THE RELATIONSHIP OF SODIUM UPTAKE, POTASSIUM REJECTION, AND SKIN POTENTIAL IN ISOLATED FROG SKIN*

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INTRODUCTION

Rather generally now it is recognized that the transport of strong electrolytes across cell barriers often requires the expenditure of metabolic energy in apparently highly specific "ion pump" mechanisms. Much of the experimental evidence for this has come and still comes from studies on the action of isolated surviving frog skin upon sodium chloride. In 1935 (5), it was found that frog skin actively takes up sodium chloride and water at the epithelial side and accumulates them at the chorion side. Studies on the influence of certain enzyme inhibitors and of certain substrates on salt accumulation and skin potential strongly suggested the existence of a correlation between the electrical and the chemical events (1, 2, 6, 8, 17–19). Through the work of several investigators (3, 4, 9, 12, 14, 20), it has now become almost certain, that the spontaneous skin potential is the result of a unique interaction of sodium ion and certain epithelial cells of frog skin. According to a widely accepted hypothesis, it is believed that the skin potential is derived from the formation and the breakdown in these cells of an uncharged sodium-carrier compound. The cell pole towards the epithelial side is believed to be the site of formation, the cell pole towards the chorion side the site of breakdown of the sodium-carrier compound. As a result of this, the epithelium attains a negative charge with relation to the chorion. Somewhere in this process of formation and breakdown of sodium-carrier compound, energy is required. As to other ions, notably chloride, potassium, and hydrogen ions, it appears that they move as free ions and are subjected, first of all, to strong electrostatic forces which have been set up as the result of sodium transport. As a consequence of all these processes, one finds net accumulation of sodium chloride at the chorion—and of potassium and hydrogen ions at the epithelial side of the skin.

Based upon results and views just mentioned, the present study is concerned with the question as to whether, in isolated skin, a positive or a negative correlation exists between net uptake of sodium chloride by the epithelium and

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skin potential. Theoretically both types of correlations are possible, depending upon whether the electric conductance in skin of sodium ion or of chloride ion is the dominating variable. This problem has already been given attention in recent publications (11, 13, 16) and will now be further examined. Besides this, studies were undertaken which would reveal the dependence of net potassium rejection from the epithelium upon skin potential. The skins include those of normal frogs and of frogs which were pretreated with various hormones; such as, an ACTH preparation, several kinds of steroid hormones, and posterior pituitary factors.

Methods

This work was carried out on isolated pieces of skins of male and female *Rana pipiens* during the months of December to March, inclusive, with the exception of a few experiments which were done in May and June; they will be specially indicated in the text.

Net Ion and Fluid Change at the Epithelial Side.—"Inside-out" skin bags were made from the skin of the hind legs. They were filled with and, for 8 hours at room temperature, immersed in 2 liters of the same kind of salt solution (0.4 Ringer's) of the following composition: NaCl = 48 μeq./ml.; KCl = 1 μeq./ml.; $NaHCO_3$ = 2 μeq./ml.; pH = 7.4–7.5. No Ca^{++} was added to the solutions, since it was found (10) that its omission does not impair active salt uptake by the skin. For further information concerning the use of skin bags in studies of the type described here, we refer to a previous publication (11), in which a detailed description of the technic has been given. Skin areas were measured with the aid of a planimeter. After the bags were emptied, they were refilled with the same volume of fluid originally put in, cut off at the fluid level, and slit along their sides, after removal of the lower tied off ends. After spreading the skins carefully on suitable paper, tracings were made and the areas were then measured.

The experiments were run in the following way: on any given day two experimental frogs (pretreated with hormones) and two control frogs were killed. Thus, as a rule, four experimental bags and four control bags (kept during the experiment in separate jars containing 2 liters of salt bath for several bags) were tested by parallel procedure. This was done in order to eliminate the possibility of recording differences in activity between experimental skins and control skins as a result of seasonal variations. After pooling the contents of two experimental bags obtained from the same frog and (separately from those) of two control bags obtained from the same frog, Na^+, K^+, and Cl^- estimations were carried out by flame photometry and by iodometry as described earlier (11).

For treatment of frogs with hormones,[1] the implantation procedure was chosen. 3 or 5 mg. of hormone was implanted in the dorsal lymph sac. While this was done, the frogs were under ether. Control frogs were either sham operated or, in addition to this, were implanted with 3 and 5 mg. of egg albumin and cholesterol respectively.

[1] The hormones were kindly supplied by the Armour Laboratories (ACTH), the Schering Corporation (DOC and DOCA), and the Merck Company (cortisone).

In a few cases the hormone was injected (see Table II). Experimental and control frogs were kept for 3 days after the implantations. They were weighed daily and sacrificed on the 4th day.

