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Oxygen Cost of Chloride Transport in Perfused Rectal Gland of *Squalus acanthias*

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Summary. In the stimulated state, with glucose as substrate, oxygen uptake by the isolated perfused rectal gland is directly related to the rate of chloride secretion. Lactate production is negligible under aerobic conditions in the stimulated gland. A stoichiometric relationship exists between chloride transport and oxygen consumption, with a Cl/O_2 ratio of about 30:1, resembling that reported for sodium in mammalian kidneys. This ratio remains constant under varying degrees and modes of stimulation. The ratio does not change when the gland is induced to secrete chloride against varying electrochemical gradients by altering the concentration of urea in the perfusate.

The transport of ions by epithelial tissues is closely linked to the provision of energy and hence to cellular respiration. The stoichiometric relationships between active sodium transport and energy metabolism has been extensively studied in a variety of tissues, including red blood cells (Whittam & Ager, 1965), frog skin and toad bladder (Zerahn, 1956; Leaf & Renshaw, 1957; Danisi and Viera, 1974), intestine (Lester & Grim, 1973), gallbladder (Martin & Diamond, 1966), and kidney (Deetjen & Kramer, 1961; Lassen, Munck & Hess-Thaysen, 1961; Kiil, 1971). The energy requirements of hydrogen ion secretion have been examined in the same way, by studying the coupling between respiration and transport in the turtle bladder (Beauwens & Al-Awqati, 1976*a*), and in gastric mucosa (Davies, 1957; Forte, Adams & Davies, 1965). Only a few published experiments, however, have investigated the connection between respiration and the active transport of chloride (Zadunaisky, Lande & Hafner, 1971; Reinach, Schoen & Candia, 1977). The isolated perfused rectal gland affords an opportunity to examine the relationship between respiration and chloride transport in a secretory organ in which secretion is accomplished by active chloride transport.

The isolated perfused rectal gland of the spiny dogfish, *Squalus acanthias,* secretes chloride against an electrochemical gradient. The process requires the expenditure of energy, depends on the activity of Na-K-ATPase, and appears to involve the cotransport of chloride with sodium into rectal gland cells across basolateral membranes (Silva et al., 1977). We have studied the relationship between chloride transport and oxygen consumption in this organ in an effort to explore the energetics of the active transport of chloride.

Materials and Methods

Dogfish of either sex were taken by hook and line from Frenchman Bay, Maine, and kept in marine livecars until they were killed, usually within three days of capture. Dogfish were killed by segmental transection of the cord and the rectal glands removed via an abdominal incision.

The rectal gland artery, vein, and duct were catheterized with PE 90 tubing. The glands were then placed in an all-glass or a plexiglass and aluminum chamber kept at 15 °C with running sea water. The glands were perfused by gravity at a pressure of 40 mm Hg. The perfusion medium contained (in mM) unless otherwise specified: Na, 280; Cl, 290; K, 5; bicarbonate, 8; phosphate, 1; Ca, 2.5; Mg, 3; sulfate, 0.5; urea, 350; glucose, 5; pH 7.6 when gassed with 99% O_2 and 1% CO_2. After perfusion, all glands were weighed so that values for oxygen consumption and chloride secretion could be related to grams wet weight. In all experiments, the arterial perfusate was sampled anaerobically through a self-sealing rubber connector attached to the arterial catheter, and the venous effluent was sampled anaerobically directly from the venous catheter. Oxygen tension was measured immediately using a polarographic oxygen electrode calibrated prior to each experiment using calibrated oxygen mixtures and Radiometer® pO_2-Zero solution. Perfusate flow was measured by collecting all venous effluent in a graduated cylinder. Oxygen content in the perfusate was calculated from the solubility coefficient of oxygen adjusted

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for temperature (15 °C) and solute content. Rectal gland oxygen consumption was calculated from the arteriovenous oxygen difference and the timed flow of the perfusate. The oxygen tension was measured in the rectal gland fluid in several glands and was the same as that of the venous effluent. Since there was no difference between the oxygen tension of the venous effluent and the secreted fluid, and the volume of the secreted fluid was on the average 33 µl/min, or about 1% of the venous flow, the oxygen content of the rectal gland fluid was not considered in the calculation of oxygen consumption.

