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Light and Electron Microscopic Studies of Experimental Hydrocephalus*

Ependymal and Subependymal Areas

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Summary. A light and electron microscopic study of the ependymal and subependymal regions of experimental hydrocephalic cats was made. Hydrocephalus was induced by injection of kaolin into the cisterna magna. Cerebrospinal fluid (CSF) turnover was measured in all experimental cats by ventricular perfusion just prior to glutaraldehyde fixation. The cats were sacrificed at 7 (acute hydrocephalus) and at 21 or more days (chronic hydrocephalus) after kaolin. The major pathological findings were: flattened and outstretched ependymal lining, detachment of ependymal cells and rarefaction of subependymal areas with increase in the extracellular space. The significant morphological alterations in acute hydrocephalus, characterized by a marked decreased rate of CSF absorption, were flattening and outstretching of ependymal cells with minimal rarefaction of subjacent tissues. In the acute animal with a measurable amount of CSF absorption, and more clearly, in the chronic animal with higher rates of CSF absorption, detachment of ependymal calls, significant rarefaction of subependymal tissues, and marked increased subependymal extracellular space were the predominant changes. It is concluded that these pathological changes provide the morphologic substrate for transventricular absorption of CSF.

Key words: Hydrocephalus, Experimental — Cats — CSF Turnover — Ependyma — Subependymal Tissue — Extracellular Space — Electron Microscopy.

Introduction

Previous studies on cerebrospinal fluid (CSF) turnover in kaolin-induced, experimental hydrocephalus in cats have shown that 7 days after the intracisternal injection of kaolin there is a marked elevation in intraventricular pressure, a moderate increase in ventricular volume, and a minimal amount of transventricular absorption of CSF (Hochwald *et al.*, 1972a). At 21 or more days after kaolin the restoration of intraventricular pressure to normal range was accompanied by an increase in both ventricular size and transventricular absorption (Hochwald *et al.*, 1972a; Hochwald and Sahar, 1972b). The transition from the acute to the chronic stage of the hydrocephalic process was associated with an increase in periventricular water content of white matter derived from the spinal fluid compartment during transventricular absorption (Lux *et al.*, 1970).

Although structural changes in the central nervous system resulting from the obstruction of the spinal fluid circulation have been recorded, a description of the

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histological and ultrastructural alterations in the brains of experimental hydrocephalic cats associated with transventricular absorption of CSF is lacking. The purpose of this study was to search for morphological correlates which may aid in explaining the development of alternate CSF absorption pathways when the normal CSF circulation is blocked. CSF turnover in these animals was measured by perfusion of the ventricular system.

Materials and Methods

Adult mongrel cats weighing 2—3 kg were used. To produce hydrocephalus, kaolin (200 mg in 0.8 ml of saline) was injected in the cisterna magna. This resulted in aseptic meningitis with severe fibrosis, obliteration of the cisterna magna, occlusion of the outlets of the fourth ventricle, and subsequent hydrocephalus (Hochwald *et al.*, 1969; Schurr *et al.*, 1953). Perfusion of the ventricular system of both normal and hydrocephalic cats was carried out as described previously (Hochwald and Wallenstein, 1967; Hochwald *et al.*, 1969; Hochwald *et al.*, 1972a) either 7 days (acute stage) or 21 or more days (chronic stage) after the injection of kaolin. Normal cats were perfused from the lateral ventricle to the cisterna magna; hydrocephalic cats from one lateral ventricle to the other. All perfusion experiments were carried out under pentobarbital anesthesia. Intravenous Flaxedil (Gallamine triethiodide) was used and the animals artificially respired. The perfusion fluid, an artificial CSF, contained inulin and radioiodinated cat serum albumin as non-diffusible indicator substances (Hochwald and Wallenstein, 1967).

