MEASUREMENTS OF RESPIRATORY PIGMENTS AND SODIUM EFFLUX IN SLICES OF AVIAN SALT-GLAND.

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In several tissues, the mechanisms by which the cells transport sodium and potassium appear to exert a controlling effect on the cell respiration (Whittam, 1962; Elshove and van Rossum, 1963; Borut and Schmidt-Nielsen, 1963). It has been suggested that this control arises because the utilisation of high-energy compounds by the ion-transporting mechanism increases the availability of high-energy acceptors for oxidative phosphorylation (Whittam, 1962) -- i.e., the metabolic state of the intracellular mitochondria approaches state 3 (see Chance and Williams, 1955). This possibility has now been examined by studying changes in the state of reduction of respiratory pigments during the treatment of slices of avian salt-gland with potassium, methacholine (which stimulates salt-secretion and respiration in the gland -- see Borut and Schmidt-Nielsen, 1963) and ouabain (which inhibits the effects of methacholine). Simultaneously, measurements were made of the rate of efflux of ^{24}Na.

METHODS. Slices (0.4-0.6 mm thick) of the salt-secreting gland of the herring gull (Larus argentatus) were soaked for 1-2 h at 1°C in a medium which contained (mM), Na^+ 170, K^+ 5, Mg^{++} 1, Ca^{++} 1, Cl^- 131, SO_4^{--} 1, phosphate 24 (pH7.4), and was labelled with ^{24}Na (10-20 μc/ml). Two slices were then rinsed for 1-2 sec. in non-radioactive medium and were mounted in a holder consisting of two, separate chambers. Oxygen-

ated, non-radioactive medium, at 25°C, was then washed continuously through each chamber; the composition of the medium bathing either slice could be changed at will during an experiment. The rate of ^{24}Na-efflux was determined by collecting the fluid emerging from the chambers and counting samples in a scintillation counter. Changes in the level of reduction of pyridine nucleotides were followed continuously by placing the slice-holder in a differential spectrofluorometer (Chance, et al., 1962) which measures the difference in intensity of the fluorescence of the two slices; the exciting light was at 366 mμ and the fluorescence emitted was measured at 470 mμ. At intervals, the slice-holder was transferred to a "split-beam" difference spectrophotometer (Yang and Legallais, 1954) in order to estimate the reduced forms of cytochromes a and c; the wavelengths and extinction coefficients used were those given by Chance and Williams (1955). The ^{24}Na remaining in the slices at the end of the experiment was determined by counting samples of a nitric-acid (0.1 N) extract of the dried tissue.

RESULTS. Fig. 1 illustrates an experiment in which both the ^{24}Na-labelled medium used for soaking at 1°C and the medium used for initial incubation at 25°C in the slice-holder were free of potassium. During the first 25 min. at 25°C some 98 per cent of the initial ^{24}Na content of the slices was washed out. At 25 min., the addition of K^+ (final concentration 5 mM) to the medium bathing one slice caused a prompt increase in the rate constant of the ^{24}Na-efflux relative to that of the untreated slice; the pyridine nucleotides underwent a transient reduction, as indicated by the temporary increase in the intensity of fluorescence. Subsequent addition of methacholine (0.33 mM) to the same slice caused a further, small increase in the rate of ^{24}Na-efflux and a reduction of the pyridine nucleotides. Addition of K^+ (5 mM) to the medium bathing the second slice, at 55 min., caused larger increases in the rate of ^{24}Na-efflux and in the level of reduction of pyridine nucleotides than did the earlier treatment of the first slice. In a series of ten such experiments,

treatment of slices with K^+ always gave an increase in the rate of ^{24}Na-efflux of the order shown in Fig. 1; the effect of K^+ on the pyridine nucleotides was more variable, however, and in three of the experiments an oxidation was seen. Methacholine invariably caused a reduction of pyridine nucleotides.

