Ependymal Abnormalities in Lissencephaly/Pachygyria

Harvey B. Sarnat, M.D., Husam Z. Darwish, M.D., Peter G. Barth, M.D., Cynthia L. Trevener, M.D., Alfredo Pinto, M.D., Suresh Kotagal, M.D., Keiko Shishikura, M.D., Makiko Osawa, M.D., and Rowena Korobkin, M.D.

Abstract. The ependyma was examined in eight children with neuroblast migratory disorders of diverse origin: three cases of lissencephaly type 1 with severe to mild degrees of agyria/pachygyria, four cases of lissencephaly type 2 in Fukuyama muscular dystrophy and the Walker-Warburg syndrome, and one case of hemimegalencephaly. Morphological and immunohistochemical abnormalities of the ependyma were strikingly similar in all. Discontinuities were disproportionate to the degree of ventriculomegaly. In some regions, the ependyma remained a pseudostratified columnar epithelium, though basal processes were absent. The poles of the horns of the lateral ventricles were replaced by extensive heterotopic ependymal rosettes. Rosettes and rows of ependymal cells were in other subventricular sites. Subependymal nodules of large astrocytes and their processes bulged into the ventricular lumen after infancy. Ependymal cells did not express glial fibrillary acidic protein, but showed persistent expression of S-100 protein, cytokeratin CK-904 and sometimes vimentin long after these proteins normally disappear. An abnormal ependyma in lissencephaly/pachygyria may contribute to disturbances in neurogenesis, guidance of axonal projections and neuroblast migrations; it may be a primary factor in pathogenesis.

Key Words: Ependyma; Hemimegalencephaly; Lissencephaly; Miller-Dieker syndrome; Neuroblast migration; Pachygyria; Walker-Warburg syndrome.

INTRODUCTION

The fetal ependyma is a much more dynamic structure than the ependyma of the adult brain. Fetal ependymal cells are columnar with long basal processes and serve an active secretory function; mature ependymal cells are simpler cuboidal cells without processes and function as a passive filter for the transport of fluid, ions and small molecules. The ependyma of the fetal brain plays an essential role in development that includes the arrest of mitotic activity in the neuroepithelium, the guidance of axonal growth cones, and perhaps the maintenance and transformation of radial glial cells that guide migratory neuroblasts (1). It differentiates in a predictable spatial and temporal sequence and differs metabolically from the mature ependyma, exhibiting immunoreactivity for a variety of intermediate filament proteins and for diffusible molecules such as S-100 protein and glycosaminoglycans (2-4).

Lissencephaly is a congenital malformation characterized by a smooth cerebral surface without convolutions and architectural alterations of the brainstem, cerebellum and cerebral cortex. The nature of the abnormal architecture indicates a primary disturbance of neuroblast migration in early gestation involving three of the four major embryonic migratory pathways (5): the corpus pontobulbare, the cerebellar external granule cell layer and the radial projections to the cerebral cortical plate; only the corpus gangliothalamicus is spared (6-9). In addition, lissencephalic brains often are of low weight and exhibit aberrant axonal pathways. Pachygyria is histologically similar to lissencephaly, but a variable number of abnormal, excessively large gyri are formed. Some brains form almost normal convolutions but show the abnormalities of cortical lamination characteristic of lissencephaly (10-12).

Because the fetal ependyma is such an important structure for developmental processes of the brain that are faulty in lissencephaly/pachygyria, this study was undertaken to determine whether the ependyma in lissencephalic brains shows evidence of defective histological differentiation or abnormal immunohistochemical features.

MATERIALS AND METHODS

Eight neuropathological specimens were available for study from five medical centers: the University of Calgary (Alberta Children’s Hospital), the University of Amsterdam in the Netherlands, St. Louis University (Cardinal Glennon Children’s Hospital), Pacific Presbyterian Medical Center in San Francisco and the Tokyo Women’s Medical College in Tokyo, Japan. Three of these brains showed varying degrees of gyration associated with the four-layered cerebral cortex of lissencephaly type 1. Type 2 lissencephaly was represented by one case of the Fukuyama variety of congenital muscular dystrophy that was previously published in Japanese (13), and three cases of Walker-Warburg syndrome. An additional form of pachygyria was a
surgical specimen from hemispherectomy for intractable epilepsy in an infant with hemimegalencephaly.

