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Ultrastructural Observation on the Wall of Fourth Ventricular Foramina Following Intraventricular Blood Infusion in the Cat

— A Transmission and Scanning Electron Microscopic Study —

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The ultrastructural changes of the wall of the fourth ventricular foramina following intraventricular autogenous blood infusion present four meaningful findings for the pathogenesis of secondary hydrocephalus. Using the transmission and scanning electron microscopy (TEM and SEM), it has found that minimal to marked separation of the intercellular cleft coincided with the intra- and intercellular vacuolation and swelling of the glial fibers in the subependymal glial sheath by the 7th day of blood infusion. A flattening of the contours of the ependymal cells and their nuclei was noted under the TEM and a seperation of ependymal cells was pronounced under the SEM during the period between the 28th and 42nd day. Ultrastructural changes of the ependymal cells correlated with the time factor and not with the blood volume infused. The supraependymal cells (SEC) seen on the ventricular surface were indicative of neuron-like structure rather than macrophages.

Key Words: Fourth ventricular foramina. Blood infusion. Hydrocephalus. Transmission electron microsocopy. Scanning electron microscopy. Ependymal cell. Supraependymal cell.

In the previous transmission and scanning electron microscopic analysis of a cat's ventricular surface following intraventricular autogenous blood infusion, the emphasis was directed chiefly toward the ependymal lining of the whole ventricular system except for the fourth ventricular foramina. The previous findings preliminarily elucidated that intraventricular autogenous blood infusion was supposed to result in an impairment of the cerebrospinal fluid(CSF) circulation by alteration of the ependymal surface and subependymal glial sheath (SEG) (Kim *et al.*, 1982). The ultrastructural changes

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on the wall of the fourth ventricular foramina following intraventricular hematoma have had little attention in the past. However, the potential pathological changes following intraventricular blood infusion are significant enough to warrant this study. The present study is a continuation of the previous study described in the above analysis and the following questions were assessed:

1. Do ultrastructural changes occur on the wall surface of narrow canals such as both foramina of Magendie and Luschka, as found in the aqueduct of Sylvius which is remote from the blood-infused frontal horn?

2. If there is any evidence of hydrocephalus, by what mechanisms and with what sequences are such changes brought about?

3. Are there any subsequent ventricular surface changes compatible with the transient but acute intraventricular pressure (IVP) increase following intraventricular autogenous blood infusion, making special note of the relationship of these changes to the unit time factor and the amount of blood infused?

4. Are there any supraependymal cells (SECs) indicative of neuron-like structures or macrophages and what is their significance following intraventricular autogenous blood infusion?

The main purpose of this study was to clarify the pathological changes involved in secondary hydrocephalus following intraventricular hematoma, focusing our attention to the ultrastructural changes on the wall of the fourth ventricular foramina. This experimental study was not done to mimic the tissue changes in human hydrocephalus. However, the observations might be applicable to elucidate the difficult problem of human hydrocephalus.

MATERIALS AND METHODS

Adult cats used in a previous study, showed a morphological alteration in the general ventricular surface.

This alteration demonstrated in the cat was similar to that observed in a human (Kim *et al.*, 1982). For this reason, we postulated that a cat's ventricle were selected because of their vulnerability and relatively critical narrow canals to pass blood.

A total of 34 mature, male and female, 10 to 12 month-old cats were used. The cats weighed 2 to 3.5kg. From the day of first contact, the cats were kept in individual cages for a minimum of 15 days. The experimental animals were placed on antibiotic premedication with ampicillin (10mg/kg. i.m.) as a precaution against intraventricular infection.

The control series consisted of three cats that were examined for the 'de novo' fourth ventricular foramina.

The experimental series were grouped according to the infused blood volume, that is, 1cc, 2cc, and 3cc. Selected cats from each group were sacrificed on the 1st, 7th, 14th, 28th, and 42nd day following intraventricular infusion of autogenous blood.

To estimate the gross amount of cat ventricular volume, a linear vertical scalp incision was made extending 2cm in front of the coronal suture line extended across the midline caudally to expose the cisterna magna. The arachnoid membrane was then opened to drain out the CSF. The right frontal horn of the lateral ventricle was punctured stereotactically with a 23 gauge long spinal needle. The dye infused into the right frontal horn was seen flow down to the opened cisterna magna amounted to less than 1cc, which was the gross ventricular volume, identical to that of Hochwald's finding (Hochwald *et al.*, 1972).

Specimens were prepared in the following manner;

The cats were anesthesized with intraperi-

toneal luminal injection (20mg/kg) and a midline thoracoabdominal incision was made. The descending aorta was clamped off at the level of the renal arteries. A cannula carrying the perfusate was inserted into a small incision made in the right atrium which served as an outlet for the blood and perfusate to drain easily.

Immediately intracardiac perfusion via the aortic arch was done. For this perfusion, 1000cc's each of normal saline and 2% glutaraldehyde was perfused for 30 minutes. The pressure which was maintained during the 30 minutes corresponded to 150cm of water.

From the control and experimental cats, tissue specimens of the brain were dissected into two blocks. Of the two block specimens, one was obtained from the wall of the foramen of Magendie and the other from the wall of the foramen of Luschka.

Except for the choroid plexus, the specimens were washed with normal saline and 2% glutaraldehyde, then fixed over-night with 3% glutaraldehyde and 0.1% cacodylate buffer at pH 7.4. The specimens were stored at 4 C throughout the procedure.

After fixation, the specimens were trimmed under the dissecting microscope to expose further the wall of fourth ventricular foramina.

