SOME MORPHOLOGICAL ASPECTS OF ACTIVE SODIUM TRANSPORT*

The Epithelium of the Frog Skin

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ABSTRACT

A method was experimentally tested which allows simultaneous morphological and bioelectrical studies of a tissue that performs active sodium transport, i.e., the isolated, surviving frog skin. In a four cell lucite chamber with four separate electric potential and current circuits, skin specimens for morphological observation (light and electron microscopy) were fixed *in situ* in well-defined functional states. The rate of active sodium transport through the epithelium of *Rana temporaria* skin was modified by changing the strength of the electric current passed through the specimens. A marked, reversible swelling of the outermost layer of the stratum granulosum was observed during short circuiting of the skin compared to the homogeneous appearance of the epithelium under open circuit conditions. Doubling the ingoing current led to an additional small increase of the swelling or the appearance of islets of cell necrosis in the same layer. There were signs of a slight shrinkage of the underlying cell layers. The observations are discussed in the light of previous bioelectrical and morphological observations.

INTRODUCTION

An enormous amount of information about active transport of sodium ions across biological membranes and its relations to electrical parameters like potential and short circuit current has accumulated in the last two decades. To some extent it has been possible to correlate functional and morphological observations with encouraging results (1, 16, 20, 22, 23), but many essential points are still open to discussion. One reason for this is that in previous investigations morphological and functional studies were carried out on different specimens. The present paper describes a technique which allows specimens for morphological studies to be fixed under well-defined functional conditions. The setup was used for investigation o the morphological changes associated with the passing of ingoing electric currents through isolated surviving frog skin. Since such currents, under appropriate conditions, are carried mainly or (under short circuit conditions) exclusively by actively transported sodium ions (2), the morphological changes may give us information about certain aspects of the active sodium transport mechanism.

Theoretical Considerations

Like many other epithelia, the epithelium of the frog skin maintains an electric potential difference between its inward and outward facing boundaries.

* Dedicated to Gunnar Björklund, Sigtuna, Sweden.

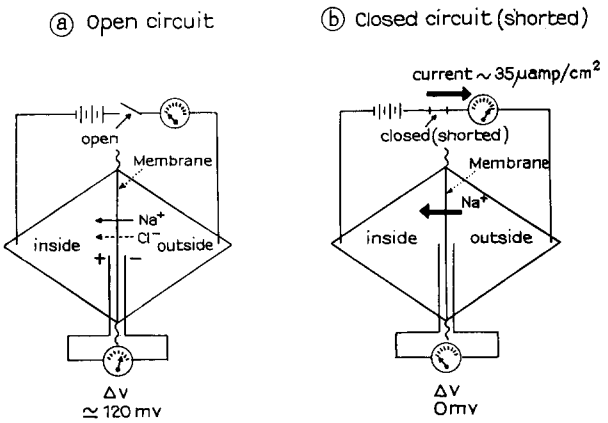


FIGURE 1 Illustration of the theoretical considerations and the methodological setup of the experiments.

If both sides of an isolated frog skin are in contact with Ringer's solution, the inside bathing solution is positive relative to the outside solution. In the species studied, *Rana temporaria*, the potential difference (called the skin potential) may be as high as 170 mv but is usually between 60 and 120 mv. According to Ussing and Zerahn (2), this potential is created and maintained by active transport of sodium ions from the outside to the inside solution. Numerous investigations in this and other laboratories have substantiated this view (references 3–6 among others). The electric potential difference across a given skin is a function of the efficiency of its "sodium pump" as well as its leakiness to anions (for details see reference 7). The operation of the active sodium transport mechanism tends to increase the potential difference, whereas the flow of passive ions like chloride under the influence of the potential difference tends to lower it. The flow of chloride ions in a way represents a shunt to the "sodium battery" so that a high chloride permeability gives a low potential and vice versa. The normally high potentials (80–120 mv) are mostly associated with a low transport of NaCl, because the low Cl^- permeability reduces the flow of chloride while the high potential opposes the inward transport of sodium. If, on the other hand, the electric potential difference is reduced, e.g. by connecting the inside and outside solutions through an external circuit, more sodium will be transported actively from the outside to the inside solution (see Fig. 1). The situation is quite simple when the external circuit has zero effective resistance. Under such conditions the potential difference is zero, and the passive ions, like chloride, have no reason for moving faster one way than the other and do not contribute to the current. The electric current, therefore, is carried exclusively by the actively transported sodium ions.

To achieve the condition of total short circuit, one can use the technique developed by Ussing and Zerahn (2).

The principle is to place a battery and a potential divider in series with the skin through suitable electrodes and to adjust the applied current to exactly the value which makes the potential drop across the skin equal to zero (measured between separate reversible electrodes). The skin now has only to overcome its own internal resistance, and so it is by definition short-circuited; but, as the skin and the external circuit are placed in series, the short circuit current can be read on a microammeter placed in the circuit. Careful measurements in instances where the inward and outward fluxes of sodium were measured with isotopes have shown that, indeed, the short circuit current is always equal to the net transport of sodium (2, 8, 9); therefore, one can safely use the short circuit current as a measure of the active sodium transport.

It has been shown independently by Zerahn (10) and Leaf and Renshaw (11) that the metabolic rate of the skin is determined by the amount of sodium transported actively. The oxygen consumption attains a basic value when sodium transport is stopped, owing to lack of sodium in the outside medium or a high opposing potential, and increases linearly with the rate of sodium transport. Metabolically, therefore, the high potential skin is in a "resting state" whereas the short-circuited skin is working harder. If the current is increased beyond the short-circuited state, the oxygen consumption continues to increase *pari passu* with the

