Experimental Brain Tumors

IV. The Incidence in Different Strains of Mice*

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Three chemical carcinogens, 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, have previously been tested for their brain tumor-inducing potentials in C3H mice (6,7,2). Each chemical, in the form of a pellet of about 1.5 mm. diameter, was usually introduced into the subcortex of the right cerebral hemisphere, but in some instances the pellets were placed so deeply that they came to lie within the lateral ventricle. In the case of methylcholanthrene deliberate attempts were also made to place certain pellets both in the cerebellum and the cerebral leptomeninges. More recent experiments with this carcinogen (8) brought the total number of animals up to 145, of which 68 (47 per cent) developed primary brain tumors. Of these, 50 were gliomas and 18 were sarcomas. Forty-seven mice treated with benzpyrene yielded 24 (51 per cent) primary brain tumors—15 gliomas and 9 sarcomas. In contrast to the rather similar results obtained with these two chemical carcinogens, dibenzanthracene yielded but 4 brain tumors in 21 mice—2 gliomas and 2 sarcomas.

The purpose of the present communication is to report the results of intracerebral methylcholanthrene implantation in different strains of mice.

METHOD

Six strains of mice (C3H, ABC albino, Bagg albino, C57 black, A albino, and dba) were utilized in these experiments with 20-methylcholanthrene. The chemical carcinogen was obtained from the Edcan Laboratories, South Norwalk, Connecticut, and was employed in the form of pellets without purification or dilution. The pellets were made by heating the crystals gently in a beaker until fusion. When cooled, the solidified material was cut into 1 mm. cubes with a knife. Male and female mice in approximately equal numbers were used in each strain. The sexes were strictly segregated throughout the experiment. The mice were 6 to 7 weeks of age when the experiments were started.

All the details of the operative procedure, anesthesia, animal care, and diet were similar to that employed in the original work with methylcholanthrene (6). The right cerebral subcortex was the site of pellet implantation in about half the mice of each strain, whereas the cerebellum was the site in the other half of the animals. It should be recognized that the chemical was undoubtedly placed more deeply in the nervous tissue in some instances than in others and that there was no absolute means of avoiding the extrusion of certain pellets through the trephine opening in the skull into the tissues of the scalp.

RESULTS

Although availability was an important factor in the choice of the particular mouse strains employed in these experiments, deliberate selections were made in two instances. Those were in the cases of the C57 black and the dba strain. The former notoriously yields a low incidence of spontaneous breast tumors, whereas the latter is equally well known for its high incidence of this particular type of neoplasm. It seemed worth while to compare these two strains in their behavior to methylcholanthrene placed intracerebrally. The results of the experiments with all 6 mouse strains are summarized in Table I.

It is apparent from this table that two mouse strains, namely, the A albino and the dba, had a surprisingly low incidence of carcinogen-induced brain tumors. Since the very pellets of methylcholanthrene that failed to induce neoplasms in the mice of these two strains did later induce them in C3H mice, it appeared that the genetic constitution of the animals was the important factor determining tumor incidence. Burdette and Strong (4) showed similar variations in extracranial tumor susceptibility in five inbred strains of mice treated with subcutaneous injections of methylcholanthrene. They found that the C3H and JK strains were most and least susceptible, respectively, to such tumors. Further data on the genetic influence on carcinogen-induced brain tumors will be presented below.

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In nearly all the instances of extracranial sarcoma the pellets were found extruded into the tissues of the scalp. Similarly, the majority of the cases of intracranial sarcoma disclosed that the pellets were in contact with the meninges.

The types of glioma encountered in the different strains of mice are listed in Table II. Included in this table are also the classifications of the gliogenous portions of the neoplasms of mixed glioma-sarcoma type. To some extent the kind of glioma was determined by the site of carcinogen implantation. Thus, in the case of the ependymoblastomas, the pellets were in contact with the ventricular ependyma, whereas the medulloblastomas generally resulted from cerebellar implantations. This has already been discussed in a previous paper (6). The one mouse of the ABC albino strain (No. 5) and one of the three A albino mice (No. 8) that developed medulloblastomas had these tumors in the cerebrum. The reason for this unusual site of medulloblastoma is as obscure as the factors that determine why subcortical pellet implantation in the cerebrum results on occasion in astrocytoma, glioblastoma multiforme, or oligodendroglioma.

A rather disappointingly large number of gliogenous neoplasms could not be classified. Formerly it often proved possible to classify such tumors by studying their growth behavior and structural characteristics in transplants. In the present experiments, however, an opportunity to transplant such a tumor occurred but once, in a C3H mouse. The tumor was a mixed glioma-sarcoma, but the gliogenous portion was apparently lost in the very first transplantation.

Further data on the genetic influence on carcinogen-induced brain tumors.—Attention was called above to the fact that the incidence of methylcholanthrene-induced brain tumors was low in the dba in comparison with the C3H mice. Both strains of mice employed in these experiments were raised in this laboratory by pen matings—several females and one male in the same cage—from stock originally obtained in 1938 from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. It seemed worth while to repeat the intracerebral implantation of methylcholanthrene in inbred mice of these two strains obtained by brother-to-sister matings. Dr. Leonell C. Strong, of the Department of Anatomy, Yale University School of Medicine, supplied such inbred C3H mice and Doctor William S. Murray, of the New York State Institute for the Study of Malignant Diseases in Springville, New York, supplied the inbred dba mice. The origin of the various strains of mice employed in these experiments is fully described in a recent paper by Strong (5). To complete the study of the genetic influence on chemically induced brain tumors, hybrids were raised in this laboratory by crossing female pen-bred C3H mice with male pen-bred dba and also male pen-bred C3H with female pen-bred dba mice. In all these experiments the carcinogen was implanted in the right cerebral subcortex and the results are summarized in Table III.

