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A Low-Salt Diet Facilitates Cl Secretion in Hen Lower Intestine

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Summary. The regulation of sodium and chloride transport in hen coprodeum by mineralocorticoids was investigated with isolated epithelia under short-circuit conditions. Unidirectional fluxes of Na and Cl were measured by isotopes and modulated by amiloride, theophylline and bumetanide. Hens were maintained either on low-NaCl diet (LS) or on high-NaCl diet (HS). Plasma aldosterone (PA) levels of these groups were measured with radioimmunoassay. A group of HS hens received injections of aldosterone on a 6-hr schedule before experiments. Another group of LS hens was resalinated, and experiments carried out on a 24-hr interval.

Salt deprivation stimulated PA levels ninefold, compared to HS hens. Na absorption was stimulated according to previous reports. Electrogenic Cl secretion was elicited by theophylline and partially inhibited by bumetanide. Modulation of PA levels by diet, resalination or aldosterone injection changed the magnitude of electrogenic Cl secretion in parallel between 0.5 μeq/cm^2 · hr (HS) and 4 μeq/cm^2 · hr (LS), with pronounced alteration in tissue resistance.

The results demonstrate a new action of aldosterone which besides stimulating Na absorption also directly or indirectly elicits Cl secretion. Evidence is presented for a hormonal adaptation of chloride transport in this epithelium. There was a morphological change of the apical plasma membrane and further experiments will have to clarify the exact cellular nature of this process.

Key Words Aldosterone · hen · large intestine · chloride · electrogenic secretion · in vitro · adaptation

Introduction

The modulation of colonic electrolyte transport by aldosterone has been investigated mainly in mammalian tissues (*cf.* Fromm & Hegel, 1978; Lückhoff & Horster, 1984; Binder, Foster & Hayslett, 1985). It was found that aldosterone stimulated sodium absorption and potassium secretion. Effects on chloride transport have not been reported. In recent years Skadhauge and co-workers (*cf.* Skadhauge et al., 1985) have conducted a number of studies on the regulation of colonic electrolyte transport in the avian lower intestine. This organ showed a pronounced dependence of sodium absorption on dietary salt intake, mediated by adaptation in plasma aldosterone concentration (Thomas & Skadhauge, 1982). Hen lower intestine is segmented into colon and coprodeum (cloaca). These segments have differential modes of electrolyte transport and undergo different modulation by aldosterone. Hen coprodeum is known as one of the most aldosterone-sensitive epithelia in the vertebrate phylum (Skadhauge, 1984). NaCl deprivation or aldosterone injections modulate sodium transport across this epithelium between zero and 14 μeq/cm^2 · hr. The effect of external aldosterone decayed substantially over five days when the dietary intake was switched from a low to a high level of NaCl (Clauss et al., 1984).

The regulation of colonic chloride transport is less well known. Similarly to Na transport, most of the knowledge about Cl transport has been obtained from studies on mammalian intestine (Frizzell, Field & Schultz, 1979). It is generally agreed that chloride absorption occurs electroneutral in exchange with bicarbonate, and that chloride secretion occurs electrogenically by distinct cells, located in the colonic crypts (Welsh et al., 1982; Halm & Frizzell, 1986). Such an electrogenic Cl secretion has also been found in hen colon (Loennroth & Munck, 1980; Andersen, Munck & Skadhauge, 1982; Voldsgaard & Bindslev, 1982; Munck, Andersen & Voldsgaard, 1984), but has not yet been demonstrated in hen coprodeum. Choshniak, Munck and Skadhauge (1977) have investigated NaCl transport across this epithelium and found almost unity of the unidirectional Cl fluxes, but have not probed on eliciting electrogenic Cl secretion.

Our study was designed to measure the regulation of Na- and Cl-transport in hen coprodeum under various dietary and hormonal states. We were

able to confirm previous findings about the hormonal regulation of Na transport (Thomas & Skadhauge, 1982; Clauss et al., 1984), and extended our investigation to the hormonal regulation of Cl transport across this tissue. Our aim was first to probe whether electrogenic Cl secretion can be elicited in hen coprodeum. Secondly we aimed at investigating the dietary and hormonal influences on the regulation of coprodeal Cl transport. Thirdly, after having found profound alterations in Cl secretion, we aimed at tracing the epithelial and cellular site of Cl secretion, e.g., to tackle the question if distinct cells are involved in either Na- or Cl-transport (Langridge-Smith, 1985, 1986).

Our study shows that aldosterone and dietary manipulations modulate sodium absorption and electrogenic Cl secretion profoundly. This is correlated to changes in the apical plasma membrane, and the occurrence of special "Cl cells." Therefore adaptational processes may be involved in the hormonal regulation of NaCl transport across this epithelium.

Materials and Methods

Animals

White Plymouth Rock laying hens, weighing 3.3 to 4.5 kg, all from the same batch were kept in two groups on either a low-NaCl diet (LS), or on a high-NaCl diet (HS) ad libitum. The LS diet consisted of a low NaCl-balanced ration (wheat, barley and soya with added vitamins and minerals) and demineralized water. The HS diet consisted of a high NaCl-balanced ration (low-NaCl ration with 1% NaCl wt/wt added) and 0.5% NaCl (wt/vol) drink. The detailed compositions of the rations are given by Skadhauge et al. (1983). The animals were adapted to these diets at least three weeks prior to the experiment. For resalination, hens which had been on LS diet received an initial oral load of 10 ml 0.75 M NaCl/kg body weight, and were then continued on the HS diet.

Aldosterone Injections and Blood Sampling

D-aldosterone (a kind gift of Ciba Geigy, Basle, Switzerland) was used in a dosage of 128 μg/kg body weight. Single injections were given at 6-hr intervals for the last 24 hr, with the last injection exactly 4 hr prior to the experiment. All injections were given intramuscularly and the hens remained in their cages until experiment. Blood samples were obtained by heart puncture immediately before decapitation, and plasma aldosterone was determined by radioimmunoassay as described by Arnason et al. (1986).

Electrical Measurements

For the experiment the hens were killed by decapitation. The coprodeum was taken out from the abdomen and a stripped preparation was obtained by dissecting the muscular layers with fine forceps and tweezers. This method of preparation has been established in several previous studies (Choshniak et al., 1977; Thomas & Skadhauge, 1982; Clauss et al., 1984). Up to six coprodeal preparations were mounted in Ussing chambers and incubated simultaneously in a circulating standard Krebs-phosphate buffer (values in mmol/liter): 140 Na, 8 K, 2.6 Ca, 1 Mg, 139 Cl, 1 SO_4, 8 phosphate, 15 glucose, with a pH of 7.3 aerated with pure O_2, and maintained at 38°C. The Ussing chambers had an opening area of 0.62 cm^2 and a soft O-ring on the mucosal side to minimize edge damage. For the electrical measurements 3 M KCl-agar bridges were connected to a multichannel, computer-controlled voltage clamp. The 3 M KCl did not significantly increase the bath K concentration after a 2 to 3-hour incubation period. The tissues were continuously short-circuited with current passed through Ag/AgCl electrodes. The computer (IBM PC) was relayed to the voltage clamp by an analog-digital and a digital-analog interface with a 12-bit resolution (IBM DACA). A computer software was developed which permitted a time-sharing on-line control and data acquisition of the six voltage clamps. The computer measured the short-circuit current (I_{sc}). The I_{sc} was reckoned positive when current flowed from the mucosal to the serosal side. For the determination of the tissue resistance (R_T) the computer posed bipolar voltage command steps (usually $\pm$ 10 mV amplitude and 300 msec duration) to the voltage clamp. Resulting clamp voltage (ΔV command) and current deflections (ΔI_{sc}) were sampled 10 msec before offset of the pulses. The current transients were regularly inspected on an oscillosope (Tektronix) to avoid interference with membrane time constants or secondary ion rearrangements. Tissue resistance was calculated according to

$$R_T = \Delta V \text{ command}/\Delta I_{sc}(\Omega \cdot \text{cm}^2). \quad (1)$$

In order to eliminate any effects of a possible rectification, the positive and negative current deflections were pooled, and the mean deflection was used for the calculation. The theoretical open-circuit potential difference (V_T) was then calculated according to Ohm's law:

$$V_T = R_T \cdot I_{sc}(\text{mV}). \quad (2)$$

V_T was calculated with the serosal side as reference. Hence it was negative when I_{sc} was positive and current (Na absorption) flowed from the mucosal to the serosal side. The computer controlled the six chambers independently in a time-sharing mode and corrected the values for series resistance, which was measured before mounting of the tissue. V_T, I_{sc}, and R_T were printed out at regular intervals on a matrix printer, and the current tracings of selected chambers were registered on a two-channel chart recorder (Kipp and Zonen BD 9).