The rates of formation and absorption of CSF were based on the dilution of the tracer substances and differences between inflow and outflow rates during steady-state perfusion (Heisey *et al.*, 1962). Perfusion time varied between $2^1/_2$—4 h. At the end of the experiment, the brain was perfused through the ascending aorta with 5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 30—45 min. The brain was removed and small blocks of tissue were cut with razor blades and immersed for an additional 2—3 h in the same fixative. They were then rinsed overnight in cold 0.1 M sodium cacodylate buffer (pH 7.4). Large sections from the cerebrum, cerebellum, and brain stem were embedded in paraffin, cut at 6 μ and stained with hematoxylin and eosin. Tissues taken for electron microscopic study included ependymal and subependymal areas from the dorsolateral wall of the lateral ventricle at the level of the parietal lobe, the corpus callosum from the frontal horn of the lateral ventricle, and the caudate nucleus. These were post-fixed for 45 min in Dalton's chrome-osmium (Dalton, 1955), dehydrated in alcohol and embedded in Araldite. 2 μ thick sections were cut, stained with toluidine blue, and studied with the light microscope. Thin sections were stained with uranyl acetate and lead citrate and were studied with a Siemens 1A electron microscope.

Four groups of experiments were carried out. In the first group, normal cats were perfused at a pressure ranging from 0—5 cm H_2O with respect to the interaural line. In the 2nd group, normal cats were perfused under an elevated pressure of 10—15 cm H_2O. In the 3rd group, acute hydrocephalic cats, 7 days after intracisternal injection of kaolin, were perfused at pressures which varied between 12—25 cm H_2O. In the fourth group, chronic hydrocephalic cats, approximately 2 months after kaolin, were perfused at pressures which ranged from 10 to 20 cm H_2O. As controls, two normal cats were killed without undergoing ventricular perfusion.

Results

Physiological Observations

The results of the cerebrospinal fluid turnover studies from both acute and chronic hydrocephalic cats were compared during steady-state lateral ventricle-to-lateral ventricle perfusion. The mean rate of CSF formation in both groups of animals was 0.009 ml/min. The capacity to absorb fluid in the acute hydrocephalic cat, however, was markedly decreased. At a perfusion pressure of 15 to

20 cm H_2O, the mean rate of CSF absorption was 0.007 ml/min. In the chronic hydrocephalic animal, perfused at a similar pressure, the mean rate of absorption was 0.046 ml/min.

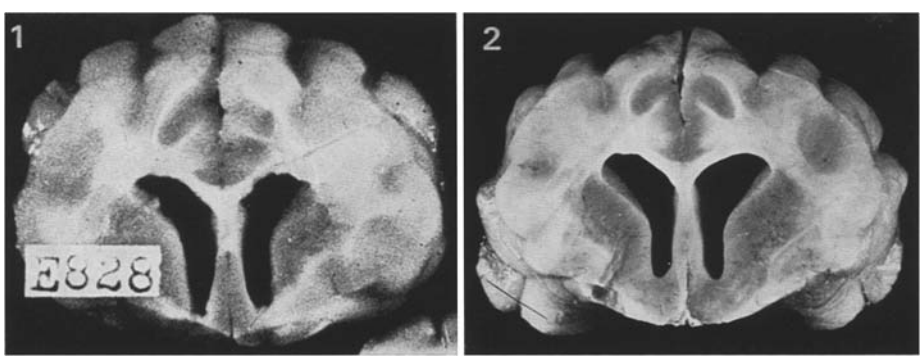
Morphology

The normal ultrastructure of the ependymal region of the cat (Figs. 3 and 6) was similar to that described by Brightman and Palay (1963) and Hirano and Zimmerman (1967) for the rat. The observations to be described were made on both paraffin and plastic embedded tissues. Although the changes noted were apparent in both types of preparations, it was thought that the plastic embedded tissues contained less artifacts, and were therefore, selected for the illustrations. Moreover, the alterations observed were not universal but multifocal, and varied in severity.

