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Avian Salt Glands

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Avian salt glands

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The processes involved at the apical and basal cell membranes in the formation of the hypertonic sodium chloride secretion in marine birds are discussed. Recent work indicates that the concentration gradient is established across the apical, i.e. luminal membrane probably by a sodium pump. There is also some evidence to suggest that during secretion, the uptake of sodium and chloride by the cell across the basal membrane may not be passive but may involve the exchange of sodium for hydrogen ions and chloride for bicarbonate. There is also the possibility that potassium secretion at high concentrations in some marine and desert species may involve an active extrusion across the apical membrane. Stimulation of secretion by acetylcholine and the possible effects of hormones on the secretory mechanism are considered.

Introduction

The nasal salt glands of marine birds secrete a hypertonic fluid consisting mainly of sodium chloride. The salt concentration is higher in the more pelagic species than in those living on estuaries and in Leach's Petrel, a concentration exceeding that of 1 mol/l NaCl has been recorded (see Schmidt-Nielsen 1960). The glands are the main route of sodium chloride excretion in marine birds and the rate of secretion can be high. For example, in this laboratory, geese have secreted up to 1.9 ml per gram of tissue per minute.

The salt gland only secretes in response to the ingestion of sea water or to the administration of hypertonic solutions (Fänge, Schmidt-Nielsen & Robinson 1958*a*; Ash 1969), and there is very good physiological, histochemical and ultrastructural evidence to support the theory that secretion is elicited and maintained by parasympathetic cholinergic nerves (Fänge *et al.* 1958*a*; Ash, Pearce & Silver 1966, 1969; Fawcett 1962). The receptors which detect excess salt in the body have recently been found to be located in the heart (Hanwell, Linzell & Peaker 1971*c*). These do not respond to expansion of the blood volume, a mechanism of stimulation suggested by Holmes (1965) and by Burford & Bond (1968), but to a raised tonicity of the blood (Hanwell, Linzell & Peaker 1971*b*, *c*) as suggested by Schmidt-Nielsen, Jörgensen & Osaki (1958) and by Ash (1969). The glands evidently require continuous stimulation with acetylcholine during secretion since interruption of nervous transmission while the glands are secreting immediately abolishes secretion (Hanwell *et al.* 1971*c*). There is no evidence to support the view that hormones may elicit normal secretion (Phillips, Holmes & Butler 1961) since denervation (Ash *et al.* 1966, 1969) and cross-circulation studies (Ann Hanwell, J. L. Linzell & M. Peaker, unpublished) show that secretion only occurs if the nerve supply to the gland is intact.

Since the discovery in 1957, by Schmidt-Nielsen, Jörgensen & Osaki that the nasal glands of marine birds secrete a hypertonic sodium chloride solution, a number of workers have used tissue slices *in vitro* to study the cellular mechanism involved in ion transport. Unfortunately, it is difficult to perform complementary studies *in vivo* because of the inaccessibility of the blood supply and the glands' inability to function under general anaesthesia. A number of investigations have involved the administration of inhibitors and hormones into the general circulation

However, many of the effects observed after giving these substances may not be direct actions on the secretory cells since plasma composition is often affected which, of course, may influence the receptors. In this laboratory techniques are being developed to study salt-gland function in conscious birds and these include measurement of blood flow (to assess the efficiency of the glands in removing ions and water from the arterial plasma), close arterial infusion of drugs and efficient methods of collecting secretion (Hanwell *et al.* 1970*a*, *b*, 1971*a*, *b*).

Species other than those inhabiting sea water may have salt glands. In arid regions, there is the same problem of a lack of free-water and the ostrich has a gland which can secrete both Na and K at high concentrations (Schmidt-Nielsen, Borut, Lee, & Crawford 1963). Moreover, the secretion of these two ions appears to be independent, in some samples [Na] and [K] were similar; in others [K] was 5 to 10 times higher than [Na]. Ion transport in the salt gland of this and other species living in arid regions obviously requires investigation.

Structure of the salt gland

The salt glands are situated in shallow depressions in the supraorbital region of the skull (figure 1). Two ducts from each gland pass through the beak and enter the nostrils. Secretion flows down the ducts, out of the nostrils and drips off the end of the beak (Schmidt-Nielsen 1960).

