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Session I

The Significance of Experimental Gliomas for Human Disease

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EPIDEMIOLOGY OF HUMAN INTRACRANIAL NEOPLASMS

In 1962 KURLAND and his associates (1) compiled the available mortality data on cerebral neoplasms for the United States and Canada as derived from the International List of Diseases and Causes of Death (2). Their study revealed a remarkably even geographic distribution for all classes of intracranial tumors in the two countries. The mortality rates ranged from 3 to 5 per 100,000 population. If this mortality estimate was correct in 1962 and if it is still valid in 1974, one can expect between 6,300 and 10,500 deaths from intracranial neoplastic disease this year in this country based on a population of two hundred and ten million.

The compilation of data of this kind is subject to innumerable inaccuracies, as pointed out by KURLAND and his associates. They noted, for example, that the rate for deaths due to brain tumors in Japan was very low, being about half that in Western countries, and they felt that at least in part this low rate could be attributed to diagnostic and reporting artifacts.

These investigators further reported the results of a 10 year survey among the population of Rochester, Minnesota. They found a prevalence ratio of intracranial neoplasms of 46 per 100,000 population and an incidence rate of about 19 per 100,000 per year. In this population, about 1 per cent of all deaths was due to primary intracranial tumors, which was about twice that reported in official mortality statistics.

Other studies have been carried out in the United States that disclose rates that vary from 3.0 - 8.4/100,000 of the population (3, 4, 5), and in other parts of the world the incidence also varies considerably (6, 7). Based upon confirmed tumor diagnosis at biopsy or autopsy, it has been estimated that primary intracranial neoplasms constitute 2-3 per cent of all neoplasms to which man is heir (8). There exist published accounts of the incidence of various expanding, space-occupying intracranial lesions, not all new-growths and not all primary neoplasms of the brain, that have been collected in different countries of the world. Some of these statistical reports have been presented in table form by this author (3), revealing wide discrepancies in the incidence of certain categories of tumors from country to country and even from series to series of cases collected by different authors in the same country.

Despite the reported variations in incidence of intracranial tumors in different series, all contributors agree that the gliomas rank first on a numerical basis. They constitute between 31 to 43 per cent of all

intracranial masses, including metastatic tumors and granulomas such as tuberculomas. Simply eliminating the latter two categories of expanding intracranial lesions from consideration has the effect of raising the glioma incidence to between 40 and 50 per cent. Thus it can be seen that for numerical considerations alone this class of brain tumors is the most important (9). But there are many additional factors, some subject to experimental probing, as will be discussed below, that make it so important.

Among the human gliomas, this author has found that the relatively malignant astrocytic tumor of the glioblastoma multiforme variety occupies first place numerically with an incidence of over 51 per cent (3). Next in frequency is the more slowly growing, hence more benign, astrocytoma (incidence of 24.5 per cent). The ependymoma is next with an incidence of slightly more than 6 per cent, followed by the oligodendroglioma (5.5 per cent). The polar spongioblastoma, confined essentially to the pons and brain stem and occasionally to the corpus callosum, was present in 3.4 per cent of the 1,633 glioma tumors of this series. Attention is drawn to the fact that 57 (3.4 per cent) of all the gliomas were mixed tumors; i.e., they contained more than one identifiable gliogenous component. Among these "mixed" gliomas were some that were composed of both ependymomatous and oligodendrogliomatous parts, and some that had additional astrocytomatous as well as sarcomatous portions.

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THE EXPERIMENTALLY PRODUCED GLIOMAS

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It is fortunate that the experimentally produced gliomas often resemble morphologically their human counterparts so closely as to be virtually indistinguishable from them. Thus the cerebral astrocytoma, produced in mice by intracerebral implantation of compact pellets of chemical carcinogens such as methylcholanthrene, dibenzanthracene or benzpyrene (10), is a microcystic tumor composed of stellate astrocytes (Fig. 1) very much as are the human astrocytomas. In general, the more slowly growing tumors of astrocytic origin are produced less frequently with the aromatic hydrocarbons than are the more malignant neoplasms.

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By far the most frequent murine glioma produced experimentally is the glioblastoma multiforme (Types III and IV astrocytoma). It is this tumor, as already mentioned, that occupies first place among the human gliomas. Like the latter, the murine neoplasm is highly pleomorphic, with foci of necrosis and marginal spongioblasts usually arranged in palisades (Fig. 2). Multinucleated tumor giant cells are common. Vascular thromboses and hemorrhages are also frequent features of this neoplasm.

