; molarity, M/131; pH 7.6. Solid line, composite of two experiments with lean cat pericardia. Rate of liquid transport, 6.00 c.mm. per milliampere per minute to cathode. Dash line, composite of three experiments with cat pericardia.

Buffer mixture of Na_2HPO_4 and KH_2PO_4; molarity, M/60; pH 7.5. Solid line, lean pericardium of male dog; runs continued throughout 6 working days. Rate of liquid transport, 1.33 c.mm. per milliampere per minute to cathode. Experimental site at end of experiment composed chiefly of bundles of collagen fibres in two more or less well defined lamellæ; elastin fibres among collagen bundles; basement membrane persistent only in places; mesothelium gone; in one region a zone of looser connective tissue containing fat cells, blood vessels, and a nerve. Fibre bundles considerably frayed out. Dot-dash line, composite of three experiments with dog pericardia; a few runs only with each membrane.

Buffer mixture of acetic acid and sodium acetate; molarity, M/50; pH 3.7. Lean pericardium of male dog; runs through 4½ working days. Rate of liquid transport, 5.76 c.mm. per milliampere per minute to anode. Experimental site of dense zones of collagen fibres with a few elastin fibres interspersed; in a part of section looser connective tissues containing fat cells and blood vessels. Basement membrane persistent in places only; mesothelium gone.

Relation of Specific Resistance of Buffer to Rate of Flow.

The buffers used were Sörensen's phosphate mixtures to which NaCl was added to give the desired conductivity. The stock KH_2PO_4 and Na_2HPO_4 solutions were mixed and diluted to give buffers of approximately 7.4 pH and M/60 concentration. Sodium chloride was added in amounts such as to give a series of M/60, M/50, M/40,

M/30, M/20, M/10, M/7, and M/6 total molar concentration. The pH was readjusted to about 7.4 by addition of dilute NaOH. The specific resistance of the buffers was determined at 21.6° ± 0.2°C. by the ordinary Kohlrausch method. The viscosity of the M/6 buffer was found to be only a little more than 2 per cent greater than that of distilled water. Viscosity measurements were thereafter

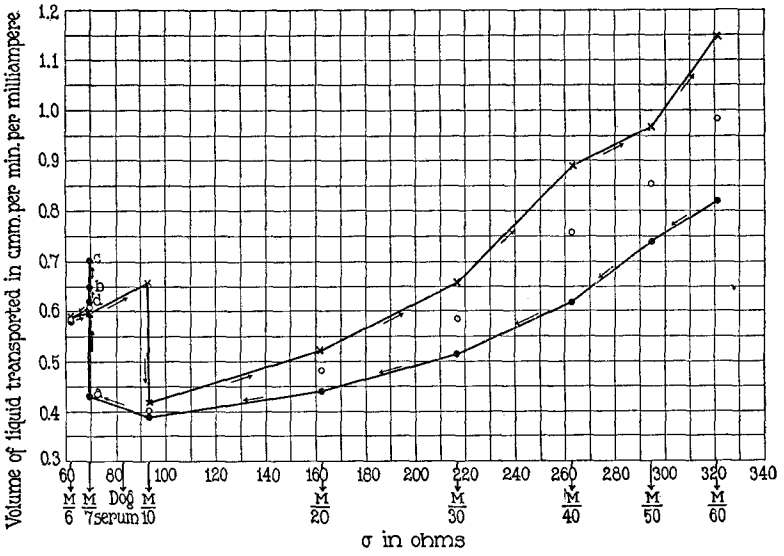

FIG. 3. Experiment 1. Relation of rate of electroendosmotic transport to specific resistance of buffers bathing membrane. Male dog pericardium. Current strength 15 milliamperes. pH of buffers 7.36 to 7.44. Arrows and letters indicate order in which buffers were used. Mean values for each buffer plotted as white circles.

discontinued, since fluctuations in room temperature and heating effects with passage of current undoubtedly caused variations in viscosity greater than this.

The experimental arrangement has already been described (Fig. 1). Eight preliminary runs with inner electrode alternately cathode and anode were routinely made with each buffer to impregnate the membrane. Three pairs of runs were then made with 15 milliamperes current. The mean rates of liquid transport for these last runs are plotted against the specific resistances of the several buffers in Figs. 3 and 4.

