

Brief Communication: Testing for Possible Effects of Cedar Wood Shavings and Bedding on Occurrence of Mammary Gland Tumors and Hepatomas in C3H-A^{vy} and C3H-A^{vy}fB Mice¹

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SUMMARY—C3H-A^{vy} and C3H-A^{vy}fB mice transferred from our colony to the laboratory of Dr. J. R. Sabine in Australia had a markedly reduced incidence of both hepatomas and mammary tumors. Sabine and co-workers (J Natl Cancer Inst 50:1237-1242, 1973) attributed the low incidence of tumors in their laboratory compared to the high incidence in this laboratory to the fact that we routinely use red cedar shavings in the bedding, whereas they use sawdust, predominantly Douglas fir. Results of tests in this laboratory showed that the difference in occurrence of tumors could not be attributed directly to either a difference in diet or bedding. There was no evidence that the cedar shavings were carcinogenic. The animals in Australia on bedding without cedar shavings were not as healthy as our animals, in that they did not grow as well and were infested with ectoparasites; apparently these factors caused the reduced occurrence of tumors.—J Natl Cancer Inst 54: 1011-1014, 1975.

MAMMARY GLAND TUMORS AND HEPATOMAS had a decreased incidence and occurred at an increased average age in colonies of strains C3H-A^{vy} and C3H-A^{vy}fB mice maintained by Sabine and co-workers (1) in Australia: both tumors developed at high incidences in these same strains in our colonies in the United States from which the Australian colonies were derived (2, 3). This reduction was noted immediately after Sabine et al. had received their stock, the incidence approaching zero by the second generation.

Two obvious differences in the care of the colonies were in the food and bedding. In Australia the mice were fed Charlick's M164 mouse cubes obtained locally, and the cages were bedded with sawdust derived predominantly from Douglas fir grown in North America. At the National Cancer Institute (NCI), the mice had been fed Purina Laboratory Chow, NCI pellets (Old Guilford), and later National Institutes of Health (NIH) rat and mouse ration; with each diet they had high incidences of tumors. NCI mice were bedded with a mixture of three-fourths pine sawdust (white, yellow, or sugar pine or a mixture of all three) and one-fourth fine shavings of the eastern red cedar (*Juniperus virginiana*). The cedar was routinely added to keep the colony free of ectoparasites.

To ascertain the cause of the reduction of tumors in the mice maintained in Australia, Dr. Sabine secured from the United States some Old Guilford food and cedar shavings such as we used. Returning some of his mice to the Old Guilford diet, he did not get an increase in tumors. But when, in addition to the Old Guilford food, he used cedar shavings for bedding, tumors increased in incidences compa-

rable with those observed in this laboratory. From these observations, Sabine et al. concluded that, although their results did not rule out other possible causes, "... the implication of cedar as a 'carcinogenic' agent was quite clear cut."

This was a serious indictment of cedar shavings in view of their widespread use as bedding for experimental animals in carcinogenic studies in the United States, their use as bedding for pets throughout the United States, and exposure of the human population to cedar wood and cedar wood products, especially cedar oil. The observation, however, was not in line with our experience with these same strains or their parent strain C3H sent to other laboratories in the United States where cedar bedding was not used and no reduction in occurrence of these tumors was noted. Furthermore, Roe and Field (4) tested cedar oil for a carcinogenic effect and obtained negative results. In addition, despite the fact that cedar wood and cedar wood products have been used by man for a long time, there has been no evidence of any carcinogenic effect in man.

We, therefore, determined to compare the occurrence of tumors in mice of these strains fed Charlick's diet with that in mice fed one of our diets. Furthermore, we particularly needed to compare the occurrence of tumors in mice on pine plus cedar bedding with that in mice on pine bedding alone. Care was taken to prevent these mice from becoming infested with ectoparasites; they remained free of any ectoparasites throughout the study.

MATERIALS AND METHODS FOR FOOD TESTS

Charlick's M164 mouse cubes were supplied to us by Dr. Sabine. This food was compared with Purina Laboratory Chow, on which our colony had formerly been maintained and had shown a high incidence of both mammary tumors and hepatomas.