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A modification of the murine glioblastoma multiforme exists in which the neoplasm, in addition to disclosing all the cytologic details just enumerated, is also characterized by numerous huge multinucleated neoplastic cells (Fig. 3). This is the tumor which in man is sometimes called "gigantocellular" or "monstrocellular" glioblastoma multiforme. The suggestion that the human tumor may be of mesodermal, hence sarcomatous, origin is not valid for this tumour in the mouse, which is unequivocally a glioma as shown by special staining methods.

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Still another variation of the malignant form of glioma of the astrocytic variety seen in the corpus callosum, pons or brain stem of the

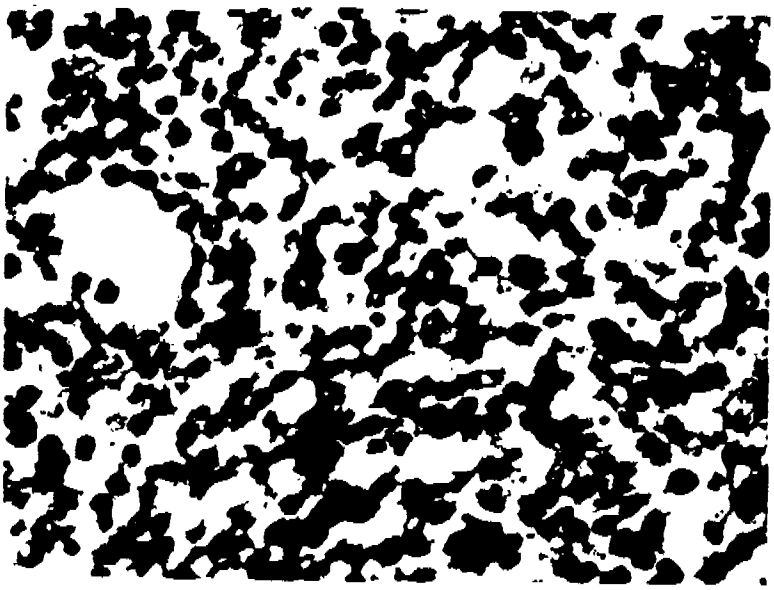


Fig.

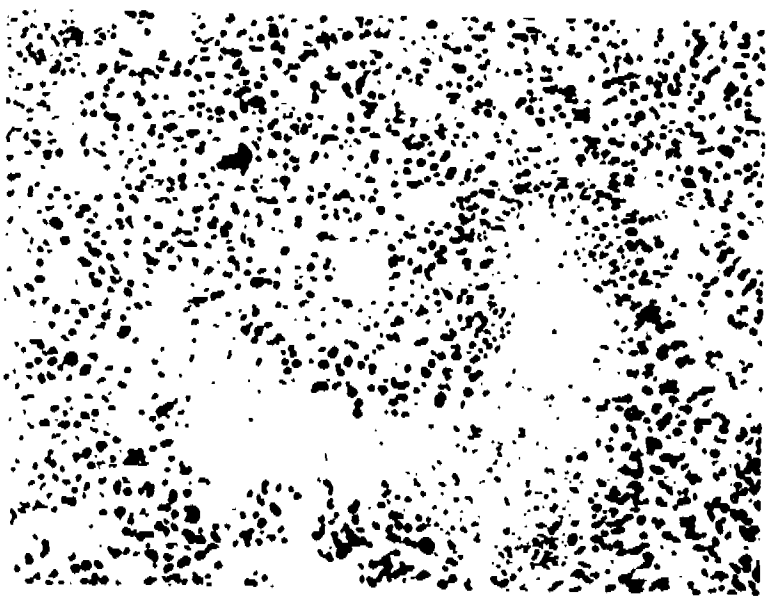
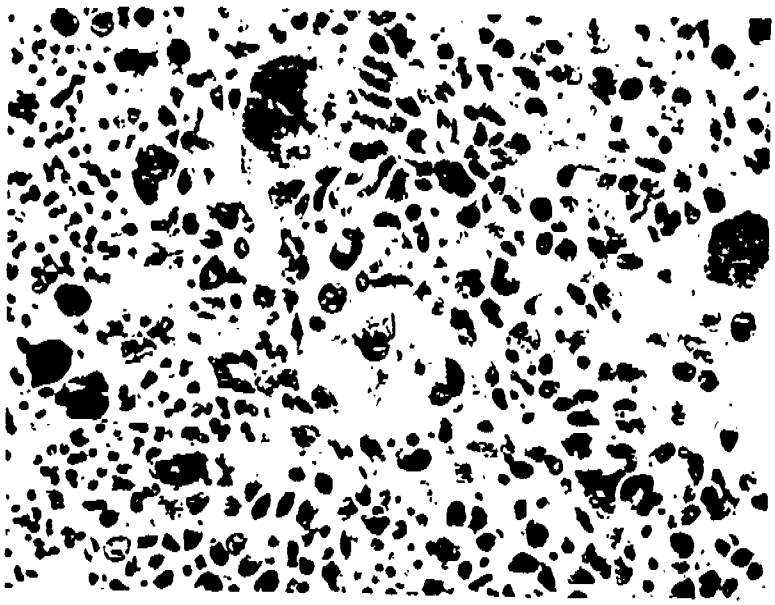