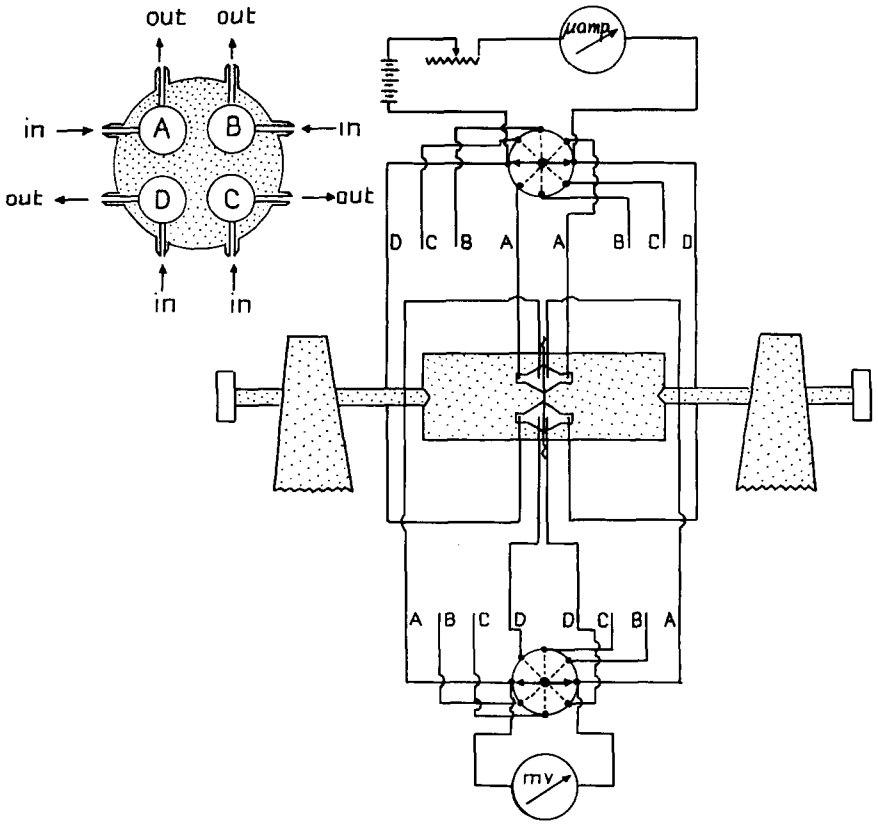


FIGURE 2 Drawing in the left upper corner illustrates the experimental chamber in a sagittal crossection. Attached in- and outlets for the circulating Ringers are shown. The drawing on the right illustrates the separate electric circuits for PD and SCC measurements.

net sodium transport until the current has reached a value of somewhat more than double the short circuit current; but in this region the sodium transport has to be measured with isotopes, if accurate figures are desired, because the passive ions give rise to a leak current of variable magnitude. From the above considerations, it will be clear that by changing the strength of an ingoing current through a frog skin one can, within limits, set the rate of active transport and the metabolic rate at any desired level.

From the morphologist's point of view there must be two questions: (*a*) are the functional states which one can define on the basis of open circuit, short circuit, and double short circuit current associated with characteristic morphological states; (*b*) is it possible to obtain trustworthy reproducible pictures of these states?[1]

[1] It may be worth mentioning that the short-circuited state should not be considered something very "unbiological." At certain times of the year, especially in the springtime, one may find skins with a rather high rate of sodium transport and a very low potential (only a few millivolts). These skins have a very low chloride resistance and can be considered internally short-circuited through the chloride shunt.

MATERIALS AND METHODS

The experimental setup, including the electric circuits, was designed on the basis of principles previously described (2). It is composed of four identical cells, allowing four 1 cm² areas of the same skin to be studied simultaneously. The volume of bathing solution necessary on either side of each skin is 5 ml (see Fig. 2).

Experimental Procedure

All experiments were performed in the months September to February on *R. temporaria* kept in shallow tap water at 4°C. The skins were obtained in the usual way and placed as gently as possible between

the two half-cells. 5 ml of frog Ringer's solution were pipetted into each half-cell. The bathing solutions were kept circulating and aerated during the entire experiment. The composition of the Ringer's used has been described elsewhere (12). Special care was taken to keep pressures as symmetrical as possible in all chambers, as judged by the vertical position of the membranes. The pressure difference across one membrane never exceeded 1 cm H_2O. The skins were kept under open-circuit conditions for 60–90 min until the initial fluctuations in potential had leveled off. Usually the four areas of one skin agreed well with respect to the bioelectric parameters potential and short circuit current. If one of them differed from the rest by more than 10 mv or 10 μamps/cm^2, it was not used.

Each skin was studied histologically with respect to the following conditions: (*a*) control, open circuit; (*b*) short-circuited for 45–50 min; (*c*) ingoing current twice the short circuit current for 20–30 min ("double current"); (*d*) recovery control (like *c*, but fixed 20 min after reopening of circuit).

The skins were fixed *in situ* by the following technique. Circulation of the bathing solutions was stopped on both sides by clamping the air admission tubes. One-half of the solution in each half-cell was removed and replaced by the same amount of 2% OsO_4 dissolved in Ringer's. The final fixative had a pH of 7.7 $\pm$ 0.1 and an osmolality of 250 milliosmol/liter $\pm$ 5. Circulation was restored symmetrically. The beginning of the fixation could be read as a sharp drop of the potential, and the potential then declined less rapidly to reach zero in times varying between 10 sec and 2 min. The time it took for the potential to vanish seemed to depend on the intensity of the circulation of the fixative. Moreover, skins with low potential tended to show a steeper decline of the potential during fixation than those with high potential. The short-circuited skins were kept short-circuited during fixation, which means that the applied current had to be reduced gradually to zero as the skin potential vanished. (In a few pilot experiments not included in this series, the current was maintained, throughout fixation, at the level of the short circuit current before the beginning of the fixation). The double current skins received a current strength of twice the short circuit current as measured immediately before the beginning of the fixation. The progress of the fixation was followed by intermittent potential readings.

When the skin potentials had dropped to zero, the skins were kept in contact with the circulating fixative for a period between 30 min and 2 hr (for obvious reasons, the fixed pieces of skin could be removed for further work-up only at the end of the whole experiment). Some control experiments were done with glutaraldehyde fixative (5% in Ringer's for 30 min to 2 hr, and 1 hr of washing in Ringer's followed by postfixation in 1% OsO_4 for 15 min).[2] The histological appearance of the specimens was grossly similar to that obtained with OsO_4 fixation. For the present purpose the glutaraldehyde fixation seemed unsatisfactory because it fixes frog skin epithelium rather slowly. This conclusion is based on the fact that the time required by the glutaraldehyde fixative to reduce the potential and the short circuit current (SCC) to zero is three to five times the time required by the osmium tetroxide fixative. In a few experiments glutaraldehyde alone was not able to reduce the bioelectric parameters to zero.

