Thymic Changes in the Magnesium-depleted Rat

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SUMMARY

The effects of a magnesium-deficient diet fed to rats for approximately 65 days have been assessed with special reference to changes in the thymus. The thymus was enlarged in 18 to 52% of deficient animals surviving more than 6 to 7 weeks in various experiments. The remainder demonstrated glands that were smaller than controls. The enlarged thymuses showed marked cellular changes with the normal structure being replaced by cells that morphologically resembled transformed lymphocytes. Of the small glands, 19% had focal or lobular cellular changes similar to those seen in enlarged thymuses. No distant metastases were found and the changes have been interpreted as hyperplastic rather than neoplastic. Prolonged magnesium depletion was accompanied by hypomagnesemia and hypercalcemia or normocalcemia. Marked leukocytosis was present during the early stages of the deficiency. Splenomegaly was consistently found in the magnesium-depleted animals.

INTRODUCTION

Thymic enlargement (in rats depleted of magnesium for approximately 65 days) has been reported by others (3—6, 11, 12) and designated as a malignant lymphosarcoma (3, 5, 6, 12). Statistics on the incidence of various changes observed have not been published, and the detailed reports of the abnormal morphology of the thymus seen with light (3, 5) or electron microscopy (6) appear to be confined to a small number of tumors.

It is now well established that magnesium deficiency induces many changes including effects on calcium and potassium metabolism (17, 24) in addition to neuromuscular abnormalities (16). These changes are particularly pronounced when the rat is maintained on a high-calcium diet, as was the case in the studies of Bois et al. (3–6). Calcium (29) and parathormone (28) have been reported to stimulate rat thymocytes in vitro. It appeared desirable to investigate further the magnesium-thymus relationship with more extensive analysis of anatomical and histological changes and nutritional influences, especially the influence of calcium. This initial paper summarizes our findings on the incidence and histology of thymic changes and changes in the blood that occurred in rats fed a diet developed in our laboratory. Some of the data have been presented previously (25).

MATERIALS AND METHODS

Male Sprague-Dawley rats (Sprague-Dawley Co., Madison, Wis.) were used in each experiment and were fed the purified diets described below. Thirty-five-day-old rats with initial mean weight of approximately 117 g were used in all but 1 experiment; in that exception the initial mean weight was 173 g. Animals were housed individually in hanging galvanized wire mesh cages during the experiments.

Magnesium-deficient Diet A was that used by Shils (24) with slight modification. Except where otherwise indicated, analytical reagent grade chemicals were used. The composition of the diet was: 56.5% dextrose monohydrate (C. P. C. International, Yonkers, N. Y.), 18.2% vitamin-free casein (Nutritional Biochemicals Corp., Cleveland, Ohio), 10.9% cornstarch and 11.9% corn oil (Embassy Grocers, New York, N. Y.), 1.0% potassium chloride, 1.4% salt mixture, and 0.1% choline chloride. To each 100 g of the above were added 2.0 mg menadione and 0.6 ml Vi-Penta multivitamin drops (Hoffmann-LaRoche, Inc., Nutley, N. J.). The salt mixture consisted of 25.74% calcium lactate pentahydrate, 20.60% dibasic calcium phosphate, 25.74% sodium chloride, 25.74% dibasic sodium phosphate, 0.26% zinc chloride, 0.26% manganous sulfate monohydrate, 1.27% ferrous sulfate heptahydrate, 0.26% cupric sulfate pentahydrate, and 0.13% sodium iodide. Control diet, A + Mg, was prepared by adding 345 mg of magnesium chloride hexahydrate to 100 g of magnesium deficient Diet A.

