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# INTRACELLULAR CONCENTRATIONS OF SODIUM, POTASSIUM AND CHLORIDE IN THE SALT-GLAND OF THE DOMESTIC GOOSE AND THEIR RELATION TO THE SECRETORY MECHANISM

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SUMMARY

1. The composition of the nasal salt-glands of geese was found to be Na $57 \pm 3{\cdot}5$ (S.E.), K $52{\cdot}3 \pm 3{\cdot}9$ and Cl $78{\cdot}3 \pm 11{\cdot}0$ m-equiv/kg fresh tissue. During secretion, the Na content was significantly raised to $72{\cdot}4 \pm 3{\cdot}4$ m-equiv/kg.

2. Salt-gland slices incubated in Krebs–Henseleit bicarbonate medium plus glucose (6 mM), in the presence of [$^{14}$C]sucrose as an extracellular marker had the following composition, Na $85{\cdot}3 \pm 3{\cdot}1$, K $37{\cdot}1 \pm 3{\cdot}1$ and Cl $74{\cdot}3 \pm 3{\cdot}6$ m-equiv/kg. The calculated intracellular concentrations were for Na $61{\cdot}5 \pm 2{\cdot}1$, K $105{\cdot}3 \pm 8{\cdot}7$ and Cl $37{\cdot}8 \pm 5{\cdot}0$ m-equiv/l. intracellular water.

3. Ouabain ($10^{-4}$ M) significantly decreased the tissue and cell K concentration and significantly increased the Na concentration.

4. Acetylcholine ($10^{-6}$ M) and eserine ($10^{-4}$ M) in the incubation medium had no effect on intracellular composition.

5. Raising the Na concentration of the medium to 172 m-equiv/l. and the Cl to 156 m-equiv/l. in two experiments had no effect on the calculated intracellular composition.

6. These results do not support reports that the cells have a very high Na concentration (about 350 m-equiv/l. intracellular water). They are compatible with the hypothesis that the hypertonic secretion is formed across the luminal membrane of the secretory cell by an active $Na^+$ pump and there are no data to suggest that $Na^+$ is concentrated across the basal membrane by a ouabain-insensitive process.

7. The data are discussed in relation to permeability studies and to electrical potential measurements within the gland by other workers.

## INTRODUCTION

During secretion, the duct of the salt-gland becomes electrically positive with respect to the blood and it has been concluded that an active transport of $Na^+$ is involved in the secretory mechanism (Thesleff & Schmidt-Nielsen, 1962). Ouabain, administered by a retrograde injection into the ducts, abolishes both secretion and the potential difference and it was therefore thought that a pump extrudes $Na^+$ across the luminal membrane of the cell. Bonting, Caravaggio, Canady & Hawkins (1964) considered that the intracellular concentrations of ions would probably be similar to those reported for other tissues and agreed that the $Na^+/K^+$-activated ATP-ase present in the avian salt-gland must be situated on the luminal membrane. Van Rossum (1966) analysed salt-gland slices and assuming intracellular K to be at a concentration of 150 m-equiv/l. calculated that intracellular Na must be in the region of 80–90 m-equiv/l., i.e. lower than the plasma concentration. He also studied $^{24}Na$ fluxes in salt-gland slices and considered that an increased permeability of the basal membrane to $Na^+$ induced by acetyl-$\beta$-methylcholine (methacholine) *in vitro* and by acetylcholine *in vivo* allows $Na^+$ to enter the cell and this in turn initiates pumping of $Na^+$ across the apical membrane of the cell into the lumen.

However, quite different data were obtained by Hokin (1967). She incubated goose salt-gland slices in the presence of an extracellular marker and calculated the intracellular concentrations of Na, K and Cl. These values for the intracellular concentrations of Na (554 m-equiv/l. intracellular water), Cl (236 m-equiv/l.) and K (236 m-equiv/l.) were very high indeed. Ouabain further raised the Na and lowered the K concentrations in the cells, and Hokin (1967) therefore postulated that $Na^+$ is concentrated in the cell by means of a ouabain-insensitive process and that a ouabain-sensitive pump driven by ATP extrudes $Na^+$ across the luminal membrane. Hokin's analyses of salt-gland tissue did not appear to be in agreement with those done by other workers (Borut & Schmidt-Nielsen, 1963; van Rossum, 1966; Fourman, 1969). The present investigation was therefore undertaken to clarify the question of the intracellular levels of Na, K and Cl. Obviously it is most important that these should be known before advancing further hypotheses on the disposition and orientation of ion pumps in the avian salt-gland.

