# ELECTROLYTE DISTRIBUTION AND ACTIVE SALT UPTAKE IN FROG SKIN*

By ERNST G. HUF, JOYCE P. WILLS, AND MARY F. ARRIGHI

*(From the Department of Physiology, Medical College of Virginia, Richmond)*

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## I

## INTRODUCTION

Essential requirements for the maintenance of active uptake of sodium chloride in isolated frog skin in Ringer's solution are: sufficient oxygen supply (8); neutral or slightly alkaline reaction of Ringer's solution (10); a reasonable temperature, *e.g.* 25°C., of the environment (10). A few years ago it was found (11) that, in addition to the above mentioned factors, the $K^+$ level in Ringer's solution greatly determines the net rate of NaCl uptake. Frog skin in salt solutions from which $K^+$ was omitted showed a relatively slow average rate of NaCl uptake. McClure (25), in 1927, called attention to the fact that water transport across skin between isotonic Ringer's solutions is smaller in the absence than in the presence of potassium. The significance of potassium for the maintenance of skin potential was demonstrated by Fukuda (6). He found that removal of potassium ions from the salt solution at the inside of the skin caused disappearance of the "asymmetry potential", especially in skins of summer frogs. According to Rubin (28), frog skin, like most other tissues, contains a relatively large amount of potassium, the bulk of which, according to Rubin and Syrocke (29), can histochemically be located in the epithelial layers of the skin. Steinbach (32) was the first to study the dependence of potassium in frog skin on the potassium concentration of the bath in which pieces of skin had been immersed for several hours. These facts, when considered together, are quite suggestive that potassium may be essentially involved in the mechanism of uptake and transport of sodium chloride and water.

The experiments to be described in the following were undertaken with the aim of demonstrating, in some detail, the dependence of several properties of isolated skin on the ionic composition of the salt solution. Both the $K^+$ and the $Na^+$ concentrations of the salt solutions were varied over a relatively wide range from levels below to levels above so called physiological ion concentra-

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tions. Properties of the skin that were studied include its ability (1) to maintain $K^+$ and $Na^+$ equilibrium within skin, (2) to take up NaCl and $H_2O$ from the salt solution at the epithelium, (3) to accumulate $K^+$ in the salt solution at the epithelium, and (4) to maintain an E.M.F. across the skin.

## II

## *Methods*

(*a*) *Skin Bags.*—All studies were carried out on skins of the hind legs of *Rana pipiens*. Net rates for uptake of ions and water by the skin were obtained using skin bags of the "inside-out" type. In these bags the corium (inside of the skin) forms the outside of the bag and the epithelium (outside of the skin) represents the inner lining of the bag. Immediately after sacrificing the animal, the skin of the legs was removed. A bag was formed of the skin from the right leg. It was filled with 4 to 6 ml. of salt solution, immersed for 8 hours in 2 liters of the same kind of salt solution, was emptied again, and the skin was then analyzed for sodium and potassium. Water content of the skin was also determined (see under section c on Sodium and potassium estimations). The skin from the other leg of the same frog served as a control. It was analyzed shortly after it was removed from the body. The skins were weighed after adherent moisture was removed by carefully blotting the skins on filter paper. For chemical analysis of skins from experimental bags, only that part was used which had actually been exposed to the solutions; therefore, in order to obtain comparable control values from the other leg, a piece of skin was cut out which corresponded, as closely as possible, to the piece of experimental skin.

The procedure of comparing homologous pieces of skins from the two sides of the animal seemed justified after it was found by appropriate investigations, that the electrolyte content of the skin of the two hind legs was, for all practical purposes, the same. The following results (with standard errors of the mean) were obtained: *Right leg*, $Na^+ = 63.7 \pm 2.4$; $K^+ = 49.5 \pm 1.8$; $Cl^- = 55.6 \pm 3.3$ $\mu$eq./gm. of wet skin; $H_2O = 73.5 \pm 0.4$ per cent. *Left leg*, $Na^+ = 62.9 \pm 2.3$; $K^+ = 49.3 \pm 1.4$; $Cl^- = 54.2 \pm 4.1$ $\mu$eq./gm. of wet skin; $H_2O = 73.4 \pm 0.5$ per cent. $Cl^-$ values are based on investigations of 4, other values on 9 frogs. These data agree well with those of other workers (28, 32).

Experiments of the various series, using salt solutions of different concentrations and compositions, were rotated throughout a period of about 18 months. This was done in order to avoid distortion of data because of possible seasonal variations in electrolyte composition and behavior of skins. (Our data do not indicate, however, that seasonal variations in skin are of significance.) Usually 4 frogs were used in one run. At the end of the experiments, the contents of each of the 4 bags were collected separately and analyzed for $Cl^-$, $Na^+$, and $K^+$, as were also the salt solutions in which the bags had been immersed.

(*b*) *Salt Solutions.*—These contained NaCl, KCl, and $NaHCO_3$. Three levels of NaCl were studied: 48, 119, 169 $\mu$eq./ml. For each of these solutions, the $K^+$ level was varied (without changing NaCl correspondingly) from approximately 0.1 to 20 $\mu$eq./ml. (The exact values are given in the tables below.) $NaHCO_3$ was added to the solutions, giving a final concentration of 2 $\mu$eq./ml. Then the salt solutions were saturated with

$O_2$. Finally, using a glass electrode, the pH was adjusted to a value close to 7.4 by carefully adding diluted HCl.

For convenience, we often refer in the following text to *"diluted saline," "physiological saline,"* and *"concentrated saline,"* which mean salt solutions containing 48, 119, and 169 $\mu$M of NaCl/ml. and various amounts of KCl. When the expression, "$K^+$-free" saline, is used, a solution containing approximately 0.1 $\mu$eq. $K^+$/ml. is meant.

(*c*) *Sodium and Potassium Estimations on Solutions and Skins.*—These were carried out by flame photometry using, mostly, a Barclay flame photometer. A series of standard solutions was included with each set of experimental samples. Skins, after weighing, were dried in platinum crucibles for 2½ hrs. at 110°C. and then, after weighing for dry weight measurements, were preheated in an electric furnace for 3 hours at 300°C. and finally incinerated by keeping them at 500–550°C. for 12 hours. Checks showed complete recovery of the alkali metals, when known amounts of a NaCl–KCl mixture, either alone or in the presence of known amounts of skin, were treated in the same way as the experimental samples.

(*d*) *Chloride Estimations.*—In salt solutions $Cl^-$ estimations were carried out iodometrically (3). The limited amount of experimental and control skin available for chemical analysis made it necessary to estimate skin chloride, if desired, in skins of frogs other than those used in the main studies referred to above. The method of chloride analysis was that described by Van Slyke and Sendroy (36).

(*e*) *The Water Content of Skin.*—This was calculated from the dry weight which was obtained as described under section (*c*).

(*f*) *Surface Areas.*—Surface areas of skins of the experimental bags were measured planimetrically after first weighing the piece of skin which was actually exposed to the salt solutions (see section *a*).

(*g*) *The Average Room Temperature.*—For the period of experimentation this was 24.7 ± 0.4°C. The laboratory was air-conditioned during the summer season.

(*h*) *Skin Potentials.*—These were measured in all experimental pieces of skin throughout the 8 hour period. Saturated KCl half cells and the usual bridge circuit were used for E.M.F. measurements. Details of our procedure have been described elsewhere (12).

## III

## *"Compartments" in Frog Skin*

Throughout this paper, the terms extra- and intracellular electrolytes will be used. It would be more correct to speak of electrolytes in the "chloride space" and the "non-chloride space," since all calculations of the distribution of ions within skin are based on Hastings and Eichelberger's "chloride space" concept (7). Today, perhaps, only a few would accept the chloride space and the non-chloride space as a true measure for the total anatomical extracellular and the intracellular compartments, respectively. Manery in her recent publication (24) has pointed out that such a general assertion seems untenable and that the various tissues have to be considered separately. The facts reported in the

literature (17, 24) suggest that, especially for skin, the non-chloride space measures only a fraction of the space occupied by the various distinct histological elements of skin. This seems also to apply to frog skin.

