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Ion and Water Balance in the Epithelium of the Abdominal Skin of the Frog *Leptodactylus ocellatus*

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Summary. Epithelia from the abdominal skin of the South American frog *Leptodactylus ocellatus* were isolated by a method consisting of trypsination and dissection. When mounted between two chambers containing Ringer's solution they show electrical properties similar to those found in whole skin. Oxygen consumption was measured. The effects of amiloride, ouabain and low temperature are studied. An analysis of the ionic distribution in the epithelium is performed. The study demonstrates that, regardless of other effects that trypsin could introduce, it constitutes a valuable tool to analyze the basic mechanisms of transepithelial transport at epithelia, cellular and subcellular levels.

This work is aimed at obtaining information on the movement and distribution of ions and water that could shed light on the process of transepithelial movement. In a previous paper (Zylber, Rotunno & Cereijido, 1973) we have described a trypsination-dissection method to obtain isolated epithelia and therefrom isolated epithelial cells. It was shown that the cells thus obtained maintain an adequate ionic balance, and are sensitive to low temperature and ouabain. The present paper is devoted to the ionic balance in epithelia and its response to factors which are known to modify transport phenomena. This information, together with the information on isolated cells obtained in the previous paper is used to reconstruct the ion distribution in the frog skin. The modifications that the trypsination-dissection method might introduce do not disturb the characteristics which are assumed to be essential for cellular and transepithelial transport. Therefore, the procedure provides a tool to continue the exploration at subcellular levels.

Materials and Methods

Since the obtainment of isolated epithelia is the first step in the separation of epithelial cells, the procedures and material used here are the same as those described in detail in the preceding paper by Zylber *et al.* (1973). There are, though, some differ-

ences, in particular if one desires to mount the isolated epithelia as a flat sheet between two chambers: (1) instead of pulling strips of epithelium from the trypsinized skin, one pulls out strips of dermis and carefully cuts with a scissors the remaining attachments between them; (2) care is taken to avoid holes in the epithelium. In this way it is possible to obtain an ample area (up to 10 to 11 cm^2) of abdominal epithelium and mount it between two chambers. Electrical potential difference ($\Delta\psi$) was measured by connecting the chambers through agar-Ringer's bridges to calomel half-cells and these cells to a Keithley 200B d–c electrometer (imput impedance, $10^{14}\,\Omega$). Current was passed using Ag–AgCl electrodes and was measured with a Simpson microammeter. These measurements were made sporadically to test the state of the isolated epithelia on the basis of their ability to work as epithelial membranes. In most cases, though, the epithelia were incubated in covered Petri dishes. The suspension was gassed by bubbling O_2. At the end of the incubation period they were put on a clean glass, blotted and weighed. All determinations made thereafter are described in the quoted paper of Zylber *et al.* (1973).

Measurements of Oxygen Consumption

A piece of epithelium was put in 5.0 ml of Ringer's solution containing (mM): 105 NaCl, 2.4 $KHCO_3$, 1.0 $CaCl_2$, 2.0 glucose, 10 Na piruvate. After a period of equilibration the oxygen consumption was measured using a polarographic electrode (YSI Model 53 Biological Oxygen Monitor). O_2 consumption was followed for 5 to 10 min. During this period the content of oxygen in the flask containing the epithelium never dropped below 70% of saturation, and the curve relating potential *vs.* time was a straight line. Once the measurement was made the piece of epithelium was removed, blotted and collected in a Teflon capsule for water and ion content determination. The measurement was repeated in the flask without the epithelium and this value was subtracted from the previous one. In this way one discards the O_2 consumption due to cells and debris that might have been lost from the trypsinized epithelium, and ensures that the O_2 consumption corresponds to the same amount of tissue used for water and ion determinations.

Details of solutions, procedures for analysis, animals and sources of chemicals, etc, are given in the previous paper.

Results

When mounted between two chambers the epithelia had a short-circuit current (SCC) of 74.3 μamps/cm^2, a $\Delta\psi$ of 18 mV, and a resistance of 242 Ω/cm^2 (Table 1). The SCC is similar to the values found in total skins of the same frogs: 62 μamps/cm^2 (Zadunaisky, Candia & Chiarandini, 1963). Epithelia of *R. pipiens* isolated by a combination of collagenase treatment plus hydrostatic pressure had a SCC of 30.4 ± 1.9 (Aceves & Erlij, 1971). Voltage and resistance are somewhat lower than in total skin. Four complete abdominal skins from the same group of frogs used to obtain isolated epithelia had 12 ± 2 (4) mV and 96.5 ± 8.9 (4) μamps/cm^2. Amiloride (10^{-4} M) was tested in three epithelia and inhibited SCC by 70%. Fig. 1 shows that upon removing the amiloride the SCC rises again to control values.