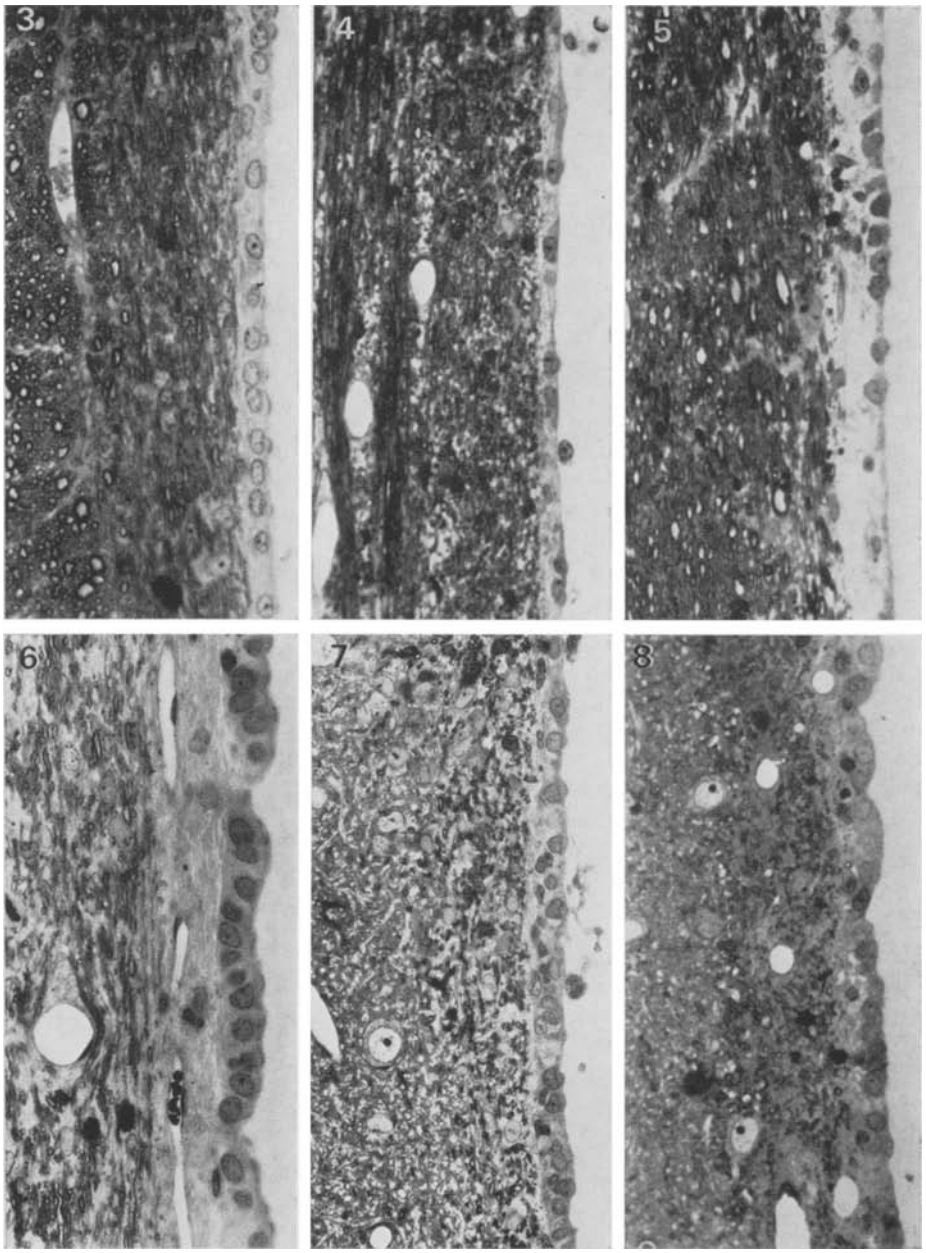
Acute Hydrocephalus

The ventricular system (lateral and third ventricles) were moderately dilated, especially, the lateral angle of the lateral ventricle which appeared rounded off (Fig. 1). Moderate flattening and stretching of ependymal cells over the white matter was observed (Fig. 4) by light microscopy. Similar changes were not seen over the gray matter (Fig. 7). The subependymal and deeper tissues did not show consistent pathological changes. The angle of the lateral ventricle showed more stretching and flattening of the ependymal cells than the rest of the ventricular lining; occasionally some discontinuity of ependymal lining, separation of ependymal cells from the subjacent white matter and slight rarefaction were noted. There were only slight focal infiltrations of polymorphonuclear leucocytes and lymphocytes around some subependymal blood vessels in half of the animals studied.