Thorough standard neuropathological examinations were carried out in the medical centers where the patients had died. Special attention was focused on the ependyma and subventricular zone. Clinical data were reviewed and correlated. Slides from each of the brains were also examined by one of us (HBS) and immunohistochemical studies for vimentin, cytokeratin CK-904, glial fibrillary acidic protein (GFAP) and S-100 protein were carried out using peroxidase-antiperoxidase techniques for formalin-fixed, paraffin-embedded sections as previously described (4).

Three normal brains of children 2 to 7 years of age and three adults who had died of non-neurological causes also were examined for ependymal reactivity for vimentin, cytokeratin CK-904, GFAP and S-100 protein. Sections of the frontal, temporal and occipital horns of the lateral ventricles, third and fourth ventricles and spinal central canal were studied. Numerous fetal brains ranging from 6 weeks gestation to term, prepared with these same immunohistochemical methods, also were available for comparison from other recent studies (4).

Six term infants ranging in age from 1 to 18 months, with hydrocephalus secondary to meningitis, were studied at autopsy to relate ventriculomegaly and the percentage of ventricular surface not covered by ependyma. None had intraventricular extension of the infection and none had cerebral malformations. The frontal horn of the lateral ventricle was measured as the septo-caudate distance, an imaginary line drawn perpendicular to the head of the caudate nucleus from the junction of the septum pellucidum and corpus callosum, in coronal microscopic sections of the cerebral hemispheres at the level of the optic chiasm. The temporal horn was measured in coronal sections at the level of the globus pallidus as an imaginary horizontal line from the lateral edge of the hippocampal formation to the recess in the lateral wall of the ventricle. The occipital horn was measured in the horizontal plane at the level of the calcar avis. Mild ventriculomegaly was considered to be 3–5 mm, moderate was 6–23 mm and severe was 26 mm or more. Variables not considered at this time included tissue shrinkage due to fixation, tissue elasticity, normal asymmetries of the occipital horns in particular, and the growth of the cerebral mantle with age not normally accompanied by a corresponding increase in the width.

Fig. 1. Case 1. Three coronal sections of the cerebral hemispheres. The frontal horns of the lateral ventricles are only minimally dilated, but less than half their surface was lined by ependyma. The temporal horns are the most dilated part of the lateral ventricles. The third ventricle also is wide. The smooth cortical surface is associated with an abnormally thick cortical plate. The corpus callosum is thin.
of the ventricular lumen. The perimeter of the ventricle was traced, and it and the ependymal-lined surface were measured separately. These preliminary results form part of the data in a larger and more detailed study, currently in progress, defining the ratio of ependymal discontinuity and ventriculomegaly in hydrocephalus not associated with major cerebral malformations.

RESULTS

The eight cases selected represent a clinically and genetically diverse group of neuroblast migratory disorders. Cases 1 and 2 are classical lissencephaly or type 1, one of whom had a chromosomal defect diagnostic of the Miller-Dieker syndrome. Case 3 is histologically similar to Cases 1 and 2, but the convolutions of the cerebral cortex were normal and no microscopic abnormalities were demonstrated in the brainstem or cerebellum. Cases 4 to 7 are lissencephaly type 2, one patient with Fukuyama muscular dystrophy and three with the Walker-Warburg syndrome. Case 8 is a surgical hemispherectomy for refractory epilepsy in an infant with hemimegalencephaly; the enlarged cerebral hemisphere showed pachygyria and abnormal cortical lamination.

