**Review Article**

Embryonal central neuroepithelial tumors and their differentiating potential

A cytogenetic view of a complex neuro-oncological problem

**Lucien J. Rubinstein, M.D.**

Division of Neuropathology, Department of Pathology, University of Virginia School of Medicine, Charlottesville, Virginia

The embryonal central nervous system (CNS) neoplasms are reviewed with special reference to their differentiating potential and in the light of current neuro-oncogenetic concepts partly derived from the experimental induction of neural tumors. The conceptual (and, occasionally, practical) distinction between adult-type and embryonal CNS tumors raises a complex problem, because neoplastic transformation essentially involves replicating stem cells in tissues of renewal and because in the human brain such cells are found mostly in the course of CNS development. A cytogenetic scheme is therefore needed to serve as a frame of reference for a classification of embryonal CNS tumors that will account for the different histological entities documented so far and for the range and the restrictions of their differentiating capabilities. Most embryonal CNS tumors can be fitted into such a scheme. The cerebral medulloepithelioma, the cerebral and cerebellar neuroblastomas, the primitive polar spongioblastoma, and the ependymoblastoma show characteristic morphological features and a correspondingly distinctive cellular differentiating potential. The differentiating capabilities of the cerebellar medulloblastoma, the pineoblastoma, and the retinoblastoma are also distinctive, and are apparently determined by the cytogenesis of the area of the CNS in which the tumors originate. The indiscriminate application of a simplistic concept that would include all the so-called "primitive neuroectodermal tumors" into a single neuroepithelial tumor entity is unlikely to bring further understanding to the problem.

**Key Words** embryonal central neuroepithelial tumor • cytogenesis • tumor differentiation • medulloepithelioma • neuroblastoma • medulloblastoma • ependymoblastoma

The object of this paper is to review the differentiating potential of the embryonal central neuroepithelial tumors, and to propose and justify a cytogenetic approach to their classification. Because it is more precise and corresponds to recognizably different tumor entities, such an approach seems to us preferable to their amalgamation under the all-inclusive term of "primitive neuroectodermal tumors" of childhood.

**Neuro-Oncogenetic Considerations**

The following considerations are necessary as an introduction to the problem of developing a system of classification.

Neoplastic Transformation: Cells of Renewal

Neoplastic disease is a process that largely involves replicating stem cells — cells that act as a source of tissue renewal but lack the differentiating characteristics of that tissue. The potential for these characteristics is carried by the stem cells and transmitted to their progeny, where it is fully realized in those postmitotic cells that have reached their terminal stage of differentiation. Only cells that are still cycling are capable of malignant transformation, and all organs actively involved in regular tissue renewal possess such a reserve population of stem cells. The transformed stem cell will dispose of a range of differentiation which, to a large extent, approximates that of the normal stem cell.
addition, some of the cells that have reached a relatively advanced stage of differentiation will in certain circumstances reenter the cycle and resume replication. Thus, while the adult brain is generally considered to have a very low neuroepithelial reserve cell population acting as a potential source of tissue renewal, it is well known that, following the appropriate stimulus, astrocytes in the G0 phase (and probably oligodendrocytes as well) are capable of resuming proliferation.

CNS Cells at Risk in Neoplastic Transformation

Experiments with oncogenic alkylating and viral agents have shown that glial precursor cells in the embryo, glial precursor cells persisting in adult life, and differentiated glial cells in the adult brain may equally be involved in neoplastic transformation. However, this capacity is not uniform. The most vulnerable cells are those in the embryo, followed by those in the neonate, whereas the least vulnerable are those in the adult brain. In the transplacental induction of nervous system tumors in the rat following a single pulse of ethylnitrosourea, the period of maximal vulnerability of the fetal neuroepithelial cells ranges from the last one-third of intrauterine life to the time of birth. According to the classical timetable of neurocytogenesis currently accepted for the mammalian brain (see below), the cells in the central nervous system (CNS) that are the most likely target in these experiments are, therefore, the migrating, still replicating subependymal cells, which are the glial precursor cells whose descendants will differentiate into astrocytes and oligodendrocytes. This might explain the great prevalence of gliomas in both spontaneously occurring and experimentally produced CNS tumors, and the frequency with which periventricular gliomas are obtained experimentally. The inference, drawn from those experimental data, that the neuroepithelial cells most likely to undergo malignant transformation might be the fetal glial cells in the later stage of pregnancy is consistent with the relatively high incidence of gliomas in childhood, and has been supported by a large statistical study of children with CNS tumors in whom age at diagnosis was correlated with the risk of later recurrence. The occurrence of congenital gliomas, a rare but well recognized event, and current kinetics data on two cerebellar medulloblastomas suggest, however, that malignant transformation may also implicate neuroepithelial cells at an earlier stage of fetal life (see below).

