J. Physiol. (1974), **237**, *pp.* 555–571
With 11 *text-figures*
Printed in Great Britain

THE SODIUM-TRANSPORTING COMPARTMENT OF THE EPITHELIUM OF FROG SKIN

BY M. CEREIJIDO, C. A. RABITO, E. RODRÍGUEZ BOULAN AND CATALINA A. ROTUNNO

From the Department of Biophysics (CIMAE), Luis Viale 2831, Buenos Aires, and the Department of Physical Chemistry, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

(*Received 6 August 1973*)

SUMMARY

1. The abdominal frog skin was mounted between two chambers containing Ringer with 1 mM-Na on the outside and 115 mM-Na on the inside. When the Na concentration of the outer solution ($[Na]_o$) is instantaneously raised from 1 to 50 mM, the short circuit current (I) increases to a new value in less than a second, and becomes essentially time-independent. Only in a few experiments was it observed to increase further, although at a much slower rate.

2. At a time t after this increase, the addition of 10^{-4} M amiloride to the outer solution produces an exponential decrease of I. The area under this exponential curve is generally taken to reflect the existence of a Na-transporting compartment (NaTC).

3. The amount of Na represented by NaTC is a function of t: it increases from $1{\cdot}7 \times 10^{-9}$ mole.cm^{-2}, at $t = 10$ sec, to $22{\cdot}8 \times 10^{-9}$ mole.cm^{-2} at $t = 10$ min.

4. In view of the fact that (*a*) I is not a function of the size of the 'NaTC' and (*b*) that whereas I reaches a steady value in a fraction of a second the size of NaTC keeps increasing for minutes, it is proposed that the 'NaTC' represents an amount of Na which is not located along the main route of transepithelial transport.

5. On the assumption that the NaTC is located in a cellular compartment and that, in order to accumulate in this compartment Na should be accompanied by a permeable anion, a series of experiments were performed with Ringer in which Cl^- was replaced by gluconate. It was observed as expected, that NaTC in gluconate is 164 times smaller than in Cl^-, but I only decreases to one half its value in Cl^- Ringer.

INTRODUCTION

The concept that, in order to be transported across the epithelium, Na penetrates passively through the outer barrier of the cells, and is pumped inwards from there across the inner barrier (Koefoed-Johnson & Ussing, 1958) has played a key role in our understanding of the process of ion movement across epithelial membranes. Ironically, this fruitful concept 'seems to present more difficulties in the case of the frog skin for which it was originally proposed than for most other epithelia' (Ussing, 1970). The main difficulties are concerned with the number and nature of the barriers traversed by Na^+, and the size and location of the Na-transporting compartment (NaTC) that would be located between these barriers.

The difficulties with respect to the nature of the barriers arise from the fact that Na can penetrate the outer border of the epithelium in a net amount from diluted solutions, and this apparently occurs in the absence of an electrochemical potential gradient (Rotunno, Pouchan & Cereijido, 1966). In order to overcome this difficulty several hypotheses have been proposed: (*a*) that Na is translocated through a series arrangement of compartments of progressively higher Na concentration, each one possessing an inwardly oriented pump (Biber, Chez & Curran, 1966); (*b*) that at the outer barrier Na^+ is exchanged for another cation (Leblanc & Morel, 1972); (*c*) that the outer barrier might possess an inwardly oriented Na pump (Biber & Curran, 1970; Cuthbert, 1972).

As regards the NaTC, the difficulties are related mainly to its size and location. The estimated size of the pool varies widely, depending on the different experimental approaches used. It ranges from less than 10^{-9} to 1720×10^{-9} mole of Na^+ per square centimetre of epithelium (Hoshiko & Ussing, 1960; Andersen & Zerahn, 1963; Curran, Herrera & Flanigan, 1963; Aceves & Erlij, 1971; Cuthbert, 1972; Moreno, Reisin, Rodriguez Boulan, Rotunno & Cereijido, 1973). Moreno *et al.* (1973) have recently compared the uptake of Na in the presence of amiloride, with the uptake in the presence of ouabain. On the basis that amiloride stops penetration of Na across the outer barrier, and ouabain stops Na extrusion at the inner barrier, the difference between the two uptakes was expected to reflect the size of the NaTC. Yet they found no difference between the two sets of measurements, and concluded that either the NaTC does not exist (except for the Na bound to the mechanism operating the translocation), or else that in the presence of ouabain the Na permeability of the outer barrier is drastically reduced, so that both amiloride (directly) and ouabain (indirectly) stop Na penetration at the outer barrier. One may conclude that the available information on the nature and size of the NaTC, if not controversial, does not offer a clear picture.

In this paper a new approach is used to evaluate the size of the NaTC. It is based on two sets of observations: (1) when the concentration of Na in the outer solution ($[Na]_o$) is suddenly increased from 1 to 50 mM, the electrical potential difference and the short-circuit current (I), which is thought to reflect the active translocation of Na^+, rise to a new steady value with a delay due mainly to unstirred layers (Kidder, Cereijido & Curran, 1964; Dainty & House, 1966; Biber, 1971). This means that, as soon as $[Na]_o$ achieves its new (higher) value at the outer barrier, I reaches 80–100 % of its new (higher) value; (2) when the penetration of Na into the epithelium is instantaneously interrupted by adding amiloride to the outer solution I decays exponentially. The area under the transient of I is generally taken to represent the NaTC (Cuthbert, 1971, 1972). The approach used in this paper consists in (*a*) increasing $[Na]_o$ from

