HISTOLOGICAL INVESTIGATIONS ON THE LIVER-DISTURBING ACTION OF 2-METHYL-4-AMINO-5-HYDROXYMETHYLPYRIMIDINE

YOSHIITO NISHIZAWA, TEISUKE KODAMA AND TOKUZO KOOKA

Department of Pediatrics, Faculty of Medicine,
Osaka University, Fukushima, Osaka

(Received October 15, 1957)

Abderhalden (1) injected the pyrimidine and thiazole moieties of thiamine in mice and rats with the object of investigating the biosynthesis of thiamine and observed the induction of running fits. Its cause was further proved to be the pyrimidine moiety, i.e., 2-methyl-4-amino-5-hydroxymethylpyrimidine (OMP). Makino and Sasaki (2) reported the induction of running fits after injecting this compound in rats maintained on a watersoluble vitamin-deficient diet (DK diet), and named this compound “toxopyrimidine”. Later, the Vitamin B Research Committee agreed to call it OMP instead of toxopyrimidine, which was to be used for a group of substances with similar pharmacological activities. Makino, together with Aramaki and Shintani (3) conducted studies on agents which would suppress the OMP-induced seizure in mice and found that vitamin $B_6$ had this activity. On the basis of their results, vitamin $B_6$ was called “atoxopyrimidine”. Shintani (4) also observed in experiments rats that long-term administration of OMP resulted in a cessation of growth with characteristic vitamin $B_6$-deficient symptoms, which disappeared after administration of vitamin $B_6$. Torigoe et al. (5) observed that the administration of OMP daily to mice with the basal diet resulted in a sharp loss of weight leading to death. Arai (6) and Shintani (4) found urinary excretion of xanthurenic acid in mice and rats after administration of OMP in tryptophan-loaded animals. Tani (7) failed to prevent the urinary excretion of xanthurenic acid by simply administering vitamin $B_6$ alone but could completely prevent the excretion of the acid by giving vitamin $B_6$ together with methionine. The disturbance in tryptophan metabolism suggests a liver-disturbing action of OMP and the effect of methionine is assumed to substantiate the view. The investigations on OMP which have hitherto been made, have dealt only with the running fits, the effect on the liver being entirely disregarded. The present study was conducted in an attempt to clarify the effect of OMP on the liver and the relationship of OMP to vitamin $B_6$. 

Studies on substances of the toxopyrimidine group. I.
EXPERIMENTAL

Methods

1. Animals Used.
   For histological studies, male mice of a pure strain, weighing 12—15 g, were
   used. For measurement of vitamin B₆ of the liver and brain, rats of a mixed
   strain, weighing 60—100 g, were used.

2. Substances Tested.
   In the former part of the study, OMP hydrochloride was used and in the
   latter part, the free base was used. The vitamin B₆ used was pyridoxine hy-
   drochloride ("Hexermin", Takeda). The methionine used was DL-methionine,
   Ishizu. N-Carbobenzyoxylglutamylcholine was "Glucin", Takeda.

3. Diets.
   The diets used are specified in each experiment.

   The animals were killed by decapitation 6 hours after feeding and 3 hours
   after injection of OMP.

5. Histological Preparation.
   The liver specimens were examined by hematoxylin-eosin (formalin-fixed, 6µ
   paraffin section), Heidenhains' iron hematoxylin (Colstar-fixed, 4µ paraffin sec-
   tion), methyl green-pyronine (alcohol-fixed, 6µ paraffin section), Best's staining
   (alcohol-fixed, 6µ paraffin section) and Sudan III (formalin-fixed, 10µ frozen
   section). Other organs were fixed in formalin, 6µ paraffin sections prepared
   and stained with hematoxylin-eosin.

6. Determination of the Vitamin B₆ Content of the Organs.
   Pyridoxal (PAL) and pyridoxine (PIN) plus pyridoxamine (PAM) were se-
   parately determined by the method of Fujita, Fujita and Fujino (8) and the sum
   was taken as the total vitamin B₆.

Results

1. Acute Intoxication Test.
   The liver of the mice in which running fits had been induced with OMP
   was examined histologically to ascertain the damage to the liver by OMP.

