Effect of Changes in Transepithelial Transport on the Uptake of Sodium across the Outer Surface of the Frog Skin

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ABSTRACT The unidirectional sodium uptake at the outer surface of the frog skin was measured by the method described by Biber and Curran (8). With bathing solutions containing 6 mM NaCl there is a good correlation between sodium uptake and short-circuit current (SCC) measured simultaneously except that the average uptake is about 40% higher than the average SCC. The discrepancy between uptake and SCC increases approximately in proportion to an increase in sodium concentration of the bathing solutions. Amiloride inhibits the unidirectional sodium uptake by 21 and 69% at a sodium concentration of 115 and 6 mM, respectively. This indicates that amiloride acts on the entry step of sodium but additional effects cannot be excluded. The sodium uptake is not affected by 10^-4 M ouabain at a sodium concentration of 115 mM but is inhibited by 40% at a sodium concentration of 6 mM. Replacement of air by nitrogen leads to a 40% decrease of sodium uptake at a sodium concentration of 6 mM. The results support the view proposed previously (8) that the sodium uptake is made up of two components, a linear component which is, essentially, not involved in transepithelial movement of sodium and a saturating component which reflects changes in transepithelial transport. Amiloride, seems largely to affect the saturating component.

Active transport of Na across the frog skin has been studied extensively in vitro ever since the initial discovery that the frog can pick up NaCl from surrounding solutions as dilute as 10^-9 M (2) and transport it across the skin (1–3). Koefoed-Johnsen and Ussing proposed a model for Na transport across epithelial cells of the frog skin (4) in which they suggested that Na enters the cells from the outside by diffusion and is extruded from the cells at the serosal side by an active transport step. The entry of Na from the outside was thought to proceed by diffusion because the outer surface of the skin behaves like a Na electrode under open circuit conditions. Since then, however, several investigators (5–7) have made observations which suggest that the entry of
Na across the outside boundary of the skin may not be due to simple diffusion. More clear-cut evidence for this view comes from direct measurements of the unidirectional uptake of Na across the outside surface of the skin (8). The method developed for these experiments allows a direct comparison to be made in a single preparation, of the sodium influx across the outer surface and the net movement of Na across the whole skin. The present experiments were carried out to determine whether changes in active transport of Na across the whole skin affect the Na influx across the outer surface of the skin. Such experiments should give us more information about the validity of the influx measurements and the relationship between active transport of Na and the Na influx. The rate of active Na transport was varied by changes of Na concentration and by the use of amiloride, ouabain, and oxygen deprivation.

METHODS

The method for the influx measurements has been described in detail previously (8). A circular piece of the abdominal skin of *Rana pipiens* was mounted in the influx chamber and bathed on both sides by isotonic Ringer solutions. The chemical composition of the solutions was always identical on both sides of the skin. At time zero, the solution in contact with the outside surface of the skin was replaced by a solution of identical composition except that 8 μCi 22Na or 5–15 μCi 24Na and 6 μCi tritiated inulin had been added. After a short exposure of 30 sec, the skin was removed from the chamber and in rapid succession blotted on filter paper and punched out of the holder. The skins were extracted in nitric acid and aliquots of the eluate were assayed for radioactive Na and inulin. Inulin served as an indicator for the volume of outside solution remaining on the outside surface of the frog skin after blotting.

The accumulation of radioactive Na in the skin is a linear function of time within the first 34 sec of exposure to the tracer, timed from beginning of injection of tracer into the chamber to blotting of the skin. During this time no significant amount of tracer appears in the solution bathing the inside of the skin. Two types of Ringer solutions were used for the influx experiments. One solution contained 115 mM NaCl, 2.5 mM KHCO₃, and 1.0 mM CaCl₂. In the other solution all but 6.4 mM NaCl was replaced by choline chloride.

