Interaction between the Effects of Inside and Outside Na and K on Bullfrog Skin Potential

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ABSTRACT The composition of the solution bathing one border of the isolated frog skin affects the response of the potential across the skin to changes in the composition of the solution bathing the opposite border. Increasing the K concentration of the inside (corium) bathing solution decreased the sensitivity of the potential to a change in outside Na concentration. Decreasing the outside Na concentration decreased the sensitivity of the potential to a change in inside K concentration. Increasing the total ionic strength of the outside bathing solution or of both bathing solutions decreased the sensitivity of the potential to a change in outside Na concentration.

INTRODUCTION

Koefoed-Johnsen and Ussing (1) proposed that the frog skin potential in the absence of penetrating anions may be regarded as the sum of a sodium diffusion potential at the outer or epithelial border and a potassium diffusion potential at the inner or corium border. Recently, Lindley and Hoshiko (2) found substantial deviations from the behavior predicted by this model under certain conditions. Increasing the total ionic strength of the bathing solution decreased the response of the skin potential to a change in the cation composition of either bathing solution. In addition, unpublished experiments of Edwards suggested that the sensitivity of the potential to a given change in the Na concentration at the outside border is affected by the K concentration of the inside bathing solution. In toad bladder, Essig and Leaf (3) suggested that the K concentration of the inside bathing solution may influence the permeability of the outside border.

The study of epithelial membranes has now reached the point at which desirable knowledge about mechanisms often seems to require the use of techniques which reach directly to the level of single cell membranes, such as

microelectrodes. However, the application of microelectrode techniques to frog skin is beset with formidable technical difficulties from the standpoint of adequate control. One must use histological control and checks for variation of resistance with position (4, 5). Because of the magnitude of the labor involved, it thus becomes important to have useful tools for narrowing down the range of possible conditions for the existence of postulated phenomena to the range of probable conditions. In the present work we have applied more simple techniques to the investigation of the conditions under which the behavior of the frog skin potential is adequately represented by the simplest version of the Koefoed-Johnsen—Ussing model. It will be shown that there is definitely interaction between the so called inner and outer components of the potential, but only under extreme conditions, and that the total ionic strength of the bathing solution markedly influences the sensitivity of the potential to changes in the ionic concentrations.

METHODS

Experiments were done at room temperature (20–24°C), on pieces of abdominal, thigh, and calf skin of the bullfrog (*Rana catesbeiana*). Plastic chambers of the type described by Ussing and Zerahn (6) with 2 cm^2 surface were used. Five ml of solution bathed each side. Potentials were monitored through 3 M KCl-agar bridges and recorded on a strip chart recorder. Skins with potentials below 80 mv in the initial Na_2SO_4 solution were rejected. A recovery potential of 50 mv or more at the end of the experiments was an additional criterion which was met in all but one experiment. In that experiment, the skins had been exposed to hypertonic solutions and high K.

In any experiment, when the K concentration was changed, Na replaced K, and when the Na concentration was changed, K replaced Na so that the total cation concentration (Na + K) was kept constant. In all experiments the sole anion was sulfate. Solutions were buffered to pH 8 with 5 millimolar tris (Sigma 7–9) buffer and were free of divalent cation. The skin potential has been observed to be well maintained in the absence of Ca (*cf.* reference 2). All solutions were analyzed for Na and K using a Baird flame photometer.

When changing solutions at least one rinse with the new solution was made before the final addition of the new solution. This made it likely that the solution in contact with the skin would be within 1 per cent of the analyzed concentration.

The solutions bathing the epidermal side of the skin are referred to as "outside solutions," those bathing the corium side, "inside solutions." The abbreviations Na^i, K^o will be used to designate the Na or K concentrations, in the inside (i) and the outside (o) solutions. All values for changes in Na or K will be designated by the ratio of the concentration of cation in the replacement solution (subscript 2) to that in the original solution (subscript 1); thus, a change from 60 Ma meq/liter to 15 meq/liter Na is denoted by $\frac{1}{4}$ ($Na_2/Na_1 = \frac{1}{4}$).