Table 1. Short-circuit current (SCC), electrical potential difference ($\Delta\psi$) and electrical resistance (R) of isolated epithelia mounted as a flat sheet between two chambers with control Ringer's (115 mM NaCl) (Exposed area 3.14 cm^2)

	SCC (μamps)	$\Delta\psi$ (mV)	R (Ω)
	300	20	67
	280	16	57
	220	12	55
	272	32	118
	236	29	123
	200	12	55
	174	12	69
	175	13	74
	300	19	63
	175	13	74
Mean	233	18	76
SEM	±16	±2	±7

Oxygen Consumption

Table 2 shows the oxygen consumption of epithelia incubated under control conditions. The value found (4.34 μliters/hr mg dry weight) is similar to the ones found by Macknight, Di Bona, Leaf and Civan (1971) in sheets of epithelial cells from urinary bladder, 4.29 to 4.71 μliter/hr mg dry weight.

There is a relationship between oxygen consumption and active transport through epithelia (Zerahn, 1956; Leaf & Renshaw, 1957; Vieira, Caplan & Essig, 1972). Ouabain, which decreases transport and short-circuit current, also inhibits oxygen consumption (Levy & Richards, 1965; Vieira *et al.*, 1972). The inhibition produced by ouabain corresponds to the component of the oxygen consumption which is associated with Na transport. In the urinary bladder this component is about one-half of the total oxygen consumption. Table 2 shows that in isolated epithelia, ouabain (10^{-4} M) decreases oxygen consumption by 33.1%. Zylber *et al.* (1973) have found that ouabain at the same concentration used here produces a large decrease of K content and a gain of Na in isolated epithelial cells.

Use of Albumin and Inulin to Calculate the Intracellular Composition

Zylber *et al.* (1973) have analyzed the suitability of ^{131}I-albumin and ^{14}C and ^{3}H-inulin as markers of the amount of Ringer's trapped in the pellet between the cells, and concluded that inulin penetrates into an extra space which is not accessible to albumin. They have not discarded the

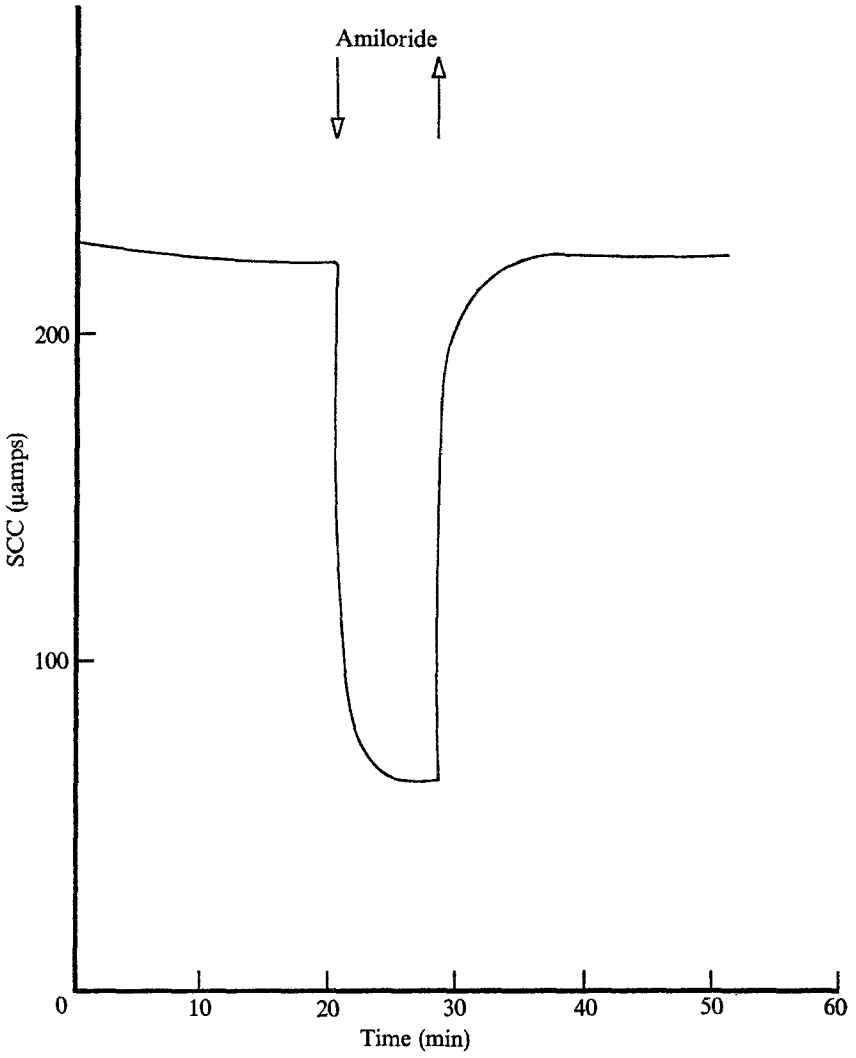

Fig. 1. Effect of amiloride (10^{-4} M) on the SCC of the isolated epithelium mounted between two identical Ringer's solutions containing 115 mM NaCl. The exposed area was 3.14 cm^2

