J. Exp. Biol. (1967), **47**, 375–391
With 8 *text-figures*
Printed in Great Britain

SEQUENTIAL CHANGES IN THE ADENOSINETRIPHOSPHATASE ACTIVITY AND THE ELECTROLYTE EXCRETORY CAPACITY OF THE NASAL GLANDS OF THE DUCK (*ANAS PLATYRHYNCHOS*) DURING THE PERIOD OF ADAPTATION TO HYPERTONIC SALINE*

BY G. L. FLETCHER, I. M. STAINER† AND W. N. HOLMES
Department of Biological Sciences, University of California, Santa Barbara, California 93106

(*Received* 19 *April* 1967)

INTRODUCTION

Animals exposed to desert and salt-water environments must be able to excrete waste products and electrolytes and at the same time maintain a state of positive water balance. The kangaroo rat for example, which is both behaviourally and physiologically adapted to its normally arid environment, excretes an extremely hypertonic urine, and even when given sea water is able to eliminate the excess electrolytes without incurring an undue loss of water (Schmidt-Nielsen & Schmidt-Nielsen, 1950). Such renal concentrating abilities are made possible by the double counter-current arrangement of the loop of Henle, the collecting ducts and the vasa recta (Wirz, Hargitay & Kuhn, 1951). The loop of Henle, however, is incompletely developed or absent in many nephrons of the bird kidney (Sperber, 1960), and although many species appear able to produce a hypertonic urine the concentrating capacity of the kidney is considerably less than that of the mammal. Consequently, we find that when birds such as the pigeon are infused with hypertonic saline they undergo a progressive dehydration (Scothorne, 1959).

Some birds, however, have successfully colonized the marine environment and can survive a continuous exposure to diets containing hypertonic saline or sea water. This is due primarily to the presence of nasal glands which constitute an extra-renal pathway for the excretion of electrolytes (Schmidt-Nielsen & Sladen, 1958; Schmidt-Nielsen, Jorgensen & Osaki, 1958; Scothorne, 1958; Holmes, Butler & Phillips, 1961). When stimulated the nasal glands secrete hypertonic sodium against a bioelectrical potential (Thesleff & Schmidt-Nielsen, 1962), suggesting that active sodium transport is involved in this excretory mechanism.

The active transport of sodium by red blood cell ghosts has been shown to depend on the presence of extracellular potassium, intracellular sodium and the hydrolysis of intracellular adenosine triphosphate (ATP) (see Skou, 1965). Furthermore, the hydrolysis of the ATP and the attendant active transport of sodium by the red blood

* This work was supported by research grants to W.N.H. from the National Science Foundation (Grant no. GB3896) and the Committee on Research, University of California.
† National Institutes of Health Pre-doctoral Fellow.

cell were shown to be inhibited in the presence of cardiac glycosides (Schatzmann, 1953). An adenosinetriphosphatase (ATP-ase) system which possessed all of the characteristics necessary for the active transport of sodium and potassium was first shown to be present in the crab nerve (Skou, 1957) and similar enzyme systems have since been described for the mammalian erythrocyte and many lower vertebrate and mammalian tissues (Post & Sen, 1964; Skou, 1965). The nasal glands of marine birds have been found to be particularly rich in sodium-and-potassium-dependent ATP-ase activity (Hokin, 1963; Bonting, Caravaggio, Canady & Hawkins, 1964).

Therefore, since the duck will readily alternate between diets either containing hypertonic saline or containing fresh water, it was considered an ideal subject in which to observe any correlation which might occur between the ATP-ase activity of the nasal glands and their capacity to excrete sodium.

MATERIALS AND METHODS

Male *Pekin white* ducks were obtained from a commercial supplier and were housed outdoors for at least 1 week before use. Each bird received a daily ration of grower food (180 g. mixed with 325 ml. tap water) and an *ad libitum* supply of fresh water (2·0 mM./l. sodium, 0·07 mM./l. potassium) or hypertonic saline (284 mM./l. sodium, 6·0 mM./l. potassium). At least 2 days before use the birds were brought indoors and maintained at 26° C, 80% relative humidity and a photoperiod of 12 hr. light and 12 hr. dark. All birds were starved for 24 hr. prior to autopsy.

When extra-renal excretory rates were to be determined, one of the brachial veins was cannulated on the previous day and a 10% solution of NaCl was intravenously infused at 0·25 ml./min./kg. body weight. Preliminary studies indicated that maximum extra-renal excretion was attained within 10 min. after the onset of nasal secretion. The rate of flow of nasal fluid, therefore, was measured 10 min. after the onset and every minute for 20 min. thereafter. The nasal fluid excreted during this period was then analysed for sodium and potassium by flame photometry and for chloride by amperometric titration against silver ions (Cotlove, 1963). The extra-renal excretory rates of water, sodium, potassium and chloride were then calculated for each bird.

The birds were killed by decapitation and the nasal glands were immediately removed, cleaned of adhering connective tissue, weighed, placed on the lid of a Petri dish containing ice and sliced. 100 mg. of nasal gland slices were then homogenized in an all-glass Potter–Elvehjem conical homogenizer containing 3·0 ml. 0·45 M. or 0·60 M. sucrose. Dry-weight determinations were made on the remaining nasal gland slices.

ATP-ase activity was determined by incubating duplicate 0·2 ml. samples of homogenate in beakers containing 3·0 ml. of the appropriate medium (see legends of figures and tables for exact composition). Incubations were continued for 5 min. in a Dubnoff metabolic shaker at 41° C. and 70 oscillations/minute. The reaction was stopped by the addition of ice-cold trichloracetic acid. The concentration of inorganic phosphate was determined by the method of Fiske & SubbaRow (1925). All values were corrected for the amount of inorganic phosphate found in unincubated samples. Disodium adenosinetriphosphate (Sigma) and ouabain octahydrate (strophanthin-G, Sigma) were used throughout these studies. The protein composition of the nasal

gland homogenates were determined by the Lowry, Rosenbrough, Farr & Randall modification (1951) of the Folin–Ciocalteau method and values were compared to standards containing bovine albumin fraction V, B grade (Calbiochem.)

Regressions were fitted by the method of least squares and compared by the analysis of covariance (Snedecor, 1956).

RESULTS

(1) *Characterization of the ATP-ase*

The effects of various components of the incubation medium on the activities of the ATP-ase preparations were studied in glands from birds maintained on fresh water and from birds which had been maintained on hypertonic saline for 14 days.

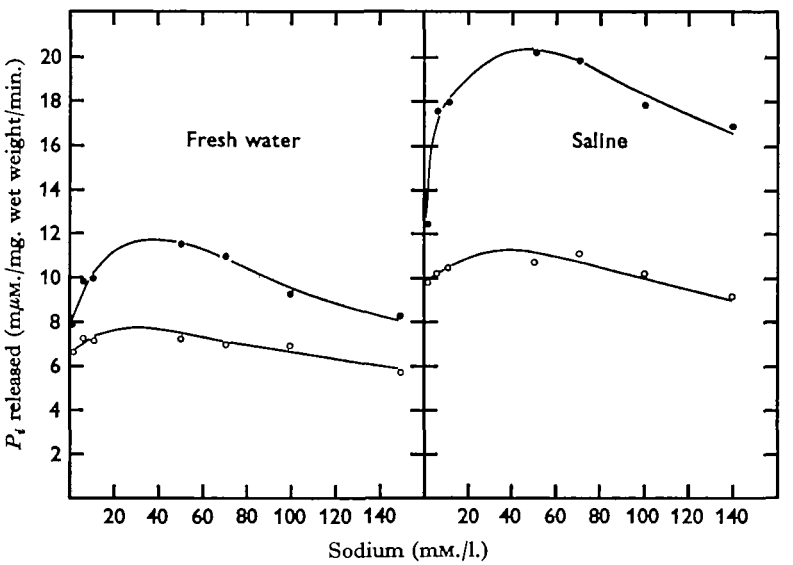

Fig. 1. The effect of Na^+ concentration on the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M./l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10 mM./l., $MgSO_4$ 0·8 mM./l., KCl 3·33 mM./l., ATP 1·0, mM./l. Alternate tubes also contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5 ml. ice-cold 13 % trichloractic acid. ●—●, Total ATP-ase activity; ○—○, ATP-ase activity in the presence of ouabain.