Magnesium-deficient Diet B was that used by Bois et al. (Ref. 2; personal communication) and consisted of 58.7% dextrose monohydrate, 23.0% vitamin-free casein, 5.0% gelatin, 0.3% DL-methionine, 5.0% corn oil, 2.0% vitamin mixture (Nutritional Biochemicals), and 6.0% salt mixture. The salt mixture contained the following anhydrous salts: 45.0% calcium carbonate, 18.0% dibasic potassium phosphate, 12.2% monobasic calcium phosphate, 20.0% sodium chloride; 4.0% ferric citrate; 0.6% manganous sulfate; 0.035% zinc carbonate; 0.13% potassium iodide; and 0.005% cupric sulfate pentahydrate. Control diet, B + Mg, was prepared by adding 120 mg of magnesium as the sulfate to 100 g of magnesium-deficient Diet B. The amounts of some components of the diets are shown in Table 1.

Animals were fed ad libitum in all experiments except...
Experiments la and lb in which controls were restricted to the dietary intake of the magnesium-deficient animals. Magnesium (10 mg) was given as the sulfate i.m. in 1 ml of 0.9% NaCl solution in a single weekly injection to some of the animals fed the magnesium-deficient Diets A and B in Experiments la and lb. All animals were weighed weekly.

The times of sacrifice of animals are shown in Table 2. Following death or at sacrifice every animal was carefully dissected and examined and its organs were weighed. Samples of tissue were fixed in phosphate-buffered formalin, and sections were prepared and stained with hematoxylin and eosin occasionally by the Giemsa or von Kossa technique. In selected cases small pieces of tissue were fixed with Karnovsky solution (14) for 2 hr, rinsed in collidine buffer for 30 min postfixed in collidine-buffered 1% osmium tetroxide for 1 hr, dehydrated in graded alcohols followed by propylene oxide, and embedded in Maraglas Dow epoxy resin 732 (8). Thin sections were stained with uranyl acetate followed by lead citrate and examined in a Siemens-Elmiskop 101 electron microscope. For orientation purposes 0.5-μm-thick sections were stained with toluidine blue.

At sacrifice, blood was collected from the dorsal aorta of ether-anesthetized animals into heparinized syringes. Plasma was stored at 4° until used for chemical analysis. White cells were counted in blood obtained from the tip of the tail of randomly selected animals after dilution with 3% acetic acid. The hematocrit was determined by a micro-method using heparinized blood. Bone marrow and blood smears were made from some sacrificed animals.

Calcium and magnesium were determined in plasma by atomic absorption spectrophotometry (21), P1 by the method of Sumner (26), and urea nitrogen by the micro-method of Kaplan (13).

A suspension of 2 × 10⁶ cells was prepared from 1 enlarged thymus in Saline A solution (22) and injected i.v. into each of five 1-day-old noninbred Wistar rats. These were sacrificed after 1 year and examined for evidence of tumor. A similar cell suspension from a 2nd enlarged thymus was injected s.c. into each of 13 one-day-old noninbred CD rats (Charles River Breeding Laboratory, Wilmington, Mass.), which were sacrificed after 7 months and examined.

RESULTS

Rats fed the magnesium-deficient Diets A and B showed characteristic signs of magnesium depletion (16, 17). There were marked differences in survival rate between the groups on the 2 deficient diets, although the magnesium content was similar (Table 1). Death occurred earlier in the animals fed Diet B and none developed an enlarged thymus. The few surviving animals on Diet B were sacrificed at 31 to 39 days; therefore, only limited discussion of the results obtained with these is given. In contrast, many of the rats on the deficient Diet A survived to the termination of the experiments and provide data for this study.

Gross Anatomy. The thymuses of animals fed the magnesium-deficient Diet A fell into 1 of 2 classes on a weight basis. They were either grossly enlarged or much smaller than those of control animals. A gland was considered to be enlarged if it was >0.3% of the body weight; this represented an increase greater than 2 S.D. above the corresponding mean weight for the thymuses from control animals.

Enlarged thymuses were usually discrete but in some cases had become attached to the chest wall anteriorly. Frequently, an enlarged thymus completely enveloped the trachea. Clear or turbid fluid was present in the thoracic cavity of a small percentage of the animals; in most cases the only thoracic abnormality detected was in the thymus.