Skin Potential.—Within the 1st hour after the bags were made, filled, and hung up in the salt bath, potential measurements were begun. They were continued at about hourly intervals. Usually 6 to 7 measurements were taken during the 8 hour experimental period. All bags were measured individually and average potential values were calculated from the 6 to 7 measurements. Then the values for each set of bags obtained from the same frog were averaged. These values will be found in the following text, tables, and graphs. It was noticed, without any exception, that the skin potentials of two bags from the same frog ran a very similar course. Changes, if any, in the P.D. with time, developed slowly. Potentials were measured with the

TABLE I

Protocols of 4 Experiments

Frog No.	Average P.D.	Volume (2 bags)		Chloride		Sodium		Potassium		pH		Skin area (2 bags)
		V_o^s	V_i^s	Cl_o^s	Cl_o^s	Na_o^s	Na_o^s	K_o^s	K_o^s	pH_i^s	pH_o^s	
	mv.	*ml.*	*ml.*	*μeq./ml.*	*μeq./ml.*	*μeq./ml.*	*μeq./ml.*	*μeq./ml.*	*μeq./ml.*			*cm.²*
Controls (frog 344, implanted with 3 mg. of egg albumin; frog 356, sham operated)												
344	82	9.82	8.72	46.8	37.8	47.0	33.7	1.00	5.70	7.8	7.2	42.4
356	25	7.86	7.03	47.9	22.6	48.7	21.5	0.95	1.06	7.7	7.2	35.6
ACTH experiments (frogs implanted with 3 mg. of a purified ACTH preparation)												
337	82	9.82	9.17	46.8	42.0	46.5	37.8	1.06	3.80	7.6	7.4	39.9
343	30	9.82	9.14	46.8	31.0	47.0	27.2	1.00	1.20	7.8	7.1	41.2

The lower indices, *o* and *i*, refer to the anatomical "outside" and "inside" of the skin, respectively. The upper indices indicate the experimental time in hours to which the given values were assigned. $pH_o^0 = pH_i^0 = 7.5$, except in 337 when it was 7.4.

aid of a bridge and a moving coil galvanometer as zero-instrument. Accuracy greater than 1 mv. was not attempted. Special care was given to the leads with which the P.D. was led off from the skin to saturated calomel electrodes. One lead (for details see below) was put into the salt bath in which four bags (experimental or controls), with their chorion sides exposed, were immersed. The other lead was put into the bag and dipped well under the surface of the fluid in it which was in contact with the epithelium. In the course of P.D. measurements, this lead was moved from one bag into another. The same set of leads was used for experimental and control bags. When no readings were taken, both leads dipped into the same 2 liter salt bath. For the most part, under this condition, no P.D. was observed. Eventually a small correction had to be applied to the skin potentials. It was found that diffusion potentials between the two symmetrical leads, resulting from the establishment of concentration differences across the skin in the course of the experiment, were small and did not call for the application of corrections on the skin potentials. The leads were made up of small

TABLE II

Height of Skin Potentials and Net Ch nges of Fluid and Ions in the Bag. Negative Values Indicate Uptake, Positive Values Indicate Rejection of Water and Ions

Series	No. of frogs	P.D.		ΔV		ΔH_2O		ΔCl^-		ΔNa^+		ΔK^+	
		Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
		mv.		*per cent*		$\mu M \times hr.^{-1} \times cm.^{-2}$		$\mu eq. \times hr.^{-1} \times cm.^{-2}$		$\mu eq. \times hr.^{-1} \times cm.^{-2}$		$\mu eq. \times 10^{-2} \times hr.^{-1} \times cm.^{-2}$	
1. Controls	19	25 to 107	56	−4.2 to −11.8	−9.0	−63 to −195	−141	−0.15 to −0.86	−0.61	−0.31 to −0.98	−0.67	−0.8 to +11.8	+3.4
2. ACTH*	19	30 to 82	50	−2.5 to −10.6	−5.5	−37 to −167	−87	−0.24 to −0.53	−0.39	−0.29 to −0.65	−0.48	+0.4 to +7.8	+4.2
3. Controls	8	35 to 72	51	−3.8 to −11.7	−8.1	−61 to −192	−129	−0.41 to −0.85	−0.57	−0.43 to −0.85	−0.61	+1.2 to +12.6	+4.9
4. Cortisone*	8	33 to 74	45	−6.7 to −14.1	−9.5	−95 to −230	−151	−0.61 to −1.09	−0.77	−0.58 to −1.23	−0.83	+0.5 to +10.7	+2.8
5. Controls	9	33 to 69	47	−6.9 to −19.2	−11.7	−110 to −307	−184	−0.46 to −1.02	−0.68	−0.51 to −1.16	−0.79	+0.1 to +6.4	+2.5
6. DOCA (DOC)*	9	35 to 82	57	−2.5 to −15.0	−10.2	−44 to −213	−161	−0.46 to −0.88	−0.66	−0.64 to −1.02	−0.81	+2.0 to +14.1	+5.8
7. Controls	8	20 to 53	39	−4.2 to −12.0	−8.4	−63 to −186	−133	−0.47 to −0.91	−0.65	−0.52 to −0.86	−0.69	0 to +20.0	+4.0
8. P.P. principle‡	8	31 to 56	45	−6.8 to −15.9	−9.5	−95 to −240	−147	−0.55 to −1.01	−0.78	−0.68 to −1.06	−0.83	+2.8 to +9.6	+5.3

* For mode of application see under Methods. Dosages: ACTH, 3 mg; steroid hormones, 5 mg. 2 frogs of series 6 were treated with DOC, the rest received DOCA. There was no striking difference in action of the two forms of the hormone.

