The Growth Potentialities of Induced Skin Tumors in Mice

The Effects of Different Methods of Chemical Carcinogenesis*

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In their original description of the induced skin tumors of the rabbit, Yamagiwa and Itohikawa (14) detailed several morphological types of neoplasm with varying life histories. These tumors have been analyzed even more closely by Roux and Kidd (12), who have shown that a series of graded lesions are produced by applications of either tar or pure hydrocarbons, ranging from warts that regress to malignant metastasizing tumors. In the case of the mouse, it has been observed many times that a typical sequence of induced hyperplasia gives way to papilloma formation and that malignant transformation of some of these papillomas occurs. Here, too, the phenomenon of regression has been recorded. It has been noted that different carcinogenic hydrocarbons give rise to a variation in the type of neoplastic skin lesions in the mouse; in particular, the potent 9,10-dimethyl-1,2-benzanthracene has been observed by Bradbury et al. (7) to give rise to more papillomas than the other compounds used; methylcholanthrene has been found to be an effective carcinogen when administered as a single large skin application (10), and the tumors so induced (6) have been found to be largely carcinomatous from the moment of their first macroscopic recognition. So far, no detailed survey of the tumors induced in the skin of the mouse, similar to that carried out for the rabbit, has been reported.

Since it was demonstrated by Mottram (11) that a single application of a carcinogenic hydrocarbon followed by repeated applications of croton oil was an effective method of tumor induction for the mouse’s skin, this method has been used extensively in a study of the phases of carcinogenesis by Berenblum and Shubik (3–5). From these experiments, much support has been gained for the concept that carcinogenesis is a two-stage mechanism consisting of a specific and irreversible initiating phase, in which normal cells are converted to latent tumor cells that lie dormant until stimulated in the promoting phase to become morphological tumors; the promoting phase can be brought about by noncarcinogenic procedures such as applications of croton oil, but it remains an ill-defined process from the standpoint of specificity (13). In previous experiments (4), quantitative surveys of the relationship of the dosage of the carcinogen to the total number of tumors induced were undertaken. All the tumors induced in the course of these experiments were charted twice weekly and eventually recorded in terms of their total number. No distinction was made among the various morphological types, the majority of tumors described being, in fact, benign papillomas. The question of early-appearing lumps that disappeared within a matter of a few weeks was considered, and these were felt to be similar to non-neoplastic lesions described by Cramer and Stowell (8). It was also noted at that time that later regression of undoubted neoplasms did occur and that the rate of malignant transformation was low. Allsopp (1), in a preliminary report, has noted similar findings with this method of carcinogenesis. In the present investigation, a comparison has been made between the tumors induced by this croton oil method and those induced by repeated applications of pure carcinogenic hydrocarbons to see if, in fact, the former do have special characteristics.

MATERIALS AND METHODS

Some of these experiments were performed at the Sir William Dunn School of Pathology, Oxford, and the remainder in this department. Fe-
male Swiss strain mice, obtained from the Medical Research Council, England, and male and female strain CF-1 mice, from Carworth Farms, were used. The mice were fed an adequate diet with water ad libitum. All solutions were applied to the interscapular region, which was kept clipped free of hair with scissors. Croton oil obtained from Boots Chemists, Nottingham, England, was used for all the experiments as a 5 per cent solution in mineral oil (liquid paraffin); the 9,10-dimethyl-1,2-benzanthracene was also dissolved in mineral oil; and both these solutions were applied with a fine glass rod. The methylcholanthrene was dissolved in acetone and applied with a glass dropper.

