Induction of Malignant Tumors by Methylcholanthrene in Transplanted Uterine Cornua and Cervices of Mice

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Spontaneous malignant epithelial tumors of the uterine cervices and cornua rarely occur in mice of most strains. One stock of mice had a high incidence of malignant uterine or cervical tumors. Cancer of the uterine cornua has not been induced experimentally although malignant tumors of the cervix and vagina have been shown, by previous investigations, to occur in mice given estrogen for long periods of time, (1, 3, 4–6, 11); or to follow the applications of carcinogens or carcinogens combined with hormones (2, 13, 14). A systematic study of the action of methylcholanthrene on the genital tissue has not been done previously.

Since the later part of the 19th century many attempts to induce cancer by transplantation of adult and embryonic tissues have been reported. Nichol, 1904 (12) apparently was the first one to transplant various kinds of adult tissues, including normal and pregnant uterine epithelium. He noted that uterine epithelium was one of the adult tissues capable of proliferation after transplantation to another host; but no cancer developed in the transplants. Recent investigations by Greene (7), and Rous and Smith (15) have shown that carcinomas and sarcomas could be produced in transplanted embryonic tissue treated with methylcholanthrene. More recently Horning (10) induced cancer by transplanting adult prostatic tissue treated with methylcholanthrene.

The present study concerns the malignant tumors induced by methylcholanthrene in transplanted young adult uterine cervical or cornual tissues in mice.

MATERIALS AND METHODS

In the first experiment 104 mice of both inbred strains and hybrid stocks were used. Most of the mice were of weaning age but some of them were 1 or 2 months older. The uterine cervices and horns were removed under aseptic conditions. In some of the animals in which the vaginas were open at the time of operation mercurichrome was used for disinfection of the cervical tissue. Small crystals of methylcholanthrene were inserted into the excised cervices and horns and the tissues were then transplanted into other hosts, usually their brothers or sisters. The transplants were placed subcutaneously in the upper abdominal areas of the mice. Some of the animals received one graft; others two or more grafts. All of the mice were kept in an air-conditioned house and were fed a diet of Purina Fox Chow and water ad libitum. All the animals were examined twice weekly. The date of the first appearance of the tumor, as a visible or palpable mass of approximately 5 mm. in diameter, was recorded. The tumors were measured at intervals during the life of the animals and the gross size was recorded at autopsy. Unusual occurrences such as bleeding and ulceration were noted in all instances. Most of the animals were killed when the tumors reached a considerable size or when the general condition of the animals had declined and death of the animal was imminent. A few of the animals died, induced tumors contributing largely to their death. At autopsy the tumor tissue, regional lymph nodes, lungs, liver, kidneys and other organs, were preserved in Bouin's and formalin fixatives, and paraffin sections were made which were stained with hematoxylin and trichrome routinely. Masson's trichrome and Laidlaw's reticulum stains have been used for demonstrating reticulum in some of the tumors. The induced tumors were transplanted in a number of instances and the transplanted tumors were carried through two or three generations.

In the second experiment a group of 18 mice was used for the study of the lesions shortly after transplantation. The animals were killed at weekly intervals up to the tenth week. The grafts were removed with the over- and underlying tissues and fixed in Bouin's solution. Two out of every 10 sections were mounted and stained with hematoxylin and trichrome.

In the third experiment 25 mice were used to study the distribution of the methylcholanthrene as
revealed by fluorescence microscopy. Most of the animals were killed at intervals of 24, 36, 48, 72 hours, etc., up to one week; others were killed 6 to 7 weeks after transplantation. The tissue removed was fixed in formalin and frozen sections were cut and mounted with glycerol. The light source used was a mercury vapor lamp, using a Ratten gelatin filter 2A to remove the longer rays. The presence or absence and the distribution of the crystals and fluorescence were recorded. Photomicrographs were taken of the fluorescent crystals and tissues, using a Leica camera and plus X film. Hematoxylin and triosin, Giemsa, trypan blue, acid fuchsin and methylene blue stains were used for orientation of the lesions in these preparations. In addition the Sudan IV stain was used for the demonstration of fat substance in some of the specimens.

Cervical or uterine tissues were not transplanted without the insertion of carcinogen, because previous investigation in this laboratory revealed that no cancer developed in such transplanted uterine tissue in mice of the A strain (5). Small pieces of spleen, liver, ovaries, bladder and rectum were transplanted after similar treatment with methylcholanthrene.

