and outside enabled calculations of the epithelial E.M.F. (E_{ion}) (Ussing, H.H., and K. Zerahn, Acta Physiol. Scand., 23, 111, 1951) for these two ions from the relation: $E_{ion} = \frac{RT}{zF} \ln \frac{J_{MS}}{J_{SM}}$. R is the universal gas constant; T is absolute temperature (283 K.); z is the valance of the ion; F is Faraday's number; J_{MS} and J_{SM} are the fluxes from lumen to bath and bath to lumen, respectively. It is seen in Table 2 that if Na^+ were transported alone one could predict a P.D. of about 40 mV mucosal side negative. If Cl^- were transported alone a P.D. of about 40 mV mucosal side positive is predicted. The average observed P.D. was about 4 mV mucosal side positive. Voltage clamping at either 0, +50 or –50 mV had no effect on unidirectional fluxes of either Na^+ or Cl^- (Table 3). These data may indicate that Na^+ and Cl^- pass through the epithelial membranes predominately as a neutral complex. Diamond (J. Physiol., London, 161, 474, 1962) explained a similar phenomenon in the gall bladder of a teleost by assuming that Na^+ and Cl^- pumps were coupled.

Water reabsorption from the bladder was shown to be closely correlated with the active NaCl reabsorption (Renfro, *op. cit.*). One probable function of this reabsorption may be seen in Table 4. When the Mg concentration of the mucosal fluid was raised to 57 mM (normal marine teleost urine contains 50-200 mM Mg) the effect of salt and water reabsorption was to increase the concentration of divalent ions in the mucosal fluid. Since divalent ions are the primary excretory products of marine teleostean kidneys the ability of the bladder to increase their concentration in the urine would be a significant asset to the water economy of the fish.

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AVIAN SALT GLAND: INTRA- AND EXTRACELLULAR ION CONCENTRATIONS IN SECRETING AND IN INACTIVE GLANDS

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The salt gland of the herring gull *Larus argentatus* secretes a fluid with Na and Cl concentrations from 600 to 800 mM and osmolalities from 1200 to 1600 mOs. Apparently the secreted fluid is produced by tubules which open into a central canal (Fänge, Schmidt-Nielsen and Osaki, Biol. Bull., 115, 162-170, 1958). The cells of the secretory tubules of ducklings raised on sea water (Ernst and Ellis, Cell Biol., 40, 305-321, 1969) are highly specialized with deeply-folded lateral and basal surfaces forming complex extracellular spaces. Active sodium transport involving Na-K-ATPase is indicated by the finding that the duct of the secreting gland is electrically positive to the blood and that ouabain injected retrograde into the duct inhibits secretion (Thesleff and Schmidt-Nielsen, Am. J. Physiol., 202, 597-600, 1962). Furthermore Ernst et al. (Biochim. Biophys. Acta, 135, 682-692, 1967) found a direct relationship between the previous osmotic stress of ducklings and the Na-K-ATPase activity in the salt gland. The findings that ouabain inhibits from the luminal side have been interpreted to mean that the transport of sodium takes place across the luminal surface of the cells.

Ernst and Ellis (Cell Biol., 40, 305-321, 1969) histologically localized the ATPase activity on the lateral and basal surfaces of the secretory cells. These findings have given rise to some controversy concerning the site of sodium transport. Does it take place across the basal and lateral membranes, or across the luminal membrane? To elucidate this problem various attempts have been made to measure the intracellular concentrations of electrolytes in secreting and nonsecreting glands. Peaker (J. Physiol., 213, 399-410, 1971), in contrast to Hokin (J. Gen. Physiol., 50, 2197-2209, 1967) found relatively low intracellular sodium and chloride concentrations. Both authors used a method of incubating the tissue slices in a medium containing an intracellular marker prior to the analysis of the tissue.

The present study was undertaken to determine the intracellular concentrations of the salt gland of the herring gull *Larus argentatus* by the method used on the mammalian papilla (Schmidt-Nielsen, et al.). Following preliminary studies to determine if the tissue of the salt gland could withstand exposure to high pressures without breaking the cells, the following protocol was adopted. Eight 4-month old herring gulls were used in the study. Four received a diet of canned cat food and herring with fresh water for drinking water. Four others were fed the same diet but were allowed only sea water for drinking. Prior to the experiment, birds were injected with 100 μCi ^{14}C-labeled PEG i.m. Gulls maintained on salt water were in addition given 100 ml of lukewarm sea water by stomach tube. An hour later when secretion from the glands was copious the bird was decapitated and the salt glands were immediately removed. One piece of each gland was frozen in dry ice and acetone; the other pieces were placed in polyethylene tubes as in the previous study (Bull., MDIBL, #44 this issue). They were centrifuged first at 4000 rpm (1900 g) for 10 min., then at 18,500 rpm (41,000 g) for 20 minutes. One piece was centrifuged without extra pressure; the other piece was placed under pressure by positioning a polyethylene cylinder with 0.5 cm water on top of the tissue. Thus the tissue with the cylinder containing 0.5 cm water was squeezed between the perforated plate and the cylinder with a pressure of 20.5 atmospheres.