## METHODS

*Animals.* Domestic geese were kept as described by Hanwell, Linzell & Peaker (1970*b*) with tap water available for drinking.

*Tissue composition* in vivo. The geese were restrained in a special holder as previously described (Hanwell, *et al.* 1970*b*) and killed by the I.V. administration of 600 mg pentobarbitone sodium. Immediately the birds became unconscious, the salt-glands were removed, cleared of adhering connective tissue, chopped into small pieces and these were placed in a tared 10 ml. volumetric flask and weighed. The Na, K and Cl content of the tissue was then determined as described below. Five geese were given 0·5 M-NaCl I.V. (18 ml./kg) (Phillips & Bellamy, 1962) which initiated nasal secretion within a few min, and were killed 25–35 min later when nasal secretion was maximal (Hanwell, Linzell & Peaker, 1971). Nasal fluid from these birds was collected as previously described (Hanwell *et al.* 1970*b*). The five birds of the other group served as controls.

*Tissue and cell composition* in vitro. For these experiments the geese were killed by decapitation. The salt-glands were quickly removed, cleared of connective tissue and placed in a Stadie-Riggs hand microtome. Slices (0·6 mm) were cut and placed on a filter paper dampened with incubation medium. When all the slices had been cut, all the tissue from one bird (approximately 0·4 g) was placed in the incubation medium.

The basic incubation medium was the bicarbonate buffered solution of Krebs & Henseleit (1932) containing glucose (6 mM). 100 ml. medium containing 1 $\mu$c [$^{14}$C]sucrose (10·4 mc/mM, Radiochemical Centre, Amersham) was used for each incubation. For 30 min before and during the 1 hr incubation, a mixture of 95 % $O_2$ and 5 % $CO_2$ was bubbled through the medium which was maintained at 41° C. At the end of the incubation, a 1 ml. aliquot of the medium was taken to determine the radioactivity. The tissue was quickly removed, gently blotted and a small piece placed in a tared plot for the determination of water content; the rest was placed in a tared 10 ml. volumetric flask which was then weighed and stoppered.

Drugs were added to the medium as 1 ml. of concentrated solutions in the incubation solution.

*Analytical procedures.* The amount of water in the tissue was determined by drying at 102° C to constant weight, with the precautions recommended by Hagemeijer, Rorive & Schoffeniels (1965). 2 ml. 10 % (v/v) redistilled acetic acid were added to the tissue in the volumetric flask which was tightly stoppered and heated for 6 hr in a bath of boiling water. After digestion of the tissue, the flasks were left overnight at room temperature. For the measurement of radioactivity a 0·5 ml. aliquot was then taken into a glass-counting vial to which was added 0·5 ml. water and 15 ml. of a mixture of toluene (2 parts) and Triton X-100 (1 part) containing 4 g 2,5-diphenyloxazole and 0·1 g 1,4-di(2-(5-phenyloxazolyl))benzene per l. toluene. The vials were shaken and allowed to stand overnight before radioactivity was determined by liquid scintillation spectrometry. Quenching in the system was determined using an external standard. The radioactivity in 1 ml. of the incubation medium was determined by the same method. To check that $^{14}$C was not lost from the tissue a known amount of [$^{14}$C]sucrose was added to unincubated fresh tissue and this was treated in the same way. No loss of radioactivity was detected.

For the measurement of the Na, K and Cl content of the digest, another 0·5 ml. aliquot was taken and to this was added 2 ml. distilled water. Tissue digests and nasal fluid were analysed in an auto-analyser (Technicon Instruments Ltd.) using flame photometry for Na and K and the colorimetric method of Zall, Fisher & Garner (1956) for Cl. For analysis of tissues digests, standards were prepared in 2 % (v/v) acetic acid.