Clinical Findings

All of the patients in this study were severely delayed in gross motor, fine motor and intellectual development. None exhibited visual fixation, but microphthalmia, optic nerve hypoplasia, cataracts and retinal abnormalities were seen only in Cases 5 to 7 with Walker-Warburg syndrome. Cases 2 and 3 showed spastic diplegia; Case 3 had axial hypotonia; Case 8 had normal muscle tone; the others were all diffusely hypotonic. Except for Case 4 who never had seizures and Case 2 who had seizures only in the first year, the others all had epilepsy. None of the patients had experienced fetal distress or birth asphyxia. All were born at term. Intrauterine growth retardation was noted in Cases 1 and 2, who weighed 2,180 and 2,690 g respectively at birth.

The immediate cause of death was aspiration or intercurrent infection not involving the nervous system; Case 8 survives in a largely vegetative state and is now 2 years of age.

Chromosomal karyotypes were normal in all cases except Case 2. DNA studies were performed on this patient and her parents using restriction fragment length polymorphism (RFLP) densitometry; five polymorphic mark-
Fig. 3. Case 3. Coronal section of cerebral hemispheres at the level of the head of the caudate nucleus. The ventricular system is not dilated. The white matter is sharply demarcated from the cortical grey matter but is thinner than expected. The corpus callosum is thin.

ers at the 17p13.3 locus were used and the patient was demonstrated to have a de novo maternal deletion of two probes (YNH37 and YN22), characteristic of the Miller-Dieker syndrome. Metabolic studies of serum amino acids, organic acids, endocrine parameters and urinary polysaccharides disclosed no abnormalities. The diagnosis of lissencephaly/pachygyria was made during life by computed tomographic and magnetic resonance imaging. The diagnosis of Fukuyama muscular dystrophy was confirmed in Case 4 by muscle biopsy at 1 year of age. Electroencephalography (EEG) was paroxysmally abnormal in all cases, including those without clinical seizures; Cases 5 and 6 had not been examined by EEG.

Family histories of cerebral dysgenesis could not be elicited in any case except Case 6 who had an older sibling with congenital hydrocephalus and cataracts, said to be secondary to a fetal rubella infection. Cases 3 and 8 have two normal living siblings; Case 6 has one normal sibling; the others have no siblings. The mother of Case 4 had a therapeutic abortion. A paternal female first cousin of Case 1 was mentally retarded; the maternal grandfather and one of his sisters had retinitis pigmentosa but were neurologically normal.

Neuropathological Findings

Grossly, the cerebral hemispheres of Cases 1, 2, 5, 6 and 7 were agyric or had a few poorly formed convolutions, chiefly in the posterior temporal and parietal regions. Deep vertical clefts, not corresponding to Sylvian fissures, were formed symmetrically in Cases 1 and 5. A shallow cobblestone pattern of the cortical surface was evident when the leptomeninges were stripped. Case 3 was unique in this series because of normal gyration of the cerebral cortex, except for minimally enlarged gyri in the posterior temporal-parietal region. Extensive pachygyria was seen in Case 4 and in the hemimegalencephalic hemisphere of Case 8. The olfactory bulbs and tracts were hypoplastic bilaterally in Case 6 and, in Case 7, the left olfactory bulb was hypoplastic and the right was absent altogether.

Coronal sections of the cerebral hemispheres disclosed
an abnormally thick cerebral cortex and correspondingly thin centrum semiovale. The lateral ventricles were mildly to moderately dilated in the cases of lissencephaly type 1 (Figs. 1, 2) and in Case 4 of Fukuyama muscular dystrophy; the ventricles were of normal size in Case 3 (Fig. 3); ventriculomegaly was demonstrated in all cases of Walker-Warburg syndrome (Fig. 4); the hemimegalencephalic hemisphere of Case 8 had mild ventricular dilatation demonstrated by imaging (Fig. 5).

The lamination of the cerebral cortex was abnormal in all cases. In Cases 1 and 2 of type 1 lissencephaly a typical four-layered cortex was found in all regions of neocortex (Fig. 6); the deepest layer consisted of columns of incompletely migrated neurons extending deeply into the subcortical white matter, almost to the periventricular region. Cases 3 and 8 showed similar abnormalities of neuroblast migration (Fig. 7), but less uniformly and, in places, a six-layered cortex approaching the normal, expected architecture was found. The four cases of type 2 lissencephaly, all had the characteristic gliomesenchymal bundles extending into the cortex from the pial surface and had severely disorganized cortical architecture without discernable lamination and with many heterotopic and disoriented neurons, including large pyramidal cells in superficial regions of the cortical plate (Fig. 8). Glial cell proliferation also was evident focally, but no infarction or hemorrhages were found.

