On the Nature of the Resting Frog Skin Potential*

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I. SHORT CONTEMPORARY HISTORY

Investigations on the electrical properties of frog muscle, nerve, and skin belong to the oldest in the history of bioelectricity. Interest in the nature of the resting P.D. of frog skin was heightened by the discovery that there occurs in the epidermis of this tissue "active ion transport" (Huf, 1935; 1936; Ussing, 1949), suggesting a possible relationship between the electrical and the chemical events. There is, as yet, no completely satisfactory explanation of the frog skin potential. Many investigators assume that there are at least two electrogenic layers within the multilayer epidermis, and numerous speculations on the nature of the skin P.D. have been offered on the basis of the two (or more) layer concept (Steinbach, 1933; Greven, 1941; Fukuda, 1942; 1944; Meyer and Bernfeld, 1946; Koefoed-Johnsen and Ussing, 1956; 1958; and others. For earlier investigators, *see* Steinbach, l.c., and Greven, l.c.). A rather penetrating analysis of the electrochemical behavior of frog skin has been presented by Linderholm (1952; 1954). He came to the conclusion that a single-layer concept was adequate to explain the electrical and diffusion properties of skin. Fukuda's work is of particular significance. He showed that the electrogenic outer layer† requires the presence of Na^+, but not of K^+, whereas the inner layer depends on the presence of K^+ in the adjacent bath. Fukuda suggested that the nature of the skin P.D. is intimately related to the preferential Na^+ permeability of the outer layer, and the preferential K^+ permeability of the inner layer.

Greven (1941) and Linderholm (1952; 1953) have proposed physico-chemical models of skin which explain quite well the experimentally found relationship between change in NaCl concentration in the outside bath and skin P.D. Both investigators calculated and found a P.D. change approximately 35 mv for a tenfold concentration change, excluding measurements at relatively high ionic strength ($\mu = 0.1$). Greven and Linderholm have not studied the electrical response of the inside to changes in ionic concentrations. The model of the skin proposed by Koefoed-Johnsen and Ussing (1956; 1958) gives emphasis to the already mentioned preferential permeability of the outer and the inner layer for Na^+ and K^+, respectively. When anion penetration was experimentally circumvented (by replacing Cl^- by ½ SO_4^{2-}), these investigators found that the skin P.D. changed by nearly 59 mv when the outside Na^+ concentration, or the inside K^+ concentration, was changed by a factor of 10. Therefore, Koefoed-Johnsen and Ussing regarded the total skin P.D. as the sum of two Nernst diffusion potentials which are generated at the Na^+ permeable, and the K^+ permeable outer and inner layer, respectively. In other words, in their experiments, the outer layer behaved like a nearly perfect reversible Na^+ electrode, and the inner layer like a nearly perfect K^+ electrode. It is interesting to note that prior to this it was claimed that the inner layer behaved like a reversible H^+ electrode (Meyer and Bernfeld, 1946). Fleming (1957) has tried to confirm this without success. Subsequent work has only in part confirmed the observations of Koefoed-Johnsen and Ussing on sulfatet skins. Disagreement exists especially about the response of the outer layer to changes in Na^+ concentration. Lindley and Hoshiko (1964) and Cereijido and Curran (1965) have reported a P.D. change of approximately 35 mv for a tenfold concentration change. This agrees with our measurements given below.

The high degree of perfection of the technique of micro-puncture and micro-P.D. measurements has, of course, attracted numerous investigators to test the two-layer concept of the nature of the frog skin P.D., reference to which was

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† In this paper the expressions "outer layer" and "inner layer" are used rather loosely. Nothing can be said with certainty about their location. The assumption of their presence is made because certain observations make it likely that such layers (or barriers) exist in the epidermis.

made above. Ottoson et al. (1953) were the first to apply this method to frog skin. They were followed by Engbaeck and Hoshiko (1957). The latest report is by Chowdhury and Snell (1964) who may be consulted for additional references on this topic. So far, the results have not been in complete agreement with each other. When a slow, inward penetration of the epidermis is made with the microelectrode, one to four P.D. steps have been observed, but their exact location in the epidermis is not certain. The electrode becomes increasingly positive with advancement of the tip, relative to the outside bath if both sides of the skin are exposed to salt solutions. Chowdhury and Snell (1964) are the only investigators who have obtained a nearly continuous and smooth potential profile. They are inclined to interpret discrete P.D. steps as the result of some distortion of the cellular and tissue structure by the advancing microelectrode. A recent statement by a group of competent and experienced investigators (Leb et al., 1965) strikes a note of warning to use great caution in the interpretation of data: ". . . the application of microelectrode techniques to frog skin is beset with formidable technical difficulties from the standpoint of adequate control." In this paper, therefore, more confidence is placed in results which were obtained with the classical technique of P.D. measurement on intact skin using agar bridges and calomel half cells.

II. STATEMENT OF PROBLEMS. EXPERIMENTS

Upon closer inspection of each of the papers cited in section I, it becomes clear that the interpretation of the data rests upon a great number of explicit and implicit assumptions. This, of course, is in the accepted tradition of scientific writing, but it also explains why the nature of the skin P.D. still is in a state of considerable controversy. The review of the pertinent literature has led us to carry out the following experiments, some of which deal with the controversial quantitative aspects of the electrical responses of the skin to changes in Na^+ and K^+ concentration, and the effect that substitution of Na^+ by Mg^{2+} has on these responses. Measurements of Q_{O_2}, and of Na^+, K^+, and Cl^- content in skin were made to evaluate the extent of damage, if any, to the skins exposed for several hours to sulfate solutions of rather unphysiological composition. Studies were also undertaken on the electrical response of osmotically and metabolically damaged skins to find out whether the Na^+ response can be diminished or abolished, if only transiently, without affecting the K^+ response, or vice versa.