Electron microscopic studies were carried out in two acute animals. Transventricular absorption of CSF was minimal in one case, undetectable in the other. In the cat with minimal absorption there was marked flattening and stretching of ependymal cells and slight increase of intercellular spaces (Fig. 9). There were also focal areas of discontinuity of ependyma and slight rarefaction of subependymal tissues. In the cat with no measurable transventricular absorption of fluid, the ependymal cells were only moderately flattened and extended (Figs. 10



Figs. 1 and 2. Coronal sections of cat brain. Fig. 1, acute hydrocephalus. 7 days post-kaolin injection. Fig. 2, chronic hydrocephalus 60 days post injection. ×2



Figs. 3—5. Ependymal region of lateral ventricles (parietal lobe, white matter). Fig. 3, normal. Fig. 4, acute hydrocephalus. Fig. 5, chronic hydrocephalus. Plastic embedding. Toluidine Blue stain. ×500

Figs. 6—8. Ependymal region of lateral ventricle (caudate nucleus, gray matter). Fig. 6, normal. Fig. 7, acute hydrocephalus. Fig. 8, chronic hydrocephalus. Plastic embedding. Toluidine Blue stain. ×500

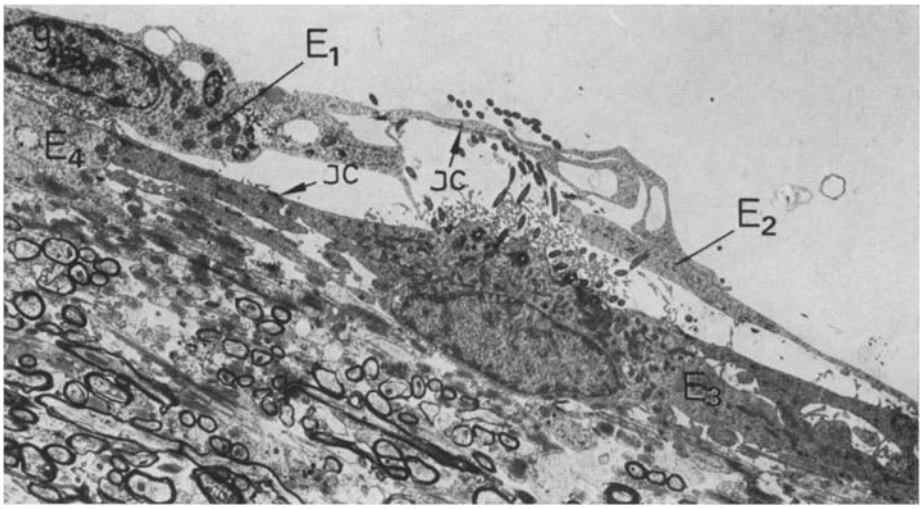


Fig. 9. Ependymal region over white matter of parietal lobe. Acute hydrocephalus with minimal absorption of CSF. The figure shows detached ependymal cells (E_1 and E_2) on the surface of other greatly outstretched ependymal cells (E_3 and E_4) which are attached to each other by junctional complexes (JC). ×5000

and 11). In both cases, however, epichoroidal-like cells were sometimes observed attached to the ependymal surface (Figs. 9 and 11). The subependymal areas underlying the gray matter showed only slight increase of intercellular spaces in both cases. There were no consistent pathological changes noted in the tissues at a depth greater than approximately 200 μ from the ependymal surface.

Chronic Hydrocephalus

The ventricular system was markedly dilated (Fig. 2) even though the intraventricular pressure was considerably lower than in acute hydrocephalus. By light microscopy (Figs. 5 and 8) there was marked flattening, stretching and separation of ependymal cells from the underlying astrocytic subependymal layer than in the acute animal. The white matter beneath the astrocytic layer was slightly rarefied. These changes over the white matter were particularly marked at the angle of the lateral ventricle. Occasionally, there were patchy areas of isomorphic gliosis of the subependymal tissues. They were seen in the absence of hyperplasia of astrocytes. The ependymal and subependymal areas over the gray matter were unremarkable except, perhaps, for an increase in glial fibers. By electron microscopy (Figs. 12 to 14) areas of stretched ependymal cells were noted. However, a diffuse separation of ependymal cells from the subjacent tissues was more frequently seen. The separated ependymal cells were occasionally anchored to the subjacent astrocytic layer by cell processes of various lengths and thicknesses. The separated cells appeared cuboidal, not stretched, and showed long and short irregular processes; their cytoplasm was denser than normal. Some of the separated ependymal cells remained attached to neighboring cells by intact junctional complexes. In the regions where the ependyma was lost,

