THE INFLUENCE OF pH UPON THE CONCENTRATION POTENTIALS ACROSS THE SKIN OF THE FROG.

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(Accepted for publication, March 15, 1928.)

INTRODUCTION.

In 1890 Ostwald (1) suggested that the electromotive phenomena in living tissues might arise because the living membranes, in common with many non-living structures, prevent or retard the passage of the ions of one electrical sign while permitting the movement of ions of the opposite sign. As a result of this selective behavior electrical stresses may arise in any situation where there is an ionic concentration gradient across a membrane and these stresses will become manifest in potential differences which can be measured by appropriate devices. While the application of this conception has proven valuable in several fields of investigation the attention of workers has in the main been focused upon the differential movement of ions through membranes, rather than upon the equally fundamental inquiry as to why membranes behave in this manner. The present communication will deal with certain observations which are believed to bear upon this latter problem.

These studies were suggested by the observations of Matsuo (2), Mond (3), and Fujita (4) that across protein membranes the concentration P.D.'s are greatly influenced by pH; that above the isoelectric point of the protein the more dilute solution is electropositive, whereas below it the polarity is reversed. We were further moved to attempt these experiments by the report of Mudd (5) that mammalian serous membranes show an electroendosmotic reversal point in a pH range (4.3–5.3) which strongly suggests that this phenomenon is controlled by a protein constituent of the membrane.

Such studies suggested to us that if, in intact animal membranes,

a similar correlation between the electromotive phenomena and pH could be demonstrated we might secure a deeper insight into the mechanism of bioelectric effects, and might be better able to decide between the views of Beutner (6) and of Höber (7) and his coworkers (2, 3) as to the particular chemical substance which is responsible for the production of P.D. in living tissues. The latter group of workers has recently contended that protein models simulate the electromotive behavior of living tissues more closely than do Beutner's oil-water systems.

We have investigated the relationship between pH and electromotive phenomena in a number of animal membranes and have so far discovered striking reversal effects in at least four different materials. The present report will deal with experiments carried out on the skin of the frog, in which the results have been particularly clear. We have been unable to discover in the literature any previous detailed study of the influence of pH upon the electrical behavior of this membrane. Uhlenbruck (8) has seen the electromotive reversals which we will discuss, but he mentions them only incidentally and does not, we believe, appreciate their significance. In a recent paper which came to our attention after the completion of our own work, Rein (9) has reported observations on another material which are in general agreement with our own results. He finds that the concentration P.D. which he can demonstrate in the human skin is reversed when the electrical charge upon it is made positive by the H ion or by Al^{+++}. These papers appear to constitute the only studies of the sort which have been carried out on animal membranes.

References to the older literature on the electromotive phenomena of the frog's skin are given by Hashida (10) and Uhlenbruck (8). Wertheimer (11) has published extensive studies on the permeability of this membrane.

When the living skin of the frog is removed from the body and so mounted that its two surfaces are bathed by an isotonic Ringer's solution, a difference of potential may be demonstrated across it, the inner surface being electropositive. Upon stimulation of the cutaneous nerves characteristic changes in this skin potential may be obtained, presumably associated with the activity of the skin glands. These intrinsic electrical effects are known to depend upon the pres-

ence, in the solutions, of Na ion, which may be replaced, in part at least, only by Li (8, 10).

Another category of electromotive phenomena is observed when salt solutions of different concentrations are applied to the two surfaces of the skin. Concentration P.D.'s of quite a high order, up to half of the theoretical maximum, are then obtained. In a membrane in which the normal skin potential persists, the actual P.D. measured in the course of such a concentration study is the algebraic sum of the normal skin potential and the concentration potential.

The present study concerns itself entirely with concentration P.D. measurements under conditions where the skin potential has been abolished. When this occurs the skin is presumably no longer alive. We have reason to believe that in this material such electromotive reversals as we shall discuss are not possible in life. Thus we find that when Ringer's or NaCl solutions are employed the skin potential diminishes as the solutions are made more acid and disappears at almost exactly the same pH (3.8–4.2) as that at which the reversal of concentration potential occurs. When the solutions are made more alkaline again the skin potential does not reappear; the concentration potential, on the other hand, assumes its original polarity and finally reaches the same value as that previously observed when skin potential was present. The ability of the skin to develop concentration potentials is obviously not dependent upon the presence of a skin potential. It depends rather upon the presence of a physical structure which may persist for many hours after the membrane has ceased to function as a living tissue. A study of such a membrane may, we believe, give us valuable information concerning the electrical behavior of that structure in life.

