MODERN CONCEPTS IN BRAIN TUMOR THERAPY

LABORATORY AND CLINICAL INVESTIGATIONS

AUDREY E. EVANS

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CASTLE HOUSE PUBLICATIONS LTD.

ISBN 0 7194 0020 1

Printed and bound at William Clowes & Sons Limited, Beccles and London

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INTRODUCTION

The conference reported in this volume was designed by members of the Cancer Clinical Investigations Review Committee to bring together those working in the numerous areas of central nervous system tumor research in the hope that an exchange of information would be beneficial and lead to new avenues of research and treatment. Recent reports of brain tumor response to chemotherapy suggested that a new era of treatment might be on the horizon and, therefore, it is important that clinicians be brought up-to-date so that the promising preliminary results can be tested in well-designed clinical trials.

The conference was divided into four general areas: 1) fundamental therapy research including animal models and pharmacokinetics, 2) neuropathology and the correlation between humans and the animal models, 3) new methods of diagnosis including biochemical markers, and 4) methods of therapy including the analysis of response. That the aims of the conference were achieved is attested by the following presentations and discussions.

In the first session on research in therapy, Dr. Swenberg presented data on chemically and virally induced tumors in animals as excellent models in which to test chemical agents. He pointed out the extraordinary variation in the histology of the resulting tumor depending on the time during the antenatal period that the fetus was exposed to the agent. Dr. Wilson also discussed the tumor models in animals and their predictability in screening chemical agents. The discussion included comments about one model that demonstrated no significant effect of procarbazine, which apparently has significant antitumor effect in humans. Possibly, the dose employed was not high enough, since no great degree of toxicity occurred. Dr. Swenberg also warned that because many of the chemicals used in therapy are carcinogens, this fact should be weighed when their use is being considered. Considerable discussion was generated by Dr. Blasberg's presentation of the factors involved in the interactions between blood, cerebrospinal fluid (CSF), and brain, since these are vital to the success of chemotherapy. Some of the discussion also dwelt on the possible advantages of intra-arterial injections or infusions and treatment via the intrathecal route. Dr. Blasberg believed that the gains of such "localized" treatment with its diminished systemic toxicity rarely outweigh the disadvantages. For example, the tumors frequently derive their blood supply from more than one artery and the circulation time from the lumbar space is slow, so that drug concentration is greatly decreased by the time the cranium is reached.

Dr. Hoshino's research with thymidine uptake by brain and tumor cells pointed out some of the problems of diffusion. The discussion centered around the possibility that there are nonproliferating tumor cells that do not take up the thymidine and that also would be insensitive to cell cycle-specific agents.

In the session on neuropathology, Doctors Rubinstein and Vogel described the gliomas as a "biological continuum" varying from benign to highly malignant and noted that subdivisions tend to be arbitrary. Dr. Rubinstein commented upon the difficulty in relating brain tumors produced in the laboratory with those found in the human. He pointed out that we do not know what factors make for either rapid or slow growth of central nervous system neoplasms. During the discussion, Dr. Vogel and others speculated on Rubinstein's finding that the most dense area of tumor proliferation is frequently around areas of necrosis as if the central hypoxia stimulated the hyperplasia.

Doctors Caveness and Mahaley reported on the changes in tumors and normal brain in monkeys and man after irradiation and/or chemotherapy. It was believed that the delayed necrosis and demyelinization seen after X-ray therapy are preceded by fibrinoid necrosis of the blood vessel wall. Endothelial damage leads to changes in perfusion; members of the audience suggested that it might be advantageous to give chemotherapy concurrently with irradiation to take advantage of the changes in the blood–brain barrier.

Several members of the panel and audience discussed the effect of irradiation to the cranium on the immune response. The possibility was raised that such irradiation alters the brain's privileged immune status, allowing it to become more susceptible to viral infections. Doctors Chang and Mahaley discussed the existence of lymphopenia in patients with malignant gliomas

prior to any treatment, and this lymphopenia probably is exaggerated by irradiation to the head.

Doctors Cooper and Kricheff presented data on the value and relative merits of nuclear scans and computerized axial tomography (CAT) with and without enhancement. These techniques mark an enormous advance in the diagnosis and accurate delineation of space-occupying lesions, but both agreed that the nuclear scan and, to a lesser extent, the CAT had limited usefulness in differentiating between tumor recurrence and postradiation changes. There seems to be little correlation between the appearance of a nuclear scan and the malignant potential of a glioma. Dr. Blasberg pointed out that nuclear scans are measuring changes in the blood–brain barrier. Such changes can be modified by concurrent therapy, especially with corticosteroids which, by their effect on diffusion, could lead to a false impression of improvement.

