Factors Modifying Experimental Epidermal Carcinogenesis in Mice

S. D. Vesselinovitch† and J. P. W. Gilman

(Division of Biology, Ontario Veterinary College, Guelph, Canada)

Studies on the induction of tumors with various chemical carcinogens by a number of workers (3, 6, 10, 12) have resulted in the concept of a two-stage mechanism of carcinogenesis. In the first stage—"initiation"—normal cells are thought to be converted irreversibly into latent tumor cells, while in the second or "promoting" stage these are brought to visible tumors.

Mottram (10) observed tumor production in mouse skin after exposing it to a single treatment with a carcinogenic hydrocarbon, followed by repeated applications of croton oil. Later, Berenblum and Shubik (8) adapted this method to a quantitative approach to the study of chemical carcinogenesis. According to this method, the actual number of tumors produced were said to measure the initiating potency of the substance under test, while the latent period constituted a measure of promoting activity. Thus, when equal amounts of two or more different carcinogens are used for the primary treatment, followed by a standard course of croton oil applications, their respective initiating potencies can be compared (3, 7). Conversely, if different concentrations of the same carcinogen are utilized, the relationship between dose and tumor response may be studied.

Berenblum and Shubik have demonstrated a direct relationship between the amount of the initiating dose and the tumor response in mice, using several concentrations of 9,10-dimethyl-1,2-benzanthracene (4). Shubik and Ritchie (14) have extended these experiments by applying the increased amounts of carcinogen in the form of two and three applications, with a time interval of 7 days between each, croton oil treatment commencing 5 weeks after the initial application of DMBA. Repetition of the initiating dose in this manner resulted in an equal or even a lower tumor response than that obtained from the single application.

MATERIALS AND METHODS

The mice used to make up the several groups of this experiment were C57BL/C3H F1 hybrids (out of C57 mothers), bred and raised in this laboratory. The sexes were divided equally within each group, and all animals were approximately 3 months of age at the beginning of the experiment.

The carcinogen used was a 1.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene in light mineral oil (BP). Croton oil was used as the promoting agent and was administered as a 5 per cent solution of Oleum Crotonis (BP) in the same mineral oil.

The entire dorsum of each mouse was kept free from hair by being clipped and shaved with an electric razor. Solutions were applied to the skin by means of a single stroke of a No. 5 camel's hair brush.

All tumors that appeared were charted, but only those that persisted for at least 4 weeks were included in final data. The latent periods of each tumor within a group, measured from the first croton oil treatment, was recorded, and the mean of all these values was designated as the average latent period. The percentage of mice with tumors

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† Research Associate Fellow, National Cancer Institute of Canada.

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was calculated on the basis of survivors at the time of the first tumor. The mice were divided into six groups, each containing twenty animals, and were treated as follows:

Group I: Single application of DMBA; 7-day interval, followed by croton oil 3 times weekly for twelve paintings and then twice weekly for 21 paintings.

Group II: Single application of DMBA, followed after 40 days by croton oil treatment as in Group I.

Group III: Two applications of DMBA, separated by a 30-day interval. Croton oil treatment was commenced 10 days after the second DMBA and continued as in Group I.

Group IV: Two applications of DMBA, separated by a 30-day interval. Croton oil treatment began 7 days after first DMBA and continued as in Group I.

Group V: Croton oil control, receiving 33 applications of croton oil as in Group I above.

Group VI: DMBA control, receiving a single application of DMBA only.

RESULTS

The tumor response obtained in each of the groups is listed in Table 1; in all instances maximal response had occurred by the 112th day after the initial croton oil treatment. Both Groups I and II, which had received a single treatment with carcinogen followed by 33 applications of the promoting agent (croton oil), showed a similar response (86 and 89 tumors, respectively), regardless of the time interval prior to the first application of croton oil. Groups III and IV, which had received double doses of DMBA, exhibited much higher tumor incidence rates when exposed to the croton oil treatment (141 and 254 tumors, respectively).

Comparisons between groups based on the average number of tumors per tumor-bearing mouse gave a similar response pattern. Thus, the lower DMBA dosage resulted in average tumor incidence rates of 5.7 and 6.8 per mouse in the single-dose Groups I and II, and 10.1 and 14.9 in the double-dose Groups III and IV.

The appreciable difference in tumor response between Groups III and IV, both of which received the same amount of carcinogen, seemed worthy of note. Essentially the only difference in treatment between these two groups was in the time at which the croton oil exposure was begun. Group III was not exposed to the promoting agent until 10 days after the second application of carcinogen. However, the Group IV mice had already received ten applications of croton oil prior to receIVING their second dose of DMBA. The effect of this difference in time and sequence of treatment was reflected in a more rapid tumor response rate in Group IV, resulting in approximately a 50 per cent increase in average tumor incidence per tumor-bearing mouse in this group over Group III (Chart 1).

No appreciable differences were observed between any of the groups in regard to their average latent periods. Neither of the control groups responded to treatment. Thus, it may be assumed that, within the limits of this experiment, the croton oil used was nontumorigenic and that the amount of 1.5 per cent concentration of DMBA in light mineral oil administered as a single application constituted a subthreshold level.