In view of the 47 per cent incidence of intracranial neoplasms that resulted from methylcholanthrene implantation in pen-bred C3H mice, an incidence of 7 such tumors out of 27 Strong inbred C3H mice is

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**Table I: Effect of Methylcholanthrene in Different Strains of Mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>Tumors</th>
<th>Negative</th>
<th>Gliomas</th>
<th>Intracranial sarcomas</th>
<th>Mixed glioma-sarcoma</th>
<th>Extranuclear sarcomas</th>
<th>Unclassified tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>42</td>
<td>34</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ABC albino</td>
<td>25</td>
<td>19</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bagg albino</td>
<td>21</td>
<td>14</td>
<td>7</td>
<td>10</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C57 black</td>
<td>35</td>
<td>21</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>-</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>A albino</td>
<td>22</td>
<td>6</td>
<td>16</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>dba</td>
<td>19</td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Table II: Gliomas in Different Strains of Mice**

<table>
<thead>
<tr>
<th></th>
<th>C3H</th>
<th>ABC albino</th>
<th>Bagg albino</th>
<th>C57 black</th>
<th>A albino</th>
<th>dba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytoma</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ependymoblastoma</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>2</td>
<td>8*</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>-</td>
<td>-</td>
<td>1†</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unclassified</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

* One tumor was a mixed glioblastoma multiforme-medulloblastoma.
† This tumor was a mixed glioblastoma multiforme-oligodendroglioma.
disappointingly low. The anticipation, not realized in this experiment, was that an even greater percentage of primary brain tumors would be found in the inbred than in the pen-bred animals. The rather large number, 8, of extracranial sarcomas in this experiment was also unexpected. Perhaps, however, the number of mice employed, 27, is too small to justify the assumption that highly inbred C3H mice are refractory to carcinogen-induced brain tumors as compared to pen-bred animals of the same strain. It should be remembered in this connection that the C3H strain was developed entirely for its spontaneous breast tumor propensities and that its high yield of chemically-induced brain tumors is coincidental.

The results with the "Murray" inbred dba mice were as gratifying as those with the inbred C3H animals were disappointing. Of 29 mice in the experiment but one animal developed an intracranial tumor—a glioma. Equally suggestive of the genetic influence on carcinogen-induced brain tumors are the results with the F1 progeny of C3H male and dba female, and C3H female and dba male animals. Of 46 mice in the former experiment, 13 developed primary brain tumors, and of 50 mice in the latter experiment, 12 had such tumors. Thus, whereas the pen-bred C3H mice yielded brain tumors in approximately 50 per cent of the animals and similarly bred dba mice yielded practically none, the hybrid offspring of these two strains had a tumor incidence of 25 per cent. Andervont (1) has already shown that C3H mice mated with I and Y mice produced a progeny whose susceptibility to induced tumors was intermediate to that of both parents. He concluded that if genetic factors were responsible for this susceptibility they were probably multiple. More recently, Burdette (3) reported that the progeny of C3H and JK mice had average and median appearance times for tumors induced by methylcholanthrene intermediate to those of the parental strains. He felt that his results were compatible with the existence of more than one gene for susceptibility to induced tumors. In so far as the incidence of carcinogen-induced brain tumors was also intermediate to that of the parental C3H and dba mice, the same conclusion is probably warranted; namely, that more than one genetic factor is responsible for the inheritance of susceptibility to such tumors.

A classification of the gliogenous neoplasms that developed in the inbred C3H and dba mice and in the F1 hybrids from these two strains is presented in Table IV. The nongliogenous intracranial tumors were all fibrosarcomas with origins, as far as could be determined, in the cerebral meninges. The classifiable gliomas were of three varieties—astrocytoma, ependymoblastoma, and glioblastoma. Since the carcinogen was invariably implanted in the cerebral subcortex, at least a partial explanation is at hand for the failure of medulloblastomas to make their appearance. The relatively small total number of gliomas, 22, is probably another explanation for the relatively few varieties of tumors of this class to develop.

In the previous contributions to the general subject of experimental brain tumors already referred to (6, 7, 2) the different types of primary intracranial neoplasms were described and illustrated in detail as regards their macroscopic and histologic features. For this reason illustrative material is not included in the present report.

**SUMMARY**

The experiments comprising the subject of this communication were devised to test the validity of the hypothesis that genetic constitution is an important factor in the incidence of primary intracranial neoplasms induced with pure methylcholanthrene implanted intracerebrally. Of six strains of mice tested, namely, C3H, ABC albino, Bagg albino, C57 black,
A albino, and dba, the first four strains yielded an incidence of 50 per cent or better in primary intracranial neoplasms, whereas the last two strains were poor with respect to carcinogen-induced brain tumors. Only 4 of 22 A albino mice and 2 of 19 dba mice developed intracranial neoplasms. The incidence of brain tumors induced with methylcholanthrene is in no way related to the propensities that the different mouse strains show in regard to the development of spontaneous mammary tumors. Both the C3H and dba strains, for example, have a high incidence of spontaneous tumors of this variety, whereas the C57 black strain yields extremely few such tumors.

Of 29 inbred dba mice (from brother-to-sister matings) only 1 developed an intracranial tumor following the intracerebral implantation of methylcholanthrene. A disappointing fact was that only 7 of 27 similarly inbred C3H mice developed primary brain tumors, in contrast to an incidence of 25 brain tumors out of 42 pen-bred mice of the same strain. Hybrids obtained by crossing pen-bred C3H and pen-bred dba mice yielded an intracranial tumor incidence of 25 out of 96 animals, or 26 per cent.

REFERENCES
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