The conditions under which the effect of amiloride, ouabain, and nitrogen was studied may affect the linearity of uptake of radioactive sodium with time. Therefore, Na influx determinations were carried out at a Na concentration of 6 mM in the presence of amiloride, ouabain, and anaerobiosis, using different times of exposure to the radioactive test solution. The Na influx values calculated from tracer uptake obtained after an exposure time of 16 sec did not differ significantly from those obtained in the same frogs after an exposure time of 32 sec. Within this time, no tracer appeared in the inside bathing solution. These measurements indicated that the uptake of radioactive Na is a linear function of time for up to 32 sec in the presence.

1 Throughout the paper this flux will be called simply influx or $J_N$.
2 In a few experiments tritiated inulin was replaced by tritiated mannitol as indicator for the test solution since mannitol is also suitable for that purpose.
of amiloride, ouabain, and nitrogen. The method may therefore be used to measure the unidirectional uptake of sodium under these conditions.

The fast time response to amiloride was studied by feeding the recording outputs (PD and SCC) of the automatic clamping device into an oscilloscope (Tektronix, Inc.) and by tracing pictures taken from the oscilloscope screen. Initially, the chamber bathing the outside surface of the skin was filled with 1.96 ml of a solution which contained 0.3 mM NaCl, 82 mM choline chloride, 2.5 mM KHCO₃, and 1 mM CaCl₂. Then, 0.14 ml of a test solution was injected very rapidly into the outside chamber. The test solutions contained appropriate amounts of NaCl, KHCO₃, and CaCl₂ to yield final concentrations of 38 mM NaCl, 77 mM choline chloride, 2.5 mM KHCO₃, and 1 mM CaCl₂. In some test solutions amiloride (N-amidino-3, 5-diamino-6-chloropyrazinecarboxamide) was added to give a final concentration of 10⁻⁴ M.

Averages are given with standard errors of the mean (SEM). Where necessary, the number of observations is added in parentheses. In order to give equal weight to each measurement the average values for short-circuit current and for sodium influx in Tables I through VI are calculated from all individual measurements rather than from the average obtained in each frog. These average values differ somewhat from the corresponding values computed from the averages obtained in each frog since the number of observations per frog varied. However, the differences between the two series of values computed by these two procedures are small and do not affect the conclusions drawn from the results. The ratios of experimental skins over control skins were calculated from the ratio obtained in each frog.

RESULTS

Two different methods were employed to test whether amiloride acts at the outside of the frog skin and therefore on the entry step of Na. The first approach made use of the fact that the transepithelial potential difference (PD) across the frog skin responds very rapidly to changes in Na concentration in the outside bathing solution (9). A similar response can be observed for the short-circuit current as shown in Fig. 1. A sudden increase of Na concentration in the outside bathing solution from 0.3 to 38 mM by injection of 0.14 ml test solution into 1.96 ml of practically sodium-free solution causes a rapid increase in transepithelial PD and SCC. The SCC (Fig. 1 A) reaches a maximum value of 100 μA within about 100 msec. The transepithelial PD was recorded simultaneously in order to test whether complete short-circuiting is maintained during this procedure. The tracing shows that the PD rises to a maximum of 4 mV when the test solution is injected. This relatively short and modest deviation from zero clamping conditions seems negligible since the transepithelial PD of the same skin reaches 98 mV under open circuit conditions (Fig. 1 B).

