J. Physiol. (1971), **213**, *pp.* 119–133
With 4 text-figures
Printed in Great Britain

THE EFFECT OF ANTIDIURETIC HORMONE ON Na MOVEMENT ACROSS FROG SKIN

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(*Received 27 July 1970*)

SUMMARY

1. The effect of antidiuretic hormone (ADH) on the movement and distribution of Na was studied. This was done using three different approaches: (*a*) the measurement of Na and ^{22}Na in slices of epithelium of skins which were exposed to Ringer of varied composition containing ^{22}Na, (*b*) the measurement of the influx of Na from the outer to the inner bathing solution with ^{22}Na added to the outside, and (*c*) the use of a recently introduced technique which permits the direct evaluation of the flux from the outer solution → epithelium, (J_{OT}), i.e. the flux across the barrier which is generally regarded as the site of ADH activity.

2. ADH increased the influx from the outer to the inner bathing solution of Na (50 %) not only when the concentration of Na on the outside was 115 mM (i.e. higher than in the epithelium) but even when the concentration was 1 mM (67 %).

3. When the skin was bathed with 1mM-Na Ringer on the outside, ADH increased the unidirectional Na flux J_{OT} by 56 % (*Rana pipiens*) and 71 % (*Leptodactylus ocellatus*). When the concentration was 115 mM a small increase (17 %) was observed in paired skins of *R. pipiens*. Under this condition no change was observed in *L. ocellatus*.

4. The amount of epithelial sodium which is labelled by ^{22}Na added to the outside was taken to reflect the amount of Na involved in Na transport across the epithelium. Depending on whether the concentration of Na on the outside was high (115 mM) or low (1 mM), ADH produced an increase, or a decrease, of both the total Na content and the amount of ^{22}Na exchanged.

5. When the concentration of Na on the outside was low, ADH increased the total influx and J_{OT} in spite of the fact that it lowers the total Na content and does not affect the exchangeable pool of Na. This observation is inconsistent with the view that the effect of ADH is due to the fact

that the increased permeability of the outer barrier allows more Na into the cell, and that the resulting increase of Na concentration in the cytoplasm accelerates the Na pumps at the inner side of the cells.

6. It is concluded that ADH speeds up Na movements at the outward facing barrier, and that this exchange which facilitates the penetration of Na into a transporting compartment produces also a gain or a loss of Na in compartments not directly involved in Na transport across the epithelium. One compartment which is not involved in Na transport might be the cytoplasm of the epithelial cells.

INTRODUCTION

Huf, Parrish & Weatherford (1951) demonstrated that neurohypophyseal hormones produce an increase of NaCl transfer across the frog skin. Fuhrman & Ussing (1951) later showed that the increased flux of the salt is due to the effect of the hormone on the process of sodium transport. Curran & Gill (1962) and Herrera & Curran (1963) suggested that the process of Na transport across frog skin was rate limited by the flux of Na from the outer bathing solution across a Na-selective barrier (J_{OT}), and that the enhancement of Na transport elicited by antidiuretic hormones (ADH) was due to an increase of the permeability of this barrier. Curran, Herrera & Flanigan (1963) later developed a kinetic technique for the evaluation of the rate coefficient for ^{24}Na movement across the individual barriers of the Na-transporting compartment, and demonstrated that ADH does in fact increase the Na permeability of the outer barrier. This conclusion can also be reached on the basis of the study of Na movement across urinary bladder (Leaf & Dempsey, 1960; Bastide & Jard, 1968) and is supported by the measurement of electrical parameters (Civan & Frazier, 1968). Yet the conclusion that ADH increases the flux of Na across the outer barrier does not rest on the direct measurement of this flux. This is due to the fact that epithelial membranes consist of a number of permeability barriers arranged in series, which makes it difficult to interpret the events at a given single stage on the basis of transepithelial measurements. Since we have developed a new method for the direct evaluation of J_{OT} (Rotunno, Vilallonga, Fernández & Cereijido, 1970) it seemed appropriate to use it to reappraise the effect of ADH on Na movement. Although the results fit, in a general way, the scheme of the mechanism of ADH activity mentioned above, they indicate that the concept that it accelerates the pumping by increasing the Na-pool might not be true. This information is discussed with respect to current models of Na transport (Ussing, 1964; Farquhar & Palade, 1966; Biber, Chez & Curran, 1966; Cereijido & Rotunno, 1968).

