J. Membrane Biol. 45, 21–35 (1979)

# Structural Simplicity of the *Zonula Occludens* in the Electrolyte Secreting Epithelium of the Avian Salt Gland

Clara V. Riddle* and Stephen A. Ernst**

Department of Anatomy, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Received 14 June 1978; revised 19 September 1978

*Summary.* The structure of the *zonula occludens* in the secretory epithelium of the salt gland of the domestic duck was determined by thin section and freeze-fracture electron microscopy. These glands secrete an effluent with a NaCl concentration four times that of plasma, and thus maintain a steep ionic gradient across their secretory epithelium. Freeze-fracture replicas from salt stressed ducks demonstrate that the *zonula occludens* is surprisingly shallow in depth (20–25 nm) and generally consists of two parallel junctional strands which are juxaposed along their entire length. In addition to the simplicity of the junction separating mucosal and serosal compartments, the ratio of junctional length to apical surface area is large since luminal surfaces of secretory cells are narrow and intermesh with one another. The *zonula occludens* in nonsecreting fresh water-adapted birds is similar to the salt stressed group except that two sets of double strand junctions are seen in addition to junctions consisting of a single set. Based on previous ultrastructural, cytochemical and physiological studies in salt glands and in other epithelia, a model for salt secretion was suggested in which intercellular space $Na^+$, generated by basolateral ouabain-sensitive $Na^+$ pumps, reaches the lumen via a paracellular route (Ernst & Mills, 1977, *J. Cell Biol.* **75**:74). The simplicity of the morphological appearance of the *zonula occludens* in the salt gland, which resembles that described for several epithelia known to be leaky to ions, is consistent with this hypothesis.

The extrarenal salt gland of marine birds has the capacity to secrete a fluid many times the salt concentration of plasma, thus providing an efficient homeostatic mechanism, allowing the animal to utilize seawater to maintain a positive water balance. In domestic ducks the main secreted salt, NaCl, is present in the effluent at a concentration four times that in the plasma [30]. In response to osmotic stress, specific ultrastructural and biochemical changes in salt gland epithelium occur [11, 12]. The predominant cell type, the principal secretory cell, devel-

* *Present address:* Department of Ophthalmology, University of Oregon Health Sciences Center, Portland, Oregon 97201.

** *To whom reprint requests should be made and present address*: Department of Anatomy, University of Michigan Medical School, Medical Science II, Ann Arbor, Michigan 48109.

0022-2631/79/0045-0021 $03.00

ops extensive amplication of its basolateral membrane and an increase in mitochondria [11]. This process of structural specialization correlates temporally with a fourfold increment in the specific activity of ($Na^+$–$K^+$)–ATPase [12], an enzyme which is considered to be equivalent to the ouabain-sensitive, ATP-dependent transport system ($Na^+$ pump) present in most tissues [34]. Salt induced *de novo* synthesis of glandular ($Na^+$–$K^+$)–ATPase [12, 36] parallels concomitant changes in the capacity of the gland to elaborate an effluent at a high salt concentration and a high secretory rate [15]. Although the concentrative step might logically be assumed to occur across the luminal interface of this secretory epithelium (*see* [27]), the amplified basolateral membrane has been shown to be the site of ($Na^+$–$K^+$)–ATPase activity by a cytochemical procedure [10, 13] and by the autoradiographic localization of $^3$H-ouabain binding sites [13].

The same pattern of ($Na^+$–$K^+$)–ATPase localization—enzyme sites present on basolateral membranes and absent on apical membranes—has been demonstrated for a variety of secretory epithelia (*see* [13] for references). Paradoxically, reabsortive epithelia not only exhibit the same distribution of ($Na^+$–$K^+$)–ATPase activity (*see* [13]), but both types of epithelia share many of the same basic ultrastructural characteristics, despite the opposed direction of salt transport, i.e., blood to lumen in secretory epithelia, but lumen to blood in reabsorptive epithelia.

Recently, Ernst and Mills [13] proposed a model for secretory salt transport based on studies with the duck nasal salt gland. The model suggests that $Na^+$, extruded into the intercellular space by basolateral plasmalemmal ($Na^+$–$K^+$)–ATPase, follows a paracellular route to reach the lumen via the *zonula occludens.*

The *zonula occludens* thus plays a central role in this hypothesis. It is the primary pathway for transepithelial $Na^+$ flux, and must therefore provide a low resistance barrier for the movement of ions. Our recent efforts have involved the use of high resolution thin section and freeze fracture electron microscopy of duck salt gland epithelium to examine morphological predictions of the model. In this paper we present evidence that the *zonula occludens* exhibits a structural simplicity similar to that described for other transporting epithelia known to be "leaky" to ions.

A preliminary account of this work was presented in abstract form [29].

## Materials and Methods

Salt glands were obtained from domestic ducks (*Anas platyrhynchos*) 2–12 months of age. All animals were randomly assigned to one of two water regimens on the sixth day following hatching. The salt stressed group was maintained on a 1% NaCl solution for 12 hr (altered to 18 hr per day after 2 months) and fresh tap water for the remainder of the day. This salt regimen leads to full glandular differentiation and maximal levels of ($Na^+$–$K^+$)–ATPase activity [11, 12]. The unstressed control group was provided with a continuous supply of tap water. Both groups were fed commercial chick starter mash *ad libitum.*

The salt glands were immediately removed following decapitation and minced in a so-

