An Experimental Mouse Testicular Teratoma as a Model for Neuroepithelial Neoplasia and Differentiation

I. Light Microscopic and Tissue and Organ Culture Observations

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The various stages of divergent neuroepithelial differentiation were studied in the solid transplants of a transplantable mouse testicular teratoma (OTT-6050) maintained in both ascitic and solid forms. They included: a) areas of undifferentiated medullary epithelium corresponding to the rare human medulloepithelioma; b) areas of neuroblastic differentiation corresponding to neuroblastoma, with more mature neuronal differentiation corresponding to ganglioneuroma or, when mixed with glial elements, to ganglioglioma; and c) more mature neuroglial areas resembling astrocytoma, oligodendroglioma or ependymoma, as well as more primitive areas corresponding to ependymoblastoma. In tissue culture using collagen-coated coverslips, astrocytic differentiation was found in the outgrowth zone after 15 days, confirmed by immunofluorescence with antibodies to an astroglia-specific protein. In organ culture systems, glial components, including ependymal structures, were preserved in tumor explants, and astrocytic differentiation, as expressed by glial fiber formation, was increased after 4 to 6 weeks *in vitro*. No neuronal differentiation was demonstrable, however. The neuroepithelial component of this experimental teratoma may provide a model for the study of neoplastic neuroepithelial differentiation. (Am J Pathol 79:147–168, 1975)

THE NERVOUS SYSTEM is the second most frequent site of primary neoplasia in children, and during the first decade of life primitive tumors of neural origin are particularly likely to occur. The embryonal tumors originating within the central nervous system (CNS) are especially complex in their identification and classification.[1] They are of special interest because they can morphologically be equated to distinct

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stages of CNS differentiation and because they may share some of the capabilities for divergent differentiation that are normally displayed in neural morphogenesis.[1,2] These capabilities, which are identifiable by specific morphologic and biochemical characteristics, could be analyzed with precision if a suitable experimental model were devised.

Numerous experimental CNS tumors have been produced by chemical carcinogens such as the resorptive *N*-nitroso alkylating agents, and by oncogenic viruses.[3–5] These animal tumors are usually comparable to human gliomas composed of adult cell types. A small number, induced by the cerebral inoculation of human adenovirus type 12, display features characteristic of an embryonal tumor.[6,7] An alternative experimental model for the study of neurooncogenesis is the neuroepithelial component of a differentiating transplantable mouse testicular teratoma. Malignant testicular teratomas that are both multipotential and transplantable may arise spontaneously in specific inbred strains of mice,[8] and their incidence can be increased by genetic and environmental factors.[9] Embryos grafted into the testis or beneath the renal capsule of adult mice of syngeneic strains before the critical stage of mesoderm formation produce similar transplantable teratomas.[10,11] Divergent differentiation from primitive stem cells into derivatives of the three classic germ layers occurs in these tumors and includes partial neural differentiation.

In the present study, detailed histologic investigations were undertaken on the ascitic and solid forms of the OTT-6050 line of transplantable mouse teratoma [10] to determine the range of differentiation of tissues derived from the classic germ layers. Tissue and organ culture technics were used to determine whether they would increase neuroepithelial differentiation; the methods included media [12,13] and systems[13,14] that have previously been found to favor this direction of differentiation. The presence in the tumors of glial fibrillary acidic (GFA) protein, a biochemical marker specific for fibrillary astrocytes,[15–17] was tested by immunofluorescence *in vivo* and *in vitro*. The electron microscopic features of the same material will be described in a subsequent report.

Materials and Methods

Teratoma Line

The line OTT-6050 of transplantable mouse teratoma was produced by Dr. Leroy C. Stevens, at the Jackson Laboratory, Bar Harbor, Me, from an intratesticular implant of a 6-day-old 129/Sv embryo into the testis of an adult F_1(A/HE × 129)

host in February, 1967 [10] and had subsequently been maintained at the Jackson Laboratory in ascitic form. Following its receipt from Dr. Stevens, the tumor has been maintained in our laboratory in both ascitic and solid forms for 24 transplant generations.

Animal Transplantation

Syngeneic hosts were 5- to 8-week-old 129/J female mice.

Ascitic Form

Two to 3 ml of sterile isotonic saline was injected intraperitoneally in the donor mouse, followed by immediate aspiration of approximately 1½ ml of tumor-containing ascitic fluid; approximately 0.3 to 0.4 ml of this fluid was injected intraperitoneally into a new host. After 4 to 5 weeks, abdominal distension developed, at which time the ascitic tumor line was transplanted again.

Solid Form

Implants from the ascitic tumor fluid which grew in the peritoneum and abdominal subcutaneous tissue were used initially to develop the solid transplantable form. For serial transplantation, tumors measuring approximately 1.5 cm in their longest dimension (or a volume of about 0.8 to 0.9 cu cm) were removed aseptically, rinsed in sterile isotonic saline, and cut into 1-mm cubes. Six to eight pieces were implanted subcutaneously into each flank of a new host, using an 18-gauge, 1½ inch trocar and were maintained by sequential flank transfers every 17 to 24 days. Palpable tumors were usually present after 14 days.

All tissue implantations and injections were aseptically performed. No antibiotics were used so as not to influence tumor growth or differentiation.

Tissue and Organ Culture Technics

Both ascitic and solid forms were cultured according to methods previously reported.[13,14,18] Briefly, small fragments of tumor were maintained in organ culture systems using three-dimensional sponge foam matrices and Millipore filter platforms, and separate explants were grown on collagen-coated coverslips to study their monolayer outgrowths. The standard medium for explantation and feeding was the same as for human and animal gliomas [13,14,18] except for the antibiotics. For the primary explantation and during the first 2 weeks of feeding, penicillin G (50 units/ml medium) and Achromycin® (tetracycline HCl, 20 μg/ml medium) were added; subsequent feeding medium contained no antibiotics. A second medium also known to favor neuroglial differentiation in monolayer culture [12] was modified as follows: unfiltered, heat-inactivated calf serum (56 C, 30 minutes) (Reheis Chemical Co, Chicago, Ill), 32.2% minimal essential medium (Eagle) with Earle's balanced salt solution (Grand Island Biological Co, Grand Island, NY), 46.4%; Simms' balanced salt solution, 2.8%; chick embryo extract, 9%; dextrose, 7 mg/ml; L-glutamine, 4.0 mM; and insulin, 0.09 units/ml (low zinc insulin, courtesy of Squibb Institute for Medical Research, New Brunswick, NJ). The chick embryo extract was prepared with PPLO-free, pathogen-free 9-day chick embryos and diluted 1:1 by volume with Simms' balanced salt solution. Penicillin G and Achromycin were added in the same concentrations and for the same time periods as in the standard nutrient medium.

