The Tumor-inhibitory Effects of 3-Methylcholanthrene on Transplantable and 3-Methylcholanthrene-induced Tumors in C3H Mice

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SUMMARY

When 3-methylcholanthrene therapy was initiated 8 days after transplantation of the myeloma X5563 in C3H mice, retardation and even complete suppression of the transplant resulted. It did not appear that a general depression of somatic growth could account for this effect. Histological examination of the carcinogen-treated myeloma revealed peripheral necrosis, reduction in mitotic figures, without a striking infiltration of leukocytes or normal plasma cells.

Dihydrotestosterone in conjunction with 3-methylcholanthrene produced a significant increase in body weight of the host mice, but appeared to have no additive effect on suppression of the myeloma's growth.

When therapy with this agent was delayed until 30 days after transplantation of the myeloma in the same strain of mice, complete regression of tumor still resulted occasionally, and the life of the tumor-bearing mice was generally prolonged.

When 3-methylcholanthrene therapy was administered to C3H mice bearing sarcomatous and/or carcinomatous tumors induced by this same carcinogen, tumor suppression and prolongation of survival were evident.

The results are discussed in relationship to previously existing theories regarding mechanisms of action of the tumor-inhibitory polycyclic hydrocarbons.

Haddow, in 1935 (8), reported that certain carcinogenic hydrocarbons retarded the growth of the transplantable Jensen sarcoma and Walker carcinoma in rats. Since that report, Haddow (8-11), Green (6), and Huggins (14) have extensively studied the tumor-retarding properties of some polycyclic hydrocarbons. Many of the potent chemical carcinogens will retard the growth of various transplantable tumors. Only a few studies (9, 10, 11) have been reported on the effect of these agents on the growth of either induced or spontaneous tumors in experimental animals. The most convincing results are those of Huggins and McCarthv (13), who found some regression of human metastatic mammary cancer following the administration of 3-methylcholanthrene. Bauer et al. (1) demonstrated regression of cancer of the skin in seven out of 22 patients treated with 3,4-benzpyrene.

This study is designed to demonstrate that a dosage of 3-methylcholanthrene effective in the treatment of a transplantable tumor is also effective in retarding the growth and lethality of induced neoplasms in the same highly inbred strain of mice.

MATERIALS AND METHODS

Preparation and administration of 3-methylcholanthrene and dihydrotestosterone.—Commercially purchased† 3-methylcholanthrene was dissolved in sesame oil by being heated to 85°C for 3-4 hours, and the final concentration was adjusted to

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† operated by the University of Chicago for the United States Atomic Energy Commission.
‡ Eastman Organic Distillation Products Industries.
so that 0.1 ml. of mixture contained 0.2 mg. of 3-methylcholanthrene. Dihydrotestosterone was dissolved in this solution or in sesame oil alone so that its concentration was 0.2 mg. 0.1 ml solution. New stock solutions were prepared at least every 2 weeks, and particular care was taken to maintain the stock in the dark.

Unless otherwise indicated, the treatment and tumor induction dosage schedules consisted of daily subcutaneous injections of 0.2 mg. 3-methylcholanthrene in 0.1 ml. of sesame oil over a period of 4 weeks. Control animals received 0.1 ml. of sesame oil subcutaneously. All injections were made into the ventral abdominal wall unless otherwise indicated.

Animals.—C3H mice were purchased either from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine (C3H/Jax) or from the Cumberland Farms, Clinton, Tennessee (C3H/Cumb). The strain, sex, and age will be indicated for each experiment. The animals were given the Rockland Mouse Diet complete and water ad libitum. When the date of birth was not identical, the mice were randomized among experimental and control groups.

Transplantable tumor experiments.—The myeloma X5563 was routinely passed subcutaneously to the dorsal surface of C3H/Jax mice by the trocar technic. Male and female mice 10-14 weeks of age were inoculated. In the first experiment, injections of sesame oil or 3-methylcholanthrene were given 3 days after transplantation. The animals were inspected and weighed at weekly intervals, and after 4 weeks were weighed and sacrificed.

In the second experiment, the mice were divided into four groups 3 days after transplantation of the myeloma. Daily treatment with sesame oil, 3-methylcholanthrene, dihydrotestosterone, or dihydrotestosterone and 3-methylcholanthrene was continued for 4 weeks. The animals were then weighed and sacrificed. The tumor mass was dissected free and weighed, but no histological studies were performed.

