THE PENETRATION OF BACTERIA THROUGH CAPILLARY SPACES

III. TRANSPORT THROUGH BERKEFELD FILTERS BY ELECTROENDOSMOTIC STREAMING

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To understand a given system it is necessary to have gained familiarity with the phenomena that occur in that system, to know what forces underlie them, and, last, and most difficult, to be able to relate these underlying forces one to the other, assigning to each its proper part in the phenomena observed. Study of the dynamics of the extraordinarily complex systems known as living organisms has made us familiar with a large array of phenomena; a number of mechanisms, too, conditioning the movements of fluids, of dissolved and suspended matter in living tissue have been brought to light. Muscular contraction with the circulation of fluids and pressure heads occasioned by it, ameboid motion of phagocytes, the beating of cilia and flagella, and protoplasmic streaming—all obviously requiring further resolution into fundamental physico-chemical forces—have been shown to play their considerable parts in the transportation of matter in living tissues. Diffusion, osmotic pressure and surface tension are known to be important kinetic forces.

It is the purpose of the present communication to consider a mechanism not so often related to the dynamics of organisms; namely, movement of liquids and suspended solids in an electric field, and to show experimentally that the forces involved

can transfer living microörganisms across a filtration membrane having certain features in common with capillary spaces found in living tissues. Whether or not such a mechanism does actually play a significant part in organisms is not under consideration in the present paper and will have to be determined by future experiment.

THEORETICAL

Two dissimilar substances, as has long been known, are seldom at the same electrical potential at their surface of contact. In biological material this interfacial potential difference is largely or wholly due to the separation of oppositely charged ions, whether by electrolytic dissociation, by adsorption in an interface (Michaelis, Freundlich and Gyemant, 1922); by unequal distribution between two phases (Beutner, 1920); or across a membrane (J. Loeb, 1922; R. F. Loeb, Atchley and Palmer, 1922; Irwin, 1922). Possibly also even at the interface of pure nonionogens, the architecture of the molecules conditions a potential difference (Frenkel, 1917).

If now there is a fall in electric potential along, i.e., tangent to, such a charged interface, the two phases will tend to be displaced with reference to each other, the positive component of the electric double layer tending to move into a region of lower, and the negative component into a region of higher, potential. In general the less firmly fixed component will be displaced with reference to the fixed component. Thus in an electric field a fluid will move through the pores of a solid membrane (electric endosmosis) and suspended particles will move through a fluid (cataphoresis).

Let us now consider a system (fig. 1) composed of a fluid with suspended particles, e.g., a culture of bacteria, bathing a diaphragm or membrane with pores of various sizes, e.g., a filter. Suppose an electric field to be set up across the filter. According to the Helmholtz-Smoluchowski equations (1914) the fluid next the pore walls will stream toward the pole of opposite sign with a velocity.

$$V_{en} = \frac{{}_F\zeta_S . K.H.}{4\pi.\eta} \qquad (1)$$

All units are C.G.S. H is the electric field strength in E. S. U. per cm. η is the coefficient of viscosity of the medium. K is the dielectric constant of the medium.[1]