The shape of the curves of the SCC or of the PD can be changed by addition of amiloride to the test solution containing Na prior to injection. A tracing showing inhibition by amiloride is presented in Fig. 2. The same preparation
was used for all the curves depicted in Figs. 1 and 2. The tracings marked as controls in Fig. 2 represent current changes resulting from injection of sodium chloride solution alone. They were obtained before (2 A) and after (2 B) carrying out the test run during which a mixture of sodium chloride and amiloride was injected. The curves with the label amiloride in Fig. 2 A and B were recorded during the test run. It was possible to superimpose the control and amiloride curves since the initial part of the curves was for all practical purposes identical. The curve obtained with amiloride departs from the control curves between 20 and 40 msec after the starting point of the rising phase. The transepithelial PD was recorded in each instance and was very similar to the curve shown at the top of Fig 1 A. Injection of 0.14 ml of test solution of the same composition as the solution in the outside chamber did not cause any significant change in transepithelial PD or SCC.
The second approach for examining the action of amiloride on the entry step involved the measurement of the unidirectional Na influx across the outer surface of the frog skin. Four frogs were used to test the action of amiloride at a Na concentration of 115 mM. Influx measurements were obtained from 25 control skins and from 22 amiloride-treated skins in an alternating fashion. Amiloride, at a final concentration of $10^{-5}$ M, was added 12 min prior to the influx determination since it was found that a stable value of SCC (slope

\[ \text{TABLE I} \]

**EFFECT OF AMILORIDE ON \( J^\text{Na} \) INFLUX AND SHORT-CIRCUIT CURRENT (SCC) AT A Na CONCENTRATION OF 115 mM**

|               | SCC       | \( J^\text{Na} \)  |
|---------------|-----------|-------------------|
|               | $\mu\text{Eq} hr^{-1} cm^{-2}$ | $\mu\text{Eq} hr^{-1} cm^{-2}$ |
| Control       | 2.82±0.20 (25) | 9.55±0.34 (25)    |
| Amiloride     | 0.35±0.03 (22) | 7.43±0.46 (22)    |
| Amiloride     | 0.13±0.02 (4)  | 0.79±0.04 (4)     |
| Control       |            |                   |

The values are given in $\mu\text{Eq} hr^{-1} cm^{-2}$.

less than 1% per min) was achieved under these conditions. The average values for SCC and Na influx (\( J^\text{Na} \)) are given in Table I. There is approximately a 20% inhibition of the Na influx but the Na transport across the epithelium is inhibited far more, by 87%. The Na influx in the control skins is more than three times as high as the net movement of Na across the epithelium.

The effect of amiloride on Na influx was also measured at a Na concentration of 6 mM. The results of 25 experimental and 27 control fluxes performed on 5 frogs are listed in Table II. The inhibition of the short-circuit current was

\[ \text{TABLE II} \]

**EFFECT OF AMILORIDE ON \( J^\text{Na} \) INFLUX AND SHORT-CIRCUIT CURRENT (SCC) AT A Na CONCENTRATION OF 6 mM**

| Experiment | SCC Amiloride | SCC Control | SCC amiloride | \( J^\text{Na} \) Amiloride | \( J^\text{Na} \) Control | \( J^\text{Na} \) amiloride |
|------------|---------------|-------------|---------------|---------------------------|------------------------|-------------------------|
| 1          | 0.08          | 2.28        | 0.04          | 0.49                      | 2.65                   | 0.19                    |
| 2          | 0.39          | 1.61        | 0.24          | 0.73                      | 2.67                   | 0.27                    |
| 3          | 0.14          | 1.21        | 0.12          | 0.65                      | 1.61                   | 0.40                    |
| 4          | 0.29          | 1.88        | 0.15          | 0.76                      | 2.65                   | 0.29                    |
| 5          | 0.32          | 1.42        | 0.23          | 0.81                      | 1.96                   | 0.41                    |
|            | Average       | 0.23±0.03   | 1.66±0.13     | 0.16±0.04                 | 0.68±0.05              | 2.27±0.15               |

The values are given in $\mu\text{Eq} hr^{-1} cm^{-2}$. 
comparable to the one seen at 115 mM but the effect on Na influx is much more pronounced (69% inhibition) than at the higher Na concentration. In the first three experiments in Table II, amiloride was added to the outside bathing solution 12 min before the influx measurement to give a final concentration of 10^-6 M. In the last two experiments amiloride was added simultaneously with the tracer. Since the influx was measured over the same 30 sec period amiloride must have an immediate effect on the sodium influx for the

![Graph](image1)

FIGURE 3
Correlation between Na influx (JNa) and short-circuit current (SCC) with a sodium concentration of 6 mM in the bathing solutions.

