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Quantitative Relationship Between Active Sodium Transport, Expansion of Endoplasmic Reticulum and Specialized Vacuoles ("Scalloped Sacs") in the Outermost Living Cell Layer of the Frog Skin Epithelium (*Rana temporaria*)

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Summary. When an isolated frog skin (*Rana temporaria*) is exposed to a hydrostatic pressure difference between inside and outside bathing solutions (inside pressure higher than outside) of 20–50 cm of H_2O and if under these conditions the skin is short-circuited electrically, small "vacuoles" appear light-microscopically in the outermost living cell layer in the epithelium. The number of such "vacuoles" shows a linear dependency on the rate of active sodium transport as measured by the short-circuit current. Electron-microscopically, the "vacuoles" are interpreted as previously undescribed organelles, the "scalloped sacs" which are about 0.5 μ in diameter, with a wrinkled surface and bounded by a unit membrane. This organelle is in intimate contact with sacs and tubules of smooth endoplasmic reticulum. The observed increase in the number of scalloped sacs usually is accompanied by a significant expansion of the whole system of endoplasmic reticulum. Some of the "vacuoles" seen light-microscopically must indeed be expanded cisternae of endoplasmic reticulum. The findings are discussed in light of the possibility that the scalloped sacs and the endoplasmic reticulum may be involved in active transport of sodium ions.

A number of morphological features, closely correlated with the functional states of the frog skin epithelium, have been described in a series of previous publications (Voûte & Ussing, 1968, 1970*a*, *b*; Voûte & Hänni, 1973).

1. Only the first reactive cell layer just underneath the stratum corneum reacts by swelling when the skin potential is short-circuited. This swelling is fully reversible within less than one minute when the current is interrupted. Since short-circuiting increases the active sodium transport, it can

be inferred that the swelling is related to an increased uptake of sodium by the cell layer in question.

2. In short-circuited skins there is a linear relationship between the current strength (and thus the rate of sodium transport) and the volume of the extracellular space of the epithelium.

3. If a hydrostatic pressure difference (inside higher than outside) is maintained between the inside and outside solutions bathing the frog skin, there is an expansion of the intercellular space of the epithelium which is closely related to the pressure head.

The quantitative relationships mentioned are all in agreement with the hypothesis that the outermost living cell layer is taking up sodium from the outside medium and releasing most or all of it into the intercellular space, thereby creating an increased osmolarity which in turn gives rise to attraction of water and expansion of the interspaces.

It is implicit in this picture that the first living cell layer ("first reacting cell layer") is performing most of the active sodium transport, although it is likely that there is some coupling (in the Loewenstein sense) between the first reacting layer and the deeper layers of epithelial cells (Farquhar & Palade, 1964; Ussing & Windhager, 1964).

In connection with the above-mentioned experiments where the hydrostatic pressure on the inside of the skin was raised above the one of the outside (Voûte & Ussing, 1970*b*), it was noticed that large, mostly perinuclear vacuoles could be observed in the cytoplasm of many cells. However, it was only after having adopted a rapid freeze-substitution technique that not only the volume of this first cell layer could be quantitatively correlated to active sodium transport (Voûte & Hänni, 1973), but also that this same cell layer could be seen to contain what appeared in the light-microscope to be multiple light dots (called "vesicles" or "vacuoles") randomly distributed in the cytoplasm. The number of dots per cell in this layer varied with the rate of active transport. Also if sodium was replaced by choline in the outside bathing solution one not only noted a shrinkage of the first living cell layer, but also an almost complete disappearance of the light dots (Voûte, 1973).

These observations obviously called for a detailed study. When examined in the electron-microscope, the light dots appeared to be vacuoles or sacs about 0.5 μ in diameter. Some of them are clearly expanded or "puffed" cisternae of endoplasmic reticulum, but most of them are well-defined structures which, in the following electron-microscopical description, we will call "scalloped sacs". These are mostly spherical with a wrinkled

surface and are bounded by a unit membrane. They are in intimate contact with sacs and tubules of smooth endoplasmic reticulum. The latter is mostly abnormally expanded whenever a hydrostatic pressure gradient (inside pressure higher than outside) is combined with short-circuiting.

This paper first treats the light-microscopic appearance of "vacuoles" in the outermost layer of living cells in the frog skin epithelium and the quantitative relationship between the number of "vacuoles" and the rate of active sodium transport as measured by the short-circuit current (Ussing & Zerahn, 1951). Then follows a description of the "scalloped sacs" and other ultrastructural features of the cell layer in question.

Materials and Methods

a. Rapid-Freeze Experiments

Two separate batches of frogs (*R. temporaria*) were used for this study. One group was kept at 4 °C in shallow tap water, whereas the other was living in a vivarium with free access to shallow tap water. The animals of the last group were fed with mealworms and new-born mice. All rapid-freeze experiments were performed during the months August through November. The experimental procedure was identical for both groups. The freshly dissected skin pieces were mounted by means of special ring holders (Voûte & Hänni, 1973) in a multichamber system as previously reported. If not specially stated (*see* Results) the bathing solutions were always identical on both sides. Ringer's solutions used were of similar composition as the ones reported elsewhere (Ussing, 1965). After an equilibration period of 60 min the skins were short-circuited for 20–30 min. Then pressure gradients varying between 20–80 cm H_2O (inside positive) were applied by raising the inside circulation funnel for 10–20 min (magnitude of applied pressure, *see* Results).

The skins were then rapidly removed from the chamber and immersed in liquid propane precooled with liquid nitrogen (−180 °C). Removal and freezing was always achieved within 1 sec. The frozen skins were then rapidly transferred into liquid nitrogen where they were cut into small pieces (1–2 mm in diameter), placed in gelatin capsules (kept in a special copper tray) and transferred into a precooled lyophilizer (−60 °C). Lyophilization was carried out for 4 hr at 10^{-4} Torr. The apparatus used was a slightly modified version of the one described by Eckert (1969).

After 4 hr the tissue was brought to room temperature in atmospheric pressure. Pre-evacuated full Epon mixture was poured into the capsules and infiltration of the tissue carried out at room temperature under a vacuum of 10^{-4} Torr. Polymerization was achieved at 60 °C overnight.