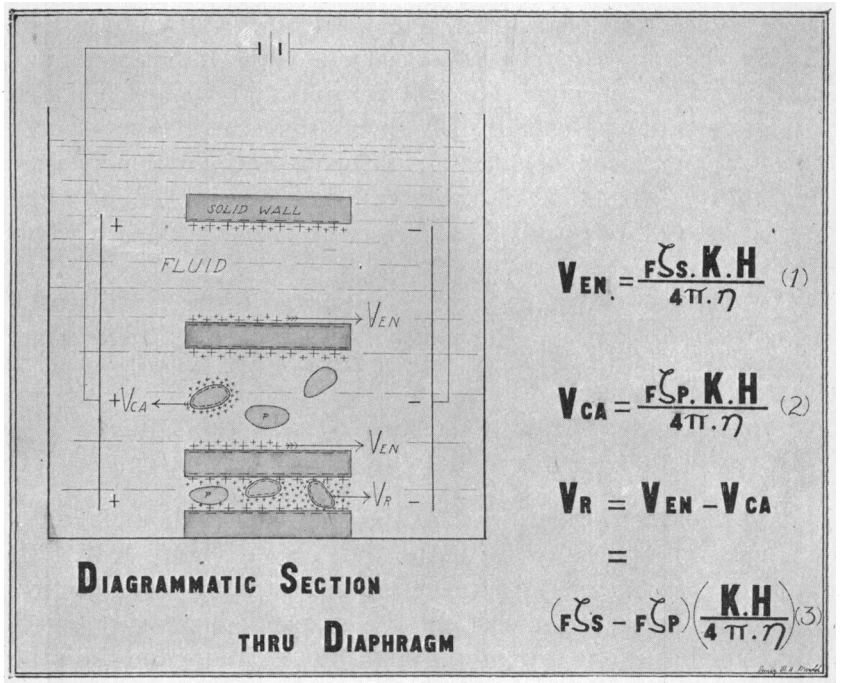

FIG. 1. SCHEMATIC REPRESENTATION OF ELECTROCAPILLARY EFFECTS

In uppermost pore of diaphragm an endosmotic current moves toward the cathode with a velocity V_{en}. In middle pore suspended particles move toward the anode with a velocity of cataphoresis, V_{ca}. In bottom pore the two effects are combined, with a resultant movement of the particles toward the cathode at an approximate velocity, $V_R = V_{en} - V_{ca}$.

${}_F\zeta_S$ is the electrokinetic p.d. between fluid and pore walls, $(\varphi i - \varphi a)$ of the Helmholtz equations, and requires a further word of explanation.

[1] This has practically always been taken as equal to the constant of the pure medium, regardless of the solute it contains, thus involving a serious uncertainty (Lattey, 1921, Keller, 1921, Errera, 1922).

The layer of water molecules next the wall is conceived in the Helmholtz theory as held immovably fixed to the wall. The displacement in an electric field takes place between this fixed fluid layer and the body of the fluid, between which there is the potential difference ζ. This ζ-potential Freundlich (1922) therefore calls the electrokinetic potential. The total p.d. between the inside of the two phases, (ϵ), which with oil-water phases containing electrolytes can be reckoned from Nernst's thermodynamically derived concentration cell formula, Freundlich calls the thermodynamic potential. He shows that the ζ- and ϵ-potentials can vary independently. ζ is, for instance, extremely sensitive to the effects of electrolytes adsorbed in the interface. Similar results for proteins have recently been obtained by Loeb (1923).

The particles shown in the middle pore of figure 1 will tend to move by cataphoresis to the positive side of the diaphragm with a velocity of cataphoresis.

$$V_{ca} = \frac{{}_F\zeta_P . K.H.}{4\pi.\eta} \tag{2}$$

${}_F\zeta_P$ is the electrokinetic p.d. at the fluid-particle boundary.

If now we suppose, as in the bottom pore of figure 1, that the capillary is so narrow that the endosmotic streams along the wall occupy its entire cross section[2] the suspended particles should tend to be borne one way by the endosmotic stream and the other way by cataphoresis. Combining the two equations we get the resultant velocity,

$$V_R = V_{en} - V_{ca} = ({}_F\zeta_S - {}_F\zeta_P)\frac{K.H.}{4\pi.\eta} \tag{3}$$

It is evident that if the particles were at a higher potential than the medium the velocity of cataphoresis would be added to that of endosmosis (v. footnote 3), and similar changes in

[2] The observations of Cotton and Mouton, (1906) suggest that glass capillaries of diameter less than 50 μ at any rate would fulfill this condition. Quartz or gypsum capillaries would have to be narrower than those of glass (1906).

sign would have to be made if the direction of the potential gradients indicated by ${}_F\zeta_S$ or H were reversed, as might well happen in a living system.

The value of V_R so derived must be regarded as qualitatively only and not quantitatively correct, since the homogeneous fall in potential along the capillary walls, ${}_F\zeta_S$, would probably be interfered with by the presence of the particles with their fields (Smoluchowski, 1914, paragraph 19).

It is also to be emphasized that the value of V_R so derived only applies to a membrane on the two sides of which the fluid pressure is and remains the same. If the ends of the capillary spaces of the membrane are closed or are at different pressures, counter currents would develop within the capillaries (Ellis, 1911).