Information on biochemical markers in the CSF was presented by Drs. Ransohoff and Paoletti. Desmosterol and polyamines correlate well with the presence of a tumor, but the need for multiple CSF samples limited their usefulness in following the progression of the disease. Dr. Bogoch presented data on the preparation of two purified brain tumor antigens: astrocytin derived from human malignant glial tissues, and malignin from malignant glial cell cultures. With these two antigens, it was possible to demonstrate the presence of an antibody in the serum of most patients with malignant brain tumors; all but 4 elderly patients so tested demonstrated the antibody. Doctors Gutin and Chang discussed the effect of chemicals and physical agents (hyperbaric oxygen) on normal brain substance and tumors. The discussion highlighted the fact that insufficient attention is being paid to the use of combined-agent chemotherapy and that drug–drug and drug–irradiation interactions are probably underestimated. Dr. Ransohoff suggested that the corticosteroid effect on the basement membrane of blood vessels might well decrease the damaging effect of irradiation.

Dr. Bleyer presented excellent data on the levels of methotrexate in the CSF, brain, and tumors depending on the rate and route of administration. He pointed out the error of basing dose calculations on surface areas because the brain size and CSF bear little relationship to body surface area. The adult brain volume is reached early in childhood. Considerable discussion ensued on the merits and disadvantages of the intraventricular administration of methotrexate via the Ommaya device. High concentrations can be maintained, but obstruction to the CSF circulation can lead to levels that produce local brain necrosis. Responding to a question regarding the value of intrathecal therapy for tumors residing within the brain substance, Dr. Bleyer stated that methotrexate in the CSF does indeed penetrate the parenchyma to a level of millimeters or even 1 cm from the surface.

Dr. Gehan presented an analysis of the prognostic factors in the survival of patients with brain tumors. He sought to define significant parameters for stratification of patient groups. Numerous variables such as age, sex, pathology, site, and signs of cranial nervous involvement were evaluated as predictions of outcome. Although it was agreed that having comparable groups in clinical studies is vital, possibly Dr. Gehan's criteria can be used to develop historical control groups to eliminate the need for untreated control patients in each new study.

Dr. Wilson discussed problems in measuring response and documenting recurrent disease. Duration of response should be included as a measurement of a drug's effectiveness in phase II studies, whereas the time to recurrence is important in phase III. Every attempt should be made to document recurrent disease with the use of several methods such as neurologic examination and nuclear and CAT scans.

Dr. Walker presented the results of the study of primary brain tumors conducted by the Brain Tumor Study Group; the Radiation Therapy Oncology Group's work on tumors metastatic to the brain was discussed by Dr. Kramer. The conference closed with short presentations of eight studies being conducted by national cooperative units including the Southwest Oncology Group, Acute Leukemia Group B, European Organization for Research on Treatment of Cancer, Western Cooperative Oncology Group, Radiation Therapy Oncology Group, Eastern Cooperative Oncology Group, and the Children's Cancer Study Groups, many of which are only in the preliminary phases of patient entry.

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Chemical- and Virus-Induced Brain Tumors[1]

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ABSTRACT—Experimental animal models resembling most human brain tumor types can be induced by exposure to oncogenic viruses or chemical carcinogens: Astrocytomas and glioblastoma multiforme can be produced experimentally by intracerebral injection of oncornaviruses, whereas medulloblastomas, choroid plexus papillomas, and ependymomas can be induced by the papovaviruses. Adenoviruses have been utilized to cause medulloepitheliomas, neuroblastomas, and retinoblastomas. All three groups of viruses can result in sarcoma production. Gliomas represent the primary tumor type induced in the brain by chemical carcinogens. These autochthonous tumor systems are reviewed, with emphasis on methods, tumor type, latency period, advantages, and disadvantages. In addition, recent investigations of molecular events involved in neoplastic transformation by chemical carcinogens are summarized.—Natl Cancer Inst Monogr 46: 3–10, 1977.

A variety of experimental brain tumor models resembling most human tumor types have been developed during the past decade. Brain tumors can be induced with intracerebral injection of viruses or chemicals, or by transplacental, parenteral, oral, or even topical exposure to chemical carcinogens. Depending on the model, such tumors arise weeks to years after exposure. The availability of suitable animal models has stimulated increased research on brain tumor kinetics, immunology, chemotherapy, and histogenesis. Recently, these models have been used in the study of molecular events responsible for neoplastic transformation. This review is intended to summarize the experimental brain tumor systems available today and to highlight some of the exciting research being done with these systems.

VIRUS-INDUCED BRAIN TUMORS

Work on experimental brain tumor induction with viruses was reviewed comprehensively in several publications (*1–6*). Intracranial tumors have been induced by representatives of most oncogenic tumor virus groups, including the adenoviruses, papovaviruses, and oncornaviruses. To date, techniques for induction and morphologic descriptions were emphasized in most studies on these models. Future investigations will probably de-emphasize morphologic classification and instead turn toward utilizing such models in basic and applied research. Morphologic studies have led to several generalizations that are summarized in table 1. For example, only oncornaviruses induce astrocytomas and glioblastomas, whereas the human papovavirus JC is the only virus known to cause the development of medulloblastomas. Choroid plexus papillomas are induced by simian virus 40 (SV40), the SV–PML papovaviruses isolated from patients with progressive multifocal leukoencephalopathy (PML) and the avian adenovirus (CELO), whereas medulloepitheliomas and retinoblastomas are only elicited with human adenoviruses. In contradistinction, sarcomas were induced by members of most tumor virus groups.