## RESULTS

*Salt-gland composition.* In geese which had not been given a salt load the tissue concentrations were, for Na, 57·0 ± 3·5 (S.E.), for K, 52·3 ± 3·9 and for Cl, 78·3 ± 11·0 m-equiv/kg tissue. In geese secreting nasal fluid, the tissue concentration of Na was raised significantly ($P < 0·05$) to 72·4 ± 3·4 m-equiv/kg. Although the mean K content was lower and the Cl content higher than in the controls, these differences were not statistically significant (Table 2). These results are compatible with the idea that the ducts

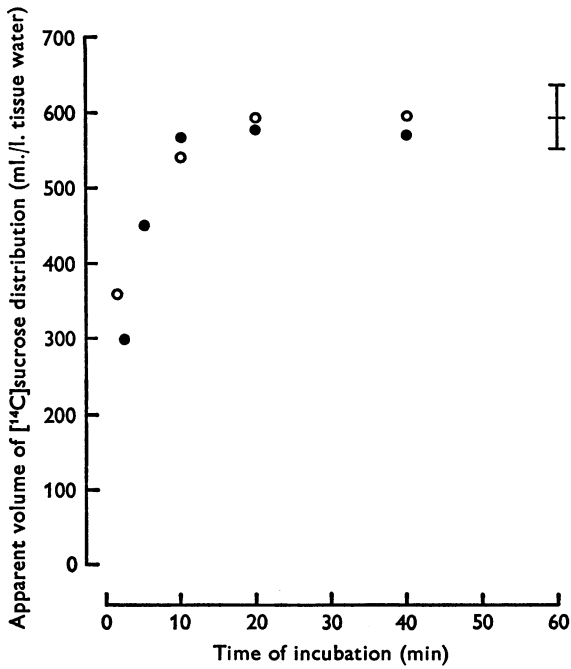


Fig. 1. Time course of uptake of [$^{14}$C]sucrose by salt-gland slices *in vitro*. The figure for 60 min shows the mean ± S.E. of five experiments and the different symbols are the results from two experiments.

of the secreting gland are full of fluid rich in Na and Cl and low in K, and that plasma and interstitial fluid levels of Na and Cl are high after salt-loading.

*Sucrose space in incubated slices.* After incubation for 1 hr the sucrose space of the slices was 593 ± 38 ml./l. tissue water. The time course of the uptake of labelled sucrose was studied by removing a slice at intervals from the incubation medium. The rate of entry into the slices was similar to that obtained by Hokin (1967) using [$^{14}$C]carboxylic acid-inulin (Fig. 1) and incubation of slices from two birds in the presence of [$^{14}$C]carboxylic

acid-inulin (Radiochemical Centre, Amersham) gave measurements of extracellular space within the range obtained with labelled sucrose.

*Composition of tissue and cells after incubation.* After incubation for 1 hr, the Na content of the slices was significantly increased ($85{\cdot}3 \pm 3{\cdot}1$ m-equiv/kg, $P < 0{\cdot}001$) and K significantly decreased ($37{\cdot}1 \pm 3{\cdot}1$ m-equiv/kg $P < 0{\cdot}01$) compared with the tissue removed from freshly killed geese (Table 2).

From a knowledge of the composition of the slices and the extracellular (sucrose) space, the Na, K and Cl concentrations in the intracellular water were calculated making the usual assumptions. The figures were: Na $61{\cdot}5 \pm 2{\cdot}1$, K $105{\cdot}3 \pm 8{\cdot}7$ and Cl $37{\cdot}8 \pm 5{\cdot}0$ m-equiv/l. intracellular water (Table 2).

TABLE 1. Composition of tissues processed in the same way and at the same time as slices of nasal salt-gland

| | No. of determinations | Mean (m-equiv/kg tissue) | | |
|---|---|---|---|---|
| | | Na | K | Cl |
| Goose sartorius muscle | 3 | 30 | 105 | 20 |
| Guinea-pig gastrocnemius muscle | 2 | 26 | 98 | 17 |
| Guinea-pig liver | 2 | 43 | 86 | 47 |
| Lactating guinea-pig mammary gland | 3 | 40 | 74 | 52 |