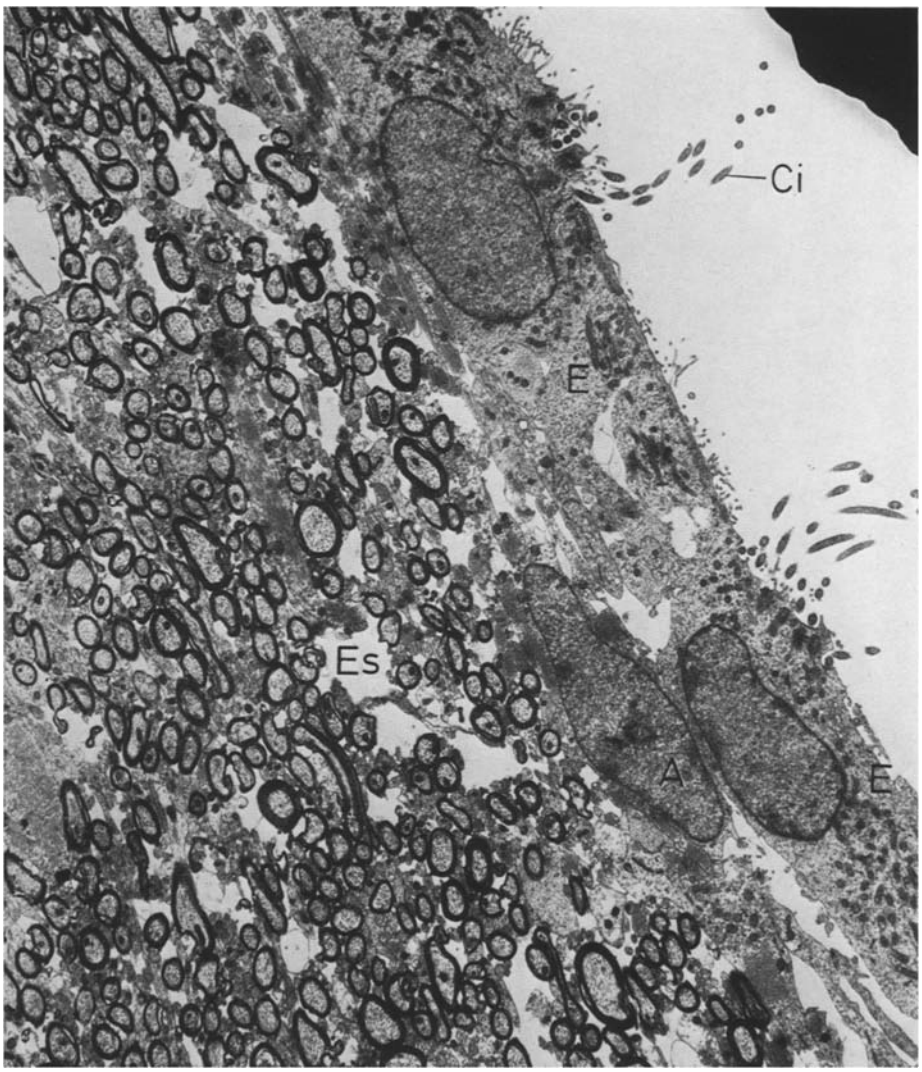


Fig. 10. Ependymal region under white matter of parietal lobe. Acute hydrocephalus with no absorption of CSF. Outstretched ependymal cells (*E*). Extracellular space (*Es*). Astrocyte (*A*). Cilia (*Ci*). ×6000

the subependymal region including capillaries was exposed directly to the ventricular cavity. Of special note was the wide separation of glial cells and myelinated fibers in the subependymal areas. An increase in extracellular space which at some points seemed to be directly continuous with the ventricular compartment was apparent (Fig. 12). The capillaries were unremarkable even through the surrounding astrocytes were often separated from them. These subependymal disruptive changes and enlarged intercellular spaces were seen to a depth of approximetaly 200 μ from the ependymal surface. Tissues deeper than this were normal.

