The Na^+, K^+, and Cl^- Content of Goose Salt Gland Slices and the Effects of Acetylcholine and Ouabain

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ABSTRACT In goose salt gland slices incubated in bicarbonate-buffered medium which contained 170 mEq of Na^+/liter, net total tissue Na^+, expressed as milliequivalents per kilogram, was, in the presence of either acetylcholine (plus eserine) or ouabain, significantly higher than that of the bathing fluid. Acetylcholine caused an increase in the tissue Na^+ content as compared with untreated slices; there was an approximately equivalent decrease in K^+ and a significant decrease in Cl^-. The calculated net intracellular concentrations of Na^+, expressed as milliequivalents per liter of intracellular water, in unstimulated, acetylcholine-stimulated, and ouabain-treated slices were 2.1, 3.1, and 2.7 times higher, respectively, than the concentration of Na^+ in the bathing fluid. The net intracellular concentration of Na^+, expressed as milliequivalents per liter of intracellular water, in slices incubated in the presence of acetylcholine was 531 mEq/liter; this is approximately the same as the concentration of Na^+ in the secreted fluid of the goose salt gland (515 mEq/liter). The results indicate that the main concentration gradient for Na^+ could be established across the basal membrane. The data do not indicate whether this involves active transport of Na^+ per se. A second stage which might involve Na-K ATPase activity at the luminal membrane is discussed. The sum of the total tissue Na^+ and K^+ was approximately 250 mEq/kg, whereas the Cl^- content was only approximately 130 mEq/kg.

INTRODUCTION

The avian salt gland is an extrarenal excretory organ which secretes a fluid which contains concentrations of NaCl which range, in different species, from 0.5 M to 1 M, the concentrations of Na^+ and Cl^- in the duct fluid being approximately 2.5 to 5 times higher than the concentrations of these ions in the plasma. The physiology of salt glands has been reviewed by Schmidt-Nielsen (1). Fänge, Schmidt-Nielsen, and Robinson (2) showed that the secretory activity of the glands is under nervous control; this appears to be mediated by

the action of increased plasma osmotic pressure on osmoreceptors in the central nervous system; this increased osmotic pressure need not be due to NaCl—secretion of NaCl by the glands also occurs in response to sucrose load (3). The immediate stimulus for secretion is the liberation of acetylcholine (ACh) at nerve endings in the gland. The nature and cellular location of the concentrating mechanisms in the gland which are involved in the secretory activity are poorly understood. The salt gland cell is clearly a polarized cell with respect to transport associated with secretion, since there is net transport of secreted material from plasma to duct. The pharmacological and morphological evidence suggests that both the luminal membrane and the basal membrane of the cell may play a role in the over-all process.

Thesleff and Schmidt-Nielsen (4) have shown that, during secretion, there is an over-all potential difference of 40–60 mv from plasma to lumen of the duct, with the duct positive. This suggests that the transport of Na^+ is against an electrochemical gradient in the over-all process and implies therefore that at some stage active Na^+ transport must be involved. Ouabain, administered by retrograde injection through the duct system, inhibits NaCl secretion by the gland and abolishes the potential difference from plasma to duct which is set up in response to stimulation of secretion (4). Ouabain also inhibits the stimulation of respiration in salt gland slices which occurs in response to ACh and which is presumably connected with the secretory response of the tissue (5, 6). These observations suggested that a $Na^+ + K^+$–dependent adenosinetriphosphatase (Na-K ATPase) might be concerned in the secretory process. Na-K ATPase activity was first demonstrated by Skou in crab nerve (7); work from many laboratories has since provided much evidence that this enzyme is, in many tissues, involved in the active transport of Na^+ out of the cell and the transport of K^+ into the cell; ouabain inhibits this transport and inhibits Na-K ATPase activity. The inhibitory action of ouabain on salt gland secretion suggests that an Na-K ATPase is concerned with NaCl secretion by the gland. If this is oriented as in other cells in which it has been studied, one would expect to find it in the luminal membrane of the salt gland cell.

