The Influence of Dietary Pyridoxine on Mortality Rate of the Magnesium Deficient Rat

BERNARD C. KORBITZ

University of Wisconsin Medical Center, 1300 University Avenue,
Division of Clinical Oncology, Madison, Wisconsin 53706, U.S.A.

(Received November 2, 1969)

The magnesium deficiency syndrome in white rats can be aggravated by the addition of high levels of pyridoxine hydrochloride to the diet. In these studies, the mortality rate of magnesium deficient rats varied in direct proportion to the dietary concentration of pyridoxine hydrochloride. This effect was not produced by increasing the thiamine hydrochloride content of the diet. The present studies did not demonstrate a consistent difference in serum magnesium concentration between the magnesium deficient rats receiving a normal pyridoxine supplement and those receiving a high pyridoxine supplement.

In the course of studying tryptophan metabolism in magnesium deficient rats, we noted that the magnesium deficiency state seemed to be accelerated in rats receiving higher than normal levels of pyridoxine in the diet. In these metabolic studies, animals which received 20 times the normal pyridoxine level in their diet had a definitely higher mortality rate than the magnesium deficient animals maintained on a normal pyridoxine intake. The present study was undertaken to more accurately corroborate the earlier observations and also to determine whether or not the enhanced mortality rate of the animals on a high pyridoxine diet could be correlated with a lower serum magnesium level.

MATERIALS AND METHODS

1. Animals

Male and female albino rats weighing 40 to 45 g were utilized in these studies. Only female rats were utilized for the mortality studies, whereas both male and female rats were used in the serum magnesium studies. The rats were housed in steel wire bottom cages in groups of 10, were fed ad libitum and received distilled water to drink.

2. Diet

A 20% casein synthetic diet was prepared which contained no added magnesium or pyridoxine (Table 1). The alcohol extracted casein, inositol, choline chloride, cystine and vitamins were purchased from Nutritional Biochemical Corporation. The mineral salts were of analytical reagent grade purity. The cerelose and corn oil were commercially available. Three diets were then prepared from the stock diet, (a) no magnesium-no pyridoxine; (b) no magnesium-normal pyridoxine, containing 0.025 mg of pyridoxine hydrochloride per 100 g of diet; and (c) no magnesium-high pyridoxine, containing 50 mg of pyridoxine hydrochloride per 100 g of diet. This concentration of pyridoxine hydrochloride is 200 times the normal dietary level.

3. Mortality Studies

Ten female rats per dietary group were maintained on the respective diets for periods of 4 to 5 weeks. The cages were checked several times daily to accurately record mortality and to prevent cannibalism. The animals were weighed every two days and the average weight per rat determined for each dietary group. The magnesium deficient rats all manifested signs and symptoms of magnesium deficiency by the 14th day of the study. These manifestations included intense vasodilation of the ears, tail, and paws, in-
creased neuromuscular irritability and occasional convulsions. The frequency of convulsions increased markedly after the 14th day of the diet and animals often expired after a prolonged convulsive seizure. The mortality studies were duplicated with two separate shipments of rats. Day 17 and 33 were arbitrarily chosen as the reference dates for calculating average mortality rates.

4. Collection of Blood for Serum Magnesium Studies

Male and female rats which had been maintained on the three synthetic diets for periods of one or two weeks were utilized. These animals were maintained separately from those animals used in the mortality studies. Five of the rats from each group were anesthetized with ether, blood obtained by cardiac puncture with a hypodermic needle and syringe and the rats then sacrificed. Blood was allowed to clot and the serum from all rats in a given dietary group pooled.

5. Serum Magnesium Analyses

The Titan Yellow colorimetric method as reported by the Brooke Army Medical Center was used (1). This is a modification of the method of Orange and Rhein (2).

RESULTS

The effect of varied levels of dietary pyridoxine on the mortality rate of magnesium deficient rats is shown in Fig. 1. It is evident that the mortality rate bears a directly proportional relationship to the dietary concentration of pyridoxine hydrochloride. There was essentially no difference in the growth rate of the normal pyridoxine group versus the high pyridoxine group. Animals which were deficient in both magnesium and pyridoxine had a rather marked inhibition of growth throughout the course of the experiment. A wide variation was encountered between serum magnesium values of the various groups within a given experiment and between a given dietary group from one experiment to the other. Serum magnesium levels of magnesium deficient rats decreased rapidly within two weeks as expected but there was no consistent difference in the serum magnesium levels of magnesium deficient rats receiving a normal pyridoxine intake compared to those receiving high levels of pyridoxine.