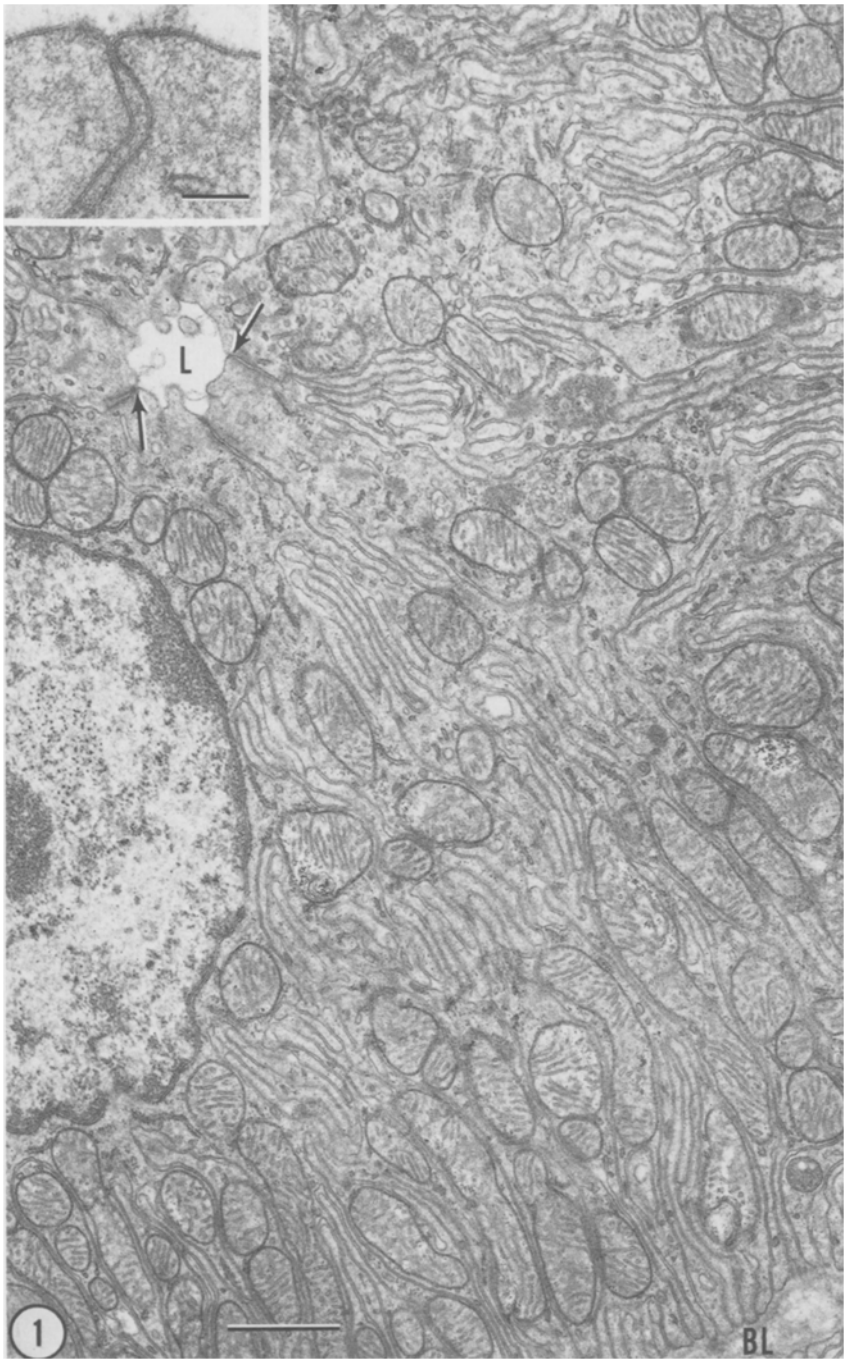



Fig. 1. Thin section view of fully specialized cells from salt stressed glands. The chief characteristics of these secretory cells are the extensive basolateral membrane amplification and the abundance of mitochondria which often pack the intracellular compartments. In contrast, the apical surfaces bordering the lumen (*L*) are limited in extent and bear only a few microvilli. The luminal space is separated from the intercellular space by *zonulae occludentes* (arrows). *BL*, basal lamina. Scale marker equals 1 μm. 16,000 ×. *Inset:* At high magnification, the *zonula occludens* appears to consist of a single junctional contact, approximately 20–30 nm in depth. Scale marker equals 0.1 μm. 93,000 ×

lution of 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for both thin section and freeze fracture electron microscopic studies.

For conventional electron microscopy, the tissue was fixed in glutaraldehyde for 2 hr at room temperature, rinsed with three 15 min changes of cacodylate buffer, and postfixed for 2 hr in a solution of 1.5% potassium ferrocyanide and 1% osmium tetroxide [22] to enhance membrane contrast. After several brief rinses in distilled water, the tissue was dehydrated and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, Pa.). For freeze fracture studies, salt gland blocks were fixed in glutaraldehyde for 30 min in cacodylate buffer and then infiltrated for 90 min in 25% glycerol in 0.1 M cacodylate buffer pH 7.4. Small tissue pieces were rapidly frozen on paper discs in Freon 22 cooled in liquid nitrogen.

Freeze-fracture replicas were prepared in a Balzers 360 M unit equipped with a platinum-carbon gun and a quartz crystal monitor. The replicas were cleaned in a solution of full strength clorox supplemented with KOH pellets and rinsed several times in distilled water. They were then picked up on coated or uncoated grids and viewed in a Phillips 300 electron microscope. The sample size for these studies included six ducks from the salt stressed group and seven ducks from the unstressed control group.

## Results

Avian salt glands are crescent shaped organs situated directly above each orbital cavity. Histological and ultrastructural characteristics of the duck salt gland were described previously [11] and are reviewed briefly here for purposes of orientation. Each gland is composed of many lobules containing numerous tubules which radiate from the central canal of each lobule to abut blindly on interlobular connective tissue at the periphery. When ducks are salt stressed, cell division of peripheral cells [6] at the ends of the secretory tubules results in an increase in tubular length. Concurrently, the increasing population of principal secretory cells lining the major extent of the expanding tubules undergoes marked cellular differentiation leading to an increase in mitochondria and an amplification of the basolateral membranes. The resulting cellular architecture is essentially identical to that of reabsorptive epithelia such as the distal tubules of mammalian kidney. The peripheral cells remain histologically unaltered, with little folding of the cell surface.