Sites of Postnatal Neurocytogenesis

Data from highly artificial animal experiments must, however, be interpreted with caution. Certain sites of cellular proliferation in the postnatal brain could play a significant role in the genesis of gliomas. The most important site appears to be the subependymal layer, which may persist in adulthood, even in primates. Another source of postnatal gliocytogenesis in the 1st year of life is the myelination glia, when a massive turnover in these cells results in a marked increase first of astrocytes and later of oligodendrocytes. The vulnerability to neoplastic transformation of glial precursor cells immediately before myelination may perhaps account for the frequency with which astrocytes and oligodendrocytes are found to participate in the development of mixed gliomas. A third important zone of postnatal neurocytogenesis is the fetal external granular layer of the cerebellum, in which it has been inferred that neoplastic transformation could be the source of at least some cerebellar medulloblastomas.

The Window of Neoplastic Vulnerability

The incidence and the types of CNS tumors occurring spontaneously or obtained experimentally are presumably contingent on the number and types of neuroepithelial cells at risk, and on their state of differentiation (potential or actual) when they undergo the first neoplastic "hit," in other words, contingent on the proportion of cells that are still cycling at that particular stage of differentiation of the CNS. If the target of transformation is the almost terminally differentiated cell that is still capable, however, of one or more rounds of DNA (deoxyribonucleic acid) synthesis, then the range of differentiation in the tumor will be correspondingly restricted. If a small number of cells only are cycling at the time of the hit, and most of them are already committed to a limited line of differentiation in their progeny, then the window of neoplastic vulnerability will be correspondingly narrow. Viewed in the context of the currently accepted stages of normal neurocytogenesis, the relative width of that window determines the great prevalence of gliomas and the striking rarity of neuronal neoplasms among central neuroepithelial tumors in general.

Cellular Displacement in Neuro-Oncogenesis

If we accept the current view that the phenotypic expression of malignant transformation results from a sequence of multiple mutational events, and if we assume that the first hit may implicate a glial precursor cell in the course of its migration from the primitive ventricular zone, then it becomes entirely conceivable, if we further assume that displacement of the targeted cells and of their progeny has occurred prior to the last stage of transformation, that such a hit could be the source of a remote superficial cortical glioma in adult life. In experimental cerebellar medulloblastomas induced in the neonatal hamster by the JC strain of human polyoma virus, the tumors originate microscopically from the internal granular neurons, but the transformed cells are presumably those of the external granular layer, which is still mitotically active when the virus is injected.

The lag time before neoplastic transformation is expressed is of course strikingly illustrated in the appearance of peripheral and CNS tumors in the adult offspring (ranging from 150 to 400 days old) of the gravid
Embyronal central neuroepithelial tumors

rat given a single injection of ethynitrosourea in the late stage of pregnancy. If the length of the latent period before tumor formation occurs is partly determined by the life span of the species, then a latency period of 20 to 40 years is conceivable for the corresponding development of a human glioma. However, such an extrapolation has been questioned in view of experimental data obtained from other animal species.36

