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SODIUM TRANSPORT ACROSS THE ISOLATED EPITHELIUM OF THE FROG SKIN

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SUMMARY

1. A method to separate the epithelium from the underlying layers of the frog skin is described. The method is based on the combined use of collagenase and hydrostatic pressures.

2. The potential difference and the short-circuit current values of isolated epithelia and whole skins are similar. Na net flux and short-circuit current are equivalent.

3. The time course of changes in potential following rapid changes in composition of the bathing solutions shows that the barrier to K diffusion at the internal surface of the isolated epithelium is larger than the barrier to Na diffusion at the external surface.

4. In the isolated epithelium there are 133 m-mole K^+ and 24·7 m-mole Na/l. cellular water. The amount of extracellular water was considered to be equal to the inulin space.

5. Arginine vasopressin (0·1 u./ml.) markedly increased short-circuit current and potential difference in isolated epithelia. The amount of Na in the epithelium that equilibrated with Na in the external solution was not increased by the hormone.

6. Ouabain (10^{-4} M) reduced short circuit current and potential difference to values close to zero. The ouabain treated epithelia contained an increased amount of Na originating in the internal solution. On the other hand the amount of Na that originated from the external solution was not increased.

7. The amount of epithelial Na that equilibrated with Na in the external solution was 0·009 μ-equiv/cm^2. This figure is about ten times smaller than the values found in whole skins.

INTRODUCTION

The anatomical complexity of the frog skin has retarded the understanding of the mechanism of transepithelial Na transport. This function

is localized in the epithelial cells that constitute only a fraction of the volume of the skin.

Several authors have estimated the amount of Na and the unidirectional fluxes of the transepithelial transport compartment assuming a one compartment model and measuring either the rate of appearance of labelled Na at the inner surface of the skin after suddenly adding the isotope to the external solution (Hoshiko & Ussing, 1960; Curran, Herrera & Flanigan, 1963) or the rate of disappearance of the label after equilibrating the skin with the isotope (Andersen & Zerahn, 1963). Hoshiko, Lindley & Edwards (1964) have shown that the contribution of diffusion delays in the connective tissue of the skin to these estimates may result in serious errors. Another limitation arises from the need to use data obtained by subjective curve fitting to a few experimental points (Myhill, 1967).

An approach for the determination of intracellular concentrations of epithelial cells has been devised by Hansen & Zerahn (1964). They cut slices of skin parallel to the surface with a freezing microtome and made determinations on samples composed mainly of epithelial cells.

We have described briefly a method in which by the use of collagenase and hydrostatic pressure the epithelium can be separated from the underlying layers of the skin. The behaviour of the transepithelial Na transport system of the isolated epithelia is similar to that observed in whole skins (Erlij & Aceves, 1969). Here we describe the method in detail and also present a survey of the physiological properties of the isolated epithelium.

Other methods have been used to separate the epithelium of the skin. Skjelvale, Nieder & Huff (1960) found that after incubating the skin for 20 hr in a 4 mM-$NaHCO_3$ solution, the corium could be easily dissected away. Recently, Fishman & Macey (1968) separated epithelia with this technique and obtained values of potential difference and short-circuit current comparable to those found in whole skins. A preparation of surviving epithelium of the toad skin can be obtained using high hydrostatic pressures alone (Rawlins, Mateu, Fragachan & Whittembury, 1970).

METHODS

Dissection and mounting

After destroying the spinal cord and the brain of the frog (*Rana pipiens*), two transverse incisions along the whole circumference of the skin of the trunk were made. One incision ran immediately behind the forelimbs, the other at the level of the pubis. The portion of skin between the incisions was carefully dissected from the underlying tissues. After the dissection, the carcass of the frog was slid out from the ring of skin which was transferred to a dissection dish filled with Ringer solution and then inverted to expose the corium. A small area of the tela subcutanea (about 1 cm^2) was removed with fine dissecting instruments under the stereoscopic microscope. The skin was reinverted to its original position (epithelium outside) and

mounted in the lucite holder shown in the inset of Fig. 1. The skin formed the lateral surface of a closed compartment, its interior was filled with Ringer solution containing collagenase, 100 mg/l. The holder with the skin was then placed in a beaker filled with Ringer solution.