**OBSERVATIONS**

Of the 104 mice of both inbred strains and hybrid stocks in the first experiment, 55 animals developed 61 tumors. The tumors consisted of 32 carcinomas and 29 sarcomas (Table I). More tumors arose from the transplanted cervical tissue and more tumors developed in mice of the A strain. No sex difference has been observed. In the present study the age of the recipients did not influence the incidence of tumor growth. The carcinomas appeared earlier than the sarcomas. About 65 per cent of the induced tumors developed before the end of the eighth week (Table II). In most instances at 3 to 5 days after transplantation an initial increase in size of grafts was observed. Some grafts disappeared later, others remained unchanged for some time or increased steadily in size. The rate of growth of the epithelial tumors was more constant and uniform whereas that of the connective tissue tumors was abrupt and rapidly progressive. Most of the tumors reached a size of 2 to 3 cm. in diameter within a period of 2 to 3 months. Invasion to the underlying muscle tissue was observed frequently. No metastases to the distant organs were observed, but lymphatic extension was noted in 2 animals. In the carcinomatous group the skin was invaded early, and this was followed by ulceration. Cystic change and hemorrhage have been observed in the methylcholanthrene-treated transplanted uterine horns.

The carcinomas were spheroid and nodular, usually invading the overlying skin and underlying muscle (Fig. 1). The cut surfaces were pale grey and slightly granular. Necrosis was noted in most tumors and grey-yellow atheromatous patches were seen in some tumors. The external surface of the sarcomas was smooth and rounded (Fig. 2). The tumor tissue was usually pink and fleshy although grey-red necrotic centers were often seen in the sarcomas.

Histologically most carcinomas were of the epidermoid type, composed of irregular masses or sheets of more or less differentiated cells with an inconspicuous stroma. In many of the tumors the cancerous cells formed epithelial pearls or small cystic areas with mucoid material in the center (Fig. 3). The tumor cells in the peripheral portion were usually less differentiated and mitoses were more frequent. The tumor cells invaded the muscle fibers (Fig. 4). One adenocarcinoma and several adenocanthomas arose in the transplanted uterine horns treated with methylcholanthrene (Fig. 5). The sar-

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**Table I: Tumors in Methylcholanthrene-Treated Transplanted Uterine Cervical and Cornual Tissues of Mice of Several Strains and Hybrid Groups**

<table>
<thead>
<tr>
<th>Strains and stocks</th>
<th>No. of mice</th>
<th>Sex</th>
<th>Average age at time of transplantation, days</th>
<th>Tissues transplanted</th>
<th>No. of mice with tumors Cervix Horn</th>
<th>No. of tumors Cervix Horn</th>
<th>Origin of tumors Cervix Horn</th>
<th>Nature of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>27 (7)</td>
<td>62 (6)</td>
<td>18 (23)</td>
<td>16 (42)</td>
<td>Ca. 3 Sar. 5</td>
</tr>
<tr>
<td>BL</td>
<td>13</td>
<td>5</td>
<td>8</td>
<td>33</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>9</td>
<td>6</td>
<td>30</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>AC2</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>32</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>AC2 X BL(G)</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>27</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>AB1 X BL(G)</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>32</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>CC1 X BL(G)</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>35</td>
<td>9</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>55</td>
<td>49</td>
<td>150</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1 (30M) 0 (25F)</td>
</tr>
</tbody>
</table>

*Ca.*: Carcinoma, *Sar.*: Sarcoma.
TABLE II

The Latent Period of the Induced Tumors in Mice of Different Strains and Stocks

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Tumor Type</th>
<th>Time in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>Carcinoma</td>
<td>14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119 126 133 140</td>
</tr>
<tr>
<td>BL(S)</td>
<td></td>
<td>Sarcoma</td>
<td></td>
</tr>
<tr>
<td>NHO</td>
<td></td>
<td>Carcinosarcoma</td>
<td></td>
</tr>
<tr>
<td>PBr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC&lt;sub&gt;3&lt;/sub&gt; x BL(S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB&lt;sub&gt;1&lt;/sub&gt; x N(C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC&lt;sub&gt;1&lt;/sub&gt; x BL(S)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AC&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td></td>
<td></td>
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</table>

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Table: The Latent Period of the Induced Tumors in Mice of Different Strains and Stocks

The tumors were for the most part composed of spindle cells; in some instances they were round, and in others, were pleomorphic (Fig. 6). In some tumors the epithelial elements were immediately surrounded by the neoplastic connective tissue, thus forming a picture of carcinosarcoma (Fig. 7). Other tumors consisted of a mixture of adenosarcomatous elements. Remnants of the cervices and horns were recognizable in the centers of some of the large tumors (Fig. 8). A few sarcomas developed in the control group and one of the transplanted bladders showed cancerous lesions.