The salt gland has a very high content of mitochondria. Their function is presumably to supply energy in a utilizable form for the great amount of osmotic work carried out by the secreting cell. However, the mitochondria are concentrated primarily near the basal surface of the cell (8, 9). The morphological picture suggests therefore that a primary energy-requiring mechanism for the elaboration of the secretory fluid might be near the basal rather than the luminal surface of the cell. Such a concentration of Na^+ from the extracellular to the intracellular fluid should result in a high concentration of Na^+ in the intracellular fluid during secretory activity of the glands. The present work was undertaken in order to investigate this possibility. The concentrations of Na^+, K^+, and Cl^- were determined in goose salt gland slices after incubation

in bicarbonate-buffered medium which contained a concentration of Na^+ approximately the same as that of the plasma of the secreting goose. The effects of acetylcholine and of ouabain were studied.

EXPERIMENTAL

Domestic geese (White China variety) were given 1.5% NaCl as their drinking water for several weeks before sacrifice. The birds were killed by decapitation.

To obtain samples of secreted fluid, geese were given an injection of 10 ml of 10% NaCl into a vein in the foot and the secretory fluid was collected by polyethylene catheter, as described by Fänge et al. (2).

Slices of goose salt gland were prepared as described previously (10). The mean thickness of the slices was 0.35 mm, SE ± 0.03, eight observations. The slices were kept in a covered dish on ice until they were placed in the incubation vessels. They were blotted lightly on Whatman No. 50 filter paper and weighed before incubation. The weight of the tissue slice per vessel was approximately 50 mg (range ± 10 mg). The volume of the incubation medium was 1 ml. Each slice was placed in its incubation vessel, the vessel was filled rapidly with the appropriate gas, and was then immediately placed in a shaking incubation bath at 38°C. The gas phase was renewed each time the vessels were opened for additions to be made to the medium. Drugs were added as concentrated solutions in 0.9% NaCl (0.01 ml per vessel); an equal amount of 0.9% NaCl was added to the control flasks when the additions were made. The vessels were placed in the bath individually at timed intervals; at the end of the incubation period they were removed at the corresponding time intervals, so that all slices were in the incubation medium for the same length of time. At the end of the incubation period, the slice from each vessel was immediately removed, blotted lightly, and weighed.

Incubation Medium The incubation medium was a modified bicarbonate-CO_2–buffered medium based on that of Krebs and Henseleit (11). The components were 170 mM Na^+, 6 mM K^+, 2.3 mM Ca^{++}, 1 mM Mg^{++}, 157 mM Cl^-, 1 mM phosphate, 1 mM SO_4^{--}, 23 mM HCO_3^-, and 6 mM glucose. The gas phase was 95% O_2 + 5% CO_2.

Volume of the Extracellular Space Inulin-carboxyl-^{14}C was obtained from Tracerlab (Tracerlab Div. Nuclear Instruments & Radioactive Chemicals, Waltham, Mass.). This was added to the medium in which salt gland slices were incubated, with shaking, at 38°C, under the same conditions as those used for estimation of the ion content of the slices. At the end of the incubation period the slices were removed from the medium, blotted lightly on Whatman No. 50 filter paper, and weighed. They were then homogenized in 2 ml of water and homogenates and rinsings were made up to 5 ml volume. The suspension was centrifuged for 20 min at 20,000 *g*. Aliquots of supernatant fluid (0.5 ml) were plated, dried, and counted in a thin end-window Geiger-Müller counter (Nuclear-Chicago Corp., Des Plaines, Ill.). This gave a measure of the inulin-carboxyl-^{14}C in solution in the tissue slice. The volume of the inulin space was calculated with reference to the counts per minute per milliliter of medium, as assayed under the same conditions; the calculation makes the

assumption that the radioactive inulin in the slice was in equilibrium with the radioactive inulin in the bathing fluid. To obtain the counts per minute per milliliter of incubation medium under these assay conditions, 50 μl of medium were added to unincubated slices in 2 ml of water and these were then homogenized and submitted to the same procedures as the incubated slices. Aliquots of the supernatant fluid (0.5 ml) after centrifugation were plated and counted. Two batches of inulin-carboxyl-^{14}C were used. They both gave essentially the same results.

Dry Weight: Wet Weight Ratios Incubated slices were blotted lightly, weighed in tared vessels, and dried to constant weight in an oven at 110°C.

Densities of Dried Tissue Densities of the dried material were obtained by measuring buoyancy in $ZnCl_2$ solutions, as described by Hagemeijer, Rorive, and Schoffeniels (12).

