EFFECT OF IRRADIATION ON THE GROWTH OF INTRACEREBRAL GLIOMAS IN MICE\textsuperscript{1,2,3}

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Because it is difficult to obtain data on the serial effects of irradiation of brain tumors in man, one is led to study the analogous situation in a laboratory animal. This report deals with the effects of x-rays on a transplantable intracerebral glioma in inbred C57BL/6 mice*.

DESCRIPTION OF TUMOR

The tumor used is one which was induced by intracerebral implantation of methylcholangthrene into a C57BL/6 mouse at the laboratories of Dr. H. M-Zimmerman, Montefiore Hospital, New York (1). Histologically, it is an ependymal, polyhedral cells with poorly defined plasma membranes and moderate quantities of eosinophilic cytoplasm. The nuclei are oval or round, with prominent mitotic figures. In some areas, the cells occur in perivascular masses arranged in a somewhat radial fashion contiguous to the vascular walls. Rarely, the central portion of the tumor is necrotic (2). The tumor is not infiltrative, but generally grows in a solid, discrete mass which displaces and compresses the surrounding brain. Occasionally, small tongues of tumor tissue extend into the adjacent brain (fig. 1). The characteristics of the tumor have not changed in over 100 transplant generations. It is maintained from one generation to the next by subcutaneous transplantation about once every 3 weeks.

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\textsuperscript{3} Presented at the meeting of the American Association of Neuropathologists on June 9, 1963, in Atlantic City, N. J., and received the Annual Award of the Association.
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\textsuperscript{*} Obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.
Fig. 1. Photomicrographs of the methylcholanthrene induced ependymoblastoma. A and B: Nests and cords of pleomorphic, polyhedral cells; $\times 100$, $\times 180$. C: Oval nuclei with prominent mitotic figures; $\times 400$. D: Areas of necrosis in the tumor; $\times 40$. 
Fig. 2. Mice immobilized in glass tubes with lead shielding for radiotherapy.

METHODS

Technic of Tumor Implantation

The tumor is minced aseptically into fragments about 1 cu. mm. in size. A fragment is then drawn into a brain trocar with a blunt tip and a diameter of an 18 gauge needle, which is fitted with a stylet. The mouse is anesthetized with pentobarbital intraperitoneally, and the scalp hair is removed with a depilatory. The scalp is incised, and a burr hole is made in the right parietal region with a dental drill. The tumor fragment is then implanted into the right cerebral hemisphere, and the scalp is closed with a silk suture. The mice recover from anesthesia in about 30 minutes.

Technic of Irradiation

The mouse is immobilized in a glass tube and all of its body except the head is shielded with a sheet of lead. X-ray therapy is then administered (fig. 2). The physical factors used were 280 kv., with a half-value layer of 2 mm. Cu or 0.17 mm. Cu and a target skin distance of 50 cm. One hundred forty roentgens per minute were delivered. The calibration of the machine was checked before each treatment by a dose meter*. Various total tumor doses which were divided into different numbers of treatments were tested. The results of therapy have all been confirmed histologically.

The histologic events which occurred during and after the administration of a curative dose of x-rays were studied by serially sacrificing mice every 2 days after the start of therapy. Mice with intracerebral tumor implants but not given radiotherapy served as controls, and were sacrificed concurrently.

RESULTS

Natural Course in Mice with an Intracerebral Tumor Implant

Within the isologous system previously described intracerebral tumor implantation is followed by the appearance of signs within 15 to 20 days, and 98

* Victoreen "r" meter, Victoreen Instrument Company, Cleveland, Ohio.
per cent of the mice are dead from tumor growth by the 28th day (3, 4). The following signs indicate that the tumor is growing: 1. The normally flat and shiny coat becomes dull and shaggy; 2. The mouse tends to walk in a hunched-up position; 3. The animal curls up when provoked instead of running about; 4. There are marked equilibratory disturbances, so that the mouse rolls over if it attempts to walk or rotates violently if held by the tip of the tail. Frequently, tumor herniates through the burr hole, and is seen as a nodule beneath the scalp.