After 2 hr of equilibrating the skins in the holder with the enzyme, the inlet tube of the holder was connected to a Mariotte bottle held to a height such that the level of the Ringer solution in it was 30 cm above the skin. After 15 min of applying the pressure one or more blisters begin to form below the epithelium. The blisters usually appeared at the spots where the tela subcutanea had been removed. When

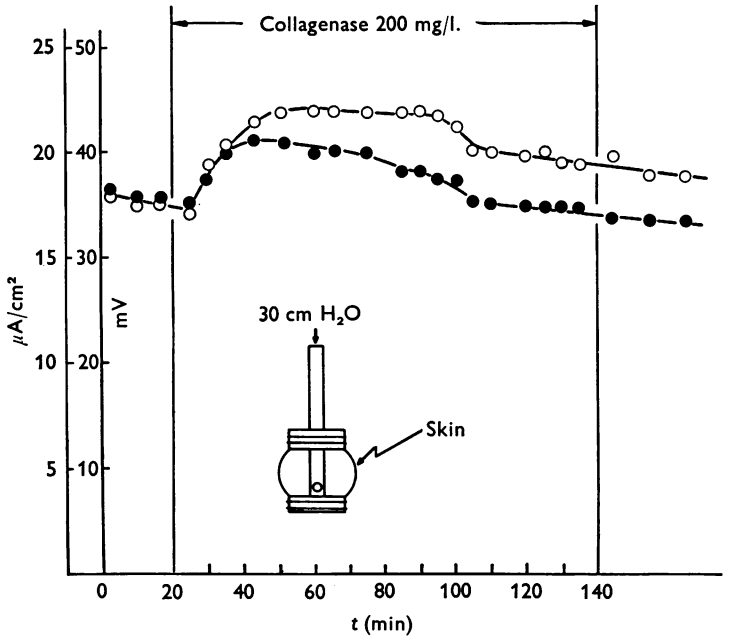

Fig. 1. The effects of collagenase on the potential difference and short-circuit current of frog skin. After 20 min of equilibration a Ringer solution containing collagenase 200 mg/l. was placed at the internal side of the skin. After 2 hr the enzyme was washed out. Abscissa: time in minutes. Ordinate: potential difference in mV. Short-circuit current in $\mu A/cm^2$. The inset shows the apparatus used to apply pressure to the skin. Open circles: short-circuit current. Filled circles: potential difference.

haematoxylin and eosin stained sections of the blister were examined with the light microscope we found that the outer wall of the blister is formed by the epithelial cells and the glands (Fig. 2), while the chromatophores, and connective tissue formed the back of the blister. Examination of these histological sections and a consideration of the probable contribution of the epidermal melanophores (Hadley & Bagnara, 1969) and the mitochondria rich cells (Farquhar & Palade, 1965) to the volume of the isolated epithelium suggest that about 80 % of it is constituted by epithelial cells in diverse stages of differentiation. Throughout this paper the term epithelial cells refers to the cells of the stratified epithelium of the skin; it does not include the epithelial cells of the glands.

Once the blisters had formed, the ring of skin was removed from the holder and opened. Under the stereoscopic microscope the corium at the back of the blister was removed with fine scissors obtaining a piece of skin with an area at its centre covered only by epithelium (Rawlins *et al.* 1970). This piece of skin was mounted between two Ussing's type of half chambers, the window (area 0·6 cm^2) between the chambers being covered only by the epithelium. The edges of the half chambers were covered with silicone grease to achieve a good seal with little lateral pressure. Symmetrical calomel half cells, connected through 3 M-KCl-agar bridges to the chambers were used for potential recording and current delivery.

