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Morphological Factors Influencing Transepithelial Permeability: A Model for the Resistance of the *Zonula Occludens*

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Summary. Epithelial cells are joined at their apical surfaces by *zonulae occludentes.* Claude and Goodenough (1973) demonstrated a correlation between the structure of the *zonula occludens* as seen in freeze-fracture preparations and the passive electrical permeability of several simple epithelia. In epithelia with high transepithelial resistance, the *zonula occludens* consisted of many strands. In epithelia with low transepithelial resistance the *zonula occludens* was much reduced, sometimes consisting of only one strand.

Evidence is reviewed here that indicates that in a number of simple epithelia the structure of the *zonula occludens* is largely responsible for the magnitude of transepithelial conductance. An equation is derived relating transepithelial junctional resistance to the number of junctional strands: $R = R_{\min} p^{-n}$ where R is the transepithelial resistance of the *zonula occludens*, $R_{\min}$ is the minimum resistance of the junction (as when there are *no* strands in the zonula occludens), p is the probability a given strand is "open" and n is the number of strands in the junction. Using published experimental values of R and n for different epithelia, the calculated value of p was found to be as high as 0.4, which suggests that the strands in the *zonula occludens* are remarkably labile.

Other morphological parameters relevant to transepithelial permeability are also considered, such as the width and depth of the intercellular spaces, and the size of the epithelial cells themselves.

Epithelial cells are joined to each other at their apices by *zonulae occludentes* (tight junctions, occluding junctions, or limiting junctions) that act as seals isolating one face of the epithelium from the other. Simple epithelia vary widely in their passive transepithelial permeability, and it appears that this variation is at least in part a function of the properties of the *zonulae occludentes* that join the cells together (Frömter, 1972; Frömter & Diamond, 1972; Moreno & Diamond, 1974). Electron microscopic examination of different simple epithelia (Friend & Gilula, 1972; Machen, Erlij & Wooding, 1972; Claude & Goodenough, 1973; Pricam *et al.*, 1974; Staehelin, 1974), and of epithelia in different phys-

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iological states (Erlij & Martínez-Palomo, 1972; DiBona & Civan, 1973; Wade & Karnovsky, 1974; Rawlins *et al.*, 1975; Humbert *et al.*, 1976) have shown that transepithelial resistance can be correlated to the morphology of the *zonula occludens.*

In freeze-fracture preparations viewed in the electron microscope, the *zonula occludens* appears as a network of anastomosing strands (*see* Fig. 1) seen either as ridges on the *A* fracture face or as grooves on the *B* fracture face of the cell membrane. In a variety of simple epithelia under normal physiological conditions, Claude and Goodenough (1973) have demonstrated a correlation between the number of junctional strands in the *zonula occludens* and passive transepithelial electrical resistance. In "tight" epithelia with a high transepithelial resistance the *zonula occludens* is made up of many anastomosing strands. In "leaky" epithelia with a low transepithelial resistance the *zonula occludens* is much reduced, sometimes consisting of only one strand (*see* Table 1).

If these junctional strands behaved as resistances in series, there would be a linear relationship between the number of strands and passive resistance across the junction. Instead, the relationship appears to be exponential (*see* Fig. 4). In this paper a model is developed that relates transepithelial junctional resistance to the number of strands in the *zonula occludens.* It is postulated that the strands contain small regions, analagous to pores, that can be either open or closed to the passage of small ions. The passive resistance across the junction in any one region will be dependent on the probability that at any moment each