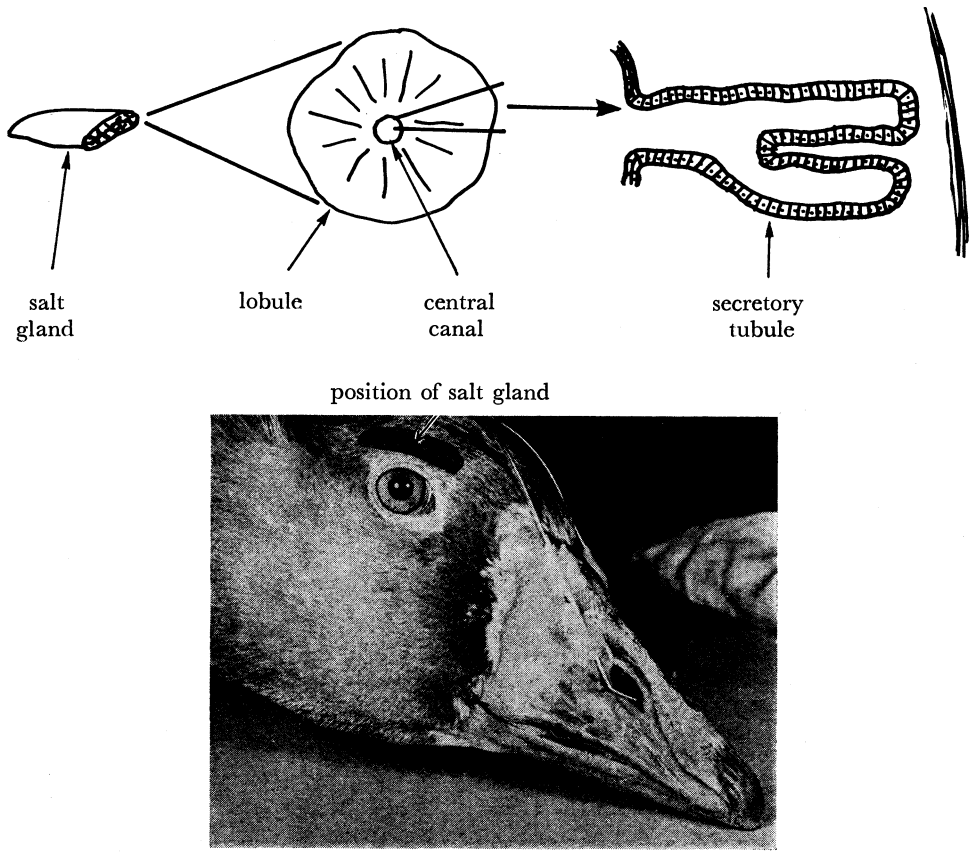

Figure 1. Position and gross structure of the salt glands in the goose. The steel cannulae in the nostrils are used to collect the secretion.

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The gland consists of longitudinal lobules, each of which has elongated secretory tubules radiating from a central canal (figure 1). In the embryo two ducts arise from each side of the anterior part of the nasal cavity. The more median duct gives rise to the anterior part of the gland while the posterior part is derived from the lateral duct (Marples 1932). Ellis, Goertemiller, Delellis & Kablotsky (1963) showed that branching of the ducts occurs to form the secretory tubules. Fänge, Schmidt-Nielsen & Osaki (1958*b*) regarded the anterior and posterior parts of the gland to be so similar in structure that they can be regarded as one functional unit.

A number of workers have now studied the ultrastructure of the salt glands and the accompanying diagram (figure 2) is largely the result of their efforts (Doyle 1960; Fawcett 1962; Komnick 1965; Ernst & Ellis 1969). The basal but not the luminal membrane is enormously infolded in birds kept on seawater, the clefts almost reaching the luminal membrane. The cells are joined by a typical junctional complex and the cytoplasm is packed with mitochondria.

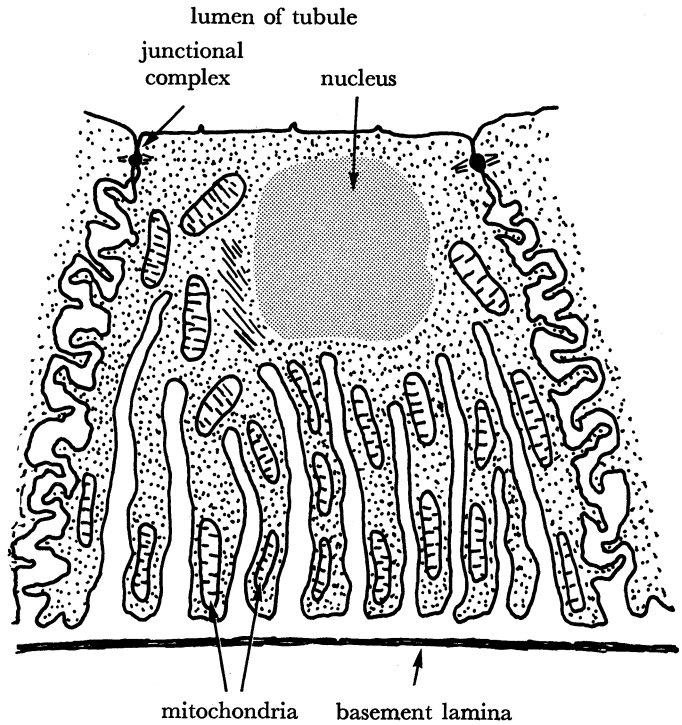

FIGURE 2. Diagram showing the ultrastructure of the secretory cell.

ION TRANSPORT

The processes involved in the formation of the nasal fluid do not involve filtration as in the kidney since neither inulin (Schmidt-Nielsen 1960) nor [^{14}C]sucrose (Ann Hanwell, J. L. Linzell & M. Peaker, unpublished) appear in the secretion while being maintained at high concentrations in the blood. Furthermore, electron micrographs show that the cells are joined by a typical complex of tight junctions and desmosomes near the luminal membrane. I do not feel that there is sufficient justification for accepting Komnick's (1965) views based only on the histochemical localization of ions that Cl^- may move, not through the cells, but between them and into the lumen. The histochemical identification of ions at best, only reveals different concentrations and not the rate of movement through any one compartment.

