

PERMEABLE JUNCTIONAL COMPLEXES

The Movement of Lanthanum across Rabbit Gallbladder and Intestine

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ABSTRACT

Ionic lanthanum has been used to study transepithelial ion permeation in *in vitro* rabbit gallbladder and intestine (ileum) by adding 1 mM La^{3+} to only the mucosal bathing solution. Transepithelial fluid transport electrical potential differences (p.d.), and resistances were measured. During La^{3+} treatment the gallbladder's rate of active solute-coupled fluid transport remained constant, the resistance increased, and the 2:1 NaCl diffusion p.d. decreased. Mucosa-to-serosa fluxes of $^{140}\text{La}^{3+}$ were measured and indicate a finite permeability of the gallbladder to La^{3+} . La^{3+} also increased the transepithelial resistance and p.d. of ileum. Electron microscopic examination of La^{3+} -treated gallbladder showed: (a) good preservation of the fine structure, (b) electron-opaque lanthanum precipitates in almost every lateral intercellular space, most frequently near the apical end of the lateral spaces close to or within the junctional complex, (c) lanthanum among the subjacent muscle and connective tissue layers, and (d) lanthanum filling almost the entire length of so-called "tight" junctions. No observations were made which unequivocally showed the penetration of lanthanum into the gallbladder cells. Electron micrographs of similar La^{3+} -treated ilea showed lanthanum deposits penetrating the junctional complexes. These results coupled with other physiological studies indicate that the low resistance pathway for transepithelial ion permeation in gallbladder and ileum is through the tight junctions. A division of salt-transporting epithelia into two main groups, those with "leaky" junctional complexes and those with tight junctional complexes, has been proposed.

INTRODUCTION

A regular feature of epithelia is the presence of a characteristic junctional complex sealing together adjacent cells. The so-called "tight" junctions (occluding zonules) of this complex impede the movement of substances along the intercellular spaces, since at this region the membranes of neighboring cells come in direct apposition forming a continuous belt around each cell (Farquhar and Palade, 1963).

In spite of the relative uniformity of structure there are several important functional differences among epithelia. Our interest was particularly aroused by the fact that when transepithelial electrical resistances are considered, epithelia can be divided into two groups. One group has resistances ranging between 5 and 200 $\Omega \cdot \text{cm}^2$, while in the second group the resistances range between 1750 and 5000 $\Omega \cdot \text{cm}^2$ (for a survey, see Rose and

Schultz, 1971). More interesting than the differences in total transepithelial resistance is that, at least for some of the low resistance epithelia, there is evidence which implies that the tight junctions are the low resistance pathway for passive ion permeation across the epithelium (proximal tubule of the kidney: Giebisch et al., 1971; gallbladder: Barry et al., 1971; ileum: Frizell and Schultz, quoted by Rose and Schultz, 1971; choroid plexus: Wright, quoted by Barry et al., 1971).

In an attempt to obtain additional evidence showing that the tight junctions of low resistance epithelia can act as a path for transepithelial ion permeation, we have studied the distribution of lanthanum in the epithelia of isolated gallbladder and ileum after exposing the mucosal side to "physiological" solutions containing a small amount (1 mM) of dissolved tracer. When used in these concentrations, all of the lanthanum in the solutions is in ionic form. Approximately 90% of the lanthanum is in the La^{3+} form and 10% in the forms $\text{La}(\text{OH})_2^+$ and $\text{La}(\text{OH})_3^+$. None of the lanthanum is precipitated as $\text{La}(\text{OH})_3$ (Moeller, 1963; Sillen and Martell, 1964). It has also been previously shown (Martinez-Palomo et al., 1971) that the distribution of lanthanum can be detected with the electron microscope in epithelia exposed to low concentrations of the metal. Furthermore, since La^{3+} is equilibrated with the living tissue, it is also possible to detect whether there are any adverse effects on it and also to use the isotope $^{140}\text{La}^{3+}$ to obtain a check on the conclusions reached from electron micrographs.

MATERIALS AND METHODS

Techniques used for obtaining *in vitro* preparations of gallbladder from anesthetized New Zealand white rabbits and for measuring transepithelial fluid transport, electrical potential differences (p.d.), and resistances were, in general, similar to those described previously by Diamond and coworkers (Diamond, 1962 *a, b*, 1964; Diamond and Harrison, 1966; Wright and Diamond, 1968; Wright et al., 1971). Transepithelial fluid transport (measured as described by Diamond, 1962 *a*, 1964), p.d., and fluxes of $^{140}\text{La}^{3+}$ were measured using a "sac" preparation of the gallbladder, cannulated in the nonverted orientation with a polyethylene cannula, filled with a Ringer's solution, and suspended in a beaker of Ringer's solution maintained at 36°–37°C by a water bath and stirred with 100% O_2 saturated with water vapor.

All transepithelial p.d. were measured to ± 0.05 mv by connecting the mucosal (i.e., the solution

facing the epithelium and junctional complexes) and serosal (i.e., the solution facing the connective tissue) bathing solutions to a Vibron electrometer (Burgess & Associates, Inc., Cleveland, Ohio) via calomel half-cells and salt bridges containing Ringer's solution immobilized by 5% agar. The p.d. were corrected for any junction p.d. arising at the agar-NaCl bridges (see MacInnes, 1961; Barry and Diamond, 1970) and have been expressed here as that of the mucosal solution with respect to the serosal solution.