Ion Measurements Incubated tissue was placed in 1 ml of concentrated nitric acid and left overnight, during which time the tissue was completely dissolved. Na^+ and K^+ concentrations were estimated by flame photometry. After suitable dilution, Cl^- was estimated by a slight modification of the titrimetric method of Schales and Schales (13) using an Aglar micrometer syringe (Burroughs Wellcome & Co., London, England).

RESULTS

Design of Experiments Four basic conditions were used in each experiment.

These were (*a*) Resting or control conditions, in which no agents were added to the bathing fluid of the vessels in which the slices were incubated. (*b*) Secretory conditions, in which ACh (10^{-6} M) plus eserine (10^{-4} M) was added to the bathing fluid; eserine was added to inhibit endogenous acetylcholinesterase activity. The secretory response of salt gland slices to ACh and other cholinergic agents is attested to by the increased efflux of ^{24}Na which occurs in response to metacholine (14), by the increased O_2 uptake which occurs in response to ACh (10, 5, 6), and by the lowered steady-state level of ATP in ACh-stimulated slices, which reflects an increased utilization of ATP by the tissue (15). (*c*) Resting conditions in which ouabain (10^{-4} M) was added to the bathing fluid in order to inhibit any ouabain-sensitive Na^+ transport in the resting tissue. (*d*) Slices incubated in the presence of both ACh (plus eserine) and ouabain. Inhibition by ouabain of ACh-stimulated secretory activity in salt gland slices is attested to by the observation that ouabain stops the stimulated respiration due to ACh (5, 6) and it reverses the lowered steady-state level of ATP which occurs in response to ACh (plus eserine) (15).

Net Tissue Concentrations of Na^+, K^+, and Cl^- Compared with the Concentrations in the Bathing Fluid In the series of experiments reported here, the Na^+ content of the incubation medium was adjusted to approximate that of plasma of

geese which had been maintained on 1.5% NaCl as their drinking water. This was found to be 176 mEq/liter (SE ± 12, four animals); the plasma K^+ value was 7.5 mEq/liter (SE ± 1.0, four animals). The medium contained bicarbonate concentrations similar to those of plasma (11); this is of considerable importance for achieving a maximum respiratory response to ACh (15).

All slices were incubated under the same conditions for 20 min before the drugs were added in order to eliminate the effects of the drugs in the initial warming up and equilibration period.

Values for the Na^+, K^+, and Cl^- levels in incubated slices, expressed as milliequivalents per kilogram final weight of tissue, and for the medium

TABLE I

SODIUM, POTASSIUM, AND CHLORIDE CONTENT OF GOOSE SALT GLAND SLICES INCUBATED IN BICARBONATE-BUFFERED MEDIUM AND THE EFFECTS OF ACETYLCHOLINE AND OUABAIN

Additions	Na^+		K^+		Cl^-	
	Medium	Tissue	Medium	Tissue	Medium	Tissue
	mEq/liter	*mEq/kg*	*mEq/liter*	*mEq/kg*	*mEq/liter*	*mEq/kg*
None	172 ± 3	181 ± 15	6.0 ± 0.1	66 ± 8	156 ± 1	141 ± 3
ACh + eserine	170 ± 2	214 ± 16	6.6 ± 0.3	40 ± 6	158 ± 2	123 ± 6
Ouabain	171 ± 2	225 ± 11	8.4 ± 0.9	12.2 ± 0.7	157 ± 2	126 ± 7
ACh + eserine + ouabain	172 ± 2	228 ± 15	9.1 ± 1.1	11.7 ± 1.2	156 ± 1	112 ± 27

Goose salt gland slices were incubated in the bicarbonate-buffered medium described in the text for 20 min before the additions were made and for 40 min after the additions were made. Initial concentrations of Na^+, K^+, and Cl^- in the incubation medium were 170, 6, and 157 mM, respectively. Concentrations of additions were: ACh, 10^{-6} M; eserine, 10^{-4} M; ouabain, 10^{-4} M. All four variables were compared in each experiment, using slices from the same goose. Values for Na^+ and K^+ are the means ± SE of values from six experiments; values for Cl^- are the means ± SE of values from three experiments. Values for tissue content were calculated using the final blotted weight of the tissue after incubation. Values for the medium are final values assayed from each vessel at the end of the incubation period.

(milliequivalents per liter), assayed after the incubation period, are shown in Table I. It can be seen from this table that the sum of the Na^+ and K^+ contents of the slices was approximately 250 mEq/liter, whereas the Cl^- content of the tissue was only approximately 130 mEq/liter. These results suggest the presence of a substantial amount of unidentified anion in the tissue under these conditions.