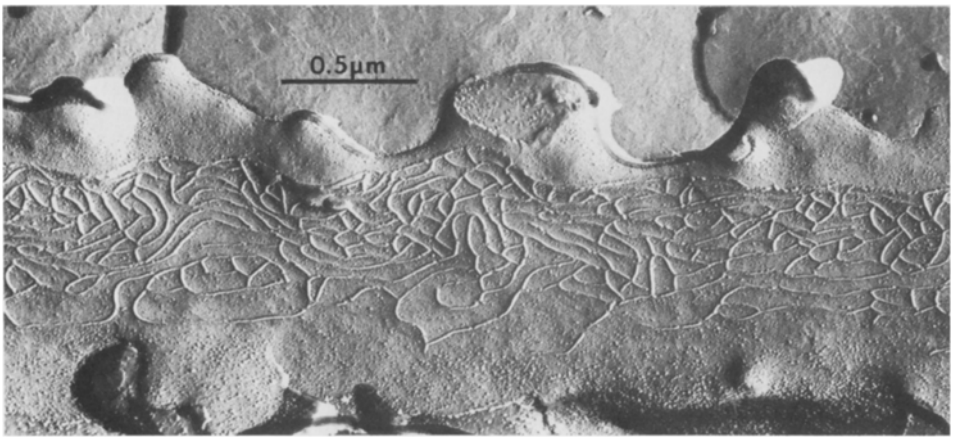

Fig. 1. A "tight" *zonula occludens* from frog urinary bladder as revealed by the freeze-fracture technique. The junction consists of a network of anastomosing strands, here seen as ridges on the *A* fracture face of the cell membrane. Magnification 40,000 ×

strand contains an open "pore" in that region. Other morphological parameters such as the the size of the cells themselves and the width and depth of the intercellular spaces are considered, and appear, at least in the cases described here, to be of secondary importance in determining overall paracellular transepithelial resistance.

Theoretical Considerations

A simple epithelium is diagrammed in Fig. 2, showing the spatial relationship of the elements that will be considered.

Figure 3 is simple circuit diagram describing the passive resistive elements in such a simple epithelium. Total transepithelial resistance (R_t) is represented by 2 resistances in parallel, the transcellular resistance (R_c) and the paracellular resistance (R_p). The passive resistance of the transcellular pathway (R_c) can be resolved into 2 resistances in series, that of the apical cell membrane (R_{m_a}) and that of the basal and lateral cell membranes (R_{m_b}) (Boulpaep, 1971). The passive resistance of the paracellular pathway (R_p) can also be divided into 2 resistances in series: the resistance of the junction itself (R_j) and the resistance of the intercellular cleft (R_i). Junctional resistance, R_j, will be compared to the structure of the junction as seen in freeze-fractured preparations.

In both tight and leaky epithelia that have been described, R_{m_a} and R_{m_b} are quite high. Estimates as high as several thousand Ωcm^2 have

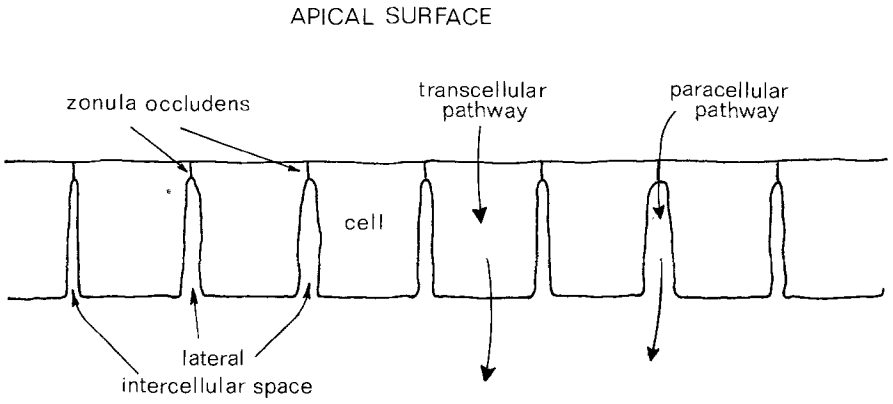

Fig. 2. A schematic diagram of a simple epithelium, showing transcellular and paracellular pathways for transepithelial current flow