![FIG. 1 Mortality rate of magnesium deficient rats on days 17 and 33 of study.](image)

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Composition of basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Content (per cent)</td>
</tr>
<tr>
<td>Casein, vitamin-test</td>
<td>20</td>
</tr>
<tr>
<td>Glucose</td>
<td>70</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
</tr>
<tr>
<td>Salts</td>
<td>4.2</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a Vitamin-Test Casein, Nutritional Biochemicals Corporation, Cleveland, Ohio.
b Providing in g/kg diet: CaCO₃, 13; CaHPO₄·2H₂O, 3; KH₂PO₄, 13; NaCl, 7; Fe citrate·6H₂O, 1; MnSO₄·2H₂O, 0.2; KI, 0.5; ZnCl₂, 0.012; and CuSO₄·5H₂O, 0.012.
c Supplies in mg/kg diet: inositol, 100; β-carotene, 0.8; calciferol, 0.0005; Ca pantothenate, 20; niacinamide, 10; biotin, 0.1; 2-methylpropylthoquinone, 8; α-tocopherol acetate, 100; thiamine·HCl, 2; folic acid, 0.2; and riboflavin, 3.

DISCUSSION

These studies firmly corroborated our earlier observations and permitted the demonstration of a definite relationship between dietary pyridoxine concentration and mortality rate of magnesium deficient rats. A correlation between mortality rate of a given dietary group and average serum magnesium levels could not be made. The mortality studies
verified the earlier observations but contributed little to the understanding of the mode of action involved. Since the average weights of the normal pyridoxine and high pyridoxine groups were nearly identical throughout the study, it cannot be argued that the increased mortality rate was due to an obligatory increase in magnesium requirements secondary to increased growth. It is possible, though, that the low mortality rate of the rats deficient in both magnesium and pyridoxine, is due to decreased magnesium requirements secondary to arrested growth. To further delineate the relationship of growth to the development of magnesium deficiency and also to determine whether other vitamins could aggravate the magnesium deficiency syndrome, an additional study was conducted. Mortality studies were repeated on the dietary groups previously mentioned but in addition a group deficient in both magnesium and thiamine and a magnesium deficient group receiving a high level of thiamine (200 times normal) were studied. This concentration of thiamine hydrochloride was 4 mg per 100 g of stock diet. In this study the high thiamine supplement had no effect either in increasing or decreasing the mortality rate of the magnesium deficient rats. The rats which were deficient in both magnesium and thiamine had a decreased mortality rate and decreased growth rate nearly identical to the group deficient in both pyridoxine and magnesium. It is concluded, therefore, that the increased mortality of the magnesium deficient-high pyridoxine dietary group, was due to a specific effect of pyridoxine, possibly mediated via an increased requirement for magnesium in the activation of the pyridoxal linked enzyme systems. In reconciling these findings with the tryptophan metabolism studies in magnesium deficient rats (3) we arrive at the following hypothesis. Many cations including Mg\(^{2+}\), Mn\(^{2+}\) and Zn\(^{2+}\) are capable of activating pyridoxal phosphate linked enzyme systems and a deficiency of one “essential” cation will not necessarily result in de-activation of the enzyme system. A high dietary pyridoxine level, however, appears to increase the demand for all “essential” activator cations. If a given cation is already deficient, the high pyridoxine load augments the deficiency state; in this case the mortality rate due to magnesium deficiency was accelerated.

The decreased mortality observed in the rats deficient in both magnesium and pyridoxine was probably due to decreased magnesium requirements secondary to the arrested growth of the vitamin deficient animals. This supposition receives support from the observation that thiamine deficiency results in a similar decrease in both growth and mortality rate of magnesium deficient rats.

Acknowledgement

This investigation was supported by the Public Health Service Research Grant No. CA 06749 from the National Cancer Institute.

Bibliography

1. Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston, Texas (1953).