**The Importance of Cellular Anaplasia**

Any attempt at an orderly classification of CNS tumors must take into account the importance of anaplasia. This extremely common event results from the fact that neoplastic cell populations, which are often heterogeneous in regard to their karyotypic and phenotypic characteristics, show considerable genetic instability, which is manifested by progressive increases in mutation rates. Consequently, clonogenic subpopulations emerge which become preferentially selected so as to form the dominant proliferating cell pool.79,80 This progression is the dynamic manifestation of the phenomenon we recognize under the microscope as cellular anaplastic change. In kinetic terms, it corresponds to an increase of the tumor growth fraction and results from the recruitment of cells from the nonproliferating, or quiescent, pool into the proliferating pool. Conversely, when differentiation takes place in a tumor cell population, there is a shift toward the decycling of that population as increasing numbers of cells from the proliferating pool enter the nonproliferating compartment. While the glioblastoma represents the classic illustration of anaplastic change in a differentiated glioma, the less frequent occurrence of a medulloblastoma as the end-stage in the anaplastic transformation of an initially more mature astrocytoma in the cerebellum or brain stem has also been described.59,75,80 In these instances, it may be virtually impossible to decide whether the poorly differentiated cells are anaplastic or embryonal. Therefore, the alternative interpretation of similar cases as exemplifying the maturation of a medulloblastoma into an astrocytoma has, quite justifiably, also been proposed.3,23 In our experience, a transitional picture between medulloblastoma and diffuse anaplastic cerebellar astrocytoma, a rare but well recognized event, is found mostly in the older age groups, an observation that perhaps favors the view that the medulloblastoma is in these cases the result of anaplasia rather than the expression of an embryonal tumor. In any event, the supervention of anaplasia in the histological makeup of gliomas of any type may cause considerable difficulty in the interpretation of embryonal CNS tumors, especially in those discovered in adults.

**Adult-Type and Embryonal Tumors**

In organs that normally have a large complement of cells of renewal and that are therefore vulnerable to neoplastic transformation in adult life, the tumors may usually be fairly easily divided on morphological grounds into neoplasms of adult type and embryonal tumors. The latter, as defined by Willis,95 are tumors that arise during embryonal, fetal, or early postnatal development from tissues which are still immature but in which differentiation is already determined and restricted. The neoplasms may be expected to display not only a capacity for differentiation similar to that of the parent developing tissue, but also some degree of divergent differentiation.

Age at onset or at discovery of the disease is not necessarily one of the parameters that define an embryonal neoplasm. Some embryonal tumors, such as sympathetic neuroblastomas and cerebellar medulloblastomas, are well known to present in adults, and conversely an adult-type tumor may make its appearance in early life. The diagnosis of an embryonal tumor should therefore rest on its histological features, not on an age-related presumption. While an obvious relationship exists between embryonal neoplasms and tumors of infancy and childhood, that relationship is largely derived from the fact that the latent period which precedes the manifestation of neoplasia now extends back to the fetal period of life. Among CNS tumors, the presumptive role of that latent period in relation to the stage of neurocytogenesis is perhaps best suggested in the medulloblastomas: the hemispheric, often desmoplastic, tumors are found most often in adolescents and young adults, presumably because they may have arisen from a neoplastic transformation of the cells of the external granular layer; the midline medulloblastomas, on the other hand, are more likely to be found in children in the first decade of life, presumably because a neoplastic hit is more likely to have implicated cells of the primitive germinal bud in the roof of the fourth ventricle (that is, those cells at an earlier stage of cytogenesis).38

A major problem arises when the distinction between adult-type and embryonal tumors becomes blurred; that is, when (as in the CNS) the cells of renewal that are the presumed target for neoplastic change are largely to be found while the tissue is still undergoing morphogenesis. In CNS tumors, this means that the morphological cell forms and the histological patterns found in embryonal tumors and those in the adult types of tumor may be so closely alike that their distinction may be difficult. The problem is at first a diagnostic one; however, it may become a conceptual one because, it might be argued, the definition that separates embryonal from adult-type tumors loses its validity in the CNS altogether. The argument could even be carried to its logical conclusion that all gliomas are basically embryonal, or primitive, neuroepithelial tumors.

However, we find in practice that in a large number of cases we are able to make the distinction, especially if the significance of cellular anaplasia is recognized. While, to be sure, the conceptual problem cannot be entirely resolved, it is desirable for such a distinction to
be maintained, but, in order to do so with as much precision and consistency as possible, it becomes necessary to apply some kind of cytogenetic scheme to our concept of embryonal CNS tumors and their histological forms. The criteria for their recognition, identification, naming, and classification should be well defined, consistent, and, ideally, conclusive. While we are a long way yet from having reached such a goal, the documentation of distinct morphological tumor entities (made possible in some ways only or largely with the electron microscope, as with the cerebellar neuroblastoma and the central neurocytoma) seems the only way of eventually ensuring an orderly classification of this complex group of tumors.