It was found (see below) that the chloride space of frog skin represents 69 per cent of the total wet weight of freshly isolated skin or approximately 94 per cent of the total water content of skin. If this represents water of the extracellular space, one would be left with 6 per cent of the total water of skin in the intracellular compartment. From histological pictures of frog skin, however, it would appear that considerably more than 6 per cent of the total water of skin must be present in the structural elements of skin. A few preliminary planimetric measurements of formol-fixed and stained frog skin have shown that the total cross-sectional area of the one or two rows of epithelial cells comprising the stratum germinativum represents approximately 9 per cent of the total cross-sectional area of the skin. The cross-sectional area of the epithelial cells of the mucous gland, which one finds embedded at certain places in the corium, could be roughly estimated at 6 per cent. Not everywhere in the skin, however, does one find these mucous glands.

This makes it rather likely that the non-chloride space is only a fraction of the anatomical intracellular space. At this time, however, it is impossible to say what the exact relationships among chloride space, non-chloride space, extracellular, and intracellular compartments in frog skin are. It is arbitrary, therefore, to speak of intracellular and extracellular electrolytes when, actually, electrolytes in the non-chloride space and chloride space, respectively, are meant. We do this in the following sections only as a matter of convenience. The more important aspect here is to apply to frog skin the chloride space concept and to see what it has to offer with regard to the distribution of $Na^+$ and $K^+$ in skin under various experimental conditions. The decision as to what the chloride space and non-chloride space mean, from a histological and a cytological point of view, is beyond the scope of this paper.

## IV

## *Data on Normal Skin*

In the following text the distinction is made between *content* and *concentration* for any one constituent in skin or salt solution. Chemical symbols in parentheses, *e.g.* $(K^+)$, express the content; *i.e.*, the number of microequivalents per gram of wet skin. $[K^+]$ and similar expressions give the number of microequivalents per gram of water or per milliliter of salt solution. The indices, *e*, *i*, *t*, are used to indicate extracellular, intracellular, and total.

*Extracellular Fluid Compartment, e.*—This was found, on an average, to be 0.697 gm./gm. of wet skin. The calculations involved are based on the work of Hastings and Eichelberger (7). It is assumed that all $Cl^-$ of the skin is extracellular. One finds $e = 0.95 \times (Cl^-)_{skin}/0.99 \times [Cl^-]_{plasma\ water}$. From 4

## TABLE I

*Extracellular and Intracellular Distribution of $Na^+$, $K^+$, and $H_2O$ in Skin*

Indices, *t*, *e*, *i*, refer to "total," "extracellular," and "intracellular" respectively. Amounts of extracellular $Na^+$, $K^+$, and $H_2O$ in the controls: $(Na^+)_e$ = 68.7 μeq./gm. wet skin; $(K^+)_e$ = 1.7 μeq./gm. wet skin; $(H_2O)_e$ = 0.690 gm./gm. wet skin.

| No. of frogs | Control skins | | | | | | $[K^+]_e$ of bath | $[Na^+]_e$ of bath | Experimental skins | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $(Na^+)_t$ | $(Na^+)_i$ | $(K^+)_t$ | $(K^+)_i$ | $(H_2O)_t$ | $(H_2O)_i$ | | | $(Na^+)_t$ | $(Na^+)_e$ | $(Na^+)_i$ | $(K^+)_t$ | $(K^+)_e$ | $(K^+)_i$ | $(H_2O)_t$ | $(H_2O)_i$ |
| | μeq./gm. wet skin | | μeq./gm. wet skin | | gm./gm. wet skin | mM/gm. wet skin | μeq./ml. | | μeq./gm. wet skin | | | μeq./gm. wet skin | | | gm./gm. wet skin | mM/gm. wet skin |
| | | | | | | | | | Average $[Na^+]_e$ = 47.9 μeq/ml. | | | | | | | |
| 16 | 68.8 | +0.1 | 44.5 | 42.8 | 0.735 | 2.5 | 0.09 | 48.3 | 39.6 | 33.3 | +6.3 | 15.9 | 0.06 | 15.8 | 0.774 | 4.7 |
| 19 | 65.5 | −3.2 | 48.5 | 46.8 | 0.741 | 2.8 | 1.11 | 48.2 | 33.6 | 33.2 | +0.4 | 26.4 | 0.8 | 25.6 | 0.776 | 4.8 |
| 15 | 65.2 | −3.5 | 39.1 | 37.4 | 0.738 | 2.7 | 5.12 | 49.3 | 37.5 | 34.0 | +3.5 | 27.3 | 3.5 | 23.8 | 0.769 | 4.4 |
| 11 | 68.4 | −0.3 | 43.8 | 42.1 | 0.741 | 2.8 | 9.92 | 48.7 | 37.8 | 33.6 | +4.2 | 33.5 | 6.8 | 26.7 | 0.768 | 4.3 |
| 12 | 65.5 | −3.2 | 44.8 | 43.1 | 0.749 | 3.3 | 17.2 | 44.1 | 34.5 | 30.4 | +4.1 | 39.8 | 11.9 | 27.9 | 0.780 | 5.0 |
| | | | | | | | | | Average $[Na^+]_e$ = 119 μeq/ml. | | | | | | | |
| 10 | 67.4 | −0.7 | 39.5 | 37.8 | 0.735 | 2.5 | 0.09 | 116 | 113 | 80.3 | +32.2 | 17.9 | 0.06 | 17.8 | 0.722 | 1.7 |
| 11 | 64.7 | −4.0 | 39.4 | 37.7 | 0.747 | 3.2 | 1.00 | 122 | 84.5 | 83.9 | +0.6 | 35.0 | 0.7 | 34.3 | 0.738 | 2.7 |
| 11 | 64.2 | −4.5 | 43.0 | 41.3 | 0.740 | 2.8 | 2.55 | 119 | 85.8 | 81.8 | +4.0 | 42.6 | 1.8 | 40.8 | 0.743 | 3.0 |
| 12 | 65.1 | −3.6 | 41.4 | 39.7 | 0.743 | 3.0 | 4.74 | 118 | 92.1 | 81.2 | +10.9 | 44.8 | 3.3 | 41.5 | 0.740 | 2.8 |
| 12 | 60.7 | −8.0 | 43.9 | 42.2 | 0.742 | 2.9 | 10.1 | 124 | 78.3 | 85.1 | −6.8 | 48.5 | 7.0 | 41.5 | 0.743 | 3.0 |
| 12 | 62.1 | −6.6 | 38.8 | 37.1 | 0.741 | 2.8 | 19.0 | 118 | 91.5 | 81.5 | +10.0 | 55.4 | 13.1 | 42.3 | 0.728 | 2.1 |
| | | | | | | | | | Average $[Na^+]_e$ = 169 μeq/ml. | | | | | | | |
| 12 | 68.1 | −0.6 | 44.9 | 43.2 | 0.739 | 2.7 | 0.11 | 166 | 164 | 115 | +49.1 | 17.8 | 0.08 | 17.7 | 0.700 | 0.56 |
| 12 | 75.9 | +7.2 | 41.4 | 39.7 | 0.734 | 2.4 | 1.08 | 169 | 143 | 116 | +26.3 | 39.4 | 0.7 | 38.7 | 0.695 | 0.26* |
| 12 | 68.8 | +0.1 | 40.2 | 38.5 | 0.743 | 3.0 | 4.91 | 169 | 137 | 116 | +20.3 | 50.0 | 3.4 | 46.6 | 0.715 | 1.4 |
| 24 | 63.1 | −5.6 | 45.0 | 43.3 | 0.728 | 2.1 | 9.88 | 174 | 121 | 120 | +1.1 | 58.1 | 6.8 | 51.3 | 0.714 | 1.3 |
| 12 | 65.8 | −2.9 | 44.4 | 42.7 | 0.750 | 3.3 | 19.5 | 165 | 140 | 114 | +26.2 | 71.9 | 13.5 | 58.4 | 0.716 | 1.4 |

* This value seems to be of little significance.

estimations on frog plasma which was collected by pooling blood of 21 frogs, $[Cl^-]_{plasma\ water}$ was found to be, on an average, 77.0 μeq./ml. This agrees well with Fenn's value of 74.3 mM/kg. of plasma (4). Chloride estimations on skin of legs from 51 frogs yielded an average value of 55.9 μeq./gm. of wet skin for $(Cl^-)$.