Images from thin sections and freeze-fracture replicas show that the basolateral membranes of salt stressed principal secretory cells are highly folded, thereby partitioning the major portion of the cell into cytoplasmic compartments filled with mitochondria (Figs. 1 and 2). The luminal surfaces, which are relatively flat, are separated from the intercellular compartments by juxtaluminal occluding junctions ("tight" junctions, *zonulae occludentes*). In thin sections, these junctions are shallow (Fig. 1) and at high magnification (Fig. 1, inset) appear to consist of a

Fig. 3. Freeze fracture view of the *P* faces of luminal membrane illustrates that the apical surfaces are narrow and intermesh with one another like interlocking fingers. *Zonulae occludentes* are not seen in this image since they lie deep to the grooves (arrows) between the adjacent luminal surfaces. Scale marker equals 0.5$\mu$m 30,000 ×

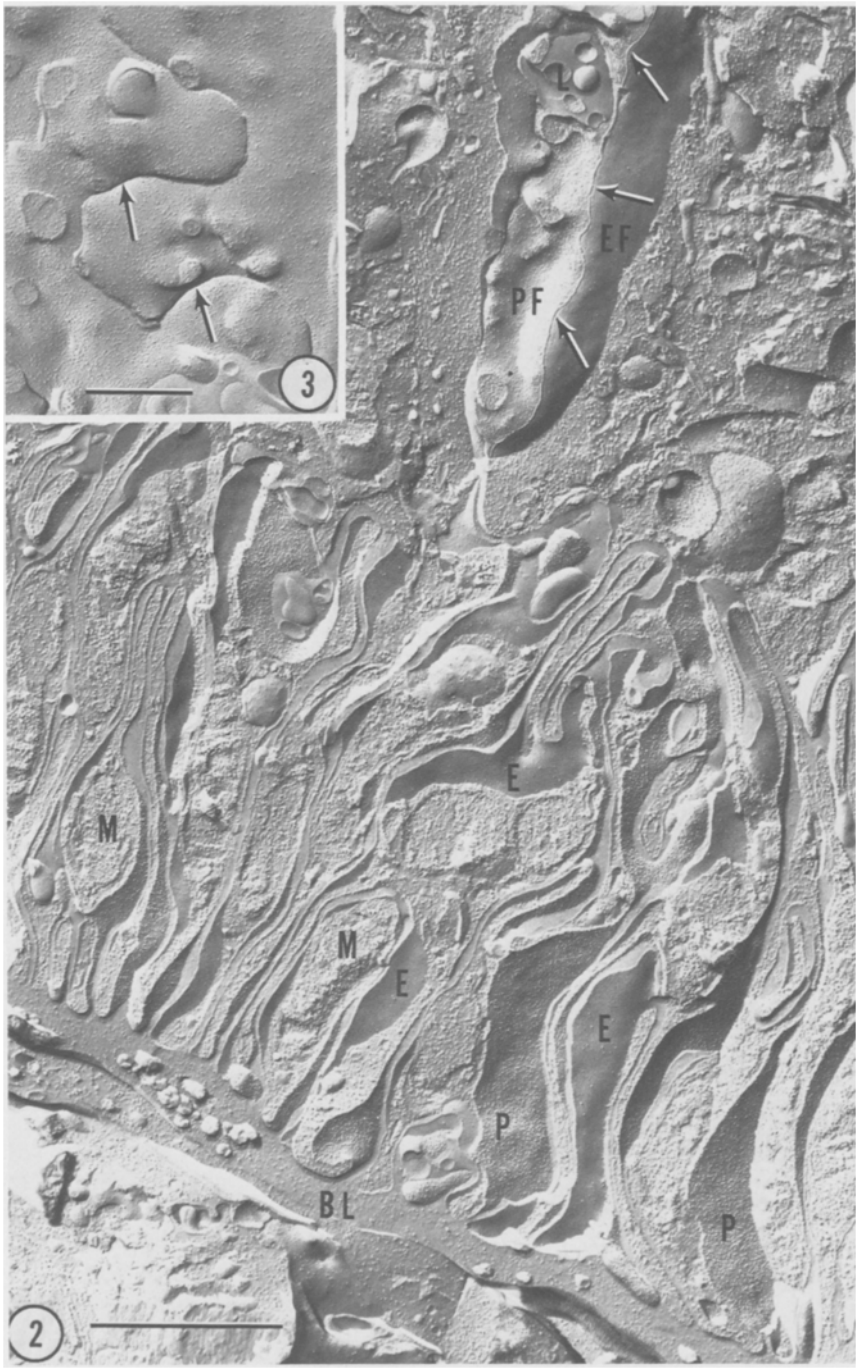



Fig. 2. This freeze fracture replica emphasizes the salient features of a secretory cell from a salt stressed duck. An extensive area between the lumen (*L*) and the basal lamina (*BL*) is occupied by the folds of the basolateral membranes which exhibit particle rich (*P*) and particle poor (*E*) fracture faces. Mitochondria (*M*) lie within the intracellular compartments. The simple *zonula occludens* (arrows) consists of a ridge and juxtaposed groove (*see* Fig. 8) at the interface of the *P* face (*PF*) of the luminal membrane and the *E* face (*EF*) of the lateral membrane. Scale marker equals 1 $\mu$m. 27,000 ×

limited focal area of contact between adjacent plasmalemmal surfaces. Freeze fracture confirms the relative simplicity of the junction in terms of its shallow depth and paucity of junctional strands (*see* Fig. 2 and below). Figures 1 and 3 show that the luminal interface of each cell is narrow and interdigitates with apical surfaces of adjacent cells, resulting in a significant enhancement of total junctional length. A similar observation was reported on the basis of scanning electron microscopy of the luminal surface [7].

The principal cells from unstressed control ducks appear less specialized when compared to the salt stressed principal secretory cells [11]. The cells possess the normal complement of mitochondria and extensive folding of the plasmalemma is not common (Figs. 4 and 5). Rather, the basal membrane is generally flush against the basal lamina. Although lateral membrane interdigitations are evident (Fig. 4), the plications are less extensive than in salt stressed cells so that large membrane faces often are present in freeze-fracture preparations (Fig. 5). As in salt stressed glands, occluding junctions in control glands are not elaborate (Fig. 5).