METHODS

Influx of sodium

The abdominal skin of the South American frog *Leptodactylus ocellatus* L. was mounted in a lucite chamber of the type designed by Curran *et al.* (1963). The electrical potential difference was measured by connecting the chambers through agar-Ringer bridges to calomel half cells and these cells to a Keithley 200B DC electrometer. The area of skin exposed to the Ringer solutions was 3·14 cm^2. Once the electrical potential achieved a steady value, 1 μc of [^{22}Na]NaCl (Amersham or New England Nuclear) was added to the outer chamber. After an equilibration period of 20–30 min (Rotunno, Pouchan & Cereijido, 1966) the inner solution was sampled every 10 min. After the fifth sample ADH (Pitressin, Parke Davis) was added to the inner bathing solution to a final concentration of 0·5 i.u./ml., and the sampling was continued. Samples of the outer bathing solution were withdrawn at the end of the experiment. The samples were counted in well-type scintillation counter (Nuclear Chicago Auto Gamma) set as a spectrometer in the ^{22}Na peak. Samples were also taken for Na measurements in a flame photometer (EEL). Four control periods and four periods after the addition of ADH were studied.

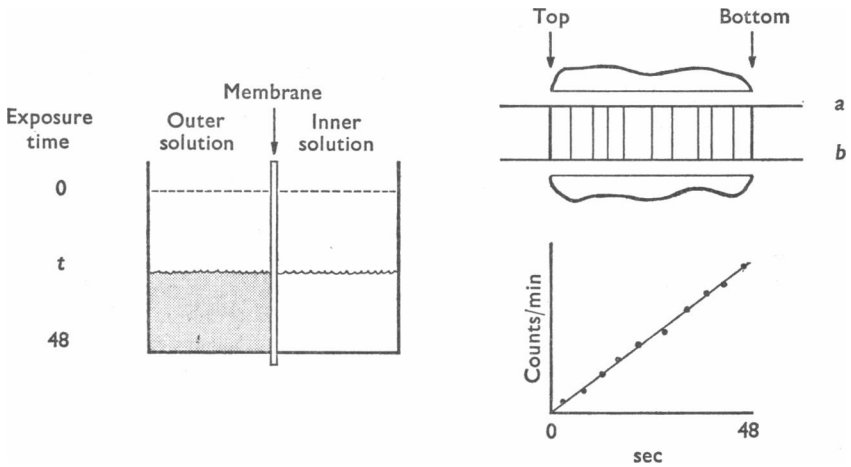

Fig. 1. Diagram of the method used for determining the unidirectional Na-flux J_{OT}. Left: the membrane is mounted in Lucite chambers with rectangular cross-sections. A dual infusion pump injects Ringer with ^{22}Na into the chamber in contact with the outer face of the skin, and non-radioactive Ringer into the other. The level of the solutions is raised steadily in 48 sec. Right: once the solution reaches the upper level, the skin is removed from the chamber and plunged deep in liquid nitrogen. A central strip of the exposed part (between lines *a* and *b*) is cut into small pieces, each one representing a certain time of exposure to tracer. The slope of the curve relating the radioactivity of each piece of skin to the time is a function of J_{OT}.

Measurement of J_{OT}

The technique used has been described in detail (Rotunno *et al.* 1970) but the following is a brief description. The skins were mounted in Lucite chambers with a rectangular cross-section (Fig. 1). After a period of pre-incubation under the