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Concentration gradients and the role of the luminal cell membrane

The first question to consider is where the concentration gradient between plasma and the secreted fluid is established. Hokin (1967) analysed goose salt gland slices after incubation in the presence of an isotopically-labelled extracellular marker and calculated the intracellular concentrations of Na, K and Cl. She found that intracellular [Na] (354 mmol./l intracellular

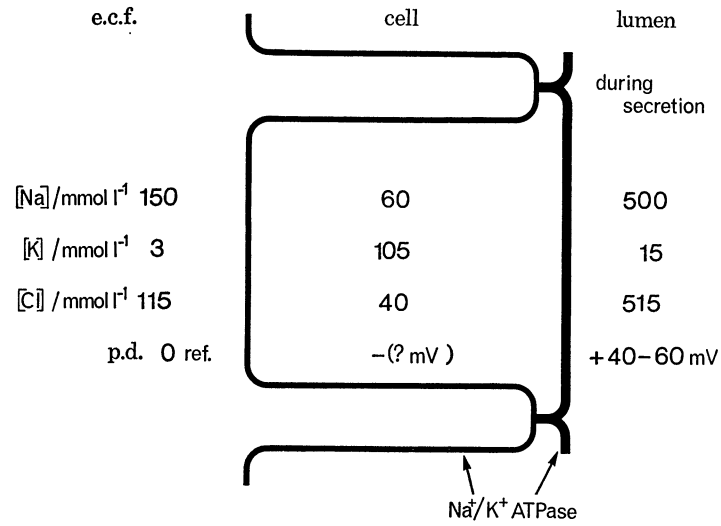

FIGURE 3. Concentration gradients (Peaker 1971*a*) and potential differences (Thesleff & Schmidt-Nielsen 1962) between extracellular fluid, secretory cells and lumen of the salt gland. The distribution of Na^+/K^+ ATPase is from Ballantyne & Wood (1970).

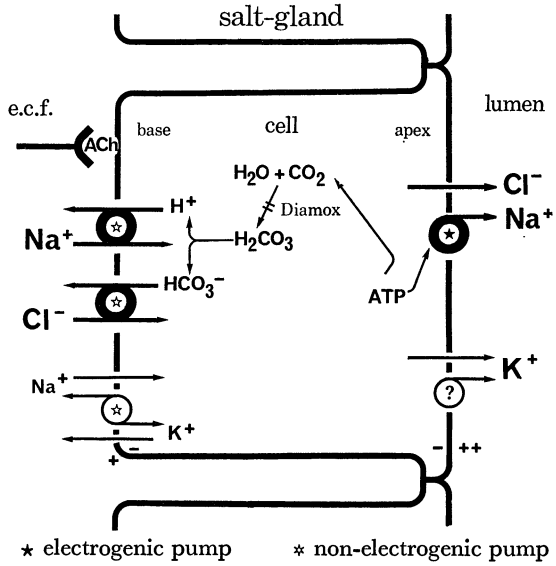

FIGURE 4. A possible mechanism to account for ion transport in the salt gland.

water), [K] (236) and [Cl] (236) were very high indeed and concluded that the concentration gradient is established not across the luminal membrane as most workers had supposed but across the basal membrane. Since the figures on the concentration of these ions in the tissue were markedly different from those obtained by other workers, both before and after incubation (Borut & Schmidt-Nielsen 1963; van Rossum 1966; Fourman 1969), Peaker (1971*a*) repeated

the work and calculated the intracellular concentrations of Na, K and Cl *in vivo* and *in vitro*. These were found to be similar to the concentrations reported in many tissues from homeothermic vertebrates (figure 3), i.e. [Na] and [Cl] considerably lower than the plasma concentrations. Therefore, it was concluded that during secretion the concentration gradient is established across the luminal membrane.

There is a good deal of evidence to suggest the presence of a Na^+ pump on the luminal membrane of the secretory cell. Thesleff & Schmidt-Nielsen (1962) found that during secretion, the duct of the gland becomes electrically positive with respect to the blood by 40 to 60 mV. Ouabain, administered into the ducts abolished both secretion and the potential difference. The salt gland contains large quantities of an ouabain-sensitive Na^+/K^+ ATPase (Hokin 1963; Bonting, Caravaggio, Canady & Hawkins 1964; Ernst, Goertemiller & Ellis 1967; Fletcher, Stainer & Holmes 1967; Ballantyne & Wood 1968, 1970), the amount of which can be related to secretory ability (Ernst *et al.* 1967; Fletcher *et al.* 1967). Moreover, Ballantyne & Wood (1970) using the Wachstein–Meisel technique have shown the Na^+–K^+/ATPase to be concentrated on the luminal side of the cell.

The proposed luminal Na^+ pump operates electrogenically with Cl^- following passively (figure 4). The extrusion of Na^+ appears not to be coupled to an influx of K^+ since the K^+ appearing in the secretion is not reaccumulated. K^+ is necessary however for stimulation of the pump (van Rossum 1966).

Hughes (1970*a*) has recently shown that the secretion of K^+ by the salt glands can be affected by the concentration of the drinking water. In gulls (*Larus glaucescens*) given full-strength sea water, the K^+ concentration of the secretion was, under certain circumstances, raised to 68 mmol/l. It seems unlikely that such a concentration could arise by a passive movement across the apical cell membrane and an active process, subject to environment changes, may be necessary to extrude K^+ in marine birds (figure 4).