A series of criteria was used to determine whether or not the fluid obtained by centrifugation was interstitial rather than intracellular fluid: (1) The PEG fluid to plasma ratio of the "interstitial" fluid obtained at 18,500 rpm was found to be close to unity. The difference between secreting and inactive glands can probably be explained by the difference in blood flow (Table 1). The lower PEG ratio for fluid obtained from the tissue under added pressure indicates dilution with cellular water. (2) The decrease in extracellular space $\left(\frac{T_{PEG}}{ItFl_{PEG}} \cdot 100 \text{ percent, Table 2}\right)$ following centrifugation at 18,500 rpm corresponded closely to the measured amount of fluid removed. (3) The K concentration of the fluid obtained at 4000 rpm and 18,500 rpm was about the same (Table 1) and was significantly lower than that of the tissue. The K concentration was slightly higher in fluid from pressed tissue, indicating some rupture of cells. (4) The tissue which had been centrifuged or centrigued with added pressure was flattened out but regained its original shape when it was subsequently immersed in water, showing that the treatment did not cause disintegration of the tissue. This was substantiated by preliminary electronmicroscopy which showed no appreciable change in the ultrastructure of the tissue following centrifugation at 18,500 rpm, and only slight changes in pressed tissue.

Assuming then that the fluid obtained at 41,000 g (18,500 rpm) represented interstitial fluid, intracellular concentrations were calculated from the equation given by Schmidt-Nielsen, et al. (Bull.,

TABLE 1

Concentrations in mM per liter in "interstitial" fluid obtained by centrifugation of salt glands, and in tissue water

		Interstitial Fluid			Tissue		
	Plasma	4000 rpm	18,500 rpm	18,500 rpm + pr.	Frozen	18,500 rpm	18,500 rpm + pr.
				SECRETING GLANDS			
PEG F1/P	1.000	-	1.21 ± 0.11	0.96 ± 0.11	0.369 ± 0.037	0.252 ± 0.020	0.171 ± 0.032
Osm	388.0 ± 20.0	402.0 ± 14.0	395.0 ± 14.0	388.0 ± 13.0	295.0 ± 25.0	358.0 ± 15.0	384.0 ± 19.0
Calc.Osm	356.0 ± 28.0	309.0 ± 26.0	381.0 ± 46.0	317.0 ± 24.0	327.0 ± 13.0	333.0 ± 14.0	328.0 ± 14.0
Na	179.0 ± 14.0	126.3 ± 8.3	148.0 ± 17.0	112.7 ± 11.0	78.7 ± 3.3	70.1 ± 53.0	62.2 ± 4.8
K	6.29 ± 0.50	31.8 ± 3.7	44.1 ± 2.91	52.3 ± 2.2	90.4 ± 4.4	101.9 ± 2.6	106.8 ± 4.4
Cl	158.2 ± 14.9	-	196.7 ± 21.0	123.3 ± 5.2	101.1 ± 15.0	83.7 ± 4.7	80.3 ± 5.0
				INACTIVE GLANDS			
PEG F1/P	1.000	-	0.87 ± 0.07	0.72 ± 0.07	0.246 ± 0.047	0.209 ± 0.029	0.114 ± 0.021
Osm	359.0 ± 2.0	391.0 ± 6.0	384.0 ± 4.0	380.0 ± 4.0	286.0 ± 22.0	348.0 ± 25.0	357.0 ± 10.0
Calc.Osm	315.0 ± 4.0	315.0 ± 13.0	332.0 ± 25.0	299.0 ± 6.0	333.0 ± 23.0	309.0 ± 4.0	320.0 ± 4.0
Na	160.0 ± 2.0	124.0 ± 3.4	130.0 ± 5.0	108.4 ± 1.5	76.4 ± 4.8	56.3 ± 1.6	52.2 ± 1.6
K	4.87 ± 0.22	41.4 ± 1.3	44.0 ± 8.2	48.0 ± 1.8	96.8 ± 4.3	103.5 ± 2.7	112.0 ± 1.3
Cl	125.9 ± 2.7	-	150.4*	136.5 ± 4.1	100.2 ± 9.2	83.1 ± 6.9	72.8 ± 6.9

Mean of 4 samples ± S.E.
*Only one sample could be analyzed for chloride

TABLE 2

Extracellular space in percent of total tissue water in salt glands uncentrifuged or after centrifugation

	Secreting glands	Inactive glands	P
Uncentrifuged	28.2 ± 0.9	25.1 ± 0.7	<0.05
Centrifuged	21.0 ± 1.1	18.8 ± 1.2	n.s.
Centrifuged plus pressure	14.0 ± 1.2	10.6 ± 1.0	<0.05

Mean of 4 gulls ± S.E.

TABLE 3

Intracellular concentrations in salt glands in mM per liter cell water

	Secreting glands	Inactive glands	P
Na	49.8 ± 1.6	43.3 ± 2.3	<0.05
K	108.2 ± 2.3	115.8 ± 2.1	<0.05
Cl	58.9 ± 5.3	66.7 ± 4.7	n.s.

Mean of 12 samples from 4 gulls ± S.E.

MDIBL, #44 this issue).

Table 3 shows the average intracellular concentrations. The differences in intracellular concentrations of Na and K between secreting and inactive salt glands are slight but significant while there is no significant difference in chloride concentrations. In secreting glands the intracellular Na was on the average 6.5 mM higher than in inactive salt glands. The K concentration was 7.6 mM lower than in the inactive salt glands.

Even though the secreting gland is producing a fluid with an osmolality of 1200 mOs and NaCl concentrations of 600 mM interstitial as well as intracellular fluids do not have higher osmolality than the blood (Table 1). How and where Na and Cl are transported across the epithelium of the secretory tubules and how the osmolality of the fluid in the tubules or possibly in the central canal is raised to four times the osmolality of the blood remain to be explained.

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