# Transplacental Production with Ethylnitrosourea of Neoplasms of the Nervous System in Sprague-Dawley Rats

A. Koestner, DVM, PhD, J. A. Swenberg, DVM, PhD, and W. Wechsler, MD

OF THE NEWLY SYNTHESIZED ONCOGENIC *N*-NITROSO COMPOUNDS, ethylnitrosourea (ENU) has been shown to be a potent resorptive carcinogen. Incidence, location and types of neoplasms produced with ENU in rats depend greatly upon age, dosage, strain, and, to a certain extent, also upon the physiologic state of the animal—*eg*, pregnancy.

Druckrey *et al*[1] produced tumors of various organs (9 of the hematopoietic system, 4 of the brain and 1 each of the spinal cord, small intestine, uterus and mammary gland) in adult BD rats with weekly intravenous injections of 10 mg/kg ENU up to a total dose of 250 mg/kg. Inoculating pregnant female rats with single intravenous doses of 20–80 mg/kg of ENU resulted in a predominance of neoplasms of the genital tract (ovaries, uterus and vagina), indicating a higher susceptibility of the genital tract to the carcinogenic effect of ENU during pregnancy.[2,3] The influence of age upon the incidence, location and tumor types was clearly demonstrated by Druckrey *et al*[4] in an experiment in which BD IX rats, in age groups of 1, 10 and 30 days, were inoculated with a single dose of 5–80 mg/kg of ENU. The significant findings of that experiment were the demonstration of the decreased susceptibility of the older age groups for the oncogenic effect of ENU and the high incidence of tumors of the central and peripheral nervous system in the 1-day-old rats. This trend of decreasing susceptibility to the oncogenic effect of ENU became even more pronounced in adult rats. Doses of 20–80 mg/kg ENU resulted in none or a few tumors of the central nervous system (CNS) reaching an incidence of approximately 20% at dose levels of 70 and 80 mg/kg. With very high single doses of 140 mg/kg, neurogenic tumors were produced in 45% of the animals and with 200 mg/kg in 60% of the animals.[5]

From the Department of Veterinary Pathology, The Ohio State University, Columbus, Ohio.

Supported in part by grant CA 11224 and training grant GM 1052 from the National Institutes of Health.

Accepted for publication December 4, 1970.

Address for reprint requests: Dr. A. Koestner, Department of Veterinary Pathology, Ohio State University, 1925 Coffey Road, Columbus, Ohio 43210.

The same authors [5-7] demonstrated for the first time that tumors can be produced transplacentally in the offspring of pregnant BD IX rats, after single or repeated intravenous injections of ENU into the pregnant mother. The transplacental induction of tumors was further confirmed by Kleihues *et al.*[8] In all of these experiments, there was a selective oncogenic involvement of the nervous system.

One can conclude from these experiments that the alkylating agent ENU is particularly suited for transplacentally inducing neoplasms of the nervous system since small doses of the carcinogen cause a high incidence of neural tumors in offspring.[1,5,6,10] Ivankovic and Druckrey,[5] using BD IX rats, demonstrated that transplacental production of tumors was not possible before the twelfth day of gestation and their initial work indicated an increased susceptibility for induction of neurogenic tumors with the advancing pregnancy, culminating toward the termination of the gestation period.

The planning of our experiments was based upon the following considerations: Previous experiments have concentrated on tumor induction primarily during the earlier stages of fetal development.[5,8,10-12] The purpose of this study was to explore the oncogenic effect of ENU on fetuses during the more susceptible late stage of gestation. In the previously reported experiments, BD IX rats were utilized exclusively. Considering the importance of possible species- and strain-dependent differences in susceptibility to chemical carcinogens, in particular to ENU, it was decided to employ in these experiments the Sprague-Dawley rat, a randomly bred rat strain.

The specific objectives of this study were (1) to test the neurooncogenic effect of 50 mg/kg of ENU on offspring of Sprague-Dawley rats intravenously inoculated on the twentieth day of pregnancy, (2) to determine the incidence and distribution of these neoplasms, and (3) to characterize and classify the tumors by correlating the results of light and electron microscopy.

## Materials and Methods

Three pregnant, female CD (Sprague-Dawley) rats free of specific pathogens (Charles River, Wilmington, Massachusetts) were inoculated with 50 mg/kg of ENU via the lateral tail vein on the twentieth day of gestation. The ENU was freshly prepared by dissolving 10 mg/ml in sterile saline and adjusting the pH to 4.5 with crystalline ascorbic acid. A total of 13 male and 12 female offspring were whelped by the inoculated mothers. The offspring were weaned at 28 days of age and housed in individual cages in isolated rooms. All animals were fed autoclaved Purina Lab Chow 5010C, and water *ad libitum*. Rats were examined daily and weighed weekly throughout the experimental period. Progressive neurologic signs and weight loss were utilized in selecting animals for euthanasia.

Most animals selected for electron microscopy were deeply anesthetized with Metophane (Pitman-Moore, Indianapolis, Indiana) and perfused via the ascending aorta with either phosphate-buffered 3% glutaraldehyde (pH 7.2) or 0.1 M cacodylate buffer (pH 7.4) and then by a solution of 4% paraformaldehyde and 2.5% glutaraldehyde for 10 minutes at 150 mmHg. Tissue specimens were diced into 1-mm cubes and immersed in the same fixative for 1 hour. The remaining rats selected for electron microscopy were anesthetized deeply with Metophane while the tumor was excised. Tissues were immediately diced in 3% glutaraldehyde or 1% osmic acid and fixed for 1 hour. Aldehyde-fixed tissues were postfixed in buffered 1% osmic acid. Samples were dehydrated using a series of graded alcohols, and embedded in Maraglas. Sections 1-μ-thick stained with toluidine blue were utilized in selecting areas for electron microscopy. Thin sections were cut with glass knives on a Porter-Blum ultramicrotome, mounted on copper grids, stained with uranyl acetate and lead citrate and examined with a Philips EM-200 electron microscope.

All experimental animals were subjected to a complete necropsy. Brains and spinal cords were fixed in Cajal's brom-formol solution, while non-neural tissues were fixed in 10% buffered formalin. All lesions, serial blocks of brain and six selected segments of spinal cord were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Wilder's reticulin, Masson's trichrome, cresyl violet and phosphotungstic acid–hematoxylin were utilized as additional histochemical or staining procedures.

## Results

### Survival Time, Incidence and Location of Tumors

All 25 offspring of the 3 Sprague-Dawley rats inoculated intravenously with 50 mg/kg of ENU on the twentieth day of gestation developed tumors. The survival time and average number of tumors per rat, separately calculated for males and females, are given in Table 1. Survival times ranged from 85 to 346 days, with a mean of 211 days. The number of tumors per animal varied from one to nine, but averaged 4.36 tumors per rat. Although male animals apparently had a higher rate of tumors and a shorter average time of survival when compared to females, these differences were not significant statistically.

