Intracellular Electrical Potentials in Frog Skin

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ABSTRACT The influence of changes in ionic composition of the bathing solutions on intracellular electrical potentials in frog skin has been examined. When the skin bathed in SO_4 Ringer's solution is penetrated with a microelectrode two approximately equal potential jumps were frequently observed and most experiments were carried out with the electrode located between these steps. Substitution of Cl for SO_4 in the bathing solutions caused a decrease in PD across both the "outer" and "inner" barriers. When the skin was short-circuited an average intracellular potential of -18 mv was found with both Cl and SO_4 Ringer's. With the skin in SO_4 Ringer's, decrease in Na concentration of the outside solution caused a decrease in PD between the microelectrode and the outside solution which was approximately the same as the decrease in total skin PD. With SO_4 Ringer's, an increase in K concentration in the inside solution caused a marked decrease in total skin PD. However, only 50 per cent of this change occurred at the inner barrier, between the microelectrode and the inside solution. The remainder of the change occurred at the outer barrier. This observation does not appear to be consistent with the model of the skin proposed by Koefoed-Johnson and Ussing (*Acta Physiol. Scand.*, 1958, **42**, 298).

The model suggested by Koefoed-Johnsen and Ussing (1) to account for the electrical potential difference (PD) across frog skin has received considerable attention since its introduction in 1958. In an effort to confirm one aspect of this model, several investigators have examined the profile of electrical potential (as a function of distance) in the skin using microelectrodes. Engbaek and Hoshiko (2), in most of their experiments, observed the two positive steps (relative to the outside solution) which were predicted, but they also found more than two steps in some cases. By marking electrode position in toad skin, Whittembury (3) has found that the two major steps are located at the outer and inner borders of the basal cells of the epithelium, the stratum germinativum. Finally, Ussing and Windhager (4) have confirmed some of these observations but they also found that three potential steps are frequently observed and have suggested that some revisions of the model may be necessary. However, a systematic study of the influence of ion composition of the

bathing solutions on the PD across individual barriers has not been carried out. The present experiments were designed to examine this behavior. Initially, our interest centered on the PD within the transporting cells under conditions of short-circuiting and on changes in this PD caused by altering Na concentration of the bathing solutions. The studies have also been expanded to include investigation of the influence of Na and K concentrations on the individual PD steps under open circuit conditions.

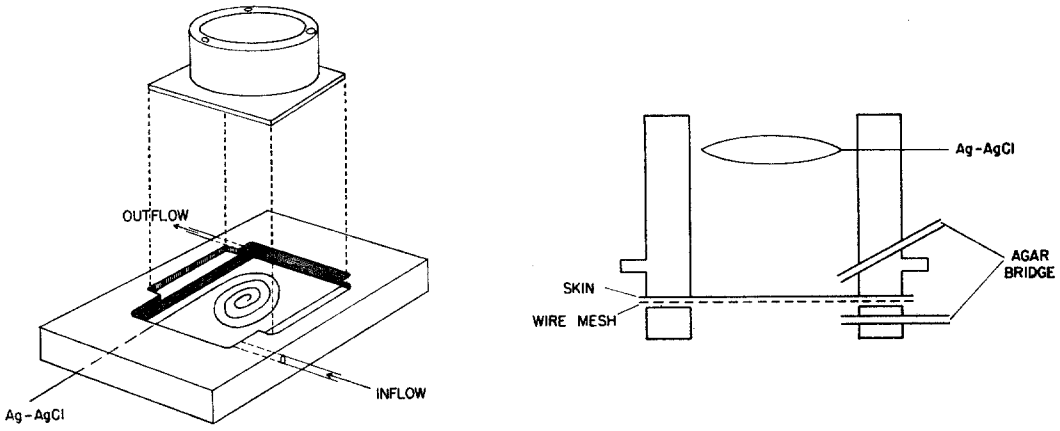

FIGURE 1. Apparatus for micropuncture experiments. A, total assembly, showing the skin chamber (above) and trough containing inside solution (below). B, schematic diagram of skin chamber showing agar bridges for potential measurements.