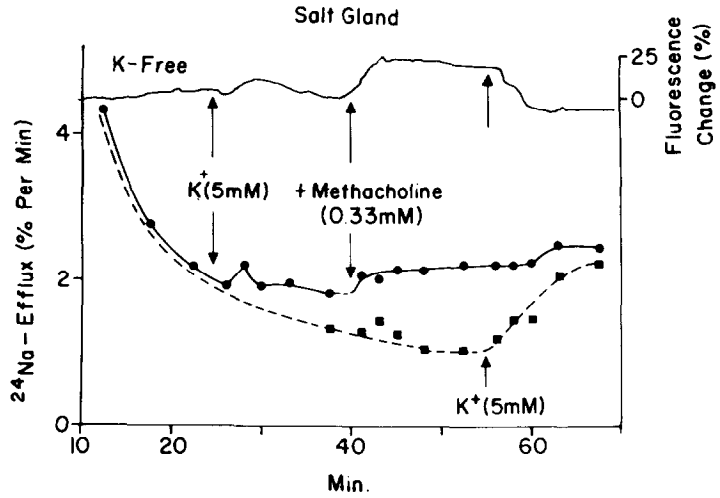

Figure 1. -- Effects of K^+ and methacholine on the fluorescence and ^{24}Na-efflux of salt-gland slices. For experimental conditions, see text. The uppermost tracing represents the difference in intensity of the fluorescence (at 470 mμ) emitted by the two slices. An increase in fluorescence intensity (reduction of pyridine nucleotides) of the slice treated with K^+ and methacholine at 25 and 40 mins., is indicated by an upward deflection of the tracing; the rate of ^{24}Na-efflux from this slice is represented by the circles and solid line. An increase in fluorescence of the other slice is indicated by a downward deflection of the tracing; the rate of ^{24}Na-efflux is represented by the squares and broken line (for the sake of clarity, only the later points on this curve are shown). Changes in fluorescence are expressed as a percentage of the absolute intensity of fluorescence of each slice in aerobic conditions. The rate of ^{24}Na-efflux is expressed as the percentage loss per min. of the total amount of radioactivity present in the slice at a given time; the total amount in the slices at each time was calculated from the slice content at the end of the experiment and the amount recovered in the effluent fluid.

Fig. 2 shows that treatment of salt-gland slices with methacholine resulted in a reduction of cytochrome c as well as of pyridine nucleotides. Cytochrome a (not shown in the figure) underwent a somewhat smaller reduction than cytochrome c. Subsequent treatment with ouabain (0.5 mM) then caused a small, further reduction of pyridine nucleotides and complete re-oxidation of cytochromes a and c. Other experiments have

shown that the reduction of pyridine nucleotides in response to methacholine also occurs in the presence of 0.5 mM iodoacetic acid, suggesting that the reduction is not due to a stimulation of glycolysis. In view of this observation, and the very high mitochondrial content of the tissue (Fawcett, 1962), it seems likely that the pyridine nucleotides involved are situated in the mitochondria rather than in the cytoplasm.

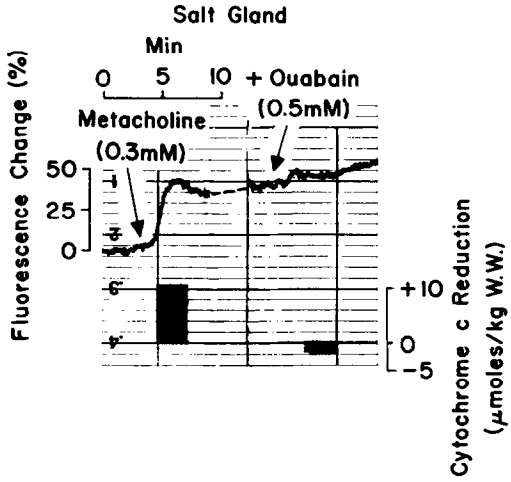

Figure 2. -- The effects of methacholine and ouabain on the pyridine nucleotides and cytochrome c of salt-gland slices. In this experiment the slices were incubated throughout at 37°C in a medium containing 5 mM K^+. For further experimental details, see text. An upward deflection of the fluorescence trace represents an increase in fluorescence; for fluorescence units, see Fig. 1. The black bars indicate the amounts of cytochrome c reduced by each treatment -- i. e., positive values represent a reduction, negative values an oxidation.

DISCUSSION. The stimulation of ^{24}Na-efflux upon addition of K^+ to the incubation medium is reminiscent of results obtained with other tissues (Hodgkin and Keynes, 1955; Essig and Leaf, 1963). Preliminary results suggest that the total sodium concentration of the salt-gland slices shows a net decrease, against a concentration gradient, after treatment with K^+, so that, in this tissue, the effect of K^+ on Na-efflux is probably not explicable in terms of a primary increase in Na-influx (contrast Essig and Leaf, 1963). Possibly the efflux of sodium is coupled to an influx of potassium (Hodgkin and Keynes, 1955).

That 5 mM K^+ stimulates ^{24}Na-efflux is also of interest in view of the rather surprising finding by Borut and Schmidt-Nielsen (1963), that it reduces the rate of respiration of salt-gland slices by 33 per cent. The effect of K^+ on the slice fluorescence has been too variable to throw any light on this observation at present.

The stimulatory effects of methacholine upon respiration and salt-secretion by the salt gland (Borut and Schmidt-Nielsen, 1963) suggest that this substance induces a change in the metabolic state of the intracellular mitochondria from a resting state, approaching state 4, towards a more active state, approaching state 3. The reduction of cytochromes c and a in the presence of methacholine can be accounted for on this basis if there is a "crossover point" (see Chance and Williams, 1955) on the substrate side of cytochrome c. The effect of subsequent treatment with ouabain is also consistent with this interpretation, since the re-oxidation of cytochromes c and a, together with some reduction of pyridine nucleotides, is characteristic of mitochondria returning from state 3 towards state 4 when the "crossover point" is between NAD and cytochrome c. However, the large reduction of pyridine nucleotides seen upon treatment of salt-gland slices with methacholine alone, indicates that in this case the transition to the active metabolic state cannot be solely described by the simple state 4-state 3 transition as observed in isolated mitochondria, since in this transition NADH becomes oxidised. Possibly, treatment of salt-gland slices with methacholine results in stimulation of the energy-linked reversal of electron transfer, in which case, as suggested by Chance and Hollunger (1961), this process may play a role in ion transport in the salt gland.

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