possibility that this extra space might be located inside the cells. This is also observed in epithelia (Table 3). The intracellular water calculated on the basis of the albumin space remains constant, but appears to decrease with time when calculated on the basis of the inulin space. Since inulin has a smaller molecular size than albumin, the results in Table 3 might, in principle, be taken to indicate that, as the incubation proceeds, inulin penetrates into regions of *extracellular* space not accessible to albumin.

Table 2. Oxygen consumption in the isolated epithelium of the frog skin

Oxygen consumption	μliter/hr mg dry wt
Control	4.34±0.31 (8)
Ouabain 10^{-4} M	2.85±0.25 (8)
% of inhibition	33.1 ±6.1 (8)

Table 3. Intracellular water and potassium in the epithelium as determined with ^{131}I-albumin and ^{3}H-inulin (n=6)

Incubation time (min)	Water		Potassium		
	^{131}I-albumin (μliter/mg dry wt)	^{3}H-inulin (μliter/mg dry wt)	Content (μmole/mg dry wt)	Concentration ^{131}I-albumin (mM)	Concentration ^{3}H-inulin (mM)
30	3.10±0.02	3.04±0.17	0.429±0.007	138.0±1.5	143.8± 7.7
60	3.12±0.05	2.67±0.20	0.415±0.008	133.5±1.4	160.1±10.6
90	3.01±0.04	2.41±0.09	0.411±0.004	136.5±1.5	173.1± 9.3

However, this possibility is unlikely if one takes K as an intracellular marker. The *content* of K remains constant throughout the incubation. If K is diluted in the intracellular water as calculated with albumin, its concentration also remains constant. This concentration is similar to the one found in isolated cells which do not have an intercellular space as the epithelia used in the present case and, therefore, permit a more accurate measurement. If, on the contrary, K were diluted in the intracellular water which can be calculated on the basis of the inulin space, its concentration appears to increase with time and achieves improbable high values. Therefore, in the experiments described hereafter, ^{131}I-albumin was adopted as extracellular marker.

Effect of Low Temperature on Ion and Water Distribution

Table 4 shows the H_2O, K and Na content of epithelia incubated 45 to 60 min in control Ringer's. The values of water and K^+ are similar to the ones found by Aceves and Erlij (1971) in epithelia isolated by the use of collagenase and pressure (4.94 μliters and 0.440 μmole per mg of dry weight). The Na^+ content is somewhat higher than the value found by these workers (0.212 μmole/mg). The overall concentrations are (mM): K^+ 112; Na^+ 75.5; Cl^- 98.8. After allowing for the measured extracellular space

Table 4. Water and ion content in isolated epithelium of the frog skin

H_2O	4.17 ±0.09 (15)	μliter/mg dry weight
K^+	0.468±0.004 (15)	μmole/mg dry weight
Na^+	0.315±0.023 (15)	μmole/mg dry weight
Cl^-	0.412±0.016 (15)	μmole/mg dry weight

(18.1% of the total water content) the cellular concentrations of these ions are (mM): K^+ 137; Na^+ 70; Cl^- 95. The high content of Na found is consistent with previous results from this laboratory (Cereijido & Rotunno, 1967; Cereijido, Reisin & Rotunno, 1968) using a completely different technique. However, it is considerably higher than the values of Na concentration found by Zylber *et al.* (1973) in the isolated cells (23.3 and 15.8 mM). It is also much higher than the values reported by Aceves and Erlij (1971) for the frog skin (*Rana pipiens*) (24.7 mM) and the ones reported by Macknight *et al.* (1972) for the toad urinary bladder (52.7 mM).

Cereijido *et al.* (1968) carried out a compartmental analysis of Na in the frog skin and concluded that the cells have an average Na concentration of 21 mM, and that an important fraction of the epithelial Na is contained in a compartment which is open towards the inside, but relatively closed to the outside. They suggested that this compartment could be identified mainly with Na in the intercellular space (ICS). The high concentration of Na that this space would thus have, may be accounted for by the existence of an intercellular substance with ion-binding properties of the kind proposed by Farquhar and Palade (1965). In this respect, the *volume* of the ICS would be correctly measured by ^{131}I-albumin, yet by assuming that Na is at the same concentration as in the Ringer's, the Na content of the ICS would be underestimated and, therefore, the cells would appear as having a higher concentration of Na. In view of this difficulty the compartmentalization of ions in the epithelium was studied with the procedure described below. Two groups of epithelia (Table 5: A and B) were incubated in control Ringer's at 20 °C for 1 hr. The epithelia were then removed, blotted, weighed, and their water and ionic composition was determined. Group A served as control. For the sake of clarity the different ionic balances calculated on the basis of data in Table 5 are schematized in Fig. 2. The dotted area corresponds to the Na contained in the albumin-determined space (ICS) at concentration equal to the Ringer's (115 mM). Epithelia in group B were treated in the same way as those in Group A except that, before blotting, they were rinsed 1 min in a Petri dish with choline Ringer's which was continuously stirred by a magnetic bar. This brief maneuver