The corpus striatum and thalamus were normally formed in all except Case 6 which showed midline thalamic fusion and also tectal “beaking.” Hamartomas and focal dysplasias of the cerebellar cortex were demonstrated in all postmortem cases except Case 3. The pontine and inferior olivary nuclei also were small, heterotopic with medial displacement, and poorly formed. The entire basis pontis was replaced by gliomesenchymal bundles in Case 5. All three cases of Walker-Warburg syndrome had an accompanying Dandy-Walker malformation.

Myelination was normal in all cases, as determined by luxol fast blue stain.

Ependymal Findings

Despite the heterogeneity of the various diseases expressed as neuroblast migratory disorders, the ependymal abnormalities were similar in all, particularly if patients of similar ages were compared (Table 1). The epithelium was discontinuous and interrupted by long and short stretches of ventricular surface lined only by glial cells and their processes or by an abrupt end of the cerebral parenchyma without gliotic transition (Figs. 8, 9).
of the lateral ventricles, though only 5 to 10 percent of the fourth ventricle was not lined by ependyma. In summary, the ependymal loss in all forms of lissencephaly/pachygyria in every case was in excess of that associated with simple hydrocephalus at corresponding degrees of ventricular enlargement.

Subventricular gliosis was more pronounced in general in the older children than in the infants, but was evident in the neonatal cases as patchy zones. Subventricular cysts lined by glial processes and without necrosis or inflammation of surrounding cerebral tissue were found only in the Walker-Warburg syndrome cases, mainly confined to the areas around the angles of the frontal horns. In the children over the age of 2 years with Miller-Dieker syndrome and Fukuyama muscular dystrophy, multiple nodules protruded from the subventricular zone to bulge into the lumen (Figs. 8, 10). These nodules were particularly numerous in regions of long ependymal discontinuities but also occurred just beneath the preserved ependyma in other places. Ependymal cells at the apices of such nodules usually were atrophic or absent. The nodules were composed of astrocytes and their processes (Fig. 11). No heterotopic neurons, multinucleated giant cells or cells with bizarre morphology were demonstrated.

The third and fourth ventricles had discontinuous ependyma and subventricular glial nodules similar to the findings in the lateral ventricles, though not as extensive, in our cases of Miller-Dieker syndrome (Cases 1, 2), Fukuyama muscular dystrophy (Case 4) and Walker-Warburg syndrome (Cases 5–7). The floor of the fourth ventricle had ependyma in the midline (floor plate) and paramedian region, but lateral to the sulci limitans it was minimal. The infundibular region of the third ventricle and the area postrema of the fourth ventricle appeared normal.

The preserved ependyma was mostly a simple cuboidal epithelium with intact cilia, but in some regions the cells were flattened and lacked cilia. In other regions, a transition was seen to a pseudostratified columnar epithelium resembling that of midfetal life, though cells were more rounded and less columnar in shape and had more spherical nuclei than normally seen in the fetus (Fig. 12). This type of organization was most common near the angles of the ventricular horns, but also was demonstrated in places along straight stretches of ventricular surface lacking angularity or even plications. Pseudostratified cells also were ciliated. The pseudostratified architecture involved the third and fourth ventricles and the cerebral aqueduct as well as the lateral ventricles.