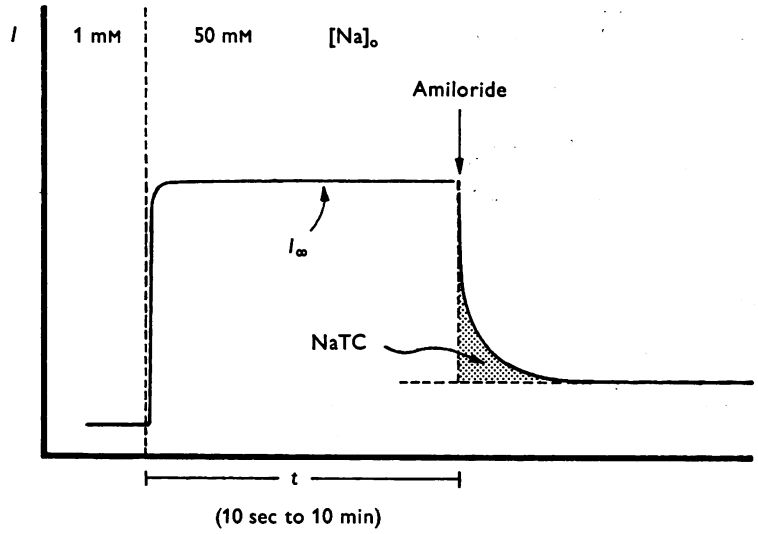

Fig. 1. Experimental protocol to study the time course of I. The skin is initially bathed in Ringer with 1 mM-NaCl on the outside and 115 mM-NaCl on the inside. At zero time (interrupted line) the concentration of Na on the outside $[Na]_o$ is instantaneously changed from 1 to 50 mM. After a given period (t) of exposure to 50 mM-NaCl, amiloride 10^{-4} M is added at the outer bathing solution. The area under the transient of the curve (shaded) is thought to reflect the size of the NaTC.

1 to 50 mM, thus eliciting an increase of I (Fig. 1) and (*b*) after a given period of time (t) ranging from 10 sec to 10 min adding amiloride with the consequent drop of I. It is observed that, whereas 80–100 % of the new value of I (I_∞ in Fig. 1) is reached in a fraction of a second, NaTC is a much slower function of t. Data and arguments are presented on the

relationship between I and NaTC which suggest that NaTC is either not directly involved in transepithelial Na transport or else not placed along the main transepithelial route.

METHODS

The experiments were performed on the abdominal skin of the local frog *Leptodactylus ocellatus*. Animals of either sex were studied at 20–22° C. The basic Ringer solution used contained (mM): 105 NaCl, 2·4 $KHCO_3$, 1·0 $CaCl_2$, and 10 Na piruvate. Other solutions used are specified in Results.

The abdominal skin was mounted as a flat sheet between a set of four individual pairs of chambers. In each pair the exposed area was 1·54 cm^2; the volume of the outer half was 0·8 ml., that of the inner half, 4·0 ml. Electrical potential difference was measured through agar bridges with 2 M-KCl, connected to calomel half-cells. These, as well as the terminals in contact with the silver plates, were connected to a voltage clamp apparatus devised by Dr Joaquín Remolina at the Centro de Estudios Avanzados (Instituto Politécnico Nacional, México).

The skin was mounted with control Ringer on the inside, and Ringer with 1 mM-Na (made isotonic with choline chloride) on the outside. Once the value of I was steady, 10 ml. Ringer with 50 mM-Na (made isotonic with choline chloride) were injected into the outer chamber in 0·3 sec. Since, as mentioned above, the volume of the outer chamber was 0·8 ml., the change of Ringer was considerably fast. It produced an increase of I in less than a second. After a given period of time, 10 ml. of the same Ringer with 50 mM-Na, but containing amiloride 10^{-4} M, was injected into the outer chamber in 0·3 sec. Amiloride produced an instantaneous drop of I. Both transients were recorded on a 7B polygraph recorder (Grass). In order to analyse in more detail the increase of I which followed the change from 1 to 50 mM-Na, a set of experiments was performed in which the transient was followed on a dual beam oscilloscope with two different sweep speeds (Tektronix Model 565).

Sources of material. Amiloride was obtained from Merck, Sharp & Dohme (West Point, Pa): choline chloride was purchased from Eastman Organic Chemicals (Rochester, N.Y.) and all other chemicals used were of A.R. grade.

Results are expressed as mean ± S.E. (number of observations).

RESULTS

Biber (1971) observed that if the solution used to change $[Na]_o$ from 1 to 50 mM contains amiloride, I climbs to almost the same level that it reaches without amiloride, but then decays back to basal level. Fig. 2 shows an experiment in which Biber's observation is confirmed. In this case, the increase of Na concentration elicits an increase of I of 104 μA; but amiloride cancels this response in less than 2 sec. Fig. 3 shows that if amiloride is added before the increase of Na concentration, the increase of I is prevented. This suggests that the transient response observed in Fig. 2 is due to a difference in diffusion speed between Na^+ and amiloride in the epithelial structures located outside the Na-sensitive barrier, i.e. is given by the Na^+ that reaches the barrier before amiloride stops the penetration. Since I is generally considered to be proportional to the size

of NaTC (Cereijido *et al.* 1964), it comes as a surprise that it should reach a near maximum level with the amount of Na^+ that can outrun amiloride in a distance of a few microns (the amount of Na represented by the area under the curve in Fig. 2 is $2{\cdot}23 \times 10^{-9}$ mole cm^{-2}). Further, if the NaTC is so small, what is the meaning of the area under the I-curve of Fig. 1 which, as discussed above, was also thought to reflect a NaTC?