The individual tumors on each mouse were recorded on charts at intervals of 2 weeks, and final histological confirmation of their nature was obtained. In any attempt to record the life histories of induced tumors many difficulties arise, making it necessary to establish arbitrary criteria that are of value more from a comparative than from an absolute standpoint. The tumors induced by the croton oil method proved by far the easiest of the induced skin tumors to record, as there tended to be fewer of them, and malignancy, if it supervened at all, occurred late. If applications of pure hydrocarbons were continued, so many tumors were produced in close proximity to one another that individual observation became extremely difficult. Most important of all is the effect of the onset of malignancy, represented macroscopically by a greatly increased growth rate, infiltration of the surrounding tissues, often ulceration, and with it regressions of many of the other benign tumors. This regression of benign tumors subsequent to the onset of malignancy in an adjacent tumor may be due to the overgrowing and obliteration of the benign tumor, or it may be secondary to ulceration and necrosis of the skin, or, lastly, it might be some other type of unknown regression phenomenon. For the purpose of this investigation, no attempt was made to determine the cause for regressions occurring subsequent to the advent of malignancy, and only those growth phenomena occurring before this were dealt with. The macroscopic diagnosis of malignancy in the mouse's skin, based on the onset of rapid infiltrative growth and often typical ulceration with erosion and raising of the edges of the lesion, was subjected to continued histological confirmation, and the two methods were found to tally remarkably. Almost all the lesions reported as malignant were anaplastic squamous-cell carcinomas with the exception of three fibrosarcomas.

The tumors observed in this investigation were found to fall quite clearly into four categories based on their growth characteristics. The classification adopted was:

1. Regressions: This constituted the largest group, and only those tumors that were present at least 4 weeks and then disappeared were included. Certain tumors were more than once found to disappear and then reappear during treatment. These were not included under "regressions," but for the purposes of the present investigation they were placed under one or another of the appropriate headings. All the regressions mentioned here did not reappear during the course of the experiments.

2. Stationary papillomas: all those tumors reaching a fixed size and remaining so for the rest of the experiment. Most of these tumors were small sessile papillomas.

3. Vigorously growing papillomas: tumors that continued growing throughout the course of the experiment in a benign fashion. Most of these were of the pedunculated papilloma variety and reached a considerable size.

4. Malignant tumors: the criteria for which have already been mentioned.

EXPERIMENTAL

Group I.—Group I consisted of 300 female Swiss mice, approximately 12 weeks old, which received a single application of 9,10-dimethyl-1,2-benzanthracene followed, after an interval of 3 weeks, by applications of 5 per cent croton oil in mineral oil for 40 weeks. This group was subdivided into three groups of 100 mice each: Group Ia received 0.17 per cent, Group Ib, 0.5 per cent, and Group Ic, 1.5 per cent of the 9,10-dimethyl-1,2-benzanthracene in mineral oil.

In Table 1 the tumor incidence for these various groups is recorded in terms of the various types of tumor. As was previously observed (4), the total tumor incidence, using this method of tumor induction, bears a direct relationship to the dosage of carcinogen. It would seem, however, that this factor does not significantly affect the distribution of tumor types. This latter conclusion cannot be stated with absolute certainty, and larger groups of mice with the lower concentrations used are needed for conclusive proof. Of the total of seven malignant tumors recorded here, six were squamous-cell carcinomas and one was a fibrosarcoma.

Group II.—Group II consisted of twenty-eight male and female CF-1 mice receiving 0.15 per cent methylcholanthrene in acetone (Group IIa) twice weekly for 30 weeks, and of thirty CF-1 mice receiving a single application of 0.3 per cent methylcholanthrene in acetone followed by twice-weekly applications of 5 per cent croton oil in mineral oil for 40 weeks (Group IIb). The results for all the
mice in this experiment are recorded in Table 2. It can be seen that, of the twenty-three mice in Group IIa developing tumors, seventeen had malignant invasive growths, of which two were fibrosarcomas and the others squamous-cell carcinomas; the eight regressions recorded occurred in three mice bearing papillomas, whose skin ulcerated and formed a thick scab that at autopsy had a purely inflammatory basis; lastly, the twelve stationary papillomas occurred in three mice, two of which died in the early stages of the experiment. The average amount of time elapsing from the time of the first recording of the tumors to the assumption of obvious invasive growth was 7 weeks. After the onset of invasive growth many regressions occurred, but these were not recorded, owing to the many complicating issues already considered. No regressions of diagnosed malignancies were seen. Of the seventeen mice with malignant tumors five developed a small papilloma giving way almost immediately to an ulcerative lesion that ultimately developed all the characteristics of malignancy. The remaining malignant tumors developed in vigorously growing papillomas.