C3H-A^{vy} and C3H-A^{vy}fB mice of the same strains as those sent to Dr. Sabine were tested. The C3H-A^{vy} mice were developed in this laboratory (2) with the A^{vy} mutation that occurred in the C3H/He strain at The Jackson Laboratory (5). Among the mice from this line with the A^{vy} mutation that increases body weight and tumor susceptibility, the females have an incidence of mammary tumors of approximately

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100% at an average age of 6-7 months and the males have an incidence of hepatomas approaching 100% by 12 months of age. The C3H-A^{vy}FB was derived from a C3H-A^{vy} litter foster-nursed on C57BL, which thus eliminates the milk-transmitted mammary tumor virus (3). Foster-nursing on C57BL does not affect the occurrence of hepatomas in males, but it reduces the incidence of mammary tumors in breeding females and greatly increases the average age of tumor development to 15 months.

Males of both strains and females of strain C3H-A^{vy} were used. At weaning (4 wk of age), littermates were divided equally into two groups, the one group thereafter being fed ad libitum the Charlick's diet and the other group the Purina Chow. They were segregated as to sex and maintained in plastic cages with 8 mice to the cage. They were kept on pine plus cedar bedding, given tap water, and weighed weekly. The females were kept until they developed a mammary tumor or became moribund from some other cause at which time they were autopsied. The males were autopsied at 12 months of age and examined for hepatomas or any other tumors. All tumors were fixed in Fekete's modification of Tellyesniczky's fixative (70% ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part), sectioned, and stained with hematoxylin and eosin for histologic examination.