In a simple homogeneous membrane of constant structure and composition the rate of electroendosmotic transport per unit of current is proportional to the specific resistance (σ) of the solution in the membrane pores. Were the present membranes of this nature (see, however,[2]) the plots of volume transported against specific resistance should be straight lines, or should deviate appreciably from straight lines only through changes in the electrokinetic P.D. How far the

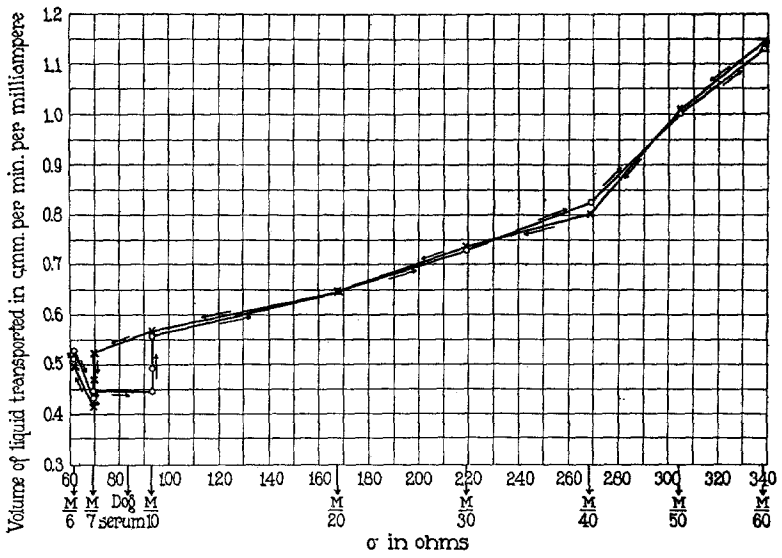

FIG. 4. Experiment 2. Rate of transport and specific resistance of buffers. Female dog pericardium. Current strength 15 milliamperes. pH of buffers 7.37 to 7.41. Arrows indicate order in which buffers were used. σ of M/7 and M/10 approximate only.

behavior of the present membranes differs from that of a simple inert membrane is shown by the discontinuity of the lines to the left-hand side of Figs. 3 and 4.

Two characteristics of the experimental curves are especially to be noted:

First, that the slope of the lines from $\sigma = 93$ ohms increases with increasing values of σ. An increase in electrokinetic P.D. between solid and solution with decreasing salt content is a general phenomenon

[2] Mudd, S., *J. Gen. Physiol.*, 1924–25, vii, 389.

in the range of concentrations here used[3] and undoubtedly contributed to the upward trend of the lines with increasing values of σ. However, in consideration of what follows this explanation seems incomplete.

Second, the remarkable discontinuity of the curves in the region in which the conductivities and osmotic pressures of the buffers are close to those of the blood. Each of the points plotted is the mean value of six 6 minute runs. The buffer in the electrode vessel was changed after each run and other delays were incurred. The several points for any one buffer are therefore separated by intervals of from 1

TABLE I.

Experiment No.	Membrane.	State of animal.	Current strength.	Buffer.	σ of buffer.	Rate of transport per milliampere.	σ of serum of experimental animal.
			milli-amperes		*ohms*	*c.mm. per min.*	*ohms*
3	Dog mesentery.	Living.	25	(Phosphates + NaCl) M/6.	61.83	0.36	83.54
	Dog mesentery.	"	25	" " " M/7.	—	0.29	83.54
	Dog mesentery.	"	25	" " " M/10.	—	0.39	83.54
4	Cat pericardium.	Dead.	15	" " " M/6.	61.83	0.57	83.27
	Cat pericardium.	"	15	" " " M/7.	69.68	0.49	83.27
	Cat pericardium.	"	15	" " " M/10.	93.09	0.57	83.27

to several hours, and an overnight stay in buffer in the ice box usually intervened between the runs with the different buffers of $\sigma = 93$ ohms or less. The "hysteresis loops" at the left-hand side of Figs. 3 and 4 thus represent slow changes with time as well as with changing buffers in the rate of electroendosmotic transport across these pericardial membranes. These changes appear to have been completely

[3] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639, 655. Northrop, J. H., and Freund, J., *J. Gen. Physiol.*, 1923–24, vi, 603. Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109, 395, 479; 1923–24, vi, 215.

reversible in Fig. 4, only partially so in Fig. 3. On what alterations in structure and composition within the membrane they depended can only be guessed. The equilibrium between membrane and enviroring medium was evidently very sensitive when the osmotic and electric conditions of the medium roughly approximated those of the blood.

The other two experiments affording data on rate of transport with the M/6, M/7, and M/10 phosphate and NaCl buffer mixtures show minimum values with M/7. See Table I.