EXPERIMENTAL

The object of the present experiments was to drive electrically a culture medium containing living bacteria through an artificial membrane. They illustrate the mechanism set forth above, and are in qualitative agreement with equation (3). The experimental arrangement was not adapted to exact quantitative treatment.

The apparatus used is shown diagrammatically in figure 2. The diaphragm is a diatomaceous earth Berkefeld filter, such as is commonly used in bacteriology for obtaining sterile filtrates. It is of the most porous or "V" type. The filter is severed from its metal mountings to prevent short circuiting, and is sealed with gold size varnish into a rubber stopper which is itself cemented with gold size into one end of a glass cylinder. In the other end of the cylinder is a second stopper fitted with a carbon electrode and four glass tubes; three open into the cylindrical vessel and are used, respectively, for introducing buffer solution into the vessel, for equalizing pressure within and without the vessel when it is inverted for withdrawal of the filtrate, and for pouring off the filtrate. The fourth tube has a platinum wire sealed into its closed tip, which contains mercury, and with a similar tube inside, serves for estimation of the p.d. acoss the filter. The inoculation

tube at its outer end is thrust through a rubber collar inside the neck of a funnel, thus facilitating sterile inoculation of the filtrate into vessels of sterile culture medium.

The cultures used for the experiments were mixed growths in hay infusion of *Erythrobacillus prodigiosus* and *Vibrio percolans*. The former is the conventional organism used in experiments with filter-passing organisms to prove the tightness of the

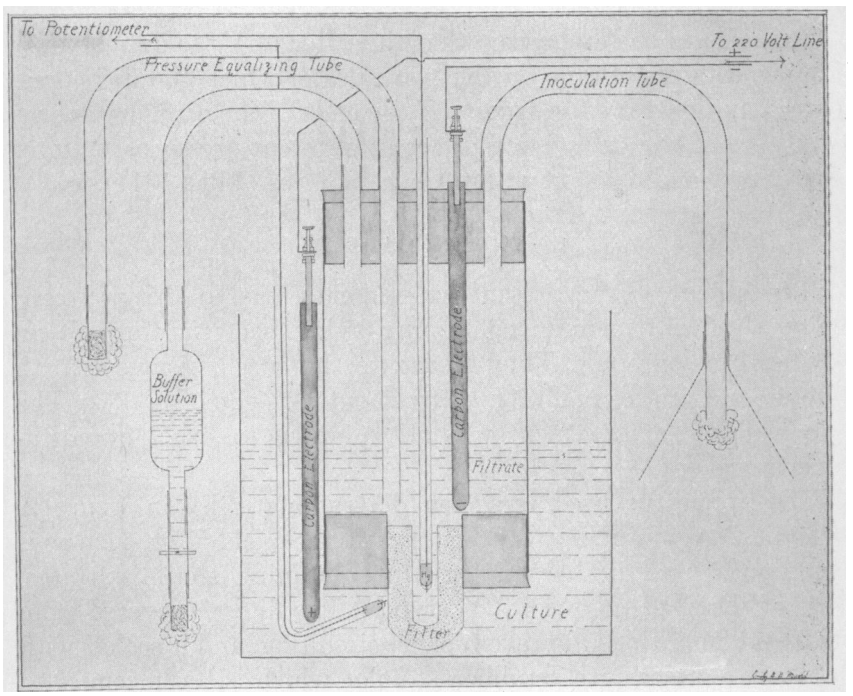

FIG. 2. SECTION THROUGH APPARATUS USED FOR ELECTROENDOSMOTIC TRANSPORT OF V. PERCOLANS (SLIGHTLY SCHEMATIC)

Filtration vessel sterile and protected from outside contamination

filter for ordinary bacteria. *V. percolans* is a non-pathogenic, motile vibrio isolated by one of us, which, under appropriate conditions, passes regularly through Berkefeld candles of the "V" type (1923).

The buffer solutions were phosphates colored by brom-thymol-blue. In the earlier experiments mixtures of the primary and

secondary phosphates were used; later the pure dihydrogen phosphate was added to the inner vessel fluid to neutralize the effects of the alkali there formed by electrolysis.

The filter as diagrammed in figure 2 is proportionately larger than in the actual apparatus. Its walls are about 4 mm. thick and are traversed by irregular and tortuous spaces narrowing down to minimal diameters of about 0.4μ (4×10^{-5} cm.). A length of filter varying in different experiments from 1 to 4 cm. was exposed below the stopper.