‡ Posterior pituitary preparations were injected into the dorsal lymph sac. Dosages: (*a*) 3 × 1 P.U. pitressin (Parke Davis & Co.) for 3 days; or (*b*) 2 × 0.3 ml. pituitrin (Squibb & Sons) for 3 days; or (*c*) 1 × 0.3 ml. pituitrin 4 hours before killing of the frogs. Control frogs were injected with preservative, chloretone and phenol respectively. No obvious differences in the activities of skins treated according to *a*, *b*, or *c* were noted. When method *c* was used the treated frogs showed significant increases in body weight, on an average of 10 per cent, whereas the controls remained practically constant in weight.

cylindrical funnels with 12 cm. long extensions put on the stem which consisted of plastic tubing of 3 mm. inside diameter. The lower half of the funnels and the tubings were filled with 0.4 Ringer's in agar-agar. The funnels were then partially filled with saturated KCl solution. From there bridges of saturated KCl in agar-agar led to the calomel electrodes. New leads were made each day that an experiment was carried out.

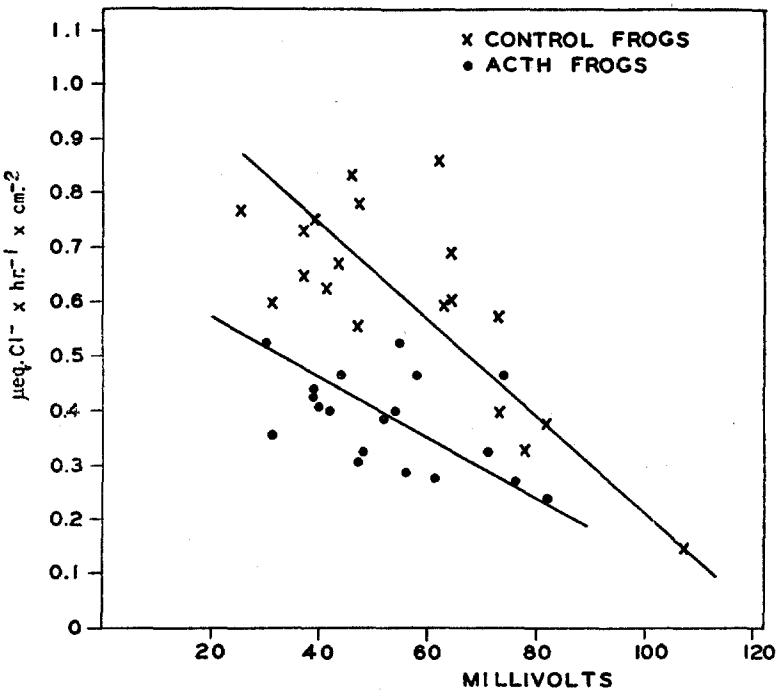

FIG. 1. Dependence of Cl^- uptake on skin potential. Skins of normal frogs and frogs pretreated with a purified ACTH preparation.

RESULTS

All analytical data obtained were recorded in protocols such as those given in Table I. There are shown, for two controls and two ACTH experiments, the results obtained from skins with a high and a low average spontaneous voltage. The lowest average spontaneous voltage ever recorded in the present study was 20 mv., the highest 107 mv. It can be seen from the table, that NaCl concentration and volume of the solution in the bag were lowered at the end of the 8 hour experimental period. K^+ and H^+ concentration, however, were increased in the fluid of the bag. Lowering in Na^+ concentration exceeded, somewhat, the lowering of Cl^- concentration. From the examples given, it seems that the lowering of the NaCl concentration in the bag is smaller in "high voltage" than in "low

voltage" skin. The opposite seems to be the case with regard to K^+ accumulation at the epithelial side. Furthermore, skins of normal (control) frogs seem to be more active than skins of frogs that were pretreated with purified ACTH preparation.