Histologic Methods

All transplanted animals were routinely autopsied. Centrifuged (500g for 10 minutes) ascitic fluid and samples of intraperitoneal and serially passaged subcu-

taneous tumor implants were fixed in phosphate-buffered 10% formalin for histology. Stains included hematoxylin and eosin; Mallory's phosphotungstic acid-hematoxylin (PTAH); Weigert's iron-hematoxylin and van Gieson; Gordon-Sweets' silver method for reticulin; periodic acid-Schiff reaction (with and without diastase); cresyl echt violet or toludine blue-borax for Nissl substance; and Lapham's phloxine-fast green-gallocyanin (PFG) for myelin and glial fibers.[19] Frozen sections at 10 to 12 μ of similarly fixed tissue were impregnated by Bielschowsky's silver method for axons.

The histologic processing technics of explants in tissue and organ cultures systems were as previously reported.[13]

Immunofluorescence Studies

Antisera against GFA protein previously produced in New Zealand albino rabbits were used.[17,20] The antisera were routinely absorbed with human serum (4:1, v/v) to remove anti-human IgA and IgG antibodies. Cryostat sections of fresh tumor and of tumor grown on sponge foam were cut at 4 to 6 μ and desiccated at 4 C for 24 to 48 hours. Saline suspensions of ascitic fluid were filtered through a nucleopore filter (8 μ diameter pore size, General Electric) and the filter attached to a glass slide with metal clips. Collagen-coated coverslips on which explants of the tumor had been grown were attached to glass slides with similar clips.

The anti-GFA protein fluorescent antibody test was carried out according to Coons' indirect procedure, as previously described.[16] Control sections were incubated with rabbit preimmunization serum. GFA protein antisera and preimmunization sera were used in 1:50 dilutions; the fluorescein-labeled goat anti-rabbit γ-globulin serum was used in 1:20 dilutions. Two fluorescent microscopes were used: an American Optical (AO) series 10 Microstar microscope with an AO Fluorome illuminator (Model 645) containing an HBO 200-watt Mercury vapor lamp, a Schott BG 12 primary exciter filter and a Schott OG1 secondary (barrier) filter, and a Zeiss fluorescent microscope with a BG12 excitor filter and either a 65 or a 50 Zeiss barrier filter.

Results

Gross Findings

Mice injected with ascitic tumor fluid developed numerous solid implants, measuring from 2 to 20 mm in their longest dimension, on all peritoneal surfaces. Hepatic and splenic serosal implants were consistently present, and retroperitoneal renal implants were frequent. Extensive nodular mesenteric implants on the lesser omentum often produced obstructive jaundice as a late complication. Phrenic implants were less frequent and did not invade the thoracic cavity. There was no lymphatic metastatic spread to the thoracic cavity or to any other extraperitoneal site.

Solid tumors in the anterior abdominal subcutaneous space, resulting from the subcutaneous injection of ascitic tumor fluid, and solid, serially transplanted flank tumors seldom infiltrated the adjacent muscle and never the peritoneal cavity. Skin ulceration sometimes occurred when

the tumors were allowed to kill the host. Metastases were never found with the transplanted solid flank tumors.

Grossly, the tumors were lobulated but not encapsulated. Their outer surfaces were gray-white and mottled, with red-gray foci. On sectioning, they were frequently cystic, with variable areas of necrosis. Necrosis was always present in tumors greater than 15 mm in their greatest dimension. Ten to 50% of the cut surfaces contained hemorrhagic foci. The tumor was divided into gray-white lobular nests mottled with tan-red foci, interrupted by scanty strands of fibrous connective tissue.

Microscopic Observations

The *ascitic fluid* contained cells arranged in two types of structure: embryoid bodies and sheets of larger, compactly arranged polygonal cells. The embryoid bodies, which ranged from 30 to 540 μ in their largest dimension, consisted of a central core of compact cells often surrounding microcystic spaces and an outer lining of epithelial cells. The overall architecture of the embryoid bodies recalled the blastocystic stage of the mouse embryo, with its typical inversion of the primary germ layers, as described by Stevens.[21] The cells in the central core had large round or oval nuclei with reticulated chromatin, a prominent nucleolus and a well-defined nuclear membrane. Their cytoplasm was scanty, occasionally with stout processes. The cells forming the outer epithelial border had similar nuclei, but their cytoplasm had an apical brush border.

Larger cells were organized in compact sheets and clumps of varying sizes, without a distinctive pattern. They were less numerous than the embryoid bodies. They were uniform and characterized by plump irregular hyperchromatic nuclei, an abundant eosinophilic cytoplasm and polygonal cytoplasmic borders. Mitotic figures were frequent. There was no evidence of cellular differentiation with routine histologic stains.

The *solid tumors that developed from ascitic tumor fluid* were similar to the spontaneous transplantable murine teratomas [22,23] and the experimental transplantable mouse testicular teratomas described by others.[8,11,24] Tissues derived from the three classic germ layers were intermixed without definite organization. Epithelial derivatives were represented by stratified squamous epithelium lining spaces and containing keratohyaline granules, by hair follicles, and by tubules or ducts lined with cuboidal and low columnar epithelium which was often ciliated or had a brush border. Glandular differentiation consisted of acini, often composed of tall columnar cells associated with mucus-secreting goblet cells. Primitive mesonephric tissue was often identified. Meso-

dermal tissue was represented by primitive mesenchymal whorls, with focal differentiation into cartilage, bone, smooth, or striated muscle. Differentiation involving derivatives of two or more germ layers in an organoid relationship, such as choroid plexus (Figure 1), peripheral ganglia (Figure 2) or intestinal villi, also occurred. Tissue showing divergent neuroepithelial differentiation was a constant finding and will be described in detail below.