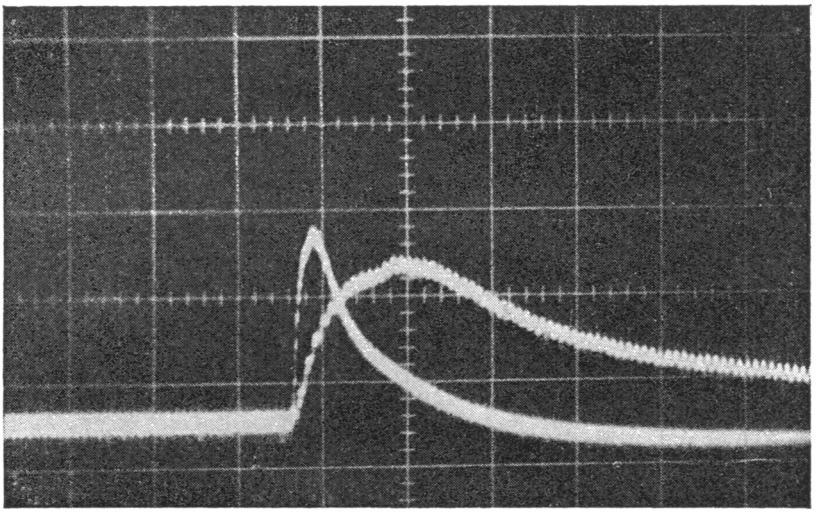

Fig. 2. Transient of I which follows a change of the outer Ringer (with 50 mM-Na and 10^{-4} M amiloride). Ordinate: slow sweep 50 μA/cm, fast sweep 42 $\mu A/cm^2$. Abscissa: slow sweep 1 sec/cm; fast sweep 0·2 sec/cm.

For the purpose of clarifying the nature of the NaTC a series of experiments were performed following the protocol already mentioned in the Introduction and depicted in Fig. 1. Fig. 4 shows a typical change in I following the increase of $[Na]_o$ from 1 to 50 mM, as well as its decay upon addition of amiloride 7 sec later. Figs. 5 and 6 show some responses that were also observed occasionally. Fig. 5 shows in a single experiment most of the pecularities observed individually in other records. I_1 is the current measured with 1 mM-Na in the outside Ringer. All other currents are measured with 50 mM-Na. I_p is the current at the peak of the overshoot. It was present in 70 % of the cases. I_{min} is a depression in the I curve which was observed around 1 sec after the overshoot in 37 % of the cases. In the remaining 63 % of the cases the current after the overshoot achieved a steady value. I_∞ is the current immediately before injecting amiloride. I_∞^A is the current recorded once the inhibition produced by amiloride has achieved a steady value. Fig. 6 shows a delayed and transient

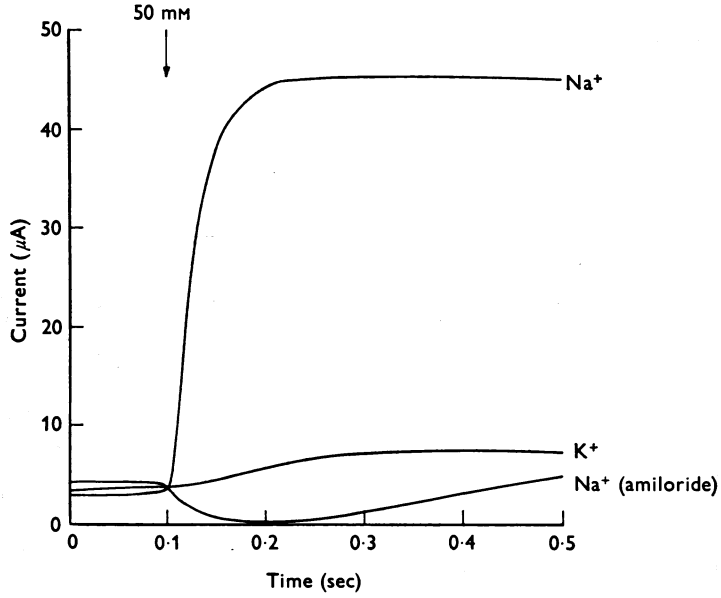

Fig. 3. Change in I which follows a change of the outer Ringer (with 1 mM-Na) for another containing 50 mM-Na^+ (upper trace) or 50 mM-K^+ (middle trace). The lowest trace corresponds to a change from 1 to 50 mM-Na, but with amiloride present throughout the experiment.

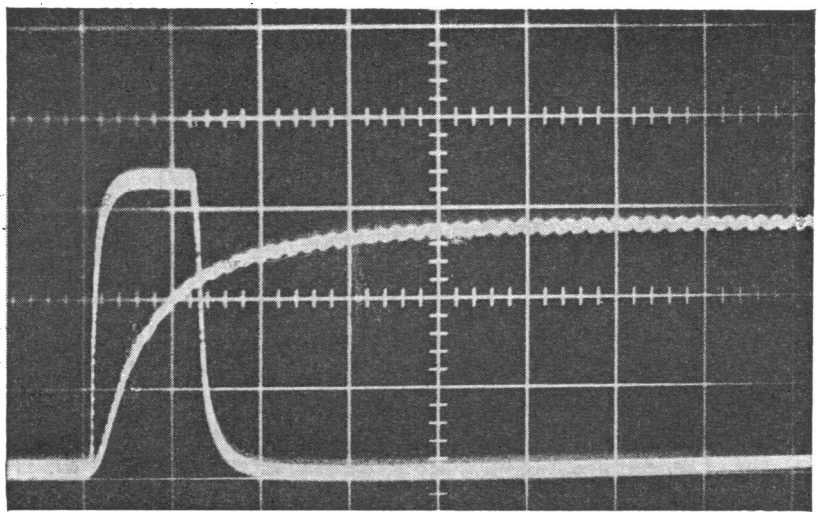

Fig. 4. Changes of I according to the protocol of Fig. 1. Ordinate: slow sweep 100 μA/cm, fast sweep 82 μA/cm. Abscissa: slow sweep 5 sec/cm, fast sweep 0·5 sec/cm.

increase of I_{∞}^{A} that was observed only in experiments of 10 sec of exposure to 50 mM-Na (see also Fig. 8, open circles). The values of the different currents are listed in Table 1.

