The Effects of Cortisone, Deoxycorticosterone and other Steroids on the Active Transport of Sodium and Potassium Ions in Yeast

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During fermentation the yeast cell can actively excrete hydrogen ions and actively absorb potassium ions, the latter with a considerable amount of specificity (Conway & O'Malley, 1942, 1943, 1946; Rothstein & Enns, 1946).

Conditions can be arranged in which the exchange of the two ions results in an external pH of 1·5. Provided the external sodium-ion concentration is high (0·2 M-sodium citrate) and in the absence of any appreciable external concentration of potassium ions, sodium is also actively exchanged for hydrogen ions and to some extent for potassium ions. In this way after a single fermentation a yeast may be readily obtained which has a sodium content of the order of 50–100 m-equiv./kg. This cannot be readily washed out, but is in fact slowly and actively excreted on suspending in water and more rapidly if the suspending fluid contains potassium ions (Conway & Ryan, 1950; Conway, 1952*a*).

The various active transports have been interpreted by the theory of the 'redox pump' (Conway, 1951, 1952*b*, 1953), which is considered to function for the active extrusion of sodium ions from cells in general and so from muscle and nerve as well as from yeast and to function in the oxyntic cells of the stomach for the active secretion of hydrogen ions.

Because of the predominant role of the adrenal cortical hormones in the regulation of sodium and potassium exchanges in animals primarily in the renal nephrons, where active transports of sodium and potassium ions are taking place, the effect of these hormones on similar active transports in the yeast cell was considered worth investigating. Interesting results were quickly obtained and are described below.

METHODS

The production of a 'sodium yeast' (Conway & Moore, 1952). Yeast is fermented in 20 vol. of 5% (w/v) glucose containing 0·2 M-sodium citrate; under these conditions the cells rapidly accumulate Na up to a concentration of 100 m-equiv./kg. or more (i.e. 230 mg./100 g. yeast); this high concentration of Na is retained by the cell after washing several times with tap water. A portion of this 'sodium yeast', suspended in 20 vol. of a solution containing KCl and NaCl (usually 0·1 N-KCl and 0·1 N-NaCl was used), will be found to excrete Na^+ into the external fluid (Conway & Ryan, 1950). The excretion of Na is much reduced in the absence of K from the external solution and takes place then with some accompanying anion.

Hormone experiments. The effects of the addition of small quantities of crystalline steroids to the suspending fluid were studied; the active excretion and uptake of Na as well as the active uptake of K were investigated. In the examination of the active uptake of Na and K the yeast was allowed to ferment in 5% (w/v) glucose containing 0·2 M-sodium citrate or 0·1 M-potassium citrate. All of the hormones investigated in this manner (cortisone, deoxycorticosterone, testosterone, progesterone, oestrone, methylandrostenediol, dehydro*epi*androsterone and 17-hydroxycorticosterone (Kendall's Compound F)) are only very slightly soluble in water and in salt solutions. For this reason ethanol at 5% (v/v) concentration was incorporated in the suspending fluid. The procedure adopted was to dissolve 5 mg. of the hormone in 5 ml. of pure ethanol and then add this to 95 ml. of the yeast suspension. The control suspension was treated similarly, omitting the 5 mg. hormone but including the 5 ml. ethanol. This incorporation of ethanol increased somewhat the solubilities of the hormones; nevertheless, the amounts going into solution were extremely small. Cortisone was used in the form of the acetate, as prepared by Merck and Co., Inc. and deoxycorticosterone as the acetate (DOCA) prepared by Organon Laboratories.

Na and K analyses were carried out on centrifuged, washed samples of the suspensions by means of the flame photometer.

RESULTS

Effects on the active excretion of sodium into 0·1 N-sodium chloride + 0·1 N-potassium chloride

Fig. 1 represents the excretion of Na by sodium yeast into 0·1 N-sodium chloride + 0·1 N-potassium chloride and the effects of cortisone and deoxycorticosterone on such excretion.