Ion movements across the basal membrane and the role of acetylcholine in initiating secretion

The mechanism by which acetylcholine stimulates salt transport is not known. While it increases oxygen consumption and has other metabolic effects (Borut & Schmidt-Nielsen 1963; Hokin 1966) this could be a consequence rather than a cause of stimulation of an ion pump or pumps. *In vitro*, parasympathomimetic drugs have been found not to affect the gross composition of salt gland slices (van Rossum 1966; Peaker 1971*a*) or the intracellular concentration of ions (Peaker 1971*a*). However, there is evidence that acetylcholine facilitates the entry of Na^+ into the cell. Van Rossum (1966) loaded slices with $^{24}Na^+$ by keeping them in cold incubation medium. After 90 min, the slices were incubated in non-labelled medium at 25 °C for 55 min. Methacholine was then added; 15 min later the slices were analysed and it was found that the specific radioactivity of Na was lower in the tissue treated with methacholine than in the control slices. This, van Rossum, suggested, might indicate that methacholine increases the passive permeability of the basal membrane to Na^+. A small increase in cell Na^+ might then initiate extrusion of Na^+ by the pump on the luminal membrane. In the inactive gland it must also be assumed that, on the basal membrane, a pump must extrude Na^+ from, and accumulate K^+ in the cell (figure 4).

Two possible mechanisms might explain the increased passage of Na^+ and Cl^- through the basal membrane during secretion. Van Rossum's (1966) suggestion that there is an increased

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passive permeability to Na^+ is an attractive one since it is the mechanism by which acetylcholine acts on excitable tissues. However, there are some objections to this idea although much more electro-physiological work needs to be done to clarify the situation. It might be expected that if Na^+ was allowed to enter passively, the potential difference across basal membrane would be altered. However, Thesleff & Schmidt-Nielsen (1962) in the only electro-physiological study on the salt gland found no consistent difference in the potential across the basal membrane whether the gland was secreting or not. In recent experiments (M. Peaker, unpublished), Li has been added to the normal incubation medium and it has been found that the rate of Li uptake by slices is not affected by the addition of methacholine. If it is assumed that as in some other tissues, Li^+ moves passively in a similar manner to Na^+ but is not accepted by pumps as Na^+ this might be thought as evidence that the passive permeability to Na^+ is not increased during secretion. Furthermore, amiloride which probably acts to decrease the passive permeability to Na^+ of some cell membranes, for example, in frog skin (see, for example, Dörge & Nagel 1970) has been found to have no effect on secretion by the salt gland of the goose (M. Peaker, unpublished observations). Simultaneous measurements of secretory rate and blood flow (Hanwell *et al.* 1971*a*) have shown that the gland can extract, at high rates of secretion, more Na^+ and Cl^- from the plasma than a simple process of diffusion into the cells would permit. Therefore, it might be suggested that as in some other secretory epithelia (see Keynes 1969), a non-electrogenic mechanism, transporting Na^+ and Cl^- into the cell might be operating.

Like Hokin (1967) but on different grounds I suggest that Na^+ crosses the basal membrane in exchange for H^+, and Cl^- in exchange for HCO_3^-. Early work by Fänge *et al.* (1958*a*) and by Nechay, Larimer & Maren (1960) supports this hypothesis. The carbonic anhydrase inhibitors acetazolamide (Diamox) and methazolamide inhibit secretion. Since large amounts of carbonic anhydrase were found in the gland by Nechay *et al.* (1960) and by Bonting *et al.* (1964) it would seem that the scheme suggested in figure 4 may be operating. CO_2 from the gland's metabolism and possibly also from the blood would form H_2CO_3 in the presence of carbonic anhydrase. This would be ionized to form H^+ and HCO_3^+ which could then exchange with Na^+ and Cl^- across the basal membrane (figure 4). The inhibition of secretion induced by Diamox can be overcome by giving large amounts of $NaHCO_3$ intravenously, i.e. metabolic alkalosis (Nechay *et al.* 1960). Under these conditions when the blood carbonic acid concentration rises it might be thought that H_2CO_3 in an unionized form may enter the cells and by-pass the Diamox block. It is also interesting that acidosis decreases secretion (Nechay *et al.* 1960; M. Peaker, unpublished observations) and a similar effect of a decreased pH has been observed in crustacean gills (Shaw 1960) where a similar mechanism is thought to operate (see Keynes 1969). Similarly, the effect of Diamox is like that on the gills of teleost fish where similar exchanges may be taking place on the membrane exposed to fresh water as Dr Maetz has explained at this meeting.

It must be admitted that parasympathomimetric drugs can induce some secretion from a gland inhibited by Diamox (Schmidt-Nielsen 1960). However, it might be postulated that these drugs stimulate the luminal Na^+ pump directly and that large doses can induce a small secretory response in this way by lowering cell [Na] and [Cl].

The exchange process on the basal membrane may also be operating in the inactive gland, at least as regards Cl^- movements. *In vitro* Diamox lowers cell [Cl^-]. With the expected decrease in intracellular [HCO_3^-] it might be assumed that an exchange is necessary to maintain the normal cell [Cl^-] (M. Peaker, unpublished observations).

