Some Factors That Influence the Growth of Neoplastic Cells*

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It has been suggested that the formation of a cancer occurs not as a continuous single process but rather as a series of biological changes (3, 6, 13, 21). The various steps involved are a matter of conjecture, but presumably neoplastic cells must first be formed and then they must proliferate to form a grossly recognizable tumor. The present communication is concerned with some of the factors that influence tumor formation, with special emphasis on the proliferation of the established neoplastic cell. The agents used included croton oil, naphthoquinone, sodium sulfide, and tissue damage as produced by mechanical wounding.

The application of certain chemical irritants (2, 3), heat (8), cold (1), or physical trauma (7, 13, 21) to a region previously treated with a subcarcinogenic amount of hydrocarbon is known to precipitate the formation of tumors in a relatively short period of time, but there is some question whether the secondary treatment completes the processes involved in the actual formation of the cancer cell or whether it is in some manner responsible for the proliferation of cancer cells already formed. Hence the effect of mechanical wounding was also determined on the growth of neoplastic cells whose existence was well established.

The effect of chemical and physical agents on carcinogenesis of the skin by methylcholanthrene.—Two series of 100 young adult albino mice were kept on wood shavings in metal box cages and were given dog biscuit and water ad libitum throughout the experiment. The mice were painted twice weekly with a solution of methylcholanthrene applied with a camel's hair brush to the interscapular area. The length of the initial treatment with the hydrocarbon varied in the two series according to the strength of the solution and the susceptibility of the mice. They were then divided into groups of 25 each. In each of the series one group was set aside as a control and received no further treatment, while in the remaining groups the area of skin that had previously been painted with the hydrocarbon was subjected to various further treatments. The animals were carefully examined every 2 weeks for the appearance of tumors. The details of dosage and time relationships are presented in Table I.

Croton oil and its resin were tested because of their pronounced augmenting effect on carcinogenesis as previously described (2, 3). A solution of naphthoquinone was used because it is an irritant known to inactivate certain enzymes (20). Sodium sulfide was applied because it represents a different type of chemical irritant, and incisions repeated at regular intervals were employed because tumors have been reported to occur in scars and healing wounds (7, 21, 22, 24). In the latter group 3 or 4 parallel skin incisions about 2 cm. in length, which penetrated the dermis, were made with a sharp scalpel.

The effect of these various forms of irritation on areas previously treated with methylcholanthrene is shown in Table I. No visible tumors were present in any of the animals when the applications of hydrocarbon were discontinued at the end of the preliminary treatment, and only a few neoplasms eventually developed in the controls. However, when a 0.1 per cent solution of naphthoquinone was applied subsequent to the hydrocarbon treatment many tumors developed. Thus 14 carcinomas and 4 papillomas arose in this group (group 8) as compared to 5 epitheliomas and 7 papillomas observed in the control group. This finding is of interest not only because it is new but also because the effect is comparable to that obtained with croton oil, a known active epicarcinogen (3). In series I, the application of croton oil resin stimulated tumor production to the extent that there were 4 epitheliomas and 14 papillomas as compared to only 1 epithelioma in the control group 4 months after discontinuing the hydrocarbon treatment; in series II, after 6 months there were 14 epitheliomas and 8 papillomas in the group that received croton oil in contrast to 5 cancers and 7 papillomas found in the control group. In fact croton oil was almost as effective in developing the tumors as the continued application of the methylcholanthrene itself (group 6). This parallels the experience of Berenblum (2, 3). Chronic physical trauma in the form of repeated incisions of the skin had a slight but definite augmenting effect on tumor formation. This effect appeared early, and in the first 2
months following the discontinuation of hydrocarbon treatment it seemed to be as effective as the croton resin applications. However, in spite of continued treatment, a regression of tumors was observed; there were 8 papillomas after 2 months and only 6 after 4 months. All tumors were localized in or very close to the area of the scar. Any experiment in which repeated incisions are attempted is apt to be complicated by infection of the area, and this could have an adverse effect on tumor formation. Furthermore, multiple incisions are apt to result in the physical destruction or elimination of small tumors. Our results, although limited, suggest that wound healing has at least a transient augmenting effect on the development of tumors, and confirm the observations of other investigators (7, 13, 21, 22).

The results of the experiment with sodium sulfide also demonstrate that it is possible to have irritation without obtaining a cocarcinogenic effect. Weekly applications of sodium sulfide to the skin in amounts sufficient to produce considerable erythema did not augment tumor formation, although we do not know whether the irritation produced by this chemical was as intense, prolonged, or penetrating as that obtained by the methods that proved to be effective.