*Extracellular Water Compartment, $(H_2O)e$.*—This was calculated as $(H_2O)_e = e \times 0.99$, which gives 0.690 gm./gm. of wet skin.

*Extracellular Sodium, $(Na^+)_e$.*—This is given by: $(Na^+)_e = e \times 0.99 \times 0.95 \times [Na^+]_{plasma\ water}$. For $[Na^+]_{plasma\ water}$, an average value of 104.8 μeq./gm. was obtained by the flame photometrical estimation of diluted frog plasma, referred to above. Fenn gives a value of 104 mM/kg. of plasma for $Na^+$ in frog plasma. Thus, using our figure, $(Na^+)_e$ = 68.7 μeq./gm. of wet skin.

*Extracellular Potassium, $(K^+)_e$.*—This was calculated from $(K^+)_e = e \times 0.99 \times 0.95 \times [K^+]_{plasma\ water}$, and was found to be 1.7 μeq./gm. of wet skin. Fenn finds, for $K^+$ in plasma, 2.5 mM/kg. of plasma, which is about 2.6 μeq./gm. of plasma water. Our own estimations of the plasma referred to above have led to a value of 4.6 μeq./gm. of plasma water. Since a slight hemolysis of our plasma may have occurred, Fenn's value was preferred.

*Intracellular Electrolytes and Water.*—The distribution of electrolytes and water between intra- and extracellular phase can be calculated from the difference between total and extracellular content; *e.g.*, $(Na^+)_i = (Na^+)_t - (Na^+)_e$. Values for extracellular sodium, potassium, and water have been given in the previous paragraphs. Values for total and intracellular electrolytes and water of normal skins are found in the "control" section of Table I.

## V

## *Results with Skins in Various Salt Solutions*

*1. Electrolyte and Water Content of Skin.*—The original chemical analytical data on the skins of the experimental bags and data on their respective controls are given in Table I, columns 2, 4, 6, 10, 13, and 16. As was pointed out under Methods, the former data were obtained from the skins that were kept for 8 hours in various salt solutions. Control values, for homologous pieces of skins, were obtained by analyzing skins shortly after removal from the animal. Information was derived from the original data for the distribution of sodium, potassium, and water between intra- and extracellular spaces. Those derived data are found in columns 3, 5, 7, 11, 12, 14, 15, and 17 of Table I.

Certain assumptions were made in the calculation of the distribution of electrolytes and water between intra- and extracellular phase in the experimental skins. They are: (*a*) that with respect to all ions the extracellular spaces were, after 8 hours, in complete diffusion equilibrium with the bath, represented by 2 liters of salt solution, and (*b*) that the cell membranes behaved as semi-

permeable membranes; therefore, if changes in the total water content/gram of skin occurred, it was assumed that the cells had taken up or had lost water and that the extracellular volume remained unchanged. With $(H_2O)_e$ constant and equal to 0.690 gm./gm. of skin (see data on normal skin), values for extracellular sodium and potassium content were calculated as: 0.690 $[Na^+]_e$ and 0.690$[K^+]_e$, respectively, when $[Na^+]_e$ and $[K^+]_e$ are the concentrations of sodium and potassium of the salt solutions in which the bags were suspended. These values are given in the 8th and 9th columns of Table I.

In order to give some evidence for the first of the two assumptions just made, the following additional studies were carried out: 94 pieces of leg skin, in groups of 5 to 6 each, from different frogs were immersed for 8 hours in salt solution containing various amounts of NaCl and KCl, as described in the section on Methods. In all cases, the $Cl^-$ content of the skins, after the period allowed for equilibration, was found to be 72.3 $\pm$ 1.1 per cent (S.E. mean) of the $Cl^-$ concentration of the bath, indicating complete chloride equilibrium.

*Sodium.*—Average values for total sodium content, $(Na^+)_t$, of control skin varied from 60.7 to 75.9 $\mu$eq./gm. Negative values for $(Na^+)_i$ shown in the third column of Table I, are of course without reality. It would appear from this that our control values for intracellular sodium are uncertain. One may say, perhaps, that normally there seems to be only little, if any, intracellular sodium present in skin. On the other hand, the increase in intracellular sodium, in many experimental skins, can hardly be questioned. High values for intracellular sodium were found when the skins were kept in $K^+$-free, physiological or concentrated salt solutions (Table I, column 12). For skins in $K^+$-free concentrated salt solution, $(Na^+)_i$ was 49.1 $\mu$eq./gm. $(Na^+)_t$ went up to 163.8 $\mu$eq./gm., as compared to a value for $(Na^+)_t$ of 68.1 $\mu$eq./gm. of wet skin in the controls.

*Potassium.*—Average normal values for $(K^+)_t$ and $(K^+)_i$ were found to vary from 38.8 to 48.5 and from 37.1 to 46.8 $\mu$eq./gm. of wet skin, respectively (Table I, columns 4 and 5). One can see, then, that approximately 96 per cent of the total potassium in skin is in the form of intracellular potassium. Skins in diluted saline were unable to maintain normal $(K^+)_t$ or $(K^+)_i$ values even when $[K^+]_e$ was increased to 17.2 $\mu$eq./ml., which is about 9 times the so called "physiological" potassium level of Ringer's solution. It can be seen from Table I, column 15, that $(K^+)_i$ varied from 25.6 to 27.9 $\mu$eq./gm. of wet skin, increasing only very little with increasing $[K^+]_e$ over the range from 1.11 to 17.2 $\mu$eq. $K^+$/ml. salt solution. The lowest value for intracellular potassium was found for skin in $K^+$-free, diluted salt solution; here, $(K^+)_i$ was found to be 15.8 $\mu$eq./gm. of wet skin. Skins in physiological saline or concentrated saline were much better able to maintain normal levels for intracellular potassium. Only when kept in $K^+$-free solutions, did the skins reach $(K^+)_i$ levels almost as low as those seen for skins in $K^+$-free diluted saline (Table 1, column 15). Higher than normal levels for intracellular potassium were seen in skins that

were kept in concentrated saline containing 10 or 20 μeq. $K^+$/ml. The highest $(K^+)_i$ value obtained was 58.4 μeq./gm. of wet skin.

*Water.*—The average total $H_2O$ content of control skins, $(H_2O)_t$, varied from 72.8 to 75.0 per cent (Table I, column 6). The same applies for experimental skins when kept in physiological saline (Table I, column 16). For skins that were kept in diluted or in concentrated saline solution, the water content varied from 76.8 to 78.0 per cent or from 69.5 to 71.6 per cent, respectively. If it is permissible to assume constancy of the amount of extracellular water, $(H_2O)_e$, one can calculate the amount of intracellular water, $(H_2O)_i$. The values obtained are shown in Table I, columns 7 and 17.

*2. NaCl and Water Uptake.*—Average values for net rate of loss of sodium, chloride, and water from the fluid in the bag are entered in Table II, columns 6, 7, and 9, respectively. It was assumed that the net rate of salt and water loss from the bag remained more or less constant throughout the 8 hour experimental period. This is undoubtedly an oversimplification of the situation. From the changes, with respect to time, in the electrical potentials that were recorded and are presented below, it seems more likely that net rates for loss of water and salt from the bags did not remain the same in all cases studied. So far, however, no experiments have been carried out to show these details.

