

LOCALIZATION OF PERMEABILITY BARRIERS IN THE FROG SKIN EPITHELIUM

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ABSTRACT

Ruthenium red and colloidal lanthanum were used to determine the site of the structural barriers to diffusion within the intercellular spaces of frog skin epithelium. Electron micrographs show that occluding zonules located at the outer border of the *stratum corneum* and at the outer layer of the *stratum granulosum* are true tight junctions since they are impermeable to these tracers. Measurement of ^{140}La uptake by the living skin shows that lanthanum moves across the external surface of the skin readily, into and out of a compartment that has a limited capacity and is bounded on its internal side by a barrier impermeable to lanthanum. Examination of these skins with the electron microscope suggests that the compartment is localized between the external membrane of the cells at the outer layer of the *s. granulosum* and at the outermost surface of the skin. These observations and other findings described in the literature indicate that the site of the external high resistance barrier of the frog skin is localized at the outer border of the *s. granulosum*.

INTRODUCTION

It is generally agreed that the transport of substances across epithelia involves the passage through two barriers that bound a cellular compartment. Most epithelial tissues in which transport has been studied are constituted by a single layer of cells sealed together by tight junctions (occluding zonules). Since tight junctions are believed to prevent the passage of substances along intercellular spaces, the selective barriers are identified with the cell membranes at either side of the junctions (Keynes, 1969).

Farquhar and Palade (1965) found that in the frog skin (a stratified squamous epithelium) there are at least two levels at which occluding zonules between adjoining epidermal cells are present. The first is at the outer front of the *stratum corneum*, and the second is at the outermost layer of the *stratum granulosum*. A third group of tight junctions

may be observed between the cells of the proximal cornified layer, when such a layer is found. In the present investigation, we have studied further these junctions to obtain some indication of their relative role in preventing the free exchange of substances across the frog skin. We have employed several electron-opaque substances that have proved to be useful in tracing extracellular spaces and defining the structure of cell junctions (Doggweiler and Frenk, 1965; Luft, 1966; Revel and Karnovsky, 1967). To facilitate the penetration of tracers through the internal surface of the epidermis, the epithelium of the frog skin was isolated by means of collagenase and hydrostatic pressure to eliminate the hindrance on the penetration of substances caused by the corium (Erlj and Aceves, 1969).

In other experiments in which the external

surface of the skin was exposed to Ringer solutions containing a low concentration of ^{140}La , a finite uptake of the cation was observed. To find out whether the distribution of lanthanum absorbed by the living tissue could be determined, the skins were fixed, sectioned, and examined with the electron microscope. A preliminary report of this work appeared previously (Bracho et al, 1970).

MATERIALS AND METHODS

Skins taken from the abdomen of adult frogs (*Rana pipiens* and *Rana palmipes*) were studied. In several experiments the epidermis was separated from the dermis by means of the procedure previously described (Erlj and Aceves, 1969; Aceves and Erlj, 1970). When solutions were applied selectively to either the external or internal surface of the skin or isolated epithelium, the tissue was mounted separating two half chambers of the Ussing-Zerahn type (Ussing and Zerahn, 1951), or in the holder used for measuring the uptake of isotope (see below). The Ussing-Zerahn chambers were connected through two pairs of KCl-agar bridges to calomel electrodes that were used to record the potential and pass current across the skin. In other experiments, pieces of skin or isolated epithelium were immersed in the fixative immediately after the dissection was completed.

Electron Microscopy

Pieces of whole skin or isolated epidermis were fixed for 1 hr with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, at room temperature and postfixated in 1% osmium tetroxide with cacodylate buffer during 1-3 hr. Other specimens were postfixated en bloc for 3 hr either in 1% osmium tetroxide to which 0.5 mg per ml of ruthenium red was added (Luft, 1966), or in the solution of osmium tetroxide and colloidal lanthanum nitrate, described by Revel and Karnovsky (1967).

To study the distribution of lanthanum in the living skin, solutions of 5×10^{-4} M lanthanum chloride were applied for 1 hr to either the internal or the external surface of the skin or the epidermis, and the specimens were fixed in the Ussing-Zerahn chambers with 2.5% glutaraldehyde in cacodylate buffer for 1 hr, stored for 8-12 hr in cacodylate buffer at 4°C, and dehydrated with or without postfixation in osmium tetroxide.

All specimens were flat embedded in Epon and sectioned for light and electron microscopy after adequate orientation of the blocks. Thin sections were examined with a Zeiss EM9A electron microscope, either without counterstain or after staining for a few seconds with lead citrate.

Uptake of Lanthanum by the Skin

The isotope flux from external solution to the skin was measured by using an experimental arrangement similar to that described by Curran and Cerejido (1965) (see inset, Fig. 9). The skin was mounted on cylindrical Lucite holders with its external surface facing down and was tied in place by a nylon thread which fitted into a groove machined in the cylinder. The cylindrical holder was attached to a metal holder that permitted its localization in a reproducible position within a beaker containing 20 ml of Ringer solution which was stirred by a magnetic stirrer. Ringer solution (2 ml) was placed within the cylinder. The area of skin exposed to the solution was 3.1 cm². The cylinder was fitted with a stopper through which passed a capillary tube for oxygenating the inside solution and an agar bridge which made contact with a calomel electrode. Another calomel electrode was connected to the solution in the beaker through an agar bridge. The potential difference between the calomel electrodes was read from a Hewlett-Packard Co. 3430 A Digital Voltmeter (Palo Alto, Calif.).