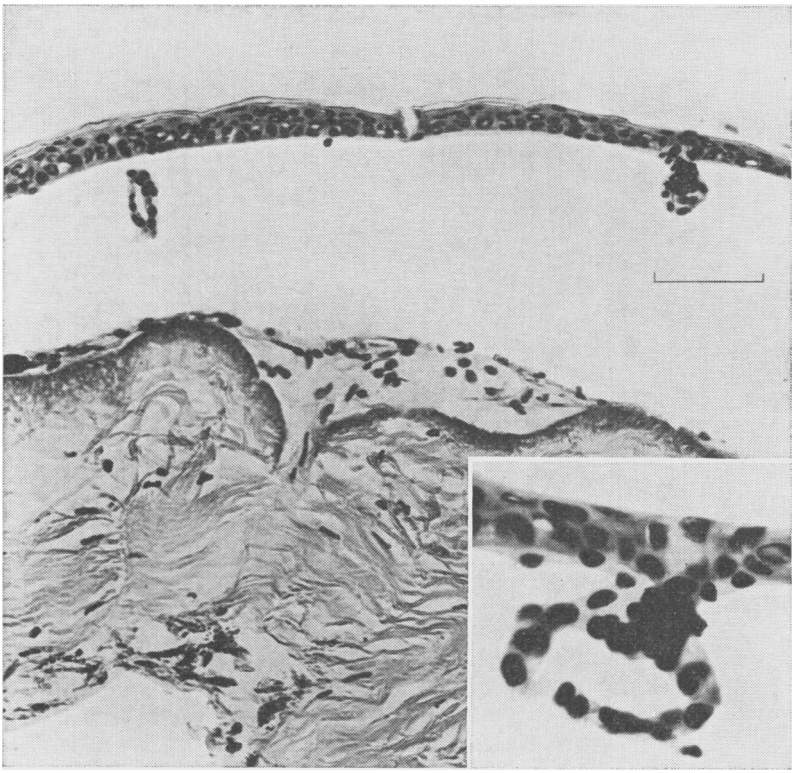

Fig. 2. An haematoxylin–eosin section of the skin after treatment with collagenase and hydrostatic pressure. The inset shows a piece of isolated epithelium with a gland on it. The horizontal calibration is 200 μ for the section of whole skin and 100 μ for the inset.

In many instances more than one epithelial preparation was obtained from a single skin. When paired epithelia were used, they were obtained from the same skin and selected so that short-circuit current differences between them were never larger than 15 % during the first hour.

Several exploratory experiments on the effects of collagenase on the potential difference and short-circuit currents of skins mounted in a Ussing type chamber, showed that the most convenient of the collagenase preparations tested was the

crude collagenase sold by Worthington Biochemical, Co. Fig. 1 illustrates a typical experiment in which the effects of the enzyme (200 mg/l.) were tested on the inside of the skin. This concentration of the enzyme did not reduce short-circuit current nor potential after 2 hr.

Isotope determinations and analysis

Na fluxes were measured with ^{22}Na (The Radiochemical Centre, Amersham) as described by Ussing (1949). To determine electrolyte concentrations and the amount of radioactivity in the epithelium after an experimental period, the piece of skin with the area of epithelium at its centre was transferred to a piece of ashless filter paper (Whatman 541). The outer surface of the skin was in contact with the paper. The area of epithelium that had been at the window between the chambers was cut with a specially designed cork borer (area 0·6 cm^2). The excess water on the inside of the epithelium was removed by gently blotting. The epithelia were then transferred within 10–20 sec to tared Teflon containers having tightly fitting Teflon lids. The containers weigh about 100 mg and successive weighings in a Mettler M5 balance over periods of several days showed that the maximum range of variation in the weight determination of the container was ± 15 μg. The wet epithelia were weighed in the containers and then they were dried in an oven to constant weight to obtain the dry weight. The containers with the epithelia were then placed in polypropylene tubes containing 0·5 ml. concentrated HNO_3 (Suprapur, Merck, Darmstadt) and extracted for 24 hr. The sample was then diluted to 3 ml. and determinations of Na and K were made by flame photometry in a SP90 Unicam Atomic Absorption Spectrophotometer. The standards contained the same concentration of HNO_3 as the samples. Appropriate amounts of Na were added to detect any interference in the K^+ determinations. For ^{22}Na counting, the samples were counted in a Nuclear Chicago Autogamma Scintillation Counter after digesting with HNO_3. Extracellular space was determined with [^{14}C]carboxynulin (New England Nuclear) and counted in a Tricarb Liquid Scintillation Counter. The scintillation solution for aqueous samples described by Swann & Magee (1968) was used. Quenching in the samples was determined with a [^{14}C]toluene standard. Since HNO_3 causes substantial quenching some independent measurements were carried out in samples leached in distilled water for 72 hr. This procedure extracts more than 95 % of the inulin in the epithelia.

Other methods

In some experiments the thickness of the epithelium of whole skins was determined following the procedure of McRobbie & Ussing (1961).

The rate of change of potential after suddenly varying the composition of either the internal or the external solution was measured following the method of Kidder, Cereijido & Curran (1964).

Solutions

Na-chloride Ringer solution had the following composition: NaCl, 115 mM; KCl, 2·5 mM; $CaCl_2$, 1 mM; Tris-maleate buffer, 3 mM; pH 7·5. Na-sulphate Ringer had Na_2SO_4, 58 mM; K_2SO_4, 1 mM; $CaSO_4$, 8 mM; Tris-maleate buffer, 3 mM; pH 7·5. To prepare K-chloride Ringer, choline chloride Ringer or K sulphate Ringer, the Na was replaced by equimolar amounts of K or choline. Ouabain and synthetic arginine vasopressin were purchased from Sigma Chemical Co.