The disappearance of salt and water from the fluid in the bag is the result of active salt uptake by the epithelium of the skin, as is well known from many earlier studies. The driving force is provided by a sodium pumping mechanism. Chloride and water follow passively. Salt and water absorbed by the epithelium are then moved across the skin and are finally released from the corium into the adjacent salt solution. It can be seen from Table II that net salt uptake was optimal when skins were kept in physiological saline solution containing 1 to 5 μeq. $K^+$/ml. One can calculate that under physiological conditions, the salt solution moving across the skin in the inward direction is approximately twice isotonic. In most cases, nearly equivalent amounts of chloride and sodium were taken up by the skin (Table II, columns 6 and 7). In a few cases, chloride uptake exceeded sodium uptake or *vice versa*. This may, in part, be the result of analytical difficulties, since at times flame photometry was not quite satisfactory. It must be recognized, on the other hand, that besides chloride and sodium ions, other unestimated anions and cations of solutions and skins may have entered, at various degrees, into the balance of cations and anions.

The pH of the solutions at the corium side usually had increased 0.1 to 0.3 unit after 8 hours. pH changes at the epithelial side were not followed. They probably had changed slightly towards the acid side (10).

The data presented in Table II (columns 2, 6, and 7) confirm and extend our earlier observation (11) on the dependence of net NaCl uptake on the potassium concentration of the salt solution in which the skin is immersed. This matter will be discussed further in the following section of this paper.

*3. Potassium Accumulation.*—Whereas, during the experiment, sodium chloride disappeared from the fluid in the bag, potassium accumulated there. Table II (column 8) gives data on the average net rate of potassium accumulation. Potassium accumulation at the epithelial side has a tendency to increase

TABLE II

*Net Amounts of Ions and Water Removed from or Gained by the Outside Fluid Compartment*

Data on thicknesses of skin and surface areas per gram of wet skin. Skin potentials. All values given are average values.

| No. of frogs | $[K^+]_o$ | $d^*$ | $\frac{cm.^2}{gm.}$ | P.D. | $-\Delta Cl‡$ | $-\Delta Na‡$ | $\Delta K‡$ | $-\Delta H_2O$ |
|---|---|---|---|---|---|---|---|---|
| | *μeq./ml.* | *mm.* | | *mv.* | *μeq.* × $cm.^{-2}$ × $hr.^{-1}$ | | | $\frac{\mu M}{cm.^{-2} \times hr.^{-1}}$ |
| | | | | $[Na^+]_o$ = 47.9 μeq./ml. | | | | |
| 16 | 0.09 | 0.221 | 41.5 | 37.3 | 0.42 | 0.43 | 0.021 | 107 |
| 19 | 1.11 | 0.220 | 43.5 | 35.2 | 0.64 | 0.69 | 0.022 | 137 |
| 15 | 5.12 | 0.196 | 48.5 | 31.6 | 0.61 | 0.73 | 0.043 | 117 |
| 11 | 9.92 | 0.212 | 43.3 | 25.6 | 0.50 | 0.50 | 0.027 | 71 |
| 12 | 17.2 | 0.236 | 38.4 | 19.6 | 0.55 | 0.55 | 0.024 | 111 |
| | | | | $[Na^+]_o$ = 119 μeq./ml. | | | | |
| 10 | 0.09 | 0.196 | 48.0 | 4.3 | 0.20 | 0.14 | 0.018 | 63 |
| 11 | 1.00 | 0.218 | 42.8 | 35.5 | 0.63 | 0.90 | 0.018 | 212 |
| 11 | 2.55 | 0.232 | 40.6 | 30.2 | 0.96 | 0.89 | 0.015 | 248 |
| 12 | 4.74 | 0.216 | 43.4 | 34.0 | 0.81 | 0.87 | 0.027 | 212 |
| 12 | 10.1 | 0.224 | 41.6 | 26.8 | 0.68 | 0.76 | 0.026 | 159 |
| 12 | 19.0 | 0.216 | 42.6 | 31.8 | 0.59 | 0.73 | 0.044 | 190 |
| | | | | $[Na^+]_o$ = 169 μeq./ml. | | | | |
| 12 | 0.11 | 0.174 | 54.0 | 2.5 | 0.17 | 0.12 | 0.019 | 41 |
| 12 | 1.08 | 0.178 | 52.0 | 9.1 | 0.43 | 0.26 | 0.025 | 69 |
| 12 | 4.91 | 0.206 | 45.5 | 12.3 | 0.36 | 0.48 | 0.034 | 77 |
| 24 | 9.88 | 0.187 | 50.2 | 16.2 | 0.59 | 0.80 | 0.035 | 141 |
| 12 | 19.5 | 0.202 | 47.7 | 14.2 | 0.48 | 0.53 | 0.052 | 117 |

* Thickness of skin as calculated from weight and surface area measured at the end of the experiment. Specific density of skin = 1.1 (9).

‡ $-\Delta Cl$ and $-\Delta Na$ indicate *loss* from outside bath; $+\Delta K$ indicates *gain* by outside bath.

with increasing potassium level of the salt solutions, at least in the series with physiological and concentrated saline solutions. At high potassium levels, the rate of potassium accumulation increases with increasing NaCl concentration of the bath. In $K^+$-free saline solutions, the rate of potassium accumulation is still relatively high regardless of the sodium chloride concentration of the bath.

Under certain circumstances, potassium of the skin will be released from the epithelial side of and from the corium side of the skin. This will be discussed in detail in the next section of this paper.

*4. Spontaneous Skin Potentials of the Experimental Skins.*—These were measured seven times, at approximately hourly intervals, during the 8 hour period. The first and the final measurements on each skin bag were made 1

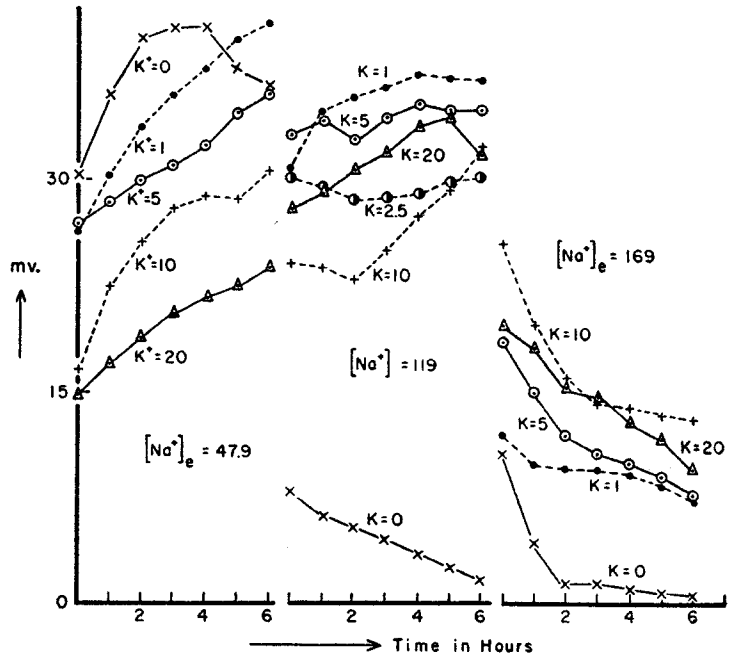


FIG. 1. E.M.F.–time course of spontaneous skin potentials for skins in salt solutions of varying potassium and sodium ion concentrations. Inside of skin positive with relation to outside.