Secreted fluid was collected from the duct at 10-min intervals. Samples of arterial perfusate and venous effluent were analyzed for oxygen content at the midpoint of each 10-min period. Experiments usually lasted 60–90 min. When an abrupt change in secretion was induced, several periods were collected to ensure a new steady state before values were recorded as representative of the new condition.

The transglandular potential difference between perfusate and duct fluid was measured with 1 M KCl agar bridges previously equilibrated with perfusate solution, using an electronic voltmeter (Model 410C, Hewlett Packard) equipped with two calomel electrodes, as previously described (Silva et al., 1977).

In some of the perfusions, labeled "stimulated" in the text, varying concentrations of theophylline (usually 0.25 mM) and of dibutyryl cyclic AMP (usually 0.05 mM) were added to the perfusate. Glands in which these compounds were not added are designated "unstimulated" or "basal." Vasoactive intestinal peptide (gift of Dr. Viktor Mutt, Karolinska Institut, Stockholm), was added to the perfusate at final concentrations of 10^{-7} and 10^{-6} M.

Ouabain (K & K), furosemide (Hoechst), and ethacrynic acid (Merck, Sharpe & Dohme) were obtained in pure powder form, dissolved immediately prior to use, and added to the perfusate as a concentrated solution in volume sufficient to give the final desired concentration.

Lactic acid was measured in the venous effluent and in rectal gland tissue by enzymatic analysis (Gutmann & Wahlefeld, 1974).

Sodium and potassium in the perfusate and secreted fluid were measured using a flame photometer. Chloride was measured in the same samples by amperometric titration. The rate of sodium and chloride secretion was calculated from their concentration and rate of fluid secretion.

Results are expressed as mean ±SEM. Statistical analysis was done using standard t test or paired t test whenever applicable.

Results

General Characteristics of the Perfused Rectal Gland (Table 1)

At a perfusion pressure of 40 mm Hg, in 742 collection periods, flow averaged 1.72 ± 0.03 ml/min/g, with an arterial pO_2 of 550 ± 11.2 mm Hg, a venous pO_2 varying from 20 to 700 mm Hg and an A-V difference ranging from 5 to 700 mm Hg.

The flow of perfusate was similar in basal and stimulated glands and did not change when secretion was stimulated with cAMP, theophylline, or vasoactive intestinal peptide. Changes in oxygen uptake were accomplished by changes in the arteriovenous difference in the oxygen content of the perfusate. When glandular secretion was kept constant by perfusing with cAMP and theophylline, oxygen consumption also remained relatively constant for 60–90 min under laboratory conditions. Table 2 illustrates three typical control experiments.

Table 1. Rate of flow, arterial oxygen tension, and venous oxygen tension in isolated perfused rectal glands

	Flow (ml/min/g)	Arterial pO_2 (mm Hg)	Venous pO_2 (mm Hg)
Unstimulated	1.79 ± 0.06[a] (242)	545 ± 5.5 (242)	446 ± 6.8 (242)
Stimulated	1.69 ± 0.04 (600)	552 ± 4.21 (500)	242 ± 6.51[b] (500)

[a] Mean ±SE. Number of observations is shown in parentheses.
[b] $P < 0.001$.