With increasing sodium concentration of the incubation medium the ATP-ase activity of the nasal glands from freshwater-adapted and saline-adapted birds increased to a maximum at approximately 50 mM./l. and progressively declined thereafter (Fig. 1).

In the presence of 50 mM./l. sodium the ATP-ase activities of both groups increased with the potassium concentration of the medium to a maximum at 10 mM./l. (Fig. 2).

When the sodium and potassium concentrations of the incubation medium were maintained at 50 and 10 mM./l. respectively a maximum inhibition of the ouabain-sensitive component of the total ATP-ase activity was observed at 0·1 mM./l. in glands from both groups of birds (Fig. 3).

The total and ouabain-insensitive ATP-ase activities of nasal glands from saline-adapted birds did not increase at magnesium concentrations in excess of 1·2 mM./l. At somewhat higher magnesium concentrations, however, there may have been a small increase in the ATP-ase activities of glands from the freshwater-adapted birds (Fig. 4).

The amount of inorgagnic phosphate released was proportional to the time of incubation and was a linear function of time up to an incubation time of 10 min. in both groups of birds (Fig. 5).

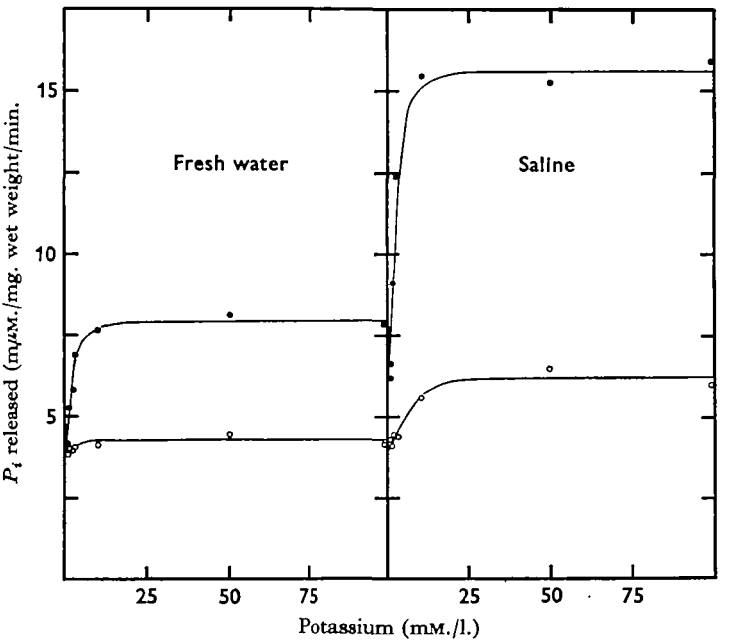

Fig. 2. The effect of potassium concentration on the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M./l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10 mM./l., $MgSO_4$ 0·8 mM./l., NaCl 50 mM./l., ATP 1·0 mM./l. Alternate tubes contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5·0 ml. ice-cold 13% trichloracetic acid. ●—●, Total ATP-ase activity; O—O, ATP-ase activity in the presence of ouabain.

An optimum ATP concentration of 0·75 mM./l. was observed when tissue from both groups of birds was incubated in a medium containing sodium, potassium and magnesium at concentrations previously observed to yield a maximal release of inorganic phosphate (Fig. 6).

(2) *ATP-ase activity and extra-renal excretion*

There was doubling of the ATP-ase activities per unit weight of tissue in the nasal glands derived from birds during the first 200 hr. of their exposure to hypertonic saline (Fig. 7). Simultaneously, there was an increase in the excretory rates of water, sodium, potassium and chloride per unit weight of nasal gland tissue which became maximal at approximately 200 hr (Fig. 8). During the remainder of the 30-day period

of exposure to hypertonic saline both the extra-renal excretory rates and the ATP-ase activities of the nasal glands were maintained at these maximal levels (Figs. 7, 8).

When the ducks were returned to fresh water at the end of the 30-day period on hypertonic saline the extra-renal excretion of water and electrolytes ceased and there was a progressive decline in the nasal gland ATP-ase activities to levels similar to those of freshwater-adapted birds which had not been exposed to the hypertonic diet (Fig. 7).

A rapid increase in nasal gland size was observed during the initial period of adaptation to saline and a maximum weight was achieved at the end of the first 72 hr. At

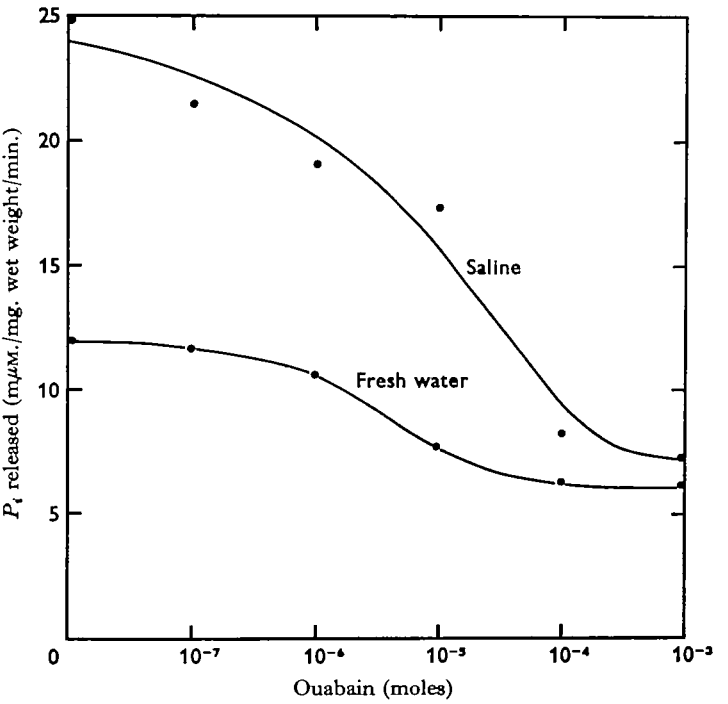

Fig. 3. The effect of ouabain concentration on the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M./l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10 mM./l., $MgSO_4$ 0·8 mM./l., NaCl 50·0 mM./l., KCl 10·0 mM./l., ATP 1·0 mM./l. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5·0 ml. ice-cold 13 % trichloracetic acid.

this time, however, the total and ouabain-sensitive ATP-ase activities were only slightly greater than the half-maximum increases ($t_{\frac{1}{2}}$ for total and ouabain-sensitive ATP-ase activities = 56 and 38 hr. respectively). No change in the percentage dry weight of nasal glands was observed during the period of adaptation to saline or in the adapted state (freshwater-adapted birds 26·0 % ± 1·72, saline-adapted birds 26·5 % ± 0·99).