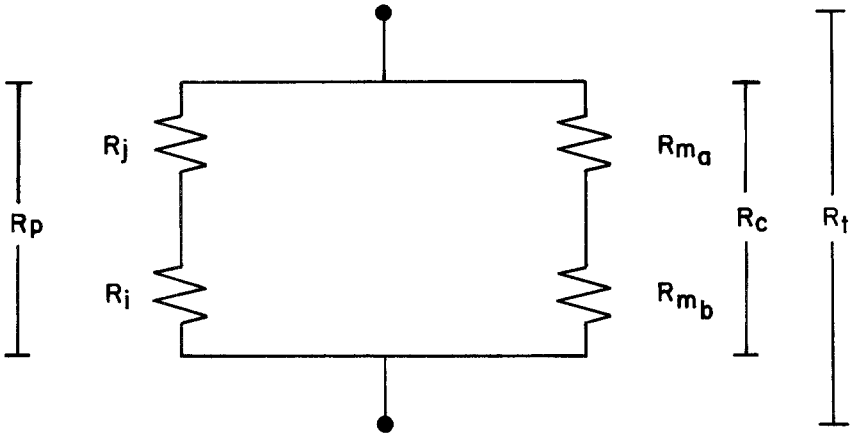

Fig. 3. Circuit diagram of a simple epithelium, showing the paracellular and the transcellular pathways in parallel, with the junctional and intercellular resistances in series in the paracellular pathway

been made, for example $3000\,\Omega cm^2$ for R_{m_a} and $4000\,\Omega cm^2$ for R_{m_b} (Boulpaep, 1971; Frömter, 1972). In many cases, R_t is anywhere from a factor of 2 or 3 to two orders of magnitude lower than either R_{m_a} or R_{m_b} and certainly much lower than R_{m_a} plus $R_{m_b}(R_c)$. (Examples of R_t are: mammalian proximal tubule, $6\,\Omega cm^2$ (Boulpaep & Seely, 1971); mammalian gallbladder, $30\,\Omega cm^2$ (Frömter & Diamond, 1972); and toad urinary bladder, $2000\,\Omega cm^2$ (Civan & Frazier, 1968). It is clear that, especially in the "leaky" epithelia, much of the transepithelial current must pass between the cells, through the comparatively low resistance paracellular pathway, R_p. For epithelia with different R_t's, assuming a fairly high R_c (say $7000\,\Omega cm^2$), one can estimate R_p using Ohm's law (*see* Table 1). As total transepithelial resistance increases, of course, the characteristics of the cell membranes themselves become more important in determining the overall characteristics of the epithelium. This is because a larger proportion of transepithelial current will flow through R_c.

Morphological Factors to be Considered

In order to relate R_p to the geometrical situation in the epithelia, one needs to know how much of the paracellular pathway is available per unit area of epithelium. There are a number of factors that can influence this.

Serosal-mucosal amplification: In tissues where the mucosa is thrown into rugae or villi, the area of the epithelial cell layer itself will be much greater than that measured on the serosal surface. An especially striking example of this is seen in the intestinal mucosa, where the amplification factor can be 10× or more (Frizzell & Schultz, 1972; Wilson, 1962). In these tissues one must correct for surface amplification in order to get a true value for R_t and therefore of R_p. This consideration is apparently not often taken into account (Frömter & Diamond, 1972; Martínez-Palomo & Erlij, 1975). Using capacitance as a measure of area may prove more satisfactory (*cf.* Lewis *et al.*, 1976).

Linear amount of paracellular element per unit area (l_p). The amount of current following the paracellular transepithelial pathway will depend not only on the specific resistance of that pathway, but also on how much of that pathway is available per unit area of epithelial surface. The linear amount of junction per cm^2 of epithelium (l_p) varies from tissue to tissue and depends on two factors. One is the size of the cells: the smaller the diameter of the cells in the epithelium, the larger l_p will be. For example, in a square centimeter of epithelium made of square cells 25 μm on a side, l_p would be 800 cm, while in an epithelium made up of square cells 5 μm on a side, l_p would be 4000 cm (*see* Table 1).

Another factor affecting l_p is the packing of the cells and the tortuosity of the intercellular junctions and interspaces. l_p will have its minimum value in an epithelium made up of smooth-sided, hexagonally packed cells, while in an epithelium made up of square cells l_p will be slightly (7%) larger. In an epithelium containing cells with wavy profiles that interdigitate with their neighbors, l_p will be even larger. Since data on the tortuosity of the junctions are unavailable for most of the epithelia under consideration, l_p will be estimated by assuming that all the cells are square.