*Effects of ouabain.* Ouabain at a concentration of $10^{-4}$ M significantly raised the Na content of the whole tissue to $116{\cdot}7 \pm 3{\cdot}2$ m-equiv/kg ($P < 0{\cdot}01$) and that of the cells to $131{\cdot}8 \pm 8{\cdot}1$ m-equiv/l. ($P < 0{\cdot}001$) and significantly lowered the K content of the tissue to $12{\cdot}5 \pm 1{\cdot}7$ m-equiv/kg $P < 0{\cdot}001$) and that of the cells to $25{\cdot}5 \pm 3{\cdot}8$ m-equiv/l. intracellular water ($P < 0{\cdot}001$). The Cl content of the tissue and cells was not significantly altered but the water content of the tissue was significantly raised to $82{\cdot}4 \pm 0{\cdot}7$ ml./100 g ($P < 0{\cdot}001$) (Table 2).

*Effects of acetylcholine and eserine.* The presence of both acetylcholine ($10^{-6}$ M) and eserine ($10^{-4}$ M) in the incubation medium had no effect on the ionic composition of the tissue or on the calculated composition of the cells (Table 2).

*Effects of increased Na and Cl concentrations in the medium.* Since, during secretion, the plasma concentrations of Na and Cl may be raised, in two experiments NaCl was added to the basic medium to bring the concentration of Na to 172 m-equiv/l. and of Cl to 156 m-equiv/l. Although the tissue concentrations of Na and Cl were outside the range of values obtained after incubation in Krebs-bicarbonate of the usual osmolarity, the calculated intracellular concentrations of Na (63·7 and 60·5), K (115·8

TABLE 2. Na, K and Cl content of goose salt-gland tissue and cells (mean ± S.E.)

| Tissue | N | Water content (ml./100 g) | Composition of tissue (m-equiv/kg) Na | K | Cl | (m-equiv/l. tissue water) Na | K | Cl |
|---|---|---|---|---|---|---|---|---|
| Control | 5 | — | 57·0 ± 3·5 | 52·3 ± 3·9 | 78·3 ± 11·0 | — | — | — |
| After 0·5 M NaCl load | 5 | — | 72·4 ± 3·4 *$^{y}$ | 44·4 ± 3·9 NS$^{y}$ | 88·6 ± 9·5 NS$^{y}$ | — | — | — |
| Incubated | 5 | 76·6 ± 0·9 | 85·3 ± 3·1 ***$^{y}$ | 37·1 ± 3·1 **$^{y}$ | 74·3 ± 3·6 NS$^{y}$ | 114·6 ± 2·8 | 48·6 ± 4·4 | 91·6 ± 3·5 |
| Incubated + ACh and eserine *t* | 5 | 77·8 ± 1·6 NS$^{z}$ | 83·2 ± 1·6 NS$^{z}$ | 36·6 ± 0·8 NS$^{z}$ | 68·1 ± 1·2 NS$^{z}$ | 107·1 ± 1·0 NS$^{z}$ | 47·1 ± 0·7 NS$^{z}$ | 87·6 ± 1·8 NS$^{z}$ |
| Incubated + ouabain ($10^{-4}$ M) | 4 | 82·4 ± 0·7 ***$^{z}$ | 116·7 ± 3·2 **$^{z}$ | 12·5 ± 1·7 ***$^{z}$ | 77·4 ± 2·6 NS$^{z}$ | 138·3 ± 3·6 ***$^{z}$ | 14·8 ± 2·0 ***$^{z}$ | 88·8 ± 4·5 NS$^{z}$ |

| Tissue | N | Water content (ml./100 g) | [$^{14}$C]sucrose space (ml./l. tissue water) | Calculated intracellular concentration (m-equiv/l. intracellular water) Na | K | Cl |
|---|---|---|---|---|---|---|
| Control | 5 | — | — | — | — | — |
| After 0·5 M NaCl load | 5 | — | — | — | — | — |
| Incubated | 5 | 76·6 ± 0·9 | 593 ± 38 | 61·5 ± 2·1 | 105·3 ± 8·7 | 37·8 ± 5·0 |
| Incubated + ACh and eserine *t* | 5 | 77·8 ± 1·6 NS$^{z}$ | 569 ± 11 NS$^{z}$ | 58·1 ± 0·7 NS$^{z}$ | 101·7 ± 1·9 NS$^{z}$ | 34·3 ± 3·3 NS$^{z}$ |
| Incubated + ouabain ($10^{-4}$ M) | 4 | 82·4 ± 0·7 **$^{z}$ | 554 ± 12 NS$^{z}$ | 131·8 ± 8·1 ***$^{z}$ | 25·5 ± 3·8 ***$^{z}$ | 38·2 ± 4·5 NS$^{z}$ |

*N* = number of determinations.