Differences between the net tissue concentration of the ions, expressed as milliequivalents per kilogram, and concentrations of ions in the medium, expressed as milliequivalents per liter, were calculated in each experiment. The mean differences are shown in Table II. The net tissue concentration of Na^+ of unstimulated slices was not significantly different from the Na^+ con-

centration in the bathing fluid under these conditions. However, in the presence of ACh (plus eserine) the net Na^+ concentration in the tissue was significantly higher than that of the bathing fluid. Since the volume of 1 kg of tissue does not exceed 1 liter (see below), these results indicate that, irrespective of the validity of the calculations of intracellular concentrations discussed below, the cells can achieve and maintain a higher net Na^+ concentration than that of the bathing medium; i.e., the cells have a mechanism for directly or indirectly concentrating Na^+ into the cell from the surrounding fluid.

The net Na^+ concentration in the tissue was also significantly higher than that of the bathing fluid in the presence of ouabain, either without or with

TABLE II

MEAN DIFFERENCES BETWEEN NET TISSUE CONTENT OF IONS AND MEDIUM CONTENT OF IONS

Additions	Na^+			K^+			Cl^-		
	mEq	SE	*P* <	*mEq*	SE	*P* <	*mEq*	SE	*P* <
None	+8.0	± 5.5	n.s.	+60.0	± 7.9	0.0005	−14.0	± 1.4	0.01
ACh + eserine	+43.3	± 15.5	0.025	+33.3	± 6.5	0.005	−35.0	± 5.0	0.005
Ouabain	+54.0	± 11.1	0.005	+3.5	± 0.7	0.005	−31.0	± 7.5	0.025
ACh + eserine + ouabain	+56.5	± 10.7	0.005	+2.5	± 1.5	n.s.	−44.0	± 20.4	n.s.

The assayed concentration of ion in the medium of each vessel (milliequivalents per liter) was subtracted from the concentration of ion (milliequivalents per kilogram) in the tissue slice from the same vessel. Values show the means ± SE of the differences. *P* values were obtained from Student's *t* test and show the probability that the mean values do not differ from zero in one direction, either positive or negative, as indicated by the sign. The degrees of freedom for Na^+ and K^+ values were five and for Cl^- values were two. The values were taken from the experiments described in Table I.

n.s., not significant ($P > 0.05$).

ACh (plus eserine). These results indicate that the mechanism which is responsible for the concentration of Na^+ into the tissue from the surrounding fluid is not blocked by the presence of ouabain in the incubation medium.

Net tissue concentrations of K^+ were significantly higher than those of the bathing fluid in unstimulated, ACh-stimulated, and ouabain-treated slices.

Net tissue concentrations of Cl^- were significantly lower than those of the bathing fluid in unstimulated, ACh-stimulated, and ouabain-treated slices.

Changes in Net Tissue Content of Ions Due to ACh Table III shows the mean change in tissue ions in ACh-treated slices when values in the individual experiments were compared with values for untreated slices in the same experiment. A significantly higher net tissue content of Na^+ was maintained in the presence of ACh (plus eserine). The increase in net tissue Na^+ was accom-

panied by an approximately equivalent decrease in net tissue K^+; there was also a significant decrease in net tissue Cl^- content.

Changes in Net Tissue Content of Ions Due to Ouabain A significantly higher net tissue content of Na^+ was maintained in the presence of ouabain and of ouabain plus ACh and eserine than in the untreated slices (Table III). Since, under these conditions, the net tissue content of Na^+ in ouabain-treated slices was higher than that of the bathing fluid (Table II), these results indicate that the gradient of Na^+ from extracellular fluid to tissue increases in the presence of ouabain as compared with the control slices. The tissue content of K^+ fell in the presence of ouabain; the Cl^- content also showed a small but significant fall.

TABLE III

MEAN DIFFERENCES BETWEEN NET TOTAL TISSUE CONTENT OF IONS IN SLICES TREATED WITH ACETYLCHOLINE OR OUABAIN AND NET TOTAL TISSUE CONTENT OF IONS OF UNTREATED SLICES

Additions	Na^+			K^+			Cl^-		
	mEq/kg	SE	*P* <	*mEq/kg*	SE	*P* <	*mEq/kg*	SE	*P* <
ACh + eserine	+32.8	± 6.8	0.005	−26.0	± 2.6	0.0005	−24.7	± 6.60	0.05
Ouabain	+44.5	± 8.5	0.005	−53.8	± 9.1	0.005	−13.0	± 3.54	0.05
ACh + eserine + ouabain	+47.3	± 8.7	0.005	−53.4	± 6.9	0.005	−25.7	± 22.5	n.s.