Another consistent finding was the presence of highly cellular foci of undifferentiated epithelial like cells (stem cells). They were similar to the central cores of the embryoid bodies described above and to previous descriptions of the so-called embryonal cells or embryonal carcinoma.[24–27] These foci, which contained mitotic figures, were probably derived from embryoid bodies that implanted on peritoneal surfaces to form solid tumors and subsequently sloughed off these tumors into the peritoneum.[25] Previous reports have demonstrated that the undifferentiated cells (stem cells) are the primordial cells from which all other tissues in the tumor arise.[21,24–26,28–30]

Neuroepithelial Differentiation

The solid tumors contained a high percentage of neuroepithelial cells. Serial sections of one entire transplanted tumor at 5-μ intervals demonstrated that approximately 80 to 90% of the cells belonged to this category. Four main patterns of neuroepithelial differentiation, which often merged into one another and which spanned the full range from embryonal to mature cells of both glial and neuronal lineage, were present as follows.

a) Foci of stratified low columnar or cuboidal epithelial-like cells were arranged in papillary and tubular formations and formed multilayered bands delineated by a well-defined internal, or luminal, limiting membrane and by a similarly well-defined external limiting membrane (Figures 3 and 4). These areas closely resemble the medulloepithelioma, a rare but well-characterized multipotential embryonal CNS tumor in man.[31–33] The cells had usually elongated pale nuclei containing a very delicate punctate chromatin network; mitotic figures were frequent and were invariably located adjacent to the internal limiting membrane (Figure 4). The lumens bordered by the internal limiting membrane were usually elongated, in the shape of canals, but occasionally circular, in the shape of rosettes (*primitive medullary rosettes*). Occasionally, pigmented single-layered columnar epithelium was present in continuity with the papillary and rosette formations.

b) Highly cellular foci of small darkly staining cells, with round or

slightly oval nuclei containing dense chromatin nodes, frequently surrounded well-defined elongated or circular canals. These structures were similar to ependymal rosettes in that the juxtaposed columnar cells had delicate but well-defined internal borders at their apices, but were frequently surrounded by multiple cell layers and often displayed one or more mitotic figures in a juxtaluminal position (Figure 5). These rosettes and tubules, which were very frequent, were similar to those seen in the rare human ependymoblastoma [34] (*ependymoblastomatous rosettes*).

c) Areas of elongated uni- and bipolar primitive neuroglial cells often showed delicate PTAH-positive processes, similar to those found in human glioblastomas and, frequently, in human medulloblastomas. These areas merged with zones containing more mature neuroglia (Figures 6 and 7), including protoplasmic and, to a lesser extent, fibrillary astrocytes (Figure 7), and oligodendrocytes, recognizable by their darkly staining nuclei surrounded by a perinuclear halo (Figure 6). In these zones, mature ependymal rosettes, similar to those frequently seen in ectopic ependymal cell nests in the normal human central nervous system and in benign ependymomas, were occasionally present. These rosettes showed a well-defined internal lumen, demonstrated variable numbers of cilia and blepharoplasts, were surrounded by one or occasionally two layers of cells, and were devoid of mitotic figures (*ependymal rosettes*) (Figure 6). The presence of fibrillary astrocytes lying singly or in clusters throughout the tumor was confirmed by immunofluorescence with GFA protein antiserum (Figure 8), the fluorescence often being most pronounced near the connective tissue stroma and adjacent to the blood vessel walls.

d) Various stages of ganglionic differentiation were present. These areas showed gradual transitions to a less densely cellular fibrillary neuropil in which progressive maturation to ganglion cells was evident, culminating in the formation of mature neurons containing Nissl substance (Figures 9, inset, and 10). The areas also included small round pale nuclei with a delicate chromatin network and small nucleoli, interpreted as neuroblasts, occasionally arranged in well-defined rosettes without a lumen but with a central delicate eosinophilic fibrillary matrix (*neuroblastic rosettes*) (Figure 9). Mitotic figures were usually not found in these foci. The Bielschowsky silver impregnation technic showed numerous delicate axons coursing through the ganglionic areas (Figures 11 and 12). Axonal processes were occasionally demonstrated to originate from the cytoplasm of one of the cells (Figure 12, inset). No myelin sheaths were found.

In summary, during the 24 transplant generations that the tumor was

maintained in this laboratory, reproducible ascitic and solid tumors were obtained. Serial sections of one subcutaneously transplanted tumor demonstrated predominantly neuroepithelial elements in all stages of differentiation.

Tissue and Organ Culture Studies

Using the standard medium, the embryoid bodies *from ascitic fluid* showed limited attachment and growth on collagen-coated and uncoated glass coverslips, and were maintained up to 17 days *in vitro*. The attached embryoid bodies became compact masses of polygonal cells whose size and morphology were similar to the core cells of the embryoid bodies and to the undifferentiated stem cells of the solid tumors. No subsequent differentiation was noted. The embryoid bodies did not survive in the modified medium or when cultured on gelfoam or Millipore filter platforms.

The serially transplanted *solid* tumors were successfully maintained, using the standard medium, on collagen-coated coverslips up to 62 days, on uncoated coverslips up to 24 days, on Millipore filter platforms up to 48 days, and on sponge foam up to 68 days *in vitro*. With the modified medium on collagen-coated coverslips, they were maintained up to 27 days. On coated coverslips, migration of the peripheral outgrowth zone occurred within 7 days and was extensive by 13 to 20 days. There was some variation in the extent of migration and in the architecture of the outgrowth zone, but two types of cellular arrangements characterized this zone. In the first type, fibroblasts predominated, identified by their polygonal or spindle shape, large pale vesicular nuclei, and single or double nucleoli. They were arranged in groups or in random webworks extending from the explant. In the second type, larger polygonal cells resembling stem cells formed compact monolayer sheets. Occasionally, stellate cells resembling astrocytes, with delicate sometimes branching processes and denser staining nuclei, were present in the less cellular fields adjacent to these monolayer sheets and were particularly luxuriant in cultures maintained in modified medium (Figure 13). Positive immunofluorescence with GFA protein antiserum was present in the processes of these cells after 11 and 27 days in both the standard and modified media (Figures 14 and 15). Scattered immunofluorescent cells were also present in the central explant. On uncoated coverslips, migration of the advancing edge of the outgrowth zone occurred within 9 days but was less extensive than on coated coverslips. No cells resembling neuroglia were present in these cultures.

Cultures on sponge foam matrices were best developed after 20 to 27

days *in vitro*. Single or small groups of cells invaded the sponge foam matrix within 12 to 16 days. The histologic pattern of multiple tissue elements found in the original tumor was usually well maintained. However, preservation of neuroepithelial elements was limited to neuroglial structures only (Figures 16–18). Ependymal rosettes were present (Figure 16). Increased glial fiber thickness and density was noted after 4 to 6 weeks, dependent on the degree of astrocytic differentiation in the original tissue (Figure 17). Positive immunofluorescence for GFA protein was demonstrated in such explants after 13 days *in vitro*.