Table 5. Effect of temperature on the water and ionic composition of isolated epithelia

Group	Temperature (°C)	Total water (μliter/mg dry wt)	I^{131}Albumin space (%)	Ion content (μmole/mg dry wt)		
				Na	K	Cl
A	20	4.28±0.16(10)	23.4±2.5(9)	0.384±0.024(10)	0.477±0.000(8)	0.438±0.026(10)
B	20	4.32±0.09(10)	22.9±1.6(10)	0.062±0.003(10)	0.437±0.008(8)	0.384±0.029(9)
C	3.5	3.78±0.08(10)	18.5±1.7(10)	0.371±0.022(10)	0.352±0.000(10)	0.315±0.016(10)
D	3.5	3.75±0.06(10)	18.6±1.3(10)	0.117±0.008(8)	0.343±0.000(8)	0.329±0.024(10)

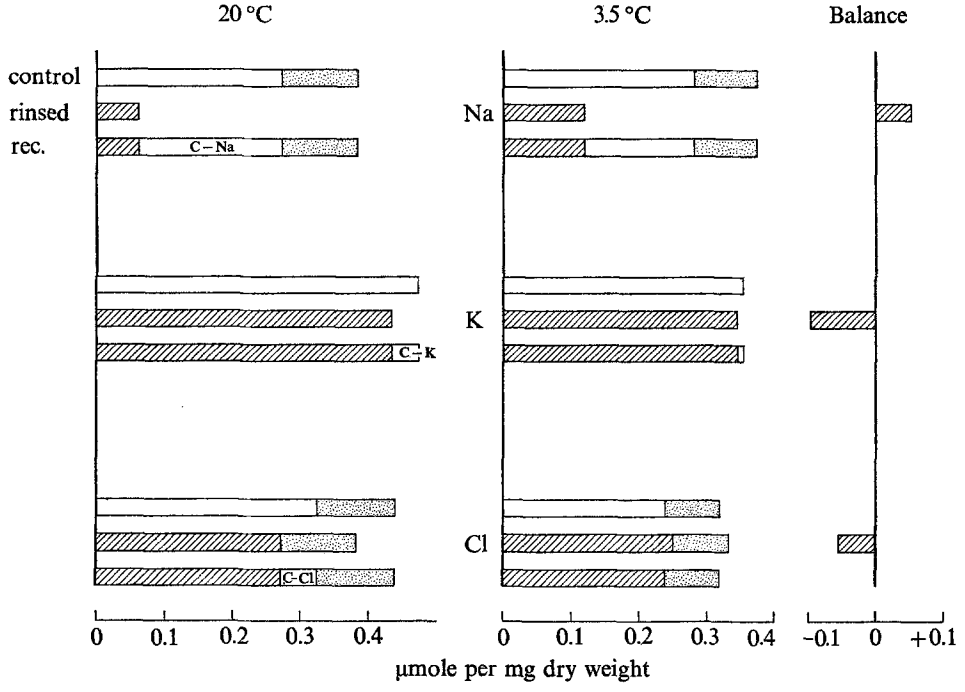

Fig. 2. Ionic balance in epithelia incubated 1 hr in control Ringer's at room (left) and at low (right) temperature. In each group of experiments the upper bar corresponds to a control experiment in which the ionic content of the intercellular space is calculated with the volume of distribution of albumin, and the ion concentration of the Ringer's (grey area). *Rinsed*: corresponds to the ionic content of the epithelia rinsed for 1 min in choline Ringer's (hatched area) and which is assumed to reflect mainly the ionic content of the cellular space. *Rec*: is the reconstruction of the ionic compartments made on the basis of the previous two sets of experiments (previous two bars). On the right-hand side is the ionic balance of the rinsed tissue which is assumed to reflect the ion content of the cellular compartment