desired conditions the chambers were emptied, and a dual infusion pump injected Ringer solution containing ^{22}Na into the chamber in contact with the outer side and simultaneously non-radioactive Ringer into the chamber in contact with the inside. The solutions were injected steadily into the bottom of the chambers, and the level rose homogeneously. The chambers were filled in 48 sec. When the solutions reach the upper border, the exposure is interrupted and the skin is plunged deep into a thermos flask containing liquid nitrogen at $-196°$ C. With this kind of exposure only the lower border was exposed for the whole time of injection (48 sec). All other parts were exposed for a fraction of 48 sec. This fraction was proportional to the distance to the floor of the chamber. The exposed part of the skin was cut into ten to sixteen transverse sections, each representing a certain uptake time of ^{22}Na. The samples were analysed for their concentration of ^{22}Na, and a kinetic curve, showing the rising amount of ^{22}Na as a function of time, was plotted. The slope of the curve together with the specific activity of ^{22}Na in the bathing solution, were used to calculate the unidirectional sodium flux from the outer solution to the epithelium (J_{OT}). The effect of ADH was studied by adding 0·5 i.u./ml. in the inside solution during pre-incubation (about 20 min). It was also present in the solution infused during the measurement of J_{OT}.

^{22}Na uptake

The skin was mounted as a flat sheet in a Curran's Lucite chamber as described above. After an equilibration period of some 20 min ADH was added to the inside, and 2·5 μc $^{22}NaCl$ was added to the outside. After another 20 min the skins were removed from the chamber, placed on a filter paper moistened with distilled water with the inner side facing the paper, and blotted with soft tissue on the outer side. A piece of skin of 1·16 cm^2 of the area exposed in the chamber was cut out with a cork borer, placed on the stage of a freezing microtome (Jung Quick-Freeze Microtome) with the outer side facing up and frozen. The elapsed time from the removal of the skin from the loading chamber until it was frozen was less than a minute.

One to three samples, weighing together around 1 mg, were taken. This constitutes roughly a half of the epithelium. A detailed description of the slicing technique was given earlier (Cereijido & Rotunno, 1967). The slices were collected in a tared small dish made of Teflon, 0·9 cm in diameter and 1 mm thick at the border. It was then dried to a constant weight at 90° C, weighed, and placed in a polystyrene test tube; the ^{22}Na was counted as described above. After counting, 2 ml. 0·1 N-HNO_3 was added. The tube was stoppered with Parafilm (Marathon) and was left overnight to be extracted in a shaker. Aliquots were then diluted and sodium was measured by flame photometry (Hitachi-Perkin-Elmer spectrophotometer with photomultiplier, with flame photometry attachment for C_2H_4 and O_2); samples of the bathing solutions were also taken for Na and ^{22}Na measurements.

Ringer solutions. The basic Ringer contained (mM): 105 NaCl; 10 Na pyruvate; 2·4 $KHCO_3$ and 1·0 $CaCl_2$. When Ringer with low concentration of Na were used, the osmolarity was kept constant with sucrose.

Unless otherwise stated the variation produced by the hormone is expressed as

$$100 \left| \frac{\text{treated-control}}{\text{control}} \right| = \text{percentage of variation.}$$

RESULTS

Na influx

The control values of the influx (Table 1) were somewhat larger than those found by Zadunaisky, Candia & Chiarandini (1963) in the same frog (3μ-equiv hr^{-1} cm^{-2}) and Curran *et al.* (1963) in *Rana pipiens* (1·40 μ equiv hr^{-1} cm^{-2}). The effect of ADH was tested at two different concentrations of

TABLE 1. The effect of ADH on the influx of sodium

Experiment	Na concentration in the bathing solution		Na influx		P
	Outer	Inner	Control	ADH	
	(mM)		(μ-mole hr^{-1} cm^{-2})		
A	115	115	4·19 ± 0·33 (30)	6·27 ± 0·42 (30)	< 0·001
B	1	115	0·237 ± 0·008 (39)	0·335 ± 0·013 (39)	< 0·001

Na in the outer bathing solution. When the skin was bathed in Ringer with 115 mM-Na on both sides the addition of ADH to the inside solution caused a significant increase of 50 % in the influx. This compares with the increase of 30 % found by Curran *et al.* (1963) in *R. pipiens*. The increase of sodium influx is not as large as the one elicited by the hormone in urinary bladder (Leaf & Dempsey, 1960; Bourguet & Morel, 1967; Bastide & Jard, 1968; Bourguet & Maetz, 1961). When the concentration of Na in the outer bathing solution was 1 mM, ADH produced an increase of the Na influx of 67 %. Since the outer solution was made isotonic by adding sucrose, there was no osmotic gradient operating across the skin. This means that the 67 % of increase cannot be ascribed to a drag of Na produced by the molecules of water, but reflects a direct effect of the hormone on the movement of Na.