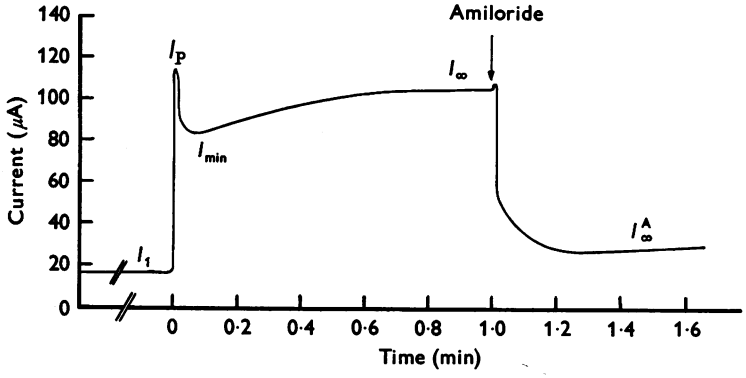

Fig. 5. Record of I as a function of time which shows together, in the same experiment, most of the pecularities of I observed (see text).

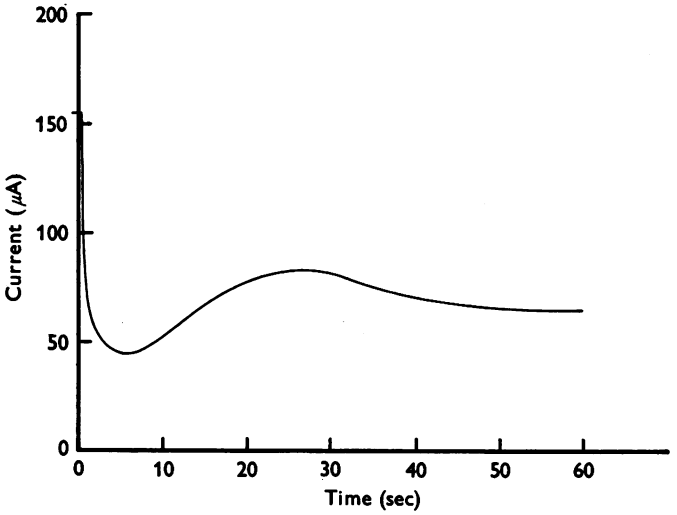

Fig. 6. Time course of I following the instantaneous addition of amiloride to the outer solution. The skin had been exposed to 50 mM-Na for 10 sec. Notice the delayed and transient increase of I which follows the initial depression.

A series of experiments was designed to explore the nature of the current response which follows the increase from 1 to 50 mM of the concentration of Na in the bathing solution. In order to compare the results obtained in the different experiments, these are expressed in terms of the increase in I as a fraction (F) of the maximal increase as detailed in the Appendix.

TABLE 1. Values of the different currents shown on Fig. 5

Time of exposure to 50 mM-Na	I_1	I_p	I_{min}	I_∞	I_∞^Δ	n
			(μA.cm^{-2})			
10 sec	16·4 ± 2·5	118 ± 13	99 ± 11	99 ± 7	31 ± 1	16
30 sec	13·9 ± 1·6	111 ± 8	88 ± 7	92 ± 7	31 ± 2	13
1 min	18·4 ± 3·2	100 ± 11	89 ± 9	95 ± 6	31 ± 2	14
5 min	18·2 ± 2·7	115 ± 17	91 ± 16	103 ± 10	43 ± 5	9
10 min	22·5 ± 5·7	104 ± 19	96 ± 22	101 ± 20	35 ± 7	6

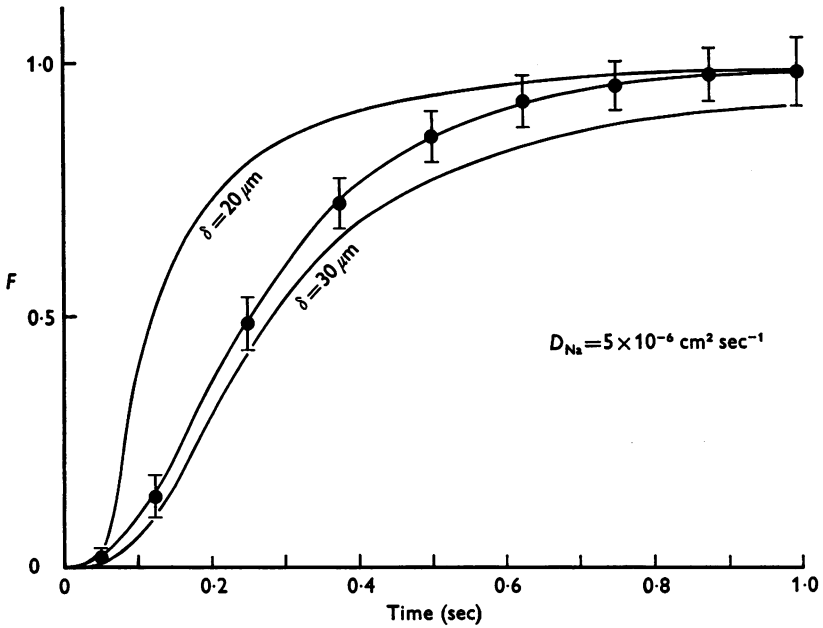

Fig. 7. Fractional change in I following an increase in Na concentration in the outside solution from 1 to 50 mM. The curves were calculated from eqn. (3) and (4) as described in the Appendix, using the values of D_{Na} and δ shown on the curves.

In agreement with previous observations on the time course of the electrical potential difference (Kidder *et al.* 1964; Dainty & House, 1966; Gebhart, Fuchs & Lindemann, 1972), it is shown (Fig. 7) that the delay is that expected for an unstirred layer some 20–30 μm thick. Thus, the site where the increase of I is achieved must be located at the outer barrier. Also, since the response is abolished by amiloride (Fig. 3), and this drug is a specific inhibitor of Na^+ sites (Eigler & Crabbé, 1969; Gebhardt *et al.* 1972; Aceves & Cereijido, 1973) I must be due to Na movement.