Experimental Protocol

After an initial period of stabilization of the short-circuit current, 0.1 mM amiloride (Merck, Sharp & Dohme) was added to the mucosal side, to block Na absorption and I_{sc} totally. After a short time period of about 4 min, 7 mM theophylline (Sigma) was added to both sides, with amiloride still present. Theophylline is known to stimulate electrogenic Cl secretion and therefore to increase the I_{sc} (Frizzell et al., 1979). After onset of stimulation and a stabilizing period of about 16 min, 0.1 mM bumetanide (Leo Pharmaceuticals) was added to the serosal side with the other drugs

still present. Bumetanide is known to inhibit electrogenic Cl secretion by blocking the basolateral Na-Cl cotransport (Heintze, Stewart & Frizzell, 1983). An example of this experimental procedure on the I_{sc} is shown in Fig. 5.

For determination of the ionic basis of the I_{sc} under various experimental conditions, the unidirectional fluxes of sodium and chloride were measured with isotopes as described previously (Choshniak et al., 1977). For that purpose 4 μCi of ^{22}Na and 8 μCi of ^{36}Cl (Amersham) were added during the equilibration period. After an isotope equilibration of 30 min, samples (10% of total volume) were taken every 10 min from the "cold" side for 30 min, and the solution replaced to maintain a constant volume. The activity of the "hot" side was determined before and after this period. ^{22}Na activity was measured in a gammacounter, and ^{36}Cl activity in a betacounter, subtracting the beta radiation of ^{22}Na calculated from the corresponding measurement of gamma activity. Accumulated unidirectional fluxes of each ion were then calculated using standard equations. Net fluxes of each experimental condition were calculated as the difference of the pooled mucosa-serosa, and serosa-mucosa fluxes.

Morphological Studies

Light Microscopy

To visualize and detect the site of Cl transport we employed an AgCl-precipitation technique (Whitear, 1975; Voute & Meier, 1978; Willumsen & Larsen, 1986). For this purpose stripped mucosal tissue from coprodeum was treated in the following way: 1) incubation for 30 min at 38°C in oxygenated standard Krebs phosphate glucose medium, 2) rinsing twice in two separate baths of a near isotonic Na-gluconate solution, without phosphate or buffer, 3) incubation (exposure of mucosal and serosal side) for 3 min in the Na-gluconate with 0.25% $AgNO_3$, 4) rinsing twice in the Na-gluconate solution, 5) blotting lightly on filter paper, 6) mounting of an area of 0.8 to 1 cm^2 at an objective slide with cover glass, 7) exposure to light for 10 min, 8) examination of the localization of precipitation of Ag, presumably as Cl, as shown by the reduction of free silver later the same day by light microscopy.

By this procedure we hoped to detect on the mucosal surface the leak of Cl out of the intercellular space and from particular cells. Silver precipitation gave these cells a black surface, and they were called "chloride cells." The number of Cl cells was quantified by counting the number of dark cell in 20 squares outlined by an ocular grid, each comprising an area of 625 μm^2. Usually a total of 50 to 100 dark cells were counted from each preparation. No precipitation occurred on the serosal side.

We employed a whole series of experiments with varying $AgNO_3$ concentrations and times of incubation and light exposure in order to detect any time or concentration dependence, which would interfere with the method and lead to artificial results. This was not the case and we could regularly detect Cl cells under the proper experimental conditions (LS diet). They were, however, not present under HS conditions, although the intercellular space was clearly precipitated under these conditions.

Staining methods (methylene blue, formazan reaction) used for supravital marking of mitochondria-rich tissue were tried without giving conspicuous differences between individual cells or differences depending on NaCl content of the diet, or did not stain at all (dimethyl-amino-styryl-ethyl-pyridinium-iodine).

Table 1. Mean values of plasma aldosterone concentration under the various experimental conditions[a]

		Plasma-aldosterone concentration (pg/ml)	
	n	LS diet	HS diet
Control	11	277 ± 62[b]	32 ± 5
Aldosterone 4 hr	4	—	436 ± 94[c]
Aldosterone 24 hr	4	—	395 ± 72[c]
Resalination 24 hr	4	85 ± 31[c]	—
Resalination 48 hr	4	42 ± 6[c]	—
Resalination 72 hr	12	44 ± 10[c]	—

[a] Values are means ± SEM, n = number of hens.
[b] Significantly different from HS diet ($P < 0.05$).
[c] Significantly different from control ($P < 0.05$).

Electron Microscopy

Coprodeal tissue for electron microscopy was prepared by routine methods. Small tissue blocks (1 mm^3) were immersion fixed rapidly after death in 3% glutaraldehyde in 0.1 M cacodylate buffer with 7% sucrose and 1 mM calcium chloride for 4 hr at 5°C, rinsed in 0.1 M cacodylate buffer and post-fixed in 1% osmium tetraoxide in the same buffer at 5°C for 2 hr. Some tissue blocks were block stained with 0.5% aqueous uranyl acetate for 1.5 hr at room temperature, immediately before dehydration and embedding in epon. Epon sections for light microscopy were stained with toluidine blue. For electron microscopy ultrathin sections of selected areas were stained with uranyl acetate and lead citrate before being examined in a Jeol transmission electron microscope 1200 EX at 60 kV.

Statistical Analysis

Results are expressed as mean ± SE, with n equaling the number of tissues from at least four hens. Statistical analyses were done by the Student's t-test with a significance level of $P \leqq 0.05$.

Results

Plasma Aldosterone Concentrations under the Various Experimental Conditions

The values for plasma aldosterone concentrations for the different experimental manipulations are given in Table 1. Hens on a regular (HS) diet had a mean plasma aldosterone concentration of 32 ± 5 pg/ml. Adaptation to the LS diet augmented the plasma level significantly to 277 ± 62 pg/ml. A single injection of 128 μg/kg D-aldosterone given to hens on HS diet exactly 4 hr before the experiment, increased the mean plasma level significantly to 436 ± 94 pg/ml. Multiple injections (4 × 128 μg/kg D-

Table 2. Transepithelial potential difference (V_T), tissue resistance (R_T), and short-circuit current (I_{sc}) of HS- and LS-tissues under the various experimental conditions[a]

	n	V_T (mV)	R_T ($\Omega \cdot cm^2$)	I_{sc} ($\mu eq/cm^2 \cdot hr$)	Δ-I_{sc} ($\mu eq/cm^2 \cdot hr$)
			HS diet		
Control	31	-0.8 ± 0.3	216 ± 13	0.19 ± 0.04	—
Amiloride	31	-0.7 ± 0.3	224 ± 13	0.16 ± 0.04	-0.01 ± 0.01
Theophylline	31	-2.7 ± 0.5^b	219 ± 15	0.62 ± 0.09^b	0.43 ± 0.09
Bumetanide	31	-1.6 ± 0.3	248 ± 16	0.43 ± 0.05^b	-0.20 ± 0.08
			LS diet		
Control	40	-28.7 ± 1.6^c	88 ± 5^c	12.40 ± 0.57^c	—
Amiloride	40	$2.9 \pm 0.6^{b,c}$	$146 \pm 9^{b,c}$	$-0.78 \pm 0.16^{b,c}$	-13.12 ± 0.61^c
Theophylline	40	$-9.2 \pm 0.9^{b,c}$	$91 \pm 6^{b,c}$	$4.62 \pm 0.51^{b,c}$	5.21 ± 0.53^c
Bumetanide	40	$-3.8 \pm 0.6^{b,c}$	$121 \pm 7^{b,c}$	$1.39 \pm 0.23^{b,c}$	-3.02 ± 0.41^c

[a] Values are means ± SEM, n = number of tissues.
[b] Significantly different ($P < 0.05$) from the preceeding experimental group.
[c] Significantly different ($P < 0.05$) from HS group.

aldosterone) given to hens on HS diet, within the last 24 hr before the experiment did not increase the mean plasma level further, which stayed at 395 ± 72 pg/ml. Resalinating animals on LS diet resulted in a prompt fall in the mean plasma level, which was already significantly lowered after 24 hr to 85 ± 31 pg/ml and decreased further within the next two days. After 72 hr, the mean plasma level had almost reached the value for long-term HS-adapted hens. The aldosterone levels during resalination (24, 48, and 72 hr) were not statistically different from the aldosterone levels in the long-term HS-diet hens. It is obvious from these results that the dietary and hormonal manipulations affect the plasma aldosterone levels in a very fast manner, similar to the earlier results reported by Clauss et al. (1984).

