J. exp. Biol. **106**, 217–229 (1983)
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FUNCTIONAL PROPERTIES OF THE PARACELLULAR PATHWAY IN SOME LEAKY EPITHELIA

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SUMMARY

We here review the functional properties of the paracellular pathway of leaky epithelia such as gallbladder and renal proximal tubule. These epithelia are characterized by leaky terminal bars between adjacent cells which allow small ions, non-electrolytes and water to leak from lumen to interstitial fluid or back. In the past 10 years a great deal of information has been obtained about the properties of the misnamed 'tight' junctions in the terminal bars, by assuming that the overall permeation pattern reflected predominantly the junctional permeation properties. Although recent trans- and intraepithelial impedance analyses indicate that this assumption is not always justified (the contribution of the lateral intercellular space to the paracellular shunt resistance is not negligible, when the spaces are collapsed) it seems that the major conclusions are correct. The properties of the terminal junctions may thus be summarized as follows. (1) Large molecules such as horseradish peroxidase are not able to pass. (2) Passage of lipophilic substances is insignificant, as these substances permeate by the cellular route. (3) Depending on the tissue, ion permeation is either governed by channels with negative fixed charges, or positive fixed charges, or both. As inferred from ion selectivity patterns the channels of different epithelia are either wide and highly hydrated or narrow and poorly hydrated, thus allowing more or less water molecules to pass besides the ions. In narrow channels single-file diffusion may occur. (4) Besides the selective channels a free solution shunt seems to be present in some epithelia. (5) When applied in millimolar concentrations 2,4,6-triaminopyrimidinium and amiloride block negatively charged junctional channels. However these substances do not simply turn leaky epithelia into tight epithelia, because they have additional effects on the cell membranes. (6) As observed in cell cultures, formation of tight junctions requires connecting particles to be present on the cell surface – which seems to be controlled by the cytoskeleton – and requires the presence of calcium ions as ligands. (7) Cellular control over paracellular permeability may be exerted through changes of intracellular calcium concentration.

INTRODUCTION

Although terminal bars had been earlier recognized as the site of close cell to cell attachment in epithelia, the question of their functional role was first asked by Bonnet

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Key words: Leaky epithelia, tight junctions, junctional permeation.