Throughout Experiments 1, 2, and 4, and only these afford data on this point, the mean rate of transport to the cathode was greater with inner than with outer electrode cathode when buffers M/6 to M/20 were used, and the rate of transport to the cathode was greater with outer than with inner electrode cathode when buffers M/30 to M/60 were used. The differences were great at the extremes of the buffer series and gradually diminished to the transition point between M/20 and M/30. With M/60, for instance, the mean rise of the meniscus toward the inner cathode was to the fall of the meniscus toward the outer cathode as 1 is to 1.6; with M/6, mean rise to inner cathode: mean fall to outer cathode:: 2.1:1. These effects no doubt indicate that the membrane was polarized during the passage of current and that the polarization was somehow correlated with the conductivities of the perfusing buffers and with the arrangement of the membrane with respect to the inner and outer fluids.

Bethe and Toropoff[4] have demonstrated the polarization of diaphragms during passage of current. Reversal with acid of the direction of electroendosmotic flow causes also reversal of the direction of polarization. The relative disturbances in ion concentrations at the membrane surfaces are diminished by increasing salt concentrations in the perfusing solutions.

The Transport Rate with Serum.

The apparatus used for serum experiments (Fig. 5) was slightly modified from suggestions made by Dr. M. Kunitz. A Zn-$ZnSO_4$ electrode connects through an L-way stop-cock with a salt bridge filled with 0.8 per cent NaCl solution. A second L-way stop-cock near the other end of the bridge facilitates control of

[4] Bethe, A., and Toropoff, T., *Z. physik. Chem.*, 1914, lxxxviii, 686; 1914–15, lxxxix, 597.

the several solutions.[5] The open-end vertical tubes are used for washing and filling. The rest of the arrangement is as already described.[2] Readings of the meniscus are made with the circuit closed. The mouth of the electrode vessel to which the membrane is applied is 6.5 mm. in internal diameter.

Serum was obtained by bleeding the animals, defibrinating, and centrifugating the blood; it contained sufficient hemoglobin to give a faint or deeper rose tint.

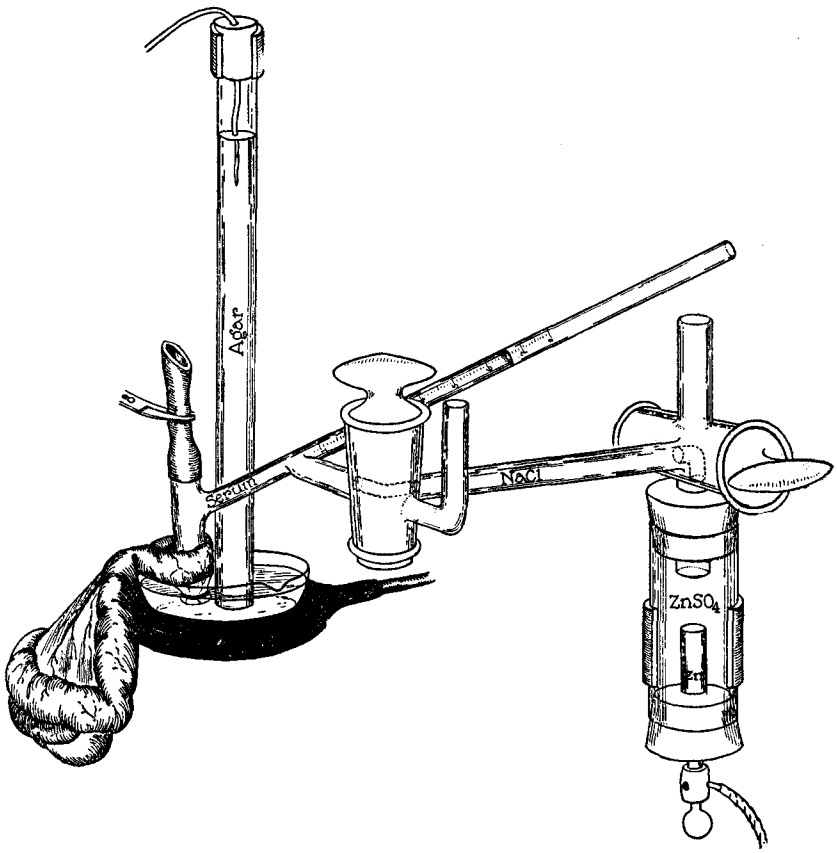

FIG. 5. Arrangement for study of electroendosmotic transport using whole blood serum and mesentery of living animal. See text.