The influx of Na across the outer border (J_{OT})

The reason that studies in this section were carried out in *L. ocellatus* and also in *R. pipiens* is twofold: (1) in order to take advantage of the large amount of information obtained with different frog species, one has to make sure that the mechanisms are similar and that comparisons are valid. While the values of most parameters discussed in this paper were measured by several authors in both species, comparative values of J_{OT} are not available because the technical possibility of a direct measurement of J_{OT} is relatively new (Rotunno *et al.* 1970; Biber & Curran, 1970), and (2) the technique for the evaluation of J_{OT} used in this paper required a large exposed area (8·9 cm^2) and this makes it impossible to obtain two pieces of

white abdominal skin for paired experiments out of the same *L. ocellatus*. *R. pipiens* are available in sizes as large as 4–5 in. (Lemberger, Wisconsin) and permit comparisons between control and ADH treated membranes from the same animal. Table 2 shows the results obtained under control conditions with the two frog species. It may be observed that *L. ocellatus* have a higher control value of J_{OT} than *R. pipiens*. The values of J_{OT} in *L. ocellatus* obtained with the present technique by Rotunno *et al.* (1970)

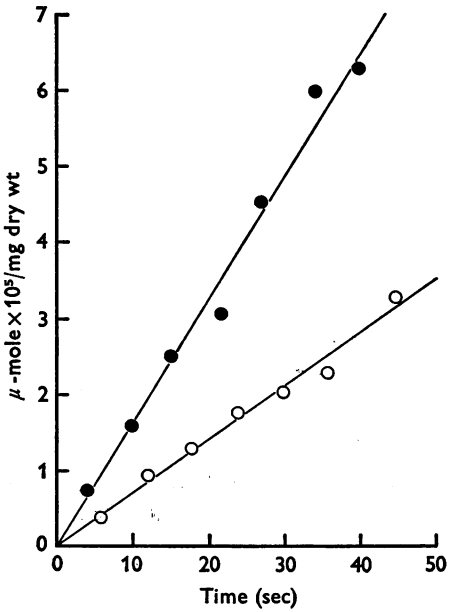

Fig. 2. J_{OT} in control (○), and ADH treated (●) skins. The corresponding fluxes are 0·180 and 0·417 μ-mole hr^{-1} cm^{-2}.

were slightly larger than those obtained by Biber & Curran (1970) in *R. pipiens* with a completely different technique (14 *vs.* 6 μ-mole hr^{-1} cm^{-2}). The measurement of J_{OT} made now with the same technique in the two species shows that the difference may be attributable to the difference in species.

As discussed below, the entrance of Na into the epithelium across the outer barrier has always been regarded as a rate limiting step in the overall process of Na transport across epithelial membranes (Civan & Frazier, 1968; Curran & Gill, 1962; Herrera & Curran, 1963; Curran *et al.* 1963; Frazier, Dempsey & Leaf, 1962). Comparison of control data in Tables 1 and 2, though, indicates that J_{OT} is larger than the transepithelial influx under all comparable conditions tested.

Fig. 2 illustrates two experiments, one in a control and another in an

TABLE 2. Effect of ADH on J_{OT}

Experiment	Frog species	Na concentration in the bathing solution (mM) Outer	Inner	J_{OT} Control	ADH	P
				(μ-mole hr^{-1} cm^{-2})		
A	*L. ocellatus*	1	115	0·465 ± 0·006 (12)	0·794 ± 0·117 (10)	< 0·05
	R. pipiens			0·268 ± 0·031 (7)	0·418 ± 0·034 (6)	< 0·01
B	*L. ocellatus*	115	115	14·01 ± 1·62 (7)	13·15 ± 1·35 (7)	—
				17·9 ± 4·5 (5) % *		< 0·05

* Control in *R. pipiens*: 5·92 ± 0·62 (5) μ-mole hr^{-1} cm^{-2}.