TABLE 1.—*Specificity of experimental brain tumor induction with oncogenic viruses*

Experimental brain tumor type	Inducing viruses	
	Group	Type[a]
Anaplastic astrocytoma or glioblastoma multiforme	RNA-Oncornaviruses	ASV, MuSV, SSV
Medulloblastoma	DNA-Papova	Human papova JC
Neuroblastomas or retinoblastomas	DNA-Adenovirus	Human adenovirus type 12
Ependymomas or choroid plexus papillomas	DNA-Papova	SSV40, human papova PML-1, JC, and BK
	DNA-Adenovirus	SA7 and CELO
Sarcomas or meningeal tumors	RNA-Oncornavirus	ASV and MuSV
	DNA-Papova	Bovine papilloma, murine polyoma, human papova JC
	DNA-Adenovirus	SA7

[a] ASV = avian sarcoma virus; MuSV = murine sarcoma virus; SSV = simian sarcoma virus; BK = human papovavirus BK; SA7 = simian adenovirus 7.

[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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[3] Dr. Darell Bigner provided many helpful suggestions during the preparation of this manuscript.

RNA Tumor Viruses

Avian Sarcoma Virus

Mammalian neuro-oncogenicity of ASV was first described by Rabotti and Raine (*7*). Subsequently, gliomas and intracranial sarcomas were induced in cats, dogs, guinea pigs, mice, rabbits, rats, and subhuman primates. The relative proportion of glial and mesenchymal elements in these brain tumors varies considerably among species. Homogeneous glial tumors have clearly been produced in the rat and dog with ASV. Bigner et al. (*8*) induced gliomas in 27 of 30 dogs, 19 of which had gliomas only, with no evidence of sarcoma formation. They later demonstrated that the site of ASV inoculation influenced tumor type; i.e., injection of ASV deep in periventricular zones resulted exclusively in astrocytomas, whereas only sarcomas were induced if the virus was injected superficially over the cerebellar vermis (*9*). Well-differentiated astrocytomas also developed in F344 (*10*) and Sprague-Dawley (*11*) rats after neonatal intracerebral injections of ASV. In addition, Copeland et al. (*12*) demonstrated that rats remained susceptible to the neuro-oncogenic effects of ASV at 100 days of age. Although the incidence of brain tumors in rats inoculated with ASV as adults was only 50%, nearly all were astrocytic.

In contrast to the rat and dog, a large percentage of the hamster, guinea pig, mouse, cat, and subhuman primate tumors induced with intracerebral ASV have a mesenchymal nature. Whereas neoplastic astrocytic elements can be observed, prominent reticulin fibers are also present. Tumors composed of large balloon-like cells (*13*) and "giant cell glioblastomas" (*14, 15*) usually contain such reticulin. This cell type probably represents a histiocytic element in a mesenchymal or mixed glial–mesenchymal tumor.

Several other features of ASV-induced brain tumors deserve comment: 1) There is generally no replication of infectious or noninfectious virus in mammalian tumor systems. However, virus structural antigens and virus-related transplantation antigens are usually produced in transformed mammalian cells. One can "rescue" ASV from such cells by growing the "nonpermissive" transformed cells with normal avian cells and allowing cell fusion to occur. 2) The dose of virus required to elicit intracranial tumors is lower than that for extracranial tumor induction. This is probably due to a greater immunologic response against the latter. 3) Improvements in the techniques for growing and concentrating ASV have allowed large pools of standardized virus to be produced (*16*). Using this virus, researchers have obtained reproducible survival curves for rats bearing a 100% incidence of astrocytomas. This system provides the first primary glioma model that is well suited for chemotherapy trials (*11*). Preliminary results of these trials have been reported (*5, 16–18*).

Other Oncornaviruses

MuSV has been reported to cause brain tumors in mice and rats following intracerebral inoculation (*5*). Gliomas, meningiomas, and hemangioendotheliomas represent the most common tumor types induced with MuSV. In contrast to ASV-induced mammalian tumors, replicating virions were easily detectable in these mouse tumors by electron microscopy (*19*). Recently, a 100% incidence of extremely vascular brain tumors containing neoplastic astrocytes and extensive endothelial proliferation was induced in Wistar-Furth rats with the Kirsten MuSV (*20*). No C-type virions were detected by electron microscopy.

Intracerebral inoculation of neonatal marmosets with SSV induced solitary tumors in 6 of 10 animals (*21*). The tumors were composed of pleomorphic neuroglial cells, areas of necrosis, hemorrhage, palisading, and prominent endothelial proliferation. These SSV-induced marmoset tumors closely resemble the human glioblastoma multiforme. Infectious virus was isolated from brain and cerebral spinal fluid of tumor-bearing animals.