After the experiment the whole skin was removed from the chamber. The four biopsies were cut in small bits with a razor blade, washed briefly in distilled water, and dehydrated with acetone in increasing concentrations. The embedding in Epon 812 was performed according to routine procedures, with prolonged infiltration steps. The biopsy pieces in the blocks were all reoriented before cutting. This was done by reembedding them in sealing wax at a 90° angle to their surface. Sections for light microscopy were cut, after a last angle correction, on a Porter-Blum MT 1 Ultramicrotome. Their thickness was 0.5 μ for microscopic evaluation and 1 μ for photographic purposes. Toluidine blue was used for staining (13). The measurement of the thickness of the different layers of the epithelium and of the thickness of the total skin were performed with a Wild microscope, with eyepieces provided with a micrometer scale (magnification of 100 for measuring total skin thickness and magnification of 1000 for measuring the thicknesses of the different layers of the epithelium).

Of every biopsy piece, three random sections were used for these measurements. Five measurements were made on each section. The measurement of the height of a cell layer was always made through a nucleus. The mean values of the measurements are given in Table I. Thin sections for electron microscopy were cut in the same way as those for light microscopy; they were picked up at a cutting thickness of 500–800 A and stained with uranyl acetate (14) and lead citrate (15). The light micrographs were taken with a Zeiss microscope with an attached Wild camera. The electron micrographs were obtained with a Siemens Elmiskop (type 1A).

RESULTS

For the general morphological description of frog skin, we would like to refer the reader to previously

[2] Glutaraldehyde fixative, even when used as a 2% solution in identical Ringer's, has an osmolality which is appreciably above that of Ringer's alone, namely ~400 milliosmols/liter.

TABLE I

Mean Values of Thickness Measurements in Skin

Only the first RCL and the total epithelium are considered

Condition	Experiments	First RCL			Total epithelium			First RCL excluded		
		μ	±	%	μ	±	%	μ	±	%
Open circuit	20	7.48	—	—	64.6	—	—	57.1	—	—
Short circuit	20	9.81	+2.33*	+31	68.5	+3.9	+6.0	58.7	+1.6	+2.7
Double-current	19	10.24	+2.76‡	+39	64.2	−0.4	−0.6	54.0	−3.1	−5.5
Recovery	6	6.72	−0.76	−10	57.4	−7.2	−11.1	50.7	−6.4	−12.7

* Statistical evaluation of this value. The numbers in brackets give the statistical evaluation of the further increase‡ under double current: mean $\bar{x}$, 2.33 (0.43; variance s^2, 11.89 (7.65); variance of the mean $s^2_{\bar{x}}$, 0.59 (0.35); standard deviation of the mean $s_{\bar{x}}$, 0.77 (1.0); degree of freedom, 19 (18); t, 3.15 (0.35; probability P, 0.01–0.001 (0.80–0.70).

published papers (16–21). Electron microscopic findings will be discussed only if they help to clarify doubtful light microscopic observations or if they offer additional information.

Qualitative description

OPEN CIRCUIT CONTROLS (FIGS. 4 *a*–6 *a*, 7 *a*, *b*): One to three layers of cornified cells are always found to separate the epithelium from the outside. These cells appear heavily and uniformly stained on light microscopic observation. Right next to an interspace of variable width bordering the cornified layer, the first layer of the stratum granulosum will be the one attracting most of our attention (we shall call it the first reacting cell layer, first RCL). Under open circuit conditions (with a high skin potential) these cells appear uniformly stained and shaped and do not show any appreciable difference compared to the underlying cells of the stratum granulosum and stratum spinosum. The stratum germinativum may easily be differentiated by the less dense cytoplasm of its cells, the columnar shape of the cells and the undulating basal surface of the cells which limits the epithelium towards the interstitial space.

A network-like system of intercellular space is observed, the separation of the cells being interrupted only by dense-appearing granules (the desmosomes) found to be especially numerous in the stratum granulosum. The first cell layer of the stratum granulosum always forms a continuous barrier, its continuity being preserved by tight locks closing the intercellular space system towards the outside medium. These attachment zones are found, with very rare exceptions,[3] at the outermost cell angles of the first RCL (Fig. 7 *a*). They are similar to the zonulae occludentes of the cornified layer previously described by Farquhar and Palade (18). The intercellular space system is more or less open throughout the epithelium and reaches the interstitial compartment; it is interrupted only by a continuous basement membrane.

SHORT-CIRCUITED SKINS (FIGS. 4 *b*–6 *b*, 8 *a*, *b*): The main morphological change is found in the cells of the first RCL. These cells appear swollen. This swelling can be observed as a more or less uniform manifestation of all the cells belonging to this layer. A decreased cytoplasmic density with a relative increase of the contrast of the cytoplasmic structures gives the cells a rather heterogeneous appearance. In general, however, the cytoplasmic organelles do not present major alterations. A slight shrinking of the underlying cells may occasionally be observed (increased nuclear and cytoplasmic density). The stratum germinativum shows neither shrinking nor swelling.

The intercellular space system seems to be open more uniformly, mainly in the stratum granulosum and stratum spinosum. No change can be found in cellular junctions, and the zonulae occludentes remain closed (Fig. 8 *a*). It should be

[3] On some rare occasions one finds an intercellular channel open all the way into the space below the stratum corneum. Since this is a rare observation and since our experimental procedure is not set to evaluate this question, this observation is mentioned only for completeness.