The data listed in Table I, and similar data of other controls and experiments not shown here, permitted the calculation of net uptake or rejection of ions

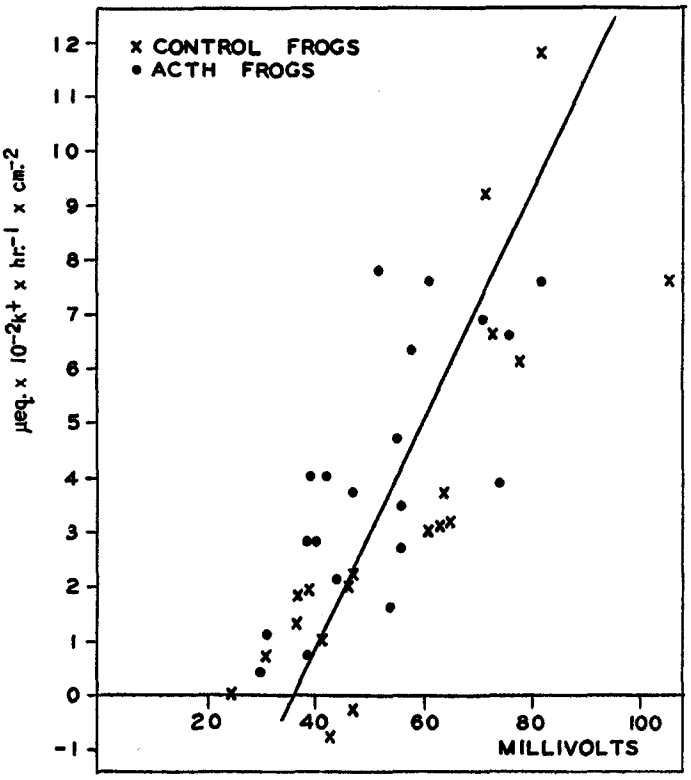

FIG. 2. Dependence of K^+ rejection on skin potential. Skins of normal frogs and frogs pretreated with a purified ACTH preparation.

in microequivalents per hour and square centimeters, under the assumption that the rate of ion uptake and ion rejection did not change appreciably over an 8 hour period. This is suggested; *e.g.*, by the maintenance for many hours of a fairly steady and eventually high skin potential. Designating net uptake (or rejection) by Δ, one has; *e.g.*, for Cl: $\Delta Cl = (V^0 \times Cl_o^0 - V^8 \times Cl_o^8)/(8 \times cm.^2)$. These calculations were carried out for all controls and experiments. The figures thus obtained are entered in Table II. Positive figures indicate absorption by, negative figures rejection from the epithelium. Values for ΔV and ΔH_2O are also listed. ΔH_2O in micromoles per hour and square

centimeters was calculated, assuming that the total net volume change (ΔV) was due to absorption by the epithelium of water only and that volume changes due to absorption or rejection of ions can be neglected. Table II is presented mainly for two reasons: (1) to give, in the same units, the order of magnitude, for simultaneously obtained values, for uptake of H_2O and NaCl and for rejection of K^+; (2) to call attention to the difference in some of the average

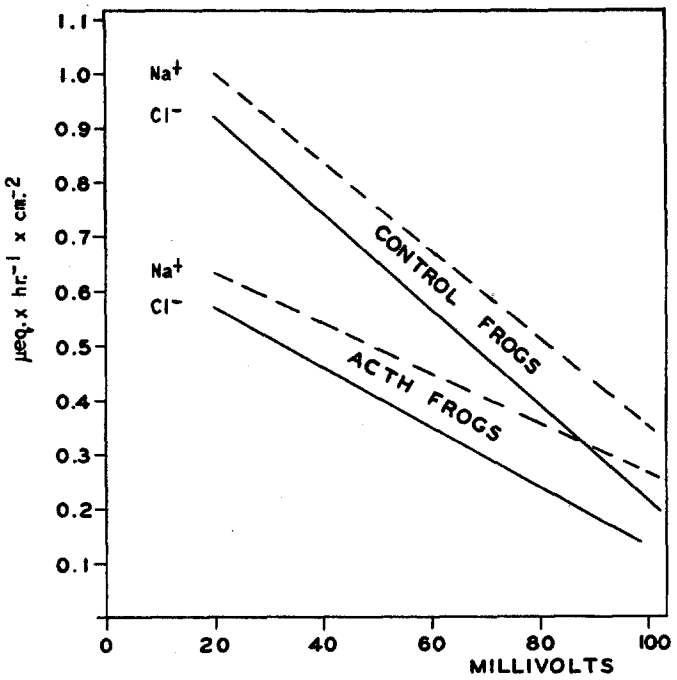

FIG. 3. Dependence of Na^+ and Cl^- uptake on skin potential. Skins of normal frogs and frogs pretreated with a purified ACTH preparation. The four linear functions were obtained independently of each other from separate graphs. The two solid lines are identical with those shown in Fig. 1, which also illustrates the scattering of individual data.

values, if one compares the results of the ACTH experiments with their respective controls and also the other mentioned controls, and experiments using other hormones, which were carried out at different times during the winter season. Obviously, skins of frogs that were pretreated with a purified ACTH preparation had the lowest activity with regard to H_2O and NaCl absorption. The significance of the difference in activity towards NaCl between ACTH-treated skins and their respective controls is obvious from Fig. 1, in which all the individual analytical data of ΔCl^- are plotted against the average voltage of the respective pieces of skin involved. Fig. 2 shows the relationship between