RESULTS

Relationship between net Na flux and short-circuit current in isolated epithelia

The values of potential difference and short-circuit current observed in isolated epithelia and in whole skins were within the same range. In twelve epithelia short-circuit current ranged from 9 to 75 μA/cm^2; the potential difference from 19 to 70 mV. In untreated skins from the same animals the short-circuit current ranged from 19 to 45 μA/cm^2 and the potential difference from 21 to 68 mV. In eight pairs of epithelia, Na flux measurements were made to find whether, as in the whole skin, the short-circuit current accounts for the net Na flux. The results are illustrated in Fig. 3.

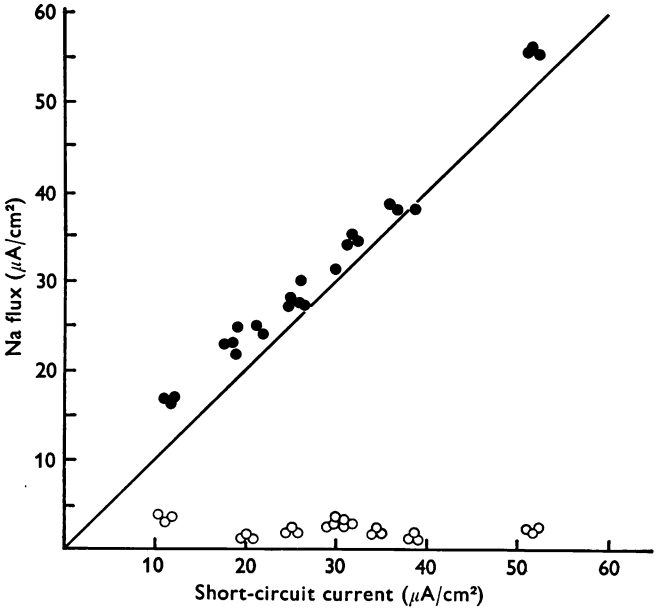

Fig. 3. The relationship between ^{22}Na flux and short-circuit current. Filled circles: Na influx. Open circles: Na outflux. Abscissa: short-circuit current in μA/cm^2. Ordinate: Na flux expressed in μA/cm^2. Epithelia bathed in Na chloride Ringer.

In the epithelia, Na influx exceeded short-circuit current by $3{\cdot}2 \pm 0{\cdot}6$ μA/cm^2, while the efflux had a value of $2{\cdot}1 \pm 0{\cdot}3$ μA/cm^2. These measurements are in remarkable agreement with the values found in whole skins (Ussing, 1969). In isolated toad epithelia separated using hydrostatic pressures alone, Rawlins *et al.* (1970) found an average efflux of 20 μA/cm^2.

The rate of action of Na^+ and K^+

Kidder *et al.* (1964) and Dainty & House (1966) have measured the rate of change of the potential difference of the frog skin immediately after a sudden change of Na concentration at the external solution or of K concentration at the internal solution. These measurements have been utilized to estimate the size of diffusion barriers at both surfaces of the skin. We have repeated these experiments on isolated epithelia.

A typical experiment illustrating the effects of a sudden rise of Na^+ in the external solution on the transepithelial potential is shown in Fig. 4. Estimation of D'/d^2 where D' is the diffusion coefficient and d is distance, was made by first plotting the fractional change in potential difference F, following a change in solution against time. F was defined as

$$F = \frac{V - V_0}{V_\infty - V_0},$$

where V is the potential difference at any time after the concentration change. Curves for different given values of D'/d^2 were calculated according to the procedure described by Olson & Schultz (1942) then these curves were compared with the experimentally determined values of F. In five epithelia a value $D'_{Na}/d_0^2 = 0{\cdot}207 \pm 0{\cdot}023$ sec^{-1} was estimated. This value is in agreement with the results of Kidder *et al.* (1964) and of Dainty & House (1966).

The rates of depolarization of whole skins and isolated epithelia after raising K of the inside solution are illustrated in Fig. 4. The curve drawn through the points obtained from whole skin experiments was calculated using $D'_K/d_i^2 = 0{\cdot}004$ sec^{-1}. This value is in agreement with the value reported by Kidder *et al.* (1964). However, it is lower than the figures reported by Dainty & House (1966), probably because in our experiments the stirring rates were lower. The line drawn on the points obtained in isolated epithelia was calculated from $D'_K/d_i^2 = 0{\cdot}012$ sec^{-1}.

The experiments of Fig. 4 were carried out in sulphate solutions. A few experiments with chloride solutions showed faster depolarization rates. In agreement with Kidder *et al.* (1964) a value of $D'_K/d_i^2 = 0{\cdot}008$ was estimated.