(3) *Improved enzyme preparation*

It is clear from the above data that the sodium transported per mole of ATP hydrolysed was considerably higher than the values previously reported for this enzyme (Hokin, 1963; Bonting *et al.* 1964). Much higher enzyme activities were subsequently obtained by incubating the homogenates in the modified medium described in the following tables. In addition, the nasal gland slices were homogenized in distilled water, lyophilized overnight at $-55°$ C and resuspended in 0·45 M. sucrose.

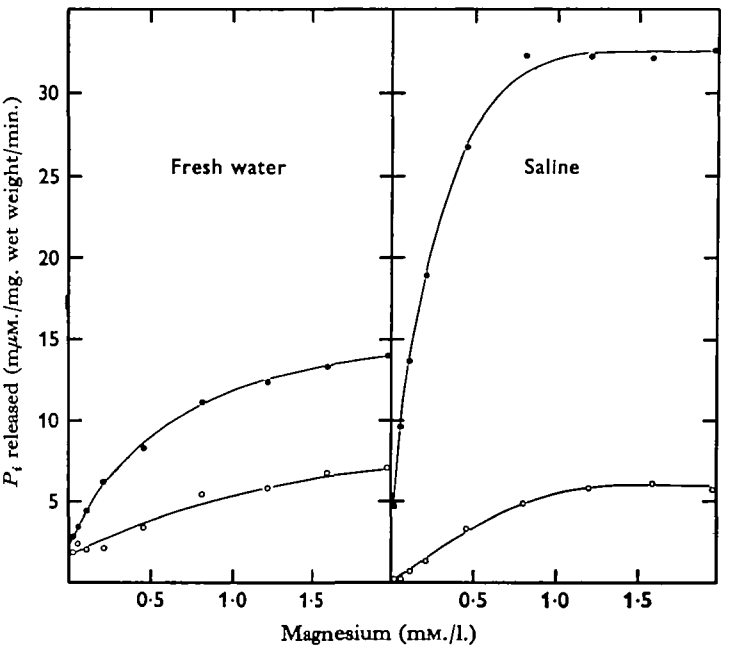

Fig. 4. The effect of Mg^{2+} concentration (as $MgSO_4$) in the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10·0 mM./l., NaCl 50·0 mM./l., KCl 10·0 mM./l., ATP 1·0 mM./l. Alternate tubes contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5·0 ml. ice-cold 13 % trichloracetic acid. ●—●, Total ATP-ase activity, O—O, ATP-ase activity in the presence of ouabain.

The characterization curves for the improved enzyme preparation were repeated and in contrast to the previous preparation (Figs. 1–6) no stimulation of the ouabain-insensitive component was observed in the presence of sodium and potassium. The optima for each substrate (Na, K, Mg and ATP) were, however, the same for both enzyme preparations. In the improved enzyme preparation the ouabain-sensitive ATP-ase activity in the freshwater-adapted birds showed a sixfold increase over the activity measured by the original method ($23·2 \pm 2·44$ *vs.* $3·88 \pm 0·38$ mμM.P_i/mg. wet weight nasal gland/min.). A similar increase in ouabain-sensitive ATP-ase activity was apparent in the 30-day saline-adapted birds ($98·2 \pm 5·49$ *vs.* $10·98 \pm 0·48$ mμM.P_i/mg. wet weight nasal gland/min.).

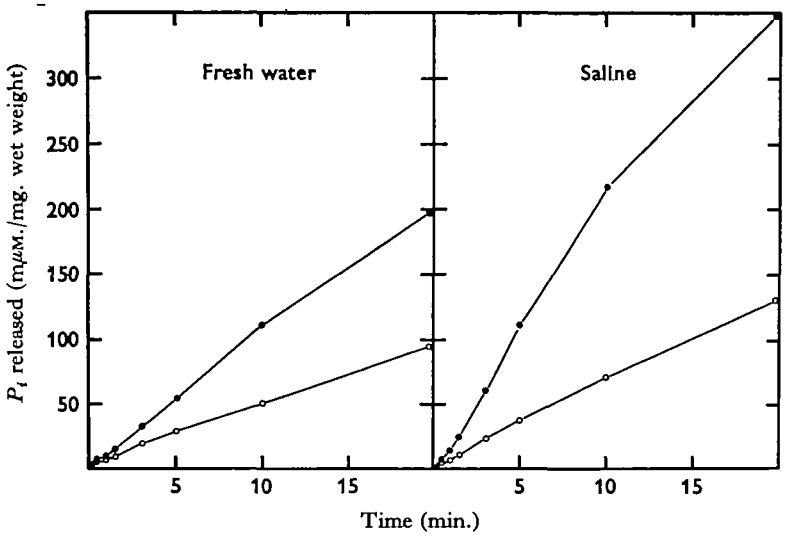

Fig. 5. The effect of incubation time on the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M./l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4), 10·0 mM./l., NaCl 50·0 mM./l., KCl 10·0 mM./l., $MgSO_4$ 1·2 mM./l., ATP 1·0 mM./l. Alternate tubes contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for various times at 41° C. and the reaction was stopped by the addition of 5·0 ml. ice-cold 13% trichloracetic acid. ●—●, Total ATP-ase activity, ○—○ ATP-ase activity in the presence of ouabain.

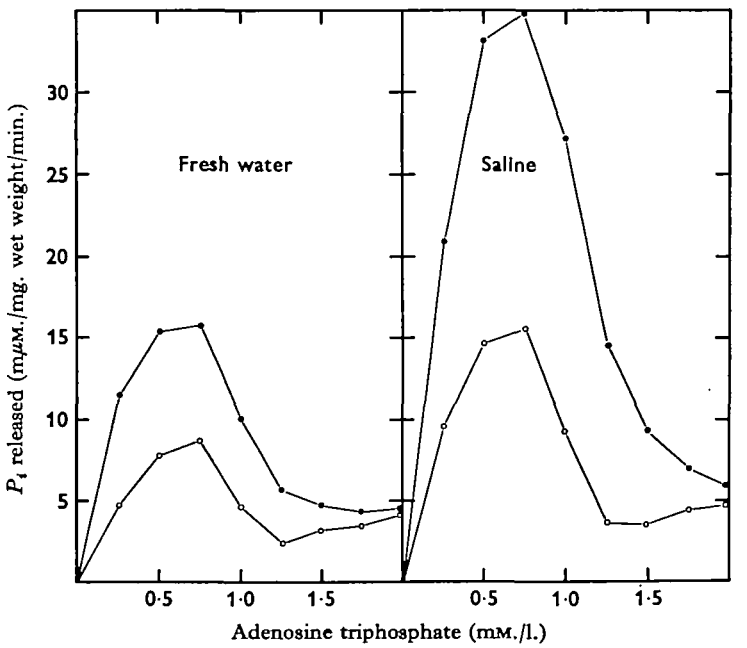

Fig. 6. The effect of ATP concentration on the ATP-ase activity of nasal gland homogenates from freshwater-adapted and saline-adapted ducks. Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. of wet tissue in 0·6 M./l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10·0 mM./l., NaCl 50·0 mM./l., KCl 10·0 mM./l., $MgSO_4$ 1·2 mM./l. Alternate tubes contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5·0 ml. ice-cold trichloracetic acid. ●—●, Total ATP-ase activity; ○—○, ATP-ase activity in the presence of ouabain.