For the TEM, the specimens were postfixed in 1% phosphate buffered osmium tetroxide at 4°C, pH 7.4 for 2 hours, dehydrated through graded concentration of alcohol, and then embedded in Epon 812. Ultrathin sections were additionally contrasted with lead citrate and uranyl acetate, then examined under transmission electron microscope and phtomicrographied recordings were made.

For the SEM, the specimens were postfixed in 1% phosphate buffered osmium tetroxide at 4 C, pH 7.4 for 2 hours, dehydrated through graded concentration of alcohol, then embedded in isoamylacetate for 30 minutes, dried in critical point drier in CO_2, mounted on stubs, plated with gold-palladium particles, and examined with the scanning electron microscope. The photomicrographs were taken with positive-negative 4x5 polaroid Land Films. The results from both TEM and SEM were based upon approximately 130 photomicrographs.

RESULTS

A. The transmission electron microscopic findings (The wall of the fourth ventricular foramina)

1. The control series (3 cats): A guide to the cross-sectional anatomy seen in TEM is provided in Fig. 1. Table 1 shows a typical ultrastructure of the wall of the fourth ventricular foramina. The ependymal cells are cubodial (or cylindrical) in shape and their cell borders have interdigitated plasmalemmata. The luminal surface of ependymal cell in covered with cilia and microvilli. Below the ependymal layer, parallel rows of glial fiber are seen in the subependymal glial sheath (SEG).

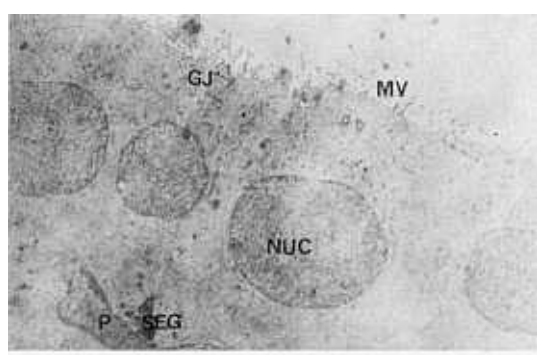

Fig. 1. Transmission electron microscopic findings of the control cats fourth ventricular formaina. Cilia (C); microvilli (MV); pial cell (P); subependymal glial sheath (SEG). Note ovoid shape of nucleus (NUC) and gap junction(GJ↓). X5000

Table 1. The transmission electron microscopic findings on the wall of the fourth ventricular foramina

Group	Unit time	Ependymal cell: Cell or nucleus contour	Ependymal cell: Intracellular vacoule	Ependymal cell: Intercellular cleft	Ependymal cell: Cilia	Ependymal cell: Microvilli	SEC****	Subependymal glial sheath: Glial fibers: Swelling or demyelination	Subependymal glial sheath: Glial fibers: Atrophy	Subependymal glial sheath: Pial cell ingrowth
Control series		Cuboid or ovoid	None	None	Intact	Numerous around cilia	Present	None	None	None
Exp. series	1st day	Moderate flattening	Present	Minimal separation	Intact	Minimal Sparsity	Present	Moderate	None	Present
	7th day	Marked flattening	Present	Marked separation (EDP***)	Minimal or moderate sparisty	Minimal Sparsity	Present (INV)	Marked	None	Present
	14th day	Moderate flattening (INV)	Present (LIB*)	Moderate separation (EDP**)	Minimal sparsity	Minimal Sprasity	Present	Minimal	Borderline	Present
	28th day	Moderate flattening	Present (LIB*)	Moderate separation (EDP**)	Minimal sparsity & clumping***	Minimal Sparsity & Clumping***	Present	Minimal	moderate	Present
	42nd day	Moderate flattening	Present (LIB*)	Minimal separation (EDP**)	Minimal sprasity marked clumping***	Minimal Sparsity Marked Clumping***	Present	Minimal	Marked	Present

LIB* : Lipoid inclusion bodv, EDP** : Electron dense particle, Clumping*** : Clumping togethr of cilia and microvilli with RBC debris,
SEC**** : Supraependymal cell, INV : Intracellular vacuole

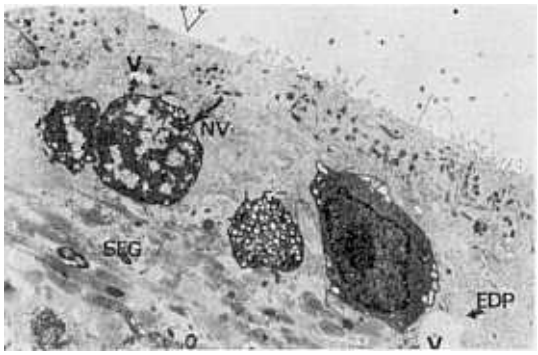

Fig. 2. Minimal flattening of ependymal cells with intranuclear vesicle(black arrow ↓) and moderate glial fiber swelling in subependymal glial sheath (SEG). Note paucity of microvilli (open arrow↓), intra-or inter-cellular vacuole (V) and electron dense particles (EDPs) in 1st day following blood infusion. x4200

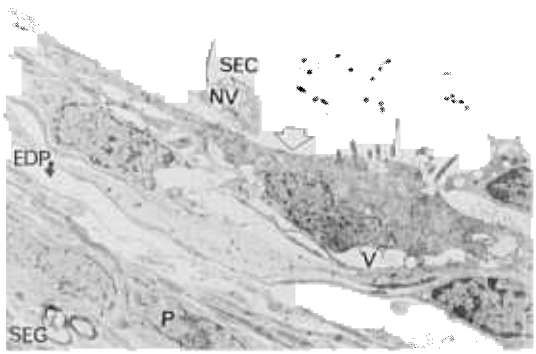

Fig. 3. Marked flattening of ependymal cells with intracellular vacuole and intercellular cleft widening. In addition on the SEC containing intracellular vacuole (NV) and paucity of microvilli (open arrow ↓), the pial cell ingrowth is well shown among markedly swollen subependymal glial sheath (SEG). 7th day following blood infusion x3800

2. The experimental series (30 cats): In the early phase (from the immediate blood infusion to the end of the 7th day), the surface cilia and microvilli were relatively sparse in population density but individually they had intact morphological characteristics. The ependymal cells were seen to lose their cell contours as well as to make a transformation of an ovoid nuclear shape to a flat one. The cellular borders became more horizontal than vertical as seen in the control series (Compare Fig. 1 with Fig. 3).

