The Carcinogenicity of \( m' \)-Methyl-\( p \)-Dimethylaminoazobenzene and of \( p \)-Monomethylaminoazobenzene*

Janet E. Giese, B.A., J. A. Miller, Ph.D., and C. A. Baumann, Ph.D.

(From the Department of Biochemistry, College of Agriculture, and The McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wisconsin)

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In a previous study the structure of \( p \)-dimethylaminoazobenzene was altered by the introduction of a methyl group into the various positions of the non-diamine ring, and it was observed that the carcinogenicity of the methylated compound depended upon the position in which the group was inserted (2). \( m' \)-Methyl-\( p \)-dimethylaminoazobenzene appeared to be more carcinogenic than \( p \)-dimethylaminoazobenzene. The removal of an N-methyl group from \( p \)-dimethylaminoazobenzene resulted in a compound, \( p \)-monomethylaminoazobenzene, that appeared to be at least as active as \( p \)-dimethylaminoazobenzene itself. The structural formulas of these azo dyes are

\[
\begin{align*}
&\text{\( p \)-dimethylaminoazobenzene} \\
&\text{\( m' \)-methyl-\( p \)-dimethylaminoazobenzene} \\
&\text{\( p \)-monomethylaminoazobenzene}
\end{align*}
\]

However, the number of animals used in the initial survey was not adequate for an evaluation of the relative potencies of such active compounds. Accordingly the carcinogenities of these three azo dyes have now been compared at several levels of administration, during several time intervals of feeding, and in two different basal rations.

METHODS

The methods employed were essentially those used previously in this laboratory (2, 3, 5). Young adult albino rats 160 to 210 gm. in weight were divided into groups of at least 12 animals and fed the rations \textit{ad libitum} that contained the three dyes being compared. The basal diets were either a semi-synthetic one containing crude casein, 120 gm.; salts, 40 gm.; corn oil, 50 gm.; rice bran concentrate, 20 gm.; glucose, 770 gm.; and riboflavin, 0.5 mgm. per kgm.; or a synthetic diet containing vitamin-free casein (3), 120 gm.; salts, 40 gm.; corn oil, 50 gm.; glucose, 790 gm.; thiamine chloride, 3 mgm.; riboflavin, 2.0 mgm.; calcium pantothenate, 7.0 mgm.; pyridoxine hydrochloride, 2.5 mgm.; and choline chloride, 30.0 mgm. per kgm. Every rat received 1 drop of halibut liver oil monthly. Both diets have been used many times in previous studies and they are known to result in a high incidence of tumors when the carcinogen fed is \( p \)-dimethylaminoazobenzene.

The carcinogens were dissolved with heat in the corn oil before incorporation in the diet. Comparisons within any series were made with molar equivalents of the three dyes; \textit{e.g.}, on a molar basis, 0.060 per cent of \( p \)-dimethylaminoazobenzene, the concentration used by most investigators, is equivalent to 0.064 and 0.056 per cent of \( m' \)-methyl-\( p \)-dimethylaminoazobenzene and of \( p \)-monomethylaminoazobenzene respectively. The exact concentrations fed and the times of feeding are indicated in Table I. At the end of the feeding period the livers were examined by laparotomy. The rats were then fed the basal diet without dye for another 2 months to permit the cirrhosis to recede, while any latent tumors originally undetected had time to develop to a recognizable size.
Cancer Research

The p-dimethylaminoazobenzene was obtained commercially (Eastman No. 338). The p-monomethylaminoazobenzene (1) and the m’-methyl-p-dimethylaminoazobenzene were synthesized in this laboratory. The details for the preparation of the latter compound are as follows: Fifty-four grams (0.5 mole) of m-toluidine are dissolved in a mixture of 115 cc. of concentrated HCl and 250 cc. of water. The solution is cooled to 0 °C. in an ice bath and stirred mechanically; and diazotization is effected by adding dropwise a cold solution of 34.5 gin. U.S.P. NaNO₂ in 150 cc. of water. The temperature of the reaction mixture should not rise above +3 °C. Sixty-one grams of N-dimethylaniline and 85 gm. of anhydrous sodium acetate are then dissolved in 1,500 cc. of 70 per cent ethyl alcohol in a water bath and the solution is cooled to 20 °C. The diazo solution is added all at once with stirring to the solution of the amine. The precipitate of the azo compound is filtered off and recrystallized from ethyl alcohol-water. The yield is approximately 100 gin. (Theor.=120 gm.) of recrystallized product, which melts at 119 °C to 120 °C.

RESULTS

Large hepatic tumors developed rapidly with each of the azo dyes fed. The monomethyl compound proved to be somewhat more active than p-dimethylaminoazobenzene, whereas the m’-methyl derivative was invariably the most active of the three (Table I). For example, when 0.060 per cent of p-dimethylaminoazobenzene or the m’-methyl-p-dimethylaminoazobenzene was fed in the semi-synthetic diet for 3 months, the mortality and degree of cirrhosis were least in the rats fed p-dimethylaminoazobenzene and greatest in those fed the m’-methyl derivative. The percentage tumor incidence at 3 months was 43 on p-dimethylaminoazobenzene, 62 on p-monomethylaminoazobenzene, and 92 on m’-methyl-p-dimethylaminoazobenzene. In the latter group the tumors were definitely larger than in those fed the other compounds.

