Effect of Diet on *N,N*-dimethyl-*p*-(*m*-tolylazo)aniline Carcinogenesis in Rats (35691)

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In azo dye carcinogenesis, it is generally agreed that a diet deficient in protein and riboflavin is essential for obtaining a high yield of hepatomas in rats in a reasonably short period of time. However, induction of liver tumors with other carcinogens, such as nitrosamines, requires a complete diet in order to maintain the rats through the induction period and obtain a good incidence of tumors. Thus, it was found desirable to study the incidence of azo dye hepatomas in rats maintained on complete diet of Purina Laboratory Chow, and establish a dose-incidence curve as a preliminary to comparative studies of the effect of different carcinogens on hepatocarcinogenesis. A dose-response curve for *N,N*-dimethyl-*p*-phenylazobenzene (DAB), when fed on Purina Laboratory Chow has been reported from this laboratory (1). The carcinogen *N,N*-dimethyl-*p*-(*m*-tolylazo) aniline (3′-Me-DAB) is reported to be more potent than DAB (2) and dietary factors, such as protein, riboflavin, and fat, seem to have less influence on the incidence of hepatomas (3). This study confirms these findings, establishes a dose-response curve for 3′-Me-DAB for male and female Osborne–Mendel rats fed on Purina Laboratory Chow, and compares the incidence and the histology of the lesions induced with those induced in rats fed on semisynthetic diet.

Materials and Methods. Experimental groups. 1. Three-month-old, inbred, Osborne–Mendel rats were fed powdered Purina Laboratory Chow, mixed with 3′-Me-DAB at three different concentrations as shown in Table I. 2. NIH black (from Long–Evans stock), Marshall 520, and Osborne–Mendel rats were fed a semisynthetic diet deficient in protein and riboflavin (4) with 0.06% 3′-Me-DAB added to it, incorporated in corn oil.

New diet was prepared every 2 weeks and stored in a cold room until used. The rats were given the carcinogenic diet for 4 months and then were fed basic diet for another 2 months, when the experiments were terminated. At the termination of the experiments, the rats were killed with ether and the livers and other organs were examined for tumors, metastases, and other lesions. Specimens for histologic studies were taken from each liver lobe, lung, tumor nodules, and other lesions. All specimens were fixed in Zenker's acetic acid fluid, embedded in paraffin, and routinely stained with hematoxylin and eosin.

The concentration of 0.12 and 0.18% 3′-Me-DAB was too toxic for male and female rats, respectively, and most of the males died in 40 and females in 80 days. They are excluded from the results.

Of the hepatomas induced in NIH black rats, three were propagated through eight transplant generations.

Results. The concentrations of the carcinogen 3′-Me-DAB used in the Purina Laboratory Chow diet and the incidences of the hepatomas induced are given in Table I. Relative incidences of various liver lesions induced and those of metastases in all the rats receiving 3′-Me-DAB either in Purina Laboratory Chow or in semisynthetic diet are shown in Table II.

The Osborne–Mendel rats on Purina chow diet developed hepatic tumors in 100% of the animals, at the highest tolerated dose of 3′-Me-DAB and almost 80% of these tumors

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TABLE I. 3′-Me-DAB Intake by Osborne–Mendel Rats for 4 Months in Purina Laboratory Chow and the Incidence of Hepatoma in 6 Months.

Effective (no., sex)	% of 3′-Me-DAB in the diet	Average food intake (gm/rat/day)	Total 3′-Me-DAB intake (gm/rat)	Hepatomas induced (%)	% Change in body weight
24 ♂	—	28	—	—	+40
10 ♂	0.06	27	1.94	40	+38
6 ♂	0.09	22	2.38	67	+33
5 ♂	0.098	21	2.48	100	+24
24 ♀	—	23	—	—	+35
6 ♀	0.06	23	1.66	0	+34
6 ♀	0.09	21	2.27	33	+31
6 ♀	0.12	18	2.59	100	+22

metastasized. At the lowest level of the carcinogen, 0.06%, 40% of the males but none of the females developed tumors. By comparison, the rats on the deficient diet, both male and female developed 100% liver tumors with 0.06% 3′-Me-DAB in their diet. However, only 30% of the males and none of the females showed any metastases on this diet. Similarly, both Marshall and NIH black rats showed very high incidence of liver tumors with 0.06% 3′-Me-DAB in deficient diet and relatively low incidence of metastasis.

In all groups of rats fed 3′-Me-DAB, regardless of the strain, sex, or the diet used, histology of the liver lesions induced was similar. These lesions ranged from well-differentiated hepatomas to poorly differentiated hepatocellular carcinomas. Areas of duct formation were noted in some cases and invasion by hepatoma cells was noted in others. Varying degree of hemorrhage and necrosis was usually present in the larger tumors. When semisynthetic diet was substituted for chow diet, not only hepatoma incidence was higher but the number of tumors per rat and the relative size of the tumors was also increased. In a single liver lobe, one sometimes found a nodule of well-differentiated eosinophilic hepatoma cells, a nodule of less-differentiated amphophilic cells, and a nodule of poorly differentiated basophilic cells which showed marked invasive qualities. Residual liver contained more or less extensive areas of cholangiofibrosis, cirrhosis, and cysts.