Table 2. Oxygen consumption, arteriovenous oxygen difference, and rate of flow in three isolated perfused rectal glands

	Time (min)	Flow (ml/min)	A-Y difference	
			pO_2 (mm Hg)	Q_{O_2} (µM/min/g)
RG 49	10	3.2	388	0.96
	20	2.8	405	0.85
	30	3.0	425	0.96
	40	3.1	405	0.95
	50	3.0	427	0.97
	60	2.9	473	1.03
	70	2.8	448	0.95
	80	3.0	455	1.01
	90	2.8	454	0.96
RG 56	10	2.5	430	1.33
	20	2.4	383	1.13
	30	2.6	400	1.28
	40	2.6	395	1.26
	50	2.6	445	1.43
	60	2.8	435	1.50
RG 107	10	3.3	300	1.53
	20	3.4	260	1.37
	30	3.3	260	1.33
	40	3.5	230	1.25
	50	3.4	245	1.29
	60	3.3	300	1.54
	70	3.4	235	1.24
	80	2.6	310	1.25

Production of lactic acid by the stimulated perfused rectal gland was negligible. The lactate content of the venous effluent was measured in eight glands and was below the sensitivity of the assay in six; in the remaining two the rate of lactate production averaged 0.2 µM/min/g.

Relationship between Oxygen Consumption and Chloride Secretion

In the stimulated state, the oxygen consumption of perfused rectal glands varied directly with the rate

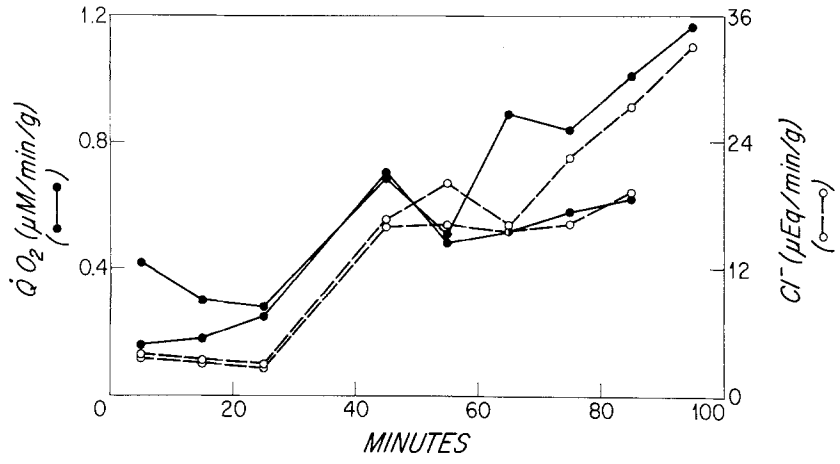

Fig. 1. Effect of progressively stimulating rectal gland secretion with concentrations of dibutyryl cyclic AMP increasing from 0 to 0.02 mM and of theophylline from 0 to 1 mM on oxygen consumption (left vertical axis, closed symbols) and chloride secretion (right vertical axis, open symbols) in two isolated perfused rectal glands. Stimulation was started at 25 min

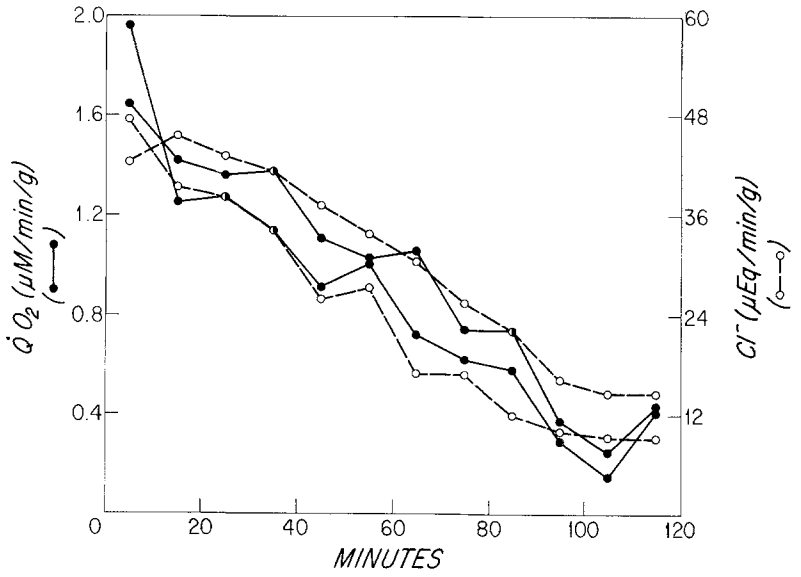

Fig. 2. Effect of reducing the stimulation of rectal gland secretion from an initial concentration of 0.2 mM dibutyryl cyclic AMP and 1 mM theophylline to zero, in two perfused rectal glands. Oxygen consumption is indicated on the left vertical axis (closed symbols) and chloride secretion on the right vertical axis (open symbols)