Splenomegaly was observed in all animals receiving either of the magnesium-deficient diets. The i.m. magnesium given to the 2 groups in Experiment 1 did not prevent this. Mean weights ± S. E. of the spleen (expressed as a percentage of the animal body weight) of animals receiving the Diets A were: in 21 magnesium-deficient, 0.389 ± 0.11; and in 6 controls, 0.18 ± 0.01. In the same experiment the heart, lungs, and kidneys of deficient animals weighed 31, 30, and 20%, respectively, more than those of controls. Only the magnesium-deficient Diet B produced an increase in liver weight.

Onset, Incidence, and Magnitude of Thymic Changes. Enlarged thymuses were first observed between the 38th and 60th day in various experiments (Table 2) in animals that died or were sacrificed while on Diet A. Enlarged thymuses were not seen in any control animal nor did they occur in rats on the deficient diets that were given small amounts of i.m. magnesium in Experiment 1 or in those fed the magnesium-deficient Diet B for the short duration of Experiment lb. Decreased thymus size was observed in other magnesium-deficient rats as early as 33 days and persisted for the duration of the individual experiments.

The incidence of thymic enlargement in magnesium-depleted younger rats was calculated from the time at which enlargement was first detected to the termination of a given experiment. It ranged from 18 to 51.8% in the various experiments (Table 2). The incidence among older animals in Experiment 3b was 16.6% compared with an incidence of 33.3% in younger animals (Experiment 3a) studied at the same time. Forty-six (23.8%) of the 193 animals entered in the combined experiments with Diet A had an enlarged thymus.

Enlarged thymuses ranged in weight from 0.69 to 9.2 g. Where thymus size was decreased in magnesium-depleted animals, the mean value was approximately 80% of the

<table>
<thead>
<tr>
<th>Component</th>
<th>% of diet</th>
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<tr>
<td>Magnesium*</td>
<td>0.001 0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.14 1.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>62 51</td>
</tr>
<tr>
<td>Protein</td>
<td>18 28</td>
</tr>
<tr>
<td>Fat</td>
<td>12 6</td>
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*The magnesium content of the control diets was: A+Mg, 0.04%; and B+Mg, 0.13%. Each batch of diet was analyzed for calcium and magnesium and the results showed little variation between batches.
Incidence of thymus enlargement

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Day of detection of 1st large thymus - Day 0</th>
<th>Period of sacrifice (days of deficiency)</th>
<th>No. of large thymuses (X)</th>
<th>No. of animals examined after Day 0 (Y)</th>
<th>X/Y (%)</th>
<th>No. of animals in experiment</th>
<th>No. of control animals</th>
<th>X/Z (%)</th>
</tr>
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<tbody>
<tr>
<td>1a</td>
<td>44</td>
<td>33-74</td>
<td>7</td>
<td>21</td>
<td>33.3</td>
<td>15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>31-39</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>43-80</td>
<td>12</td>
<td>67</td>
<td>18</td>
<td>9</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>40</td>
<td>60-61</td>
<td>9</td>
<td>27</td>
<td>33.3</td>
<td>5</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>60</td>
<td>61-80</td>
<td>4</td>
<td>24</td>
<td>16.7</td>
<td>6</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>52-66</td>
<td>14</td>
<td>27</td>
<td>51.8</td>
<td>6</td>
<td>46.7</td>
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</table>

a Forty-six (23.8%) of the 193 animals started on Diet A developed large thymuses.

b Animals were pair-fed in this experiment.

c Older animals were used in this experiment.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of thymuses examined</th>
<th>Diet</th>
<th>No. of enlarged thymuses</th>
<th>No. of thymuses with grossly abnormal histology</th>
</tr>
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<tr>
<td>1a</td>
<td>34</td>
<td>Mg-deficient A</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Mg-deficient A + i.m. Mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>A + Mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>Mg-deficient A</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>A + Mg</td>
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<td>0</td>
</tr>
<tr>
<td>3 (a + b)</td>
<td>53</td>
<td>Mg-deficient A</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>A + Mg</td>
<td>0</td>
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by electron microscopy. The glands from control rats had lymphocytes with relatively large nucleus to cytoplasm ratios. Their nuclei contained abundant chromatin, much of which was margined and often contained a small nucleolus (Fig. 5). The cytoplasm contained very few mitochondria, little endoplasmic reticulum, and relatively few ribosomes (Fig. 5, inset). Large lymphocytes were scattered throughout the sections. These latter cells had larger nuclei and slightly larger nucleoli than the smaller lymphocytes; chromatin was less condensed and the cytoplasm of these cells contained numerous polyribosomes. Scattered dark reticular cells were also found; these spindle-shaped cells were smaller and more electron dense than the other cells. Their nuclei were of moderate size, often elongated, and contained areas of heterochromatin and fairly well-developed nucleoli; the cytoplasm was irregular in outline and long slender processes extended between other cells (Fig. 5). Very few Hassall’s corpuscles were noted.

