STUDIES OF INTERRELATIONSHIPS OF THYROXINE, MAGNESIUM, AND VITAMIN B₁₂*

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The increased requirement for vitamin B₁₂ in thyrotoxic animals is well established (1-3). Vitale et al. (4) have demonstrated that magnesium will also partially overcome the growth-inhibiting action of thyroxine administration. Uncoupling of oxidative phosphorylation occurs in rats made magnesium-deficient either by dietary deprivation of magnesium (5) or by thyroxine administration (4).

In view of these observations, the present study of interrelationships between thyroxine, magnesium, and vitamin B₁₂ was undertaken.

EXPERIMENTAL

Male Charles River rats, averaging 57 gm. and housed in individual cages, were used in this study. The basal diet consisted, in per cent, of glucose 70, devitaminized casein 20, corn oil 5, cod liver oil 1, Jones-Foster salt mix (6) with MgSO₄ and CaCO₃ removed 2.25, CaCO₃ 1.5, and choline 0.3. Vitamin supplements of 4 mg. of thiamine, 8 mg. of riboflavin, 4 mg. of pyridoxine, 40 mg. of niacin, and 25 mg. of calcium pantothenate were added per kilo of diet. MgO was added as required so that all diets contained either 20 or 160 mg. per cent of Mg. Where used, thyroxine supplements were 20 mg. and vitamin B₁₂ 20 γ per kilo. In the first experiment six groups of rats were used. After 4 weeks on the experimental diets, the rats were decapitated and blood was collected for serum magnesium determinations (7). Serum protein fractions were determined electrophoretically (8). Samples of liver and small intestines were homogenized in phosphate buffer, autoclaved for 5 minutes according to the method of Scheid et al. (9), and then analyzed microbiologically for vitamin B₁₂ (10). After fixation in 10 per cent formalin, sections of one kidney and of stomach

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showing both rumen and glandular stomach from each animal were stained with hematoxylin and eosin and examined microscopically.

A second series of rats was fed certain of the experimental diets for 24 days. During the succeeding 3 days, the rats were decapitated and oxidative phosphorylation efficiency of heart mitochondria was determined according to the method of Hogeboom et al. (11).

Results

The data obtained from the first series of rats are shown in Tables I and II. As previously described (4), increasing the dietary Mg from 20 to 160 mg. per cent partially protected against the growth inhibition induced by thyroxine administration. Growth stimulation of thyroxine-treated animals by vitamin B$_{12}$ might have been greater, had larger quantities of the vitamin been used. However, a lower level of vitamin B$_{12}$ supplementation was preferred to prevent the masking of a Mg or thyroxine effect on the tissue concentrations of vitamin B$_{12}$. The liver vitamin B$_{12}$ values obtained when Diets 20MB and 20MBT were fed indicate that this precaution was not completely successful. Other than in this instance the administration of thyroxine markedly lowered the vitamin B$_{12}$ content of liver and small intestines. This was particularly true with Diets 20M and 20MT. The lowering of tissue vitamin B$_{12}$ concentrations by thyroxine was reversed by increasing the Mg content of the diet (Diets 20MT and 160MT).

As previously reported (4), thyroxine administration lowered serum Mg. Vitamin B$_{12}$ did not have any significant effect on serum Mg. It must be pointed out that the complexometric titration method used for determining Mg in this work (7) consistently gives higher serum Mg values than those obtained with the commonly used Titan yellow method (12). However, in our hands duplication of results is accomplished more easily by using the titrometric method.

Table II shows the effect of thyroxine and vitamin B$_{12}$ on rat serum proteins. The control values obtained with the group on Diet 20M are essentially the same as those obtained from the group on Diet 20MB. The administration of thyroxine resulted in a lowering of total serum protein and in $\alpha_1$-globulins and an elevation of $\alpha_2$-globulins. There also was a trend toward a lowering of $\gamma$-globulins. The addition of vitamin B$_{12}$ with thyroxine partially reversed the effect of thyroxine on these serum proteins. There was no effect of thyroxine on serum albumin. It is uncertain whether the $\beta$-globulin values in groups on Diets 20MT and 20 MBT are actually raised by the thyroxine treatment. In uncompleted experiments in this laboratory, Mg as well as vitamin B$_{12}$ appears to reverse the effect of thyroxine on serum proteins. The results obtained in this study are
similar to those of Mulgaonkar and Sreenivasan (13), who fed rats purified
diets containing 10 per cent casein plus an additional 0.15 per cent iodinated
casein and found that, when vitamin B<sub>12</sub> and folic acid supplements were