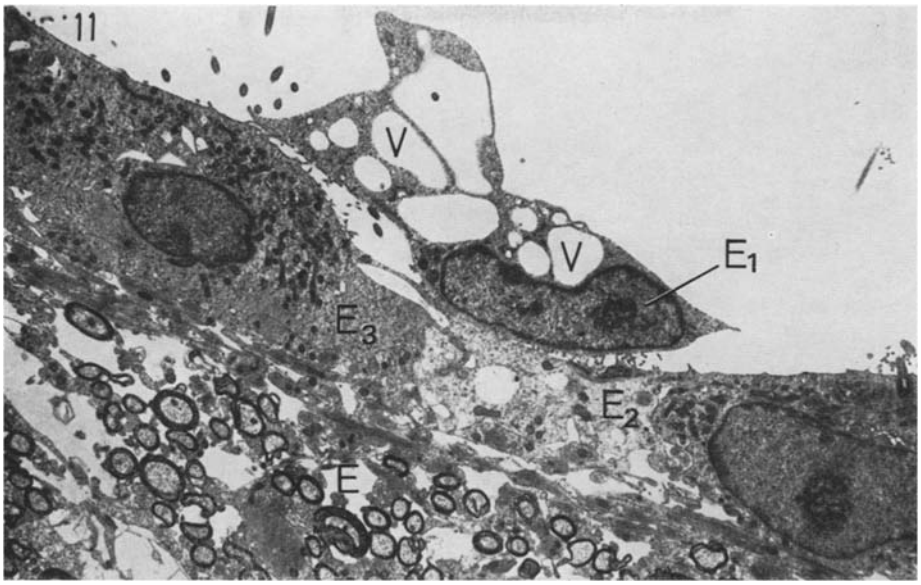


Fig. 11. Ependymal region white matter of parietal lobe. Acute hydrocephalus with no absorption of CSF. Detached ependymal cell (E_1) with vacuolated cytoplasm (V). Extracellular space (E). Other ependymal cells (E_2 and E_3) $\times 6000$

The ependymal region over the gray matter showed no particular disruptive changes except for occasional necrotic ependymal cells and a slight to moderate increase in the number of astroglial filaments (Fig. 14).

Discussion

The effects of the hydrocephalic process on CSF turnover were similar to those previously described (Hochwald *et al.*, 1972a). Although the rate of bulk formation of fluid was similar in both acute and chronic hydrocephalic cats, the rate of bulk absorption of fluid was much smaller in the acute animals. This coincided with previous observations that in the acute hydrocephalic cat, the opening pressure, that is the pressure measured upon penetration of the ventricle, is several times greater than in the chronic hydrocephalic cat (Hochwald *et al.*, 1972a). It was concluded previously that during the change from the normal to the chronic hydrocephalic cat, a transient period of elevated intraventricular pressure occurred during which transventricular absorption developed and resulted in a decreased intraventricular pressure.

The pathological changes observed in kaolin-induced hydrocephalus are considered to be due to the hydrocephalic process itself and not to inflammation. The major alterations in the acute stage were limited to a flattening and stretching of ependymal cells with slight rarefaction of subjacent tissues. This correlates well with the absence of a significant amount of transventricular absorption (Hochwald *et al.*, 1972a). In the acute hydrocephalic cat with a small degree of absorption, slight vacuolization of the subependymal region was noted. In the