As can be seen the excretion of Na is markedly inhibited by deoxycorticosterone and to a smaller extent by cortisone. None of the other steroids mentioned above had any effect, nor had bile salts. Deoxycorticosterone always showed this inhibiting action; usually cortisone had a much smaller and scarcely appreciable effect.

Effects on the active excretion of sodium into tap water

Three experiments were carried out with sodium yeasts excreting into tap water (Na concn. 0·3 m-equiv./l., K concn. 0·01 m-equiv./l.). The mean Na

and K concentrations of the sodium yeasts were 211 and 210 mg./100 g. yeast, respectively, and after 20 min. in tap water the curve of Na loss flattened out, so that over the next 70 min. very little extra Na was lost.

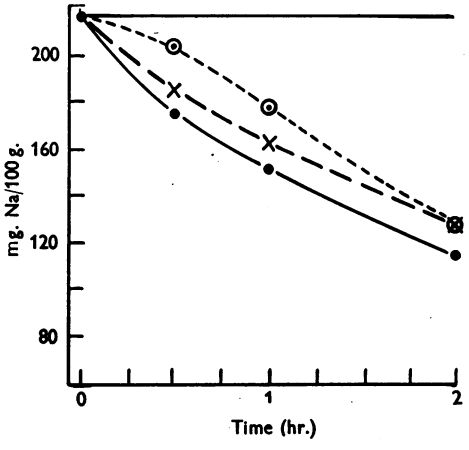

Fig. 1. Effects of cortisone and deoxycorticosterone on excretion of Na by Na-yeast into 0·1 N-NaCl + 0·1 N-KCl; the unbroken line represents the excretion of Na^+ into 0·1 N-NaCl with no external K. Untreated suspension(●); 5 mg. cortisone/100 ml. (×); 5 mg. deoxycorticosterone/100 ml. (⊙).

From 20 to 90 min. the mean loss of Na from the yeast was 75 ± 1·4 mg./100 g. yeast and in presence of deoxycorticosterone it was 79 ± 1·9 mg./100 g. These are the means for eight results with and without the hormone, the samples being taken at similar times. Here the hormone effect appears as a slight but not significant increased loss of Na.

The mean K values for the eight samples were 202 ± 0·9 and 201 ± 0·7 mg./100 g. with and without the hormone, which indicated no significant change.

From these results deoxycorticosterone does not appear to affect the excretion of Na when this occurs into tap water, i.e. in the virtual absence of external K^+.

Effects on the active uptake of potassium

Fig. 2 shows the inhibiting action of deoxycorticosterone on the active K uptake. Cortisone has practically no effect here. Of the other hormones 17-hydroxycorticosterone alone showed any definite result, and this was an activation and not an inhibition, the effect being relatively small but statistically significant. Owing to the small effect of 17-hydroxycorticosterone, the conditions of comparison had to be carefully standardized with respect to timing at all stages; it was found necessary to carry out the individual fermentations in centrifuge tubes, all the suspensions being then centrifuged and washed at the same time so that the fermentation times of the treated and untreated specimens were exactly the same. Table 1 gives the results of six experiments showing K uptake for control and

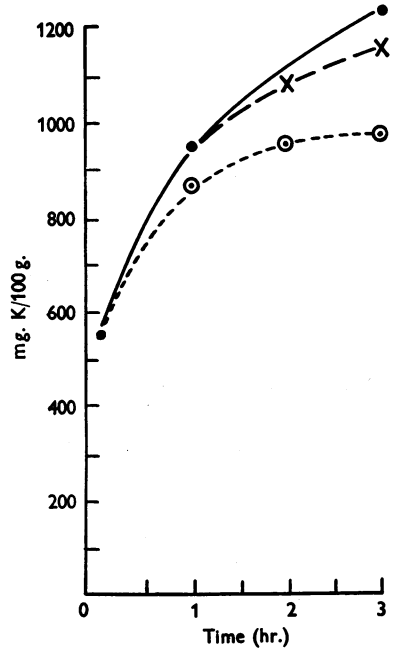

Fig. 2. Effects of cortisone and deoxycorticosterone on active uptake of K by yeast during fermentation in 0·1 M-potassium citrate. Untreated suspension (●); 5 mg. cortisone/100 ml. (×); 5 mg. deoxycorticosterone/100 ml. (⊙).

