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Tumor Susceptibility in Two Mouse Strains with Varying Doses of Carcinogen (41393)

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Abstract. Administration of 3-methylcholanthrene to C3H/HeJ and C57BL/6J mice at four dose levels over a 1:125 range resulted in a practically equal tumor response in both strains at all dose levels. Liisa Prehn and E. M. Lawler (Science **204**: 309–310, 1979), using a different method of carcinogen administration, found a reversal of tumor susceptibility when varying the dose of carcinogen, a phenomenon not observed by us. Our study suggests that Prehn and Lawler's results may have been influenced, at least in part, by the practice of the latter authors to exclude data of substantial numbers of tumor-free mice from the evaluation.

The present work was prompted by a report of Liisa Prehn and Lawler (1) who observed an unusual dose–response relationship of tumor formation in mice treated with 3-methylcholanthrene (MC). According to these authors, the rank order of tumor susceptibility in 10 mouse strains was reversed when the dose of carcinogen was reduced drastically. Prehn and Lawler suggested that these unexpected results were due to a correlation between the immunogenicity of the induced tumors and the concentration of carcinogen used. Such a conjecture would invalidate the notion generally accepted in toxicology of using high doses of carcinogens in animal exposure experiments to draw conclusions on the response to lower doses in actual human exposure. Consequently, it would become necessary to revise our present concept of what constitutes an appropriate *in vivo* carcinogenesis assay.

In the present study, the carcinogen was administered by the sc technique most widely used in bioassays. This contrasts with the mode of administration of Prehn and Lawler who gave the carcinogen embedded in paraffin disks as carriers, thus introducing the complicating factor of solid-state carcinogenesis into their experiment and further leading these authors to discard between 32 and 50% of the mice from the experiment, all without tumors, where the paraffin disk could not be found at autopsy.

Materials and Methods. Twenty female mice of each of the C3H/HeJ and C57BL/6J strains received a single sc injection into the groin of 0.2 ml MC solution in tricaprylin at each of the following dose levels: 625, 125, 25, and 5 μg of MC per mouse. An additional group of 20 female mice of each strain received 0.2 ml of tricaprylin alone. The two mouse strains were those which behaved in an extreme fashion in the study of Prehn and Lawler. Mice were palpated weekly to detect tumors and were sacrificed when a tumor measured 1 cm or more. All survivors were sacrificed after 73 weeks and were autopsied.

Results and Discussion. We found tumors at the injection site in 36 C3H/HeJ mice and in 50 C57BL/6J mice. No additional tumors were seen at necropsy. All tumors were subjected to histopathologic study. Seventy-eight of the eighty-six tumors were poorly or moderately differentiated fibrosarcomas, three were squamous cell carcinomas (all in C3H mice at the highest dose of MC), and five had been lost for histopathological study through cannibalism of their hosts (all in C3H mice). In addition, there occurred three adenocarcinomas, two of the mammary glands and one of a salivary gland (all in C3H mice). These latter three tumors were not consid-

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ered to constitute a response to MC administration.

A fair to good dose–response relationship was obtained for each of the two mouse strains, as shown in Fig. 1. At all four dose levels of carcinogen, the C57BL/6J mice exhibited a more marked response and, hence, a higher tumor susceptibility than the C3H mice. The difference between the response in the two mouse strains was not statistically significant at any of the four dose levels, as determined by the χ^2 test ($\chi^2 = 0.28$, 0.57, 0.53, and 0.47, respectively, by order of decreasing dose). There were only minor differences in the time sequence of tumor appearance between the two mouse strains at the same dose of MC, as seen in Fig. 2 for the highest and lowest doses.

When our data on tumor response were expressed as the "average numbers of tumor-free days," and the points for different strains at equal dose were connected by straight lines, according to Prehn and Lawler, it is again apparent that the C57BL/6J mice remained more responsive to the effects of MC than the C3H/HeJ mice at all four dose levels of carcinogen, as shown in Fig. 3. The slopes of the lines in the negative control groups (0) and at the lowest dose of MC (5 μg) are due to the spontaneous mortality of the mice, being very slightly higher in the C57BL/6J strain. Consequently, no reversal of tumor susceptibility took place in our experiment, unlike in that of Prehn and Lawler.

