

Effect of Aging in Two-Stage Carcinogenesis on Mouse Skin with Phorbol Myristate Acetate as Promoting Agent¹

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ABSTRACT

Life table analysis curves have been constructed for some of the data presented in a previous report on the effect of aging and of the interval between primary and secondary treatment in two-stage carcinogenesis on mouse skin. These curves are compared with those in a recent report from another laboratory also concerning aging and skin carcinogenesis. While comparison was difficult due to differences in experimental protocol, our conclusion that age at the onset of secondary treatment is of overriding importance in determining final tumor yields is substantiated.

INTRODUCTION

A recent report (9) described an extensive experiment with 560 mice in which 2-stage skin carcinogenesis experiments were carried out for the life span of the animals. In this experiment the effects of varying the interval between initiation and promotion and of the age of the animals at the beginning of promoting treatment were examined. From the results presented it was concluded that there was a general decrease in tumor yield with increasing age at the time of beginning of promotion. The experiments also clearly showed that the initiating effect persisted even when the interval between initiation and promotion was 56 weeks, which confirmed earlier experiments on the irreversibility of initiation (1, 7, 8).

The above findings attracted interest from other workers in the area of effect of aging on chemical carcinogenesis and 2-stage carcinogenesis in laboratory animals and man (5, 11). Much of the interest was expressed in personal communications to the authors, requesting additional information. The original animal records used for our published report (9) were used for the preparation of life table analyses. These life table analyses are presented and discussed in this report.

MATERIALS AND METHODS

Chemicals and Bioassay Procedure. The test methods and origin of the chemicals were described in detail in our earlier reports (9, 10). The initiating agent was 7,12-dimethylbenz(a)anthracene, applied in a single dose, 20 μ g/0.1 ml

acetone, to the dorsal skin of female ICR/Ha mice, followed at various intervals by application of PMA,² 2.5 μ g/0.1 ml acetone, 3 times weekly for the life span of the animals. In all, there were 11 groups, which included the necessary age-adjusted control groups that received initiator only, promoter only, solvent only, and no treatment. The criteria for classification of tumors, *i.e.*, papillomas or carcinomas, time to first tumor, etc., were all described in detail in earlier reports (9, 10). The details of 3 of these groups are shown diagrammatically in Chart 1.

Life Table Analysis. The actuarial or life table analysis as used previously in studies at this Institute (4) was used for the preparation of the results presented here. This procedure takes into account tumor incidences as well as survival of mice, *i.e.*, animals at risk, in all experimental groups.

RESULTS AND DISCUSSION

The life table analyses, expressed as percentage of mice without tumors plotted against duration of exposure to PMA or against age of the mice in days, are presented in Charts 2 and 3, respectively.³ The results from the life table analyses of Groups 1, 3, and 5 are compared here since they are representative of the range of tests in our original study (9).

A recent report by Peto *et al.* (5) drew attention to the lack of life table analysis in our study. Our results were plotted as percentage of animals with tumors (*i.e.*, number of animals with tumors per number of animals at risk), and our conclusions were based on these curves (9).

From their work on the effect of age on mouse skin carcinogenesis as induced by benzo(a)pyrene, Peto *et al.* (5) concluded that the skin cancer incidence rates depended only on duration of carcinogenic insult and not at all on the age of the animals when regular exposure to the chemical began (10 to 55 weeks). It is difficult to make a valid comparison between their experiments (carcinogenesis by a single chemical) and ours (2-stage carcinogenesis). However, a number of observations can be made. In our experiments first tumors arose between 4 and 9 weeks after the beginning of promoting treatment and were recorded when they reached a size of 1 cu mm and persisted for 30 days or more (9). In the work of Peto *et al.* (5), tumor size was 10 mm, and their first tumors were not recorded until

² The abbreviation used is: PMA, phorbol myristate acetate (trivial name for *Chemical Abstracts Registry No.* 16561-29-8).

³ The exact numbers used for these computations can be obtained from the authors in tabular form.