The frog's skin has, of course, a somewhat complex structure, consisting of at least two distinct layers. The outer epithelial layer, containing many glands, is almost exclusively responsible for the electromotive phenomena. When this outer layer has been removed the inner portion, composed of fibrous tissue and smooth muscle, and containing nerves and blood vessels, is unable to develop concentration P.D.'s of any magnitude.

Preliminary reports (12, 13) of this work have been published and may be consulted for a brief summary of the results which have been obtained upon other materials.

Method.

Fig. 1 presents, in diagrammatic form, the experimental arrangements used for the determination of P.D.'s, except in certain special cases later to be described in which some modification was necessary. In general the experiments follow a familiar procedure, and offer no exceptional features. The membrane, consisting

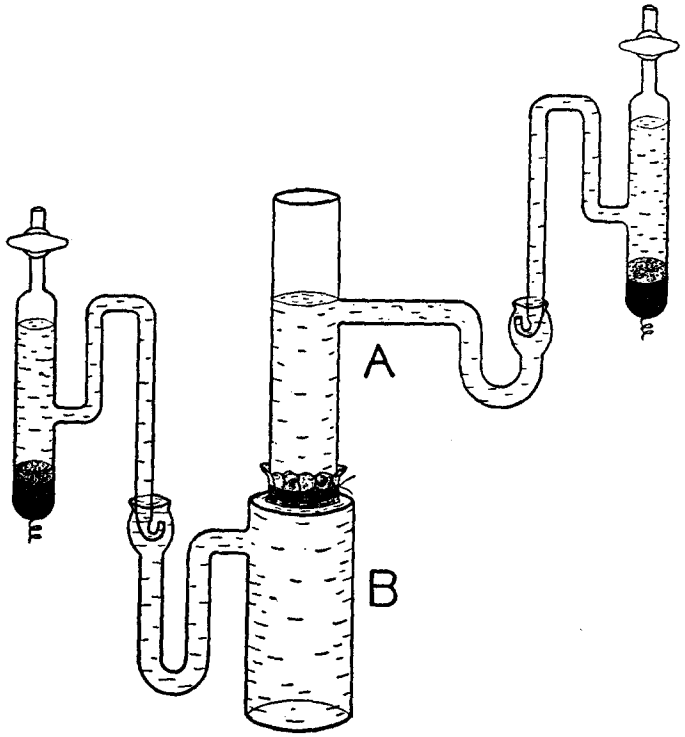

FIG. 1. Diagram of apparatus. The skin, mounted over the end of vessel *A*, is brought in contact with the salt solutions in vessels *A* and *B*. Electrical connections to the potentiometer are made through saturated KCl calomel half-cells.

of a piece of skin cut from the back, is mounted on the lower end of a glass cylinder, *A*, the inside of the skin being turned toward the inside of the chamber. One solution fills the chamber and extends through the side tube to the smaller vessel at its end. Electrical connection to one pole of the potentiometer is made through a saturated KCl calomel half-cell. The other solution is placed in a glass vessel, *B*, with a similar side tube. Electrical connection to the other pole of the potentiometer is made through a second calomel half-cell. The instrument used is a

Leeds and Northrup portable hydrogen ion potentiometer. A capillary electrometer is used as the null instrument.

RESULTS.

The phenomena now to be described may be studied in a variety of ways. We have taken at least three distinct steps in the analysis of the problem, beginning with a roughly qualitative demonstration, proceeding through a more careful study with unbuffered solutions, and ending in a series of determinations with acetate buffers, in which the pH is more rigidly controlled, and in which the electromotive effects are shown to be closely related to the electrical charge of the skin. These three portions of the study all contribute to the argument and will be described in order.

1. When solutions of .1 N KCl, made up in the usual manner, and at a pH above 5.0, are brought in contact with the two surfaces of the skin, it is observed that the skin potential originally present diminishes rapidly and finally disappears after a few minutes. The P.D. of the system thereupon becomes zero. If now the solution in vessel *B* is replaced with .001 N KCl and the system again examined, a concentration potential is observed, the voltage often reaching 60 millivolts. The more dilute solution is electropositive. This P.D. is a membrane potential in the strict sense. It is very much greater than any diffusion potential which could possibly arise between the two solutions. The magnitude of the diffusion potential between .1 N and .001 N KCl may be calculated, and comes out to be .8 millivolts only; the polarity is the reverse of that actually observed when the membrane is present. The membrane itself must obviously be in some way responsible for the P.D. produced.

