LIPOTROPIC ACTIVITY AND TOXICITY OF METHOXININE (OXYMETHIONINE)

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Methoxinine (DL-2-amino-4-methoxybutanoic acid) has been synthesized by Roblin and coworkers (1), who studied its action on Escherichia coli and Staphylococcus aureus. The growth-inhibitory effect of this compound was prevented by L-methionine but not by the D isomer. 1 mole of DL-methionine reversed the antibacterial action of 500 to 1000 moles of DL-methoxinine. In combination with sulfonamides a synergistic bacteriostatic effect was produced by methoxinine.

Methoxinine was of interest to this laboratory as a tool in an investigation of the protective action afforded by methionine against the hepatotoxic effects of certain chemicals in protein-deficient states. Provided certain conditions were fulfilled, it might be possible by the use of the oxygen analogue to distinguish between any effect of methionine due to its sparing or replenishment of sulfhydryl enzyme systems (2) and such effect as might result solely from its rôle as a lipotropic factor (3). Before such a demonstration might be made, however, it was first necessary to establish that methoxinine does possess lipotropic activity, in addition to being devoid of toxic manifestations that might otherwise complicate the experiment. The work described in the present report shows that the lipotropic activity does indeed exist, but that methoxinine exhibits an order of toxicity that would probably preclude its use in the connection indicated.

EXPERIMENTAL

DL-Methoxinine was synthesized1 by a modification of the procedure of Roblin et al. (1). The final product melted in a range of 249–251° (corrected) with decomposition and effervescence. The rate of heating was about 2° per minute above 230°. Elementary analysis gave the following results.

\[
\text{C}_4\text{H}_8\text{O}_2\text{N. Calculated. C 45.10, H 8.33, N 10.52}
\]

\[
\text{Found. " 44.46, " 8.06, " 10.39}
\]

The experimental animals were Rockland rats of the Sherman strain, weighing between 130 and 180 gm. Equal numbers of both males and fe-

1 The authors are indebted to Mr. Joseph Fugger, Department of Chemistry, University of Pittsburgh, for the synthesis of the DL-methoxinine, which was performed under the direction of Dr. Klaus Hofmann of that Department.
males were used. In Series I four groups of ten rats each were fed a lipogenic diet for a period of 21 days, and the supplements of methoxinine or DL-methionine (or both) were given in distilled water solution by stomach tube. In Series II, the amino acids were incorporated into the basal diet to the extent of 0.5 per cent of the latter.

The basal diet consisted of lard 40, casein 5, sucrose 46, salts (4) 4, and cellulose 5. Each animal received daily a tablet supplying approximately 25 \( \gamma \) of thiamine hydrochloride, 100 \( \gamma \) of calcium pantothenate, 25 \( \gamma \) of pyridoxine hydrochloride, and 25 \( \gamma \) of riboflavin. In addition, 3 drops of a mixture of 50 ml. of Natola and 1 gm. of \( \alpha \)-tocopherol were given weekly.

In Series I, Group I served as controls, and the others were given the following supplements daily by stomach tube in about 2 ml. of distilled water solution: Group II, 50 mg. of methoxinine; Group III, 50 mg. of methionine; and Group IV, 50 mg. of methoxinine plus 50 mg. of methionine. The last group was included for the purpose of observing any possible antagonistic effect between the two compounds. The experiments of Series II became necessary when it was found that the animals in Group II above did not survive the 21 day experimental period. In Series II, therefore, the amino acids were fed in the diet. Group I of Series II is likewise a control; Group II received a diet containing 0.5 per cent methionine; and Group III, one containing 0.5 per cent methoxinine. Because of the limitation of the quantity of methoxinine available, no group comparable to Group IV of Series I could be included.

All groups were maintained on the experiment for 21 days. Food intake and body weight were recorded weekly. At the end of this period, the animals were exsanguinated by decapitation under light nembutal anesthesia, and the liver was removed, weighed, and analyzed for total fatty acids plus cholesterol by chromate oxidation. The kidneys were weighed (combined weight) and portions of each fixed for histological examination.