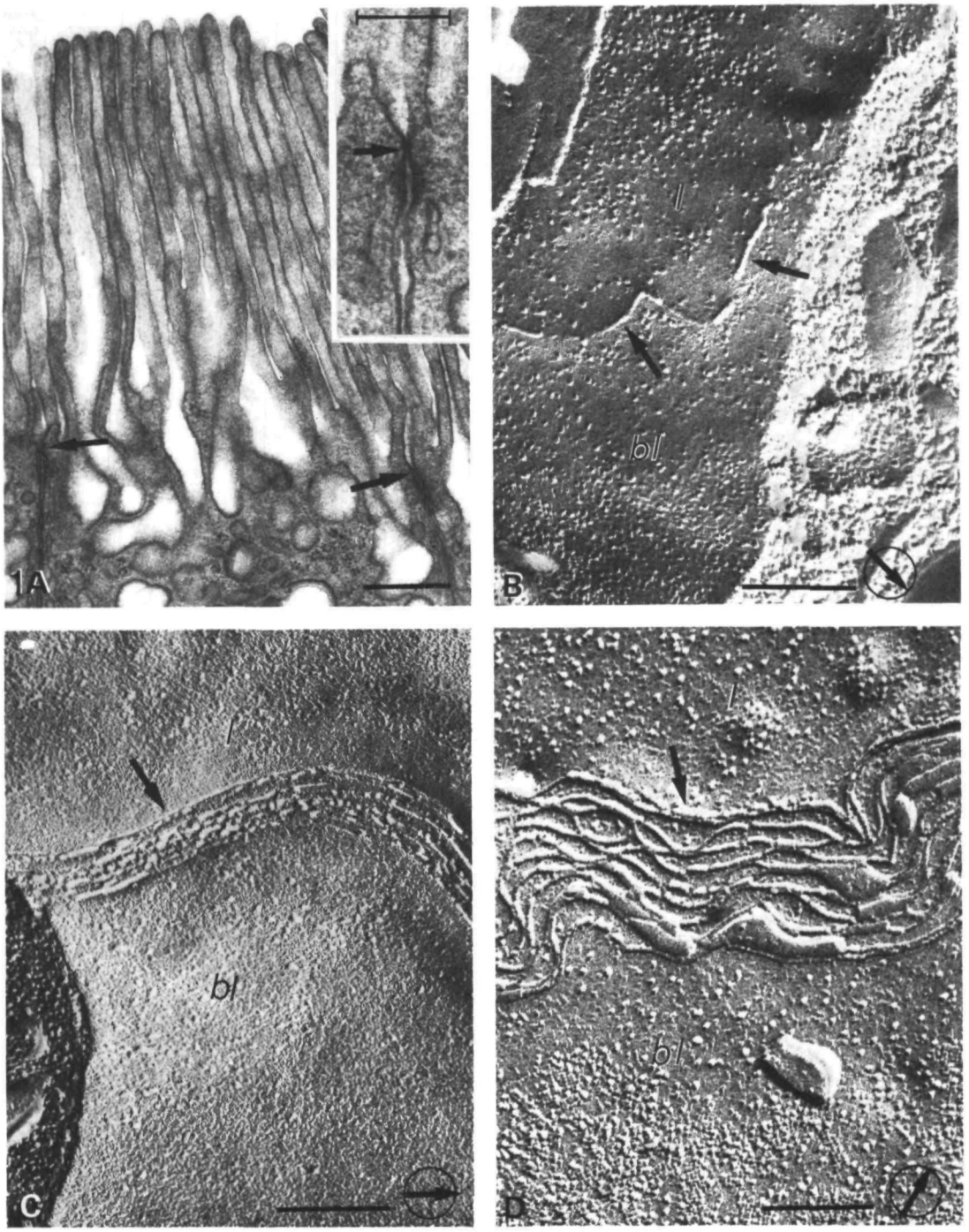
(1895). From studies on various gastrointestinal epithelia of an executed man inferred that such terminal bars might be present in all epithelia, but was cautious not to infer anything about their function. He simply writes that the role which these bars play in osmotic processes requires further intensive studies. The first systematic study with the electron microscope appeared almost 70 years later (Farquhar & Palade, 1963). The authors observed that the cell membranes of adjacent cells apparently fuse over longer distances in the region of the terminal bars and – from studies with an electronmicroscopic tracer – came to the conclusion that these bars constitute a tight seal between the apical and basal surface of epithelia. This lead to the now widespread use of the term 'tight junction'. Simultaneously, however it was observed by physiologists (Ussing & Windhager, 1964; Clarkson, 1967; Hoshi & Sakai, 1967; Windhager, Boulpaep & Giebisch, 1967) that a shunt path existed in various epithelia. Although the anatomical nature of the shunt path (gaps from cell shedding, 'tight junctions', different cell types, edge leaks of the preparations) remained initially unclear, it was soon shown by the use of ionic lanthanum as an electronmicroscopic tracer (Whittembury & Rawlins, 1971; Machen, Erlij & Wooding, 1972) and by voltage scanning with a microelectrode (Frömter, 1972) that in some epithelia the 'tight junctions' were indeed leaky, providing a highly conductive paracellular shunt between apical and basal fluid compartments. This led to the distinction between tight and leaky epithelia (Frömter & Diamond, 1972).

Since that time a decade of intensive investigations has followed, in which the structure and function of tight and leaky 'tight junctions' have been studied. In this review we try to summarize the major functional aspects which have emerged. Unfortunately space does not permit us to refer to every detail and to every publication, for which we ask pardon. To avoid confusion between the words 'tight' and 'leaky' we shall as a rule use the words *terminal junctions*.

MORPHOLOGY AND GENERAL ASPECTS OF JUNCTIONAL PERMEATION

Although space does not allow us to review the results of electronmicroscopic studies in detail, we must at least mention that cell-to-cell attachment in the junction is achieved by strands of intramembraneous fibrils or rows of particles of around 10 nm width (Staehelin, Mukhergee & Williams, 1969; Chalcroft & Bullivant, 1970; Goodenough & Revel, 1970; Wade & Karnovsky, 1974; Staehelin, 1974; see also Fig. 1) which may be interrupted by small gaps of similar width. It is generally observed that the number of strands correlates with junctional tightness (Claude & Goodenough, 1973; see also Fig. 1). However exceptions have been reported (Martinez-Palomo & Erlij, 1975). A conclusive analysis of this correlation will require additional and more precise information.