$^{y}$ with respect to control tissue; $^{z}$ with respect to tissue incubated without the addition of drugs; *t* acetylcholine $10^{-6}$ M and eserine $10^{-4}$ M.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$; NS = not significant.

and 101·2) and Cl (39·3 and 43 m-equiv/l. intracellular water) were very similar to, and within the range of, values from tissue incubated in the basic medium.

*Composition of the nasal fluid.* The mean composition of the fluid was in the expected range (Na 433 ± 14, K 10·8 ± 0·6 and Cl 443 ± 13·6 m-equiv/l.); this is dealt with more fully by Hanwell, Linzell & Peaker (1971).

## DISCUSSION

*Validity of the procedures.* It will be seen that the basic disagreement between the results described in this paper and those of Hokin (1967) is in the figures for the Na, K and Cl content of the tissue slices. Therefore, the following procedures were carried out.

1. Goose sartorius muscle, guinea-pig gastrocnemius muscle, guinea-pig liver and lactating guinea-pig mammary gland were treated in exactly the same way as the slices of salt-gland and the determinations were made at the same time and under the same conditions. The analyses of all these tissues gave results in excellent agreement with published data for these organs (Table 1).

2. When known amounts of Na, K and Cl were added to tissue in the volumetric flasks recoveries of the added salts were between 97 and 102 %.

3. There was the slender possibility that the 10 % acetic acid used to digest the tissues did not liberate all the salts from the tissue. Therefore, digestion was carried out using concentrated nitric acid at 100° C, a procedure which completely digested the tissue within 15 min. The figures for Na and K in salt-gland slices and guinea-pig gastrocnemius muscle were almost identical to those obtained after acetic acid digestion. Cl cannot be measured after digestion in nitric acid because of the risk of loss of volatile Cl (see Peters & van Slyke, 1932), and it should be pointed out that Hokin's figures for Cl in the salt-gland were obtained after digestion in concentrated nitric acid.

4. The analytical procedures used could accurately detect concentrations of Na, K and Cl down to a level of 0·5 m-equiv/l. However, the actual concentrations present were well above this limit, usually between 2 and 4·5 m-equiv/l. and, at these levels, the apparatus was arranged so that the pen recorders were working within their most sensitive range.

5. Larger amounts of tissue were analysed than Hokin (1967) used in order to reduce the risk of any small degree of contamination markedly affecting the calculated concentration in the tissues.

*Intracellular concentrations of ions and the secretory mechanisms.* The results obtained here on the ionic composition of goose salt-gland cells are clearly not in agreement with those of Hokin (1967) (Table 3). It is not

merely on the calculated intracellular composition that the results do not agree with Hokin's; the composition of the tissue slices was also completely different (Table 3). Unfortunately Hokin (1967) included no data on the composition of the slices before incubation but the figures reported in this paper are similar to those obtained on the Na and K content by van Rossum (1966) in the gull, *Larus argentatus*, and on the Na content by Fourman (1969) in the duck, *Anas platyrhynchos*. Similarly, the gross composition of the incubated slices is now found to be similar to that reported by Borut & Schmidt-Nielsen (1963) and by van Rossum (1966). The reason

TABLE 3. Comparison of tissue and cell content of Na, K and Cl obtained in the present work with those of Hokin (1967) (data from *in vitro* experiments)

| Tissue composition (m-equiv/kg) | Present work | Hokin's results |
|---|---|---|
| Na | 85 | 181 |
| K | 37 | 66 |
| Cl | 74 | 141 |
| Cell composition (m-equiv/l. intracellular water) | | |
| Na | 62 | 354 |
| K | 105 | 236 |
| Cl | 38 | 236 |

why Hokin (1967) found such large amounts of Na and Cl in salt-gland slices is not apparent. Her geese had been given salt water to drink but then so had the gulls and ducks used by Borut & Schmidt-Nielsen (1963), van Rossum (1966) and Fourman (1969).