To keep up with the IVP increase, the intracellular vacuolation associated with moderate to marked flattening of ependymal cells and a minimal to a marked separation of the intercellular cleft was demonstrated (Compare Fig. 1 with Fig. 2 and Fig. 3). The 7th post-infusion day specimens showed the most significant separation in the intercellular junction.

This separation revealed the morphological severity was, in part, correlated with the unit time factor (Fig. 3 and Table 1).

The vacuoles in the ependymal cell nucleus and electron dense particles (EDPs) at the intercellular celft were identified in the 1st and 7th post-infusion day specimens (Fig. 2, 3 and Table 1). Their appearance was presumed to be from the degradation of red blood cells (RBCs) from the infused blood and from IVP increase following blood infusion.

In the subependymal glial sheath (SEG), wide separation of glial fibers and pial ingrowth was demonstrated (Fig. 3 and Table 1). The glial fiber swelling or demyelination was the most prominent in the 7th post-infusion day specimen (Fig. 3 and Table 1). The SECs-suggested to be

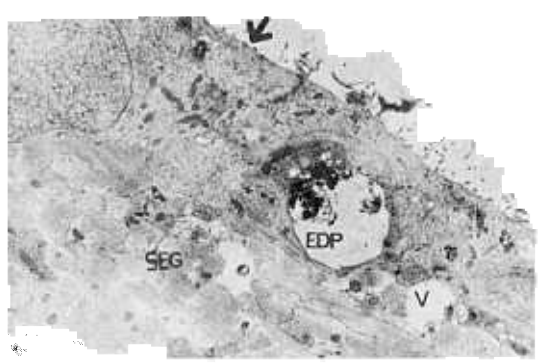

Fig. 4. Moderate flattening of ependymal cells with intracellular electron dense particles (EDPs) contained in them. Marked swelling of glial fibers in the SEG. 14th day following blood infusion. x5700

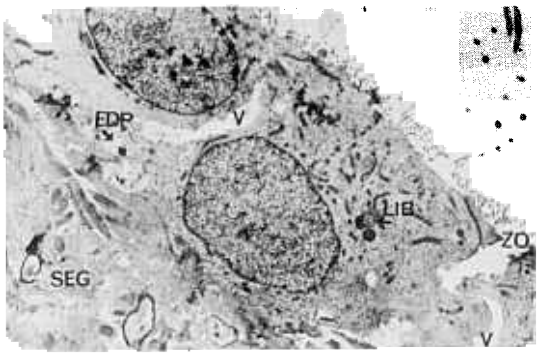

Fig. 5. Moderately separated intercellular cleft (ZO) with still moderately flattened ependymal cells. Also note the lipoid inclusion body (LIB) seen in the ependymal parenchyme, electron dense particle (EDP) and atrophic stage of SEG. 28th day following blood infusion. x5300

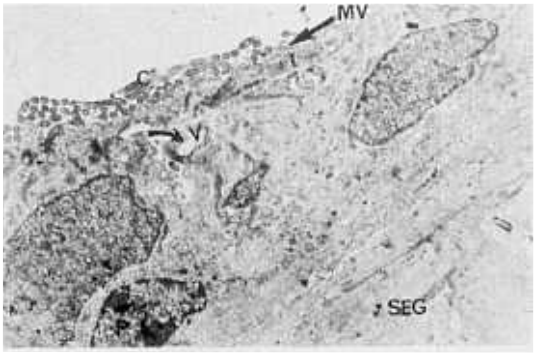

Fig. 6. Marked clumping together of cilia and microvilli with RBC debris (black straight arrow ↓). Still moderate flattening of ependymal cells as well as markedly atrophied SEG is identified. Note pial cell (P) ingrowth. 42nd day following blood infusion. x6700

neuronal in character-were found from the 1st day to the end of 42nd post-infusion day specimens, as found in the control series (Fig. 3 and Table 1). Specifically, they had intracellular vacuoles in the 7th post-infusion day specimens (Fig. 3).

In the intermediate phase (from the 7th to the end of 28th post-infusion day), the surface morphology of ependymal cells showed minimal sparsity in the density of cilia and microvilli indicative of more prominent bare areas (Fig. 4 and Table 1).

When the ependymal cells, with the same nuclear variation, began to have moderate flattening contours again, the intercellular electron dense particles were still observed to have intracellular lipoid inclusion bodies (LIBs) (Fig. 4, Fig. 5 and Table 1). In accordance with the above findings, intercellular cleft separation was in a stationary state (moderate separation) with a horizontal intercellular border (Fig. 4 and Fig. 5). In the SEG, the glial fiber swelling or demyelination was progressive to an atrophic state and therefore the axonal borders could not be confirmed definitely with the constant pial cell ingrowth (Fig. 4, Fig. 5, Fig. 6 and Table 1).

In the late phase (from the 28th to the end of 42nd post-infusion day), it was demonstrated that marked clumping of cilia and microvilli with RBC debris was associated with minimal sparsity of cilia and microvilli (Fig. 6 and Table 1).