Transepithelial Electrical Properties under Various Dietary and Experimental Conditions

The transepithelial potential difference (V_T), tissue resistance (R_T) and short-circuit current (I_{sc}) measured under control conditions corresponded essentially to the values shown previously by several investigations (Skadhauge et al., 1983; Clauss et al., 1984). Table 2 shows that under HS conditions V_T and I_{sc} are low in the vicinity of zero and that R_T is high with a mean value of 216 ± 13 $\Omega \cdot cm^2$. This indicates very little Na transport and a tight epithelium. In contrast under LS conditions, V_T and I_{sc} increased significantly to very high levels, whereas R_T decreased significantly to about one third. These values verify that the LS diet has stimulated electrogenic Na transport tremendously as shown previously (Choshniak et al., 1977; Clauss et al., 1984). Addition of 0.1 mM amiloride to the mucosal side had no significant effect in the HS group, whereas it abolished V_T and I_{sc} in the LS group and nearly doubled R_T. Adding theophylline (7 mM) to both solutions had only a minor effect in the HS group. In the LS group, however, it stimulated significantly V_T and I_{sc}, presumably by an onset of Cl secretion, and decreased R_T. Bumetanide (0.1 mM serosal) nearly reversed this effect in the LS group, whereas it had little effect in the HS group. These data suggest that the different dietary manipulations, which lead to profound alterations in plasma-aldosterone (*see* Table 1) have not only influences on electrogenic Na absorption, but do also markedly influence electrogenic Cl secretion.

Transepithelial Fluxes of Sodium and Chloride under the Dietary Conditions

In order to confirm this interpretation of the electrical data, we performed on some tissues a series of isotope measurements of unidirectional Na- and Cl-fluxes. These data are presented in Table 3 and show that our interpretation is correct. Whereas under HS conditions the net fluxes of Na and Cl are not significantly different from zero, the LS diet causes a significant increase in net Na absorption entirely due to a stimulation of the *m*-to-*s* Na flux. This Na absorption is significantly blocked by amiloride. Under LS condition theophylline (after amiloride) elicites a net Cl secretion of about 4 $\mu Eq/cm^2 \cdot hr$, solely by a significant increase in J_{sm}^{Cl}. This effect is closely matched by the I_{sc}. Bumetanide inhibits this electrogenic Cl secretion significantly. In addition it can be seen from Table 3 that theophylline may have secondary effects on Na transport, because J_{ms}^{Na} significantly decreased after theoph-

Table 3. Short-circuit (I_{sc}) and the unidirectional fluxes of sodium and chloride of HS- and LS-tissues under the various experimental conditions[a]

	I_{sc} (μeq/cm^2 · hr)	J^{Na}_{net} (μeq/cm^2 · hr)	J^{Na}_{ms} (μeq/cm^2 · hr)	J^{Na}_{sm} (μeq/cm^2 · hr)	J^{Cl}_{net} (μeq/cm^2 · hr)	J^{Cl}_{ms} (μeq/cm^2 · hr)	J^{Cl}_{sm} (μeq/cm^2 · hr)
			HS conditions (n = 10, n fluxes = 5)				
Control	0.04 ± 0.06	−0.8 ± 0.8	2.3 ± 0.7	3.1 ± 0.5	−0.7 ± 0.9	3.1 ± 0.8	3.8 ± 0.5
Amiloride	0.05 ± 0.06	−0.8 ± 0.6	2.3 ± 0.5	3.2 ± 0.3	−0.8 ± 0.9	3.4 ± 0.7	4.2 ± 0.6
Theophylline	0.62 ± 0.09[b]	−0.2 ± 0.8	2.6 ± 0.5	2.8 ± 0.6	−0.4 ± 1.1	3.5 ± 0.8	3.8 ± 0.6
Bumetanide	0.29 ± 0.04[b]	0.2 ± 0.8	2.6 ± 0.7	2.5 ± 0.3	0.1 ± 1.3	3.6 ± 1.2	3.5 ± 0.5
			LS conditions (n = 16, n fluxes = 8)				
Control	13.2 ± 1.0[c]	15.5 ± 1.6[c]	17.8 ± 1.5[c]	2.3 ± 0.5	0.2 ± 1.1	3.7 ± 0.6	3.5 ± 1.0
Amiloride	−1.0 ± 0.2[b]	2.6 ± 1.1[b]	6.5 ± 0.6[b,c]	3.9 ± 1.0	−0.3 ± 1.4	4.8 ± 1.0	5.0 ± 1.1
Theophylline	6.1 ± 0.9[b,c]	0.02 ± 0.9	3.1 ± 0.6[b]	3.1 ± 0.7	−4.2 ± 1.8[b,c]	4.5 ± 1.2	8.7 ± 1.2[b,c]
Bumetanide	1.8 ± 0.3[b,c]	0.6 ± 0.9	3.5 ± 0.7	2.9 ± 0.7	−1.7 ± 1.2[b]	4.1 ± 1.0	5.8 ± 0.7

[a] Values are means ± SEM; n refers to tissues in electrical measurements (I_{sc}) and tissue pairs in flux measurements.
[b] Significantly different ($P < 0.05$) from the preceding experimental group.
[c] Significantly different ($P < 0.05$) from HS group.

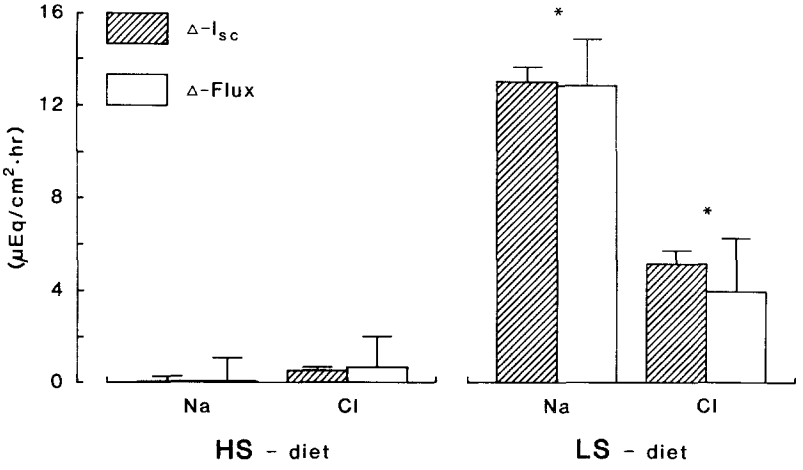


Fig. 1. Ion-specific short-circuit current (Δ-I_{sc}) and ion-specific net fluxes for sodium (Δ-amiloride), and for chloride (Δ-theophylline), under HS conditions and LS conditions. Note the excellent agreement between the electrical measurement (Δ-I_{sc}) and the isotopic measurements (Δ-flux) under all experimental conditions. Measurements under LS conditions are significantly higher than under HS conditions

ylline application. This finding is surprising and cannot be explained, because 0.1 mM amiloride should have already completely blocked the Na channels, and thereby Na absorption. We have summarized the effects of amiloride (ΔNa) and theophylline (ΔCl) on I_{sc} and net fluxes in Fig. 1 in order to illustrate the electrogenic nature of the system. The ΔI_{sc} closely matches the change in net fluxes of Na and Cl and is therefore a good measurement of the effect. In addition Fig. 1 demonstrates the difference between the two dietary states, indicating significant effects only in the LS group.

Cytological Features

Location of "Chloride Cells"

The location of free silver on the mucosal surface of coprodeum from high- and low-NaCl-diet hens will appear in Fig. 2. The outlines of the cells are clearly visible presumably due to diffusion of Cl^- from intercellular spaces. Some cells, "chloride cells," are intensively stained in tissues from low-NaCl hens (Fig. 2*A*) and these cells are absent from the coprodeum of high-NaCl-diet hens (Fig. 2B).

The chloride cells disappeared gradually following the time after resalination and their appearance could be induced by 24-hr treatment of aldosterone to hens on high-NaCl diet, but not following 4-hr aldosterone treatment. The quantification of the cell number is reported in Table 4. The total number of cells was on both high- and low-NaCl diet around 20 per ocular grid, giving a mucosal surface area per cell of around 30 μm^2. In coprodeum of low-NaCl diet around 15% of the cells were represented by chloride cells.

