THE INFLUENCE OF DIET ON THE RIBOFLAVIN CONTENT AND THE ABILITY OF RAT LIVER SLICES TO DESTROY THE CARCINOGEN N,N-DIMETHYL-p-AMINOAZO-BENZENE*

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The production of liver tumors in the rat by the oral administration of N,N-dimethyl-p-aminazobenzene (DMB) is markedly influenced by diet. Our early work (1, 2) showed that the maintenance of normal hepatic riboflavin levels was of importance in protecting rats against tumor formation. The work of Griffin and Baumann (3) and Miller, Miller, Kline, and Rusch (4) has emphasized the relationship between hepatic riboflavin levels and the carcinogenic action of DMB and related compounds. Riboflavin supplements are not effective in maintaining hepatic riboflavin levels or preventing tumor formation in the absence of adequate protein intake (2).

In a study (5) from this laboratory of the effect of diet on the ability of rat liver slices to destroy DMB it was found that the maintenance of rats on a brown rice-carrot diet, which favors tumor production by DMB (6, 7), decreased this activity of rat liver. It was also observed that the addition of protective supplements such as dried brewers’ yeast or riboflavin and casein prevented this decrease. Measurement of the riboflavin content of the livers of rats on these diets indicated that, as the riboflavin level fell, the ability to destroy DMB also decreased.

The use of brown rice diets in the preceding study (5), while suggesting that the changes observed were due to inadequate riboflavin and protein intake, made it necessary to rule out the presence of a “toxic” factor in the rice as the responsible agent. For this reason, and for the purpose of controlling the intake of other dietary constituents, the experiments have been repeated with diets which did not contain rice or carrot. As the Wisconsin group (8, 9) have published extensively on the effect of casein-dextrose diets on the carcinogenic action of DMB, the diets selected were modeled on the ones investigated by that group.

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The destruction of DMB by liver slices was measured as described in our earlier report (5) and depended on the recovery and spectrophotometric estimation of the DMB that was not destroyed. 100 γ of DMB were incubated with 150 mg. of liver slices in all experiments. Under the conditions of these experiments the destruction of DMB was proportional to the period of incubation. The recovery of added DMB at zero time was good, 90 to 95 per cent.

The basic diets used were of the casein-dextrose type described by Miller (9) for medium tumor incidence. All vitamin supplements, except those varied in the different experiments, were administered as specified (9). Halibut liver oil was omitted in view of the short time involved. In the 4 per cent protein diet the dextrose was increased to 87 per cent of the diet. The diets were fed ad libitum.

The rats used were of the Wistar strain, weighing between 100 and 150 gm. at the start of the experiment. Similar results have been obtained with Sherman strain rats obtained from the Rockland Farms (but are not included in this paper).

The riboflavin was measured by a modification of the fluorometric method of Hodson and Norris (10).

Results

The data obtained on hepatic riboflavin levels and the destruction of DMB by liver slices from rats maintained on various diets are shown in Table I. It can be seen that, as the riboflavin content of the diet was reduced, the riboflavin level in the liver and the in vitro destruction of DMB both decreased (Diets 1, 2, 3, and 4). In marked contrast, when thiamine (Diet 5) or choline (Diet 6) was omitted from the diet, there was no change in DMB destruction, and the hepatic riboflavin level was in the normal range, although in the absence of thiamine these values were slightly higher and in the absence of choline slightly lower than the controls. The failure of the omission from the diet of thiamine or choline to affect the ability of rat liver to destroy DMB thus supports the observation made when brown rice diets were used that a riboflavin-containing component of hepatic cells is either primarily or secondarily involved in the destruction of DMB.

The riboflavin level in the liver of rats is lowered also when the protein content of the diet is reduced (11, 12). As is shown in Table I (Diets 7 and 8), when the protein content of the diet used was reduced from 12 to 4 per cent, both the riboflavin level and the ability of liver to destroy DMB were decreased. Thus the protein level of the diet as well as its riboflavin content has been found to be of importance in maintaining hepatic riboflavin levels and high activity with respect to DMB destruction.
Biotin has been reported (13, 14) to increase tumor incidence when certain partially protective diets were used and adenine has been reported (15) to increase liver damage associated with DMB administration. The addition of adenine (Diet 9) and biotin (Diet 10) to the basal diet was without effect on riboflavín level or DMB destruction.