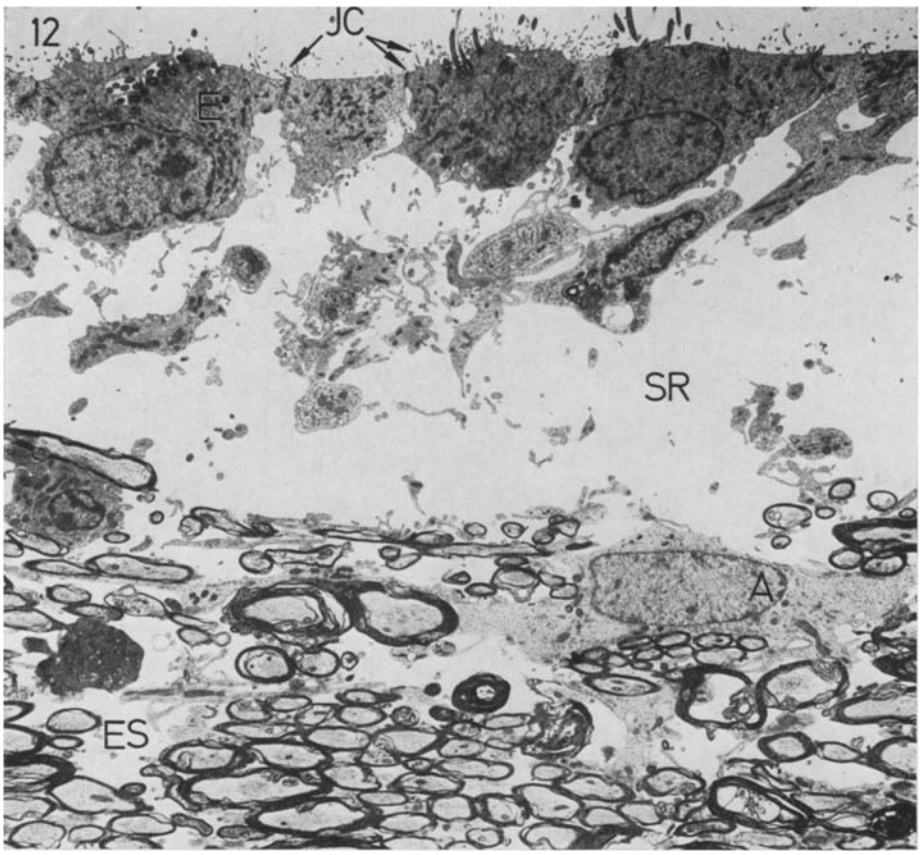


Fig. 12. Ependymal region, white matter of parietal lobe. Chronic hydrocephalus. Partially detached but not outstretched ependymal cells (*E*) still connected to each other by junctional complexes (*JC*). The subependymal region (*SR*) is rarefied. The extracellular space (*ES*) of white matter is considerably enlarged. Astrocyte (*A*). ×4000

chronic hydrocephalic cat in which a much greater rate of bulk absorption of CSF was shown to occur, there was a partial multifocal separation and destruction of ependymal cells, and a considerable increase in extracellular space in the subjacent white matter.

There are few histological and ultrastructural studies of brain tissue in experimental hydrocephalus. Observations on the olfactory bulb of adult rabbits made hydrocephalic by the subarachnoid infusion of silicone oil by Weller and Wisniewski (1969) have shown a stretching of ependyma and compression of periventricular tissue. Severe hydrocephalus was characterized by splitting of ependyma, gross extracellular edema of white matter and destruction of nerve fibers. It was proposed that splitting of the ependymal lining and ensuing free flow of CSF into the white matter could enhance tissue damage caused by the compression effects of hydrocephalus. A generalized stretching of ependymal surface with flattening and compression of ependymal epithelium and edema of the