A sudden change in pH may now be produced by adding a few drops of .1 N HCl to the dilute solution. When, by such an addition the pH is lowered to 3.0 or thereabouts a striking change is observed in the concentration potential. The P.D. previously present rapidly diminishes to zero, to be succeeded by a P.D. in the opposite direction which builds up to a maximum which is usually not greatly different from the value originally found in the other direction before the addition of the acid, although in general the terminal values tend to be less.

The ionic concentrations in vessel *B* are of course affected by the addition of acid. Thus at pH = 3.0 the Cl ion concentration is no longer .001 N in the dilute solution but .002 N, nearly. An ionic concentration gradient still exists, therefore, in the same direction as before, and the P.D. observed after acid treatment is a concentration potential whose polarity is determined by some change within the membrane induced by the H ion.

The sudden reversal in P.D. may be observed by setting the potentiometer to zero and allowing the full force of the unbalanced membrane potential to deflect the meniscus of the electrometer to one side of its zero position. Upon the addition of the acid the meniscus is seen to move rapidly through the zero position to the other side, indicating the electromotive reversal.

By successive adjustments of the pH, now to the alkaline and now to the acid side, the P.D. may be reversed many times at will. The pH of the internal solution has little or no effect upon the behavior. It may be lowered and raised simultaneously with that in the external solution, or it may be left more alkaline throughout; in each case the electromotive reversals are practically identical. Very definitely the phenomena are connected with the epithelial layer of the skin which is in contact with the more dilute solution.

2. More complete information as to the form of the reversal curve and as to the reversal point is obtained when the pH is shifted more gradually by the application of a series of solutions at different pH's. By the addition of HCl the pH of a .1 N KCl, and of .001 N KCl series is adjusted to cover the range from 3.0 to 5.6 in steps of .2 pH. As has been pointed out, the ionic concentration difference between concentrated and dilute solutions is not constant in such a series, but even at pH = 3.0 the ionic ratios are still 50 to 1, nearly, and the gradient is always in the same direction over the whole range. When the concentration P.D.'s across the skin are measured by the application of such a series of solutions, the pH being the same on both sides of the membrane at each reading, typical reversal curves such as those shown in Fig. 2 are secured. The reversal points vary considerably from skin to skin, and are influenced to some extent by the direction from which the reversal point is approached. This is almost certainly due to a failure to secure a rigorous control of pH within the membrane

by the application of such an unbuffered series. This effect disappears in the determinations with acetate buffers, later to be described. The form of the curves obtained with KCl solutions and the reversal points indicated must therefore be accepted as approximate only, but as giving a correct picture of the general nature of the phenomena, and as demonstrating that the effects cannot possibly be due to diffusion