To measure the gallbladder's permeability to La^{3+} , $^{140}\text{LaCl}_3$ plus enough nonradioactive La^{3+} to make a final concentration of 1 mM was added to the mucosal solution, the final specific activity of which was 5 $\mu\text{Ci/ml}$.

Gallbladder resistances, measured as described previously by Wright and Diamond (1968) and Wright et al. (1971) by mounting the gallbladder between two lucite chambers, were corrected to account for the resistance of the Ringer's solution bathing both sides of the epithelium. The exposed area of the gallbladder was 1.13 cm^2 . The resistances are expressed here as the total measured transepithelial resistance. The solutions were maintained at 35°–37°C by water jacketing of the fluid-containing chambers (see Schultz and Zalusky, 1964; Smulders and Wright, 1971).

Since the gallbladder is more permeable to cations than to anions (Wheeler, 1963; Pidot and Diamond, 1964; Dietschy, 1964; Wright and Diamond, 1968), when a concentration gradient of NaCl is established across the epithelium, the dilute solution becomes electrically positive with respect to the concentrated solution. The size of this p.d. is determined by the NaCl concentration gradient and by the gallbladder's selectivity for Na^+ over Cl^- . Transepithelial diffusion p.d. thus provide an indication of the gallbladder's permeability properties. Previous workers (Machen and Diamond, 1969; Barry and Diamond, 1970) had used a p.d. of about 8 mv resulting from a 2:1 concentration gradient of NaCl (e.g., 150 mM NaCl as the serosal solution and 75 mM NaCl as the mucosal solution) as a criterion for a viable *in vitro* gallbladder preparation. We, therefore, measured a diffusion p.d. at room temperature (20°–23°C) resulting from a 2:1 NaCl concentration gradient (using solution 1, described below, containing 132.5 mM NaCl, as the serosal solution, and solution 2, containing 66.25 mM NaCl, as the mucosal solution) at the beginning of each experiment. If this initial p.d. measurement was less than 8.80 mv, the gallbladder was discarded. All further experimental procedures were carried out at 35°–37°C.

Techniques used for obtaining *in vitro* preparations of rabbit intestine (ileum) were as described by Schultz and Zalusky (1964). The intestine was mounted with the serosal muscle layer left intact in

the resistance-measuring chambers described above, and transepithelial resistances and p.d. were measured at 35°–37°C.

The experimental solutions, all of which were pH 7.3–7.5 (measured with a glass electrode) and 280–284 mosmolal (measured with a Fiske Osmometer, Fiske Associates, Inc., Uxbridge, Mass.), were: (1) 132.5 mM NaCl, 7.0 mM KCl, 1.0 mM CaCl₂, 1.2 mM MgCl₂, 11.1 mM glucose, and 5.0 mM Tris buffer; (2) 66.25 mM NaCl, 121.6 mM mannitol, 7.0 mM KCl, 1.0 mM CaCl₂, 1.2 mM MgCl₂, 11.1 mM glucose, and 5.0 mM Tris buffer; (3) as in solution 1 except that all the glucose was replaced by mannitol.

For use as an electron-opaque substance, La³⁺ was added as LaCl₃ to a final concentration of 1 mM to only the mucosal bathing solution of either the gallbladder or intestine. The epithelium was incubated under these conditions for 1 hr and then fixed by carefully replacing the mucosal and serosal bathing solutions with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.7–7.9). During this and all procedures involving the changing of the solutions, care was taken that there was no undue distension of either the gallbladder or intestine. The epithelium was fixed for 1 hr and then washed 4–5 times and allowed to stand overnight in 0.1 M cacodylate buffer alone. The tissue then was either embedded without further fixation or else postfixed for 1 hr in 1% OsO₄ in pH 7.2 Veronal-acetate buffer and then stained for 1 hr in 2% aqueous uranyl acetate and lead citrate. All specimens were dehydrated through an alcohol series, embedded in Araldite or Epon at 60°C, sectioned on an LKB ultratome (Laboratoric Och Kemikaliska Produkter, Stockholm), and examined in an AEI EM6B microscope at 60 kv. Some sections were stained on the grid with uranyl acetate followed by lead citrate before examination.

RESULTS

Physiological Studies

To test whether 1 mM La³⁺ had any adverse effects on the gallbladder, we first measured its effects on active solute-linked fluid transport, the main function of this tissue.

The average (\pm SEM) rate of fluid transport in three gallbladders bathed with solution 1 on both sides of the epithelium was initially $24.4 \pm 6.7 \mu\text{l}/\text{cm}^2 \cdot \text{hr}^1$. The average rate of fluid transport

¹ This rate is slower than the rates obtained by other workers (Wheeler, 1963; Diamond, 1964; Dietschy, 1964; Tormey and Diamond, 1967; Machen and Diamond, 1969) because NaHCO₃, which is known to stimulate the gallbladder's fluid transport (Diamond, 1964; Wheeler et al., 1969), was omitted from the bathing solutions.