Table I: The Comparative Carcinogenicities of m’-Methyl-p-dimethylaminoazobenzene, p-Monomethylaminoazobenzene, and p-Dimethylaminoazobenzene in Rats

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Per cent in diet</th>
<th>Average weight, gm.</th>
<th>Average weight increment at end of feeding, gm.</th>
<th>Diameter of tumor, inc.</th>
<th>Time dye was fed, mos.</th>
<th>Survival * at end feeding dye</th>
<th>Tumor incidence † at end feeding dye</th>
<th>Circrhosis at end of feeding dye</th>
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<tbody>
<tr>
<td>m’-Methyl-DAB ‡</td>
<td>0.064</td>
<td>189</td>
<td>1</td>
<td>9.2</td>
<td>3</td>
<td>12/20</td>
<td>11/12</td>
<td>12/12</td>
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<tr>
<td>MAR ‡</td>
<td>0.056</td>
<td>190</td>
<td>8</td>
<td>9.8</td>
<td>3</td>
<td>16/20</td>
<td>10/16</td>
<td>12/12</td>
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<tr>
<td>DAB ‡</td>
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<td>168</td>
<td>24</td>
<td>9.4</td>
<td>3</td>
<td>14/20</td>
<td>6/14</td>
<td>11/14</td>
</tr>
<tr>
<td>m’-Methyl-DAB</td>
<td>0.056</td>
<td>201</td>
<td>19</td>
<td>9.3</td>
<td>2½</td>
<td>13/17</td>
<td>0/13</td>
<td>13/13</td>
</tr>
<tr>
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<td>190</td>
<td>23</td>
<td>11.7</td>
<td>2½</td>
<td>11/12</td>
<td>2/11</td>
<td>11/13</td>
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<tr>
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<td>186</td>
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<td>2½</td>
<td>11/12</td>
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<td>11/13</td>
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<tr>
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<td>172</td>
<td>18</td>
<td>9.5</td>
<td>2½</td>
<td>15/16</td>
<td>1/15</td>
<td>12/15</td>
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<td>176</td>
<td>50</td>
<td>11.0</td>
<td>2½</td>
<td>15/16</td>
<td>1/15</td>
<td>8/15</td>
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<tr>
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<td>202</td>
<td>13</td>
<td>10.8</td>
<td>2½</td>
<td>15/15</td>
<td>8/15</td>
<td>14/15</td>
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<td>3</td>
<td>8/15</td>
<td>7/8</td>
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<td>3½</td>
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<td>1/14</td>
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<td>3</td>
<td>13/15</td>
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<tr>
<td>DAB</td>
<td>0.045</td>
<td>193</td>
<td>47</td>
<td>11.5</td>
<td>3</td>
<td>15/15</td>
<td>1/15</td>
<td>5/15</td>
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</table>

* Survival = number living over number at start.  † Tumor incidence = number with tumors over number surviving the period during which the dye was fed.  ‡ DAB = p-dimethylaminoazobenzene.  MAB = p-monomethylaminoazobenzene.
the semi-synthetic one containing the rice bran concentrate. After 3½ months of feeding the dye, the tumor incidence in the group fed p-dimethylaminoazobenzene was only 14 per cent 2 months later as compared to 50 per cent when the monomethyl compound was fed. In the same series, rats were fed the m'-methyl compound for only 3 months and the incidence of tumors reached 100 per cent shortly thereafter. When 0.045 per cent of p-dimethylaminoazobenzene was fed for 3 months in the synthetic diet, the incidence of tumors 2 months later, was 33 per cent, as compared to an incidence of 61 per cent when the m'-methyl derivative was fed.

It is thus evident that m'-methyl-p-dimethylaminoazobenzene was more carcinogenic than the other twoazo dyes. At all levels of administration, at all periods of feeding, and on both basal rations, the degree of cirrhosis caused by this compound was also invariably more severe than that caused by p-monomethylaminoazobenzene or p-dimethylaminoazobenzene. This greater carcinogenicity of the m'-methyl derivative was evident in spite of the fact that the average daily food intake, and therefore the intake of dye, was less on this compound than when the other two carcinogens were fed. The high carcinogenicity of m'-methyl-p-dimethylaminoazobenzene is therefore regarded as real.