The fact that an occasional mouse survives intracerebral tumor implantation is ascribed to technical failure, such as the implantation of a necrotic tumor fragment.

In mice which die as a result of tumor growth, examination of the brain sections reveals that the tumor frequently occupies almost half of the cerebral hemisphere.

**Effect of X-Ray Therapy**

In evaluating the effects of x-ray therapy in mice with intracerebral tumor implants, different total tumor doses administered fractionally over varying periods of time were tested (table 1). As can be seen, a total tumor dose of 1,100 or 1,840 r administered in 5 divided doses between days 10 and 24 after tumor implantation is curative for only a small number of mice, and retards the rate at which the other animals die. With the same schedule of irradiation, a total dose ranging from 2,200 to 3,000 r is essentially curative. In the few mice in this category which did not survive, the cause of death was tumor growth. A total dose of 3,500 r causes death from irradiation.

The data on the effect of fractionation on the result of therapy are summarized in Table 2. A total dose which is curative if administered in 5 divided doses from days 10 to 26 after implantation causes death from radiation if administered in 3 divided doses between days 10 and 15 after implantation.

In mice with intracerebral gliomas, a nodule representing herniating tumor or brain is commonly present over the burr hole beneath the scalp. In mice receiving a curative dose of irradiation, the nodule regresses in size and eventually disappears. Later in the course of therapy, at about day 25 to 30 after the first x-ray treatment, mice begin to show periorbital and scalp epilation. The mice which received a curative total tumor dose of 2,200 r appeared healthy throughout

<table>
<thead>
<tr>
<th>Tumor dose</th>
<th>Fractionation on day post implantation</th>
<th>Number of mice</th>
<th>% surviving at day</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>28</td>
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<td>10–13–17–20–24</td>
<td>11</td>
<td>100</td>
<td>100</td>
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<tr>
<td>3000</td>
<td>11–14–18–22–26</td>
<td>8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3500</td>
<td>10–12–14–19–21</td>
<td>9</td>
<td>88</td>
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</table>
while those which received a curative total tumor dose of 3,000 r began to look ill on about day 30 after the first treatment, but once again began to look healthy about 10 days later. The signs attributed to radiation sickness were: 1. Dull and shaggy hair, and 2. a hunched posture. Figure 3 shows a mouse which had received a toxic dose of radiation.

Figures 4 and 5 compare the growth of an intracerebral tumor in untreated mice to that in mice exposed to a 2,200 r curative dose of x-rays.

Histologic examination of untreated mice killed serially after the implantation of a tumor fragment reveals that the highly cellular tumor rapidly proliferates

### TABLE 2

*The Effects of Fractionation of X-Irradiation on Mouse Cerebral Glioma*

<table>
<thead>
<tr>
<th>Tumor dose</th>
<th>Fractionation on day post implantation</th>
<th>Number of mice</th>
<th>% surviving at day</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
<td>10–13–15</td>
<td>8</td>
<td>100 0 0 0</td>
<td>Radiation “Cured”</td>
</tr>
<tr>
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<td>10–13–17–20–24</td>
<td>11</td>
<td>100 100 100 91</td>
<td>Radiation “Cured”</td>
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<tr>
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<td>10–12–14</td>
<td>9</td>
<td>88 0 0 0</td>
<td>Radiation “Cured”</td>
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<tr>
<td>3000</td>
<td>11–14–18–22–26</td>
<td>8</td>
<td>100 100 100 88</td>
<td>Radiation “Cured”</td>
</tr>
</tbody>
</table>

Fig. 3. A mouse which has received a toxic dose of x-irradiation. There is depigmentation and epilation of the scalp hair.
and provokes a variable lymphocytic response in the adjacent brain, ranging from slight to moderate. Some of the vessels in this area occasionally show cuffing with small numbers of lymphocytes.

Often, there is marked sponginess of the adjacent brain, indicating edema, and sometimes large vacuoles, containing fluid, separate the proliferating neoplastic cells from the surrounding brain. Cords of neoplastic cells are commonly separated from one another by small amounts of edema fluid. Immature or proliferating glia were not seen in the neighborhood of the tumor. Without ever eliciting a greater response from the host, the tumor grows rapidly and kills the animal.