The changes in potential reported in the Results are the differences between the

steady state values during the experimental period and the steady state control values. A steady state was assumed to be established when the change in potential did not exceed 1 mv per 5 minutes. If no steady state was achieved during the experimental period, it was terminated at a predetermined time (15 to 35 minutes depending on the experiment). Previous experience showed that more than 90 per cent of the potential change occurred during this time. The change in potential, Δv, in response to a given change in K^i or Na^o (in Fig. 1 of $\frac{1}{8}$) was the measurement of interest. Δv was taken as the steady experimental potential minus the control potential immediately preceding. The sensitivity of the potential to the ion concentration change will be defined as the ratio of the two, $F\Delta v/RT\Delta \ln K^i$ or $F\Delta v/RT\Delta \ln Na^o$ for a given

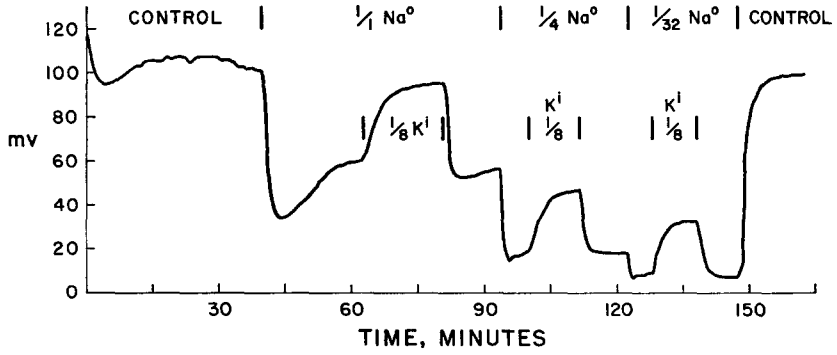

FIGURE 1. Effect of Na^o on bullfrog skin potential response to a change in K^i. This graph (redrawn from the original record) shows the time course of a typical balanced incomplete block experiment. This first sharp drop in potential is due to replacement of 55 Na, 5 K in the inside solution with 60 K and replacement of the outside control solution with 60 Na. Na^o is indicated above, the vertical lines represent points where changes in solution were made. The $\frac{1}{8}$ K^i, below, is the test replacement of 60 K. The final sharp increase in potential represents replacement of both solutions with 55 Na, 5 K to return to the control conditions.

change in K^i or in Na^o. A discussion of the meaning of Δv is given in Lindley and Hoshiko (2).

Two types of statistical design were used, the split plot design (7) and the balanced incomplete block design (8). In either type, multiple pieces of skin from each frog were used, and several experimental treatments were done on each piece of skin. A sample protocol and a figure showing a typical split plot experiment will be found in Lindley and Hoshiko (2). For the case of a balanced incomplete block design a typical experiment is illustrated in Fig. 1. The solution assignments are those given by Cochran and Cox (reference 8, Table 11.4, p. 471). Fractional Na concentrations in the test solutions were 2^0 to 2^{-6}, except 2^{-1}; number of treatments, $t = 6$; number of units per block, $k = 3$; number of replications, $r = 5$; number of blocks, $b = 10$; repetition of entire experiment $\lambda = 2$; and number of times 2 treatments appear in the same block, $p = 2$.

RESULTS

A. *Effect of K^i on Na^o Sensivity*

1. EFFECT OF K^i IN THE ABSENCE OF AN OSMOTIC GRADIENT (MAY, DECEMBER, FEBRUARY)

Three sets of experiments were done to study the effect of altering K^i on the sensitivity of the skin potential to variations in Na^o. In the first (May), a split plot design, each of six pieces of skin from a single frog was assigned a different K^i. The change in skin potential in response to a reduction in Na^o is shown

TABLE I

EFFECT OF K^i ON CHANGE OF SKIN POTENTIAL IN RESPONSE TO CHANGE OF Na^o (NO OSMOTIC GRADIENT)

Potential change, Δv

	K^i, *milliequivalents/liter*					
	0	2	5	15	30	60
Na^o, fraction of control	*mv*	*mv*	*mv*	*mv*	*mv*	*mv*
1/4	−26.8	−25.0	−25.7	−25.0	−23.3	−24.3
1/8	−38.3	−38.0	−39.3	−35.2	−35.8	−33.7
1/16	−49.2	−51.7	−52.8	−47.5	−45.2	−43.0