Methods

The experiments were performed during all seasons, except winter, on belly skin of large frogs (*R. pipiens*). The skin was mounted in a two chamber (each 18 ml) cell made of lucite. The skin area was 4.9 cm^2. Continuous mixing of the fluid (25 C) was achieved by using circulating pumps (20 ml per min). Skin P.D.'s were measured in the conventional way with calomel half cells, millivolt recorders (Varian Associates, Model G-10; Sargent, Model SR) and occasionally Keithley Electrometer, Model 600A. Careful attention was given to asymmetry and junction P.D.'s in the system. They were either absent or played only a minor role, and when used for corrections did not significantly alter the observations and conclusions drawn from the data. Measurements on skins were started about 1 hour after mounting of the skins while exposed to sulfate solutions, pH 8, containing Na = 100; K = 10; Tris (hydroxymethyl) amino methane = 10, μeq per ml. Keeping constant the composition of the solution at one side of the skin, the Na^+ and K^+ concentration of the solution at the other side was altered, lowering $[Na^+]$ and elevating $[K^+]$, but keeping $[Na^+] + [K^+]$ constant. Total osmolarity: 135 milliosmols per liter by the method of freezing point depression. $[Na^+] =$ 110 (no K) will be designated as Na_1; lower $[Na^+]$ will be designated as Na_2, and Na_2/Na_1 will be designated as r. $[Na^+]_0$ and $[Na^+]_1$ stand for sodium concentration in the solutions at the outside and at the inside of the skin, respectively. Solutions were changed at about 10 min intervals when fairly stable new P.D. levels were usually seen. The data on skins which gave less than 90% recovery in Na = 100, K = 10 were discarded. Usually the response of the outside was tested before testing the inside, but no differences in results were found due to the order of testing. *P.D.* will designate the potential difference across the whole skin (inside +). $\Delta V = (P.D.)_2 - (P.D.)_1$, *i.e.*, the difference in P.D.'s at Na_2 and Na_1. Oxygen uptake measurements (20 C) on fresh skin samples (120 mg) were carried out with the Warburg method. The belly skin was cut into several pieces which were randomly placed into Warburg flasks containing solutions of various compositions. Estimations of Na^+ and K^+ in skin were done as described earlier (Huf et al., 1955). For Cl^- estimations, the method of van Slyke and Sendroy (1923) was employed. A drop of picric acid was added to the standard solutions to simulate the yellow color of skin digests.

Electrical Response of the Outside (Outer Layer of the Epidermis; June 1963 through June 1964)

Studies on 19 skins (63 measurements) gave results (table 1, col. 3) which fitted the computer calculated regression equation:

$$P.D. = 35.7 \log [Na^+]_0 + 17.9 \quad (1)$$

TABLE 1

Dependence of frog skin P.D. on varying composition of salt solution at the epithelial side. Belly skin of Rana pipiens. $Na_1 = 110$; $K = 0$. Na_2 = lower Na^+ concentration, as given in column 1. Composition at the dermal side of the skin was kept constant: Na 110; K = 10. THAM 10; pH 8, 25 C. Common anion SO_4^{2-}.

1			2	3	4	5
Solution pH 8						
Na^+	K^+	THAM*	$r = Na_2/Na_1$	P.D. (inside +)	ΔV	$\alpha\dagger = P_K/P_{Na}$
μEq/ml				mv	mv	
110	0	10	1.000	92		0.410
75	35	10	0.682	84	−8	0.365
35	75	10	0.318	73	−19	0.280
10	100	10	0.091	53	−39	0.167
2	108	10	0.018	30	−62	0.077‡

* Tris (hydroxmethyl) amino methane.

† Calculated from $\alpha = (r^{0.59} - r)/(1 - r)$; see section IIIa.

‡ Comparable to the value given by Lindley and Hoshiko (1964); see introduction.

TABLE 2

Electrolyte composition of fresh skin and experimental skin (after use). Experimental skins were soaked for one hour in sulfate solutions containing, in μEq/ml, Na: 110; K 10. During the experiments the skins were exposed, in sequence, to sulfate solutions pH7 of decreasing Na concentration (110 → 0 μEq/ml), and increasing K concentration (10 → 60 μEq/ml). Once or twice in each experiment Mg SO_4 (50 μEq/ml) replaced Na or K. All solutions were isosmotic. Time of study, March and April 1963.

	Experiment No.	Testing inside (i) outside (o)	Duration of experiment (hrs)	μEq/gm dry wt. at end of experiment			% H_2O
				Na	K	Cl	
Fresh Skins (Huf et al., 1955)				254	164	215	74
Experimental Skins	2	i	3	321		7.2	79.8
	3	i	4		195	12.0	80.6
	4	i	22	471*		19.0*	83.1*
	5	i	6		196	21.8	82.2
	6	o, i	6		181	6.0	79.8
	7	i, o	4	324			80.3
	14	o, i	5	229	210		78.6
	15	o, i	6	366	210		81.0
Average				310	198	11.7	80.5

* Not included in the average value.

with confidence limits of about ± 4 mv. In 3 of the 19 cases, the salt solutions contained 2 mM per liter $CaSO_4$. The results were not different from those seen when Ca^{++}-free solutions were used. Another series on six skins (24 measurements) was performed with Mg^{++} containing solutions of the following composition (μosmols per ml): $Na^+:K^+:Mg^{++}$ = 75:10:25; 35:10:65; 10:10:90; 2:10:98. The regression line was:

$$P.D. = 23.3 \log [Na^+]_0 + 17.4 \quad (2)$$

Substituting Mg^{++} for Na^+ increased the ionic strength of the solutions by factors varying from 1.4 to 2.5. No significant difference in freezing point depression was found, however, between solutions with and without Mg^{++}. A Beckman sodium electrode 39278 in conjunction with a Beckman Model 76 Expanded Scale pH meter was used to check the Na^+ activities in the solutions. We consistently found that for a tenfold change in $[Na^+]$, the P.D. of the Na^+ electrode changed by 53 to 54 mv.

