The Carcinogenicity of Certain Compounds Related to *p*-Dimethylaminoazobenzene*

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Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat (1-7). N,N-dimethyl-*p*-aminoazobenzene and N-methyl-*p*-aminoazobenzene have been found to be equally carcinogenic (4, 7). They produced cholangiomas and hepatomas in all animals tested in approximately the same period of time. N,N-dimethyl-3'-methyl-4-aminoazobenzene was more carcinogenic than the parent compound N,N-dimethyl-*p*-aminoazobenzene (4); but the N,N-dimethyl-2'-methyl-4-aminoazobenzene and N,N-dimethyl-4'-methyl-4-aminoazobenzene were very much less active. N,N-diethyl-*p*-aminoazobenzene and all other higher alkyl homologues of N,N-dimethyl-*p*-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the liver of the rat when fed in equimolecular amounts (7).

The investigation has been extended to several compounds of this series which have not been previously tested.

EXPERIMENTAL

A 3 per cent solution of N,N-dimethyl-*p*-aminoazobenzene or molar equivalent amounts of the other compounds were prepared with cottonseed oil, and 20 cc. were evenly mixed with 1,000 gm. of coarsely ground, unpolished rice; *i.e.*, 0.6 gm. of the compound per kgm. 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 died or were sacrificed at the end of the experimental period of 250 days. The rats used in this study were Sherman strain males weighing about 150 gm.

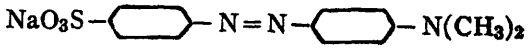
The results obtained are summarized in Tables I and II. In all cases the gross diagnosis was confirmed by microscopic examination.

Although the number of days of feedings was the same, individual rats consumed different amounts of azo compounds (6). Therefore, we have calculated the approximate amounts of azo compounds consumed daily by each animal from the average amount ingested by a group of rats at different times during the experimental period. These figures are given in the fifth column of Table II.

As may be seen from the data in Tables I and II, N-methyl-3'-methyl-4-aminoazobenzene was at least as carcinogenic as the N-methyl or N,N-dimethyl-*p*-aminoazobenzene. The animals fed N-methyl-3'-methyl compound developed typical liver cancer in all cases during experimental periods of 141 to 225 days. However, the N-methyl-2'-methyl-4-aminoazobenzene and the N-methyl-4'-methyl-4-aminoazobenzene were very much less carcinogenic, incidence of liver cancer being 27 and 20 per cent, respectively, during the experimental period of 100 to 250 days.

N,N-diethyl-*p*-aminoazobenzene failed to produce cirrhosis or liver tumors, but N-methyl-N-ethyl-*p*-aminoazobenzene was definitely carcinogenic (67 per cent liver cancer by 250 days), an indication of the importance of the methyl radical for carcinogenesis. N,N-diethanol-*p*-aminoazobenzene was similarly tested for its carcinogenicity. None of the 10 rats fed the compounds showed cirrhosis or neoplastic changes in the liver during 313 days.

It is of interest that addition of a hydroxy group in the para prime position of *p*-dimethylaminoazobenzene resulted in a compound possessing no carcinogenicity. The livers of rats fed N,N-dimethyl-4'-hydroxy-4-aminoazobenzene 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 alteration. Introduction of a water-soluble group in the 4' position may change the character of a carcinogenic compound, *p*-dimethylaminoazobenzene. Thus, methyl orange,



which is water-soluble, is not carcinogenic (2).

Unfortunately many of the animals fed N,N-dimethyl-4'-hydroxy-4-aminoazobenzene died within 150 days without noteworthy liver change or other visceral changes except congestion of the lungs. Therefore, the experiment was repeated using heavier rats, about 200 gm., but the toxic effect

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of the compound on the animal did not diminish. Of the 10 rats, 8 died between 95 and 150 days after the beginning of the experiment and the remaining 2 rats were sacrificed on the 212th day. Microscopic examination of the liver revealed neither cirrhosis nor tumor formation.

The failure of N,N-dimethyl-4′-hydroxy-4-aminoazobenzene to produce liver tumors may lie in the fact that only 4 animals lived over 150 days. A small probability of liver tumor production may have been missed because of the small number of survivors. Of course the compound may be noncarcinogenic.

Nutrition of young adult rats during ingestion of the N,N-dimethyl-*p*-aminoazobenzene, N-methyl-*p*-aminoazobenzene, N-methyl-2′-methyl-4-aminoazobenzene, N-methyl-3′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene in addition to the rice diet with supplement of fresh carrots, may be briefly summarized as follows: The majority of the rats fed N,N-dimethyl-*p*-aminoazobenzene, N-methyl-*p*-aminoazobenzene and N-methyl-3′-methyl-4-aminoazobenzene lost body weight from the beginning of the experiment. Some gained weight slightly during the first 2 weeks, and maintained their weight for about 2 months. At this time, the general appearance of the animals was good, but thereafter, when malig-