Cytogenetic Approach to Embryonal CNS Tumors

Current Scheme of Normal Neurocytogenesis

The classic neurocytogenetic timetable of events occurring in the mammalian forebrain, favored in the last two decades, is characterized by the sequence of three stages (Table 1). The first stage consists in the replication of undifferentiated, uncommitted cells of the primitive neural tube. The second stage is defined by the migration of committed, nonreplicating neuroblasts. The third stage consists in the migration of replicating glial cell precursors which are the committed (or uncommitted) ancestors of astrocytes and oligodendrocytes. At the onset of the third stage the residual committed (or undifferentiated) neural cells, which were committed to become ependymoblasts, begin to acquire the differentiating features of ependymal cells.

The cytogenetic events in the cerebellum are in some respects quite different from those in the forebrain. While the Purkinje cells, the Golgi type-II neurons, and the basket cells of the molecular layer are derived from the primitive ventricular neuroepithelium, undifferentiated cells which originate in the roof of the fourth ventricle (especially from the ventrolateral and dorsal midline areas of the rhombic lip) migrate over the surface of the fetal cerebellar cortex to form the future external granular layer. The cells of that layer, especially those of the outer zone, continue to replicate as they cover the surface of the cerebellum. While they have been regarded by some workers as giving rise to both neurons and glia, more recent studies have supported the early view of Cajal that the cells of that layer are the source of granular neurons only. The Bergmann glia are usually believed to arise from the primary germinal zone of the cerebellum, but their origin from the fetal granular layer has also been proposed. The more recent autoradiographic studies on the neuroglia of the internal granular layer have not thrown any light on their cytogenesis.

Possible Modifications to the Current Scheme

The scheme of neurocytogenesis in the forebrain shown in Table 1 may have to be modified in the light of recent observations on the fetal monkey brain using glial fibrillary acidic protein (GFAP) as a marker for glia-committed cells. According to these recent observations, well before neuroblasts have become postmitotic the replicating ventricular cells already consist of a coexisting mixed population of glia-committed cells and neuroblasts; moreover, the majority of ventricular cells at the peak of neuroblast production (that is, at midgestation), including those in mitosis, are GFAP-positive. Other data further suggest that GFAP-positive radial glia, presumably responsible for guiding developing neurons in their migration, already appear in the first third of gestation. Therefore, cells committed to glial differentiation could be the target of oncogenesis at a considerably earlier age of fetal life than has so far been inferred from the transplacental induction of neural tumors with alkylating agents. Moreover, the radial glia, rather than undifferentiated glial cell precursors, are currently regarded as capable of generating both stellate astrocytes and myelin-forming oligodendrocytes, and immature spinal oligodendrocytes have been shown transiently to express GFAP, an interesting observation that may be relevant to the presence of GFAP in the cells of some oligodendrogliomas.

Insight into the apparent paradox raised by the existence of the cerebral neuroblastoma is provided by recent studies in the chick embryo, using markers such as neurofilament protein and neuronal cell surface-specific antigens. These studies have suggested that some replicating primitive neuroepithelial cells, probably in their last mitotic round, are already neuron-
Embryonal central neuroepithelial tumors

committed. Thus, the window of neoplastic vulnerability, normally closed to postmitotic neuroblasts, would admit a small number of already neuron-determined cells in the course of their terminal round of DNA synthesis. The existence of such a narrow window would therefore account both for the great rarity and the occasional occurrence of cerebral neuroblastomas in man.

The range and restrictions in the differentiating capabilities of the replicating immature central neuroepithelial cells in their different stages of cytogenesis will presumably determine the range and restriction of differentiating potential that will be expressed in their respective neoplastic counterparts.

**Application of a Cytogenetic Scheme**

**Classification of Embryonal CNS Tumors**

Irrespective of whether the classic scheme of normal central neurocytogenesis in the forebrain shown in Table 1 may have to be modified in the future, the only logical approach to embryonal CNS tumors would be to classify them according to the differentiating potential of their constituent cells, an approach possible only if they can be related to an acceptable scheme of neurocytogenesis. Such a relationship has previously been suggested, and the different types of tumor that can be so defined have been confirmed. Embryonal CNS tumors that can be fitted within an orderly neurocytogenetic framework, essentially derived from the concepts of Fujita, include (Table 2): the medulloepithelioma, the cerebral neuroblastoma, the primitive polar spongioblastoma, the ependymoblastoma, and, in the cerebellum, the medulloblastoma, with the cerebellar neuroblastoma as a more recently defined subgroup. In the pinal parenchyma, the pineoblastoma and, in the retina, the retinoblastoma provide a different range in their respective differentiating potential that is related to the morphogenesis of the area of the CNS in which they originate.