Electrical Response of the Inside (Inner Layer of the Epidermis; June through August 1963)

From experiments on nine skins (31 measurements) it was found that a tenfold change in $[K^+]_1$, changed the skin P.D. on the average by 57 mv. In five additional experiments (20 measurements) with solutions containing 10 K^+, and Na^+ and Mg^{++} in varying proportions, but totaling 110 milliosmols per liter (see under I), the skin P.D. was always 64 mv. In contrast to the response of the outside to Na^+, the response of the inside to K^+ was little if at all affected by Mg^{++}. There seems to be no objection, therefore, to the use of Mg^{++} as a substitute for Na^+ when testing the electrical properties of the inside of the skin.

TABLE 3

Oxygen consumption of frog skin in solutions of varying composition. All solutions were buffered with 5 mM/1 THAM. Each solution was tested on six to eight skins of different frogs. Q_{O_2} data are given as mean values ± one standard deviation of the mean.

Series	Time of Exp.	pH	Composition of Solutions						Milliosmoles/1 from ΔC°	Ionic Strength μ	Q_{O_2}
			Na	K	Mg	SO_4	$[Fe(CN)_6]$	Sucrose			
			Milliosmoles/liter								ml × mg dry wt.$^{-1}$ × hr^{-1}
H	August	6	100	10	0	55	0	0	135	0.165	0.54 ± 0.033
H	1963	7	100	10	0	55	0	0	135	0.165	0.53 ± 0.043
H		8	100	10	0	55	0	0	135	0.165	0.52 ± 0.040
1	April	8	100	10	0	55	0	0	135	0.165	0.55 ± 0.033
2	June	8	50	60	0	55	0	0	136	0.165	0.51 ± 0.039
3	1963	8	0	60	50	80	0	0	137	0.290	0.32 ± 0.028
4		8	0	60	0	30	0	55	135	0.090	0.34 ± 0.026
6	May	8	50	10	50	80	0	0	138	0.290	0.36 ± 0.047
7	1963	8	50	10	0	30	0	55	136	0.090	0.41 ± 0.019
9		8	0	110	0	55	0	0		0.165	0.28 ± 0.022
8	April	8	50	10	0	0	15	75	138	0.150	0.52 ± 0.037
5	1963	8	0	60	0	0	15	75	136	0.150	0.45 ± 0.036

Metabolic, Electrolyte Measurements on Skin (March and April 1963)

Seven skins which had been used in 3 to 6 hours experiments were analyzed for Na^+, K^+ and Cl^- (table 2). The average results were: Na^+, 310; K^+, 198; Cl^-, 11.7 μeq per gm dry wt, and H_2O, 80.5%. Except for the per cent water, which was about 9% above control values, (Huf et al., 1955) there was no significant alteration in the Na^+ and K^+ content of whole skin. It should be noted that about 5% of the Cl^- in fresh skin remained in skin which was kept for several hours in chloride-free sulfate solutions. Skins in $Na^+ + K^+$ sulfate solutions showed normal respiration rate 0.53 ± 0.04 ml O_2 per mg per hr (table 3). No significant decrease in Q_{O_2} was seen during a period of five hours. The lowest Q_{O_2} (0.28 ± 0.02) was seen in Na^+ free K_2SO_4 solution. This is in agreement with Zerahn's work (1956). Skins in solutions containing 50 Na, 10 K, and either 50 milliosmols per liter Mg^{++} or sucrose had the same Q_{O_2}: 0.36 ± 0.05 and 0.41 ± 0.02 for skins in Mg^{++} and sucrose solutions, respectively. In the foregoing, all errors are expressed as one standard error of the mean of eight measurements for each case. These observations suggest that skins in sulfate solutions do not suffer severe metabolic alterations which, in other media, are readily detectable by the method of whole skin analysis (Huf et al., 1955; 1957; Huf and Doss, 1959).

Electrical Response of Osmotically or Chemically Damaged Skin (March through May 1964)

Results obtained on 5 of 11 experiments with skins which were osmotically damaged by soaking for several hours in bicarbonated water (Winn et al., 1964) are shown in figure 1. The experimental conditions, other than those mentioned under *Methods,* are given in the legend. At the end of the experiments the epidermis could easily be separated from the corium. In some of these experiments the outside (normally negative relative to the inside) became slightly positive. This was the case when the K_2SO_4 concentration at the inside was high, giving rise, probably, to a K^+ diffusion potential. Similar results were obtained on 30 skins which were treated before and during the testing with 0.02 M diethyl malonate, or 0.02 M NaF, or 0.001 M quinone (Eubank et al., 1962). In all experiments both sides of the skin failed *concurrently* to respond in the manner typical for fresh skin.