DNA Tumor Viruses

Papovaviruses

Several members of the papovavirus group cause tumors when inoculated intracerebrally into animals (*5*). Bovine papilloma virus induced meningiomas, fibromas, and fibrosarcomas in hamsters and calves following latency periods ranging from 20 days to nearly a year. No virus particles were detected in the tumors by electron microscopy. Fibrosarcomas were also detected in the leptomeninges after polyoma virus was injected intracerebrally into newborn rats, hamsters, and rabbits. Intracerebral inoculations of SV40 into newborn hamsters induced fibrosarcomas in the leptomeninges and choroid plexus papillomas in the ventricles.

Of greatest interest, however, are the tumors that developed in hamsters after intracerebral

injections of JC virus, a human papovavirus isolated from a patient with PML (*22–24*). Brain tumors developed in 50 of 63 hamsters inoculated with JC virus, with medulloblastomas representing the most common tumor type. These appear to arise from the internal granule cell layer of the cerebellum and frequently are multifocal. Several other tumor types have been induced with JC virus, including primitive gliomas, papillary ependymomas, meningiomas, and pineocytomas. Virus was recovered from 5 of 7 tumors tested. The JC virus is morphologically indistinguishable from SV40 and SV40-PML strain 1, but differs from them in its cell culture host range and antigenic properties (*5, 25*).

Adenoviruses

The experimental induction of neurogenic tumors in rats, hamsters, and mice after the animals received intracerebral, intraocular, or ip inoculations of human adenovirus 12 provides an excellent model for future research on neuroblastomas, medulloepitheliomas, and retinoblastomas (*26–31*). Medulloepitheliomas were induced in 88% of the rats given intracerebral injections of human adenovirus 12. These appeared to develop from the subependymal plate and were characterized by the formation of rosettes, high levels of cholinesterase following in vitro cultivation, and the presence of cilia containing 9+0 tubules (a hallmark of normal sensory neuronal cells). Similar 9+0 tubules were demonstrated ultrastructurally in the cilia of retinoblastomas induced in rats and hamsters following neonatal intraocular injections of human adenovirus 12.

Other members of the adenovirus group that elicited intracranial tumors (*5*) include the simian and avian adenoviruses (SA7, SV20, and CELO). SA7 induced poorly differentiated tumors of the choroid plexus, trigeminal nerve, and dura mater. Injection of SV20 into hamsters caused extremely undifferentiated tumors, which had the same morphology whether induced sc or intracerebrally. Intracerebral tumors produced with CELO had a morphology distinctly different from neoplasms induced with either the human or the simian adenoviruses; they were composed of papillary arrangements of cuboidal and columnar cells that filled the ventricles. Although originally thought to be ependymal tumors, the CELO tumors probably represent choroid plexus papillomas.

CHEMICALLY INDUCED TUMORS OF THE CENTRAL NERVOUS SYSTEM (CNS)

The susceptibility of neuroectodermal tissue to the oncogenic effects of chemical carcinogens was first demonstrated in 1939 (*32*). Since then, brain tumors have been induced by local implantation of several polycyclic hydrocarbons (PCH). More recently, additional classes of carcinogens were shown to be oncogenic for the nervous system following systemic exposure. These ranged from agents such as 2-acetylaminofluorene, which caused a few tumors of the nervous system along with many tumors elsewhere, to the nitrosoureas that caused neuroectodermal tumors in 100% of the animals. Several reviews (*33–36*) and a monograph (*5*) have been published on the chemical induction of brain tumors. In addition to reports by some scientists on the induction and characterization of various brain tumor models, other investigators used animal models for neuro-oncogenesis to study the molecular basis for neoplastic transformation. Whether similar mechanisms are involved in human brain tumors is unknown. It has been estimated that 80–90% of all human cancer is caused by chemicals; however, no chemical carcinogen has been clearly linked to human brain tumors.

In the section to follow, various brain tumor models have been arranged by the class of carcinogen used to induce them.

Polycyclic Hydrocarbons

Over 200 years ago, Sir Percival Pott first theorized the potential carcinogenic hazard of PCH. Therefore, it was fitting that the first experimental tumors of the nervous system were also induced with PCH (*32*). Since that time, brain tumors have been produced with several PCH, including 3-methylcholanthrene, benzo[*a*]pyrene, dibenzanthracene, dimethylbenz[*a*]anthracene (DMBA), and trimethyl benzanthracene. Such tumors have been induced in frogs, toads, mice, rats, hamsters, and dogs. However, birds, guinea pigs, rabbits, cats, and monkeys were resistant to the neuro-oncogenic effect of PCH. In general, brain tumors occur only when the PCH are in direct contact with the CNS. To accomplish this, the carcinogens are directly implanted in the brain. The incidence and type of tumor produced by PCH are greatly influenced by the positioning of the pellet (*37*). Few gliomas but many sarcomas and meningiomas result from superficial placement of the pellet near the dura. Ependymomas were primarily induced when the

pellet was placed in the ventricles, whereas oligodendrogliomas were associated with implantation in the frontal white matter. Astrocytomas arose near pellets placed in the subcortical regions of the parietal lobe, and medulloblastomas formed after pellet implantation into the cerebellum.