Fig. 4.

* The first eleven figures are of tumors embedded in paraffin and stained with hematoxylin-eosin.



[illegible] glioblastoma multiforme. x 215.

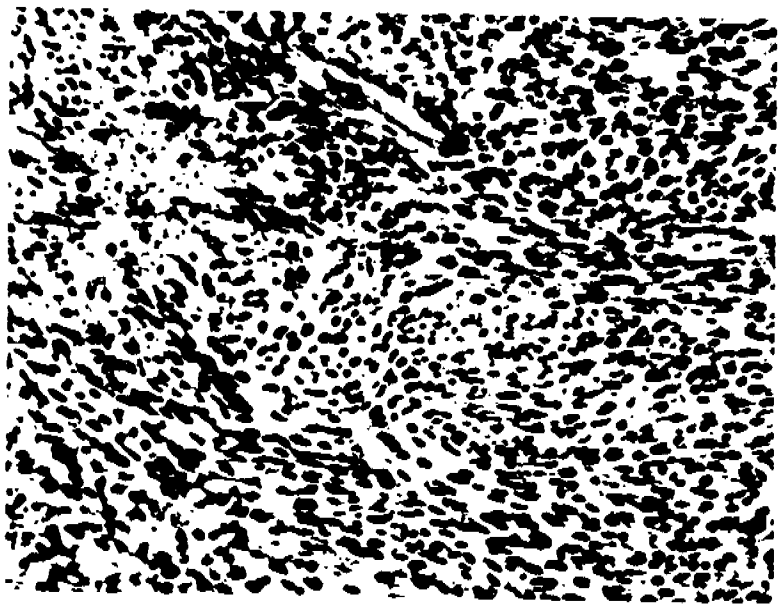


Fig. 4. Polar spongioblastoma in corpus callosum. Note interlacing bundles of spongioblasts. x [illegible]

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mouse is characterized by large interlacing bundles of bipolar and unipolar spongioblasts (Fig. 4). There are present occasional necrotic foci in some of these tumors and also a few giant cells with multiple nuclei. A considerable degree of cellular pleomorphism is always present. In man, this tumor is designated by the term "polar spongioblastoma."

With pellets of carcinogen implanted in the subcortical white matter of the animal brain, there is often produced a glioma of distinctive cellular appearance. The rather large uniform tumor cells are compartmentalized into lobules, almost gland-like in appearance, by vascularized connective tissue septa (Fig. 5). The individual neoplastic cells have clear cytoplasm between the chromatin-rich, round nuclei and the cellular membranes. The pale cytoplasm creates the appearance of "haloes" around the nuclei. This tumor is the easily recognized oligodendroglioma. Less frequently than the similar tumor in man, this murine glioma has calcium salt deposits.