Table 1. *Effect of 17-hydroxycorticosterone on potassium uptake by yeast cells*

(Fermentation for 1 hr. in 20 vol. potassium citrate-glucose solution, as described in Methods section. The figures are increases in K content expressed as mg./100 g. yeast.)

Expt. no.	Untreated yeast	Yeast treated with 17-hydroxy-corticosterone	Difference due to hormone
1	294	326	+32
2	310	330	+20
3	270	300	+30
4	260	290	+30
5	250	330	+80
6	280	320	+40
Mean	277	316	+38·7 ± 8·7

17-hydroxycorticosterone suspensions during fermentation for 1 hr. In each case there was an increased uptake of K in the presence of the hormone, the mean of such increases being 38·7 ± 8·7 mg. K/100 g. yeast.

Fig. 3 shows the effect of 17-hydroxycorticosterone on K uptake during a 2 hr. fermentation period.

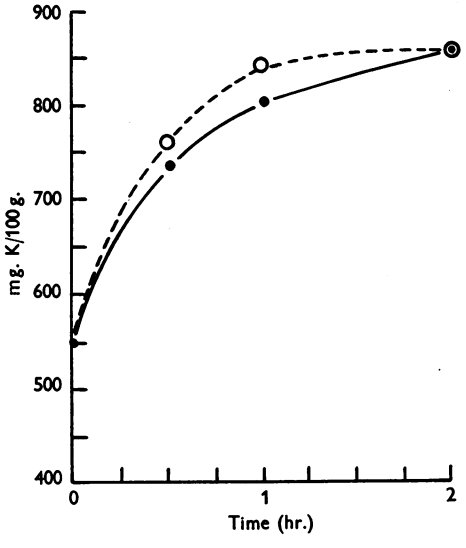

Fig. 3. Effect of 17-hydroxycorticosterone on active uptake of K by yeast during fermentation in 0·1 M-potassium citrate. Untreated suspension (●); 5 mg. 17-hydroxycorticosterone/100 ml. (○).

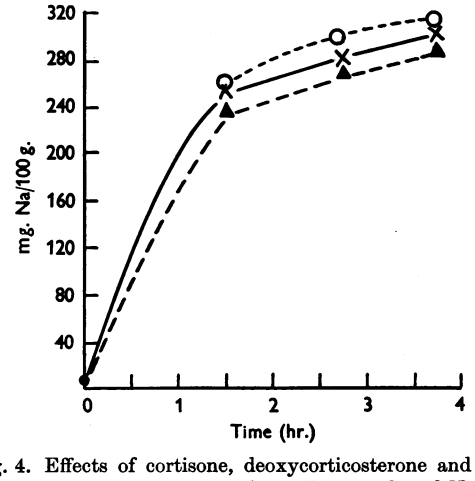

Fig. 4. Effects of cortisone, deoxycorticosterone and 17-hydroxycorticosterone on the active uptake of Na by yeast during fermentation in 0·2 M-sodium citrate. Untreated suspension and 5 mg. cortisone/100 ml. (×); 5 mg. deoxycorticosterone/100 ml. (▲); 5 mg. 17-hydroxycorticosterone/100 ml. (○).

The active uptake of sodium

Here the inhibiting effect of deoxycorticosterone again appears. Fig. 4 shows the results of an experiment in which Na is being actively absorbed from 0·2 M-sodium citrate. Cortisone was not found to have any significant effect. 17-Hydroxycorticosterone increased the uptake slightly.

In these experiments deoxycorticosterone produced a marked inhibition though the external concentration was as low as about $2{\cdot}5\text{–}3{\cdot}3 \times 10^{-5}$ M. The relative concentrations of deoxycorticosterone, cortisone, and 17-hydroxycorticosterone, when used in the external or suspending solutions, was roughly 1:2:5.