Although several investigators have successfully induced brain tumors in rats with PCH, mice appear to be the most susceptible animal species (*34*). Tumor incidence varies from less than 10 to nearly 100%, with an average of 40–60% of the animals developing tumors. Most animals die between 200 and 300 days; however, latency periods have varied from as short as 68 to as long as 750 days. The primary advantage of this model is that the site of tumor formation can be localized. Disadvantages of the system include the variable incidence and latency period of the experimental tumors. The model is poorly suited for quantitative dose–response studies and investigations of early biochemical processes, since the time of exposure can vary from minutes to months because of the extremely long half-life of the pellet. The importance of unique doughnut-shaped virions detected in macrophages surrounding pellets of carcinogens prior to tumor formation requires further elucidation (*38*). Whether these virions represent activated viruses that play an important role in tumor induction remains unknown. However, they have never been detected in experimental gliomas and have induced fibrosarcomas rather than gliomas when they were injected intracerebrally into mice.

When pregnant rats were exposed to DMBA iv on day 21 (*39*) or orally on days 14, 17, and 20 of gestation (*40*), the most frequently detected neoplasms induced in the offspring were tumors of the nervous system. Transplacental exposure to additional PCH will be necessary to determine whether this is a general phenomenon or a specific effect of DMBA. In any event, it should now be possible to design pulse exposure experiments and to demonstrate dose–response relationships for DMBA.

Nitroso Compounds

A landmark in experimental neuro-oncology occurred in 1964 when Druckrey and co-workers (*41*) selectively induced tumors of the rat nervous system after iv injections of methylnitrosourea (MNU). This provided the first reproducible brain tumor model that did not traumatize the brain. Since then, several additional carcinogens have been discovered that primarily induce neuroectodermal tumors following systemic administration. These animal models represent some of the most powerful tools for modern research in chemical neuro-oncogenesis.

Methylnitrosourea

This acyl-alkyl-nitrosamide is among the most potent of all the chemical carcinogens known today. When administered iv at repeated low doses, the nervous system clearly represents the target organ. The highest incidence of neurogenic tumors is obtained with repeated iv injections of MNU at a dose schedule of 5 mg/kg/week for 32–36 weeks (*42*). This dosage has consistently produced a 90–100% incidence of grossly detectable brain tumors in Sprague-Dawley rats. Well-differentiated neurinomas are also induced; however, the incidence of these is much lower. This low incidence of tumors of the peripheral nervous system (PNS) can be reversed if F344 rats are used instead of Sprague-Dawley (*43*). The rat and the rabbit appear most susceptible to brain tumor induction with MNU (*5*). Dogs have been more variable; some brain and peripheral nervous system tumors have been induced in various breeds. Mice were quite resistant to neuro-oncogenesis with MNU. Denlinger et al. (*44*) induced 2 gliomas in 19 C3H mice exposed iv to 25 mg/kg every 4 weeks (total dose, 175 mg/kg). In spite of observation periods exceeding 6 years, attempts to induce brain tumors in rhesus monkeys have been unsuccessful (*5*). When cats were exposed to iv or oral doses of 25 or 20 mg/kg, respectively, all animals died within 10 days from severe bone marrow toxicity and septicemia (*35*). Additional resistant species include guinea pigs, sheep, and swine.

Anaplastic gliomas, mixed gliomas, and oligodendrogliomas represent the most common brain tumors induced with MNU. These are frequently located near periventricular regions, in subcortical white matter, and in the hippocampus. If the dose of MNU is raised from 5 mg/kg/week to 10 or 20 mg/kg twice a week, a shift from glial to mesenchymal cell types becomes apparent, with most tumors being sarcomas or gliosarcomas (*45*). MNU is also a potent carcinogen when administered locally, as shown clearly in experiments utilizing the sc, ip, or oral routes. Tumors develop at the site of administration and to a lesser extent in the nervous system. The lower incidence of tumors detected in the nervous system can best be explained by the decreased levels of circulating MNU. In addition to tumors at the site of injection and in the nervous system, a high incidence

of thymic lymphomas can be induced after oral or sc administration of 20 mg/kg twice a week for 9 weeks. These types of experiments have clearly shown that MNU is not "neurospecific." Rather, MNU is a potent carcinogen for many tissues. Potential mechanisms involved in determining which target site is affected by this and related carcinogens will be discussed later.