This is further indicated by the experiment in Fig. 3, where K fails to elicit an increase of I similar to the one elicited by Na. Furthermore, an increase of the concentration from 1 to 50 mM-Na produces an increase of the Na influx from 0·2 to 1·8 μmole h^{-1} cm^{-2} (Moreno *et al.* 1973). In order to increase the concentration of Na^+ in the outer chamber, Na^+ was injected as Cl^- salt. However, it is unlikely that the variation of I was due to Cl^-, because the concentration of this ion is kept constant. For the same reason the fact that the skin of *Leptodactylus ocellatus* has a small inward

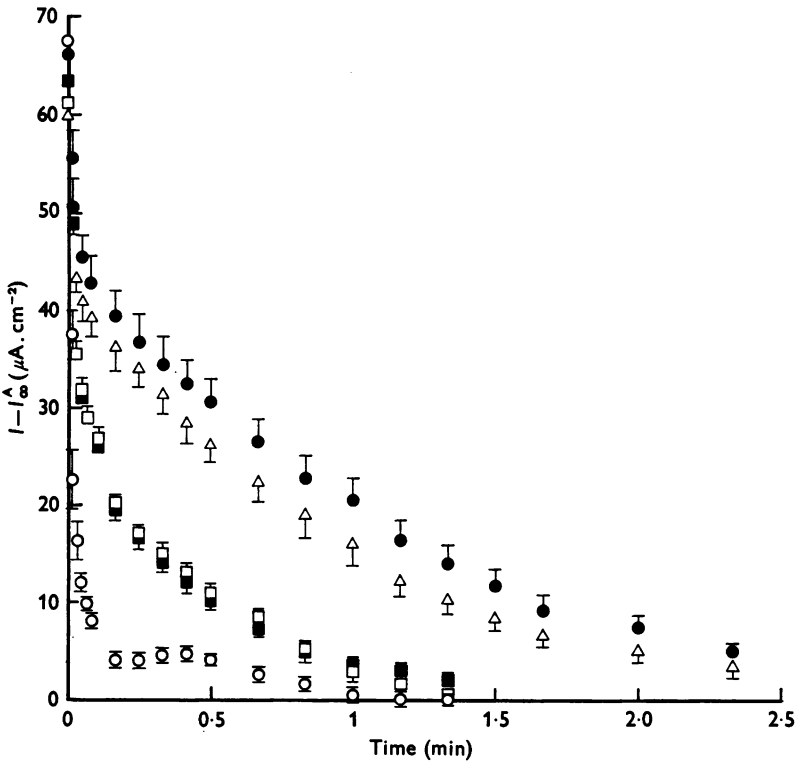

Fig. 8. Time course of I (expressed as $I_\infty - I_\infty^A$) following the addition of amiloride to the outer solution. The curves correspond to experiments where the skin has been exposed to 50 mM-Na for 10 sec (○); 30 sec (□); 1 min (■); 5 min (△) and 10 min (●).

transport of chloride (Zadunaisky, Candia & Chiarandini, 1963) should not modify the change in I elicited by Na. One may conclude that the increase of I when one changes from 1 to 50 mM corresponds to an increase of Na penetration across the outer barrier.

The value of I following the addition of 10^{-4} M amiloride very seldom dropped to zero, or to the value it had in 1 mM-Na. In a few experiments

in which higher doses were tested, the inhibition of I was not total either. The data in Table 1 shows that the effect of amiloride does not depend on the length of exposure to 50 mM-Na.

No systematic study of the nature of the overshoot (I_p) and of the depression (I_{min}) was performed. The current just before the addition of amiloride (I_∞) is essentially the same in skins exposed to 50 mM-Na for 10 sec and for 10 min. The lowest I_∞ recorded (at 30 sec) and the highest (at 5 min) differ by 10 % ($0{\cdot}15 < P < 0{\cdot}20$).

Fig. 8 shows the time course of I^A, recorded in the different experiments. As first pointed out by Cuthbert (1971), the transient following the addition of amiloride is either a single or a double exponential. This was also observed in the present study. In most cases there was more than one exponential.

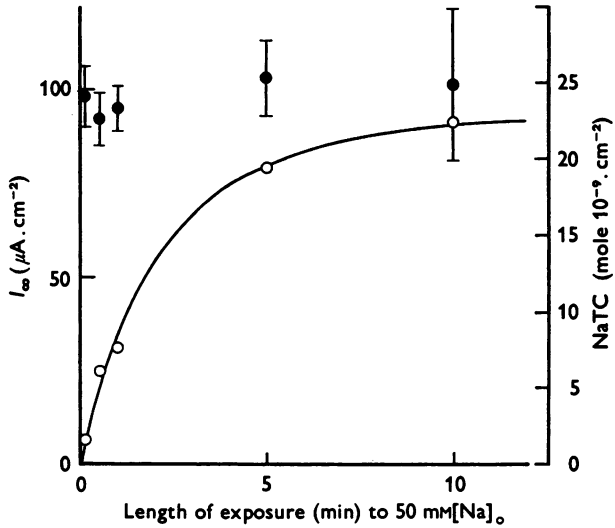

Fig. 9. Comparison between the value of the short-circuit current (I_∞) just before adding amiloride (filled circles) and the NaTC (open circles). The values of NaTC were calculated by weighing the area under the transient curve (see Fig. 8) as described in the text.

Fig. 9 shows the area under the transient of the current curve after adding amiloride, as well as the value of I.

The results in Figs. 8, 9 and Table 1 indicate (1) that, except for the small delay attributed to the unstirred layer (Fig. 7) the steady-state value of I in 50 mM-Na (I_∞) is not a function of the time of exposure, (2) that the area under the I curve (NaTC) increases with the length of exposure to Na with a time course much slower than the establishment of a steady I_∞, (3) part of the transient must be due to the delay in amiloride effect

due to its diffusion in unstirred layers on the outside. If one takes the area under the curve of Fig. 2 as an expression of this delay, and subtracts the $2{\cdot}23 \times 10^{-9}$ mole.cm^{-2} that it represents, the value of NaTC achieved is $2{\cdot}3 \times 10^{-8}$ mole.cm^{-2}. Assuming that the concentration of Na in the epithelium is around 20 mM (Aceves & Erlij, 1971; Zylber *et al.* 1973; Rotunno *et al.* 1973) the NaTC of $2{\cdot}3 \times 10^{-8}$ mole.cm^{-2} corresponds to a piece of epithelium 1 cm^2 wide and 12 μm thick. This would correspond to the Na contained in a fraction of epithelium somewhat thicker than the outermost cell layer which is not yet cornified (*stratum granulosum*).

