

THE EFFECT OF THYROXINE ON MAGNESIUM REQUIREMENT*

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It has recently been demonstrated that animals maintained in the cold (12.8°) have an increased requirement for magnesium (1). Animals fed a purified diet grew maximally when the diet contained 25 mg. per cent of magnesium, whereas animals maintained in the cold required approximately 50 mg. per cent of magnesium to achieve maximal rates of growth although such animals never attained the weight of their controls in the warm room (23.9°) (1).

The effect of cold in increasing thyroid activity and production of thyroid hormone is well known (2). It was suggested that the increased requirement for magnesium in the cold might be related to thyroid activity (1), since thyroxine uncouples oxidative phosphorylation *in vitro*, and that magnesium prevents this uncoupling effect (3, 4). Furthermore, the concentration of serum magnesium is lower in thyrotoxic patients and, upon treatment, serum magnesium levels return to near normal.¹ Such patients may require higher intakes of magnesium than normal to attain magnesium balance (5).

This study deals with the effect of thyroxine on the growth of animals fed various levels of magnesium and with the capacity of the hearts of these animals to carry out oxidative phosphorylation.

EXPERIMENTAL

21 day-old rats obtained from the Charles River Breeding Laboratories, Inc., Boston, Massachusetts, and weighing approximately 50 gm., were fed a purified diet previously described (1). The rats were divided into sixteen groups, twelve animals in each, and fed the purified diet supplemented with thyroxine to supply 0, 1, 2, or 4 mg. per 100 gm. In addition,

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¹ Vitale, J. J., Ingbar, S., and Dowling, T., unpublished data.

tion, each of these diets was further supplemented with various levels of magnesium: 20, 40, 80, and 160 mg. per 100 gm. of diet. The animals were fed *ad libitum* and housed in individual cages. The temperature was maintained at $25.6^{\circ} \pm 2^{\circ}$. The rats were weighed twice weekly and observed daily for signs of magnesium deficiency for 4 weeks. Approximately 4 months later a portion of the experiment was repeated. Groups of twelve animals each were given the same diet containing 20, 40, 80, or 160 mg. of magnesium and 2 mg. of thyroxine per 100 gm. of diet. A control group with no thyroxine received 25 mg. of magnesium per 100 gm. of diet. At the end of 24 days six animals from groups fed the following diets were sacrificed by decapitation: (1) no thyroxine and 25 mg. per cent of magnesium, (2) 2 mg. per cent of thyroxine and 20 mg. per cent of magnesium, and (3) 2 mg. per cent of thyroxine and 160 mg. per cent of magnesium. Serum magnesium levels reported in Table II for these three groups were determined in animals other than those sacrificed for oxidative phosphorylation studies. The hearts were quickly removed and washed in ice-cold KCl (0.154 M) and oxidative phosphorylation studies were done on mitochondria prepared in 0.88 M sucrose as described elsewhere (6). The reaction flasks contained 6 μ moles of adenosine triphosphate, 40 μ moles of phosphate buffer, pH 7.4, 22.5 μ moles of MgSO_4 , 0.12 μ mole of cytochrome *c*, 30 μ moles of α -ketoglutarate, 50 μ moles of glucose, hexokinase, and mitochondria from 3 to 4 gm. of heart tissue. The mitochondria were suspended in 1 ml. of sucrose per gm. of wet weight of heart used. Final volume per flask was 3.2 ml. Equilibration was for 5 minutes in an air phase and the reaction was run at 37° . The center well contained 0.2 ml. of 20 per cent NaOH. Serum magnesium levels were determined according to the method of Orange and Rhein (7).

Results

Table I illustrates the effects of thyroxine on the growth of rats fed various levels of magnesium for 16 days. Rats fed no thyroxine grew maximally on a diet containing 20 mg. per cent of magnesium. The addition of more magnesium to the diet resulted in no increased growth over the 16 day period. It is apparent that, as the thyroxine in the diet increased from 1 to 4 mg. per cent, more magnesium was required for maximal weight gain. The animals fed the 1 mg. per cent of thyroxine diet gained 60 gm. when the magnesium content was 40 mg. per cent. However, rats fed the 4 mg. per cent of thyroxine diet required 160 mg. per cent of magnesium to attain the same weight gain.

Table II demonstrates the effect of thyroxine and magnesium on oxidative phosphorylation of heart tissue. With control animals fed no thyroxine and a diet containing 25 mg. per cent of magnesium, a P:O ratio of

2.20 was obtained, whereas the hearts from animals fed a diet containing approximately the same level of magnesium and 2 mg. per cent of thyroxine had a P:O ratio of 1.06. When 2 mg. per cent of thyroxine and 160 mg. per cent of magnesium were fed, a P:O ratio of 2.14 was found. The average serum magnesium level in control animals fed 25 mg. per cent of magnesium was approximately 1.8 mg. per cent. Thyroxine markedly decreased the serum magnesium level to 0.5 when the diet contained only

TABLE I
Effect of Magnesium on Growth of Thyroxine-Fed Rats

| Magnesium, mg. per cent of diet | Thyroxine, mg. per cent of diet | | | | |
|---------------------------------|---------------------------------|----|----|----|----|
| | 0 | 1 | 2 | 2* | 4 |
| | Weight gain, gm. | | | | |
| 20 | 89 | 44 | 47 | 41 | 31 |
| 40 | 79 | 60 | 46 | 53 | 39 |
| 80 | 98 | 66 | 52 | 59 | 57 |
| 160 | 85 | 60 | 66 | 59 | 66 |

* A second experiment repeated 4 months later.

