

THE EFFECT OF MAGNESIUM DEFICIENCY ON OXIDATIVE PHOSPHORYLATION*

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The uncoupling of oxidative phosphorylation in mitochondrial preparations by thyroxine and its reversal are now well known (1-6). More recently we have shown that high levels of dietary magnesium completely prevent the uncoupling effect on heart mitochondria of thyroxine-fed animals, and partially overcome the growth inhibition caused by thyroxine. Thyroxine thus raises the magnesium requirement. The gross signs observed in the thyroxine-treated animals were in many respects similar to those seen in magnesium deficiency (6).

These observations suggested that magnesium deficiency alone might produce a similar uncoupling of oxidative phosphorylation. The results of this present paper demonstrate that such is the case. We have also studied the effects of thyroxine injection. The mitochondria of cardiac tissue are much more sensitive to the effects of thyroxine administration and magnesium deficiency than are those of liver and kidney.

EXPERIMENTAL

Twenty-two day-old male rats, obtained from the Charles River Breeding Laboratories, Inc., Boston, and weighing approximately 50 gm., were placed in individual cages and fed the magnesium-deficient diet previously described. At intervals, the animals were killed and the heart, liver, and kidneys of each removed for study. The magnesium requirement for optimal growth on this diet is approximately 20 to 24 mg. of magnesium per 100 gm. of diet. Control animals had sufficient MgO added to the diet to supply 24 mg. per cent of Mg. In acute experiments, rats receiving the control diet containing 24 mg. per cent magnesium were injected with thyroxine, 10 mg. per kilo of body weight, and killed after 10, 15, 30, and 180 minutes. In order to study the effects of magnesium, a similar

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group of six animals received an injection of 10 mg. of magnesium 10 minutes before the thyroxine injection. These rats were killed 10 minutes after the dose of thyroxine.

Mitochondria of the heart, kidney, and liver were prepared and the oxidative phosphorylation efficiency was determined as described previously (6). The Warburg vessels contained the following: main compartment, 0.5 ml. of mitochondrial suspension, 0.042 μ mole of cytochrome *c*, 22.5 μ moles of MgSO_4 , 6 μ moles of adenosine triphosphate (ATP), 40 μ moles of potassium phosphate buffer, pH 7.3, 20 μ moles of α -ketoglutarate, and 4 μ moles of NaF. The side arm contained 5.0 mg. of crystalline hexokinase and 50 μ moles of glucose. The final volume was made up to 3.0 ml. with 0.154 M KCl. The center well contained 0.2 ml. of 20 per cent KOH. The phosphorus determinations were carried out by the method of Taussky and Shorr (7).

RESULTS AND DISCUSSION

Magnesium deficiency develops rapidly in young animals. After 4 days on the deficient diet the typical symptoms of hyperexcitability, hyperemia of the ears, and tonic convulsions were seen. At this time the P:O of heart mitochondria was approximately half that of the control animals (Table I). The P:O ratio of liver and kidney mitochondria had not changed and values similar to those of the controls were obtained. The P:O ratio for heart mitochondria was further decreased after 8 days on the deficient diet and a similar value was obtained after 11 days. The P:O ratio for kidney and liver mitochondria was probably below normal after 11 days and perhaps below normal for the kidney after 8 days. However, it is clear that there was much less change in these tissues than in the heart in spite of the fact that many animals died of magnesium deficiency before this time.

A single injection of 10 mg. of thyroxine per kilo of body weight produced a marked fall in the P:O ratio of heart mitochondria within 10 minutes (Table II). The effect was still evident, but smaller after 15 minutes, and had disappeared within 30 minutes. The group of animals which received magnesium injections immediately before the thyroxine showed no fall. The P:O ratio of liver mitochondria was evidently slightly affected within 10 minutes, but no effect was observed after 15 minutes. As with magnesium deficiency, the liver is apparently considerably more resistant to the uncoupling effect of thyroxine.

It is obvious that the effects of magnesium deficiency and thyroxine excess produce a similar uncoupling of oxidative phosphorylation. Both effects are reversible by magnesium administration. Heart mitochondria are peculiarly susceptible to both. It has been suggested that the action of

thyroxine might be related to its effect on the structural integrity of the mitochondria (8, 9). Magnesium may, of course, somehow prevent such changes. However, this does not seem to provide an adequate explanation for the production of typical magnesium deficiency by continued thyroxine

TABLE I

Effect of Magnesium Deficiency on Oxidative Phosphorylation (P:O) of Heart, Liver, and Kidney Mitochondria

No. of days		Heart		Liver		Kidney	
		Control rats	Deficient rats	Control rats	Deficient rats	Control rats	Deficient rats
4	$Q_{O_2}(N)^*$	404	548	220	271	424	444
	P:O	2.7	1.4	2.8	2.3	2.6	2.4
8	$Q_{O_2}(N)$	334	303	175	183	328	415
	P:O	2.3	1.0	2.5	2.8	2.4	1.9
11	$Q_{O_2}(N)$	490	593	156	172	323	327
	P:O	2.3	1.1	2.8	2.2	2.5	2.0

* $Q_{O_2}(N)$ = microliters of O_2 per mg. of nitrogen per hour.

TABLE II

Effect of Injected Thyroxine on Oxidative Phosphorylation (P:O) of Heart and Liver Mitochondria

Time after injection	Heart (P:O)		Liver (P:O)	
	Control	Treated*	Control	Treated
<i>min.</i>				
10	2.3	1.3 (2.3)†	2.7	2.2
15	2.4	1.8	3.2	3.4
30	2.2	2.4	2.9	3.1
180	2.3	2.4		

* 10 mg. of thyroxine per kilo of body weight were injected intraperitoneally.

† Average P:O of five animals injected with magnesium (10 mg. per kilo of body weight) 10 minutes before the injection of thyroxine.

administration, the latter being characterized by low serum magnesium values, poor growth, and typical deficiency symptoms (6).

SUMMARY

Magnesium deficiency in young rats produced uncoupling of oxidative phosphorylation in heart mitochondria within 4 days and a maximal effect within 8 days. Liver and kidney mitochondria were much less

sensitive to dietary magnesium deprivation and only small decreases in the P:O ratio were observed.

Heart mitochondria are also particularly sensitive to thyroxine administration. A maximal decrease in the P:O ratio was observed within 10 minutes after a thyroxine injection and lasted less than 30 minutes. The uncoupling action of thyroxine was completely prevented by a preliminary injection of magnesium.

BIBLIOGRAPHY

1. Martius, C., and Hess, B., *Arch. Biochem. and Biophys.*, **33**, 486 (1951).
2. Mudd, S. H., Park, J. H., and Lipmann, F., *Proc. Nat. Acad. Sc.*, **40**, 909 (1954).
3. Bain, J. A., *J. Pharmacol. and Exp. Therap.*, **111**, 2 (1954).
4. Klemperer, H. G., *Biochem. J.*, **60**, 122 (1955).
5. Maley, G. F., and Lardy, H. A., *J. Biol. Chem.*, **204**, 435 (1953).
6. Vitale, J. J., Hegsted, D. M., Nakamura, M., and Connors, P., *J. Biol. Chem.*, **226**, 597 (1957).
7. Taussky, H. H., and Shorr, E., *J. Biol. Chem.*, **202**, 675 (1953).
8. Tapley, D. F., *J. Biol. Chem.*, **222**, 325 (1956).
9. Tapley, D. F., and Cooper, C., *J. Biol. Chem.*, **222**, 341 (1956).