Observations on Rats Fed with Compounds Related to Dimethylaminoazobenzene


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(Received for publication, November 6, 1944)

Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat and to a lesser extent in other species (1, 3-5). N,N-dimethyl-p-aminoazobenzene has been found to be the most potent carcinogen of this type so far tested (3, 6).

It is our purpose in this paper to report observations concerning relative carcinogenic activity in the rat of several compounds of this series that have not been previously tested.

EXPERIMENTAL

A 3 per cent solution of N,N-dimethyl-p-aminoazobenzene or molar equivalent amounts of other compounds was prepared, and 20 cc. were evenly mixed with 1,000 gm. of coarsely ground, unpolished rice; i.e., 0.6 gm. of the compound per kg. The rats were permitted to eat as much of the mixture as they desired; all received a small amount of fresh carrots daily and unlimited quantities of water were permitted. Feeding was continued until the animals either succumbed or were sacrificed. The rats used in this study were of the Sherman stock, with an initial body weight of about 150 gm.

The results obtained are summarized in Table I. In all cases the gross diagnosis was confirmed by microscopic examination.

As may be seen, the daily ingestion of large amounts of N,N-diethyl-p-aminoazobenzene, N,N-dipropyl-p-aminoazobenzene, N,N-dibutyl-p-aminoazobenzene, and N,N-diamyl-p-aminoazobenzene, the higher homologues of N,N-dimethyl-p-aminoazobenzene, failed to produce liver cirrhosis or tumors in rats during a feeding period lasting 78 to 511 days. On the other hand, the animals fed N,N-dimethyl-p-aminoazobenzene developed typical liver cancer in all cases in comparatively shorter periods of time.

It is interesting to note that approximately the same degree of carcinogenic activity is attained by the N,N-dimethyl-p-aminoazobenzene compound as by the N,N-dimethyl derivative. Cholangiomas (bile duct carcinoma) or hepatomas developed in all 10 rats fed N,N-dimethyl-p-aminoazobenzene for 108 to 228 days (Fig. 1). Two of the rats also had metastases to the mesentery and omentum.

It was found, however, that when the methyl group is attached to the second phenyl ring (p) instead of to the amino group, the carcinogenicity is greatly reduced. Of 12 rats that received 4-methyl-4'-aminoazobenzene orally only 1, which died 427 days after the beginning of the experiment, showed a liver with neoplastic growth. The organ was covered with large cancer nodules, and metastases to the mesentery, omentum, and diaphragm were found; 11 rats, or 92 per cent, had smooth, practically normal livers.

Although 3,2'-dimethyl-4-aminoazobenzene (o-aminoazotoluene) is highly carcinogenic, 2',3',4',5',6'-pentamethyl-4-aminoazobenzene is noncarcinogenic.

During the course of this study the effect upon the rat liver of feeding a combination of aniline and N,N-dimethyl-p-phenylene diamine, split products of N,N-dimethyl-p-aminoazobenzene, was investigated. Molecular equivalent amounts of these substances were well mixed with unpolished rice and fed in the usual manner. The results of this experiment showed that the daily ingestion of large amounts of these substances failed to produce liver cirrhosis or tumors in rats during experimental periods of 147 to 515 days.

Diacetyl-p-phenylene diamine, one of the metabolites of N,N-dimethyl-p-aminoazobenzene, was similarly tested for its carcinogenicity. None of the 10 rats fed the acetylated paraphenylenediamine showed cirrhosis or neoplastic changes in the liver. Two of these animals lived more than 240 days.

The liver of a rat fed N,N-diethyl-p-aminoazobenzene showed a few flecks. Microscopic sections of the liver showed neither cirrhosis nor tumor.

While the livers of animals fed N,N-diethyl-p-aminoazobenzene and N,N-dipropyl-p-aminoazobenzene showed no great changes in color, size, or shape many of the livers of animals fed N,N-dibutyl-p-aminoazobenzene and N,N-diamyl-p-aminoazobenzene were pale and yellowish in color. The livers had smooth surfaces and histological examination showed no evidence of tumors, bile duct changes, or abnormal regeneration of the ducts and liver cells, or any abnormal nuclear alterations.
The only visceral changes seen were those arising from bronchopneumonia. No tumor was found in the visceral organs other than the liver or elsewhere, except that the stomach of one rat fed N,N-dibutyl-N-methyl-p-aminoazobenzene, was found to be highly carcinogenic. An isomer of this compound, 4'-methyl-4-aminoazobenzene, was only slightly carcinogenic (only 1 rat out of 12 showed cirrhotic and neoplastic changes). The carcinogenic potency of these 3 compounds correlates relatively well with the in vitro "toxicity" of the diamine split products (2).

All the higher alkyl homologues of N,N-dimethyl-p-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the rat when fed in equimolar amounts.