The number, location and size of neoplasms are given in Table 2. A total of 102 neural tumors and 7 non-neural tumors were produced in

Table 1. Survival Time and Average Number of Tumors in Offspring of CD Rats Treated with 50 mg/kg ENU on the Twentieth Day of Gestation

| Sex | No. of animals | Survival time (days) | | No. of tumors per rat |
|---|---|---|---|---|
| | | Average | Range | |
| Male | 13 | 201 | 134–256 | 4.69 |
| Female | 12 | 222 | 85–346 | 4.00 |
| Total | 25 | 211 | 85–346 | 4.36 |

Table 2. Number, Location, and Size of Tumors in Offspring of CD Rats Treated with 50 mg/kg ENU on the Twentieth Day of Gestation*

| Location | Gross tumors | | | Micro tumors | | | Early neoplastic proliferation | | | Totals | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | Total | M | F | Total | M | F | Total | M | F | Number | % |
| Brain | 6 | 8 | 14 | 23 | 16 | 39 | 3 | 1 | 4 | 32 | 25 | 57 | 52 |
| Spinal cord | 7 | 5 | 12 | 2 | 4 | 6 | 0 | 0 | 0 | 9 | 9 | 18 | 17 |
| Peripheral NS† | 14 | 5 | 19 | 5 | 3 | 8 | — | — | — | 19 | 8 | 27 | 25 |
| Extraneural organs | 1 | 5 | 6 | 0 | 1 | 1 | — | — | — | 1 | 6 | 7 | 6 |
| Total No. | 28 | 23 | 51 | 30 | 24 | 54 | 3 | 1 | 4 | 61 | 48 | 109 | — |
| % | 26 | 21 | 47 | 27 | 22 | 49 | 3 | 1 | 4 | 56 | 44 | 100 | 100 |

*M indicates male; F, female.

†Peripheral nervous system includes cranial nerves, spinal roots and peripheral nerves.

the 25 animals exposed transplacentally to the carcinogen. All animals developed one or more tumors of the nervous system. More than half of the tumors occurred in the brain, although only 25% of these were grossly visible. Two thirds of the tumors of the spinal cord and peripheral nerves were detected grossly.

Non-neural tumors comprised 6.4% of the total. This heterogenous group included one each of the following: myelogenous leukemia, ductular adenocarcinoma of the mammary gland with metastases to the lung, mesothelioma of the peritoneum, renal fibrosarcoma, arrhenoblastoma of the ovary, and a papilloma of the cardiac portion of the stomach.

#### Classification of Tumors of the Nervous System

The classification of neural tumors induced by ENU is given in Table 3. The classification of the neural tumors in this study was based upon histologic and ultrastructural features of the cells comprising the neoplasms. The frequency of cell types comprising the neoplasm ultimately determined the classification. When several clearly distinguishable cell types formed the tumor, it was named according to the predominant cell types, such as mixed glioma (oligoastrocytoma) and glioependymoma. Poorly differentiated tumors were designated as anaplastic. Oligodendrogliomas were the most common tumors, followed by mixed gliomas, anaplastic neurinomas and ependymomas. Each of the remaining tumor types represented less than 5% of the neural tumors.

The designation *early neoplastic proliferation* was applied to focal cell proliferations at sites of predilection for neoplastic development, yet

Table 3. Incidence and Classification of 102 ENU-Induced Tumors of the Nervous System.

| Type of Tumor | Number |
|---|---|
| Tumors of the central nervous system | |
| Astrocytoma | 3 |
| Oligodendroglioma | 31 |
| Mixed Glioma | 19 |
| Anaplastic Glioma | 5 |
| Anaplastic Glioependymoma | 4 |
| Anaplastic Ependyoma | 10 |
| Meningioma | 1 |
| Total | 75 |
| Tumors of the peripheral nervous system | |
| Neurinoma | 3 |
| Anaplastic Neurinoma | 24 |
| Total | 27 |

criteria commonly associated with neoplasms (such as destructive or invasive growth) were absent. Microtumors, however, possessed the basic characteristics of neoplasms, and were only distinguished from macrotumors by their size. Early neoplastic proliferations, microtumors and macrotumors were considered developmental stages of the neoplastic growth process.

### Tumors of the Central Nervous System

Rats with brain tumors were usually acutely depressed in the terminal stage of disease. The affected animals became comatose over a period of hours and died shortly thereafter. Definite sites of predilection for tumors were present in brains of rats treated with ENU. The hippocampus and adjoining periventricular tissues, subcortical white matter, basal ganglia, cerebral cortex and rarely cerebellum and medulla were involved with decreasing frequency. Three fourths of the brain tumors were microscopic in size, while the remaining brain tumors varied from 3 to 13 mm in diameter. Large tumors extended to the surface and caused displacement of midline structures. Multiple tumors were present in ENU-treated rats more often than single neoplasms. No extraneural metastases were observed.

Clinical signs of involvement of the spinal cord usually developed over a period of days to weeks. Most commonly, a unilateral posterior paresis was followed by bilateral paralysis with urine retention. Tumors occurred in most segments of the cord, but were located most frequently in the lower thoracic and lumbar regions.

*Oligodendroglioma.* Oligodendrogliomas were the most common tumors induced by ENU. The majority of these were microscopic in size

and located periventricularly and within the subcortical white matter of the hemispheres. There was a transition from focal proliferation of oligodendrocytes (Fig 1) to large, grossly visible neoplasms. Neoplastic cells were small to medium in size; they had infrequent mitoses, and the highest degree of differentiation during the initial growth phase and, in larger tumors, at the periphery of the neoplastic growth. Extension to the meninges was present in two cases. Larger tumors frequently contained small aggregates of proliferating astrocytes and had moderate degrees of vascular proliferation. In the spinal cord, most gliomas were located in the white matter and consisted of well-differentiated oligodendrogliomas. Morphologically, they resembled their counterpart in the brain.

Neoplastic oligodendrocytes were characterized ultrastructurally by a round to slightly oval electron-dense nucleus with patchy distribution of chromatin and by a distinctly demarcated cytoplasm. They were equipped moderately with cytoplasmic organelles. Profiles of rough endoplasmic reticulum were regularly present and aggregations of free ribosomes were prominent throughout the cytoplasm. Individual microtubules were observed in some cells. Thin processes evolved from the perikaryon but glial filaments were absent (Fig 2). In less differentiated cells, the nucleus was proportionally larger, the nucleolus more prominent, and the cytoplasmic rim narrower with fewer cytoplasmic organelles.

*Astrocytoma.* Tumors consisting primarily of astrocytes occurred much less frequently than oligodendrogliomas and mixed gliomas. The site of predilection for astrocytomas corresponded to that of oligodendrogliomas. Isomorphic populations of medium-sized and large cells with round-to-oval nuclei and moderately abundant cytoplasm comprised the predominant cell type. Mild-to-moderate degrees of vascular proliferation occurred in the neoplasms. The neoplastic astrocyte ultrastructurally resembled the normal astrocyte in its main features. The cells were usually large and had round, ovoid or pleomorphic nuclei with nucleoli usually prominent. The nucleus was moderately electron dense and had an even distribution of chromatin. Many large cytoplasmic processes evolved from the perikaryon. These cells were rich in cytoplasmic organelles, particularly in mitochondria and endoplasmic reticulum and often contained glial fibrils (Fig 3).

*Mixed Glioma.* Mixed gliomas consisting of a mixture of neoplastic oligodendrocytes and astrocytes were the second most common neoplasm of the CNS. They varied in size from microtumors to grossly detectable tumors, with microtumors being most common (Fig 4).