Incubations were also tried in the presence of the concentrations as used in the Ussing chamber

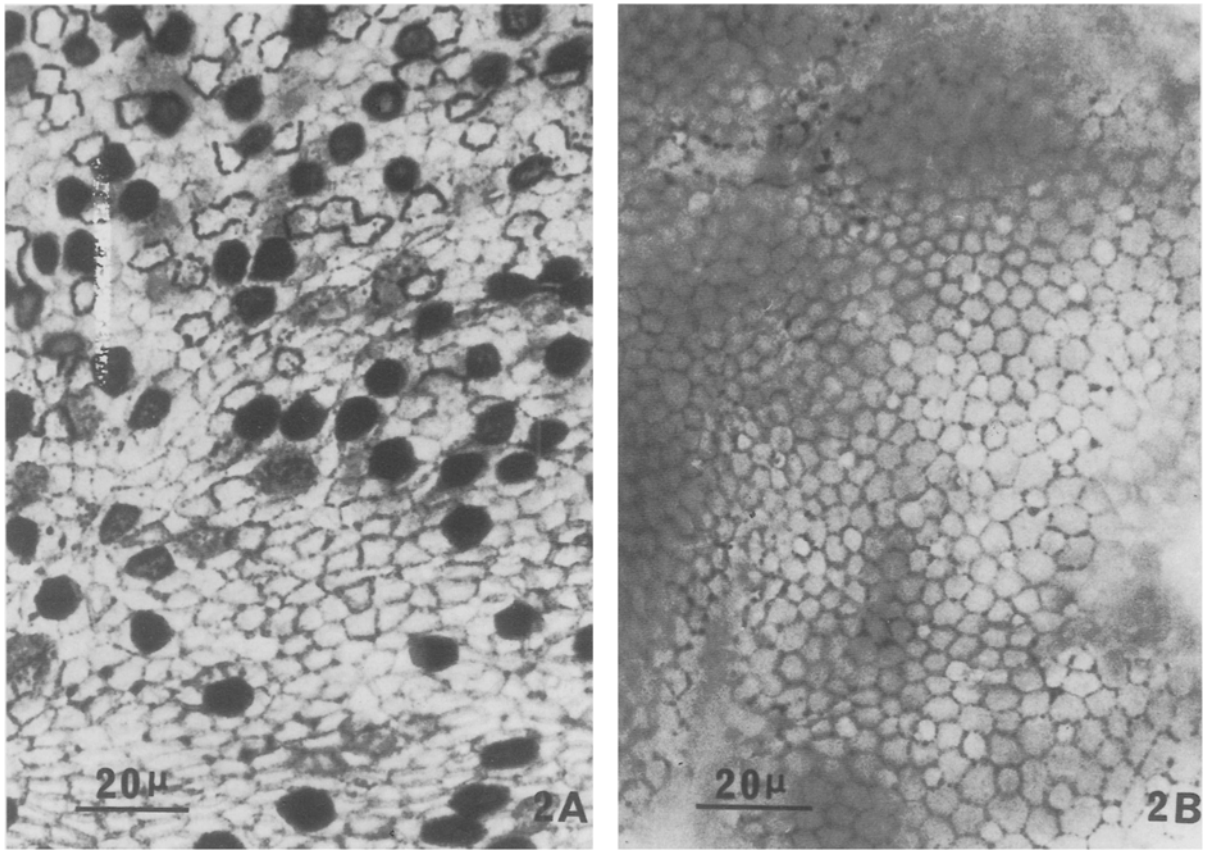


Fig. 2. Localization of chloride cells by the AgCl-precipitation method (mag. 750×, bar represents 20 μm). (*A*) Appearance of dark cells in the LS state. (*B*) Absence of dark cells in the HS state. Note the precipitation at the cell boundaries in both states.

Table 4. Density of "chloride cells"[a]

Diet and treatment	No. of cells/area[b]	% of LS state
Low NaCl (LS)	3.13 ± 0.37	100
High NaCl (HS)	0	0
HS + 4-hr Aldo	0	0
HS + 24-hr Aldo	1.88 ± 0.71	60
LS → HS (24-hr resal.)	3.03 ± 0.48	97
LS → HS (48-hr resal.)	1.03 ± 0.43[c]	33
LS → HS (72-hr resal.)	0.48 ± 0.29[c]	15

[a] Values are means ± SEM, n = 10 to 12 in LS and HS, n = 4 elsewhere.
[b] Number of stained cells per 625 μm^2 surface area; total cell count was approx. 20 per 625 μm^2.
[c] Significantly different ($P < 0.05$) from LS group.

study of amiloride, theophylline amiloride + theophylline, and amiloride + theophylline + bumetanide, but no conspicuous difference was observed in the intensity of staining or the number of chloride cells; neither did incubation with or without indacrinone (1 mM), a chloride channel blocker, result in any difference.

Electron Microscopy

The coprodeal epithelium from LS-diet hens showed a cell surface with well developed microvilli. The few areas with cells intensively stained with toluidine blue (the dark cells) were selected for electron microscopy (Fig. 3*A*). These cells were electron-dense cells and contained many mitochondria as well as the neighboring lighter cells. The microvilli of the dark cells seemed to be slightly higher than of the neighboring light cells.

The epithelium from HS-diet hens showed a surface almost depleted of microvilli (Fig. 3*B*). In the apical part of the cells several membrane-bound granules with varying density of their content were seen. Some of the granules were in close contact with the luminal surface (Fig. 3*B*, inset). The mitochondria in epithelial cells from LS-diet hens seemed to be larger than from HS-diet hens.

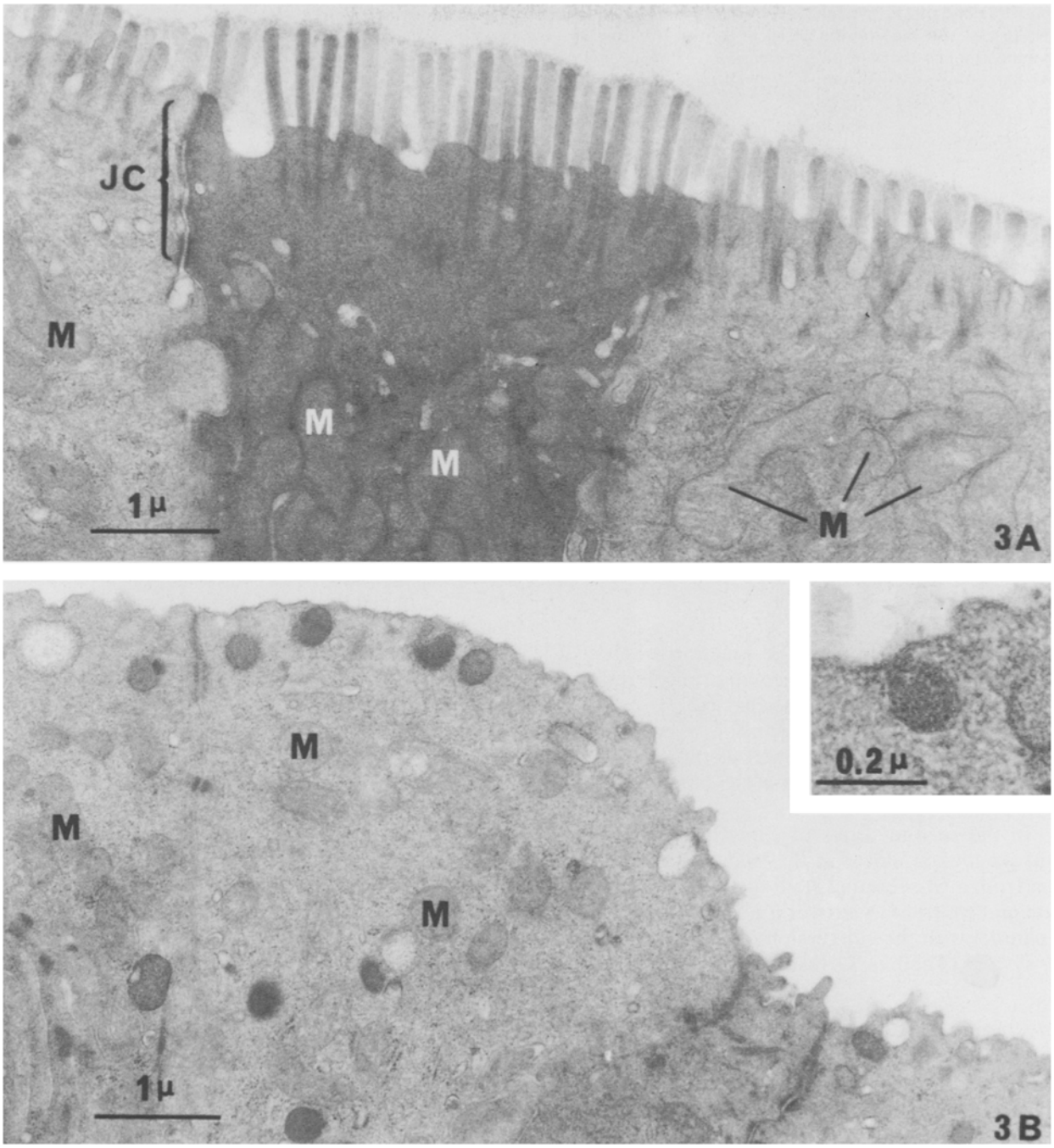


Fig. 3. Electron micrograph of the apical part of coprodeal epithelium, perpendicular to the cell surface. (*A*) The epithelium from a low-NaCl hen shows its densely arranged microvilli. The electron-dense cell in the middle as well as the neighboring cells contain many mitochondria (*M*). Notice the well-developed glycocalyx of the microvilli. Junctional complex (*JC*). 20,000×; bar represents 1 μm. (*B*) The epithelium from a high-NaCl hen. Several mitochondria are seen. Membrane-bound granules with variable density of their content are seen close to the luminal surface. 20,000×; bar represents 1 μm. *Inset*. Here the membrane of a granule is closely related to the apical plasma membrane. 87,500×; bar represents 0.2 μm

These observations together with the electrical and the flux data led us to the suggestion that dietary manipulation from a high- to a low-salt diet provokes the adaptation or development of cells which may secrete Cl into the coprodeal lumen. The question whether these cells are specialized groups, or if a larger amount of the principal cells are involved, needs further investigation.