III. DISCUSSION AND INTERPRETATIONS

Response of the Outer Layer

It has been found by Koefoed-Johnsen and Ussing (1956; 1958) that the epidermis of the brown frog (*R. temporaria*) behaves like an almost ideal Na^+ electrode over a wide range of concentrations (1 to 100 mM). Our experience and that of others (Lindley and Hoshiko, 1964; Cereijido and Curran, 1965) is that this is not the case

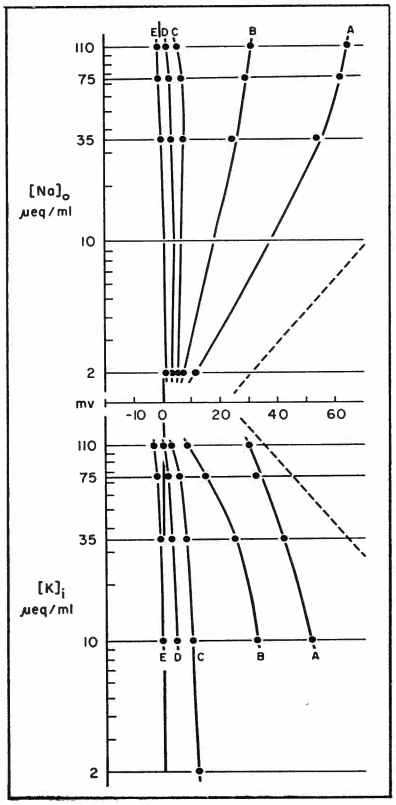

Fig. 1—Results obtained on five skins which were osmotically damaged by soaking for several hours in bicarbonated water. Semi-log plot of change in P.D. (abscissa) with changing outside $[Na^+]_o$ (keeping inside solution constant at 110 Na 10 K), and, following this, with changing inside $[K^+]_i$: (keeping outside solution constant at 110 Na 10K). Sum of $[Na^+] + [K^+]$ in the test solutions was kept constant 110. Soaking periods: *A* and *D*, 2 hours; *B* and *C*, 1½ hours; *E*, 4 hours.

for the skin of the leopard frog (*R. pipiens*) and the bullfrog (*R. catesbiana*). When placed in sulfate Ringer's solution, these skins gave a P.D. change of only about 36 mv (23 mv, if Mg^{2+} was present in the solutions) for a tenfold change in Na^+ concentration, instead of the expected P.D. change of 59 mv. The results of the experiments presented above suggest that this is typical for the normal fresh skin and is not related to a poor physiological condition of the skin membrane. Skins which deviated greatly from the ideal Na^+ electrode behavior (*see* above) performed quite well when the dermal side was tested for response to potassium, *i.e.*, a P.D. change of almost 59 mv for a tenfold change in K^+ concentration was obtained. In experimentally damaged skin, both sides failed *concurrently* to respond in the manner typical for fresh skin. This, it would seem, rules out the possibility that an increased "sulfate-shunt" (Ussing and Windhager, 1964) was the cause for the non-Nernstian behavior of the outer layer of fresh skin. One would expect that skins in poor physiological condition leading to increased anion permeability would fail in their Na^+ and K^+ responses, like the experimentally damaged skins. It should be mentioned here that, in the experiments with metabolic inhibitors, every stage and degree of damage was applied, reasoning that in mildly poisoned skins a transient isolation of the Na^+ from the K^+ response might occur. This, however, was never achieved. The skins exposed for many hours to solutions of rather unphysiological composition had a respiration rate and a Na^+ and K^+ content comparable to control skins. It must be granted that the method of whole skin analysis is not a very sensitive method to detect alterations in skin electrolytes. On the other hand, the same method permits a demonstration of gain in Na^+ and loss in K^+ in metabolically poisoned skins. It cannot completely be ruled out that the skin of *R. temporaria* behaves differently from the skin of the other species mentioned above. For instance, the skin of *R. temporaria* is thinner than the skin of *R. pipiens*. The same is true, however, for the skin of *R. pipiens* as compared to the skin of *R. catesbiana*, and yet, there is no difference in the Na^+ response in the skins of the last mentioned species. An entirely satisfactory explanation for the electrical behavior of the outer layer of frog skin when the outside Na^+ concentration is changed is not available at the present time. Any discussion of this topic should include the following thoughts: a) Significance of K^+ leakage from the epidermis; b) Greven's skin model; c) Linderholm's skin model; d) Koefoed-Johnsen and Ussing's skin model. The role of K^+ leakage is discussed first because this relates more immediately to the experiments described above.

a) *K^+ leakage from the epidermis.* It is well known that the epidermis shows leakiness to potassium (Steinbach, 1937; Huf et al., 1952; Huf and Wills, 1953; Bricker et al., 1963; Klahr and Bricker, 1964). A skin with K^+ leakage would be analogous to a glass Na^+ electrode with a "potassium error." It would explain qualitatively why a P.D. change of less than 59 mv per tenfold change in Na^+ concentration is to be expected. The following quantitative considerations will show that K^+ leakage seems to play some role, but it does not fully account for difference between the ideal 59 mv and the actual 36 mv P.D. change.