If the mechanism outlined here is operating in the salt gland, secretion may be under the influence of a positive feed-back system. As Na^+ is pumped out across the luminal membrane, more O_2 is used, more CO_2 is formed, H^+ and HCO_3^- are produced which then exchange for more Na^+ and Cl^-, supplying more Na^+ to the luminal pump. This scheme could explain why the maximal stimulus for secretion, an intravenous injection of sodium chloride does not induce secretion at an immediately high rate. Instead, secretion increases to reach a maximum after about 15 to 20 min in the goose even though blood flow has been found to be very high 5 min after salt loading (Hanwell *et al.* 1971*a*).

Where acetylcholine acts primarily to induce secretion is not known. Bonting *et al.* (1964) suggested that acetylcholine may act only to increase blood flow and thereby increase the amount of Na^+ available for transport. However, this suggestion neglects the *in vitro* effects of acetylcholine already referred to. Moreover, recent work has shown that, as in some mammalian salivary glands, atropine inhibits secretion, but not the increase in blood flow through the gland (Hanwell *et al.* 1971*a*). Therefore, on these grounds this theory should be abandoned.

It might be thought that acetylcholine may stimulate the luminal Na^+ pump. The response elicited by large doses of parasympathomimetics while the gland is inhibited by Diamox may suggest this and the situation is not without precedent since Pinsker & Kandel (1969) found that an electrogenic Na^+ pump is activated by acetylcholine in a neuron of the mollusc *Aplysia*. However, not all the events which occur in the salt gland in response to acetylcholine are inhibited by ouabain. While the increase in respiration is blocked (Borut & Schmidt-Nielsen 1963), the phosphatidic acid cycle is not (Hokin & Hokin 1966). It is also interesting to note that tissue respiration is not affected by methazolamide (Borut & Schmidt-Nielsen) which may also suggest that the effect of carbonic anhydrase inhibitors is on ion transport acting via an inhibition of the formation of H^+ and HCO_3^- rather than by altering intracellular pH which has been suggested as the mechanism of inhibition (Nechay *et al.* 1960) since an alteration in pH might also be expected to affect respiration.

It seems possible that acetylcholine may have several stimulatory effects on the secretory cell rather than a single, primary action but the question remains whether acetylcholine stimulates directly or whether some intracellular messenger is involved. If there is such a messenger, it seems that it is not cyclic-AMP, since dibutyryl cyclic-AMP infused into the carotid artery does not initiate secretion (M. Peaker, unpublished observations).

Effects of hormones on secretion

Adrenocortical hormones

The administration of glucocorticoid hormones or ACTH has been found to enhance secretion by the salt glands of the domestic duck (Holmes, Phillips & Butler 1961; Phillips & Bellamy 1962; Holmes, Phillips & Chester Jones 1963; Peaker, Peaker, Phillips & Wright 1971). It was suggested that the naturally occurring glucocorticoid in birds, corticosterone, may play some direct role in the control of the salt gland. However, it has been found that in ducks given ACTH, the plasma K^+ and blood glucose concentrations are increased. If these effects on the plasma were mimicked by other means, secretion by the salt glands was also enhanced (Peaker *et al.* 1971). Therefore the action of glucocorticoids in enhancing secretion can be attributed to indirect effects on blood composition which then influence the receptors controlling secretion and so far there is no evidence of a direct involvement in stimulating ion transport in the salt

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glands. Similarly, the effect of aldosterone in enhancing secretion (Holmes *et al.* 1961) was later attributed to the retention of high levels of Na^+ in the blood by the kidney (Phillips & Bellamy 1962), or in promoting the intestinal absorption of salt (Douglas 1970).

Adrenalectomy abolished secretion in response to a salt-load (Phillips *et al.* 1961) but again it is not known whether this was due to an absence of corticosterone from the gland or to the well-known metabolic and circulatory consequences of adrenalectomy. It seems possible that like perhaps most, if not all, vertebrate tissues, the salt gland requires adrenocortical hormones but there is no firm evidence that, as in the toad bladder, they play any part in its control.

Adenohypophysial hormones

Prolactin has been shown to enhance secretion by the salt glands of ducks given a minimal stimulatory load of sodium chloride. The effect is rapid, a significant change in secretory rate being recorded in the first five minute collection period after prolactin was given i.v. This rapid effect could not be related to changes in plasma composition and therefore there is the possibility that prolactin may directly affect the salt gland (Peaker & Phillips 1969; Peaker, Phillips & Wright 1970).

Adenohypophysectomy almost completely abolished secretion in response to a salt load (Wright, Phillips & Huang 1966) but it is not known which hormone was involved since replacement treatment was not attempted. In view of the effects of adrenalectomy, it was interpreted that the absence of ACTH was responsible. However, might it not, on equal grounds, be proposed that prolactin may be necessary for salt gland secretion? Prolactin is necessary for the ion movements essential for the survival of euryhaline teleost fish in freshwater (see reviews by Meites & Nicoll 1966; Bern & Nicoll 1968) and the possibility that prolactin is necessary for extra-renal salt excretion in birds should also be considered.

Neurohypophysial hormones

The presence of the posterior lobe of the pituitary is not essential for secretion, nor is the concentration of the fluid affected by neurohypophysectomy (Wright, Phillips, Peaker & Peaker 1967). However, recent work has shown that a naturally-occurring neurohypophysial hormone in birds, arginine vasotocin (AVT) can have marked effects on secretion.