The total and ouabain-sensitive ATP-ase activities of the 30-day saline-adapted birds were at least three times higher than the corresponding values for the 30-day freshwater-adapted group (Table 1). When the 30-day saline-adapted birds were returned to fresh water for 21 days the ATP-ase activities declined to the level of the birds which had been maintained on fresh water for a similar period (Table 1). In

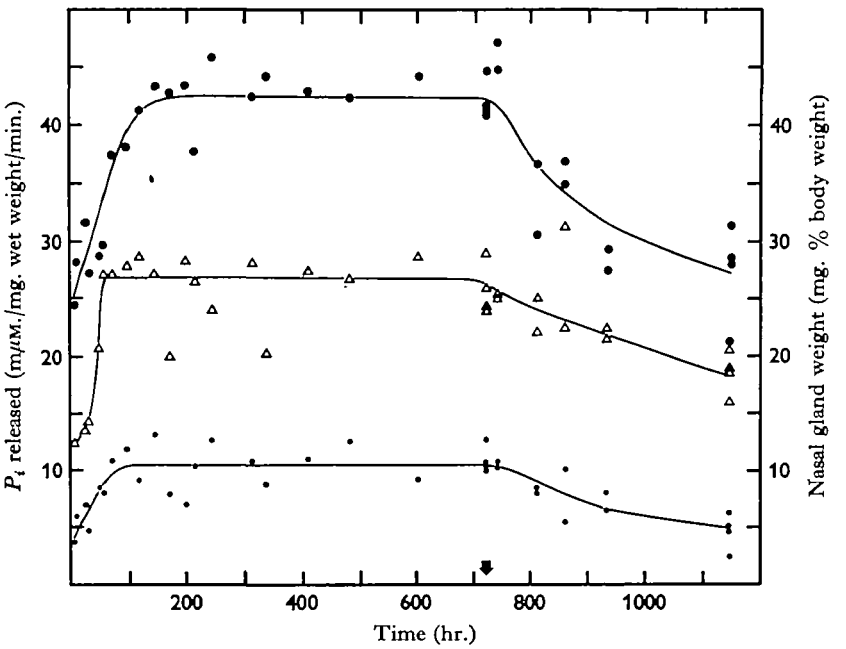

Fig. 7. Changes in the total ATP-ase activity, ●, the ouabain-sensitive ATP-ase activity, •, and the relative nasal gland size, △, of the duck during the period of adaptation to saline, and their subsequent return to fresh water. The abscissa represents the time each animal was exposed to the experimental drinking water. Each point represents a value taken from a single bird. At time zero the ducks were given saline (284 mM./l. Na^+, 6·0 mM./l. K^+) as their sole source of drinking water. At 720 hr. (indicated by the arrow on the abscissa) the saline was removed and the ducks were given fresh water to drink. At the indicated times the ducks were killed and the nasal glands were removed, weighed, sliced and homogenized. Weight specific ATP-ase activities were determined using the optimum incubation medium previously established. (Each 0·2 ml. aliquot of homogenate, containing 6·46 mg. wet tissue in 0·6 M/l. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 10 mM./l., NaCl 50 mM./l., KCl 10 mM./l., $MgSO_4$ 1·2 mM./l., ATP 0·75 mM./l. Alternate beakers contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 5·0 ml. 13% ice-cold trichloracetic acid.)

the group of birds which had been exposed to saline for 30 days, returned to fresh water for 21 days and re-adapted to saline for an additional 7-day period, the ATP-ase activities were significantly greater than those of the birds which had been continuously maintained on fresh water for 58 days (Table 1). The differences between the ATP-ase activities of the freshwater-adapted and saline-adapted birds were apparent when the activities were expressed either in mμM.P_i/mg. wet weight of nasal gland/min. or in mμM.P_i/mg. protein/min. (Table 1).

The extra-renal excretory rates of water, sodium and potassium were significantly greater in the 30-day saline-adapted birds than in the 30-day freshwater-adapted

controls (Tables 2 and 3). Similarly, birds which had been returned to saline for 7 days following a 21-day period of readaptation to fresh water also showed significantly higher excretory rates when compared to controls which had been continuously maintained on fresh water for 58 days (Tables 2 and 3). Thus, comparing Tables 1 and 3 it can be seen that upon adaptation to saline both the weight-specific ATP-ase activity and the sodium excretory rates increased three- to fourfold over the control freshwater-adapted values.

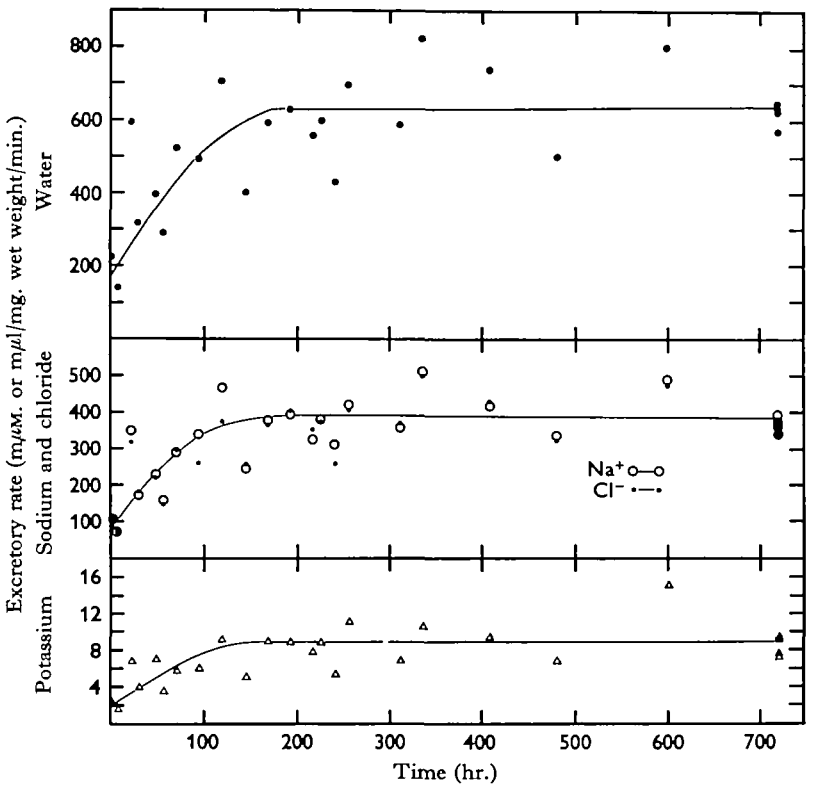

Fig. 8. Changes in the maximum nasal gland excretory rates of sodium, chloride, potassium and water during the period of adaptation of ducks to saline. (The abscissa represents the time each bird was exposed to saline and each point represents a value taken from a single bird. At zero time ducks were given saline (282 mM./l. Na^+ and 6·0 mM./l. K^+) as their sole source of drinking water and at the indicated times the maximum extra-renal excretory rates were determined by the intra-venous infusion of a 10% sodium chloride solution. Following this, the birds were immediately killed and the nasal glands were removed, weighed, homogenized and ATP-ase determinations were made (see Fig. 7). The sodium, potassium and chloride concentrations in the nasal fluid were determined and the maximum excretory rates were expressed in mμM. or mμl./mg. wet weight of nasal gland tissue/minute.)