The ependymal cells and their nuclei had a moderate flattening contour with minimal intercellular cleft separation. The SEG demonstrated a minimal degree of glial fiber swelling or demyelination and marked atrophy of the axonal processes (Fig. 6).

Lastly, the above findings shown on the wall of both foramina of Magendie and Luschka were nearly similar in appearance. It was supposed that the ultrastructural changes of the two foramina were the same because the wall structure of both foramina of Magendie and Luschka was, in a sense, an extension of the fourth ventricular floor.

B. The Scanning Electron Microscopic Findings (The Wall of Fourth Ventricular Formaina)

1. The Control Series (3 cats): A typical view taken under the SEM was punctuated by numerous ependymal cells that possessed trichomatous tufts of long cilia. But the cellular

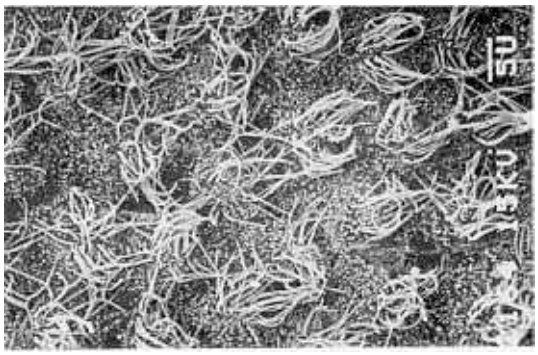

Fig. 7. Scanning electron microscopic view of control cat fourth ventricular floor. There are trichomatous tufts of long cilia and relatively dense microvilli. The distance among ciliary tufts is somewhat sparse. x2000

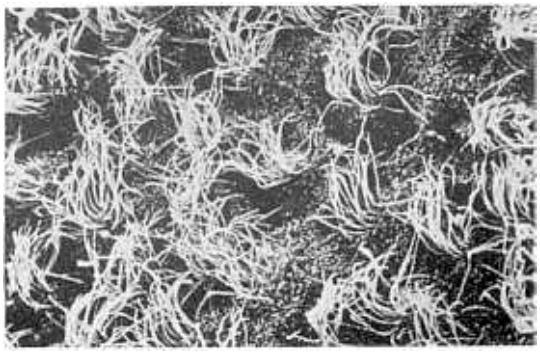

Fig. 8. SEM of the 1st post-infusion cat revealed minimal sparsity of microvilli without definite deminution of ciliary tuft density. x2000

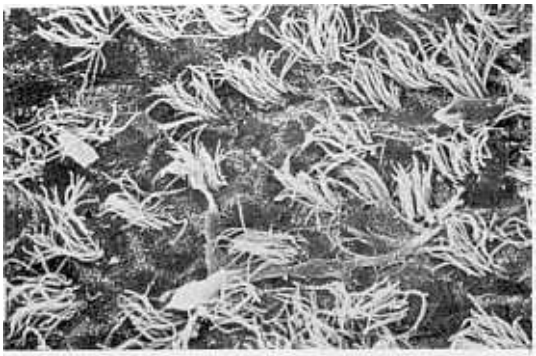

Fig. 9. SEM of the 7th post-infusion day cat. Note deformed RBC debris adhering to cilia and microvilli. The SECs are definitely identified among tufts of cilia and microvilli. x2000

boundaries were not definitely outlined by densely matted microvilli.

It was noted that the tufts of cilia had a multidirectional wave pattern (metachronal activity) and some ends of ciliary tufts had rounded knobs.

The distance among the ciliary tufts was relatively sparse, but the remaining larger spaces were mostly covered with microvilli except for some bare areas.

In addition, the axonal processes of the curious, stellate and free cell (SEC) were found to be surrounded by cilia and microvilli.

The above findings were observed on the wall of both foramen of Magendie and Luschka (Fig. 7 and Table 2.).

2. The Experimental Series (30 cats): In the early phase (from the immediate blood infusion to the end of the 7th day), the tufts of cilia seemed to be intact until the 1st post-infusion day. But the microvilli became progressively sparse in population density on the cell surface between widely spread tufts of cillia (Fig. 8 and Fig. 9). The metachronal activity of cilia decreased minimally as the RBC debris began to adhere to tufts of cilia and microvilli in the 7th post-infusion day specimens (Fig. 9 and Table 2). The surface anatomy of SEC (maybe axonal process in character) was more apparent in the 7th post-infusion day specimens (Fig. 8 and Fig. 9).

In the intermediate phase (from the 7th day to the end of 28th day), the individual morphology of cilia and microvilli was still unchanged with an equal degree of moderate sparsity (Compare Fig. 9 with Fig. 10), but specifically microvilli showed a moderate sparsity (Fig. 10, Fig. 11 and Table 2). The pattern of cilia was significantly altered in metachronal activity: here cilia and microvilli were not randomly distributed over the surface compared with the control series, but they appeared to be

Table 2. The scanning electron microscopic findings on the wall of the fourth ventricular foramina. (The ependymal cell surface)

Group	Unit time	Tuft of cilia		Microvilli	Distance between ciliary tuft	Cellular boundary	RBC debris	SEC
		Density	Metachronal activity					
Control series		Profuse	Present	Profuse	Relatively compact	Not identifiable	None	Present
Exp. series	1st day	Profuse	Present	Minimal Sparsity	Minimal separation	Identifiable	Present	Present
	7th day	Profuse	Minimal decrease	Moderate Sparsity	Minimal separation	Identifiable	Present Adehsion with cillia and microvilli	More Apparent
	14th day	Profuse	Moderate decrease	Moderate Sparsity	Moderate separation	Identificable	Present Adhesion with cilia and microvilli	Present
	28th day	Profuse	Marked decrease	Moderate Sparsity	Moderate separation	Identifiable Minimal inter-cellular cleft	Increase in number and dense adhesion with cilia and microvilli	Present
	42nd day	Profuse	Marked decrease	Moderate Sparsity	Marked separation	Identifiable Marked inter-cellular cleft	More dense adhesion with cilia and microvilli	More Apparent