ΔK^+ and P.D. The straight lines in Figs. 1 and 2 drawn through the scattered experimental points are arbitrary. They seem to express, however, at a first approximation, the correlations between Cl^- uptake and skin potential and K^+ rejection and skin potential, respectively. A detailed graph, showing the relationship between individual values of ΔNa^+ and P.D.'s, is not given for the reason that it is very nearly the same as that shown in Fig. 1. All ΔNa^+ values, however, were higher than their respective ΔCl values. The broken lines in Fig. 3, referring to Na^+ uptake were obtained from separate graphs and independently of the solid lines expressing (approximately) the ΔCl^--P.D. relationships. In each series control and experimental, the ΔNa^+-P.D. and the ΔCl^--P.D. functions have very nearly the same slope.

Graphs similar to those described, were made for the results obtained from series 3 to 8 of Table II. They are not shown here, since they only confirm what has been stated above. The graphs did not suggest any significant differences between the experiments of series to 3 to 8 and their respective controls.

DISCUSSION

In this paper, the emphasis is laid on the relationship that exists in normal skin among uptake of NaCl, rejection of K^+, and skin potential. The results of experiments with various hormones suggest, rather than conclusively demonstrate, for ACTH that it may alter some of the normal relations referred to above.

From the results obtained in this study, it may be stated that in isolated surviving frog skin a negative correlation exists between spontaneous skin potential and active uptake of NaCl by the epithelium. A high potential is associated with a low active NaCl uptake and *vice versa*. On the other hand, a positive correlation exists between K^+ rejection from the epithelium and skin potential; *i.e.*, high potential is associated with high K^+ rejection and *vice versa*. These observations agree well with earlier results from this laboratory (11), which showed that skins in NaI-Ringer's have a considerably higher potential than control skins in NaI-free Ringer's. The NaI-treated skins had also a diminished active NaCl uptake and a considerably increased K^+ rejection as compared to untreated control skins. Ussing and coworkers have also found the existence of a negative correlation between skin potential and uptake of NaCl (13, 20).

In the following, some of the pertinent problems of the present study will be further discussed.

1. Active Uptake of Sodium Chloride.—Linderholm (16) finds that the spontaneous skin potential, φ, and net active salt uptake, Φ^{salt}, can be expressed by:

$$\varphi = \frac{G_a^+ \cdot \varphi_a^+ - (G_a^+ - G^-)(RT/F) \ln (a_2/a_1)}{G_a^+ + G^-} \tag{1}$$

$$\Phi^{salt} = \frac{G_a^+ \cdot G^-[(\varphi_a^+ - 2(RT/F) \ln (a_2/a_1)]}{F(G_a^+ + G^-)} \tag{2}$$

The designation given to the symbols in these equations is as follows: G_a^+ and G^-, electric conductance in skin of the actively and passively transported ions, chiefly Na^+ and Cl^-, respectively; a_1 and a_2, the total chemical activities of the electrolytes in the solutions at the two sides of the skin; φ_a^+, the active transport potential; R, T, and F, gas constant, absolute temperature, and Faraday's number, respectively.

Although one may criticize the unrestricted application of Linderholm's equations (1, and 2) because of the great complexity of frog skin, we have found them useful in interpreting our findings. The oversimplification in the following calculations, however, is well realized.

If one considers the case of $a_1 = a_2$, which applies, approximately, for the present studies, (1) and (2) become:

$$\varphi = \frac{G_a^+ \cdot \varphi_a^+}{G_a^+ + G^-} \qquad (3) \qquad\qquad \Phi^{salt} = \frac{G_a^+ \cdot G^- \cdot \varphi_a^+}{F(G_a^+ + G^-)} \qquad (4)$$

It can be seen that φ and Φ^{salt} are functions of three possible variables. Considering for a moment φ_a^+ and G_a^+ as constant and G^- as the only variable, one finds:

$$\frac{\partial \varphi}{\partial G^-} = -\frac{G_a^+ \cdot \varphi_a^+}{(G_a^+ + G^-)^2} \qquad (5); \qquad\qquad \frac{\partial \Phi^{salt}}{\partial G^-} = \frac{(G_a^+)^2 \cdot \varphi_a^+}{F(G_a^+ + G^-)^2} \qquad (6)$$

From (5) and (6) it follows also that:

$$\frac{\partial \Phi^{salt}}{\partial \varphi} = -\frac{G_a^+}{F} \qquad (7)$$

and

$$\Phi^{salt} = \frac{G_a^+}{F}(\varphi_a^+ - \varphi) \qquad (8)$$

According to Linderholm, φ_a^+ is approximately 0.075 volt. He gives for G_a^+ an average value of $0.55 \times 10^{-3}\ \Omega^{-1} \times cm^{-2}$. By using these data one finds for:

$$\frac{\Phi^{salt}}{\mu M \times hr.^{-1} \times cm.^{-2}} = 20.5(0.075 - \varphi_{volt}) \qquad (9)$$