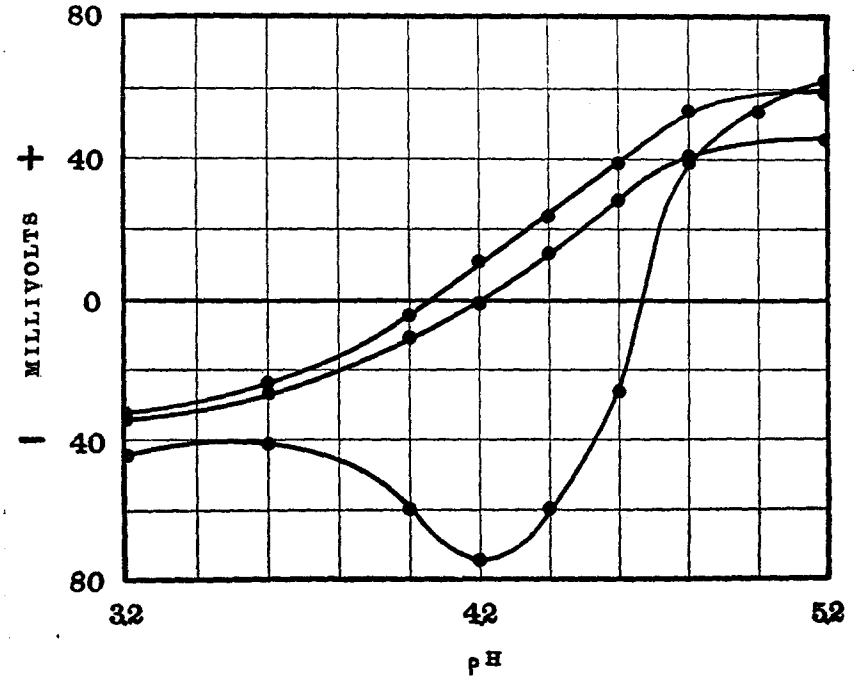

FIG. 2. Concentration P.D.'s between .1 N and .001 N KCl, as influenced by pH. The three curves represent the electromotive behavior of three different skins. The sign is that of the dilute solution.

potentials, or to any other factors involving the solutions only, but must be referred to changes in the membrane achieved through the influence of a varying pH.

The magnitude of the voltage observed at any pH is influenced by the manner in which the dilute solutions are applied. Throughout all determinations it is the custom to wash the outer surface of the skin through three changes of the dilute solution and to stir that

solution during the whole time in which the electromotive readings are being secured. If such precautions are not taken the P.D.'S, while definite, are considerably lower in value than when the dilute solution is continually renewed against the membrane. This effect is probably due to diffusion of salt through the membrane from the more concentrated solution, with a consequent reduction in the magnitude of the ionic gradient immediately across the membrane, and an associated diminution of electromotive force.

During the period in which the outer surface of the membrane, newly removed from contact with .1 N KCl, is adjusting itself to the dilute solution, many curious transient electromotive effects have been observed which we are at present quite unable to interpret. These effects were much less marked in the experiments with buffered solutions, described below. Immediately after the application of dilute KCl there may appear a large P.D. in a direction opposite to that which is found later after the system has come more nearly into a new equilibrium. This P.D. rapidly vanishes, to be replaced, within a minute or 2, by a P.D. in the opposite direction which builds up to a fairly constant maximum after 5 minutes. The readings here reported are in all cases these terminal values.

3. The final and most carefully controlled experiments have been carried out in Na acetate-acetic acid buffers. Preliminary tests demonstrated that a satisfactory concentration P.D. can be developed across frog skin when two different concentrations of these buffers are applied to the two surfaces. Inasmuch as over the whole effective range of this buffer (pH 3.6 to 5.8) the dissociation of the acetic acid is very slight and since we have every reason to believe that the undissociated molecule can make no contribution to the production of electromotive effects of the sort under discussion the concentration P.D. observed must be referred almost entirely to the acetate.

In the first experiments a series of buffers, .2 M in strength with respect to acetate, were employed as the more concentrated solutions. When such a solution at pH = 5.8 is applied to both surfaces of the skin, the skin potential initially observed rapidly disappears and the P.D. of the system becomes zero. If, now, the solution in contact with the outside of the skin is replaced by a tenfold dilution of the stock solution, a P.D. appears, the dilute solution being electropositive.

Similar readings may now be repeated in solutions becoming progressively more acid, and reversal curves similar to those already described are secured. These curves, however, are very far from being symmetrical with respect to the line of zero potential, the voltages on the acid side of the reversal point reaching much higher values than those obtained on the alkaline side.

It soon became evident that it is not sufficient to maintain a constant ion ratio, as the pH is varied, but that the total ion concentration must also be controlled. It has long been recognized that the magnitude of electrokinetic effects and allied phenomena are markedly influenced by the ionic concentration, being maximal over a certain dilute range of solutions, diminishing in more concentrated solutions, and finally disappearing entirely at or near moleculor values. It seemed reasonable to suppose that some similar effect was involved in the present study. Accordingly a new series of buffers was made up, by appropriate dilution of the stock solutions, having in every case a Na acetate concentration equal to that of the stock solution at pH 4.0. In this solution that concentration is .036 M. This series is used for the more concentrated solutions. A second series of dilute solutions is made up by a twentyfold dilution of the first series. An ionic gradient is therefore secured in which, over the whole range of the determinations, total ion concentration as well as ion ratio is very nearly constant.