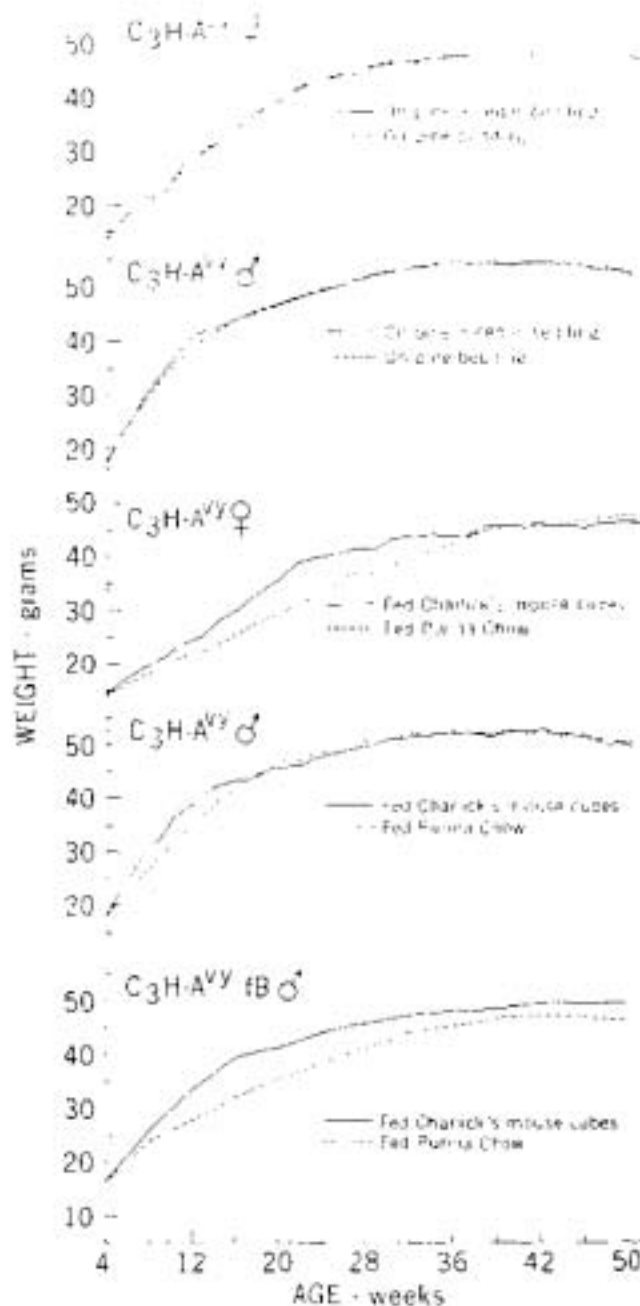
RESULTS OF FOOD TESTS

The growth curves (text-fig. 1) showed that all groups fed the Charlick's diet grew more rapidly during the early part of their life than did those fed the Purina Chow, probably because the mice ate more Charlick's cubes, since they were softer. The more rapid growth in the males fed Charlick's cubes was reflected in a slightly higher incidence of hepatomas or higher average number of hepatomas (table 1). The more rapid growth in the females fed Charlick's cubes was associated more with a lower age of developing mammary tumors, although the actual mammary tumor incidence was also slightly higher.

The fact that the mice fed the Charlick's cubes developed more tumors than those fed Purina Chow showed that the diet was not a factor in reduction of tumor occurrence in the mice in Australia. This is in line with the observations made on the diet by the Australian group.

MATERIALS AND METHODS FOR TESTS ON CEDAR SHAVINGS

To test the possible effect of cedar shavings on occurrence of tumors, the breeding colony of C3H-A^{vy} mice was divided in the following way: Half of the new matings were maintained on three-fourths pine sawdust plus one-fourth cedar shavings and the other half on pine sawdust alone. When their offspring were in turn mated, they were kept on the same kind of bedding as the parents. Additional groups of males and females maintained on pine plus cedar and on pine alone were at the time of weaning segregated as to sex, with 8 mice to each cage. These segregated animals and the breeders were fed the NIH open-



TEXT-FIGURE 1.—Growth curves of C3H-A^{vy} and C3H-A^{vy}FB mice.

formula diet and given tap water. This diet formulated by Knapka et al. (6) affords excellent growth of this strain. All females, except 2 in the segregated groups, 1 on pine, and 1 on pine plus cedar, developed mammary tumors and were autopsied. Due to lack of space, the breeding males were autopsied when their mates were and, since they were only about 6 months of age, were too young to provide data on tumor occurrence. The segregated males were autopsied at 12 months of age, and their hepatomas were recorded. All tumors were fixed, sectioned, and stained like those from the animals in the diet test.

TABLE 1.—Effect of food on occurrence of tumors in virgin mice of strains C3H-A^u and C3H-A^u/fB

Food	Strain	Sex	Number	Age (mo)	Number with hepatomas	Average No. of hepatomas	Number with mammary tumors	Average age (mo) at development of mammary tumor
Charlick's cubes	C3H-A ^u /fB	♂	48	12	31	1.632		
Purina Chow	C3H-A ^u /fB	♂	48	12	21	1.476		
Charlick's cubes	C3H-A ^u	♂	8	12	7	2.714		
Purina Chow	C3H-A ^u	♂	8	12	8	2.250		
Charlick's cubes	C3H-A ^u	♀	24		2		24	7.04
Purina Chow	C3H-A ^u	♀	24		4		22	9.27

TABLE 2.—Effect of bedding on occurrence of tumors in strain C3H-A^u mice

Bedding	Number of mice	Sex	Age (mo)	Number with hepatomas	Average No. of hepatomas	Number with mammary tumors	Average age (mo) at development of mammary tumor
Pine	56	♂	12	53	2.953		
Pine + cedar	56	♂	12	55	2.517		
Pine	54	♀ (virgins)	—	2		53*	6.09
Pine + cedar	55	♀ (virgins)	—	1		54	6.47
Pine	68	♀ (breeders)	—	2		68	6.25
Pine + cedar	64	♀ (breeders)	—	0		64	6.23

* 1 living with no tumor.

RESULTS OF TESTS ON CEDAR SHAVINGS

The weight curves (text-fig. 1) showed that the mice maintained on pine bedding alone did not differ in growth rate from those on pine plus cedar bedding. Similarly, the males maintained on pine bedding alone did not differ in hepatoma incidence from those maintained on pine plus cedar (table 2). Furthermore, females (of the breeding colony or virgins) on pine bedding alone did not differ in mammary tumor incidence from those on pine plus cedar. Thus in our laboratory, when the mice placed on pine bedding alone were kept free of ectoparasites so that their growth rates were not reduced, the hepatoma or mammary tumor incidence was unchanged from the high incidence we have formerly recorded for the C3H-A^u colony.