Numerous rosettes and rows of displaced ependymal cells were found in the subventricular zone of all ventricles, both in regions where the ependyma was absent from the overlying ventricular surface and in regions where it remained intact. The cells forming rosettes had abundant cytoplasm and often were ciliated, the cilia extending into

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**Fig. 5.** Case 8. Computed tomographic image of a 2-month-old infant girl with hemimegalencephaly. The larger hemisphere has a smooth-appearing surface but actually showed pachygyria at the time of surgical hemispherectomy. The ventricles are asymmetrically dilated, more on the left. A thin and distorted corpus callosum is present.
the narrow, empty lumen of the rosette (Fig. 13). At the extremities of the occipital, temporal and frontal horns of the lateral ventricles, the ventricular cavity was completely obliterated or coapted, and a “ghost ventricle” was outlined by extensive ependymal rosettes (Fig. 14). In places, partial rosettes or rows of subventricular ependyma were formed (Figs. 13, 15). Fresh erythrocytes were seen in relation to some subventricular partial rosettes or rows of ependymal cells, probably due to fresh terminal petechiae. They were not enclosed by endothelial cells.

Ependymal cells at the ventricular surface showed immunoreactivity for GFAP only in scattered cells, as normally expected in the neonatal brain. Astrocytes and glial processes within the subventricular nodules were strongly reactive (Fig. 11). In one case of Walker-Warburg syndrome in a neonate, the ependyma was strongly reactive for vimentin, but this fetal protein was not demonstrated in the other cases. Cytokeratin CK-904 was demonstrated in most ependymal cells in all brains studied, the immunoreactivity being weak or strong in an unpredictable fashion along the ependyma. S-100 protein was consistently strongly expressed in ependymal cells of all brains studied, regardless of age (Fig. 16). Ependymal cells forming rosettes were nonreactive for the antibodies tested, except in the infant with hemimegalencephalic pachygyria in which about half the cells forming partial rosettes were reactive for S-100 protein.

**DISCUSSION**

Ependymal aberrations in disorders of neuroblast migration are remarkably similar despite the diversity in genetic traits and of cortical architecture in the Miller-Dieker syndrome, Fukuyama muscular dystrophy and the Walker-Warburg syndrome. The ependyma was similarly involved in six cases of cerebro-hepato-renal (Zellweger) disease (14), another genetic disease with abnormal neuroblast migration. The principal differences in these cases were that subventricular glial nodules were absent and ependymal rosettes were strongly reactive for S-100 protein, but the younger ages of the Zellweger disease patients may have been a factor (14).

Some ependymal alterations in lissencephaly might be...
interpreted as exaggerated normal variations. Sulcation of the ventricular walls, particularly the lateral walls of the cerebral aqueduct and the floor of the fourth ventricle lateral to the sulcus limitans, occurs during normal growth and persists to some degree after growth ceases. Minor forking of the aqueduct, asymmetries of the horns of the lateral ventricles with coaptation of ependymal surfaces at the angles, and a few subventricular rosettes and rows of ependymal cells have been described for many years as developmental anomalies in otherwise normal brains (15–17). Small areas of focal ependymal loss increasing with advancing gestational age are even found in late fetal life (18). The extent of these nonspecific changes in lissencephaly/pachygyria is far greater than can be attributed to normal variation.

One explanation of the similarity of changes is that the ependyma has such a limited repertoire of responses to injury that the end-result of many conditions is similar. Some features, such as discontinuities of the ependyma at the ventricular surface and subventricular ependymal rosettes and gliosis, are shared with acquired conditions of ventricular enlargement that stretch the ependyma. Quantitative data relating degrees of ventriculomegaly to ependymal discontinuities are preliminary, but even these comparisons between normally laminated hydrocephalic brains of comparable ages and our cases of lissencephaly/pachygyria clearly demonstrate that ventricular dilatation alone cannot account for the long stretches of ventricular surface devoid of ependyma and extensive subventricular rosette formation in lissencephaly.

The persistence of a pseudostratified columnar epithelial organization of the ependyma in lissencephaly/pachygyria suggests maturational arrest. The multiple large glial nodules at the ventricular wall are probably less specific. Subependymal gliosis and proliferative glial nodules in regions of local ependymal loss usually follow inflammatory processes such as “ventriculitis” in septic neonates. Such granular ependymitis with or without focal
loss of ependymal cells occurs to a minor degree in as many as 65 percent of brains of both young and elderly adults that contain a variety of acquired neuropathological lesions or are otherwise normal (19). These changes are attributed to "banal viral infections" involving ependymal cells, such as childhood mumps (19), though this explanation cannot be invoked in genetic diseases. In our cases and in Zellweger disease (14) the glial nodules are absent or minimal in early infancy and become prominent in later childhood, an attempted reparative process over years. The extensive rosettes outlining "ghost ventricles" in our cases of lissencephaly suggest that the extremitiy of the ventricle either never formed or became obliterated early in fetal life. Whether abnormal ependymal organization was a cause or a result of ventricular obliteration cannot be ascertained from the end morphology.

