Observations on the Carcinogenicity of 1,2,3,4-Dibenzophenanthrene and Its 9-Methyl and 10-Methyl Derivatives*

Paul N. Harris, M.D., and Charles K. Bradsher, Ph.D.

(From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Indiana, and the Department of Chemistry, Duke University, Durham, North Carolina)

(Received for publication June 10, 1946)

In 1940 Badger *et al.* (1) reported that 1,2,3,4-dibenzophenanthrene was carcinogenic for mice when applied by painting the skin twice weekly with a 0.3 per cent solution in benzene, or by subcutaneous injection of 2.5 to 5 mgm. doses in 0.2 cc. of sesame oil, with repetition of the injection at intervals of 3 to 5 weeks according to the rate of disappearance of the quantity given previously. Of 20 mice painted with the compound, 5 developed cutaneous papillomata within a period of 212 to 258 days, and 8 developed cutaneous carcinomas within the same period. Of 30 mice that received the compound subcutaneously (the number of injections and the total dose administered were not stated), 5 developed sarcomas within a period of 144 to 185 days, and one other developed a spindle celled tumor of doubtful malignancy. The authors did not report upon methyl derivatives of this compound.

METHODS

In our experiments, suspensions of 1,2,3,4-dibenzophenanthrene and of its 9-methyl and 10-methyl derivatives were injected subcutaneously into young mice of the New Buffalo strain. This strain has been maintained by inbreeding in the Research Laboratories of Eli Lilly and Company since 1940, and was obtained from Dr. William S. Murray, of the New York State Institute for the Study of Malignant Diseases. A ratio of 100 mgm. of hydrocarbon to 1 cc. of menstruum was used. Tricapryllin was first employed as the suspending agent, but when our supply was exhausted, the ethyl ester of sesame oil was substituted. No difference in results attributable to this substitution could be detected. No mouse received a second injection.

The usual dose of 1,2,3,4-dibenzophenanthrene and of its 10-methyl derivative was 5 mgm. However, 8 mice received 10 mgm. and 2 received 15 mgm. of the parent compound; and 1 mouse received 15 mgm., 1 received 40 mgm., and 2 received 50 mgm. of the derivative. As anyone who has tried to inject such a suspension knows, these figures are not absolutely accurate, but they are close approximations. The dose of the 9-methyl derivative varied widely; 850 mgm. were injected into 32 mice, and some received very large doses. The structural formulas of these compounds are shown in Fig. 1.

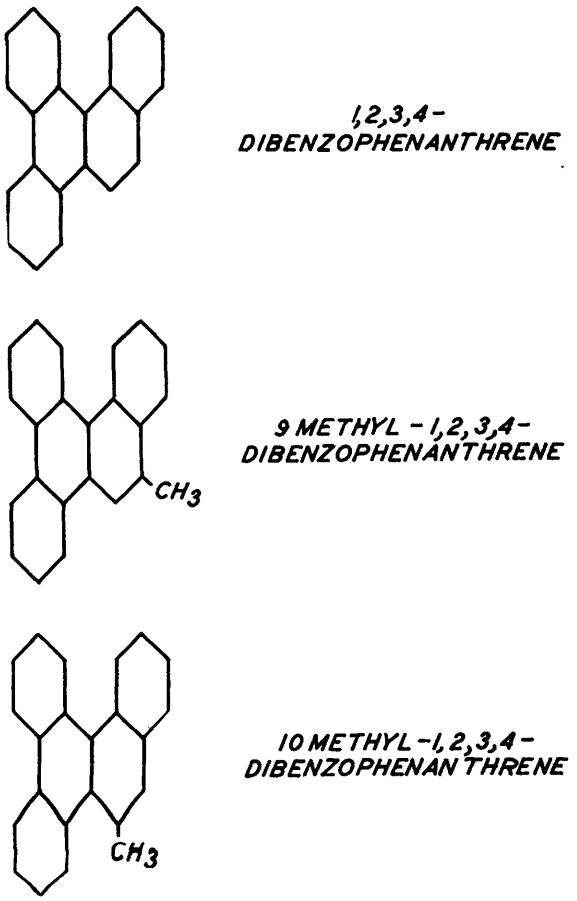

FIG. 1.—Structural formulas of 1,2,3,4-dibenzophenanthrene and its 9-methyl and 10-methyl derivatives.

* Presented at the 37th Annual Meeting of the American Association for Cancer Research at Atlantic City, New Jersey, March 11, 1946.