AVT greatly enhances secretion in ducks and geese and when given without a salt load, initiates a prolonged secretion at a low rate. The mechanism by which this effect is mediated is not known but there is the possibility of a direct action since the intravenous administration of AVT into geese already secreting in response to salt loading results in a rapid and prolonged decrease in the Na, K and Cl concentrations in the secretion which might suggest that the permeability of the cell membranes to water is increased. (Peaker 1971*b*).

Whether these findings on the effect of exogenous hormone indicate any physiological role for AVT in the control of the concentration of the secreted fluid is not known but it would seem that a lowering of the concentration is opposite to what a bird short of free water would need.

There is therefore at present, no direct evidence that hormones influence or control secretion by direct actions on the secretory cells, even though such actions have been claimed. Even in the long-term adaptation to salt-water conditions when the salt glands undergo considerable

development, there is no direct evidence that these events are initiated by hormonal means, although in this case, the argument is a good deal stronger even if only based on the necessity for hormones of osmoregulatory systems in other animals. It seems likely that as in many, if not all, vertebrate tissues, a hormonal environment may be necessary for the glands' operation. This does not imply a control or special hormonal requirement but simply the maintenance of cells in a normal condition.

Adaptation of the salt gland to saltwater conditions

Although the salt gland of birds which have been maintained in fresh water will respond immediately to salt water, continuous exposure to a saline environment results in growth and interesting changes in the cell of the salt gland. Heinroth & Heinroth in 1927 observed that in marine Eider ducks, kept in the Berlin Zoological Gardens, the size of the nasal-glands decreased when the birds were given fresh water to drink. Schildmacher (1932) then described waxing and waning in the size of the glands in domestic ducks alternatively given sea water and fresh water. Since 1957, a number of workers have described the changes associated with growth and development of the secretory cells and the efficiency of secretion in birds transferred from fresh water to salt water. Of most interest in this discussion is the change in efficiency of the salt glands and the tissue levels of Na^+/K^+ ATPase.

It has been shown that with the increase in size of the glands, the excretory capacity and the Na^+ concentration of the fluid are increased (Schmidt-Nielsen & Kim 1964; Fletcher *et al.* 1967). Even though part of the increased secretory capacity is connected with the increase in size, Fletcher *et al.* (1967) found that the weight-specific sodium transporting capacity increased fourfold in ducks given saline drinking water. Therefore, the efficiency of the glands, both in terms of the secretory capacity and of the concentration of the secretion is increased. This increase in secretory capability was accompanied by a concomitant increase in Na^+/K^+ ATPase in the gland but not by an increase in the ratio Na transported: ATP hydrolysed. Similarly other authors have shown that the amount of this enzyme in the gland is increased by salt feeding (Bonting *et al.* 1964; Ernst *et al.* 1967; Ballantyne & Wood 1970). The changes in secretory capacity (Fletcher *et al.* 1967) and Na^+/K^+ ATPase (Fletcher *et al.* 1967; Ernst *et al.* 1967) could be reversed by giving fresh water.

Accompanying these changes, Ernst & Ellis (1969) in an excellent ultrastructural study found that the cells undergo considerable development. The basal and lateral membrane become extensively and deeply infolded and the number of mitochondria increases. There is no infolding of the apical membrane, across which it has been postulated the concentration gradient is established by a Na^+ pump. However, apical infolding would seem not to be desirable in glands forming a hypertonic secretion since if the pump was situated in these folds water from the cells would have more chance of passing into and thereby diluting the solution on the lines suggested by Dr Diamond for the formation of isotonic fluid in forwards-facing channels. It seems possible that the increased basal infolding accounts for the necessarily increased uptake of ions and oxygen from the extracellular fluid to supply the increased pumping capacity of the apical membrane.

While the stimulus for the changes induced in the salt gland by salt feeding appears to be osmotic stress (Goertemiller & Ellis 1966), the actual trigger mechanism is not known. Development of the salt gland is initiated (as judged by a rise in RNA in the gland) by as little as

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6 h exposure to salt water (Holmes & Stewart 1968). It seems possible that hormones responsible for the changes and in this connexion it is interesting to note that in ducks the prolactin content of the pituitary is increased by 50 % on the second day of salt feeding, thereafter declining to subnormal levels, by day 5 (Ensor & Phillips 1970). Since prolactin is often involved in the development of a specific organ (e.g. the mammary gland) it is possible in view of the effect of this hormone in enhancing secretion that it may be responsible for initiating the cellular changes in the salt gland induced by a saline environment.

Efficiency of the salt glands

At high rates of secretion, the salt gland can remove large quantities of salt from the arterial plasma (Hanwell *et al.* 1971*a*). For example, in one goose, 57 % of the Na and 80 % of the Cl was removed from the arterial plasma and secreted. It seems likely that the large surface area of the basal membrane formed by infolding may be partly responsible for the magnitude of the uptake from extracellular fluid. There is also a counter-current arrangement of blood capillaries and secretory tubules (Fänge *et al.* 1958*b*) which suggests that blood passes the bases of many cells before entering a vein. This arrangement could obviously increase the quantity of ions extracted from the blood passing through the gland.