If one chooses the Goldman-Hodgkin-Katz equation for calculations of ratios of the permeability coefficients, $\alpha = P_K/P_{Na}$, one obtains from equation (3) below (Lindley and Hoshiko, 1964):

$$\Delta V = \frac{RT}{F} \ln \left[\frac{Na_2}{Na_1} (1 - \alpha) + \alpha \right]$$

$$= 59 \log [r(1 - \alpha) + \alpha] \quad (3)$$

Equating (3) with (1), applied to Na_1 and Na_2, one obtains

$$\alpha = \frac{r^{0.59} - r}{1 - r}$$

The limiting value of α for $r = 1$ can be obtained as:

$$\lim_{r \to 1} \frac{r^{0.59} - r}{1 - r} = \lim_{r \to 1} \frac{d(r^{0.59} - r)}{d(1 - r)}$$

$$= 1 - 0.59 = 0.41$$

The thin curves in figure 2 show the predicted relationships between ΔV and r, calculated from equation (3) by assuming several values for α. The heavy line is the regression line fitted to the experimental data (table 1, col. 4). The graph suggests that the linear semi-log relationship between $Na_2/Na_1 = r$, and ΔV may be the result of a decrease of α with decrease of r. Whereas this possibility cannot be excluded, it is, intuitively, a somewhat remote possibility. A better argument against this possibility comes from the following facts. From the studies of Cereijido et al. (1964) and those of Winn et al. (1964), a P_{Na} for the outer layer of the order of 8×10^{-6} cm per sec may be calculated, valid for $[Na^+]_o = 100$. P_{Na} increases rapidly with decreasing $[Na]_o$. From the data of Huf and Wills (1953) and those of Klahr and Bricker (1964), a rough estimation of P_K (corrected for skin P.D. where needed) shows that P_K is probably below 1×10^{-6} cm per sec. From these data, values for α may be calculated over a wide range of $[Na]_o$, all of which are far below the α values shown in table 1, col. 5. It is doubtful, therefore, that the high values for α shown in table 1 have any real meaning.

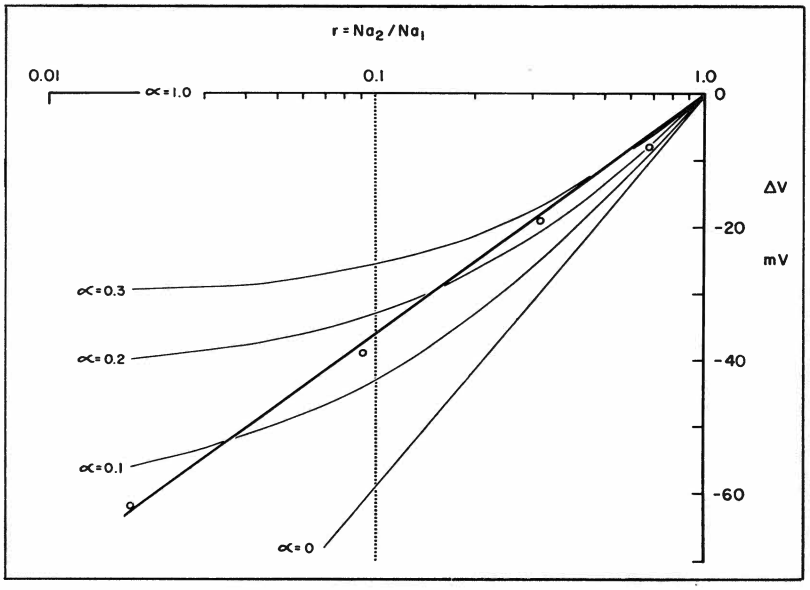

Fig. 2—Semi-long plot (heavy line) of the change of difference in skin P.D. (ΔV) with changing $r = Na_2/Na_1$ in the outside solution. Na_1 is the original Na^+ concentration = 110, K = 0; Na_2 is the Na^+ concentration of the subsequently used test solutions. The sum of $[Na^+] + [K^+]$ was kept constant at 110. Inside solution 110 Na, 10 K. $\alpha = P_K/P_{Na}$.

b) *Greven's skin model* (1941). Greven made the assumption that the outer layer of the frog skin contains "Festions," A, so that the "membrane" condition may be represented as $m^+A^+n^-$, where m^+ and n^- are the diffusible ions, *e.g.*, Na^+ and Cl^- in the skin. When a NaCl gradient is imposed upon the skin in the direction: outside → inside ($c_1 > c_2$), a P.D. (or E) is generated which can be expressed as the sum of a diffusion potential and two Donnan potentials (one on each side of the membrane). This P.D. can be calculated by applying the theory of Teorell-Meyer-Sievers. One obtains:

$$E = \frac{RT}{F}\left[u \ln \frac{x_2 + Au}{x_1 + Au} + \frac{1}{2} \ln \frac{(x_1 + A)(x_2 - A)}{(x_1 - A)(x_2 + A)}\right]$$

in which

$$u = \frac{U_K - U_A}{U_K + U_A};$$

$$x_1 = \sqrt{4C_1^2 + A^2};$$

$$x_2 = \sqrt{4C_2^2 + A^2}$$

To test the validity of this model, values for U_K and U_A were taken from physicochemical tables. c_2 was kept constant, 120 mM per liter NaCl; c_1 was varied from 120 to 0.47 mM per liter NaCl. Values for A were assumed, ranging from 60 to 0.001 meq per liter. All measured P.D. values were corrected for the asymmetry potential which existed when $c_1 = c_2 = 120$ mM NaCl per liter. It was found (*see* fig. 9 of Greven's paper) that the behavior of the model was in remarkably good agreement with the behavior of the skin. The model also predicted one maximum in the P.D./log c_1 curve. This maximum was also seen in experiments, if NaCl was used. It occurred in solutions of NaCl ≧ 30 mM per liter. Below this concentration, a tenfold change in c_1 gave a P.D. change of about 40 mv. It is known that maxima are not seen if sulfate Ringer's of comparable ionic strength solutions are used. An explanation for this has been given by Linderholm (1952; 1954; see below).