Cerebral cortex and hippocampus were affected most frequently. By using electron microscopy, the two glial cell types, oligodendrocytes and astrocytes, were detected in varying proportions and varying degrees of differentiation.

*Anaplastic Glioma.* Tumors with prominent regressive changes, a high mitotic index and cellular pleomorphism varying from immature cells to poorly differentiated astrocytes or polymorphous oligodendrocytes were classified as anaplastic gliomas. These neoplasms were usually associated with necrosis, marked vascular proliferation, hemorrhage and extensive peritumoral edema. Diffuse meningeal invasion from the subdural space of the forebrain to the cauda equina occurred in 1 rat. Upon electron microscopic examination, astrocytic as well as oligodendrocytic cells in various stages of differentation were found to be present. Some of the cells ressembled, in size and shape, neoplastic oligodendrocytes but lacked cytoplasmic organization; others resembled astrocytes but were almost devoid of glial filaments. In addition, there were highly anaplastic glial cells, characterized by large nuclei with one to two nucleoli and a rim of cytoplasm in which only a few cytoplasmic organelles and numerous free ribosomal aggregates were discernable (Fig 5). Lysosomal bodies were present in varying numbers but were most prominent in anaplastic gliomas and ependymomas.

*Anaplastic Glioependymoma.* Two tumors of the brain and two of the spinal cord contained definite areas of ependymoma and glioma. The ependymomatous areas were characterized by columns and small groups of cells that occasionally formed rosettes (Fig 6). One tumor from each location contained areas of well-differentiated neoplastic oligodendrocytes, while the remaining tumors contained pleomorphic glial cells. The mitotic index was moderately high in all tumors, but regressive changes were only evident in the neoplasms with pleomorphic glial cells. Cellular anaplasia seemed an important feature of this type of neoplasm, prompting the designation as anaplastic glioependymoma.

*Anaplastic Ependymoma.* Anaplastic ependymomas were the most common tumor of the spinal cord, but were rare in the brain. A large cerebellar tumor extending along the aqueduct was characterized by medium-sized hyperchromatic cells that formed columns, palisaded and were associated with extracellular ground substance. The tumor contained extensive necrosis and a moderate degree of vascular proliferation. In contradistinction to the ependymoma of the brain, no direct connection between tumors and the ependymal lining of the central canal was demonstrated in ependymomas of the spinal cord.

Most ependymomas involved the white matter, with compression of adjacent gray matter (Fig 7). In the light microscope, tumor cells were characterized by an oval nucleus, a rim of eosinophilic cytoplasm and numerous fine processes. They formed small aggregates or columns and were surrounded by eosinophilic extracellular ground substance. Large cysts containing similar ground substance were present in many of the tumors. Extensive vascular proliferation, forming glomerulus-like structures, surrounded most ependymomas. Neoplastic cells occasionally spread along intrathecal routes.

The ependymoma cells were characterized by a large, eccentrically located nucleus of moderate density; ultrastructural study revealed evenly distributed chromatin (Fig 8). Usually one prominent nucleolus was present. The cytoplasm was rich in rough endoplasmic reticulum, which consisted mostly of short profiles lined by ribosomes. Ribosomal rosettes were distributed densely throughout the cytoplasm. A fair number of mitochondria and lipid droplets were also present. The Golgi apparatus was prominent. Occasionally centrioles, but no cilia were observed. Cells were attached to each other, forming short cell cords. At the site of attachment, their plasma membranes followed a straight course with only occasional uncomplicated interdigitations and intercellular formation of cysts (Fig 9). Desmosomes were observed only infrequently. Free segments of the cytoplasmic membrane formed numerous small processes and villous projections interdigitating with similar processes of adjacent cells or extending freely into the extracellular space, which was filled with a fine granular precipitate.

*Meningioma.* A perisagittal tumor mass of the meninges, causing compression of the frontal lobe and olfactory bulb, occurred in 1 rat. Histologically, the tumor mass consisted of two different types of tumor. One was circumscribed, restricted to the meninges and diagnosed as a meningioma. The other was interconnected with the olfactory bulb and diffusely disseminated over the meninges. This tumor appeared to be of neuroectodermal origin but has not been classified yet. The meningioma was composed of two cell types, a slender fibroblastic component producing abundant whorls of collagen, and a small oval hyperchromatic cell with indistinct cytoplasm (Fig 10). The neoplasm was not invasive and had a low mitotic index. No psammoma bodies were observed. Ultrastructurally, the majority of the tumor cells were of the fibroblastic type. In addition, there were smaller cells with rounded nuclei and irregular cellular outlines. Processes of these cells formed interdigitating bundles and whorls similar to those described for endotheliomatous meningiomas in man (Fig 11). No basal membrane

or desmosomes were demonstrated. The cytoplasm of all cells was moderately well equipped with organelles but contained no filaments.

### Tumors of the Peripheral Nervous System

The trigeminal nerve was the most common single site for tumors of the peripheral nervous system. Head tilt, exophthalmus, impairment of facial movements and depression were the main clinical signs. Tumors of the spinal roots occurred most commonly in those roots forming the lumbosacral plexus. Clinical signs were similar to those of tumors of the spinal cord. Tumors also involved large and small peripheral nerves predominantly deriving from the brachial and lumbosacral plexuses.

*Neurinomas.* Differentiated neurinomas were not common in rats treated with ENU (Fig 12). When present, they were characterized by oval nuclei and interconnecting elongated processes. Moderate amounts of collagen and reticulin were produced. Mitoses were rare.

*Anaplastic Neurinomas.* Most neurinomas of ENU-treated rats were anaplastic (Fig 13). Even early stages of tumor development, consisting of hypercellular spinal or trigeminal nerves, contained poorly differentiated cells. Larger neurinomas were composed of small, oval, hyperchromatic cells with indistinct cytoplasm, or thin spindle-shaped cells with dark elongated nuclei. Anaplastic neurinomas commonly invaded the skull and muscle. The brain and spinal cord were frequently compressed, but not invaded by neurinomas (Fig 12). Cysts containing eosinophilic material were the most common regressive change, especially in tumors of the trigeminal nerve. In large tumors of peripheral nerves, cyst formation and hemorrhage were a prominent feature. The mitotic index of these tumors was moderate to high. The ultrastructure of the ENU-induced neurinoma was characterized by a pleomorphic cell population that had one or several of the following features. It contained a plump, roughly oval, indented nucleus with 1 to 2 nucleoli in a slightly eccentric position. The cytoplasm was moderately rich in organelles. Prominent processes were characteristically intertwined with processes of other cells (bundling). A complete basement membrane, a characteristic feature of Schwann cells, was rarely demonstrated; however, varying numbers of cells had partial basement membranes (Fig 14). Cells without a basement membrane increased with advancing anaplasia. In the majority of neurinomas, extracellular collagen fibers or their precursors were rarely observed. Occasionally, tumor cells contained axis cylinders without detectable myelination (Fig 15). While the cell of origin of the neurinoma could not be identified conclusively with the

light microscope, electron microscopy clearly indicated that poorly differentiated Schwann cells participate in the formation of the neurinoma. Whether or not mesenchymal cells are involved remains to be determined.