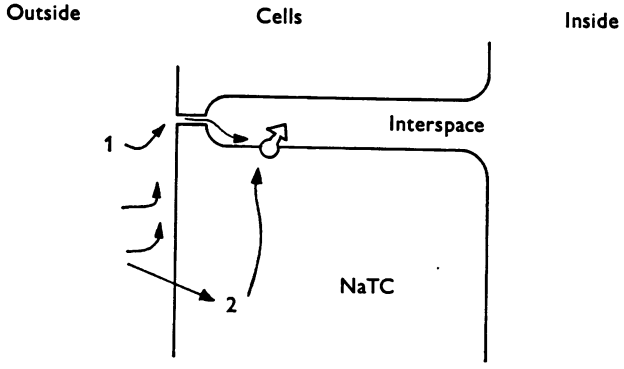

Fig. 10. Schematic representation of a portion of the epithelium with the two routes of Na transport discussed in the text. Route 2 crosses through the cytoplasm of the epithelial cells. The NaTC would correspond to the Na contained in the cytoplasm of these cells.

The fraction of the epithelium occupied by NaTC may be in fact somewhat larger, because the 12 μm refers to the fraction of NaTC inhibited by amiloride and, as mentioned above, this inhibition is not total. The fact that on one hand a steady current (I_∞) is achieved as soon as $[Na]_o$ reaches a stable concentration at the outer barrier, and that on the other the NaTC is a much slower function of time, suggests that the Na in this NaTC is not the one primarily involved in transcellular Na movement.

Fig. 10 offers a provisional interpretation of the results presented above, and may be used to describe the rationale behind the experiments which will follow. It proposes that the Na represented by the area under the I-curve (Fig. 1 and 8) corresponds to Na in the epithelial cells, and that the main route of transcellular Na movement avoids the cytoplasm of these cells. One of the requirements of this view is that Na^+ accumulates in the NaTC with an accompanying anion. A corollary of this requirement is that if one performs the experiment with an anion larger than Cl^-,

the NaTC should be greatly diminished. Accordingly, several experiments were performed with Ringer in which Cl^- was substituted by gluconate.

Fig. 11 shows the results obtained in paired observations using Cl^- (open circles) and gluconate (filled circles) in both the outer and the inner bathing solution. The experiments were performed in skins exposed 5 min to 50 mM-Na on the outside. Two points are evident: (1) gluconate reduces the value of the steady-state current (I_∞) obtained with 50 mM-Na^+. This was expected as several authors have demonstrated that Na

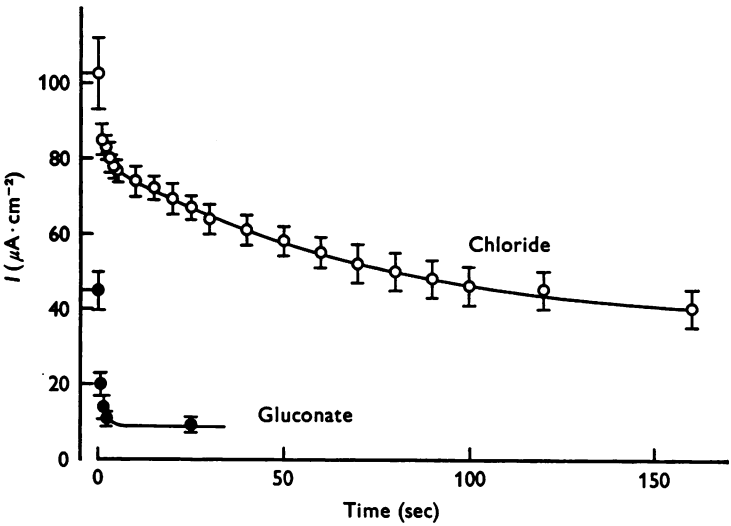

Fig. 11. Time course of I following the injection of amiloride in a group of skins bathed in Ringer with Cl^- (open circles) and in another group bathed in Ringer with gluconate as anion (filled circles).

transport and I are sensitive to the nature of the anion (Lindley, Hoshiko, 1964; Gill Ferreira, 1968; Huf, 1972), (2) the area under the curve in the case of Cl^- is $19{\cdot}7 \times 10^{-9}$ mole Na cm^{-2} and in the case of gluconate is $0{\cdot}12 \times 10^{-9}$ mole cm^{-2}. Hence while current drops by a factor of 2, the 'NaTC' decreases by a factor of 164.

DISCUSSION

As soon as the Na concentration reaches a steady value at the outer barrier, the current I, which reflects Na penetration, achieves a value that in most cases is independent of time. This current is not a function of the size of the NaTC (Fig. 9). The size of the NaTC, in turn, is not a function of I but a function of the time of exposure to high Na on the

outside (Fig. 9). This suggests that I might not be directly related to NaTC.

It seems therefore necessary to re-define NaTC. For the sake of discussion we may adopt the following definition: NaTC is an amount of Na located somewhere in the epithelium that, upon interruption of Na^+ supply from the outside, will be pumped into the inner side, thus originating an I. The evidence obtained both in frog skin and urinary bladder is convincing enough to suppose that these membranes have a NaTC located intracellularly (Schwartz & Snell, 1968; Nagel & Dörge, 1970; Dörge & Nagel, 1970; Vanatta & Bryant, 1970; Finn & Rockoff, 1971). The delay in achieving a maximum steady value of NaTC (Fig. 9) might be interpreted as the time required to fill up the cellular compartment through route 2 (Fig. 10). In this case one would expect the I curve to be like the one in Fig. 5, i.e. one in which I keeps increasing as the contribution of route 2 becomes more important. Yet, as discussed above, the curve in Fig. 5, although observed in several opportunities, is not the most frequent one. The general case is that I_{min} and I_∞ are similar (Table 1) and that the curves look like those in Figs. 1 and 4. Therefore, two main possibilities are open: (1) that the contribution of route 2 slowly increases I but this increase becomes masked by the spontaneous variation of the I curve over the period of 10 min; (2) that the pump was rate-limiting. In this case, only after the penetration across the outer barrier is blocked with amiloride could the pump extrude the Na contained in the NaTC. However, this interpretation must be taken with reservations, as Cereijido *et al.* (1964) have obtained some evidence that the active mechanism is not rate-limiting.