The body weights of the saline-adapted birds did not differ significantly from the corresponding freshwater-adapted controls (Table 2). The relative nasal gland weights, however, approximately doubled in the saline-adapted birds (Table 2). This doubling of nasal gland weight combined with the three- to fourfold increase in the weight-specific sodium excretory capacity resulted in a seven to eightfold increase in the extra-renal sodium excretion (Table 2).

Although the increased volume of nasal fluid accounted for most of the increased ion output, there was also a significantly higher concentration of sodium chloride present in the nasal fluid of the saline-adapted birds. Potassium, however, did not show a clear pattern of concentration changes, indicating that the differences observed are possibly independent of those observed for sodium chloride (Table 3).

The maximum rates of sodium excretion per unit wet weight of nasal gland (Table 3) may be considered to be a measure of the rate of active sodium transport by the

Table 1. *The ATP-ase activities in nasal glands from ducks maintained on various freshwater and/or saline regimes*

(Each 0·2 ml. aliquot of homogenate, containing 0·334 mg. wet tissue in 0·45 M. sucrose, was added to 3·0 ml. of the following incubation medium: tris chloride buffer (pH 7·4) 90 mM./l., NaCl 50·0 mM./l., KCl 10 mM./l., $MgSO_4$ 1·2 mM./l., ATP 1·25 mM./l. and (ethylenedinitrilo)-tetra-acetic acid (EDTA) 0·1 mM./l. Alternate tubes contained 0·1 mM./l. ouabain. The homogenate was incubated in the medium for 5 min. at 41° C. and the reaction was stopped by the addition of 0·8 ml. ice-cold 40% trichloracetic acid (TCA). Enzyme activities were expressed in terms of inorganic phosphate released pet unit wet weight of nasal gland or per unit of protein in the homogenate. All values are expressed as means ±S.E.)

		P_i release					
		(mμM./mg. wet wt. nasal gland/min)			(mμM./mg. protein/min.)		
Group	No. of birds	Total	Ouabain insensitive	Ouabain sensitive	Total	Ouabain insensitive	Ouabain sensitive
30 days fresh water	5	68·7 ±5·8	45·5 ±5·15	23·2 ±2·44	432 ±25·1	283 ±20·2	149 ±18·5
30 days saline	5	209*** ±15·8	111*** ±11·3	98·2*** ±5·49	1100*** ±68·2	579*** ±55·8	517*** ±17·0
51 days fresh water	5	85·3 ±5·9	50·4 ±5·9	34·9 ±4·7	474 ±27·5	280 ±31·6	194 ±24·7
30 days saline+21 days fresh water	5	70·2 ±7·5	41·1 ±5·8	29·0 ±3·0	380 ±41·9	223 ±31·6	158 ±17·2
58 days fresh water	5	56·1 ±8·6	28·8 ±6·5	27·2 ±2·4	315 ±40·7	161 ±31·8	154 ±10·2
30 days saline+21 days fresh water+7 days saline	7	161* ±33	86·7*** ±10·2	74·2*** ±6·2	898*** ±89·6	479*** ±53·3	419*** ±41·1

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$ with respect to value for corresponding freshwater-maintained birds.

gland. Likewise, the corresponding nasal gland ATP-ase activities may have represented the rate of ATP hydrolysis necessary for the active transport of this sodium (Table 1). Thus, it should be noted that the moles of sodium transported per mole of ATP hydrolysed by each component of the ATP-ase system studied was constant irrespective of whether the birds were adapted to fresh water or to saline (Table 4).

In the saline-adapted birds a significant positive correlation was observed between the ATP-ase activities and the sodium excretion rates (Table 5). The ouabain-sensitive ATP-ase activities correlated more closely and more significantly with the sodium excretory rates than did either the ouabain-insensitive or the total ATP-ase components

Table 2. *Relative nasal gland weight and maximum daily extra-renal excretion of water and electrolytes from ducks maintained on various freshwater and/or saline regimes*

(Maximum extra-renal excretory rates were determined by the intravenous infusion of 10% sodium chloride solution immediately prior to autopsy. Excretory rates were then expressed per kg. body weight per day. All values are expressed as means ±S.E.)

Group	No. of birds	Body weight (g.)	Nasal gland weight (mg.)	Relative nasal gland weight (mg. % B.W.)	Maximum extra-renal excretion (ml. or mM./kg body weight/day) Water	Na	K	Cl
30 days fresh water	5	2535 ±101	403 ±23	15·9 ±0·9	47·2 ±6·1	22·3 ±3·5	0·54 ±0·11	21·3 ±3·6
30 days saline	5	2405 ±117	673** ±53	28·7** ±3·4	284** ±51	166** ±28	4·14*** ±0·67	165*** ±29
51 days fresh water	5	2340 ±121	408 ±24	17·5 ±1·0	72·1 ±11·8	34·1 ±5·1	0·43 ±0·06	34·6 ±2·3
30 days saline+21 days fresh water	5	2823* ±127	487 ±34	17·2 ±0·6	79·4 ±9·8	37·9 ±5·2	0·66* ±0·08	39·1 ±5·2
58 days fresh water	6	2347 ±94	338 ±27	14·5 ±1·1	58·4 ±3·2	26·6 ±2·2	0·69 ±0·07	26·4 ±2·2
30 days saline+21 days fresh water +7 days saline	6	2518 ±64	684*** ±42	26·9** ±1·3	231** ±20	134** ±11·6	3·24** ±0·29	136** ±11·8

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ with respect to value for corresponding freshwater-maintained birds.

Table 3. *Concentration of electrolytes in the nasal gland fluid and the maximum nasal gland excretory rates in ducks maintained on various freshwater and/or saline regimes*

(Maximum extra-renal excretory rates were determined by the intravenous infusion of 10% sodium chloride solution. Excretory rates were then expressed per mg. wet weight of nasal gland tissue. All values are expressed as means ±S.E.)

Group	No. of birds	Concentration of nasal fluid (mM./l.) Na	K	Cl	Maximum excretory rates (mμl. or mμM./mg./min.) Water	Na	K	Cl
30 days fresh water	5	466 ±17	12·9 ±0·66	461 ±14	209 ±29	99·0 ±15·7	2·74 ±0·43	98·1 ±15
30 days saline	5	592*** ±8·0	14·8* ±0·52	585*** ±5·0	668*** ±54	395*** ±29	9·88*** ±0·82	390*** ±28
51 days fresh water	5	480 ±11	12·8 ±0·46	488 ±15	278 ±34	132 ±15	3·49 ±0·36	134 ±14
30 days saline+21 days fresh water	5	474 ±16	15·2** ±0·46	491 ±13	318 ±31	151 ±16	4·79* ±0·42	156 ±16
58 days fresh water	6	455 ±9·0	11·8 ±0·63	454 ±8·0	284 ±15	129 ±6·0	3·33 ±0·16	129 ±6·0
30 days saline+21 days fresh water+7 days saline	6	581*** ±10·7	14·0* ±0·50	589*** ±15·4	593*** ±36·5	344*** ±18·8	8·31*** ±0·55	365*** ±16·9

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ with respect to value for corresponding freshwater-maintained birds.

(Table 5). The slopes of these lines represent the moles of sodium transported per mole of inorganic phosphate released from the medium by each component of the enzyme system.