hour after filling and 1 hour before emptying the bag. No potential measurements were carried out on control skins, since they were prepared for chemical analysis shortly after removal. In all cases reported here, the outside of the skin was electrically negative with relation to the inside. Changes in skin potentials with respect to time of exposure to various salt solutions are shown in Fig. 1. Each point of the graphs represents the average value of 10 to 24 experiments (see column 1 of Table I). Certain features of these graphs may be summarized as follows: (*a*) In diluted saline, potentials increased, more or less gradually, with increasing time. Skins in $K^+$-free saline made an exception, in that the potential first increased, but decreased during the later hours of the

experiments. Raising the $K^+$ level in the salt solutions resulted in lowering skin potentials. The highest skin potentials, at least for the first 4 to 5 hours, were obtained in $K^+$-free saline. Conversely, the lowest potentials were recorded in saline solutions containing 20 $\mu$eq. $K^+$/ml. (*b*) In physiological saline with 2.5 and 5 $\mu$eq. $K^+$/ml., skin potentials were fairly steady throughout the period of measurements. Skins in solutions with 1, 10, and 20 $\mu$eq. $K^+$/ml. showed clearly, although moderately, the development of higher potentials with increasing time. In contrast to what has been said under (*a*), however, skin potentials from skins in $K^+$-free saline solution were very low and potentials further diminished with increasing time. Thus, at the end of the experimental period, skin potentials were almost zero. Within the range of 1 to 20 $\mu$eq. $K^+$/ml., there was no regularity as to influence of $K^+$ level on height of skin potential. (*c*) In concentrated saline, skin potentials fell continuously with increasing time. The situation is just the opposite of what happened in diluted saline. The reversal of behavior of skin potentials with skin in concentrated as compared to skin in diluted saline appeared also with respect to the effect of various $K^+$ levels. Potentials were lowest (rather than highest) in $K^+$-free saline, and they were highest (rather than lowest) in saline solutions with high $K^+$ levels.

No measurements were made of ion fluxes and of the conductances of skins for ions (15, 20–23, 33, 35), under the various conditions chosen in this study. Since lack of such important information makes it impossible to give a conclusive interpretation of the correlation among spontaneous skin-potential, ion distribution in skin, and ion transport, purely speculative comments concerning the correlation will also be omitted from this paper.

## VI

## DISCUSSION

*1. NaCl Uptake and Potassium Balance of Skin.*—The main objective of this study was to show the correlations between the distribution of sodium and potassium in frog skin and its ability to take up sodium chloride and water from the salt solution which touches the epithelial side (outside) of the skin. Information concerning this question is given in Tables I and II.

In all our studies, analytical data were obtained for (1) net uptake of sodium chloride and water by the outside of the skin by estimating the loss of these constituents from the outside bath; (2) net release of potassium from the outside by estimating the gain of potassium in the outside bath; (3) net changes in sodium, potassium, and water content of the skin. No estimations were made to show the amount of sodium and water released from the inside of the skin into the inside bath and of potassium taken up by the inside from the inside bath. Information concerning these changes may be readily obtained from the fact that, for any one constituent of the system, the sum of what is present in

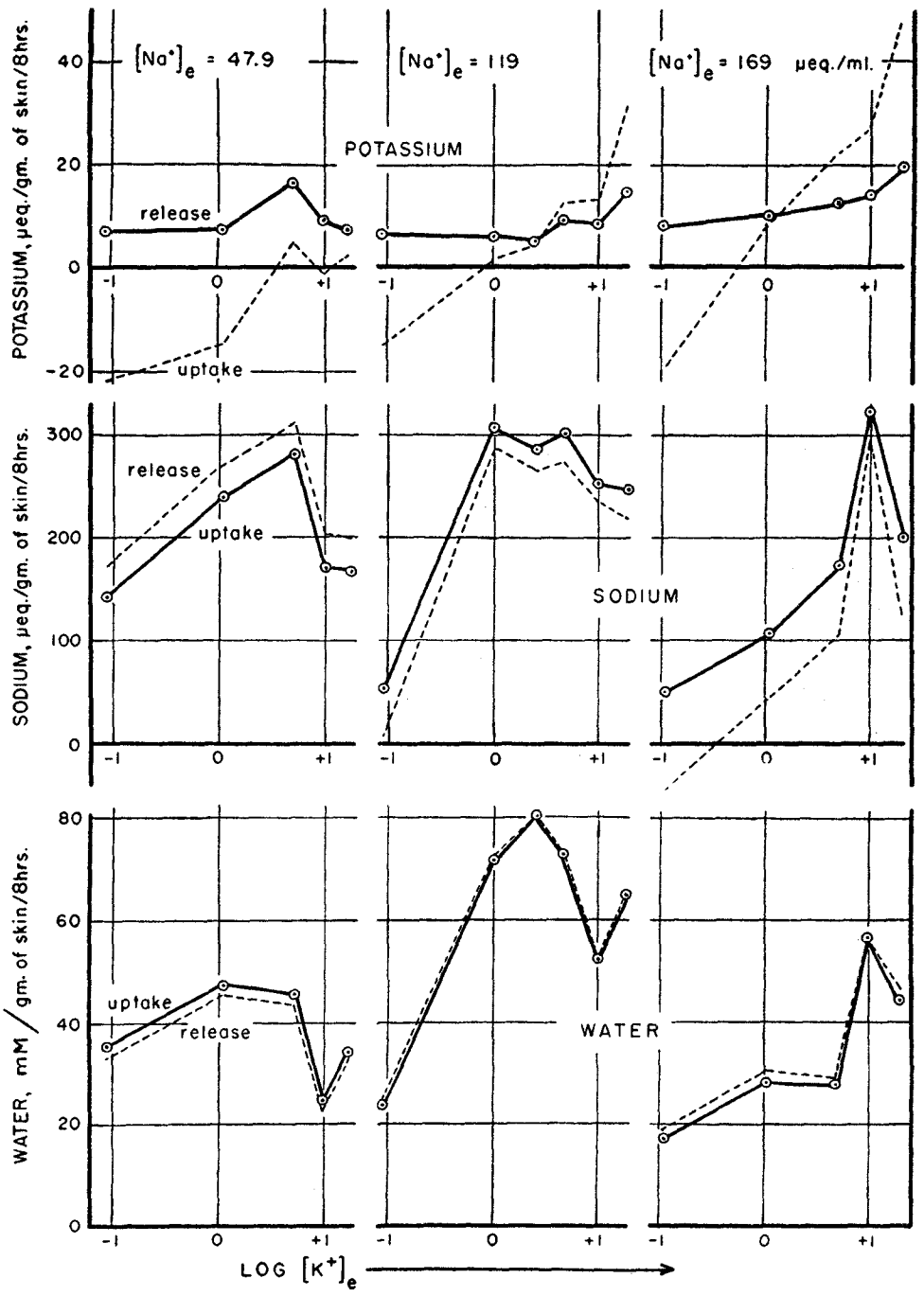
POTASSIUM, μeq./gm. of skin/8hrs.
SODIUM, μeq./gm. of skin/8hrs.
WATER, mM / gm. of skin/8hrs.
[Na+]e = 47.9
[Na+]e = 119
[Na+]e = 169 μeq./ml.
POTASSIUM
SODIUM
WATER
release
uptake
40
20
0
-20
300
200
100
80
60
-1
0
+1
LOG [K+]e

FIG. 2

the inside bath, in the outside bath, and in the skin remains constant. Values for net uptake of potassium by the inside of the skin from the inside bath were obtained by adding to the net amount of potassium gained by the skin[1] the net amount of potassium released from the outside of the skin into the outside bath. Data for net amount of sodium (or water) released from the inside of the skin into the inside bath were calculated by subtracting the net amount gained by the skin[1] from the net amount taken up by the outside of the skin from the outside bath.

With this information on hand, Fig. 2 was drawn which gives a complete picture of the net sodium, potassium, and water shifts in the skin and the salt solutions on either side of the skin. The broken lines in this figure refer to the calculated uptake and release data. The points on the solid lines represent those data that were obtained by measurements.

It can be seen from the upper set of graphs that skin in diluted saline was found to be in negative potassium balance over the whole range of $[K^+]_e$, that is from 0.09 to 17.2 $\mu$eq. $K^+$/ml. If extrapolation to a point of intersection of the broken and the solid lines in the graph (upper left-hand corner) be permissible, one would find that at a potassium ion concentration in the bath of about 22 $\mu$eq./ml., the skin would be in potassium balance. For skins in physiological saline and concentrated saline the potassium concentrations of the baths necessary for maintenance of $K^+$ equilibrium in skin are 2.7 and 1.3 $\mu$eq./ml., respectively.