of chloride secretion. When secretion was progressively stimulated by increasing concentrations of theophylline and dibutyryl cAMP, oxygen consumption rose in parallel (Fig. 1), and when secretion was allowed to decline spontaneously by removing these agents, oxygen uptake fell (Fig. 2).

In eight further experiments, secretion and oxygen uptake were stimulated by cAMP and theophylline. The ratio of chloride secreted to O_2 utilized was 29.2 ± 4.1 μeq/μM. After stimulation of 10 glands with vasoactive intestinal peptide, the putative hormonal agent that activates the rectal gland (Stoff et al., 1979), a similar ratio was apparent, 29.9 ± 4.5 μeq/μM.

Figure 3 illustrates the relationship between chloride secretion and oxygen uptake in 500 collection periods obtained in 140 stimulated glands. The straight line that best fits these points has a slope of 0.031 ± 0.001 with a correlation coefficient (r) of 0.83 and an intercept on the y axis of 0.145 ± 0.02 μM O_2/min/g. The average Cl/O_2 ratio in these experiments was 30.1 ± 0.5 μeq/μM.

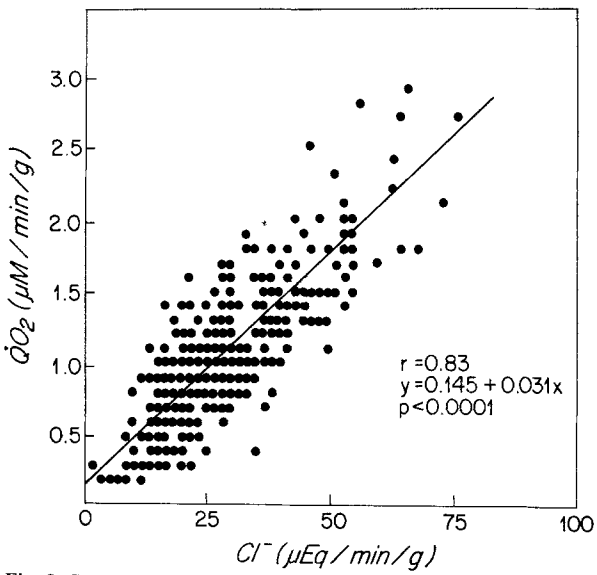

Fig. 3. Summary of the relation between oxygen consumption and chloride secretion in 190 isolated perfused rectal glands. A total of 500 collection periods is represented here. The line was calculated using least squares regression analysis

Table 3. Effect of inhibition by furosemide, and ethacrynic acid and by ion substitutions on oxygen consumption in the stimulated isolated perfused rectal gland

Inhibitor	Ion substitution	Q_{O_2} (μM/min/g)	Cl (μeq/min/g)	Na (μeq/min/g)
None (control)	None	1.23 ± 0.11 (9)	28.74 ± 2.88 (9)	29.13 ± 2.86 (9)
Furosemide 10^{-3} M	None	0.10 ± 0.06 (4)	4.23 ± 0.99 (4)	4.28 ± 1.44 (4)
Ethacrynic acid 10^{-3} M	None	0.45 ± 0.04 (6)	12.89 ± 3.94 (6)	11.73 ± 4.17 (6)
Ouabain 10^{-4} M	None	0.15 ± 0.04 (17)	2.75 ± 0.46 (18)	2.89 ± 0.54 (16)
None	Lithium	0.43 ± 0.11 (4)	0.85 ± 0.13 (4)	0.33 ± 0.12 (4)
None	Nitrate	0.43 ± 0.06 (9)	5.24 ± 0.85 (9)	7.07 ± 1.06 (9)
None	Acetate	0.32 ± 0.11 (3)	0.32 ± 0.12 (3)	3.45 ± 1.10 (3)
None	Sulfate	0.20 ± 0.05 (5)	1.08 ± 0.33 (6)	2.73 ± 1.15 (6)