Each potential change in millivolts is the mean of six experiments. The mean initial potential for the thirty-six pieces of skin used was 115 mv (range 101 to 137 mv). Mean recovery potential was 110 mv (range 57 to 129 mv). Total cation inside and outside was 60 meq/liter during the experiment. Control Na^o was 60 meq/liter.

in Table I. Analysis of variance showed a significant difference ($P < 0.05$) in the response of the potential among different K^i for a given Na^o, and two further experiments were done to pin-point the effect by using the balanced incomplete block design. The response to the same change in Na, 1/8 in December, and 1/64 in February, was tested at six different K^i: 1, 1/4, 1/8, 1/16, 1/32, 1/64. Total cation concentration was 60 meq/liter in all solutions. The results are illustrated in Fig. 2. No effect of K^i was seen when the Na^o change was 1/8. However, when the Na^o change was 1/64, a significant effect was found. The slope of the regression line for the 1/64 change in Na differed from zero ($P < 0.01$) and did not depart from linearity.

2. EFFECT OF K^i IN THE PRESENCE OF AN OSMOTIC GRADIENT (MAY, JUNE)

Two sets of experiments were done to determine the effect of K^i on the sensitivity of the potential to a change in Na^o in the presence of an osmotic gradient. The outside control solution for these experiments was 20 Na. Three

pieces of skin from a single frog were bathed on the inside with solutions containing 60 meq/liter total cation; K^i was 0.6, 6.0, or 60. Three other pieces of skin from the same frog were bathed on the inside with solutions containing 160 meq/liter total cation and K^i of 0.6, 6.0, or 160. Since other experiments (see section A.1) suggested that a significant effect might be obtained at lower Na^o, concentration changes of $\frac{1}{4}$, $\frac{1}{16}$, and $\frac{1}{64}$ were used. Again the potential change (Table II) was clearly dependent on the change in Na^o. With either 60 or 160 total cation, the change in potential was greater at low K concentration. However, the response with $\frac{1}{4}$ Na was the same for all K. The mean difference of 7.2 mv between 0.6 K^i and 6.0 K^i at 160 total cation

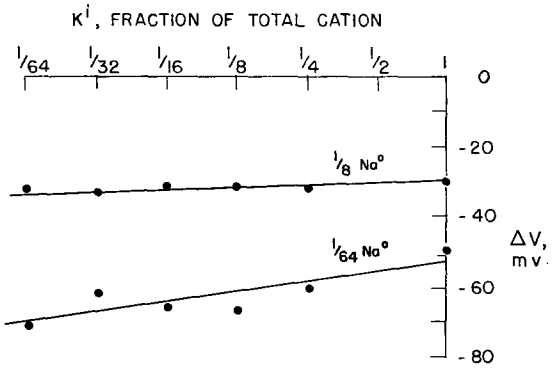

FIGURE 2. Effect of K^i on response of the potential to change in Na^o: Total cation both sides, 60. Each point is the mean of ten experiments. SEM for $\frac{1}{8}$ curve ± 1.1 mv; SEM for $\frac{1}{64}$ curve ± 3.2 mv. The line shown is the regression line for each experiment. The mean initial control potential for the $\frac{1}{8}$ experiment was 94 mv (range 82 to 106 mv), mean recovery, 91 mv (range 73 to 112 mv); for the $\frac{1}{64}$ experiment mean initial control was 106 mv (range 90 to 118 mv), mean recovery was 102 mv (range 82 to 127 mv).

was not significant. With $\frac{1}{16}$ Na and $\frac{1}{64}$ Na, the potential change was smaller as K^i increased. When K was the sole alkali metal cation in the inside solution, there were no differences in the responses between 60 and 160 total cation. These results agree with those of the previous section that K^i affects the sensitivity of the skin potential to changes in Na^o, the effects being more pronounced with the larger changes in Na^o of ($\frac{1}{16}$ and $\frac{1}{64}$). In addition, the Na sensitivity was greater when the inside solution was 160 total cation.

B. *Effect of Na^o on Sensitivity of Skin Potential to K^i (December)*

To test for an effect of Na^o on the sensitivity of K^i, a set of experiments analogous to those in the previous section was done. The results of the experiments are summarized in Fig. 3. Increased Na^o increased the K sensitivity of the

inside border, thus the potential change due to a change in K^i, was in part a function of Na^o.