Route 1 in Fig. 10 would behave essentially as a single asymmetrical 'barrier', i.e. a step or series of steps without the involvement of an appreciable amount of Na. This view would agree with previous results obtained in this laboratory (Moreno *et al.* 1973) as well as those of Mandel & Curran (1973).

As mentioned above, NaTC corresponds to a band of epithelium somewhat thicker than the amount of Na contained in the first reactive layer of the epithelium. Na inside the cells is thought to diffuse to inner cell layers via cell contacts (Ussing & Windhager, 1964). When the concentration of Na in the outer solution is high enough, or when the intracellular potential inside the cells is made negative by passing a short-circuit current (Cereijido & Curran, 1965) Na^+ is expected to penetrate passively into the NaTC. This would agree with (*a*) the analysis of Frömter & Diamond which suggests that the characteristics of *passive* fluxes across the frog skin correspond to those expected across a cell membrane (these passive fluxes should not be confused with those occur-

ring through the shunt pathway demonstrated by Mandel & Curran (1972) and which are thought to pass extracellularly), (*b*) the fact that the first reactive layer swells when Na is being translocated (Vôute & Ussing, 1970) and (*c*) with the data in Fig. 11 which indicates that, in order to penetrate into the NaTC, Na^+ depends on a permeable co-ion.

Cereijido & Rotunno (1968) have proposed a working model with two transepithelial routes: one transcellular and another extracellular. The data in this paper agree with these views. Cuthbert (1972) has presented a theoretical model of epithelium which assumes the existence of an active step at the outer border. Such a model would also agree with the data here presented. However, epithelial membranes are very rich in physical variables, possible interpretations are numerous, and a thorough discussion would be considerably lengthy. It seems advisable to postpone such a discussion pending experimental evidence on other aspects of the phenomena described here.

We wish to express our indebtedness to Professor Joaquín Remolina from the Centro de Estudios Avenzados (ITN) of Mexico who designed and built the voltage clamp apparatus used in this study, and to Mrs Marisa B. de Gonzalez and M. Gonzalez A. de Carman for their valuable technical assistance. M. Cereijido and C. A. Rotunno are Career Investigators of the National Research Council of Argentina (CNICT). This work was supported by research grants from the CNICT and the Public Health Service of U.S.A.

APPENDIX

When the concentration of Na in the outer bathing solution $[Na]_o$ is changed from 1 to 50 mM, the current changes from I_1 to I_∞. The fractional change of current may be expressed as

$$F = \frac{I - I_1}{I_\infty - I_1}. \tag{1}$$

The relationship between I as well as the influx of Na across the outer barrier and $[Na]_o$ is given by an equation of the form

$$I = \frac{I_{max}\,[Na]_o}{[Na] + K_m}, \tag{2}$$

where I_{max} is the value of I at infinite $[Na]_o$, and K_m is the value of $[Na]_o$ at which I is equal to $0{\cdot}5\,I_{max}$. Combining eqns. (1) and (2) and taking the value of K_m as 9 mM (C. A. Rabito *et al.* unpublished results) one obtains

$$F = \frac{[Na]_o}{0{\cdot}75\,([Na]_o + 9)} - 0{\cdot}133 \tag{3}$$

$[Na]_o$ at the outer barrier as a function of time after an instantaneous change of concentration in the outer solution is given by

$$[Na]_o = [Na]_{o\infty} + \frac{4}{\pi}([Na]_{o1} - [Na]_{o\infty}) \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp - \left(\frac{(2n+1)^2 \pi^2 D_{Na} t}{4\delta^2}\right), \quad (4)$$

where $[Na]_{o1}$ and $[Na]_{o\infty}$ are the concentrations of Na before (1 mM) and after (50 mM) the change, D_{Na} is the effective diffusion coefficient of Na and δ is the length of the diffusion path (Crank, 1956). By combining eqns. (3) and (4) and assigning values to D_{Na} and δ, a series of theoretical curves of F as a function of time can be drawn. In Fig. 7 these curves are compared to the experimental points.

REFERENCES

ACEVES, J. & CEREIJIDO, M. (1973). The effect of amiloride on sodium and potassium fluxes in red cells. *J. Physiol.* **229**, 709–718.

ACEVES, J. & ERLIJ, D. (1971). Sodium transport across the isolated epithelium of frog skin. *J. Physiol.* **212**, 195–210.

ANDERSEN, B. & ZERAHN, K. (1963). Method for non-destructive determination of sodium transport pool in frog skin with radio-sodium. *Acta physiol. scand.* **59**, 319–329.

BIBER, T. U. L. (1971). Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. gen. Physiol.* **58**, 131–144.

BIBER, T. U. L. & CURRAN, P. F. (1970). Direct measurement of uptake of sodium at the outer surface of the frog skin. *J. gen. Physiol.* **56**, 83–99.

BIBER, T. U. L., CHEZ, R. A. & CURRAN, P. F. (1966). Na transport across frog skin at low external Na concentrations. *J. gen. Physiol.* **49**, 1161–1176.

CEREIJIDO, M. & CURRAN, P. F. (1965). Intracellular electrical potentials in frog skin. *J. gen. Physiol.* **48**, 543–557.

CEREIJIDO, M., HERRERA, F. C., FLANIGAN, W. J. & CURRAN, P. F. (1964). The influence of Na concentration on Na transport across frog skin. *J. gen. Physiol.* **47**, 879–893.

CEREIJIDO, M. & ROTUNNO, C. A. (1968). Fluxes and distribution of sodium in frog skin: a new model. *J. gen. Physiol.* **51**, 280s–289s.