TABLE 3. The effect of ADH on the content and labelling of sodium

Experiment	Na concentration in the bathing solution (mM) Outer	Inner		Na content Control	ADH	P
				(μ-mole/mg wet wt.)		
A	115	115	Total	0·242 ± 0·008 (21)	0·262 ± 0·008 (14)	—
			Exchanged	0·050 ± 0·004 (21)	0·080 ± 0·014 (14)	< 0·05
B	115	5	Total	0·154 ± 0·006 (22)	0·162 ± 0·009 (26)	—
			Exchanged	0·041 ± 0·004 (22)	0·047 ± 0·006 (26)	—
C	5	5	Total	0·156 ± 0·013 (25)	0·082 ± 0·013 (32)	< 0·001
			Exchanged	0·030 ± 0·001 (25)	0·028 ± 0·001 (32)	—

ADH treated skin, and Table 2 summarizes the effect of ADH on J_{OT}. ADH increased the entrance of Na from the outer bathing solution even from solutions in which Na was as diluted as 1 mM. The fact that the outer solution was isotonic with the Ringer on the inside would prevent the viscous drag of Na molecules by H_2O molecules being the possible origin of the observed increase of J_{OT}. When the skin was bathed with 1 mM-Na on the outside ADH raised the value of J_{OT} by 71 % in *L. ocellatus* and by 56 % in *R. pipiens*. While this confirms the evidence obtained on a different basis that ADH speeds up Na transport by increasing the permeability of the outer barrier, the observation that, anyway, the influx in the ADH treated skins is below the control value of J_{OT} (0·335 *vs.* 0·465) does not permit one to conclude that the Na influx increases as a consequence of an easier penetration across the outer barrier. When the skin was bathed with 115 mM-Na Ringer on both sides, the control value of J_{OT} (14·01) was also larger than the total influx in ADH treated skins (6·27). Under this condition ADH produced a 17·9 % increase of J_{OT} in *R. pipiens*. This difference was too small to be significant with a reasonable number of observations in control and ADH treated skins from different frogs. As explained above, the size of *L. ocellatus* does not permit the study of paired samples of skins from the same animal.

^{22}Na uptake

When ^{22}Na was added to the outer bathing solution the influx of ^{22}Na reached equilibrium in less than 20 min (Hoshiko & Ussing, 1960; Curran *et al.* 1963; Cereijido, Herrera, Flanigan & Curran, 1964; Cereijido & Rotunno, 1967). It can be demonstrated that by the time the influx of ^{22}Na equilibrates, the Na transporting compartment should have its ^{22}Na-specific activity in a steady state (Solomon, 1960). It can be demonstrated that, if the fraction of the total Na in the epithelium that participates in the process of Na transport is contained in a compartment bound on the inside by a Na-impermeable barrier, the value of its specific activity in the steady state should be identical with that in the bathing solution where the tracer was added. Hence the number of counts per minute of ^{22}Na in the epithelium divided by the specific activity of ^{22}Na in the outer chamber can be taken as an estimate of the amount of Na in the transporting compartment, in particular if, as it is the case in the present paper, only comparative studies are intended. There is a relationship between the amount of Na in this compartment and the rate of Na pumping (Curran *et al.* 1963; Cereijido *et al.* 1964). It was suggested (Curran *et al.* 1963; Crabbé & de Weer, 1965), that the increase in Na permeability of the outer barrier elicited by ADH, produces a rise in the concentration of Na in the transporting compartment, and that the Na pump located at the inner

barrier reacts to this rise in concentration by pumping more Na toward the inside. The aim of this section was to obtain information on this process. Table 3 shows the amount of Na and the amount of Na labelled after 20–30 min of adding ^{22}Na to the outer bathing solution. Since the slicing technique cannot avoid including some connective tissue in the samples, the total amount of Na in the epithelium bathed on the inside by 115 mM-Na Ringer is probably overestimated. In skins bathed in Ringer with 115 mM-Na on both sides ADH elicited a small increase of labelled Na (0·080 *vs.* 0·050). This increase is reflected in the total Na content (0·262 *vs.* 0·242) but is not big enough to be significant. This effect of ADH on the pool of labelled Na confirms previous observations in frog skin (Curran *et al.* 1963) as well as in toad bladder (Crabbé & de Weer, 1965).