### Table I: Incidence of Hepatic Tumors in Rats Fed Various Azo and Related Compounds

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Structural formula</th>
<th>No. of animals</th>
<th>Amount ingested daily, mgm.</th>
<th>No. of days fed</th>
<th>Liver findings *</th>
<th>Incidence of liver cancer, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-Dimethyl-p-aminoazobenzene</td>
<td></td>
<td>34</td>
<td>4.8</td>
<td>100-280</td>
<td>0 0 3 20 11 100</td>
<td></td>
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<tr>
<td>N,N-Diethyl-p-aminoazobenzene</td>
<td></td>
<td>10</td>
<td>4.8</td>
<td>88.511</td>
<td>10 0 0 0 0 0</td>
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<tr>
<td>N,N-Dipropyl-p-aminoazobenzene</td>
<td></td>
<td>10</td>
<td>4.8</td>
<td>78.207</td>
<td>10 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>N,N-Dibutyl-p-aminoazobenzene</td>
<td></td>
<td>10</td>
<td>4.8</td>
<td>97.496</td>
<td>10 0 0 0 0 0</td>
<td></td>
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<tr>
<td>N,N-Diamyl-p-aminoazobenzene</td>
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<td>10</td>
<td>4.8</td>
<td>134.468</td>
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<tr>
<td>N-Methyl-p-aminoazobenzene</td>
<td></td>
<td>10</td>
<td>4.8</td>
<td>108.228</td>
<td>0 0 2 6 2 100</td>
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<tr>
<td>2',3',4',5',6'-Pentamethyl-4'-aminoazobenzene</td>
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<td>12</td>
<td>4.8</td>
<td>73.768</td>
<td>11 0 0 0 1 8</td>
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<tr>
<td>N,N-Dimethyl-p-phenylenediamine</td>
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<td>15</td>
<td>4.4</td>
<td>147.515</td>
<td>15 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>N,N-Dibutyl-p-phenylenediamine</td>
<td></td>
<td>5</td>
<td>6.5</td>
<td>146.493</td>
<td>5 0 0 0 0 0</td>
<td></td>
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<td>Diacetyl-p-phenylenediamine</td>
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<td>10</td>
<td>5.0</td>
<td>100.240</td>
<td>10 0 0 0 0 0</td>
<td></td>
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<tr>
<td>N,N-Dimethyl-p-phenylenediamine plus aniline</td>
<td></td>
<td>15</td>
<td>4.4</td>
<td>147.515</td>
<td>15 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

* - indicates smooth, practically normal liver; + + indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma or both; ++ indicates extensive liver cancer without metastasis; +++ indicates extensive liver cancer with metastasis.

1 N,N-Dimethyl-p-aminoazobenzene, No. 338, Eastman Kodak Company.

All the alkyl radicals were of the normal series.
Fig. 1.—Gross appearance of livers (A and B) of rats fed N-methyl-p-aminooazobenzene-rice diet for 150 and 228 days respectively, showing cholangioma and hepatoma nodules.

Fig. 2.—A. Section of liver showing cholangiomatous type of tumor. Hematoxylin and eosin. Mag. × 90. (For gross liver see Fig. 1-A.) B. Same section at higher magnification. Mag. × 210.
Thus whereas methylation of the amino nitrogen of p-aminooazobenzene renders the molecule highly carcinogenic, alkylation with ethyl, propyl, butyl, and amyl groups does not.

To date no attempts to produce liver cancer in the rat by the administration of split products and related compounds of N,N-dimethyl-p-aminooazobenzene have been successful.

SUMMARY

1. All rats fed 4.8 mgm. of N,N-dimethyl-p-aminooazobenzene daily for a period of 150 days developed liver cancer.

2. Daily ingestion of 4.8 mgm. of N,N-diethyl-p-aminooazobenzene, N,N-dipropyl-p-aminooazobenzene, N,N-dibutyl-p-aminooazobenzene, or N,N-diamyl-p-aminooazobenzene failed to produce liver cancer, an indication of the importance of the methyl radical for carcinogenesis.

3. Although N-methyl-p-aminooazobenzene is highly carcinogenic its isomer, 4'-methyl-4-aminooazobenzene, is only feebly so.

4. Daily ingestion of 2',3',4',5',6'-pentamethyl-4-aminooazobenzene, p-sulfamylamidodimethylaniline, N,N-dibutyl-p-phenylenediamine, diacetyl-p-phenylenediamine, and combined feeding with N,N-dimethyl-p-phenylenediamine and aniline, failed to produce liver cirrhosis or tumors in rats.

The authors wish to thank Professor L. F. Fieser and Dr. E. Berliner, of Harvard University, for the samples of the N-methyl-p-aminooazobenzene, 4'-methyl-4-aminooazobenzene, and 2',3',4',5',6'-pentamethyl-p-aminooazobenzene used in this investigation. We wish also to thank Dr. M. L. Crossley, of Calco Chemical Division, American Cyanamid Company, for N,N-diethyl-p-aminooazobenzene, N,N-dipropyl-p-aminooazobenzene, N,N-dibutyl-p-aminooazobenzene, N,N-diamyl-p-aminooazobenzene, N,N-dibutyl-p-phenylenediamine, and p-sulfamylamidodimethylaniline; Dr. Leonor Michaelis, of The Rockefeller Institute for Medical Research, for N,N-dimethyl-p-phenylenediamine; and Dr. E. S. Stevenson, of Memorial Hospital, for diacetyl-p-phenylenediamine.

REFERENCES


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Cancer Res 1945;5:235-238.

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