METHODS

The abdominal skin of *Rana pipiens* was mounted as a flat sheet in the chamber shown in Fig. 1. The skin (8.3 cm^2 in area) was mounted with the outer side upward and supported by a grid (lectromesh, C. O. Jelliff Manufacturing Corp.) placed against the inner surface. The inside bathing solution was contained in the trough into which the chamber holding the skin was placed. In many experiments, the lower chamber consisted of a second lucite cylinder rather than the trough shown. This arrangement was satisfactory but was later abandoned because, under these conditions, changing the inside bathing solution with a microelectrode in the skin was impossible. Whenever possible during an experiment, solutions were bubbled with air to provide oxygenation. However, even if the solutions were not aerated for relatively long periods of time the drop in total skin PD, or short-circuit current, which is characteristic of anoxia (5) was never observed. The upper and lower chambers contained current-passing electrodes of Ag-AgCl in the form of tight spirals. The size and shape of these electrodes insured a uniform current field over the skin area.

The normal Ringer's solution contained 115 mM NaCl, 2.5 mM $KHCO_3$, and 1.0 mM $CaCl_2$ and, after equilibration with air, had a pH of 8.2. In many experiments,

sulfate Ringer's, in which all Cl was replaced by SO_4, was used. Na and K concentrations in these solutions were often altered by replacing Na by K, but in some experiments choline was used as a replacement cation. Choline sulfate was prepared by titrating choline bicarbonate with H_2SO_4 to a pH of 5. Choline concentration was estimated from the amount of standardized acid required for the titration. The pH of the choline Ringer's prepared with this solution was adjusted to 8.2 by addition of choline bicarbonate. Measurements of freezing point depression indicated that the choline Ringer's solution had approximately the same osmotic activity as the Na and K solutions. By using choline as an inert cation, the concentration of either Na or K in the solutions could be altered without changes in the other alkali metal ion or in total anion concentration. In experiments in which Na or K concentrations were altered, samples were removed directly from the chambers for determination of concentration by flame photometry.

Microelectrodes were drawn from 2 mm pyrex tubing and filled with 3 M KCl by boiling under vacuum. In preliminary experiments, electrodes having a long tapered shank and a tip resistance of 10 to 20 MΩ were prepared. These, however, proved unsatisfactory since they were almost inevitably broken during puncture of the skin. Consequently, electrodes having a very short taper and tip resistance of 1 to 5 MΩ were used. Microscopic examinations indicated that the tips were less than 0.5 micron in diameter.[1] With these electrodes the skin could be penetrated easily and the observed PD's were stable for long periods indicating that the cells were not irreversibly damaged. Tip potentials in Cl and SO_4 solutions were generally small and differences between Na- and K-containing solutions were less than 5 mv. The skin was punctured from the outer side with the electrode moving perpendicular to the surface. Attempts to penetrate the skin from the inner side were unsuccessful, apparently because of the mechanical resistance of the connective tissue.

The electrode was mounted in a hollow lucite holder which was clamped to a steel holder equipped with a fine screw to provide vertical motion. The lucite holder was filled with 3 M KCl and an agar–3 M KCl bridge made contact with a calomel electrode. Two additional bridges connected the outer and the inner solutions to calomel electrodes so that total skin PD could be measured. The potential of the microelectrode was determined relative to that of the electrode in the outside solution. Both total PD and electrode PD were measured with Keithley model 200B DC electrometers (input impedance, 10^{14} Ω). In many cases, the outputs of the electrometers were fed into a dual channel recorder (Texas Instrument Co.) for simultaneous recording. Current was passed through the skin from an external battery *via* the Ag-AgCl electrodes and measured with a Simpson microammeter. The resistance and tip potential of the microelectrode were determined before and after penetration of the skin and the puncture was discarded if there were changes greater than 0.2 MΩ in resistance or greater than 4 mv in tip potential.

In many experiments, the PD within a single cell was measured under several different conditions. In order to do this, a puncture was made and the electrode was left undisturbed for 10 to 20 minutes. The solution in the upper (outer) or lower

[1] Since the electrodes had an unusually short shank, the relatively low resistance does not indicate a large tip diameter as would be the case with electrodes having a long shank.

(inner) chamber could then be carefully changed without altering the position of the electrode. The solution in the lower chamber was altered by flowing 1000 ml of new solution through slowly, taking care to exert no upward pressure on the skin. To change the solution in the upper chamber, approximately 28 of the 30 ml was removed by suction and new solution injected *via* a syringe at a rate sufficient to insure mixing. This procedure was repeated 5 times. The solution in the upper chamber could be bubbled gently with air to aid mixing without disturbing the electrode. All measurements following solution changes were made after the PD had reached a new steady level. At the end of most experiments, the original bathing solutions were restored to the system in order to confirm that neither the position of the electrode nor the properties of the cell had been appreciably altered.