It is not clear from the results whether the cells of the salt-gland have the same composition *in vitro* that they normally maintain *in vivo*. The slices incubated *in vitro* had gained Na and Cl and lost K compared with unincubated tissue. This could mean either that, as in some other tissues (see Ussing, 1960), the cells gained Na and lost K *in vitro* or that the change in composition was due to Na and Cl passing into, and K passing out of, cells damaged during the preparation of the slices. No data are available for the intracellular concentrations *in vivo* owing to the seemingly insurmountable problem of determining the volume of the fluid in the lumen during secretion. However, a rough estimate can be made of intracellular Na in the duck salt-gland from the inulin space of inactive glands (Bellamy & Phillips, 1966) and their composition (Fourman, 1969). Calculations from these figures give an intracellular Na concentration of 44 m-equiv/l., a figure much more similar to that obtained *in vitro* in this work than to

that of Hokin's (Table 3). It must therefore be concluded that the normal intracellular concentrations of Na, K and Cl are similar to those in many other tissues of homoeothermic vertebrates, i.e. Na is about one-third of its concentration in the plasma.

Acetylcholine and eserine at the same concentration as Hokin (1967) employed had no detectable effect on intracellular composition and this is in agreement with van Rossum's (1966) findings. On the other hand, Hokin (1967) found that cell Na was increased and cell K decreased. Since Hokin used only 1 ml. of medium in which to incubate the tissue it might be suggested that the tissue became short of $O_2$ when respiration increased under the influence of acetylcholine (Hokin, 1966). This could then lead to a fall in cell [K] and a rise in [Na]. However, Hokin's initial cell [Na] was much higher than the Na content of the medium so that this explanation might not apply. It should be pointed out, however, that to maintain the normal ionic composition of mammary cells a large volume of medium through which $O_2$ is continuously bubbled is necessary (J. L. Linzell & M. Peaker, unpublished observations).

Concerning the mechanism of salt-gland secretion, the following findings seem significant.

1. The intracellular concentrations of Na, K and Cl (Table 2) are similar to those of a number of other tissues, i.e. Na approximately 60, K 105 and Cl 38 m-equiv/l.

2. Ouabain *in vitro* decreases cell K and increases Na.

3. The inside of the cell is electrically negative with respect to the blood. The exact potential difference could not be measured but no changes were evident when the glad was secreting (Thesleff & Schmidt-Nielsen, 1962).

4. The duct becomes electrically positive with respect to the blood during secretion and the retrograde injection of ouabain into the duct abolishes both secretion and the potential difference (Thesleff & Schmidt-Nielsen, 1962).

5. The gland contains a $Na^+/K^+$-activated ATP-ase (see, for example, Bonting *et al.* 1964), the amount of which can be related to secretory capacity (Ernst, Goertemiller & Ellis, 1967; Fletcher, Stainer & Holmes, 1967; Ballantyne & Wood, 1970).

6. Histochemical evidence strongly suggests that $Na^+/K^+$-activated ATP-ase is concentrated on the luminal side of the cell (Ballantyne & Wood, 1970).

7. Parasympathomimetic drugs increase respiration *in vitro* (see Borut & Schmidt-Nielsen, 1963; Hokin, 1966) and stimulate $^{24}Na$ efflux from slices (van Rossum, 1966).

8. In $^{24}Na$-loaded slices, methacholine decreases the specific activity of the Na although the Na content of the tissue does not change and as

van Rossum (1966) has suggested it is likely that methacholine alters the permeability of the cell membrane to $Na^+$.

A scheme for the movements of ions in the salt-gland is proposed in Fig. 2. It seems that a pump must be present on the basal membrane both to extrude $Na^+$, which has leaked in from the extracellular fluid, and to accumulate $K^+$. When the gland is stimulated by parasympathetic nerves