If we select, as Dainty & House (1966) did, $D'_K = 4 \times 10^{-6}$ cm^2 sec^{-1} we obtain $d_i = 182\ \mu$ in the isolated epithelia. A reasonable value for the unstirred layer in frog skin is $50\ \mu$ (Dainty & House, 1966). This figure suggests that after crossing the unstirred layer, K has to diffuse some distance within the epithelium to produce its full effect.

It is apparent that it is not easy to select with certainty values for D'_K and d_i for the isolated epithelium. However it is clear that the barrier to

K^+ diffusion at the internal surface of the isolated epithelium is much larger than the barrier to Na^+ diffusion at the external surface.

The Na^+ and K^+ content of isolated epithelia

Table 1 lists the Na^+ and K^+ content of eight epithelia. The values of intracellular water used to calculate the intracellular concentrations in Table 1 were estimated by subtracting the values for dry weight and extra-

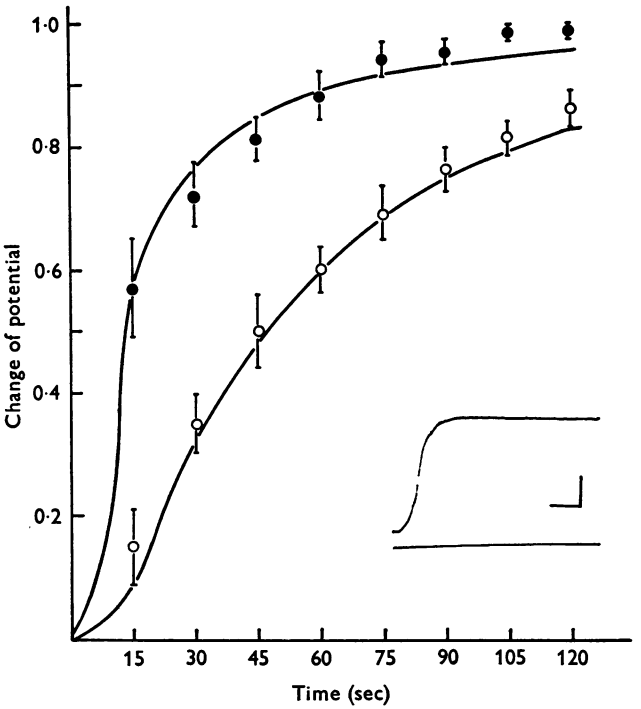

Fig. 4. The rate of action of K^+ and Na^+ on the transepithelial potential difference. The graphs represent the fractional change of potential after suddenly changing K concentration at the internal surface of eleven isolated epithelia (filled circles) and seven whole skins (open circles). Abscissa: time in sec. Ordinate: fractional change of potential. The inset shows an oscilloscope tracing obtained during an increase in Na concentration from 0·5 to 58 mM at the external surface of the isolated epithelium. The horizontal line corresponds to 0 potential difference. The vertical calibration is 10 mV. The horizontal calibration is 300 msec. All the experiments were performed in sulphate solutions.

cellular space from the wet weight. We found values of 133·1 m-mole K^+ and 24·7 m-mole Na^+/l. cell water. The inulin space was measured after equilibrating with inulin for an hour either at the internal or the external

surface of the epithelium; the assumption was made that Na^+ and K^+ concentrations of the water in the inulin space were the same as in the Ringer solution. Recently, Zerahn (1969) determined ion concentration in slices of epithelium in which he also measured the extracellular space and found 119·5 m-mole K^+ and 35 m-mole Na/l. cell water.

TABLE 1. Na and K content in epithelia immersed in normal Ringer

Expt.	Wet weight* (μg)	Dry weight (μg)	Total Na† (μ-mole/g) wet weight	Cell Na^+ (m-mole/l.) cell water	Total K^+† (μ-mole/g) wet weight	Cell K^+ (m-mole/l.) cell water	SCC (μA/cm^2)
1	2624	505	30	11·9	95	149·8	33·4
2	3036	500	45	35·5	92	133·7	30·0
3	2487	397	40	23·2	88	133·8	36·5
4	1870	355	38	10·3	79	142·5	38·6
5	2537	439	34	12·7	86	132·0	25·0
6	2534	401	54	44·2	77	116·2	27·7
7	2608	395	47	35·1	76	112·7	31·0
8	2136	345	43	24·9	89	144·2	21·4
$\bar{Y}$	2479	417	41	24·7	85	133·1	30·4
S.E.	±115	±20·1	±4	±4·17	±3	±4·3	±1·9

* Of 0·6 cm^2 epithelium.

† These values represent Na and K content before allowing for the extracellular space.