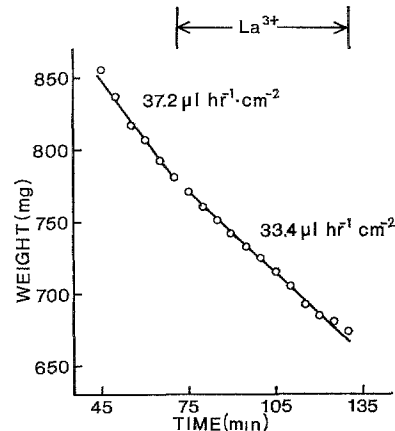


FIGURE 1 Effect of 1 mM La³⁺ on gallbladder fluid absorption. Weight of gallbladder plus contents has been plotted versus time after dissection. Both sides of the gallbladder were initially bathed in solution 1. At $t = 71$ min, the mucosal bathing solution only was changed to one containing 1 mM LaCl₃.

during the hour after addition of 1 mM LaCl₃ to the mucosal solution of the same three gallbladders was $21.3 \pm 6.0 \mu\text{l}/\text{cm}^2 \cdot \text{hr}$. One of these experiments is illustrated in Fig. 1. The fluid transport rate, as measured by a progressive loss in weight of the gallbladder, was not significantly affected by the presence of 1 mM La³⁺ in the mucosal bathing solution.

We were also interested in the effect of 1 mM La³⁺ on 2:1 NaCl diffusion p.d. in the gallbladder. In five gallbladders prepared as "sacs" (two of these were fixed for electron microscopy, and three were used for measuring mucosa-to-serosa fluxes of ¹⁴⁰La³⁺) a 2:1 diffusion p.d. was measured just before (average = 9.42 ± 0.37 mv) and after (average = 2.04 ± 0.93 mv) 1 hr treatment with 1 mM La³⁺.

Polyvalent cations, including La³⁺, are known to cause a decrease in gallbladder 2:1 NaCl diffusion p.d., presumably by causing a decreased cation and an increased anion conductance (Wright and Diamond, 1968). However, to ensure that the decrease in the 2:1 dilution p.d. caused by 1 mM La³⁺ was not due to nonspecific damage or the opening of a free-solution shunt, the effect of La³⁺ on both 2:1 diffusion p.d. and resistance was measured over the course of an hour in three experiments. One of these experiments is shown in Fig. 2.

1 mM La³⁺ added to the mucosal bathing solu-

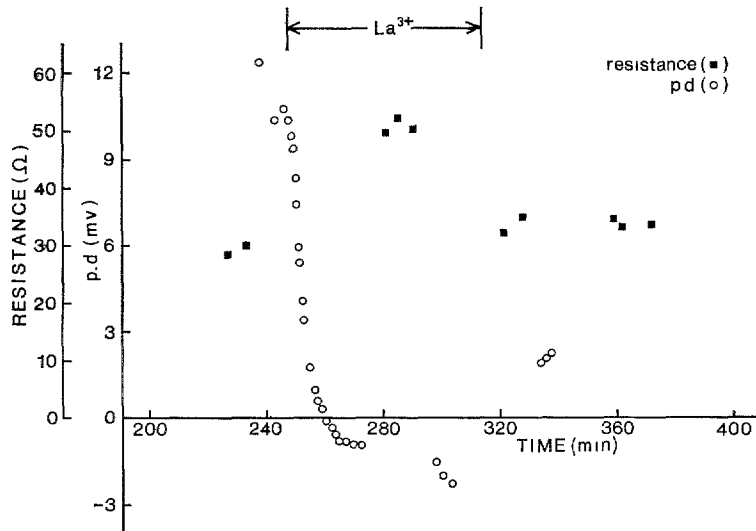


FIGURE 2 Effect of 1 mM La^{3+} on 2:1 NaCl dilution p.d. and resistance of gallbladder. Resistance (measured with solution 1 bathing both sides of the epithelium) and 2:1 dilution p.d. (measured when only the mucosal solution was changed to solution 2) were measured before, during, and after addition of 1 mM LaCl_3 to the mucosal bathing solution. La^{3+} caused the 2:1 dilution p.d. to decrease and the transepithelial resistance to increase.

tion caused. (a) A decrease in the 2:1 NaCl diffusion p.d. The average 2:1 diffusion p.d. in the three gallbladders immediately before adding La^{3+} was 12.83 ± 0.85 mv. The average minimum p.d. reached after La^{3+} treatment was -0.28 ± 3.10 mv. La^{3+} required 15–30 min to exert its maximal effect on the diffusion p.d. (b) A concomitant increase in the transepithelial resistance. The average resistance before La^{3+} treatment in these same three gallbladders was $29.2 \pm 2.6 \Omega$; after La^{3+} was added to the mucosal solution, the resistance increased to a maximum (average = $43.8 \pm 7.1 \Omega$) and then slowly decreased, with a time course similar to that seen in previous studies (Wright et al., 1971), to a final average of $33.5 \pm 6.3 \Omega$.

After 1 hr treatment, the effects of La^{3+} on the gallbladder's diffusion p.d. and resistance were only poorly reversible. After washing the epithelium repeatedly with La^{3+} -free solutions the average 2:1 NaCl diffusion p.d. and resistance were 4.12 ± 1.29 mv and $29.5 \pm 6.3 \Omega$, respectively.

If La^{3+} was decreasing the 2:1 diffusion p.d. by causing nonspecific damage or the opening of a free-solution shunt (e.g., by disruption of the structure of the tight junctions), a decreased transepithelial resistance should have resulted; La^{3+} increased the resistance. Thus, the effect of 1 mM La^{3+} in causing a decrease in the gallbladder's 2:

NaCl diffusion p.d. is due not to nonspecific damage or the opening of a shunt but presumably to a decrease in the cation and an increase in the anion conductance of the epithelium as postulated for other polyvalent cations (Wright and Diamond, 1968; Machen and Diamond, 1972).