The effect of tissue damage as produced by wounding on the growth of established tumors.—Forty rats having palpable liver tumors induced by feeding p-dimethylaminoazobenzene were selected, and midline abdominal incisions extending to the sternum were made under ether anesthesia in such a manner that the livers could be easily inspected (17). The presence of large hepatomas having been confirmed, the wounds were closed with silk sutures. Six of the rats were given no further treatment following the first incision, and were killed after 40 days. In the remaining 34 rats, periodic irritation of the wounded area was repeated from 1 to 5 times. This treatment consisted of cutting, perforating, and drawing a threaded needle through the healing wound in numerous places at weekly intervals. The animals subjected to repeated

<table>
<thead>
<tr>
<th>Strain of mice</th>
<th>Initial treatment</th>
<th>Group</th>
<th>Subsequent treatment</th>
<th>Incidence of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC Stock</td>
<td>0.3% methylcholanthrene in benzene applied twice weekly for 8 weeks</td>
<td>1</td>
<td>None</td>
<td>1 mo. 2 mo. 3 mos.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Sat. sol. of NaS once weekly for 7 weeks</td>
<td>0 0 25 0 25 1 0 24 1 0 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Incisions of skin twice a month for 4 months</td>
<td>0 3 19 1 8 13 1 6 15 1 6 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0.1% croton oil resin twice weekly for 4 months</td>
<td>0 2 23 2 8 15 3 11 11 4 14 7</td>
</tr>
<tr>
<td>Strain C</td>
<td>0.2% methylcholanthrene in benzyl alcohol applied twice weekly for 16 weeks</td>
<td>5</td>
<td>None</td>
<td>1 0 23 0 3 21 1 4 19 5 7 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Continuation with methylcholangthrene for 6 months</td>
<td>0 1 24 1 12 12 4 10 11 22 0 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>1% croton oil resin twice weekly for 6 months</td>
<td>0 5 20 4 6 15 4 9 12 14 8 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.1% naphthoquinone twice weekly for 6 months</td>
<td>0 6 19 4 4 17 4 5 16 14 4 7</td>
</tr>
</tbody>
</table>

* Number of months after discontinuing the initial treatment.
† The results listed for series I are for 4 months and those in series II for 6 months.
Ca = Carcinoma.
P = Papilloma.
N = Negative.
nodules were also seen in these adhesions but no metastases were observed to any other part of the abdominal cavity. It is probable, therefore, that the tumor nodules developed from cells that reached the wound by direct contact. Histological examination demonstrated that the nodules were hepatomas identical with the primary liver tumor with which they were in contact (Fig. 2). Microscopic study of the wounds also revealed the presence of many nests of similar tumor cells of varying sizes. In fact, some wounds were literally sprinkled with nests of hepatoma cells. These tumors grew most vigorously in the fresh wounds and attained their largest size about 2 to 3 weeks after the injury. After this period the nuclei of the tumor cells became pyknotic.
(Fig. 3), and regression in the older wounds was rapid (Fig. 4). No tumors were found after 40 days in the wounds of the 6 control rats whose midline incisions were not subjected to postoperative interference, and with one exception hepatomas were absent in the fully healed wounds that had been subjected to repeated injury. In one animal the hepatoma continued growing until death. The conclusion should not be drawn that all experimental tumors will regress in healed wounds. Generally the rat hepatoma does not metastasize widely, nor is it extremely invasive. It is very likely that a more malignant tumor would not be so apt to regress in a healed wound once it had attained the size achieved by some of the hepatomas observed in this experiment.

**DISCUSSION**

The present experiments are in harmony with the general idea that cancer formation occurs following a sequence of biological changes (3, 6, 13). These changes might be classified as follows:

I. Induction period. This is the period during which neoplastic cells are formed.

II. Critical or reversible period. This starts at the moment the neoplastic cells are formed, and represents that stage in which cellular proliferation is delicately balanced. Presumably during this phase the cells may proliferate for a time, some may die, and others may lie quiescent for varying periods. This is the transitional period, during which the cells are most susceptible to the influence of their environment. It could vary in time from a few moments to periods of weeks, months, or even years. Whether a tumor cell dies or proliferates depends upon the balance between the proliferative capacity of the cell and the local tissue resistance.

III. Period of progression. This may be considered as that period when the neoplastic cells have gained ascendancy over the forces that hold them in control. There is no sharp line between the second and third periods, but in general the latter is one of relatively unrestricted, invasive growth, during which regressions are infrequent. The exact size that a neoplasm must attain before it reaches this stage varies, no doubt, with different tumors. Indeed the separation of tumor formation into various periods is only an arbitrary scheme, intended to assist in the clarification of our views on carcinogenesis. Actually the different stages are probably not clearly separable, and within a neoplastic focus, at any period, tumor cells are being formed, others are dividing, and still others are dying. The final outcome is the result of a balance of the various factors involved.

The postulation of a second, or critical, period in the development of a tumor is supported by several types of experimental evidence. First, when methylcholanthrene is applied to the skin of mice for a time short of that necessary to induce tumors, the subsequent resumption of the hydrocarbon treatment will precipitate tumor formation even though 3 to 4 months intervene between the 2 periods of hydrocarbon application (10). Such observations suggest that the initial application of hydrocarbon produced alterations in the tissues that persisted for at least 4 months, although there is no proof that any neoplastic cells had been completely formed as the result of the initial treatment with hydrocarbon.