## Discussion

The intravenous injection of a single dose of ENU (50 mg/kg) into pregnant Sprague-Dawley rats during the twentieth day of gestation resulted in inducing neurogenic tumors in 100% of the offspring after an average survival time of 211 days (range of 85–346 days). Multiple tumors, at various stages of development, were present in most animals. Non-neural tumors comprised 6.4% of the total number of neoplasms.

When the results were compared to those of a similar experiment [5] using inbred BD IX rats inoculated intravenously with 60 mg/kg of ENU on the twentieth day of gestation, they differed in several respects. Although all 28 offspring in the earlier experiment developed tumors of the nervous system, the total number of tumors was only 77 with an average of 2.7 tumors/rat as compared to 102 tumors in 25 offspring with an average of 4.36 tumors/rat in our series. The difference is even more pronounced in regard to location of the neurogenic tumors. If only the macrotumors of our series were included in this comparison with the BD IX experiment, the percentage ratio was 31 to 12% for brain tumors, 26 to 9% for tumors of the spinal cord and 42 to 79% for tumors of the peripheral nerves. When microtumors were included, brain tumors in our series increased to 56% followed by tumors of the peripheral nerve with 26% and tumors of the spinal cord with 18%. These results suggest that Sprague-Dawley rats have a higher susceptibility to the oncogenic effect of ENU and a greater tendency to develop CNS tumors than BD IX rats under the conditions described in these experiments.

Since all conditions in these two experimental series were identical except for a slightly different dose level (50 versus 60 mg/kg), the genetic variations between these two strains of rats are the most likely explanation for the different results. This view was recently supported by Druckrey *et al* [11] who demonstrated, in 10 inbred strains of BD rats, significant differences in the susceptibility to transplacental induction of tumors with ENU.

A study of transplacental induction of tumors in Sprague-Dawley rats at an earlier gestation period is not available for comparison, but experiments with BD IX rats by Ivankovic and Druckrey [5] clearly demonstrate a decreased susceptibility during earlier stages of prenatal development. When BD IX rats were inoculated with 60 mg/kg ENU on the fifteenth

day of gestation, instead of the twentieth day, the total number of tumors decreased from 2.7 to 1.5 tumors/animal. Comparing the distribution of tumors obtained by transplacental induction at the fifteenth day of gestation with that of the twentieth day of gestation, the most obvious difference was the higher proportion of brain tumors in the group inoculated on the fifteenth day. Development and functional stages of various parts of the nervous system may play a role in the susceptibility to the oncogenic effects of chemical carcinogens but the true nature of this range of susceptibility is not known.

By examining multiple sections from each animal, various stages of tumor development, from early neoplastic proliferation to microtumors and, finally, to grossly detectable neoplasms, were evident. Most of these were located near the lateral ventricle, suggesting the subependymal region as site of origin. Since it is known that carcinogenic *N*-nitroso compounds are metabolized rapidly and the induction phase is completed within hours after exposure to the compound, it was surprising to find tumors in varying stages of development after a single injection of ENU. This may be explained by variations in the onset of neoplastic proliferation or by different rates of growth between tumors. Support for the latter mechanism included the high mitotic index and cellular pleomorphism in the majority of macrotumors compared to the isomorphic character and low mitotic rate of microtumors.

Although the number and distribution of various types of neurogenic tumors varied with the stage of prenatal development during which they were induced, the basic morphologic characteristics of the tumor types in transplacentally induced neoplasms at different stages of gestation were similar.[5,10,12] The preliminary classification proposed by previous workers [5,9,10,12] for neurogenic tumors transplacentally induced with ENU was considered acceptable and adopted with only slight terminologic modifications. The CNS tumors in this experiment were either gliomas or ependymomas or mixtures of both (glioependymomas). Since anaplasia was an important feature, particularly of advanced tumor growth, this expression was used to designate the immature characteristics of such tumors of the central and peripheral nervous system. The term glioblastoma was not included in our classification and glioblastoma-like tumors were classified as anaplastic gliomas. This classification avoids using precommitted terms of human neuro-oncology.

The classification was based on the morphologic features of the predominant cell type (or types) comprising the neoplasm. Electron microscopy proved to be an important aid in tracing the neoplasms to their cell of origin. Of the transplacentally induced neurogenic tumors, the

neurinomas have probably been most thoroughly investigated ultrastructurally.[8,10] In those studies, tumor cells were described that, in more differentiated neurinomas, maintained characteristic features of the Schwann cell such as a basement membrane and multiple interdigitating processes. Differentiated neurinomas are, however, more commonly produced in adult rats with methylnitrosourea.[13,14] In our experiments, the majority of neurinomas were of the anaplastic type. In spite of their anaplasia, the presence of at least a partial basement membrane and interdigitating processes, and the occasional incorporation of axons suggested a poorly differentiated Schwann cell as the prevalent cell of the anaplastic neurinoma.

The anaplastic ependymoma of brain and spinal cord was composed of cells retaining few characteristics of mature ependymal cells. Most cells had a marginated nucleus, multiple processes, and villous projections occasionally resembling microvilli. They formed cell cords with scattered desmosomes between adjacent cells. The presence of occasional centrioles and the lack of cilia correspond to similar findings by Wechsler *et al.*[10] The high degree of anaplasia makes this type of neoplasm the most difficult to classify.

Neoplastic astrocytes and oligodendrocytes in various stages of differentiation were demonstrated electron microscopically in ENU-induced gliomas. As the gliomas grew in size, the number of anaplastic cells generally increased and tumors were then classified as anaplastic gliomas or glioependymomas. Medulloblastomas or mixed tumors composed of gliomatous and sarcomatous elements, as observed in a few instances by Ivankovic and Druckrey,[5] were not demonstrated in this study. There were also no indications of neoplastic growth of neuronal elements comparable to gangliocytomas or neuroblastomas.

Meningeal tumors were only rarely reported in association with experiments using *N*-nitroso compounds as carcinogenic agents. In one summary report comprising 952 tumors,[9] only 0.7% were of meningeal origin and in another report,[12] 1.8% of 1.731 tumors involved the meninges. These meningeal tumors were classified as meningeal sarcomas. In contrast, the tumor described in this study contained structural and cytologic features characteristic of a fast-growing meningioma; however, the possibility that this tumor may be spontaneous and unrelated to the treament can not be excluded. Jänisch and Schreiber [15] reached a similar conclusion for the 2 meningiomas they observed among 1432 tumors experimentally induced in adult rats with methylnitrosourea.

When the possibility is considered that, under natural conditions, brain tumors may be the result of prenatal exposure to a carcinogen, the

ENU model seems ideally suited for the study of various parameters of neoplastic transformation of neuroectodermal cells. A single injection of the compound induces, under optimal conditions, neurogenic tumors in 100% of the offspring. Different tumor types occur mostly at predictable locations in the nervous system.

## Summary

Three Sprague-Dawley rats were inoculated intravenously with a single dose of 50 mg/kg of ethylnitrosourea on the twentieth day of gestation. All 25 offspring developed tumors of the nervous system. A total of 102 neural tumors and 7 non-neural tumors were produced. The number of tumors/animal averaged 4.36. The survival time ranged from 85 to 346 days (with a mean of 211 days) after the carcinogen was injected.