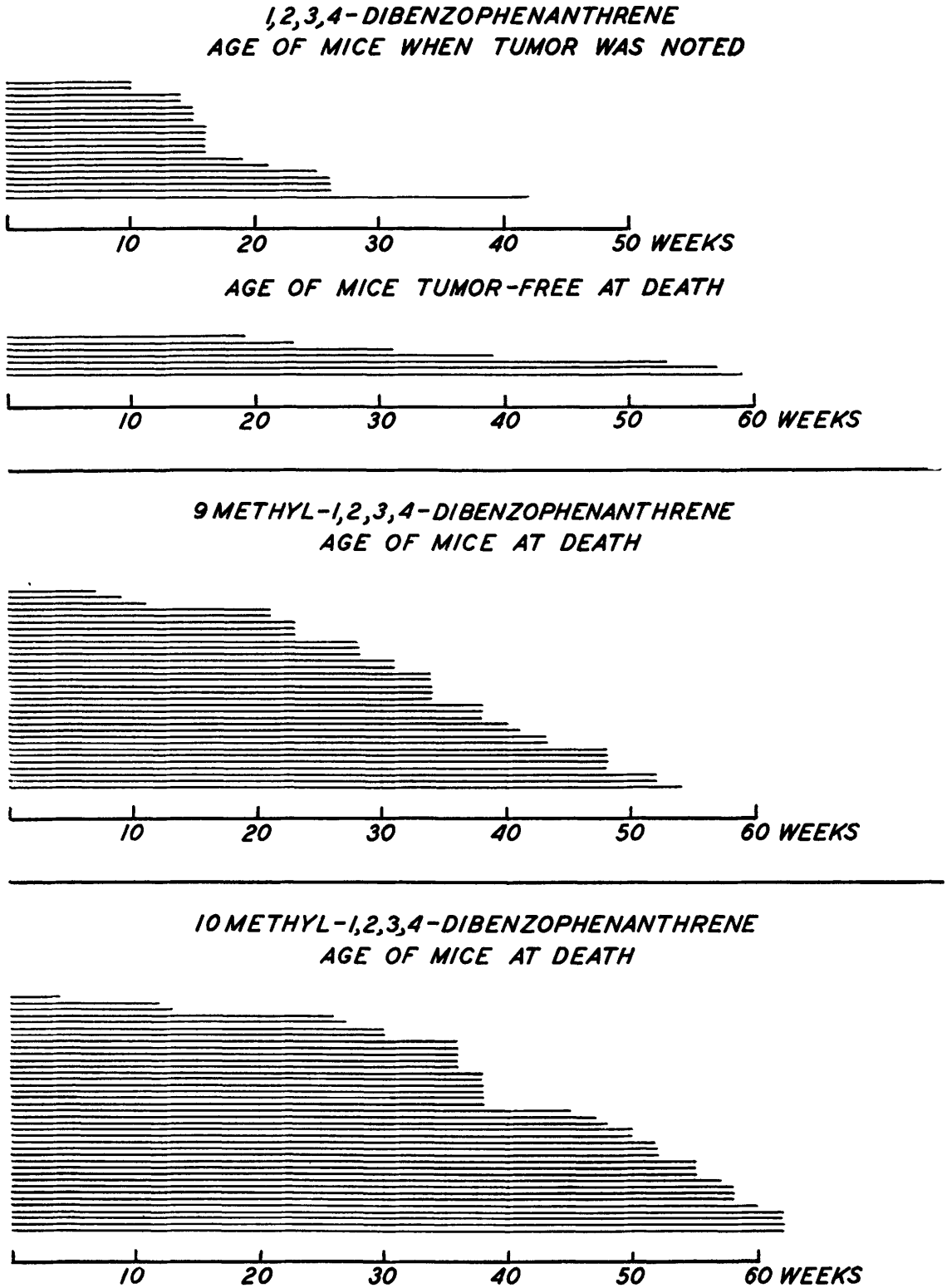

FIG. 2.—Graphical record of results of injections of 1,2,3,4-dibenzophenanthrene and its 9-methyl and 10-methyl derivatives. Each horizontal line represents 1 mouse.

RESULTS

Twenty-six mice were injected with 1,2,3,4-dibenzophenanthrene, and 19 ultimately developed tumors. One animal developed epidermoid carcinoma at the site of injection after a latent period of 26 weeks, and 3 developed both epidermoid carcinoma and sarcoma at the site of injection after latent periods of 25, 26, and 42 weeks. The other 15 mice developed sarcomas after latent periods of 10, 14, 15, 16, 19, 21, and 26 weeks. This is shown in Fig. 2, in which each horizontal line represents 1 mouse. The sarcomas, in general, were uniform in structure and were composed of fusiform cells arranged in bundles and whorls. Giant cells were relatively uncommon in most tumors, but were abundant in a few. Mitoses were usually numerous, and invasion of the abdominal muscle and of the panniculus carnosus was common. All these tumors appeared to be of fibroblastic origin. Fourteen mice developed ulcers at the site of inoculation, and in 10 of these, tumors appeared later. The 4 that developed carcinomas were in this group. The mice were not killed immediately upon the discovery of tumors, but were kept for about three weeks in order to obtain tumors of larger size.

Seven mice injected with hydrocarbon failed to develop tumors and lived from 19 to 59 weeks after inoculation (see Fig. 2). Four of these soon developed ulcers at the site of inoculation and possibly thereby lost enough hydrocarbon to render the dose ineffective. These mice died at 19, 23, 31, and 59 weeks after injection.

No tumors developed in mice that received the two methyl derivatives of 1,2,3,4-dibenzophenanthrene, and ulceration did not develop at the site of inoculation of either compound. Neither did tumors develop in control mice injected with tricapryllin or the ethyl ester of sesame oil. Thirty-two mice were inoculated with the 9-methyl derivative. Their survival ranged from 7 to 54 weeks and averaged 34 weeks. Thirty-eight mice received the 10-methyl derivative. Their survival ranged from 4 to 62 weeks and averaged 43 weeks (see Fig. 2).

DISCUSSION

Although the phenomenon is well known, it is nevertheless of considerable interest that addition of a methyl group to the 9 or 10 carbon atom of 1,2,3,4-dibenzophenanthrene changes the compound from a highly carcinogenic one to an apparently innocuous one. The fact that in our experiment tumors appeared earlier and in much larger percentage than in the study of Badger *et al.* (1) is probably due to our use of a suitable inbred strain instead of market mice.

SUMMARY

1. 1,2,3,4-Dibenzophenanthrene was found to be a highly potent carcinogen when injected subcutaneously into mice.

2. The 9-methyl and 10-methyl derivatives of this hydrocarbon failed to produce tumors when injected subcutaneously into mice.

REFERENCE

1. Badger, G. M., Cook, J. W., Hewett, C. L., Kennaway, E. L., Kennaway, N. M., Martin, R. H., and Robinson, A. M. The Production of Cancer by Pure Hydrocarbons. V. Proc. Roy. Soc. London, s. B, **129**:439-467. 1940.