Table 6. Ion content in the epithelium as a function of the length of washing

Epithelium	Na (μmole/mg dry wt)	K (μmole/mg dry wt)
Control	0.366 ± 0.012 (6)	0.477 ± 0.000 (6)
Washed 1 min	0.068 ± 0.009 (6)	0.433 ± 0.004 (6)
Washed 30 min	0.050 ± 0.007 (4)	0.359 ± 0.009 (6)
Washed 60 min	0.041 ± 0.007 (6)	0.325 ± 0.000 (6)

caused a considerable drop in Na content. Table 6 shows that, if the washing is continued, the ion content drops further, but with a much slower rate, indicating that the amount lost in the first minute was contained in a relatively open compartment. The assumption is made that the Na contained in the ICS is part of the Na lost in this procedure. The amount of Na remaining in the washed tissue divided by the amount of cellular water (calculated as Total Water minus the ICS) is 18.7 ± 1.3 (10) mM, a value in satisfactory agreement with the values found in the isolated cells: 15.8 ± 1.8 (14) by Zylber *et al.* The third band in each group in Fig. 2 labeled "Rec" (for "reconstruction") expresses the interpretation of the ionic balance. There is a large amount of easily rinsed Na (C–Na), whose nature is discussed below.

If the same set of experiments is performed with cold Ringer's (Groups C and D) two main changes may be observed: an increase of the Na remaining after rinsing, and a decrease in the amount of C–Na. The comparison of experiments with Na at the two temperatures supports the view that Na remaining after rinsing and which increases upon cooling represents Na in the cytoplasm.

The amount of K contained in the ICS at the same concentration as in the Ringer's (2.4 mM) is a negligible fraction of the total K^+ content. However, rinsing produces a slight decrease in K^+ content (C–K). The amount of K in the rinsed epithelia divided by their cellular water yields a concentration of 143.8 ± 3.5 (8) mM, which is similar to the one found in isolated cells 143.0 ± 4.0 (14) mM by Zylber *et al.* Incubation at low temperature decreases the K constant and concentration of the epithelia, and also of C–K. Therefore, these observations with K support further the view that Na and K retained after rinsing correspond to an intracellular compartment.

Rinsing with choline chloride is not expected to modify noticeably the amount of Cl^- content in the epithelium (C–Cl). The concentration of Cl^- in the epithelia rinsed with choline Ringer's was 80.1 ± 5.7 (9) mM, which compares closely with the value in the isolated cells washed with choline:

83.5 ± 4.7 (14) mM found in the previous paper. Low temperature decreases the content of chloride. At low temperatures, rinsing does not decrease it further. The fact that the amount of Cl^- lost is roughly equal to the difference between Na gained and K lost suggests that the epithelia incubated in cold Ringer's lose KCl (0.055 μmole/mg) and also lose K in exchange for Na. It may be noticed that they also lose water. In experiments B and D the cells have 3.33 μliters (i.e., 77.1% of 4.32 μliters) and 3.05 μliters (i.e., 81.4% of 3.75 μliters) of water, respectively. This indicates that cooling produces a loss of 3.33 to 3.05 = 0.28 μliter of water. The KCl lost would therefore have a molarity equal to 0.055 μmole per mg/0.28 μliter per mg = 140 mM; i.e., the same concentration of K in the cells (143 mM as measured in the cells, and 143.8 mM as calculated in the cellular compartment of the isolated epithelium).

Effect of Ouabain on Ion and Water Distribution

The aim, the conceptual framework and the experimental approach of these experiments are similar to those in the previous section. This time a more specific inhibitor of Na and K transport is used: ouabain 10^{-4} M. Table 7 shows the experimental results, and Fig. 3 schematizes the calculated ionic balances. Ouabain produces a large increase in Na and a marked decrease of K content at expenses of the nonwashable fractions of these ions. The content of Cl^- is not significantly varied. These results are therefore in keeping with those obtained with cold and also with those obtained with isolated cells (Zylber *et al.*, 1973). These effects produced by ouabain are also similar to those observed by Herrera (1968) and Aceves and Erlij (1971).

It is interesting that one of the main effects produced by ouabain is a decrease of the Na content in the C-compartment. Models of Na transport

Table 7. Effect of ouabain (10^{-4} M) on the water and ionic composition of isolated epithelia (n = 6)

Group	Total water (μliter/mg dry wt)	^{131}I-albumin space (%)	Ion content		
			Na^+ (μmole/mg dry wt)	K^+	Cl^-
A	4.19 ± 0.08	17.6 ± 1.4	0.366 ± 0.012	0.477 ± 0.000	0.388 ± 0.021
B	3.76 ± 0.11	19.6 ± 1.3	0.068 ± 0.009	0.433 ± 0.042	0.287 ± 0.011
C	3.65 ± 0.09	17.1 ± 1.1	0.476 ± 0.026	0.250 ± 0.028	0.348 ± 0.022
D	3.48 ± 0.08	26.0 ± 1.6	0.269 ± 0.020	0.207 ± 0.022	0.322 ± 0.015