### *Structure of the Zonula Occludens*

In the freeze-fracture replicas of salt glands from salt stressed ducks, the *zonula occludens* generally consists of two closely apposed parallel strands on the *P* face (parallel grooves on the *E* face) of the lateral membrane (Figs. 6 and 7), forming a doublet. The width of the doublet (approximately 20–25 nm) is equal to the sum of the width of two individual strands. Short focal widenings of the junction to 80 nm are seen often (Fig. 6) and consist of either one doublet and a single strand or two doublets; almost always one or more interconnecting cross bars are present (Fig. 6). Rarely are two sets of doublets present for any considerable distance and no discontinuities in strands were evident. Free ends or spurs are only seen in areas where three or more cells meet. In such areas the junction is more elaborate (Fig. 5) and is similar to that described by Staehelin (35) for other epithelia.

The doublet nature of the junction can be difficult to discern because of the way the membranes fracture. More than half the time the fracture makes a transition from one cell to another between the two strands of the *zonula occludens.* The most common transition is from the *P* face of the luminal membrane to the *E* face of the basolateral membrane of the adjacent cell (Fig. 8). These replicated surfaces present a ridge on the *P* face and a groove on the *E* face, but due to the close apposition of the two strands, the ridge and groove arrangement is not always easy to resolve. The complementary fracture face was seen less frequently. Nearly one fourth of all replicas show a fracture from the *P* face of the luminal membrane of a cell to the *P* face of its lateral membrane. Such replicas always have the two juxtaposed ridges (Fig. 6). In a few cases the complementary fracture, from the *E* face of the luminal membrane to the *E* face of the lateral membrane was observed; this situation resulted invariably in the presence of two parallel grooves (Fig. 7).

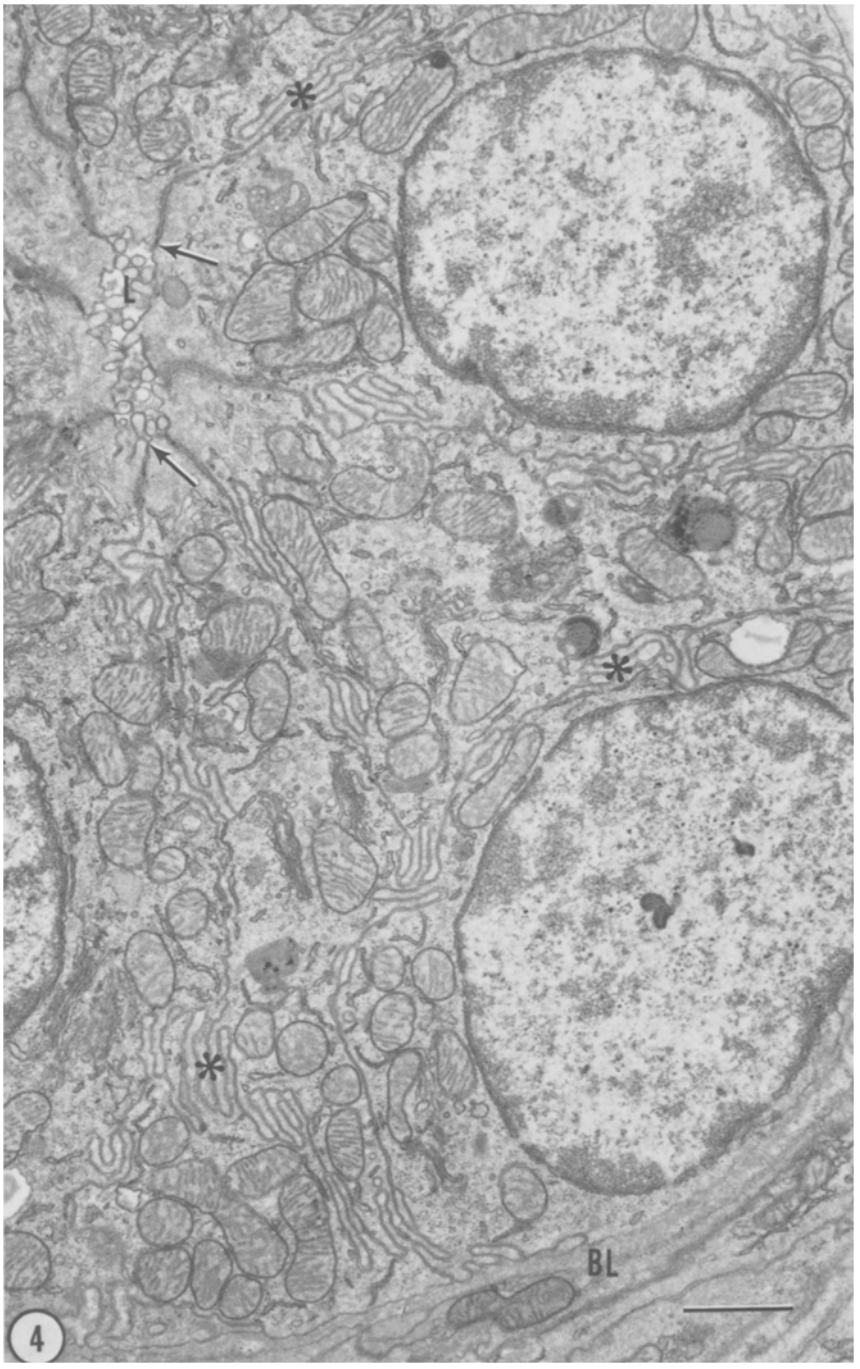
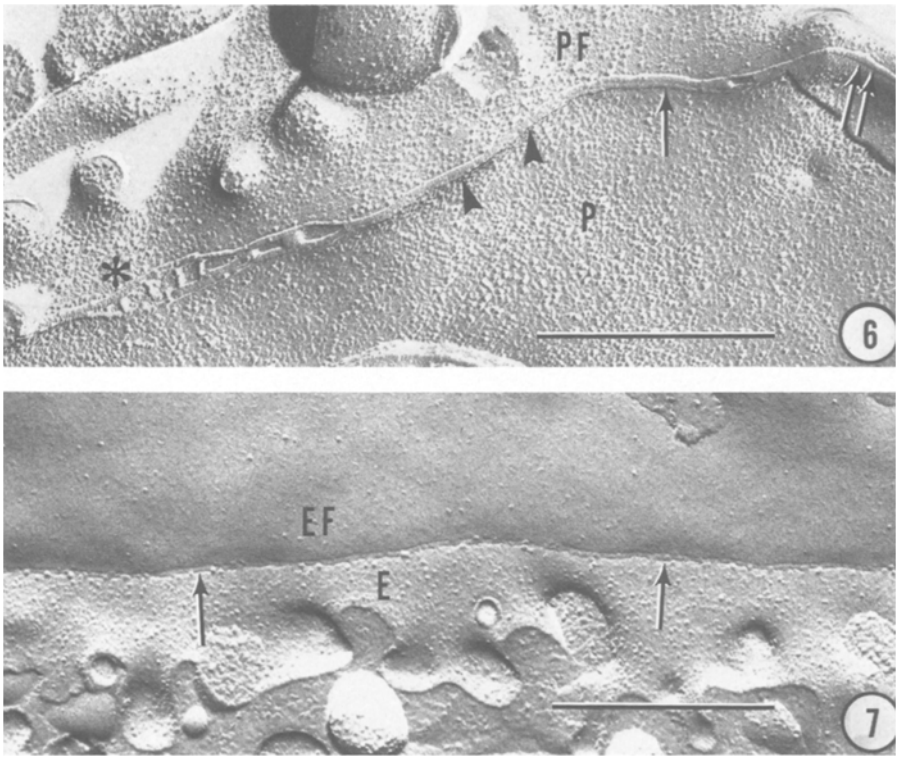



