The Role of Magnesium in Adrenal Insufficiency

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After injection of magnesium salts into rabbits (Du Bois, Albaum & Potter, 1943) or rats (Stoner, 1950) there occurs an increase in the adenosine triphosphate content of muscle tissue. This effect of magnesium, which may be due to inhibition by excess of Mg^{2+} ions of adenosine triphosphatase or phosphokinases or both, suggests that any condition leading to increased magnesium content of muscle tissue may lead to severe disturbances in normal function. Such an increase in muscle magnesium is seen in a number of conditions; in general, changes in muscle potassium, whether increases or decreases, are paralleled by similar changes in the magnesium content (Baldwin, Robinson, Zierler & Lilienthal, 1952). In adrenal insufficiency therefore one would expect an increase in magnesium proportional to the increase in potassium usually seen in this condition. Such an increase in muscle and plasma magnesium of adrenalectomized rats was reported from this laboratory (Conway & Hingerty, 1946) and similar increases in plasma magnesium after adrenalectomy were found by Harrop, Soffer, Ellsworth & Trescher (1933) in dogs, and by Zwemer & Sullivan (1934) in cats.

Among other effects of adrenalectomy seen in the above-mentioned group of rats (Conway & Hingerty, 1946) were an increase in phosphocreatine and a marked decrease in hexose esters of muscle tissue. These changes were confirmed in later experiments (Conway & Hingerty, 1953), and a small rise in adenosine triphosphate was also found. The results of Albaum, Hirshfeld, Tonhazy & Umbreit (1951) also show some increase in adenosine triphosphate and phosphocreatine of rat muscle after adrenalectomy.

From the above it would appear possible that the changes seen in adenosine triphosphate and phosphocreatine (and possibly also those seen in the hexose esters) of muscle after adrenalectomy are due to the increased magnesium content of the muscle found in this condition. To test this hypothesis, intact rats were injected with magnesium sulphate solution to bring the muscle magnesium to approximately the same level as that seen in adrenal insufficiency, and adenosine triphosphate, phosphocreatine and hexose esters of the muscle tissue were then estimated. Because of the marked depressing effects of increased Mg^{2+} ions only short-term experiments could be performed; the results, nevertheless, appear to be of interest.

METHODS

Young, male albino rats of the Wistar strain were used in all the experiments. Each rat was injected intraperitoneally with $MgSO_4$ solution (1 mg. of $MgSO_4$ /g. body wt.), controls being injected with water. Control experiments were also carried out on a group of rats paralysed by intramuscular injection of Myanesin [3-(2-methylphenoxy)propane-1:2-diol; 500 mg./kg.]. After an interval of about 15–30 min. (when the animals had become quiet but before unconsciousness had set in), the rats were killed and approx. 1 g. quantities of muscle were removed quickly to 10% (w/v) ice-cold trichloroacetic acid and fractionated as previously described (Conway & Hingerty, 1946). Quantities of about 0.3 g. of muscle were removed to platinum crucibles, dried after treatment with a few drops of 4N- H_2SO_4 and dissolved in hot N-HCl. The magnesium was precipitated from this solution (after neutralization) by 8-hydroxyquinoline. It was then centrifuged, washed, redissolved in 3 ml. of 0.5N-HCl and estimated in the Beckmann flame photometer at 371 m μ .

RESULTS

The injected rats showed an average increase in muscle magnesium of 23% as compared with an increase of 17% in the adrenalectomized rats. At the same time there occurred changes in phosphocreatine, adenosine triphosphate and hexose esters of muscle which in general paralleled those seen in the adrenalectomized animals. These changes are shown in Table 1, which includes data obtained in the work previously referred to (Conway & Hingerty, 1946, 1953). The table also includes data for the rats injected with Myanesin.

The changes in the various phosphate compounds investigated may be summarized as follows.

