neys. Use of hamster kidney tissue cultures as a selective system in identification of agents from cases of aseptic meningitis was reported.

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Incidence of Liver Tumors in Male and Female Rats Fed Carcinogenic Azo Dyes. (24613)

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Sex and sex hormones have an influence on carcinogenesis in the liver. Female mice develop more liver tumors than males following administration of *O*-aminoazotoluene(1). Testosterone administration lowers this incidence (2), and stilbesterol-treated female mice develop liver tumors more readily than untreated females when fed diets containing *O*-aminoazotoluene(3). However, male rats of several strains are more susceptible to development of liver tumors than females, when they are fed diets containing acetylaminofluorene (4,5,6). Working with carcinogenic azo dyes. Rumsfeld *et al.*(7) find a higher incidence of liver tumors in male than in female rats fed 3'-methyl-4-dimethylaminoazobenzene or 4'-fluoro-4-dimethylaminoazobenzene. The present report describes the relative incidence of liver tumors in male and female Osborne-Mendel rats fed azo dyes, *p*-dimethylaminobenzene-1-azo-1-naphthalene (DAN), *p*-dimethylaminobenzene-1-azo-2-naphthalene (DA-2-N), or *p*-dimethylaminoazobenzene (DAB).

Materials and methods. Male and female Osborne-Mendel rats, 3-4 months old, were fed a semisynthetic diet containing *p*-dimethylaminobenzene - 1 - azo - 1 - naphthalene (DAN), *p* - dimethylaminobenzene - 1-azo-2-naphthalene (DA-2-N), or *p*-dimethylaminoazobenzene (DAB). The diet consisted of cerulose sugar 78%, vitamin-low casein 12%, salt mixture 4%, corn oil 5%, and vitamin mixture 1%. The composition of the vitamin mixture in mg % was thiamine hydrochloride 30, riboflavin 20, choline chloride 300, calcium pantothenate 70, pyridoxine hydrochloride 25, and nicotinic acid 50. In addition each animal received one drop of halibut liver oil once a week. Treatment of rats in each experiment is tabulated in Table I. *DAN*: Seventy-five male and 60 female rats received 0.075% DAN in the diet for 300 days. Ten rats each received respectively, 0.15, 0.3, and 0.6% DAN for 300 days. *DA-2-N*: Forty male and 24 female rats were fed a diet containing 0.075% DA-2-N for 230 days. *DAB*: Eighty male and 40 female rats were fed diets containing 0.06% DAB. Another group of

TABLE I. Incidence of Liver Tumors in Osborne-Mendel Rats Fed Carcinogenic Dyes.

Treatment				Tumors induced			
Carcinogen			Induction	♂		♀	
	%	Days fed	period (days)	Incidence	%	Incidence	%
DAN	.075	300	270	43/57	75	8/48	17
	.15	"	"			1/9	11
	.3	"	"			3/5	60
	.6	"	"	2/4	50		
DA-2-N	.075	230	80	39/40	98	24/24	100
DAB	.06	280	150	56/66	85	6/31	19
DAB and protein	.06 8	"	"			2/52	4

60 female rats receiving 0.06% DAB were given 8% protein in place of the usual 12% in their diet. All animals were placed on a diet of purina chow pellets, when their carcinogenic diet was discontinued. Daily observations were made on diet consumption and general health of each rat. Animals were palpated for tumor growth and weighed every 2 weeks, and killed for study when tumors became large, or when rats appeared very sick. All clinical diagnoses of tumor were confirmed by histologic diagnosis after postmortem examination. Multiple sections were examined from all livers and from all conspicuous tumors. Tissues were fixed in Zenker-acetic and microscopic sections were stained routinely with hematoxylin and eosin.

Results. Number and percentage of liver tumors induced in each experiment with azo compounds DAN, DA-2-N, and DAB are shown in Table I. DAN and DAB induced more liver tumors in male than in female Osborne-Mendel rats; while DA-2-N fed rats showed no significant sex difference in incidence of these tumors.

The first liver tumor was palpated on 270th day in DAN fed rats, on 150th day in DAB fed, and on 80th day in DA-2-N fed rats, after start of the carcinogenic diet. "Induction period" is defined as the interval between beginning of experiment and appearance of first palpable tumor. All incidence figures are based on number of animals that survived the induction period.