Table 5. Transepithelial potential difference (V_T), tissue resistance (R_T), and short-circuit current (I_{sc}) of HS tissues after aldosterone injections (4 hr before experiment and 3 times during 24 hr before the experiment) under the various experimental conditions[a]

	n	V_T (mV)	R_T ($\Omega \cdot cm^2$)	I_{sc} ($\mu eq/cm^2 \cdot hr$)	Δ-I_{sc} ($\mu eq/cm^2 \cdot hr$)
		4 hours Aldosterone (one injection)			
Control	13	−2.3 ± 0.5[b]	238 ± 32	0.36 ± 0.07[b]	—
Amiloride	13	−0.1 ± 0.4[c]	259 ± 38	0.01 ± 0.07[c]	−0.35 ± 0.11[b]
Theophylline	13	−5.4 ± 0.7[c]	218 ± 26	0.99 ± 0.13[c]	0.98 ± 0.19[b]
Bumetanide	13	−2.2 ± 0.4[c]	259 ± 34	0.31 ± 0.02[c]	−0.68 ± 0.13[b]
		24 hours Aldosterone (three injections)			
Control	16	−8.6 ± 1.8[b]	177 ± 16	1.93 ± 0.35[b]	—
Amiloride	16	0.5 ± 0.5[c]	199 ± 20	−0.19 ± 0.10[c]	−2.12 ± 0.37[b]
Theophylline	16	−5.9 ± 0.8[c]	155 ± 13	1.48 ± 0.14[c]	1.67 ± 0.14[b]
Bumetanide	16	−2.7 ± 0.5[c]	213 ± 19	0.48 ± 0.06[c]	−1.00 ± 0.14[b]

[a] Values are means ± SEM, n = number of tissues.
[b] Significantly different from HS group (data shown in Tables 2 and 3).
[c] Significantly different from the preceding experimental group.

Investigations of the Time Course and the Mediator

In order to look closer at the functional behavior and the time course of the cellular adaptation we performed two series of experiments:

In the first series (aldosterone injections) we tried to stimulate electrogenic Cl secretion in coprodeum of HS hens and establish the putative mediator.

In the second series of experiments (resalination) we looked closer at the switch of the epithelium from a Cl-secreting state to a non-Cl-secreting state and we tried to answer if this conversion goes in parallel with the conversion of the Na-transporting system (Thomas & Skadhauge, 1982).

Aldosterone Injection (Adaptation)

Injecting HS hens with 128 μg/kg D-aldosterone 4 hr before the experiment resulted in a small but significant increase in V_T and I_{sc} (Table 5) compared to the HS-control group. R_T remained unchanged. Amiloride inhibited the aldosterone-induced I_{sc} significantly to zero, and theophylline elicited a current response of about 1 $\mu eq/cm^2 \cdot hr$, which was inhibited 7% by bumetanide. Injecting aldosterone 4 times over the last 24 hr prior to the experiment increased the response further (Table 5). There was a baseline I_{sc} of 1.9 $\mu eq/cm^2 \cdot hr$ which was inhibited by amiloride to slight negative values. The theophylline-induced increase in I_{sc} was further augmented to about 1.7 $\mu eq/cm^2 \cdot hr$ and significantly inhibited by bumetanide. From these experiments we conclude that aldosterone is a likely mediator of the alteration in electrogenic Cl secretion in hen coprodeum. In Fig. 4 we compare the modulation of the Na-dependent I_{sc} (ΔI_{sc}-amiloride) and of the Cl-dependent I_{sc} (ΔI_{sc}-theophylline) of the several experimental groups ranging from the HS state over the aldosterone experiments to the LS state. It seems likely that either by exogenous application of aldosterone which elevates the plasma aldosterone level to about 400 pg/ml (*see* Table 1) or by a chronic LS diet which elevates plasma aldosterone levels to 277 pg/ml, both Na-absorption systems and Cl-secretory systems may be induced. Figure 4 displays, however, that whereas the stimulation of the two transport systems is somewhat similar under short-time exogenous aldosterone exposure, it is significantly increased in favor of Na under LS conditions.

Resalination Experiments (Readaptation)

In order to study the time course of the readaptation of the coprodeal tissue to a HS diet we resalinated LS hens and performed experiments after 24, 48 and 72 hr. The results are shown in Table 6. After 24 hr the I_{sc} is already close to zero, and there is only a slight amiloride-inhibition present. Theophylline, however, stimulates the I_{sc} significantly by about 2 $\mu eq/cm^2 \cdot hr$, an effect significantly inhibited by bumetanide.

After 48 hr there is no amiloride effect detectable but still a theophyline effect of 0.9 $\mu eq/cm^2 \cdot hr$, which is also maintained after 72 hr. Figure 5 compares original I_{sc} tracings of resalination experi-

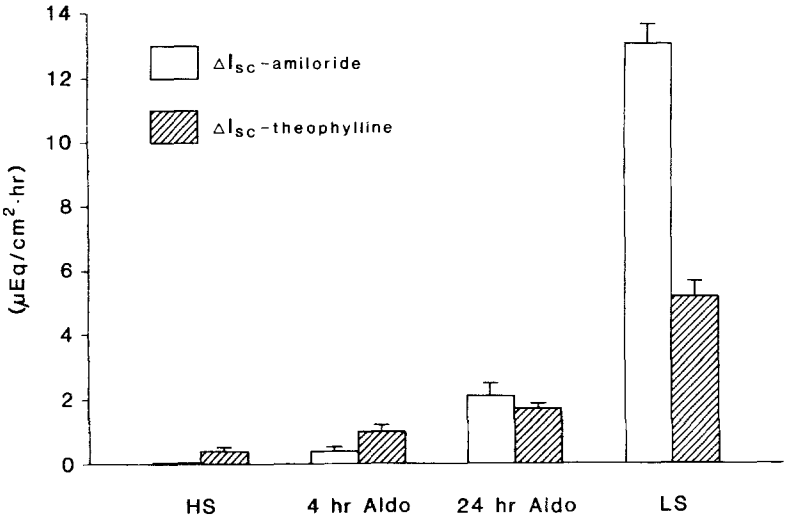


Fig. 4. Ion-specific short-circuit current for sodium (ΔI_{sc}-amiloride), and for chloride (ΔI_{sc}-theophylline) over a variety of experimental conditions, spanning from HS diet to measurements after 4-hr aldosterone treatment, 24-hr aldosterone treatment, to LS conditions

Table 6. Transepithelial potential difference (V_T), tissue resistance (R_T), and short-circuit current (I_{sc}) of LS hens on resalination after 24, 48 and 72 hr, under the various experimental conditions[a]

	n	V_T (mV)	R_T ($\Omega \cdot cm^2$)	I_{sc} ($\mu eq/cm^2 \cdot hr$)	Δ-I_{sc} ($\mu eq/cm^2 \cdot hr$)
			24 hours resalination		
Control	13	0.7 ± 1.1	140 ± 13	−0.30 ± 0.31	—
Amiloride	13	3.5 ± 0.6[b]	145 ± 13	−1.06 ± 0.17[b]	−0.76 ± 0.28
Theophylline	13	−2.0 ± 0.8[b]	108 ± 11[b]	0.97 ± 0.32[b]	1.96 ± 0.33
Bumetanide	13	1.3 ± 0.6[b]	164 ± 13[b]	−0.38 ± 0.15[b]	−1.11 ± 0.32
			48 hours resalination		
Control	16	1.9 ± 0.5	145 ± 8	−0.50 ± 0.12	—
Amiloride	16	2.8 ± 0.4	148 ± 8	−0.72 ± 0.11	−0.22 ± 0.10
Theophylline	16	−0.5 ± 0.4[b]	129 ± 7[b]	0.16 ± 0.10	0.88 ± 0.09
Bumetanide	16	−0.3 ± 0.5	207 ± 18[b]	0.09 ± 0.09	−0.17 ± 0.10
			72 hours resalination		
Control	13	3.2 ± 0.4	144 ± 9	−0.82 ± 0.10	—
Amiloride	13	3.5 ± 0.4	147 ± 9	−0.89 ± 0.10	−0.07 ± 0.08
Theophylline	13	0.0 ± 0.2[b]	115 ± 6[b]	0.08 ± 0.08[b]	0.81 ± 0.10
Bumetanide	13	1.1 ± 0.5[b]	180 ± 14[b]	−0.34 ± 0.16[b]	−0.23 ± 0.14