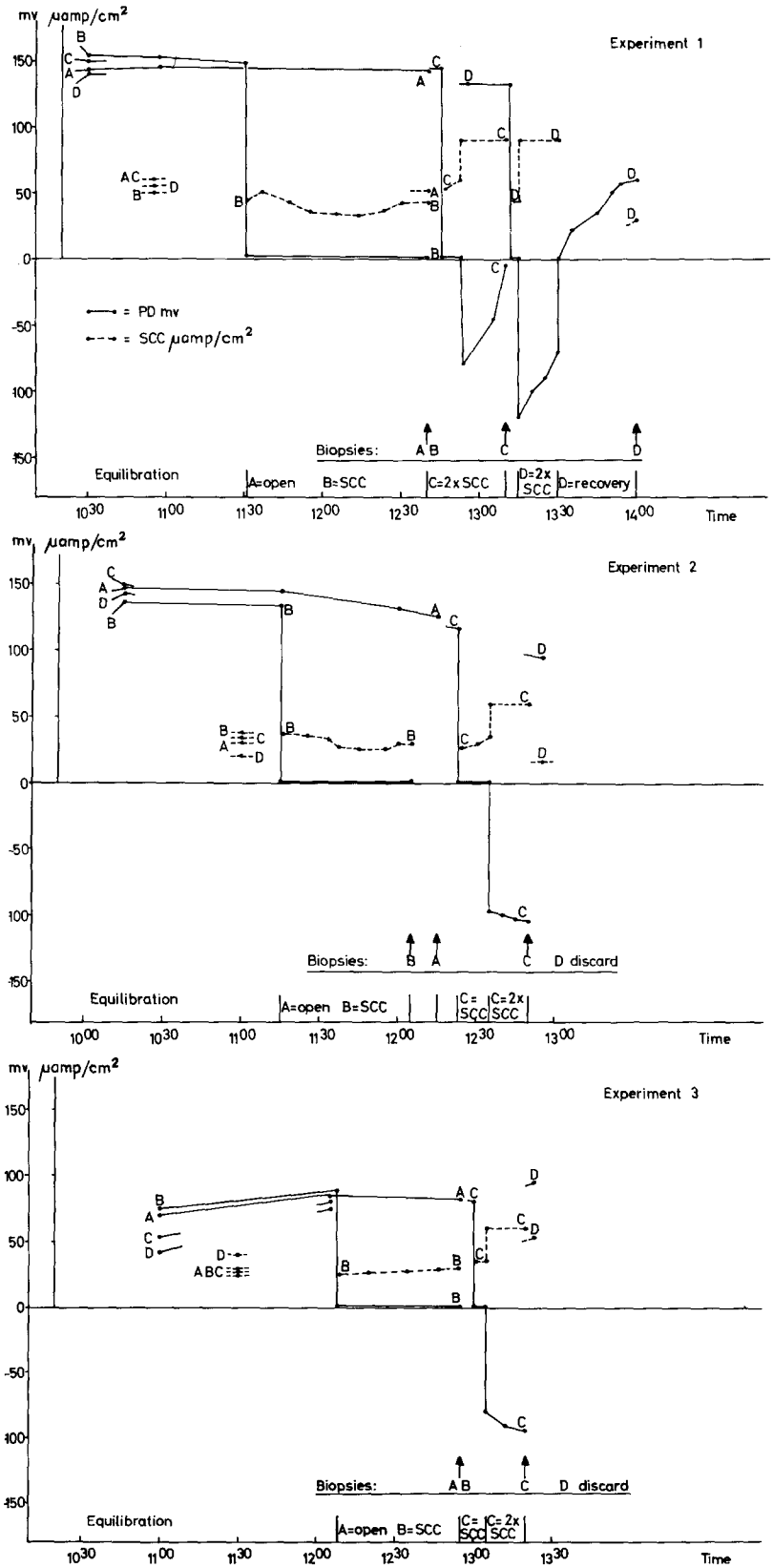
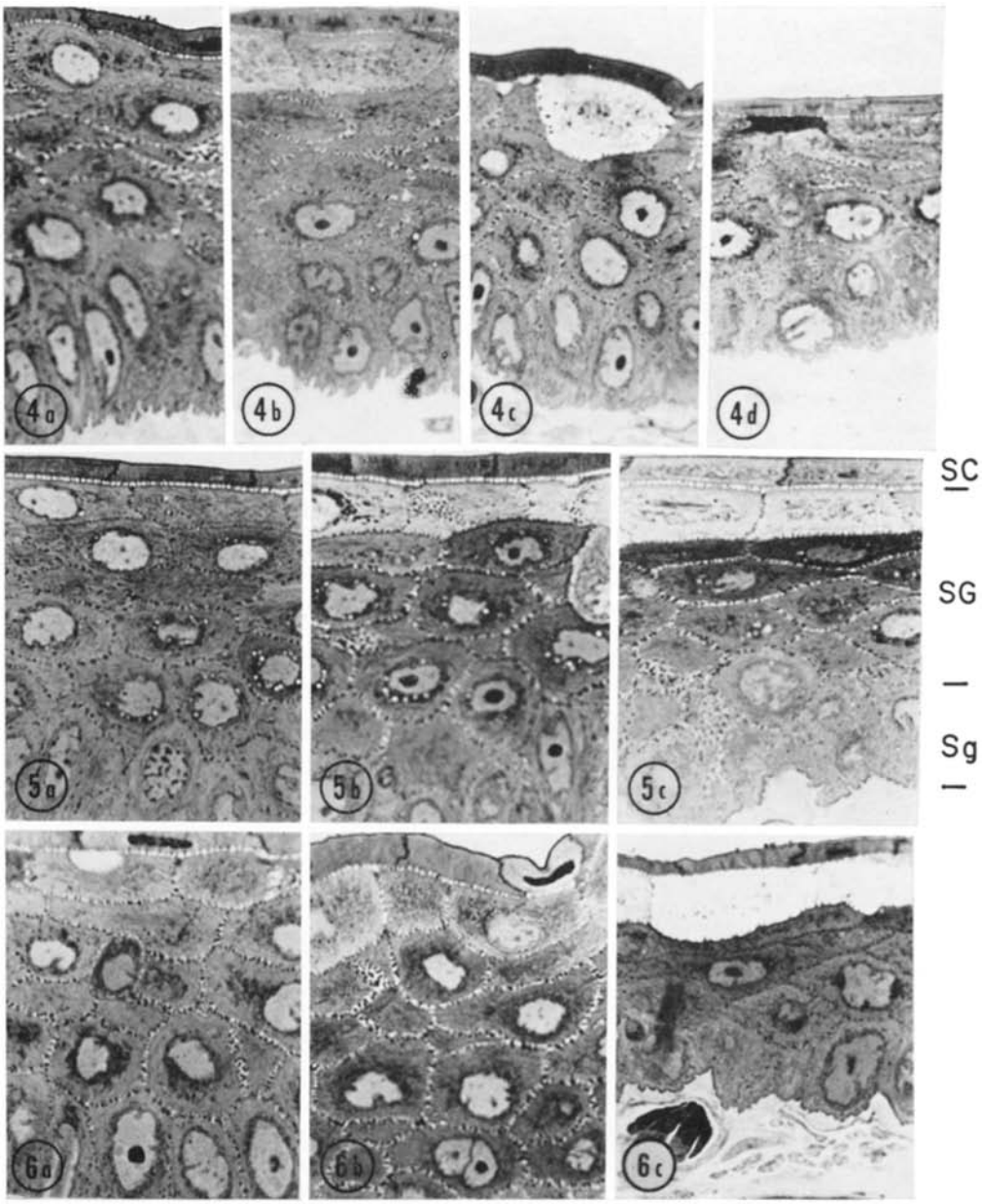