Fig. 4. This electron micrograph shows a portion of a secretory tubule from the salt gland of an unstressed duck. The basolateral membranes are less tortuous than in salt stressed cells. The lateral plasma membranes are folded and interdigitate with adjacent cells (asterisks), while the basal plasma membranes are flush against the basal lamina (*BL*). The intercellular spaces are separated from the luminal space (*L*) by occluding junctions (arrows). Scale marker equals 1 $\mu$m. 15,000 ×


BF
E
E
P
EF
EF
I
L
E
P
5

← Fig. 5. A freeze fracture replica of secretory cells from a fresh water adapted duck illustrates the comparatively smooth contours of the cell surfaces. The lateral folds are seen as sheets of membranes exposed on their *E* faces (*E*) and *P* faces (*P*). The basal membrane is flush against the basal lamina (*BL*). A cross fracture of the luminal compartment (*L*) as well as a surface view of the *E* faces (*EF*) of the luminal membrane are seen. The *zonula occludens* between *E* fracture faces of luminal and lateral membranes consists of two juxtaposed grooves (arrows). Junctional morphology is more elaborate where three cells are in apposition (arrowheads). Scale marker equals 1 μm. 15,000 ×




Figs. 6–9. High magnification freeze fracture views of the *zonulae occludentes* of secretory cells from salt stressed (Figs. 6–8) and unstressed (Fig. 9) ducks. Fig. 6. A junctional doublet, 20–25 nm in width and consisting of two adjacent strands (arrow), is always present when the fracture is from the *P* face (*PF*) of the luminal membrane to the *P* face (*P*) of the lateral membrane. Due to the angle of shadowing, the doublet nature of the junction is obscured in some places (arrowheads). When the fracture passes from the luminal *P* face (*PF*) to the lateral *E* face (*E*), a closely juxtaposed ridge and groove are present (double arrow). This fracture pattern is seen more clearly in Fig. 8. Local modifications of these two-stranded junctions consist of a widening of the *zonula occludens* to about 80 nm with cross bars interconnecting the strands (asterisk). Scale marker equals 0.5 μm. 64,000 ×

Fig. 7. Two closely parallel grooves (arrows) are evident when the fracture is from the *E* face (*EF*) of the luminal membrane to the *E* face (*E*) of the lateral membrane. Apical *E* faces are generally as particle rich as the complementary *P* faces. Scale marker equals 0.5 μm. 60,000 ×

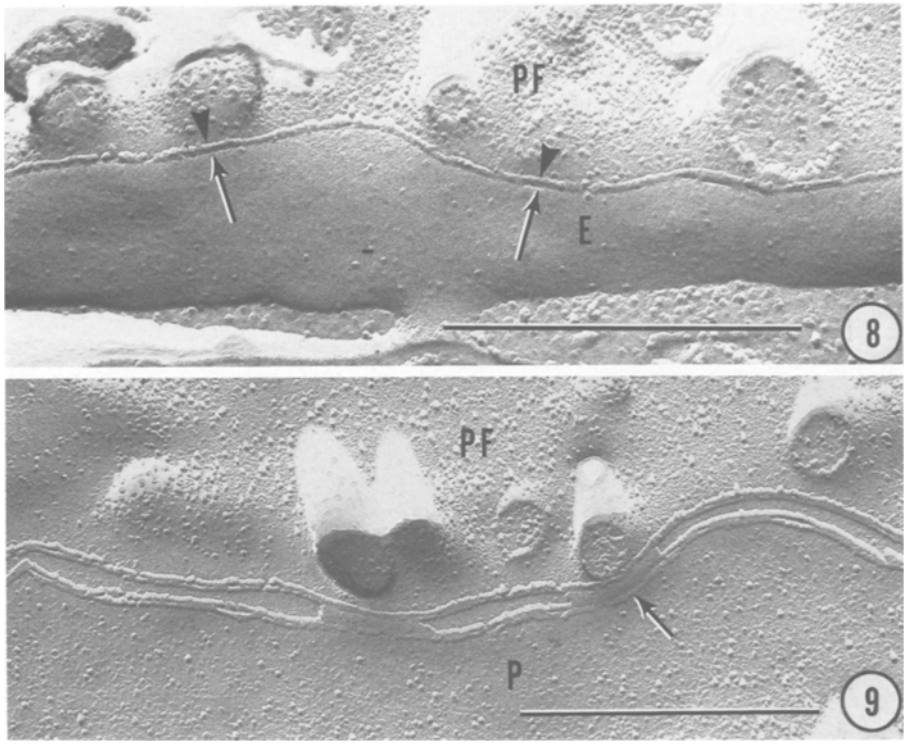



Fig. 8. The most frequently observed fracture occurs between the strands of the doublet as the fracture passes from the *P* face (*PF*) of the luminal membrane to the *E* face (*E*) of the lateral membrane, thus producing a ridge (arrowheads) and parallel groove (arrows). Scale marker equals 0.5 μm. 95,500 ×