Histologic examination of the brains of mice killed serially during a curative course of therapy reveals that x-rays alter the development of the implanted tumor by decreasing its cellular activity, which is followed by a reduction in size of the neoplastic nodule. Thus, 2 days after the first dose, in animals being given a curative course in 5 divided doses, there is widespread early degeneration of neoplastic cells. These changes include hyperchromatism, pyknosis, and, occasionally, karyorrhexis. Foci of acellular, eosinophilic fibrillary material, surrounded by degenerating tumor cells and nuclear debris, are scattered throughout the tumor. As therapy is continued, the degenerative changes in the neoplastic cells become more prevalent. In addition to pyknosis and karyorrhexis, some cells assume giant proportions. Their nuclei become large and hypochromatic, and have an irregular outline and a grotesque appearance (fig. 6). Multinucleated
Fig. 5. Coronal sections of brains of treated and untreated mice 18 and 22 days after tumor implantation.

giant tumor cells are seen occasionally. Regardless of this atypical pleomorphism, the neoplastic potential of these elements appears questionable. They are seen exclusively in tumors undergoing involution and probably represent reactive forms to x-ray injury in a degenerating phase.

A few days after the onset of degenerative changes in the neoplastic cells, lymphocytes begin to infiltrate in and around the tumor, and the vessels in the adjacent brain exhibit lymphocytic cuffing. As tumor necrosis becomes more widespread, the number of lymphocytes increases.

With decreasing cellularity of the tumor, there is a corresponding increase in the amount of edema fluid which separates groups of neoplastic cells. Concomitantly, there is an over-all decrease in the size of the tumor nodule. By day 8 after the start of therapy, many reactive, swollen astrocytes are seen in the adjacent edematous cerebral tissue, as well as in areas of the tumor where necrosis is well advanced. From the astrocytes, long, cytoplasmic processes extend between the neoplastic cells. Occasionally, some fibroblasts invade the tumor (fig. 7). As more of the neoplastic cells die, the tumor nodule progressively contracts, and increasing numbers of reactive astrocytes appear among the neoplastic cells, enclosing them within a meshwork of cytoplasmic processes.

Twelve days after the start of therapy, at a time when tumor necrosis is almost complete, and when lymphocytic infiltration of the tumor is prominent, macro-
Fig. 6. Section of tumor from a mouse after the start of x-ray therapy, showing giant neoplastic cells with hyperchromic nuclei. The cell at the lower left contains two large nuclei and a cytoplasmic vacuole. Lymphocytes are scattered about the field; X 2000.
Fig. 7. Sections of the tumor at 12, 16, 18 and 22 days after implantation (respectively 2, 6, 8 and 12 days after starting x-ray therapy). There is progressive decrease in the cellularity of the tumor; × 100.

Macrophages swollen with granular debris appear in the adjacent cerebral tissue (figs. 8 and 9). Thereafter, the neoplastic cells disappear completely, the numbers of lymphocytes decrease progressively, and the former site of the tumor is occupied by large numbers of macrophages and reactive glia. Over the next several weeks the macrophages disappear and the glia assume a mature aspect. The final result
Fig. 8. (a) Lymphocytes clustered around degenerating tumor cells, 6 days after starting therapy; (b) Infiltration of the degenerating tumor with lymphocytes and reactive astrocytes, 8 days after starting therapy; (c) Macrophages and lymphocytes in brain tissue adjacent to the degenerating tumor, 10 days after starting therapy; (d) Glial scar with cholesterol clefts, 18 days after starting therapy; all $\times$ 200.
Fig. 9. Section from treated animal showing macrophages laden with granular debris in brain adjacent to degenerating tumor; × 900.
Fig. 10. Resultant cerebral scars after curative x-ray therapy; (a) Cholesterol clefts are present among macrophages laden with granular debris and hemosiderin; (b) Lymphocytes, macrophages and reactive glia; (c) Degenerating tumor cells enmeshed in a fibrous and glial scar; (d) Mature glial and fibrous scar; all X 45.
is a discrete, contracted, glial scar (fig. 10). Fibroplasia is usually minimal, but occasionally it is prominent. In a limited number of “cured” mice, there were small cholesterol clefts, foci of dystrophic calcification, and hemosiderin granules. Vascular changes, including early proliferation of the endothelium, were rarely seen.