When the skin was bathed on the inside with iso-osmotic Ringer with only 5 mM-Na, the total amount of Na in the epithelium was lower than the value found in epithelia bathed with 115 mM-Na on both sides (0·154 and 0·156 *vs.* 0·242). Cereijido, Reisin & Rotunno (1968) have shown that this decrease corresponds to the variation of the amount of Na in a large compartment which is open toward the inside but relatively closed toward the outside, and which seems to contain mainly fixed Na. These authors have suggested that this compartment is constituted by Na in the intercellular space of the epithelium, and in the connective tissue that might be included in the samples. It may be noticed that when the skin was bathed in Ringer with low concentration of Na on the inside, but control 115 mM-Na Ringer on the outside, ADH did not alter significantly the total sodium content (0·162 *vs.* 0·154) nor the amount of exchanged Na (0·047 *vs.* 0·041). When the skin was bathed in 5 mM-Na Ringer, the amount of Na in the epithelium did not seem to be modified by changes in the concentration of Na on the outside (0·156 *vs.* 0·154 Expts. B and C). If the concentration of Na in the outer Ringer was low (experiment C), ADH produced a decrease in the amount of Na in the epithelium (0·082 *vs.* 0·156) but did not vary the amount contained in the transporting compartment (0·028 *vs.* 0·030).

DISCUSSION

The results of the present paper, in particular those obtained with the direct measurement of the flux from the outer bathing solution to the epithelium (J_{OT}) confirm in a general way the assumptions that the effect of ADH on Na^+ movement is evidenced at the outer border and that it is not elicited by virtue of a coupling with the water movement (Hays & Leaf, 1962; Curran *et al.* 1963; Bourget & Morel, 1967; Bastide & Jard, 1968; Civan & Frazier, 1968). However, the value of the influx of Na under control conditions and once ADH is added, are smaller than J_{OT} under

control conditions, and therefore it does not seem necessary that J_{OT} should increase in order to allow a larger influx across the whole epithelium. In trying to afford an interpretation to this fact, one has to consider that J_{OT} is an *unidirectional* flux and, in spite of its relatively large value, the *net* amount of Na penetrating the epithelium might be actually much smaller and constitutes a rate limiting step. Large unidirectional fluxes with small net fluxes are characteristic of mechanisms of facilitated diffusion. Rotunno *et al.* (1970) have presented some evidence that J_{OT} is in fact carried by a mechanism of facilitated diffusion. This suggests that the effect of ADH on J_{OT} might be due to a modification of a system of facilitated diffusion.

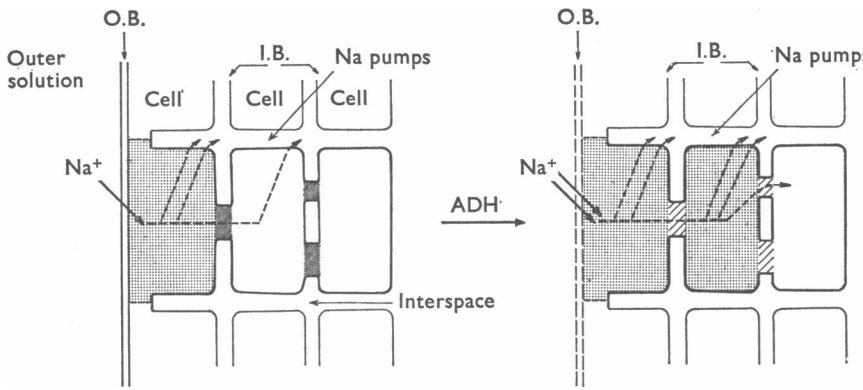

Fig. 3. Transcellular model to account for Na distribution and movement across epithelial membranes. Left: the sodium penetrates by diffusing down its gradient across an outer barrier (O.B.) and is actively extruded towards the interspace across an inner barrier (I.B.). Part of the sodium crosses intercellular bridges and is extruded by inner cells. Right: ADH increases the permeability of the outer barrier and would also permit an easier passage to inner layers of cells.

Although the over-all process of Na transport across the frog skin is inhibited by ouabain (Koefoed-Johnsen, 1958; Zadunaisky *et al.* 1963), J_{OT} is not affected by this drug (Rotunno *et al.* 1970) so that J_{OT} is carried by an essentially passive mechanism, i.e. one that is not directly coupled to the supply of metabolic energy through an ouabain-sensitive ATPase. Therefore the effect of ADH on J_{OT} observed in these experiments supports the assumption that ADH acts primarily on a passive mechanism (Civan, Kedem & Leaf, 1966).