The isolated perfused rectal gland thus appears to secrete approximately 29–33 μeq of chloride for every μM of oxygen consumed, and the oxygen cost of transporting 1 mole of chloride is about 0.03 moles of O_2.

Basal Oxygen Consumption by the Rectal Gland

In contrast to the direct correspondence between transport and oxygen consumption in the stimulated rectal gland, there was no clear relationship between the much lower levels of chloride secretion and oxygen consumption observed in unstimulated perfused glands. Average oxygen uptake of resting glands was 0.30 ± 0.01 μM/min/g and chloride secretion 4.88 ± 0.30 μeq/min/g in 242 collections carried out in 81 experiments. The correlation coefficient between these terms is zero. Oxygen consumption in the resting state was approximately twice that calculated by extrapolation of the regression line depicted in Fig. 3 to a state of zero secretion (0.145 ± 0.02 μM/min/g, $p < 0.001$), an excess of 0.155 μM/min/g.

Although much weight should not be attached to individual measurements in the unstimulated state because of the low levels of oxygen consumption and chloride secretion, it is of interest that the ratio of the mean resting chloride transport (4.88 μeq/min/g) to this small suprabasal oxygen consumption (0.155 μM/min/g) is 31, thus comfortingly consistent with the stoichiometry of chloride secretion observed during stimulated secretion.

Table 4. Cl/O_2 ratio during stimulated secretion against different electrical and chemical gradients

	Duct fluid Cl^- (meq/l)	Cl^- secretion (μeq/min/g)	Cl/O_2 Ratio	Transglandular PD (mV)	Electrochemical gradient (mV)
No urea	297 ± 2[a] (14)	42 ± 17 (14)	28 ± 8 (14)	− 6.2 ± 1.2 (4)	− 6.8
350 mM	422 ± 7 (8)	29 ± 9 (8)	26 ± 7 (8)	−16.1 ± 1.3 (4)	−25.5
550 mM	531 ± 7 (12)	28 ± 9 (12)	25 ± 6 (12)		
700 mM	570 ± 12 (7)	23 ± 6 (7)	31 ± 10 (7)		

[a] Values are means ± SE. The number of experiments is shown in parentheses.

Secretion of chloride can be inhibited in the presence of cAMP and theophylline by perfusing with ouabain or furosemide, and by substituting other ions for sodium or chloride (Table 3). Oxygen consumption under these circumstances varied from 0.10 to 0.40 μM/min/g.

Effect of Altering the Electrochemical Gradient Opposing Chloride Secretion

The electrochemical gradient against which the rectal gland transports chloride can be varied by experimental manipulation. Fluid secreted by the rectal gland is isosmotic with the perfusing solution. Since urea is virtually excluded from the secretion (Siegel et al., 1975), changing the concentration of urea in the perfusate results in a change in the concentration of electrolytes in the secreted fluid. As urea concentration is increased in the perfusate from 0 to 350, 550, and 700 mM, chloride concentration in the secreted fluid rises from 297 through 422, 531, and 570 mM, $r = 0.95$. Reducing the osmolarity of the perfusate by omitting urea lowers the concentration of sodium chloride in the duct. In the complete absence of urea there is no chemical gradient for chloride across the gland. Under these circumstances (Table 4) the electrical potential of the duct becomes significantly less negative in relation to the perfusate so that the electrical gradient against which chloride moves is also reduced. The electrochemical gradient against which chloride is secreted appears, therefore, to be a function of the urea concentration of the perfusate.