When both inside and outside solutions were replaced simultaneously, the potential initially decreased rapidly, then increased slowly, and finally

TABLE II

EFFECT OF K^i ON CHANGE OF SKIN POTENTIAL IN RESPONSE TO CHANGE OF Na^o (OSMOTIC GRADIENT PRESENT)

Potential change, Δv

Total cation inside K^i, *milliequivalents/liter*	60 meq/liter 0.6	6.0	60	160 meq/liter 0.6	6.0	160
Na^o, fraction of control	*mv*	*mv*	*mv*	*mv*	*mv*	*mv*
1/4	−26.5	−27.2	−27.2	−35.5	−28.7	−25.2
1/16	−58.0	−54.8	−43.7	−68.5	−60.2	−46.7
1/64	−84.5	−78.5	−49.8	−91.8	−83.2	−53.5

Each potential change in millivolts is the mean of six experiments. Mean initial control potential of the eighteen pieces of skin at 60 total cation inside was 94 mv (range 80 to 105 mv). Mean recovery potential was 89 mv (range 50 to 110 mv). For the eighteen pieces of skin in 160 total cation solution, the mean initial control potential was 99 mv (range 80 to 111 mv), and the mean recovery potential was 78 mv (range 61 to 101 mv). Total cation outside 20 meq/liter. Control Na^o was 20 meq/liter.

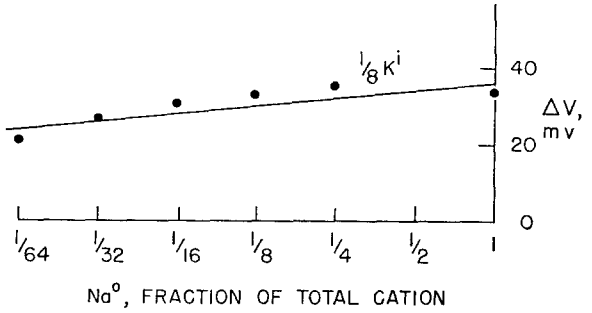

FIGURE 3. Effect of Na^o on response of the potential to change in K^i. Total cation both sides, 60. Each point is the mean of 10 experiments. SEM ± 1.2 mv. The line shown is the regression line for the experiment. The mean initial control potential was 106 mv (range 95 to 120 mv), for the twenty pieces of skin used. The mean recovery potential was 102 mv (range 82 to 127 mv).

reached a steady level (Fig. 1). In other experiments (unpublished) such "overshoots" were occasionally observed. In particular, they appeared on changing the inside bathing solution from Na to K but not when changing from Cs, Li, or Rb to K. This effect may be due to a K stimulation of skin glands, perhaps *via* the nerves.

C. *Effect of Ionic Strength on the Sensitivity of the Potential to Change in Na°* (*July, August*)

Previous experiments (2) and those above suggested that the ionic strength may affect the response. To test this possibility a set of experiments was done in which the ionic strength of the outside solution was varied. The outside surface of each of six pieces of skin from one frog was bathed with solutions containing 15, 30, 60, 120, 180, and 240 meq/liter total cation. The initial outside solution was all Na_2SO_4. The inside solution was 55 meq/liter Na, 5

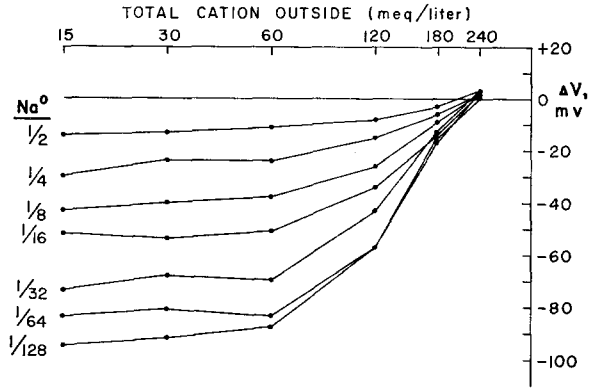

FIGURE 4. Effect of varying ionic strength of outside solution on the sensitivity of the potential to change in Na°. Mean initial control potentials for the thirty six pieces of skin from six frogs was 108 mv (range 80 to 130 mv); mean recovery potential was 107 mv (range 50 to 140 mv). Total cation inside 60. Each point is the mean of six experiments. SEM for the entire experiment ± 0.3 mv. Rather than the usual plot of Δv *vs.* log Na_2/Na_1, the plot is Δv *vs.* ionic strength for each fractional concentration of Na°. This shows the effect of ionic strength more clearly; however, the 59 mv slope predicted by the Nernst equation cannot be plotted. The log scale is used for the abscissa since the total cation of the outside solutions was chosen at equally spaced logarithmic intervals, except for the 180 point.