As a check on the electron microscopic observations, mucosa-to-serosa fluxes of $^{140}\text{La}^{3+}$ were measured in three gallbladders. When 1 mM La^{3+} was added to the mucosal solution, the average transepithelial flux was 0.03 ± 0.01 n-moles/cm² hr. Compared with the passive fluxes of Na^+ in fish gallbladder ($8.4 \mu\text{mole/cm}^2 \cdot \text{hr}$, Diamond, 1962 b) the rabbit gallbladder has a very low, yet finite, permeability to La^{3+} .

Finally, the effects of 1 mM La^{3+} solutions on intestine were studied by measuring the transepithelial p.d. and resistance before and after La^{3+} treatment. In two experiments the p.d. and resistances were measured upon setting up the ilea with solution 3 bathing both sides. These were -4.50 and -5.00 mv and 61.0 and 42.0Ω , respectively.

When solution 3 was replaced by solution 1, the characteristic glucose-dependent increase in the transepithelial p.d. (Barry et al., 1964; Schultz and Zalusky, 1964; Gilles-Baillien and Schoffeniels, 1965; Wright, 1966; Rose and Schultz, 1971) was measured: the p.d. increased from -4.50 and

-2.60 mv to -6.00 and -7.60 mv, respectively. The transepithelial resistances remained nearly constant at 61.0 and 40.0 Ω . 1 mM La^{3+} added to the mucosal solution caused, within 4 min, a further increase (to -6.80 and -8.70 mv) in the transepithelial p.d. These p.d. decayed at a constant rate over the course of an hour to -1.60 and -2.30 mv. The transepithelial resistance also increased, the maximum effect being observed after 12-30 min. In the two intestines, the resistances increased to 82.0 and 55.0 Ω , respectively, and, in contrast to the p.d. remained fairly constant over the course of 1 hr La^{3+} treatment. The final resistances were 76.0 and 47.0 Ω .

Electron Microscopy

Fig. 3 summarizes observations made in unstained sections from five gallbladders fixed after 1 hr incubation in solution 1 with 1 mM La^{3+} added only on the mucosal side. The electron-opaque precipitates, characteristic of lanthanum, were observed in almost every lateral intercellular space and most frequently near the apical end of the lateral spaces close to or within the junctional complex. Lanthanum deposits were also observed beyond the epithelial layer among the subjacent muscle and connective tissue layers. The tracer was rarely seen in the lumen and infrequently observed among the microvilli of the tissue, probably because the bulk of the lanthanum in the gallbladder lumen was washed away during the fixation and embedding procedures.

No observations were made showing unequivocally the penetration of lanthanum into the cells. On occasions, lanthanum deposits surrounded by a continuous membrane, forming what appeared to be a small vesicle, were observed near the cell surface. It was, however, difficult to discount that they were only recesses in the cell surface sectioned

in a plane more or less parallel to their opening. Serial sectioning to settle the point was not undertaken since the sparseness of the vesicles indicates that, regardless of their true nature, the vesicles do not represent a major route of transepithelial movement of lanthanum.