Fig. 1. Terminal bars of rat renal tubular epithelia. (A) Proximal tubule; cross section of cell apex with microvilli and terminal bars (arrows). Inset shows terminal junction (arrow) at higher magnification. (B) Proximal tubule; freeze fracture image of a cleaved terminal bar which consists of a single interrupted strand. *l*, lumen compartment; *bl*, lateral space. (C) Cortical thick ascending limb and (D) collecting duct also show freeze fracture images of the respective terminal bars. The circled arrow indicates direction of shadowing. Note increasing width and increasing number of strands from (B) to (D). (Original photograph from Dr W. Haase, Max-Planck-Institut für Biophysik, Frankfurt, F.R.G.). Calibration bars = 0·5 μm in A and 0·2 μm in B, C and D, and in inset to A.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Electronmicroscopic observations allow two conclusions to be drawn about the mechanisms of junctional permeation:

(1) Permeation depends on molecular size and on the properties of the individual junction. Bulky tracer molecules such as haemoglobin (Farquhar & Palade, 1963) or horseradish peroxidase (Bentzel, Parsa & Hare, 1969) do not pass the junctions of any epithelia under control conditions, while smaller molecules such as ionic La^{3+} do pass in leaky epithelia such as renal proximal tubule, gallbladder and small intestine, but not in tight epithelia such as frog skin or toad bladder (Whittembury & Rawlins, 1971; Machen *et al.* 1972).

(2) Since the molecules do not cross a lipid bilayer on their route across the terminal junctions one may predict that junctional permeation differs from that of the cell membrane insofar as lipophilic substances may be expected to bypass the junctions. This fits with experimental observations (see for example Moreno, 1975*b*).

Regarding ion permeation, no specific predictions can be made from electronmicroscopic studies. The fact that La^{3+} does not pass the terminal junctions of tight epithelia may either indicate that these junctions are completely tight for all ions or just for cations, or just for La^{3+}. One electrophysiological study on rabbit urinary bladder indicates that its junctions may act as virtually perfect seals with resistances of 300 $k\Omega cm^2$ or more (Lewis, Eaton & Diamond, 1976) while other studies on other tight epithelia report 10 to 100 times lower junctional resistance values (Ussing & Windhager, 1964; O'Neil & Helman, 1976; Saito, Lief & Essig, 1974; Reuss & Finn, 1974), which may in part reflect sealing problems of the preparation inside the chamber (Helman & Miller, 1971) or inadequate analysis techniques. Space does not permit us to discuss this point further here.

PERMEATION PROPERTIES OF LEAKY TERMINAL JUNCTIONS

To study junctional permeation requires techniques which allow us to quantify fluxes or current flows across the junctions and to separate them precisely from transcellular fluxes and currents. Strictly speaking such techniques – insofar as a precise distinction between both components is concerned – are not available today or have not been available until recently. Most approaches which have been used in the past have considerable shortcomings or rely on unproven assumptions, so that the results must be considered with caution. Space does not permit us to elaborate this point here in detail. Reports in the literature about junctional permeabilities in leaky epithelia rely almost exclusively on the electrophysiological finding (which in itself is only of qualitative significance) that the paracellular conductance is one or two orders of magnitude greater than the cellular conductance in newt and *Necturus* proximal tubule (Hoshi & Sakai, 1967; Windhager *et al.* 1967; Guggino, Windhager, Boulpaep & Giebisch, 1982), in *Necturus* gallbladder (Frömter, 1972; Reuss & Finn, 1975) and in the renal proximal tubule (Frömter, 1977*a*). On the basis of these findings it is usually assumed that cellular transport can be neglected and transepithelial potential and resistance data are interpreted as reflecting exclusively junctional permeabilities. Although this approach is more-or-less correct under control conditions, there can be no doubt that it leads to serious troubles in three cases. (1) When the experimental conditions are associated with large changes of cell membrane conductances – during

replacement of Na^+ by K^+ the cell membrane conductances can increase by one order of magnitude (G. Kottra and E. Frömter, unpublished observations). (2) When the lateral space collapses (Frömter, 1972; Bindslev, Tormey & Wright, 1974; Lim, Kottra, Kampmann & Frömter, 1983) and the resistance of the space can become equal to or greater than the resistance of the terminal junctions. (3) When the preparation is not tightly sealed inside the chamber. The latter point is less problematic in leaky than in tight epithelia but may play an important role even in gallbladder (G. Kottra, L. G. M. Gordon and E. Frömter, unpublished observations, see also below).

We now summarize what is known about junctional permeation properties in various leaky epithelia.

Gallbladder

The most extensive studies were performed by Diamond and collaborators on rabbit and frog gallbladder. They are summarized in a review paper by Moreno & Diamond (1975*a*). Although some of their conclusions must be considered with some reservation, since we know that it is not always possible to extrapolate from transepithelial to junctional properties (see above and Fig. 2 below), we assume that these studies yield a quite reliable picture of the permeation properties of the terminal junctions of gallbladder. In their experiments Diamond and collaborators found evidence for two distinct pathways: a cation-selective pathway and a non-selective shunt pathway (which allows, for example, Cl^- to pass together with some Na^+ and other substances).