meq/liter K, in all cases. The Na sensitivity of the outside border of each piece of skin was tested with seven concentrations of Na. (A few skins were tried at 480 meq/liter total cation; however, the recovery of the potential at the end of the experiment was poor and the results therefore are not included. Small increases rather than decreases in potential were noted when Na° was decreased.) The change in potential for a given change in Na° is decreased at total cation concentrations above 60 meq/liter (Fig. 4). The constant field equation did not fit the data well. In these experiments an osmotic gradient occurred from inside to outside at 240, 180, and 120 meq/liter total cation, where the change in response was most prominent.

Measurements of the response of the potential at the outside border in the absence of an osmotic gradient proved difficult. The isolated frog skin did not recover well when the solutions bathing the skin were hypertonic, and not at all when the inside solutions were less than 40 meq/liter total cation. Therefore, the experiment was limited to four concentrations of 240, 180, 120, and 60 meq/liter total cation on each side. Only three changes in Na^o were used since the skins did not recover at all from long exposure to 180 and 240 total cation. The mean initial control potential was comparable to that of the previous experiment, 107 mv (range 27 to 135 mv). The results (Fig. 5) were similar to those of the previous set of experiments. The response to a given change in Na^o increased as the ionic strength of the bathing solution decreased. The constant field equation again did not fit the data well. In

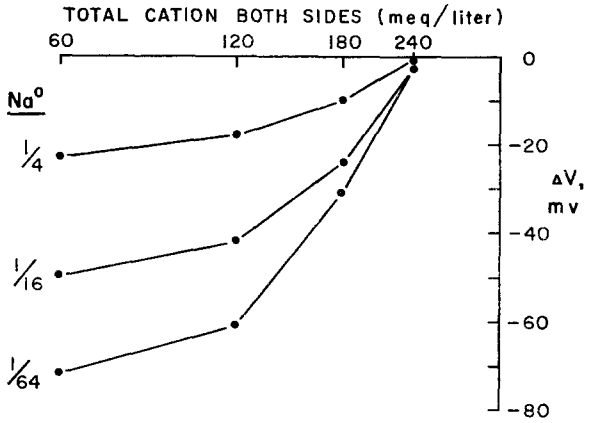

FIGURE 5. Effect of varying ionic strength of both bathing solutions on sensitivity of the potential to change in Na^o. Each point is the mean of six experiments. SEM for the entire experiment was ± 0.8 mv.

neither experiment did the change in Na sensitivity have a linear relationship to the ionic strength.

The effect of ionic strength on the response of the potential to changes in K^i could not be determined over a great enough range of ionic strength to obtain data comparable to those obtained for Na^o.

DISCUSSION

The results for bullfrog skin indicate a surprising latitude of conditions under which the Koefoed-Johnsen—Ussing model is a good approximation. To explain the observations it is necessary to investigate the types of postulates required to adopt the Koefoed-Johnsen—Ussing model. There are two general categories: membrane complexities beyond those embodied in either the

Nernst equation or the constant field equation, and anatomical complexities rooted in the fact that the structure of the skin is not simply two large membranes in parallel.

It may be possible to account for the behavior of the frog skin potential in these experiments in terms of an electrogenic pump (9, 10). Such an explanation would require that the pump rate be a function of Na^o, K^o, Na^i, or K^i, or a combination of these. Thus, the present experiments represent an additional constraint upon the postulation of electrogenic pumps as an explanation of the potential across isolated frog skin. Actually, at least two explanations are possible within the framework of the Koefoed-Johnsen—Ussing model of frog skin and are considered below.