The tissue content of ion (milliequivalents per kilogram) of slices incubated without additions was subtracted in each individual experiment from the tissue content of ions (milliequivalents per kilogram) of slices incubated with the indicated additions. Values show the means ± SE of the differences. *P* values were obtained from Student's *t* test and show the probability that the mean values do not differ from zero in one direction, either positive or negative, as indicated by the sign. The degrees of freedom for Na^+ and K^+ values were five and for Cl^- values were two. The values were taken from the experiments described in Table I.
n.s., not significant ($P > 0.05$).

The fall in tissue K^+ in the presence of ouabain can be explained by inhibition of Na-K–dependent transport of K^+ into the cell. Such transport would result in untreated slices in the removal of some Na^+ from the cell and this would tend to offset the concentration of Na^+ into the slice. Inhibition by ouabain of this K^+-linked movement of Na^+ out of the cell, accompanied by lack of inhibition of the mechanism for concentrating Na^+ into the cell, could account for the observation that the gradient of Na^+ from extracellular fluid to tissue increases in the presence of ouabain as compared with the control slices. (The inhibition by ouabain of Na-K–dependent transport activity which maintains the "normal" low levels of Na^+ and "normal" high levels of K^+ in cells in other tissues leads to an increase in the intracellular Na^+ and a decrease in intracellular K^+ due to the approach to equilibrium of the concentrations of these ions in the extracellular and intracellular fluids. The

situation in the salt gland slices is quite different from this in respect to Na^+, since the Na^+ concentration in the slices is maintained higher than that of the extracellular fluid and this gradient increases rather than decreases in the presence of ouabain. It is necessary to conclude therefore that ouabain in the bathing fluid does not inhibit the mechanism for maintaining this gradient.)

Volume of the Extracellular Space Calculation of intracellular concentrations involves having a reasonably accurate estimate of that volume of the tissue which is in equilibrium with the bathing fluid under the relevant ex-

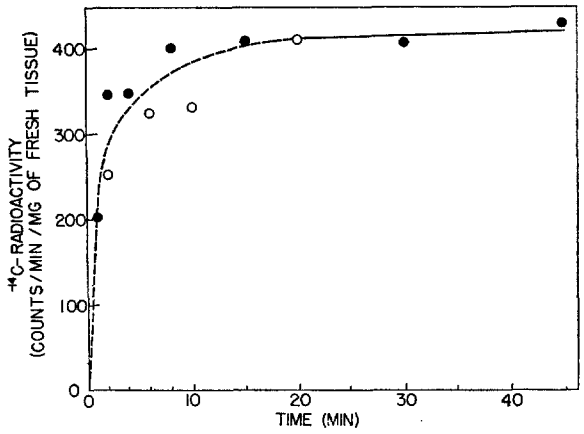

FIGURE 1. Time course of the uptake of inulin-carboxyl-^{14}C in goose salt gland slices. Solid circles and open circles show results from two separate experiments. Slices (weight range, 48–64 mg) were incubated at 38°C, with shaking, in 1 ml of Krebs and Henseleit bicarbonate medium which contained Na^+, 143 mM; Cl^-, 130 mM; inulin-carboxyl-^{14}C, 0.87 mg per ml, specific activity, 2.6 μc per mg; and other constituents as described in the text. Under the assay conditions described in the text, the medium gave 1.00×10^6 (solid circles) and 1.04×10^6 (open circles) counts per minute per milliliter in the two experiments. Values given are the averages of duplicates which did not differ by more than 10%; they are expressed as counts per minute per milligram initial fresh weight of tissue slice.

perimental conditions. In the experiments reported here, this was estimated by measurement of the inulin-carboxyl-^{14}C space.