To measure lanthanum uptake, the solution in the beaker was removed and replaced by a similar solution that contained the desired lanthanum concentration. Lanthanum was added as a mixture of unlabeled La^{+++} and about 20 μCi of ^{140}La . The skin was allowed to remain in the radioactive solution for a carefully timed period, then removed, rinsed for 10 sec in a large volume of nonradioactive Ringer solution, and blotted carefully. The holder was then placed in a scintillation counter fitted with a special holder to insure that the skin was always at the same position over the crystal detector. The preparation was counted for 1 min, replaced in the radioactive solution, and the process was repeated. Usually the uptake of ^{140}La was measured for four 80-sec periods, then for ten 1-min periods, and then for several 5- and 10-min periods to complete 1 or more hr of exposure to the radioactive solution.

In some experiments, we also measured the rate of washout of ^{140}La from the loaded skin into different solutions. The beaker with radioactive solution was removed and replaced with a beaker containing unlabeled solution. After a period in this unlabeled solution, the holder was removed, and the skin was carefully blotted and counted. The skin was then replaced in a beaker with fresh solution and the process was repeated for several periods. The solution in the washout beakers was also counted to compare its radioactivity with the decrease in counts observed in the skin. The specific activity of the loading solution at the beginning and at the end of the experiment was determined by counting a 0.5 ml sample of the loading solution placed inside the holder after the skin had been replaced by a thin polyethylene membrane.

At the end of every uptake experiment, the skin

was cut off the holder just below the nylon thread. The holder was then counted, and it was found that between 4 and 7% of the activity measured with the whole skin in place could be detected.

To determine whether any lanthanum crossed the skin, the solution inside the holder was counted at the end of the experiments. This point was also investigated by measuring to determine whether any radioactive lanthanum crossed the skins mounted in Ussing-Zerah type of chambers after different periods of adding the isotope to the external solution.

Ringer solution had the following composition (in mmoles/l): NaCl, 115; KCl, 2.5; CaCl₂, 1; Tris maleate buffer, 3, pH 7.5.

RESULTS

The Structural Barriers of the Skin

The isolated epithelium preparation of the frog skin obtained by means of collagenase and hydrostatic pressure is composed of the whole squamous stratified epithelium (Fig. 1), and the skin glands. The general organization and fine structure of the isolated epithelium is similar to that of the whole skin (Fig. 2), (Voûte, 1963; Parakkal and Matoltsy, 1964; Farquhar and Palade, 1965). No basal lamina was observed in these preparations (Fig. 2).

In specimens of the isolated epithelium treated en bloc with ruthenium red, an electron-opaque reacting product is seen along the surface coat of the outer cornified cells of the *s. corneum* and also along the border of cell membranes throughout the *s. germinativum*, *s. spinosum*, and *s. granulosum*. Ruthenium red-positive material was not found in the space between the cells of the *s. corneum*

and *s. granulosum* nor in the cytoplasm of any of the epithelial cells (Figs. 2 and 3). The barriers that limit the movements of ruthenium red along the intercellular spaces into the compartment formed between the *s. granulosum* and *s. corneum* appear to be the occluding zonules joining the cells in these layers. No marker was found within these tight junctions. Fig. 3 and 4 show typical images of the farthest penetration of ruthenium red from the internal side.

In all the isolated epithelia, colloidal lanthanum produced images of the penetration through the internal side identical to those described for ruthenium red. Also, in agreement with the findings with ruthenium red, we observed in most instances, that colloidal lanthanum did not penetrate from the outside beyond the surface coat at the external border of the *s. corneum*. However, in a few cases (Figs. 5, 6, and 7), the electron-opaque reaction of colloidal lanthanum was observed within the cytoplasm of the cornified cells and the space between the *s. corneum* and *s. granulosum*. It appears that lanthanum can cross the membranes of the cornified cells in these preparations since no tracer was found inside the tight junction between apposing cornified cells (Figs. 6 and 7). In these preparations, lanthanum was never found within the tight junctions between the cells of the *s. granulosum*, nor in the cytoplasm of cells of this or deeper layers. In all preparations, penetration of the tracers to the intercellular spaces along the lateral cut edges of the epidermis was limited to only a few microns in depth.

Typical gap junctions (Revel and Karnovsky, 1967) were not observed in our preparations of



FIGURE 1 Photomicrograph of a portion of an isolated epidermis of the abdominal skin of the frog. Notice the adequate preservation of epidermal cells, the dense precipitate induced by ruthenium red at the intercellular space of the *stratum granulosum* (SG) and the *stratum spinosum* (SS), and the absence of basal lamina. SC: *stratum corneum*; SGe: *stratum germinativum*. The specimen is fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide with ruthenium red, and embedded in Epon. 1 μ unstained section. Phase contrast optics. $\times 800$.