Eight carcinomas and 4 sarcomas that arose in the transplanted uterine tissue were transplanted into other hosts. The transplants of the carcinomas were histologically identical with the original tumors. In some of the transplanted pleomorphic cell sarcomas the subsequent transplants were composed of spindle cells resembling fibroblasts.

A study of the serial sections from the grafted tissue removed shortly after transplantation, revealed that from the end of the second week on there was suggestive evidence of neoplastic growth of the epithelial cells of the cervical mucosa. In a hybrid mouse that was killed at the end of the second week, localized areas of small and irregular strands of epithelial cells had penetrated the basement membrane of the cervical mucosa and grown into the underlying tissue (Fig. 9). The irregularly stratified epithelium was partially cornified and surrounding areas filled with leukocytes and sloughed cells. These neoplastic changes were associated with inflammatory reactions in the stroma. The remaining portion of the cervical mucosa revealed either diffuse increase in thickness of the epithelial cells or no alteration. Sometimes necrosis of the epithelium was associated with cellular exudate. In a hybrid mouse that was killed at the fourth week the grafted cervical tissue exhibited definite localized areas of neoplastic change; in some areas the epithelial cells had lost their polarity, were deeply stained and had penetrated the basement membrane (Fig. 10). In association with these neoplastic cells small collections of polymorphonuclear cells were found among the superficial layers of the degenerated epithelial cells. Another animal of the A strain had 2 tumors 6 weeks after transplantation; one consisted of an epidermoid carcinoma arising from the grafted cervical tissues and measured 2 cm. in size. The other was composed of several small carcinomatous nodules. One of the 2 tumors was composed of epidermoid cells, apparently arising from the proximal portion of the uterine horn; the other growth showed initial neoplasia of the endometrium of the distal end of the horn. These tumors were composed of small and irregular cystic structures, lined by 2 or
more layers of cuboidal or cornified cells. The architecture of the stroma of the uterine horn tissue was recognizable. The lumen of the uterine horn toward the distal end was partly lined by a stratified epithelium.

At one week after transplantation fluid and cellular exudate had accumulated in the lumen of the grafted cervices, and localized areas of thickening of the epithelium had occurred. Groups of leukocytes in the lumen were arranged radially around structures resembling crystals. In the thickened epithelium disintegrated phagocytes and polymorphonuclear leukocytes were surrounded by both degenerating and proliferating epithelial cells.

The subcutaneous tissue of the host showed marked inflammatory reactions during the first or second weeks and then the inflammation gradually subsided. The epidermis of most of the hosts revealed no change, but in some instances the areas adjacent to the tumor were inseparable from the neoplastic tissue. In some animals the tumor tissue merged with the sebaceous glands. The adjacent mammary tissue of the female hosts usually remained unaltered but were hyperplastic in one animal.

The sections examined with the use of ultraviolet light showed both green-yellow fluorescence of the methylcholanthrene crystals and the blue fluorescence of the dissolved carcinogen in the lumen of the grafted tissue. In some instances the crystals were attached to the epithelium. In association with the rounded blue masses of fluorescence noted in the lumen were small light blue granules. In 5 animals small fluorescent granules were also present in the epithelium (Figs. 11 and 12). In one animal, killed at the end of one week, the transplant had attached firmly to the skin and fluorescence was observed in the sebaceous glands of the host's skin. Blue fluorescence was observed in the surrounding adipose tissue. Crystals and fluorescence decreased gradually toward the end of the first week; however, in two animals that were killed at the end of the sixth and seventh weeks respectively, a few crystals and small areas of fluorescence were noted in the grafted tissue. One of these 2 grafts showed a small cancerous lesion of the epidermoid type. The blood vessels and lymphatics and their contents in the transplanted tissues revealed no fluorescence. The lymph nodes, livers and gall bladder were examined in some instances and revealed no detectable fluorescence.

Acid fuchsin stain did not interfere with the visualization of the fluorescence of the methylcholanthrene; while preparations stained with Giemsa, methylene blue, or hematoxylin revealed decreased intensity or failed to show any fluorescence. Crystals could be seen in frozen preparations thus stained and mounted in glycerol without passing through the fat solvents. Some of the preparations were stained with hematoxylin and trisoin after they had been studied under ultraviolet light in an unstained state (Fig. 11). The close relationship of the crystals and fluorescent substance, accumulated leukocytes and the local tissue could be determined. Using Sudan IV many fat droplets could be seen in the superficial layers of the epithelium and also in the cellular exudate.