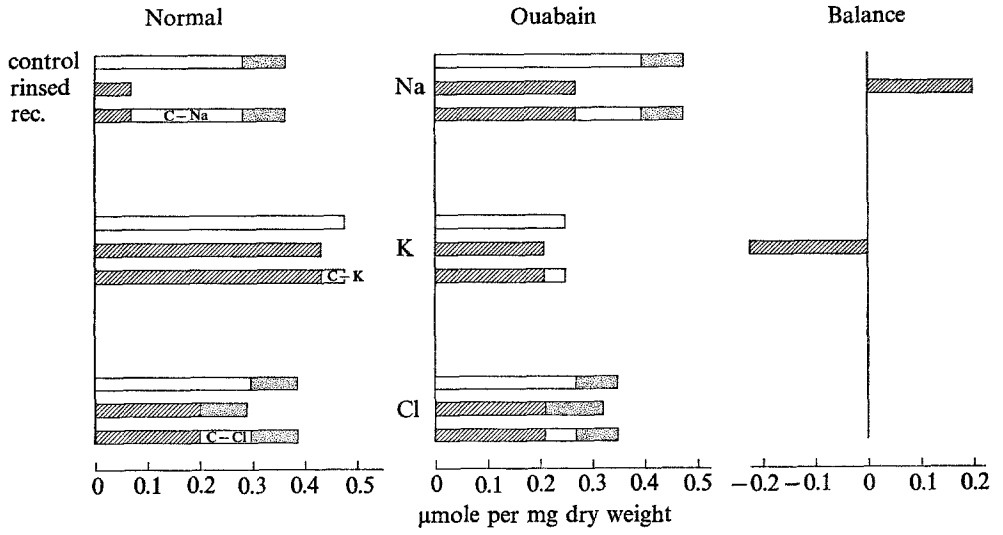

Fig. 3. Ionic balance in epithelia incubated 1 hr in Ringer's (115 mM NaCl) under control conditions (left) and in the presence of ouabain 10^{-4} M (right). The schedule of this study as well as the meaning and distribution of the bars are similar to those in Fig. 2

across epithelia propose that the Na taken from the outer solution is pumped into the intercellular space. The high concentration that Na would thus achieve may play a role in water transport in some epithelia (Diamond & Bossert, 1967). Since this Na is not intracellular it is expected to be "washable" but, since its high concentration depends on continuous pumping, it is expected to decrease upon treatment with ouabain. These expectancies agree with the present observations on the C-compartment.

Na Content as a Function of the Na Concentration in the Rinsing Ringer's

The ion content of the C-space is so high and the volume that it probably occupies (the ICS) is so small that one wonders whether it could be entirely due to a dynamic accumulation elicited by pumping, or that it might be in part related to ion binding. To characterize this space somewhat further, the epithelium was incubated 1 hr in control Ringer's (115 mM NaCl) and then washed 1 min with Ringer's with a Na concentration ranging from 0 to 115 mM. NaCl was replaced by an equivalent amount of choline chloride. As in previous cases, ^{131}I-albumin was present both during the incubation and in the washing Ringer's at the same specific activity. The overall con-

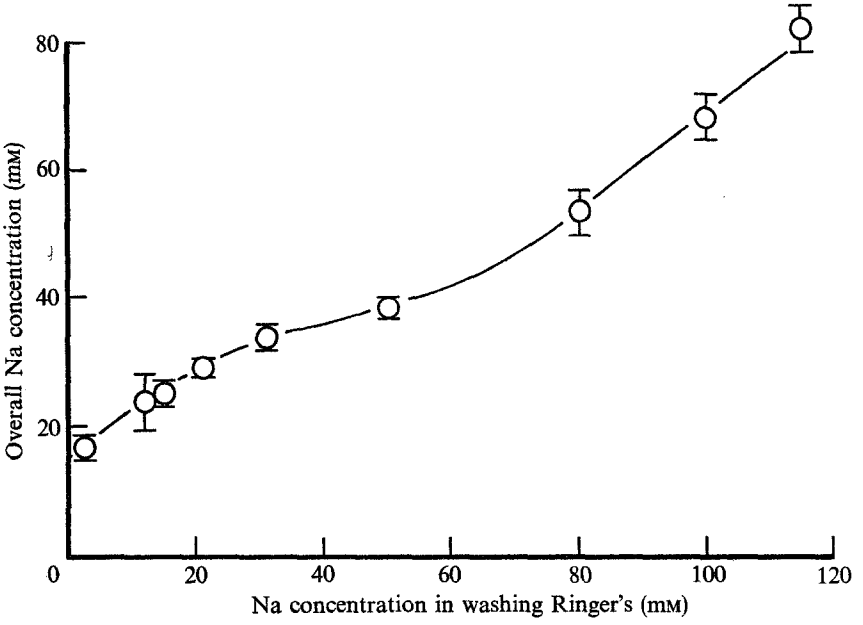

Fig. 4. Overall Na concentration of epithelia incubated 1 hr in control Ringer's and washed 1 min in Ringer's of different concentration of Na (abscissa). The overall Na concentration was calculated with Eq. (1). The curve was drawn by eye