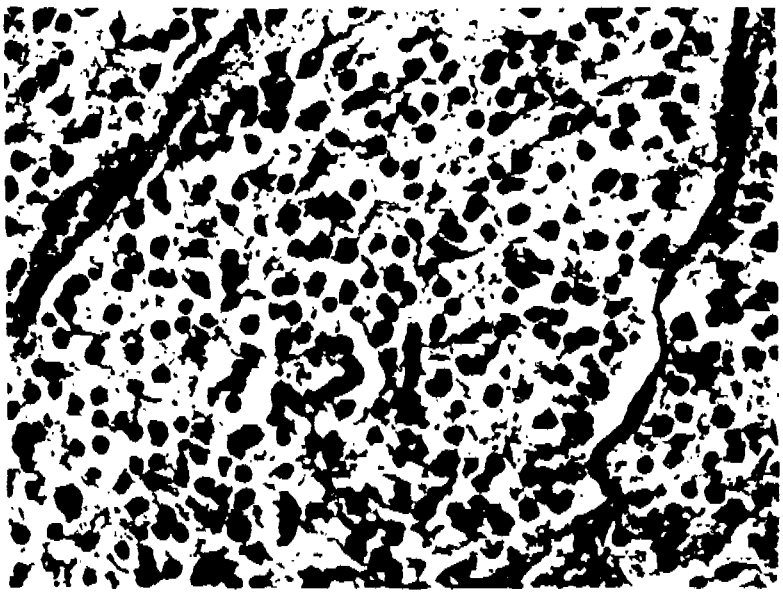

Fig. 5. Oligodendroglioma. The characteristic cells have round nuclei with clear cytoplasm that imparts an appearance of perinuclear haloes. Vascularized connective tissue septa divide the tumor into lobules. x 450

When the chemical carcinogen is implanted intraventricularly, there very often appears a tumor whose origin can be traced to the lining ependymal cells. Characteristically, the tumor cells form rosettes either around blood vessels or around acellular centers that are filled with delicate fibrils whose origin is from the neoplastic cells (Fig. 6). Electron microscopic study of these ependymomas discloses the same junctional complexes and cilia (or their precursors known as "basal bodies") that are diagnostic of the human tumor of this variety (11, 12).

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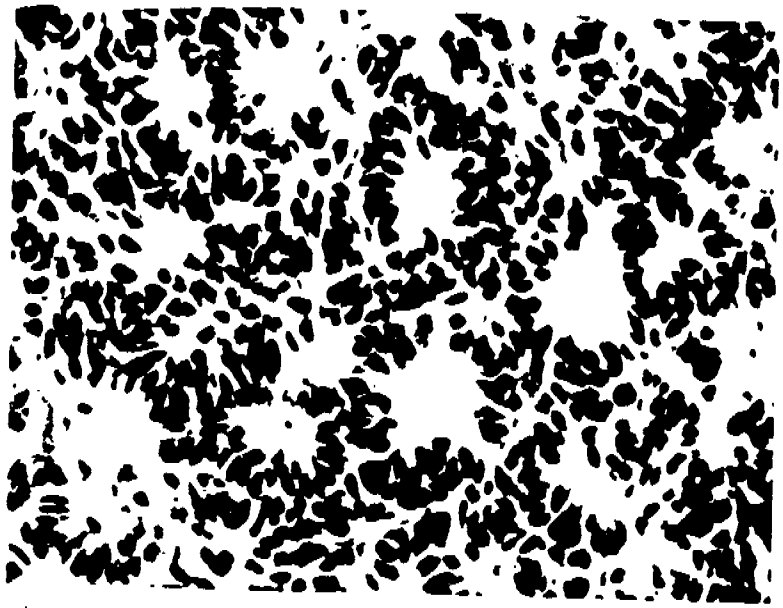

Fig. 6. Ependymoma. Tumor with the formation of small concentric centers of glial fibrils. x 150

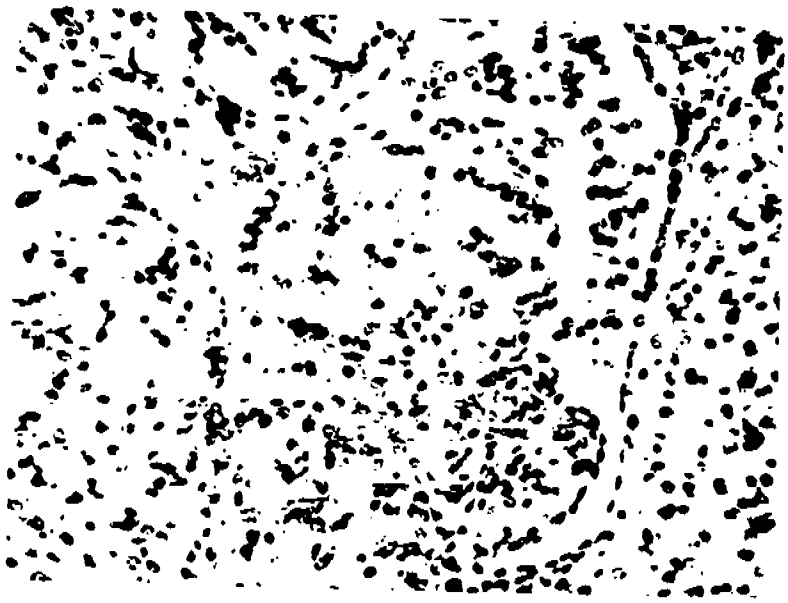

Fig. 7. Myxopapillary ependymoma with the formation of cysts to take origin from ependyma lining the central canal. x

There is a variation of this tumor in man that is called a myxopapillary ependymoma, and precisely this morphologic variant is sometimes seen in the experimental animal (Fig. 7). Its origin from the ventricular ependyma is clear. It is composed of tumor cells arranged in papillae between which is deposited a proteinaceous, myxomatous, PAS-positive substance.