[5] Replacement of this L-way cock by a T-way cock adapts the apparatus also to the determination of the H ion reversal points of membranous tissues. This form of the apparatus may be had from Arthur H. Thomas Company of Philadelphia.

The serum from animals fasted before use was clear. If the animals had been newly fed the serum was turbid even after prolonged centrifugation, presumably due to lipemia. Dark-field examination of such turbid serum showed myriad bright spherical objects ranging from the limit of visibility to droplets of a micron

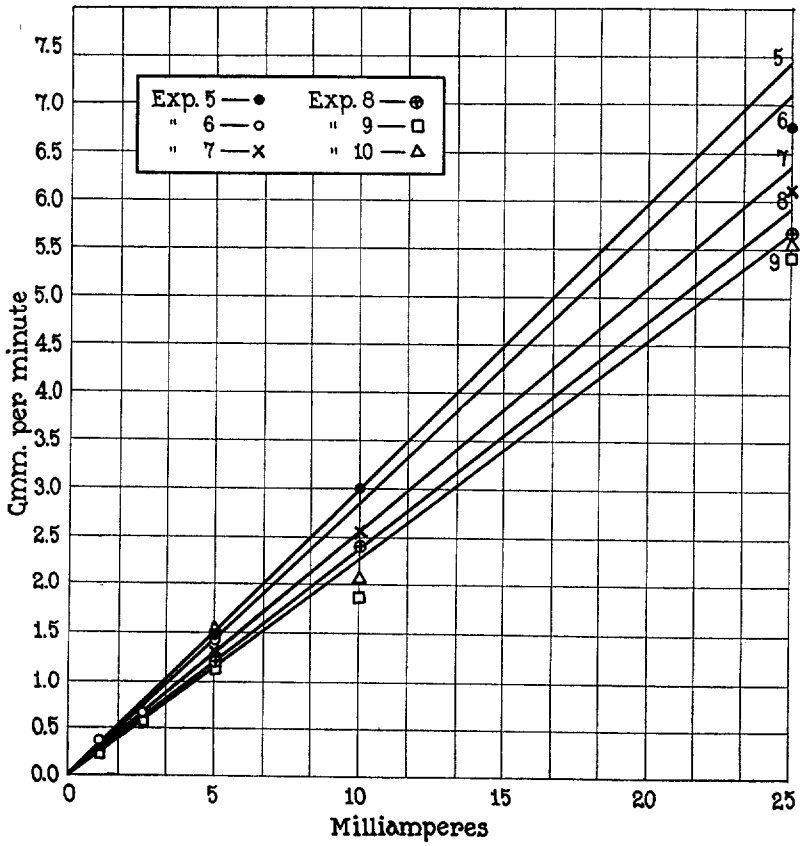

FIG. 6. Rates of transport of liquid plotted against current strengths. Fluid bathing membranes, undiluted dog blood serum. Experiment 5, living dog mesentery; Experiment 6, living dog mesentery; Experiment 7, dog mesentery, post mortem; Experiment 8, dog pleura, post mortem; Experiment 9, dog pericardium, post mortem; Experiment 10, dog pericardium, post mortem.

or more in diameter. With the mesenteries of the living animals the fresh serum of another animal of the same species was used as perfusing fluid; precautions were taken against injury of the living mesentery. With membranes used post mortem the serum of the animal from which the membrane came was used.

In general the points obtained with current strengths up to 10 milliamperes are seen to lie satisfactorily along straight lines passing through the origin (Fig. 6).

TABLE II.

Experiment No.	Serum. From	Serum. State.	Membrane.	State of animal.	Current strengths (in order used).	Rate of transport in c.mm. per min. per milliampere.
					milli-amperes	
5	Dog B.	Clear.	Mesentery, Dog C.	Living.	10, 5, 25	0.30
6	" D.	"	" " E.	"	5, 2.5, 1	0.28
7	" C.	"	" " C.	Dead 23 hrs.	5, 10, 25	0.25
11	" A.	Turbid.	" " A.	" 3 "	25	0.27
9	" F.	Clear.	Pericardium, Dog F.	" 3 "	5, 2.5, 1 10, 25	0.23
11	" A.	Turbid.	" " A.	" 1½ "	25	0.23
8	" E.	Clear.	Pleura, Dog E.	" 18 "	5, 10, 25	0.24
12	" A.	Turbid.	" " A.	" 22 "	25	0.19*
13	Cat A.	Clear.	Mesentery, Cat. B.	Living.	25	0.22
14	" C.	Turbid.	" " D.	"	25	0.24
15	" C.	"	" " C.	Dead 7 hrs.	25	0.22
16	" B.	Clear.	Pericardium, Cat B.	" 4 "	25	0.26
17	" C.	Turbid.	" " C.	" 6 "	25	0.23
18	" B.	Clear.	Pleura, Cat B.	" 2 "	25	0.28
19	" D.	Slightly turbid.	" " D.	" 2 "	25	0.24
Average.....						0.25

* The site of Experiment 12 seems to have been pleura from the anterior mediastinum rather than from the fibrous sheet between apex of pericardium and diaphragm ordinarily used. The section showed strands of atrophic thymus tissue and cysts filled with a coagulum between the pleural leaves.