FIGURE 4
Correlation between Na influx (JNa) and short-circuit current (SCC) with a sodium concentration of 115 mM in the bathing solutions.

inhibition of SCC and Na influx is not significantly different from the corresponding values seen in the first three experiments.

The individual flux values of the five experiments listed in Table II are used in Fig. 3 for a plot of the influx against the short-circuit current measured simultaneously. The open circles represent the control values. The influx values for the controls varied from around 1 to 4 μEq hr⁻¹ cm⁻². The plot of influx vs. short-circuit current reveals a surprisingly close relationship between the two parameters for the control values. Additionally, three points are of
interest. First, the slope of the regression line is 1.08. Since the SCC represents net movement of Na (that is influx minus outflux) across the skin, the transepithelial influx would be expected to be about 10% higher than the net flux. Therefore, the slope of the unidirectional Na influx across the outside surface measured here may be very close indeed to the slope expected for the transepithelial Na influx. Second, the intercept is somewhat greater than zero. Third, inspection of Fig. 3 suggests that the points obtained from amiloride-treated skins fall in line with the points obtained from control skins. In fact, the slope and intercept of the regression line obtained from the 25 amiloride-treated skins alone is 1.02 (±0.27) x +0.45 (±0.07) and has a correlation coefficient of 0.620. The regression line calculated from all the points on Fig. 3 is very similar (1.08 (±0.04) x +0.45 (±0.055)).

The averages of the four experiments which are summarized in Table I have been used to plot in Fig. 4 the Na influx ($J_N^A$) obtained at 115 mM against the short-circuit current (SCC) of the same skins. The average of the control values is connected by a line with the average of the amiloride-treated skins of the same frog. Average values rather than individual values were used because the scatter is much more pronounced than at 6 mM. The regression line calculated from all 47 measurements has a slope which is somewhat smaller but not significantly different from one. The intercept lies at 7.07 μEq hr⁻¹ cm⁻² and is, therefore, 16 times larger than the intercept obtained at a Na concentration of 6 mM.

Four frogs were tested for an effect of ouabain (final concentration 10⁻⁴ M) on Na influx at a Na concentration of 115 mM. The 21 control influxes and 23 experimental influxes do not differ significantly (Table III). In these experiments, as in the ones carried out at 6 mM Na, ouabain was added to both sides of the frog skin 40–70 min before measurement of the influxes. Experimental and control measurements were performed in an alternating way. Using the same experimental procedures, the effect of ouabain was tested at a Na concentration of 6 mM. The results of 51 control measurements and 47

| Table III | EFFECT OF OUABAIN ON Na INFLUX ($J_N^A$) AND SHORT-CIRCUIT CURRENT (SCC) AT A Na CONCENTRATION OF 115 mM |
|-----------|---------------------------------------------------------------------------------------------------------|
| SCC       | $J_N^A$                                                                                                  |
|           | μEq hr⁻¹ cm⁻² | μEq hr⁻¹ cm⁻² |
| Control   | 1.93±0.28 (21) | 7.27±0.47 (21) |
| Ouabain   | 0.15±0.02 (23) | 7.78±0.48 (23) |
| Ouabain   | 0.12±0.04 (4)  | 1.07±0.07 (4)  |
| Control   | 7.07 ±0.28 (4) | 1.07±0.07 (4)  |
Determinations in ouabain-treated skins carried out in 10 frogs are listed in Table IV. The inhibition of the net movement of Na across the epithelium by ouabain is similar to the one observed at a Na concentration of 115 mM (SCC ratio of 0.17 compared to 0.12 at 115 mM), but in this case, there is a clear reduction of Na influx in the ouabain-treated skins. The average influx is significantly decreased from 1.20 to 0.68 µEq hr⁻¹ cm⁻² and the average of the ratios of ouabain-treated over control skins obtained for each experiment is 0.60.