Fig. 3 depicts in a very schematic manner a transcellular model of Na transport across epithelia. Cells are connected to each other through low resistance paths (Ussing & Windhager, 1964). The Na pumps are located at the cell-intercellular space boundary (Farquhar & Palade, 1966; Rotunno

et al. 1966). When Na from the outer bathing solution penetrates into the cells it follows mainly two routes: (1) some Na ions are actively extruded toward the interspace. Since this interspace is closed toward the outside, but open toward the inside, the pumped Na ions will diffuse toward the inner bathing solution; and (2) the rest of the Na will pass through the intercellular paths into the inner layers of cells to be, in turn, pumped toward the intercellular space. ADH might, in principle, act in three different stages: (1) by increasing the penetration of Na across the outer barrier, (2) by stimulating the active step, and (3) by increasing the ionic conductivity of the intercellular paths, thus recruiting inner layers of cells for the process of Na transport.

The concentration of Na in the cellular compartment has been estimated at 9 mM (Biber *et al.* 1966) to 21 mM (Cereijido *et al.* 1968). When the concentration of Na in the outer medium is 115 mM the concentration gradient is oriented towards the cell and an increase in the Na permeability elicited by ADH would enhance the penetration of Na into the cells, a fact that, as mentioned above, is claimed to be the mechanism by which ADH increases the transport of Na. However, this view would fail to explain not only how the skin will transport Na from a diluted Na solution as living skins do, but would not explain the increase of Na transport produced by ADH when the concentration of Na on the outside is 5 mM. In this case the concentration gradient of Na is oriented towards the outside and, as expected, the increase of the Na permeability of the outer barrier produced by ADH *decreases* the Na pool (compare experiments C and B: 0·082 *vs.* 0·162). The change of the concentration of Na in the outer solution from 115 to 5 mM does not modify the total Na content (0·154 expt. B *vs.* 0·156, expt. C) but does lower the amount of Na exchanged (0·041 − 0·030 = 0·011). This would indicate that under these conditions ADH produced the loss of Na from a compartment which is not directly involved in Na-transport. It is hard to afford an explanation to these facts on the basis of the model in Fig. 3.

Fig. 4 depicts the model of Na transport across the frog skin introduced by Cereijido & Rotunno (1968). It is based on the fact that the permeability of bilipidic membranes to ions is so poor that when an ion from the outer bathing solution approaches the cellular membrane it would tend to migrate by jumping from fixed polar group to fixed polar group rather than pentrate twice the hydrophobic component (Cereijido & Fraidenraich, 1970). The transporting compartment of this model is constituted by the polar groups of the outer leaflet of the plasma membrane of epithelial cells. These fixed groups are sandwiched between two barriers of low Na permeability: (1) the hydrophobic components of the membrane and (2) a barrier that confers to the 'inner facing membrane' its low Na perme-

ability (Koefoed-Johnsen & Ussing, 1958; MacRobbie & Ussing, 1961) and that the model assumes to be located between the fixed groups and the intercellular space. This second barrier does not exist at the outer border where Na ions can leave or enter the transporting compartment by adsorbing or desorbing to the fixed polar groups. The pumps located all over the inner facing membranes translocate Na from fixed groups to the intercellular space. Because of the two barriers of low permeability the site left by a pumped Na is only refilled when the Na in a neighbour polar