### Table I

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of rats</th>
<th>Weight gain per 4 wks.</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; content†</th>
<th>Serum Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Small intestine</td>
</tr>
<tr>
<td>20M</td>
<td>10</td>
<td>134 ± 9</td>
<td>54 ± 8</td>
<td>71 ± 7</td>
</tr>
<tr>
<td>20MB</td>
<td>10</td>
<td>131 ± 8</td>
<td>90 ± 8</td>
<td>97 ± 8</td>
</tr>
<tr>
<td>20MT</td>
<td>8</td>
<td>71 ± 7</td>
<td>29 ± 5 (7)</td>
<td>44 ± 6 (7)</td>
</tr>
<tr>
<td>20MBT</td>
<td>8</td>
<td>81 ± 11</td>
<td>102 ± 9 (6)</td>
<td>71 ± 8 (6)</td>
</tr>
<tr>
<td>160MT</td>
<td>8</td>
<td>85 ± 5</td>
<td>49 ± 11 (7)</td>
<td>64 ± 6 (7)</td>
</tr>
<tr>
<td>160MBT</td>
<td>7</td>
<td>91 ± 5</td>
<td>131 ± 10</td>
<td>100 ± 11</td>
</tr>
</tbody>
</table>

* 20M, 20 mg. per cent of Mg; 20MB, 20 mg. per cent of Mg + 2 γ per cent of vitamin B<sub>12</sub>; 20MT, 20 mg. per cent of Mg + 2 mg. per cent of thyroxine; 20MBT, 20 mg. per cent of Mg + 2 γ per cent vitamin B<sub>12</sub> + 2 mg. per cent of thyroxine; 160MT, 160 mg. per cent of Mg + 2 mg. per cent of thyroxine; 160MBT, 160 mg. per cent of Mg + 2 γ per cent of vitamin B<sub>12</sub> + 2 mg. per cent of thyroxine.
† Expressed as millimicrograms of vitamin B<sub>12</sub> per gm. of fresh tissue. The numbers in parentheses refer to the number of samples analyzed when different from the number of rats in the group. The values include the standard error of the mean.

### Table II

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of rats</th>
<th>Total protein</th>
<th>Albumin</th>
<th>α&lt;sub&gt;1&lt;/sub&gt;</th>
<th>α&lt;sub&gt;2&lt;/sub&gt;</th>
<th>β</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per cent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20M</td>
<td>9</td>
<td>6.3 ± 0.2</td>
<td>247.3 ± 1.1</td>
<td>14.9 ± 0.6</td>
<td>10.7 ± 1.6</td>
<td>17.8 ± 1.1</td>
<td>0.3 ± 1.4</td>
</tr>
<tr>
<td>20MB</td>
<td>8</td>
<td>6.2 ± 0.1</td>
<td>47.3 ± 2.4</td>
<td>13.2 ± 0.9</td>
<td>11.7 ± 0.9</td>
<td>19.1 ± 1.2</td>
<td>1.28 ± 0.7</td>
</tr>
<tr>
<td>20MT</td>
<td>7</td>
<td>5.7 ± 0.1</td>
<td>37.5 ± 3.8</td>
<td>8.3 ± 1.2</td>
<td>15.6 ± 0.7</td>
<td>21.3 ± 1.5</td>
<td>1.57 ± 0.1</td>
</tr>
<tr>
<td>20MBT</td>
<td>7</td>
<td>6.0 ± 0.2</td>
<td>43.0 ± 4.4</td>
<td>10.2 ± 1.3</td>
<td>13.7 ± 0.7</td>
<td>23.1 ± 1.6</td>
<td>1.68 ± 1.2</td>
</tr>
</tbody>
</table>

The values are presented as percentage of serum protein plus the standard error of the mean.

not given, there were decreases in total serum proteins, decreases in serum albumin and α<sub>1</sub>-globulin, and increases in α<sub>2</sub>-globulin and β-globulin on a per cent serum protein basis. No differences were found in the percentage of γ-globulin. The differences in these two studies, particularly in the absolute values obtained, are probably the result of the hypoproteinemia obtained with 10 per cent casein diets.
Table III shows the effect of magnesium, vitamin B₁₂, and thyroxine administration on oxidative phosphorylation of rat heart mitochondria. Cardiac mitochondria were used because they are more sensitive to the effects of thyroxine administration and magnesium deficiency than are those of liver and kidney. Each day's determinations were made simultaneously since control values vary somewhat from day to day. The values obtained each day are presented separately. The previously demonstrated effect of dietary Mg on the thyroxine-induced uncoupling of phosphorylation is seen. These data show that, like Mg, vitamin B₁₂ is at least partially effective in reversing the effect of thyroxine in uncoupling oxidative phosphorylation.