Such dilutions cause a shift in the pH toward the alkaline side, but even when the original stock solutions are diluted a hundredfold the resulting pH change is not more than .2 of a pH unit. As a result of this shift the dilute solution is always slightly more alkaline than the concentrated solution, but in both the H ion concentration is negligible as compared with the salt, and no effect on the direction or magnitude of the concentration potential is produced, except in so far as the changing pH modifies the character of the membrane itself. In every case the actual pH values in the solutions, after both the first and the second dilution, are determined by the quinhydrone electrode. Contact with the tissue sometimes produces a slight change in the pH of the applied solutions. The determination of pH in the more dilute solution, therefore, is carried out on that sample last applied to the membrane, immediately after the electromotive reading has been

taken. The pH values here given for the electromotive measurements are always those thus determined, since we know that it is the pH of this dilute solution which controls the electromotive reversals.

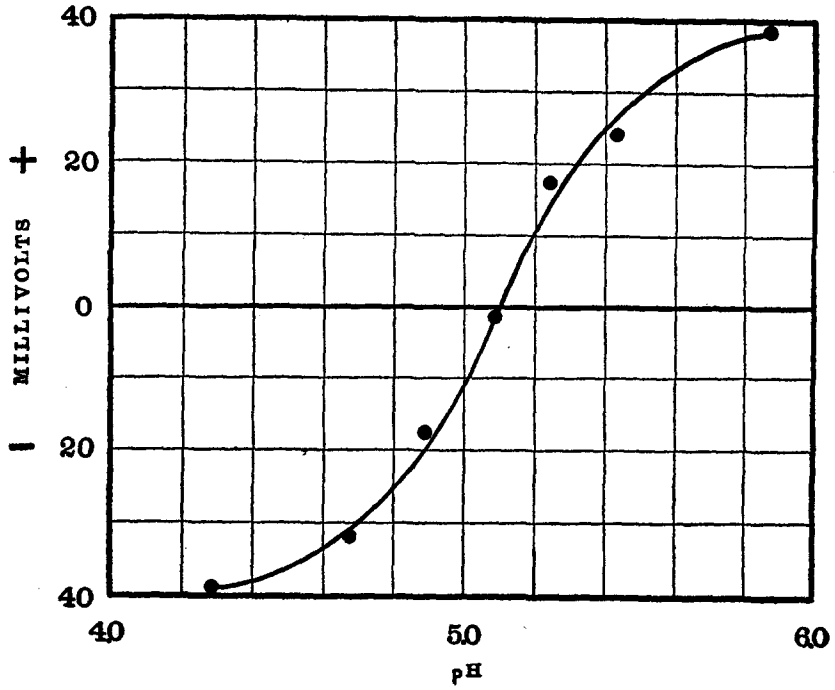

FIG. 3. Concentration P.D.'s between 0.036 N and 0.0018 N Na acetate, as influenced by pH. The sign is that of the dilute solution. The readings are as follows:

pH	P.D.
5.88	+38.8 mv.
5.43	+23.9 "
5.24	+17.0 "
5.09	− 1.4 "
4.89	−17.7 "
4.68	−31.6 "
4.29	−39.0 "

When the ionic concentrations are kept constant in the manner just discussed the reversal curves assume a more symmetrical form. A very good example of such a curve is shown in Fig. 3. At every point the concentration gradient is .036–.0018 M Na acetate. The

reversal or "isoelectric" point comes at pH = 5.1. Above this point the dilute solution is electropositive; below it this solution is electronegative.

These electromotive reversals are closely correlated with the membrane charge. It has been found possible, in a group of experiments, to secure an electroendosmotic determination of this charge. In these experiments the method and apparatus described by Mudd (14) have been used. The determination is not easy to make since the movement of water through the membrane tends to disintegrate it. Particularly is there a tendency for the outer epithelial layer to become separated from the inner layer of the skin, and in experiments on electroendosmosis we have never been successful in approaching the reversal point from the acid side without having this separation appear. All trustworthy determinations have been secured by approaching the reversal point from the alkaline side.