Ethylnitrosourea (ENU)

The exquisite sensitivity of the fetal rat's nervous system to the oncogenic effects of ENU was first demonstrated in the experiments of Ivankovic and Druckrey (*46*). A 100% incidence of neurogenic tumors developed in offspring exposed to a single dose of 20 mg/kg or more during the last trimester of pregnancy. Clear dose–response relationships exist with single doses of 1, 5, 20, or 50 mg/kg inducing a 12, 79, 100, and 100% incidence of neurogenic tumors, respectively (*47*). While the tumor incidence increased, the latency period for tumor induction decreased from a mean of 655 days for 1 mg/kg to 211 days for 50 mg/kg. Age of the individual greatly influences the susceptibility to the neuro-oncogenic effects of ENU. The rat fetus is resistant to the carcinogenic effects of ENU before the 12th day of gestation; however, it is susceptible to teratogenic effects before this stage of development. Susceptibility to neuro-oncogenesis increases from the 12th day of gestation to birth, after which it begins decreasing (*33*). By 30 days of age, the rat has a susceptibility comparable to the adult; i.e., the nervous system no longer represents the target organ and much higher doses of ENU are required to induce a similar incidence of neoplasia.

ENU has usually been administered iv to pregnant rats, although it can be given orally, ip, or sc. Neurogenic tumors were even reported in 38% of the offspring whose mothers had 50 mg/kg of ENU applied topically to the skin (*48*). It has also been possible to induce tumors by administration of precursors of ENU in the food and water of pregnant rats. Under the mildly acidic conditions of the rat's stomach, ethylurea and sodium nitrite undergo nitrosation and form ENU in vivo (*49*).

Rats are the species most sensitive to the neuro-oncogenic effects of ENU. Hamsters, mice, and opossums have also developed neurogenic tumors. In hamsters, tumors have been confined to the PNS, whereas in mice a low incidence of CNS and PNS tumors develops. Mixed gliomas and oligodendrogliomas represent the most common brain tumors induced with ENU, whereas anaplastic neurinomas are the type occurring most frequently in the PNS. Anaplastic gliomas and sarcomas are rare. Animals bearing anaplastic neurinomas die significantly earlier than those bearing gliomas. When the sequential development of these tumors was investigated, evidence of neoplastic proliferation was detected in trigeminal nerves as early as 3 weeks after exposure to ENU (*50*). Comparable changes were not evident in the brain until 4 months after ENU exposure. Mechanisms involved in this differential triggering of neoplastic proliferation between glial and Schwann cells remain unknown.

Other Nitroso Compounds

Additional nitroso compounds possessing neuro-oncogenic properties have been described since the original discovery of MNU (*5, 33*), including dimethylnitrosourea, trimethylnitrosourea, propylnitrosourea, butylnitrosourea, dinitrosopiperazine, nitrosopiperidine, nitrosomorpholine, ethylnitrosobiuret, methylnitrosobiuret, ethylnitrosourethane, and methylnitrosourethane. In general, these offer no major advantages over MNU and ENU and will not be discussed in detail.

Other Carcinogens

Triazenes

Several of the dialkyl-aryl-triazenes have been shown to be carcinogenic for the nervous system as well as for other tissues (*51*). Neuro-oncogenesis has been successful both in adult rats and transplacentally. As noted for MNU and ENU, the methyl compounds were most effective in adults, whereas the corresponding ethyl compounds were highly oncogenic to the fetal rat's nervous system.

1,2-Diethylhydrazine, Azoethane, and Azoxyethane

Transplacental neuro-oncogenesis has been induced in the rat with these three carcinogens (*33*). In contrast to the triazenes, adult rats developed multiple intestinal tumors instead of neurogenic neoplasms when the methyl counterpart was administered.

INVESTIGATIONS ON THE MECHANISMS OF CHEMICAL NEURO-ONCOGENESIS

Scientists conducting studies at several laboratories have suggested a possible mechanism for

the neurospecificity of some chemical carcinogens. That chemical carcinogens are electrophilic reagents has been known for several years (*52*). Those carcinogens that are not electrophilic must be metabolized to form electrophiles, which interact with macromolecules such as DNA, RNA, and protein. Neoplastic transformation represents a heritable change at the cellular level, with the most likely site for this change being the DNA of the originally transformed cell (*53*).

Investigations of several carcinogens and tissues demonstrated alkylation of DNA bases. The greatest amount of this alkylation occurred at the N-7 position on guanine, with lesser amounts being detected at the N-3 position of adenine and the O^6-position of guanine. When the extent of *N*-7 alkylguanine formation was determined in brains and livers of adult and 10-day-old rats given injections of ENU, no correlation was found between DNA alkylation and carcinogenicity (*54*). Subsequent studies determined the degree of purine alkylation at various times after ENU exposure. Goth and Rajewsky (*55, 56*) demonstrated surprising differences between the repair rates of O^6-ethylguanine and those of *N*-7 ethylguanine or *N*-3-ethyladenine. O^6-Ethylguanine persisted for long periods in the target organ (brain) but was repaired much more rapidly in the liver. *N*-7-Ethylguanine was removed rapidly in both tissues. If one determines the brain:liver ratio for each of the major sites of purine alkylation, a high degree of correlation is evident between alkylation at the O^6-position of guanine and carcinogenicity.