Though the rate of chloride secretion diminished as the gradient opposing secretion rose, the oxygen cost of transporting 1 mole of chloride remained unchanged, as indicated by the constant ratio of Cl/O_2 (Table 4).

Discussion

The present studies show that chloride secretion by the rectal gland is tightly coupled to oxygen consumption. Both secretion and oxygen utilization rise and fall in parallel when stimulation by cAMP or VIP is initiated or discontinued. While in the unstimulated state, a close correlation between the low levels of electrolyte transport and oxygen uptake is not observed, it may be that it is masked by the high proportion of Q_{O_2} devoted to nontransport functions under these circumstances. It is noteworthy that in the basal state the low rate of secretion is not inhibited by ouabain or furosemide (Solomon et al., 1974). The mechanism of secretion under these circumstances is not well understood.

The secretion of sodium chloride by the rectal gland is accomplished primarily by the active transport of chloride against its electrical and chemical gradient (Silva et al., 1977). A schematic model for which there is much supporting evidence is shown in Fig. 4. A sodium chloride carrier located in the basolateral membrane effects uphill transport of chloride into the cell, coupled with the downhill movement of sodium from the extracellular fluid along its electrochemical gradient. Chloride diffuses passively from the electrically negative cell interior across the lumenal membrane, impelled by electrical forces. Sodium moves down its electrochemical gradient through the paracellular pathway into the tubular lumen. In this model chloride movement is linked indirectly to ATP hydrolysis, Na-K-ATPase and active sodium transport, via its cotransport into the cell with sodium. The energetics of chloride secretion by the gland can be examined in the light of this hypothesis.

The ratio of chloride secreted to oxygen consumed by the rectal gland is approximately 30 to 1. It is important to appreciate that this is a minimum value, calculated in most cases without taking into account the low and uncertain rate of basal oxygen consumption, which, if subtracted from total Q_{O_2}, would raise the Cl/O_2 ratio even higher than 30:1. This resembles the ratio of sodium reabsorption to oxygen consumption observed in the mammalian kidney (Deetjen & Kramer, 1961; Kiil, 1971; Lassen et al., 1961) and the 25:1 ratio in gall bladder (Martin & Diamond, 1966), but is higher than the ratio of sodium transport to oxygen consumption, varying between 14:1 and 19:1, found in frog skin and toad bladder. It is interesting that the electrochemical gradient opposing the secretion of chloride could be varied widely in the present experiments, from about 25.5 mV when urea was present to 6.8 mV when it was omitted, without substantially changing the oxygen cost of transport per mole of chloride secreted. Analogous results have been reported in other systems in which the energetics of transport against chemical gradients have been measured. For example, the ratio of sodium transported inwardly across the frog skin to oxygen consumed by the skin is constant regardless of the gradient against which sodium moves (Danisi & Viera, 1971). In the toad bladder as well, the number of moles of sodium transported from mucosa to serosa per mole of CO_2 produced is unchanged when the electrochemical gradient for sodium across the bladder is altered (Canessa et al., 1978). Secretion of hydrogen ions by the turtle bladder similarly bears a constant relation to the production of CO_2, whether or not H^+ is transported against a pH gradient (Beauwens & Al-Awqati, 1976*b*). Finally, the extrusion of