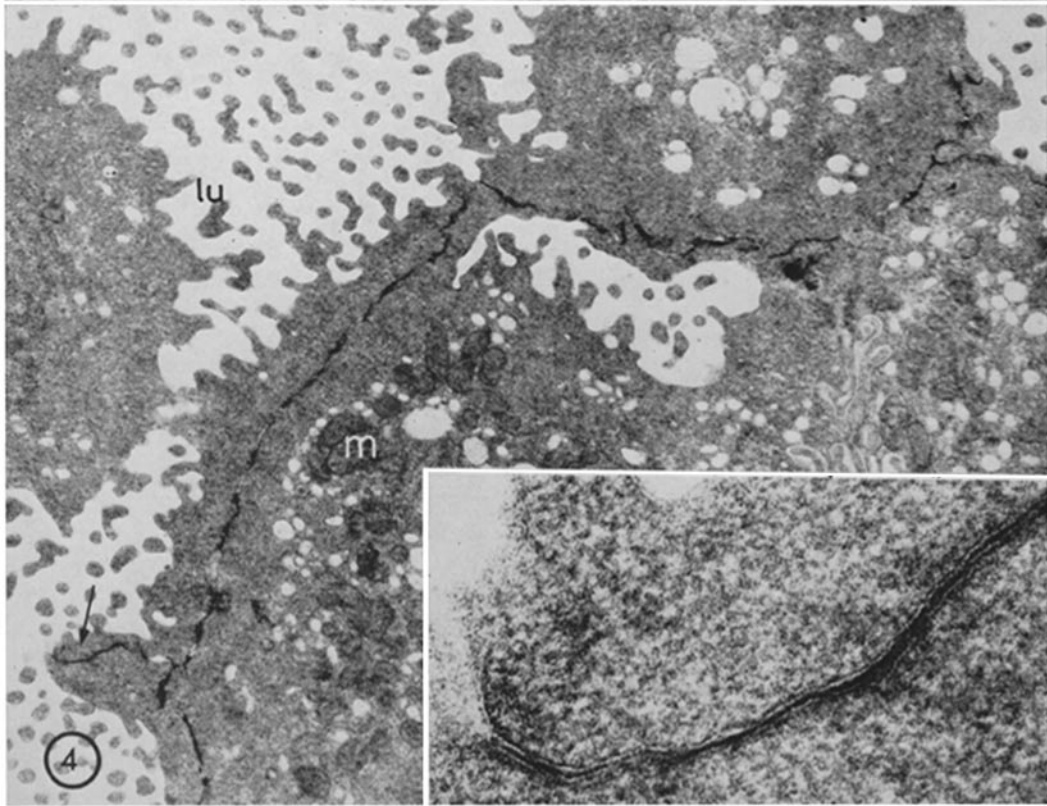
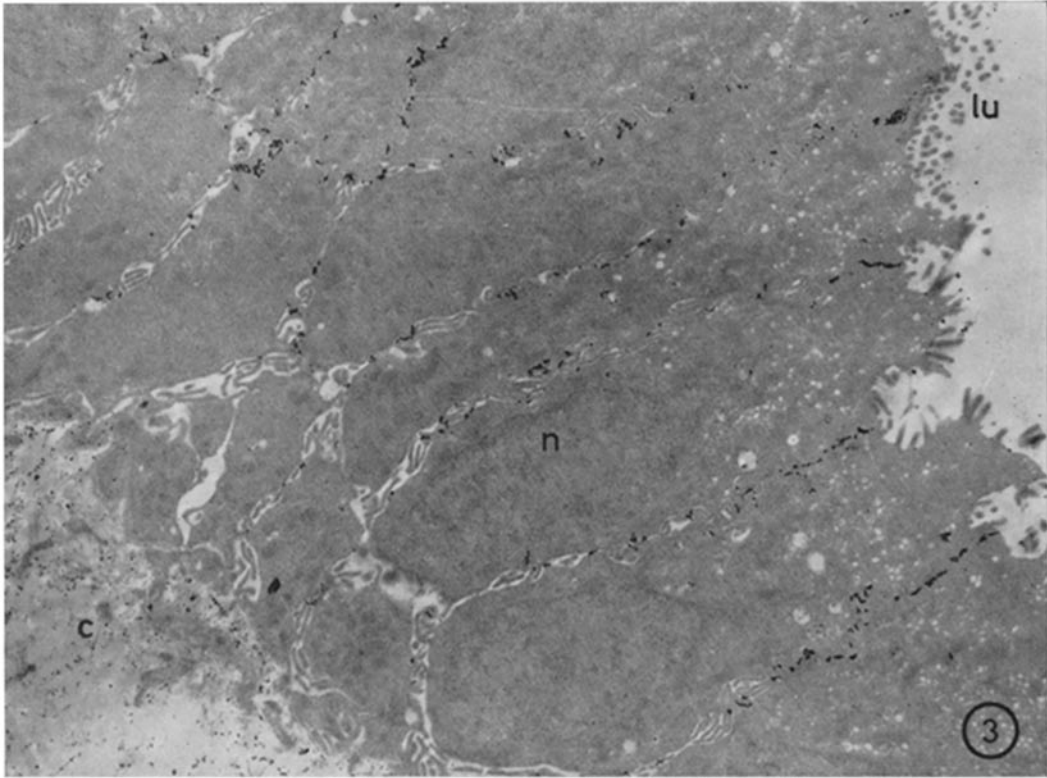
Further evidence indicating the widespread penetration of lanthanum into the junctional complex region was obtained when oblique or grazing sections that cut close to the luminal surface of the epithelium were examined (Fig. 4). Considerable lengths of the junctional complex region almost uniformly filled with lanthanum were observed.

Examination at high magnification of the junctional complex of gallbladders not treated with La^{3+} shows that most frequently the outer leaflets of the apposed membranes are not fused throughout the entire length of the occluding zonules (Fig. 5). Instead, there is a series of fusion sites between which the membranes are separated for short distances by narrow spaces. This pattern has already been described in the tight junctions of the gallbladder (Tormey, quoted by Barry et al., 1971), the bile canaliculi (Goodenough and Revel, 1970), and the vertebrate brain (Brightman and Reese, 1969).

When the junctional complexes of gallbladders, fixed after equilibration with La^{3+} in their lumens, were examined with the electron microscope, electron-opaque deposits of lanthanum were observed filling almost the entire length of the region that is normally identified with the tight junction (Figs. 6-8). In many cases there were small focal regions in which it was impossible to discern whether or not lanthanum deposits were present between the outer membranes of the junction (Fig. 6, between arrows), while in others lanthanum could be followed throughout the length of the junction (Fig. 7).

FIGURE 3 Transverse section of rabbit gallbladder bathed for 1 hr with 1 mM La^{3+} in the mucosal solution only. Although very little lanthanum is seen in the lumen (*lu*) or on the surface of the apical plasma-lemma, the junctional complexes at the apex of the cells are filled. The lanthanum has also been deposited throughout the lateral intercellular spaces and the connective tissue (*c*). Cell nucleus (*n*). Sections fixed in glutaraldehyde only, without postossmication. Unstained. $\times 6500$.