DISCUSSION

The mechanism of secretion by the nasal glands of marine birds would appear to involve the active transport of sodium. This is evidenced by the fact that the nasal

Table 4. *Moles of sodium transported per moles of ATP hydrolysed in ducks maintained on various freshwater and/or saline regimes*

(The ratios were derived from the individual values of the sodium excretory rates and the rates of ATP hydrolysis shown in Tables 1 and 3. All values are expressed as means ±S.E.)

		Na transported (moles) per ATP hydrolysed (moles)		
Group	No. of birds	Total	Ouabain insensitive	Ouabain sensitive
30 days fresh water	5	1·50 ±0·26	2·35 ±0·49	4·48 ±0·65
30 days saline	5	1·93 ±0·16	3·77 ±0·43	4·03 ±0·26
51 days fresh water	5	1·59 ±0·22	2·78 ±0·37	4·17 ±0·74
30 days saline + 21 days fresh water	5	2·21 ±0·26	3·93* ±0·18	5·45 ±0·82
58 days fresh water	5	2·41 ±0·31	5·18 ±0·91	4·66 ±0·37
30 days saline + 21 days fresh water + 7 days saline	6	2·21 ±0·15	4·38 ±0·66	4·70 ±0·16

* $P < 0.05$ with respect to value for corresponding freshwater maintained birds.

Table 5. *Correlation between the ATP-ase activities and the sodium-secretory capacities of nasal glands from saline-adapted ducks*

(The ATP-ase activities and sodium-secretory rates were determined according to the procedures outlined in Tables 1 and 2 respectively.)

Enzyme	No. of birds	Regression* ($Y = a + bX$)	$S_{y.x}$†	S_b‡	r§	P value
Total ATP-ase	11	$Y = 188{\cdot}1 + 0{\cdot}98X$	48·5	0·33	0·70	< 0·02
Ouabain-insensitive ATP-ase	11	$Y = 237{\cdot}4 + 1{\cdot}33X$	53·7	0·57	0·61	< 0·05
Ouabain-sensitive ATP-ase	11	$Y = 146{\cdot}4 + 2{\cdot}59X$	44·7	0·75	0·75	< 0·01

* $Y = a + bX$ where Y = sodium secreted in mμM./mg. wet weight nasal gland/min.; b = mμM. odium secreted/mμM. inorganic phosphate released; X = enzyme activity in mμM. inorganic phosphate released/mg. wet weight nasal gland/min.; and a = the ordinate intercept.

† $S_{y.x}$ = standard deviation from the regression.

‡ S_b = standard error of the regression coefficient (b).

§ r = the correlation coefficient.

fluid is composed primarily of a hypertonic sodium chloride solution, and that whilst the gland is secreting the nasal fluid in the duct is positively charged with respect to the blood (Thesleff & Schmidt-Neilsen, 1962). The movement of sodium therefore is against both an electrical and a chemical gradient which, by definition, would involve

active transport (Ussing, 1960). Furthermore, when strophanthin was injected into the duct of the nasal gland of the gull, both the secretion of sodium and the establishment of a positive potential were abolished (Thesleff & Schmidt-Nielsen, 1962). Thus the active transport of sodium by the nasal gland appears to be inhibited in the presence of a cardiac glycoside, a characteristic it shares with all other sodium-transporting tissues so far studied (Skou, 1965). This inhibition of sodium transport was presumably due to an action of the cardiac glycoside on the sodium-and-potassium-dependent ATP-ase system.

If an ATP-ase system is responsible for the active transport of sodium, then the activity of this enzyme system should correlate with the sodium-transporting capacity of the tissues in which it is found. Evidence for this may be found in red blood cells where the species-specific sodium-transporting properties have been shown to be directly proportional to their ATP-ase activities (Tosteson, Moulton & Blaustein, 1960; Bonting, Simon & Hawkins, 1961). Similar correlations have also been shown for a variety of other tissues (Bonting, Caravaggio & Hawkins, 1962; Bonting & Caravaggio, 1963). The nasal gland is of particular interest in this regard since it represents a tissue in which the sodium-transporting capacity varies according to the time the bird has been exposed to hypertonic drinking water.

In the present experiments the nasal gland tissue from the saline-adapted birds was always observed to release inorganic phosphate from the ATP in the medium at a higher rate than tissue derived from the freshwater-adapted birds; this was true regardless of the composition of the incubation medium. Therefore, if the characterization curves for each parameter (Figs. 1–6) can be interpreted to describe the dependence of the ouabain-sensitive ATP-ase system on the various substrates (Na, K and ATP), the differences observed between the saline-adapted and freshwater-adapted ducks cannot be attributed to differences in substrate optima.

The ATP-ase system of the nasal gland fulfills certain of the requirements necessary for an enzyme associated with the active transport of sodium. It specifically catalyses the hydrolysis of ATP and it appears to reside within the membranous components of the cell (Hokin, 1963). Also, its catalytic action is dependent upon the presence of both sodium and potassium and is inhibited by ouabain. The present study indicates that changes in the sodium-transporting capacities of nasal glands from birds in different physiological states correlate both temporally and quantitatively with changes in the ATP-ase activity. It should be noted, however, that although the ouabain-sensitive ATP-ase activities show a closer correlation with sodium transport than do the total or ouabain-insensitive ATP-ase actitives, all three parameters follow the same qualitative pattern. This may indicate that they are part of the same enzyme system which function differently in the presence or absence of ouabain (Skou, 1965).

The increase in weight-specific ATP-ase activity in the nasal glands of the duck adapted to saline could be due to (*a*) an increase in the specific activity of the enzyme molecule, (*b*) an increase in the amount of enzyme present per unit membrane, and (*c*) an increase in the amount of membrane per cell and its associated ATP-ase system.

Since the ATP-ase enzyme system appears to require an organized lipoprotein structure (Skou, 1965; Tanaka & Abood, 1964) and recent observations by Ellis, Geortemiller, DeLellis & Kablotsky (1963) have shown that the concentration of

phospholipid rises in the nasal glands of ducks exposed to salt water, an increase in the membranous components of those cells involved in the active transport of sodium seems likely. This increase in membrane could thus accommodate a greater amount of the associated ATP-ase enzyme system. It would also provide an increased surface area for the presumably passive movements of chloride and water along the osmotic and electrical gradients created by the active transport of sodium.

The concentration of sodium chloride in the nasal gland fluid of the saline-adapted duck is approximately 120 mM./l. greater than that from the freshwater-adapted ducks. If one presumes that the cells involved in sodium transport are maintaining a steady electro-chemical gradient, then such an increase in sodium chloride concentration could be produced by a similar rise in concentration at the basal side of the cell. There is no evidence that this is so, for the plasma levels of sodium chloride are the same in both groups of ducks and a counter-current multiplier system does not seem to be present (Fänge, Schmidt-Nielsen & Osaki, 1958; Schmidt-Nielsen, 1960). If this is the case then the cells of the nasal glands from saline-adapted ducks have developed the ability to maintain a larger electro-chemical gradient than the corresponding cells in the freshwater-adapted duck. Such an increase implies a change in some property of the membranes of the transporting cells. This may involve their permeability to water or an increase in the ATP-ase activity per unit membrane. The cells of the nasal gland, like those of the frog skin and toad bladder, are polar in that they are capable of effecting the net transfer of sodium chloride and water from the blood to the duct lumen. If the model proposed by Koefoed-Johnsen & Ussing (1958) for the unidirectional movements of sodium across the frog skin, and the known stoichiometry of the enzyme system responsible for sodium transport, can be applied to the nasal gland cells, then it would appear that the ATP-ase system resides on the luminal side of the secreting cells. The changes found in the ATP-ase activity of nasal glands from ducks adapted to saline would therefore presumably take place in the apical part of the cells, while changes in permeability to the passive components of the nasal fluid could occur both basally and apically.