The question may be raised as to whether this change in the potassium concentration of the bath, for maintenance of potassium balance of the skin, towards smaller values with increasing NaCl concentration is a function merely of the ionic strength or a specific ion function, especially of sodium ions. The ionic strengths of the three salt solutions maintaining potassium equilibrium in skin are: $\mu = 0.070$ (diluted saline); $\mu = 0.146$ (physiological saline); $\mu = 0.182$ (concentrated saline). The sodium ion concentrations (equivalents per liter), of these solutions are: $[Na^+] = 0.048, 0.119$, and 0.169, respectively. One can see that these figures do not answer the question raised above as to which factor, ionic strength or sodium ion concentration, was the more important

[1] A net loss is treated as a "negative" net gain.

FIG. 2. Net uptake by and net release from skin of potassium, sodium, and water, in dependence upon extracellular potassium ion concentration. For potassium the solid lines refer to potassium release from the epithelium (outside of skin), the broken lines to potassium uptake by the inside of the skin. For sodium (water), uptake and release graphs refer to sodium (water) uptake by the outside and sodium (water) release from the inside, respectively. Points on solid lines represent data obtained directly from measurements of gains or losses of electrolytes of the outside bath. Broken lines are indirectly arrived at from calculations (see text).

one in maintaining potassium balance of skin. In the present study, in preparing salt solutions, we decided against reducing sodium chloride in the solutions by an amount equivalent to the potassium chloride that was added in increasing amounts. The reason for this was that sodium is the principal ion acted upon by the skin; therefore, in studying the dependence of net NaCl uptake on potassium ion concentration of the bath, a change of both sodium and potassium chloride concentrations of the bath would have led to inconclusive results. By increasing the potassium concentration of the bath from approximately 0.1 to 20 μeq./ml., *i.e.* 200 times, and by maintaining the concentration of sodium chloride, it was clear that changes in potassium concentrations rather than changes in ionic strengths would be the decisive factor in altering net NaCl uptake.

From Fig. 2, the following conclusions may be drawn with regard to the dependence of net NaCl and water uptake on the potassium balance of skin: (1) Decidedly negative as well as positive potassium balance of skin results in relatively small rates of NaCl and water uptake. In other words, whenever skin loses a fair amount of potassium or gains an excess of it, the mechanism of NaCl uptake in skin does not function properly. (2) In salt solutions of physiological NaCl level, optimal rate for NaCl uptake is found over a relatively wide range of potassium ion concentration of the bath, resulting in a slightly negative, a complete, or slightly positive potassium balance of skin. (3) For skins in diluted or concentrated saline optimal rates for NaCl uptake are reached only at moderately negative or at a somewhat more than moderately positive potassium balance in skin, respectively.

*2. Ion Exchange Processes in Frog Skin.*—Of the two cases, namely, decrease of net NaCl uptake at a decidedly negative potassium balance of skin and decrease of net NaCl uptake at a decidedly positive potassium balance, only the former will be discussed further, at the present time.

Combining the information given in Tables I and II and Fig. 2, one can say that at very low potassium concentrations of the bath (extracellular fluid), intracellular potassium, which is normally, high, is diminished. At the same time, intracellular sodium, which is normally low, increases. This is associated with diminished net NaCl uptake. The decrease is less in solutions of relatively low extracellular sodium than in solutions of physiological or relatively high extracellular sodium. The changes in the distribution of intracellular electrolytes may conveniently be referred to as ion exchange processes in which intracellular potassium is replaced by extracellular sodium. Whereas it seems that such an exchange reaction can easily be forced upon the skin, under loss of ability to actively take up sodium, and with it chloride, as described above, it is also quite obvious from the behavior of normal skin in which, under proper conditions, vigorous uptake of sodium chloride occurs, that provisions are made in order to prevent this ion exchange from taking place. Active sodium trans-

port in frog skin has been visualized (13) as a two step process, involving two adjacent histological structures with different biochemical features. Trapping of $Na^+$ ions in an ion exchange system may lead to diminished supply of $Na^+$ to the mechanism which transports $Na^+$ ions across the skin. This could explain why net uptake of NaCl by skin from $K^+$-free salt solutions is diminished.

From the data on intracellular sodium and potassium presented in Table I, one can calculate the exchange ratio for the two ions under consideration. It is found that, in $K^+$-free diluted saline, for every 4 potassium ions that leave the intracellular compartment, one sodium ion enters it. For skin in $K^+$-free physiological saline, the ratio of number of potassium ions lost/number of sodium ions gained is 4:6 and for concentrated saline it is 4:8.

*3. The Physicochemical State of Potassium in Skin.*—It can be shown that the data on intracellular potassium concentration in frog skin, $[K^+]_i$, as a function of the potassium concentration of the bath, $[K^+]_e$, satisfy the equation,

$$[K^+]_i = 10^b[K^+]_e^{1-a}. \quad (1)$$

This expression represents a case of Freundlich's isotherm, and it would appear, therefore, that potassium in frog skin may be said to be in a state of adsorption. $[K^+]_i$, the number of microequivalents of potassium per gram of intracellular water, is obtained from $[K^+]_i = (K^+)_i/(H_2O)_i$ (see Table I). $[K^+]_e$ is the potassium concentration of the bath in microequivalents per milliliter. In the above equation, $a$ and $b$ are constants, values for which can easily be found graphically. The best way to do this is to plot the log $([K^+]_i/[K^+]_e)$ against the log $[K^+]_e$ (Fig. 3). The three almost parallel descending straight lines shown in the figure are the regression lines, the equations for which are given in the upper right corner of the Figure, together with some pertinent statistical information. The intersection of the regression lines with the abscissa and ordinate will lead to values for $a$ and $b$. It, then, is possible to plot the Freundlich isotherm for potassium in frog skin (Fig. 4).

Perhaps one may be disturbed by the high values for $[K^+]_i$ obtained in these calculations and plotted at the ordinate. Values of the order of 1 to 2 molar for the "intracellular" potassium ion concentration, naturally, have little appeal to physiologists. It must be borne in mind, however, that the calculations do not suggest the interpretation that potassium ions are rather free. On the contrary, the fact that the data approximately fit Freundlich's isotherm suggests that the ions are in part bound and, hence, that their activity is diminished.

It can also be shown that the relationship between log $[K^+]_i$ and log $[K^+]_e$ is a linear one, as it should be for a process of adsorption that follows Freundlich's isotherm. The slope of the isotherms, however, is rather small, and it becomes,

therefore, more difficult to obtain good values for the constants in the isotherms.

