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Letter to the Editor

Post-Carcinogen Interval in Carcinogenesis

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It is generally accepted that the induction of an experimental tumor depends on the dose of carcinogen administered. In previous publications, we also reported that by increasing the total topical dose of 1,2-dibenzopyrene or diethylnitrosamine, an increasing number of epithelial dysplasias and invasive carcinomas developed in the uterine cervix and in the esophagus of mice after several months [1–4]. Similarly, by increasing the total parenteral administration of 1,2-dimethylhydrazine to rats, an increasing number of colon tumors occurred after several months [5, 6].

Recently, we studied the time dependency of tumor induction in the esophagus of mice [7]. The advantage of this model resides in the fact that not only one but up to 30 tumors may develop in the esophagus of the C57B1 mouse. A total of 274 C57B1 mice were investigated. Diethylnitrosamine (DEN) was administered in drinking water at a concentration of 0.04 ml/1000 ml water. From Table 1, it may be deduced that while some groups of mice were killed immediately after DEN administration for 1 day, 2 weeks, 1, 2, 3, 4 and 6 months, other groups of mice receiving the same DEN treatment were then allowed to survive (without DEN treatment) to complete 7 months. For mice receiving DEN for 6 months, the alloted survival time without administration of DEN was 9 months. The mice were killed by allotane anesthesia, their esophagus cut, opened wide and placed on a Millipore filter. The preparations were fixed in methanol–acetic acid and scanned 'wet' in a dissection microscope by means of diffuse monochromatic light from a fluorescent tube placed underneath the dissection microscope. This method of transillumination gives reproducible,

Table 1. The number of tumors per cm of esophageal mucosa (tumor index = TI) after DEN treatment at various time intervals in 274 C57B1 mice

	Killed immediately after DEN administration				Allowed to live without DEN to complete 7 months				Allowed to live without DEN to complete 9 months			
DEN treatment	No. of animals	No.of tumors	Length in cm	TI	No. of animals	No. of tumor	Length in cm	TI	No. of animals	No. of tumors	Length in cm	TI
1 day	10	0	33.2	0	31	1	99.2	0.10				
2 weeks*	10	0	34.1	0	30	22	96.8	0.23				
1 month*	19	0	64.6	0	29	80	95.2	0.84				
2 months*	18	0	59.4	0	18	42	55.8	0.80				
3 months*	18	0	51.0	0	26	282	85.8	3.3				
4 months*	20	63	62.8	1.0	25	425	84.2	5.0				
6 months*	20	202	68.0	3.0					10	397	57.5	6.9

*3 days/week.

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reliable results in our model [4]. By dividing the amount of tumors recorded by the total length of the esophagus in each animal (measured with a conventional ruler), the tumor index (TI = tumors occurring/cm of esophageal mucosa in each esophagus) was estimated. This method permits the comparison of results in all tested animals despite possible variations in the length of resected esophagus.

The results condensed in Table 1 indicate that mice killed immediately after DEN administration had no esophageal tumors during the first 3 months of daily treatment. Esophageal tumors developed only after 4 months of DEN treament, the highest TI was found after 6 months of DEN treatment. On the other hand, mice reacted differently when given the same dose of DEN but allowed to survive without that treatment for 7 or 9 months. The results in Table 1 show that one of the 31 mice treated for 1 day only with DEN demonstrated one esophageal tumor at autopsy 7 months later. When 30 mice were allowed to survive 7 months after 2 weeks of DEN administration, 22 esophageal tumors were demonstrated. A significantly larger number of esophageal tumors developed after initial DEN treatment for 1, 2 and 3 months, but surviving 7 months without additional treatment.

From the above, we conclude that daily administration of DEN for long periods of time is not necessary to induce large numbers of esophageal tumors in the C57B1 mouse. Esophageal tumors are bound to occur even after a short period of DEN treatment. Provided that the carcinogen-free follow-up period is sufficiently long, a significantly large number of tumors will develop without additional carcinogen treatment. The implications of this investigation should be borne in mind when evaluating the effect of certain drugs on experimental carcinogenesis.

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