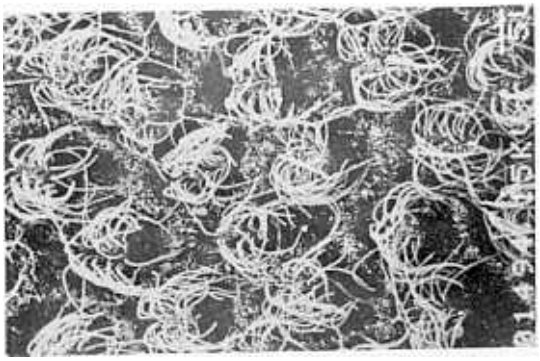

Fig. 10. SEM of the 14th post-infusion day cat. There is no definite interval change in the distance among ciliary tufts and microvilli. The microvilli are seemed to be aggregated at the intercellular border. x2000

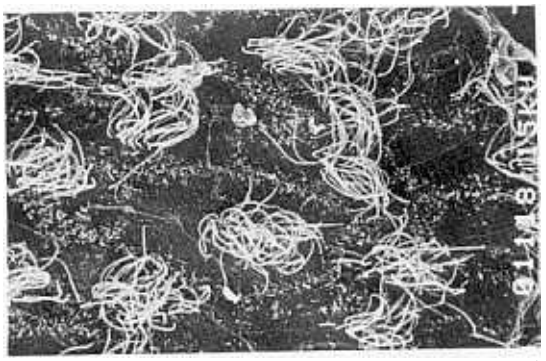

Fig. 11. SEM of the 28th post-infusion day cat showed marked decrease of ciliary metachronal activity. RBC debris are seen to adhere to the ciliary tufts and microvilli. x2000

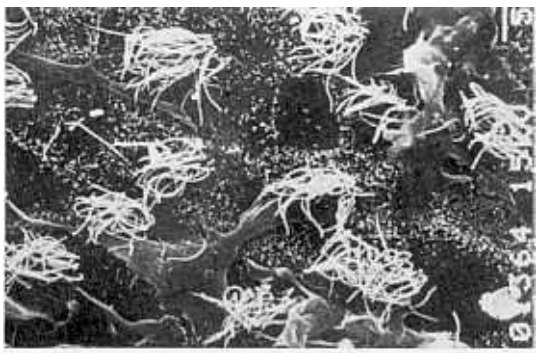

Fig. 12. SEM of the 42nd post-infusion day cat. Marked separation of ciliary tufts, moderate sparsity of microvilli and widened intercellular cleft. The clumping together of cilia and microvilli due to RBC debris without definite appearance of these deformed features as scavenger cells. x2000

more adherent to and to be covered with RBC debris (Fig. 11).

In the 28th post-infusion day specimen, the intercellular clefts were seen as the first evidence of a surface destructive process at the zonula occludens (ZO) (Compare Fig. 5).

In the late phase (from the 28th post-infusion day to the end of the 42nd day), the tufts of cilia were not so heavily compacted, but microvilli were moderately sparse (Fig. 12).

Also the 42nd post-infucion day specimen revealed that most of the cilia and microvilli were covered with RBC debris in addition to apparent intercellular cleft separation and axonal processes of SECs (Fig. 12).

Another noteworthy TEM and SEM findings revealed that the ultrastructural changes shown in this experimental study were correlated with the unit time factor, not with the transient IVP increase due to a small volume of autogenous blood infusion (less than 3cc).

DISCUSSION

Basically, under the TEM, we could observe the permeability change between the ependymal cells and subependymal glial sheath, while under the SEM, the three dimensional surface features including structural and permeability changes could be studied.

Any interpretation of the foregoing observations must be tempered with caution due to the obvious limitation of TEM and SEM technique. Clearly, the critical identification and confirmation of substructures such as cilia, microvilli and other membranous modifications, is difficult to estabilli and other membranous modifications, is difficult to estabilish with certainty even though with paralled cross-sectional analysis of the same specimens with TEM. Further, within this context, lies the obvious pitfall of misinterpretation due to distortion of physical

alteration of the ventricular wall surface through preparative technique, postmortem autolysis, and/or pontential sexual difference (Scott *et al.*, 1973; Bruni, 1978). Nevertheless, it would appear that there are subtle regional variations in the surface architecture of the adult ventricular wall paralleled by more sharply defined structural diversities in fetal brains (Scott *et al.*, 1973).

Since Burr and McCarthy (1900) conducted the first studies on experimental internal hydrocephalus, many investigators have created internal hydrocephalus in numerous animals by various methods. Most of the methods used to create the hydrocephalus are mechanical, chemical or by using an infectious agent, For example, a mechanical agent makes an inflated balloon type of obstruction within the ventricular system and/or subarachnoid space. The balloon effect can be made by an intracisternal injection of kaolin (Fishman and Greer, 1963; Hochwald *et al.*, 1969; Clark and Milhorat, 1970; Lux *et al.*, 1970; Milhorat, 1070a; Milhorat *et al.*, 1970b; Sahar *et al.*, 1970; Dohrmann, 1971; Weller *et al.*, 1971; Hochwald *et al.*, 1972; Ogata *et al.*, 1976; Page, 1975; Go *et al.*, 1976; Torvik *et al.*, 1976; Gopinath *et al.*, 1979) or by subarachnoid infusion of inert, non-inflammatory silicone oil (Wisniewski *et al.*, 1969). Other agents include intraventricular injection of India ink or lampblack (Wisniewski *et al.*, 1969) leading to such an aqueductal stenosis as found in secondary phenomenon following radiation (Hicks, 1953) or viral infections (Nielsen and Gauger, 1974).