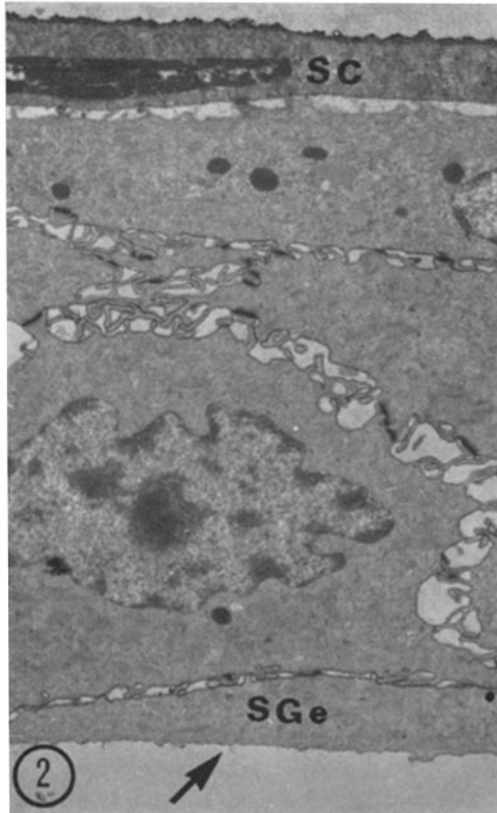


FIGURE 2 Isolated epidermis of the frog skin. In this preparation, only epidermal cells are present. No basal lamina (arrow) or other fibrillar or cellular components of the connective tissue remain after treatment with collagenase and hydrostatic pressure. SC: stratum corneum; SGe: stratum germinativum. The specimen is fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide with ruthenium red, and embedded in Epon. The section is stained with lead citrate. $\times 5500$.

isolated epithelium or whole skin. Short regions of close membrane apposition similar to those described by Farquhar and Palade (1965) as *maculae occludentes* were found only occasionally in the deeper layers.

Action and Movements of Lanthanum in the Living Skin

As an initial step to find out whether lanthanum could be used as a tracer to detect the permeability barriers in the living skin, we determined its effects on the electrical properties of the skin and also measured the movements of ^{140}La into and

across the skin. Fig. 8 shows that the addition of $5 \times 10^{-4}\text{M La}^{+++}$ to the external solution caused a large increase in short circuit current and potential. In other experiments in which ^{22}Na and ^{36}Cl fluxes were measured (Bracho, Erij, and García; data to be published), we found that these increases result from a selective stimulation of the inward movement of sodium across the skin. Lanthanum (10^{-4}M – 10^{-3}M) had no effect on the short circuit current when added to the inside solution. It is clear that lanthanum does not impair transport of sodium across frog skin.

Two representative experiments in which the movements of ^{140}La into and out of the skin were measured are illustrated in Fig. 9. The uptake of lanthanum was found to reach a maximum value within 5 min of exposure to the isotope. Most of the lanthanum is washed out readily from the skin when nonradioactive Ringer solution is placed in contact with the external surface of the skin. However, when the skin is washed with glutaraldehyde solution, almost all of the isotope remains within the skin.

The average uptake from $5 \times 10^{-4}\text{M La}^{+++}$ Ringer solution in 12 skins was $1 \times 10^{-8}\text{M cm}^{-2}$. This amount of lanthanum was contained in $20\ \mu\text{l}$ of the external solution. This volume is larger than the quantity of water adhering to the external surface of the skin after blotting (between 0.100 and $0.350\ \mu\text{l/cm}^2$; Biber and Curran, 1970; Aceves and Erij, 1970). Furthermore, the wash in inactive Ringer solution before each period of counting ought to have removed most of the adhering radioactive solution. Indeed, it is very likely that we are underestimating the maximum values of uptake since some La^{+++} may have been removed during the 10-sec wash period as suggested by the desorption part of the curve.

Lanthanum did not move across the skin. No radioactivity was detected in the inside solution even after exposing skins for 5 hr to a 10^{-3}M lanthanum Ringer solution at the outer surface.

Distribution of Lanthanum

Fig. 10 illustrates an electron micrograph from a skin exposed to $5 \times 10^{-4}\text{M}$ lanthanum on the external surface. A dense, finely granular or microcrystalline precipitate was present at the surface coat and randomly distributed within the cytoplasm of the cells of the *s. corneum* and also in the intercellular space between the *s. corneum* and *s. granulosum*. The dense precipitates were never

observed within the cells of the *s. granulosum* or at deeper layers when the specimens were treated with lanthanum from the outside. These findings were constant in all specimens examined. Skins treated only with Ringer solution and processed similarly for electron microscopy did not show any precipitate.