For morphological evaluation 1-μ sections were cut at a right angle to the skin surface. They were stained with an aqueous 1% toluidine blue solution. Counting of "vacuoles" was performed at a total magnification of 2000 × whereas a primary magnification of 320 × was used for photographic work. The microscope was a Leitz Orthoplan with periplane apochromatic optics. The counting of "vacuoles" was performed on three sections from each of three samples from each piece of skin. The counting was then repeated by a different person. Neither of the operators knew the prehistory and the electrophysiologic parameters of the samples in question. On the average, vacuoles were counted in 100 cells per section. This corresponds to a total number of close to 1000 cells for each functional state (i.e. short-circuit current).

b. Material for Ultrastructural Survey

The skins were fixed with osmium tetroxide in Ringer's solution in the experimental chambers under the appropriate experimental conditions (pressure, short-circuit, etc.) as described previously (Voûte & Ussing, 1968, 1970*b*). The further procedure was identical to the one described in these papers.

Another series of experiments (control and experimental conditions) was performed in order to obtain a reasonably good membrane preservation. These experiments were performed in late autumn. The skins were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer in the experimental chambers for 2 hr. They were then removed and suitable specimens were washed in buffer and then postfixed for 2 hr at 4 °C in 1% osmium tetroxide in the same buffer; they were stained *en bloc* in 0.5% aqueous uranyl acetate for 1 hr, dehydrated in increasing concentrations of ethanol, transferred to propylene oxide and embedded in Epon.

Areas suitable for electron-microscopy were located by examining 1-μ sections stained with toluidine blue. Silver to grey sections were stained with uranyl acetate and lead citrate. The sections were viewed and photographed with a Hitachi HS 8 electron-microscope operated at 50 kV.

Results

Rapid-Freeze Experiments

Optimal Conditions for "Formation of Vacuoles"

In the preliminary experiments it was not always possible to reproduce the phenomenon of "vacuole formation". The first problem therefore was to find the optimal experimental conditions for the phenomenon.

Magnitude of Pressure Gradient Applied. As already mentioned, the "vacuole formation" in the epithelial cells was strongly stimulated by a hydrostatic pressure gradient (inside higher than outside).

Skins with high electric resistance generally exhibit a high pressure tolerance, i.e. the epithelium can stand high pressures without changes in its bioelectric parameters or separation from the corium (blister-formation). Skins with low electric resistance do not tolerate pressure gradients higher than 20–30 cm H_2O.

Similarly "vacuole formation" depended on the electric resistance, so that the higher the resistance, the higher the pressure needed to produce "vacuoles" visible in the light-microscope.

Dependence of "Vacuole Formation" on Temperature and Season. In our laboratories like in most others, frogs are routinely kept in cold rooms (4 °C) in shallow tap water. Under such conditions the skins usually exhibit high electric resistance values, especially in the winter months. In spring and summer, however, there is a spectacular drop in skin resistance, even when the animals are kept in the cold.

Frogs kept at room temperature also exhibit seasonal variations in skin resistance, although with less pronounced fluctuations than seen in "cold-adapted" animals.

On the basis of these findings it was decided to do all subsequent experiments on animals kept at room temperature.

Importance of the Main Anion of the Bathing Solutions. If the expansion of the endoplasmic reticulum, seen in connection with "vacuole formation", was in some way associated with transport of sodium into the reticulum, one might expect that the phenomenon would to some extent depend on the availability of a suitable counter-ion to sodium. In the literature there are certain hints, indicating that the active transport of sodium is highly dependent on the main anion of the solution bathing the inside of the skin. Thus replacement of chloride in the inside solution by the less diffusible gluconate ion reduces the rate of active sodium transport drastically, although the skin potential remains high due to the fact that gluconate has a lower shunting effect than chloride (Ussing, 1965). Quite recently Huf (1972) has found that sulfate, replacing chloride in the inside solution, depresses the short-circuit current and thus the sodium transport, whereas there is no such depressing effect if the replacement is done in the outside solution.

These observations made it desirable to study also the effect of the anion on the "vacuole formation".

Quantitative Aspects of the "Vacuole Formation"

Fig. 1 shows the results from a series of experiments with NaCl-Ringer's on both sides of the skin. The number of "vacuoles" (abscissa) is plotted against the corresponding values for the short-circuit current (ordinate). The point corresponding to zero short-circuit current (no active sodium transport) was obtained from a number of experiments where sodium in the outside solution was replaced by choline. The regression line for all values is dotted, whereas the full line is based on those SCC values which are lower than 50 μAmps/cm^2.

It is easily seen that there is a close correlation ($R = 0.93$) between the number of vacuoles per cell in the first living cell layer and the corresponding values for active sodium transport as measured by the SCC.

Fig. 1 is obtained by pooling all experimental values, both those obtained with warm-adapted and with cold-adapted frogs, but there is no indication that the two "families" of points separate. Thus, in principle, there is no difference between the low resistance and high resistance skins with respect to the phenomenon of "vacuole formation". Fig. 2 shows the

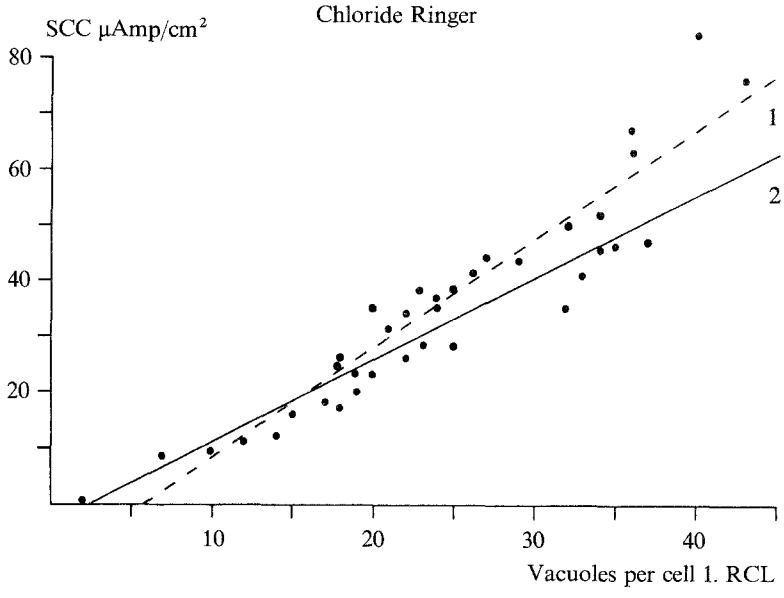


Fig. 1. Relation between active sodium transport and number of "vacuoles" in the 1st living cell layer next to the stratum corneum. Chloride Ringer's as bathing fluid. Ordinate: Sodium transport as short-circuit current (SCC). Abscissa: Number of "vacuoles" per cell of the 1st living cell layer (1. RCL). Broken line: Regression line for all points (2). Full line: Regression line for SCC 50 μAmps/cm² and less. *Statistics:* Line 1: $n=33$, $p<0.001$, $R=0.939$; SCC $=(0.152 \cdot 01) \times$ number of "vacuoles" -4.432. Line 2: $n=37$, $p<0.001$, $R=0.935$; SCC $=(0.185 \cdot 01) \times$ number of "vacuoles" -10.458