The lesions resulting from the above methods manifested ventricular dilatation proximal to the obstruction, especially in the narrow lumen of the aqueduct of Sylvius and cisternal portion at the brain base. These lesions are a major cause of hydrocephalus in the form of inflammatory and/or adhesive reaction followed by atresia, simple stenosis or gliosis (Nielsen and Gauger, 1974).

In this experimental study, we extensively observed the ultrastructural changes on the wall of fourth ventricular formaina to define further the role of intraventricularly infused blood in the development of hydrocephalus.

The cerebrospinal fluid (CSF) in the cerebral ventricles is separated from the nervous tissue by the ependymal cell layer (Brightman and Reese, 1963; Worthington and Cathcart, 1963; Weindl and Joynt, 1972; Fleischhauer, 1973; Scott *et al.*, 1973; Scott *et al.*, 1974; Dempsey and Nielsen, 1976). The ependymal cells constitute not only a part of the barrier between the CSF and the nervous tissue but are also responsible for the exchange of fluid substances and information from the CSF to the brain (Oksche, 1956; Fawcett, 1962; Brightman and Palay, 1963; Dobbing, 1963; Fleischhauer, 1964; Leonhardt, 1968; Wittkowski, 1968; Noack and Wolff, 1970; Lee, 1971; Clementi and Marini, 1972; Noack *et al.*, 1972; Westergaard, 1972; Leonhardt and Lindemann, 1973; Scott *et al.*, 1974; Bruni *et al.*, 1978).

The human ependyaml cells are nearly identical in their ultrastructural features to the ependyma of other animals, even though the morphological features of cilia and microvili are different according to the species of animal (Worthington and Cathcart, 1963; Clementi and Marini, 1972; Fleischhauer, 1973; Scott *et al.*, 1973; Bruni, 1974; Scott *et al.*, 1974).

Because the whole ventricular system consists of craters, folds, ridges and polipoid protrusions, and ultrastructurally consists of cilia, microvilli and the still functionally unidentified supraependymal cells (SECs), the relatively smooth to granular appearing plasmalemmata are completely hidden (Friede, 1961; Brightman and Palay, 1963; Clementi and Marini, 1972; Fleischhauer, 1973; Page, 1975; Bruni, 1978).

The cilia can be observed projecting from the center of the ependymal cell with a tuft of rough-surfaced cilia which emanate also from the central part of the cell. The ciliary tips have bleb-like protrusions which are different from species to species (Worthington and Cathcart, 1963; Scott *et al.*, 1973; Dempsey and Nielsen, 1976; Bruni *et al.*, 1978; Sturrock, 1978);

In addition to cilia, there are microvilli which appear as a surface modification. The microvilli are numerous, especially, at the periphery of the ependymal cells and cover the remaining or background surface (Oksche, 1956; Friede, 1961; Fawcett, 1962; Bering and Sato, 1963; Fleischhauer, 1964; Wittkowski *et al.*, 1968; Weller and Wisniweski, 1969; Noack and Wolff, 1970; Weller *et al.*, 1971; Clementi and Marini, 1972; Noack *et al.*, 1972; Weindl and Joynt, 1972; Fleischhauer, 1973; Scott *et al.*, 1973; Bruni, 1974; Scott *et al.*, 1974; Go *et al.*, 1976; Bruni *et al.*, 1978; Gopinath *et al.*, 1979). Over all the region of the fourth ventricular floor between isolated groups of cilia, the densely matted microvilli are situated around a tuft of cilia (Scott *et al.*, 1973).

The supraependymal cells (SECs) are small, triangular-shaped cells that have stout palmate processes with a ruffled border. The cell bodies have processes that measure about 40mμ in length and have diameter 1-1.5mμ. The processes cross the ventricular lumen in a winding path that varies in extension and frequency by dilating "en passant" or more often at the ends. Some of processes apparently also traverse the ependyma into deeper layers. This assumption is supported by the crater-like depressions in the ventricular surface where these processes appear to end (Brightman and Palay, 1963; Leonhardt and Lindemann, 1973; Chamberlain, 1974; Page, 1975; Bruni *et al.*, 1978; Sturrock, 1978; McKenna and Chairetakis, 1980). There are two types of specialized structures between the ependymal cells (Dobbing, 1963; Lee, 1971). One is an open gap junction between the ciliated ependymal cells associated with the other characteristics of the ciliary patterns which are related to the gray matter. The other is over white matter where the ciliary density is sparse and there is a tight junction instead. The morphologic and functional significance is not constant among animals of the same species or from species to species (Bering and Sato, 1963; Brightman and Reese, 1969; Weller and Wisniewski, 1969; Wisniewski *et al.*, 1969; Milhorat. 1970b; Noack and Wolff, 1970; Weller *et al.*, 1971; Go *et al.*, 1976; Torvik *et al.*, 1976; Torvik and Stenwig, 1977; Gopinath *et al.*, 1979).

From the functional point of view, the coordinated beating of the dense standing cilia appears as an important factor in propulsion of CSF and its mixing. The CSF flow 'in vivo' is understood to be related to the summation of secretory pressure gradients from choroidal and extrachoroidal sources, osmotic pressure gradients and ciliary action of ependymal cells (Sahar *et al.*, 1970; Scott *et al.*, 1973).