In addition, nests of undifferentiated stem cells (Figure 18) and epithelial structures derived from ectoderm and endoderm were present in the organ cultures. Maintenance of mesodermal differentiation was chiefly limited to the presence of primitive mesenchyme, which occasionally showed early chondrogenic differentiation.

Single explants maintained for 15 or 19 days on gelfoam were subsequently transplanted intraperitoneally and subcutaneously into the flanks of syngeneic mice. Solid tumors in recipient animals developed 4 to 16 weeks later. They demonstrated the wide range of somatic differentiation characteristic of the other solid tumors of the line.

Discussion

Neural differentiation is a frequent finding in spontaneously occurring as well as in experimentally induced teratomas. The phenomenon has been noted in over 75% of cases in man [35] and in over 50% of gonadal teratomas occurring spontaneously in those inbred mouse strains with a normally low incidence of teratomas.[22,36–39] In mouse strains (LT and 129) with a high incidence of teratomas,[9,40] as well as in experimentally induced testicular [10,41] and renal subcapsular [11] teratomas, the neuroepithelial component often predominates; this high incidence is maintained in their derived transplants. In transplantable teratomas derived from embryonic implants, the incidence of neural components composing more than half of the tumor may be as high as 70%.[10] Neuroepithelial differentiation occurs early and consistently in developing spontaneous testicular teratomas in newborn mice of the 129 strain [26] and in tumors derived from *in vivo* and *in vitro* cloning of undifferentiated stem cells obtained from either ascitic embryoid bodies [28–30,42] or solid tumors.[43,44] Further evidence that neuroepithelial determination is an early and independent occurrence in the ectoderm is provided by the experiments of Artzt and Bennett,[2] in which grafts from 8-day old embryos from mice with a specific genetic defect gave rise to a notable incidence of primitive neuroepithelial tumors corresponding to the medulloepithe-

lioma, medulloblastoma and neuroblastoma. Experimental observations of this kind, in which a mutation compromises a specific morphogenetic event, emphasize the possibility that the occurrence of embryonal CNS neoplasia may arise from a defect in differentiation.[1,2]

The chief characteristics of neuroepithelial differentiation in our tumor line are summarized as follows: a) It was found in solid tumors only, where it was part of a divergent differentiation from multipotential stem cells. In ascitic fluid, the stem cells formed embryoid bodies only. b) It was very extensive. By serial sections in one tumor it was estimated to involve 80 to 90% of the cells. However, differentiation in other somatic directions was still present; this was confirmed by the development of new teratomatous tumors after animal-reimplantation of primary explants that had been maintained *in vitro* in a medium known to favor neuroepithelial differentiation and in which neuroglial elements had been well preserved. c) With the exception of myelin formation, all stages of divergent neuroepithelial differentiation, ranging from medullary epithelium to mature ganglion and glial elements, were present. Criteria for neuronal cell types included the demonstration of Nissl substance in their cytoplasm; the identification of axons by specific silver methods; and the demonstration that axonal processes originated from these cells. The presence of specific ganglionic organelles, such as synapses and dense-core vesicles, has subsequently been confirmed by electron microscopy.[45] Criteria for glial differentiation included affinity for stains specific for neuroglial fibers, and positive immunofluorescence with GFA protein antiserum. Ependymal differentiation was expressed by the demonstration of cilia and blepharoplasts and the formation of characteristic rosettes with well-defined central canals. These rosettes were of two types: a more primitive type, in which the lumen was surrounded by several cell layers and in which mitotic figures were present in the juxtaluminal layer; and a type in which the lumen was surrounded by one or occasionally two cell layers, which were devoid of mitotic figures. The ependymal differentiation of the cells adjacent to the internal lumen was confirmed by electron microscopy.[45] In our organ culture systems, we were unable to observe preservation or differentiation of neuroblastic elements, but neuroglial components, including ependymal structures, were well preserved. On collagen-coated coverslips, astrocytic differentiation was also observed after 2 weeks *in vitro* and was confirmed by positive immunofluorescence against GFA protein. Increased glial fiber formation after 4 to 6 weeks on gelfoam matrices suggested an increased degree of differentiation in fibrillary astrocytes, a finding which is in agreement with previous stud-

ies in this laboratory on the *in vitro* differentiation of human [13,14] and experimental [18] astrocytic gliomas maintained in similar organ culture systems. On the other hand, it stands in contrast to previous studies on cultures of transplantable teratoma maintained on cellulose sponge foam matrices, which reported either an absence or very low preservation of neuroepithelial differentiation.[43,46,47]

The neuroepithelial differentiation demonstrated in the present tumor resembles that in a recently described teratocarcinoma in which the differentiation was restricted solely to neuroectodermal derivatives.[48] However, there are a number of differences between the two tumors. First, in the teratocarcinoma described by Damjanov *et al*,[48] the differentiation was restricted solely to neuroepithelial components. Second, the stem cells of the restricted tumor failed to form ascitic embryoid bodies after intraperitoneal injection. Whereas this finding might suggest a possible relationship between the inability to form further embryoid bodies and the lack of divergent somatic differentiation, cloning experiments indicate that such a relationship is not constant.[30] Third, the stem cells in the restricted teratocarcinoma often metastasized through the lymphatic pathways to the abdominal cavity after subcutaneous implantation. Fourth, they did not develop neuroepithelial tubes resembling the embryonal medullary epithelium and were not reported to differentiate into large populations of mitotically active cells that were gradually progressing into various stages of neuroepithelial maturation. Fifth, neither myelin nor microglia, present in the restricted tumor, were found in our tumor.

Neuroepithelial differentiation from embryoid bodies has been reported by others, using various cell or tissue culture technics.[29,30,42,47,49,50] In the experiments described in this report, using media known to favor neuroglial differentiation, we were unable to achieve this, presumably because these media were unfavorable for the maintenance and growth of embryoid bodies. With the use of new media developed in this laboratory, we have more recently been able to obtain neuroepithelial differentiation from embryoid bodies maintained *in vitro;* these findings will be described in a subsequent report.