p-Monomethylaminoazobenzene also appeared to be somewhat more carcinogenic than p-dimethylaminoazobenzene. An extreme example of this difference was noted when nine-tenths of the molar equivalent of the original 0.060 per cent of p-dimethylaminoazobenzene was fed for 2½ months. The incidence of tumors 2 months later was 82 per cent when the monomethyl derivative was fed, as compared to only 18 per cent for the dimethyl compound. At other levels of administration, and on other diets, the superiority of the monomethyl derivative was less noteworthy, although it always existed. The degree of cirrhosis was also greater in the rats fed p-monomethylaminoazobenzene. However, the rats fed the monomethyl compound invariably ate more food, and therefore more molecules of azo dye, than those fed p-dimethylaminoazobenzene. Hence the question is still open whether p-monomethylaminoazobenzene really induces tumors more rapidly than p-dimethylaminoazobenzene when all other factors are adequately controlled. But that the monomethyl compound is at least as active as the dimethyl derivative is evident from the present experiments. Kensler 1 has also observed that p-monomethylaminoazobenzene is highly carcinogenic. Nevertheless, more data will be needed before the relative activities of the monomethyl and dimethyl dyes can be regarded as established. The question is of interest, since the two compounds appear to be interconvertible in the liver of the rat (4); roughly 3 μg of p-dimethylaminoazobenzene and 1 μg of p-monomethylaminoazobenzene are present per liver either when 0.060 per cent of the dimethyl compound is fed alone or when the molar equivalent (0.054 per cent) of the monomethyl derivative is given. Hence either compound, or the mixture of the two, or one of their metabolic derivatives could be the true carcinogen.

A somewhat higher tumor incidence was noted with p-dimethylaminoazobenzene when 0.045 per cent of the dye was fed than when 0.054 per cent was given in the rice bran concentrate diet; a similar situation occurred on the synthetic diet with dye levels of 0.060 and 0.045 per cent. While it is likely that the animals employed may have varied in their susceptibility to the carcinogen from series to series, it is also possible that the less extensive cirrhosis encountered on lower levels of dye allowed the tumors present to proliferate more rapidly than in the more severely damaged livers.

**Toxicity of dyes.**—In addition to being the most carcinogenic of the three compounds, m'-methyl-p-dimethylaminoazobenzene was also the most toxic. This was demonstrated in a number of ways. In the prolonged experiments in which tumors were induced in adult animals, the percentage survival and the gains in weight were invariably the least in groups fed the m'-methyl derivative. When the dyes were fed to younger animals, m'-methyl-p-dimethylaminoazobenzene also proved to be the most toxic, as determined by the number of deaths and by the changes in weight (Table II). p-Monomethylaminoazobenzene appeared to be about as toxic to young rats as p-dimethylaminoazobenzene (Table II), but adult rats fed the mono-

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1 C. J. Kensler, personal communication.
methyl dye consumed more food and usually gained more weight than comparable animals on the other dyes. Their survival was also better (Table I). Experiments with 90 mice revealed that all three azo dyes are toxic at levels as low as the molar equivalent of 0.03 per cent p-dimethylaminobenzene, but the distribution of deaths was much more erratic in the mouse than in the rat, and no conclusions could be drawn as to the relative toxicity of the three compounds in mice.

Variation in diet.—In view of the high carcinogenicity of m'-methyl-p-dimethylaminobenzene, the question arose whether the formation of liver tumors with this compound was as sensitive to variations in diet as the carcinogenicity of p-dimethylaminobenzene. If so, it would be possible to save time, labor, and food by using the more active compound in dietary studies. Some sensitivity to diet was suggested by the data in Table I. When 0.048 per cent of m'-methyl-p-dimethylaminobenzene was incorporated in the semisynthetic diet containing the rice bran concentrate and fed for 2 months, the incidence of tumors 2 months later was at least 80 per cent. However, when this amount of the dye was incorporated in the synthetic ration and fed for 3 months, the incidence of tumors 2 months later was only 61 per cent. Since the ration containing the rice bran concentrate definitely increases the rate at which hepatic tumors are formed when the carcinogen is p-dimethylaminobenzene (3, 5, 6), it would follow that the carcinogenicity of the more potent m'-methyl derivative may also be modified by diet to some extent. But a very noticeable effect of diet should not be expected in experiments in which tumors are produced so rapidly. It is recognized that modifying cocarcinogenic or anticarcinogenic influences are best demonstrated when the dosage of carcinogen employed is minimal.

SUMMARY

1. m'-Methyl-p-dimethylaminobenzene proved to be the most potent carcinogenic azo dye hitherto reported for the liver of the rat. On equivalent concentrations of dye rats fed the m'-methyl derivative invariably lost more weight, developed a more severe cirrhosis, and formed large hepatic tumors more rapidly than when p-dimethylaminobenzene was fed. When 0.048 per cent of m'-methyl-p-dimethylaminobenzene (\(\frac{3}{4}\) molar equivalent of the usual 0.060 per cent p-dimethylaminobenzene) was fed for 2\(\frac{1}{4}\) months, the incidence of hepatic tumors 2 months later was 100 per cent.

2. p-Monomethylaminobenzene was at least as carcinogenic as p-dimethylaminobenzene. Actually more tumors developed when rations containing the monomethyl compound were fed ad libitum than when p-dimethylaminobenzene was fed, but the rats fed the monomethyl compound ate more food, and therefore consumed more dye.

3. In young rats m'-methyl-p-dimethylaminobenzene proved to be the most toxic of the three dyes.

REFERENCES


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