[a] Values are means ± SEM, n = number of tissues.
[b] Significantly different ($P < 0.05$) from the preceding experimental group.

ments, showing the LS state, 24-hr resalination, and the HS state. It is clearly obvious that resalination not only suppresses Na current within 24 hr, but also decreases the rate of electrogenic Cl secretion. Figure 6 compares the time courses of these effects by giving the percent of I_{sc} alteration by amiloride (Na current) or theophylline (Cl current). It is obvious that the decrease in both is caused by resalination but that they do not go in parallel, i.e. after 24 hr the Na current is almost zero, whereas there is still about 40% Cl secretion. We conclude from these two experimental variations (adaptation and readaptation) that both conductive Na and Cl transport may be modulated by dietary changes elicited directly or indirectly by aldosterone.

Discussion

Sodium and Chloride Transport

Modulation of Sodium Transport

The stimulatory effect of dietary manipulations or exogenous aldosterone applications on coprodeal sodium transport has already been demonstrated by Thomas and Skadhauge (1982). The time course of single aldosterone injections has been further investigated by Clauss et al. (1984). These investigators demonstrated also the I_{sc} decline after resalination, in spite of delayed aldosterone injections. It is clear from these studies that aldosterone not only has a

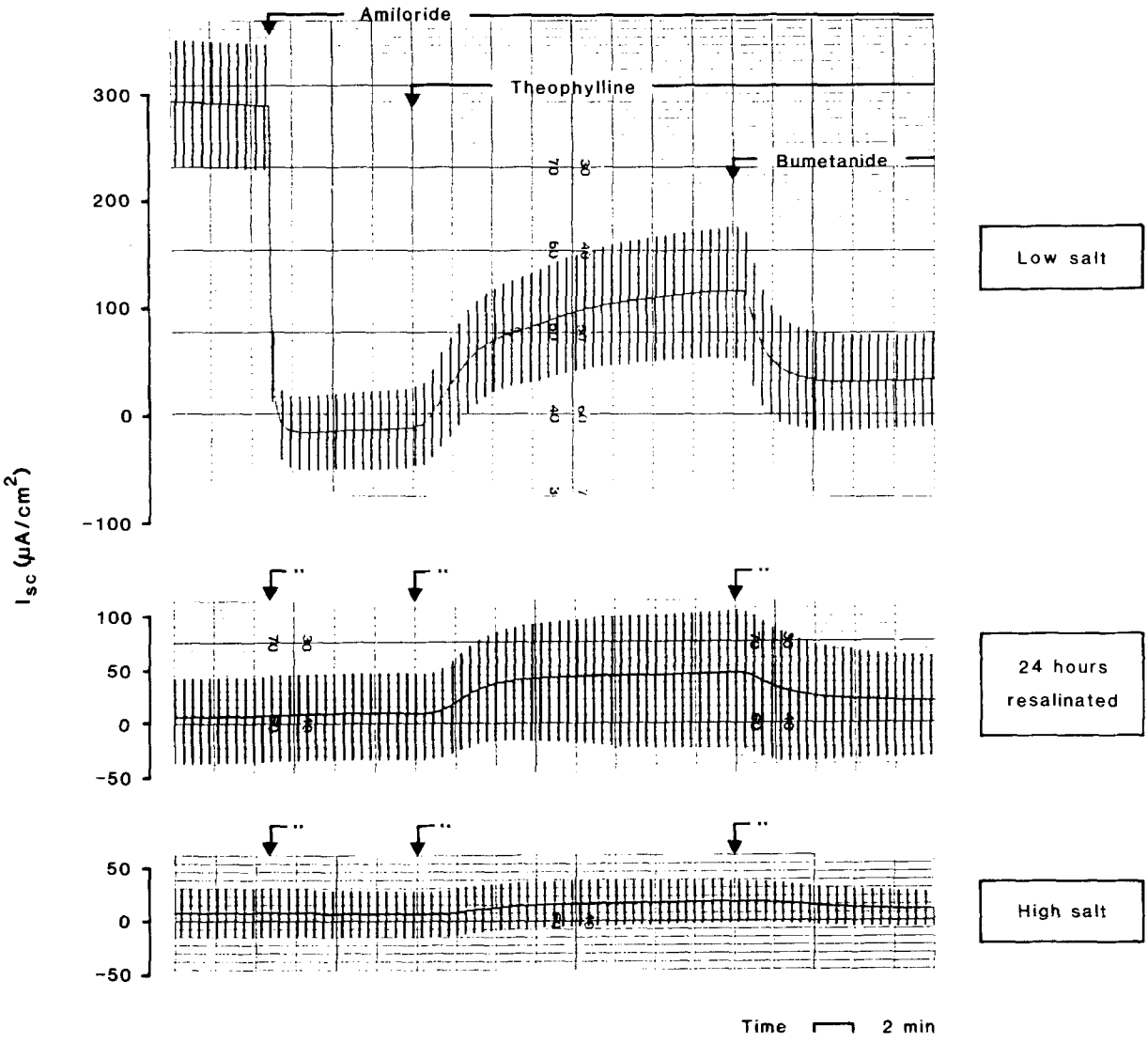


Fig. 5. Original tracings of short-circuit current in three experiments. The application of the appropriate drugs is indicated by arrows, and is similar in all experiments. Note the disappearance of the amiloride effect, and the decrease of the theophylline effect by the switch from the LS state to the HS state

short-term effect, maximal within 4 to 8 hr, but that other mediators or secondary cellular adaptational processes must be involved, to result in the maximal stimulation of I_{sc}, seen in hens kept on an LS diet. Our present results on sodium transport fully confirm these earlier studies and indicate an epithelial adaptation after long-term aldosterone application.

Earlier studies by Christensen and Bindslev (1982) demonstrated that Na entry across the apical membrane of the coprodeal cells occurs by diffusion through selective Na channels. These seem to originate from preformed cytoplasmic material (Cuthbert et al., 1982), which is incorporated as rod-shaped particles in the apical cell membrane (Eldrup, Møllgard & Bindslev, 1979). The electrical properties of the apical Na conductance are analyzed in detail by a recent investigation, as it shows that the Na permeability is stimulated by mineralocorticoids, as well as glucocorticoids, and is inhibited by adrenal antagonists like spironolactone (Clauss et al., 1987). The intracellular Na activity is not varied in spite of a huge change in apical Na permeability. This shows that apical and basolateral Na-transporting systems are closely linked in order to allow a continuous flush-through of sodium, and to avoid any disturbances of the internal electrolyte composition (Schultz, 1984).

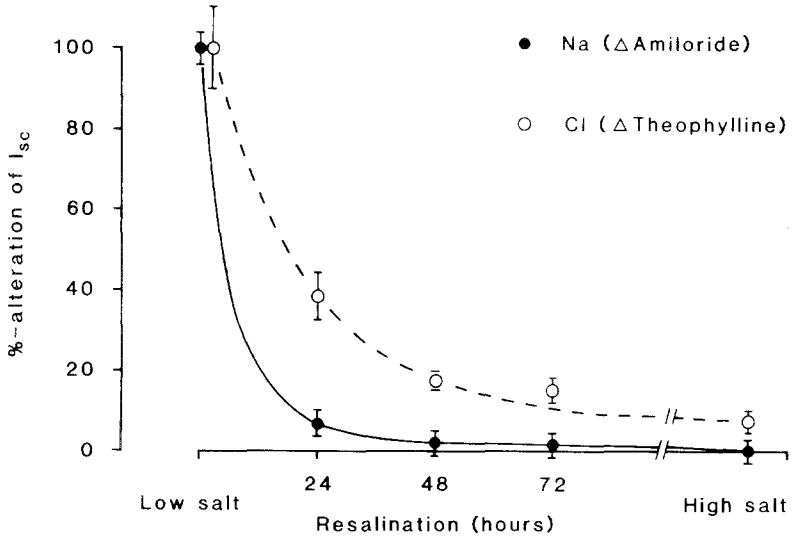


Fig. 6. Time course of the ion-specific short-circuit current (shown as % of LS state) in the resalination experiments over 72 hr. Note the different time course of the Na current and the Cl current

Modulation of Chloride Transport

In earlier studies on sodium and chloride transport across coprodeal epithelium, Choshniak et al. (1977) found unity of unidirectional chloride fluxes across this epithelium in both dietary states. They noted, however, an approximately doubling of the fluxes in the LS state. We cannot confirm this observation in our study, as we note similar rates of Cl transport in both dietary states in the nonstimulated preparation.