c) *Linderholm's skin model* (1952; 1954). Greven's assumption of fixed charges in frog skin has been criticized by Linderholm, who gives reasons which make it un-

likely that fixed charges are of significance (*see also* Linderholm, 1960). Considering the well-known specificity of the response of the outer layer to Na^+, Linderholm suggested that the form of the P.D./log c_1 curve may have something to do with the active transport of Na^+ ion involving a specific carrier. His skin model is described as follows: "The frog skin membrane is supposed to be inhomogeneous in so far as there are some parts of the membrane, where active transport does not take place but where both Na and other ions diffuse through the skin as passive ions, maybe through fine pores. . . . The other part of the membrane contains a sodium carrier, and here the active transport takes place. It may be thought of as a liquid membrane, essentially impermeable to other ions than those transported by the carrier." Applying principles of electrochemistry to this model, Linderholm could derive the following equation for the P.D. (or φ) of a "hypothetical frog skin" separating two NaCl solutions:

$$\varphi = \varphi_a^{Na} \frac{G_a^{Na}}{G_a^{Na} + G^{Cl}} - \frac{G_a^{Na} - G^{Cl}}{G_a^{Na} + G^{Cl}} \frac{RT}{F} \ln \frac{a_2}{a_1}$$

The meaning of the symbols is as follows: φ_a^{Na} = effective active transport potential; G's = partial ion conductances; a_1 and a_2 = activities of the NaCl solutions at the outside and the inside of the skin. The model expresses the P.D. as the algebraic sum of a fraction of φ_a^{Na}, and a diffusion potential. φ_a^{Na} is itself c_1 dependent; it decreases with increasing c_1, although not quite linearly with respect to log c_1. Linderholm has shown that the behavior of model and skin are in good agreement. The model also predicts a maximum in the φ/log c_1 curve (*see* previous section). Linderholm found that in skins with high total conductance the maximum was often at low c_1, and vice versa. The model has not yet been tested for the case $G^{Cl} \ll G_a^{Na}$, or $G^{SO_4} \ll G_a^{Na}$. Applying simple algebra, however, it can be seen that φ remains the algebraic sum of (c_1-dependent) φ_a^{Na} and a diffusion potential. This feature of the model makes it useful for a quantitative analysis of the electrical response of the outside of the skin to changes in the outside electrolyte concentration.

d) *The Koefoed-Johnsen and Ussing skin model* (1956; 1958) The attractiveness of this two-layer concept lies in the fact that it attempts to explain the skin P.D. in terms of two intra-epithelial Na^+ and K^+ diffusion potentials (*see also* Andersen and Zerahn, 1963; Hansen and Zerahn, 1964). The claim that skin in sulfate Ringer's solution gives a nearly 59 mv change for a tenfold change in Na^+ concentration of the outside bath has never been confirmed (see sections I and II). In other words, it still has to be shown that the φ/log c_1 relationship is quantitatively predictable from the model for skin in sulfate, or in chloride Ringer's.* All investigators seem to agree that the φ/log c_1 relationship for skin in sulfate Ringer's does not have a maximum in a solution approaching an ionic strength of $\mu = 0.1$. The reason for this may be found in the much higher total conductance of sulfated skins, as compared to skins in chloride Ringer's (Cereijido and Curran, 1965). This would shift a possible maximum to a higher c_1 (see Linderholm, 1952; 1954).

Response of the Inner Layer

When Linderholm (1952; 1954) proposed his version of the one-layer concept of the skin P.D., he did not consider the interesting work of Fukuda (1942). He showed that, upon removal of K^+ from chloride-Ringer solution at the inside of the skin, the total skin P.D. rose and, upon stepwise increase of the K^+ concentration, the P.D. stepwise decreased. This is perhaps better explained if one assumes the involvement of a second electrogenic layer in the generation of the total skin P.D. Koefoed-Johnsen and Ussing have extended Fukuda's work, using sulfate-Ringer's instead of chloride-Ringer's. They noticed that the inner layer behaved very nearly like a reversible K^+ electrode. A tenfold change in K^+ concentration at the inside of the skin gave a skin P.D. change of about 59 mv. This result was confirmed by Cereijido and Curran (1965); our own measurements reported in section II are also in agreement with those of Koefoed-Johnsen and Ussing. Any hypothesis on the nature of the electrical response of the inner layer must, of course, take into consideration the mechanism of active Na^+ transport, which may be located in this region. Studies on electrolyte distribution and active ion transport in frog skin under varying metabolic conditions (Huf et al., 1957) have suggested that a metabolically forced 1:1 $Na^+ \rightleftarrows K^+$ exchange may be an essential step in the mechanism of active sodium transport according to the following sequence of reactions:

$$\text{outside bath} \rightarrow Na^+ + RK_{m+1}Na_{n-1}$$
$$\rightarrow RK_mNa_n + K^+ \leftarrow \text{inside bath}$$
$$\text{leakage} \leftarrow K^+ + RK_mNa_n$$
$$\rightarrow RK_{m+1}Na_{n-1} + Na^+ \rightarrow$$

R is a "carrier," which may have a definite but as yet unknown chemical entity, or it is perhaps simply a special compartment where certain energy transformations take place which do not occur in ad-