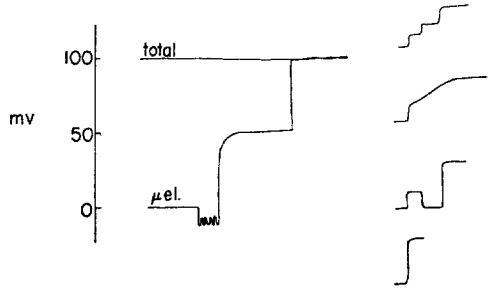

FIGURE 2. Potential profile in frog skin showing two positive potential jumps. Other types of profiles observed are shown schematically on the right.

RESULTS

Potential Profile in SO_4 and Cl Ringer

As described below, we have been primarily interested in the situation in which the microelectrode potential is approximately 50 per cent of the total PD and have not carried out a systematic investigation of the various potential profiles. However, a few general comments can be made based largely on preliminary experiments. A representative record of microelectrode potential relative to outside solution, drawn from the original trace, is shown in Fig. 2 for a skin bathed in SO_4 Ringer's. In most experiments, the first change in PD was a negative deflection (relative to the outside solution) which varied in magnitude from a few millivolts to 50 mv. This was followed, upon advancement of the electrode, by a positive deflection which often stabilized at a value of 40 to 60 per cent of the total PD. Further movement of the electrode gave a second positive step with the microelectrode PD approaching, within a few millivolts, the total PD. This pattern occurred in approximately 60 per cent of the punctures in which a complete profile was obtained in SO_4 Ringer's but different profiles were also observed. In approximately 30 per cent of these punctures, there appeared to be three or more steps of potential while in 10 per cent of the cases the electrode appeared to penetrate the skin completely giving only one step. In a few instances, there was an initial positive step but when the electrode was advanced the PD first fell toward

zero and then increased to a value consistent with the total PD. These patterns are illustrated schematically on the right of Fig. 2. Since the epithelium of the skin is a complex structure containing many cells, the observation of different patterns of PD is, perhaps, not surprising. The possible significance of some of these has been discussed by Ussing and Windhager (4). The work of Whittembury (3) has indicated that the "normal" two step pattern arises when the electrode penetrates the basal cells of the epithelium. On the basis of these findings, we have assumed that observation of a stable first step of about 50 per cent of the total skin PD represents penetration of one of these basal cells and have taken this PD as the one across the "outer barrier or membrane." In many experiments, we have then taken the PD across the "inner membrane" as the difference between the total PD and the first step.[2] This assumption seems reasonable and it has been confirmed by further advancing the electrode in at least one experiment of each series discussed. This

TABLE I

ELECTRICAL POTENTIAL STEPS IN FROG SKIN

Bathing solution	Total PD	1st step	2nd step
	mv	*mv*	*mv*
SO_4 Ringer's	117.5±4.2	65.8±1.9*	51.7±1.3
Cl Ringer's	66.9±5.0	35.9±2.2	31.0±1.7

* Errors are given as standard error of the mean. The number of observations were, in SO_4 Ringer's, 65, in Cl Ringer's, 40.

operation often led to breaking of the electrode, making a final resistance measurement impossible, and it was not, therefore, always carried out.

Determination of the potential profile with the skin in Cl Ringer's is more difficult since no information is available regarding the relationship between PD and location of the electrode. The following procedure was, therefore, used in order to insure that the electrode was in the same position as it was in experiments with SO_4 Ringer's. The skin was initially bathed in SO_4 Ringer's and punctured several times to obtain an estimate of the mean value of the first step. The inside solution was then changed to Cl Ringer's and the skin punctured again. The electrode was left in place and the outside solution changed to Cl Ringer's. Changing the inside solution from SO_4 to Cl Ringer's appeared to have little effect on the first step so that this method of positioning the electrode between the two steps in SO_4 Ringer's appears valid. The

[2] Throughout the following discussion, we shall refer to the PD across the outer barrier as the PD between outside solution and microelectrode when the microelectrode is in a position such that it records approximately 50 per cent of the total PD with the skin bathed in normal SO_4 Ringer's. The term inner membrane PD will refer to the difference between the microelectrode and the inside solution.