Both nervous and hormonal factors have been implicated as regulators of sodium excretion by the avian nasal gland. Fänge, Schmidt-Nielsen & Robinson (1958) have shown that stimulation of the cut branch of the facial nerve supplying the nasal gland resulted in the excretion of sodium. Furthermore, cholinergic drugs have been found to have a similar stimulatory effect when parenterally administered, whereas cholinergic-blocking agents and sympathomimetic drugs were found to be inhibitory (Fänge, Schmidt-Nielsen & Robinson, 1958). The role of the parasympathetic innervation appears to be at least in part vasomotor (Fänge, Krog & Reite, 1963; Phillips & Bellamy, 1967). However, Fawcett (1962) has claimed that nerves actually terminate on some of the principal cells within the nasal gland of the gull (*Larus argentatus*) and recently van Rossum (1966) has shown *in vitro* that methylcholine chloride stimulated the efflux of $^{24}Na^{+}$ from the nasal gland cells.

Evidence is also available to suggest that the adrenocortical steroids are involved in nasal gland excretion (Holmes, Phillips & Butler, 1961; Phillips, Holmes & Butler, 1961; Phillips & Bellamy, 1962; Donaldson & Holmes, 1965; Wright, Phillips & Huang, 1966). Although the role of the adrenocorticoids has still to be elucidated, the recent observations that an intracellular accumulation of corticosterone occurred

in the secreting nasal glands of the salt-loaded freshwater-adapted ducks (Bellamy & Phillips, 1966) may indicate their possible mode of action.

Recently, studies on the toad bladder have led to the hypothesis that the aldosterone-stimulated active sodium transport is preceded by the induced synthesis of protein (Sharpe & Leaf, 1966; Edelman, 1965). The exact nature and role of this newly synthesized protein is at present not clear although it does not appear to be a Na-K-dependent ATP-ase system (Sharpe & Leaf, 1966). Studies on the rat kidney, however, (Chignell & Titus, 1966; Landon, Jazab & Forte 1966) seem to suggest that the level of ATP-ase activity may be regulated by the glucocorticoids.

It has been well established that the marine birds rely upon the presence of functional nasal glands to remove the excess sodium chloride injested with their drinking water. The concentration of sea water and the maximum daily intake of sodium chloride which these birds can tolerate, however, is still largely a matter of conjecture.

If we assume that the kidneys of marine species have roughly the same excretory capacity, then the amount of sodium chloride which these birds may ingest will depend upon several properties of the nasal glands; these include the size of the gland, the concentrating ability of the tissue and the rate of sodium transport. Thus the penguin (*Pygoscelis adeliae*) with a relative nasal gland weight of 500 mg. % body weight (Douglas, 1964) would be expected to be capable of excreting more sodium than the duck with a nasal gland weight of 28·7 mg. % body weight. The gull (*Larus glaucescens*) with a nasal gland weight of 102 mg. % body weight would be intermediate in this regard (Holmes, Butler & Phillips, 1961). The concentrating ability of the nasal gland tissue determines the volume of free water the bird can obtain from the ingested sea water. Thus, Leech's petrel (Schmidt-Nielsen, 1960) which secretes a nasal fluid containing 1000 m-equiv./l. sodium will obtain 53 ml. of sodium-free water from every 100 ml. of standard seawater (470 mM./l. sodium) drunk. The herring gull (Schmidt-Nielsen, 1960) and the duck, on the other hand, would gain only 33 and 22 ml. of water under similar circumstances. The rate at which the nasal gland can transport sodium also appears to be an important parameter controlling the bird's ability to live on hypertonic saline for the nasal gland of a saline-adapted duck can transport sodium at four times the rate of the freshwater-adapted duck. Preliminary studies have indicated that the ouabain-sensitive ATP-ase activity of a herring gull was 50% greater than that of the saline-adapted duck (153 *vs.* 98·2 mμM.P_i released/mg. wet weight nasal tissue/min., unpublished observation) suggesting that the sodium-transporting capacity of the gull nasal gland is greater than that of the duck.

In conclusion, therefore, these data suggest that during the period of adaptation to sea water, the duck develops an increased ability to survive on a saline diet. This increased ability to survive appears to be in part due to developmental changes occurring in the nasal gland. These include a doubling in size, a 27% increase in the sodium concentration and a fourfold increase in the weight-specific sodium-transporting capacity of the tissue.

SUMMARY

1. Regardless of the composition of the incubation medium, the nasal gland tissue from ducks adapted to hypertonic saline always released inorganic phosphate from

the ATP in the medium at a higher rate than tissue derived from freshwater-adapted ducks.

2. Changes in the nasal gland ATP-ase activities during the period of adaptation to saline followed the same time course as the changes which occurred in the sodium-excretory capacity of the tissue.

3. Adaptation to saline resulted in a three- to fourfold increase in the weight-specific ATP-ase activity which was accompanied by a three- to fourfold increase in the weight-specific sodium-excretory capacity.

4. In the saline-adapted birds a positive correlation was found between the sodium-excretory capacity of the nasal gland tissue and the corresponding ATP-ase activity.

5. The moles of sodium excreted per mole of ATP hydrolysed was constant irrespective of whether the birds were adapted to either fresh water or salt water.

6. Concommitant with the changes in weight-specific sodium excretion and ATP-ase activity, an increase in the sodium concentration of the nasal gland fluid was observed. An approximately twofold increase in nasal gland weight also occurred during this period.

7. All changes which were observed to occur during the period of adaptation to saline were reversed when the birds were returned to fresh water.

8. The observed changes in enzyme activity and sodium excretory rates of the nasal glands are discussed in relation to possible cellular changes which might occur and their importance relative to the birds's ability to adapt to a marine environment.

REFERENCES

BELLAMY, D. & PHILLIPS, J. G. (1966). Effect of the administration of sodium chloride solutions on the concentration of radioactivity in the nasal glands of ducks (*Anas platyrhynchos*) injected with ^{3}H-corticosterone. *J. Endocrin.* **36**, 97–8.

BONTNG, S. L., SIMON, K. A. & HAWKINS, N. M. (1961). Studies on sodium-potassium-activated adenosinetriphosphatase. I. Quantitative distribution in several tissues of the cat. *Archs. Biochem. Biophys.* **95**, 416–23.

BONTING, S. L., CARAVAGGIO, L. L. & HAWKINS, N. M. (1962). Studies on sodium-potassium-activated adenosinetriphosphatase. IV. Correlation with cation transport sensitive to cardiac glycosides. *Archs. Biochem. Biophys.* **98**, 413–19.

BONTING, S. L. & CARAVAGGIO, L. L. (1963). Studies on sodium-potassium-activated adenosinetriphosphatase. V. Correlation of enzyme activity with cation fluxes in six tissues. *Archs. Biochem. Biophys.* **101**, 37–46.

BONTING, S. L., CARAVAGGIO, L. L., CANADY, M. R. & HAWKINS, N. M. (1964). Studies on sodium-potassium-activated adenosine triphosphatase. XI. The salt gland of the herring gull. *Archs. Biochem. Biophys.* **106**, 49–56.