The dimensions of the intercellular cleft. Assuming that the lateral spaces between the epithelial cells are filled with a solution with the same resistivity as the bulk solutions, and knowing l_p and the width and length of the interspaces, R_i can be calculated:

$$R_i = \rho L / \omega l_p \tag{1}$$

where ρ is the resistivity of the bulk solution, L is the height of the interspace, ω is the width of the interspace, and l_p the linear amount of paracellular element per cm^2 of epithelium (Claude, 1968). For example, in the case of *Necturus* proximal tubule where the cells are approximate-

ly 25 μm high ($L = 25\,\mu m$), the resistivity (ρ) of the bulk solution is approximately 100 Ωcm, l_p is approximately 800 cm/cm^2, and ω has an average value of 0.5 μm (Claude, 1968):

$$R_i = (100\,\Omega cm)\,(25 \times 10^{-4}\,cm)/(5 \times 10^{-5}\,cm)\,(800\,cm/cm^2)$$
$$= 6.25\,\Omega cm^2 \text{ or less than } 10\,\% \text{ of } R_p.$$

In most tissues considered here, R_i will be even smaller; the cells are smaller so L is smaller and l_p is larger. In addition, in mammalian tissue the resistivity of the bulk solution is lower than in the above example ($\rho \simeq 55\,\Omega cm$) so that R_i would be smaller still. R_i will also vary with ω; if the lateral cell membranes are tightly apposed (ω less than 10–20 nm), R_i could become quite large and dominate R_p. Conversely, if ω is larger than 0.5 μm, R_i will be quite small. Unfortunately, it is extremely difficult to be sure of the true dimensions of ω, both because of possible fixation artifacts and because it is not known exactly where the physical limits of the cell membrane really are with respect to its "unit membrane" image in the electron microscope. However, in some experimental situations where the epithelium is subjected to artificial electrical or osmotic gradients, the intercellular spaces do collapse, and transepithelial resistance increases (Frömter, 1972; Smulders, Tormey & Wright, 1972; Bindslev, Tormey & Wright, 1974). In general, estimates of R_i based on morphological data are small with respect to R_p, so that for the purposes of this paper, R_j will be approximated by the value of R_p.

The Specific Resistance of the *Zonula Occludens* ($R_j\,l_p$)

Using data available in the literature for R_t and R_c, one can estimate R_j for a number of epithelia. We will use the values for R_t and the data on junctional structure that were tabulated in Claude and Goodenough (1973) (*see* Table 1). R_c was assumed to be 7000 Ωcm^2 in all cases. R_i was assumed to be negligible because the height of the interspace (L) is less than 10 μm in most cases and because the interspace (ω) usually appears to be quite wide, 50 nm to 0.5 μm. The width of the interspace at the level of the *zonula adhaerens* and the desmosome is usually equal to or greater than 20 nm, and the depth (L) of these elements in the junctional complex is very small, so their calculated resistance is also very small. Values for l_p for the various epithelia are estimated on the basis of cell diameters from

Table 1. A comparison of junctional resistance with number of junctional strands for several simple epithelia

Epithelium	Approximate diameter of cells (μ)[a]	l_p(cm/cm^2)	R_t(Ωcm^2)[b]	R_p(Ωcm^2)[c]	$R_j l_p$(Ωcm)[d]	Mean No. of strands[e]
Mammalian proximal tubule	10 [16, 25]	2000 (mouse)	6 (5) (dog)	6	1.2×10^4	1.2
Rabbit gallbladder	10 [2, 20]	2000	30 (15)	30	6.0×10^4	4.1
Necturus proximal tubule	25 [7]	800	70 (4)	70	5.6×10^4	3.3
Necturus gallbladder	30[g]	666	300 (14)	310	2.0×10^5	6.2
Rat intestine	10 [30]	2000	300 (1)[f]	310	6.2×10^5	5.3
Mammalian distal tubule	10 [9]	2000	300 (5) (dog)	310	6.2×10^5	5.8
Necturus distal tubule	15 [7]	1300	300 (4)	310	4.0×10^5	4.8
Toad bladder	10[h]	2000	2000 (6)	2800	5.6×10^6	8.0