Still another variation of the ependymoma is seen in the experimental animal and is similar to the rare human medulloepithelioma (13). This neoplasm has a tubular and papillary arrangement of its columnar cells (Fig. 8). In part it appears to be well differentiated, but some portions of the tumor are quite primitive in appearance and contain numerous cells in mitotic division.

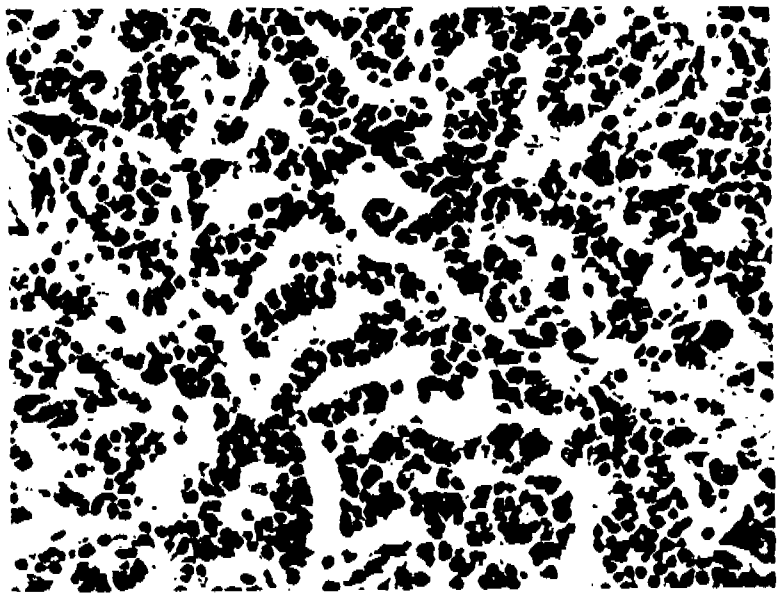

Fig. 8. Medulloepithelioma. The columnar neoplastic cells form tubules and papillary structures. x 175

CYTOGENESIS AND ETIOLOGY OF EXPERIMENTAL GLIOMAS

The murine gliomas induced with hydrocarbons are derivatives of the three basic glial cell types of the adult animal brain: the astrocyte, the oligodendrocyte and the ependyma. It is emphasized that in 35 years of experimentation during which several thousand mouse brain tumors were produced, not a single neoplasm developed whose origin could be traced to the fourth glial element of the brain; namely, the microglial cell. It is further emphasized that all the gliomas were invariably induced in adult animals and that the tumorigenic process was started by chemical implantation in the brains of young adult animals (from 6 weeks to 6 months of age). This was done for the purpose of

reasonably excluding the possibility of the interaction of the carcinogens with immature or embryonic glia, or embryonic cell rests. It may account for the fact that not a single ganglioglioma was ever produced, presumably because after neurons reach maturity and are fully differentiated they no longer are capable of neoplastic transformation. In a few instances when tumors developed in the cortical gray matter or the basal ganglions, a superficial appearance of gangliogliomas resulted (Fig. 9). The malignant glial cells in such tumors were easily recognizable and mature neurons were found among them, but the neurocytes were not neoplastic. They appeared merely to have been entrapped by the proliferating glia.