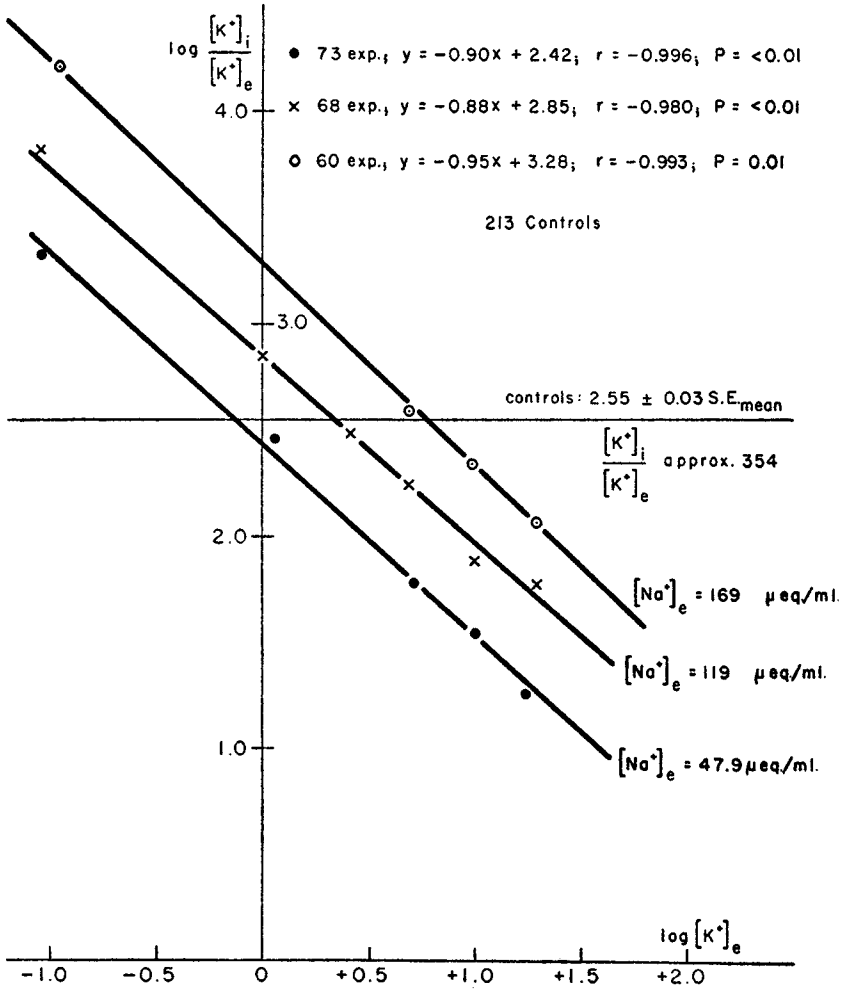


FIG. 3. Dependence of log $[K^+]_i/[K^+]_e$ (intracellular/extracellular potassium ion concentration) on log of $[K^+]_e$ for three extracellular sodium levels of the bath.

A similar analysis of the results, based on $(K^+)_i$, the intracellular potassium content of skin (Table I), leads one to suggest also that potassium is adsorbed in frog skin and that Freundlich's isotherm applies. The relationships between log $(K^+)_i$ or log $\{(K^+)_i/[K^+]_e\}$ and log $[K^+]_e$, however, appear in graphs as

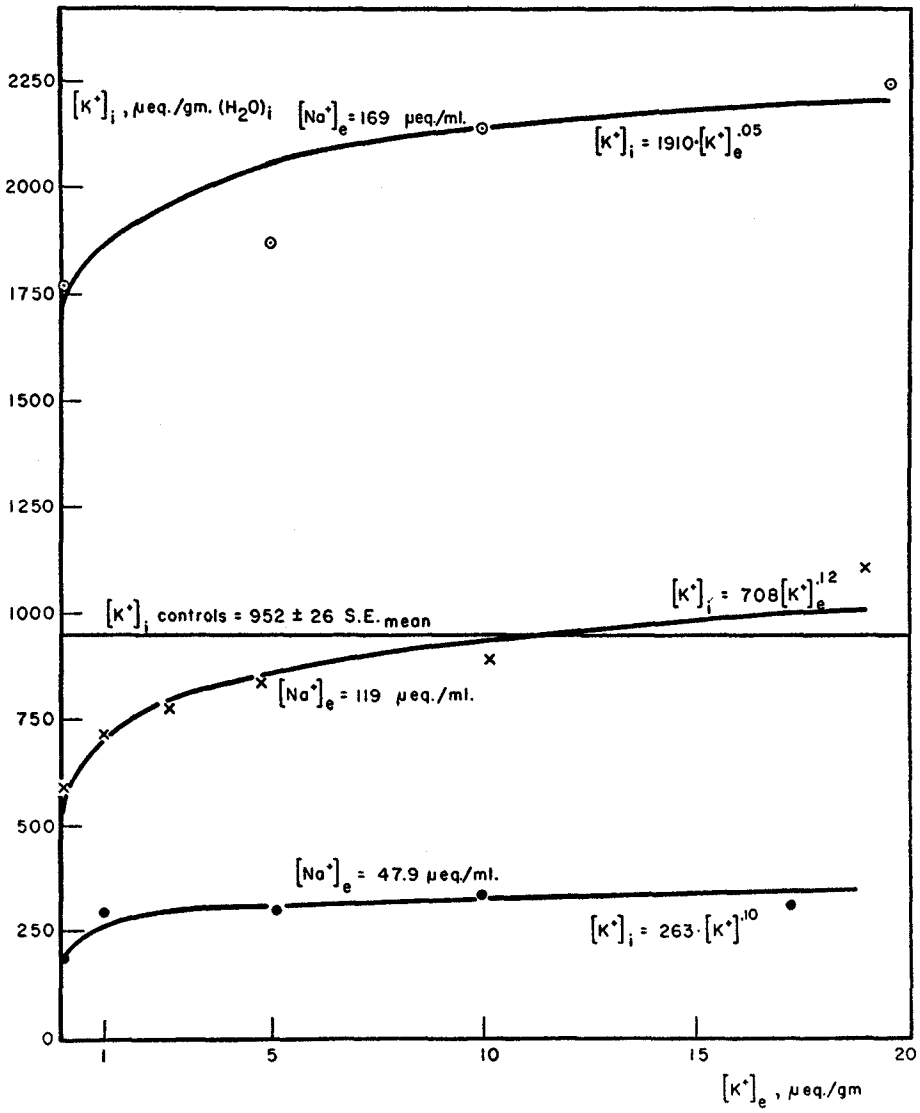


FIG. 4. Potassium isotherms (Freundlich) in frog skin. $t$ = 24–25°C.

slightly curved lines; therefore, this type of analysis leads to somewhat less satisfactory conclusions.

The data of potassium in frog skin were also used in an attempt to see whether they could be fitted to a Langmuir isotherm. This attempt, however, failed. Neither the $(K^+)_i$ nor the $[K^+]_i$ data yielded acceptable values for the "asso-

ciation constant," $k$ (14), in Langmuir's isotherm. *E.g.*, $k$ would vary from $12 \times 10^3$ to $0.9 \times 10^3$, decreasing with increasing $[K^+]_e$, when this is expressed in equivalents per liter.

Of the two mentioned isotherms, Langmuir's has a rational basis and, therefore, must be preferred in the interpretation of data such as presented here. It will be recalled that Langmuir's isotherm can be arrived at by considering adsorption as a bimolecular chemical reaction (14). Such ideal circumstances probably do not exist in the process of binding of potassium by frog skin.

Recently, Sips (31) suggested a variation of Freundlich's isotherm, namely,

$$\theta = \frac{A \cdot p^c}{1 + A \cdot p^c}. \tag{2}$$

$\Theta$ stands for the fraction of the surface (such as a catalyst surface) covered, $p$ for gas pressure. $A$ and $c$ are constants. Sips has investigated the type of distribution of active centers for $c = \frac{1}{2}$ and found a near Gaussian distribution of sites with respect to the energy of adsorption. It has also been pointed out (16) that for $c = 1$, one deals with Langmuir's isotherm. The significance of Sips' isotherm lies in the fact that it provides for a rational transition from the Freundlich isotherm into a Langmuir isotherm or *vice versa.*

In applying these ideas to the case of potassium binding in frog skin, we have calculated values for the "association constant," $k$, from the equation,

$$\frac{(K^+)_i}{(K^+)_i^{max}} = \frac{k\sqrt{[K^+]_e}}{1 + k\sqrt{[K^+]_e}}, \tag{3}$$

in which $(K^+)_i^{max}$ is the saturation value for potassium adsorption or binding in skin. Figures for $(K^+)_i^{max}$ were obtained by extrapolation from a plot of the $(K^+)_i$ data (Table I) against $[K^+]_e$. We have thus found $(K^+)_i^{max} = 0.030$, 0.046, and 0.068 eq./kg. of wet skin for skins in diluted, physiological, and concentrated saline solutions, respectively. All these figures are approximate. It is doubtful whether more reliable data could be obtained experimentally, by keeping skins in solutions of much higher potassium concentrations than those used here. Such procedures might lead to unpredictable changes in the behavior of skin towards potassium. The calculations have given $k$ values of $101 \pm 21$ (standard error of the mean), $104 \pm 14$, and $36 \pm 3$, for skins in diluted, physiological, and concentrated saline, respectively. The accuracy of these values is admittedly not too great. Considering, however, the nature of the studies, involving an *Adsorbens* (skin) which is rather inhomogeneous, one can readily see why the $k$ values cannot be accurate. For the same reason, it is really not appropriate to attach to these figures the meaning of "association constants," as we have done. The nature of the potassium-binding material in skin is as yet unknown and one cannot describe the process in the form of a specific chemical reaction. It may be permissible, however, to refer to $k$ as the "apparent association constant" in potassium binding.