The potential source was the laboratory 220 volts d.c. line. The drop in potential across the filter wall was irregularly distributed over its surface, and varied widely according to position of electrodes and distribution of electrolyte through the system. The limits in the most intense part of the field were about 10 to 70 volts. This p.d. had to be measured with a potentiometer; voltmeters, because of the high resistance of the solutions, gave too low readings. The current strength varied from 0.02 to 0.1 ampère. The rate of filtration (i.e., electroendosmotic transport) under the above circumstances was about 2 or 3 cc. per minute.

The experimental procedure was as follows:

The apparatus was assembled as described. Cotton was adjusted to protect the exposed ends of all tubes and the funnel of the inoculation tube was covered with a towel. The whole was autoclaved, a Diack control being used to insure sterilization. When cool, the lower end of the filtration vessel and filter were soaked in distilled water until the liquid level inside was just high enough to cover the tip of the carbon electrode. A few cubic centimeters of M/25 NaH_2PO_4 solution containing bromthymol-blue were allowed to run into the filtration vessel. In the later experiments, i.e., with three exceptions from experiment E_{18} through E_{36}, the amount necessary to neutralize the alkali produced by electrolysis was added to the filtration vessel before the closing of the circuit and no further additions were made.

A suspension of *V. percolans* and *E. prodigiosus* was prepared either, in the early experiments, by suspending surface agar growths in dilute culture medium, or later (E_{15} through E_{36}), by

using diluted or undiluted hay infusion cultures strained to remove flocculi and surface pellicle. The filter was then immersed in the suspension up to the inner fluid level. The circuit was closed. As the inside fluid level slowly rose the filtration vessel was lowered to keep inside and outside levels approximately the same. The circuit was broken, the filtration vessel removed from the culture and the filtrate was inoculated into vessels containing sterile hay infusion. Inoculation was made in a chemical "hood" whose metal floor was the top of an electrically heated steam bath. By previously washing the air within this chamber with steam, contaminations were reduced to a minimum. Finally one tube was inoculated as a control with the unfiltered culture. All inoculated vessels were kept in an incubator at about 30°C. If the filtrate contained vibrios one or more of the inoculated vessels showed clouding after twenty-four to sixty hours. The control tube, inoculated usually with 3 loops of unfiltered culture, regularly showed clouding in twenty-four hours due to a mixed growth of the two organisms, and often of others introduced by air contamination of the open outer vessel.

The organisms in the clouded vessels were routinely identified by examination with the dark field illuminator, by Gram-stained preparations and by plating out on agar. Vessels in which no clouding appeared after a week were designated negative(−), (table 1). Vessels in which *V. percolans* was identified in pure culture were called positive (+). The control tubes were called positive (+), when a mixed growth of *V. percolans* and *E. prodigiosus* was demonstrated in them.

The results are given in table 1. During the early experiments results were somewhat variable, chiefly because of imperfect technique. Later it may be seen that, with the exception of an occasional negative experiment, vibrios were quite regularly grown in pure culture from the filtrates of runs of six and one-half minutes or longer. About 15 cc. of fluid were filtered in this time. Experiments of less than six and one-half minutes duration were negative.

Though it is perhaps sufficiently evident that the vibrios were not passing by their own unaided efforts from the nutrient