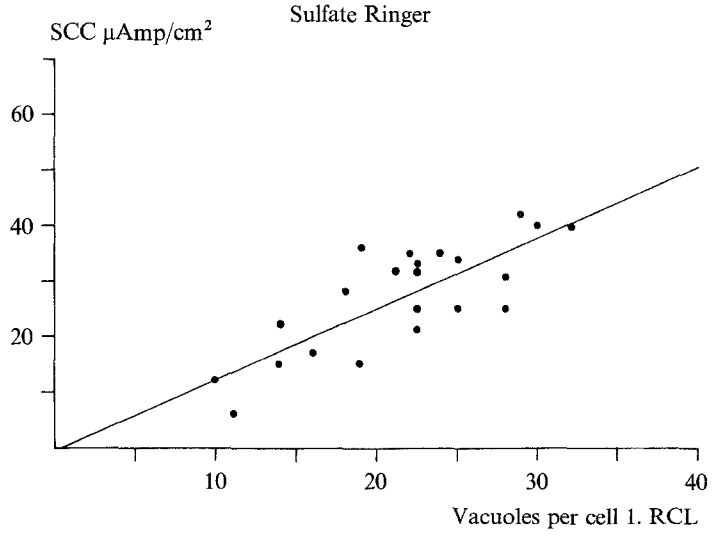


Fig. 2. Same relation as Fig. 1 but in the presence of sulfate as major anion in the bathing fluid. No transport values above 50 μAmps/cm². *Statistic:* $n=22$, $p<0.001$, $R=0.785$; SCC $=(0.128 \cdot 01) \times$ number of "vacuoles" -0.377

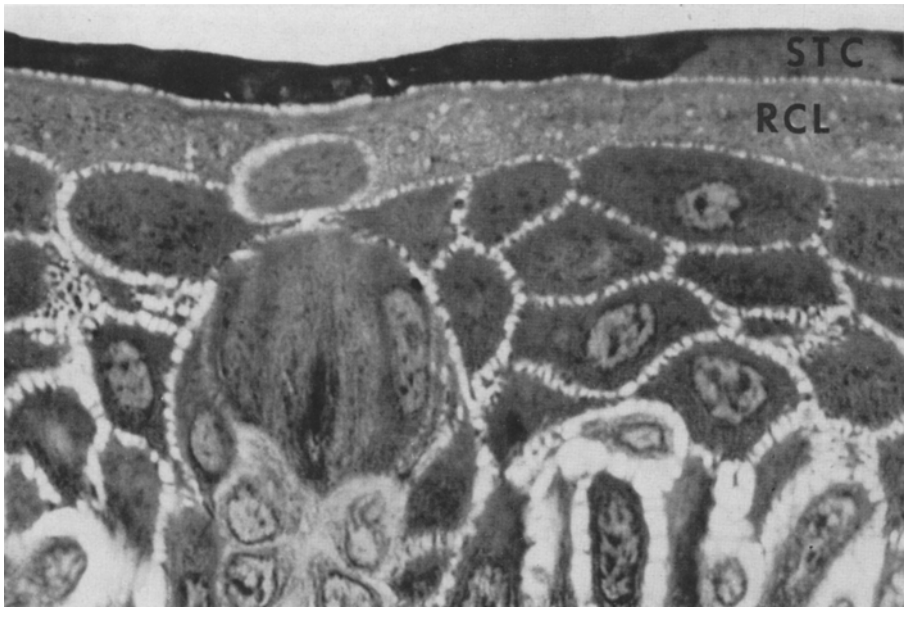


Fig. 3. Light-micrograph of a frog skin epithelium. Short-circuit conditions and 20 cm H_2O pressure gradient (inside positive). Note the swelling of the 1st living cell layer (RCL) next to the str. corneum (STC). Appearance of multiple cytoplasmic "vesicles" or "vacuoles". 1,600 ×

results of a series of experiments where the more slowly permeating anion sulfate was used instead of chloride. It is seen that, again, we obtain a linear relationship between the number of "vacuoles" and the SCC. Even the slope of the line is practically the same as in the chloride experiments. The only difference is that no skin yielded a short-circuit current higher than 50 μAmps/cm^2. Thus the phenomenon can be obtained with different anions, whereas it seems to require sodium.

The phenomenon of "vacuole formation", as observed under the light-microscope, is shown in the light-micrograph Fig. 3. It is seen that the "vacuoles" are found almost exclusively in the first living cell layer.

Ultrastructural Observations

In an attempt to further characterize the "vacuoles" observed in the light-microscope an electron-microscopic study was undertaken. Only observations pertinent to the light-microscopic findings will be considered in the subsequent description. Therefore, the attention was directed primarily to the changes in the first reactive cell layer.

Open Circuit Conditions (Control Skins)

The cells of the first reacting cell layer have an abundance of granular and agranular endoplasmic reticulum. However, the flattened cisternae and tubules are often difficult to detect due to the presence of numerous cytoplasmic filaments and clusters of ribosomes. The filaments are anchored along the lateral cell membrane in the cytoplasmic attachment plaques of abundant desmosomes. Few mitochondria are present – often interspersed between bundles of filaments. A relatively small Golgi complex is located in the vicinity of the nucleus. Large dense granules, most of which are concentrated in the perinuclear cytoplasm, and sporadic lipid droplets are also found. Occasionally, typical "scalloped sacs" (*see* description below) are observed.