* We have found that when the sodium concentration on the outside was lowered from Na_1 to Na_2, skins in sulfate-Ringer's almost followed the law $\Delta V = RT/F \ln \sqrt{Na_2/Na_1}$, and $\Delta V = RT/F \ln \sqrt[3]{Na_2/Na_1}$ in the presence of Mg^{2+} (equations (1) and (2) respectively), rather than the Nernst law $\Delta V = RT/F \ln(Na_2/Na_1)$.

jacent areas. The reasons for the assumption of attachment of several atoms of Na^+ and K^+ to R are given in the quoted paper. Operation of a carrier system involving the structure RK_mNa_n implies that no active transport takes place if either the K^+, or the Na^+ concentration, or both, are too low in the transport compartment. This is in agreement with the experimental data of Huf and Wills (1951), Ussing (1954), and Curran and Cereijido (1965). Figure 3*A* is a simplified version of the model shown in figure 4 of the paper of Huf et al. (1957). On the basis of these experimental results and assumptions, several speculative models describing the electrical response of the inner layer to changes in the K^+ concentration on the inside of the skin may be constructed.

a) Figure 3*B* shows the well-known model of Koefoed-Johnsen and Ussing (1958). It is assumed that the inner layer is the seat of the Na^+ pump, generating the force E_{Na} on Na^+ crossing this border. Because of the K^+ electrode behavior of the inner border and also because of the equivalence rule (short circuit current = net Na^+ flux, Ussing and Zerahn, 1951), Koefoed-Johnsen and Ussing have assumed that E_{Na} is kept electroneutral by means of a 1:1 $Na^+ \rightleftarrows K^+$ exchange across the inner border (cell membrane). For a sulfated skin in steady state, therefore, this model visualizes the existence of an electroneutral, K^+-coupled Na^+ pump and a Nernst-type K^+ diffusion potential across the inner border.

b) An alternative model, equally lacking unequivocal experimental support but preferred by us, is the following one.† Implicit in the

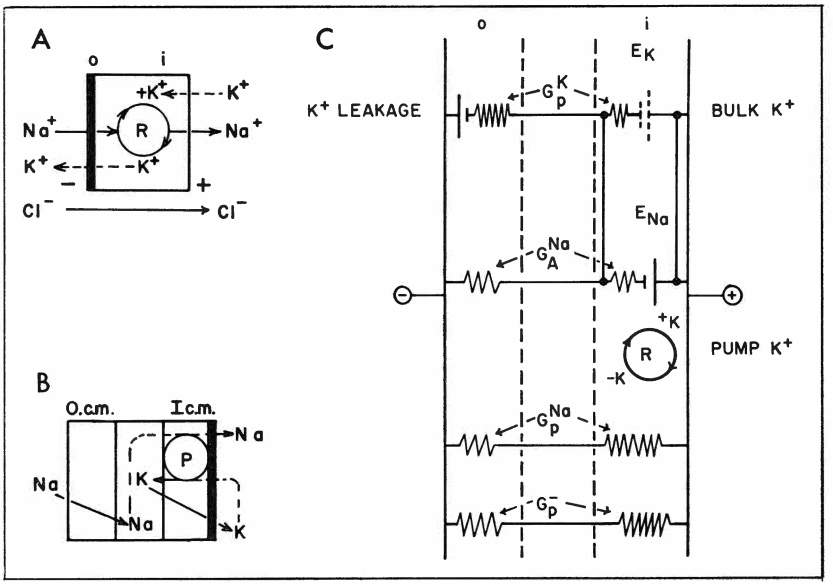

Fig. 3*A*—Active Na^+ transport model by Huf et al. (1957). R is a hypothetical polyvalent metabolically supported carrier forming a complex RK_mNa_n which can exchange one ion for the other when energy transfer occurs. 3*A* depicts an electrogenic Na^+ pump, since K^+ is assumed to recycle only within the transport compartment, and does not cross the "inner layer." See also the similar Klahr and Bricker model (1964). 3*B*) Frog skin model by Koefoed-Johnsen and Ussing (1958) depicting an electroneutral Na^+ pump. O.c.m. = outer cell membrane. I.c.m. = inner cell membrane. 3*C*) Hypothetical electrical equivalent circuit representing the open frog skin. The scheme is essentially a combination of the model proposed by Linderholm (1952; 1954), and the active Na transport model suggested by Huf (3*A*). The two-layer concept ("o" and "i"), rather than Linderholm's one-layer concept has been adopted. (Section I and III of this paper). Without the K^+ parameters, the scheme is identical with Linderholm's model (1954), with the main resistances (or conductances, G) located in both layers. The subscripts A and p indicate "active" and "passive" respectively. E_{Na} (Linderholm's ϕ_A^{Na}) is the true transport potential of the sodium pump. In accordance with the data of Huf et al. (1957), K^+ is treated as if it were present in two compartments: pump potassium, and bulk potassium. The K battery (E_K) is shown in dashed lines to indicate that for the skin in steady state (K^+ influx = K outflux) this battery is inoperative. A K^+ leakage system in the outer layer is also indicated.