When lanthanum was added to the Ringer solution bathing the internal surface of isolated epithelia, a precipitate was present almost exclusively in the intracellular channels of the epidermis. This precipitate never extended beyond the region where occluding zonules between cells of the *s. granulosum* are present (Fig. 11). Occasionally a precipitate was seen within the cytoplasm of some cells of the *s. germinativum*. No precipitates were detected within the cytoplasm of other epidermal cells when lanthanum was added to the internal solution.

DISCUSSION

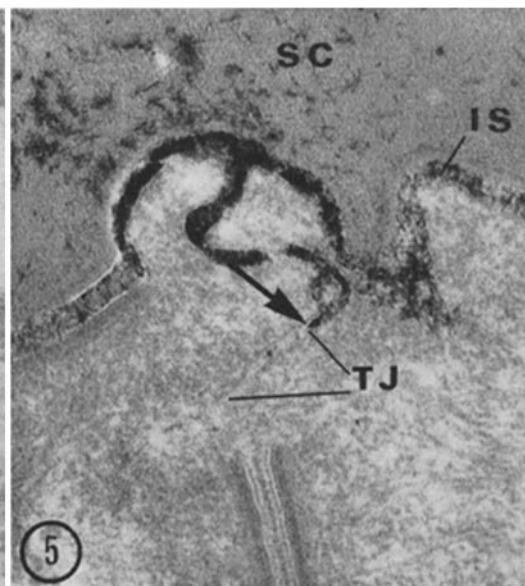
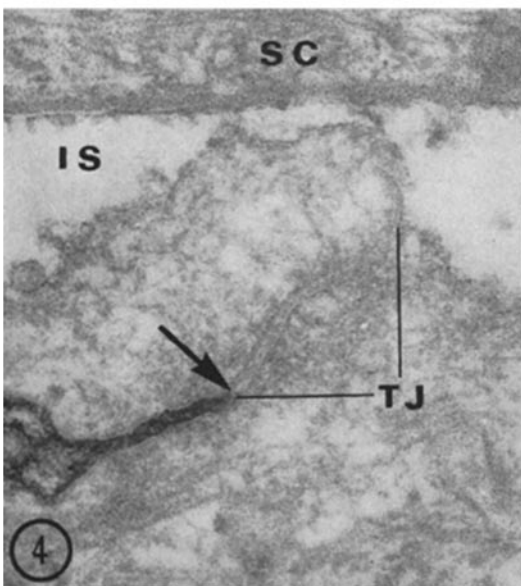
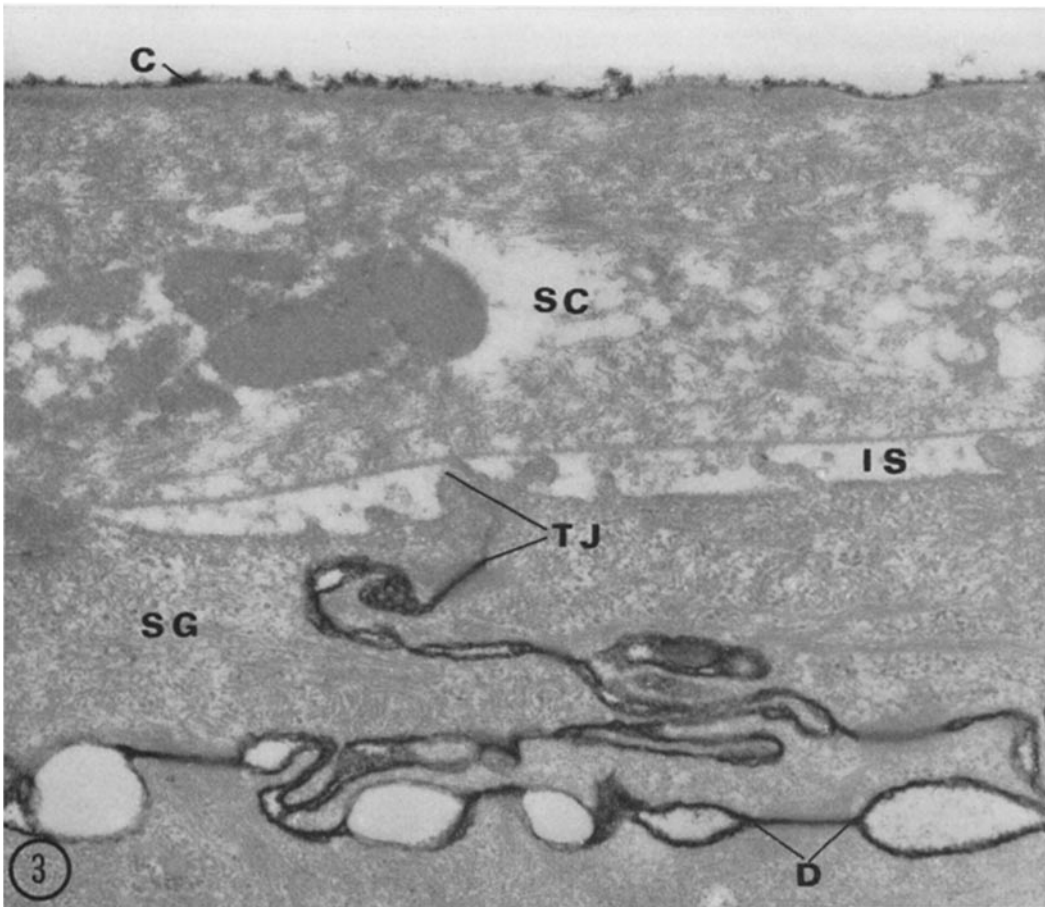
Perhaps the two most interesting conclusions that can be reached from the experiments described in this study are: first, both sets of occluding zonules present in the frog skin, i.e., those which seal together the cells of the *s. corneum* and those which join the cells of the *s. granulosum*, are "true" tight junctions; second, the diffusion barriers thus formed have different permeability properties.