The persistence of S-100 protein and cytokeratin expression in ependymal cells years after it should have been suppressed is a constant and perhaps the most specific feature of the ependyma in lissencephaly and may provide a clue to a pathogenetic role of the ependyma in altering neuroblast migration. Its precise function cannot be stated until more data become available on the influence of the ependyma on the maintenance and transformation of radial glial cells of the subventricular zone. The loss of these products from the ependyma coincides with the time of retraction of radial glial processes and the conversion of these cells to mature astrocytes in the course of normal maturation (4). The dynamic role of the ependyma in the development of the nervous system, including its participation in neural induction and its active secretory function, is well documented (1). Regional differences in the differentiation of the fetal ependyma are inconsistent with the obsolete concept that it is a homogeneous structure (4).

The capacity of the mature ependyma to regenerate or repair itself is very limited, unlike most epithelia. In experimental hydrocephalus induced by intracisternal silicone or kaolin in animals, the ependyma becomes discontinuous and gaps are filled by glial processes; remaining ependymal cells do not undergo mitosis and subventricular glial cells do not exhibit metaplasia to become new ependymal cells (20, 21). Subependymal cells are mobilized within hours after ependymal injury and mature astrocytes proliferate in about 6 days (22). The primitive neuroepithelium with a partially differentiated roof plate in the chick embryo is able to close surgical incisions in the dorsal midline to restore both epithelial integrity and

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Fig. 8.  Case 4. (A) Thin coronal section of the left cerebral hemisphere of a 6-year-old boy with Fukuyama congenital muscular dystrophy. The surface of the brain shows pachygyria. The lateral and third ventricles are moderately dilated. Letters within Figure 8A correspond to regions shown at higher magnification in 8B–E. (B) Neurons comprising the frontal cortex are disoriented and haphazardly arranged; the molecular layer is at the top. (C) A large subventricular glial nodule at the inferior angle of the frontal horn has discontinuous ependyma over its surface and heterotopic ependymal rosettes beneath. (D) The ependyma overlying the hippocampus is interrupted by a series of subventricular glial nodules that protrude into the lumen; ependymal cells are absent at the surfaces of these nodules. (E) The neocortex of the inferior surface of the temporal lobe shows lack

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of laminated architecture and gliomesenchymal bundles extending from the pial surface (at the bottom of the figure). (F) A large subventricular nodule containing many astrocytes and their processes is at the surface of the third ventricle. Hematoxylin-eosin. (B, F) ×400; (C, D) ×250; (E) ×40.

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Fig. 9. Case 3. The angle of the frontal horn of the lateral ventricle shows a discontinuous ependyma with less than half of the ventricular surface covered. A large ependymal rosette is seen within the subventricular zone (arrow). Hematoxylin-eosin. ×40.

<table>
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<th>Discontinuities</th>
<th>Glial nodules</th>
<th>Pseudostratification</th>
<th>Subventricular rosettes</th>
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<th>Cytokeratin</th>
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++ = mild ventriculomegaly, discontinuities involve less than half the ventricular surface, few glial nodules, pseudostratification involves less than half the ventricular ependyma, few rosettes, immunoreactivity weak or only in scattered cells. ++ = moderate or severe ventriculomegaly, discontinuities involve more than half of ventricular surface, extensive glial nodules, pseudostratification involves more than half the ventricular ependyma, extensive rosettes, immunoreactivity strong in most cells. n/a = not available. da = days; mo = months; yr = years of age. M = male; F = female.
the shape of the neural tube, but wounds of young embryos heal faster and more consistently than do those of older embryos (23, 24). The transplantation of fetal cerebral cortical homografts into the spinal cord of adult rats, however, is associated with mitotic proliferation of new ependymal cells to line the cysts that form within the graft (25). In none of our cases of human lissencephaly or other neuroblast migratory disorders with enlarged ventricles did we observe mitotic activity of ependymal cells to suggest regeneration.

