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POSSIBLE EPITHELIAL SODIUM CHANNELS VISUALIZED BY FREEZE-FRACTURE

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Summary

The coprodeum is a very efficient Na^+-retaining epithelium. Coprodeum from birds on a high Na^+ diet has virtually no ion transport, while an Amiloride-sensitive Na^+ absorption of 10—12 $\mu equiv \cdot cm^{-2} \cdot h^{-1}$ is induced in the coprodeal epithelium from birds on a low Na^+ diet. Both measurements of the Na^+ influx and Na^+-diffusion potentials across the luminal cell membrane have revealed a selective opening of this membrane to Na^+ in birds on a low Na^+ diet. Freeze-fracture P faces of the luminal membrane in coprodea taken from birds on a low Na^+ diet have rod-shaped particles, 100 × 240 Å, in more than 20% of the principal cells. Rod-shaped particles appear in less than 1% of these cells in coprodea from high Na^+-diet birds. Thus a low Na^+ diet induces rod-shaped particles in the luminal cell membrane of the hen coprodeum. These new particles may function as Na^+-channels mediating the increased Na^+-influx across the apical cell membrane.

Sodium retaining epithelia have specific sodium binding sites on their luminal cell membrane. Evidence has appeared recently in favour of a channel rather than a carrier mechanism for Na^+ entry at the luminal surface [1,2]. Epithelium from the midportion of the hen cloaca, the so-called coprodeum, exhibits a very high Na^+ absorption in birds on a low Na^+ diet compared with birds on a high Na^+ diet [3]. In the coprodeum flux measurements and electrophysiological results have revealed an opening of the luminal cell membrane for Na^+ in the Na^+-depleted state [4]. The present freeze-

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fracture study was undertaken in order to investigate whether the increased Na^+ absorption was related to morphological changes in the luminal cell membrane. The results suggest that sodium depletion stimulates an insertion of a new class of rod-shaped particles in the luminal membrane. Such particles which have also been found in other Na^+-retaining epithelia may well function as Na^+-channels since they span the apolar region of the membrane. Additional and supportive results from a combined light and electron microscopic study will be published elsewhere [5].

The methods for isolating the hen coprodeum and measuring fluxes have been presented in detail [4]. Pieces of stripped coprodeum were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, infiltrated with glycerol, frozen in liquid Freon 22 cooled by liquid nitrogen and fractured and replicated in a Balzer's freeze etch unit (BAF 301) equipped with an electron beam gun and a quartz thin film monitor. After tissue digestion with chromic acid and cleaning, the replicas were examined without knowledge of the tissue status.

Morphological examination and transport studies were carried out on the same material. Table I shows the flux of Na^+ from the mucosal side to the cell, J^{Na}_{mc}, from the mucosal side to the serosal side, J^{Na}_{ms}, and the short-circuit current, I_{sc}, in coprodea taken from birds on a high and a low Na^+ diet. The fluxes and the I_{sc} were within the ranges found earlier [4].