TABLE 1

EXPERIMENT NUMBER	FILTER NUMBER	NUMBER OF TIMES FILTER USED PREVIOUSLY	DURATION OF EXPERIMENT IN	NUMBER OF VESSELS POSITIVE	NUMBER OF VESSELS NEGATIVE	CONTROL TUBE
			minutes			
E1	14 or 15	0	30	Leak about stopper; all tubes mixed growth		
E2	14 or 15	0 or 1	36	1?	3	+
E3	14 or 15	1?	40	1?	4	+
E4*	17?	0	30	1?	2	+
E5	17	1?	31	7	1	+
E6	11	0	32¾	0	11	+
E7	15	2?	26	0	8	+
E8	14	2?	15	0	9	+
E9	17	2?	28	0	8	+
E10	11	1	36	5	0	+
E11	18	0	27	1	8	+
E12	16	0		3	5	+
E13	20	0	22	2	0	+
E14	21	0	25	3	4	+
E15	22	0	30	7	4	+
E16	23	0	25	0	8	+
E17	24	1 (for control)		8	0	+
E18	25	1 (for control)	25?	0	9	+
E19	26	1 (control)	38	3	1	+
E20	24	1 control 1 experiment	25	3	1	+
E21	27	1 control	28	9	0	+
E22	27	1 control 1 experiment	30½	5	0	+
E23	26	1 control 1 experiment	15	2	0	+
E24	16	1 control 1 experiment	8½	0	3	+
E25	11	1 control 2 experiments	10+	2	0	+
E26	22	1 control 1 experiment	10	2	1	+
E27	17	1 control 3 experiments	11¼	1	1	+
E28	21	1 control 1 experiment	9¼	2	0	+
E29	25	1 control 1 experiment	10	0	3	

*The apparent negative results of the first experiments were due partly to the fact that the free alkali in the filtration vessel was not neutralized by the addition of buffer. From Experiment E5 on, buffer was added in all experiments.

TABLE 1—*Continued*

EXPERIMENT NUMBER	FILTER NUMBER	NUMBER OF TIMES FILTER USED PREVIOUSLY	DURATION OF EXPERIMENT IN	NUMBER OF VESSELS POSITIVE	NUMBER OF VESSELS NEGATIVE	CONTROL TUBE
			minutes			
E30	28	1 control	9	2	0	+
E31	29	1 control	9⅓	2	0	+
E32	29	1 control 1 experiment	6⅓	0	2	+
E33	28	1 control 1 experiment	8⅔	2	0	+
E34	30	1 control	7	1	1	+
E35	32	1 control	5 7/60	0	1	+
E36	33	1 control	6	0	2	+
E37	31	1 control	6½	1	1	+

medium outside the filter into acidified distilled water within it, nevertheless a series of control experiments were run to obtain more definite evidence on this point. For these the apparatus and culture were prepared exactly as for an experiment except that the circuit was not closed. The fluid levels inside and out naturally remained unaltered and the vibrios had to pass, if at all, by their own motility from culture medium without to distilled water within the filter.

In old filters, i.e., filters in whose pores nutrient substances remained from previous filtrations, vibrios were cultivated from the fluid within the filtration vessel after exposures of nineteen hours and some minutes, of ninety minutes, and of seventy-seven minutes. Negative results were obtained with exposure of old filters for ninety-two and ninety minutes. With new filters no positive fluids resulted from exposures of less than two hours (table 2.)

Behavior of the motile vibrios in an electric field has been observed a number of times in a simple cataphoresis chamber made by conducting current through electrolyte-soaked cotton threads to an ordinary blood counting chamber of the Levy single field type. Endosmotic streaming may be observed in the fluid immediately adjacent to the top and bottom of such a chamber and cataphoresis in its more central parts. No evidence of orientation of the motile organisms in the electric field has been observed, however.

It is thus evident that the vibrios are borne passively through the filter by the endosmotic stream as they would be by streaming produced by gravity or other source of energy. In this case, however, the inward movement of the organisms in the endosmotic stream is partially opposed by the outward movement by cataphoresis of the organisms through the stream, so that they are not so rapidly conveyed into the filtrate as they would be by streaming at a similar rate produced by gravity. This is

TABLE 2

CONTROL EXPERIMENT NUMBER	FILTER NUMBER	NUMBER OF TIMES FILTER USED PREVIOUSLY	DURATION OF EXPERIMENT	NUMBER VESSELS POSITIVE	NUMBER VESSELS NEGATIVE	CONTROL TUBE
I	17	2 or 3	1 hour 30 minutes	0	3	+
II	20	1	19 hours 20 minutes	3	0	+
III	22	1	1 hour 32 minutes	0	3	+
IV	21	1	19 hours 50 minutes	2	0	+
V	25	0	1 hour 38 minutes	0	2	+
VI	24	0	1 hour 43 minutes	0	1	+
VII	26	0	1 hour 3 minutes	0	3	+
VIII	27	0	2 hours 43 minutes	3	0	+
IX	11	2	1 hour 30 minutes	3	0	+
X	16	1	1 hour 17 minutes	2	0	+
XI	28	0	2 hours (approx.)	1	1	+
XII	29	0	2 hours 10 minutes	1	3	+
XIII	30	0	1 hour 31 minutes	0	2	+
XIV	31	0	1 hour 45 minutes	0	2	+
XV	32	0	1 hour 30 minutes	0	2	+
XVI	33	0	1 hour 45 minutes	0	3	+