The tumors were classified on the basis of light and electron microscopy into the following groups: 32 oligodendrogliomas, 4 astrocytomas, 19 mixed gliomas, 5 anaplastic gliomas, 4 glioependymomas, 10 ependymomas, 1 meningioma and 27 neurinomas (3 differentiated and 24 anaplastic). The electron microscopic examination proved to be a valuable aid in tracing neurogenic tumors to their cells of origin.

Sites of predilection for tumors of the central nervous system were the cerebral hemispheres, particularly the periventricular areas of the lateral ventricles, and the white matter of the lower thoracic and lumbar cord. Tumors of the peripheral nervous system were most frequently located in the trigeminal nerve and the spinal roots forming the lumbosacral plexus. The majority of gliomas were well differentiated, while the neurinomas and ependymomas were anaplastic.

This experiment demonstrated that a single transplacental exposure to a neuro-oncogenic compound is capable of inducing neuroectodermal tumors in all offspring. When compared with similar transplacental experiments using different strains of rats, the Sprague-Dawley rat was shown to be highly susceptible to the oncogenic effect of ENU during the terminal stage of intrauterine development.

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Dr. Wechsler is visiting professor in this department. His home address is the Max-Planck-Institute für Hirnforschung, 5 Köln-Merheim, Ostmerheimerstr. 200, Germany.

## Legends for Figures

**Fig 1.**—Early neoplastic proliferation of oligodendrocytes in packets and in satellite position around neurons (S) in cerebral cortex. (H&E, × 294).

**Fig 2.**—Neoplastic oligodendrocyte with eccentrically located, moderately electron-dense nucleus, a fair number of organelles and a few fine processes (× 9000).

**Fig 3.**—Many glial filaments (*f*) comprise large portion of cytoplasm of neoplastic astrocyte. Note large number of cytoplasmic organelles (× 8500).

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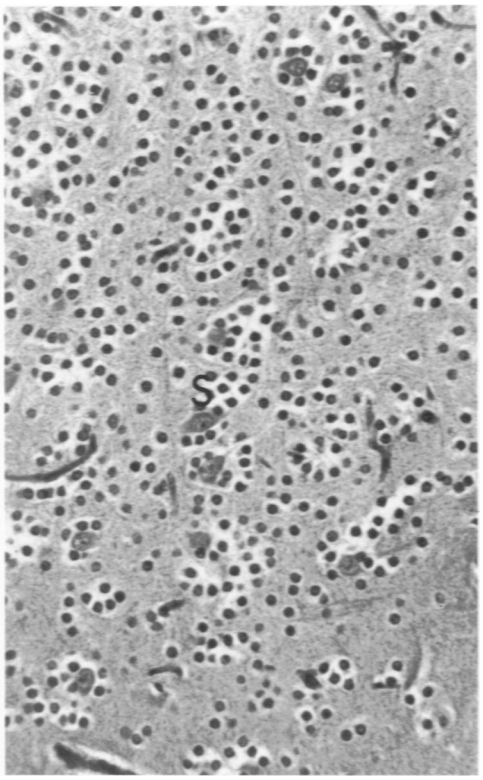


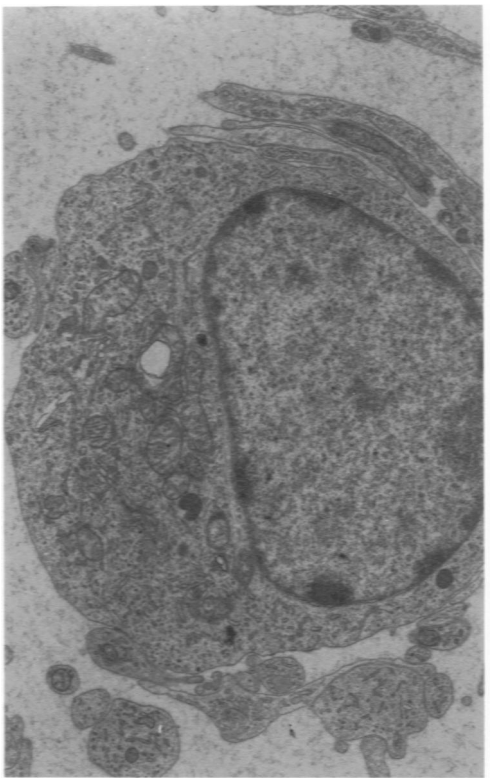

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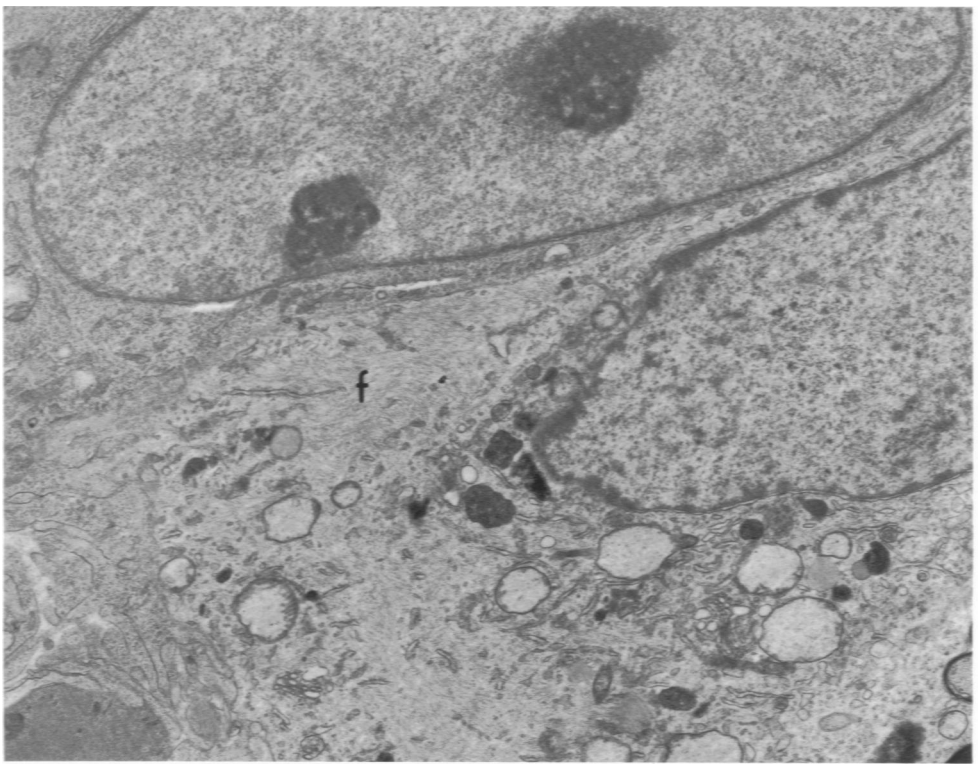