This difficulty appears to arise mainly from the fact that the two layers of the skin are very different in their electrical characteristics. It has previously been stated that the electromotive forces observed across the whole skin are almost entirely produced by the epithelial layer. It is also very apparent that the electroendosmotic transport of water through the whole skin is quite small in amount, compared with that which occurs when the epithelial layer has been removed. It follows, as a result of this difference, that, as long as the two membranes remain bound together in the normal way, the epithelial layer dominates the electroendosmosis, even as it does the electromotive phenomena. When the two membranes are separated, and particularly when actual openings appear in the epithelium, the amount of water which passes is determined almost entirely by the inner membrane. As a result the epithelial layer, if still intact, may be raised up in the form of a blister. A reversal in the direction of the current brings about a removal of this collection of fluid. In both cases the readings actually secured represent only the amount of fluid which has passed the inner membrane. Curiously enough the pH reversal point for this inner membrane is very different from that for the epithelium, and does not lie within the whole range of the acetate buffers, but at some higher pH value. A few determinations with Sørensen's phosphates have indicated an electroendosmotic reversal point at about 6.2–6.4.

When the two membranes have separated, therefore, no correlation can be established between the electroendosmotic and the electromotive behavior. But when they remain attached, so that the epithelium controls both phenomena, a close correlation is discerned. Table I gives the pH reversal points for both as determined in three experiments in which electroendosmosis was studied alone, eight experiments

TABLE I.

Reversal Points for Electroendosmosis and Potential Difference.

Experiment	Electroendosmosis	Potential difference
	pH	*pH*
1		5.09
2		5.12
3	5.19	
4		5.09
5	4.94	
6	5.24	5.20
7	5.19	5.15
8		5.08
9		5.20
10		4.97
11	5.03	5.10
12	5.07	4.96
13	5.23	
14	5.08	4.93
15	5.09	5.04
16		5.18
17	5.21	5.00
18		5.07
Average	5.12	5.07

in which the electromotive forces were measured alone, and seven experiments in which both were measured simultaneously.

For this last group of experiments a modification of the above described technique was used. It was found possible, after securing an electroendosmotic determination in the more concentrated solution at each pH (Na acetate always = .036 M) to read the concentration P.D. at that pH without removing the membrane from the electroendosmosis apparatus. This was done by bringing the more dilute solution (Na acetate = .0018 M) into contact with the epithelium and making electrical contact to the potentiometer through two saturated

KCl calomel half-cells, one dipping into the dilute solution, the other making connection with the inside more concentrated solution through a side tube on the electroendosmosis apparatus. After the attainment of approximate constancy in the electromotive reading the final value was accepted as giving the concentration P.D. at this pH. The

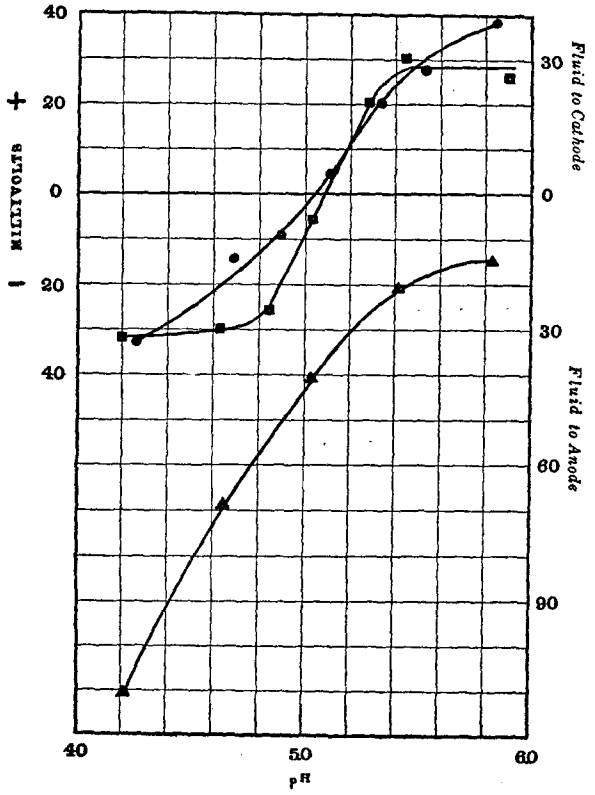

FIG. 4. Correlation between concentration P.D.'s and electroendosmosis. Ordinates at right represent the c. mm. of fluid transported in 15 minutes by a current strength of 6 ma.

● = P.D. in whole skin.

■ = Electroendosmosis through whole skin.

▲ = Electroendosmosis through skin after removal of epithelium.

solutions were then both replaced by the concentrated solution at the next lower pH and again an electroendosmotic and an electromotive reading were taken in succession at the new pH.