TABLE II

ELECTRICAL PROPERTIES OF FROG SKIN

Bathing solution	Short-circuit current	Cell potential	Barrier conductance	
			Outer	Inner
	$\mu a/cm^2$	*mv*	*mmho/cm²*	
SO_4 Ringer's	64±4*	−17.7±2.0	0.77	1.88
Cl Ringer's	61±3	−18.2±1.7	1.13	4.76

* Errors are standard error of the mean.

results of a series of punctures in 13 skins are summarized in Table I. Measurements were made with both Cl and SO_4 Ringer's on each skin. Substitution of Cl for SO_4 reduces the PD across both inner and outer membranes by approximately 40 per cent.

Potential Profile under Short-Circuit Conditions

During each puncture included in Table I, sufficient current was passed through the skin to reduce the total PD to zero, and the PD between the cell and the bathing solutions was observed. The results are summarized in Table II. In 40 separate punctures with the skins bathed in Cl Ringer's, a PD of -18.2 ± 1.7 mv was observed. The distribution of the observed values of this negative well is shown in Fig. 3. Although the spread is large, the majority of values fall between -6 and -25 mv; in only two cases was a positive potential observed under short-circuit conditions. Skins in SO_4 Ringer's showed a negative well of -17.7 ± 2.0 mv under these conditions.

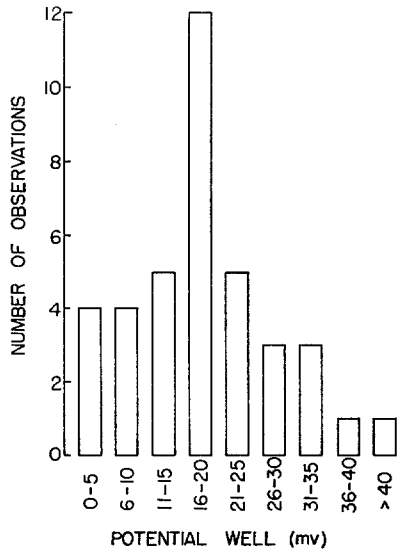

FIGURE 3. Distribution of observations on the negative potential well within the skin during short-circuiting. The skins were bathed in Cl Ringer's solution. Each observation represents a separate puncture. Two observations in which the potential in the cells was positive are not included.

These results can be utilized to estimate the electrical conductances of the outer and inner barriers of the skin. This calculation requires the assumptions that the current field is entirely uniform, that all current passes through both barriers, and that puncture of a cell does not seriously alter the membrane resistance. Since these assumptions are open to question, the results are at best approximate, but they may be useful for comparative purposes. The values obtained are included in Table II. As expected, the conductance of both barriers is decreased in the presence of the impermeable SO_4 ion, but the effect is considerably greater at the inner barrier. It is of interest to note that in SO_4 Ringer's the position of the microelectrode was such that approximately 70 per cent of the skin resistance was between the electrode and the outside solution.

TABLE III

EFFECT OF Na CONCENTRATION ON CELL POTENTIAL UNDER SHORT-CIRCUIT CONDITIONS

Na concentration	PD (cell-outside)	Short-circuit current
mM	*mv*	*μa/cm²*
115	−16.0±4.8*	58
15	−16.7±5.1	36
8	−16.5±5.0	26
3	−16.9±4.6	12

* Errors are standard error of the mean.

Ten experiments were carried out to determine whether the magnitude of the potential well under short-circuit conditions was dependent on the Na concentration in the outside solution. In each skin, Na concentrations of 3, 8, 15, and 115 mM were tested during a single micropuncture. The experiments were carried out in Cl Ringer's and choline was used to replace Na. The results, summarized in Table III, indicate that there is no significant change in the magnitude of the well despite a 40-fold change in Na concentration and a five-fold change in short-circuit current. In two experiments, the skin was kept short-circuited continuously while in the others, current was passed only intermittently. Identical results were obtained under both conditions. The large standard errors are due mainly to an appreciable spread in the magnitude of the potential well under control conditions (115 mM Na). In individual experiments, the size of the well differed by only a few millivolts at the different Na concentrations and, as indicated by the averaged data, there was no consistent relationship between PD and concentration.

The data from these experiments also permit estimation of the conductance of the two barriers at different outside Na concentrations. The calculations

led to the expected result that conductance of the outer barrier decreased as Na concentration was lowered and to the unexpected result that there is also an appreciable decrease in conductance of the inner barrier under these conditions. Examination of the data showed that there is an approximately linear relationship between conductance of both barriers and short-circuit current as illustrated in Fig. 4.

