Induction of Hepatomas by Thiouracil in Inbred Strains of Mice.* (28406)

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In strain C3H mice spontaneous hepatomas occur more frequently in males than in females. In his last screening of more than 300 animals, Andervont found an incidence of 5-11% in females and 6-55% in males (1). Hepatoma can be induced in rats and mice with several carcinogenic agents(2,3), the most common of which are the azo dyes. Paschkis *et al.*(4) discovered that when thiouracil is administered simultaneously with 2-acetaminofluorine (AAF), the thiouracil protects the rat liver from the hepatoma induced by the dye.

In studies on the influence of a 0.3% thiouracil (TU) diet on adrenals of gonadectomized C3H mice(5), a high frequency of hepatoma was incidentally found. Since the etiologic factors in hepatoma are obscure it seemed worthwhile to study this finding in more detail.

Materials and methods. Two inbred strains of mice were used in this experiment. One was a subline of C3H mice maintained in our laboratory since 1948. The other was an inbred strain developed in our school and named TM. The inbreeding of the TM mouse was begun also in 1948. The experiment was started when the animals were weaned at the age of one month. Litter mates were distributed among 8 groups. Males were separated from females and all animals were housed 4 or 5 animals to a cage. The 8 groups were: females on TU diet; females on stock diet; males on TU diet and males on stock food of both C3H and TM strains.

The diet containing 0.3% thiouracil (Lederle) was prepared by grinding Rockland rat diet and blending with the drug in a ball mill for 2 hours. Controls were fed the Rockland food which is the regular colony diet. Food and tap water were offered to all animals *ad libitum*. Mice were numbered and weighed weekly. They were sacrificed at the age of 18 months when the TU fed groups had been taking the goitrogenic diet for 17 months. Their livers were carefully inspected and specimens of the hepatomas found were fixed in Bouin's fluid, sectioned, and stained with hematoxylin and eosin for microscopical studies.

Results. The hepatomas found in the animals given TU appeared to be identical to those found in their controls. Macroscopically the tumors were multiple, well limited, lobulated nodules. In some animals they were present in all of the lobes of the liver. Microscopically they consisted of areas of atypical cells which compressed the adjacent normal tissue. These cells were enormously enlarged and filled with basophilic material. They were often vacuolated, and the hyperchromatic nuclei contained multiple nucleoli. The areas of atypical cells were not encapsulated.

The incidence of hepatomas in C3H and TM mice studied is summarized in Tables I and II.

The weight of the animals lost significance after 10 months because at this time some of the C3H females on stock diet had mammary tumors which were large enough to increase body weight. In general weight and growth rate of the animals fed TU diet were slightly lower than those of stock fed animals of both sexes and strains.

These results show that TU raised considerably the incidence of hepatoma in C3H mice of both sexes. However a similar effect was not obtained in strain TM in which spontaneous hepatoma has not been observed.

Discussion. The incidence of spontaneous hepatoma observed in female and in male mice in the control groups generally corresponds to that reported by Andervont for the C3H mouse. The somewhat lower incidence in females did not prove to be significantly different from that found in the males, therefore probably does not differ from that reported by Andervont for the female. The

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TABLE I. Incidence of Hepatoma in C_3H and TM Mice.

	Females				Males			
	TU diet		Stock diet		TU diet		Stock diet	
Strain	No. mice	Hepatoma	No. mice	Hepatoma	No. mice	Hepatoma	No. mice	Hepatoma
C_3H	16	14	24	0	13	12	32	2
TM	22	0	20	0	22	0	20	0

incidence of hepatoma produced by TU was so high in both males and females that the differences related to sex that would be expected from other studies is not apparent.

The production of hepatoma by TU in the C3H mouse is probably due to the high susceptibility of this strain for hepatomas evinced by an appreciable incidence of spontaneous hepatoma and by production of this tumor by other agents(2,3). TU was not found to produce the liver tumors in the experiment with TM mice. This suggests that genetic differences between strains of the same species are influential factors in induction of hepatomas by agents such as TU, as is true in other types of tumors.

Tannenbaum and Silverstone(7) found that caloric restriction inhibits spontaneous hepatoma in the male C3H mouse. This is in contrast to the present results in which the animals grew less, presumably because of the goitrogenic effect of TU, but still developed more hepatomas. Likewise the protective action of thiouracil against AAF induced cancer of the liver in rats is in contrast to the high rate of induction of hepatomas by TU in the C3H mouse.

TABLE II. Percentage Hepatoma in C^3H and TM Mice.

	Females		Males	
Strain	TU diet	Stock diet	TU diet	Stock diet
C_3H	87	0	92	6.6
TM	0	0	0	0

Summary. Feeding a diet containing 0.3% of thiouracil (TU) by dry weight for 18 months to a subline of C3H mice produced a nearly 100% incidence of hepatoma in both sexes. Controls given no TU had definite but significantly lower incidence of this tumor. When the inbred strain of mice TM was used, no hepatomas were observed in experimental nor in control animals. Apparently genetic susceptibility must be present for development of hepatomas and for their increased incidence in mice treated with TU.

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