CRANK, J. (1956). In *The Mathematics of Diffusion*, p. 45. London: Oxford University Press.

CURRAN, P. F., HERRERA, F. C. & FLANIGAN, W. J. (1963). The effect of Ca and antidiuretic hormone on Na transport across frog skin. II. Sites and mechanisms of action. *J. gen. Physiol.* **46**, 1011–1027.

CUTHBERT, A. W. (1971). Neurohypophyseal hormones and sodium transport. *Phil. Trans. R. Soc.* B **262**, 103–109.

CUTHBERT, A. W. (1972). A double (series) pump model for transporting epithelia. *J. theor. Biol.* **36**, 555–568.

DAINTY, J. & HOUSE, C. R. (1966). 'Unstirred layer' in frog skin. *J. Physiol.* **182**, 66–78.

DÖRGE, A. & NAGEL, W. (1970). Effect of amiloride on sodium transport in frog skin. II. Sodium transport pool and unidirectional fluxes. *Pflügers Arch. ges. Physiol.* **321**, 91–101.

EIGLER, J. & CRABBÉ, J. (1969). Effects of diuretics on active sodium transport in amphibian membranes. In *Renal Transport and Diuretics*, ed. THURAU, K. & JAHRMÄRKER, H., pp. 195–208. Berlin: Springer-Verlag.

FINN, A. L. & ROCKOFF, M. L. (1971). The kinetics of sodium transport in the toad bladder. I. Determination of the transport pool. *J. gen. Physiol.* **57**, 326–348.

FRÖMTER, E. & DIAMOND, J. (1972). Route of passive ion permeation in epithelia. *Nature, Lond.* **235**, 9–13.

GEBHARDT, U., FUCHS, W. & LINDEMANN, B. (1972). Resistance response of frog skin to brief and long lasting changes of $(Na)_0$ and $(K)_0$. In *Role of Membranes in Secretory Processes*, ed. BOLIS, L., p. 284. Amsterdam: North Holland Publishing Co.

GILL FERREIRA, K. T. (1968). Anionic dependence of sodium transport in the frog skin. *Biochim. biophys. Acta* **150**, 587–598.

HOSHIKO, T. & USSING, H. H. (1960). The kinetics of Na^{24} flux across amphibian skin and bladder. *Acta physiol. scand.* **49**, 74–81.

HUF, E. G. (1972). The role of Cl^- and other anions in active Na^+ transport in isolated frog skin. *Acta physiol. scand.* **84**, 366–381.

KIDDER, G. W., CEREIJIDO, M. & CURRAN, P. F. (1964). Transient changes in electrical potential differences across frog skin. *Am. J. Physiol.* **207**, 935–940.

KOEFOED-JOHNSEN, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

LEBLANC, G. & MOREL, F. (1972). Kinetics of sodium and lithium accumulation in isolated frog skin epithelium. *Symposium on Membrane Biology* (Copenhagen).

LINDLEY, B. D. & HOSHIKO, T. (1964). The effects of alkali metal-cation and common anions on the frog skin potential. *J. gen. Physiol.* **47**, 749–771.

MANDEL, L. J. & CURRAN, P. F. (1972). Response of the frog skin to steady-state voltage clamping. I. The shunt pathway. *J. gen. Physiol.* **59**, 503–518.

MANDEL, L. J. & CURRAN, P. F. (1973). Response of the frog skin to steady-state voltage clamping. II. The active pathway. *J. gen. Physiol.* **62**, 1–24.

MORENO, J. H., REISIN, I. L., RODRÍGUEZ BOULAN, E., ROTUNNO, C. A. & CEREIJIDO, M. (1973). Barriers to sodium movement across frog skin. *J. membrane Biol.* **11**, 99–115.

NAGEL, W. & DÖRGE, A. (1970). Effect of amiloride on sodium transport of frog skin. I. Action on intracellular sodium content. *Pflügers Arch. ges. Physiol.* **317**, 84–92.

ROTUNNO, C. A., POUCHAN, M. I. & CEREIJIDO, M. (1966). Location of the mechanism of active transport of sodium across the frog skin. *Nature, Lond.* **210**, 597–599.

ROTUNNO, C. A., ZYLBER, E. A. & CEREIJIDO, M. (1973). Ion and water balance in the epithelium of the abdominal skin of the frog *Leptodactylus ocellatus*. *J. membrane Biol.* **13**, 217–232.

SCHWARTZ, T. L. & SNELL, F. M. (1968). Nonsteady-state three compartment tracer kinetics. II. Sodium flux transients in the toad urinary bladder in response to short circuit. *Biophys. J.* **8**, 818–841.

USSING, H. H. (1970). Structure and function of epithelia. In *Electrophysiology of Epithelial Cells* (Chairman Prof. Dr G. GIEBISCH), pp. 3–16. Stuttgart: F. K. Schattauer Verlag.

USSING, H. H. & WINDHAGER, E. E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta physiol. scand.* **61**, 484–504.

VANATTA, J. C. & BRYANT, L. A. (1970). Compartmentation of the sodium transport pool of the toad bladder. *Proc. Soc. exp. Biol. Med.* **133**, 385–393.

VÔUTE, C. L. & USSING, H. H. (1970). Quantitative relation between hydrostatic pressure gradient, extracellular volume and active sodium transport in the epithelium of the frog skin (*R. temporaria*). *Expl Cell Res.* **62**, 375–383.

ZADUNAISKY, J. A., CANDIA, O. A. & CHIARANDINI, D. J. (1963). The origin of the short-circuit current in isolated skin of the South American frog *Leptodactylus ocellatus*. *J. gen. Physiol.* **47**, 393–402.

ZYLBER, E. A., ROTUNNO, C. A. & CEREIJIDO, M. (1973). Ion and water balance in isolated epithelial cells of the abdominal skin of the frog *Leptodactylus ocellatus*. *J. membrane Biol.* **13**, 199–216.