Further support for this hypothesis has been reported by Kleihues and co-workers (*57–59*), who demonstrated persistence of O^6-methylguanine in brains of rats treated with MNU and methylmethanesulfonate (MMS). Both methylating agents caused brain tumors in rats; however, MMS has a much weaker neuro-oncogenic effect. When equimolar doses of MNU and MMS were administered to rats, only 0.05 as much O^6-methylguanine was present after exposure to MMS. Thus the extent of initial O^6-alkylguanine formation and its persistence over time correlates with carcinogenicity. A possible mechanism for this correlation was suggested by Loveless (*60*). Alkylation at the O^6-position of guanine forces the base into the enol form, which causes anomalous base pairing during DNA replication. Instead of pairing with cytosine, O^6-alkylguanine pairs with thymine. Fixation of anomalous base pairing requires that at least one cell replication take place before repair of the damaged DNA. The lack of this replication in end-stage neurons may explain why no neuronal tumors have been induced with MNU, even though DNA alkylation occurs to a greater extent in neurons than in glia (*61*). It also helps to explain the predilection sites for ENU- and MNU-induced gliomas, since these areas contain the glia with the greatest propensity for cell replication. Enzymic repair systems for O^6-alkylguanine have recently been demonstrated (*62, 63*). If these systems are deficient in rat brain, the exquisite susceptibility of the fetal rat brain to carcinogens may thus be explained. Over half the 35 or more compounds that caused transplacental carcinogenesis induced tumors of the rat nervous system.

In summary, pathogenetic mechanisms involved in experimental neuro-oncogenesis seem to be as well or better known than those of other neoplasms. Delayed repair of O^6-alkylguanine appears to be a major factor in sensitizing the rat nervous system to carcinogenesis. Whether this mechanism is responsible for human brain tumors remains to be investigated. Sensitivity to neuro-oncogenesis may be a general phenomenon for many carcinogens if effective tissue concentrations reach the nervous system. At present, the somatic mutation theory of cancer induction best explains chemical neuro-oncogenesis.

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Animal Models of Brain Tumors [1, 2]

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ABSTRACT—Although no single model of the numerous animal models of brain tumors developed in recent years perfectly represents the spontaneous human tumors, different ones do have specific advantages for certain types of studies. Chemical induction in adult animals, transplacental chemical induction, viral induction, and transplantation are effective methods that allow choice of numerous histologic types of tumors which, to a greater or lesser extent, simulate human tumors. Reproducibility of location, cell type, and time of tumor appearances; expense; ability to grow in tissue culture; safety for personnel; trauma to brain; nature of vasculature, and amount of brain and tumor tissue available for examination are among the variables to be considered in choosing a model appropriate for a particular study.—Natl Cancer Inst Monog 46: 11–17, 1977.

As chemical oncolytic agents came into use in the 1940's and early 1950's, the National Institutes of Health Screening Committee established a panel of animal tumor models. It was soon apparent that standard models could not be used to predict the potential effectiveness of brain tumor drugs; compounds such as nitrogen mustard, potent against extraneural tumors, proved ineffective in clinical trials. Then in 1962, Chirigos et al. (*1*) demonstrated that cyclophosphamide was effective against mouse L1210 leukemia if the tumor implant were placed subcutaneously but ineffective if placed intracerebrally. To complicate the situation even more, Shapiro (*2*) later showed that implants of VM-26 inhibited tumor DNA production whether they were placed in the brain or subcutaneously. VM-26 also inhibited growth at both sites, although a subcutaneous tumor developed less than an intracerebral one. However, VM-26 did not improve survival in animals bearing the brain tumor (*2*).

The importance of cellular growth kinetics suggested that glial tumors would predict response of human glial tumors better than would high growth fraction tumors such as L1210 leukemia, Dunning leukemia, B16 melanoma, or Ehrlich carcinoma, even though the efficacy of 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) was originally demonstrated on intracerebral L1210 (*3*). Whereas it was unlikely that any given model could perfectly simulate all human brain tumors, physiologically and in response to any treatment, tumors of brain tissue were essential. Furthermore, since the vasculature of a tumor originating from brain differs depending on whether it is placed in brain or subcutaneous tissue, such a tumor model should be located in the brain (*4*). To be useful in evaluating drug and other therapies, such a model should also be consistent in tumor type from animal to animal and predictable in disease onset and course (table 1).