† First presented on May 7, 1965 at the 43rd Meeting of the Virginia Academy of Sciences, Richmond, (*Va. J. Sci.* 16: 391, 1965). It is interesting to note that Cross et al. have presented similar arguments and supporting data on frog muscle (*J. Physiol.* 181: 865–880, 1965).

model discussed above seems to be the assumption that all cell potassium is functionally in one compartment. It has been shown, however, (Huf et al., 1957; 1959) that transcellular active Na^+ transport and cellular Na^+-K^+ balance are separable (*e.g.,* with fluoroacetate, or changing temperature), but not entirely separate mechanisms, suggesting the presence of K^+ in the cell in at least two functionally different compartments: *pump potassium* which need not cross the inner border, and *bulk potassium* which, if it crosses this border freely, may account for the K^+ electrode behavior of the inner border of the epidermis. For the normal skin in steady state, bulk K^+ may be kept in electrochemical balance by an *electrogenic* Na^+ *pump* in accordance with the Ussing-Teorell equation

$$\phi_{in}/\phi_{out}(K^+ \text{ flux ratio}) = \exp.\ (E - E_K)F/RT.$$

When the membrane potential

$$E = E_{Na} = E_K = 59 \log [K^+]_{cis}/[K^+]_{trans}$$

(bulk K^+ concentration across the inner border), the K^+ fluxes are equal. The P.D. drop seen at the inner border when $[K^+]_i$ is increased is a transient, not a steady state phenomenon, as the important experiments of Klahr and Bricker (1964) have shown. In their studies, using sulfate solutions, skins regained 40 to 120% of the original steady state P.D. within about 1 hour.‡ Huf et al. (1955) also have observed recovery and maintenance of the P.D. of skins in chloride Ringer's at elevated $[K^+]$. This was associated with elevation in K^+ accumulation in the non-chloride space. These observations are consistent with the interpretation of the electrical response of the inner layer to K^+ as follows: Steady state P.D.: $E_{Na} \pm 0$; P.D.: shortly after tenfold increase of $[K^+]_i$: E_{Na}-59, transiently, leading slowly to the original steady state P.D.: $E_{Na} \pm 0$ (ideal recovery). For steady state conditions, this model, therefore, visualizes the existence of an *electrogenic, K^+-coupled* Na^+ *pump* which operates with a fraction of the total cell K^+. In doing so, this mechanism effects transcellular active Na^+ transport and maintenance of cellular K balance without the appearance of a K^+ diffusion potential. Under non-steady state conditions a Nernst-type K^+ diffusion potential does appear, which, however, is transient in nature.

c) There is no proof that there exists any coupling, tight, ionic (a) or loose, electrical (b), between active transcellular Na^+ transport and cellular K^+ balance. Both processes may occur independently of each other. This view is supported by several facts, among them the observation (Huf et al., 1957; Curran and Cereijido, 1965) that certain drugs when applied in low concentration inhibit only Na^+ transport. When used in higher concentration, however, the skins loose K^+ and gain Na^+. This suggests active uptake of K^+ into the cells, independent of active transcellular Na^+ transport, to balance K^+ loss via a diffusion pathway. Steinbach (1937) over 30 years ago had already published data in favor of "potassium secretion" in the inside → outside direction. A model of the skin such as this would not be incompatible with the K^+ electrode behavior of the inner layer.

IV. SUMMARY

1. In this paper some of the highlights of research on the nature of the resting frog skin potential have been presented. Reviewing a period of about 30 years, it was the intention to show that several key problems have been recognized by a number of investigators who,

‡ The short circuit current also fell sharply and transiently when $[K^+]_i$ was raised.

through their experimental work, have tried to find unequivocal solutions to such problems as follows: a) The number and location of electrogenic layers (barriers) within the rather complex epidermis. b) The characterization of these barriers in terms of specific permeability properties. c) The electrical response of the two sides of the skin to changes in ionic concentrations in the solutions at the skin surfaces. d) The role of intra-epidermal active ion transport in the generation of the skin P.D. e) The correlation between active ion transport, skin P.D. and intra-epithelial (intracellular) electrolyte distribution.

Highly refined methods of study are now widely in use. This, of course, is unavoidable and necessary, to find conclusive answers to the problems mentioned. On the other hand, one must be on guard about possible pitfalls when applying such refined techniques. The study of the P.D. profile within the epidermis, using microelectrodes is beset with difficulties (*see* section I). Another example is the study of permeabilities of diffusion barriers within the epidermis by the method of applying radioisotopes to opposite surfaces of the skin. The analysis of data requires the consideration of such knotty problems as coupled flows and isotope interactions (Kedem and Essig, 1965). Although progress is made in these areas, it must be admitted that at this time no completely satisfactory explanation of the resting frog skin P.D. can be given.

A summary of viewpoints presented in this article is shown in Figure 3*C*. This tentative skin model is essentially a modification of the scheme suggested by Linderholm (1954). If the skin is in steady state, K^+ movement may not contribute to the skin P.D., except that the small outward K^+ leakage may be a factor. As has been pointed out in section III, the Linderholm skin model gives a reasonably good quantitative explanation for the electrical response of the outside of the skin to changes in the Na^+ concentration. The model shown in 3*C* raises the problem of the nature of the coupling between E_{Na} and E_K at the inside (inner layer) of the skin; it may vary from an electrical (in open skin) to an ionic (shorted skin) coupling if cellular K^+ is to be maintained in either case. The model describes the P.D. as a function of ion concentrations in the solutions at the two sides of the skin membrane. The model does not explain the skin P.D. in terms of intra-epidermal electrolyte gradients and the P.D. profile. The solution of this problem still lies in the future.

2. A reinvestigation was made on the electrical response of the outside and the inside of the skin to Na^+ and K^+ sulfate in the presence and absence of Mg^{++}. Fresh skins, metabolically poisoned skins, and osmotically damaged skins were used. Skin electrolytes and skin respiration were measured to evaluate possible tissue damage in skins kept for hours in sulfate solutions of rather unphysiological composition. The results are briefly summarized in section II.

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