FIGURE 3 Simplified graphical illustration of bioelectric protocols belonging to the morphological illustrations shown in this paper.



FIGURES 4–6 Light micrographs of frog skin epithelium under different bioelectric conditions (for corresponding functional data, see corresponding graphics Fig. 3). Each horizonal row pertains to the same experiment (1–3). The letters indicate the experimental condition (*a–d*): *a*, open circuit; *b*, short circuit; *c*, double current inwards; *d*, recovery after double current 1μ sections stained with toluidine blue. × 2000.

Fig. 4. *a*, Note uniform appearance of all cell layers; *b*, a slight swelling of the first RCL can be observed; *c*, single necrotic cell in the first RCL; *d*, the whole epithelium is shrunken. Pyknotic cell in the first RCL.

Fig. 5, *a*, same as Fig. 1 a; *b*, again, marked swelling of first RCL and shrinking of lower cells are apparent; *c*, same findings as in 5 *b* but more marked. *SC*, stratum corneum; *SG*, stratum granulosum and stratum spinosum; *Sg*, stratum germinativum.

Fig. 6 *a*, First RCL seems already slightly swollen (compared to Figs. 4–5 *a*) (Note lower PD readings than in the two previous experiments); *b*, Marked increase of swelling of first RCL as compared to Fig. 6 *a*; *c*, Extreme swelling of first RCL with pronounced shrinking of underlying epithelium.