In the third experiment, the tumor was allowed to grow to detectable size, and the animals were then randomized on the basis of tumor size. Therapy was instituted 30 days after tumor transplantation, and at weekly intervals thereafter the tumors were measured. A continuous record of time of death was maintained. At the end of 4 weeks, the methylcholanthrene or sesame oil treatment was stopped, but the survivors were kept to follow the course of any possible "cures."

Induced tumor experiments.—In these studies only C3H/Cumb females of 12-14 weeks of age were used. In the first group, 30 mice received sesame oil, and 30 received 0.2 mg. of 3-methylcholanthrene injected subcutaneously into the ventral abdominal area daily for 4 weeks.

Fourteen weeks after the institution of therapy all the surviving animals were weighed. Total white blood count and hematoctrit determinations were performed, and the mice were autopsied. The tumors were dissected free from the surrounding normal tissue. The liver, spleen, kidney, and tumor were weighed and sections taken for histological examination.

In the second group of 80 mice treatment was similar to that in the first group, but injection of the carcinogen was begun 2 weeks later, and no sesame oil control group was included. Approximately 14 weeks after institution of induction treatment, the survivors were divided into two groups on the basis of general condition, body weight, and tumor size. Tumor size was estimated as the product of two dimensions measured with calipers. Therapy with sesame oil or 3-methylcholanthrene was instituted 10 weeks after the end of the induction period. The injections were made subcutaneously into the dorsal surface away from the tumors, and the therapeutic program was continued for 4 weeks. The mice were inspected regularly and mortality recorded accurately until the last animal died. Tumor size was measured on the 7th, 15th, and 28th days of the therapeutic regimen.

RESULTS

The influence of 3-methylcholanthrene on the transplantable myeloma X5563.—Table 1 summarizes the data pooled from three separate experiments, all performed in identical fashion. It can be seen that growth of the myeloma X5563 in C3H mice was consistently depressed by 3-methylcholanthrene if treatment with this carcinogen was instituted within 3 days of tumor transplantation. Furthermore, among those mice with a palpable tumor mass the average weight of the resected tumors treated with sesame oil was twice as great as that of the 3-methylcholanthrene-treated tumors.

Histological examination revealed a distinct contrast between tumors in mice treated with 3-methylcholanthrene and those from control mice. In the control group, necrosis was usually not
a prominent feature. It sometimes occurred in very large tumors but always in a central location, sparing the peripheral borders. Mitotic figures were prominent throughout, and almost no fibrous tissue was present. The carcinogen-treated tumors revealed a great reduction in mitotic figures, many of which were bizarre. Peripheral necrosis and fibrosis were prominent features. Passing from these areas of frank necrosis, bizarre cells with disrupted nuclei and vacuolated cytoplasm were seen. A distinct infiltration with leukocytes or normal plasma cells, however, was not characteristic. Figures 1 and 2 demonstrate some of these features.

Table 1 further demonstrates that the tumor-inhibitory effect of the carcinogen was not associated with general retardation of body growth of the host. Excluding the weight of the excised tumors, the net difference in body weight between the control and 3-methylcholanthrene-treated animals is negligible.

Further evidence that the tumor inhibition was not dependent on a general growth suppressive effect of 3-methylcholanthrene is demonstrated in Table 2. Administration of dihydrotestosterone led to a slight increase over the expected net weight change in sesame oil or 3-methylcholanthrene-treated mice. A striking increase in weight resulted when this androgenic hormone was combined with 3-methylcholanthrene for 4 weeks of therapy, although it had no demonstrable influence on the transplanted myeloma.

The phenomena of tumor retardation and/or regression induced by the carcinogenic agent are demonstrated in the third experiment. Those animals with palpable masses 30 days after inoculation were divided into two groups on the basis of tumor size. Chart 1 shows that the last control animal died 29 days after the beginning of treatment, when 42 per cent of the carcinogen-treated animals were still alive. Furthermore, the four animals which still survived at 65 days gave no evidence of the presence of a transplantable tumor. If the tumor was considered “small” at the onset of therapy, complete regression occurred.

Some gross and histological characteristics of the induced tumors.—Of the initial group of 30 CSH 9 mice which received 3-methylcholanthrene subcutaneously for a period of 28 consecutive days, eighteen animals were alive 14 weeks after institution of therapy. These animals appeared chronically ill with hunched posture, diarrhea, and ruffled hair. The ventral abdominal surface was nearby replaced by a poorly defined tumor mass(es). Frequently, bleeding necrotic ulcers were present in the tumor.