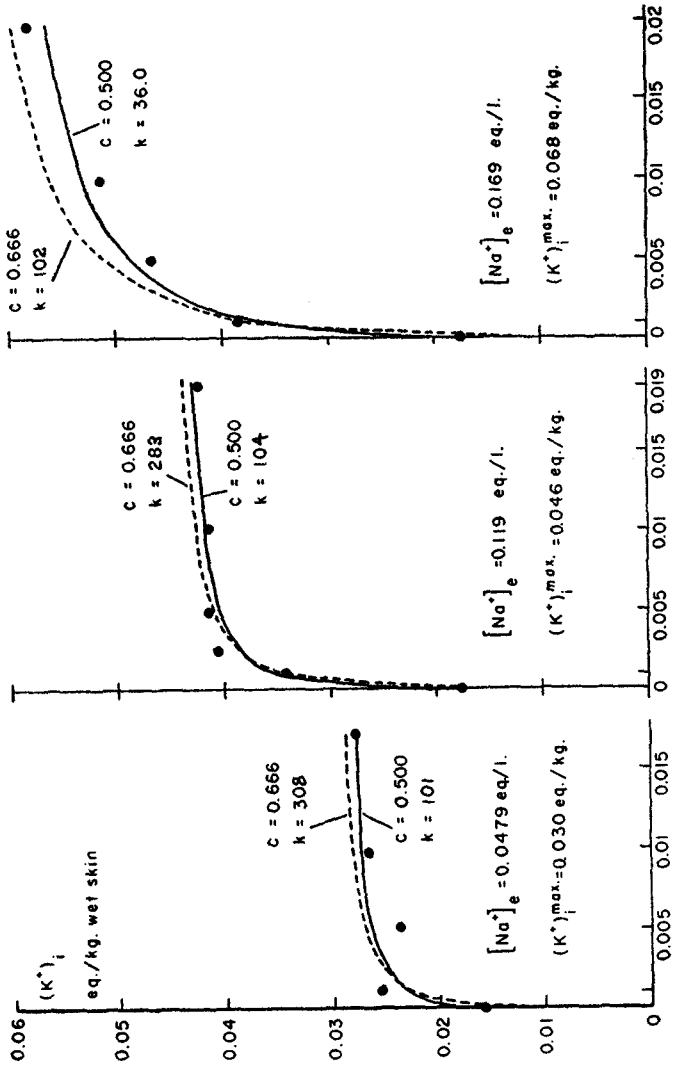


FIG. 5. Potassium isotherms (Sips) in frog skin. $t$ = 24–25°C.

Fig. 5 shows three curves (solid lines) for potassium binding which are based on the $k$ values given above. It can be seen that the experimental data, at a first approximation, seem to satisfy Equation 3. It can also be seen from the dotted lines that values for $k$ and $c$, other than those discussed, lead to isotherms which lie within the range of some of the experimental results. Since the accuracy of those latter $k$ values is smaller than that of the former and since $k$ values, based on calculations with $c = \frac{1}{2}$ probably have a better theoretical foundation, it may be more useful, at present, to describe potassium binding in skin quantitatively, applying isotherm Equation 3.

In considering potassium adsorption in frog skin, the question arises as to the nature of the surfaces involved. This question is related to the problem of the "chloride space" and the "non-chloride space" of skin, which was discussed in section III of this paper. Electron microscopic studies of frog skin have revealed a large surface represented by numerous protoplasmic extensions reaching from cell to cell in the epidermis (27). One may visualize that potassium is adsorbed at this surface. Indirect evidence points to a possible function of the mitochondria in potassium adsorption. Mitochondria in the epithelial cells of frog skin were photographed (27), but their ultrastructure has not yet been observed. It is known that 1,4-dinitrophenol releases potassium from frog skin and inhibits NaCl uptake (5, 19). Dinitrophenol is, furthermore, one of the best known chemicals that uncouple oxidative phosphorylation, a process which appears to be largely localized in the mitochondria (18, 30). It seems to us that these facts should be taken as a hint of a possible participation of frog skin mitochrondria in potassium adsorption and active sodium transport across the skin. The importance of mitochondria in relation to the electrolyte metabolism of secretory cells has also been stressed by others (1, 2, 26, 34).

## SUMMARY

1. The "chloride space" in frog skin was determined and found to be 69.7 per cent by weight of wet skin. The chloride space occupies about 94 per cent of the total water space of skin. From this and other information, it appears that the "non-chloride space" measures only a part of the space occupied by the structural elements of skin. This space is referred to here as the intracellular compartment and the remainder as the extracellular compartment of frog skin. On this basis, potassium and sodium in skin are distributed as follows: total sodium, 60 to 75 $\mu$eq./gm. of wet skin; all sodium is probably extracellular; total potassium, 39 to 49 $\mu$eq./gm.; intracellular potassium, 37 to 47 $\mu$eq./gm.

2. Skins were immersed in solutions differing from each other in their sodium and potassium concentrations. Three levels of NaCl were studied: 48, 119, and 169 $\mu$eq./ml. For each of these solutions (referred to below as diluted, physiological, and concentrated saline), the potassium levels were varied from 0.1 to 20 $\mu$eq./ml. For skins in solutions low in potassium and high in sodium, it was

found that an exchange of intracellular potassium against extracellular sodium occurs. The ratio for the number of potassium ions lost/number of sodium ions gained was 4:1, 4:6, and 4:8 for skin in $K^+$-free diluted, physiological, and concentrated saline, respectively.

3. Uptake of NaCl by the epithelium of frog skin is dependent on the potassium concentration of the environment. For skins in physiological saline, net uptake of NaCl was optimal (0.90 $\mu$eq. $\times$ cm.$^{-2}$ $\times$ hr.$^{-1}$) at 1 to 5 $\mu$eq. $K^+$/ml. For skins in diluted and concentrated saline optimal NaCl uptake was seen at potassium concentrations of approximately 5 and 10 $\mu$eq. $K^+$/ml., respectively. Net uptake of NaCl by the skin is also discussed, with relation to the potassium balance of skin.

4. Skin potentials decreased with increasing extracellular potassium concentration when diluted saline solutions were used. The opposite of this was found for skins in concentrated saline. For skins in physiological saline, skin potentials rose sharply from rather low values, when placed in solutions very low in potassium, to relatively high values, when immersed in solutions containing 1 to 5 $\mu$eq. $K^+$/ml. Further increase in potassium concentration of the bath led to slight reductions in skin potentials. The highest potentials observed were of the order of 40 mv. In all cases studied, the inside was positive with relation to the outside.

5. It can be shown that values for intracellular potassium concentration as a function of extracellular potassium concentration satisfy, at a first but good approximation, Freundlich's isotherm. A modification of Freundlich's isotherm, recently introduced by Sips, may also be used to correlate the experimental data quantitatively. Since the latter isotherm has a rational interpretation, it is suggested that this be used, rather than Freundlich's isotherm, to express quantitatively the dependence of intracellular on extracellular potassium in frog skin.

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