The cation selective pathway

From ion substitution experiments (involving measurements of conductances, dilution potentials and biionic potentials and their concentration and temperature dependence) it was initially concluded that this pathway acted as a long pore in a membrane, which was thick enough for electroneutrality to hold, and which was lined by neutral fixed sites (such as C = O groups). Later, however, when the electronmicroscopists showed that the actual permeation barrier in the junctions was very short (possibly between 5 and 10 nm, i.e. the diameter of an individual strand or fibril in the junction) the model of a negatively-charged, short pore was preferred, in which electroneutrality does not hold. This model can also explain the observed concentration dependence of the transference numbers and of the conductance and, in addition, accounts for the fact that the cation selective pathway can be blocked by positively charged ions [such as H^+, Ca^{2+}, La^{3+} or Th^{4+} and by the organic cation 2,4,6-triaminopyrimidinium (TAP) which may be expected to bind to fixed negative charges on the pore and thus obliterate it]. From proton-titration curves it was concluded that the fixed charges have a pK of 4·5 (in both rabbit and frog) and hence may be either COO^- or PO_4^- groups. Furthermore, it was concluded that the pores also contain NH_2 groups which become protonated, if the pH is lowered to below pH 3, under which condition the pores become Cl-selective. From studies with nitrogenous cations it was, in addition, concluded that each pore contains at least four proton-acceptor sites which discriminate more sharply among proton donors than does water. From the latter studies an effective pore diameter was also calculated (0·88 nm in rabbit and 1·62 nm in frog gallbladder). Such pores would allow three, or more, wa

[redacted]ecules to be present beside a small cation within a given cross section of the pore (Moreno & Diamond, 1975*b*). This fits with the concept that the cation pathway is well hydrated and not an extremely discriminative pathway, as are the Na^+ or K^+ channels in nerve and muscle membranes. This conclusion is also supported by a comparison of the ion selectivity sequences (Moreno & Diamond, 1975*b*). Frog and rabbit gallbladder exhibit Eisenman's sequence II ($Rb^+ > Cs^+ > K^+ > Na^+ > Li^+$) and IV ($K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$) respectively, while for comparison squid axon Na^+ channel (Meves & Chandler, 1965) exhibits sequence XI ($Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$). (Note that sequence I is the sequence of free solution mobilities while sequence XI is that of the crystal radii, Eisenman, 1961.)

This picture of the cation selective pathway was recently extended by Salas & Moreno (1982) who re-evaluated the concentration dependence of the partial conductance of the alkali cations and compared the latter with unidirectional tracer fluxes in toad gallbladder. Contrary to previous investigators, they concluded that the cation-selective pores exhibit single-file diffusion. This phenomenon is typical of pores which accommodate two or more ions inside the channel, where they cannot bypass each other (Hodgkin & Keynes, 1955). According to Salas & Moreno (1982) the cation channel should have two internal binding sites for cations. Comparing this data with other single-filing channels, such as the gramicidin A channel (which has a pore diameter of 0·4 nm), it is difficult to envisage, however, why the pores of toad gallbladder tight junctions, which are supposedly four times wider, should not allow two cations to bypass each other. Moreover, some criticism can be raised against Salas & Moreno's data, since most of their experiments were performed in the presence of high K^+ concentrations, which, according to our experience, leads to a collapse of the lateral spaces with increasing resistance of the paracellular pathway and to a drastic decrease of the cell membrane resistances (G. Kottra and E. Frömter, unpublished observations). It is thus questionable whether the observed flux and conductance phenomena can be related to the terminal junction alone. This point needs further investigations with more appropriate techniques.

Attempts to identify terminal junction pores of *Necturus* and frog gallbladder by fluctuation analysis (noise analysis) have not yet provided insight into the permeation mechanism. Gögelein & van Driessche (1981) observed a low frequency ($f < 10$ Hz) noise component, the spectral density of which exhibited a $1/f^2$ pattern but had no Lorentzian component. It increased in the presence of ion gradients across the bladder but was practically independent of the nature of the diffusing ions and disappeared in the presence of TAP. These observations suggest that this noise component arose from ion diffusion across the relatively poorly selective terminal junctions, which may have permanently open pores.