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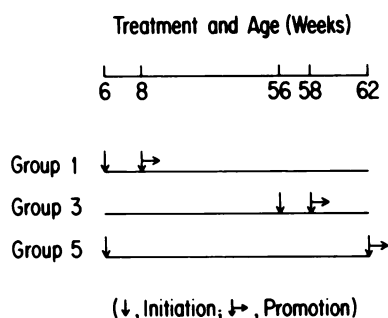


Chart 1. Protocol for establishing the effect of aging and of the interval between initiation and promotion in 2-stage carcinogenesis. In Group 1, only 100 of the original 119 animals were used for life-table analysis. ↓, initiation with 20 μg 7,2-dimethylbenz(a)anthracene, ↔, promotion with 2.5 μg PMA 3 times weekly.

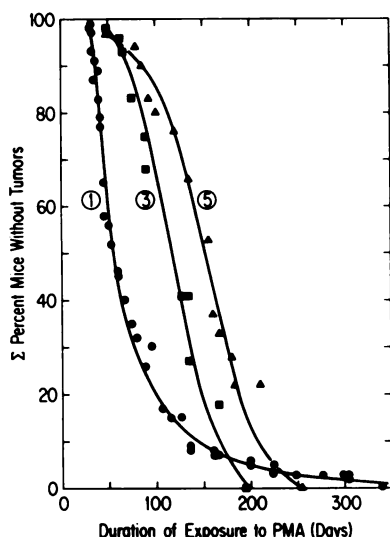


Chart 2. Life table analysis; percentage of animals without tumors compared with duration of exposure. Groups 1, 3, and 5 (representing 100, 51, and 35 mice, respectively) refer to those given in Chart 1.

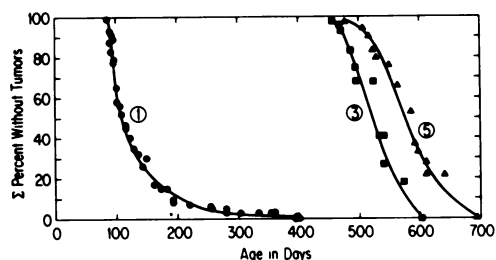


Chart 3. Life table analysis; percentage of animals without tumors compared with age. Groups 1, 3, and 5 (representing 100, 51, and 35 mice, respectively) refer to those given in Chart 1.

at least 40 weeks. Nevertheless, the life table analyses shown in Charts 2 and 3 are comparable with those given in Fig. 2 of the paper by Peto *et al.* (5) and indicate that the percentage of tumor bearers decreases as the age of the animals at the onset of promotion increases. Furthermore, our earlier conclusion remains that the tumor yield decreases when there is a long interval between initiation and promotion (comparing Groups 1 and 5) or when both initiation and promotion occur in older animals (comparing Groups 1 and 3). The average number of tumors per tumor

bearer likewise decreases with increasing age at the onset of promotion (9.4, 4.2, and 3.2 for Groups 1, 3, and 5, respectively).

Several earlier studies have been concerned with the effect of aging in chemical carcinogenesis (1-3, 5, 6). Only some of these relate to 2-stage carcinogenesis in mouse skin (1, 6-8, 9). This subject was also discussed recently in a comprehensive review on cutaneous chemical carcinogenesis (11). The findings of Roe *et al.* (6) cannot be considered conclusive because of the short duration of the experiment (20-week treatment). Furthermore, these authors omitted the necessary age-matched controls for initiation experiments; these controls were included in our earlier study (9). Treatment of the animals with urethan by i.p. injection and "quaternary" treatment with PMA complicate a comparison of their work with our 2-stage carcinogenesis studies. Thus, their experiments were difficult to interpret, and their conclusions cannot be considered valid. These authors also claimed to have observed a loss of initiating effect with aging and gave various possible reasons for this observation. Among these was that the loss of initiating effect not seen in other earlier experiments (1, 8) may have been due to lower doses of initiator used by Roe *et al.* (6). This explanation is no longer applicable because of our subsequent observations (9); we used lower doses of initiator and promoter than did Roe *et al.* (6).

Our earlier conclusion that the age of the animals at the beginning of promoting treatment is important and is an overriding factor in determining final tumor yields remains unchanged.

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