The first conclusion is based mainly on the observations made on sections of frog skin impregnated with colloidal lanthanum or ruthenium red. In these experiments, no penetration of the tracers into the occluding zonules at the *s. corneum* and the *s. granulosum* was observed. This finding extends earlier observations of Farquhar and Palade (1965) and differentiates between occluding zonules and gap junctions. At occluding zonules, the intercellular space is obliterated and no extracellular markers are detected within these specialized contacts. On the other hand, gap junctions are permeable to colloidal lanthanum and to ruthenium red (Revel and Karnovsky, 1967; Brightman and Reese, 1969; Goodenough and Revel, 1970; Martínez-Palomo, 1970 a, 1971).

The second conclusion is based on the results of ionic ^{140}La uptake and the distribution of electron-opaque material in the skins exposed to lanthanum. After an initial period of rapid uptake, no additional lanthanum was absorbed by the skin. Once loaded with ^{140}La , the skins rapidly lost most of the isotope when the external surface was washed with

a nonlabeled Ringer solution. These findings indicate that there is at the outer region of the skin a compartment bounded at its outer surface by a barrier that imposes little restrictions to the movements of ionic lanthanum. The inner boundary of this compartment restricts markedly the movements of lanthanum since no isotope could be found in the internal solution even after exposing the skins to high ^{140}La concentrations for several hours.

The fine structural observations on the skins exposed before fixation to lanthanum suggest that the inner barrier of the compartment is constituted by the outer membrane of the cells of the *s. granulosum* and the occluding zonules that obliterate the intercellular spaces between them. This conclusion is based on the assumption that the precipitates observed represent essentially the true distribution of lanthanum in the living tissues. We feel that it is unlikely that an important fraction of lanthanum would have gone beyond the outer border of the *s. granulosum* without being detected with the electron microscope, because it appears from our results that electron-opaque precipitates are not associated exclusively with a preferred type of structure. The precipitates were observed at the external surface coat, in the space between the *s. corneum* and *s. granulosum*, and randomly within the cytoplasm of cornified cells, when lanthanum was added to the external solution. However when lanthanum was added to the internal solution, precipitates were found in the intercellular spaces up to the level of the occluding zonules at the *s. granulosum* and occasionally within some cells of the *s. germinativum*. An alternative explanation would be that the observed lanthanum acts both as a tracer and as a stain that binds with specific sites present only in some cells; however, since the interaction of La^{+++} with ligands is fundamentally one of electrostatic forces (Moeller, 1963), groups able to react with lanthanum will probably be widely distributed within all types of cells. These considerations imply that only the cornified cells are permeable to lanthanum. The presence of lanthanum in a few of the cells of the *s. germinativum* of isolated epithelia may result from an altered membrane permeability caused by the separation procedure. The concentration of precipitate along the surface coat and in the intercellular space above the *s. granulosum* suggests that lanthanum is staining some



component of the cell surface (Martínez-Palomo, 1970 b).

Provided that the above considerations are correct, our results indicate that of the two sets of barriers present in the frog skin, the first one, formed by the cells of the *s. corneum* and its occluding zonules, is the less selective, at least as far as lanthanum is concerned. The second, formed by the outer membranes and occluding zonules of the cells in the *s. granulosum*, is probably the site of the selective external barrier of the skin.

The problem of the localization of the outer selective barrier of the transport system of the amphibian skin has received frequent attention. Kidder et al., (1964) and Dainty and House (1966) measured the rate of change of potential difference across the skin immediately after a sudden change in Na concentration in the external solution. From these measurements, they estimated the size of the diffusion distance for sodium to the sodium selective external barrier. The conclusion was that this distance is of 25–50 μ . These values are close to the depth of the unstirred layer to be expected at the interface between the skin and the solution. However, the method lacks precision to distinguish whether the barrier is at the outer anatomical border of the skin itself or a few microns deeper, i.e. at the outer border of the *s. granulosum*.

Whittembury (1964) measured the resistance between a microelectrode introduced from the outer surface of the skin and the external solution.