Fig. 11. (a) Characteristic appearance of tumor from an untreated animal; (b), (c), and (d). Tumors from insufficiently treated animals which died from tumor growth, showing large serpentine areas of necrosis; all $\times 45$. 
Untoward effects of irradiation upon the neuron population were not observed in "cured" mice.

In animals dying from tumor growth after a course of therapy which was not curative but was sufficient to retard death (i.e., animals receiving a total tumor dose of 1,100 or 1,840 r), a consistent feature on histologic examination was the presence of large serpentine areas of necrosis in the midst of an otherwise healthy tumor. These necrotic areas contained a fibrillary or amorphous, acellular, eosinophilic material upon which were superimposed densely basophilic granules of varying size, probably representing nuclear debris. Healthy neoplasm grew immediately adjacent to these areas, which probably represented the remains of portions of tumor which were destroyed by the x-rays (fig. 11). Lymphocytic infiltration of these tumors was not seen, and, except for an occasional collection of lymphocytes in the adjacent brain, there was no evidence of any other cellular reaction in response to tumor growth.

The extent of these necrotic areas was much greater than is ever seen in untreated animals, in which the tumors occasionally show small areas of necrosis. They occurred characteristically in inadequately irradiated animals dying from tumor growth between days 35 and 56 after tumor implantation (untreated animals generally die between days 15 and 25 after tumor implantation).

The organs of the head and thorax of the mice dying from an overdose of x-rays were examined histologically. The tumors were necrotic, the brains were normal. However, there was radiation reaction with superficial ulceration of the oropharyngeal epithelium, and focal interstitial hemorrhages in the lungs. Thus, it is unlikely that these mice died from the effects of radiation to the brain, but rather from the effects of x-rays on contiguous organs which could not be adequately shielded.

**DISCUSSION**

Various investigators have attempted to document the effect of irradiation on intracerebral gliomas in man by comparing portions of tumor removed at surgery with portions removed at reoperation or at necropsy after x-ray therapy (5-9). Dyke and Davidoff (10) have reviewed this material. The consensus of opinion of the workers cited was that the primary effect of x-rays was on the neoplastic cells. These became swollen or shrunken, while the nuclei exhibited various forms of degeneration, such as hyperchromatism, pyknosis, disruption of the nuclear membrane, and karyorrhexis. The pyknotic nuclei frequently contained large coarse clumps of chromatin, superficially resembling mitotic figures (5). Bizarre, multinucleated giant cells of a degenerative type were noted, the cell cytoplasm developing a hyaline appearance, the nuclei shrinking and becoming necrotic, and an appreciable reduction in the cellularity and in the number of mitoses occurring, as determined by cell counts (6).

An increase in glial and fibrous stroma, and hyperplasia of the endothelial layers of blood vessels was also attributed to irradiation.

The reports on human material pointed out that the attempt to attribute the histologic changes to the effects of irradiation was often difficult and for several
reasons conclusions had to be guarded: 1. Relatively long periods of time commonly elapsed between irradiation and examination of the postradiation specimen, so that the effects of irradiation might have been overwhelmed by regrowth of tumor; 2. The nature of a glioma commonly changes spontaneously with time; 3. Frequently, only small biopsy specimens were available for examination, whereas it is known that the nature of a glial neoplasm may vary greatly from one area to another; 4. The total dosage and schedule of x-ray administration varied a great deal from one patient to another.

Netsky et al. (11), working with a methylcholanthrene-induced ependymoblastoma in isologous C57BL/6 mice, exposed tumors growing subcutaneously to single doses of x-rays ranging from 200 to 5,000 r. Doses of 200 and 400 r did not affect tumor growth, as compared to controls. However, within a few days of exposure to 3,000 or 5,000 r, the tumors began to regress in size. Some tumors never recovered, while others again began to grow after 2 to 3 weeks, and eventually killed the host. In such cases, it was believed that regrowth stemmed from portions of the tumor which had been out of the field of irradiation. This was proved by exposing tumor in vitro to various single doses of x-rays and then implanting the tumor subcutaneously. Tumors which had received 400 r grew as did those in unirradiated controls, while tumors which received 3,000 r failed to grow.