The Effect of Cation Concentration on PD

Koefoed-Johnsen and Ussing (1) have suggested that the outer barrier of the skin is permeable to Na and not to K, while the inner barrier is permeable to K but not to Na. The present microelectrode technique makes possible a

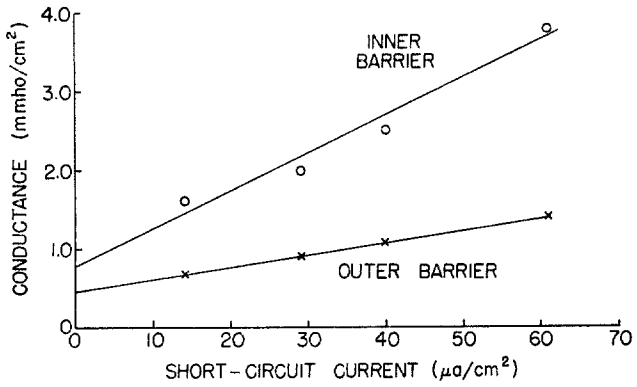

FIGURE 4. Conductance of outer and inner barriers of the skin as a function of short-circuit current. Each point represents the average of 8 experiments. Short-circuit current was altered by changing Na concentration of the outside bathing solution.

more direct test of this hypothesis. The experiments were carried out using SO_4 Ringer's since the skin is nearly impermeable to this anion. In order to avoid possible complications which might arise from changing both Na and K concentrations at the same time, choline was used as a cation for replacement. In experiments involving changes in Na concentration of the outside solution, 4 Na concentrations were tested in each experiment and the measurements were made with a single micropuncture, the electrode remaining in place throughout. The averaged results of experiments on 8 skins are summarized in Fig. 5 in which total PD and PD across the outer and inner barriers are shown as functions of the logarithm of outside Na concentration. As expected from the work of Koefoed-Johnsen and Ussing (1), both the total PD and the PD across the outer barrier decrease with decreasing Na concentration. There is only a slight change in the PD across the inner membrane. Although the total PD is approximately a linear function of log $[Na]_o$, the slope of the line is considerably less than 58 mv per tenfold change in concentration, the value expected if the outer barrier were permeable only to Na.

In experiments testing the effect of K in the inside solution, the basic SO_4 Ringer's solution contained 4 mM K, 80 mM choline, and 30 mM Na; K concentration was increased at the expense of choline. The averaged results of experiments on 7 skins testing the effect of 5 K concentrations are summarized in Fig. 6 in which PD is plotted against the logarithm of inside K concentration. The total PD is approximately a linear function of log $[K]_i$ for the four higher concentrations and the line has a slope of 54 mv per decade, in reasonable agreement with that expected if the inner membrane were permeable only to K. However, the data obtained with the microelectrode indicate that a considerable portion of the PD change occurs across the outer membrane. The PD between the electrode and the inside solution changes by only 22 mv per decade.

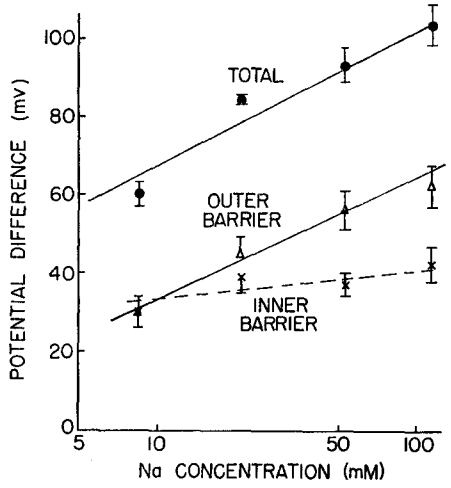

FIGURE 5. Total PD and PD across outer and inner barriers as a function of logarithm of Na concentration in the outside solution. Each point represents the average of 8 experiments. The bars represent ± one standard error of the mean.