   Experimental Conditions — Mice were fed a millet and corn diet for 7 days,
   then given a water-soluble vitamin-deficient diet for 9 days, after which 0.2 mg/g
   OMP was injected subcutaneously. Running fits were induced in 1—2 hours
   in all of the 14 animals given OMP. After the occurrence of the seizure, the
   animals were sacrificed by decapitation and the liver examined histologically.

   Histological Findings — Severe congestion in the liver was observed in the
   majority of the cases. Little pathological change was noted in the picture but
degeneration of the mitochondria was apparent from the central part of the lob-
bules to the periphery: The mitochondria in the central part are distorted in
shape, stained poorly and, in severe cases, have disappeared. The mitochondria
in the periphery of the lobules are stained well but with clear pathological
changes. The degeneration appears to progress from the center. Glycogen is
markedly reduced, glycogen granules being scarcely observed. Fat increased
centrally or diffusely. These findings indicate clearly an acute intoxication picture.

2. Chronic Intoxication Test.

The effect on the liver of frequent administration of OMP in doses lower than that for inducing the running fit, was examined. The conditions of the experiment are described in Table I. Animals were divided into three groups,

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. OMP</td>
<td>Ordinary diet; 1.5 mg OMP once daily, 8 subcut. injections</td>
</tr>
<tr>
<td>2. OMP + Vitamin B₆ (A)</td>
<td>Ordinary diet; 0.3 mg vitamin B₆ a.m.; 1.5 mg OMP p.m., 8 subcut. injections</td>
</tr>
<tr>
<td>3. OMP + Vitamin B₆ (B)</td>
<td>Ordinary diet; 4.5 mg vitamin B₆ a.m.; 1.5 mg OMP p.m., 8 subcut. injections</td>
</tr>
<tr>
<td>4. Vitamin B₆-deficient (A)</td>
<td>No treatment; vitamin B₆-deficient diet, 10 days</td>
</tr>
<tr>
<td>5. Vitamin B₆-deficient (B)</td>
<td>No treatment; vitamin B₆-deficient diet, 30 days</td>
</tr>
</tbody>
</table>

Ordinary diet: Whole wheat flour, vegetable, fish meal.
Vitamin B₆-deficient diet (according to Aramaki): Sucrose 68.4, casein 22.0, peanut oil a 5.0, McCollum salt 4.0, choline hydrochloride 0.1, multivitamin without vitamin B₆ b 0.5 per cent.

a Vitamin A 2000 I.U., and vitamin D₃ 200 I.U. were added
b Consisting of thiamine 0.2, riboflavin 0.3, Ca pantothenate 2.0, niacin 2.0, vitamin K₃ 0.5, folic acid 0.5, inositol 10, and glucose 484.5 mg.

t.e., (a) an OMP group, (b) OMP plus vitamin B₆ group and (c) a vitamin B₆-deficient group. The histological changes in the liver were compared. Mortality was predominantly high in the OMP group, i.e., 11 of the 15 cases, but was negligible in other groups. The OMP and OMP plus vitamin B₆ groups were normal in outward appearance, whereas in the vitamin B₆-deficient group, the fur became rough from about the fifth day but the symptoms for vitamin B₆ deficiency, such as dermatitis, reddening and edema of the extremities, were not noted. The gain in body weight was less in the OMP and vitamin B₆-deficient groups than in the OMP plus vitamin B₆ group. Especially in the vitamin B₆-deficient group, a loss in weight became apparent from about the fifth day, coinciding with the appearance of the roughness of the fur (Fig. 1).