Conversely, the presence of microvilli appears to be most closely associated with cells whose primary role is that of absorption. However, they do occur in limited numbers upon the surface of numerous epithelial cells which demonstrate a secreatory capacity as well (Oksche, 1956; Fleischhauer, 1964; Leonhardt, 1968; Wittkowski, 1968; Noack and Wolff, 1970; Noack *et al.*, 1972; Hochwald *et al.*, 1973; Loenhardt and Lindemann, 1973; Scott *et al.*, 1973; Bruni, 1973; Scott *et al.*, 1974; Bruni *et al.*, 1978).

The presence of a variety of SECs has been reported in the mammalian ventricular system and their significance has been controversial among investigators (Carpenter *et al.*, 1970;

Clementi and Marini, 1972; Leonhardt and Lindemann, 1973; Chamberlain, 1974; Sturrock, 1978; McKenna and Chairetakis, 1980). In some species, glial cells lie freely in the ventricles (Leonhardt, 1968; Noack and Wolff, 1970; Clementi and Marini, 1972; Noack *et al.*, 1972; Westergaard, 1972; Leonhardt and Lindemann, 1973), while in others, microglia-like "spider cells" lie on the epndymal surface (Carpenter *et al.*, 1970; Chamberlain, 1974; Sturrock, 1978; Mckenna and Chairetakis, 1980). Recently, the presence of these curious SECs with branching processes within the nonciliated ventricular floor of a number of species, has been confirmed as being similar in shape to neuronal structures (Leonhardt, 1968; Noack *et al.*, 1970; Noack and Wolff, 1970; Clementi and Marini, 1972; Westergaard, 1972; Leonhardt and Lindemann, 1973). It is speculated that they may represent intraluminal processes, although at present the precise nature and function of these conceivable neuronal elements is obscure. However in the processer, there seems to be an exchange of information as a sensor of CSF to occur from the CSF to the nervous tissue and vice versa, thus actively modifying the chemical nature of the CSF. The SECs, on the other hand, act as sentinels at the transition of cell debris and latex beads for renewal of ependymal cells (Carpenter *et al.*, 1970; Chamberlain, 1974; Sturrock, 1978; McKenna and Chairetakis, 1980).

The morphological characteristics of the SECs which are round and small bodies, appearing in single or cluster arrangement, long or slender in appearance, have 3 to 4 processes. The intermittent dilatation of the processes shows a deep cystoplasmic fold and much ruffled appearance. In view of the morphological variety, the SECs have been assumed to have a special functional capacity other than a morphological one. In the ventricular cavity, some SECs are attached to the choroid plexus (Kolmer cell) (Carpenter *et al.*, 1970; Chamberlain, 1974) and others are attached to ependymal cells. Their appearance is dependent on multiple factors; species, the ventricles examined, the age of the animal and the technique for preparing specimens (Scott *et al.*, 1973). However, we can speculate that there are two different possible factors in the SECs as intraventricular axon processes or macrophages.

Despite the voluminous literature with regard to experimental hydrocephalus, many investigators (Burr and McCarthy, 1900; De, 1950; Fishman and Greer, 1963; Weller and Wisniewski, 1969; Wisniewski *et al.*, 1969; Clark and Milhorat, 1970; Dohrmann, 1971; Weller *et al.*, 1971; Clementi and Marini, 1972; Torvik *et al.*, 1976; Torvik *et al.*, 1976; Torvik and Stenwig, 1977) injected various materials into the subarachnoid cistern of experimental animals and it has been long recognized that all parts of the brain are not equally affected.

In our previous report (Kim *et al.*, 1981), it appeared as if intraventricular blood infusion had made the most significant ultrastructural changes in the narrow lumem, i.e., the aqueduct of Sylvius. With the same assumption, it was speculated that the intraventricular hematoma might induce the secondary hydrocephalus by means of morphological alteration of the wall of the fourth ventricular formaina and in the basal and communicating cisternal pathways toward the archnoid villi. By using cats in this study, we hoped to induce the secondary hydrocephalus as suggested by other investigators (Foltz and Ward, 1956; Grinker *et al.*, 1960; Ojemann and New, 1963; Narva and Riekkinen, 1972; McQueen *et al.*, 1974; Wilkinson *et al.*, 1974; Blaylock and Kempe, 1978; Vassilouthis and Richardson, 1979; Choi *et al.*, 1982). Thereby we tried to verify further in this study, whether saline perfusions of the spinal subarach-

noid space of animals is impeded by an admixture of red cells or the addition of red cells to CSF maintains increased intracranial pressure in animals (Loeser *et al.*, 1968).

Similarly, a comparable degree of obstruction of the arachnoid villi throughout the cerebrospinal axis was demonstrated to constitue a major obstacle to absortion and to help explain the increased CSF pressure commonly observed in acute SAH (Vassilouthis and Richardson, 1979).

Literature review revealed that, if bioactive substances were infused intraventricularly, there were variegated ultrastructural changes of the ependymal surface which meant their secretory and/or absorption function (Oksche, 1956; Fleischhauer, 1964; Leonhardt, 1968; Leonhardt and Lindemann, 1973; Bruni *et al.*, 1978).

The mechanical impairment of the CSF flow at the level of formaina of the fourth ventricle and/or within the basal cisterns and brought about significant hydrocephalus. It was assumed that this experimental model might elicit a mechanical, chemical and/or other stressful reactions of the wall of the fourth ventricular formaina as in a human with intraventricular hematoma.

We could postulate that this experimental model would bring about, in addition to generalized increased intracranial pressure, several important factors which would provoke ultrastructural changes of the wall of the fourth ventricular formaina.