**Table IV**

**Effect of Ouabain on Na Influx (JNa) and Short-Circuit Current (SCC)**

|        | SCC       | JNa       |
|--------|-----------|-----------|
|        | µEq hr⁻¹ cm⁻² | µEq hr⁻¹ cm⁻² |
| Control| 0.73±0.08 (51) | 1.20±0.08 (51) |
| Ouabain| 0.10±0.01 (47) | 0.68±0.05 (47) |
| Ouabain| 0.17±0.03 (10) | 0.60±0.03 (10) |

**Table V**

**Effect of Nitrogen on Na Influx (JNa) and Short-Circuit Current (SCC)**

|        | SCC       | JNa       |
|--------|-----------|-----------|
|        | µEq hr⁻¹ cm⁻² | µEq hr⁻¹ cm⁻² |
| Control| 0.90±0.11 (14) | 1.19±0.09 (14) |
| Nitrogen| 0.23±0.02 (14) | 0.71±0.04 (14) |
| Nitrogen| 0.29±0.06 (4)  | 0.60±0.05 (4)  |

When the air bubbling the chambers is replaced by pure nitrogen, a decrease in SCC and PD occurs within a few minutes due to lack of oxygen. Four frogs were used to test the effect of such nitrogen replacement on the Na influx at a Na concentration of 6 mM. Influx measurements were alternated between control skins (14 observations) and nitrogen-treated skins (14 observations). Exposure time to nitrogen was 40–60 min. The results are listed in Table V. The SCC was reduced to about one-third in the experimental skins and the Na influx was 60% of the control values.
DISCUSSION

Biber and Curran (8) obtained evidence that the Na influx measured by the method used in this paper was composed of the sum of a linear component and a saturating component. Their results also raised the possibility that only the saturating component contributes to the transepithelial movement of Na. They found that the linear component accounted for most of the influx at a sodium concentration of 115 mM but for only 20% at a sodium concentration of 6 mM. The present results were analyzed for the relation between the Na influx and the SCC (Figs. 3 and 4) and appear to be entirely consistent with this view.

If there is a linear component that is exclusively a function of sodium concentration but not of net sodium movement across the epithelium, it should raise all points on such a plot of Na influx vs. SCC by an equal amount above the line of identity and the intercept of the regression line should be a measure of this linear component of the influx. Moreover, the intercept should be proportional to the sodium concentration. The intercept of the regression line calculated from the points obtained at 6.4 mM Na is 0.45 µEq hr⁻¹ cm⁻². The values for the amiloride-treated skins fall in line with the controls suggesting that the linear component is not greatly affected by amiloride. At a Na concentration of 115 mM the intercept on a corresponding plot should be about 18 times greater than at 6.4 mM. In fact, the intercept of the regression line calculated from individual fluxes obtained at 115 mM Na is 16 times as high as the one obtained at 6.4 mM Na.

As shown in Fig. 3 changes in Na influx are closely correlated with changes in net Na transport (SCC). However, the average of the influx values obtained under control conditions here and in a previous publication (8) at a Na concentration of 6 mM is 40% larger than the SCC measured simultaneously during the influx determinations. Under steady-state conditions the influx across the outer surface of the skin has to be at least as large as the transepithelial influx of Na. This transepithelial influx of Na is usually up to 20% greater than the SCC (8, Table II). Thus, the measurements of the Na influx across the outer surface of the skin are about 20% larger than the transepithelial influx estimated from the SCC. Such a difference could be accounted for by the linear component provided that this component is not contributing to transepithelial movement of sodium.

The fact that the values for the influx measurements are larger than those predicted for the transepithelial influx and that these measurements show a good correlation with the SCC rules out a suggestion made by Rotunno et al. (10) that the estimates of Na influx obtained with this method may be artificially low due to an effect produced by damaged edges. It should be added
in this context that the measurements of the SCC obtained in these chambers are comparable or even higher than those obtained by others (6, 11-13).