All the 30 control animals were alive at this time.

### Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>SEX</th>
<th>No. Mice</th>
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<td>No. + total*</td>
<td>Effect on tumor growth</td>
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<td></td>
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<td>With tumor (%)</td>
<td>Without tumor (%)</td>
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<tr>
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<td>19</td>
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<td></td>
<td></td>
</tr>
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<td>M</td>
<td>36</td>
<td>23</td>
<td>36</td>
</tr>
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<td></td>
<td>36</td>
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</tr>
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<td>16</td>
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</table>

* Number of mice with tumors/total number injected.
† Average weight based only on those mice with tumors.
‡ Gross weight change = the weight of the mice with or without tumor after 28 days of therapy less their weight at the onset of therapy.
§ Net weight change = the weight of these mice minus the weight of the tumor when present less their weight at the onset of therapy.
time and grossly appeared to be completely normal. The average body weight of the mice with chemically induced tumors was 20.5 gm., the hematocrit 36.4 per cent, and the white blood count 30,200. Compared with these values were an average body weight of 23.1 gm., hematocrit of 51.7 per cent, and a white blood count of 12,700 in the control group.

Autopsy was performed on all the surviving mice with induced tumors and on the control mice. No gross or histological lesions were present in the control animals. The tumors in the carcinogen-treated group were locally quite invasive. Involvement of the skin, subcutaneous tissue, muscle, and even extension into and through the peritoneum were found, but no gross distant metastases were detected. The spleen was usually enlarged, averaging more than 3 times the weight of the control animals. No other gross abnormalities were detected. Histological examination revealed that in thirteen of the eighteen tumors the induced neoplasm was a fibrosarcoma. Squamous-cell carcinoma was present in one case. In two animals it was difficult to ascertain whether the lesion was an epithelioma or, in fact, epidermal carcinoma. Finally, mixed carcinosarcomatous lesions were present in two of the animals given injections subcutaneously of 0.2 mg. 3-methylcholanthrene for 28 days. Figure 3 illustrates one of these tumors classified as a carcinosarcoma. Metastases were not demonstrated microscopically in the organs examined.

Extraduillary

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Sex</th>
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<tr>
<td></td>
<td></td>
<td>Without tumor (%)</td>
<td>Weight of tumor† (gm.)</td>
<td>Gross weight change‡ (gm.)</td>
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* Number of mice with tumors/total number injected.
† Average weight based only on those mice with tumors.
‡ Gross weight change = the weight of the mice with or without tumors after 28 days of therapy less their weight at the onset of therapy.
§ Net weight change = the weight of these mice minus the weight of the tumor when present less their weight at the onset of therapy.
myelopoiesis was greatly increased in the spleens of all the tumor-bearing mice. The liver was involved to a lesser degree, and in some of these animals myelopoiesis was present in the kidneys.

The influence of 3-methylcholanthrene on the induced tumors.—The explosive onset of induced tumors was again remarkable in the second group of carcinoen-treated animals. Fourteen weeks after the beginning of the induction phase, 67 of the initial group of 80 mice were found to possess tumors grossly identical to that in the autopsied group.

Table 3 demonstrates a slight suppression of tumor growth in the 3-methylcholanthrene-treated animals. When one considers that only the surviving animals were measured on the 7th, 15th, and 25th days, the effect may be more dramatic than these figures indicate. It should be stressed that accurate measurement of these induced tumors was not possible, owing to their highly invasive character. In general, those animals with larger tumors or with tumors that ulcerated early died more rapidly. A definite prolongation of life was achieved by the carcinogen therapy. After a 28-day course of this treatment, 21 of 33 animals survived, whereas the last of the 34 controls expired on this day. No further treatment was given, and all the experimental animals died within another 27 days.

DISCUSSION

Green (6, 7) has suggested that the tumor-inhibitory properties of the polycyclic hydrocarbons are dependent on their ability to enhance the natural immunity of the host. He has pointed out that the highest incidence of transplantable tumor regression following therapy with one of these agents occurs when the incidence of natural regression is itself the highest (6). Although this information has been gathered almost entirely from studies involving transplantable tumors in relatively noninbred strains of rats (6, 8, 10–12, 14), Rubin (17) has recently reported data which suggest that this is also the case in mice.