**DISCUSSION**

The morphological evidence presented here indicates that malignant tumors, including carcinomas, adenocarcinomas, adenoscarcomas, carcinomas and sarcomas arose in transplanted uterine cervical and cornual tissue treated with methylcholanthrene. Evidence was sufficient to establish the origin of the tumor tissue both in the grafted tissue removed shortly after transplantation and in the fully developed tumors. The histological characteristics of the epidermoid carcinomas were similar to the cancers seen in the untreated and estrogen-treated mice (3, 4, 6). Tumors of similar types appear to develop under the influence of different inciting agents.

Although approximately equal numbers of transplants of the uterine cervices and horns have been made, only a small percentage of the uterine cornual transplants showed malignant tumors. The reasons for the relative infrequency of the epithelial tumors of the endometrium are unknown but the epithelium of the endometrium may be refractory to the carcinogen used or the quantity of methylcholanthrene may have caused excessive necrosis of the epithelium.

The tendency for the initial neoplastic lesions to be localized in certain areas of the epithelium in the treated transplant tissues has been observed. This was also true in the gastric cancers (18).

Due to the frequent association of these initial lesions with the accumulated leukocytes it has been assumed that there might have been some relationship between the applied carcinogen, cellular exudate and the reaction of the local tissue. Fluorescence of carcinogens in skin has been studied (16, 17). Graffi (8, 9), using carcinogenic hydrocarbons dissolved in glycerin and serum, demonstrated fluorescence in many cultured tumors and normal tissues including leukocytes. As a result of the present fluorescence study it seems that the localization of the initial neoplasia was the result of a concentration of crystals of methylcholanthrene in the trans-
planted tissue. It is also possible that the cellular elements in the exudate might have facilitated or altered the action of the carcinogen. The presence of fluorescence in the fat tissue has been observed by many investigators. The significance of this observation is not certain.

The high incidence of these induced carcinomas in mice of the A strain is of interest. There is no evidence that mice of the A strain are more susceptible to spontaneous cancers of the genital tissue. The increased susceptibility to tumor formation may be partly due to the fact that the mice of the A strain are highly inbred animals in which the homologous grafts showed a higher degree of survival. It might also be possible that these mice react to the carcinogen in a different manner than the other strains do. Further investigation is necessary to elucidate this problem.

SUMMARY AND CONCLUSION

Fifty-five of the 104 mice of both inbred strains and hybrid stocks developed 61 malignant tumors in transplanted uterine cervical and horn tissues treated with methylcholanthrene. The malignant tumors consisted of epidermoid carcinomas, adenocarcinomas, adenocanthomas, carcinosarcomas and sarcomas. Fewer tumors arose from the transplanted cornual tissue than from cervices. More cancers developed in mice of the A strain than the other strains used.

REFERENCES

DESCRIPTION OF FIGURES 1 TO 6

Fig. 1.—Gross appearance of an induced carcinoma that developed from the transplanted cervical tissue in a mouse of the NHO strain.

Fig. 2.—An induced sarcoma with rounded and smooth surface is seen in the lower chest wall of a hybrid mouse.

Fig. 3.—Photomicrograph of an epidermoid carcinoma arising from the transplanted cervical tissue. The cervical mucosa was seen in the center of the tumor.

Fig. 4.—Photomicrograph of an epidermoid carcinoma arising from the transplanted cervical tissue of a mouse of the A strain. Small and irregular solid strands of tumor cells invaded the muscle fibers.

Fig. 5.—Photomicrograph of an adenocarcinoma of a hybrid mouse. The tumor is composed of irregular glandular structures. Many mitoses are seen.

Fig. 6.—Photomicrograph of a pleomorphic cell sarcoma.
DESCRIPTION OF FIGURES 7 TO 12

Fig. 7.—Photomicrograph of a carcinosarcoma arising from the transplanted cervical tissue in a hybrid mouse.

Fig. 8.—Low power view of the same tumor revealing the original architecture of the transplanted cervical tissue.

Figs. 9 and 10.—The localized initial neoplasia of the epithelium in the transplanted cervical tissue.

Fig. 11.—Photomicrograph of a preparation examined with the ultraviolet light. The spindle shaped crystals were green yellow in color and the small granules in the inflamed epithelium were blue and fluorescent.

Fig. 12.—The same preparation stained with hematoxylin and eosin. Accumulation of polymorphonuclear leukocytes is seen in the areas where the crystals and fluorescent granules were present.
Figs. 7-12
Induction of Malignant Tumors by Methylcholanthrene in Transplanted Uterine Cornua and Cervices of Mice

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