illustrated by comparison of the results in table 1 above, with tables 1, 2, and 3, of a previous communication on filtration of *V. percolans* (Mudd, 1923). Rates of streaming of the same order were produced by the electric field in one case and by suction in the other. With suction filtrates were uniformly inoculated with vibrios (when normal) in two to six minutes; by endomosis at least six and one-half minutes were required.

Had the vibrios been positively instead of negatively charged their velocity of cataphoresis would have been added to the endosmotic current instead of subtracted from it, as is evident from equation (3).

It will be noted that the potentials used in the above experiments were high. Forty volts, for instance, over a path of 8 mm. through the filter wall, would be equal in intensity to a potential difference of 200 millivolts over a path of 40μ through a membrane. This is a larger p.d. than is to be anticipated in a living system. The pressures developed by the endosmotic current under the conditions of the experiments were correspondingly high, 60 to 220 cm. of water (measured by rise of filtrate in a manometer tube projecting from the filtration vessel). Moreover as previously shown (1923), the mechanical difficulties of the vibrios passing the filter at all are great. We were breaking new ground and used forces much larger than we should expect under the conditions of nature.

DISCUSSION

The experiments reported above were undertaken under the stimulus of certain observations regarding passage of bacteria and other particles through the mucous membranes of the tonsils. A brief résumé of these observations may serve as an example of a concrete medical problem in the further investigation of which it is hoped the mechanism outlined above may possibly serve as a guide.

Jonathan Wright in 1896 (1896) figured tubercle bacilli passing through the intact laryngeal epithelium of a patient with tuberculous laryngitis. Wood (1914) later gave hogs tuberculosis and anthrax by swabbing cultures of the specific organisms lightly on the animals' tonsils, and demonstrated the anthrax bacilli penetrating between the cells of the tonsillar epithelium. The crypt bacteria were kept out until the epithelium had been devitalized by the invading anthrax organisms. J. L. Goodale (1897) in 1897 dusted carmine on the surface of tonsils shortly before excision and on subsequent microscopic examination, found the carmine particles passing in between the tonsillar epithelial cells and in the direction of the efferent lymph channels. The indigenous bacteria of the crypts, though of about the same size as the carmine granules, could only rarely be found in or beneath the epithelium. He believed that the carmine was

carried in by a stream of buccal fluids resorbed through the tonsillar epithelium into the cervical lymph vessels. M. Hendelsohn (1898) reported similar experiments with several types of particles penetrating both the faucial and pharyngeal tonsils. He reported the granules as being both intercellular and intracellular as they passed the epithelium. Jonathan Wright subsequently confirmed and extended these observations; the foreign particulate matter was taken in between the cells, the cryptal bacteria were kept out. A. S. Warthin (1923) has recently confirmed both the selective transfer through the tonsillar epithelium and the intercellular course of the particles admitted.

Wright (1909) has especially urged the importance of seeking physico-chemical mechanisms capable of explaining such selective action by the first line of defense against infectious disease. What forces act at membranes and are capable of importing foreign particles and excluding the crypt bacteria?

One naturally thinks of osmotic pressure. It is hard to understand a *selective* influence of osmotic pressure. Surface tension has been especially mentioned by Wright. That surface tension can transport bacteria along phase boundaries in a striking manner has been shown by us elsewhere. The electrocapillary mechanism described would also seem for the present at least a possible working hypothesis. The question as to whether or not it will be found to play a significant part in the phenomena under consideration will have to wait upon further investigation.