Histological Findings — With the hematoxylin-eosin staining, a moderate degree of congestion was found in the OMP group. A large number of small vacuoles were present in the cells in the central part of the lobules in this group (Fig. 2), whereas in the OMP plus vitamin B₆ group, the number of cells containing fairly large vacuoles increased (Fig. 3). In the vitamin B₆-deficient group, the cells containing the large vacuoles made up a large part of the lobule (Fig. 4).
FIGS. 1-4  Hematoxylin-Eosin Double Staining  (× 280)
FIGS. 5-8  Heidenhain’s Iron-Hematoxylin Staining  (× 720)
1, 5: normal group; 2, 5: OMP group; 3, 7: B₆ plus OMP group.
4, 8: B₆ deficiency group.
Figs. 9-12  Methyl Green-Pyronine Staining (x 720)
Figs. 13-16  Best's Staining

9, 13: normal group;  10, 14: OMP group;
11, 15: B6 plus OMP group;  12, 16: B6 deficiency group.
Degeneration of mitochondria was definitely observed in the OMP group: The mitochondria were stained poorly, reduced in number and disappeared almost completely in the center part of the lobule (Fig. 6), whereas in the periphery, they were irregular in size and form. In the parts with intensive pathological changes, mitochondria could hardly be observed in the whole section and the cell boundaries were indistinct. In the OMP plus vitamin B₆ group, some reduction in mitochondria was also found, but compared with the OMP group, the changes were slighter (Fig. 7): In the periphery of the lobules, the mitochondria were mostly almost normal. In the vitamin B₆-deficient group, mitochondria remained close to normal up to 10 days, but at 30 days the cells were swollen to about twice the normal size by formation of large vacuoles and the mitochondria were more affected as compared with 10 days (Fig. 8). The cell nuclei in the OMP group were irregular in size and shape; the nuclear membrane stained deeply, and the nucleolus was distorted and stained deeply (Fig. 6). The nuclei in the OMP plus vitamin B₆ group were grossly normal (Fig. 7). In the vitamin B₆-deficient group, a similar finding was observed as in the OMP group (Fig. 8).

Examination of the ribonucleic acid showed an irregular network with pyronine in the OMP and OMP plus vitamin B₆ groups but it was less in number in the OMP group, many cells having lost the pyronine-staining network or perinuclear staining (Figs. 10—11). In the vitamin B₆-deficient group, the pro-
toplasm is filled with a number of pyronine non-staining vacuoles so that the pyronine network was very coarse when examined on the 10th day, but greater in amount as compared with the other two groups. After 30 days of vitamin B₆ feeding, however, the ribonucleic acid content was somewhat reduced, forming a gross reticular structure in the intervaculolar spaces with greater granule formation (Fig. 12).

Glycogen is markedly reduced in the OMP group: Glycogen-containing cells are very few (Fig. 14). In the OMP plus vitamin B₆ group, glycogen is also reduced in amount, only a small quantity occurring in the periphery (Fig. 15). In the vitamin B₆ deficient group, on the other hand, glycogen granules exist diffusely in the lobules of the liver after 30 days, the cells were enlarged due to the excessive storage of glycogen (Fig. 16).

Fat is moderately deposited in the OMP group and the size of the fat particles is small (Fig. 18). In the OMP plus vitamin B₆ group, some fat particles are found in the central part of the lobule (Fig. 19), but in the vitamin B₆-deficient group, only a small quantity is observed in the periphery after 10 days. After 30 days, however, there is a light increase in fat granules; the increase is more marked in the periphery than in the central portion. The granules appear as particles of various sizes from minute to moderate, but the majority are moderately large (Fig. 20).

The above findings show that a disturbance in liver function is incurred by eight subcutaneous injections of 0.1 mg/g each of OMP in mice maintained on an ordinary diet. This disturbance can be partially, but not totally, inhibited by administering vitamin B₆ in a dose 1/5 that of OMP. The changes in the liver differ from those caused by vitamin B₆ deficiency. The dose of the vitamin B₆ used was based on the finding of Makino et al. (3) that the running fit induced by OMP could be prevented by the administration of pyridoxine in a dose 1/5—1/10 that of OMP. But raising the dose of vitamin B₆ indiscriminately is of no use, since the mouse dies with a dose of pyridoxine 5 times that of OMP. A dose 3 times that of OMP was tried, but no difference in result was noted as compared with the case in which pyridoxine 5 times that of OMP was used. Even a large dose of pyridoxine could not completely suppress the liver disturbance incurred by OMP. Feeding a vitamin B₆-deficient diet for 10 days, resulted in no detectable liver disturbance, but after feeding for 30 days, a slight liver disturbance was apparent.