Phosphocreatine levels are increased in both adrenalectomized animals and animals injected with magnesium; the adrenalectomized animals showed an increase, from 24.7 ± 0.27 to 28.1 ± 0.44 (14% increase, $P < 0.01$), and the injected animals showed an increase from 24.7 ± 0.27 to 26.2 ± 0.62 (6% increase, $P 0.02$). No significant change is seen in the group injected with Myanesin.

Adenosine triphosphate increases slightly in both the adrenalectomized animals (from 19.6 ± 0.38 to 20.3 ± 0.30 , $P 0.2$) and animals injected with magnesium (19.6 ± 0.38 to 21.2 ± 0.58 , $P 0.02$). These changes represent increases of 4 and 8% respectively. There is no significant increase above normal in the group injected with Myanesin.

Glucose 6-phosphate shows the most marked changes, the mean value being 6.08 ± 0.57 in the normal rats, which falls to 1.52 ± 0.34 in the adrenalectomized rats (a decrease of 75 %, $P < 0.01$) and to 3.96 ± 0.44 in the rats injected with magnesium (a decrease of 35 %, $P < 0.01$). The effect of the magnesium injection, although marked, is thus much smaller than that of adrenalectomy, but this is not surprising when one considers the differences in experimental conditions, particularly the much shorter time of action of the injected magnesium. In the group injected with Myanesin the G 6-P falls but to a lesser extent (26 % decrease, $P 0.02$).

Glucose 1-phosphate shows some increase in the mean value after adrenalectomy (from 3.05 ± 0.21 to 3.40 ± 0.16 , $P 0.2$) and also after injection of

MgSO₄ (from 3.05 ± 0.21 to 4.16 ± 0.50 , $P 0.05$). Here the group injected with Myanesin shows the greatest effect, an increase of 54 % ($P < 0.01$).

Fructose 6-phosphate, constituting only a small fraction of the total hexose esters, is very markedly decreased in the adrenalectomized animals (from 0.92 ± 0.03 to 0.27 ± 0.04 , i.e. a fall of 71 %, $P < 0.01$); in the injected rats, however, the change was in the opposite direction, there being a small increase from 0.92 ± 0.03 to 1.12 ± 0.08 ($P 0.02$). The values after Myanesin are not altered significantly.

The changes seen after adrenalectomy and after MgSO₄ injection are perhaps best illustrated by the distribution data (Fig. 1). It appears that there is a general parallelism between the effects of adrenalectomy and of MgSO₄ injection on the levels of

Table 1. *Changes in magnesium and in phosphorylated compounds of skeletal muscle of rats after magnesium sulphate injections and after adrenalectomy*

Values are given in mg.atoms of Mg or of P/kg. of muscle (wet wt.), followed by the s.e.m. and (in parentheses) the number of analyses. Included are results previously obtained for normal and adrenalectomized rats (Conway & Hingerty, 1946, 1953).

Normal	Magnesium	Phospho- creatine	Adenosine triphosphate	Glucose 1-phosphate	Fructose 6-phosphate	Glucose 6-phosphate
Normal	10.6 ± 0.24 (16)	24.7 ± 0.27 (30)	19.6 ± 0.38 (28)	3.05 ± 0.21 (27)	0.92 ± 0.03 (29)	6.08 ± 0.57 (21)
Magnesium injected	13.1 ± 0.43 (14)	26.2 ± 0.62 (18)	21.2 ± 0.58 (18)	4.16 ± 0.50 (17)	1.12 ± 0.08 (17)	3.96 ± 0.44 (17)
Adrenalectomized	12.4 ± 0.44 (8)	28.1 ± 0.44 (20)	20.3 ± 0.30 (19)	3.40 ± 0.16 (23)	0.27 ± 0.02 (17)	1.52 ± 0.34 (19)
Myanesin injected	—	24.8 ± 0.38 (15)	20.0 ± 0.60 (15)	4.70 ± 0.50 (15)	0.88 ± 0.11 (15)	4.50 ± 0.44 (8)