A major problem is that, with the exception of the cerebellar medulloblastoma and the retinoblastoma, we are dealing here with very rare entities. However, all have been documented in almost half the cases. Some examples show a considerable connective tissue stroma in which metaplasia to cartilage, bone, and striated muscle has been described. It is not impossible, as suggested, that such a primitive neuroepithelial tumor might be capable of expressing a myogenic differentiating potential, as exemplified in the rare cerebellar medulloblastoma and as reported in some in vitro studies performed on neuronal and glial animal cell lines. Further studies will be needed to confirm such a widening of the limits of neoplastic neuroepithelial differentiation.

**The Cerebral Medulloepithelioma**

The cerebral medulloepithelioma, of which more than 20 have now been reported, is the prototype of embryonal CNS tumors. Its hallmark features are its architectural pattern, which recalls that of the primitive medullary epithelium, and its capacity for multiple divergent differentiation, which may span the entire range of central neuroepithelial cytogenesis, from the most primitive to the most differentiated cell forms. Differentiation along glial and/or neuronal lines has been documented in almost half the cases. Some examples show a considerable connective tissue stroma in which metaplasia to cartilage, bone, and striated muscle has been described. It is not impossible, as suggested, that such a primitive neuroepithelial tumor might be capable of expressing a myogenic differentiating potential, as exemplified in the rare cerebellar medulloblastoma and as reported in some in vitro studies performed on neuronal and glial animal cell lines. Further studies will be needed to confirm such a widening of the limits of neoplastic neuroepithelial differentiation.

**The Cerebral Neuroblastoma**

Both isolated reports and larger clinicopathological series, including a small number of electron microscopic studies, have permitted a more precise delineation of the cerebral neuroblastoma, of which 80% of the cases present in the first decade of life. Characteristically, almost all are well defined, even sharply circumscribed, often firm and lobulated, with a histological pattern that ranges from a classical form demonstrating neuroblastic (Homer Wright) rosettes to a highly desmoplastic form, analogous to the corresponding variant of cerebellar medulloblastoma. The different histological patterns have, in our experience, no predictive significance in regard to postoperative survival. About 25% of the tumors show ganglionic differentiation, but neither the age of the patient nor postoperative survival could be correlated with that
feature. Electron microscopy has in some cases revealed the presence of dense-core vesicles in the perikarya and processes of the tumor cells, but the demonstration of synapses is exceptional. Exceptionally also, raised levels of catecholamines have been found in the urine and cerebrospinal fluid. Bioassay of a large upper cervical intramedullary neuroblastoma in a 35-year-old man, referred to us, disclosed raised levels of catecholamines, and the patient had increased urinary excretion of metadrenaline and vanillylmandelic acid, which returned to normal after removal of the tumor. From the clinical point of view, it is apparent that a substantial number of these patients have a rather more favorable prognosis for long-term survival than other patients with malignant central neuroepithelial tumors. In our series, eight patients survived 7 years after diagnosis, and three for more than 10 years. The overall 3-year survival rate was 60%. A conservative estimate suggests that 30% or more of the patients have a 5-year postoperative survival time. The period of risk for tumor recurrence or death was a function of the age at initial diagnosis, and Collins' law (which is based on the unproven assumptions that in embryonal tumors neoplastic transformation occurs at an early stage of development and that the subsequent rate of tumor growth is constant) was applicable to the overwhelming majority of cases. Most deaths or fatal recurrences occurred within the first 3 years after operation.

The Primitive Polar Spongioblastoma

The great rarity of the primitive polar spongioblastoma, assumed on morphological grounds to have arisen from migrating primitive glial precursor cells, is quite paradoxical in view of the presumptive vulnerability of the same cells to neoplastic transformation in experimental transplacental tumor induction. Its histological pattern of palisading, poorly differentiated, unipolar glial cells is diagnostic, and differentiation along astrocytic and/or oligodendrogial lines may be present. We have noted in several examples that the tumor cells in the more primitive areas are negative for GFAP. Although this neoplasm is instantly recognizable under the microscope, data on its electron microscopic features, tissue culture characteristics, or animal model equivalent are lacking. In some cases, as noted also by Becker and Hinton, a similar pattern may be demonstrated by neuroblasts, as confirmed by silver impregnation.