In the Miller-Dieker syndrome, represented by Cases 1 and 2, a microdefect at the 17p13.3 locus has been identified (26-28). This gene probably encodes a protein important in neuroblast migration, perhaps involving the radial glial cell membrane or even the migratory neuroblast itself. Though it is plausible that this locus also programs ependymal differentiation in some way, genetic heterogeneity such as translocation occurs in some cases (29). Similar ependymal abnormalities occur in other disturbances of neuroblast migration in which the locus on chromosome 17 is not implicated. Several homeotic genes may have a similar expression. Lissencephaly may also
result from nongenetic diseases, such as congenital cytomegalovirus infection, that interfere with neuroblast migration in fetal life (30).

Lissencephalic brains may be excessively small or enlarged. The most severely agyric brains are most often of low weight and hypoplastic because of a paucity of cells. Evidence of an encephaloclastic process or of accelerated cellular death is lacking and deficient neumogenesis is a more likely pathogenesis. In normal development, most mitotic activity occurs at the ventricular surface of the neuroepithelium and is arrested when the ependyma differentiates. It is therefore advantageous to delay ependymal development as long as possible in some regions to permit the requisite number of mitotic cycles to be completed (31). Precocious formation of an ependyma reduces the total number of neuroblasts generated and microencephaly results (9). Mild disturbances of gyration with histological evidence of a migratory disorder also may be associated with a small brain, as in Case 3. If the number of neurons is not markedly reduced, the brain may be of normal size or even pathologically enlarged, as in Case 4. A large cerebral volume, megalencephaly, is an alternative to convolutions for maintaining the surface area of the cortex.

Most cases of type I lissencephaly and pachygyria show a similar four-layer neocortex with more of a columnar than laminar histological appearance; layer 1 is the molecular zone, similar to its counterpart in normal cortex; layer 2 is a mixture of nonlaminated large and small neurons corresponding to layers 2 through 6 of the normal neocortex; layer 3 is a narrow cell-sparse zone; and layer 4 consists of columns of incompletely migrated nerve cells of variable maturity that extend deeply into the subventricular zone (6–9). The origins of layers 1, 2 and 4 are clear, but that of the cell-sparse layer 3 is less apparent. It probably represents a persistent "subplate zone" of the fetal cerebrum. The cortical plate forms within the embryonic marginal zone as neuroblasts migrate from the subventricular zone (9, 32); the portion of the marginal zone remaining superficial to the cortical plate becomes the mature molecular zone or layer 1 of both normal six-layered and lissencephalic four-layered cortices; the portion of the marginal zone lying beneath the cortical plate is the subplate zone which is later incorporated into the deepest layer of the cortical plate and is, therefore, transient in the normal fetal brain but persists as layer 3 in lissencephaly.

**Fig. 11.** Case 1. GFAP is not expressed in ependymal cells on either side of the subventricular nodule, but large astrocytes and their processes within the nodule exhibit strong immunoreactivity. ×400.

**Fig. 12.** Case 3. Some portions of the ependyma lining the lateral ventricle retained a fetal pseudostratified organization, but the nonciliated cells and nuclei were more rounded than elongated, unlike true fetal ependymal cells. Hematoxylin-eosin. ×250.
Fig. 13. (A) Case 2. The ependyma lining the temporal horn of the lateral ventricle is discontinuous and atrophic. A large ependymal rosette is seen deep within the subventricular zone (arrows); the cells forming this rosette are taller and better ciliated than those at the ventricular surface. They were nonreactive for GFAP and S-100 protein. (B) Case 6. The surface of the lateral ventricle is not lined by ependyma, but a row of subventricular ependymal rosettes lies beneath a zone of gliosis. (C) Case 7. A row of pseudostratified ependymal cells lies beneath the lateral ventricular surface and parallel to it; it is separated from the ventricle by a zone of cerebral parenchyma; the apparent cavity adjacent to this heterotopic ependyma may have been artifactually produced during tissue processing. Hematoxylin-eosin. (A, B) ×250; (C) ×100.
Fetal ependymal cells have an active secretory function rather than their passive filtration role at maturity. Ependymal secretory products include glycosaminoglycans and proteoglycans, such as keratan sulfate, that repel axonal growth cones (33) and other small molecules, including S-100 protein, that may attract growing axons (4). The fetal ependyma plays an important role in axonal guidance during the formation of longitudinal tracts. In lissencephaly, the corticospinal and other long pathways often exhibit aberrant fiber projections (34).