The free-solution shunt

The observation that protonation of the membrane, down to pH 3, reduces the Na^+ conductance to close to zero, without increasing the Cl^- conductance, suggests that Cl^- ions cannot pass the cation-selective pathway in the terminal junctions of rabbit and frog gallbladder. (They may do so, however, if the pH is further decreased, see above.) However, in most gallbladder preparations, in both species, a significant Cl^- [redacted]nductance is observed which must thus follow a different route. As observed by

Diamond and collaborators (see Moreno & Diamond, 1975*a*) the Cl^- conductance amounts to 7·5 % 'or less' of the total transepithelial conductance, but it may be as high as 25 %. This conductance has the properties of a free solution shunt. Its temperature dependence equals that of the Cl^- mobility in free solution. The selectivity to bulky anions also resembles the respective pattern of free-solution mobilities. However, it is uncertain whether this free-solution shunt Cl^- conductance is of any physiological significance. The fact that it was observed to increase with duration of the experiments (particularly in phosphate-free solutions) suggests that it represents possibly an artifactual leak-pathway, resulting either from disintegration of junctions, from damaged cells or from leaks at the chamber edges. Unfortunately no attempts were made to separate leak contributions from physiological shunts in any of the experiments. We have demonstrated that conventional preparations of *Necturus* gallbladder can have considerable edge leaks (G. Kottra, L. G. M. Gordon and E. Frömter, unpublished observations). It would thus seem as likely that the gaps between tight junction particles, which have been recognized in the freeze fracture replicas of the terminal bars (see above), act as internal free-solution shunts. As shown by Moreno (1975*b*) besides Cl^- (and by inference also some Na^+) the free-solution shunt also allows sucrose to pass. Although the above description suggests that water molecules should also be able to pass through the junctional route, it seems that the water permeability of the cell membranes is so high (Persson & Spring, 1982) that the contribution of the junctions to transepithelial water flow is negligible (van Os & Slegers, 1973; Moreno, 1975*b*; Frederiksen, Møllgård & Rostgaard, 1979).

Renal proximal tubule

The ion permeation properties of the terminal junctions of other leaky epithelia are less well known today than those of gallbladder. However, there is enough convincing evidence available to indicate that different leaky epithelia do not have identical junctional properties and that the properties may even change in a given tissue as a function of anatomical location. This is best documented by the data for the renal proximal tubule. On the one hand a number of observations were made which resemble those in gallbladder. Measurements of dilution potentials and biionic potentials on surface tubules of dog and rat kidney (Boulpaep & Seely, 1971; Frömter, Müller & Wick, 1971) showed a small cation over anion selectivity with Na^+/Cl^--permeability ratios of 1·38: 1 and 1·56: 1 respectively (against 12: 1 or more in gallbladder). There is also a small but significant discrimination among alkali cations which obeyed sequence IV of Eisenman as in gallbladder (E. Frömter, unpublished observations) and evidence for the involvement of a small net concentration of negative fixed charges (between 2 and 5 mmol l^{-1} of pore fluid) was obtained, which was supported by inhibition studies with Ca^{2+} and with low pH (Frömter, 1977*b*). These results suggest that the proximal tubules contain a similar cation selective pathway to gallbladder but, in addition, have a much larger free solution shunt. This idea would fit with the freeze fracture images of proximal tubule terminal bars, which show single or double connecting strands that are frequently interrupted by small gaps (see Fig. 1). On the other hand, it has been observed that anion selectivities do not exactly follow the free-solution mobilities (compare Frömter, *et al.* 1971 and Boulpaep & Seely, 1971). Although this could still be accounted for by postulati

But some extra restriction occurs with the bulkier anions, the idea of a simple free-solution shunt breaks down if one considers that proximal tubules of juxta-medullary origin, have anion selective junctions (Kawamura, Imai, Seldin & Kokko, 1975) with Na^+/Cl^- permeability ratios that are often smaller than the free-solution mobility ratio (Warnock & Yee, 1982).

A similar or even more pronounced case of $P_{Na} < P_{Cl}$ is found in *Necturus* renal proximal tubule, where the permeability ratio P_{Na}: P_{Cl} is 1:7 (Edelman & Anagnostopoulos, 1978). The junctions of *Necturus* proximal tubule have a distinct anion selectivity pattern, which clearly differs from the pattern of free solution mobilities (Anagnostopoulos, 1975). The selectivity sequence of the halides is sequence II or III of Eisenman. This implies that the binding sites are more selective for the halides than for water. (Note that sequence I is the sequence of free solution mobilities and sequence VII that of the crystal radii.) At present, however it is not possible to decide whether these binding sites represent positive fixed charges or not. Regarding the question of tracer flux coupling or single-filing, no evidence for a significant discrepancy between the tracer permeabilities and the respective partial conductances was found (Frömter, Rumrich & Ullrich, 1973). Noise analysis has not yet been performed on kidney tubules.