Changes in the First Reacting Cell Layer After SCC and Hydrostatic Pressure

(A) *Light-microscopy of material processed for electron-microscopy.* The appearance and distribution of "vacuoles" in 1 μ, toluidine blue-stained sections of the present conventionally fixed material are comparable to the size and distribution of the "vacuoles" following the freeze-substitution method.

(B) *Electron-microscopy of the freeze-substituted material* also demonstrates the "vacuoles". However, the morphology cannot be worked out due to the severe changes caused by ice crystal formation.

(C) *Electron-microscopy of conventionally fixed material.* By far the majority of the light-microscopic "vacuoles" corresponds to what we have termed scalloped sacs. Characteristically, these sacs are ruffled, fuzzy-edged, rounded structures containing a flocculent material of low electron density (Figs. 4–7). In many sections it is possible to distinguish a limiting unit membrane (Fig. 5*B* and *C*). The scalloped sacs are found randomly distributed in the cells of the first reactive cell layer often in very close apposition to mitochondria, small vesicles and tubules and to cisternae of granular or agranular endoplasmic reticulum. The small vesicles seem to fuse with the sacs, whereas the small tubules of endoplasmic reticulum seem to terminate directly on the surface of the sacs separated from them only by a very thin fibrillar layer (Fig. 6). Scalloped sacs completely encaged by filaments are also found but frequently the filaments weave in between both scalloped sacs and perisaccular organelles. The scalloped sacs are never seen directly apposed to the lateral or basal interspaces and neither are they seen close to the outward facing cell membrane.

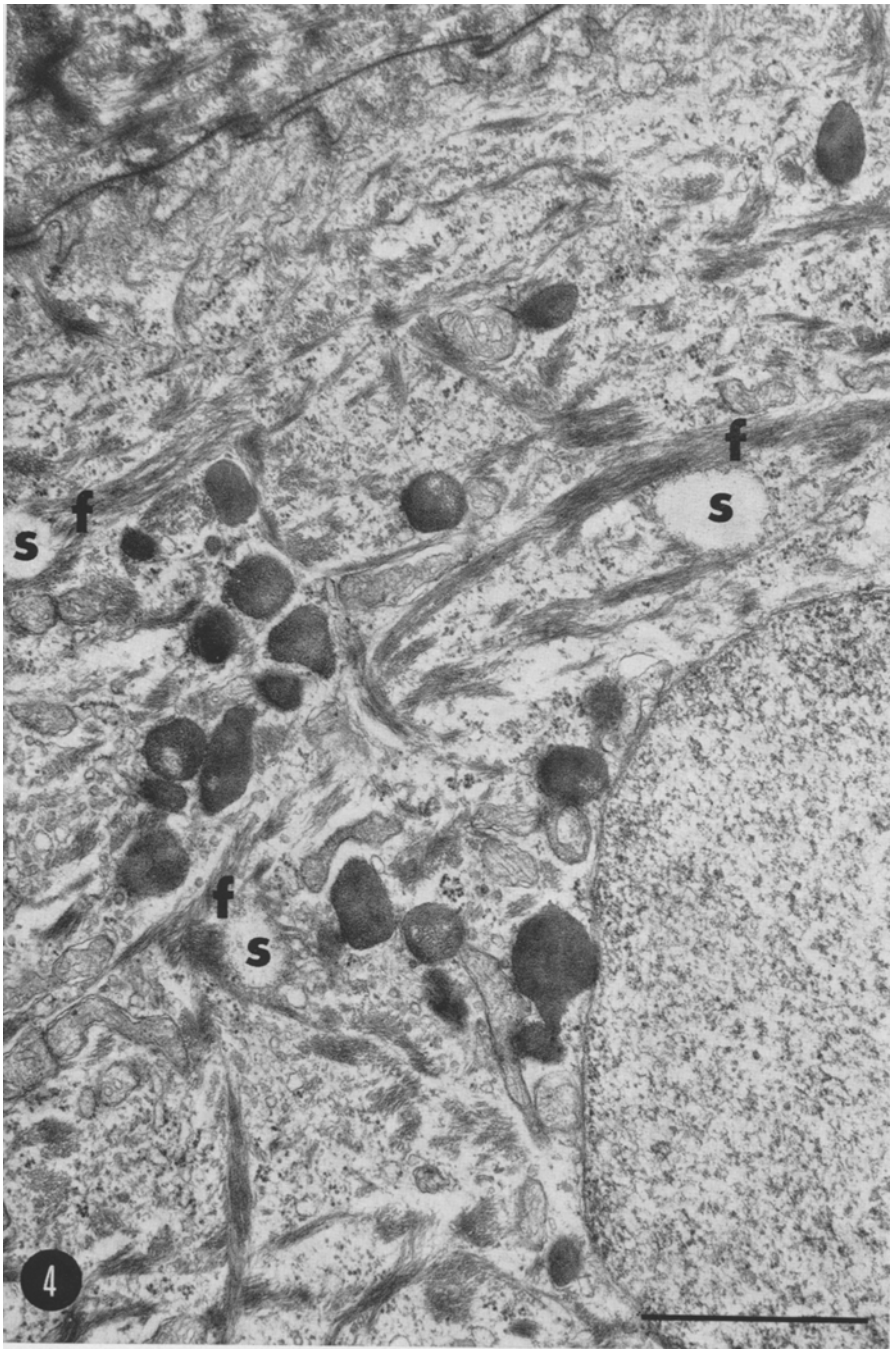


Fig. 4. The appearance of scalloped sacs (*s*) in the first reactive cell layer in a low power electron-micrograph. Note the closely associated microfilaments (*f*). Short-circuit conditions and 20 cm H_2O gradient (inside positive). 30,000 ×. Bar indicates 1 μ