The position of the tip was localized by injecting carmine through it. This procedure showed that the resistance between the microelectrode and the solution was negligible when the tip is localized within the cells of the *s. corneum*. This finding suggests that the ionic permeability of the *s. corneum* is large. However, since during penetration of the skin from the external surface the micropipettes are considerably deformed, they may damage the outer layer of cells. To avoid this problem, the localization of the external resistance was reinvestigated in Whittembury's laboratory (Rawlins et al., 1970) by using a preparation of the isolated epithelium of the toad skin in which microelectrodes can be inserted through the internal surface with little deformation. With this technique, these authors found that the external high resistance barrier is localized between 5 and 15 μ from the outer anatomical border of the skin. Unfortunately, it was not possible to determine the layer in which the micropipette tip was localized since the conclusion of that investigation was that the high resistance was registered from the cells of either the *s. corneum* or the *s. granulosum*.

Lindemann and Thorns (1967) have also measured the resistance between a microelectrode tip and the external solution, and they localized the tip by positioning it with a precision stop motor and also by direct observation with a high power microscope. They concluded that, in most of the skins, the main resistive barrier was at the outer-

FIGURE 3 Outer region of an isolated frog epidermis treated with ruthenium red during postfixation. Notice the presence of ruthenium red-positive material at the surface coat (C) of a cornified cell (SC). Ruthenium red penetrates from the interior of the epidermis and forms dense deposits at the outer surface of cell membranes in the *stratum granulosum* (SG) but it does not permeate a tight junction (TJ) located beneath the intercellular space (IS) separating the *stratum corneum* (SC) from the *stratum granulosum* (SG). D, desmosome. The specimen is fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide with ruthenium red, and embedded in Epon. The section is stained with lead citrate. $\times 23,000$.

FIGURE 4 Tight junction (TJ) between two cells of the *stratum granulosum*. The junction is not permeated by ruthenium red (arrow) which has penetrated the intercellular space from the basal region of the epidermis. The intercellular space (IS) between the *stratum granulosum* and the *stratum corneum* (SC) is devoid of ruthenium red precipitate. The specimen and section preparation is the same as for Fig. 3. $\times 90,000$.

FIGURE 5 Tight junction (TJ) in a location similar to that shown in Fig. 4. Colloidal lanthanum has penetrated into the cytoplasm of a cornified cell (SC) and into the intercellular space (IS) separating cornified cells from the *stratum granulosum*. However, the tracer is prevented from entering into the lateral intercellular spaces between two cells of the *s. granulosum* by the tight junction (TJ). The specimen is fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide with 1% lanthanum nitrate, pH 7.7, and embedded in Epon. The section is stained with lead citrate. $\times 80,000$.