The Ependymoblastoma

The ependymoblastoma, of which we have now examined 11 examples, shows a distinctive microscopic picture in which highly cellular primitive glial cells demonstrate clear-cut ependymal differentiation, with the development of numerous ependymoblastic rosettes in both the primary growth and the subarachnoid metastases. Within the context of the cytogenetic scheme of Table 1, the term "ependymoblast" has been suggested as appropriate for the cells in the primitive neural tube which already display, at least in their early stages, the differentiating hallmark features of ependymal cells while exhibiting at the same time evidence of persistent mitotic activity. The rosette-forming cells in the tumor often contain mitotic figures, thus resembling the Flexner-Wintersteiner rosettes of retinoblastomas.

A slight variation in that cytogenetic scheme assumes, in addition, the existence of a primitive "polar ependymoblast," that is, a cell intermediary between a primitive glioblast and an ependymoblast, which would allow interconversion between these cells. This hypothesis seems supported by the occasional demonstration, by silver impregnation, of poorly differentiated cells with the features of polar spongioblasts in ependymoblastomas. Whatever cytogenetic concept is adopted, it implies, in these tumors, a restriction of differentiation to glial precursor cells and to cells with the differentiating features of ependymal cells only.

In contrast to most ependymomas, focal microscopic invasion is frequent, and leptomeningeal involvement may be extensive. Widespread cerebrospinal metastases may occur in the manner of a medulloblastoma. Confusion is still apparent in recent histological classifications of CNS tumors on the distinction between this tumor and ependymomas which have undergone anaplastic change. Yet on morphological grounds the separation is relatively easy. In contrast also to ependymomas, of which three-quarters occur below the tentorium, most ependymoblastomas have so far been supratentorial. Seven of our 11 cases were separate from the ventricles, suggesting an origin from ectopic ependymal precursor cells.

The Cerebellar Medulloblastoma

The cerebellar medulloblastoma is, with the retinoblastoma, by far the most common of the embryonal tumors of the CNS and has therefore been extensively studied, including its histogenetic aspects and differentiating potential. Its relative frequency suggests that the window of neoplastic vulnerability for the cells at risk must be wider than for any other embryonal central neuroepithelial cells. It is reasonable to relate this difference to the fact that the cytogenesis of the cerebellum differs in some important respects from that of the forebrain and to the much longer period of time during which germinative cells remain mitotically active in that part of the CNS.

It is therefore understandable that the fetal granular layer of the cerebellar cortex, which maintains its mitotic activity up to the end of the 1st year of postnatal life, has long been regarded as a possible source of origin for cerebellar medulloblastomas. Neoplastic proliferation of the cells of that layer accompanying a congenital medulloblastoma has been documented, but the observation remains exceptional. However,
Embryonal central neuroepithelial tumors

since that report was published in 1970, we have seen two further examples in which an abnormal proliferation of that layer was associated with a neuroepithelial tumor in the cerebellum. More frequent is the observation of apparent continuity between subpial aggregations of tumor cells, sometimes regarded as persistent remnants of the fetal granular layer, and infiltrating forms of medulloblastoma which most often present in the lateral lobes of adolescents and young adults. Here the interpretation of tumor origin from that layer is more dubious because these neoplastic surface localizations beneath the pia could equally be a manifestation of secondary structures of tumor growth, a phenomenon that is commonly seen in infiltrating gliomas other than medulloblastomas. The morphological similarities of medulloblastoma cells to the cells of the fetal granular layer have also been used in support of a histogenetic link. One of the difficulties in confirming the hypothesis is the rarity of animal experimental models for the study of this relatively common tumor, especially the failure to obtain cerebellar medulloblastomas in rats after the injection of alkylating agents.

However, other histogenetic sites are likely to be at least as important as the fetal granular layer, or even more so. The midline tumors may have arisen from nests of primitive germinal cells in the posterior medullary velum, a hypothesis based on the examination of embryonic, fetal, and infantile brains in which persistence of these cell nests has often been demonstrated. A third possible site of origin, namely the internal granular neurons, has been postulated from experimentally induced tumors obtained in adult mice with the topical application of carcinogenic hydrocarbons, and in hamsters following the inoculation at birth of the JC strain of human papovavirus. As noted above, the transformed cells in the latter experiments may well originally have been those of the fetal granular layer.