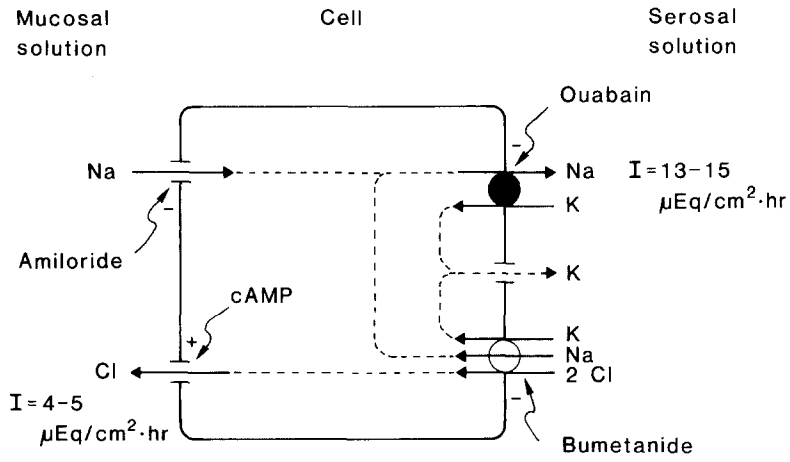
Although electrogenic Cl secretion has been demonstrated in hen colon (Voldsgaard & Bindslev, 1982; Munck et al., 1984) it had not yet been observed in the coprodeum, and our present study provides the first demonstration of this mechanism in hen coprodeum. Furthermore, we observed a correlation between the magnitude of stimulation and the hormonal status of the animal. Electrogenic Cl secretion could be elicited only when the endogenous aldosterone level was high.

Our experimental sequence, arranged in the classical way of probing the cellular mechanism of the secretory process (*see* Table 3), shows that the mechanism of electrogenic Cl secretion in avian lower intestine closely resembles that of mammalian intestine (Frizzell et al., 1979). The stimulation of Cl secretion by theopylline, an inhibitor of the phosphodiesterase, reveals the involvement of the cellular cAMP system (Bindslev, 1987). It is generally believed that cAMP triggers the opening of an apical Cl conductance, which allows in turn the efflux of cellular Cl to the mucosal solution, down a favorable electrochemical gradient (Frizzell, Welsh & Smith, 1981; Shorofsky, Field & Fozzard, 1984). Recently, Greger and Schlatter (1985) and Greger, Schlatter and Gögelein (1985) have demonstrated in elegant patch-clamp experiments the induction of such Cl channels by cAMP in the apical cell membrane of the rectal gland. Further evidence for such channels derives from biochemical work on membrane vesicles, isolated from bovine tracheal epithelium (Langridge-Smith, Field & Dubinsky, 1984). Most recently, Frizzell, Rechkemmer and Shoemaker (1986), and Welsh and Liedtke (1986) demonstrated the activation process of the Cl channel by cAMP in detail.

From the marked differences in tissue resistance after theophylline stimulation in the various experimental groups (Table 2), we are tempted to conclude on a variation in apical Cl-channel density, dependent on the hormonal status. There are, however, other possible explanations; for example, alterations of intracellular Cl activity and electromotive driving force across the apical cell membrane, or impairment of the trigger system to open the Cl channel, as shown recently for pathophysiological conditions by Frizzell et al. (1986). The question about those details of the Cl-secreting mechanism cannot be answered with the technique employed in our study, and requires further experiments on isolated cells employing the patch-clamp technique.

Cl secretion was markedly inhibited by serosal bumetanide, a well-established blocker of the Na-2Cl-K cotransporter (Hoffmann, Schiodt & Dunham, 1986). Although the details and Na dependence of this system have still to be investigated, there is reasonable evidence for such a system present in the basolateral membrane of the coprodeal cells.

In summary we can adopt the classical model



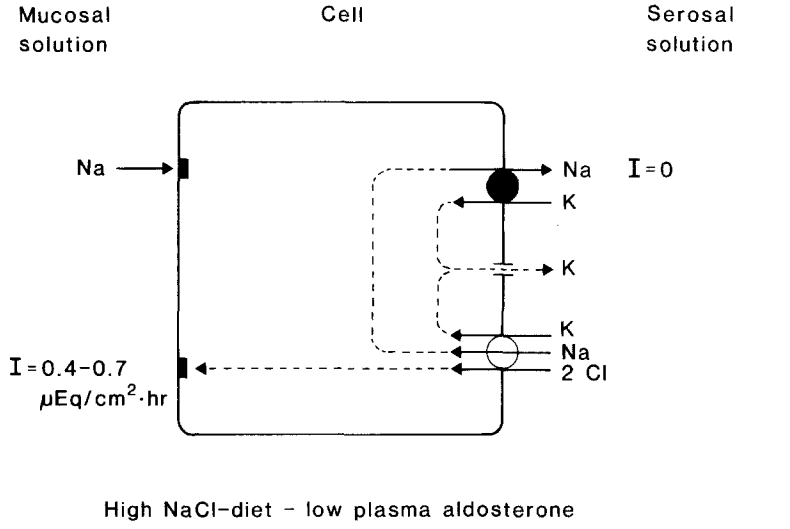


Fig. 7. Tentative model of Na- and Cl-transport under the two dietary states, which for simplicity have been drawn in the same cell. Two separate cell types with segregated transport characteristics are equally likely

for electrogenic Cl secretion from the mammalian lower intestine (Heintze et al., 1983) and present it for the two dietary states together with the mode of electrogenic sodium absorption (Fig. 7). We would like to stress, however, that this is a preliminary model, that aldosterone may have influenced other electrically silent transport systems as well, and that other mediators and secondary cellular adaptational processes may have been involved.

Cytological Features

Chloride Cells

The silver localized to the cell boundaries and to a fraction of the epithelial cells may represent precipitated AgCl or other ions with low solubility product like CO_3 and PO_4. In other Cl-secreting epithelia (Willumsen & Larsen, 1986) the method with $AgNO_3$, used in our study, demonstrated clearly Cl-transporting cells. They presented convincing evidence that the stained cells secrete chloride. If we apply this assumption to our data, the rate of Cl^--transport per each cell would be around 0.2 nA, which seems a reasonable number. On the other hand our correlation is not as good as in toad skin, and therefore the question is whether Cl^- has really diffused from the cells and the lateral intercellular spaces or has been trapped locally, for example, at the mucosal surface of cells with well-developed glycocalyx of their microvilli. It is possible that the Ag ion has augmented the permeability (Curran, 1972), or that the chloride cells arise as a conse-

quence of diffusion of Cl^- from per se particularly permeable cells. The absence of conspicuous effects of stimulators and inhibitors of Cl transport does not support the latter notion. "Cl cells" may therefore also indicate a cell type with other precipitable anions. The possibility that we have revealed a class of cells with special surface properties therefore remains open.

The capability of aldosterone treatment to induce chloride cells, supports the notion that this hormone in addition to its well-known effects on apical Na permeability and basolateral pump activity, also may induce a cellular development leading to a higher rate of Na transport in special cells. Whether the chloride cells are also mitochondria rich, i.e., having the ability to "fuel" a high rate of transcellular Na transport, and in fact do represent a class of cells with more apical transport sites remains to be elucidated.

The Cytological Change

The most prominent cytological changes in the coprodeal epithelium from low- to high-NaCl diet were, respectively, a change in size and number of microvilli of the apical plasma membrane from many to very few, and in the size and amount of membrane-bound bodies from few to many in their apical cytoplasm (*see* Fig. 3). These observations are in agreement with earlier investigations of apparently less well-fixed material by Eldrup et al. (1979). However, they also found dark cells rich in mitochondria, which is not supported by our investigation.

The suggestion of specialized Cl-secreting cells, based on our experiments with $AgNO_3$ for light microscopy, as being identical to dark or electron-dense cells rich in mitochondria (Eldrup et al., 1979) has not been supported by electron microscopy in our study, as all principal cells have many mitochondria. However, it cannot be excluded either, as the dark cells often had more well-developed microvilli than the lighter cells. Further studies using different methods have to be undertaken to clarify if the high capacity of Cl secretion in coprodeum is due to specialized cells, visualized as chloride cells by the silver nitrate incubation, or if this pattern is due to an intermittent transport over the apical plasma membrane of the principal cells.