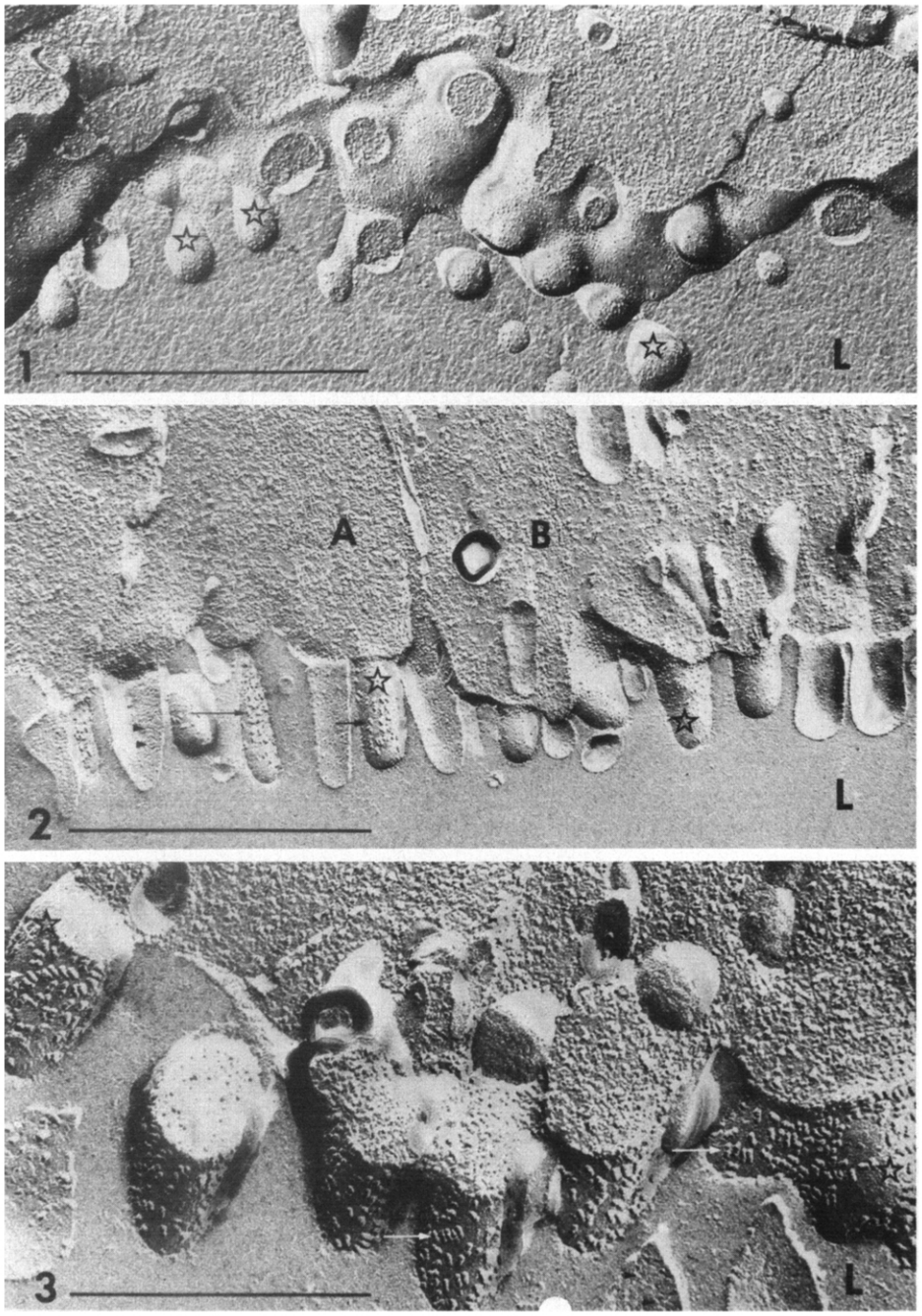
TABLE I

The sodium influx, J^{Na}_{mc}, the sodium transmural flux, J^{Na}_{ms}, and the short-circuit current, I_{sc}, in coprodea from birds on a high and a low Na^+-diet. J^{Na}_{mc} and J^{Na}_{ms} are given as ranges and the I_{sc} is given as the mean of 4 different tissues. The numbers in parenthesis are S.E.M. The Ringer contained 130 mM Na^+.

	J^{Na}_{mc} (μequiv·cm^{-2}·h^{-1})	J^{Na}_{ms} (μequiv·cm^{-2}·h^{-1})	I_{sc} (μA·cm^{-2})	No. of observations
H	0.7— 1.4	1.3— 1.8	10.5 (6.0)	4
L	6.4—25.1	8.2—17.6	288 (127)	4

In freeze fracture replicas from coprodeum two different cell types can easily be distinguished at the luminal surface of the epithelial lining: (1) goblet cells with a rather smooth apical surface and a cytoplasm containing large numbers of mucous droplets and (2) high columnar cells with short and plump microvilli. The latter cell type, to be called the principal cell, is the most frequent representing some 75% of all the cells in the mucosal lining.

Figs. 1 and 2 show the freeze fracture appearance of luminal membranes of principal cells from two different coprodea. The principal cell to the left in Fig. 2 exhibits some characteristic intramembranous rod-shaped particles which are present neither in the neighbouring cell nor in the principal cell in Fig. 1. Both the frequency of cells with rod-shaped particles in their luminal cell membrane and the density of these particles vary from tissue to tissue, but when present the rod-shaped particle is the predominant type of intramembranous particle.



Figs. 1, 2 and 3. Electron micrographs of the freeze-fractured luminal surface of principal cells from hen coprodeum. Fig. 1 demonstrates the appearance of intramembranous particles in birds on a high Na^+-diet. Note the absence of rod-shaped particles. The luminal surface is more microvillous than what is generally found in coprodea from birds on a high Na^+-diet. The cell was selected to facilitate comparison with microvillous cells in coprodea from birds on a low Na^+-diet. Figs. 2 and 3 which are obtained from birds on low Na^+-diet show the characteristic rod-shaped intramembranous particles (black arrows on Fig. 2) on the P face. The E face contains relatively few particles, but some rod-shaped depressions can be recognized (arrow-heads). Note that only one of the two principal cells (A and B) in Fig. 2 exhibits rod-shaped particles. Rows of rod-shaped particles, in some of which subunits can be distinguished, are indicated by white arrows in Fig. 3. Some microvilli P faces are indicated by asterisks. L = lumen. Bars in Figs. 1 and 2 = 1 μm. Bar in Fig. 3 = 0.5 μm.