The effect of liver-protecting agents, e.g., methionine and of N-carbobenzyloxy-glutamylcholine (Pro-Glc), whose lipotropic action was found in the laboratory of the authors (9), on the liver disturbance due to OMP was examined.

The prophylactic effect of methionine and Pro-Glc in acute OMP intoxication was compared with that of vitamin B₆. Table II shows the experimental conditions and the results. All three agents had no preventive effect on mortality, but with methionine and Pro-Glc, the survival time was prolonged. It is assumed to be a result of a prophylactic effect due to the accelerated detoxifying function of the liver. Preliminary administration of vitamin B₆ failed
to show a prophylactic effect, but concomitant administration of the vitamin resulted in a definite prevention (Table III).

**Table II**

*Effect of Liver-Protecting Agents on OMP-Induced Liver Disturbance (1)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage per mouse per day</th>
<th>Method of administration</th>
<th>OMP Mouse mg/g</th>
<th>Death after hr 0.5-1</th>
<th>1-3</th>
<th>3-9</th>
<th>9-15</th>
<th>15-24</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Water 0.3 ml</td>
<td>Intragastric 30 times</td>
<td>1.0</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>7</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>B6 group</td>
<td>PIN 0.3 mg (ca. 20 mg/kg)</td>
<td>Subcutaneous 30 times</td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>6</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Pro-Glc group</td>
<td>Pro-Glc 0.06 mg (ca. 4 mg/kg)</td>
<td>Intragastric 30 times</td>
<td>1.0</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Methionine group</td>
<td>dl-Met 3 mg (ca. 200 mg/kg)</td>
<td>Intragastric 30 times</td>
<td>1.0</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

The liver of the animals, in which running fits had been induced by OMP, was studied histologically. Aside from congestion, little pathological changes were observed with the hematoxylin-eosin stain in the OMP group. The chief change was in mitochondria; it was most severe in the OMP group, slightest in the vitamin B6 group, and intermediate in the methionine and Pro-Glc groups. The changes in the OMP group were the same as described above, *i.e.*, reduced stainability and decrease in number or disappearance of mitochondria from the central vein to the peripheral part of the lobule. The well-stained mitochon-
dria existing in the peripheral part were mostly pathological. The changes in other groups were similar but such a severe case as complete disappearance of mitochondria was not observed. Investigation of the ribonucleic acid showed that in the OMP group, there was a reduction in pyronine-staining substances and poor formation of granules in the central part of the lobule, whereas in the vitamin B₆ group, the pyronine-staining substances formed granules and were rich in the perinuclear region. In the methionine and Pro-Glc groups, pyronine granules are also found but not as abundantly as in the vitamin B₆ group. As for glycogen and fat, no significant differences were noted between the groups (Table IV).

**Table IV**

**Histological Findings in Liver**

<table>
<thead>
<tr>
<th>Individual</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, central; M, medial; P, peripheral (Also in Table VI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The effect of these liver-protecting agents on chronic OMP intoxication was studied. Methionine or Pro-Glc was first given orally for 20 days and from the 20th day, 1.5 mg of OMP (ca 0.1 mg/g) was administered. The prophylactic

**Table V**

**Effect of Liver-protecting Agents on Chronic OMP intoxication**

<table>
<thead>
<tr>
<th>Group</th>
<th>Method</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Vitamin B₆ + OMP</td>
<td>Ordinary diet; 4.5 mg B₆ a.m. 1.5 mg OMP p.m., 8 subcutaneous injections</td>
<td>0/12</td>
</tr>
<tr>
<td>2 Methionine + OMP</td>
<td>Ordinary diet; 3.0 mg methionine (200 mg/kg) for 20 days, then 1.5 mg OMP, 8 subcutaneous injections</td>
<td>1/11</td>
</tr>
<tr>
<td>3 Methionine + B₆ + OMP</td>
<td>Ordinary diet; 3.0 mg methionine for 20 days, then 4.5 mg B₆ a.m. and 1.5 mg OMP p.m., 8 subcutaneous injections</td>
<td>0/11</td>
</tr>
<tr>
<td>4 Pro-Glc + OMP</td>
<td>Ordinary diet; 0.06 mg Pro-Glc (4 mg/kg) for 20 days, then 1.5 mg OMP, 8 subcutaneous injections</td>
<td>3/13</td>
</tr>
<tr>
<td>5 Pro-Glc + B₆ + OMP</td>
<td>Ordinary diet; 0.05 mg Pro-Glc for 20 days, then 4.5 mg B₆ a.m. and 1.5 mg OMP p.m., 8 subcutaneous injections</td>
<td>1/11</td>
</tr>
</tbody>
</table>