The concentrations at which deoxycorticosterone is effective on the active transport of Na or K is of a lower order than that of cyanide or azide on cell respiration.

DISCUSSION

Of the eight hormones studied for their effect on active Na and K transport across the yeast-cell membrane, three showed a significant effect, namely deoxycorticosterone, cortisone and 17-hydroxycorticosterone. The most pronounced was that of deoxycorticosterone. In a large number of experiments it never failed to show an inhibitory action, the effect on the active excretion of Na being the most pronounced.

Cortisone had a weaker effect and occasionally none could be shown but any exhibited was likewise of an inhibitory kind.

On the other hand, 17-hydroxycorticosterone showed a small but stimulating action on the active transports.

The nature of the inhibition produced by deoxycorticosterone

The inhibition of the sodium excretion could be considered either as a direct effect or an indirect one, in the latter case the primary action being on the K^+ entrance, with which the excreted Na^+ exchanges under the conditions of the experiments (no appreciable net H^+ exchange occurs). The fact that no effect of deoxycorticosterone is evident when the Na is excreted into tap water, indicates that the real effect on the Na excretion into a solution containing 0·1 M-KCl (as well as 0·1 M-NaCl) is on the K-carrier mechanism and this is in harmony with the view developed in the Discussion of the previous paper (Conway & Hingerty, 1953). An effect on free K^+ permeability seems a most unlikely cause of the inhibition as a tenfold change in the external concentration of K^+ outside the cell has scarcely any effect on the rate of active Na extrusion (Conway & Ryan, 1950).

Turning to the inhibition of the active K and Na uptake, the mechanism of such uptake may be considered to be the same, as by including only small amounts of K in the suspending fluid one can inhibit almost entirely the Na absorption. On the

other hand, the mechanism of active Na extrusion is not the same as that of K uptake, for the former is largely Na specific and the latter largely K specific.

Deoxycorticosterone may be taken then to inhibit indirectly active Na extrusion by its effect on the active K uptake or on the K carrier.

SUMMARY

1. Sodium ions accumulated by the yeast cell during fermentation in a medium containing a high concentration of sodium can be actively excreted into a medium containing potassium ions with which the sodium ions exchange. In the absence of external potassium, sodium is excreted much more slowly with an accompanying anion.

2. Of eight crystalline steroids (cortisone, deoxycorticosterone, testosterone, progesterone, oestrone, methylandrostenediol, dehydro*epi*androsterone and 17-hydroxycorticosterone) investigated for a possible effect on such sodium and potassium exchanges in the yeast cells, as well as on active potassium uptake during fermentation only three (deoxycorticosterone, cortisone and 17-hydroxycorticosterone) showed significant effects; deoxycorticosterone showed the most pronounced effect, namely an inhibitory action on the active transport of sodium and potassium; cortisone also showed a slight inhibiting action whereas 17-hydroxycorticosterone had a small stimulating effect on the active transport.

3. Deoxycorticosterone does not inhibit appreciably the active excretion of sodium from yeast suspended in tap water and it is concluded that its marked effect on sodium excretion into a solution containing 0·1 M-potassium chloride is on the potassium-carrier mechanism which has been interpreted in terms of a 'redox pump' (Conway, 1951, 1952*b*, 1953).

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The Separation of the Phosphate Esters of Muscle by Paper Chromatography

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Knowledge about the phosphorus metabolism of muscle depends on the separation and determination of the phosphate esters in muscle extracts. In the early work, such as that done by Eggleton & Eggleton (1929), methods are described whereby the adenosine polyphosphate, creatine phosphate, orthophosphate and monophosphate ester fractions can be separated and determined. These methods have given valuable information about the large changes in these esters in muscle after long periods of activity, but they are difficult to use, particularly for investigating short periods of activity in which the changes are small.

In this work the chromatographic method described by Hanes & Isherwood (1949), which has not so far been used for the esters in muscle extracts, has been examined. The esters in frog muscle have been compared with those in tortoise muscle and, to compare this method with the others, some of the early work on the changes during long periods of

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