In the lipide analyses, the livers were ground in a Waring blender with a small quantity of ethyl alcohol, and the pulp transferred quantitatively to a volumetric flask with additional alcohol. Sufficient ethyl alcohol and ethyl ether were then added to fill the flask to the mark with a 3:1 proportion of the two solvents, respectively. The flasks were selected to give a minimum volume of 20 ml. of alcohol ether mixture to 1 gm. of wet weight of liver tissue, and the tissue pulp was allowed to remain in contact with the extracting fluid, with occasional shaking, for at least several hours be-
fore aliquots of the latter were removed for saponification of the contained lipide. No heating was involved in the extraction procedure. The equivalence, 1 mg. of fatty acid plus cholesterol equals 3.7 ml. of 0.1 n dichromate, which is employed in blood analysis, was arbitrarily taken for use in this work. The total fatty acids plus cholesterol are expressed as percentage of wet weight of the liver.

**Table I**

*Influence of Methionine and Methoxinine Supplements, by Stomach Tube, on Rats Receiving Lipogenic Diet (Series I)*

Group I animals were the controls, Group III received 50 mg. of methionine daily and Group IV received 50 mg. of methionine and 50 mg. of methoxinine daily.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, gm. per rat per day</td>
<td>4.16</td>
<td>4.11</td>
<td>4.24</td>
</tr>
<tr>
<td>Weight loss, gm.</td>
<td>16.60</td>
<td>13.20</td>
<td>34.40*</td>
</tr>
<tr>
<td>Liver weight, % of body weight</td>
<td>6.10</td>
<td>6.33</td>
<td>5.13*</td>
</tr>
<tr>
<td>Kidney % of body weight</td>
<td>0.87</td>
<td>0.92</td>
<td>1.08*</td>
</tr>
<tr>
<td>Liver lipide, % of wet organ weight</td>
<td>19.90</td>
<td>14.90*</td>
<td>7.02†</td>
</tr>
</tbody>
</table>

* Deviation from Group I statistically significant.
† Deviation from Groups I and III statistically significant.

**Table II**

*Effect of Incorporating 0.6 Per Cent Methionine and 0.5 Per Cent Methoxinine, Respectively, in Lipogenic Diet (Series II)*

Group I animals were the controls, Group II received the methionine-supplemented diet, and Group III the methoxinine-supplemented diet.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, gm. per rat per day</td>
<td>6.22</td>
<td>5.76</td>
<td>4.38</td>
</tr>
<tr>
<td>Weight loss, gm.</td>
<td>24.10</td>
<td>26.50</td>
<td>52.30*</td>
</tr>
<tr>
<td>Liver weight, % of body weight</td>
<td>6.28</td>
<td>4.77*</td>
<td>5.80</td>
</tr>
<tr>
<td>Kidney % of body weight</td>
<td>0.82</td>
<td>0.87</td>
<td>1.03*</td>
</tr>
<tr>
<td>Liver lipide, % of wet organ weight</td>
<td>18.10</td>
<td>5.60*</td>
<td>8.70*</td>
</tr>
</tbody>
</table>

* Deviation from Group I statistically significant. The liver lipide values of Groups II and III are not significantly different.

**Results**

Table I presents the means of the results obtained in Series I in which the amino acid supplements were given in fixed quantity by stomach tube. All the animals in Group II of this series died between the 12th and 20th days of the experiment, and are therefore excluded from any comparison. Table II sets forth similar data for the animals in Series II, in which the
basal diet was supplemented to the extent of 0.5 per cent methionine and 0.5 per cent methoxinine, respectively.