TABLE 1.—*Types of studies*

1. Drug screening and evaluation
a. Individual drugs, dose schedules, and combinations
b. Radiation therapy schedules
c. Immunotherapy trials
2. Pharmacokinetics
3. Tumor-cell population kinetics
4. Immunology
5. Biochemistry, blood–brain barrier

In 1939, Seligman and Shear (*5*) demonstrated that 3-methylcholanthrene implanted in the brains of mice induced neuroectodermal tumors. This was repeated by Zimmerman and Arnold in 1941 and studied subsequently (*6*, *7*). Several other polycyclic hydrocarbons, such as dibenzanthracene, benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and trimethylbenzanthracene, also induced tumors similarly. Susceptibility to tumor induction varied; mice, frogs, hamsters, and dogs were susceptible, whereas birds, guinea pigs, rabbits, cats, and monkeys were not (*8*). Some mouse strains proved more susceptible than others. The type of tumor induced varied; in general, implantation of the pellet or powder superficially in the brain caused sarcomas and meningiomas, whereas placement in frontal white

[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Supported in part by Public Health Service grant CA13525 from the National Cancer Institute, and grants from the Phi Beta Psi Sorority and the Association for Brain Tumor Research.

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matter elicited a high percentage of oligodendrogliomas. Subcortical parietal lobe placement produced many astrocytomas, cerebellar placement induced medulloblastomas, and intraventricular placement gave rise to ependymomas (*7*).

Latency periods varied from 68 to 750 days, with a tumor incidence of approximately 40–60%. Obviously, this makes evaluation of any treatment difficult because the tumor is not apparent until a near lethal mass has been attained, and the mortality distribution is so scattered that statistical evaluation is nearly impossible. This model has allowed study of several interesting issues in tumorigenesis, such as the presence of virus particles (capable of producing fibrosarcomas on reinjection) in macrophages near the chemical implant shortly before, but not after, the tumors develop (*9*). These neoplasms also have the disadvantage of a traumatic introduction of the inciting agent at the site of the tumor, which may alter tissue character and vasculature.

In 1964, Druckrey et al. (*8*) and Swenberg and co-workers (*10–13*) found that methylnitrosourea (MNU) injected iv would cause a high incidence of neural tumors. Repeated iv doses weekly for 32–36 weeks can produce tumors in as many as 90–100% of the Sprague-Dawley rats. In general, rats and rabbits have been most susceptible, with dogs, mice, cats, and monkeys much less so. This carcinogen produces various tumors, including anaplastic gliomas, mixed gliomas, and oligodendrogliomas, although at higher dose levels, an increasing incidence of sarcomas, gliosarcomas, and peripheral nerve tumors such as neurinomas occur. These tumors also have cell-cycle time and histologic similarities to human tumor types. Again, there is variability between strains because F344 and Sprague-Dawley rats are more likely to develop neurinomas and gliomas, respectively (table 2).

ENU given to a pregnant rat in the last trimester by virtually any route will cause a high incidence of nervous system tumors in the offspring (*14, 15*); unlike MNU, it has little central nervous system oncogenic effect on adult rats, which tend to develop leukemias instead (*12*).

Induction with transplacentally administered ENU commonly produces mixed gliomas and oligodendrogliomas, as well as anaplastic neurinomas of the fifth cranial nerve, whereas anaplastic gliomas and sarcomas are rare. Anaplastic ependymomas occur in the spinal cord. This model has the advantage of inducing tumors with single injections in animals of the same age, thereby saving the considerable effort of multiple iv administration needed with MNU and allowing more precise estimates of neoplastic onset.

TABLE 2.—*Classification of models*[a]

Type	Chemical	Viral
Autochthonous	Polycyclic hydrocarbons 3-Methylcholanthrene and others MNU given iv to adult rats, rabbits ENU given transplacentally to rates	ASV, MuSV, SV40, Human JC papovavirus Human adenovirus 12
Transplanted	3-Methylcholanthrene-induced ependymoblastoma, glioma 26, glioma 261 Rat MNU-induced tumor lines, e.g., 9L	ASV-induced gliosarcoma in beagles

[a]ASV = avian sarcoma virus; MuSV = murine sarcoma virus; SV40 = simian virus 40; ENU = ethylnitrosourea.

The advantage of the MNU and ENU models is the production of tumor types similar to human gliomas without mechanical trauma to the brain. Disadvantages are: 1) lack of localization of the tumor, 2) inconsistency of histologic tumor type, 3) multiplicity of tumors, and 4) the induction of tumors outside the nervous system; also, 5) some scatter in tumor induction times (and hence of survival) is present. Nevertheless, some chemotherapy experiments have been performed. For example, Swenberg (*16*) was able to demonstrate some increase in survival time of MNU-treated rats that had received 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU).

A number of different compounds have been used similarly; these include other nitroso compounds, triazenes, 1,2-diethylhydrazine, azoethane, and azoxyethane, all of which have little advantage over MNU and ENU as chemical carcinogens.

Variability of tumor type and unpredictability of time of tumor onset and death can be avoided by the direct implantation of tumor cells or fragments. Since 1891, many attempts have been made to implant human tumors in the brains of various animals, with mixed success. It has usually proved difficult to maintain serial transplantation, but even when transplantation is successful fairly consistently, such as that of human choriocarcinoma in the monkey brain, the xenogeneic nature of the tumor creates several new variables. Notable examples are host-immune response and differences in the nature of the supporting stroma. Consequently, conclusions based on these models are difficult to generalize (*17*).