**Table I**

**Riboflavín Content and Destruction of DMB by Liver Slices from Rats Fed Casein-Dextrose Base Diets for 14 to 20 Days**

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of rats</th>
<th>Riboflavín per gm. liver (wet weight)</th>
<th>DMB destruction by 150 mg. liver slices in 60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>16.2-25.0</td>
<td>21.5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>16.2-22.8</td>
<td>18.7</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>12.3-18.8</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>10.7-14.4</td>
<td>13.0</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>21.8-25.2</td>
<td>22.8</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>13.3-25.0</td>
<td>19.7</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>11.0-21.8</td>
<td>16.5</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>9.2-19.4</td>
<td>14.0</td>
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<tr>
<td>9</td>
<td>5</td>
<td>21.0-25.2</td>
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<tr>
<td>12</td>
<td>10</td>
<td>11.0-14.8</td>
<td>12.2</td>
</tr>
</tbody>
</table>

* Riboflavín.

The relationship between the riboflavín level in the liver and the ability to destroy DMB is shown more clearly in Fig. 1. Rats fed Diets 1 through 8, Table I, have been plotted. The data show that the decrease in the ability to destroy DMB is most marked as the riboflavín content falls from 22 to 15 γ per gm. of liver tissue. Rats maintained on a ration of dog chow usually have between 25 and 30 γ of riboflavín per gm. of liver tissue but
the destruction of DMB is not much greater than for livers containing 22 to 25 \( \gamma \) per gm. Conversely it has been found that rats maintained on a brown rice-carrot diet for periods of 80 to 100 days containing 8 to 10 \( \gamma \) of riboflavin per gm. of liver destroyed only slightly less DMB than the livers containing 12 to 15 \( \gamma \) per gm. Thus the striking drop in the ability to destroy DMB occurs to a large extent as the riboflavin content is decreased from 22 to 15 \( \gamma \) per gm. and the curve flattens out at both high and low riboflavin levels.

The results presented in the preceding paragraphs were obtained without the inclusion of the carcinogen DMB in the diet. The inclusion of DMB in diets of the brown rice type was shown to depress the riboflavin level more than the diet alone (1). This observation has been extended to other diets and azo compounds by Griffin and Baumann (3). As is shown in Table I, the inclusion of 0.06 per cent DMB in the 0.001 and 0.0001 per cent riboflavin diets (Diets 11 and 12) decreases both the riboflavin level and the ability of slices of the liver to destroy DMB, more than the diet alone.

**Fig. 1.** Relationship between the riboflavin content and destruction of DMB by liver slices from rats fed casein-dextrose base diets for 14 to 20 days. The solid points connected by the dotted line represent the average values for each 2 \( \gamma \) range of riboflavin level; i.e., the point at 15 \( \gamma \) per gm. includes all animals containing from 14.0 to 15.9 \( \gamma \) per gm. of liver.
DISCUSSION

The data presented extend the observation (5) originally made on rats maintained on brown rice diets that the rate of destruction of the hepatocarcinogen DMB by rat liver slices is related to their riboflavin content. Although the metabolism of DMB in vivo is not completely worked out, it involves a complex series of reactions leading to cleavage of the azo linkage (16), demethylation of the amino group (16, 17), oxidation of the p' position (16, 17), the accumulation of an unknown but colored derivative in the liver (18), as well as the excretion of the metabolites in conjugated form (16, 17). As neither the end-products nor the intermediary products of the destruction of DMB by liver slices have been identified, it is difficult to assess the significance of the apparent correlation between riboflavin content and DMB destruction. However, it appears likely that the azo linkage is the grouping attacked in the slice experiments, as the destruction of p-aminoazobenzene is also decreased by diets lowering the riboflavin content of the liver (5).

Mueller and Miller (18) have recently reported that diphosphopyridine nucleotide (DPN) is involved in the destruction of DMB in liver homogenates. This has also been observed in our laboratory. Measurement of DPN content (1, 19) of the livers of rats fed high and low tumor incidence diets of the brown rice type showed that the DPN level was depressed by the administration of DMB in the diet but not by the diet alone, in contrast to the variation of riboflavin levels as a function of these diets. Thus it would appear that DPN is not a limiting factor in the liver slice experiments reported in this paper.

SUMMARY

1. The limitation of the riboflavin or protein intake but not the thiamine or choline intake has been found to reduce both the riboflavin concentration in rat liver and the ability of rat liver slices to destroy the liver carcinogen N,N-dimethyl-p-aminoazobenzene.

2. The addition of biotin or adenine to the diet was without effect on either the riboflavin level or the destruction of the carcinogen by liver slices.

3. Data showing the relationship between the riboflavin level in the liver and the ability to destroy the carcinogen are presented.

4. The inclusion of the carcinogen in the diet further reduced the hepatic riboflavin level and the ability of liver slices to destroy it.

BIBLIOGRAPHY

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