DISCUSSION

In any study of carcinogenesis, strict attention must be paid to the animal's condition. Any factor that will increase growth of the animal will increase occurrence of tumors and any factor that will decrease growth will decrease occurrence of tumors, all other factors being equal. Many papers could be cited for illustration, but one is particularly cited in which reciprocal F₂ hybrid mice were studied in this laboratory (7). In these animals, the incidence of hepatomas could be changed from 0 to 100% by varying of three factors: strain of mother, sex, and genotype (A^uA vs. Aa). Each of these factors had some effect on occurrence of hepatomas. Each of these factors similarly affected growth, causing an average weight at 1 year to vary from 59.2 g in the group with the combination of factors that caused

100% of them to have hepatomas to 36.8 g in the group with the combination resulting in no hepatomas. Any change in diet that will effect a change in growth is reflected in a similar change in tumor response. The Purina pellets fed in this study tended to be extra hard, and the animals given these pellets grew less well than those given Charlick's cubes. The best growth was observed in mice fed the NIH diet. Likewise, the incidence of hepatomas in the males and mammary tumors in the females, although relatively high in all groups, was highest in animals fed the NIH diet, slightly less in those fed Charlick's cubes, and lowest in those fed Purina Chow.

Our results indicate that the great reduction in occurrence of hepatomas and mammary tumors in the C3H-A^u and C3H-A^u/fB strains when transferred from our laboratory to Dr. Sabine's laboratory in Australia was not due directly to either change in diet or elimination of the cedar from the bedding. These studies revealed no evidence that cedar shavings are carcinogenic.

The addition of some cedar shavings to the bedding of experimental animals has had widespread use because they prevent infestation with ectoparasites. During the past 29 years that we have used cedar, our colonies have been free of ectoparasites. Six male and 6 female C3H-A^u/fB mice returned by Dr. Sabine to this laboratory were found on their arrival to be so heavily infested with small mites that they could hardly live. One had to be autopsied on the day of arrival. Sabine et al. stated that their animals on bedding without cedar weighed less than their U.S. counterparts and had a higher degree of infestation with ectoparasites; they acknowledged that these factors might have contributed to the lowered inci-

dence of tumors in their laboratory. When Sabine et al. returned their animals to cedar shavings and the tumor incidence was high again, the animals again grew better. It would appear that the great reduction in occurrence of tumors in the animals of Sabine et al. was caused by the lower weight gain and ectoparasitic infestation rather than by food or bedding.

REFERENCES

- (1) SABINE JR, HORTON BJ, WICKS MB: Spontaneous tumors in C3H-A^y and C3H-A^y/B mice: High incidence in the United States and low incidence in Australia. *J Natl Cancer Inst* 30:1237-1242, 1971
- (2) HESTON WE, VLADARIS G: C3H-A^y—a high hepatoma and high mammary tumor strain of mice. *J Natl Cancer Inst* 49:1161-1166, 1968
- (3) VLADARIS G, HESTON WE, SMITH GH: Strain C3H mice: Ninety percent incidence of mammary tumors transmitted by either parent. *Science* 170:185-187, 1969
- (4) ROE FJ, FIELD WE: Chronic toxicity of essential oils and certain other products of natural origin. *Food Cosmet Toxicol* 3:311-324, 1965
- (5) DICKE MM: A new viable yellow mutation in the mouse. *J Hered* 53:84-86, 1962
- (6) KNAPKA JJ, SMITH KP, JONES FJ: Effect of open closed formula rations on the performance of strains of laboratory mice. *Lab Anim Sci* 21:680-684, 1974
- (7) HESTON WE, VLADARIS G: Factors in the causation of spontaneous hepatomas in mice. *J Natl Cancer Inst* 37:839-843, 1966