Inulin space (μl. 0·6 cm^2) { Internal 0·410 ± 0·020, $n = 8$; External 0·060 ± 0·008, $n = 10$; Total 0·470

Since the data of Hansen & Zerahn (1964) and of Cereijido, Reisin & Rotunno (1968) for electrolyte concentrations in slices of epithelium, did not take into account the extracellular space, they can only be compared with our average values for the total epithelium of 41·3 μ-mole Na^+/g wet wt. and 85 μ-mole K^+/g wet wt. Hansen & Zerahn (1964) found in the epithelial slice of skins exposed to Li^+ on the external side the following values: Li^+, 11·7; Na^+, 35·6 and K^+, 74·5 μ-mole/g wet wt. Cereijido *et al.* (1968) found 44 and 60 μ-mole Na/g wet wt. when they used 115 mM-Na^+ Ringer at the internal side of the skin.

The average value for the wet weight of the epithelium was 2479 μg. We tried another estimate of epithelial volume of the skin by measuring its thickness using the technique of McRobbie & Ussing (1961) in whole skins. The average thickness was 49 ± 6 μ ($n = 15$). The volume of 0·6 cm^2 of epithelia of this thickness is 2·940 μl. It is possible that the difference between the values obtained with both techniques is caused because the microscopic measurements overestimate the thickness of the

epithelial layer, since other layers (xanthopheres and iridophores) are located between the deepest epithelial layer and the melanophores (Hadley & Bagnara, 1969; Noble, 1954) that are a reference point in the method of McRobbie & Ussing (1961).

The effects of antidiuretic hormone

The effects of arginine vasopressin 0·1 u./ml., on potential difference and short-circuit current are illustrated in Fig. 5. The hormone increased both potential difference and short-circuit current. In isolated epithelia from toad skin bathed in sulphate Ringer solution, Rawlins *et al.* (1970) found that antidiuretic hormone increased the short-circuit current but not the potential difference. Results of experiments undertaken to explore

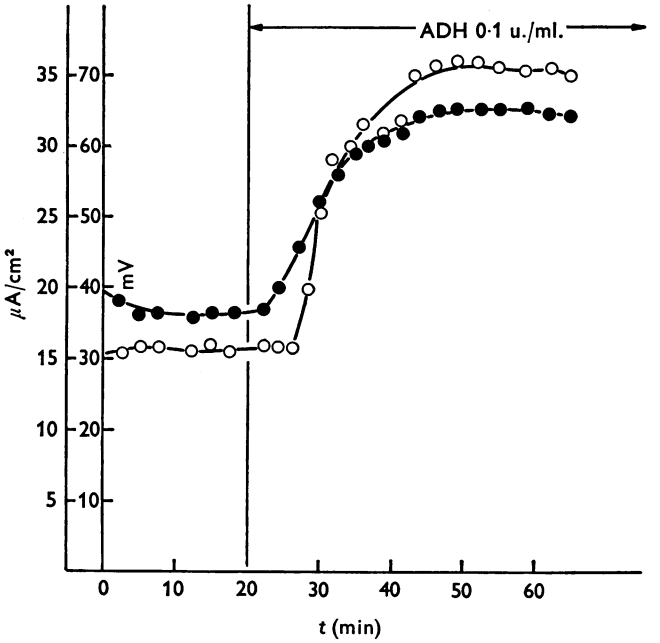

Fig. 5. The effects of antidiuretic hormone (0·1 u./ml.) on the short-circuit current and potential difference of the isolated epithelium. Abscissa: time in min. Ordinate: potential difference in mV. Short-circuit current in $\mu A/cm^2$. Na chloride Ringer on both surfaces of the skin. Open circles: short-circuit current. Filled circles: potential differences.

TABLE 2. The effects of arginine vasopressin (0·1 u./ml.) on the amount of intracellular Na that equilibrates with the ^{22}Na present in the external solution during 1 hr exposure

	$[Na]_i$ equilibrated (m-mole/l.)	n	SCC ($\mu A/cm^2$)
Control	1·86 ± 0·9	7	13·0 ± 1·5
Vasopressin	1·60 ± 0·7	7	24·3 ± 3·9

whether a relationship between the effects of antidiuretic hormone on Na transport and Na uptake by the skin could be found are listed in Table 2. The amount of epithelial Na that equilibrated with Na in the bathing solution was calculated by subtracting the counts in the extracellular space from the total counts in the epithelium and then dividing the difference by the specific activity of the loading solution.

In contrast with the findings of Curran *et al.* (1963), we did not observe any significant difference in Na uptake between control and hormone treated epithelia, in spite of Na transport being almost doubled during the action of the hormone.