FIGURE 4 Oblique section through the apical portion of La^{3+} -treated (mucosal solution only) gallbladder at the level of the junctional complex. The complex can be seen to be filled with lanthanum along most of its length. Inset shows the junctional complex ending at the lumen (see arrow). Mitochondrion (*m*); lumen (*lu*). Specimen fixed in glutaraldehyde, postfixed in OsO_4 and uranyl acetate, and sections stained on the grid with uranyl acetate and lead citrate. $\times 14,000$. Inset $\times 165,000$.



The width of the tight junctions with or without La^{3+} seemed to be equivalent. Because of the irregularity of the junctions' focal dilatations, the width is very difficult to measure. In all junctions with membranes resolved (with or without lanthanum) there was at least one position where the adjacent plasmalemmas were fused to give a common central leaflet. The impression given was of lanthanum filling the focal dilatations rather than causing an increase in distance between the two plasmalemmas.

Control gallbladders (incubated without La^{3+}) fixed in glutaraldehyde as above and postfixed in osmium tetroxide or potassium permanganate solutions containing 1 mM La^{3+} showed no penetration of the tight junctions by lanthanum.

Electron microscopic examination of sections of two ilea fixed after incubation in solution 1 with 1 mM La^{3+} added only to the mucosal bathing solution showed frequent coarse lanthanum deposits lying between the microvilli in the intestinal lumen (Figs 9 and 10). Lanthanum also penetrated the junctional complexes of intestinal epithelium (Figs. 9 and 10). Lanthanum deposits were observed in about half of the junctional complexes examined in transverse sections. As in the case of the gallbladder, it was possible to find images showing lanthanum present in almost the entire length of the tight junction (Fig. 9).

DISCUSSION

The experiments described in this communication show that lanthanum moves across the wall of the gallbladder, permeating the epithelium through the junctional complexes. Observations with the electron microscope on rabbit ileum show that lanthanum also penetrates the junctional complexes of this epithelium. The penetration of lan-

thanum through the junctional complexes of ileum and gallbladder epithelia is in marked contrast with findings in frog skin epithelium (Martinez-Palomo et al., 1971) and toad urinary bladder (Bracho, Erlij, and Martinez-Palomo, unpublished observations) where lanthanum was never observed on the opposite side of the epithelia nor within the tight junctions.

A number of findings suggest that La^{3+} does not have adverse effects on the gallbladder. The fine structure of the epithelium was well preserved after incubation in La^{3+} -containing solutions, and the active solute-coupled water transport was not altered by the tracer.

In addition, after 1 hr treatment with La^{3+} , the resistances of the gallbladder and ileum were always above the initial levels in La^{3+} -free solutions, even though there was, after the initial increase in resistance caused by La^{3+} , a slow decrease in the resistance. This slow decrease in gallbladder resistance was similar to that measured previously in control gallbladders by Wright et al., (1971) and which has been attributed to changes, with time, of wall charges or dipoles along the route of transepithelial ion permeation (Barry et al., 1971). It is clear, though, that as far as tissue resistance changes are concerned the La^{3+} -treated gallbladders and ilea are in a condition at least as satisfactory as that of control epithelia.

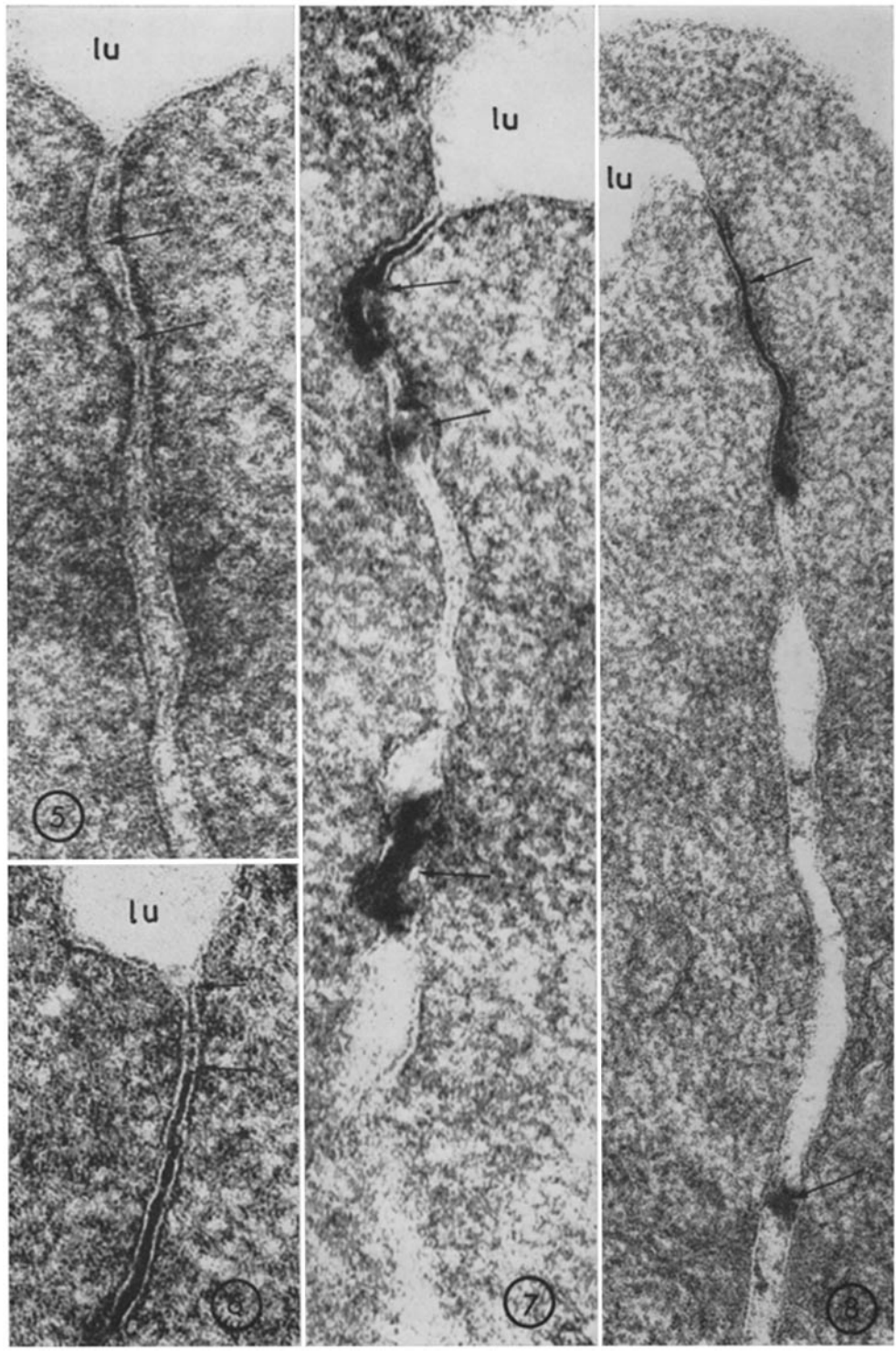
The difference in the distribution of lanthanum in the gallbladder and ileum on the one hand and frog skin and toad urinary bladder on the other hand, taken together with their differences in physiological properties, suggests that salt-transporting epithelia may be divided into two main groups, those with leaky junctional complexes and those with tight junctional complexes. In the first group, junctional complexes have low resistance to