Methionine and Pro-Glc were administered by gastric gavage with vinyl tube.
effect was compared with the group in which vitamin B₆ had been given concomitantly. The experimental conditions are shown in Table V.

There was neither apparent effect in any of the five groups nor significant difference in the growth curves. The difference in mortality following administration of OMP was also insignificant.

**Histological Findings** — No marked pathological change was found with the hematoxylin-eosin stain (Figs. 3, 5, 6). The pathological changes in nuclei were slight in all the groups. Stainability of mitochondria was reduced and a number of abnormal cells were present in all the groups. Each group could not be differentiated on the basis of mitochondrial picture (Figs. 9, 11, 12). The mitochondria in the methionine plus vitamin B₆ plus OMP group were closest to normal.

There was also little difference in the ribonucleic acid among the groups but in the methionine group, relatively homogenous granular ribonucleic acid was present and cells containing ribonucleic acid were found in the perinuclear region. (Figs. 15, 17, 19).

Glycogen was somewhat reduced in all the groups as compared with the normal control (Figs. 21, 23, 24). Fat staining revealed a slight to moderate deposition of small granules in all the groups (Figs. 27, 29, 30). The mortality rate suggested that not only vitamin B₆ but also methionine and Pro-Glc had some protective effect. It was also observed histologically that the liver disturbance due to OMP could not be completely suppressed by vitamin B₆, methionine or Pro-Glc. The concomitant administration of vitamin B₆ and methionine, however, appeared to have the greatest preventative effect. This finding explains the results obtained above that the concomitant administration of methionine and vitamin B₆ is required for preventing urinary excretion of xanthurenic acid.

The above results show that vitamin B₆ has the greatest preventative action against acute and chronic OMP intoxication but a certain degree of prevention is possible with other liver-protecting agents alone.
4. Minimum Liver-Disturbing Dose of OMP.

In order to find out the minimum liver-disturbing dose of OMP, animals were given 8—9 subcutaneous injections of 0.1, 0.08, 0.06, and 0.04 mg/g, respectively, of OMP\(^2\) and the liver was examined histologically. As can be noted in Fig 2, mortality seems to run parallel with the dosage.

**Histological Findings** — With the hematoxylin-eosin stain, the protoplasm of many cells is filled with large vacuoles so that the cells were enlarged. Liver congestion, derangement of hepatic cell cords or signs of cellular degeneration were, however, absent. The stainability of mitochondria was good in all the groups, but pathological changes were clearly seen in the group given 0.1

<table>
<thead>
<tr>
<th>Table VI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histological Findings in Liver</strong></td>
</tr>
<tr>
<td><strong>Damage of mitochondria</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Amount of OMP (mg/g)</td>
</tr>
<tr>
<td>No cholesterol was detected.</td>
</tr>
<tr>
<td>V, vacuole; M, mitochondrion.</td>
</tr>
</tbody>
</table>

\(^2\) The free base was used in all the succeeding experiments.
and 0.08 mg/g. The nuclei were grossly normal in all the groups. The ribonucleic acid was in the form of a coarse network due to large vacuoles, but no abnormal findings were present. Glycogen and fat were clearly increased and fatty degeneration was observed (Table VI).

As a constant change is found after 8 injections of 0.04 mg/g OMP, experiments were conducted with still smaller doses, i.e., 15 injections of 0.04, 0.004 or 0.0004 mg/g. Findings similar to the previous experiment were observed in the 0.04 mg/g group but in the latter two groups, the findings were normal. The minimum liver-disturbing dose, therefore, is assumed to be between 0.04 and 0.004 mg/g.