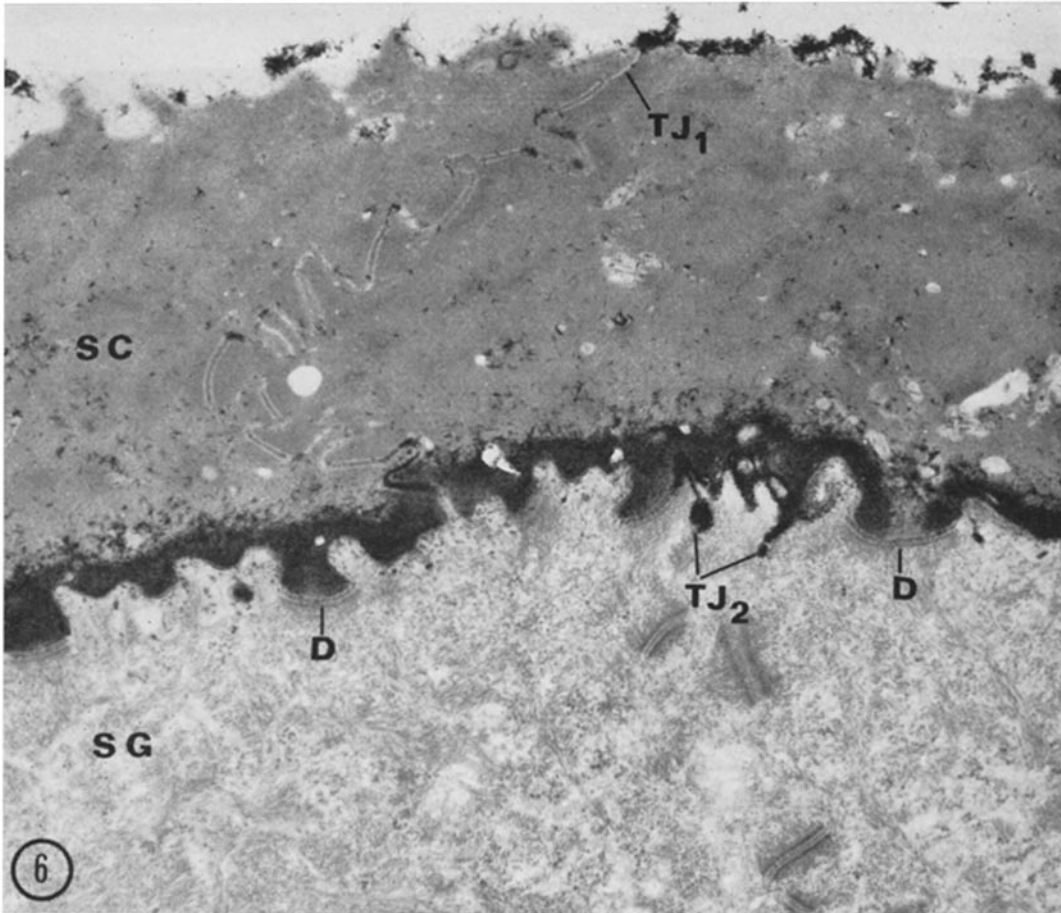


FIGURE 6 Outer region of the frog epidermis treated with colloidal lanthanum during postfixation. A dense precipitate is seen along the surface coat of a cornified cell (SC), in the cytoplasm of two cornified cells, and at the intercellular space between the *stratum corneum* (SC) and the *stratum granulosum* (SG). The precipitate has accumulated at the basal region of cornified cells where it forms a dense, irregular band. No lanthanum is seen either at a tight junction between two cornified cells (TJ₁), or below two tight junctions (TJ₂) between adjacent cells of the *stratum granulosum* (SG). D, desmosome. The specimen and section preparation is the same as for Fig. 5. $\times 21,000$.

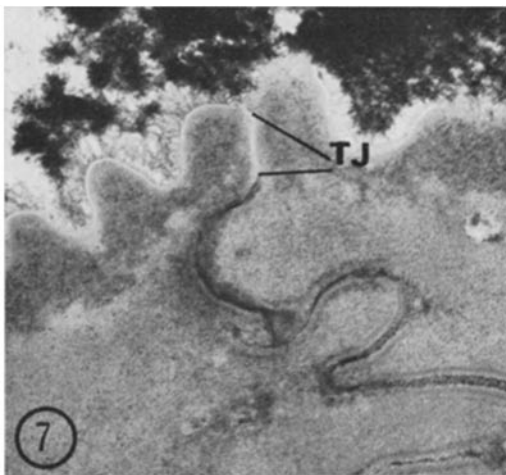


FIGURE 7 Tight junction (TJ) between two cornified cells, showing the region of membrane fusion of apposing cell membranes. Notice the absence of lanthanum precipitate at the level of the junction. The specimen and section preparation is the same as for Fig. 5. $\times 57,000$.

most surface of the epithelium. They were careful, however, to point out that perhaps the cornified cells were missing in their preparations, due to the possibility that the skins could have been in a particular stage of the monthly regeneration cycle. Probably, heavily cornified cells make precise measurements with microelectrodes impossible.

For this reason, the skins studied by Lindemann and Thorns (1967) may represent a necessary selection. The conclusion of these authors, then, may be based mainly on observations on "young cornified cells" and possibly may not apply to "old cornified cells".

From the results of microelectrode experiments, a sodium selective barrier was located underneath the cornified cell layer of the frog skin by Ussing and Windhager (1964), while Farquhar and Palade (1966) suggested, on morphological and cytochemical grounds, that the "outward facing membrane" was localized on the outer front of the *s. corneum* or in the external aspect of the *s. granulosum*. More recently, Voûte and Ussing (1968) have determined the changes in cell volume in different layers of the skin during sodium transport. A reversible swelling associated with high rates of transport was detected only in the cells of the outermost layer of the *s. granulosum*, suggesting that all the sodium that moves across the skin has to cross the first layer of the *s. granulosum* and that a great part of the transport is performed by this layer.

Considering the above mentioned information and the results of the present investigation, we feel that the most satisfactory conclusion that can be reached, for the time being, is that the important external barrier to movement of substance across the epithelium of the frog skin is constituted by the outer membrane of the cells in the *s. granulosum* which are sealed together by occluding zonules. An additional barrier is formed by the cells of the

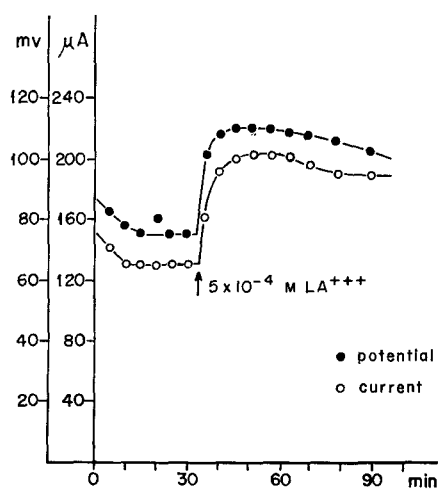


FIGURE 8 The effects of lanthanum ($5 \times 10^{-4}M$) on the short circuit current and potential difference of a frog skin mounted in a Ussing-Zerahn chamber. The arrow marks the addition of $LaCl_3$ to the external chamber. Abscissa: time in minutes. Ordinate: potential difference in mv. Short circuit current in μA . Area of skin in chamber: 3.1 cm^2 . Empty circles: short circuit current. Filled circles: potential difference.