In order to test the possibility that this unexpected result might be due to the use of choline, 3 experiments using normal Na_2SO_4 Ringer's solution were carried out in which Na was removed as the K concentration was raised; similar results were obtained. The slopes of the straight lines drawn through the points were, in millivolts per decade, total PD, 47, outer membrane, 26, and inner membrane, 21. In these experiments the highest K concentration used was 115 mM. Under these conditions, a mean PD of 28 mv remained between the tip of the electrode and the inside solution. In addition, the possibility that the results could be influenced by leaving an electrode in the skin was examined in 3 experiments. In these, 10 separate punctures were made at each of 3 K concentrations and the results averaged. The electrode was left in the skin for no more than 1 minute and a different area was used for each puncture. The mean slopes obtained were, in millivolts per decade, total, 53, outer membrane, 27, inner membrane, 26. Although the values of the slopes are somewhat different from those obtained in the larger series in which

choline was used, the fact remains that a large proportion of the total PD drop caused by elevated K in the inside solution occurred in the PD assumed to be localized at the outer barrier. Finally, 2 experiments were carried out on the European frog, *Rana esculenta*, and again similar results were obtained. The average slopes were, in millivolts per decade, total PD, 60, outer membrane 31, and inner membrane, 29.

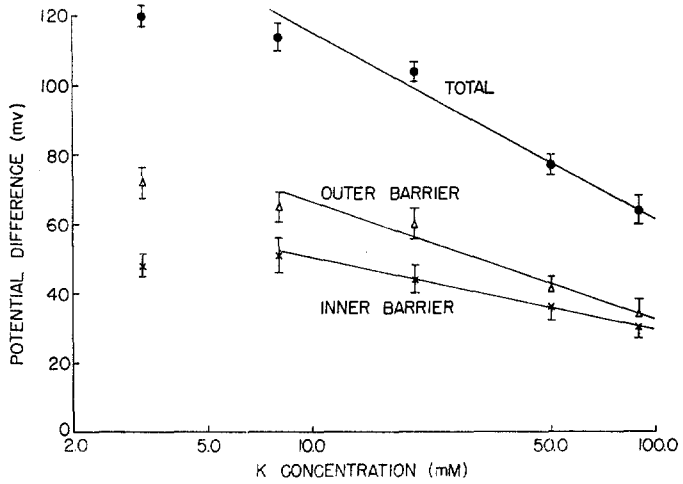

FIGURE 6. Total PD and PD across outer and inner barriers as a function of logarithm of K concentration in the inside solution. Each point is the average of 7 experiments. The bars represent ± one standard error of the mean.

DISCUSSION

Short-Circuiting Experiments

The observation of a negative well within the cell under short-circuit conditions in SO_4 Ringer's is in agreement with the results of a single experiment published by Hoshiko (6) and with the observations of Ussing and Windhager (4). In Cl Ringer's, the presence of such a well might be expected from consideration of the behavior of the Cl ion. Chloride appears to be passively transported (7) since there is no net flux of this ion when the skin is short-circuited.[3] However, if cell Cl concentration is lower than that in the bathing solutions, Cl should enter the cells from both sides during short-circuiting and the cells should swell. Since appreciable swelling is not observed under these conditions (9), a negative well of sufficient magnitude to prevent Cl entry should be present. Assuming that the Cl is passively distributed across both membranes, cell chloride concentration can be estimated using the expression

[3] The present considerations probably do not apply to the frog *Leptodactylus ocellatus* which shows a significant active Cl transport (8).

$$-\Delta\psi = 58 \log \frac{[Cl]_{out}}{[Cl]_{cell}}$$

in which $\Delta\psi$ is the PD between the bathing solutions and the cell in the short-circuited skin. Introducing the values $\Delta\psi = -18.2$ mv (Table II) and $[Cl]_{out} = 115$ mM we find that the equilibrium concentration of Cl in the cells would be 56 mM. This figure is in good agreement with the value of 49 mM estimated by MacRobbie and Ussing (9) on the basis of osmotic experiments. Thus, the present results offer a simple explanation of the observation that under short-circuited conditions the total current crossing the outer barrier is carried by Na ions (the short-circuit current is equal to net Na flux) even though the membrane is permeable to Cl ions. If, under short-circuit conditions, Cl is at equilibrium across the outer barrier, the net flow of Cl will vanish and this ion will not contribute to the current.

These observations on the short-circuited skin are also of importance in interpreting the results reported by Cereijido *et al.* (10). They found that the rate coefficients for unidirectional Na movements across the outer barrier were markedly altered by changes in Na concentration in the outside solution. Since these coefficients include any effect of PD, the observed changes could be attributed, at least in part, to changes in PD across the barrier following alteration of Na concentration. These experiments were carried out with the skin short-circuited. The data presented in Table III show that the PD across the outer barrier under these conditions is independent of Na concentration and, therefore, confirm the suggestion that the observed variation in rate coefficients cannot be ascribed to changes in PD.