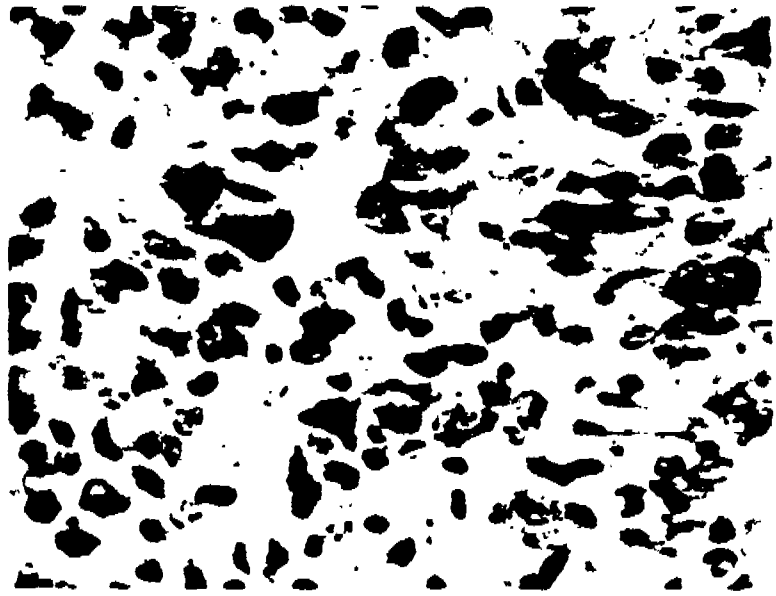

Fig. 9. Undifferentiated cortical glioma entrapping a pair of neurons whose cytoplasm contains lipoid bodies. This is the closest cytological approach to a ganglioglioma yielded by the experimental tumors. x 680

The situation regarding the production of medulloblastomas is rather of a different order. These tumors, too, were produced with some ease and by chemical carcinogens in the cerebella of adult mice (14). The experiments reveal that these tumors have their origin in the cerebellar cortex, evidently from the small granular cells and not from glia (Figs. 10, 11). This would make the medulloblastoma a neuroblastoma, and not a glioma at all. It would seem that in the process of neuronal differentiation in the cerebellar folia the small granular cells had not achieved the degree of maturation as to preclude neoplastic transformation, as is the case with the larger ganglion cells of the cerebellar cortex.

The problem of the "mixed" gliomas, as well as of the mixed gliomasarcomas, seen both in man and the mouse seem to be readily explained on the basis of the following consideration. The small carcinogenic

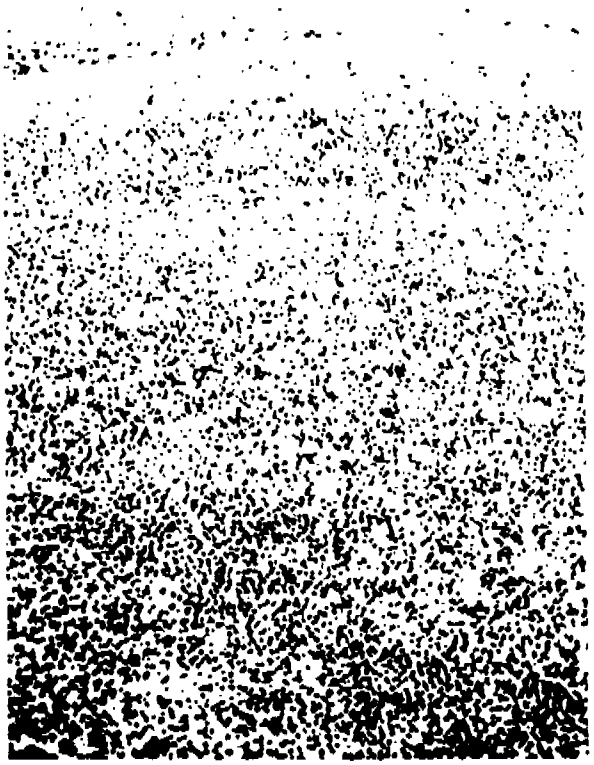

Fig. 10. Medulloblastoma. The tumor cells occupy the inner granular cell layer of the cerebellum. At the top of the figure, the vacuolar cleft represents the interfolial subarachnoid space. Note that there is no outer granular cell layer in this adult mouse. x 140

pellets, which measure approximately 1 mm in diameter and are implanted in the brains for the purpose of producing the neoplasms, are many times larger than any of the cells in whose midst they are placed. In this way many different cells of the same type, and possibly also of different types, are stimulated by the carcinogenic agent to undergo neoplastic proliferation. Thus the simultaneous proliferation of cells of different types gives rise to the mixed tumors. Perhaps the marvel is that such tumors do not actually occur more frequently than they do, for "pure" tumors, i.e. those of one cell type, can evidently occur if only a single cell gives rise to the neoplasm or if only cells of the same and not other types are affected.