Equation (7) expresses what has been found experimentally in this study, namely the negative correlation that exists between active salt uptake and spontaneous skin potential. Equation (9) has been plotted in Fig. 4 from which it can be seen that the theoretical function is similar to the one that was found experimentally. Scattering of experimental data and the dependence in the calculations on average values found in the literature for G_a^+ and φ_a^+ may be the chief obstacles in obtaining at this time, a better agreement between theory and experiments. The similarity of the two functions, nevertheless, seems to support the interpretation that although Na^+ is the leading partner in active NaCl uptake by isolated frog skin (19, 20), it is the electric conductance of the

passively moving Cl^- which essentially determines the rate of net salt uptake. This may be suggestive of one's recognizing two main features of the salt pump in isolated frog skin: the existence of a sodium engine, working at a rather fixed energetic level, and a variable chloride brake.

By differentiating in equations (3) and (4), φ and Φ^{salt} with respect to G_a^+, (considering other possible variables as constant) it will be seen that a positive correlation should exist between active NaCl uptake and skin potential. It is

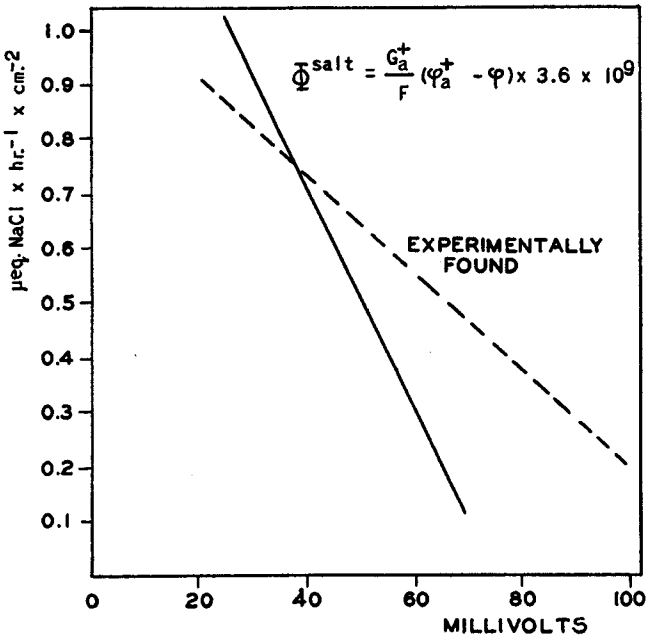

FIG. 4. Dependence of salt (NaCl) uptake on skin potential. Comparison between theoretical and experimental functions. The broken line is identical with the upper solid line of Fig. 1.

possible that this may be found for skin kept under conditions which are different from those applied here. Other tissues which are able actively to take up NaCl, *e.g.* the tubules of the kidney, may also show the positive rather than the negative type of correlation. A few facts point in this direction. We refer to Wilbrandt's studies (22) in which it was shown that in *Necturus* the lumen of the proximal tubule is negatively charged with relation to the vascular end of the epithelial cells. Furthermore, in higher animals, the typical alterations in urinary excretion of Na^+ and K^+, as a result of adrenalectomy, could be well interpreted on the basis that a positive correlation exists between active NaCl uptake and tubular potential.

Fig. 3 shows that net uptake of Na^+ exceeds net uptake of Cl^-. This has been

a consistent observation (8–11). Na^+ uptake is a more complex phenomenon than Cl^- uptake. Some Na^+ is actively pumped into and across the skin (9, 19, 20); the rest of the total Na^+ taken up most likely comes from an exchange reaction in which Na^+ at the epithelial side is exchanged for H^+ produced in metabolism at the chorion side. Acidification of the solution at the epithelial side is an almost consistent observation in studies of active salt uptake by frog skin (8–11; see also Table I). Since the results of the present study (Fig. 3) suggest that one consider the $Na^+ \rightleftarrows H^+$ exchange, in contrast to active Na^+ uptake, as being less dependent upon or even independent of skin potential, it would follow that more Na^+ is actively taken up than exchanged in "low voltage" skins, whereas the opposite seems to hold true for "high voltage" skins. From this discussion it seems to emerge also, that the values of ΔCl^-, as used here in text and illustrations, express more correctly than the values of ΔNa^+ the net amount of salt (NaCl) that is pumped into the skin. This has been applied in Fig. 4 in which the values of $\Delta NaCl$, plotted on the ordinate, are actually those of ΔCl^-, as found experimentally and given in Fig. 1.