The aim of the present work was to develop an experimental model for the study of embryonal central nervous system tumors in which further differentiation is or is not expressed. The human adenovirus-induced embryonal CNS tumors reported by Mukai and Kobayashi [6,7] as embryonic gliomas in hamsters or as medulloepitheliomas in Sprague-Dawley rats appear to be primitive tumors of neuroblastic origin, as suggested by the presence of neuroblastic rosettes and of neurosensory

9 + 0 type cilia, and by the induction of typical retinoblastomas following intraocular injection of the virus. The viral target in that experimental model of embryonal tumor therefore appears to be exclusively neuronal. By contrast, the stages of neuroepithelial differentiation in the mouse testicular teratoma described in this report correspond to three general types of human CNS tumors: a) The areas of bipotential medullary epithelium correspond to the human medulloepithelioma. b) Areas of neuroblastic differentiation, including Homer Wright rosettes, correspond to the neuroblastoma, with the more mature neuronal differentiation corresponding to the ganglioneuroma or, when mixed with glial elements, to the ganglioglioma. c) In the neuroglial cell lines, astrocytomas, oligodendrogliomas and ependymomas in various stages of differentiation are represented. The areas of rosettes containing mitotic figures correspond to the primitive ependymoblastoma,[34] whereas the more mature rosettes can be equated with the better differentiated ependymoma. The neuroectodermal component of this experimental mouse teratoma may therefore serve as a model for neuroepithelial differentiation and for the study of both normal and neoplastic neurocytogenesis.

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Acknowledgments

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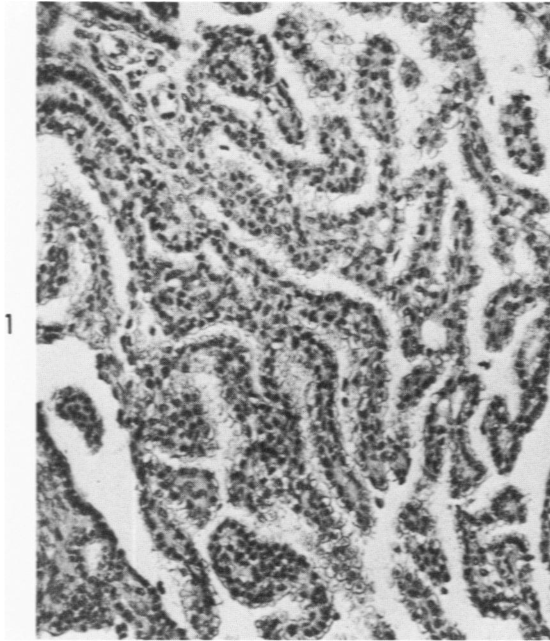
[*Illustrations follow*]

Figures 1-4 show fields from solid mesenteric implants derived from OTT-6050 mouse testicular teratoma in ascitic form (43 days after intraperitoneal inoculation).

1



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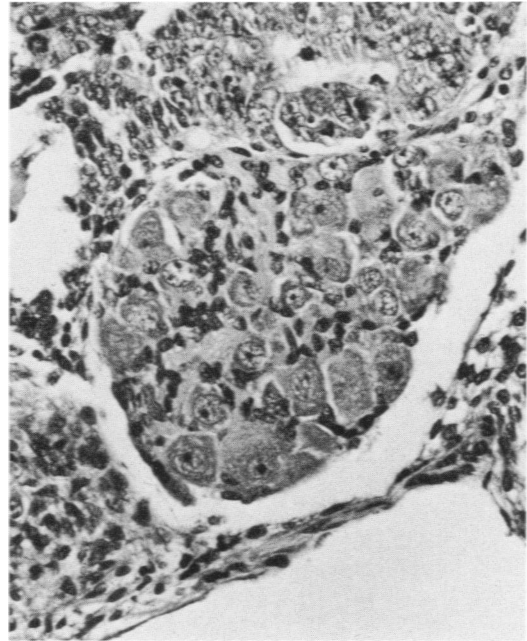

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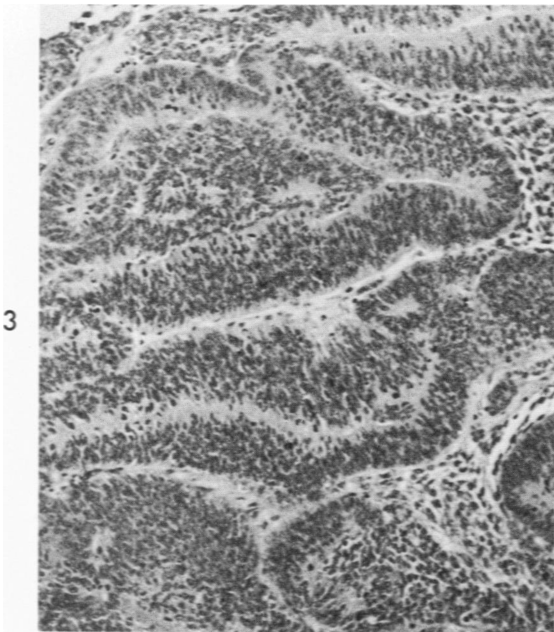

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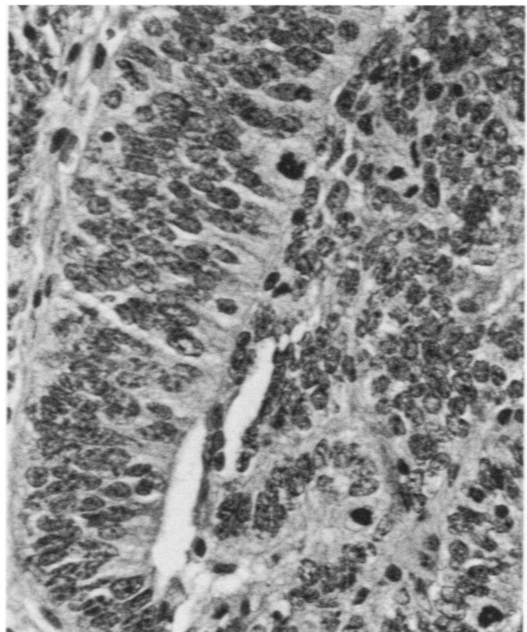


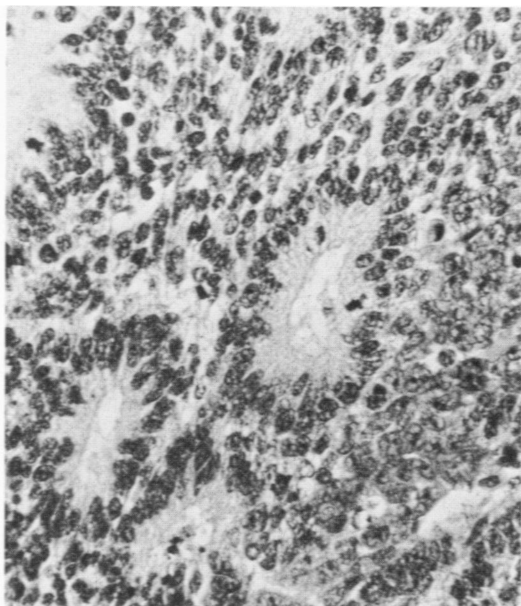
Fig 1—Tissue forming choroid plexus (H&E, × 150). **Fig 2**—Collection of peripheral ganglion cells with satellite cells, resembling dorsal root ganglion (H&E, × 300). **Fig 3**—Papillary arrangement of multilayered cells resembling medullary, or primitive neural, epithelium (H&E, × 120). **Fig 4**—Higher magnification of medullary-like epithelium, demonstrating well-defined external and internal limiting membranes, and mitotic figures near the internal membrane (H&E, × 480).