The averages of the ten reversal points for electroendosmosis and of the fifteen reversal points for P.D. are in close agreement, differing by .05 of a pH unit only. The tendency for the electroendosmotic reversal point to be higher than that for P.D. is probably due to the fact that the inner membrane is able to exert a slight effect upon the electroendosmosis through the whole skin, shifting the reversal point toward the alkaline side, but is unable similarly to modify the P.D. In spite of this tendency, the agreement is so good that there can be no doubt that the two phenomena are closely related.

The relationships discussed in the last paragraphs are graphically shown in Fig. 4. The circles give the P.D. readings, the squares the electroendosmotic readings, secured by the method just detailed. The two reversal curves are similar in form, and pass through the line of zero potential at very nearly the same point. The triangles show the form of the electroendosmotic curve secured when the epithelial coat has been scraped off. The close correlation between electromotive force and electroendosmosis in the intact skin and the dependence of both upon the epithelial layer, is very evident.

DISCUSSION.

The definite character of these electromotive reversal phenomena permits certain conclusions concerning the chemical nature of that substance in the membrane which is responsible for them. We may be quite certain that we are dealing with an ampholyte, or group of ampholytes, whose state of dissociation, controlled by the pH, is in some way responsible for the electrical behavior. This ampholyte cannot be identified with absolute certainty, but it seems to be reasonably certain that we are dealing with a protein. As Mudd (5) has pointed out, the only known ampholytic lipoids, the phosphatides, have a broad isoelectric zone in which the molecules are electrically neutral, and sharp reversal phenomena are not to be expected. The electromotive effects which we have described are obviously similar to the titration curves of typical animal proteins (Cohn (15)), to electroendosmotic phenomena in protein membranes, to the cataphoretic behavior of protein particles, and to the P.D. determinations which Matsuo (2), Mond (3), and Fujita (4) have obtained in protein models.

The electromotive reversal points are also in a pH range characteristic for animal proteins. These similarities indicate that in this material at least proteins are responsible for the electrical effects, and lend support to the views of Höber (7) and his coworkers.

In some of the systems which Beutner (6) has described electromotive reversals have been found which at first glance might be thought to be similar to, or identical with, the effects which we have discussed. Beutner has found that when such a substance as salicylaldehyde is used for the "oil" or water-immiscible phase in his models, the more dilute solution, in a KCl concentration series, is electropositive. When a basic substance, such as toluidine, is used the polarity of the concentration effect with KCl is in the opposite direction, the more dilute solution being electronegative. In more complicated systems in which the water-immiscible phase contains both an acid and a basic component the polarity is in one direction when an HCl concentration series is applied, and in the other direction when NaOH is used. Such effects are not, we believe, identical with the phenomena here described. In the first place it has not been shown that such electromotive reversals as Beutner secures are sharply defined within a narrow pH zone. Furthermore it comes out in his experiments that when the water-immiscible phase is acid the dilute solution is electropositive, when alkaline it is electronegative. This is the reverse of our own findings in which the dilute solution is electropositive on the alkaline side of an isoelectric point, electronegative on the acid side.

Certain it is that neither lipoids, nor proteins, nor any other particular chemical substances are necessary for the development of concentration P.D.'s which may simulate bioelectric effects. The glass electrode of Haber and Klemensiewicz (16) and the paraffin, wax, and collodion membranes of Michaelis and his coworkers (17–19) across which concentration P.D.'s of a high order may be demonstrated, are sufficient to show that we are dealing with some very general phenomenon. The mere imitation of bioelectric effects by models is therefore not sufficient to elucidate the specific mechanisms present in the living membrane. When it is found that animal membranes act as do protein membranes in respect to the influence of pH upon their electromotive behavior, we are justified in inferring that proteins are responsible in a specific way for bioelectric effects.