In Group IIb the tumor distribution was very similar to that obtained in the previous croton oil experiment. The two malignant tumors were squamous-cell carcinomas.

Group III.—In Group III a total of ninety CF-1 female mice were used. All these mice received a single application of 1.5 per cent 9,10-dimethyl-1,2-benzanthracene in mineral oil; the mice were then divided into three equal groups and after an interval of 3 weeks treated as follows: Group IIIa received twice-weekly applications of 0.1 per cent 9,10-dimethyl-1,2-benzanthracene in mineral oil for 20 weeks; Group IIIb received twice-weekly applications of croton oil for 35 weeks, and Group IIIc received a mixture of 0.1 per cent 9,10-dimethyl-1,2-benzanthracene and 5 per cent croton oil in mineral oil for 20 weeks. This last group was instituted to determine the existence of any possible anti-carcinogenic action on the part of croton oil giving rise to the high regression rate.

The results for this group are recorded in Table 3, where it can be seen that the Group IIIb treated with a single application of carcinogen followed by croton oil has reproduced a picture very similar to that seen previously. The Group IIIa, receiving repeated applications of 9,10-dimethyl-1,2-benzanthracene, was rather different from Group IIa receiving only methylcholanthrene, in that no regressions were observed, more papillomas were induced, and all the malignancies occurring began in vigorously growing papillomas and not in ulcerated areas. Group IIIc, receiving both croton oil and

<table>
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<tr>
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<tr>
<td>DISTRIBUTION PATTERN OF TUMORS INDUCED BY SINGLE APPLICATION OF DMBA* FOLLOWED BY REPEATED APPLICATIONS OF CROTON OIL</td>
</tr>
<tr>
<td>CONCENTRATION* OF CARCINOGEN</td>
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<tr>
<td>GROUP</td>
</tr>
<tr>
<td>Ia</td>
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<td>Ib</td>
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<td>Ic</td>
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* 9,10-dimethyl-1,2-benzanthracene in mineral oil applied once only.
† A single application of carcinogen followed, after an interval of 3 weeks, by applications of 5 per cent croton oil in mineral oil twice weekly for 40 weeks.

<table>
<thead>
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<th>TABLE 2</th>
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<tr>
<td>A COMPARISON OF THE DISTRIBUTION OF TUMORS INDUCED BY MC* APPLIED REPEATEDLY, WITH THOSE INDUCED BY A SINGLE APPLICATION OF MC* FOLLOWED BY REPEATED APPLICATIONS OF CROTON OIL</td>
</tr>
<tr>
<td>GROUP</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>IIa</td>
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<td>IIb</td>
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* MC: Methylcholanthrene. † Due to ulceration. ‡ The extra number of tumors represented here and not accounted for in the tumor distribution disappeared subsequent to malignancy and are therefore not counted, as mentioned in the text. Results were at 40 weeks from the first application of MC in Group IIa and 40 weeks from commencement of croton oil in Group IIb.
9,10-dimethyl-1,2-benzanthracene, showed an increase in the number of tumors over either Group IIIa or Group IIIb and, with this, a slightly increased regression rate. The cause of this becomes difficult to interpret and might presumably be due either to the elicitation by croton oil of added papillomas not possessing sufficient growth-potentiality to survive or to a specific anti-carcinogenic effect of this substance.

CONCLUSIONS

These experiments show without doubt that there is a considerable difference between carcinogenesis by a single application of a carcinogen followed repeatedly by croton oil and carcinogenesis by repeated applications of the carcinogen alone.

TABLE 3
A COMPARISON OF THE INDUCED TUMORS*

Tumors induced by: (a) DMBA* applied repeatedly, (b) a single application of DMBA* followed by repeated applications of croton oil, and (c) a mixture of DMBA* and croton oil applied repeatedly.