Green (6) has expressed the view that, if the tumor grows without natural regression in only one highly inbred strain of mice, its inhibition by the distal application of polycyclic hydrocarbons is much less likely.

The data we have presented militate against this theory. The myeloma X5563 arose as a spontaneous tumor in the C3H mouse and has since been serially passaged in this inbred strain by Potter (15). Growth in any other strain has not been reported. When treatment with the carcinogen was begun within the first 3 days of transplantation, growth failed to occur in 39 per cent of the animals. In the control animals growth was encountered in all but one instance. Furthermore, suppression and complete regression were still accomplished when therapy was delayed until 30 days after transplantation. In contrast, we have not encountered any instance of spontaneous regression of this tumor once established in several hundred C3H/Jax mice.

Green (6, 7) has stated that inhibition of either

<table>
<thead>
<tr>
<th>TABLE 3</th>
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<tr>
<td><strong>The Effect of 3-Methylcholanthrene on the Growth of 3-Methylcholanthrene-induced Neoplasms in C3H Mice</strong></td>
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<tr>
<td>Therapy Group</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sesame oil</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
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</tbody>
</table>

* = Days after institution of therapy.
N = Total number of surviving mice.
T = The average of the product of two dimensions measured with calipers.
spontaneous or induced tumors by a polycyclic hydrocarbon should not occur, because there is no host-tumor resistance under these circumstances. Carcinogenic agents have now been described, however, which occasionally alter growth of chemically induced sarcomas in rats (10), suppress growth of spontaneous mammary carcinomas in mice (9, 12), and sometimes initiate regression of mammary carcinomas (13) and skin cancer (1) in the human. We have further demonstrated a suppressive effect exerted by 3-methylcholanthrene on epidermoid carcinomas and fibrosarcomas induced in CSH mice.

Although the tumor-inhibitory action of carcinogenic agents may sometimes be due to a general depression of somatic growth (12, 19) or to the dietary protein content (3), this is not always so. Our studies, designed with reference to the findings of Huggins and Pollic (14) and Glenn and co-workers (4), reveal a potentiation by 3-methylcholanthrene and dihydrotestosterone, as shown by change in body weight of mice inoculated with the myeloma X5563. In this connection, it is interesting to note that potentiation of weight increase (9) did not extend to growth of the myeloma, thus suggesting that the tumor-inhibitory property of 3-methylcholanthrene is distinct from its capacity to influence somatic growth.

To conclude that some degree of immunological incompatibility cannot occur between a host and its spontaneous or externally induced tumor would be extremely hazardous. If such exists, tumor inhibition may in part be due to an enhancement of this immunity, although the data of Rubin (16, 17) suggest that 3-methylcholanthrene depresses the immunological response in mice, rather than augments it.

Finally, the possibility that there is a more direct attack on the cancer cell must be carefully considered. Shubik and Porta (18) have presented strong evidence that the effects of 3-methylcholanthrene, 3-4-benzpyrene, and 9,10-dimethyl-1,2-benzanthracene, administered as large single doses to normal adult mice, are strikingly akin pathologically to those induced by x-rays (2) or nitrogen mustard (5). Our tentative opinion is that the characteristics of peripheral necrosis, reduction of mitotic figures, without a prominent infiltration of host leukocytes and plasma cells, are similar to those seen when tumors are exposed to other cytotoxic agents.

ACKNOWLEDGMENTS

We are indebted to Dr. Charles B. Huggins, whose encouragement and advice stimulated the completion of these experiments.

REFERENCES

10. ———. The Influence of Carcinogenic Substances on Sarcoma Induced by the Same and Other Compounds. Ibid., pp. 891-91.
Fig. 1.—Photomicrograph of the myeloma X5563 excised after 28 days of therapy with control sesame oil. Tumor weighed 4.4 gm. Note, at least fourteen mitotic figures are present. (Hematoxylin-eosin-azure, X470.)

Fig. 2.—Photomicrograph of the myeloma X5563 excised after 28 days of therapy with 3-methylcholanthrene. Tumor weighed 2.1 gm. Note the reduced mitotic figures and the large bizarre cells. (Hematoxylin-eosin-azure, X470.)

Fig. 3.—Photomicrograph of a tumor indicated by 3-methylcholanthrene. Note the mixed elements of fibrosarcoma and epidermoid carcinoma. (Hematoxylin-eosin-azure, X153.)
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