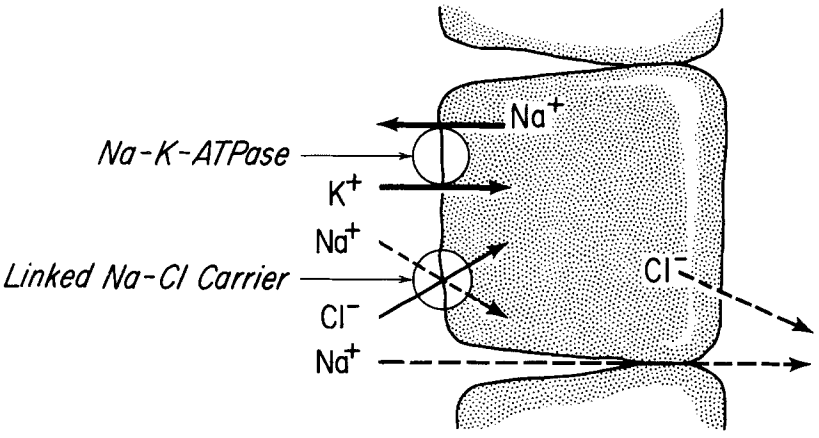

Fig. 4. Schematic model for transport of chloride across rectal gland epithelium (modified from Silva et al., 1977). Passive ion movements are shown by dotted lines, active transport by solid arrows. A sodium chloride carrier located in the basolateral cell membrane couples the uphill movement of chloride into the negatively-charged interior of the cell with the parallel downhill movement of sodium. A large downhill gradient for sodium influx is maintained by activity of Na-K-ATPase. Chloride diffuses passively from cell into tubular lumen down an electrical gradient. Sodium moves down its electrochemical gradient into tubules through paracellular pathways

Na^+ by human red cell ghosts is accomplished at a constant metabolic cost in the face of changes in the gradient opposing sodium transport (Whittam & Ager, 1965). In all these situations, the free energy of the driving reaction must be greater than that required to overcome the electrochemical gradient opposing ion movement. A reduction in the opposing electrochemical gradient results in an increase in the number of transported ions without changing the energy utilized per mole of ion transported.

The transport of chloride by the rectal gland is linked to that of sodium. The process indirectly depends on Na-K-ATPase, in a way suggesting the cotransport of Cl^- with Na^+ into cells across basolateral membranes (Silva et al., 1977). Direct evidence for such cotransport has been obtained in experiments with membrane vesicles derived from the rectal gland (Eveloff et al., 1978). Whether the cotransport is electrically neutral or involves the transfer of more than one Cl^- per Na^+ has not been determined. It is instructive to calculate the energy cost of active chloride transport according to this hypothesis and to compare it with the oxygen consumption actually observed. Such calculations, it should be understood, depend on a number of assumptions, including a fixed ratio of Na ions translocated to ATP hydrolyzed by Na-K-ATPase in intact cells, and a constant ratio of oxygen consumption to the production of high energy phosphate bonds in ATP in living cells, extrapolated from the optimum P/O ratio of isolated mitochondria.

If 3 moles of Na^+ are transported per mole of ATP hydrolyzed by basolateral membranes of the rectal gland (Glynn & Karlish, 1975; Hilden & Hokin, 1975), and the P/O ratio of rectal gland mitochondria is 3:1, the ratio of Na^+ transported to O_2 consumed should be 18:1. If 1 mole of Cl^- were transferred for every mole of Na^+ transported, the ratio of Cl^- to Q_{O_2} would also be 18:1, clearly lower than the figure of 30:1 that was observed in the present experiments. Derivation of energy for transport from nonoxidative metabolism (glycolysis) cannot be invoked because the production of lactic acid by the gland was minimal.

Any explanation of this discrepancy must be highly speculative at present. The role of solvent drag and other factors, such as diffusion down local standing gradients created by active transport, are completely undetermined. If passive transport of fluid containing chloride across the rectal gland epithelium is hypothesized, it would have to be by a mechanism that leaves urea behind, because of the consistently low concentration of urea in duct fluid, averaging 10–15% of its concentration in the perfusate. The high ratio of Cl^- to O_2 might suggest that more than 3 moles of Na^+ are transported per mole of ATP hydrolyzed in the rectal gland, or that more than 1 chloride ion might be transported for each sodium extruded from the cell by Na-K-ATPase. It should be noted that even at a Cl/O_2 ratio of 30:1, the free energy theoretically available from oxidative metabolism would exceed that required to overcome the electrochemical gradient found in the present experiments to oppose the movement of chloride from perfusate to duct lumen.

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