[a] The numbers in parentheses indicate the sources from which these data were taken.
[b] Transepithelial resistance. The numbers in parentheses indicate the sources from which these data were taken.
[c] Paracellular resistance. *See text.*
[d] "Specific resistance" of junction. *See text.*
[e] From Claude and Goodenough (1973).
[f] Adjusted for serosal-mucosal surface amplification. *See text.*
[g] A.J. Hudspeth, *personal communication.*
[h] D.R. DiBona, *personal communication.*

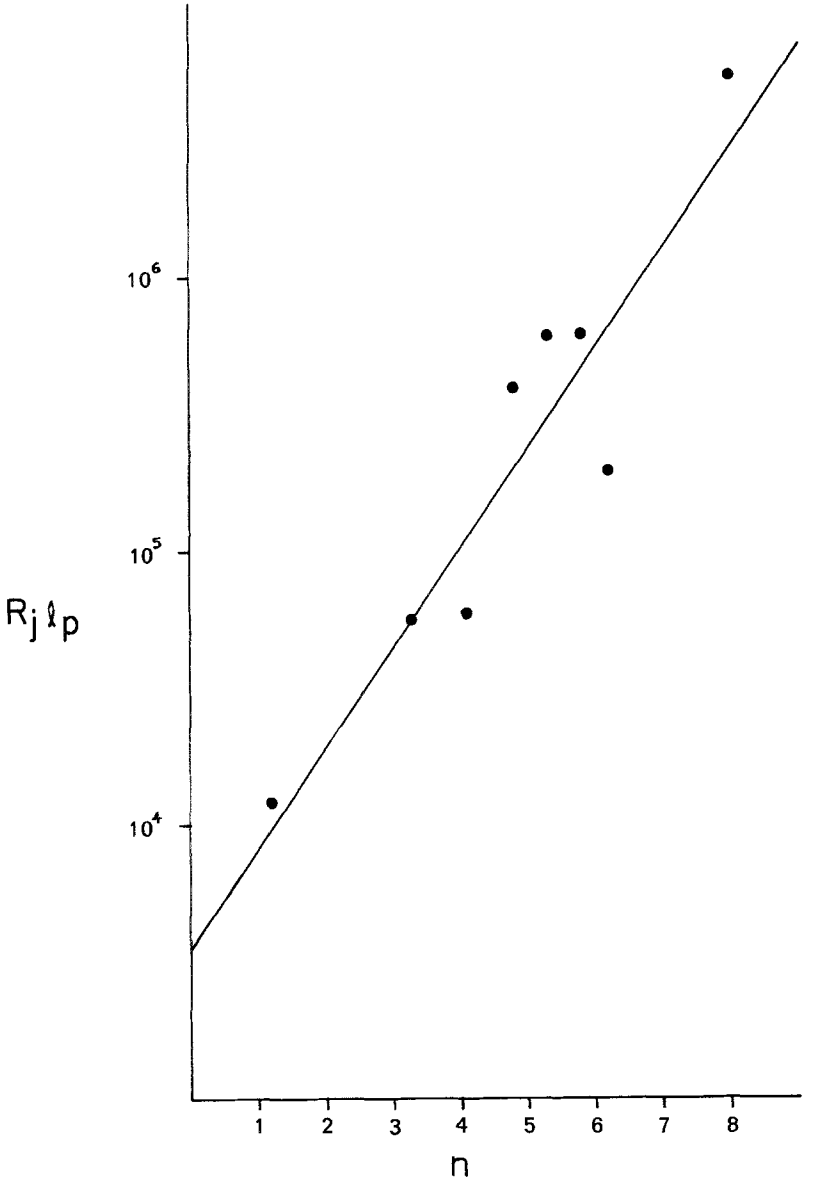

Fig. 4. Graph of $R_j l_p$, junctional "specific resistance" (expressed as Ω cm) as a function of n, the number of junctional strands in the *zonula occludens*. The points shown are derived from published data. The regression line is fitted to the data. The correlation coefficient is 0.93

micrographs in the literature and by assuming the cells to be square (*see above*).