Fig. 1. Frequency distribution of phosphocreatine (PC), adenosine triphosphate (ATP), glucose 6-phosphate (G 6-P), fructose 6-phosphate (F 6-P) and glucose 1-phosphate (G 1-P) values for muscles of normal, adrenalectomized and magnesium-injected rats. Abscissae (class intervals) are given as mg.atoms of P/kg. of muscle (wet wt.) and ordinates as number of observations.

hexose esters and of adenosine triphosphate and phosphocreatine in rat muscle, so that the changes in these substances after adrenalectomy could be interpreted as being secondary (at least in part) to the increased magnesium levels seen in this condition. As indicated by Stoner (1950), these changes may be partly due to the effect of the Mg^{2+} ion in eliminating movements during the killing of the animals; the generally smaller effect of Myanesin suggests that the Mg^{2+} ions have another action besides a paralysing one.

DISCUSSION

The experiments show a certain parallelism between the effects of adrenalectomy and of $MgSO_4$ injection in so far as phosphocreatine is increased in both groups of animals and glucose 6-phosphate is markedly decreased. Besides the increase in phosphocreatine there appears a very slight increase in adenosine triphosphate, as shown by the frequency-distribution diagrams. These similarities in the effects of excess of Mg^{2+} ions and of adrenal insufficiency suggest that magnesium may be the connecting link between the inorganic changes seen in adrenal insufficiency and certain of the disturbances of organic metabolism seen in this condition. In a previous communication (Conway & Hingerty, 1953) evidence was advanced for the view that the increases in plasma and muscle potassium occurring after adrenalectomy were secondary effects, the primary effect being renal and presumably associated with loss of a normal inhibition by cortical hormones of potassium reabsorption. As the changes in magnesium paralleled closely the changes in potassium we may assume that the increases of magnesium in plasma and muscle are also secondary. Such changes in muscle magnesium might have very profound metabolic effects owing to inhibition of adenosine triphosphatase activity and consequent reduction in the utilization of energy-rich phosphate.

An explanation of the effects of excess of Mg^{2+} ions in causing the marked changes seen in the hexose esters is less readily forthcoming unless one considers that the ions in excess may inhibit also other enzymes concerned with the transfer of energy-rich phosphate. If such an inhibition of the phosphokinases occurred in the muscle after adrenalectomy the result would be a decreased conversion of glucose into glucose 6-phosphate (and glycogen) and a decreased conversion of fructose 6-phosphate into fructose 1:6-diphosphate. With adrenalectomized rats a fall occurs in both glucose 6-phosphate and fructose 6-phosphate, also (Conway & Hingerty, 1946) in fructose 1:6-diphosphate. The glucose 6-phosphate falls also in the animals injected with magnesium but here the fructose

6-phosphate shows a slight increase. This is explicable when the greater glycogen stores of these animals are considered; with adequate glycogen the reactions could proceed as far as fructose 6-phosphate, even in the absence of the energy-rich phosphate from adenosine triphosphate, but here a blockage would occur since the fructose 6-phosphate could not undergo further conversion into fructose 1:6-diphosphate. In the adrenalectomized rats, on the other hand, insufficient glycogen (and blood glucose) would be available and consequently both glucose 6-phosphate and fructose 6-phosphate would suffer depletion.

Much further information on the effects of excess of Mg^{2+} ions on enzyme systems in the living muscle is desirable in this connexion; nevertheless, the retention of energy-rich phosphate in the phosphocreatine and adenosine triphosphate of the muscle would provide an explanation for the interference with muscular contraction, growth, heat production (and other physiological functions dependent on readily available energy-rich phosphate) seen in adrenal insufficiency.

SUMMARY

1. Certain disturbances of carbohydrate metabolism seen in adrenalectomized rats can be brought about by raising the muscle magnesium content of intact rats to the level found in adrenal insufficiency.

2. In both conditions there is a marked fall in the glucose 6-phosphate content, and there is an increase of phosphocreatine. There is also in both conditions some increase of adenosine triphosphate.

3. The evidence is in accordance with the hypothesis that the increased magnesium levels occurring in adrenal insufficiency are largely responsible, through inhibition of certain enzyme activities, for the metabolic disturbances seen in this condition.

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