Influence of Dimethylaminoazobenzene on Leukocyte Production in the Rat

By James S. Dinning, Ph.D., Lou Dewees Payne, B.S. and Paul L. Day, Ph.D.

In previous reports² we have shown a requirement for methyl groups in the production of leukocytes. It was found that with the soybean protein basal diet used, methionine alone, or betaine or choline with vitamin B₁₂ could satisfy this methyl group requirement. The present report shows that p-dimethylaminoazobenzene (DAB) plus vitamin B₁₂ will prevent the leukopenia which appears in rats fed the methionine-deficient basal diet.

Table 1.—Growth Rate, Mortality Rate and Leukocyte Counts of Rats Fed Alpha Protein Diet with Various Supplements

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ave. weight increase in 64 days</th>
<th>Mortality during 64 days</th>
<th>Incidence of hepatomas*</th>
<th>Total white blood cells</th>
<th>52nd-56th exp. days</th>
<th>65th-69th exp. days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (8)†</td>
<td>6.2</td>
<td>50</td>
<td>0</td>
<td>9.1</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Basal + B₁₂ (8)</td>
<td>15.1</td>
<td>0</td>
<td>0</td>
<td>12.8</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Basal + DAB (9)</td>
<td>5.6</td>
<td>11</td>
<td>17</td>
<td>13.2</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Basal + DAB + methionine (9)</td>
<td>119.2</td>
<td>0</td>
<td>11</td>
<td>21.5</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Basal + DAB + B₁₂ (9)</td>
<td>20.2</td>
<td>0</td>
<td>78</td>
<td>18.4</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>Basal + DAB + B₁₂ + methionine (9)</td>
<td>119.1</td>
<td>0</td>
<td>33</td>
<td>22.2</td>
<td>20.2</td>
<td></td>
</tr>
</tbody>
</table>

† Number of animals originally present.

Experimental

Weanling female Sprague-Dawley rats were fed the basal diet previously described. This diet contains 18 per cent isolated soybean protein* as the only protein. The diet was deficient in methionine, as it contained only 0.27 per cent of this essential amino acid. One group of rats received this basal diet without supplement, others were given the basal diet with the following supplements per 100 Gm. of diet: vitamin B₁₂ (Rubramin), 5 μg.; DAB, 70 mg.; DAB plus DL-methionine, 0.6 Gm.; DAB + vitamin B₁₂ + methionine. Blood studies, including red blood cell counts, hemoglobin, total white blood cells, and differential counts, were made between the fifty-second and fifty-sixth experimental days and again between the sixty-fifth and sixty-ninth experimental days. The only differences between the groups were in white blood cell numbers, hence only these data are given. The rats were autopsied after 170 days on the diets and the incidence of hepatomas noted. These data have been previously reported.³

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* "Alpha Protein," obtained from the Glidden Company, Chicago.
RESULTS

The results are given in table 1. Only methionine was effective in restoring growth rates while all the supplements were effective in reducing mortality. Rats receiving the basal diet alone or the basal diet supplemented with DAB or with vitamin B₁₂ were leukopenic. Supplementation of the basal diet with DAB plus vitamin B₁₂ maintained the peripheral leukocytes at normal levels, as did supplementation with DAB plus methionine. The incidence of hepatomas, taken from the previous report,² is included as an aid to interpretation of the data.

DISCUSSION

The fact that DAB plus vitamin B₁₂ maintained the peripheral leukocytes of methionine-deficient rats at normal levels does not prove that the methyl groups of DAB were labile under those conditions. However, in view of our previous experiments¹ ² this interpretation seems to be the most reasonable. Du Vigneaud and associates⁴ reported that the methyl groups of DAB were not labile; their diet differed from ours in two important respects: it contained adequate methionine, and no vitamin B₁₂ was added. These differences could explain the divergent results. If one is willing to assume that the observed leukocyte response is evidence that the methyl groups of DAB are labile in the presence of vitamin B₁₂, the fact that vitamin B₁₂ also increased the carcinogenicity of DAB offers another apparent conflict. It has been shown that the completely demethylated derivative of DAB is noncarcinogenic.⁵ One possible reconciliation of these apparent discrepancies is that the carcinogenicity of DAB is related to the process of demethylation. The protection which methionine offered in incidence of hepatomas could then have been due to a reduced demand for DAB methyl groups.

SUMMARY

1. Supplementation with p-dimethylaminoazobenzene plus vitamin B₁₂ prevented leukopenia in rats fed a methionine-deficient basal diet. p-Dimethylaminoazobenzene was ineffective in this regard in the absence of supplementary vitamin B₁₂.
2. Possible relationships of this observation to the theory of p-dimethylaminoazobenzene carcinogenesis are discussed.

REFERENCES

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