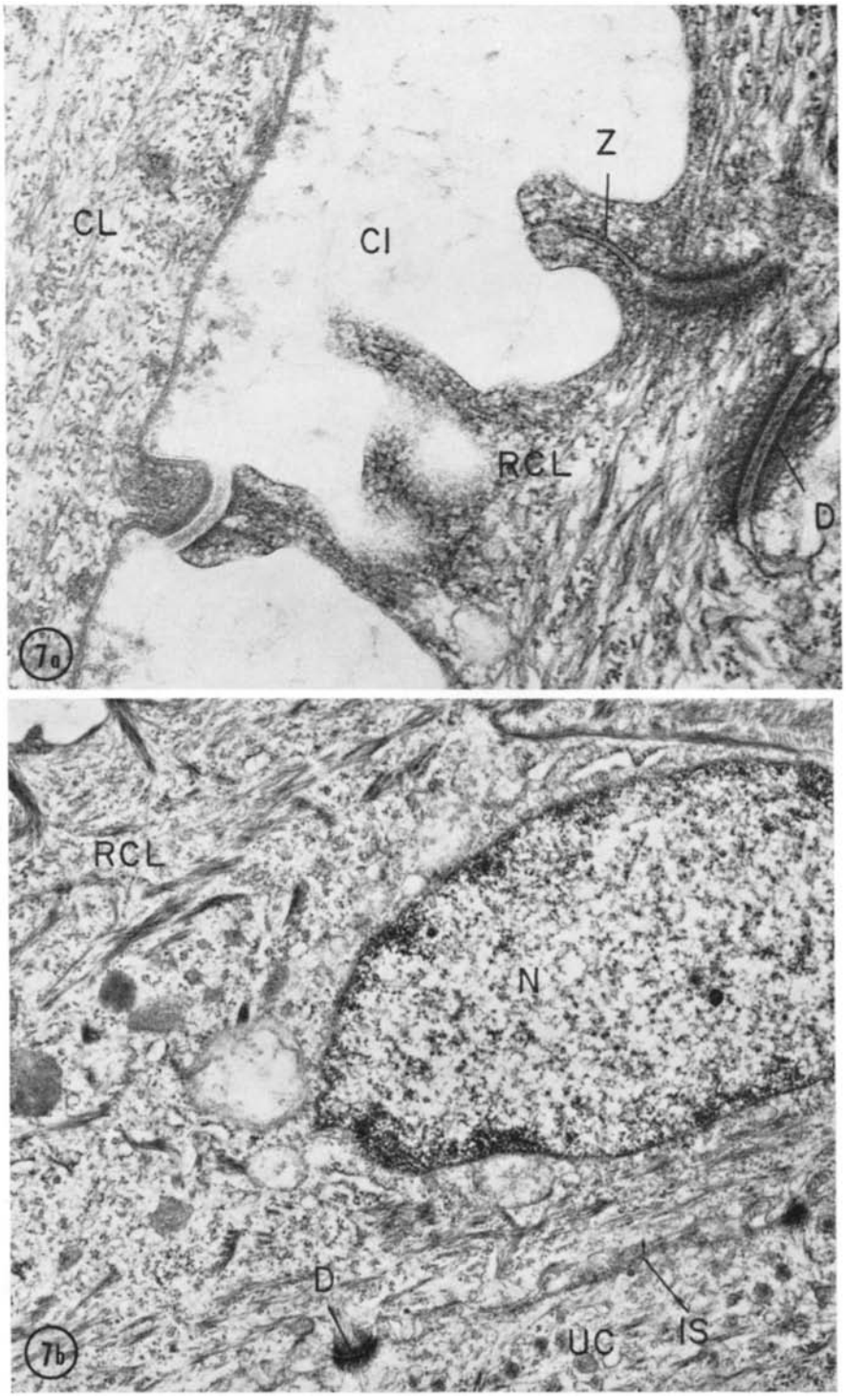


FIGURE 7 Open circuit. *a*, Electron micrograph illustrating the cellular interspace (*CI*) between cornified layer (*CL*) and first reacting cell layer (*RCL*). *D*, desmosome; *Z*, zonula occludens. × 60,000. *b*, This micrograph shows a cell (*RCL*) of the first *RCL* in its full diameter. Compare cytoplasmic densities of this cell and the next cell (*UC*). *N*, nucleus; *IS*, interspace; *D*, desmosome. × 20,000.

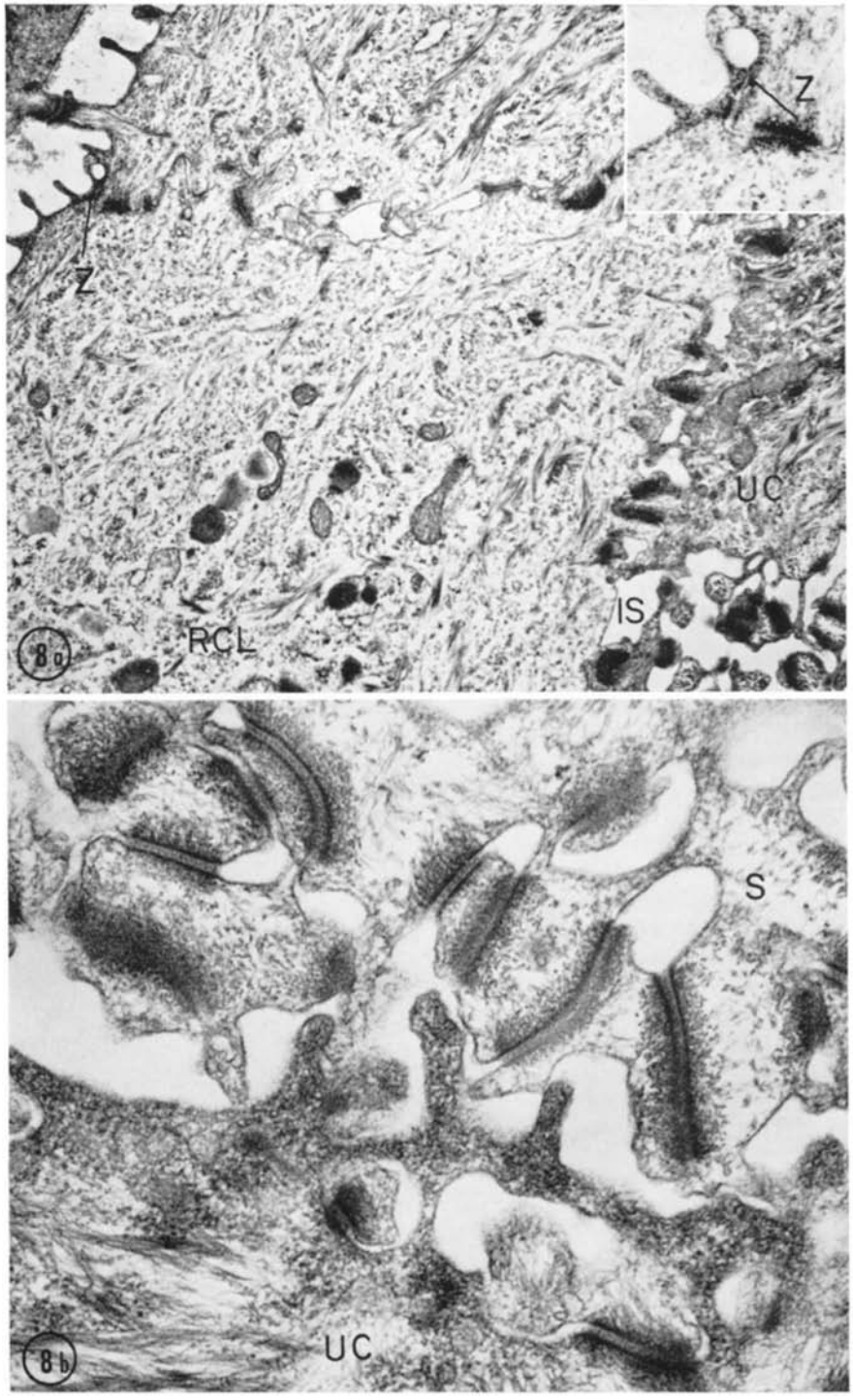


FIGURE 8 Short circuit. *a*, As compared to that in Fig. 7 *a*, the cytoplasmic density of the two cells is now clearly different. Cellular organelles seem to be unaltered; the zonula occludens region (*Z*) remains closed. Same region is shown at a higher magnification in inset. *RCL*, first reacting cell; *UC*, underlying cell; *IS*, interspace. Fig. 8, × 15,000; inset, × 40,000. *b*, Electron micrograph illustrating the cellular junctions between swollen cell (*S*) and underlying cell (*UC*) with normal cytoplasmic density. Note open interspace system. × 40,000.