In separate experiments, time course showed that the uptake of inulin-carboxyl-^{14}C was essentially complete after 10 min in incubated slices (Fig. 1). Values for inulin space obtained after 20 and 60 min of incubation in other experiments were, within experimental error, the same. Values for inulin space determined with a range of concentrations of inulin-carboxyl-^{14}C of 0.22 to 1.66 mg per ml were also, within experimental error, the same. In another series of experiments, the inulin-carboxyl-^{14}C space was compared with the volume of the extracellular space calculated from kinetic uptake

data to be in radioactive equilibrium with orthophosphate-^{32}P added to the medium (16). The value for inulin space under these conditions, expressed as milliliters per kilogram initial fresh weight of tissue was 430, SE ± 23, five experiments; the value for the extracellular space as determined by the ^{32}P uptake method was 422, SE ± 51, three experiments.

These results were taken to indicate that measurement of the volume of the extracellular space by the uptake of inulin-carboxyl-^{14}C was a reasonably valid procedure in salt gland slices under these conditions.

The inulin-carboxyl-^{14}C spaces of goose salt gland slices incubated under the conditions of Table I (in the same experiments) are shown in Table IV. The values are expressed as milliliters per kilogram final blotted weight of tissue. For this reason they are somewhat higher than the values quoted

TABLE IV

Additions	Inulin-carboxyl-^{14}C space	Dry weight / Wet weight (final)	Density of dry material	Change in weight during incubation
	ml/kg			%
None	503 ± 54	0.229 ± 0.004	1.18 ± 0.05	−11
ACh + eserine	551 ± 8	0.223 ± 0.007	1.17 ± 0.04	−11
Ouabain	466 ± 30	0.220 ± 0.007	1.16 ± 0.03	−4
ACh + eserine + ouabain	473 ± 26	0.218 ± 0.012	1.19 ± 0.05	−7

The concentration of inulin-carboxyl-^{14}C in the medium was 1.60 mg/ml, specific activity, 1.20 μc/mg. Values for inulin-carboxyl-^{14}C space were calculated using the final blotted wet weight of the tissue after incubation. Values for inulin-carboxyl-^{14}C space, dry weight:wet weight ratios, and densities are the means ± SE of results from three experiments; values for per cent change in weight are the means of values from six experiments. Values were taken from parallel incubations in the same experiments described in Table I.

above, as there was some loss in tissue weight, also shown in Table IV, during the incubation period.

It should be pointed out that in incubated slices the apparent volume of the extracellular space includes not only that space which is extracellular space in vivo but also the volume of broken and damaged cells whose contents may be in equilibrium with the constituents of the bathing fluid.

The amount of ion in the extracellular space was calculated using the mean observed values for the inulin-carboxyl-^{14}C space under each of the four basic experimental conditions and the values for the concentrations of ions in the bathing fluid at the end of the incubation period. These calculations make the usual assumption that the ions of the inulin space are in equilibrium with the ions of the incubation medium.

Calculation of the Intracellular Content of Ions The amount of ion in the intracellular space (milliequivalents per kilogram final weight of tissue) was

obtained by subtracting the amount of ion in the extracellular space from the total tissue ion content.

Two values were calculated for the net concentration of ions in the intracellular space. One was a maximum value; this was obtained by expressing the intracellular ion content as milliequivalents per liter of intracellular water, which represents the minimum aqueous intracellular volume. The volume of the intracellular water (milliliters per kilogram final weight of tissue) was obtained by subtracting the volume of the inulin-carboxyl-^{14}C space from the volume of the total tissue water. The volume of the total tissue water was determined by measuring the dry weight:wet weight ratio of the incubated tissue; the values for these ratios are shown in Table IV.

The other value for net concentrations of ions in the intracellular space was a minimum value which was obtained by expressing the intracellular ion content as milliequivalents per liter of total nonextracellular space. This space includes both the volume of the intracellular water and the volume of solutes and insoluble material in the cell; it represents a maximum limit for the possible volume available to the intracellular ions. To obtain the volume of the nonextracellular space, the density of the dried tissue was measured. The results are shown in Table IV. The volume of the dry material (milliliters per kilogram final weight of tissue) was calculated from its weight (grams per kilogram final weight of tissue) and its density. Addition of this volume to the volume of total tissue water gave the total tissue volume (milliliters per kilogram final weight of tissue). From this value the volume of the inulin-carboxyl-^{14}C space was subtracted to give the total nonextracellular volume (milliliters per kilogram final weight of tissue). (The solutes of the extracellular fluid make a negligible contribution to the dry weight of the tissue.)