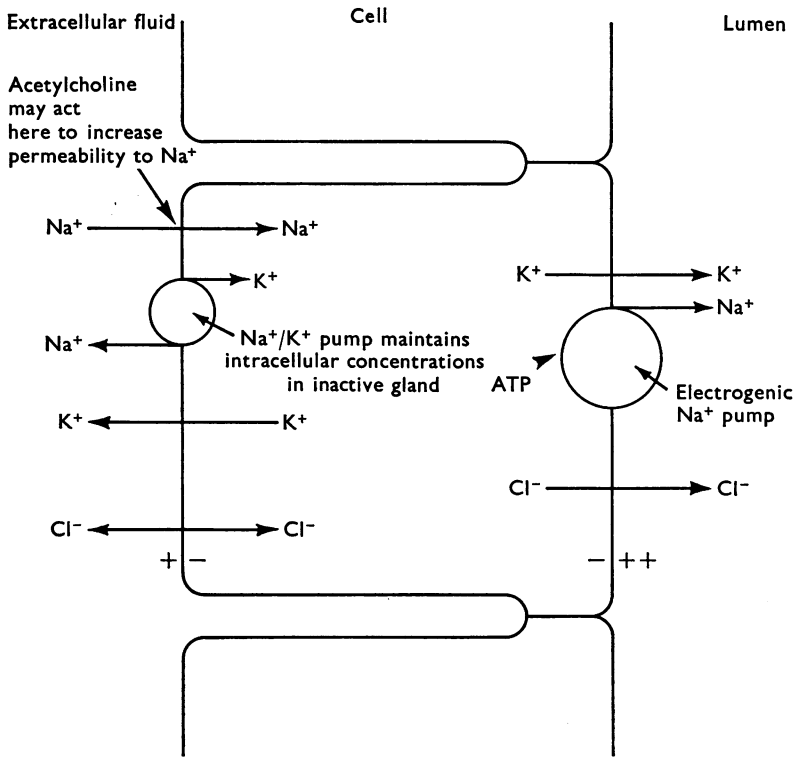


Fig. 2. Scheme for ion transport in the salt-gland.

(Ash, Pearce & Silver, 1969), acetylcholine may act to increase the passive permeability of the basal membrane to $Na^+$ to an extent beyond that which the basal pump can counter (van Rossum, 1966). With an increase in cell $Na^+$ and an increase in respiration (induced perhaps by the raised $Na^+$ or by acetylcholine directly), the pump on the apical membrane starts to extrude $Na^+$ from the cell to maintain the intracellular level. It might be expected that treatment with cholinergic drugs *in vitro* would result in a higher internal $Na^+$ content. However, the rise in cell $Na^+$ would presumably only be small when the apical pump is operating efficiently and van Rossum (1966) has found that $^{24}Na$ efflux from slices is only temporarily

increased in response to methacholine, an effect he attributes to the desensitizing effect cholinergic agents have on cell membranes.

It seems that there are three possible mechanisms by which salt secretion could be initiated: (1) an increase in the permeability of the basal membrane to $Na^+$; (2) an inhibition of the $Na^+$ pump on the basal membrane; and (3) a direct stimulation of the $Na^+$ pump on the apical membrane. While van Rossum's evidence might suggest that acetylcholine acts on the permeability of the basal membrane, it is possible that other agents which may have a direct effect on the salt-gland, for example, prolactin (Peaker, Phillips & Wright, 1970) act in a different manner. In this scheme, Cl movements could be entirely passive but to account for the hypertonicity of the secretion, the passive permeability of the apical membrane to $Na^+$, $K^+$, $Cl^-$ and water must be limited.

There appears to be little or no coupling between $Na^+$ efflux and $K^+$ influx across the apical membrane. The K:Na ratio of the nasal fluid varied from 1:41 to 1:49 and it might, therefore, be assumed that some $K^+$ is lost from the cell by small passive losses and possibly is carried out instead of $Na^+$ by the pump. That the cells fail to reabsorb $K^+$ argues in favour of the absence of coupled $Na^+/K^+$ transport.

On the basis of Hokin's (1967) suggestion that the basal membrane concentrates $Na^+$ in the cell, Ernst & Ellis (1969) suggested that this might be related to the greatly infolded basal and lateral membranes seen in the electron microscope. However, this folding may serve only to increase the surface area available for the passive entry of ions and the uptake of metabolic substrates from the extracellular fluid which has now been shown to be a very efficient process (Hanwell *et al.* 1970*a*, 1971).

## REFERENCES


Ash, R. W., Pearce, J. W. & Silver, A. (1969). An investigation of the nerve supply to the salt gland of the duck. *Q. Jl exp. Physiol.* **54**, 281–295.