centration of Na was calculated as

$$(Na_T - Na_{Alb})/(W_T - W_{Alb}) = [Na] \quad (1)$$

where Na_T is the total amount of Na in the tissue, and Na_{Alb} is the Na contained in the albumin space at the same concentration as in the washing Ringer's. W_T and W_{Alb} represent the amount of water in the tissue and in the albumin space, respectively. Thus, the numerator of Eq. (1) represents the Na contained in the cells plus the Na in the C-space, and the denominator represents the cellular water. Fig. 4 shows the concentration of Na as a function of the Na concentration in the washing Ringer's. The value of the intercept is 16 mM. On the basis of the considerations made with respect to Tables 5 and 6 and Figs. 2 and 3 one may consider that this concentration represents the one in the cells.

This curve does not correspond to the one expected on the basis of an extracellular space where the only variable is the concentration of Na, and suggests that other factors might be involved. However, the information available is not sufficient to characterize such factors.

Discussion

As mentioned in the Introduction the aims of our work were twofold: (a) to obtain information on the distribution of water and ions in the epithelium that could shed light on the process of transepithelial transport, and (b) to develop a procedure that, while preserving the transport properties of the different levels of organization (i.e., cellular and epithelial), would permit studies of ionic distribution at subcellular levels. The previous paper (Zylber *et al.*, 1973) was concerned with the state and ionic balance of the isolated cells. The present paper undertakes the next level of organization, that of the cells assembled in the epithelium, and tries to reconstruct the picture. The main effort was to obtain evidence that, in spite of the technical procedure to isolate the epithelium and the modifications it might have introduced, the preparation conserves unaltered those characteristics directly involved in ion distribution and transport. In this respect the electrical behavior of the isolated epithelium, and its response to amiloride, ouabain and low temperature, seem convincing enough as to encourage further studies and discussion of this preparation.

Fig. 5 summarizes the results obtained with cells and attempts also an interpretation in terms of the anatomical peculiarities of the epithelium. It is based on the assumption that the trapping space may be adequately evaluated by the use of labeled albumin. (1) *The cells* (Fig. 5*a*) have a concentration of K^+, Na^+ and Cl^- of 142.0, 15.8 and 57.2 mM, respectively. The trapping space has 21% of the total water of the samples (Zylber *et al.*, 1973) and the concentration of its different ions is assumed to be equal as in Ringer's. This leaves a certain amount of Na^+ (0.074 μmole/mg) and of Cl^- (0.110 μmole/mg) in a space easily connected with the bathing solution which can be quickly washed away. (2) *The isolated epithelium* (Fig. 5*b*): if the fraction of ions which, after cooling or treating with ouabain, loses K and gains Na can be taken as the cellular compartment, then the cellular concentrations of K and Na are 132.3 and 18.7 mM, respectively. The epithelium has an intercellular space of 23% of the total water. This leaves 0.040 μmole of K, 0.211 μmole of Na, and 0.030 μmole of Cl^- per mg of dry weight which do not have an obvious location (see C-compartment of Figs. 2 and 3). (3) *The epithelium of the frog skin* (Fig. 5*c*): the sketch on the right-hand side of Fig. 5 speculates as to the possible distribution of ions in the epithelium bathed in control Ringer's on both sides. The concentration of ions in the cornified layer is taken to be equal to the ones found in moulting layers (Zylber *et al.*, Table 10). The concentrations of K and Na in the cells are taken as the average of their concentrations in the cells,