The changes in sensitivity of the potential observed in these experiments may be related to changes in intracellular electrolyte concentration. Huf *et al.* (11) estimated changes in intracellular electrolyte concentrations when Na^o and K^i were altered, but in their experiments the anion was chloride. Alternatively, isotope techniques (3, 12–14) have been used, but none of the methods tried to date will provide quantitative data with the necessary precision (15). Hansen and Zerahn (16) made serial sections of the skin parallel to the outside surface, but their values for Na and K in the epithelial layer varied widely. Thus, the levels of Na and K in epithelial cells cannot be obtained with the necessary precision and accuracy with presently available methods. However, intracellular cation composition changes, if they occur, might be responsible for the changes in potential sensitivity. It is necessary to assume that the permeabilities of the cell borders are dependent on the intracellular ion composition, and that different species of ions compete for the same sites in the membrane, as proposed by Sjodin (17).

An explanation for the observed interaction can be sought in the existence of a significant parallel shunt pathway in addition to the intracellular pathway (18, 19). Recent electron microscope studies of frog skin by Farquhar and Palade (20) have provided anatomic evidence for such shunt pathways. A simple equivalent circuit for the frog skin following the models of Ussing and Zerahn (6) and of Koefoed-Johnsen and Ussing (1) is shown in Fig. 6. With a finite shunt, current will flow in the closed loop. The total skin potential will be

$$V_2 = \frac{R_3(E_1 + E_2)}{R_1 + R_2 + R_3} \tag{1}$$

The interactions might possibly be explained by appropriate changes in the three resistances. Changes in the skin resistance occur when the ionic compostion of bathing solutions is altered. It is not possible at present however, to identify which of the above resistances change.

From the model the potential measured across the outside permeability barrier will be

$$V_1 = \frac{(R_2 + R_3)\, E_1 - R_1 E_2}{R_1 + R_2 + R_3} \tag{2}$$

It is clear that V_1 is not a simple function of E_1 and $dV_1/dE_1 \neq 1$. The potential change measured across the outer permeability barrier is in part a function of E_2 and all the resistances. Many consequences follow; for example, the micropuncture measurement of the potential across the outside border of the frog skin with changing Na^o may not measure an effect of E_{Na} alone.

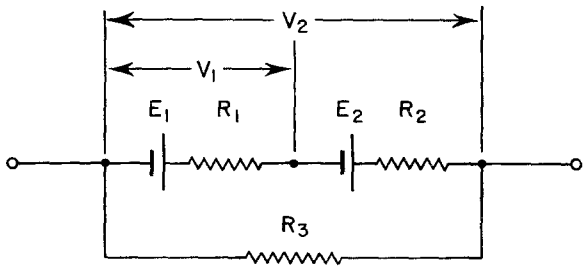

FIGURE 6. Simple equivalent circuit for frog skin. The anatomic correspondences are:
V_1 = potential change across outer Na-selective permeability barrier.
V_2 = total transepithelial potential.
E_1R_1 = outer Na-selective permeability barrier.
E_2R_2 = inner K-selective permeability barrier.
R_3 = extracellular shunt.

A possible explanation for the changes in the sensitivity of the potential to Na^o when the total ionic composition of the bathing solution is increased may lie in the increased shunting. Ussing reported increased Na and SO_4 shunting when the outside solution is made hypertonic with urea. Previous experiments (21) demonstrated decreased resistance when the outside solutions are made hypertonic. This would cause partial short-circuiting of the potential and therefore decreased sensitivity.

The foregoing analysis raises the question of the meaning of the potential change, Δv. In the presence of a finite shunt Δv is a complicated function of both diffusion potentials and the various conductances. Thus, the meaning of Δv does closely depend upon the particular model chosen to represent the skin. However, it would appear from these experiments that it is possible to select conditions under which Δv does closely approximate a change in the appropriated diffusion potential. Under these conditions, the constant field equation can be fitted to the data with little difficulty and the relative permeability ratio, α, is meaningful in describing selectivity. It was seen that

high ionic strength, high K^i, and low Na^o all cause significant alteration in the behavior of the skin and complicate the interpretation of the skin potential. Based on these experiments, it seems desirable that the K concentration of the inside bathing solution be low and remain constant when testing the outside border. The Na concentration of the outside bathing solution should be high when testing the inside border. The ionic strength of the outside solution should be made as low as practical, but the inside solution should be isotonic or only slightly hypotonic. Note that the above stated conditions are quite similar to the physiological conditions existing *in situ*. Under these conditions the behavior of the isolated skin approaches that expected for two independent potential steps connected in series as proposed by Koefoed-Johnsen and Ussing (1).

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