Another possible application of the electrocapillary mechanism developed above may be worth mentioning here. An electrical disturbance is known, from the studies of Bayliss and Bradford (1885), of Cannon and Cattell (1916), and of Gesell (1919), to result from stimulation of glands. This electric effect is strikingly correlated with the outflow of secretion from the glands, and is not necessarily correlated with changes in blood supply. Bradford (1887), by ligating the efferent ducts of the glands did not significantly alter the electric variation, and thus showed that the flow of secretion along the gland duct was not the cause of the disturbance in potential. The converse possibility, namely that an electric p.d. between the ends of the cells bordering the lumen

and the peripheral ends next the blood supply might cause the outflow of secretion by a mechanism analogous to that developed above, does not seem to have been considered in the literature. It seems to have sufficient possibility at least to be worth testing.

An alternative possibility in secretion is that stimulation of the gland may cause an increased permeability to osmotically active cell components of that part of the cell membrane bordering the lumen (Bayliss, 1920). In that case, as Bayliss has pointed out, osmotic pressure would cause a streaming of water through the cells into the lumen. On the other hand, such an osmotic mechanism does not seem adequate in such a case as resorption in the kidney tubules where water is passed from tubules into blood stream against osmotic pressure. The possibility that electroendosmotic streaming is here involved is at least to be considered.

Be it understood: this paper does not, and does not aim to, prove anything in regard to what actually happens in the organism in infection, resorption, secretion or other process. It is simply an exposition of a mechanism, one among many, by which fluid and suspended particles may be moved through capillary spaces, and as such it may or may not turn out to have a significant part in vital processes. It is perhaps of interest, first because overlooked by most biologists, and, second, because it is *selective* in nature.[3] Should future study reveal suitable potential gradients, (expressed as H in equation 3 above), and evaluate the charges between particles and their environing medium, $({}_F\zeta_P)$, and between the walls of intercellular spaces and their contained

[3] As an example of its selectivity suppose the following circumstances:

The walls of the capillary spaces are charged negatively to −30 millivolts against the fluid in them, $({}_F\zeta_S = -30)$. Suppose one type of particle to be charged also to −30 millivolts, $({}_F\zeta_P = -30)$. Suppose a second type of particle to be charged positively to 20 millivolts, $({}_F\zeta'_P = +20)$. From equation (3) we have $V_R = (-30 + 30)\frac{K.H}{4\pi\eta} = 0$, and $V'_R = (-30 - 20)\frac{K.H}{4\pi\eta} = -50\frac{K.H}{4\pi\eta}$. Thus the first particles would undergo little or no movement, whereas the second particles would move toward the cathode at a velocity about $\frac{5}{8}$ that of the endosmotic stream. It is known that the charges of cells vary according to their constitution and to the hydrogen and other ion concentrations of the fluids bathing them, (Coulter; Northrup and DeKruif; Eggerth and Bellows, 1921–22).

fluids ($_F\zeta_S$), it is hoped that the relations described by the above equations may at least show how the electrokinetic effects would tend to move the fluids and particles. Whether the actual movement would be approximately that given by V_R in any case would of course depend on whether electrokinetic effects alone were operative, or were combined with movements due to osmotic pressure, gravity, muscular contraction, etc.

SUMMARY

Experiments are reported in which a filterable microörganism, *Vibrio percolans*, was drawn through artificial membranes by electroendosmotic streaming. The membranes were Berkefeld "V" filters. Their wall thickness was about 0.4 cm. Their pores were irregular and tortuous, narrowing down to minimal diameters of about 0.4μ, (0.4 + 10^{-4} cm.)

The difference in potential across the membranes varied from ten to seventy volts. The source was carbon electrodes connected to the laboratory 220 volt, direct current, line. The endosmotic transport of fluid across the filter under these circumstances was 2 to 3 cc. per minute. The pressures developed were those of 60 to 220 cm. of water.

Vibrio percolans is negatively charged relative to its medium, and thus tends to move by cataphoresis to the anode. Water streams by electroendosmosis through the negative filter pores to the cathode. The resultant movement of the microorganisms under the combined influence of cataphoresis and endosmosis is in this case with the endosmotic stream to the cathode. Appearance of vibrios in the filtrate about the cathode is much quicker than when they penetrate the filter by their own motility alone and somewhat slower than when they are filtered by suction. These results are in qualitative agreement at least with the equations ofthe Helmholtz-Smoluchowski theory set forth above.

Possible applications of this mechanism of electroendosmosis and cataphoretic movement are indicated in relation to penetration of bacteria through epithelia and in relation to secretion and resorption.

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