2. K^+ Rejection.—According to Linderholm (16) the net transport of an inactive ion can be obtained from:

$$\Phi^i = \frac{G^i}{F}\left(\frac{RT}{F} \ln \xi^i\right) \tag{10}$$

G^i is the electric conductance and z^i is the electrovalency of the inactive ion i. ξ^{z^i} the contribution of the electric potential to the total activity of this ion, is given by:

$$\xi^{z^i} = e^{\varphi \cdot z^i \cdot F/RT} \tag{11}$$

From (10) and (11), as applied to K^+, it follows:

$$\Phi^K = \frac{G^K}{F} \cdot \varphi \tag{12}$$

G^K in frog skin is not known. Following Linderholm's suggestions (16, page 88), however, we were led to assume for it a value of $0.009\ \Omega^{-1} \times cm^{-2}$. With this it was then possible to write for the rejection of K^+ from the epithelium:

$$\underset{\mu eq. \times hr.^{-1} \times cm.^{-2}}{\Phi^K} = 0.336 \cdot \varphi_{volt} \tag{13}$$

Empirically one finds that the linear function in Fig. 2 can be expressed by

$$\underset{\mu eq. \times hr.^{-1} \times cm.^{-2}}{\Phi^K} = 2.0 \cdot \varphi_{volt} - 0.07 \tag{14}$$

Equation (13) infers that on theoretical grounds, a positive and linear function ought to exist between net K^+ rejection and skin potential. This has been found experimentally. There are, however, differences between theory and experiment: (1) The slope of the experimentally established function (14) is about 6

times greater than the slope of the theoretical function (13). This may not be surprising, in view of the great uncertainty that is attached to the value of G^K as given above. (2) More significance must be attributed to the fact that the experimental function—in contrast to the theoretical function—does not run through the point of origin of a system of coordinates; rather, it cuts through the voltage axis somewhere between 30 and 40 mv. (Fig. 2). This latter fact strongly suggests that one consider also the possibility of active uptake of potassium. This would become analytically more obvious, if the skin potential were so low that the force of a hypothetical potassium pump, pumping K^+ inwards, would not become overshadowed by the stronger electrostatic force, rejecting K^+ towards the outside of the skin. It may well be that Na^+ and K^+ are carried from the epithelium towards the chorion by the same carrier. It seems unlikely that any one carrier acts entirely specifically upon one kind of ions only, although in frog skin the carrier appears highly specific for Na^+ ions. From the results shown in Fig. 2, it would seem that at a voltage of 30 to 40 mv., K^+ is neither rejected by nor taken up by the epithelium. Using the tracer technic, Levi and Ussing (15) have found that at about 50 mv., influx and outflux of tracer potassium are equal. These authors also suggested the possibility of active K^+ uptake by the epithelium of skin. Later, however, Ussing (21) has viewed his earlier interpretation rather critically.

3. Ratio of Na^+ Taken Up to K^+ Rejected.—In an earlier paper (10) it was stated that for each 100 μeq. Na^+ which is taken up by the epithelium, 4 to 1 or even less μeq. K^+ are given off from this layer. It is now possible to show that the ratio of Na^+ taken up to K^+ rejected is a function of the skin potential. Fig. 5 combines the results of Figs. 1 and 2. It will be seen that the ratio of sodium taken up to potassium rejected is 84 when the skin potential is 40 mv.; it is only 5 to 6, however, for a skin of 80 mv. Ratios for other voltages may be read from the graph. All numerical values of these ratios are approximate.

4. Fluid Shifts.—All net fluid shifts were small, about 10 per cent or less of the original volume; they occurred in the direction of active NaCl uptake; *i.e.*, from the epithelium towards the chorion. Values for these shifts are given in Table II. No correlation between net fluid transport and P.D. could be found. It is likely that net fluid transport is a complex phenomenon. Water may be moved osmotically and also as water of hydration of the moving ions. The question must be raised as to whether chloride is not more important as a carrier of water than sodium, since part of the chloride may permeate inwards through the extracellular spaces. Sodium, if it reacts with a carrier within the cell, may be partly deprived of its water-shell. Active transport of water remains to be proved or disproved. The functional significance, in water transport, of "pores" *versus* colloidal phases in frog skin, has been repeatedly discussed (7, 21). Nevertheless, the mode of water transport across frog skin which is associated with active salt uptake is still obscure.