The rod-shaped particles are confined to the P face and corresponding rod-shaped depressions are present on the E face, Figs. 2 and 3. In all coprodea the rod-shaped particles are very homogenous in size and shape although more than half of these particles could be resolved into 2 or 3 subunits, Fig. 3. The dimensions of the rod-shaped particles were measured on 13 electron micrographs of apical fracture faces obtained from 5 different coprodea of unknown tissue status. The dimensions, i.e. mean-width and mean-length of the rod-shaped particles, are 97 Å (range 71—126 Å, 60 measurements) by 238 Å (range 219—277, 126 measurements), when measured normal to the shadowing direction. The rod-shaped particles are often arranged in a specific pattern with the long axis parallel to each other, Figs. 2 and 3. The membrane face between the rod-shaped particles is remarkably smooth with only very few globular particles, Fig. 3. The rod-shaped particles dominate the appearance of the P face of the luminal membrane in one fourth of the coprodeal principal cells, a finding which suggests that the principal cells with rod-shaped particles perform a highly specific transport function in the coprodeum.

When the code for the functional status of the different epithelia was broken, both the density of rod-shaped particles and the number of principal cells containing these particles were found to be highly dependent on the Na^+ content of the diet given to the birds. From coprodea of 4 birds on high Na diet we looked at well over 1000 fracture faces of apical membranes from principal cells. Less than 1% of these fracture faces contained rod-shaped particles or impressions of such particles, Fig. 1. It was difficult to identify real microvilli on the luminal surface of the principal cells from high Na^+-diet coprodea. Out of more than 2000 apical fracture faces of the principal cells from 4 coprodea of birds on a low Na^+-diet, between 20 and 30% of the cells exhibited rod-shaped particles. In these coprodea the apical membrane of the principal cells had short but distinct microvilli in contrast to the apical membrane of principal cells from coprodea of birds on a high Na^+ diet [5]. In a fifth coprodeum from a bird on a low Na^+ diet rod-shaped particles were observed in a high density in about 40% of the envisioned principal cells. This coprodeum had a rather high short-circuit current, I_{sc} = 410—495 $\mu A/cm^2$.

Characteristic rod-shaped particles are also found on P faces of some intracellular membranes close to the luminal membrane of microvillous cells from stimulated coprodea, but never in the baso-lateral membranes of coprodeal cells.

The goblet cells have a similar appearance in coprodea from different experimental situations and intramembranous rod-shaped particles were never found in association with these cells. Also the freeze fractured tight junction networks were similar in the various experimental situations. Both strand number, 6—9, and junctional depth 0.3—0.5 μm were similar to those of tight epithelia, e.g. toad urinary bladder [6], although the direct-current resistance of the coprodeum is only between 140 and 260 Ωcm^2 [4]. In a combined light- and transmission electron microscopic study we have found that virtually all the principal cells exhibit a light cytoplasm in birds on a high Na^+ diet, whereas one fourth of the principal cells demonstrate a dark cytoplasm in coprodea from birds on a low Na^+ diet [5].

The finding of new intramembranous particles can be taken as strong evidence in support of the permease theory [7,8]. The intracellular membranes containing similar rod-shaped particles may also be involved in Na^+ transport or may reflect a step in the process of inserting new transport molecules in the microvillous luminal membrane [9].

The size of the rod-shaped particles indicates that they span the hydrophobic phase of the cell membranes. This feature is a prerequisite for the Na^+ channel hypothesis brought forward by Lindemann and van Driessche [1,2]. It is conceivable that the dormant cells for sodium transport in the coprodeal epithelium of birds on a high Na^+-diet has a low sodium content [10]. The K_t for the Na^+ pump, i.e. the ouabain-sensitive (Na^+-K^+)-ATPase activity of mucosal scrapings from the coprodeum, is about 20 mM Na^+ in both dietary states (Bindslev, N., unpublished). Thus an opening of the luminal membrane would stimulate a transmural Na^+ transport by an increase in the Na^+ concentration of the cytoplasmic transport pool.

The sodium transport in Na^+-retaining epithelia is blocked specifically by Amiloride when tested, and the epithelia are characterized by the presence of dark and mitochondria-rich cells which exhibit rod-shaped particles in their plasma membranes [11—17]. Thus the induced rod-shaped particles in the luminal cell membrane during increased Na^+ absorption may be the same transport molecule for all species and epithelia.

Aldosterone induces new protein molecules in the plasma membranes of mitochondria-rich cells, microvillous cells, of the toad urinary bladder. The molecular weight ranges from 12 000 to 170 000 [18]. Combined with the results we have obtained, it may be inferred that aldosterone inserts sodium selective channels in the luminal membrane of some cells in Na^+-retaining epithelia.

The results of this study indicate that Na^+ depletion triggers an insertion of a specific class of intramembranous particles in the luminal cell membrane of principal cells of the coprodeum. These particles may well function as the specific Na^+-sites that were demonstrated physiologically in the coprodeal epithelium during Na^+ depletion [4].

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