Fig 5—Ependymoblastomatous rosettes surrounded by poorly differentiated neuroglial cells in the sixth subcutaneous transplant derived from a subcutaneous needle tract implant. Note the juxtaluminal mitotic figures (H&E, × 300).

Fig 6—Ependymal rosette in a field of well-differentiated glial cells, some of which are oligodendroglial, in the sixth subcutaneous transplant of a mesenteric implant (H&E, × 300).

Fig 7—Fibrillary astrocytic differentiation in an omental implant from ascitic tumor cells (31 days postinoculation) (van Gieson, × 300).

Fig 8—Intense immunofluorescence of glial cells and their processes with GFA protein antiserum in the eighth subcutaneous transplant derived from a subcutaneous needle tract implant (× 450).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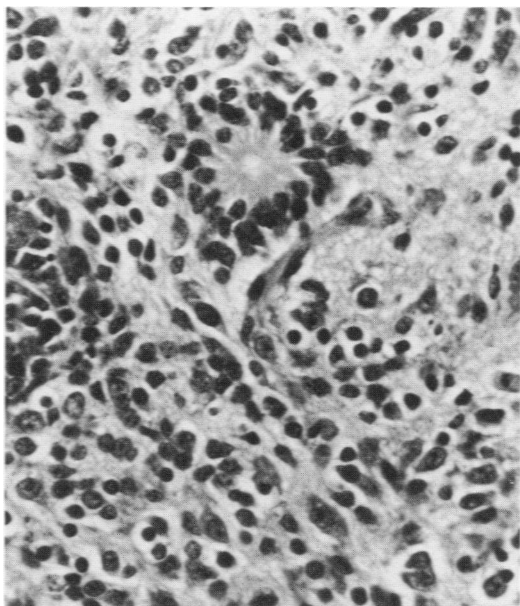
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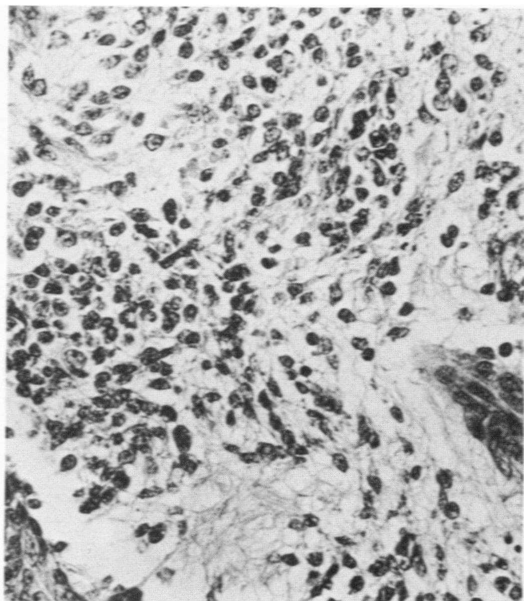
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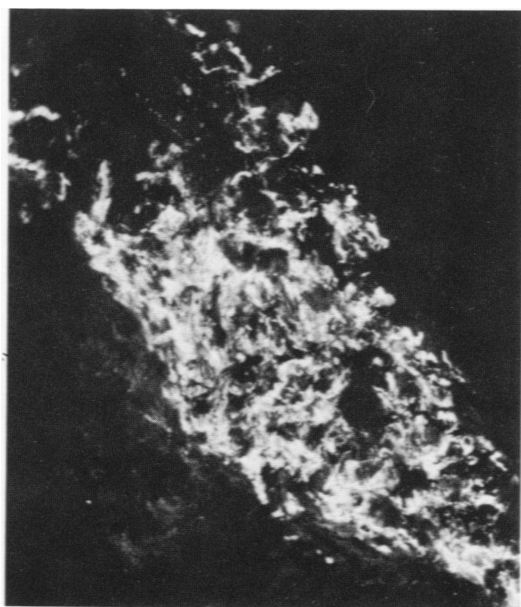
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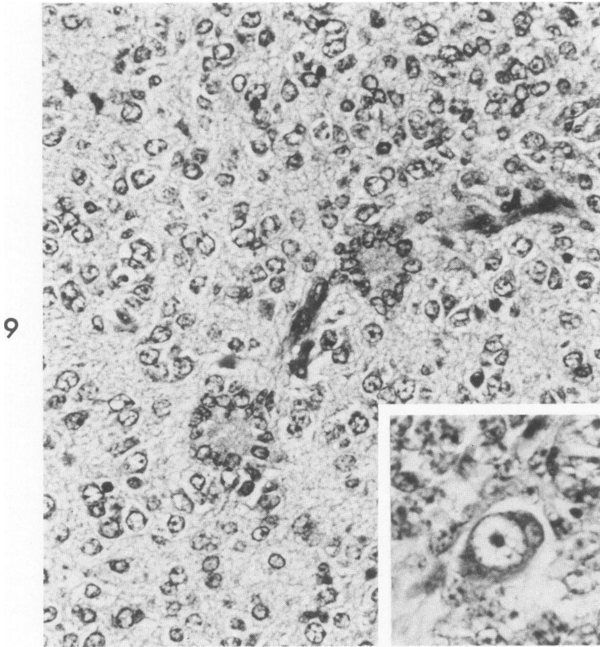