5. The Effect of OMP Injections on Pyridoxal and Pyridoxine plus Pyridoxamine of the Liver and Pyridoxal of the Brain.

Shintani (4) reported that long term administration of OMP in mice resulted in a decrease in weight and appearance of vitamin B₆ deficiency symptoms but he did not determine the vitamin B₆ content in these animals. Studies were therefore conducted on the changes in the vitamin B₆ content of the organs following long term administration of small doses of OMP. Daily 0.02 mg/g of OMP was given for 25 days to mice raised on an ordinary diet. When the appearance of the animal became poor or a loss in weight became apparent during the course of the experiment, the dose of OMP was reduced to one-half and if even this failed to improve the condition, the injections were stopped.

The appetite became poor in about one week but was recovered after about 3 weeks. In the first week of OMP treatment, the animals became violent and bit each other but from the second week, they became quiet and listless. Three of the animals showed erosion of the ears in the third week. The animals which died showed a roughening of the fur but characteristic signs of vitamin B₆ deficiency were not seen. Seven of the 24 animals died and the majority of these weighed less than 70 g at the beginning of the experiment. Most of them died 5—13 days after the start of injections. In the surviving animals, no increase in weight was observed up to 15 days, but a slight gain was observed thereafter. When the dosage was reduced to one-half, an improvement in outward appearance was usually noted within several days accompanied with a gain in weight. These findings suggest that the minimum disturbing dose of OMP is in the neighborhood of 0.02 mg/g.

The vitamin B₆ content of the brain and liver of these animals was then measured. Fig. 3 shows the results. The pyridoxine plus pyridoxamine of the liver was somewhat decreased as compared with the control, whereas the pyridoxal was definitely decreased so that the total vitamin B₆ content was decreased. Pyridoxal level of the brain, however, showed no change.

6. Effect of OMP on Other Organs.

Atrophy of the thymus and spleen was observed microscopically after OMP administration. Reduction of the red pulp of the spleen and decrease in megalocytes of the bone marrow were also noted but these were not found in the vitamin B₆ group. Intermediate changes were present in the methionine and Pro-Glc groups.

3 Wheat flour, fish meal and vegetables ad libitum.
DISCUSSION

The above results show that OMP has a liver-disturbing effect. It has already been shown by Shintani (4) and others that the administration of OMP results in urinary excretion of 4-pyridoxic acid and develops vitamin B₆ deficiency symptoms. The vitamin B₆ content of the organs following long term administration of OMP was determined by the authors and the reduction in the vitamin level was clearly demonstrated. Therefore it is easily conceivable that a slight liver disturbance due to OMP may resemble the pictures in vitamin B₆ deficiency. But the picture of OMP intoxication proper differs somewhat from that of vitamin B₆ deficiency. The changes induced by feeding the animal a vitamin B₆-deficient diet for one month are clearly different from those induced by OMP. The effect of OMP, moreover, is not limited to the liver but changes as atrophy of the thymus and spleen are found and the whole body is affected. The toxic effect becomes apparent, furthermore, with very small doses.

Vitamin B₆ is most effective in suppressing the toxic effect of OMP as reported by Makino et al., but it was also found that other liver-protecting agents alone had some suppressive action. None of the above, however, is able to prevent the toxic effect of OMP completely.

SUMMARY

1. OMP is found to have a liver-disturbing effect.
2. The toxicity of OMP is most effectively prevented by vitamin B₆, but liver-protecting agents, as methionine and N-carbobenzoxy glutamylcholine, also have some effect even when administered alone.
3. The minimum disturbing dose of OMP is between 0.04 and 0.004 mg/g.
4. The pyridoxine plus pyridoxamine content and the pyridoxal content of the liver are reduced when OMP is administered.

ACKNOWLEDGEMENT

The kindness of Dr. Taizo Matsukawa, Takeda Research Laboratory, who supplied the samples is gratefully acknowledged.

REFERENCES