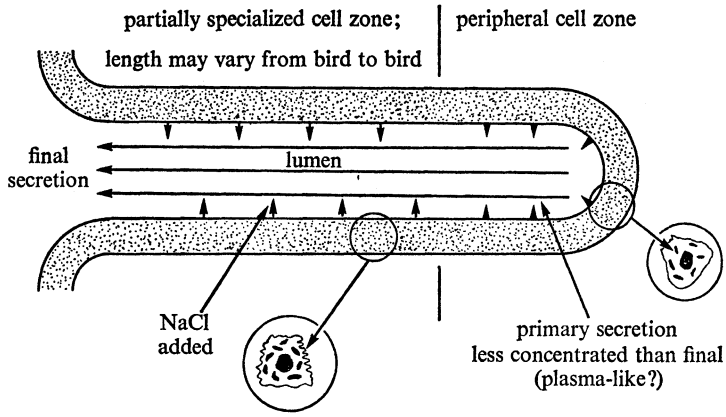

Figure 5. A possible scheme for the determination of the concentration of the secretion in any one bird. The cell types (ringed) are from Ernst & Ellis (1969).

Concentration of the secretion

Recent work has shown that the rate of secretion and its concentration are related. The overall efficiency of the secretory mechanism was found to be greater in geese (on fresh water) which excreted a larger volume of fluid in response to an intravenous injection of 0.5 mol/l NaCl since the concentrations of Na and Cl were positively correlated with the rate of secretion (Hanwell *et al.* 1971*a*). A similar finding has recently been reported by Hughes (1970*b*) in the gull, *Larus glaucescens*. When ducks were given only salt water to drink, Fletcher *et al.* (1967) found that the maximum rate of secretion and the concentration of Na in the fluid increased. Ernst & Ellis (1969) have, as referred to earlier, shown that the secretory cells change considerably in ducklings when salt is added to the drinking water. In this ultrastructural study, several types of cell were found to be present including generative, partially specialized and

fully specialized cells. In birds kept on fresh water, partially specialized cells were predominant but in birds given salt water, fully specialized cells with deep basal infoldings developed. The more highly specialized cells were situated at the central end of the tubules (figure 5).

It seems possible that different birds under fresh water conditions show different degrees of development of the cells in the tubule and that the more specialized type of cell might be able to produce a more concentrated secretion.

Within any one individual goose, the rate of secretion has been found to be inversely related to the concentrations of Na and Cl in the fluid (Hanwell *et al.* 1970*b*, 1971*a*). A scheme which could account for this relationship is that the relatively unspecialized cells towards the peripheral, blind end of the tubule might produce a fluid containing much less NaCl than the final secretion and that Na^+ and Cl^- are then added at a high concentration to the primary secretion by more highly developed cells further along the tubule. At low rates of flow, the fluid would be in contact with the more central cells for a longer period and therefore the final secretion would be more concentrated. With a high rate of flow, the primary secretion would have less time for NaCl to be added and a less concentrated fluid would be secreted (figure 5).

There is some evidence to support his hypothesis. Staaland (1967) in a study of wading birds, found that a more concentrated secretion was formed by those species having longer secretory tubules suggesting that the final concentration is established along the secretory tubule in a step-wise manner. It might be assumed from the work of Ernst & Ellis (1969) that the longer tubules had more fully specialized cells which in this scheme would account for the more concentrated secretion.

The possibility that cells other than those in the secretory tubules are involved in elaborating the secretion should not be overlooked, for example, recent histochemical work has shown that the cells of the central canal of each lobule appear highly active (Kühnel, Petry & Burock 1969).

The factors which control and influence the concentration of the secretion are therefore in need of investigation and it is hoped that micro-puncture techniques will lead to interesting developments in this important aspect of salt gland function.

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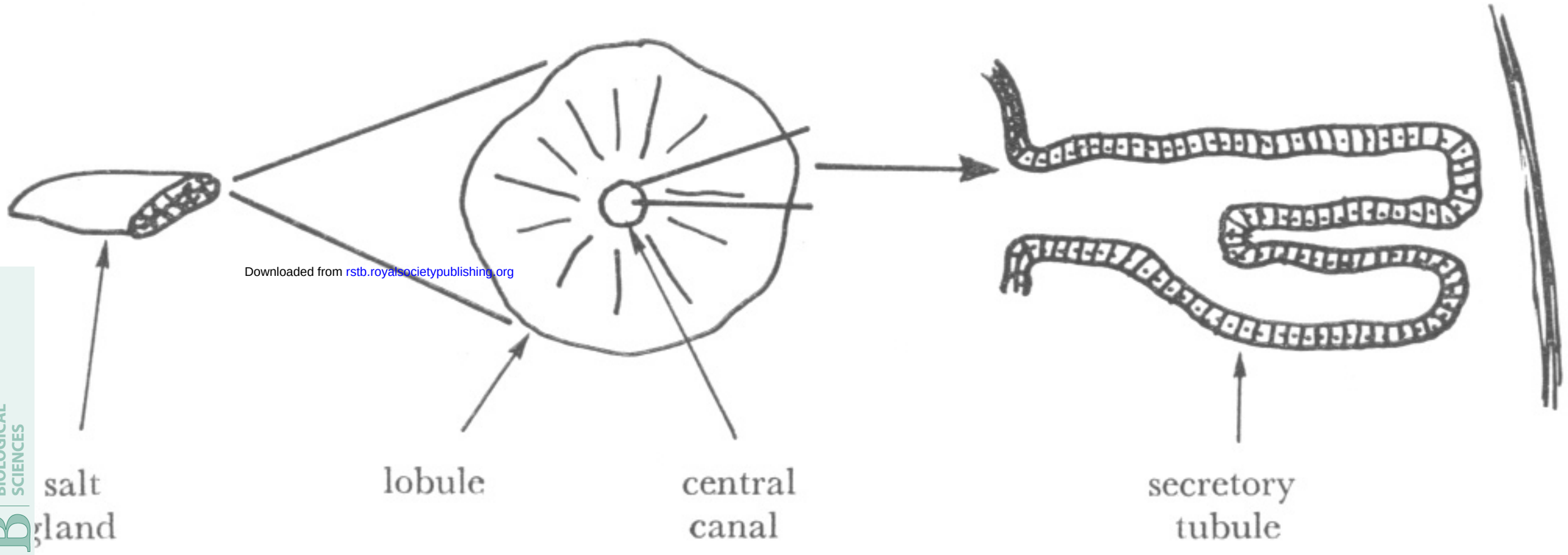