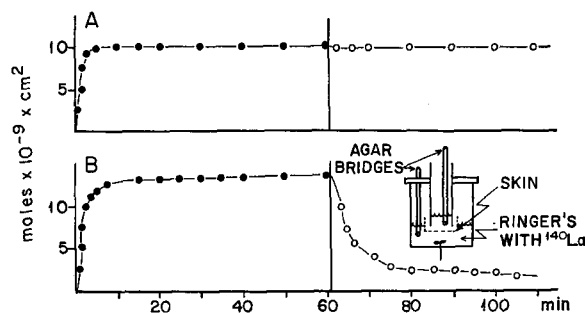


FIGURE 9 The uptake and washout of lanthanum through the external surface of the frog skin. In both curves *A* and *B*, the uptake (filled circles) was followed during 60 min from a $5 \times 10^{-4} M$ La Ringer solution. The vertical lines indicate the moment when the skins were transferred to a beaker containing nonlabeled solution, without cold La. In *A*, the empty circles illustrate the change in lanthanum content of the skin when a nonlabeled glutaraldehyde solution was used to wash the skin. In *B*, the skin was washed with normal Ringer's solution. Abscissa: time spent by the skin in either the loading or washing solution. Ordinate: amount of lanthanum per cm^2 of skin.

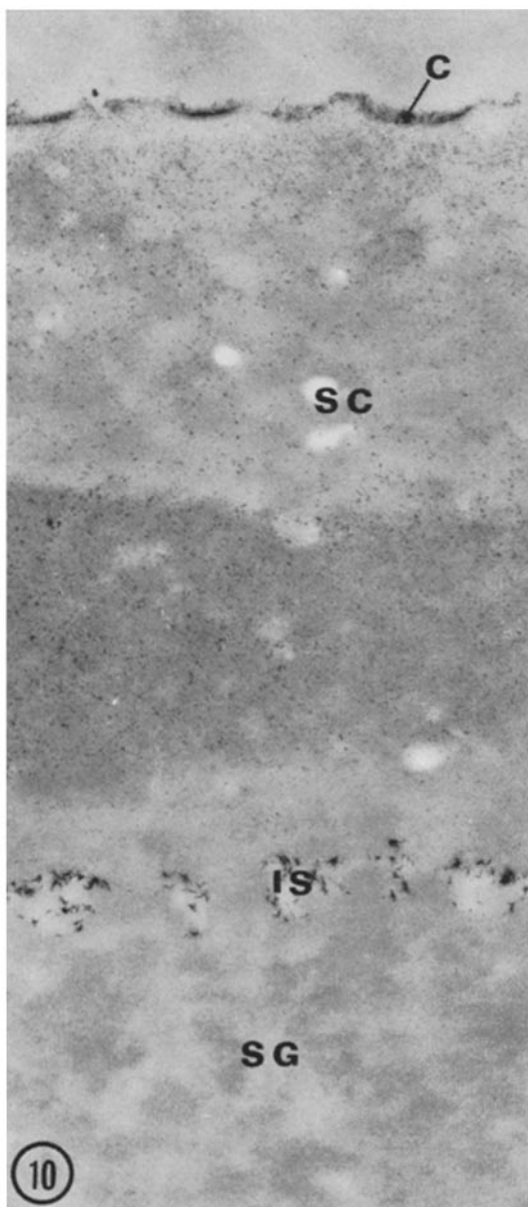


FIGURE 10 Outer region of the frog epidermis treated from the outside with 5×10^{-4} M lanthanum chloride during 1 hr. A dense precipitate is seen at the surface coat of the cornified cells (C), in the cytoplasm of a cornified cell (SC), and at the intercellular space (IS) between the *stratum corneum* (SC) and the *stratum granulosum* (SG). No precipitate is seen in the cytoplasm of the *stratum granulosum* cell. The specimen is fixed in 2.5% glutaraldehyde with 0.1 M cacodylate buffer pH 7.3, and embedded in Epon without postfixation. The section is unstained. $\times 57,000$.

FIGURE 11 Junction between two cells of the outer layer of the *stratum granulosum*. The epidermis was treated from the inside with 5×10^{-4} M lanthanum chloride during 1 hr. The dense precipitate is seen along the intercellular space of the *stratum granulosum*, but is not present either in the intercellular space (IS) between the *stratum granulosum* and the *stratum corneum* (SC) or in the cytoplasm of a cornified cell (SC). The specimen fixation is the same as for Fig. 10. The section is stained with lead citrate. $\times 21,000$.

s. corneum, whose resistance and selectivity may vary significantly with the stage of monthly regeneration cycle of the skin, but probably are low during a large part of the cycle. The possibility that these cells constitute a compartment at the outer surface of the skin with a relatively high permeability towards the outside has to be considered for the interpretation of experiments in which the uptake of substances through the external surface of the skins is measured as in the

recent observations on sodium uptake of Biber and Curran (1970) and Rotunno et al. (1970).

Apart from their implications for the physiology of the frog skin, the data presented in this paper indicate that ionic lanthanum used in relatively low concentrations may be a useful tracer in electron microscopy.

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