Fig. 9. In the fresh water-adapted salt glands, two sets of parallel doublets, each approximately 20 nm in width, are seen in addition to junctional areas consisting of a single doublet (*also see* Fig. 5). The fracture shown here is between luminal and lateral *P* faces, except for two focal areas where the cleavage plane skips from *P* face to the *E* face of the adjacent cell and back to the *P* face along the junctional strands. As best seen in the area on the right (arrow), this type of fracture reveals a strand juxtaposed to a groove, a strip of *E* face, and then a groove adjacent to a strand. Scale marker equals 0.5 μm. 81,000 ×

Principal cells from salt glands of unstressed control ducks (2–12 months of age) have the same basic type of *zonula occludens* as those in glands from stressed animals. However, the junctions are usually somewhat more complex, consisting of two, three, or four strands. These strands are generally present as a single doublet (Fig. 5) or as two sets of doublets (Fig. 9), separated from each other by a width of about 20 nm to give a total junctional depth of 60–80 nm (Fig. 9). Preliminary freeze fracture data from newly hatched ducks maintained on fresh water for 1–2 weeks demonstrate, however, a preponderance of multistranded junctions.

Every effort was made to distinguish the principal cells from the unspecialized peripheral cell and the junctions were only analyzed if enough of the cell was present to definitely identify it as a principal cell. Such distinctions were some-

what more difficult in the unstressed controls since the differences between principal cells, peripheral cells, and the stages in between are less obvious. With the aid of other criteria such as increased amounts of connective tissue which are present at the blind ends of the tubules where the peripheral cells lie, it was apparent that a clear differentiation could be made between principal cells and peripheral cells on the basis of their *zonulae occludentes.* In both stressed and unstressed ducks the *zonula occludens* of the peripheral cells consisted of a loose meshwork of interlocking strands with a total junctional depth always exceeding 80 nm.

## Discussion

The *zonula occludens,* first described by Farquhar and Palade [14], was assumed initially to restrict the passage of small molecules such as electrolytes and water across epithelia. Physiological data, however, demonstrated that occluding junctions in some epithelia might be leaky, thereby providing a paracellular route for ion permeation [1, 16–18]. More recently, Claude and Goodenough [3] showed by freeze-fracture electron microscopy that junctional tightness, as judged by transepithelial resistance values, was correlated positively with the number of anastomosing junctional strands and, to a lesser extent, with junctional depth. Other studies [4, 9, 20, 24, 28, 35, 39] generally lend credence to a model in which the extent of transepithelial conductance may be viewed, in part, as a function of junctional morphology [2].

Considering current interest in paracellular pathways for ion permeation in reabsorptive epithelia, surprisingly little attention has been paid to the structure of occluding junctions in ion secretory epithelia and their role in the formation of isotonic and, in particular, hypertonic secretions. The avian salt gland serves as a useful model system from such studies. These glands respond to osmotic stress by elaborating a hyperosmolar secretion ranging from two to eight times the NaCl concentration of plasma [30]. It has been assumed on empirical grounds that electrolyte flux across this epithelium is entirely transcellular [27], since the maintenance of steep solute gradients would appear to be incompatible with a low resistance pathway across cellular junctions [18]. However, ultrastructural studies of avian salt glands [25], as well as of other secretory epithelia [5, 38], indicate that the apical junctions are not elaborate.

The present study, employing thin section and freeze-fracture electron microscopy of duck salt glands, shows that the *zonula occludens* is remarkably simple. In salt stressed ducks, the junction invariably consists of two parallel contiguous strands (Figs. 6–8). The appearance of the junction in unstressed salt glands is similar except that two sets of doublets, separated by a 20-nm space, were seen frequently (Fig. 9). Discontinuities in the junctions were not observed in either the salt or fresh water condition. The doublet nature of the junction in salt stressed duck salt glands differs from the single strand junction described by Ellis *et al.* [7] in the herring gull. However, the junctional element shown in their published image uniformly measures 20 nm in width, suggesting that the doublet nature of the junction may have been obscured by heavy metal shadowing in a manner similar to that shown for a portion (arrowheads) of the two-stranded junction illustrated in Fig. 6.

The relative simplicity of junctional morphology in salt stressed glands resembles that described for leaky epithelia and contrasts markedly with the extensive junctional network of anastomosing strands exemplified by high resistance epithelia. Accordingly, if one assumes that salt gland junctions must be impermeable to electrolytes and water in other to maintain a steep salt gradient during hypertonic secretion, then it follows that paracellular permeability is not necessarily related to the number of junctional elements. Most studies, however, confirm that the number of junctional ridges in transporting epithelia is proportional to electrical measurements of transmural resistance [2, 3, 8, 28]. This relationship does not always obtain when the relative tightness of some epithelia is assessed electron microscopically on the basis of junctional permeation by lanthanum solutions [8, 26, 28, 37]. Such exceptions, however, have been reported only in a few cases where epithelia (rat distal tubule, rabbit ileum) with moderate resistance values (100–300 $\Omega cm^2$) demonstrate relatively well developed junctions (range 5–10 strands). We are unaware of any examples of high resistance epithelia which exhibit simple junctions (1-2 strands) such as those of salt gland epithelium. Although studies with lanthanum-containing fixatives in salt glands [25] and in elasmobranch rectal gland [38] suggested possible junctional permeation by this probe, definitive measurements of electrical parameters such as transepithelial resistance have not been possible due to the lack of easy access to the mucosal compartment.

Considering the steep solute gradient across salt gland epithelium and the hypertonicity of the transported fluid (four times the concentration of plasma NaCl in the duck), the simplicity of the occluding junction is perplexing. Furthermore, the intermeshing of the narrow apical surfaces of individual cells bordering the lumen (Fig. 3) provides a large ratio for the linear amount of junction per unit of luminal surface area. Taken together, these morphological observations suggest that junctional conductance may contribute significantly to the total transepithelial ionic flux across the epithelium.