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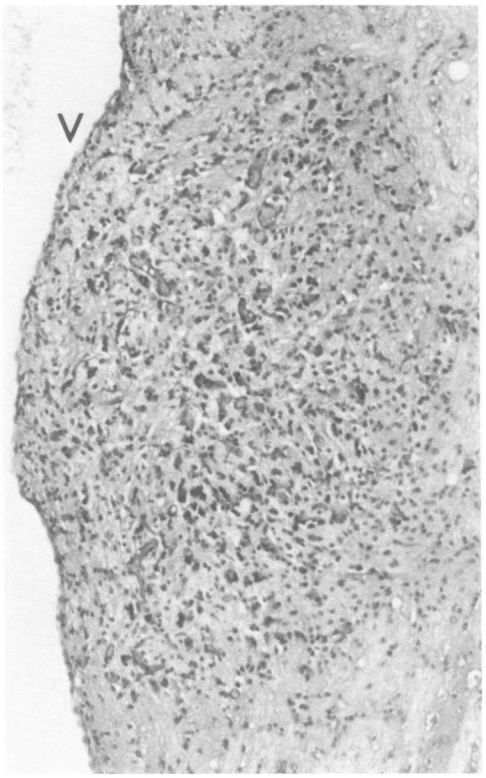


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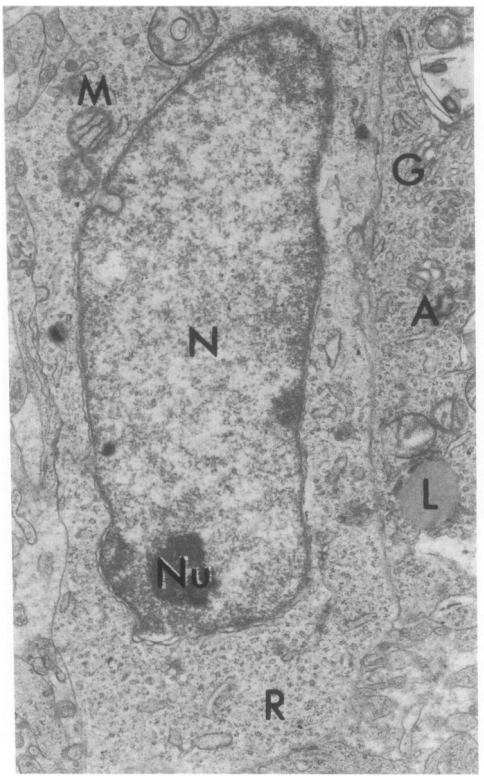


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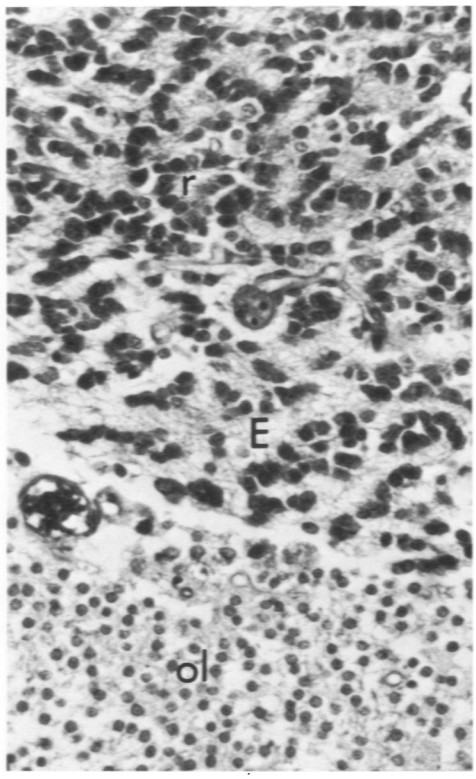
**Fig 4.**—Mixed glioma microtumor adjacent to lateral ventricle (*V*) (H&E, × 115).

**Fig 5.**—Anaplastic glial cell next to protoplasmic astrocyte (*A*). Note poor organization of cytoplasm, with few organelles and numerous free ribosomal aggregates (*R*). *G* indicates Golgi apparatus; *N*, nucleus; *Nu*, nucleolus; *L*, lipid droplets; *M*, mitochondria (× 15,500).

**Fig 6.**—Cerebellar glioependymoma consisting of oligodendroglial (*ol*) and ependymal (*E*) regions. Neoplastic ependyma cells form cords and rosettes (*r*) (H&E, × 292).

**Fig 7.**—Anaplastic ependymoma of spinal cord containing cysts (*C*) and many blood vessels (*BV*) (H&E, × 45).

**Fig 8.**—Anaplastic ependymoma cells with large oval nuclei, a thin rim of cytoplasm and many fine processes (*P*) (× 10,220).

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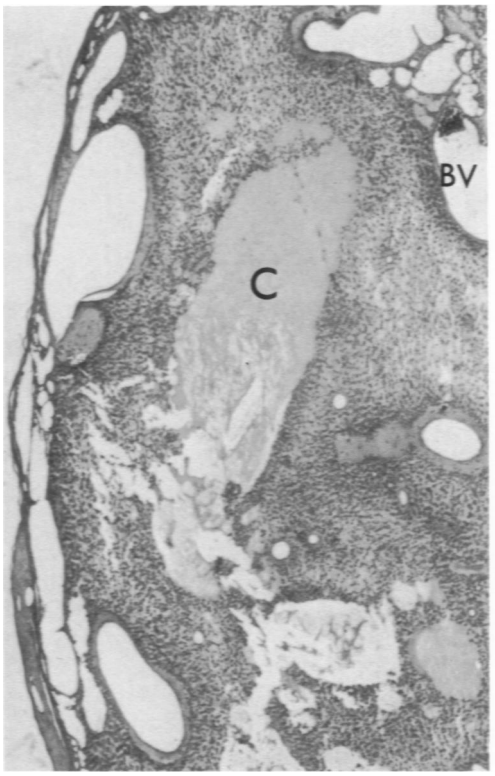


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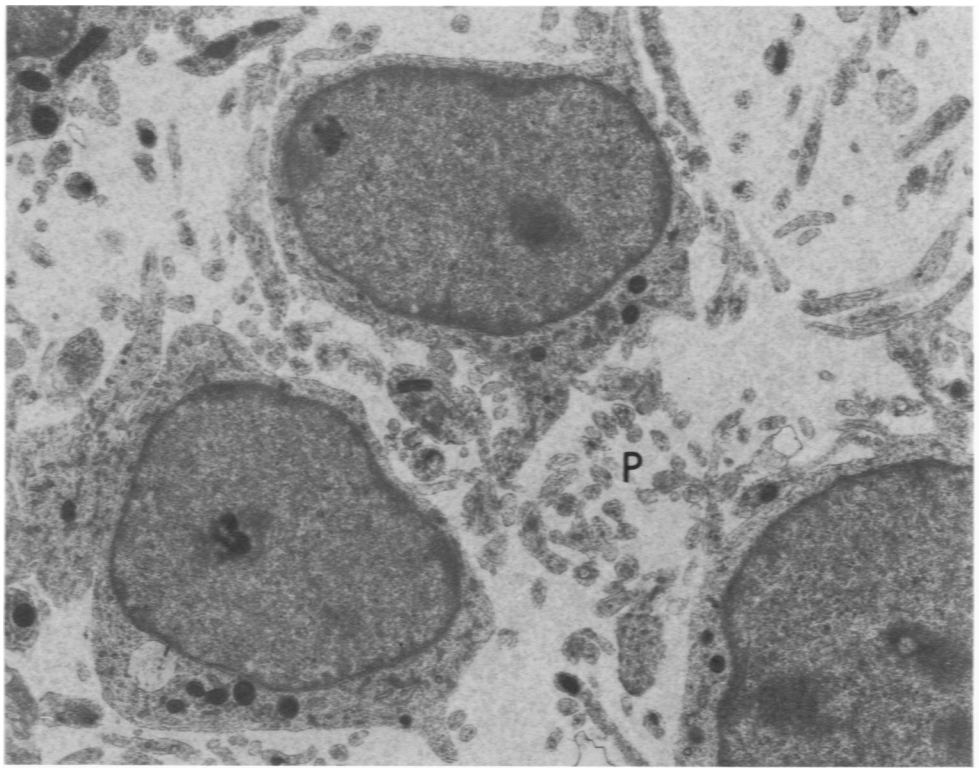