In summary, we conclude that – depending on species or anatomical location – the terminal junction in proximal tubules may be either slightly cation or slightly anion selective. The channels which determine this property may bear either a small number of negative or of positive fixed charges. They are certainly highly hydrated and thus impose properties which are not very different from a free-solution shunt. However, important questions remain. Are we dealing with a single pore or with a population of different pores? What are their structure and their effective diameters? Earlier estimates of pore diameters between 0·64 and 1·06 nm (Baumann, Frömter & Ullrich, 1967) were based on reflection coefficients of a limited number of non-electrolytes. Their interpretation in terms of the sieving theory of Goldstein & Solomon (1960) however requires re-evaluation, both from a theoretical and an experimental point of view, because one is probably dealing with a mixed population of pores which do not have cylindrical shapes (Bentzel & Reczek, 1978). This also raises the important question of the water permeability of the terminal junctions in renal proximal tubules which cannot be answered with certainty at present. However, the presence of a significant solvent drag effect on NaCl movement, which we have observed previously (Frömter *et al.* 1973), would favour a higher junctional water permeability, at least in rat proximal tubules, although opposite views have also been expressed (see for example Rector & Berry, 1982).

Other leaky epithelia

In other leaky epithelia (such as other segments of the kidney, the intestine, the choroid plexus or the corneal endothelium) the information on tight junction properties is rather scanty. Generally it is found that junctional permeation is governed by a similar type of cation-selective channel as described by Diamond and collaborators (see above) for gallbladder. This is true for small intestine (Frizzell & Schultz, 1972; Munck & Schultz, 1974), choroid plexus (Wright, 1972) and, to some extent, also for the cortical thick ascending limb of the renal nephron (Greger, 1981). The latter case,

however, is peculiar since its alkali cation permeability sequence falls in betw[illegible] Eisenman's sequences VI and VIII or IX, while all other leaky epithelia mentioned above exhibit only sequence II to IV. This difference means that the channel must be considerably less hydrated in the cortical thick ascending limb than it is in the other epithelia, a conclusion that fits precisely with the fact that the cortical thick ascending limb has a very low water permeability. Such a finding would imply that not only the cell membranes, but also the tight junctions are relatively tight to water in this tissue. This implies that the terminal junctions of the other leaky epithelia may have some significant water permeability. Another interesting feature of the terminal junctions in cortical thick ascending limb is that they exhibit single-filing (Andreoli & Hebert, 1982). This would be understandable if the channel was so narrow as to restrict even the entry of water molecules. Single-filing was also reported in the terminal junctions of rabbit colon (Fromm & Schultz, 1981), which seem to discriminate better between Na^+ and K^+ than any other junctions so far studied and may therefore also be more narrow.

ALTERATION OF JUNCTIONAL PERMEABILITY

Opening

In tight epithelia it has long been known that the terminal junctions can be disrupted under the influence of hypertonic solutions on the contravascular surface. This leads to increasing anion and cation conductances and non-electrolyte permeabilities (Ussing & Windhager, 1964; Ussing, 1966). The mechanism of disruption is not well understood. It is often associated with blistering within the terminal bars (DiBona, 1972). This suggests that there is a development of high pressure areas in the junctional slits, but the occurrence of blistering depends also on the chemical nature of the osmotic stimuli (Martinez-Palomo & Erlij, 1975).

Widening of the terminal junction and increased passage of La^{3+} was also observed in response to luminal hypertonicity in renal proximal tubules (Rawlins, Gonzáles, Pérez-Gonzáles & Whittembury, 1975). In addition, it seems that small hydrostatic pressure changes, such as those encountered in volume expansion (saline diuresis) or ureteral or renal venous occlusion, change the permeability of the paracellular pathway to ions and small non-electrolytes (Boulpaep, 1972). These changes seem to be associated with widening of the terminal junctions in renal proximal tubules (Bentzel, 1972; Bulger, Lorentz, Colindres & Gottschalk, 1974).