In the above calculations, the final weight of tissue refers to the wet weight of the tissue at the end of the incubation period.

It should be pointed out that neither of these calculations of the net intracellular concentrations of ions makes any allowance for the compartmentalization of fluids inside the cell such as might occur in mitochondria and nuclei; nor do they provide information as to what proportion of the ions may be bound or in the free state in the tissue.

Net Intracellular Concentrations of Ions Values for the net intracellular concentrations of Na^+, K^+, and Cl^-, calculated for each variable from the mean values shown in Tables I and IV, are shown in Table V.

These values magnify the observations on the relationship of tissue ions to ions of the bathing fluid and changes due to ACh (plus eserine) and ouabain which are discussed above in reference to the net total tissue content of ions. The minimum net concentrations of intracellular Na^+ were higher in slices treated with ACh (plus eserine) and in slices treated with ouabain than in

untreated slices; the maximum net concentrations of intracellular Na^+ in unstimulated, ACh-treated, and ouabain-treated slices were 2.1, 3.1, and 2.7 times higher, respectively, than the concentration of Na^+ in the bathing fluid.

One of the most interesting results to emerge from the calculations of net intracellular concentrations was that the maximum net intracellular concentration of Na^+ in the presence of ACh (plus eserine) was 531 mEq/liter of intracellular water. This is similar to the concentration of Na^+ in the secreted fluid of the goose salt gland in vivo (515 mEq/liter). The observed concentrations of Na^+, K^+, and Cl^- in goose secreted fluid are shown in Table VI. The net intracellular concentrations of K^+ were higher than those of secreted

TABLE V

CALCULATED MAXIMUM AND MINIMUM NET INTRACELLULAR CONCENTRATIONS OF IONS

Additions	Intracellular water			Total nonextracellular space		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
	mEq/liter	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*
None	354	236	236	206	136	136
ACh + eserine	531	152	159	289	82	87
Ouabain	461	27	175	288	17	109
ACh + eserine + ouabain	475	24	149	300	15	94

Values were calculated as described in the text from the mean values shown in Tables I and IV.

TABLE VI

SODIUM, POTASSIUM, AND CHLORIDE CONCENTRATIONS IN SECRETED FLUID FROM GOOSE SALT GLANDS

Na^+, *mEq/liter*	515 ± 5
K^+, *mEq/liter*	28 ± 4
Cl^-, *mEq/liter*	515 ± 5

Values are the means ± SE of results from three animals.

fluid in resting and ACh-stimulated slices. Intracellular concentrations of Cl^- were less than in secreted fluid and less in ACh-stimulated than in resting slices.

DISCUSSION

Role of the Basal Membrane The data presented here indicate that the avian salt gland is capable of establishing and maintaining a net concentration of Na^+ in the cell which is higher than that of the bathing fluid. The apparent intracellular concentrations of Na^+ indicate that the main concentration gradient for Na^+ could be established across the basal membrane. The data do not indicate whether the concentration of Na^+ into the cell involves the active

transport of Na^+ or whether the entry of Na^+ is along an electrochemical gradient established by some other process. The study of Thesleff and Schmidt-Nielsen (4) has indicated that the cytoplasmic fluid may be electrically negative with respect to the interstitial fluid, which would support the possibility that the entry of Na^+ across the basal membrane might be a passive rather than an active process. The data presented here indicate that the mechanism involved in this process is not blocked by the addition of ouabain to the bathing fluid.

Role of the Luminal Membrane The observation that ouabain does not block the capacity of the cells to maintain an intracellular concentration of Na^+ which is higher than that of the bathing fluid indicates that the action of ouabain on the secretory process is at some stage other than the mechanism at the basal membrane. Although the maximum net intracellular concentration of Na^+ in ACh-stimulated slices was as high as that of the secreted fluid of the geese, in the absence of definitive data on the magnitude and direction of the potential difference across the membrane, it is not possible to predict whether Na^+ leaves the cell across the luminal membrane by a passive rather than an active process. However, the observation of Thesleff and Schmidt-Nielsen (4) that a potential difference from plasma to duct, with duct fluid positive, is set up when secretion is initiated by nerve stimulation, together with their indication that the cytoplasmic fluid may be electrically negative with respect to the interstitial fluid, suggests that the exit of Na^+ across the luminal membrane may be against its electrochemical gradient. The effects of ouabain in abolishing secretion in vivo (4) and the respiratory response to ACh in slices (5, 6) have been taken to indicate the participation of a ouabain-sensitive Na-K ATPase in the over-all secretory process. A role for ouabain-sensitive Na-K ATPase activity in the secretory activity of the gland is also suggested by the high levels of this enzyme in salt gland tissue (6, 17). If this enzyme is oriented to participate in the pumping of Na^+ out of the cell, as it is in other tissues, then, if its activity is part of the over-all secretory process, it would be expected to be involved in the transport of Na^+ across the luminal membrane.