The effect of amiloride on the Na transport across the amphibian skin and bladder has been studied extensively by various investigators (12-19) and a number of experiments have led to the suggestion that amiloride acts on the entry step of Na across the outside (mucosal) surface of the epithelial cells. In all cases, however, such conclusions were based on indirect approaches such as effects on vasopressin action, on exchangeable intracellular Na concentration or active Na transport pool or indirect kinetic methods. In principle, some objections can be raised to any of these conclusions and more direct information seems desirable.

The present experiments indicate clearly that amiloride acts on the Na influx across the outside surface. Furthermore, two points suggest that amiloride has its primary effect on the saturating component of the influx. First, the average inhibition of the influx is only 21% at a Na concentration of 115 mM but increases to 69% at a Na concentration of 6 mM and thus approaches the 84% reduction in SCC. In other words, at a Na concentration of 115 mM the effect of amiloride on the saturating component may be partly masked by the large and essentially unchanged linear component. Second, as pointed out above, at a Na concentration of 6 mM the individual points for Na influx obtained in the presence of amiloride fall in line with the control values when plotted against the SCC (Fig. 3). On the other hand, the results do not permit one to rule out a small effect on the linear component of the influx or an additional effect further along the active Na transport pathway, for example, on the extrusion of Na at the intercellular or serosal boundaries of the cells or in the intercellular spaces.

Additional support for an action of amiloride at the outside of the frog skin is given by the oscilloscope tracings obtained from experiments in which NaCl or a combination of NaCl and amiloride was injected rapidly into the outside solution. It is not known whether under such conditions the SCC represents actual movement of Na across the epithelium. Nevertheless, superimposition of control and experimental curves indicates that amiloride acts only about 20-40 msec after the beginning of the effect due to addition of Na ions alone. Presumably, amiloride and Na have to diffuse through a thin unstirred layer located at the outside surface of the skin and possibly through one or two cornified layers to reach the site of action. The difference in molecular size between amiloride and hydrated Na ions could explain the delayed action of amiloride on such grounds since diffusional delay will cause a slower increase in concentration of amiloride than in concentration of Na.

Previous experiments suggested that the entry of Na into the frog skin is not due to simple diffusion alone but that both Na and Li interact, at least partly, with a membrane component during influx (8). Three possibilities of inter-
action were considered for the portion of the influx exhibiting saturation kinetics and inhibitory effects, namely an active transport process, a "carrier"-mediated facilitated diffusion, and a "carrier"-mediated exchange diffusion. It is unlikely that exchange diffusion is entirely responsible for this interaction since the experiments carried out so far with this method (8 and present paper) suggest strongly that the saturating component is linked to a net movement of sodium and exchange diffusion cannot bring about a net movement of ions. An active transport step for entry of Na at the outer surface of the skin has been considered (5, 7). Using the technique described by Andersen and Zerahn (20) Zerahn observed "within the limitations of the methods used that the Na pool behaves as if it has passed the transport mechanism" (7). He concluded that "the mechanism for active transport of Na is effective at the outer surface of the frog skin." However, the presence of a small amount of tracer in a compartment which precedes the active transport step may not be detected by this method. Recent experiments carried out in Ussing's laboratory (21) suggest that most, if not all, active transport of Na takes place in the outermost living cell layer. A few seconds after removal of the tracer from the outside surface the amount of tracer in the outermost living cell layer may be very small indeed since the volume of these cells is small compared to the total volume of the epithelium and since the tracer may proceed extremely rapidly from the outside solution into and across the first cell layer into the deeper layers of the epithelium. Hence, the method used by Zerahn may not allow the distinction between an active transport step and diffusion for entry of sodium across the outside surface of the skin since it may not distinguish between an active transport step at either side of the first living cell layer. On the other hand, recent observations by Biber and Curran (8) do support the possibility that the entry of Na across the outside surface may proceed by an active transport step.