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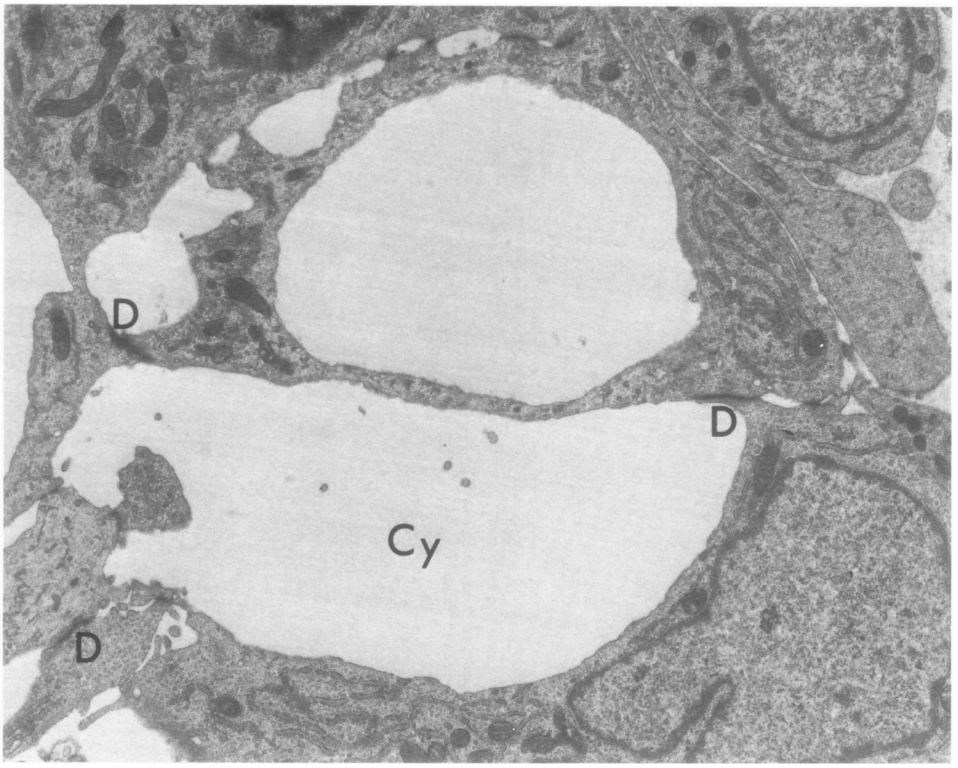


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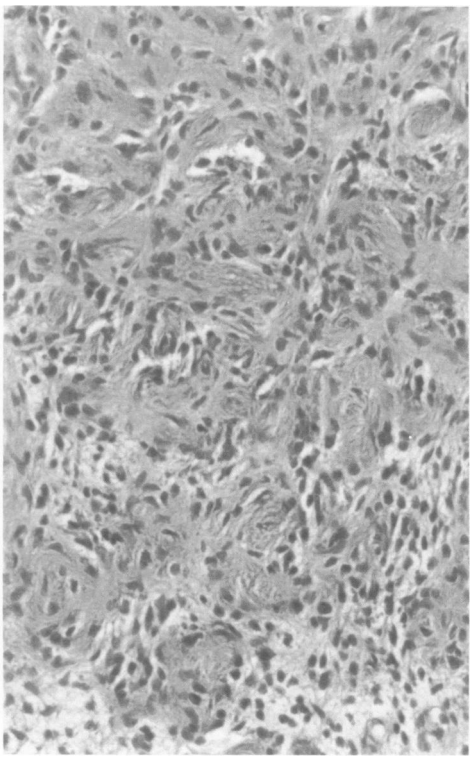

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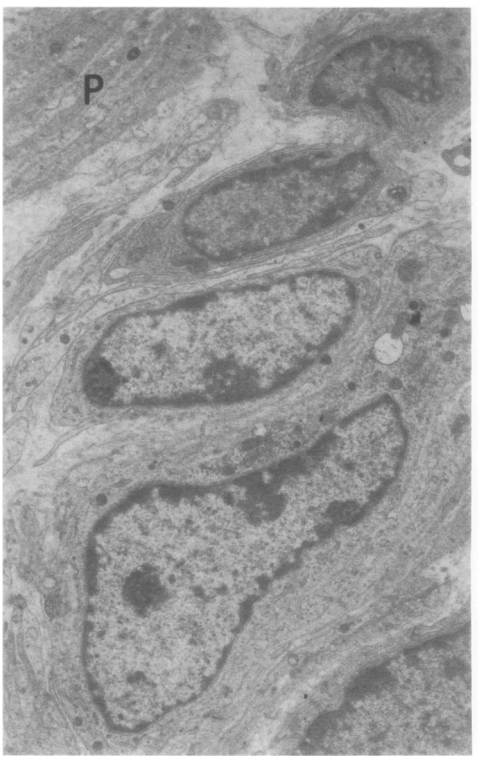


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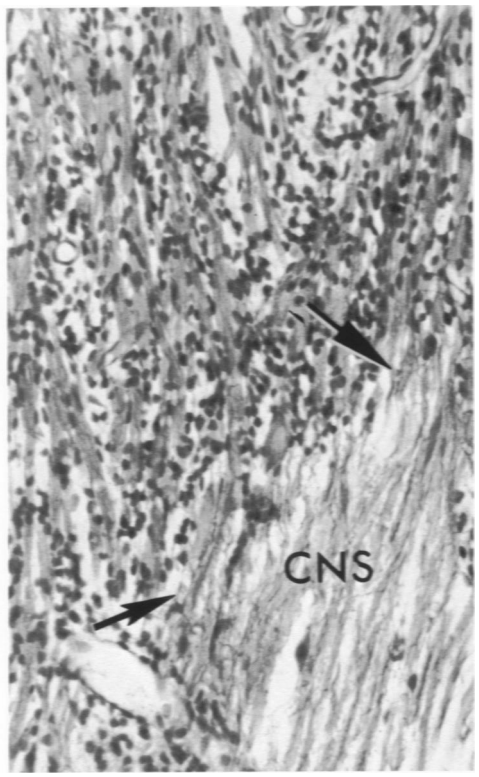
**Fig 9.**—Prominent desmosomes (*D*) connect several neoplastic ependymal cells forming microcysts (*Cy*) (× 11,300). **Fig 10.**—Meningeal neoplasm composed of small spindle cells wrapped around each other, forming whorls. Cytoplasmic border is outlined indistinctly (H&E, × 286). **Fig 11.**—Ultrastructure of meningeal tumor suggestive of meningioma with characteristics of fibromatosis and endotheliomatous cellular elements. Cells depicted show fibromatous features while processes in upper left corner (*P*) show bundle formations characteristic of endotheliomatous cells (× 7000).