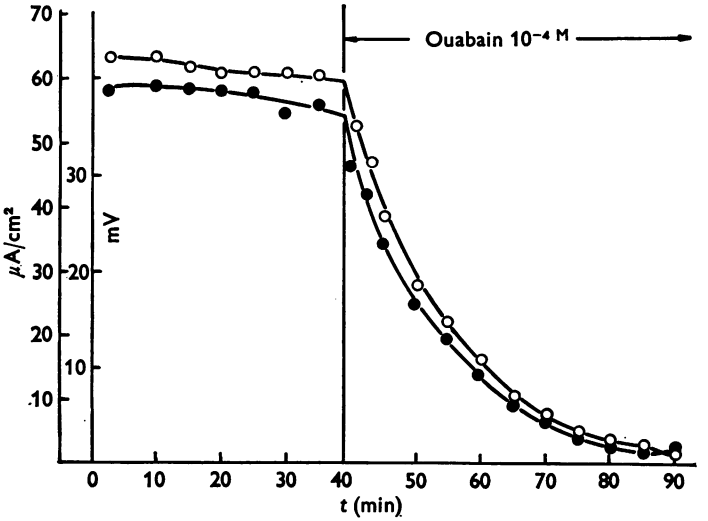

Fig. 6. The effects of ouabain (10^{-4} M) on the short-circuit current and potential difference of the isolated epithelium. Abscissa: time in min. Ordinate: potential difference in mV. Short-circuit current in $\mu A/cm^2$. Na chloride Ringer on both surfaces throughout the experiment. Open circles: short-circuit current. Filled circles: potential difference.

The effects of ouabain

A typical experiment of the action of ouabain on the isolated epithelium is illustrated in Fig. 6. Approximately 90 min after the addition of the glycoside the short-circuit current and the potential had values close to zero.

Herrera (1968) found that the Na accumulated by the toad bladder during the action of ouabain originated only from the serosal solution. The experiments listed in Table 3 show that the isolated epithelium behaves like the toad bladder. In these experiments 30 min after the addition of ouabain to one of the paired epithelia, ^{22}Na was included either in the

external or in the internal solution of the control and of the ouabain-treated epithelia. 3 hr after labelling both the control and the ouabain-treated epithelia were removed for counting.

We did not observe any differences in Na uptake between ouabain-treated and control epithelia when ^{22}Na was in the external solution. On the other hand, the amount of Na accumulated through the internal surface was about five times larger in ouabain-treated epithelia than in control epithelia.

TABLE 3. The effects of ouabain (10^{-4} M) on the amount of intracellular Na that equilibrates with ^{22}Na added either to the outside or to the inside bathing solutions

	Solution labelled	$[Na]_i$ equilibrated (m-mole/l.)	n	SCC ($\mu A/cm^2$)
Control	Inside	11·1 ± 1·34	6	40·3 ± 3·8
Ouabain	Inside	55·9 ± 7·6		0·5 ± 0·2
Control	Outside	3·0 ± 1·4	6	37·1 ± 4·0
Ouabain	Outside	3·0 ± 1·8		0·6 ± 0·3

DISCUSSION

One of the most interesting features of this study is the description of a simple method for separating the epithelium of the skin from the corium with relatively little damage to the transepithelial transport process. Although the isolated epithelium is less complicated than the whole skin, it still contains, apart from the epithelial cells in various stages of differentiation, the skin glands and also the epidermal melanophores as well as the mitochondria rich cells. In the discussion that follows we have assumed that our measurements reflect the composition of the epithelial cells. This assumption is based on an estimate that showed that epithelial cells constitute about 80 % of the volume of the isolated epithelium. Our assumptions may have to be modified when the amount of electrolytes in the lumen and cells of resting glands are known. Frog skin secretions have 26 m-equiv Na/l. (Campbell, Aiyawar, Berry & Huf, 1967). This value suggests that the error implicit in these assumptions should be small.

Comparison of uptake and Na concentration determinations

An estimate of the fraction of epithelial Na that equilibrates with the Na either in the external or the internal solutions can be made by comparing the data of Table 1 with the control uptake determinations of Tables 2 and 3. After 3 hr, about 45 % of the total Na of the epithelium equilibrated with the Na in the internal solution, while only 7–12·5 % of the total Na equilibrated with the Na in the external solution.