The unexpected appearance of the *zonula occludens* in this secretory epithelium is paralleled by the seemingly paradoxical localization of ouabain-sensitive $Na^+$ pumps which are generally considered to drive salt transport in this and other epithelia. Empirically, one would expect a concentrative extrusion step at the apical interface of secretory cells, yet cytochemical localization of ($Na^+$-$K^+$)-ATPase at the light and electron microscopic levels [10, 13] and autoradiographic localization of $^3$H-ouabain binding sites [13] both indicate that ouabain-sensitive $Na^+$ transport is restricted to the basolateral cell surfaces. Nonluminal localization of ($Na^+$-$K^+$)-ATPase is common to other secretory epithelial as well (*see* [13] for references). On the basis of these studies, and considering physiological data on ion secretion across teleost gill and operculum [21, 23] and elasmobranch rectal gland [31], a model was proposed [13] which suggests that NaCl secretion depends, in part, on paracellular movement of $Na^+$. In this model, basolateral $Na^+$ pumps are oriented to extrude $Na^+$ from the cell into the intercellular space. We suggested that this establishes a favorable electrochemical gradient for the inward diffusion of $Na^+$ and provides the driving force for the co-transport of $Cl^-$ into the cell against its electrical gradient. Provided that the electrochemical potential of intracellular $Cl^-$ exceeds that of the luminal solution, movement of $Cl^-$

across the apical interface may proceed passively without intervention of an apical pump. $Na^+$, pumped into the intercellular space, traverses the paracellular pathway in response to the favorable potential (lumen negative) created by the transepithelial transport of $Cl^-$. Similar models were proposed to account for NaCl secretion in teleost gill [32] and in shark rectal gland [33]. This model predicts a high conductance shunt pathway in salt gland epithelium and is entirely consistent, therefore, with the simple junctional morphology observed in the present study.

An alternative model to account for hypertonic salt secretion by salt glands was proposed by Ellis *et al.* [7]. They suggested that a primary isotonic secretion is elaborated by the peripheral cells at the blind ends of the secretory tubules. The secretion is concentrated secondarily, as it passes along the tubule, by passive extraction of water across permeable junctions into intercellular spaces of principal secretory cells rendered hypertonic by basolateral $Na^+$ pumps. This model does not explain the mechanism of secretion, but rather proposes a method for solute concentration. Moreover, it is not applicable to secretory epithelia which lack ducts (e.g., teleost gill and opercular epithelia). If the peripheral cells are responsible for generating all of the transported NaCl, then electrolyte secretion must occur by a ouabain-insensitive mechanism since peripheral cells do not bind ouabain or exhibit ($Na^+$-$K^+$)-ATPase activity [13]. Furthermore, peripheral cells are mitotically active [6], show little evidence of morphological specialization (e.g., membrane amplification, abundant mitochondria) normally associated with high levels of electrolyte transport [11] and represent a rather small percentage of the salt gland cell population to account for the extraction of NaCl from the blood at the highly efficient rates reported by Hanwell *et al.* [19].

Definitive support for a secretory mechanism involving paracellular ion permeation [13] requires measurement of critical electrical parameters across salt gland epithelium. Histological restraints which preclude easy access to the luminal compartment have impeded such measurements. However, the proposed model is applicable to several other hypertonic secretory systems (teleost gill and operculum, elasmobranch rectal gland) where such data is available. Thus consistent with this model, transepithelial potential (lumen negative) and "active" chloride efflux in these epithelia appear to be intimately associated with basolateral ($Na^+$-$K^+$)-ATPase since these parameters are inhibited by serosal ouabain. If junctional structure plays an integral part in determining the conductance properties of an epithelium, then in order for this model to have general validity, other epithelia which produce hypertonic secretions should exhibit a similar junctional simplicity as that described in the present communication for the salt gland. In this regard, preliminary observations on the junctions in the opercular epithelium of sea-water-adapted fish [10*a*] are particularly encouraging. In this low resistance epithelium (140 $\Omega cm^2$, [21]) freeze fracture indicates that junctions between pavement epithelial cells are elaborate whereas those between ion transporting chloride cells consist of one or two junctional strands.

The excellent technical assistance of Mr. Joseph Severdia is gratefully appreciated. We also wish to thank Dr. John W. Mills and Dr. Karl J. Karnaky, Jr., for their helpful comments concerning this manuscript.

This research was supported by United States Public Health Service grant GM 23659

and Biomedical Research Support grant RR 05417 awarded to Temple University School of Medicine. Stephen A. Ernst is a recipient of United States Public Health Service Research Career Development Award 5 KO4 GM00072.

## References

1. Boulpaep, E.L. 1971. Electrophysiological properties of the proximal tubule: Importance of cellular and intercellular transport pathways. *In*: Electrophysiology of Epithelial Cells. Symposia Medica Hoechst. 1970. G. Giebisch, editor. Schattauer Verlag, Stuttgart
2. Claude, P. 1978. Morphological factors influencing transepithelial permeability: A model for the resistance of the *zonula occludens*. *J. Membrane Biol.* **39:**219
3. Claude, P., Goodenough, D.A. 1973. Fracture faces of *zonulae occludentes* from "tight" and "leaky" epithelia. *J. Cell Biol.* **58:**390
4. DiBona, D.R., Civan, M.M. 1973. Pathways for movement of ions and water across toad urinary bladder. I. Anatomic site of transepithelial shunt pathways. *J. Membrane Biol.* **12:**101
5. Dunson, W.A., Packer, P.K., Dunson, M.K. 1971. Sea snakes: An unusual salt gland under the tongue. *Science* **173:**437
6. Ellis, R.A. 1965. DNA labelling and X-irradiation studies of the phosphatase-positive peripheral cells in the nasal (salt) glands of ducks. *Am. Zool.* **5:**648
7. Ellis, R.A., Goertemiller, C.C., Jr., Stetson, D.L. 1977. Significance of extensive "leaky" cell junctions in the avian salt gland. *Nature (London)* **268:**555
8. Erlij, D. 1976. Solute transport across isolated epithelia. *Kidney Int.* **9:**76
9. Erlij, D., Martínez-Palomo, A. 1972. Opening of tight junctions in frog skin by hypertonic urea solutions. *J. Membrane Biol.* **9:**229
10. Ernst, S.A. 1972. Transport adenosine triphosphatase cytochemistry. II. Cytochemical localization of ouabain-sensitive, potassium-dependent phosphatase activity in the secretory epithelium of the avian salt gland. *J. Histochem. Cytochem.* **20:**23