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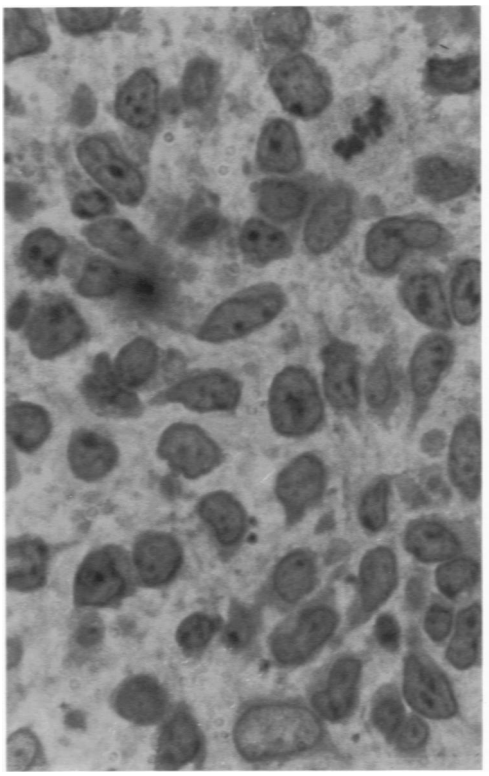

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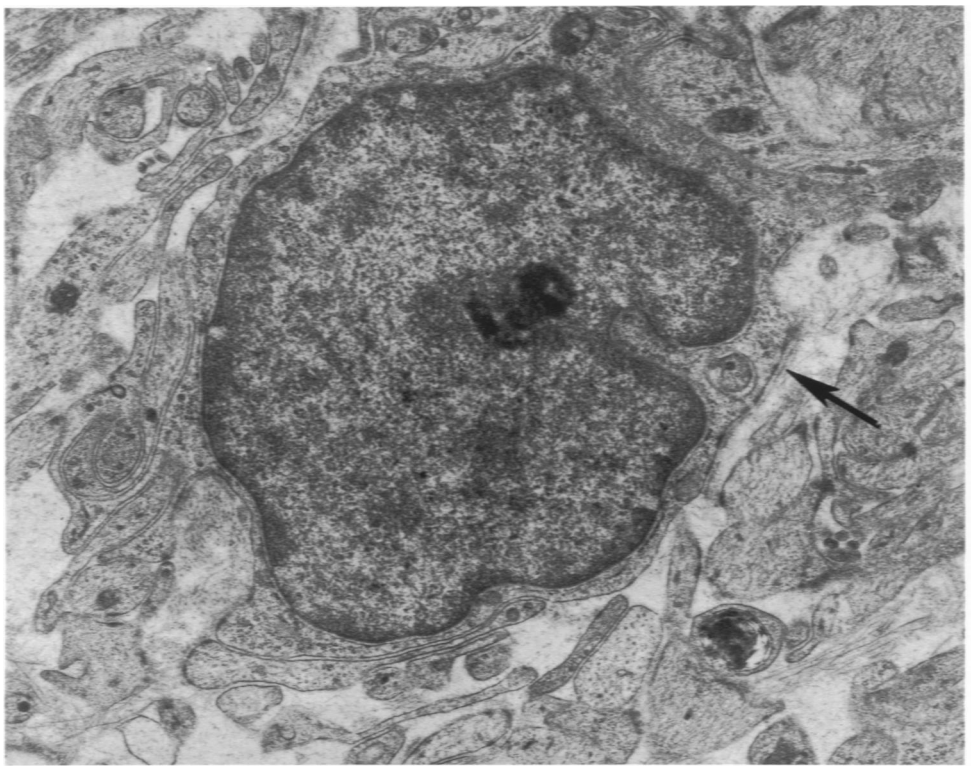

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**Fig 12.**—Neoplastic Schwann cells have invaded trigeminal nerve up to its junction with brain (*arrows*). CNS invasion is restricted to perivascular spaces (H&E, × 286). **Fig 13.**—Anaplastic neurinoma consisting of poorly differentiated pleomorphic cell population (toluidine blue, × 1168). **Fig 14.**—Short segment of basement membrane (*arrow*) is opposed to neoplastic Schwann cell. Note intertwining processes (× 20,122).

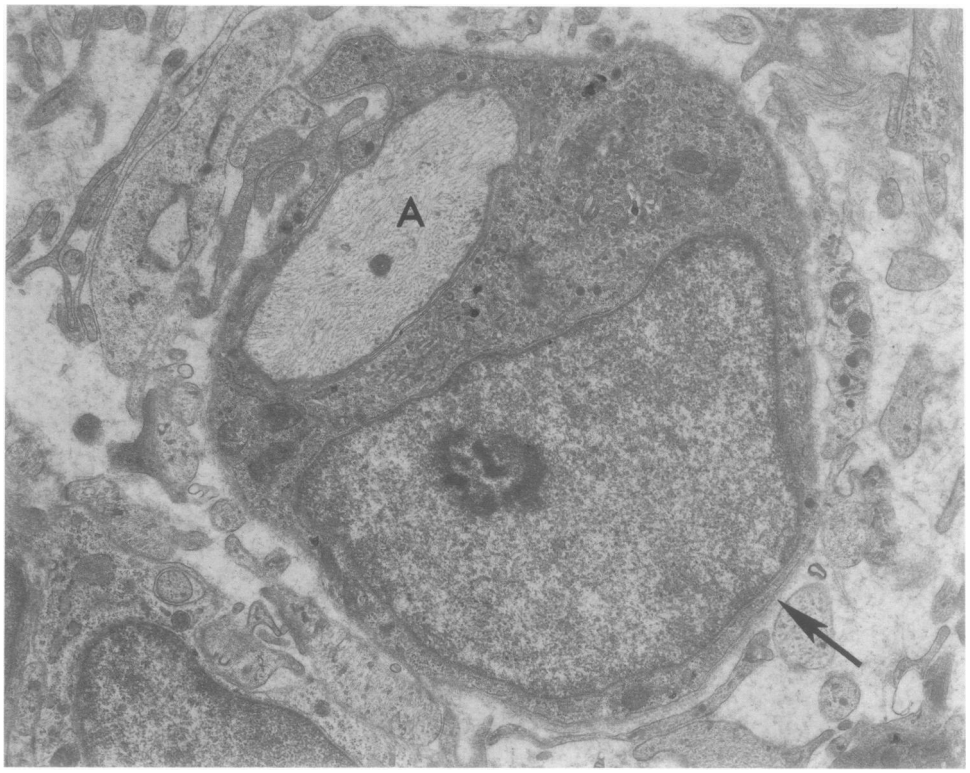


**Fig 15.**—Poorly differentiated Schwann cell has incorporated nonmyelinated axon (*A*) and has partial basement membrane (*arrow*) (× 16,600).