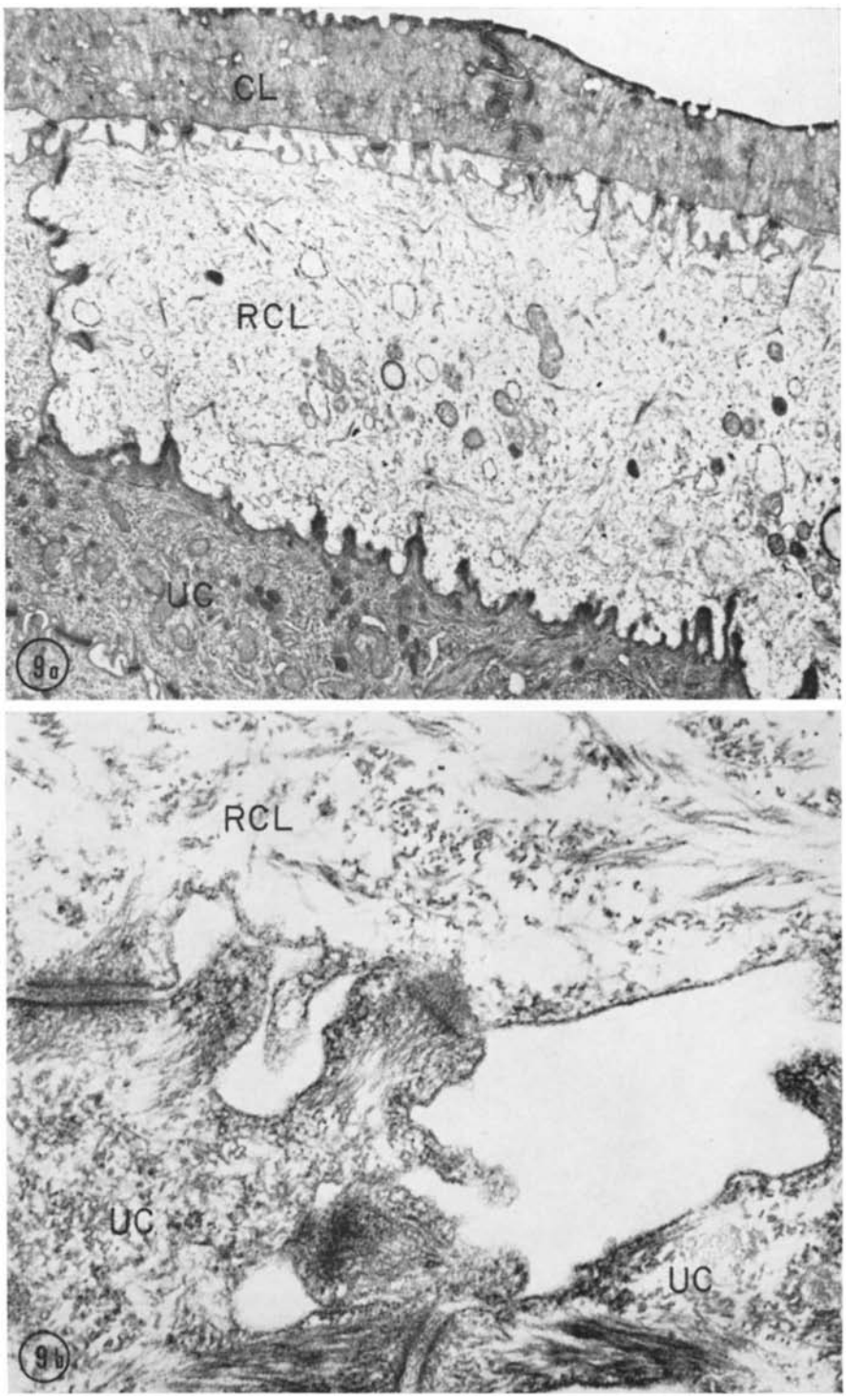


FIGURE 9 Double current. *a*, Cell of the first RCL appears to be dying, since the cytoplasm is loosened especially along the cell membranes and all cellular organelles show more or less marked alterations. Highly shrunken underlying cells. Labelings as in Fig. 9 *b*. × 8,000. *b*, Analogous to Fig. 8 *b*; this electron micrograph illustrates the cellular junctions and interspaces between a highly swollen cell (*RCL*) of the first RCL and two lower cells of normal appearance (*UC*). Note open interspaces. × 60,000.

noted that the interspaces between the swollen cells have, in most cases, practically vanished and that dotted lines of aligned desmosomes merely indicate their location.

SKINS UNDER DOUBLE-CURRENT CONDITIONS (2 × SCC) (FIGS. 4 *c*–6 *c*, 9 *a*, *b*): At this stage of forced Na transport, two different types of cell reactions can be found. One type is a further slight but uniform increase of the swelling (Figs. 4–5 *c*) with more or less marked cytoplasmic changes in all cells of the first RCL. In most cases the swollen cells appear damaged or necrotic. Some skins, however, seem to tolerate forced Na transport better than others (Fig. 5 *c*).

The other type of reaction is heterogeneous. Between normal appearing cells of the first RCL one finds, here and there, single swollen cells which show the same changes as the cells with uniform behavior, but the degree of damage is more severe (Fig. 4 *c*). In some sections the underlying cells of the stratum granulosum and stratum spinosum look shrunken and denser (Fig. 5 *c*). The rest of the epithelium is unaltered.

TABLE II

Behaviour of All Skins (Swelling and Shrinking)

	Increase		No change		Decrease	
	No.	%	*No.*	%	*No.*	%
First RCL	16	80	3	15	1	5
Total epithelium	8	40	6	30	6	30
Skin	8	42	7	37	4	21

RECOVERY (FIG. 4 *d*): In some short-term experiments, the cells of the first RCL exhibit a slight remaining swelling. If the skin is allowed to recover for more than 15 min after the circuit has been reopened, one will find an epithelium as illustrated in Fig. 4 *d* (a more or less collapsed epithelium of uniform density comparable to that of open-circuit skins). Now the first RCL will be interrupted, here and there, by flat, dark cells resembling the cells of the cornified layer. These cells obviously represent the cells damaged during forced Na transport. The intermediate cells of this layer which survived the procedure have again become comparable to the cells of the rest of the stratum granulosum. In some rare skins the open circuit state control and the recovery state do not show any major difference. However, this can be observed only in skins with no drop in potential under forced Na transport and with good short circuit current and potential readings after doubling the current.