The importance of the apical plasma membrane has been indicated by experiments with applied steroids compared to low-NaCl diet (Clauss et al., 1987). The size of the mitochondria from low NaCl-fed hens seems to be larger than from high NaCl-fed hens, thus indicating an implication of the mitochondria with apical NaCl transport in coprodeal epithelium.

Conclusion

In summary we have shown that hen coprodeum posesses similar systems for sodium and chloride transport as found in the mammalian colon. There may be specialized cells, responsible for Cl secretion. The new finding of our study is the modulation of this secretory system by the NaCl content of the diet and by adrenal steroids. It remains to be investigated in further studies which cellular events are responsible for such a modulation.

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References

Andersen, V., Munck, B.G., Skadhauge, E. 1982. Sodium and chloride-dependence of theophylline induced chloride secretion across the colon of the hen. Modulation by dietary intake of sodium. *Proc. 4th Conf. ESCPB*, Bielefeld, Abstract volume:121

Arnason, S.S., Rice, G.E., Chadwick, A., Skadhauge, E. 1986. Plasma levels of arginine vasotocin, prolactin, aldosterone and corticosterone during prolonged dehydration in the domestic fowl: Effect of dietary NaCl. *J. Comp. Physiol. B.* **156**:383–397

Binder, H.J., Foster, E.S., Hayslett, J.P. 1985. Electrolyte transport in distal colon of the rat: Role of aldosterone. *Mol. Physiol.* **8**:3–10

Bindslev, N. 1987. Transporter for cAMP in Cl-secretion. *Acta Physiol. Scand.* **129**:15A

Choshniak, I., Munck, B.G., Skadhauge, E. 1977. Sodium chloride transport across the chicken coprodeum. Basic characteristics and dependence on sodium chloride intake. *J. Physiol. (London)* **271**:489–504

Christensen, O., Bindslev, N. 1982. Fluctuation analysis of short-circuit current in a warm-blooded sodium-retaining epithelium: Site current, density, and interaction with triamterene. *J. Membrane Biol.* **65**:19–30

Clauss, W., Arnason, S.S., Munck, B.G., Skadhauge, E. 1984. Aldosterone-induced sodium transport in lower intestine. Effects of varying NaCl intake. *Pfluegers Arch.* **401**:354–360

Clauss, W., Dürr, J.E., Guth, D., Skadhauge, E. 1987. Effects of adrenal steroids on Na transport in the lower intestine (coprodeum) of the hen. *J. Membrane Biol.* **96**:141–152

Curran, P.F. 1972. Effects of silver ion on permeability properties of frog skin. *Biochim. Biophys. Acta* **288:**90–97

Cuthbert, A.W., Edwardson, J.M., Bindslev, N., Skadhauge, E. 1982. Identification of potential components of the transport mechanism for Na in the hen colon and coprodaeum. *Pfluegers Arch.* **392:**347–351

Eldrup, E., Møllgard, K., Bindlsev, N. 1979. Possible sodium channels in the luminal membrane of the hen lower intestine visualized by freeze fracture. *INSERM* **85:**253–262

Frizzell, R.A., Field, M., Schultz, S.G. 1979. Sodium coupled chloride transport by epithelial tissues. Editorial review. *Am. J. Physiol.* **236:**F1–F8

Frizzell, R.A., Rechkemmer, G., Shoemaker, R.L. 1986. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science* **233:**558–560

Frizzell, R.A., Welsh, M.J., Smith, P.L. 1981. Hormonal control of chloride secretion by canine tracheal epithelium: An electrophysiologic analysis. *Ann. N.Y. Acad. Sci.* **372:**558–569

Fromm, M., Hegel, U. 1978. Segmental heterogenicity of epithelial transport in rat large intestine. *Pfluegers Arch.* **378:**71–83

Greger, R., Schlatter, E. 1985. cAMP increases the apical Cl conductance in the rectal gland of *Squalus acanthias*. *In:* Transport Processes, Iono- and Osmoregulation. R. Gilles and M. Gilles-Baillien, editors. pp. 9–13, Springer, Berlin

Greger, R., Schlatter, E., Gögelein, H. 1985. Cl-channels in the apical cell membrane of the rectal gland induced by cAMP. *Pfluegers Arch.* **403:**446–448

Halm, D.R., Frizzell, R.A. 1986. Active K transport across rabbit distal colon: Relation to Na absorption and Cl secretion. *Am. J. Physiol.* **251:**C252–C267

Heintze, K., Stewart, C.P., Frizzell, R.A. 1983. Sodium-dependent chloride secretion across rabbit descending colon. *Am. J. Physiol.* **244:**G357–G365

Hoffmann, E.K., Schiodt, M., Dunham, P. 1986. The number of chloride-cation cotransport sites on Ehrlich ascites cells measured with 3H bumetanide. *Am. J. Physiol.* **250:**C688–C693

Langridge-Smith, J.E. 1985. Na absorption and Cl secretion in bovine tracheal epithelium may take place in different cells. *J. Physiol. (London)* 71*P*

Langridge-Smith, J.E. 1986. Interaction between sodium and chloride transport in bovine tracheal epithelium. *J. Physiol. (London)* **376:**299–319

Langridge-Smith, J.E., Field, M., Dubinsky, W.P. 1984. Cl transport in apical plasma membrane vesicles isolated from bovine tracheal epithelium. *Biochim. Biophys. Acta* **777:**84–93

Loennroth, I., Munck, B.G. 1980. Effect of chloropromazine on ion transport induced by cholera toxin, cyclic AMP and cyclic GMP in isolated mucosa from hen intestine. *Acta Pharmacol. Toxicol.* **47:**190–194

Lückhoff, A., Horster, M. 1984. Hormonal regulation of electrolyte and water transport in the colon. *Klin. Wochenschr.* **625:**555–563

Munck, B.G., Andersen, V., Voldsgaard, P. 1984. Chloride transport across the isolated hen colon. *In:* Intestinal Absorption and Secretion. E. Skadhauge and K. Heintze, editors. pp. 373–386. MTP Press, Lancaster

Schultz, S.G. 1984. A cellular model for active sodium absorption by mammalian colon. *Annu. Rev. Physiol.* **46:**435–451

Shorofsky, S.R., Field, M., Fozzard, H.A. 1984. Mechanism of Cl secretion in canine trachea: Changes in intracellular chloride activity with secretion. *J. Membrane Biol.* **81:**1–9

Skadhauge, E. 1984. Introduction of an epithelium with huge variation in sodium transport and novel aldosterone effects. *In:* Intestinal Absorption and Secretion. E. Skadhauge and K. Heintze, editors. pp. 201–208. MTP Press, Lancaster

Skadhauge, E., Clauss, W., Arnason, S.S., Thomas, D.H. 1985. Mineralocorticoid regulation of lower intestinal ion transport. *In:* Transport Processes, Iono- and Osmoregulation. R. Gilles and M. Gilles-Baillien, editors. pp. 118–133. Springer, Berlin, Heidelberg

Skadhauge, E., Thomas, D.H., Chadwick, A., Jallageas, M. 1983. Time course of adaptation to low and high NaCl diets in the domestic fowl. Effects on electrolyte excretion and on plasma hormone levels (aldosterone, corticosterone and prolactin). *Pfluegers Arch.* **396:**301–307

Thomas, D.H., Skadhauge, E. 1982. Time course of adaptation to low and high NaCl diets in the domestic fowl. *Pfluegers Arch.* **395:**165–170

Voldsgaard, P., Bindslev, N. 1982. Bumetanide-sensitive chloride secretion in the hen lower intestine. Evidence for separate cellular pathways for Cl-secretion and -absorption. *Proc. 4th Conf. ESCPB*, Bielefeld, 149

Vóûte, C.L., Meier, W. 1978. The mitochondria-rich cell of frog skin as hormone-sensitive "shunt-path." *J. Membrane Biol.* **Special Issue:**151–165

Welsh, M.J., Liedtke, C.M. 1986. Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature (London)* **322:**467–470

Welsh, M.J., Smith, P.L., Fromm, M., Frizzell, R.A. 1982. Crypts are the site of intestinal fluid and electrolyte secretion. *Science* **218:**1219–1221

Whitear, M. 1975. Flask cells and epidermal dynamics in frog skin. *J. Zool. (London)* **175:**107–149

Willumsen, N.J., Larsen, E.H. 1986. Membrane potentials and intracellular Cl^- activity of toad skin epithelium in relation to activation and deactivation of the transepithelial Cl^- conductance. *J. Membrane Biol.* **94:**173–190

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