DISCUSSION

The fact that the tumor response rates following a single application of DMBA were not observed to be affected by the time interval between this initiating treatment and the first croton oil painting is in agreement with earlier reports by Berenblum and Shubik (5) and by Graffi (7).
The observations on the apparent effect of the time interval used between the equal parts of a total initiating dose of DMBA, as well as those on the influence of initiator-promoter sequence, seem to be of some interest. A time lapse of 30 days between two successive applications of DMBA resulted in additive initiation. This response was considerably enhanced when treatment with the promoting agent partially preceded the second application of carcinogen (Group IV). Although these observations are at variance with the results obtained by Shubik and Ritchie (14), they may be interpreted in part in the light of the hypothetical explanations of their findings suggested by these workers. Thus, it might be assumed that the increased time lapse between carcinogenic treatments from 7 days (Shubik and Ritchie) to 30 days was sufficient to outlast the “refractory state” induced by the first application of carcinogen. This would seem to be an adequate explanation, in view of the fact that the whole initiation process itself must be considered to be influenced by the responsive state of the exposed cells as well as the potency of the carcinogen used.

The results reported above appear to be in agreement with those of Berenblum and Shubik (4) in which increasing concentrations of a single carcinogenic treatment resulted in correspondingly higher tumor responses; however, a comparison between these two groups of experiments is not completely justifiable even though both made use of graded doses of carcinogen. When a single-dose technic is used, the carcinogen, regardless of its concentration, is applied only to previously untreated skin. On the other hand, when the carcinogen is applied in more than one application, only the first exposure comes into contact with untreated skin, while the subsequent exposures must act upon cells whose responsiveness is almost certainly altered, temporarily at least, by the preceding treatment.

That the ability to alter the responsiveness of epidermal cells to a second exposure with a carcinogen is not restricted to an “initiator” is suggested by the response observed in Group IV. Here, the difference in tumor response is seemingly associated with the sequence of initiator-promoter exposures. From these results, the following two questions immediately pose themselves:

1. Has the promoting agent, croton oil, a sensitizing effect when it precedes a second carcinogenic treatment? Mottram (11) claimed such an effect for croton oil when it was applied to the epidermis prior to the first carcinogenic treatment: however, Berenblum and Shubik (2) and, more recently, Berenblum and Haran (1) were unable to confirm this finding.

2. Does the primary exposure to the carcinogen give rise to several levels of cellular initiation, only some of which are of sufficient degree (sensitivity) to respond directly to the promoting agent as visible tumors, while others may be so conditioned by this subsequent exposure to this promoting agent that they become capable of responding rapidly to a second carcinogenic exposure?

The observations reported here are not sufficiently extensive to provide definite answers to the questions at present. However, the reported data suggest an affirmative answer to both of these questions. Particularly is this so when one notes (Chart 1) that the very sharp increase in the tumor incidence of Group IV occurred so soon after the second DMBA treatment that it could not possibly have resulted from cells initiated de novo by that exposure. This increase, then, would appear to be associated with the added tumorigenic stimulus of the second DMBA exposure acting on foci of cells previously so conditioned that they immediately responded as visible tumors. Thus, it would

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**Chart 1.** Patterns of tumor induction—number of tumors plotted against time, measured from the first croton oil application.

- **Group II (G-2):** Single application of DMBA, followed by croton oil treatments after 40-day interval.
- **Group III (G-3):** Two applications of DMBA, separated by a 30-day interval. Croton oil treatment commenced 10 days after the second DMBA.
- **Group IV (G-4):** Two applications of DMBA, as in Group III. Croton oil treatment was begun 7 days after first DMBA.
not seem as if the promoting action of croton oil necessarily brings all initiated cells to the level of visible tumors, as originally stated by Berenblum and Shubik (3). In this regard, both Shubik (18) and Klein (8, 9) have since presented data which suggest that there may be more than one level of initiation and therefore that this process is not necessarily a single step, as originally conceived.

SUMMARY

1. Mice were treated with either single applications of a 1.5 per cent solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) or two such applications separated by a 30-day interval.

2. In all groups the initial treatment was followed by 33 applications of croton oil, beginning either on the 7th or 40th day after the first DMBA exposure.

3. The time lapse between the initiating treatment with the carcinogen and commencement of promoting treatments with croton oil did not affect either the number of tumors or the latent period in groups exposed only once to DMBA.

4. Two exposures to DMBA, when separated by a 30-day interval, resulted in an increase in the number of tumors, but there was no appreciable alteration in the percentage mice developing tumors.

5. An even greater increase in the number of tumors was obtained when ten croton oil paintings preceded the second DMBA exposure.

6. The significance of these findings were discussed in connection with the initiation-promotion concept of the process of carcinogenesis.

REFERENCES


3. ———. A New, Quantitative Approach to the Study of the Stages of Chemical Carcinogenesis in the Mouse's Skin. Ibid., pp. 588-91.


5. ———. The Persistence of Latent Tumor Cells Induced in the Mouse's Skin by a Single Application of 9,10-Dimethyl-1,2-benzanthracene. Ibid., pp. 384-88.


9. ———. Induction of Skin Tumors in the Mouse with Minute Doses of 9,10-Dimethyl-1,2-benzanthracene Alone or with Croton Oil. Cancer Research, 16:123-27, 1956.


11. ———. A Sensitizing Factor in Experimental Blastogenesis. Ibid., pp. 391-402.


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