The tumors were examined histologically at various intervals after exposure to x-rays. Within a few days after a dose of 5,000 r, the tumor cells became swollen, and their nuclei swollen or pyknotic. At 9 days, scattered macrophages and mononuclear cells were present at the periphery; at 14 days, the site of the tumor was marked only by macrophages and mononuclear cells, some bizarre-looking remnants of tumor occasionally being present. The histologic changes were proportionately slighter after exposure to smaller doses.

Tansley and Wilson (12) gave single doses of radiation, ranging from 1,623 to 3,247 r, to the heads of mice bearing an intracerebral transplantable glioma. They recorded no cures, although tumor growth in some cases was temporarily inhibited. The characteristic histologic picture in such cases was the presence in the tumor of multinucleated giant cells which frequently contained abnormal mitoses.

In an attempt to approximate more closely the clinical situation of a patient with a brain tumor, we studied the effects of fractionated doses of x-rays directed at the heads of mice with intracerebral gliomas. Thus, it was possible to observe the differential sensitivity to x-rays of the tumor and the brain, and the histologic changes in the surrounding brain, as well as in the tumor.

All of the changes in the neoplastic glia, which various workers describing human material have attributed to the effects of irradiation, have been observed during a course of x-ray therapy in our mice. Thus, pyknosis, hyperchromatism, karyorrhexis, and metamorphosis of the neoplastic cells into bizarre, multinucleated giant forms were all seen in the irradiated mice. In mice with intracerebral gliomas, the primary effect of x-rays was on the neoplastic cells only; this agrees with the conclusions of those working with human material. The effect of x-rays on the blood vessels of the tumor and of the adjacent brain was negligi-
ble. In the human material, this effect was somewhat more prominent, but these changes (endothelial and adventitial hyperplasia) were not thought to be a significant cause of tumor destruction.

An analogy may be made between the clinical course and the pathological findings in the mice inadequately treated with x-rays and most human patients with gliomas who die of tumor growth after x-ray therapy. The lives of the treated mice were prolonged, but eventually they succumbed to the tumor. It is generally agreed that in patients with gliomas, x-ray therapy, although rarely curative, usually prolongs life. As has been noted, histologic examination of the brains of inadequately treated mice consistently revealed large areas of necrosis immediately adjacent to areas of healthy tumor. The amount of necrosis was much greater than is generally seen in the tumors of untreated animals. Alpers and Pancoast (5) described proliferating, healthy tumor cells adjacent to areas of tumor necrosis in patients with medulloblastoma who died after a course of x-ray therapy. Tarlov (9) noted massive focal areas of necrosis in astroblastomas following x-ray therapy. These features in the human material were interpreted in the same way as were similar features in our inadequately treated mice. It was judged that the necrotic areas represented the remains of portions of neoplasm destroyed by x-rays, and that tumor cells, which survived subsequently proliferated and encroached upon the areas of necrosis so that the tumor eventually reached a lethal size.

In irradiated mice, the mechanisms of repair which accompany and follow tumor necrosis can be followed serially. With human material, these processes can only be intimated, since tissue is examined at reoperation or necropsy; the lapse of time after x-ray administration is too long for the transient cellular changes of repair to remain manifest.

**SUMMARY**

It is possible to cure mice bearing intracerebral gliomas with appropriate fractionated doses of irradiation. X-rays alter the neoplastic cells first by reducing their cellular activity. The tumor necrosis which follows is accompanied by the appearance of lymphocytes, and then of macrophages. Reactive astrocytes and, occasionally, fibroblasts infiltrate the necrotic tumor. Vascular changes are not significant. Untoward effects of irradiation in the neuron population are not observed in "cured" mice.

The histologic picture in mice which have been cured is an area of discrete gliosis at the site formerly occupied by the tumor.

**REFERENCES**


In Memoriam

SAUL R. KOREY

April 12, 1918 — September 27, 1963