Undifferentiated neuroepithelial cells and fetal ependymal cells express a few (4, 35) of the more than 20 distinct polypeptides that comprise the complex family of cytokeratins in various epithelial cell lines (36, 37). Those cytokeratins exhibiting immunoreactivity in the fetal ependyma have a relatively high molecular weight: CK-904, here shown to be expressed in lissencephaly and also in normal fetal ependyma (1, 4), has a molecular weight of 68 kDa; other cytokeratins weighing 54 to 58 kDa have weak or no reactivity in fetal ependymal cells. Vimentin molecules, by contrast, weigh 55 kDa and those of GFAP weigh 50 kDa. Apart from demonstrating another feature of metabolic immaturity of the ependyma in disorders of neoblast migration, the persistence of fetal cytokeratin in this neuroepithelium and its expected disappearance after early infancy is of uncertain functional importance.

The Miller-Dieker syndrome is type 1 lissencephaly or pachygyria associated with characteristic dysmorphic facies (6, 9, 38), best exemplified by Case 2. Some children with type 1 lissencephaly do not show abnormal facies or other dysmorphic features (39). Type 2 lissencephaly is limited to certain other genetic diseases such as Fukuyama muscular dystrophy (40, 41) and the Walker-Warburg syndrome (42). It differs histologically from type 1 lissencephaly because the four distinctive layers of type 1 are replaced by a less clearly defined mixture of neurons of various sizes and orientations and by large pyramidal cells displaced in superficial parts of the cortical plate. Gliomesenchymal bundles extending into the cortex from the pial surface are characteristic of type 2 but not of type 1 lissencephaly (41, 42). In a 23-week fetus with type 2 lissencephaly associated with Fukuyama muscular dystrophy, the ependyma of the lateral ventricle appeared intact with normal basal processes and GFAP reactivity as expected at this age (43).

Hemimegalencephaly is associated with several syndromes, some with and others without somatic hemihypertrophy of the face, trunk and extremities. Two neuropathological patterns are found: in the first, a cytological dysgenesis resembles the cerebral lesions of tuberous sclerosis; in the second, often seen in the epidermal nevus syndrome, a disturbance of neuroblast migration to the cerebral cortex is accompanied by abnormal gyration (9, 44, 45). Case 5 belongs to this latter category; it resembles the cases of bilateral lissencephaly/pachygryia not only because of the poor cortical laminar and the columns of incompletely migrated neurons in the subcortical white matter, but also because of similar alterations in the ependyma including the persistence of S-100 protein and cytokeratin immunoreactivity.

We would conclude that the ependyma in various types

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Fig. 14. Case 3. The extremity of the occipital horn of the lateral ventricle is replaced by a series of ependymal cell clusters and rosettes that outline a ghost occipital horn without a ventricular cavity. Hematoxylin-eosin. (A) \( \times 25 \); (B) \( \times 100 \).

Fig. 15. Case 8. (A) The ependyma is discontinuous at the ventricular surface of the temporal lobe in this surgical specimen of hemimegalencephaly. In the subventricular region, many complete and incomplete (arrows) ependymal rosettes are found. Fresh blood cells adjacent to these partial rosettes may be due to minor surgical trauma. (B) Neurons are arrested in migration within the subventricular white matter. Hematoxylin-eosin. (A) \( \times 100 \); (B) \( \times 250 \).
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