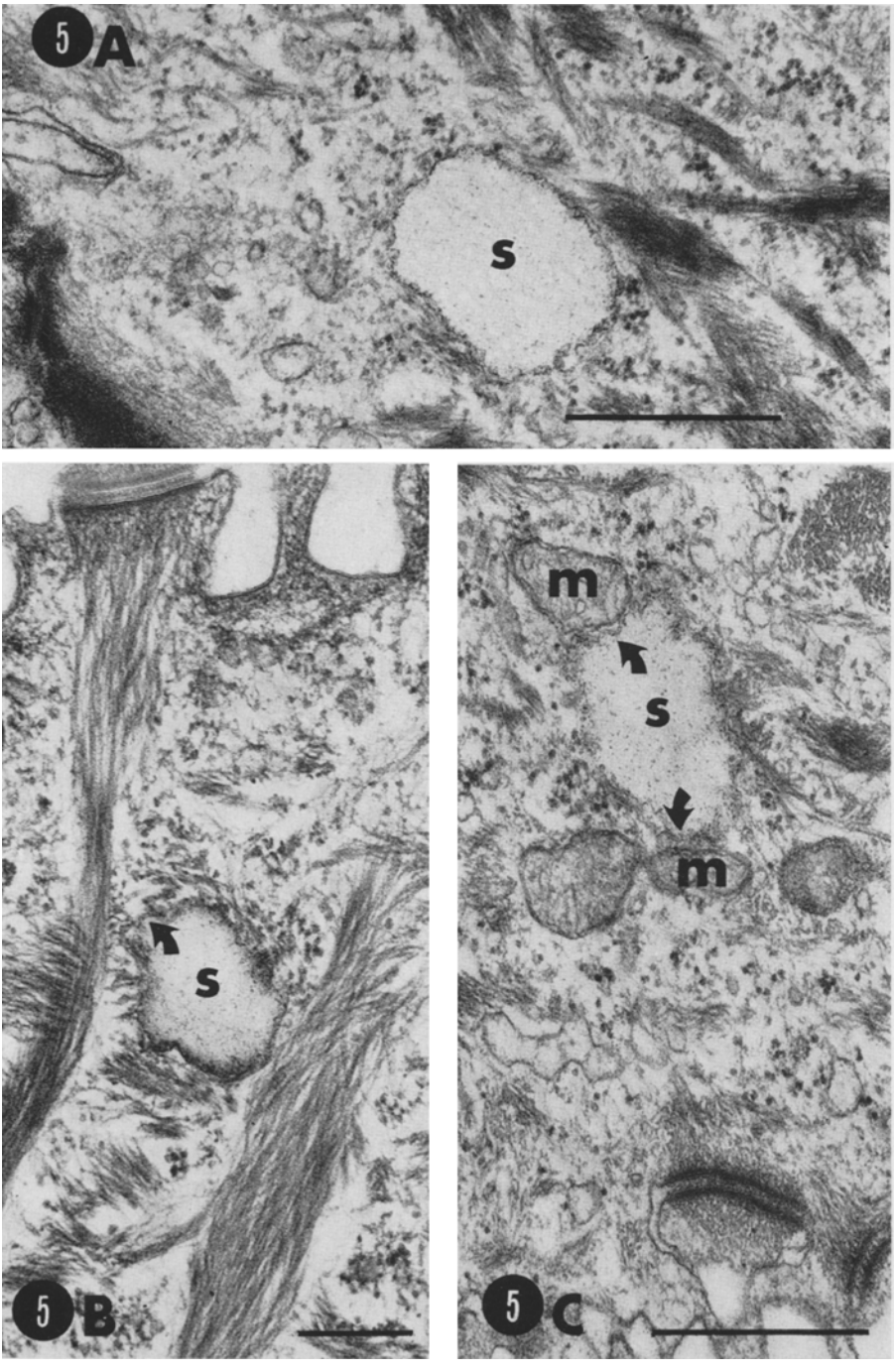


Fig. 5*A* – *C*. Portion of the middle region (5*A*), the apical region (5*B*) and the basal region (5*C*) of the first reacting cell layer showing scalloped sacs (*s*). The scalloped surface makes it difficult to determine whether the sacs are limited by a membrane. However, when the plane of sectioning is perpendicular to the surface, it is possible to distinguish a unit membrane (arrows in Fig. 5*B* and *C*). Note the close relationship to mitochondria (*m*) in Fig. 5*C*. In Fig. 5*A* and *C* small tubules are seen adjacent to the sacs. Short-circuit conditions and 20 cm H_2O gradient. *A* and *C* 57,000 × ; *B* 30,000 ×. Bar indicates 0.5 μ