**DISCUSSION**

All groups of animals in both series lost weight during the experimental period, and both the weight loss and food intake were unaffected by supplements of methionine. The addition of 0.5 per cent methoxinine to the basal diet did not alter food consumption, but it markedly accelerated the loss in weight. On the basis of the mean food intake of the group, a level of 0.5 per cent methoxinine in the diet represents an intake of 22 mg. per rat per day as compared with the 50 mg. per rat per day that proved lethal to the animals in Group II of Series I under similar conditions. While no attempt has been made to compare the initial food consumption of this group with that of the others, it may be stated that the appetite of the animals (Group II) was severely depressed. The combination of a nearly equimolar amount of methionine with the methoxinine (Group IV, Series I) brought the food intake up on a par with that of the other groups and permitted survival for the experimental period, but did not accomplish a reversal of the weight loss.

A direct evaluation of the lipotropic activity of methoxinine is not possible from the data in Series I because all the animals in Group II died before completion of the experiment. But the fact that the combination of methoxinine and methionine, as used in Group IV of this series, brought about a significantly greater reduction in liver lipide than the methionine alone is indicative of a lipotropic effect of the oxygen analogue. Direct evidence is afforded in Series II, in which the addition of 0.5 per cent methoxinine to the basal diet produced an effect on liver lipide equal to that of methionine. In both series the food intake of each group was statistically identical with that of the others, so that variation in food consumption is not a factor in the results obtained.

That methoxinine possesses considerable toxicity under the conditions of these experiments is manifest in the complete mortality in 12 to 20 days of the group receiving 50 mg. per rat per day and the extreme weight depletion of the animals ingesting it as 0.5 per cent of the diet. The mechanism of such a toxic effect is not apparent from this work. It may be pointed out, however, that, if this effect represents an interference with methionine metabolism, it is clearly not the transmethylation function of the latter that is blocked. Moreover, the fact that the toxicity is only slightly counteracted by the simultaneous administration of a practically equimolar quantity of \( \text{D}-\text{methionine} \) leads one to believe that some factors other than this specific metabolite antagonism are operative.

The pathology observed microscopically in sections of the liver correlated reasonably well with the amount of fat found by chemical analysis.
It is worthy of note that the analytical results revealed a greater lipide content of the liver when methionine was given by stomach tube (50 mg. per rat per day) than when it was added to the diet, even though the dietary supplement calculated on the basis of the food intake was only 29 mg. per rat per day. The microscopic sections showed, correspondingly, a more extreme fatty infiltration in the former instance. The sections of kidney were particularly interesting in that those from the animals that succumbed to the 50 mg. per day dose of methoxinine exhibited varying degrees of tubular damage, frequently with glomerular involvement. Some tubular, and occasionally glomerular, changes were found in many of the animals, irrespective of grouping, but the changes in Group II of Series I were more extensive and severe than in any other. This condition seems to have been ameliorated to some extent by the simultaneous administration of methionine as judged from the pathology in Group IV of Series I.

SUMMARY

1. With rats fed a diet containing 5 per cent casein and 40 per cent lard, a supplement of 50 mg. of methionine plus 50 mg. of methoxinine per rat per day effected a significantly greater reduction in the lipide content of the liver than did 50 mg. of methionine alone.

2. Addition of either 0.5 per cent methoxinine or 0.5 per cent methionine to the basal diet lowered the lipide content of the liver to the same degree.

3. The administration of 50 mg. per rat per day of methoxinine to animals fed the basal diet produced complete mortality of the group in 12 to 20 days, with significant kidney pathology evident microscopically. Addition of 50 mg. of methionine to this dose raised the level of food consumption, permitted survival over the experimental period, and reduced the severity of kidney damage, but did not prevent a significantly greater depletion of body weight as compared to the controls or to the group receiving methionine alone. This accelerated rate of weight loss was also observed in those animals fed the basal diet containing 0.5 per cent methoxinine (22 mg. per rat per day), although no mortalities occurred in this group over the period indicated.

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BIBLIOGRAPHY

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