A predominant role in junctional sealing seems to be played by Ca^{2+} ions. Prolonged incubation in calcium-free media and chelation of Ca^{2+} by EGTA have been

Fig. 2. Impedance analysis of *Necturus* gallbladder. Effect of amiloride. Abscissa and ordinate are real and negative imaginary components of the impedance in Ωcm^2. Symbols ×× and ▶▶ denote measured transepithelial low and high frequency data points and ◇◇ and ++ the respective intraepithelial data points. The continuous lines are the transepithelial and apparent intraepithelial impedance of a distributed model equivalent circuit obtained by least square fitting. The best fit parameters are listed at the top. RA, RBL, RJ and RLIS are resistances of the apical and basolateral cell membrane, the terminal junction and the lateral intercellular space in Ωcm^2 and CA and CBL are respective capacitances in $\mu F\,cm^{-2}$. The data were obtained at various times after addition of 10^{-3} mol l^{-1} of amiloride to the lumen compartment. Note that the approximate doubling of transepithelial d.c. resistance from ~98 to ~155 Ωcm^2 reflects contributions from different resistance barriers (G. Kottra and E. Frömter, unpublished observations).

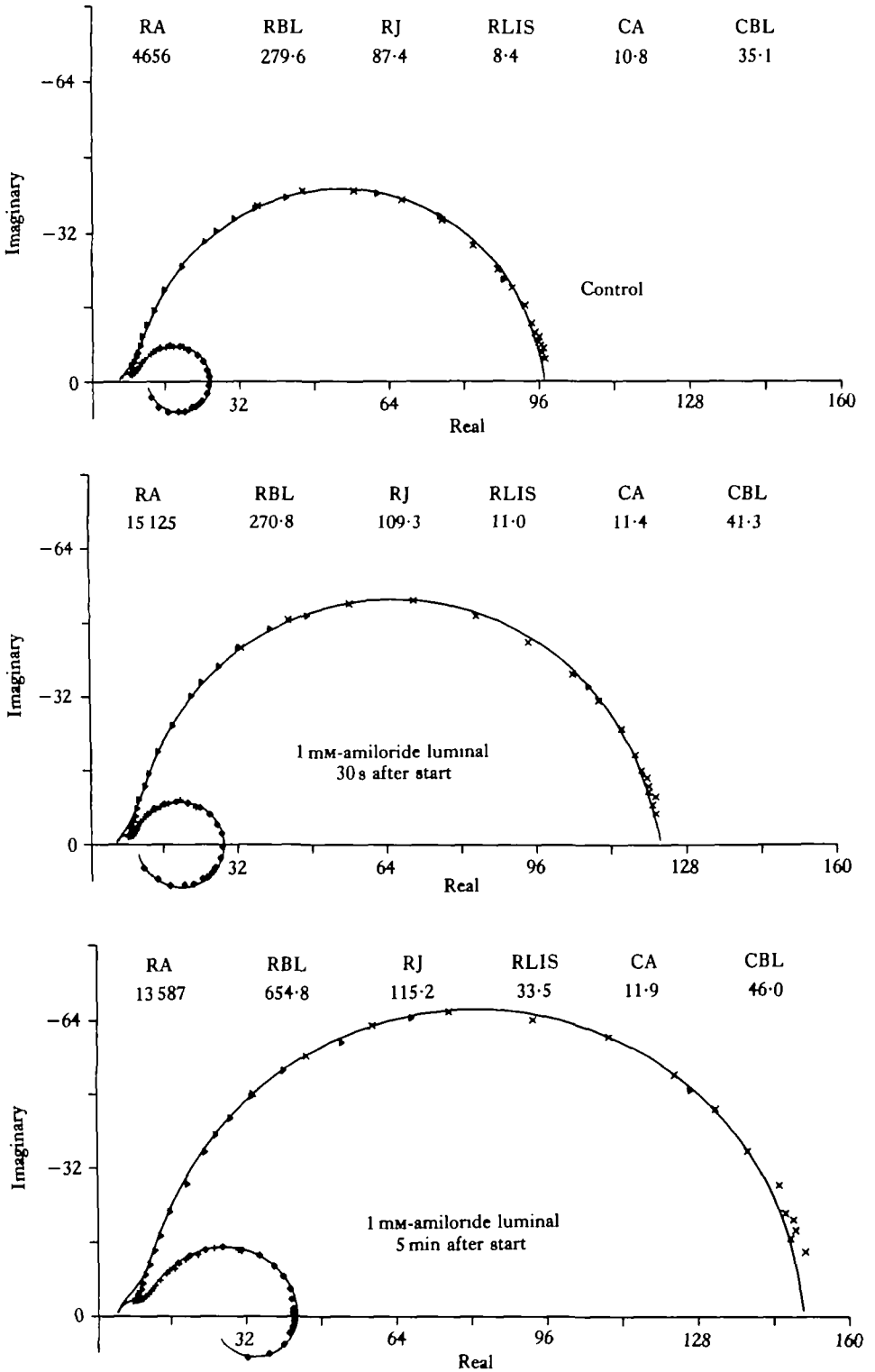
RA
4656
RBL
279·6
RJ
87·4
RLIS
8·4
CA
10·8
CBL
35·1
Control
RA
15 125
RBL
270·8
RJ
109·3
RLIS
11·0
CA
11·4
CBL
41·3
1 mM-amiloride luminal
30 s after start
RA
13 587
RBL
654·8
RJ
115·2
RLIS
33·5
CA
11·9
CBL
46·0
1 mM-amiloride luminal
5 min after start
Imaginary
Real
−64
−32
0
32
64
96
128
160