10.*a*. Ernst, S.A., Dodson, W.B., Karnaky, K.J., Jr. 1978. Structural diversity of *zonulae occludentes* in seawater-adapted killifish opercular epithelium. *J. Cell Biol.* **79:**242*a*

11. Ernst, S.A., Ellis, R.A. 1969. The development of surface specialization in the secretory epithelium of the avian salt gland in response to osmotic stress. *J. Cell Biol.* **40:**305
12. Ernst, S.A., Goertemiller, C.C., Jr., Ellis, R.A. 1967. The effect of salt regimens on the development of $(Na^+ - K^+)$-dependent ATPase activity during growth of salt glands of domestic ducklings. *Biochim. Biophys. Acta* **135:**682
13. Ernst, S.A., Mills, J.W. 1977. Basolateral plasma membrane localization of ouabain-sensitive sodium transport sites in the secretory epithelium of the avian salt gland. *J. Cell Biol.* **75:**74
14. Farquhar, M.G., Palade, G.E. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* **17:**375
15. Fletcher, G.L., Stainer, I.M., Holmes, W.N. 1967. Sequential changes in the adenosinetriphosphatase activity in the electrolyte excretory capacity of the nasal glands of the duck (*Anas platyrhynchos*) during the period of adaptation to hypertonic saline. *J. Exp. Biol.* **47:**375
16. Frizzel, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. Influence of shunt on transmural sodium transport and electrical potential differences. *J. Gen. Physiol.* **59:**318
17. Fromter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8:**259

18. Fromter, E., Diamond, J. 1972. Route of passive ion permeation in epithelia. *Nature New Biol.* **235:**9
19. Hanwell, A., Linzell, J.L., Peaker, M. 1971. Salt gland secretion and blood flow in the goose. *J. Physiol. (London)* **213:**373
20. Humbert, F., Grandchamp, A., Pricam, C., Perrelet, A., Orci, L. 1976. Morphological changes in tight junctions of *Necturus maculosus* proximal tubules undergoing saline diuresis. *J. Cell Biol.* **69:**90
21. Karnaky, K.J., Jr., Degnan, K.J., Zadunaisky, J.A. 1977. Chloride transport across isolated opercular epithelium of Killifish: a membrane rich in chloride cells. *Science* **195:**203
22. Karnovsky, M.J. 1971. Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. *Proc. 11th Annu. Meet. Am. Soc. Cell Biol.* P. 146
23. Kirschner, L.B. Greenwald, L., Sanders, M. 1974. On the mechanism of sodium extrusion across the irrigated gill of sea water-adapted rainbow trout *(Salmo gairdneri). J. Gen. Physiol.* **64:**148
24. Machen, R.E., Erlij, D., Wooding, F.B.P. 1972. Permeable junctional complexes. The movement of lanthanum across rabbit gallbladder and intestine. *J. Cell Biol.* **54:**302
25. Martin, B.J., Philpott, C.W. 1974. The adaptive response of the salt glands of adult mallard ducks to a salt water regime: An ultrastructural and tracer study. *J. Exp. Zool.* **186:**111
26. Martinez-Palomo, A., Erlij, D. 1975. Structure of tight junctions in epithelia with different permeability. *Proc. Nat. Acad. Sci. USA* **72:**4487
27. Peaker, M., Linzell, J.L. 1975. Salt glands in birds and reptiles. Cambridge University Press, Cambridge
28. Pricam, C., Humbert, F., Perrelet, A., Orci, L. 1974. A freeze-etch study of the tight junctions of the rat kidney tubules. *Lab. Invest.* **30:**286
29. Riddle, C.V., Ernst, S.A. 1977. Structure of the zonula occludens in salt gland epithelium. *J. Cell Biol.* **75:**68*a*
30. Schmidt-Nielson., K. 1960. The salt-secreting gland of marine birds. *Circulation* **21:**955
31. Siegel, N.J., Schon, D.A., Hayslett, J.P. 1976. Evidence for active chloride transport in dogfish rectal gland. *Am. J. Physiol.* **230:**1250
32. Silva, P., Solomon, R., Spokes, K., Epstein, F.H. 1977. Ouabain inhibition of gill Na-K-ATPase: Relationship to active chloride transport. *J. Exp. Zool.* **199:**419
33. Silva, P., Stoff, J., Field, M., Fine, L. 1977. Mechanism of active chloride secretion by shark rectal gland: Role of Na-K-ATPase in chloride transport. *Am. J. Physiol.* **233:***F*298
34. Skou, J.C. 1965. Enzymatic basis for active transport of $Na^+$ and $K^+$ across cell membranes. *Physiol. Rev.* **45:**595
35. Staehelin, L.A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* **39:**191
36. Stewart, D.J., Semple, E.W., Swart, G.T., Sen, A.K. 1976. Induction of the catalytic protein of ($Na^+$ + $K^+$)-ATPase in the salt gland of the duck. *Biochim. Biophys. Acta* **419:**150
37. Tisher, C.C., Yarger, W.E. 1975. Lanthanum permeability of tight junctions along the collecting duct of the rat. *Kidney Int.* **7:**35
38. Van Lennep, E.W. 1968. Electron microscopic histochemical studies on salt-excreting glands in elasmobranchs and marine catfish. *J. Ultrastruct. Res.* **25:**94
39. Wade, J.B., Karnovsky, M.J. 1974. Fracture faces of osmotically disrupted *zonulae occludentes. J. Cell Biol.* **62:**344