We have been led by the correlations above described and by many other similar observations on other materials to the view that there is some fundamental relation between the electrical charge present upon the surfaces and pore walls of an organized membrane and the ability of ions to penetrate the structure. In the frog skin it is clear that when the charge on a membrane is negative, above an isoelectric point, the penetration of cations is more easy than that of anions. This is true if we accept the polarity of the concentration effect as a trustworthy criterion for the determination of which ion penetrates more readily. Michaelis and his coworkers (20, 21) have secured very satisfactory evidence that this view is correct. Above the isoelectric point, therefore, the membrane appears to retard the movement of those ions which are of the same electrical sign as itself, and to permit the movement of ions of the opposite electrical sign. This differential effect disappears when the membrane charge has been reduced to zero. At more acid values the relationships are reversed; the polarity of the concentration effect now indicates that negatively charged anions pass more readily through a positively charged membrane than do positively charged cations. We are led to the view that electrostatic forces between charged surfaces and ions in the solution immediately in contact with those surfaces are responsible for these effects, the membrane charge operating to retard or prevent the entrance of ions of the same sign into the narrow pores through the membrane which are believed to constitute the pathways for ionic movements.

A different interpretation has been given by Bethe and Toropoff (22) and accepted by Michaelis (23) and Rein (9). According to this view the retardation of the ion of one sign is due to its selective adsorption upon the pore walls of a membrane, its companion ion being still free to move. Membrane charge, determined by such an adsorption, is thus a result rather than a cause of the ionic immobilization. We are unable to harmonize this conception with our own findings. In a membrane whose electrical behavior is determined by an amphoteric component the membrane charge is sufficiently explained in terms of the dissociation state of the ampholyte; the selective adsorption of ions from the solution need not be invoked. Above the isoelectric point, in such membranes, anions appear to be retarded, and, accord-

ing to the view of Bethe and Toropoff, this must occur because anions are selectively adsorbed. We must therefore imagine the adsorption of negatively charged anions upon surfaces which are already negatively charged by virtue of their own intrinsic ionogenic tendency. Such a situation seems to us to be without precedent, and we are led to suggest the alternative view which has been outlined. Even in other membranes in which membrane charge unquestionably arises from selective ionic adsorptions we feel that this charge, once developed, may operate to retard or prevent the movement, into the membrane, of other ions of the same sign still free in the solutions.

It will have been recognized that this conception as to the mechanism of bioelectric effects is not original with us. Similar views have been suggested by Girard (24–26), Haynes (27), Loeb (28), Risse (29) and others. Bayliss (30) and Jacobs (31) have recognized the possible intervention of such a factor in the control of permeability. The conception can readily be associated with the observations of many workers (32–34) that electrically neutral molecules penetrate living membranes more readily than do ions.

It may be remarked, in conclusion, that the electrical charge of a membrane is certainly not the only physical factor involved in the determination of electromotive effects or of permeability relations. The size and number of the pores, the thickness of the membrane, and other physical characteristics are of great importance. In many very porous membranes the P.D.'s developed are not membrane potentials in the strict sense, but diffusion potentials in large part, somewhat modified by the membrane, as Loeb (28) has pointed out. In those fairly porous membranes which Loeb, and Bartell (35) and his collaborators have used for studies of anomalous osmosis, membrane charge appears to be of slight influence in determining the P.D. across the membrane. The correlation which we have discussed becomes evident only in less permeable membranes in which, we believe, the dimensions of the channels through the membrane are so far reduced that electrostatic effects between the charged surfaces and ions in the solution dominate the electromotive behavior.

SUMMARY.

The production of concentration P.D.'s across the skin of the frog is very intimately related to the pH of the applied solutions. On the alkaline side of an isoelectric point the dilute solution is electropositive; on the acid side this solution becomes electronegative.

When the pH is suddenly lowered from a value more alkaline than this isoelectric point to one considerably more acid the change in polarity may occur within a few seconds. The effect is reversible.

When a series of unbuffered solutions at different pH values are applied reversal curves may be obtained. When the concentration gradient is .1 N–.001 N KCl the reversal points lie between pH 4.1 and 4.8.

When studied in acetate buffers this electromotive reversal is found to be closely correlated with the electrical charge upon the membrane, as determined by electroendosmosis through it. Reversal occurs between pH 4.9 and 5.2.

It is concluded that the electromotive behavior of this material is controlled by some ampholyte, or group of ampholytes, within the membrane. This ampholyte is probably a protein.

On both sides of their isoelectric point these membranes, in common with protein membranes, behave as if they retarded or prevented the movement through them of ions of the same electrical sign as they themselves bear, while permitting the movement of ions of the opposite sign. It is suggested that this correlation arises because of electrostatic effects between the charged surfaces and ions in the solution.

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