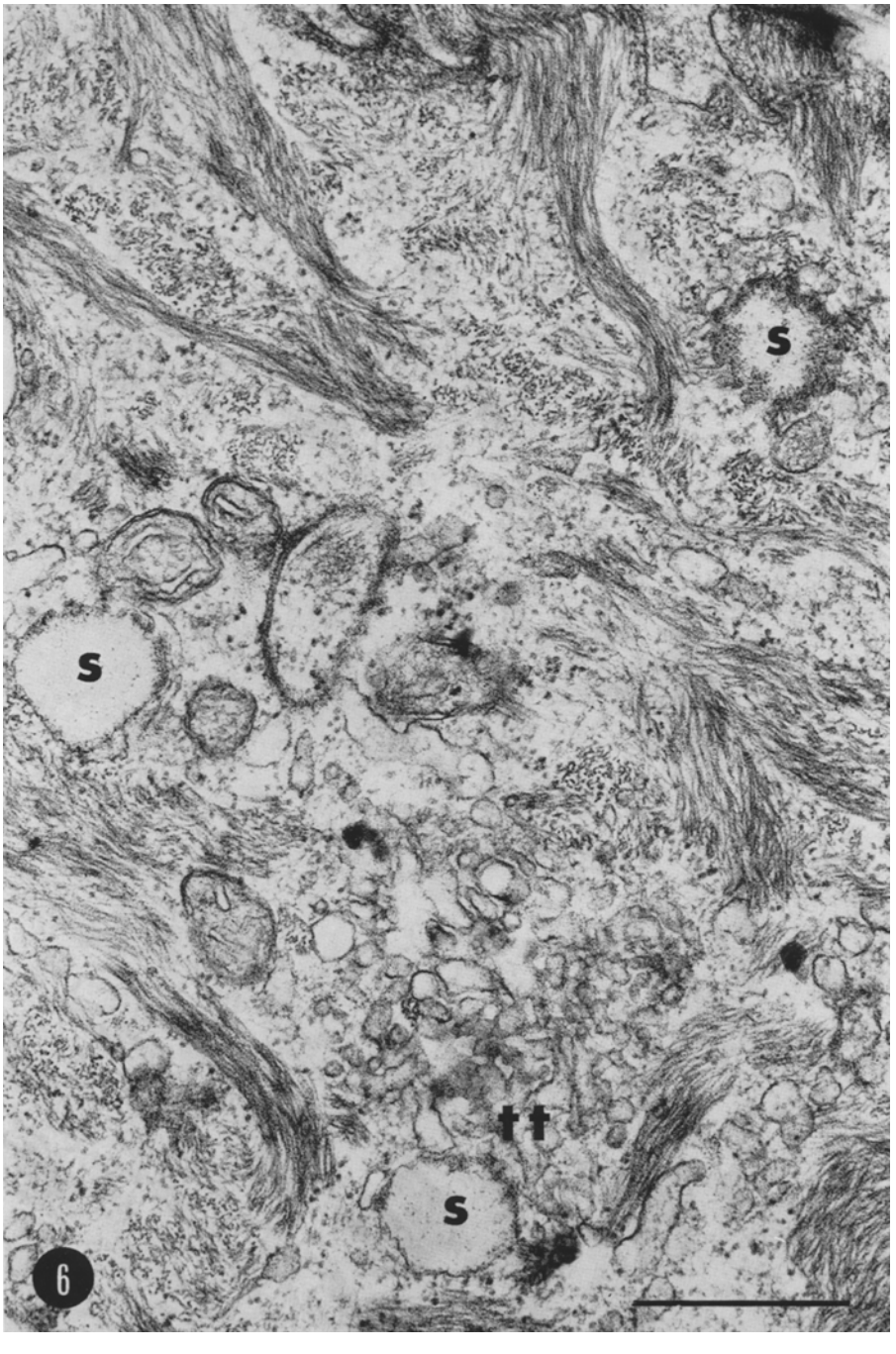
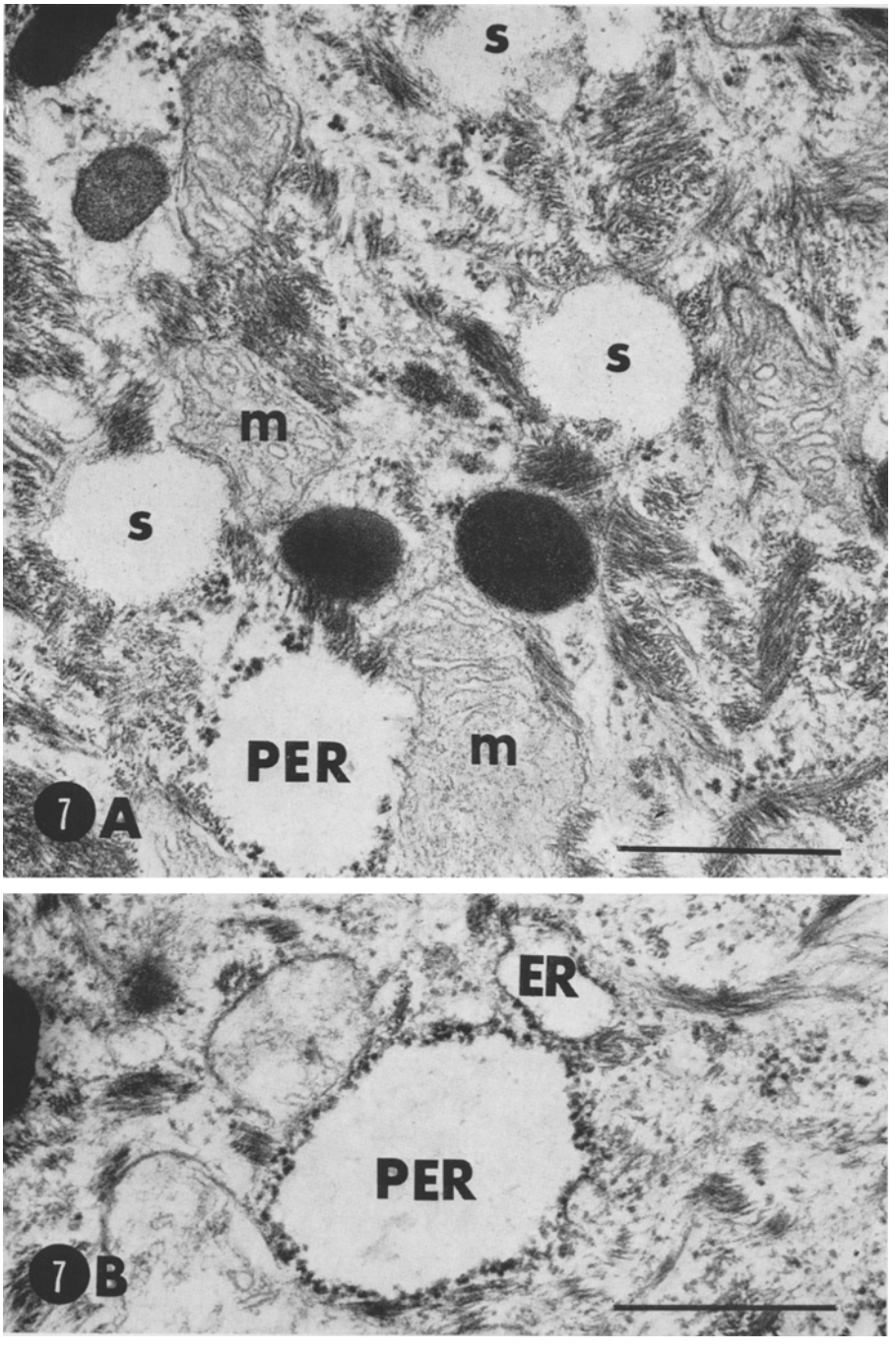


Fig. 6. Small tubules of agranular endoplasmic reticulum are characteristically found very close to the scalloped sacs (*s*). However, 60 min after removal of short-circuit conditions and pressure gradient an extensive network of expanded tortuous tubules (*tt*) is surrounding the scalloped sacs. 57,000 ×. Bar indicates 0.5 μ



Figs. 7*A* and *B*. These electron-micrographs demonstrate that some of the "vacuoles" observed by the light microscope probably are "puffed" cisternae of granular endoplasmic reticulum. In Fig. 7*A* note three scalloped sacs (*s*) without associated ribosomes, as opposed to the structure which we tentatively have named "puffed" endoplasmic reticulum (*PER*). Also observe the very close relationship between mitochondria (*m*) and both scalloped sacs and "puffed" endoplasmic reticulum. Compare in Fig. 7*B* the expanded cisterna of granular endoplasmic reticulum (*ER*) to the closely related structure (*PER*). Short circuit conditions and pressure gradient. 57,000 ×. Bar indicates 0.5 μ