R_j is calculated for each epithelium, and the figure $R_j l_p$ (expressed in Ωcm) is used as a "specific resistance" figure adjusted for l_p, the amount

of junction per unit area. This figure is compared to the mean number of strands present in the *zonula occludens* as seen by the freeze fracture technique (Claude & Goodenough, 1973) in Table 1.

If one plots $R_j l_p$ against the mean number of junctional strands demonstrated by freeze-fracture methods, one can see that the log of $R_j l_p$ is proportional to the number of strands in the junction (Fig. 4). This is not what one would expect if the strands were behaving as simple resistances in series, in which case $R_j l_p$ would be directly proportional to the number of strands.

Interpretation

In Fig. 5, a diagram of a *zonula occludens* with 4 strands is shown in cross section (Fig. 5*a*) and *en face*, as in a freeze-fractured preparation (Fig. 5*b*). Each strand represents a barrier to the flow of ions between the cells in the apical to basal direction (or vice versa) (Fig. 5*a*). Since the strands are not arranged in simple parallel lines but in an irregular network (Figs. 1 and 5*b*), n, the number of strands in the effective pathway across the junction, is calculated by counting the minimum number of strands an ion or other solute would have to cross (arrows) in getting from the apical to the basal edge of the junction. If the strands were functioning as simple resistors in series, the total resistance for each pathway across the junction would be the sum of the resistances of the individual strands in the pathway. However, as shown above, the relationship appears to be logarithmic.

What is the physical basis for this nonlinear relationship between $R_j l_p$ and the number of junctional strands? An interpretation might be that the strands contain labile porelike structures that can be either open or closed to the movement of small ions. If these "pores" open and close randomly for short periods of time, the local resistance of each strand will be related to the probability (p) of the strand having an open "pore" in that region. When there are two or more similar junctional strands in series, the "specific resistance" of the junction ($R_j l_p$) will be proportional to the probability (p) of each strand having an open "pore" in each pathway, raised to the negative power of the number of strands in the junction (n). An equation for "specific" junctional resistance can thus be devised:

$$R_j l_p = R_j l_{p_{\min}} \cdot p^{-n}$$

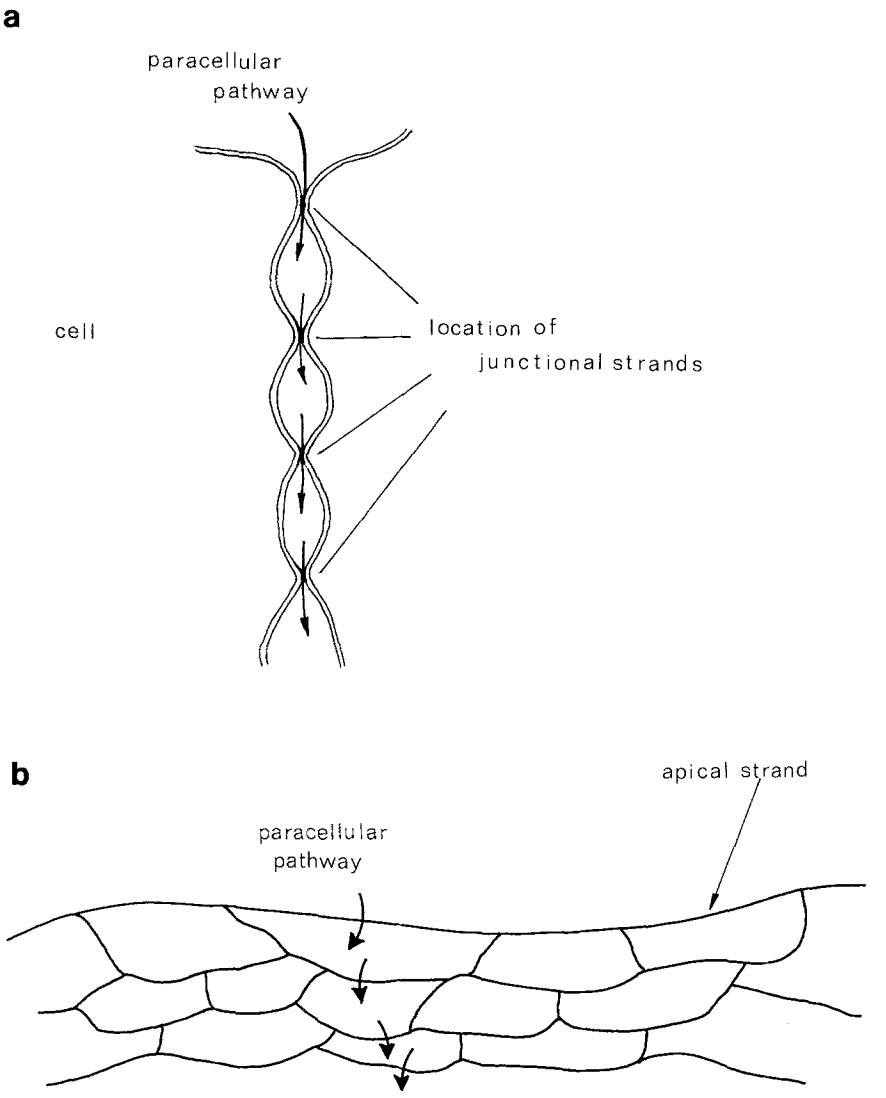

Fig. 5. Schematic representation of the *zonula occludens* as a barrier in the paracellular pathway. (*a*): *Zonula occludens* in cross section, as seen in thin sections. (*b*): *Zonula occludens* seen *en face*, as in freeze-fractured preparations

or,

$$\log R_j l_p = \log R_j l_{p_{\min}} - n \log p \tag{2}$$

where $R_j l_{p_{\min}}$ represents the specific resistance of a junction with no strands at all; a narrow but open cleft at the level of the *zonula occludens.*

If the probability of the strands having open pores in each pathway is $1 (p = 1)$, or if there are no strands $(n = 0)$, the junction will be "open",

and $R_j l_p$ will equal $R_j l_{p_{\min}}$. If the probability p is less than 1, as the number of strands in the junction increases, $R_j l_p$ will increase as a function of p^{-n}.

From the data in Fig. 4 ($R_j l_p$ is plotted against n), one can calculate the intercept (log $R_j l_{p_{\min}}$), the slope ($-\log p$) and the correlation coefficient (r) describing the relationship between $\log R_j l_p$ and n. Log $R_j l_{p_{\min}}$ is 3.53, so $R_j l_{p_{\min}}$ is approximately 3400 Ωcm, or the specific junctional resistance when all the strands are "open". The slope, $-\log p$, is 0.37, so p is 0.426 or the probability of each strand in the junction being in an "open" state. The correlation coefficient (r) describing the relationship between log $R_j l_p$ and n is 0.93.

Recent evidence indicates that the values for R_j used here may be too low in the case of the tighter epithelia (Walser, 1970; Reuss & Finn, 1974; Higgins *et al.*, 1975). If this is so, it would have the effect of increasing the slope of the line in Fig. 4 and therefore of decreasing p or the probability of each pore being open. In fact, a small change in p can have a large effect on junctional resistance. For example, consider an epithelium with an 8-stranded junction, in which $p=0.4$. If $R_j l_{p_{\min}} = 3400\,\Omega\text{cm}$, $l_p = 2000\,\text{cm/cm}^2$ and $R_c = 7000\,\Omega\text{cm}^2$, then $R_j l_p$ would equal $5.2\times10^6\,\Omega\text{cm}$, R_j would equal $2600\,\Omega\text{cm}^2$, and R_t would equal $1895\,\Omega\text{cm}^2$. If p were 0.3, however, $R_j l_p$ would equal $5.2\times10^7\,\Omega\text{cm}$, R_j would equal $26{,}000\,\Omega\text{cm}^2$ and R_t would equal $5510\,\Omega\text{cm}^2$.