The results also lend support to the suggestion of Hansen and Zerahn (11) that accumulation of Li in the epithelium of short-circuited skins could be due to a potential well. Assuming that the well is not appreciably altered by relatively low Li concentrations, the observed maximum value of -45 mv would be sufficient to account for the observed maximum eightfold concentration of Li.

Dependence of PD *on Cation Concentration*

The effects of Na concentration in the outside solution on the PD pattern agree, at least qualitatively, with those predicted by the model of Koefoed-Johnsen and Ussing (1). The decrease in total PD occurring as a result of lowered Na concentration is due almost entirely to a change in the PD between the microelectrode and the outside solution. However, the observed slope of 35 mv for a tenfold change in Na concentration suggests that even in SO_4 Ringer's some other ion or ions must have sufficient permeability at the outer barrier to influence the PD appreciably. Measurements with $S^{35}O_4$ in several skins showed that the over-all flux of this ion is very small (of the order of

0.002 μeq/hr. cm^2). However, these results cannot rule out the possibility that either the inner or outer barrier may have significant SO_4 permeability. Permeability of the barrier to the cations choline or K could also contribute to the low slope. Finally, Ussing and Windhager (4) have recently suggested that there may be an appreciable passive shunt, possibly between cells, in skins bathed in SO_4 Ringer's. Such a shunt could also contribute to a reduction in slope even if the barrier designated as the outer membrane were a nearly perfect Na electrode. Thus, there are sufficient variables involved in the determination of the PD at the outer barrier to make exact interpretation extremely difficult. However, the present observations do indicate that this PD is determined mainly by Na concentration.

Although the effect of K concentration in the inside solution on total PD agrees well with that observed by Koefoed-Johnsen and Ussing, the findings based on microelectrode studies are unexpected. Only about 40 to 50 per cent of the change in total PD can be ascribed to a change at a barrier between the tip of the microelectrode and the inside solution, with the remainder occurring between the electrode and the outside solution. Thus, the inner barrier does not appear to function as a K electrode, and the observed theoretical slope for the total potential may be fortuitous. The complete explanation of these observations remains uncertain. It is possible that they could arise if the microelectrode were in a position other than that assumed, in the cells of the stratum germinativum. Previous studies, particularly those of Whittembury (3) and of Ussing and Windhager (4) suggest that this is not the case. A potential step of approximately 50 per cent of the total PD appears to be associated with penetration of cells near the base of the epithelium. Further, experiments involving changes in composition of both solutions were carried out with the microelectrode in the same position. Since the equivalent of Na electrode behavior was found for the outer barrier under these conditions, it seems unlikely that failure to observe K electrode behavior at the inner barrier can be ascribed simply to electrode position.

There are, however, several possible factors which could account for the rather strong dependence of PD across the *outer* barrier on *inside* K concentration. In terms of the model, such an effect could arise as a result of changes in cell composition. An increase in cell Na would lead to a decrease in PD across the Na-sensitive outer barrier. The cell Na pool exchanges rapidly, having a half-time of 2 to 5 minutes (10), so that significant change could occur within the time required for the PD change and could account for the effect at the outer membrane. Such a change in cell Na would require either increased Na permeability of the outer membrane or inhibition of the active step as a result of increased K concentration. Although there have been suggestions that such effects may exist (6, 12), there are no compelling reasons to assume that they are necessarily involved in the phenomenon considered here.

A second possible explanation is offered by the suggestion of Ussing and Windhager (4) that an appreciable passive shunt conductance may exist in skins bathed with SO_4 Ringer's. If the conductance of such a shunt were increased markedly in the presence of high K in the inside solution, the increased local current through it could give rise to a decrease in PD across the outer barrier. An increased shunt conductance would be consistent with the observation of Bricker *et al.* (13) that skins bathed with high (120 mM) K on the inside have a very high conductance. On the other hand, Hoshiko (6) has reported only minimal conductance changes following an increase of inside K from 4 to 40 mM. In order to explain our observations, the shunt pathway, presumably located between the cells, must be altered by changes in K but not in Na concentration in the inside solution.