A more detailed study of the mechanism of carcinogenesis with chemical agents than was afforded by light microscopy was made possible with the availability of the electron microscope. It had been shown that the induction of cerebral neoplasms with carcinogens followed the process of phagocytosis of the chemical crystals by those cells destined to become neoplastic (15). What ensued within the chemical-bear-

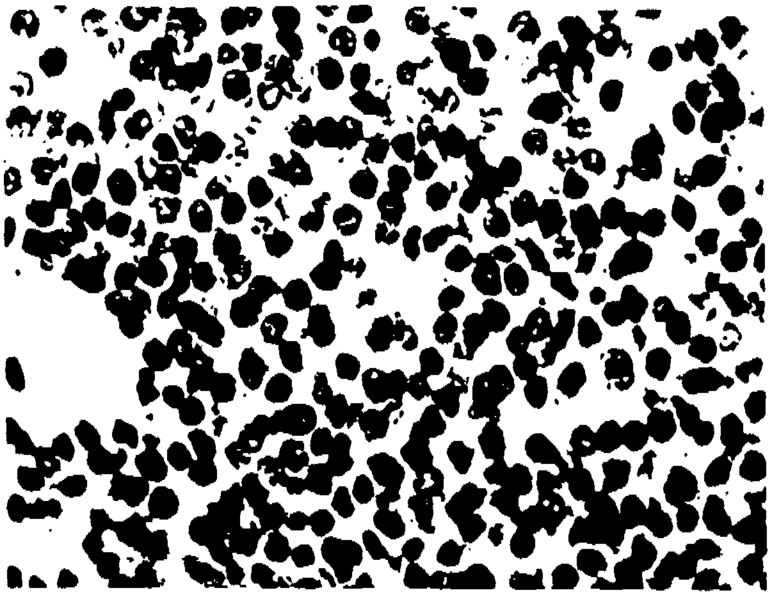

Fig. 11. [illegible] higher magnification of tumor cells in figure 10. The [illegible] blasts ("[illegible] blasts") are uniform in appearance and there is practically no stroma, either glial or mesodermal. In the very center of the figure, some tumor cells are arranged so as to suggest a rosette. x [illegible]

ing cells that resulted in their neoplastic transformation was suggested by several electron microscopic studies (16, 17, 18). Within the cytoplasm of the cells reacting to the carcinogenic crystals there appeared uniform filamentous and spherical particles with an outer diameter of about 77 m$^{\mu}$ (Fig. 12). These intracellular particles were present only during the precancerous stages of the cellular response to the pellet implantation; they disappeared from the cells when a glioma could first be recognized microscopically. Efforts to produce tumors with these virions in saline suspensions that were free of whole tumor cells and of carcinogen resulted in the production of malignant neoplasms at the injection sites in 8 of 14 animals (8). Additional confirmatory evidence is still necessary for the establishment of these virions as agents in experimental gliomatogenesis. No evidence of any kind is as yet available that would implicate viruses as etiologic agents in human gliomas.

From time to time it was suggested in the past that the human glioblastoma multiforme was caused by an infectious agent (19). It was the following considerations that were responsible for this view: 1, this tumor frequently has extensive necrosis; 2, it often has a partial mesodermal stroma; 3, there are vascular proliferative changes suggestive of granulation tissue; 4, there are vascular thrombotic occlusions; 5, lymphocytic accumulations may surround some blood vessels; and 6, some multinucleated giant cells may resemble those seen in granulomas. Of course, none of these features prove an infectious etiology -

Fig. 12. Intracytoplasmic filamentous and spherical virions in a pre-cancerous lesion produced with dibenzanthracene in the brain of an adult mouse. Electron micrograph x 100,000

all early attempts to culture significant organisms in these tumors failed. In more recent times, it was demonstrated that some nuclei in the tumor cells contained structures that were somewhat suggestive of viral inclusion bodies. But electron microscopy proved conclusively that these intranuclear bodies were merely cytoplasmic invaginations.

Other chemical agents in addition to the aromatic hydrocarbons have been employed in the production of experimental brain tumors. The resorptive carcinogens such as methylnitrosourea (MNU) and ethylnitrosourea (ENU) have the advantage of employability in the rat, a larger animal than the mouse, and can be fed or injected intravenously (20, 21). Employed in the pregnant rat, ENU passes through the placenta and produces neural tumors in the offspring (22). These tumors are frequently multiple and their locations in the nervous system are quite unpredictable; among favorite sites are the Gasserian ganglion and the spinal cord. Of the gliomas produced with these compounds, there seem to be high incidences of oligodendrogliomas and mixed gliomas. Of the non-glial tumors, both intra- and extracranial neurilemomas are prominent.

