Tryptophan Metabolism in the Magnesium Deficient Rat

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Quantitative determinations of urinary metabolites of tryptophan were conducted in weanling white rats which had been maintained on a synthetic diet completely free of magnesium. No significant differences could be determined in the pattern of urinary metabolites of tryptophan in the magnesium deficient animals as compared to rats maintained on a synthetic controlled diet. Although magnesium is capable of catalyzing pyridoxal phosphate enzyme systems, the present study suggests that there is a "latitude" in metal requirements by these enzyme systems and that several other cations such as manganese or zinc may function to activate the system when magnesium is absent. It is concluded that deficiency of magnesium per se does not significantly affect tryptophan metabolism.

The role of metal ions in tryptophan and pyridoxine metabolism was initially demonstrated by Jakoby and Bonner (1) who in 1953 reported the activation of Neurospora kynureninase by Mg$^{2+}$ ions. Yanofsky (2) also made the interesting observation that the inhibition of D-serine dehydrase by Zn$^{2+}$ or Co$^{2+}$ ions could be partially overcome by Mg$^{2+}$ ions. These studies therefore indicated that pyridoxal phosphate-requiring enzymes could be both activated and inhibited by various cations. In 1954 Metzler, Ikawa and Snell (3) proposed that pyridoxal phosphate functions as a complex with a substrate and a metal ion. Similarly, McCormick, Gregory and Snell (4) have demonstrated that purified pyridoxal kinase from various sources required metal ions for activation. Mg$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ ions activated the kinase, while Cu$^{2+}$ and Hg$^{2+}$ ions were found to be inhibitory.

In addition to the above evidence of definite effect of metal ions on pyridoxine metabolism, it appears that pyridoxine affects metal metabolism. Gubler, Cartwright and Wintrobe (5) in 1949 reported that intestinal absorption of iron was significantly increased in pyridoxine deficient rats and total body iron and copper values in these deficient animals were higher than controls. Hsu and Kawin (6) have shown that pyridoxine deficiency in rats produced abnormal radioactive manganese uptake by various organs. Gershoff and Andrus (7) have delineated a more definite magnesium-pyridoxine relationship to tryptophan metabolism. While studying renal calculi formation in pyridoxine deficient rats, these investigators noted that the rats had elevated urinary levels of oxalic acid and xanthurenic acid while urinary citrate was decreased. The administration of high levels of dietary magnesium to the pyridoxine deficient animal caused citrate levels to return to normal and brought about a significant decrease in the amount of xanthurenic acid.

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excreted. It is therefore evident that a relationship exists between pyridoxal phosphate formation and activation, tryptophan metabolism and the metabolism of a number of metals, including magnesium in microorganisms as well as experimental animals. The purpose of this study was to study the effects of magnesium deficiency on tryptophan metabolism in the albino rat.

**Material and Methods**

1. Animals

Female albino rats weighing 40 to 45 g were purchased from the Holtzman Company of Madison, Wisconsin. The animals were housed in steel wire cages in groups of 5, and 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). Three experimental diets were then prepared by adding varying levels of magnesium and/or pyridoxine to the magnesium and pyridoxine deficient synthetic diet. The control diet was prepared by adding 52 mg of magnesium in the form of MgSO$_4$·7H$_2$O and 0.25 mg of pyridoxine hydrochloride per 100 g of stock diet. A magnesium deficient normal pyridoxine group diet was prepared by adding 0.25 mg of pyridoxine to the magnesium deficient stock diet and a no magnesium-high pyridoxine group diet was prepared by adding 5 mg of pyridoxine hydrochloride per 100 g of the magnesium deficient stock diet. The alcohol extracted casein, inositol, choline, cystine and the vitamins were purchased from Nutritional Biochemical Corporation. The mineral salts utilized were of analytical reagent grade purity. The cerelose and corn oil were commercially available.

3. Urine Collections

Five rats per group were maintained on the 3 respective diets for 14 days, then placed in stainless steel metabolism cages in groups of 5. 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, increased neuromuscular irritability and occasional convulsions. Urine was collected in Erlenmeyer flasks under toluene. A basal 24 hour pooled urine sample was obtained for each group. Each rat then received an intraperitoneal injection of 40 mg of L-tryptophan in aqueous solution and again a 24 hour pooled urine sample was obtained from each group. The rats were then continued on their respective diets for one additional week after which time they were again returned to the metabolism cages and the above urine collections carried out prior to and after the second intraperitoneal injection of 40 mg of L-tryptophan. These studies were then duplicated with a second shipment of rats.