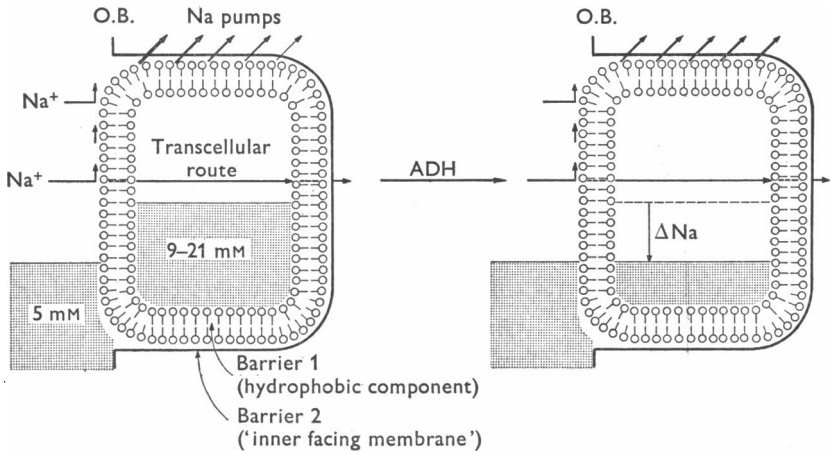

Fig. 4. Non-transcellular model for Na movement across epithelial cells. The transporting compartment between barrier 1 and barrier 2 contains Na bounded to polar groups of the outer surface of the plasma membrane of the cells. The lipidic component of the plasma membrane is represented in a much larger scale to illustrate the point that for a Na ion it is far easier to migrate around the cell rather than to penetrate twice the hydrophobic component of the membrane. Na is adsorbed and transported in spite of the fact that the concentration of Na in the outer solution is lower than in the cells. ADH (*right*) increases the permeability of the outer barrier (O.B.) and produces a loss of cellular Na. This does not impair the increase of J_{OT} elicited by the hormone.

group jumps into the empty site. The over-all effect is to move the empty site towards the outward facing barrier where another Na can be adsorbed. This model in which Na migrates essentially on a flat layer of polar groups affords a plausible explanation to the fact that the skin takes up Na, not just when it has a Ringer with 115 mM-Na on the outside, but even when the frog is swimming in a pond with fresh water. Some features of this model might have some relevance for the discussion of the present results with ADH. (1) The amount of Na contained in the transporting compartment is small as compared with the total Na contained in the epithelium.

The epithelial cells have their own mechanism to maintain their Na balance with the internal environment. The Na in their cytoplasm does not get involved in Na transport *across* the epithelium. If the permeability of the outer face of these cells were increased by ADH, they would lose or gain Na depending on the concentration in the outer solution. The Na they contain under these circumstances would get labelled with ^{22}Na. Although the fate of this Na is not central to the mechanism of Na transport *across*, if the outer solution has a high (115 mM) concentration, the cells would gain Na and pump it toward the intercellular space. The facts that this mechanism would work at high concentrations of Na, and that for technical reasons (the study of short circuit currents) the effect of ADH is generally studied with identical Ringer on both sides, might lead one to believe that it was the key feature of ADH activity. (2) As discussed above, ADH can act even at low concentrations of Na on the outside increasing J_{OT}, which is considered to be a passive flux across a system of facilitated diffusion. These characteristics are the ones expected from the mechanism of Fig. 4. Although it is difficult to explain with the information available at present how ADH would affect the Na migration through such a transporting compartment, it is relevant to point out that: (1) lipid monolayers may discriminate Na^+ from other ions (Cereijido *et al.* 1969; Vilallonga, Fernández, Rotunno & Cereijido, 1969), (2) monolayers formed with lipids extracted from the frog skin possess ion-selectivity (Fernández *et al.* 1970), (3) that there is a relationship between lipid composition, and ion transport across the frog skin (Wathlington & Harlan, 1969) and the toad urinary bladder (DeGraefe, Dempsey Lameyer & Leaf, 1965), (4) ADH affects the metabolism of phospholipids in epithelial membranes (Hestrin-Lerner & Hokin, 1964) and finally (5) ADH modifies the characteristics of lipid monolayers (Snart & Sanyal, 1968). Although there is some ground to suspect that Na migration, lipid composition, and ADH effect are somehow associated, it would be too audacious to venture that the effect of ADH can be understood in terms of modifications of the diffusion path of Na. However, it might be useful to point out that the phenomena listed above can be ordered along such lines of thought, and that the results of the present paper would be in keeping with such lines.

We wish to thank Mrs Marisa Battelli de Gonzalez and Miss Graciela Ansaldi for their competent assistance, and Parke Davis Laboratories for the generous supply of ADH. This work was supported by research grants from the Public Health Service of U.S.A. and the National Research Council of Argentina (CNICT). The authors are Career Investigators from the CNICT.

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