Beginning in the mid-1930s work was started with viral agents in attempts to produce brain tumors in animals. First, the Rous sarcoma virus (RSV) was injected in chicks with some success and later, in the 1960s, the same agent was employed in hamsters, rabbits and dogs. The astrocytoma was the most common glioma produced in mammals with the RSV, but spongioblastomas and glioblastomas multiforme were not uncommon. Soon other viruses were put into use in brain tumor production by an increasing number of investigators. The results of these experiments were well summarized by IKUTA and KUMANISHI (23), who reported significant results of their own with the human adenovirus type 12 in hamsters. That the human adenovirus type 12 is oncogenic for animals is of considerable importance and has already stimulated intensive search for other human oncogenic viruses. Most recently MUKAI and KOBAYASHI (24) described the results in newborn hamsters that re-

ceived intracerebral inoculations of the human adenovirus. Their animals developed cerebral and spinal tumors of remarkable cellular uniformity that with light and electron microscopy were classified as ependymomas. It is this glioma that other investigators also have reported when employing adenovirus type 12.

IN EXPLANATION OF CERTAIN GLIOMA PROBLEMS

In 1957 this writer touched on the significance of experimental gliomatogenesis in explaining some problems with human gliomas (25). The more important of these problems are the following:

1. Differentiation and Dedifferentiation of Tumor Cells. It has long been established that the cells of the nervous system including glia reach maturity by a process of differentiation morphologically and functionally. In neoplasia, it has been assumed that a process of dedifferentiation accounts for the anaplastic cells associated with malignancy. As part of this concept it has also been assumed that in the formation of a glioma a single differentiated cell is somehow stimulated to proliferation and that the resulting neoplasm has its origin from that one cell. The cellular pleomorphism of any glioma is attributed, on the basis of this theory of the unicellular origin of tumors, to dedifferentiation. But it has already been shown in the discussion of the origin of mixed gliomas in animals that the diverse cellular composition of these tumors is a function of multicellular stimulation to proliferation. Dedifferentiation undoubtedly accounts for the presence of astroblasts and spongioblasts in a tumor of astrocytic origin, but it cannot conceivably account for the presence of astrocytes in an ependymoma, or oligodendroglioma, or medulloblastoma, nor of ependymal cells in an oligodendroglioma. The concept of dedifferentiation as the explanation for pleomorphism in human gliomas therefore has restricted applicability. Like the extracranial neoplasms of the malignant lymphoma group that have a multicellular origin, this is also the case of many gliomas, both human and experimental.

2. Extracranial Metastasis of Gliomas. It is a well known fact that spontaneous extracranial metastasis of human gliomas practically never occurs, even with the most malignant of the tumors such as the glioblastoma multiforme. This is also true for the experimental neoplasms. From time to time it has been reported that small numbers of tumor cells have been found in the blood aspirated from the superior longitudinal sinus of patients with gliomas, but these tumor cells evidently fail to survive in sufficient numbers to provide active growth in other organs of the body. When gliomas in man have been found in metastatic foci extracranially, they have occurred almost without exception following surgery, when it was assumed that inadvertent transplants of tumor cells occurred intravascularly. This writer has seen metastatic human gliomas in the lungs and liver following surgery. He has also seen such tumors lining the right pleural and the peritoneal cavities in patients who have had ventriculopleural and ventriculoperitoneal shunts for obstructive hydrocephalus due to tumors.

The experimental glioma studies have helped validate the clinical assumptions regarding extracranial metastases. First, it has been shown that experimental tumors of this class are incapable of invading cerebral blood vessel walls, unlike primary intracranial mesodermal tumors, and hence they are sealed off from effective pathways of communication between the brain and the other organs of the body. Then it was shown in

numerous experiments (26, 27) that such primary experimental gliomas when provided with an egress from the skull will grow with ease in extracranial locations. Such egress is provided by the mechanical transplantation of fragments of glioma with a trochar in the subcutaneous tissues of the flank in homozygous mice. Or they will grow readily in the anterior chamber of the eye in mice and other animals. In experiments performed in my laboratory by Dr. N. KAGEYAMA some years ago, it was shown that saline suspensions of tumor homogenates when injected into the jugular veins of mice produced pulmonary gliomatous metastases with great ease. Likewise, such suspensions injected into the internal carotid artery resulted in intracerebral metastases. These experiments proved conclusively that gliomas will grow extracranially if only they are provided the necessary transport system.

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