A valid objection against the derivation of cerebellar medulloblastomas from the cells of that layer is that, while the tumors frequently show glial differentiation, the fetal granular layer gives rise, according to current views, to granular neurons only. However, most of the work on the differentiating potential of that layer has so far been concerned with events occurring in its later stage of maturation. The question whether it could not be the source of glial cells at an earlier stage has not been investigated — an inquiry that would be relevant since there is evidence that the development of astrocytes in the internal granular layer precedes that of the neurons.

Inferences on normal cytogenesis drawn from the differentiating features expressed by a tumor should in any case be qualified by the fact that in neoplasms the differentiating potential may be less (or, because of the development of anaplasia, often more) restricted than in normal tissues. Moreover, divergent (or heteroplastic) differentiation is a feature of embryonal tumors in general. In any event, irrespective of the differentiating capabilities of the fetal granular layer, numerous observations in situ and in vitro, corroborated by electron microscopic and immunohistochemical studies, have collectively shown that the medulloblastoma is capable of differentiation along neuronal and glial lines, and sometimes of divergent differentiation to both. Electron microscopy has, moreover, permitted the identification, within the group, of a morphologically distinct, pure cerebellar neuroblastoma that can be aligned with the cerebral neuroblastoma referred to above. From the clinical point of view this separation seems to have little significance so far, but the possibility of subsequent maturation to adult ganglion cells has been suggested in one case.

The bipotential differentiating capacity of the medulloblastoma, probably best demonstrated in tissue culture, suggests that it may arise from a primitive neuroepithelial cell capable of two-directional differentiation, presumably at an earlier stage of ontogenesis than those cells of the fetal granular layer destined to become internal granular neurons. Such an assumption is currently gaining support from the cell kinetics data of Hirakawa, et al. (unpublished data). They studied the cases of two children aged, respectively, 5 years and 4 months, and indicated that, based on the regression of the growth curves of the tumors, the medulloblastomas may have had their onset between the 15th and the 24th week of intrauterine life.

The question could certainly be raised whether the cerebellar medulloblastoma represents a single entity or several cytogenetically distinct forms of primitive CNS tumors. It is highly likely that, like other types of glioma, these tumors are composed of heterogeneous cell populations, despite their apparent morphological uniformity. As suggested by the recent recognition of the cerebellar neuroblastoma within the group, separate cytological entities might result from the neoplastic transformation of different cerebellar neuroepithelial cells at various stages of ontogenesis. The study of relevant experimental animal models might throw further light on that point.

In any event, the well recognized bipotentiality of the medulloblastoma is a feature that separates it from many other embryonal CNS tumors, especially from most supratentorial neoplasms, among which, save for the highly distinctive medulloepithelioma, divergent glial and neuronal differentiation is exceptional in our experience. This implies that the window of neoplastic vulnerability for bipotential primitive neuroepithelial cells in the supratentorial compartment must be narrow, a hypothesis consistent with the data, referred to above, that suggest that commitment to a neuronal or glial line is an early event in the neurocytogenesis of the forebrain. Consequently, embryonal tumors are considerably less frequent in the supratentorial compartment than in the cerebellum, and our experience
has been that their differentiating potential is usually restricted to either the neuronal or the glial line. We exclude from this discussion the gangliogliomas, which, by reason of their differentiated features, are not regarded as embryonal CNS tumors in the present context.

The Pineoblastoma

The pineoblastoma represents another example of embryonal tumor in which the differentiating capabilities correspond to the cytogenetic potential of the area of the CNS in which it originates. While many examples are poorly differentiated, a few display divergent astrocytic and ganglionic differentiation and, in some cases, an additional capacity for retinoblastic differentiation with the formation of Flexner rosettes and fleurettes. The latter are a remarkable demonstration of the ontogeny of an organ being recalled in its neoplastic development, since the structure and function of the pineal gland in lower vertebrates are well known to be those of a photoreceptor organ. Such a differentiating feature is found in no other embryonal CNS tumor, except of course in the retinoblastoma. The special morphological characteristics of the pineoblastoma are, as expected, expressed even more precisely by electron microscopy in the more differentiated pineocytoma as well as in the experimental hamster pineocytoma induced by the JC strain of human papovavirus. The close morphological relationship between pineoblastoma and pineocytoma supports the separate identification of the former as a specialized example of embryonal CNS tumor.