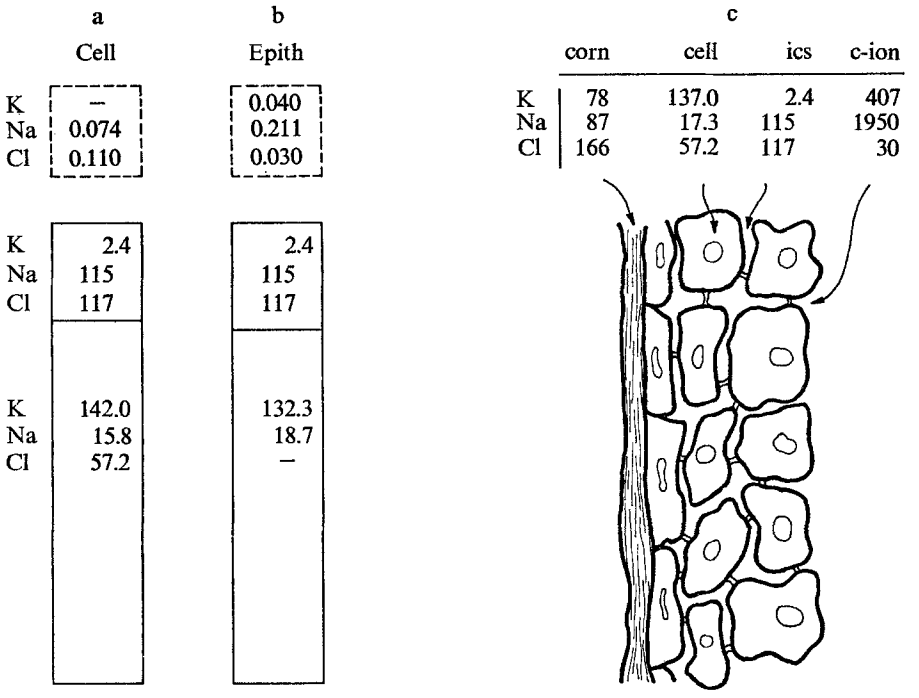

Fig. 5. (*a*) Ion concentration in the cells (bottom) and in the trapping space of 21% of the total volume (middle). The numbers in the dashed block on the top correspond to the amount of ions that can be easily (1 min) washed but which are in excess of the amount of ions contained in the trapping space at the concentration of the Ringer's. (*b*) Same parameters in the epithelium. In this case the middle block represents the ICS occupying 23% of the total volume instead of the trapping space. (*c*) Reconstruction of the ion concentration in the epithelium. The concentration in the cornified layer (*corn*) is assumed to be the same as in the moulting layer

and the cellular compartment of the epithelium. The concentrations in the ICS are equal to those in the Ringer's. If the amount of ions in "compartment C" are assumed to be contained in the ICS they would have the very high concentrations listed in the small table inserted in Fig. 5. In favor of this location of at least part of the C-compartment are the facts that: (*a*) it may be washed in one minute; (*b*) the intercellular space of some epithelia (gallbladder, intestine) is assumed to be the locus of a high concentration of Na due to the continuous imput of the Na-pumps located on the cellular borders facing the ICS. In this connection we may notice that, by incubating the epithelia in cold Ringer's or in the presence of ouabain, the amount of Na in the C-compartment decreases (Tables 5 and 7 and Figs. 2 and 3) as

if it were actually a relationship between the Na pumping and the size of this compartment. Vôute and Ussing (1970) have studied the quantitative relation between short-circuit current and interspace volume, and concluded that the active transport from the outside into the interspace determines the volume of the ICS; (*c*) the intercellular space seems to be filled with material that can complex ions (Farquhar & Palade, 1965); (*d*) a large fraction of the Na in the epithelium is contained in a space which is closed on the outside but open towards the inside (Cereijido *et al.*, 1968).

However, the ICS might not be the *unique* place where the epithelium may complex Na. Thus, studies carried out by combining electron-microscopy with the electron probe have found accumulation of Na not only in the borders of the cells and in the interspace, but in the nuclea as well (C. M. Libanatti & C. J. Tandler, *personal communication*). With the information available it is difficult to explain how Na in the nucleus would exchange easily with the bathing solutions. A substance with a Na binding property was described by Vanatta (1966) in the urinary bladder. Rotunno, Kowalewski and Cereijido (1967) and Reisin, Rotunno, Corchs, Kowalewski and Cereijido (1970) have observed that the NMR spectrum of Na in the frog skin is different from that in free solution, and suggested that it is due to Na complexation. However, Shporer and Civan (1972) have recently demonstrated that the modifications found can also be due to factors other than complexation.

Since even the isolated cells have a compartment with quickly washable Na they are expected to contribute to the C-compartment. As discussed by Zylber *et al.* (1973) a possibility exists that this space was produced by trypsin. If this were so, the change induced by trypsin, which did not interfere with the ionic balance of the cells, nor with their response to cold and ouabain, does not seem to interfere with these functions of the epithelium either. The elucidation of the nature of this large amount of epithelial Na contained in an openly connected compartment must await further studies.

As discussed elsewhere (Rotunno, Pouchan & Cereijido, 1966; Cereijido & Rotunno, 1967; Cereijido *et al.*, 1968; 1973) one of the basic problems in Na transport through frog skin is the penetration of Na in a net amount from an outer solution with low Na concentration (1 mM) into a cell with a higher *overall* Na concentration (15.8 mM). Since the bulk of the information suggests that this step is passive, the possibility arises that the intracellular Na was compartmentalized (e.g., bound in organella like the nucleus). In this respect, the facts that trypsin permits the obtainment of isolated epithelia and isolated cells, and that it does not disturb their basic transport

properties suggest that the procedure used here can be used as a step to subcellular studies.

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