Rous and his associates (13, 22) have shown that wounding rabbit ears caused the appearance of papillomas at sites from which they had previously regressed. The wounding process was therefore thought to stimulate the growth of quiescent neoplastic cells. The growth of hepatoma cells in wounds in the present experiment likewise suggests the stimulating influence of irritation on the proliferation of established neoplastic cells.

It seems likely that the noncarcinogenic agents that favor the growth of neoplastic cells during the critical period exert their effects on established cancer cells rather than on the actual formation of such cells, since croton oil applied to the skin of mice prior to benzpyrene had no effect on tumor formation (3). Furthermore, if heat, cold, or trauma was applied during the induction period the process of carcinogenesis was actually retarded, presumably by an interference with some of the biochemical changes necessary in the development of the neoplastic cell (23).

In an investigation in which the growth rates of experimental tumors were closely followed, Blum (4) demonstrated that the growth rate of the neoplasm did not remain constant throughout the development nor did it follow the same pattern in all tumors of the same type. His data suggest that opposing growth and regressive processes determine the time at which a tumor becomes established, and indicate that tumor cells do not escape and assume their own essential proliferation rates, but that the rates of growth are to a great extent dominated by the controlling influence of the tissue.

The experiments of Fischer also suggest that quiescent neoplastic cells may be stimulated to activity if certain favorable conditions are imposed (9). He transplanted pieces of apparently normal mammary tissue to other parts of the same mouse. After a few days these pieces were removed and reinoculated into other areas of the same animal and the procedure was repeated many times. Several tumors eventually grew in the region of the transplant. These results might be explained on the basis that the frequent wounding incident to the inoculations had exerted a favorable in-
Apparently irritation and yet the development of tumors in old burns or inflammation between irritation or injury and cancer formation. It is a well known fact that injury to tissues is followed by an improved blood supply, an elevated temperature, and an increase of nutritive substances to the region. Conditions favorable for cellular proliferation probably persist until the injury has been repaired. Loofbourow has described growth-promoting nucleotides produced by injured cells (11, 12), while Menkin has demonstrated stimulating agents in inflammatory exudates (14). The latter investigator injected an exudate from inflamed pleura into the ear of a rabbit and observed striking proliferation, characterized by hyperplasia and metaplasia of the normal epithelial layer and by foci of keratinization. Small nodules in the cartilage were also observed. Among other things, the exudate contained lactic acid and glucose (15, 16).

These findings support the suggestion by Potter that injury and irritation result in the breakdown of adenosinetriphosphate (18, 19). The adenosine and inorganic phosphate resulting from this primary reaction could lead to an increased glycolysis and a localized vasodilatation (18), and thus would favor the proliferation of fibroblasts as well as any neoplastic cells that might be present in the involved area. As the healing process neared completion the breakdown of adenosinetriphosphate would diminish until, finally, tissue oxidations would be able to esterify phosphate and adenosine at concentrations that are below the growth threshold. On this basis the continuation of any factor, be it gross irritation or some more subtle agent, that would accelerate the breakdown of adenosinetriphosphate or hamper its resynthesis would lead to a milieu favorable for proliferation. The subsequent growth of neoplastic cells in a healed tissue would then depend upon the inherent malignancy of the cells and the size the tumor had attained before the healing process was completed. A short period of irritation with the subsequent increase in connective tissue and decrease in blood supply would restrain the continued proliferation of the cells, whereas a longer period of healing would aid the tumor to attain a size that would be less dependent upon the local tissue environment.

There has been much uncertainty as to the connection between irritation or injury and cancer formation. Apparently irritation per se is not carcinogenic (23), and yet the development of tumors in old burns or other wounds is more frequent than could be accounted for by the mere laws of chance (24). These observations could be explained on the basis of the suggestions just offered.

**SUMMARY**

Methylcholanthrene was applied to the skin of mice for a period just short of the formation of visible tumors. The skin was then treated with naphthoquinone, croton oil, croton oil resin, or sodium sulfide, or it was subjected to frequent incisions. Many tumors developed following exposure to naphthoquinone, croton oil, croton oil resin, or to the processes of wound repair. The sodium sulfide was ineffective.

Frequent incisions made through the abdominal wall of rats with primary hepatomas resulted in nests of proliferating hepatoma cells in the regions of the wound. These neoplasms regressed during the later stages of the healing process. It is suggested that such transplanted cancer cells found a favorable environment for growth in the region of the healing wounds, and that the defense mechanism of tissues that inhibit such growth normally is decreased in areas where reparative processes are active.

These results are correlated with the suggestion that tumor formation may be divided into three phases: (a) period of induction, during which the neoplastic cell is formed; (b) critical or reversible period, in which the growth of the neoplastic cell is in delicate balance and depends upon the local tissue environment; and (c) the final period of progression, during which growth is relatively unchecked.

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