A third explanation may be based on another suggestion of Ussing and Windhager (4). They have proposed that the functional cells in the epithelium are arranged in series, with those in the outer part of the epithelium contributing to the total potential. This proposal is based on the relatively frequent observation of a third potential step in the profile with the extra step located in the outer part of the epithelium. As mentioned above, such profiles have also been observed in the present experiments. If the skin contains two (or more) layers of cells functioning in a manner analogous to that proposed by Koefoed-Johnsen and Ussing, the present results can be explained. We must further assume that (*a*) the electrode is in a basal cell and (*b*) that K in the inside solution has access to most of the cells in the epithelium. The basis of the first assumption is discussed above. The second seems reasonable in the light of recent studies of K exchange (14) which indicate that most of the K in the skin exchanges readily with K^{42} in the inside solution. In such a system, increase of K concentration of the inside solution will cause a depolarization at the inward facing membrane of all cell layers. The depolarization of the outer cells will be measured as a change in PD between the microelectrode and the outer solution. If such an effect is involved, the inward facing membranes of the cells cannot function as nearly perfect K electrodes; the slope of the line relating the PD at the inner membrane to K concentration is much too low. Thus, the inner membrane must be significantly permeable to some other ion or ions in the system such as Na, SO_4, or choline.

A distinction among the possible explanations of the observed effects of K on the potential profile cannot be made on the basis of the data available at present. It is entirely possible that all three effects (changes in cell composition, shunt pathways, several cell layers) are involved, each contributing in part to the over-all phenomenon, and other explanations cannot be excluded. The results do, however, indicate that the inner membrane of the skin does not function in the simple manner proposed by Koefoed-Johnsen and Ussing (1).

The present experiments also raise an important point regarding the mecha-

nism of active Na transport. In agreement with the observations of Frazier and Leaf (15) in toad bladder, we have found that a substantial PD exists across the inner membrane in the presence of high K in the inside solution. According to the model of Koefoed-Johnsen and Ussing (1) this PD should vanish when inside K is raised to the level of cell K. The mean concentration of K in the total water of the skin is approximately 55 mM; after correction for inulin space, a figure for cell K concentration of 100 mM is obtained.[4] However, extrapolation of various curves available indicates that in SO_4 Ringer's, a PD of 10 to 30 mv remains when inside K is 100 mM. If the "inner barrier" is permeable to ions other than K as appears possible from the above considerations, the residual PD might be explained. On the other hand, it is possible, as suggested by Frazier and Leaf (15) for toad bladder, that the PD across the inner membrane is caused, in part, by an electrogenic mechanism of active Na extrusion rather than by the non-electrogenic forced Na-K exchange system proposed by Koefoed-Johnsen and Ussing. The presence of such a system would be consistent with the observations of Bricker *et al.* (13). They found a short-circuit current across the skin under conditions in which they felt that K could not be carrying current at the inner barrier and suggested, as one possible explanation, an electrogenic Na transport system at the inner membrane. Such a transport system would be consistent with the observation of Curran and Cereijido (14) that a simple Na-K exchange at the inner membrane does not appear to be involved in the process of active Na transport across the skin and might also offer an explanation of the relationship between short-circuit current and conductance of the inner barrier (Fig. 4). If the Na transport system were carrying current across this barrier, a decrease in transport rate might well lead to a decrease in conductance as observed. Further investigation of many of these points is essential before any final conclusion can be drawn, but it is apparent that an electrogenic pump would require complete reevaluation of events at the inner barrier.

The present results indicate that the profile of electrical potential across frog skin cannot be entirely explained by the model proposed by Koefoed-Johnsen and Ussing (1), at least in its original form. They also raise some questions regarding the nature of the active Na transport system itself. Finally, the concept of inner and outer membranes seems less clear cut than originally proposed. Although it is convenient to retain the nomenclature of inner and outer, these "membranes" may well be rather complex structures made up of several barriers in series. There is not as yet sufficient information available to propose a self-consistent modification of the model of the skin, but it seems apparent that interpretation of observations in terms of the original model must be viewed with caution.

[4] Unpublished observations; see also Huf, Wills, and Arrighi (16).

We are indebted to Miss Margaret Moore and Mrs. Margery Blacklow for valuable technical assistance.

This work was supported in part by a Public Health Service research grant (AM-06540) and a research career program award (AM-K3-5456) to Dr. Curran, both from the National Institute of Arthritis and Metabolic Diseases.

Dr. Cereijido was a Public Health Service International Research Fellow.

Received for publication, September 25, 1964.

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