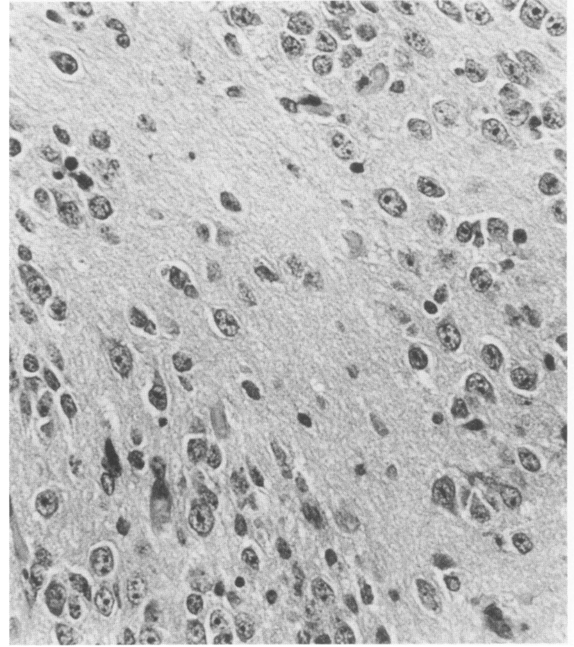

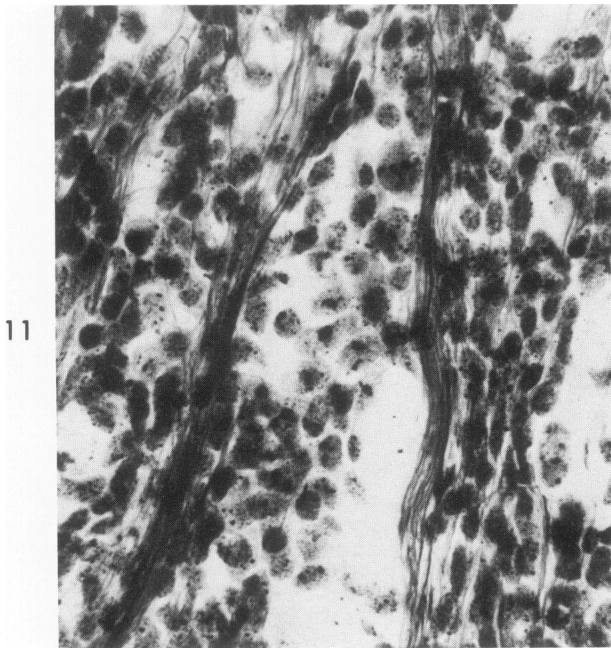

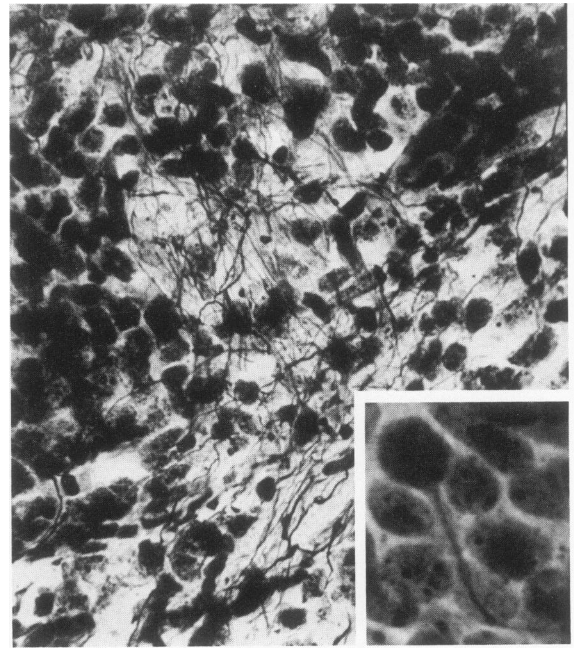

Fig 9—Two neuroblastic rosettes in a field showing early neuronal differentiation, in the eighth subcutaneous transplant derived from a subcutaneous needle tract implant (H&E, × 270). **Inset**—A mature ganglion cell surrounded by primitive mesenchymal cells in the same tumor as Figure 7 (van Gieson, × 480). **Fig 10**—Ganglionic differentiation with mature neurons and well-developed neuropil in the twelfth subcutaneous transplant derived from a subcutaneous needle tract implant (H&E, × 300). **Fig 11**—Leashes of parallel axons traversing a field of neuroblasts, in the seventh subcutaneous transplant of derivation similar to that of Figure 10 (Bielschowsky silver impregnation, × 480). **Fig 12**—Webwork of delicate neurites in a field showing neuronal differentiation, in same tumor as Figure 11 (Bielschowsky silver impregnation, × 480). **Inset**—Axonal process originating from the cytoplasm of a neuroblast (Bielschowsky, × 1500).

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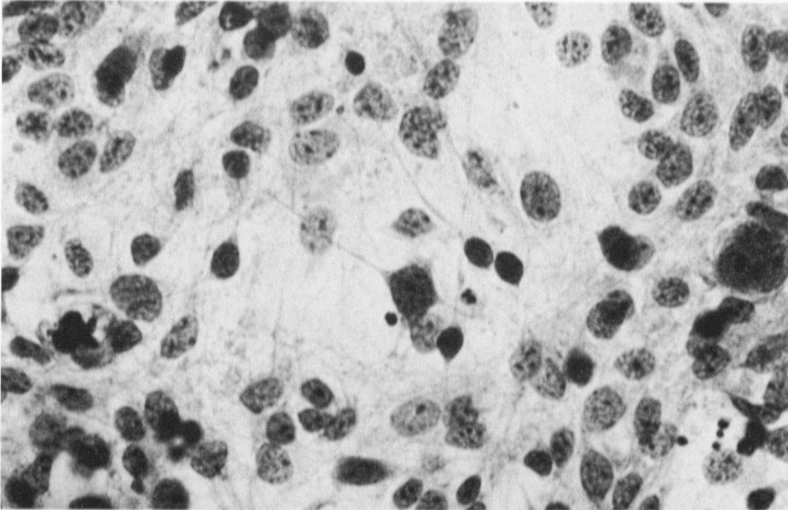

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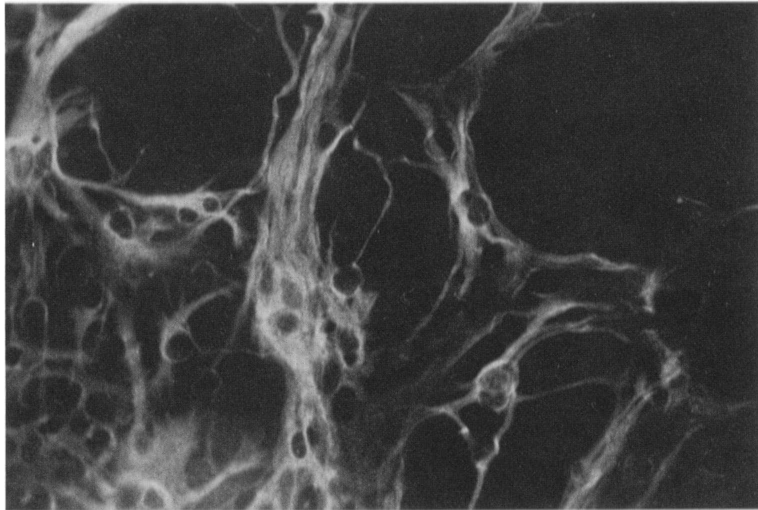