The Retinoblastoma

The retinoblastoma is yet another instance of an embryonal CNS neoplasm in which the direction of differentiation is, according to most investigators, restricted to that of the cell from which it is assumed to arise. It is generally agreed that, by light microscopy, differentiation in retinoblastomas is expressed by the development either of neuroblastic (Homer Wright) rosettes or of Flexner-Wintersteiner rosettes and fleurettes, the latter two formations mimicking the cyto- and architectural features of rod and cone cells. This photosensory line of differentiation has been confirmed by electron microscopy and in tissue culture. With few exceptions, most in vitro studies have failed to demonstrate neuroblastic differentiation in retinoblastomas. Taylor, et al., interpreted their tissue cultures of six retinoblastomas examined by scanning electron microscopy as showing divergent glial and neuronal differentiation. However, the development of an abundant fibrillary gliosis, either adjacent to the tumor or incorporated within it, is well known, and it may be difficult to establish unequivocally that the tumor cells have differentiated along glial lines unless the presence of gliosis in the original explants has been ruled out.

"Primitive Neuroectodermal Tumors" of Childhood

Separate Entity or Incomplete Documentation?

In agreement with isolated case reports, we have noted a few supratentorial examples composed of a mixture of neuroplastic neuronal and glial elements, thus resulting in a complex tumor, partly ganglioneuroblastoma, partly anaplastic astrocytoma. These cases, which are exceptional, have not so far been incorporated into the scheme of Table 2, and are currently being studied. The demonstration of divergent glial and neuroblastic differentiation in primitive CNS tumors has been facilitated by immunohistochemical techniques, and the growing use of these sensitive methods is expected to uncover an increasing number of such cases. However, caution is needed in their interpretation, especially in distinguishing neoplastic from included reactive elements. Interpretative problems also hinge on the criteria that clearly define a single entity, and, particularly in the case of limited fragments obtained at operation, on whether the latter are representative of the rest of the tumor, and on whether they can be studied by techniques more sophisticated than routine histological stains. Under the term “primitive neuroectodermal tumors of the brain” a number of poorly differentiated cerebral neuroepithelial neoplasms have been reported which do not appear to represent a clear-cut histological entity. The criteria for their recognition lack precision. Some of these tumors may be anaplastic small-cell gliomas, others could represent cerebral neuroblastomas. Neuroblastic differentiation has indeed been suggested by electron microscopy in one example, but usually the documentation is too incomplete to permit the assignment of these cases to a definite category. It seems to us that if the diagnostic uncertainties are such as to preclude their identification, it would be better to leave them unclassified. The creation of a separate tumor entity largely based on an inability to achieve diagnostic precision seems to us a retrograde step because, while it may provide to the less experienced a convenient repository for tumors that cannot be diagnosed, it is only too tempting to include in such a category cases that can be classified more accurately when more detailed study is possible.

The Case Against Oversimplification

The complexity of the cytogenetic problem raised by the embryonal CNS tumors is illustrated in Tables 1 and 2. It is therefore not surprising that an attempt has been made to simplify it by amalgamating all these tumors within the overall designation of “primitive neuroectodermal tumors,” irrespective of their location in the CNS, and to propose a simple subdivision according to whether the tumor cells are completely undifferentiated or whether they show evidence of one or more lines of differentiation along glial, ependymal, or neuronal derivatives. Such a proposal rests on the largely erroneous assumption that the subependymal primitive cells are, at all times and irrespective of their
Embryonal central neuroepithelial tumors

different stages of cytogenesis, capable of differentiating along any or all cell lines, and it therefore fails to relate the incidence and the types of embryonal tumors to the restrictions of differentiating potential that they usually demonstrate. By blurring the distinctive histological patterns that have been documented so far, it carries the implication that the different tumor entities are interchangeable. Confusion may therefore arise in their recognition, and diagnostic errors may be introduced. That a problem of identification exists in some of these cases is undeniable, but a simplistic approach based on a somewhat superficial view of the question is unlikely, in our opinion, to provide a useful frame of reference for the further study of these complex tumors.

References
34. Horten BC, Rubinstein LJ: Primary cerebral neuroblas-
56. Masuko S, Shimada Y: Neuronal cell-surface specific antigen(s) is expressed during the terminal mitosis of cells destined to become neuroblasts. Dev Biol 96: 396–404, 1983
74. Markesbery WR, Haugh RM, Young AB: Ultrastructure

J. Neurosurg. / Volume 62 / June, 1985