### Table III

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>25th day</th>
<th>26th day</th>
<th>27th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>20MB</td>
<td>3.26</td>
<td>2.12</td>
<td>2.42</td>
</tr>
<tr>
<td>20MT</td>
<td>1.10</td>
<td>1.09</td>
<td>1.68</td>
</tr>
<tr>
<td>20MBT</td>
<td>1.73</td>
<td>1.79</td>
<td>1.33</td>
</tr>
<tr>
<td>160MBT</td>
<td></td>
<td></td>
<td>2.56</td>
</tr>
<tr>
<td>160MT</td>
<td></td>
<td></td>
<td>2.18</td>
</tr>
</tbody>
</table>

Each value represents data obtained on pooled hearts of three rats.

**DISCUSSION**

The interrelationships of thyroxine, vitamin B₁₂, and Mg shown in this study cannot be fully interpreted at this time. The specific roles of thyroid hormones in metabolism are obscure, although apparently related to activation of enzyme systems involved in energy production. The increased requirement of hyperthyroid rats for Mg may in part be related to the increased oxygen consumption of their tissues, since Mg is an essential part of respiratory enzyme systems. Additional Mg may also be needed to overcome thyroxine toxicity by antagonizing the effect of thyroxine on mitochondrial structure (14).

The relationship between vitamin B₁₂ and thyroxine is even more obscure. du Toit (15) has shown that thyroxine is effective in promoting protein synthesis in athyroid rats. Protein synthesis in these animals slightly exceeded that observed in normal rats. The administration of excess thyroxine results in growth inhibition and, therefore, in impaired utilization of protein for growth. Vitamin B₁₂ partially reverses thyroxine-
induced growth inhibition (1–3) and vitamin B₁₂ has been shown to play a role in the capacity of the normal mammal to utilize protein (16). It seems conceivable that thyroxine and vitamin B₁₂ are related through their respective roles in protein metabolism. This is supported somewhat by the action of vitamin B₁₂ in partially reversing the effect of thyroxine on serum proteins. In addition thyroxine appears to affect vitamin B₁₂ economy. Tissue vitamin B₁₂ concentrations of thyroxine-treated rats were markedly lower than those of controls. The mechanism by which this reduction of vitamin B₁₂ took place is unknown. Increased catabolism or excretion of vitamin B₁₂ or decreased intestinal synthesis of the vitamin may have occurred. In any event, the loss of tissue vitamin B₁₂ could be prevented by Mg administration. This was not a reciprocal relationship. Vitamin B₁₂ supplementation did not protect against the loss of serum Mg in thyrotoxic rats. In one instance (Diets 20M and 20MB) the addition of vitamin B₁₂ resulted in a statistically significant lowering of serum Mg. Histologic examination revealed that two of the control rats (on Diet 20M) and one of the animals receiving the control diet plus vitamin B₁₂ (Diet 20MB) showed some calcium deposition within the tubules of the outer portion of the renal medulla. This lesion, which is indicative of borderline Mg deficiency, was not observed in any of the other animals and is further evidence that vitamin B₁₂ does not spare Mg. As previously reported from this laboratory (17), thyroxine administration appeared to offer protection against the renal injury associated with Mg deficiency. Both in this work and in a previous study (4), thyroxine lowered serum Mg. No explanation for this apparent paradox is presently available.

Although the administration of vitamin B₁₂ or Mg resulted in a similar reversal of thyroxine toxicity effects, there is no reason for believing that this was entirely the result of their functioning in the same metabolic systems. Mg is an essential part of both respiratory and phosphorylation systems, but no evidence for a similar role for vitamin B₁₂ is available. Whether or not the effect of vitamin B₁₂ on oxidative phosphorylation is direct or indirect awaits further work.

No stomach epithelial abnormality was observed in any of the rats. Although marked morphologic changes of the stomach are found in pernicious anemia (18), a disease caused by a systemic deficiency of vitamin B₁₂, there is no evidence that vitamin B₁₂ deficiency in experimental animals results in gastric mucosal lesions. The gastric lesions of pernicious anemia are probably the cause of impaired vitamin B₁₂ absorption in this disease and are not the result of vitamin B₁₂ deficiency. This does not preclude the possibility that vitamin B₁₂ plays an important role in the normal functioning of the gastrointestinal mucosa which is metabolically one of the most active of animal tissues. The high concentration of intestinal
vitamin B₁₂, as evidenced by the similarity in the vitamin B₁₂ content of intestines and liver of the same animals, argues for this possibility.

**SUMMARY**

Interrelationships between thyroxine, Mg, and vitamin B₁₂ have been studied. Thyroxine administration results in a loss of tissue vitamin B₁₂ which can be prevented by Mg supplementation. Lowered serum Mg levels in thyrotoxic rats are not raised by vitamin B₁₂ administration. The effects of thyroxine in depressing growth, uncoupling oxidative phosphorylation, and altering serum protein fractions can be reversed at least in part by Mg or vitamin B₁₂.

**BIBLIOGRAPHY**

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