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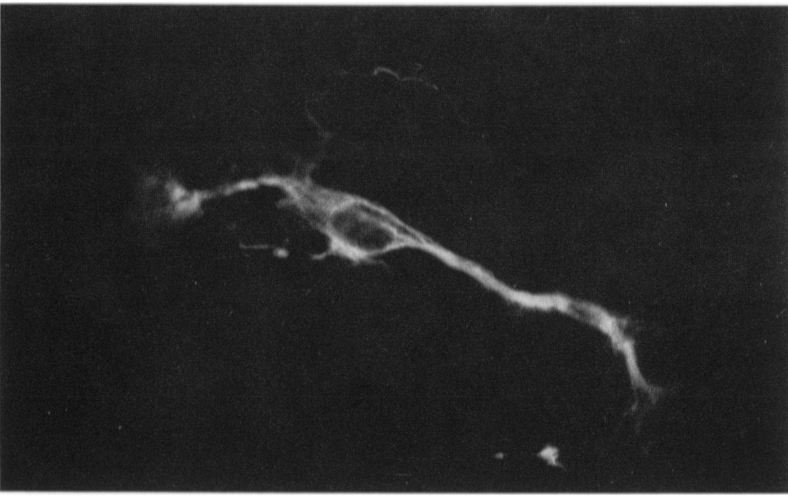

Figures 13-15 show explants grown on collagen-coated coverslips in modified medium, from the eighth subcutaneous transplant derived from a subcutaneous needle tract implant.

Fig 13—Astrocytic differentiation in the outgrowth zone after 14 days *in vitro* (H&E, × 615). **Fig 14**—Numerous astrocytic cell bodies and processes showing positive immunofluorescence for GFA protein in the outgrowth zone of an explant after 27 days *in vitro* (× 720). **Fig 15**—Single elongated astrocyte with positive immunofluorescence for GFA protein in the outgrowth zone of an explant after 27 days *in vitro* (× 1800).

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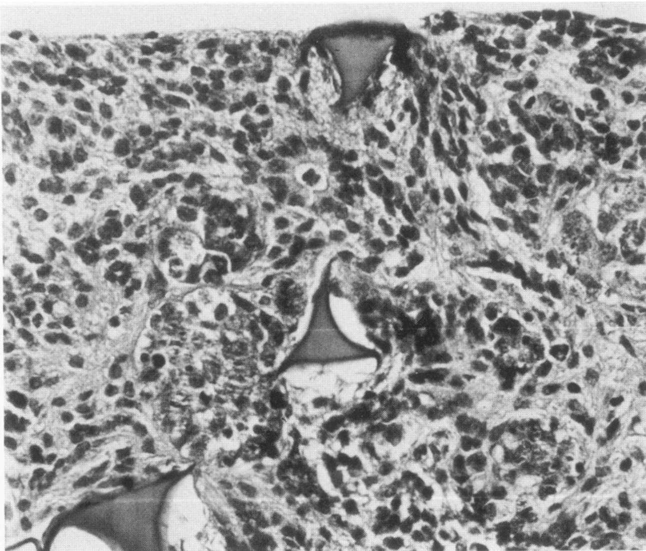

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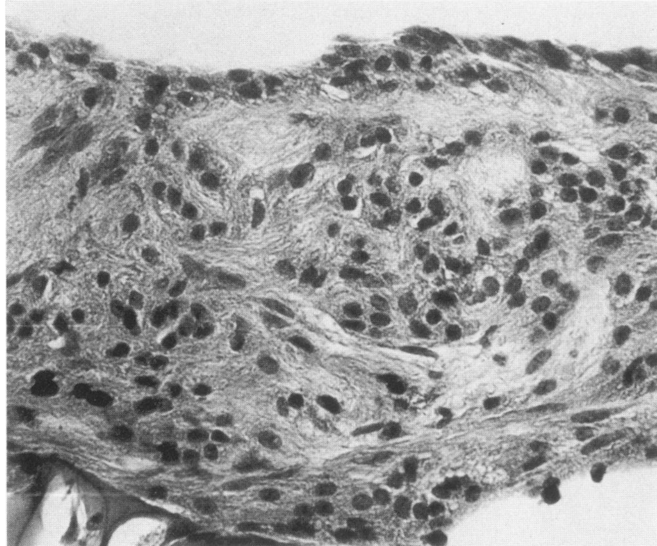

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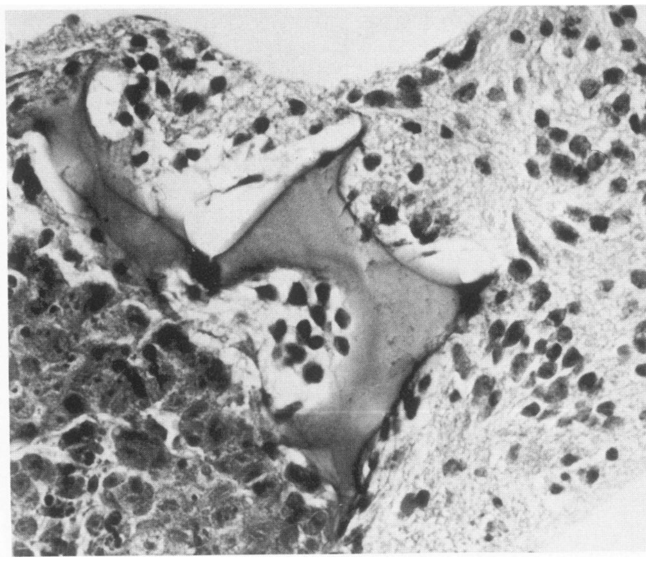

Figures 16-18 show gelfoam cultures of explants on standard medium, from the first (**17**) and the eighth (**16** and **18**) subcutaneous transplants derived from a subcutaneous needle tract implant.

Fig 16—An ependymal rosette in the center of a field of glial tissue after 28 days *in vitro*. Note invasion of the gelfoam (H&E, × 300). **Fig 17**—Increased glial fiber formation in astrocytic area of an explant after 48 day *in vitro* (H&E, × 300). **Fig 18**—Nest of undifferentiated stem cells (*lower left*) adjacent to astrocytic area after 34 days *in vitro* (H&E, × 300).