QUANTITATIVE APPROACH: As Table II demonstrates, a measurable swelling of the first RCL occurred in 80% of all experiments, whereas swelling of the total epithelium as determined by measurements could be found in only 40% of the experiments. The first RCL, which has an average thickness of 7.48 μ under open circuit, increases to 9.81 μ in thickness after short circuiting; this means an average increase of 2.33 μ or 31%. The increase in thickness of the total epithelium has been found to be 3.9 μ or 6.0%, or, if the first RCL is excluded, 1.6 μ or 2.7%. Thus about 60% of the total increase is due to the first RCL; or, if an average of five to seven cell layers is taken, a mean swelling of 0.2–0.3 μ per remaining cell may be calculated. This corresponds to an increase of 3–5% per cell for the underlying layers as compared to an average of 31% for the first RCL. (Statistical evaluation below Table I.)

DISCUSSION

It appears from the material presented above that short circuiting is associated with only one manifest and reproducible morphological effect, namely a swelling of the outermost layer of the stratum granulosum (first RCL).

Even when the skin specimens are fixed as fast as possible during short circuiting, there is no evidence of vacuole formation, pinocytotic activity, channel formation, or rearrangement of the cytoplasmic organelles. It is also of interest that the zonula occludens region, which closes the interspaces towards the outside medium, remains closed during current flow.

The changes seen in the short-circuited skins are completely reversible. As a matter of fact, in an initial series of 50 experiments in which the skins were fixed during open circuit conditions after various times of short circuiting, the skins which had been short-circuited did not appear significantly different from the controls. The fact that, in the 20 experiments in which the skins were fixed *during* short circuiting, all skins showed cytological changes suggests that the changes take place within minutes. The reversibility of the phenomenon is an indication that we are dealing with a physiological reaction to current flow rather than with a result of cell damage.

If we make the reasonable assumption that the

epithelial cells are in nearly osmotic equilibrium with the bathing solutions, the swelling must mean that the number of osmotically active particles in the first RCL is increased during short circuiting. (This applies whether or not the swelling is due to cell damage.) One possibility is that the increase in metabolic rate, which is associated with the increased active transport of sodium during short circuiting, leads to an augmentation of the number of osmotically active organic particles in the cells. However, this would mean that such particles should constitute some 20% or more of the total osmolarity of the cells during short circuiting; this is much more than one finds in, say, working muscle. A hypothesis which perhaps is more likely is that there is an increase in inorganic electrolytes in the cells of the first RCL during short circuiting. Let us assume, for the sake of argument, that the outward facing boundary of the cells in question is permeable to sodium but less so to chloride, and that the inward facing boundary is more permeable to chloride and less so to sodium. It can then be seen intuitively, and can easily be shown mathematically, that there will be an accumulation of electrolyte (NaCl) between the membranes when an ingoing current is passed. In an artificial, two membrane system, the accumulation would go on as long as the current was running. In the living cell a steady state might be established if the sodium pump were stimulated by the increase in cellular sodium concentration so that the swelling stopped when the entry of Na through the outer barrier was balanced by pumping through the inward facing barrier. The argument can be carried through with essentially the same result for a more sophisticated cell model containing nondiffusible colloid anions and potassium ions (24). It is thus clear that a relatively simple cell model might give the volume response observed during short circuiting, but a detailed evaluation of the merits of the hypothesis requires more knowledge about the relative permeabilities of the inward and outward facing membranes of the first RCL than we have at present.

The fact that only one cell layer shows changes with relation to short circuiting might be taken to mean that this layer alone is performing the active sodium transport. This may be; however, it is quite conceivable that sodium which has entered the first RCL from the outside solution can pass from one epithelial cell to the other through intercellular connections of the type described by Kanno and Loewenstein (22, 23) (as in the case of the cells of the salivary gland of *Drosophila*). If so, all epithelial cells, even the deeper layers, might participate in the transport. Such an arrangement has, in fact, been suggested independently by Farquhar and Palade (18–20) and by Ussing and Windhager (7). Both groups assume, furthermore, that the intercellular space system is relatively tight toward the outside medium but is open towards the inside of the epithelium. This means that sodium, which is transported actively out of a cell, will end up in the intercellular space system and from there pass to the inside solution, irrespective of whether it has been transported by a cell in the first or in the last cell layer.

If this hypothesis is correct, one may ask why only one cell layer swells during short circuiting? The reason may be that all of the sodium ions have to pass this layer whereas an ever decreasing amount is received by the other layers, the farther they are from the surface. Another reason might be that the first RCL is approaching the end of its life and is losing its capacity for osmotic regulation. But then one might ask why an aged cell should respond with an absolutely reversible phenomenon.

If the strength of the ingoing current is doubled, the swelling of the first reacting cell layer becomes more pronounced and, in contrast to the result with simple short circuiting, there is evidence of cell damage in many skins. As has already been mentioned, the oxygen consumption is still closely correlated with the sodium transport under such conditions.[4] Therefore, double current must mean a very heavy drain on the energy reserves of the cells, and one may speculate that this may lead to exhaustion and premature death of some of the cells. However, there may be other reasons for cell necrosis, for instance membrane damage owing to excessive swelling, or direct electrical damage to the membranes. After recovery from double current, the cells of the whole epithelium look slightly shrunken, and many cells of the first RCL have died. This latter observation is of interest because it shows that the cell damage in the double current experiments is brought about by this treatment per se and is not an artefact brought about by OsO_4 fixation during current flow.

This brings us to the general question whether the pictures we have obtained of the cellular

[4] Andersen, B. Unpublished observations.

changes during current flow are correct. Among the factors which determine the volume of living cells, the osmotic effect of electrolytes plays a dominating role. With short circuit current, often more than 1 μmole of Na passes 1 cm^2 of skin per hour. This means that an amount of Na corresponding to the total electrolyte content of one cell layer passes the skin in a few minutes. Therefore, unless the fixation is very fast, one might anticipate that the electric current would bring about a redistribution of electrolytes and hence of water if, for instance, the sodium pump were inhibited before the whole cell had been fixed.

While such fixation artefacts cannot be excluded, there is some indication that they are not important. Thus in one series of short circuit experiments not presented here, the current was kept at a prefixation level during fixation instead of being lowered during fixation to keep the potential at zero. The histological appearance of skins in that series of experiments was comparable, in all respects, to that of the material presented here, the swelling being, perhaps, slightly more pronounced. This indicates that the amount of current passing during fixation is not of decisive importance for the histological appearance of the specimens.

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