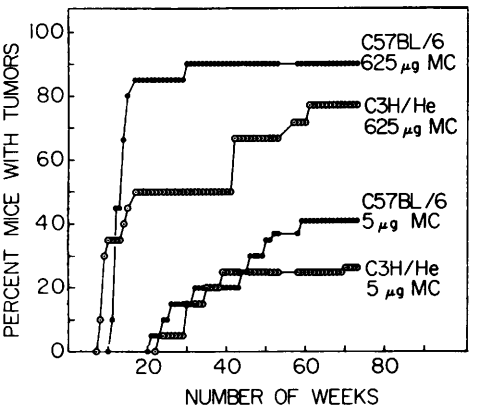

FIG. 2. Time–tumor incidence progression in two mouse strains, after administration of 5 and 625 μg 3-methylcholanthrene.

This disparity may have been due to the different techniques of MC administration in the two studies or to Prehn and Lawler's handling of their data. These authors state that they examined "all tumors and all tumor-free mice, at the end of each experiment . . . during autopsy for the presence of the 3-methylcholanthrene disk. If the disk was missing, the mouse was discarded from the experiment because it was impossible to know how long the mouse had been ex-

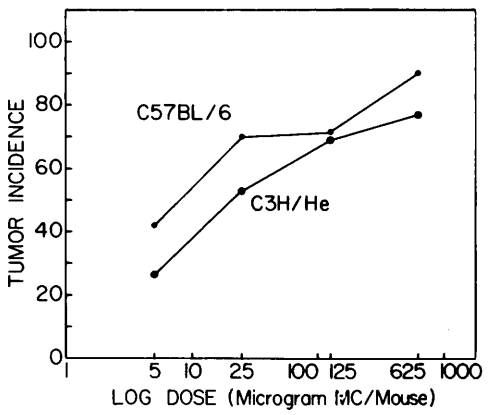

FIG. 1. Dose–tumor incidence relationship in two mouse strains, 73 weeks after 3-methylcholanthrene administration. Tumor incidence is expressed as numbers of mice with tumors in percentage of effective numbers of mice, the effective number being equal to the sum of the number of survivors plus the number of dead mice with tumors (2).

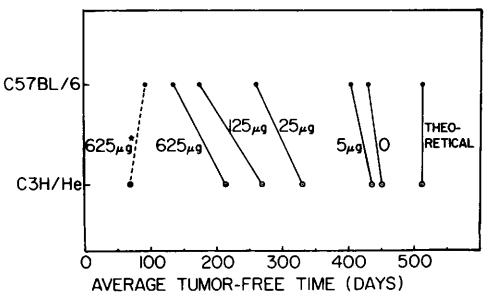

FIG. 3. Average tumor-free days in two mouse strains, 73 weeks after 3-methylcholanthrene administration at four dose levels (shown in μg) and in control mice (0) exhibiting no tumors. *The dotted line represents another plot of the data at 625 μg MC, after excluding tumor-free mice, in analogy to Prehn and Lawler (1).

posed to the 3-methylcholanthrene." Prehn and Lawler continue by saying that "mice of strains C3H/HeJ, C57BL/6J, and A/J rejected from 32 to 50% of the 5% 3-methylcholanthrene disks."These authors stated that the C3H/HeJ and C57BL/6J strains were equal in the rejection of the MC disks, and they claimed that "the sloughing of the 3-methylcholanthrene disks did not appear to have been a factor in the results." They did not, however, document that assertion. Nevertheless, Prehn and Lawler rejected only tumor-free and no tumor-bearing mice from their experiment. We see no justification for the rejection of these or of any animals from any study. In fact, if tumor-free mice were deleted from our own experiment, the illusion of a reversal in tumor susceptibility would actually be created (dotted line in Fig. 3). This manipulation, thus, illustrates the dangers of such an exclusion of animals.

Our results also show that there is little, if any, difference between the susceptibility of MC-mediated sarcoma induction in C3H/He and C57BL/6J mice. This conclusion confirms observations reported by Nebert and Jensen (3).

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