As a general rule, hyperplastic nodules and areas of pseudotubule proliferation were found more often in the livers of rats that came to autopsy while still on the carcinogenic diet. The liver biopsies, done on four female rats at the end of the period of feeding carcinogenic diet, showed many hyperplastic nodules and areas of pseudotubule proliferation. The same animals when autopsied after 2 months on the basic diet, showed very few of these lesions, but instead three rats had well-developed hepatoma nodules.

Discussion. In the present study dose-response curve of tumor incidence has been established for male and female Osborne–Mendel rats fed *N,N*-dimethyl-*p*-(*m*-tolylazo)aniline (3′-Me-DAB) in Purina Laboratory Chow. Other workers (3, 5, 6) used a combination of 3′-Me-DAB and complete diet in their experiments. However, they do not yield any dose-response information, as their experiments had different objectives. When working with *N,N*-dimethyl-*p*-phenylazoaniline (DAB) fed in chow diet it was reported (1) that it took 0.18% DAB (maximum tolerated dose) to give 50% hepatoma incidence, in male Osborne–Mendel rats. This was three times the concentration required to give 100% incidence on deficient diet. In the present study, it took 1.6 times as much 3′-Me-DAB to give 100% tumor incidence in male Osborne–Mendel rats on chow diet as it took to get the same incidence with deficient diet. In female O–M rats on chow diet, 0.06% carcinogen which gives 100% tumor incidence on deficient diet, did not induce any tumors. It took 0.12% carcinogen to get 100% incidence. These results are consistent with those of Giese *et al.* (3) who

TABLE II. Incidence (%) of Liver Lesions in Rats Fed 3′-Me-DAB in Either Purina Laboratory Chow or Semisynthetic Diet.

Treatment (% 3′-Me-DAB in diet)	Strain	Effective (no., sex)	Hyperplastic nodule	Hepatoma	Prolif. pseudotubules	Cholangiofibrosis	Metastasis lung
Purina Laboratory Chow diet							
0.06	O–M	6 ♀	17	—	67	33	—
0.06	O–M	10 ♂	—	40	10	70	—
0.09	O–M	6 ♀	67	33	67	67	17
0.09	O–M	6 ♂	—	67	—	83	50
0.098	O–M	5 ♂	—	100	—	100	80
0.12	O–M	6 ♀	—	100	—	100	83
Semisynthetic diet							
0.06	O–M	9 ♀	33	100	—	100	—
0.06	O–M	10 ♂	—	100	—	70	30
0.06	Marshall	7 ♂	14	100	28	85	57
0.06	NIH black	10 ♂	40	90	—	60	30

found that protein and riboflavin are less effective in altering tumor incidence with 3′-Me-DAB than that with DAB. Cortell (6) found that if 3′-Me-DAB-containing deficient diet was followed by adequate diet, more malignant tumors developed that metastasized more freely than when it was followed by deficient diet. Our results also show a higher rate of metastasis in tumors of the rats on show diet than of those on deficient diet.

The hyperplastic liver nodules, which show some of the early cytological changes seen in the carcinomas, were more frequent in the rats still on carcinogenic diet than in the animals after a period of carcinogen-free diet. Biopsy studies in this connection suggest a complete disappearance of these nodules in some cases and their progression to carcinoma in others. Farber (7) described similar hyperplastic nodules induced in rat livers with ethionine. It is postulated that the hyperplastic nodules in early stages of development, when the carcinogen is removed from the diet, disappear and those in later stages of development progress to hepatoma.

Summary. *N,N*-Dimethyl-*p*-(*m*-tolylazo) aniline (3′-Me-DAB), mixed either in powdered Purina Laboratory Chow or in semisynthetic diet was fed to rats for 4 months. The animals were then returned to their respective basic diet for the next 2 months, when the experiment was terminated and the animals examined for possible lesions. Male Osborne–Mendel rats on chow diet showed a hepatoma incidence of 40, 67, and 100% in rats receiving 0.06, 0.09, and 0.098% 3′-Me-DAB, respectively, and female rats showed a hepatoma incidence of 0, 33, and 100% in animals receiving 0.06, 0.09, and 0.12% 3′-Me-DAB, respectively. The carcinogen concentration of 0.12 and 0.18% was too toxic for male and female rats, respectively. In the rats receiving 0.06% 3′-Me-DAB in semisynthetic diet, hepatoma incidence was 100% in all groups except NIH black where it was 90%.

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