The larger altered lymphocytes in the glands from magnesium-depleted rats had a smaller nucleus to cytoplasm ratio than the usual lymphocyte, and the cytoplasm was characterized by large numbers of polyribosomes with few endoplasmic reticulum profiles (Fig. 6). The nuclei contained dispersed chromatin and the prominent nucleoli often showed evidence of margination; some of the nuclei were irregular in outline. Scattered mitotic figures were evident and dark reticular cells were more numerous than in the control animals (Figs. 6 and 7). In glands that were grossly enlarged, the typical round or oval shape of the lymphocytes was lost and the lymphocytes were elongated. These cells often showed sharp angulation, which appeared to be associated with the presence of bundles of collagen adjacent to the cells. These glands also had increased numbers of pleomorphic cells with oddly shaped nuclei and abundant cytoplasm (Fig. 8).

Changes in Plasma or Blood. Plasma magnesium was markedly depressed in all animals fed the magnesium-deficient Diets A or B. Animals receiving supplemental magnesium injections that were sacrificed 3 to 7 days following the last injection also had very low plasma magnesium levels. Plasma calcium in the magnesium-depleted animals was variably normal or slightly elevated, and plasma inorganic phosphate was variably normal or slightly lower than in controls.

All animals fed the magnesium-deficient Diet B, including those receiving i.m. magnesium, had elevated plasma urea nitrogen levels. Six of the 9 control animals on the B + Mg diet sacrificed at 31 to 39 days were azotemic; the remainder maintained on this diet for 75 days had normal plasma urea levels. Magnesium-deficient Diet A had no significant effect on plasma urea levels at any time.

The hematocrit measured in Experiment 2 was slightly but significantly reduced in magnesium-depleted animals. Total white cell count (Experiment 1) showed a peak of elevation 5 times the count for control animals 5 days after the onset of vasodilation and erythema of the extremities in all animals fed the magnesium-deficient Diets A or B. The white cell count thereafter gradually fell to a value approximately 1.5 to 2.0 times the control values and remained fairly constant for the following 20 to 30 days. At 55 to 70 days on the magnesium-deficient Diet A, the white cell count was normal. Supplemental magnesium did not alter the pattern of changes in white cell count in animals fed the magnesium-deficient diets, but cessation of the i.m. magnesium injections on the 65th day was followed by an abrupt increase in white cell count.

Changes in the Kidney. Von Kossa staining of sections of kidney from animals fed the magnesium-deficient Diet B showed heavy deposits of calcium salts. Kidney calcification was not detected in sections of kidney from animals fed the magnesium-deficient Diet A.

Attempts to Transfer “Tumors.” None of 5 rats given i.v. injections of a cell preparation from an abnormal thymus showed any signs of tumor when sacrificed 1 year after having subsisted on a stock diet. No gross abnormality was found in any of the 13 animals given the s.c. injection of the 2nd cell preparation when these were sacrificed after 7 months on a stock diet.

DISCUSSION

Symptoms and abnormalities in the blood and in spleen size of the magnesium-depleted rats reported here are consistent with those reported by others (1, 9, 15–18, 30). Since the spontaneous occurrence of thymic lymphoma in rats is very unusual (7, 23), the occurrence of thymic enlargement as a consequence of magnesium depletion is significant. Data presented here confirm the finding of thymic enlargement following deprivation of magnesium for 6 or more weeks. The severity of magnesium depletion appears to be important in the development of the thymic abnormality since i.m. injections of small amounts of magnesium prevented its occurrence; the incidence of thymic enlargement was decreased also when older rats were fed the diet.