5. Influence of Hormones.—The data presented in Fig. 1 show that there were just about as many "low" and "high" voltage skins in the ACTH as in the control series. In other words, the pretreatment of frogs with the ACTH preparation did not result in a change of the spontaneous skin potential. This explains why no difference in K^+ rejection in the two series was observed (Fig. 2). K^+, as has been pointed out under (2), is primarily treated by the skin as a passive ion that moves down-hill in the electric field. It is obvious, however,

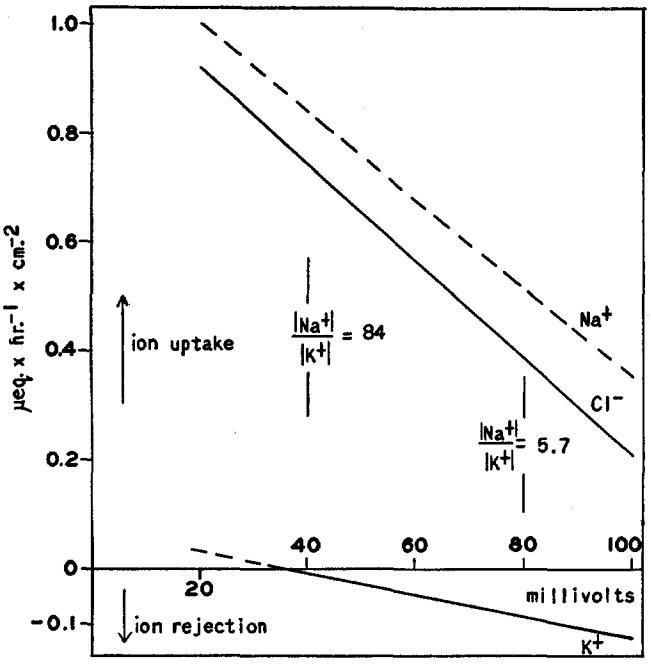

FIG. 5. For Na^+, Cl^-, and K^+, the dependence of ion uptake and ion rejection upon skin potential. Ratios of Na^+ taken up to K^+ rejected. Scattering of individual data may be visualized from Figs. 1 and 2.

from Fig. 1, that for a given skin voltage, less NaCl has been taken up by the treated than by the untreated skins. The linear function, expressing the dependence of NaCl uptake on skin potential, has a considerably smaller slope for the treated than for the untreated skins. There are two possible explanations for this: (1) According to equation (7) the slope of the function under consideration depends essentially on the electric conductance, G_a^+, of the active ion, Na^+. The action of the hormone preparation on skin may, therefore, have resulted in a true lowering of G_a^+. (2) Another explanation would be to assume that the hormone preparation has opened the "pores" of the skin and thereby increased the permeability of the skin for the back diffusion of NaCl from the

chorion to the epithelial side. This would mean that the action of the hormone may have resulted in an apparent rather than in a true lowering of G_a^+.

Implantation of DOCA, DOC, or cortisone did not lead to significant alterations in isolated skin with regard to skin potential, active NaCl uptake, and K^+ rejection. This fact raises the question as to whether ACTH itself was the factor in the hormone preparation, that brought about the above described changes. Of course, ACTH may have acted directly upon the skin; or other hormones than those applied here were produced and acted in the frog's body. Results of experiments with various posterior pituitary factors (carried out in May and June) seem to exclude the possibility that their presence in the ACTH preparation accounted for its effect on net active uptake of NaCl. The hormone preparation used had an activity of 45 per cent of the international standard preparation of ACTH. It will be necessary, to repeat these studies with chemically pure ACTH.

SUMMARY

1. Isolated surviving frog skin, when bathed with the same kind of diluted Ringer's solution on both sides, shows a negative correlation between net active salt uptake by the epithelium and spontaneous skin potential. Average values of 0.15 to 0.86 μeq. $\times$ hr.$^{-1}$ $\times$ cm.$^{-2}$ were measured and correlated with average skin potentials ranging from 107 to 25 mv.

2. Sodium uptake exceeded chloride uptake by about the same amount, irrespective of the height of the skin potential.

3. The same skins which exhibited a negative correlation between net uptake of sodium chloride and skin potential showed a positive correlation between net potassium rejection from the epithelium and skin potential, for voltages above 30 to 40 mv. In skins of voltages lower than this, potassium ions were taken up rather than rejected. Average values for rejection of +11.8 to −0.8 centi-μeq. $\times$ hr.$^{-1}$ $\times$ cm.$^{-2}$ were measured.

4. Net fluid uptake, associated with active uptake of sodium chloride, was small and occurred in the direction of the salt uptake. No dependence of net fluid uptake upon skin potential was observed.

5. Skins of winter frogs, pretreated with a commercial purified ACTH preparation, were less active than their respective controls with regard to uptake of sodium chloride. Rejection of potassium was the same in treated and untreated skins. Posterior pituitary factors, as possible contaminants, did not account for the effect of the ACTH preparation.

6. DOCA, DOC, and cortisone did not alter the normal correlation referred to under (1) and (3).

7. In interpreting the experimental results on theoretical grounds, it is suggested (*a*) that in normal skin, it is the variation in the electric conductance in skin of chloride ions which essentially, although not exclusively, determines

the rate of net uptake of sodium chloride, (*b*) that a factor in the ACTH preparation used, possibly ACTH itself, may have lowered the electric conductance in skin of sodium ions either truly or apparently, (*c*) that potassium ions are treated by the skin primarily as passive ions. There is some indication that potassium ions are also actively taken up by the epithelium of skin.

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