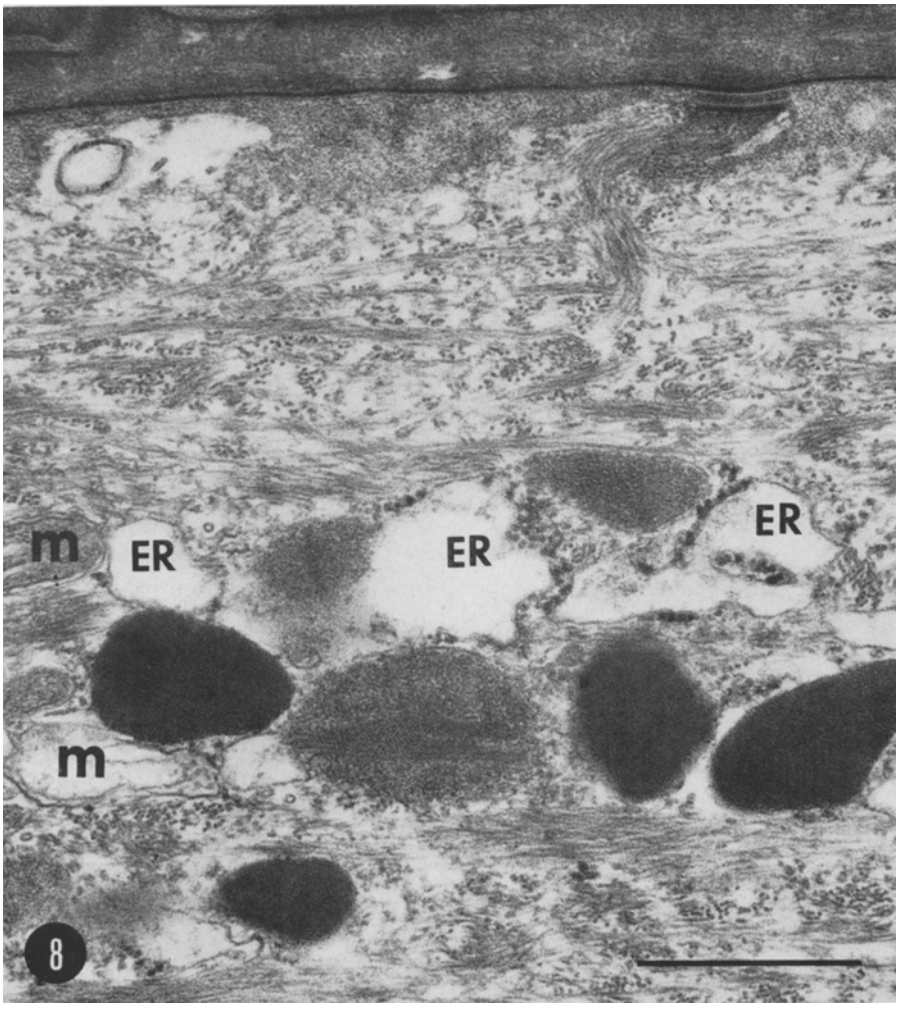


Fig. 8. In this electron-micrograph the ultrastructural equivalents of the "vacuoles" are distended elements of endoplasmic reticulum (*ER*). This specimen is fixed in glutaraldehyde and uranyl block-stained. Characteristic "scalloped sacs" are not seen in this material, but it is quite conceivable that a faster fixation, as can be achieved by using osmium tetroxide, might have shown more distended sacs with typical "scallop sac"-appearance. Note also here the close relationship between mitochondria (*m*) and endoplasmic reticulum. 57,000 ×. Bar indicates 0.5 μ

The entire endoplasmic reticulum of the first reactive cell layer seems to be expanded (e.g. Fig. 7*A* and *B*), and small cisternae are often seen very close to the basal cell membrane.

Electron lucent rounded areas without identifiable limiting membranes were sometimes observed in the cytoplasm. Lipid droplets were not identi-

fied with certainty but large dense granules were present. Autophagic vacuoles, multivesicular bodies and swollen mitochondria which all can simulate "vacuoles" in the light-microscope were not more frequent after altering the experimental condition.

Glutaraldehyde-Osmium-Fixed and Uranyl Block-Stained Control and Experimental Skins

Apart from a better preservation of all membranes the morphology of the control skins of the present material cannot be distinguished from that of the control skins fixed only in osmium tetroxide. No lipid droplets were observed in the first reacting cell layer in these experiments.

Following SCC and hydrostatic pressure the endoplasmic reticulum was significantly expanded. In the case of glutaraldehyde fixation and uranyl block-staining there is no doubt that the ultrastructural equivalent of the "vacuoles" present light-microscopically in the first reacting cell layer is distended sacs of endoplasmic reticulum since limiting membranes, sometimes studded with ribosomes, can be clearly identified (Fig. 8).

Discussion

The "vacuoles" as seen in osmium-fixed specimens may be reminiscent of lipid inclusions from which the lipid has been extracted or removed. However, both from morphological and physiological points of view this possibility can be disregarded. In the first place, even in cases where a skin shows a large number of scalloped sacs during the experiment, untreated control samples of the same skin contain only few lipid inclusions of the size and type which might have served as "precursors" for the "scalloped sacs". Secondly, frog skin contains only insignificant amounts of glycogen from which lipid might be formed during the experiment. Thirdly, specimens fixed and stained with the application of optimum conditions for membrane preservation show the "vacuoles" to be provided with well-defined membranes of the same type as the one exhibited by endoplasmic reticulum. Finally, freeze-fracturing experiments (*to be published*) show that the "scalloped sacs" are membrane-covered expansions, intimately associated with the endoplasmic reticulum.

It appears from the foregoing observations that the light dots or "vacuoles" which can be observed in the outermost living cell layer ("first reacting layer") of the frog skin epithelium are well-defined anatomical structures. This, in fact, is the rationale for relating their number to the

rate of sodium transport (*see below*). After osmium fixation most of the "vacuoles" are of the type described as "scalloped sacs" and a lesser number look like expansions of the endoplasmic reticulum, whereas after glutaraldehyde fixation and uranyl block-staining all "vacuoles" look like distended cisternae of endoplasmic reticulum. In most skins they can be observed only when a critical hydrostatic pressure drop is established between the inside and outside bathing solutions, and if, on top of that, the skin is short-circuited. But it should be stressed that scalloped sacs are found even in the absence of a pressure gradient.