The results presented here and those discussed above suggest that the over-all secretory process in the avian salt gland may be divided into two stages—one at the basal membrane which is ouabain-insensitive and which may be driven by some process which generates a potential difference across the cell membrane sufficient to allow passive entry of Na^+ against its chemical gradient; and one which is ouabain-sensitive and, presumably driven by ATP, which results in the active exit of Na^+ against its electrochemical gradient across the luminal membrane.

The author is indebted to Professor Lowell E. Hokin for the data on the Na^+ and K^+ content of goose plasma.

The technical assistance of Mr. Khosrow Sadeghian is gratefully acknowledged.

This work was supported by grants NB-01730 and NB-06745-01 from the National Institutes of Health.

Received for publication 21 November 1966.

REFERENCES

1. SCHMIDT-NIELSEN, K. 1965. Physiology of Salt Glands. Sekretion und Exkretion, Springer-Verlag, New York. 269.
2. FÄNGE, R., K. SCHMIDT-NIELSEN, and M. ROBINSON. 1958. Control of secretion from the avian salt gland. *Am. J. Physiol.* **195**:321.
3. SCHMIDT-NIELSEN, K., C. B. JORGENSEN, and H. OSAKI. 1958. Extrarenal salt secretion in birds. *Am. J. Physiol.* **193**:101.
4. THESLEFF, S., and K. SCHMIDT-NIELSEN. 1962. An electrophysiological study of the salt gland of the herring gull. *Am. J. Physiol.* **202**:597.
5. BORUT, A., and K. SCHMIDT-NIELSEN. 1963. Respiration of avian salt-secreting gland in tissue slice experiments. *Am. J. Physiol.* **204**:573.
6. HOKIN, M. R. 1963. Studies on $Na^+ + K^+$-dependent, ouabain-sensitive adenosine triphosphatase in the avian salt gland. *Biochim. Biophys. Acta.* **77**:108.
7. SKOU, J. C. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta.* **23**:394.
8. DOYLE, W. L. 1960. The principal cells of the salt gland of marine birds. *Exptl. Cell Res.* **21**:386.
9. FAWCETT, D. W. 1962. Physiologically significant specializations of the cell surface. *Circulation.* **26**:1105.
10. HOKIN, L. E., and M. R. HOKIN. 1960. Studies on the carrier function of phosphatidic acid in sodium transport. *J. Gen. Physiol.* **44**:61.
11. KREBS, H. A., and K. HENSELEIT. 1932. Untersuchungen über die Harnstoffbildung im Tierkörper. *Z. Physiol. Chem.* **210**:33.
12. HAGEMEIJER, F., G. RORIVE, AND E. SCHOFFENIELS. 1965. The ionic composition of rat aortic smooth muscle fibres. *Arch. Internat. Physiol. Biochim.* **73**:453.
13. SCHALES, O., and S. I. SCHALES. 1941. A simple and accurate method for the determination of chloride in biological fluids. *J. Biol. Chem.* **140**:879.
14. VAN ROSSUM, G. D. V. 1964. Measurements of respiratory pigments and sodium efflux in slices of avian salt gland. *Biochem. Biophys. Res. Commun.* **15**:540.
15. HOKIN, M. R. 1966. Respiration and ATP and ADP levels during Na^+ transport in salt gland slices. *Life Sci.* **5**:1829.
16. HOKIN, M. R., and L. E. HOKIN. 1967. The formation and continuous turnover of a fraction of phosphatidic acid on stimulation of NaCl secretion by acetylcholine in the salt gland. *J. Gen. Physiol.* **50**:793.
17. BONTING, S. L., L. L. CARAVAGGIO, M. R. CANADY, and N. H. HAWKINS. 1964. Studies on sodium-potassium–activated adenosine triphosphatase. XI. The salt gland of the herring gull. *Arch. Biochem. Biophys.* **106**:49.