FIGURES 5-8 Transverse sections of junctional complexes between rabbit gallbladder cells.

FIGURE 5 Control gallbladder which was placed in fixative immediately after dissection. Regions of focal separation (arrows) are visible in the tight junction region. Lumen (*lu*). Specimen fixed in glutaraldehyde, postfixed in OsO_4 , and stained on the grid with uranyl acetate and lead citrate. $\times 250,000$

FIGURE 6 La^{3+} -treated (mucosal solution only) gallbladder. Lanthanum is not present in the most apical part of the junction (between the arrows). Lumen (*lu*). Specimen fixed in glutaraldehyde, postfixed in OsO_4 , and stained on the grid with uranyl acetate and lead citrate. $\times 250,000$.

FIGURES 7 and 8 La^{3+} -treated (mucosal solution only) gallbladder. Lanthanum is present (arrows) throughout the entire length of the region of the tight junction and below. Lumen (*lu*). Specimens fixed in glutaraldehyde, postfixed in OsO_4 , and stained on the grid with uranyl acetate and lead citrate.



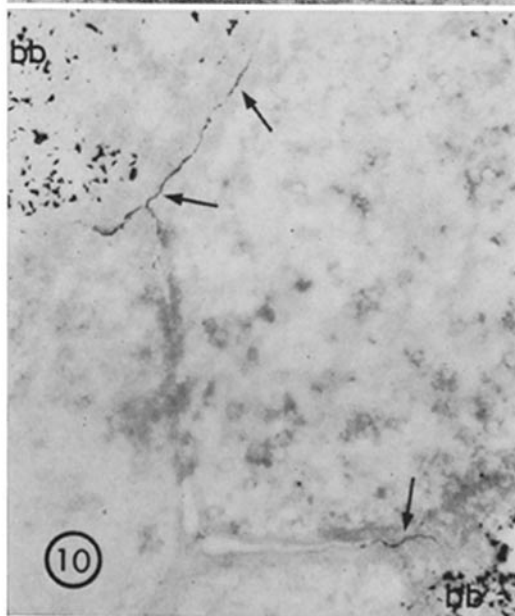
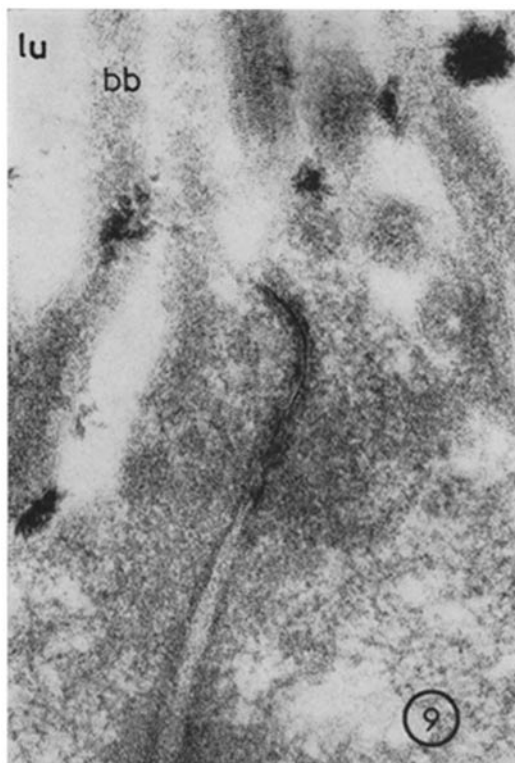