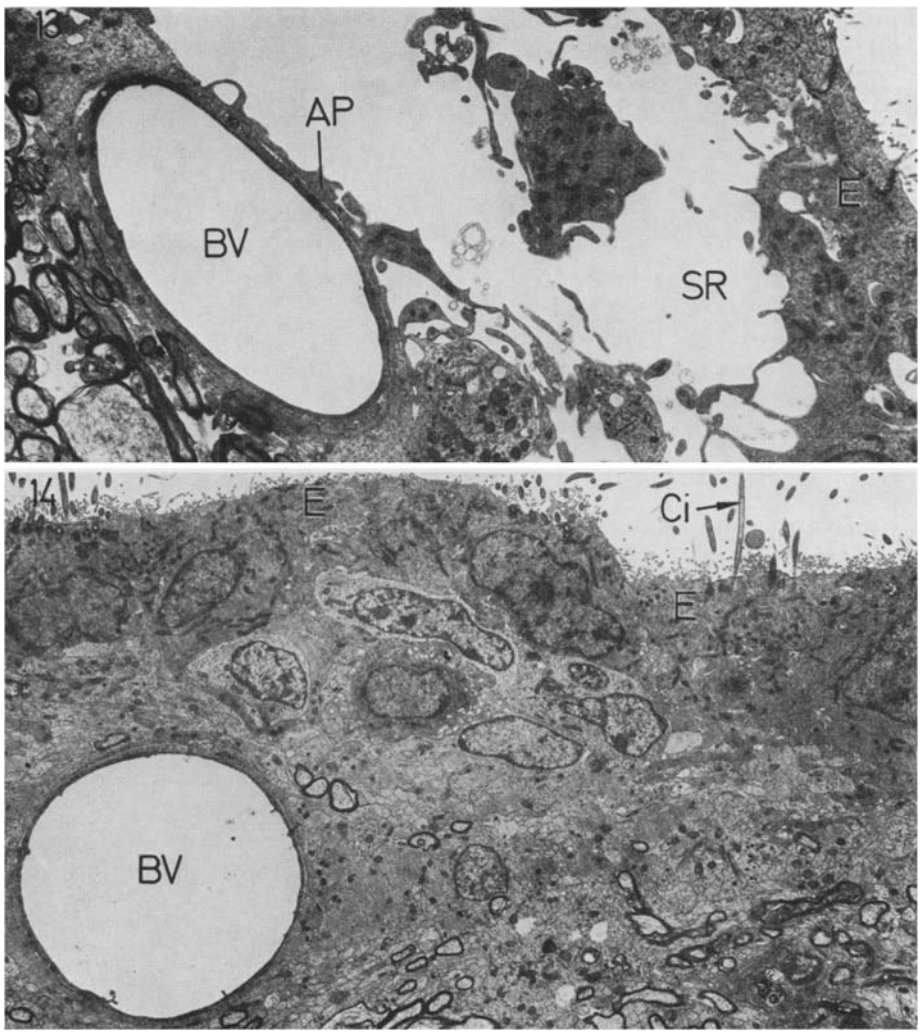


Fig. 13. Subependymal region (*SR*), white matter of parietal lobe. Chronic hydrocephalus. Ependymal cells (*E*). Blood vessel (*BV*) surrounded by thin astrocytic processes (*AP*). ×4000

Fig. 14. Ependymal region gray of caudate nucleus. Chronic hydrocephalus. Ependymal cells (*E*). Cilia (*Ci*). Blood vessel (*BV*). Note the absence of rarefaction. ×4000

damaged ependyma of the lateral ventricle has also been demonstrated by obstructing the cerebral aqueduct of monkeys with an inflatable balloon (Clark and Milhorat, 1970).

Movement of CSF into the periventricular tissue (CSF edema) would be expected to play an important role in establishing pathological alterations other than those resulting from an expanding ventricle. It should be emphasized that compression of brain tissue and stretching of the periventricular region due to increased ventricular pressure is modified by infusion of CSF into the tissue.

The histological changes found in the periventricular region were almost exclusively limited to the ependymal lining of the white matter. This same area in the gray matter remained virtually unchanged. This could be attributed to a greater vulnerability of the white matter to the pressure of edema fluid.

The major morphological difference between acute and chronic hydrocephalus lies in the severity of the structural changes in the periventricular region. The enlarged extracellular spaces are in direct communication with the ventricular compartment. This has been called "CSF edema" in comparison to other forms of cerebral edema where excessive extracellular fluid is apparently derived from the blood (Wisniewski, *et al.* 1969). Since there is no increase in permeability of blood-brain-barrier to protein in these experimental animals (Hochwald *et al.*, 1972a), the possibility of the fluid being derived from the intracerebral blood vessels seems less likely. It has been suggested from previous results (Hochwald *et al.*, 1972a; Lux *et al.*, 1970) that this extracellular fluid is cerebrospinal fluid undergoing transventricular absorption. An increase in intraventricular pressure results in the bulk flow of fluid to the blood possibly through the endothelium of periventricular capillaries. In normal animals, these same spaces are thought to contain extrachoroidal sources of newly formed cerebrospinal fluid flowing from the brain tissue to the ventricles (Hochwald and Wallenstein, 1967). In normal animals with intact ependymal lining, there is no evidence of transventricular absorption, and CSF flows from the ventricles to the cranial subarachnoid space where it passes into the blood via the arachnoid villi (Welch and Friedman, 1960). The present studies with hydrocephalic cats suggest that the morphological changes noted in the ependymal and subependymal tissue result from the obstruction of the cerebrospinal fluid circulation, which in turn, gives rise to alternate pathways for cerebrospinal fluid absorption.

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