Exceptions are the points plotted in triangles and the 10 milliampere point in Experiment 9. The points in triangles are, for reason not understood, so irregular that no attempt has been made to draw a line through them; they are not included in Table II.

All of the 25 milliampere values and the 10 milliampere point of Experiment 9 are lower than expectation. The disturbing factor is not known. The temperature of the serum bathing the membrane was raised a few degrees by the passage of the 10 and 25 milliampere currents, and this may have been of influence. Liquid flow may have become turbulent with the higher current strengths. However, the mean departure of observed values at 25 milliamperes from the straight lines amounted to only 5.4 per cent. The values of transport rate given in Table II for the experiments plotted in Fig. 6 are the slopes of the straight lines. For the experiments in which only 25 milliampere points are available the tabulated values for transport rate are probably about 5 per cent too low.

No certain correlation was detected between rate of transport and the thickness of the several membranes; this is in harmony with other electroendosmotic experiments.[6]

DISCUSSION.

A number of animal membranes have been shown to be negatively charged relative to their environing medium when that medium is blood serum or other buffer of neutral or slightly alkaline reaction. The existence of this electric potential difference necessitates that the liquid in the membrane pores should tend to move toward the cathode when the membrane is traversed by an electric current. The rate of liquid flow has been shown to be proportional to the current strength and to amount when serum is used to 0.2 to 0.3 c. mm. per minute per milliampere.

The functional activity of glands[7] and muscles is known to be accompanied by electric current flow, and numerous other sources of current in the body are either known or may be confidently inferred from analogy with non-living systems. The suggestion has already been made[8] that the action current of glands might influence the

[6] von Smoluchowski, M., in Graetz, L., Handbuch der Elektrizität und des Magnetismus, Leipsic, 1914, ii, pt. 2, 380.

[7] Hermann, L., and Luchsinger, B., *Arch. ges. Physiol.*, 1878, xvii, 310. Bayliss, W. M., and Bradford, J. R., *J. Physiol.*, 1885, vi, p. xiii; 1886, vii, 217. Bradford, J. R., *J. Physiol.*, 1887, viii, 86. Cannon, W. B., and Cattell, McK., *Am. J. Physiol.*, 1916, xli, 39. Gesell, R., *Am. J. Physiol.*, 1918–19, xlvii, 411.

[8] Mudd, S., and Mudd, E. B. H., *J. Bact.*, 1924, ix, 163.

process of secretion. Consideration of the facts of the preceding paragraph, the writer believes, endows this possibility with a degree of probability amounting almost to certainty. For in such a system, in which liquid is being transported through capillary channels which are at the same time the site of an "action current" the electric current must at least modify if it does not control the liquid flow. Knowledge as to whether the electroendosmotic effect plays a major or minor part will have to await further study of the orientation and magnitude of the electric disturbances.

A number of the experiments here reported were performed by my technical assistant, Mr. Leo S. Hrdina.

SUMMARY.

The rate of electroendosmotic flow through dog and cat pericardia is found to be proportional to the current strength. The plots of current strengths against volumes of liquid transported in unit time are, in the better experiments, straight lines passing *through the origin;* the slopes of the lines are characteristic of the several systems.

Data on transport rate with buffers of different specific resistances showed the following phenomena:

1. Decrease of the observed transport rate to a minimum between σ values of 95 and 60 ohms.
2. Changes in the membrane markedly affecting transport rate, at conductivities and osmotic pressures close to those of the blood.
3. Polarization of the membrane during the passage of current.

The mean rate found for electroendosmotic transport across dog and cat serous membranes bathed in serum has been 0.19 to 0.30 (average, 0.25) c.mm. per minute per milliampere.[1] The best experiments with dog serum and the living mesenteries of dogs under ether gave a mean rate of 0.29 c.mm. per minute per milliampere.

These data, together with data from other sources, are believed to indicate a probability approaching certainty that electroendosmotic effects are a factor in glandular secretion.