CHIGNELL, C. F. & TITUS, E. (1966). Effect of adrenal steroids on Na^+- and K^+-requiring adenosine triphosphatase from rat kidney. *J. biol. Chem.* **241**, 5083–9.

COTLOVE, K. (1963). Determination of the true chloride content of biological fluids and tissues. II. *Analyt. Chem.* **35**, 101–5.

DONALDSON, E. M. & HOLMES, W. N. (1965). Corticosteroidogenesis in the fresh water and saline-maintained duck (*Anas platyrhynchos*). *J. Ednocrin.* **32**, 329–36.

DOUGLAS, D. S. (1964). Extra-renal salt excretion in the Adélie penguin chick. *Biologie Antartique, C. R. Premier Sym.* (Ed. R. Carrick, M. Holdgate and J. Provost). Paris.

EDELMAN, I. S. (1965). Relationship of protein synthesis to ion transport in mammalian systems. *Proc. XXIII Int., Contr. Physiol. Sci., Tokyo, Exerpta Medica Int. Congr.* (Series no. 87), pp. 587–95.

ELLIS, R. A., GOERTEMILLER, C. C., DELELLIS, R. A. & KABLOTSKY, Y. H. (1963). The effect of a salt-water regimen on the salt glands of domestic ducklings. *Devl Biol.* **8**, 286–308.

FÄNGE, R., SCHMIDT-NIELSEN, K. & OSAKI H, (1958). The salt gland of the herring gull. *Biol. Bull. mar. biol. Lab. Woods Hole*, **115**, 162–71.

FÄNGE, R., SCHMIDT-NIELSEN, K. & ROBINSON, M. (1958). Control of secretion from the avian salt gland. *Am. J. Physiol.* **195**, 321–6.

FÄNGE, R., KROG, J. & REITE, O. (1963). Blood flow in the avian salt gland studied by polarographic oxygen electrodes. *Acta Physiol. Scand.* **58**, 40–7.

FAWCETT, D. W. (1962). Physiologically significant specializations of the cell surface. *Circulation* **26**, 1105–32.

FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorous. *J. biol. Chem.* **66**, 375–400.

HOKIN, M. R. (1963), Studies on a $Na^+ + K^+$-dependent, ouabain-sensitive adenosine triphosphatase in the avian salt gland. *Biochim. biophys. Acta* **77**, 108–20.

HOLMES, W. N., BUTLER, D. G. & PHILLIPS, J. G. (1961). Observations on the effect of maintaining glaucous-winged gulls (*Larus glaucescens*) on freshwater and seawater for long periods. *J. Endocrin.* **23**, 53–61.

HOLMES, W. N., PHILLIPS, J. G. & BUTLER, D. G. (1961). The effect of adrenocortical steroids on the renal and extrarenal responses of the domestic duck (*Anas platyrhynchos*) to saline loading. *Endocrinology* **69**, 483–95.

KOEFOED-JOHNSEN, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

LANDON, J. E., JAZAB, N. & FORTE, L. (1966). Aldosterone and sodium-potassium-dependent ATP-ase activity of rat kidney membranes. *Am. J. Physiol.* **211**, 1050–6.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–75.

PHILLIPS, J. G., HOLMES, W. N. & BUTLER, D. G. (1961). The effect of total and subtotal adrenalectomy on the renal and extrarenal response of the domestic duck (*Anas platyrhynchos*) to saline loading. *Endocrinology* **69**, 958–69.

PHILLIPS, J. G. & BELLAMY, D. (1962). Aspects of the hormonal control of nasal gland secretion in birds. *J. Endocrin.* **24**, vi.

PHILLIPS J. G. & BELLAMY, D. (1967). The control of nasal glands function, with special reference to the role of adrenocorticosteroids. *Proc. 2nd Int. Symp. Hormonal Steroids. Excerpta Medica Int. Congr.* (Series no. 132), pp. 877–81.

POST, R. L. & SEN, A. K. (1964). An enzymatic mechanism of active sodium and potassium transport. *J. Histochem. Cytochem.* **13**, 105–12.

VAN ROSSUM, G. D. V. (1966). Movements of Na^+ and K^+ in slices of herring-gull salt gland. *Biochim. biophys. Acta* **126**, 338–49.

SCHATZMAN, J. H. (1953). Herzglykoside als Hemmstoffe fur den activen Kalium und Natrium transport durch die Erythrocytenmembran. *Helv. physiol. pharmac. Acta* **11**, 346–54.

SCHMIDT-NIELSEN, B. & SCHMIDT-NIELSEN, KNUT (1950). Do Kangaroo rats thrive when drinking sea water? *Am. J. Physiol.* **160**, 299–4.

SCHMIDT-NIELSEN, K., JORGENSEN, C. B. & OSAKI, H. (1958). Extrarenal salt excretion in birds. *Am. J. Physiol.* **193**, 101–7.

SCHMIDT-NIELSEN, K. & SLADEN, W. J. L. (1958). Nasal salt secretion in the Humbolt Penguin. *Nature, Lond.* **181**, 1217–18.

SCHMIDT-NIELSEN, K. (1960). The salt secreting gland of marine birds, *Circulation* **21**, 955–67.

SCOTHORNE, R. J. (1958). A histochemical study of the nasal (supraorbital) gland of the duck. *Nature, Lond.* **182**, 732.

SCOTHORNE, R. J. (1959). On the response of the duck and the pigeon to intravenous hypertonic saline solutions. *Q. Jl exp. Physiol.* **44**, 200–7.

SHARPE, G. W. G. & LEAF, A. (1966). Mechanism of action of aldosterone. *Physiol. Rev.* **46**, 593–633.

SKOU, J. C. (1957). The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. biophys. Acta* **23**, 394–401.

SKOU, J. C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. *Physiol. Rev.* **45**, 596–617.

SNEDECOR, G. W. (1956). *Statistical Methods.* Ames, Iowa: Iowa State College Press.

SPERBER, I. (1960). In vol. 1 of *Biology and Comparative Physiology of Birds*, chap. XII, pp. 469–92. (Ed. A. T. Marshall.) Academic Press.

TANAKA, R. & ABOOD, L. G. (1964). Phospholipid requirement of Na^+, K^+-activated adenosine triphosphatase from rat brain. *Archs Biochem. Biophys.* **108**, 47–52.

THESLEFF, S. & SCHMIDT-NIELSEN, K. (1962). An electrophysiological study of the salt gland of the herring gull. *Am. J. Physiol.* **202**, 597–600.

TOSTESON, D. C., MOULTON, R. H. & BLAUSTEIN, M. P. (1960). An enzymatic basis for the difference in active cation transport in two genetic types of sheep red cells. *Fed. Proc.* **19**, 128.

USSING, H. H. (1960). The alkali metal ions in isolated systems and tissues. *Handb. exptl Pharmakol.* **13**, 1–195.

WIRZ, H., HARGITAY, B. & KUHN, W. (1951). Concentrating mechanism in the mammalian kidney. *Acta Helv. Physiol.* **9**, 196–207.

WRIGHT, A., PHILLIPS, J. G. & HUANG, D. P. (1966). The effect of adenohypophysectomy on the extrarenal and renal excretion of the saline-loaded duck (*Anas platyrhynchos*). *J. Endocrin.* **36**, 249–56.