The gross appearance of the enlarged thymuses resembled that described by the earlier workers, but larger glands noted with Diet A were appreciably heavier than those reported. However, a fundamental difference between the work described here and that of the earlier authors is our failure to demonstrate either the presence of metastases in any animal with an enlarged thymus or to transfer tumor to normal rats.

A predominance of large lymphocytes or lymphoblasts (5) and increased mitotic figures (5, 11, 12) have been reported. The presence of numerous ribosomes in enlarged lymphocytes noted here is consistent with the observation by Bois et al. (6), but we failed to see any structures resembling viruses reported by them.

The resemblance of the predominant cells in the affected thymus of our study to lymphocytes antigenically stimulated in vitro (20, 27) is based upon the common properties of a decreased nucleus to cytoplasm ratio, large accumulation of polyribosomes in the cytoplasm, transformation of nuclear heterochromatin into euchromatin, and prominent...
nucleoli that were frequently seen in contact with nuclear envelopes. However, in humans similar cells may be seen in both hyperplastic and neoplastic lymph nodes (P. H. Lieberman, personal observation). The significance of the presence of dark reticular cells in the thymus is not known (10) but may reflect degenerative changes as suggested by Mollo et al. (19). In the absence of objective evidence of cancer, we prefer to adopt a conservative attitude and presently regard the lesion that we have observed as hyperplastic.

The sequence of events by which magnesium deficiency in the rat causes the changes described is unknown. Furthermore, the present work suggests that 2 distinct populations of abnormal thymuses exist among the magnesium-depleted rats. The very small thymuses seen in some deficient animals may represent a further stage in thymic alteration. The abnormalities may be related to some immunological change in the thymocytes leading to transformation and division of cells with consequent enlargement of the gland and loss of normal architecture. The possibility of reversibility of the altered enlarged thymus toward normal when magnesium is restored to the diet is currently under investigation.

ACKNOWLEDGMENTS

We wish to express indebtedness to the technical staff of the Metabolic and Nutrition Laboratory for assistance with animal care and technical procedures during the course of this work and to Dr. Chester Southam for the i.v. injection of thymic cells.

REFERENCES

Fig. 1. Section of control thymus gland. H & E, × 400.

Fig. 2. Section of very large thymus gland. H & E, × 400.

Fig. 3. Section of partially altered thymus gland from a magnesium-deficient rat. One lobule shows normal-appearing lymphocytes; the other lobule shows the altered lymphocytes referred to in the text. H & E, × 250.

Fig. 4. Hyperplastic lymphoid tissue from an enlarged thymus gland which is infiltrating muscle adjacent to the gland. H & E, × 250.

Fig. 5. Low magnification electron micrograph of a normal rat thymus gland. Most of the cells are typical small lymphocytes, but scattered medium and large lymphocytes are also seen. Long cytoplasmic processes from dark reticular cells extend between the lymphocytic cells (arrow). Inset, polysomes are not abundant in the sparse cytoplasm in these lymphocytes. Fig. 5, × 3,800; inset, × 21,600.

Fig. 6. Electron micrograph of an enlarged thymus gland from a magnesium-deficient rat. In contrast to the control glands, most of the cells are large altered lymphocytes as referred to in the text. Note the large nucleoli, dispersed chromatin, and abundant cytoplasm. Dark reticular cells are prominent. A dividing lymphocyte can also be seen (lower right). Inset, numerous polysomes are evident in these large cells at high magnification. Fig. 6, × 3,800; inset, × 21,600.

Fig. 7. A cluster of predominantly altered lymphocytes and dark reticular cells in a thymus from a magnesium-deficient rat. × 5,800.

Fig. 8. Typical cells found in a very large thymus from a magnesium-deficient animal. In addition to the altered lymphocytes, pleomorphic cells with a patchy (marginated) chromatin pattern are also seen. Note the scattered areas of fibrosis. × 5,800.
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