FIGURE 9 Transverse section of junctional complex between rabbit intestine (ileum) epithelial cells. Intestines were bathed with 1 mM La^{3+} on the brush border (*bb*) side of the epithelium only. Lanthanum is present throughout the entire length of the tight junctions.

ion permeation and are penetrated by La^{3+} . The low junctional resistance results in low transepithelial resistance and p.d. values. In addition, these epithelia have high hydraulic conductivities and large active solute-coupled water fluxes. In the second group, junctional complexes have high resistance, and no penetration of La^{3+} is detected. Transepithelial resistance and p.d. are essentially a function of the ionic fluxes across the cell membranes. Furthermore, hydraulic conductivities and active solute-coupled water fluxes of these epithelia are low. Although the division is still based on a limited number of examples, an enlargement of the list will decide whether or not salt-transporting epithelia can be separated into two main groups according to the properties of their junctional complexes.

Two other questions arise from the presence of epithelia with leaky junctional complexes. Since in a suspension of $\text{La}(\text{OH})_3$ a certain amount of the lanthanum is present in ionic form (Moeller, 1963; Sillen and Martell, 1964), the first question is whether the same results can be obtained by equilibrating tissues with both ionic and colloidal lanthanum. The answer seems to depend on the method of fixation.

In tissues in which colloidal lanthanum was added during fixation, high resolution electron micrographs of tight junctions (between endothelial and epithelial cells of the mouse brain: Brightman and Reese, 1969; between mouse liver cells: Goodenough and Revel, 1970) which are very similar in appearance to those in the gallbladder (Tormey, quoted by Barry et al., 1971) have given no evidence that these junctions are permeable to colloidal lanthanum. Overton (1968) obtained similar negative results in a study of mouse small intestine. However, when colloidal lanthanum was injected into the common bile duct of living animals, Schatzki (1971) found ^{140}La moving across the wall of the biliary canaliculi and in some cases electron microscopic evidence (Schatzki, 1969, 1971) suggesting the passage of

Lumen (*lu*). Section fixed in glutaraldehyde only, without postosmication. Unstained. $\times 100,000$.

FIGURE 10 Oblique section through the apical portion of La^{3+} -treated (mucosal solution only) ileal cells. Coarse deposits of lanthanum are present between the microvilli of the brush border of the cells (*bb*) and within the obliquely cut tight junctions (arrows). Fixed in glutaraldehyde only, without postosmication. Unstained. $\times 11,000$.

lanthanum through tight junctions. In sections obtained from tissue that had been exposed to the tracer and glutaraldehyde simultaneously, such evidence was almost uniformly absent. The implication from these studies is that ionic and colloidal lanthanum may give similar results if the tissues are incubated with the tracer before fixation.

The second question concerns the relationship between high hydraulic conductivity and leaky junctional complexes. Although it is not yet possible to determine with precision the proportion of the total water flow that passes between the cells (Bentzel et al., 1969), it must be pointed out that, to obtain an isotonic transported fluid, part of the water flow must be across the cells and lateral cell membranes into the lateral spaces (see Diamond and Bossert, 1967, for discussion of standing osmotic gradients in the lateral intercellular spaces).

Finally, we would like to stress that these considerations are only guesses based on our knowledge of the chemical properties of lanthanum, since the nature of the process by which the lanthanum deposits are formed is still obscure. We selected this method because it had been previously found (Martinez-Palomo et al., 1971) that lanthanum added in ionic form to unfixed tissues penetrated consistently into regions in which no reproducible penetration with the "colloidal" lanthanum technique could be detected.

Furthermore, since during exposure to 1 mM La^{3+} none of the lanthanum is in the insoluble $\text{La}(\text{OH})_3$ form, we believe that our results are due to the movements of ionic lanthanum. The deposits observed with the electron microscope could be formed by being bound by some material within the junctional complex and the lateral intercellular spaces. Indeed, the fact that extended (1 hr) incubation in lanthanum (present study) and thorium (Machen and Diamond, 1972) solutions alters in a poorly reversible manner the passive ion permeability characteristics of the gallbladder's tight junctions suggests some form of irreversible combination between these polyvalent cations and the junction. This suggestion could also explain, at least in part, the low value of the lanthanum flux across the gallbladder, since an important fraction of the lanthanum passing through the junctional complex could be trapped within the tissue and never appear in the serosal solution.

These difficulties in understanding the process by which lanthanum penetrates the tight junctions of some tissues do not, however, alter the main conclusion of this investigation, i.e., that the tracer penetrates the junctions of epithelia that by physiological criteria appear to be leaky while it does not penetrate the junctions of epithelia that seem to be tight.

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Note Added in Proof. After submitting our manuscript, two publications have appeared with a direct bearing on the subject discussed here. Frömter and Diamond (*Nature (Paris)*, 235:9, 1972) based on measurements of the electrical properties of *Necturus* gallbladders and on a survey of the literature have proposed, as we do here, a division of epithelia into two types depending on the tightness of their junctional complexes. Also in agreement with our conclusion are the observations of Whitembury and Rawlins (*Pfluegers Arch. Eur. J. Physiol.* 330:302, 1971) on proximal tubules of toad kidney. They have also used ionic lanthanum as a tracer and found that it penetrated through the junctional complex, as expected for a "leaky" junction.

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