Fig. 2

reported to split terminal junctions in natural epithelia (Sedar & Forte, 1964; Hay & Singer & Malamed, 1965) and in artificial epithelia grown from cell cultures (Martinez-Palomo, Meza, Beaty & Cereijido, 1980).

The early phase of junctional opening is not associated with striking changes in the pattern of junctional strands. It is readily reversible, upon re-addition of Ca^{2+} to the bathing media, without the need for *de novo* protein synthesis. Re-sealing is obviously an extracellular surface reaction in which Ca^{2+} ions act as ligands between negative fixed charges on the adjacent cell membranes.

On the other hand it seems also that *intra*cellular calcium is important for junctional tightness. This follows from the observation of Martinez-Palomo *et al.* (1980) that the terminal junctions of cultured epithelia open after application of the calcium ionophore A 23187, which presumably increases intracellular Ca^{2+} concentration. This effect could be related to changes in anchoring of the connecting particles to the cytoskeleton. It could also explain reports of increasing junctional leakiness after addition of neurotransmitters such as adrenaline (Peaker & Taylor, 1975) or acetylcholine (Jansen, Fleuron-Jakobs, DePont & Bonting, 1980) or of cAMP (Lorentz, 1974; Jacobson, 1979) or theophylline (Mandel, 1975) to various tissues.

Blockage

As mentioned above, high extracellular concentrations of Ca^{2+} ions may partially block the ion permeation across the cation selective channel in gallbladder terminal junctions. The same is true for H^+ ions. 2,4,6-Triaminopyrimidinium (TAP) is, however, a more convenient blocker (Moreno, 1975*a*). Since only the protonated form is active, the drug must be applied at pH 6 where a concentration of 19 mmol l^{-1} (of the protonated form) inhibits cation conductance by 92 % with Michaelis-Menten type saturation kinetics and a half inhibition constant of 2·6 mmol l^{-1}. Besides TAP, amiloride (the specific inhibitor of Na^+ channels in frog skin) has also been reported to block the terminal junctions of *Necturus* and frog gallbladder (Schifferdecker, 1977; Balaban, Mandel & Benos, 1979). In both preparations, concentrations of 3×10^{-3} M increased the transepithelial resistance, slowly but reversibly, by about twofold. In frog gallbladder it was shown that amiloride inhibited the partial Na^+ conductance, again with Michaelis-Menten type kinetics, with a half inhibition constant of 1 mmol l^{-1}. However, amiloride does not only block the terminal junctions. We have recently observed (using impedance analysis with intra- and transepithelial recording of the potential response to transepithelial alternating current signals) that amiloride has four effects (G. Kottra and E. Frömter, unpublished observations). It increases the resistance of (1) the terminal junctions, (2) the apical cell membrane, (3) the basal cell membrane and (4) the lateral intercellular space. As shown in Fig. 2 effects (1) and (2) occur rapidly, while (3) and (4) occur only slowly. The two latter effects are probably secondary responses to inhibition of transepithelial NaCl transport.

Besides TAP and amiloride, a number of plant cytokinins and other microfilament-active substances (such as cytochalasin B and phalloidin) have been reported to increase transepithelial resistance, presumably through a partial blockage of cation channels in the terminal junctions (Bentzel *et al.* 1980), and a similar effect was reported for an anionic dye (Frederiksen *et al.* 1979).

CONCLUSION

We have reviewed and summarized the functional properties of the terminal junctions of leaky epithelia. Many of these points are convincingly documented, but we have also discussed some new findings which call for repetition of some experiments with improved techniques. This applies mainly to some transepithelial measurements of partial ion conductances, which have been interpreted as reflecting properties of the terminal junctions but may in fact at least partially arise from other resistive barriers, such as the cell membranes or the lateral intercellular space. Re-evaluation of such experiments with modern impedance analysis techniques (Lim *et al.* 1983) may be expected to yield more reliable information, to provide additional insight, and to help resolve previously observed inconsistencies. It is quite possible that the present picture of the functional properties of the terminal junctions may then require modification.

The authors thank Dr M. Frömter for reading and typing the manuscript.

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