$R_j l_{p_{\min}}$ can also be estimated by assuming that the junction has no strands at all, but consists only of a narrow slit filled with a solution similar to the bulk solution (*see* Eq. (1)]. If one assumes that the slit is 10 nm wide ($\omega = 1\times10^{-6}$ cm) (one occasionally sees cell membranes approach that closely, without touching, at the level of the *zonula occludens*), and that the bulk solution is amphibian Ringer's ($\rho = 100\,\Omega\text{cm}$) and that the height of the junction is 0.5 μm ($L = 5\times10^{-5}$ cm), then

$$R_j l_{p_{\min}} = \rho L/\omega = (100\,\Omega\text{cm})(5\times10^{-5}\text{ cm})/(1\times10^{-6}\text{ cm}) = 5000\,\Omega\text{cm}. \quad (3)$$

By making different assumptions about the dimensions of the variables, of course, the value of $R_j l_{p_{\min}}$ can be varied. For example, if a slit width of 20 nm is assumed, then $R_j l_{p_{\min}}$ will be half as large; or if mammalian Ringer's ($\rho = 55\,\Omega\text{cm}$) is assumed, $R_j l_{p_{\min}}$ will be 45% lower; or if l_p is 1000 rather than 2000 cm, $R_j l_{p_{\min}}$ will be half as large; or if the depth of the junction is taken to be 100 nm rather than 0.5 μm, $R_j l_{p_{\min}}$ will be one-fifth as large. However, the assumptions made here are compatible with the structure of most *zonulae occludentes* as seen in sectioned

material and the value for $R_j l_{p_{min}}$ derived in this way is consistent with the value derived from the data in Fig. 4; 1000–5000 Ω cm vs. 3400 Ω cm.

Conclusion and Discussion

Data available about the morphology and physiology of the *zonula occludens* are compatible with a model of the junction in which each junctional strand contains "pores" that can be open or closed to small ions, with a certain probability of each strand in each pathway having an open pore. In physical terms this might simply mean a periodic rearrangement of the molecules making up the strands, such that in one state certain ions could pass, and in the other state they could not. Since the permeability of an ion through a pore or channel is highly dependent on the charge density of the pore, slight modifications in the molecular arrangement of the strand might have a profound effect on the permeability of the strand to that ion. It is known that some *zonulae occludentes* are cation selective (Moreno & Diamond, 1974), so that the bulk of the transepithelial current measured must be carried by sodium ions; small changes in the charge density of the junctional strands might cause transitions from high to low sodium permeability or from "open" to "closed" states. Similar considerations may apply in epithelia where the permeability of the junction is modified experimentally without concomitant changes in the arrangement of junctional strands (Martínez-Palomo & Erlij, 1975). A variety of experimental conditions might modify the ionic environment of the junctional strands and change their permeability characteristics.

The structure of the *zonula occludens* is certainly not the only factor determining passive transepithelial permeability, but in the cases examined here it appears to dominate the permeability characteristics of the paracellular pathway. The other morphological factors considered in this paper, such as l_p and the dimensions of the interspaces, are related to transepithelial resistance in a linear manner, so the overall effect of modifying one or another of them is less profound than that of modifying the number of strands in the *zonula occludens*. Given the importance of the *zonula occludens* in determining transepithelial permeability characteristics, it would not be surprising if subtle changes in the chemical structure of the junctional strands would profoundly affect the overall permeability or the ion selectivity of an epithelium.

The calculations described here are based on physiological and morphological data from a variety of different sources. Ideally, com-

parisons should be made between physiological and morphological data obtained from identical preparations. In addition, if the opening and closing of small regions of the strands are discrete on-off events, it might be possible to analyze them by observing fluctuations in transepithelial conductance. The magnitude and duration of the conductance increment due to an opening in a single strand could thus be estimated by "noise analysis" similar to that carried out at the neuromuscular junction by Katz and Miledi (1972) and Magleby and Stevens (1972).

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