Although we are discussing variations in the number of "vacuoles" it may really be the question whether one merely makes them visible by blowing them up. The solution of this problem as well as the problems of the possible connections between the endoplasmic reticulum, the scalloped sacs, and the cell membrane will have to await serial sectioning of specimens which are processed through other fixatives in order to obtain better preservation of membranes. However, it is worthwhile pointing out that mitochondria are always found in very close relationship to the scalloped sacs and not to the outward facing cell membrane or any other part of the cell membrane. The membrane-limited scalloped sac is, therefore, from a theoretical point of view, a good candidate for a site where pumping activity can be performed.

The linear correlation found between the number of "vacuoles" observed and the rate of active sodium transport makes it very likely that the scalloped sacs and endoplasmic reticulum are more or less directly involved in the active sodium transport. The involvement might, of course, be indirect, in the sense that the reticulum could be involved in the synthesis of some protein which is necessary for the active sodium transport, or there may be a more direct type of involvement so that sodium is being transported from the cytoplasm into the endoplasmic reticulum and the scalloped sac. For the time being it seems more attractive to ascribe the phenomena to an active uptake of sodium than to the synthesis of some specific protein, mainly because the expansion is seen so rapidly (within less than 10 min after application of pressure and short-circuit) that there does not seem to be time for a significant protein synthesis. If sodium is really transported into the reticulum, one might speculate that the sodium containing fluid in the scalloped sacs and the tubular system would empty intermittently or continuously into the intercellular space. For the time being one can only guess that an opposing hydrostatic pressure in some way interferes with the emptying of the vesicular and tubular contents. Granted that the appearance of scalloped sacs and the expansion of the endoplasmic

reticulum is due to active sodium uptake, one is still faced with the question whether this is only an auxiliary sodium pumping system which takes over when the cell is being flooded with sodium under adverse conditions or whether it is a normal step in transepithelial sodium transport. The latter possibility must be viewed in light of the well-established fact of identity between short-circuit current and net active sodium transport. Clearly, if the active part of the sodium transport is a transfer into the endoplasmic reticulum and a subsequent transfer of the fluid to the intercellular space, sodium must be followed by an anion, and there is no net transfer of electric charge. Therefore, the process can only work as a net sodium transport system if the anion involved is recycled between the inside bathing solution and the transporting cell. The anion cannot stem from the outside bathing solution because that would reduce the number of electric charges associated with a given amount of sodium. In this context it is interesting to note that the rate of active sodium transport is strongly and positively correlated with the diffusibility of the anion of the inside solution but largely independent of the anion bathing the outside of the skin (Ussing, 1965; Huf, 1972).

Indeed, there are some hints in the literature that the endoplasmic reticulum is in some way involved in active sodium transport. Thus a general expansion of the system is seen when an electric current double the short-circuit current is passed inward through the frog skin (*compare* Voûte & Ussing, 1968; Fig. 9*a*). Saladino, Bentley and Trump (1969), studying the effect of the polyene antibiotic Amphotericin B on toad bladder, found that this substance induces an expansion of the endoplasmic system in the presence, but not in the absence, of sodium. It may also be worth mentioning that in protozoans the contractile vacuole (which may be considered part of the endoplasmic system) is involved in the expulsion of sodium (*see* Elliot, 1973).

Certain findings would find a natural explanation if it should turn out that part of the cellular sodium is located in the endoplasmic reticulum and the scalloped sacs rather that in the cytoplasm.

Thus Zerahn (1969) obtained kinetic data which could be interpreted to mean that the transport pool of sodium (i.e., sodium with access to the sodium pump) is considerably smaller than the total cellular concentration of sodium.

In conclusion one can say that the hypothesis of the involvment of the endoplasmic reticulum and the scalloped sacs in active sodium transport cannot be considered proved, but it seems worth studying.

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References

Eckert, H. 1969. Distribution of soluble substances at the ultrastructural level. *In:* Autoradiography of Diffusible Substances. L. J. Roth and W. E. Stumpf, editors. p. 321. Academic Press, New York-London

Elliot, A. M., editor. 1973. Biology of Tetrahymena. Dowden, Huchison and Ross, Inc., Stroudsburg, Pennsylvania

Farquhar, M. G., Palade, G. E. 1964. Functional organization of amphibian skin. *Proc. Nat. Acad. Sci.* **51**:569

Huf, G. E. 1972. The role of Cl^- and other anions in active Na^+ transport in isolated frog skin. *Acta Physiol. Scand.* **84**:366

Saladino, A. J., Bentley, P. J., Trump, B. F. 1969. Ion movement in cell injury. *Amer. J. Pathol.* **54**:421

Ussing, H. H. 1965. Relationship between osmotic reactions and active sodium transport in the frog skin epithelium. *Acta Physiol. Scand.* **63**:141

Ussing, H. H., Windhager, E. E. 1964. Nature of shunt path and active transport path through frog skin. *Acta Physiol. Scand.* **61**:484

Ussing, H. H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110

Voûte, C. L. 1973. Morphophysiological evidence for a two step active transport path for sodium in the epithelium of frog skin (*Rana temporaria*). (Abstract) *Experientia* **29**:749

Voûte, C. L., Hänni, S. 1973. Relation between structure and function in frog skin. *In:* Transport Mechanisms in Epithelia. *Proc. A. Benzon Symp. V.* H. H. Ussing and N. A. Thorn, editors. p. 87. Munksgaard, Copenhagen

Voûte, C. L., Ussing, H. H. 1968. Some morphological aspects of active sodium transport. The epithelium of the frog skin. *J. Cell Biol.* **36**:625

Voûte, C. L., Ussing, H. H. 1970*a*. The morphological aspects of shunt-path in the epithelium of the frog skin (*R. temporaria*). *Exp. Cell Res.* **61**:133

Voûte, C. L., Ussing, H. H. 1970*b*. Quantitative relation between hydrostatic pressure gradient, extracellular volume and active sodium transport in the epithelium of the frog skin (*R. temporaria*). *Exp. Cell Res.* **62**:375

Zerahn, K. 1969. Nature and localization of the sodium pool during active transport in the isolated frog skin. *Acta Physiol. Scand.* **77**:272