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<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>NO. OF SURVIVORS AT TIME OF FIRST TUMOR</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIIa</td>
<td>One application of 1.5 percent DMBA,* then, after 3 weeks, 0.1 percent DMBA* twice weekly</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>IIIb</td>
<td>One application of 1.5 percent DMBA,* then 5 percent croton oil twice weekly</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>IIIc</td>
<td>One application of 1.5 percent DMBA,* then mixture of 0.1 percent DMBA* and 5 percent croton oil twice weekly</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

* DMBA: 9,10-dimethyl-1,2-benzanthracene. All solutions made up in mineral oil. Results at 38 weeks from beginning of experiment.
† Previous to the onset of invasive growth.
‡ Two malignancies appearing in 48th week from beginning of experiment.

These differences are threefold: there is, first, an apparently increased regression rate; second, a marked decrease in the number of malignant transformations; and, third, vigorously growing benign tumors, not noted following application of the carcinogen alone, and closely resembling the benign tumors of man, are induced. These differences can be summarized by saying that the croton oil method yields a graded series of lesions ranging from tumors that only persist for a matter of months and then disappear, on through those that only reach a limited size, to frankly invasive tumors, with intermediate types being represented. With the repeated applications of the carcinogen, various types of tumor are induced too; but eventually malignant transformation occurs in almost all animals and obscures the fate of some of the other lesions. In particular, the fate of the regressions becomes obscured. With the croton oil method these occur between the 12th and 25th week of the tumor's existence, and, thus, experiments using carcinogen continuously cannot be expected to demonstrate this. The malignancies reported from the use of the carcinogens alone occurred, on an average, after the tumor had been present 7 weeks; and, therefore, any further regressions after this time were obscured. Nevertheless, there is no doubt that regression of some of the benign lesions does occur after this time, although what proportion simply merge into the rapidly growing malignant lesions cannot be determined. Thus, no true comparison can be made. It would seem that the most reasonable explanation of this picture, within the limits of the previously proposed two-stage mechanism of carcinogenesis, is to consider initia-
mas to carcinomas. From the data presented in this investigation, the changes necessary to alter the distribution of the tumor types and thus influence the rate of malignant transformation seem to be identical with the change involved in initiation. The inclusion of additional stages, involving changes of a nature different from those already considered, does not seem to be indicated. In the case of the tumors induced with a single application of carcinogen followed by croton oil, their particular natures would appear to have been determined by the initial treatment. Increasing the dosage of single applications of the carcinogen to be followed by a promoting agent does not appear to alter the tumor distribution, whereas repeated applications of the carcinogen do. It might be presumed that the first application alters the susceptibility of the cells involved to the second.

In their most recent report, Friedewald and Rous (9) have studied in detail the fate and time of appearance of tumors induced in five rabbits treated twice weekly for 250 days with methylcholanthrene and then with intermittent wound healing (disking) for up to 5 years. They observed a linear accumulation of large numbers of tumors during the whole of this period, and, in attempting to explain this phenomenon, they found it necessary to modify the previous concept of the mechanism of carcinogenesis. They feel that the substitution of latent neoplastic potentialities for latent tumor cells is necessary to explain these new observations.

The present experiments are felt to remain consistent with the view that carcinogenesis is begun by the conversion of some few cells to latent tumor cells. This is demonstrated with clarity by the use of a single application of a carcinogen as the initiating agent, and undoubtedly the use of repeated applications would result in a conversion of most of, if not all, the cells in the area to latent tumor cells. It is therefore difficult to compare the two types of experiments, although in the view of the present author the linear accumulation of tumors over a period of time can be considered consistent with the previous concept of carcinogenesis, with the modification that the latent tumor cells do not constitute a uniform change and that they represent differing grades of growth-potential.

SUMMARY
1. Skin carcinogenesis by a single application of 9,10-dimethyl-1,2-benzanthracene or methylcholanthrene followed by repeated applications of croton oil has been compared with carcinogenesis by repeated applications of either of the hydrocarbons alone.
2. The croton oil method yields only a small number of malignant tumors and gives rise to a very high regression rate.
3. The tumors induced have been classified as either (a) regressions, (b) stationary papillomas, (c) vigorously growing papillomas, or (d) malignancies.
4. From a consideration of the results obtained it is suggested that the process of initiation should be considered as a graded one, inducing graded changes in growth-potentiality in the latent tumor cells.

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REFERENCES
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