Ballantyne, B. & Wood, W. G. (1970). ATPase and $Na^+$–$K^+$ transport in the avian nasal gland. *Cytobios* **5**, 41–51.

Bellamy, D. & Phillips, J. G. (1966). Effects of the administration of sodium chloride solutions on the concentration of radioactivity in the nasal gland of ducks (*Anas platyrhynchos*) injected with [$^3$H]corticosterone. *J. Endocr.* **36**, 97–98.

Bonting, S. L., Caravaggio, L. L., Canady, M. R. & Hawkins, N. M. (1964). Studies on sodium-potassium-activated adenosinetriphosphatase. XI. The salt gland of the herring gull. *Archs Biochem. Biophys.* **106**, 49–56.

Borut, A. & Schmidt-Nielsen, K. (1963). Respiration of avian salt-secreting gland in tissue slice experiments. *Am. J. Physiol.* **204**, 573–581.

Ernst, S. A. & Ellis, R. A. (1969). The development of surface specialization in the secretory epithelium of the avian salt gland in response to osmotic stress. *J. cell Biol.* **40**, 305–321.

ERNST, S. A., GOERTEMILLER, C. C. & ELLIS, R. A. (1967). The effect of salt regimens on the development of ($Na^+$ $K^+$)-dependent ATPase activity during the growth of salt glands in ducklings. *Biochim. biophys. Acta* **135**, 682–692.

FLETCHER, G. L., STAINER, I. M. & HOLMES, W. N. (1967). Sequential changes in adenosinetriphosphatase activity and electrolyte excretory capacity of the nasal-glands of the duck during the period of adaptation to salt-water. *J. exp. Biol.* **47**, 375–391.

FOURMAN, J. (1969). Cholinesterase activity in the supra-orbital salt secreting gland of the duck. *J. Anat.* **104**, 233–239.

HAGEMEIJER, F., RORIVE, G. & SCHOFFENIELS, E. (1965). The ionic composition of rat aortic smooth muscle fibres. *Archs int. Physiol. Biochem.* **73**, 453–475.

HANWELL, A., LINZELL, J. L. & PEAKER, M. (1970*a*). Avian salt-gland blood flow and the extraction of ions from the plasma. *J. Physiol.* **207**, 83–84*P*.

HANWELL, A., LINZELL, J. L. & PEAKER, M. (1970*b*). Salt-gland function in the domestic goose. *J. Physiol.* **210**, 97–99*P*.

HANWELL, A., LINZELL, J. L. & PEAKER, M. (1971). Salt-gland secretion and blood flow in the goose. *J. Physiol.* **213**, 373–387.

HOKIN, M. R. (1966). Respiration and ATP and ADP levels during $Na^+$ transport in salt gland slices. *Life Sci. Oxford* **5**, 1829–1837.

HOKIN, M. R. (1967). The $Na^+$, $K^+$ and $Cl^-$ content of goose salt gland slices and the effects of acetylcholine and ouabain. *J. gen. Physiol.* **50**, 2197–2209.

KREBS, H. A. & HENSELEIT, R. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. physiol. Chem.* **210**, 33–66.

PEAKER, M., PHILLIPS, J. G. & WRIGHT, A. (1970). The effects of prolactin on the secretory ability of the nasal salt-gland of the domestic duck (*Anas platyrhynchos*). *J. Endocr.* **47**, 123–127.

PETERS, J. P. & VAN SLYKE, D. D. (1932). *Quantitative Clinical Chemistry*. Vol. 2. *Methods*. Baltimore: Williams and Wilkins.

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

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

USSING, H. H. (1960). The alkali metal ions in isolated systems and tissues. In *Handbuch der experimentellen Pharmakologie*, vol. 13, pp. 1–195, ed. EICHLER, O. & FARAH, A. Berlin: Springer-Verlag.

VAN ROSSUM, G. D. V. (1966). Movements of $Na^+$ and $K^+$ in slices of herring-gull salt gland. *Biochim. biophys. Acta* **126**, 338–349.

ZALL, D. M., FISHER, D. & GARNER, H. Q. (1956). Photometric determination of chlorides in water. *Analyt. Chem.* **28**, 1665–1668.