The 3 carcinogens used, differ from one another in having a benzene, an *a*-naphthalene, or a *b*-naphthalene radical, and are similar in having *p*-dimethylaminobenzene with an azo linkage. When an equimolal weight of each carcinogen was fed to different groups of rats, no significant difference was observed in diet consumed, general health, or weight during the induction period.

Differences in induction periods of these compounds probably reflect the differences in metabolism, detoxification, and elimination of the compounds and capacity of liver to carry on these functions. There have been a number of reports about metabolism of DAB by the rat liver, but no such information is yet available for DAN and DA-2-N.

In DAN fed rats, 57 males survived the induction period and of these 43 developed liver tumors, an incidence of 75%, while only 8 out of 48 female rats had liver tumors, an incidence of 17%. The average time for appearance of palpable tumors in males and females was 445 and 538 days respectively. Microscopic examination of liver tissue showed no significant difference in incidence or extent of cirrhosis, fatty changes, bile duct cysts or cholangiofibrosis, between livers of male and female rats. However, as female rats came to necropsy about 3 to 4 months later than male rats, their lesions have taken that much longer time to develop.

Female rats receiving twice as much DAN (0.15%) as in the first experiment, did not show any increase in incidence of liver tumors. However, when the dose of carcinogen was further increased to 0.3 and 0.6%, only about half of the animals survived the induction period, and none survived over a year. In rats fed 0.3 and 0.6% DAN, about half of survivors developed liver tumors. Increasing the dose of DAN failed to shorten the induction

TABLE II. Time of Appearance of Palpable Liver Tumors in Rats Fed DA-2-N.

	Tumors palpated			
	♂		♀	
Time in days	No.	%	No.	%
Under 210	4	10	3	12
211-270	27	69	4	17
271-330	6	15	10	42
>331	2	5	7	29

period of 270 days for this compound. Daily food consumption by rats fed higher doses of DAN was not significantly different from that of rats on 0.075% DAN in the diet. Since effective higher doses of the carcinogen failed to shorten the induction period, it appears that concentration of DAN was not the limiting factor in induction of tumors in these experiments. Rates of metabolism of the azo dye, detoxification and elimination of metabolites, and depletion of reserve capacity of liver to perform these functions are some factors which may determine the pace of tumor induction.

In DA-2-N fed rats 39 out of 40 males and all 24 females developed liver tumors, an incidence of 98 and 100% respectively. Tabulation of time of first appearance of palpable tumors (Table II) reveals that in 9 months 79% of male rats had palpable tumors while only 29% of females developed palpable tumors.

Relative delay in appearance of tumors in female rats indicates a higher resistance to carcinogenesis induced by DA-2-N. If the experiment had been terminated at 270 days, it may have reflected some of this resistance in incidence figures.

In rats fed DAB, 56 out of 66 male rats and 6 out of 31 females developed liver tumors, an incidence of 85 and 19% respectively.

Since a low protein diet gives a high incidence of tumors in azo dye carcinogenesis, the protein in the diet was lowered from 12 to 8% in the hope of increasing the incidence of tumors in female rats. However, these female rats had a lower tumor incidence (4%) than females on a 12% protein diet. This reduction of protein to 8% proved to be too low to support growth, as shown by poor weight gains. Thus low incidence of tumors in this group may be a starvation phenomenon. However, these rats lived to an age of 24 to 30 months and more.

Summary. Osborne-Mendel rats fed *p*-dimethylaminobenzene - 1-azo - 1-naphthalene (DAN) and *p*-dimethylaminoazobenzene (DAB) showed a higher incidence of liver tumors in males than in females. In DAN fed rats the respective incidence was 75 and 17% and in DAB fed rats 85 and 19%. Feeding higher doses of DAN (0.3%) to rats, failed to shorten the induction period for this compound. Rats fed *p*-dimethylaminobenzene-1-azo-2-naphthalene (DA-2-N) showed no significant difference in incidence of liver tumors between males (98%) and females (100%). However, tumors were much slower in appearing in females than in males, indicating relative resistance of the female liver to carcinogenesis induced by DA-2-N.

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