Effect of Insulin on Sodium in Muscle

WHEN diaphragm muscle is suspended in oxygenated Krebs solution at 38° C., it remains viable for many hours. Analysis, however, has shown that prolonged immersion of the muscle in physiological saline is associated with a small loss of potassium from the tissue and a considerable increase in sodium[1]. Preliminary experiments suggested that this increase in tissue sodium could be largely avoided if the muscle was suspended in rat serum instead of in saline. Later, reconstituted human freeze-dried serum was found to be as effective as rat serum, and has been used in the experiments reported here.

Diaphragm muscle was quickly removed from decapitated male rats weighing 110–120 gm. and suspended for 2 hr. at 38° C. in aerated saline which was similar to that employed previously by one of us[1], except that 5 m*M* potassium was used. The sodium content of these muscles was measured[1] and compared with that of muscles immersed for 2 hr. in the other aerated fluids listed in column 2 of Table 1. The reconstituted human serum used in experiment 1 was dialysed before use for 18 hr. against repeated changes of saline.

Table 1. SODIUM CONTENT OF SOAKED DIAPHRAGMS (M.MOLES/KGM. WET TISSUE ± S.E.)

Exp. No	Test solution	Diaphragm soaked in: Test solution	Saline	No. of pairs	*P*
1	Dialysed human serum	35·1±0·6	48·6±1·6	12	<0·01
2	Saline plus serum proteins	35·4±0·8	54·3±3·4	6	<0·01
3	Saline plus insulin (0·01 unit/ml.)	42·6±1·5	53·0±1·5	9	<0·01
4	Saline plus insulin (0·01 unit/ml.) after cysteine	54·4±1·6	56·0±1·1	5	>0·2
5	Serum after cysteine	36·9±0·8	50·7±1·9	6	<0·01

The sodium content of diaphragms removed and not soaked was 32·7 m.moles/kgm. wet tissue ± 0·4 (S.E. of thirty-three measurements).

Table 1 shows that muscles suspended in human serum which has been dialysed against saline (experiment 1) have a lower sodium content than control muscles soaked in saline. There was no loss of potassium from these muscles. Measurement of the extracellular space with inulin showed that the difference in sodium content shown in experiment 1 was not attributable to alterations in the extracellular sodium. Control experiments in which sucrose was used (0·05 gm./100 ml. saline) did not support the view that the action of serum was attributable to osmotic effects.

The proteins of reconstituted human serum were salted out by saturated ammonium sulphate, redissolved and dialysed against saline, the final volume being adjusted to that of the original serum. Table 1 (experiment 2) shows that the presence of the proteins (or of other substances precipitated by ammonium sulphate) preserved the low sodium content of soaked muscles, as did serum. Extraction[2] of the serum with lipid solvents (ethanol/ether 3 : 1 or chloroform/methanol 2 : 1) did not significantly alter the ability of the serum to maintain a low sodium content in soaked muscles.

Saline containing insulin (0·01 unit/ml.) was effective in maintaining a low sodium content (experiment 3), and it prevented loss of potassium. The insulin/saline was also treated anaerobically at 18° C. with cysteine/hydrochloric acid (20 mgm./ml.) for 12 hr., and the cysteine was later removed by dialysis against saline. After such treatment the insulin failed to maintain muscle sodium at a low value (experiment 4), which is in accord with the observation[3,4] that insulin is inactivated by cysteine. It was therefore of interest to try the effect of cysteine on serum. Experiment 5 shows that serum after treatment with cysteine (and dialysis) was still able to maintain a low sodium content of test muscles.

The ability of plasma to maintain muscle sodium at a low value has been found in the case of the frog[5]. Experiments with radioactive sodium have indicated that the presence of insulin and glucose in saline gives a lower sodium content of test muscles as compared with controls in which these are both absent[6]. The effect of insulin in maintaining low sodium in muscle is consistent with the higher potassium and raised membrane potential of rat muscle soaked in insulin/saline[7]. It seems that proteins or other substances, in concentrations found in serum, can maintain a low sodium content of soaked muscles even with the insulin inactivated. It is therefore not certain whether insulin is a physiological factor which is responsible for the low sodium content of diaphragm muscles *in vivo*.

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R. CREESE
J. L. D'SILVA
JEAN NORTHOVER

Department of Physiology,
London Hospital Medical College,
London, E.1.
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Hypothalamus and Oxytocin

EVIDENCE has recently been produced that, in the rat, oxytocin is synthesized in the paraventricular nucleus[1]. After small electrolytic lesions in this region a decrease in the amount of oxytocin was found in the posterior pituitary with no change in its vasopressin content. Robertson and Hawker[2] have demonstrated the presence of a second oxytocic substance in the hypothalami of cows, oxen, cats (and kittens), rats and mice, but this substance was not present in pituitary extracts. This oxytocic substance differs from naturally occurring oxytocin in its stability towards 0·01 *M* thioglycollate; this procedure inactivates the hormones of the posterior pituitary[3].

There is some evidence that the second oxytocic substance recovered from blood extracts by Hawker and Robertson[4] and Hawker and Roberts[5] may be synthesized in the paraventricular nucleus of the hypothalamus together with oxytocin. First, the correlation of percentages of second oxytocic substance with the total oxytocic activity in extracts of