TABLE II
Effect of Dietary Magnesium on Oxidative Phosphorylation of Hearts from Thyroxine-Fed Rats

| Group | Mg ⁺⁺ , mg. per cent of diet | Thyroxine, mg. per cent of diet | P:O | Serum Mg ⁺⁺ , mg. per cent |
|--------------------|---|---------------------------------|------|---------------------------------------|
| Controls | 25 | 0 | 2.20 | 1.87 ± 0.10* |
| Treated | 20 | 2 | 1.06 | 0.50 ± 0.08 |
| “ | 160 | 2 | 2.14 | 1.67 ± 0.13 |

* Mean ± standard error.

20 mg. per cent of magnesium. The feeding of 160 mg. per cent of magnesium restored the serum magnesium concentration to near normal levels.

Magnesium deficiency in rats is characterized by vasodilatation and hyperemia of the vascular bed which is particularly evident in the ears, tails, and feet. In surviving animals, trophic changes of the epidermal structures and edema of the extremities occur. These gross signs of magnesium deficiency were observed in many of the animals receiving thyroxine. Although no attempt was made to grade the degree of vasodilatation and hyperemia among the several groups, it was apparent that the animals fed the lower levels of magnesium (20 to 40 mg. per cent) had a higher and more severe incidence of vasodilatation and hyperemia of the

ears, tails, and feet than those fed the higher concentrations of magnesium (160 mg. per cent). Without thyroxine supplements, diets containing 20 mg. per cent of magnesium completely prevented the appearance of such signs.

DISCUSSION

The data demonstrate unquestionably a marked increase in the magnesium requirement caused by the administration of thyroxine. As the thyroxine level is increased, more magnesium is required to obtain maximal weight gains. At the highest level of thyroxine used (4 mg. per cent) some degree of vasodilatation and hyperemia of the ears was seen even with 8 times the normal magnesium level in the diet, suggesting that the requirement of magnesium had not yet been reached under these conditions. However, with a lower level of thyroxine (1 mg. per cent) maximal growth was obtained with 40 mg. per cent of magnesium. This was still appreciably below the growth achieved by animals not receiving thyroxine. The same effect was observed in previous studies at cold temperature; *i.e.*, although the magnesium requirement was elevated, no level of magnesium fed produced weight gains equivalent to those obtained in the warm temperature. Undoubtedly, other factors than magnesium are involved. The original work of Ershoff (8) demonstrated that whole liver insoluble material was partially capable of overcoming the growth depressant effects of thyroid substance. This has been confirmed many times. More recently Emerson *et al.* (9) have reported that, in animals receiving 0.1 per cent iodinated casein (Protamone) in the diet, growth responses were obtained by increasing the level of casein, replacing part of the hydrogenated fat fed by corn oil, and adding either cholesterol or bile acids to the diet. A further response above that obtained with the combination of the above three was obtained with liver or soy bean proteins.

The diets we have used contained 20 per cent casein, 5 per cent corn oil, and no liver, cholesterol, or bile acids. The role that magnesium might play in combination with the factors discussed by Emerson *et al.* (9) is, of course, unknown. The report of cholesterol or bile acids as partially overcoming thyroxine-induced growth inhibition is surprising, particularly since these materials definitely raise the magnesium requirement as does thyroxine (10).

The finding of the reversibility of the uncoupling effect of thyroxine on oxidative phosphorylation by dietary magnesium not only confirms the previously observed effect on isolated mitochondrial preparations (3, 4), but also demonstrates a similar effect *in vivo*. According to the papers of Tapley and Cooper (11) it is not yet clear whether the effect of thyroxine and magnesium is related to the biochemical or to the physical integrity of the mitochondrial system.

In our experiments high levels of dietary magnesium were capable of completely reversing the measurable uncoupling effect of thyroxine, although growth was not restored to normal and signs suggestive of magnesium deficiency persisted. The implication, of course, is that the effects of thyroxine are probably not completely explicable in terms of the uncoupling action.

SUMMARY

Growth inhibition in young rats produced by the addition of thyroxine to the diet is partially overcome by extra supplements of magnesium. The amount of magnesium required is related to the amount of thyroxine added, clearly demonstrating an elevation of the magnesium requirement by thyroxine. Magnesium is not, however, capable of completely preventing the thyroxine-induced growth depression.

The oxidative phosphorylation of mitochondrial preparations from the hearts of these animals was impaired in the thyroxine-treated animals and raised to normal by high levels of dietary magnesium.

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