This limited equilibration of epithelial Na with Na in the external

solution could be accounted for by a single compartment model constituted by all the cells in the epithelium (Ussing & Windhager, 1964; Farquhar & Palade, 1965), if the radioactive Na entering from the external surface were diluted by non-radioactive Na entering through the internal surface. If some seven times as much Na flowed into the epithelium through the internal surface and then were recirculated back into the internal solution, the difference between the amount of epithelial Na equilibrating with the Na in the external solution and the chemically determined amount of Na would be explained. The possibility that there is a high Na entry into the epithelial cells through the internal surface is not supported by several observations. When the amount of Na in the non-inulin water of the experiments used to construct Table 1 is expressed per unit of area 68×10^3 p-mole cm^{-2} are found. This amount of Na ought to exchange completely after a few minutes of equilibration with radioactive Na across a barrier with a proposed flux seven times higher than the influx through the external barrier. This prediction is not supported by the data in Table 3. Furthermore, Cereijido & Rotunno (1967) found that after 90 min of equilibration with ^{22}Na through the internal surface, only about 15 % of the Na in the epithelial cells had exchanged. More recently J. Aceves, T. U. Biber, P. Curran & L. Mandel (personal communication) found that the Na uptake through the internal surface of the isolated epithelium is very small when compared with the uptake through the external surface.

These considerations suggest that the transport compartment constitutes only a fraction of the total epithelium. Whether it is formed only by a single layer of cells—the 'first reacting cell layer' of the experiments of Voute & Ussing (1968)—or perhaps it is associated with a given cellular component as suggested by Cereijido *et al.* (1968) is a question that so far is not possible to answer.

TABLE 4. Na transport pool in μ-equiv/cm^2 of frog skin determined by different methods

Hoshiko & Ussing (1960)	0·07	Curran *et al.* (1963)	0·15
Andersen & Zerahn I (1963)	0·172	Present results*	0·009
Andersen & Zerahn II (1963)	0·093		

* This value was calculated from the values in Table 1 that show that there are 2750 μl. cell water/cm^2 epithelium, and from the amount of epithelial Na that equilibrates with the Na in the external solution shown in Table 3.

Table 4 shows that the amount of tissue Na that equilibrates with external Na in isolated epithelia is small when compared to determinations made in whole skins (Hoshiko & Ussing, 1960; Andersen & Zerahn, 1963; Curran *et al.* 1963). It is possible that the discrepancy could be due to an

underestimation of the quantity of Na present in the corium of the skin. This could lead to a large error if it is considered that the corium can bind Na (Imamura, Takeda & Sasaki, 1965). Furthermore, the amount of extracellular Na not subtracted from the transport compartment would be greater in skins with a high rate of transport, because when radioactive Na is at the external surface of the skin, the specific activity of the internal solution is proportional to the rate of transepithelial transport. Recently Zerahn (1969) concluded that his determinations of the transport compartment in whole skins represented mostly Na that had already been transported.

The action of antidiuretic hormone

Previous findings (Curran *et al.* 1963; Cereijido, Herrera, Flanigan & Curran, 1964; Frazier, Dempsey & Leaf, 1962) indicated that the rate of Na transport across the frog skin and toad bladder is proportional to the amount of Na in the tissue and that antidiuretic hormone stimulates transport by increasing the amount of Na in the transport compartment derived from the external solution. The experiments in Table 2 show that no increase in the amount of Na derived from the external solution could be detected during the action of antidiuretic hormone on isolated epithelia immersed in 115 mM-Na solutions.

Our findings can be explained if we accept that antidiuretic hormone simultaneously increases Na entrance from the external solution into the transport compartment and active extrusion from the compartment into the internal solution. This explanation is probably the most acceptable for the time being since the failure to detect an increased amount of Na in the epithelium during the action of antidiuretic hormone does not overrule evidence, independent of studies of movements of Na isotopes in the skin, that shows that the hormone increases the permeability of a barrier at the outer surface of the epithelium (Andersen & Ussing, 1957; Cuthbert & Painter, 1968; McRobbie & Ussing, 1961; Whittembury, 1962; Rawlins *et al.* 1970).

The effects of ouabain

Our finding that ouabain poisoned epithelia do not accumulate Na from the external solution is in agreement with the observations of Herrera (1968) in the toad bladder. From this observation we can conclude, as McRobbie & Ussing (1961) did, that ouabain in addition to blocking the Na pump reduces the Na permeability of the epithelial cells. This conclusion ought to be qualified since after ouabain treatment the cells of the epithelium do accumulate Na from the internal solution; therefore if ouabain reduces permeability it reduces mainly the permeability of the external surface of the epithelium. Recently Biber (1970) found that the

Na uptake from the external solution was depressed by ouabain when $[Na]_o$ was 6 mM, but it was not reduced when $[Na]_o$ = 115 mM. This result suggests that other explanations for our results in a 115 mM-NaCl solution are possible; for example, it can be suggested that the transport compartment has a fixed capacity. Obviously our knowledge of the path through which Na crosses the skin is very limited and detailed speculations of its organization are still difficult.

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