TABLE I: INCIDENCE OF HEPATIC TUMORS IN RATS FED VARIOUS AZO COMPOUNDS

Compound fed	No. of animals	Liver findings at autopsy*
N,N-Dimethyl-*p*-aminoazobenzene	15	+ 95, ± 101, + 115, + 137, + 150, ++ 150, ++ 150, +++ 153, ++ 166, ++ 168, +++ 168, +++ 168, + 192, ++ 200, +++ 250 days
N-Methyl-*p*-aminoazobenzene	14	+ 132, +++ 147, +++ 155, ++ 157, + 171, ++ 177, +++ 181, ++ 182, + 183, + 190, +++ 200, ± 237, + 250, +++ 250 days
N-Methyl-2′-methyl-4-aminoazobenzene	15	− 98, − 107, − 124, − 127, + 138, + 184, − 204, − 211, + 230, +++ 230, − 234, ± 237, − 247, − 247, − 250 days
N-Methyl-3′-methyl-4-aminoazobenzene	15	++ 141, +++ 141, + 145, + 147, +++ 151, ++ 156, ++ 156, + 161, + 167, ++ 171, + 172, ++ 173, ++ 174, + 175, +++ 225 days
N-Methyl-4′-methyl-4-aminoazobenzene	15	± 100, + 189, − 199, ± 202, − 234, + 237, − 247, ± 247, − 250, − 250, − 250, − 250, − 250, ± 250, + 250 days
N,N-Diethyl-*p*-aminoazobenzene	20	− 123, − 162, − 166, − 171, − 228, − 235, − 238, − 240, ± 240, − 289, − 313, − 313, − 313, − 313, − 313, − 313, − 313, − 313, − 313, − 313, − 313 days
N,N-Diethanol-*p*-aminoazobenzene	10	− 162, − 165, − 175, − 228, − 289, − 313, − 313, − 313, − 313, − 313 days
N-Methyl-N-ethyl-*p*-aminoazobenzene	15	− 110, + 133, ++ 151, + 182, − 199, + 200, + 202, ++ 232, ++ 236, − 250, − 250, − 250, − 250, − 250, − 250 days
N,N-Dimethyl-4′-hydroxy-4-aminoazobenzene	16	− 87, − 90, − 98, − 98, − 106, − 111, − 112, − 115, − 116, − 116, − 120, − 121, − 121, − 121, − 250, − 250 days

* − 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.

nant changes began to appear in the liver, the condition of the animals was poor. On the other hand, animals on N-methyl-2′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene gained weight or maintained their body weight during the first 2 months. About 70 per cent of the animals presented a healthy appearance throughout the experimental period of 250 days.

Nutrition of young adult rats during ingestion of N,N-diethyl-*p*-aminoazobenzene and N,N-diethanol-*p*-aminoazobenzene was good. Practically all animals gained weight and presented a healthy appearance throughout the experimental period of 313 days. Rats fed N-methyl-N-ethyl-*p*-aminoazobenzene-rice diet maintained their body weight during the first 2 months, and thereafter their physical appearance was poor except in about 30 per cent of the animals, which did not develop liver tumors.

Table II. Incidence of Hepatic Tumors in Rats Fed Various Azo Compounds

Chemical compound (Structural formula)	No. of animals	Per cent in diet	Amount ingested daily, mgm.	No. of days fed	Liver findings*					Incidence of liver cancer, per cent
					–	±	+	++	+++	
N,N-Dimethyl-*p*-aminoazobenzene	15	0.06	4.8	95–250	0	1	5	5	4	93
N-Methyl-*p*-aminoazobenzene	14	0.056	4.8	132–250	0	1	5	3	5	93
N-Methyl-2′-methyl-4-aminoazobenzene	15	0.06	6.0	98–250	10	1	3	0	1	27
N-Methyl-3′-methyl-4-aminoazobenzene	15	0.06	4.5	141–225	0	0	6	6	3	100
N-Methyl-4′-methyl-4-aminoazobenzene	15	0.06	6.0	100–250	8	4	3	0	0	20
N-N,-Diethyl-*p*-aminoazobenzene	20	0.067	7.4	123–313	19	1	0	0	0	0
N,N-Diethanol-*p*-aminoazobenzene	10	0.076	8.4	162–313	10	0	0	0	0	0
N-Methyl-N-ethyl-*p*-aminoazobenzene	15	0.064	4.8	110–250	5	0	6	4	0	67
N,N-Dimethyl-4′-hydroxy-4-aminoazobenzene	16	0.064	4.0	87–250	16	0	0	0	0	0

* See Table I for explanation of symbols.

liver cancer, but daily ingestion of about 4.8 mgm. of N-methyl-N-ethyl-*p*-aminoazobenzene produced liver cancer, an indication of the importance of the methyl radical for carcinogenesis.

4. An hydroxy group in the para prime position of *p*-dimethylaminoazobenzene resulted in a compound possessing no carcinogenicity under these conditions.

SUMMARY

1. Nearly all of the rats which were fed about 4.8 mgm. of N,N-dimethyl-*p*-aminoazobenzene or N-methyl-*p*-aminoazobenzene daily for periods of 100 to 250 days developed hepatic cancer.

2. Although N-methyl-3′-methyl-4-aminoazobenzene is at least as carcinogenic as the N-methyl or N,N-dimethyl compound, is isomers: N-methyl-2′-methyl-4-aminoazobenzene and N-methyl-4′-methyl-4-aminoazobenzene, are much less carcinogenic.

3. Daily ingestion of about 7.4 mgm. of N,N-diethyl-*p*-aminoazobenzene or about 8.4 mgm. of N,N-diethanol-*p*-aminoazobenzene failed to produce

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