**Analytical Methods**

**Diazotizable amines** — Indoxyl sulfate, anthranilic acid glucuronide, *O*-aminohippuric acid, acetylkynurenine, anthranilic acid and kynurenine were determined by the previously reported method of Brown and Price (8).

**Hydroxykynurenine** — This compound was determined by the method of Brown (9).

**Kynurenic acid and xanthurenic acid** — The fluorometric determination of kynurenic and xanthurenic acids was carried out according to the method of Satoh and Price (10). *N*-methyl-2-pyridone-5-carboxamide was not determined, since an earlier study by Brown and Price (8) had shown it to be a trace metabolite in rats.

**Results**

The quantities of the various urinary
TABLE 2

Summary of the average values of urinary tryptophan metabolites from 4 experiments

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>No magnesium Normal pyridoxine</th>
<th>No magnesium High pyridoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Basal</td>
<td>Post</td>
</tr>
<tr>
<td>Indoxyl-sulfate</td>
<td>3.3±1.3</td>
<td>0.2±0.1</td>
<td>2.3±2.3</td>
</tr>
<tr>
<td>Anthranilic acid glucuronide</td>
<td>3.1±1.2</td>
<td>0.2±0.1</td>
<td>2.3±2.3</td>
</tr>
<tr>
<td>o-Aminoisopropionic acid</td>
<td>0.1±1.1</td>
<td>0.2±0.1</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>Acetylkynurenine and anthranilic acid</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>1.3±1.3</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Hydroxykynurenic acid</td>
<td>1.5±0.7</td>
<td>2.3±2.4</td>
<td>2.3±1.4</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>0.4±0.3</td>
<td>0.4±0.3</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
<td>0.2±0.2</td>
</tr>
</tbody>
</table>

* Standard deviations shown after each average value.

metabolites of tryptophan are tabulated in Table 2 and arranged according to dietary group. These values are all expressed as micromoles of metabolite per rat per day and represent data obtained from duplicate experimental groups after 2 and 3 weeks on the respective diets. Since within any given dietary group there was no consistent difference in the urinary excretion of metabolites at 2 weeks compared to 3 weeks, these values were considered as duplicate experiments. The value for each metabolite in Table 2, therefore, represent the average of 4 values. It is readily obvious from Table 2 that magnesium deficiency per se had no appreciable effect on the tryptophan metabolites studied. It was noted, however, that the magnesium deficient rats receiving the high pyridoxine supplement, had a higher mortality rate than the animals on a regular pyridoxine supplement.

**DISCUSSION**

The failure to demonstrate an appreciable effect of magnesium deficiency on tryptophan metabolism in this study raises a number of highly speculative questions regarding metal activation of pyridoxal phosphate enzyme systems concerned with tryptophan metabolism. It might be argued that the results of this study indicate that in the rat, magnesium is not involved in the activation of pyridoxal kinase and pyridoxal phosphate-containing enzymes and that some other cation is involved. Since Mg$^{2+}$ is one of the major cations of the body and is known to activate a wide variety of enzymatic reactions *in vitro*, it is difficult to believe that this cation does not play a role in the metabolism of pyridoxine and tryptophan. Furthermore, the work of Jakoby and Bonner (1) as well as that of McCormick, Gregory and Snell (4), has definitely established the fact that magnesium activates a number of these enzymes. The demonstration that pyridoxal kinase can be activated by several cations including Mg$^{2+}$ provides support for the view that possibly the pyridoxal phosphate-containing enzyme systems involved in tryptophan metabolism can similarly be activated by several different cations (4). Snell (11) has recently commented on this "latitude" in metal requirements by enzyme systems and he concluded that it may be meaningless to inquire which cation is the "essential" cation. This concept of cation activation of "multi-receptive" enzyme systems seems to be a reasonable approach to reconciling these experimental findings with the pre-existing data. One can, therefore, interpret the results of this study in the following way. Mg$^{2+}$ may be one of only several cations which are capable of activating pyridoxal kinase and pyridoxal phosphate-containing enzyme systems in the rat. In experimental magnesium deficiency no abnormality in tryptophan metabolism was observed because other "activator" cations such as Mn$^{2+}$ or Zn$^{2+}$ were
still present in normal concentrations. According to this hypothesis, even when the "preferred" cation is deficient, less active cations are capable of successful enzyme activation.

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REFERENCES