In the present experiments, an attempt was made to obtain further information on the nature of Na movement across the outer barrier by examining the effects of ouabain and anoxia on the process. Cardiac glycosides are thought to block active transport of Na and K selectively in many systems including the frog skin. Therefore, it seemed of interest to test the effect of this drug on the influx of Na. Ouabain inhibits the Na influx at a Na concentration of 6 mM but not at a Na concentration of 115 mM. Such a difference in action is to be expected if ouabain affects the saturating portion of the influx only. Considering the ratios of experimental over control values one finds an important difference between the action of ouabain and amiloride though. The inhibitory effect of ouabain is much less pronounced than that of amiloride although the inhibition of the SCC is very similar. The cause of this difference is not known but three possibilities are of special interest. First, the linear component may be increased not only in the ouabain-treated skins.
but also in the preparations used as controls thus masking a more pronounced effect on the saturating component. A comparison of the control SCC obtained in the amiloride experiments at a Na concentration of 6 mM (Table II) with the control SCC measured in the ouabain (Table IV) and nitrogen (Table V) experiments shows that the values in the latter two groups are considerably lower than in the first one. This difference can be explained by the delay of control and experimental influx measurements caused by incubation in ouabain or nitrogen since the SCC declines with time. Such a decrease in SCC may not be accompanied by a corresponding decrease in the linear component. The linear component may therefore contribute proportionally more to the influx in the ouabain and nitrogen experiments than in the amiloride experiments and a comparison of the fractional inhibition between the groups may be misleading. For this reason, a direct comparison has been made in Table VI between the decrease of the SCC and the reduction of the influx. The changes in influx are smaller (about 20% for amiloride and ouabain and about 40% for nitrogen) than the changes seen in net Na flux (SCC). Such a difference could be explained either by a relatively modest increase of the linear component of the influx in the experimental group or, as will be pointed out below, by an additional effect of these drugs at a different step of the sodium transport system.

Second, ouabain may act on two distinctly different steps of the Na transport pathway, on the entry across the outside surface and on the extrusion of Na across the serosal and intercellular boundaries of the cells. Ouabain may diffuse much more readily to the sites located at the serosal and intercellular boundaries than to the site of action for the entry step. In fact, an effect of ouabain on the SCC can only be shown when ouabain is added to the serosal side of the skin. The third possibility is that the ouabain acts on the Na influx indirectly. Ouabain could have an effect on Na influx via a change in intracellular potential or an increase in Na concentration induced by an inhibition of the active transport across the serosal and intercellular boundaries of the

| Table VI | COMPARISON OF DECREASE OF SCC AND \( J^{N_a} \) AT A SODIUM CONCENTRATION OF 6 mM |
|---|---|
| SCC \( \mu \text{Eq hr}^{-1} \text{cm}^{-2} \) | \( J^{N_a} \mu \text{Eq hr}^{-1} \text{cm}^{-2} \) |
| Amiloride* | 1.43 | 1.59 |
| Ouabain* | 0.63 | 0.52 |
| Nitrogen* | 0.67 | 0.48 |

* Average of the experimental values subtracted from the average of the control values.
epithelial cells. Na entry by means of a carrier-mediated transport system across the outside cell surface could be affected either by a change in potential difference across this boundary or by a rise in intracellular Na concentration.

The effect of nitrogen is interesting because it seems to be readily reversible. In most instances reintroduction of air into the system causes a rise in SCC within seconds, even after an exposure to nitrogen as long as 20–40 min. Such findings suggest that the active transport of sodium across the skin is reversibly suppressed by anaerobiosis. This reversible depression of net transfer of sodium across the skin is accompanied by a fall in the uptake of sodium at the outer surface of the epithelium indicating that not only the transepithelial transport but also the unidirectional uptake of sodium is at least partly dependent on oxidative metabolism.

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