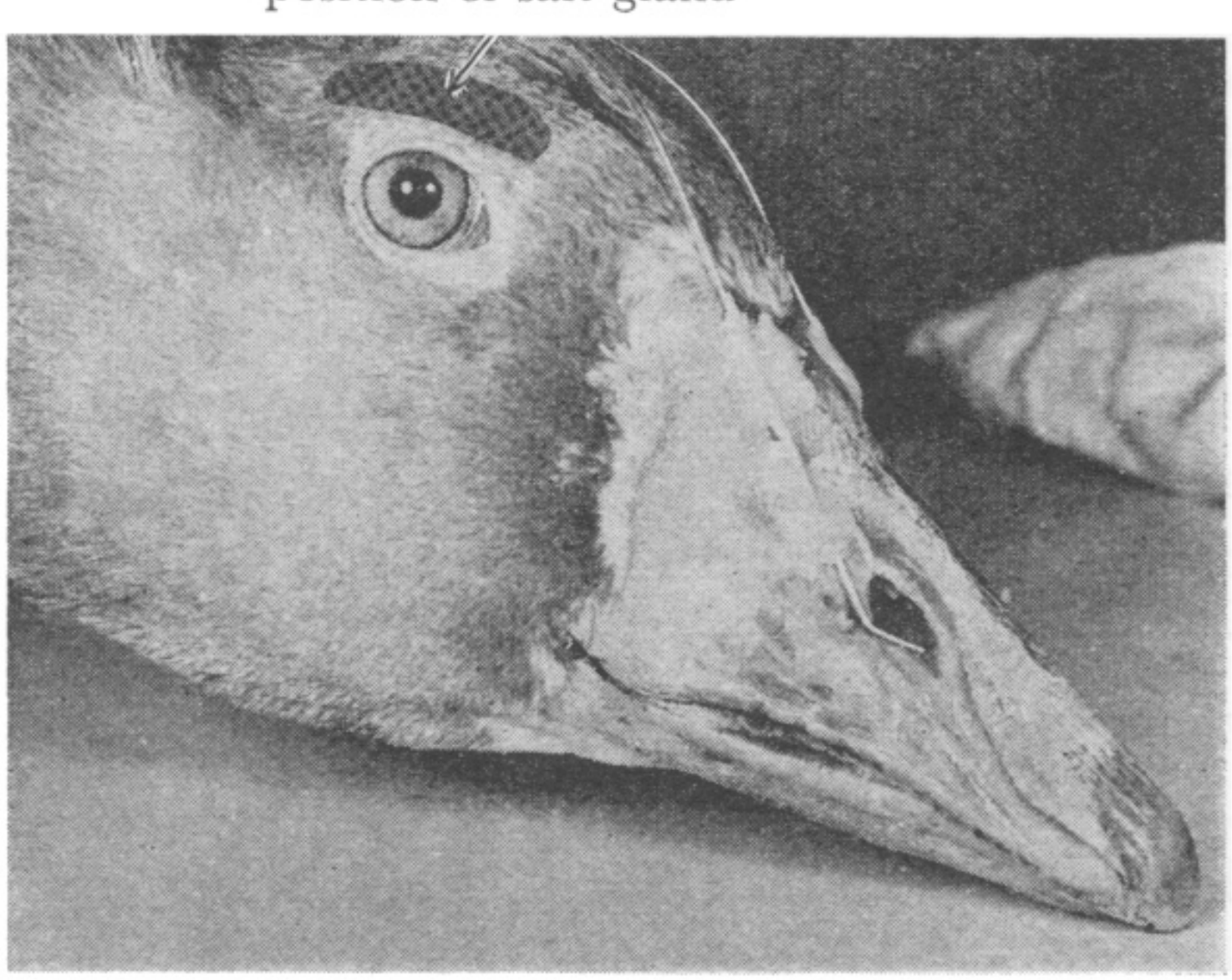

FIGURE 1. Position and gross structure of the salt glands in the goose. The steel cannulae in the nostrils are used to collect the secretion.

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