The first change anticipated was mechanical. The mechanical change, because of the increased volume of blood infused, formed an element which caused a direct insult to the ependymal surface structure thereby producing the acute and chronic phase of hydrocephalus. The combination of the mechanical insult by the RBCs with their debris induced an inflammatory reaction. According to Foltz and Ward (1956), the first investigators to recognize the two phases(acute and chronic) of ventricular enlargement following SAH, the acute phase occured immediately after the bleeding and was associated with rapid deterioration. Within 3 to 6 hours, hydrocephalus was pronounced.

Mechanical insult to the wall of the fourth ventricular formaina and/or within the basal cistern and arachnoid villi by RBCs, appeared as a subsequent impairment of the CSF flow and its absorption from the wall of the fourth ventricular foramina to even superior sagittal sinus has also been suggested (Foltz and Ward, 1956; Ellington and Margolis, 1969; McQueen *et al.*, 1974; Wilkinson *et al.*, 1974; Blaylock and Kempe, 1978; Vassilouthis and Richardson, 1970; Choi *et al.*, 1982). The pathogenesis of the second "chronic phase" is explained on the basis of basal arachnoidal adhesions, the result of leptomeningeal adhesions to the presence of blood products, preventing the CSF from reaching the site of its absorption. It is this second phase that often forms the underlying pathoanatomical basis of the clinical syndrome known as "communicating hydrocephalus" (Foltz and Ward, 1956; Editorial, 1977; Vassilouthis and Richardson, 1979).

We supposed that this experimental model could be applied to the category of the acute and chronic phase of hydrocephalus because the previous observation demonstrated by the RBCs and their cell debris appeared to be caught in the array of cilia around the aquenduct of Sylvius. The present observation also showed that the deformed RBCs on the wall of fourth ventricular formaina was also seen to adhere to the cilia and microvilli under the SEM, but subependymal changes were shown only under th TEM.

The chemicals released from RBCs or other blood components might be supposed to contribute to ependymal surface changes, to function

as an osmotic pressure gradient inducer and to make specific chemical reactions on the ependymal structures to which this experiment was not directed. Finally, questions might be raised whether the infections were relevant, in a broader manner, to the pathogenesis of hydrocephalus. If, for example such as in this study, resolution of intraventricular hematoma might leave proximally basilar cistern and distally arachnoid villi scarred and obstructed, the likelihood of such a sequel would be for greater following low-grade bacterial or viral ventriculomeningitides (Nielsen and Gauger, 1970). This sequel would be true if these infections were of extended duration after blood-induced adhesive arachnoiditis. It was frequently observed clinically that blood in the CSF containing space elicited a polymorphonuclear inflammatory response in the CSF. This may be an important mechanism of obstruction, especially in the acute phase of post-SAH hydrocephalus (Ellington and Margolis, 1969; Narva and Riekkinen, 1972; NcQueen *et al.*, 1974; Vassilouthis and Richardson, 1979).

It is well known that the acute and chronic phase of hydrocephalus develops in case of imparied CSF pathway (Vassilouthis and Richardson, 1979). Other experimental studies reported that perfusion with the CSF volume of more than 8cc was significant to develop the IVP increase in cats (Hochwald *et al.*, 1973), but we used 3cc as a maximum blood volume infused. Therefore, we could observe significant ultrastructural surface changes on the wall of fourth ventricular formaina, due to an infused blood volume of less than 8cc (Kim *et al.*, 1981). The unit time factor seemed to be more meaningful than the maximal blood volume of 3cc.

The mechanisms by which CSF is capable of being removed from the isolated ventricles in naturally-occuring obstructive hydrocephalus are as yet unknown (Foltz and Ward, 1956; Ojeman and New, 1963; Butler *et al.*, 1972; Vassilouthis and Richardson, 1979). Some factors instrumental to the absorption of fluid must include intraventricular pressure (IVP), ventricular surface changes providing a larger surface area, chemicals available for flow of fluid from the ventricle to the blood, and a relationship between IVP and periventricular venous pressure which would favor absorption (Lux *et al.*, 1970).

It has been repeatedly demonstrated that bulk absorption of CSF is linearly related to the pressure gradient in the CSF system. The dependence of CSF barrier, where pressure related clearance of CSF, could be detected only above 5 to 10cm H2O pressure. Therefore, the pressure-volume relationship is very suggestive (Fishman and Greer, 1963; Lux *et al.*, 1970).

Another possibility is the presence of an active transport mechanism that is capable of removing small amounts of portein from the CSF system (Sahar *et al.*, 1970). In this study, we didn't apply the perfusion method but a simple infusion method in order that this experimental model might be a similar model to naturally-occuring intraventricular hematoma which could incur in an acute and/or chronic phase of hydrocephalus.

The variables of ventricular ependymal cell modification to IVP increase are as follows; the height and the arrangement of the ependymal cell, the absorption capacity of the ependymal cell, and whether the surrounding structure is gray or white matter (Milhorat, 1970b). For example, the height of the ependymal cell from apex to base in the experimental cat was nearly one third to one fourth of the control size, the cellular nuclei were parallel to the basement membrane, and the pial cells were newly regrown to compensate for the IVP increase exerted on the ventricular surface (Clark and Milhorat, 1970b; Dohrmann, 1971). There are two areas of the ventricular surface, namely, the ependy-

mal cells over white or gray matter masses (Dobbing, 1963). In the presence of hydrocephalus, the ependymal cells over gray matter were relatively unchanged, while those over white matter were altered when the ICP was increased. Therefore, the ependymal cells over gray matter of the fourth ventricular floor and the dorsolateral portion of the third ventricle showed less severe disorganization than the ventral portion of the third ventricle (Milhorat *et al.*, 1970b; Go *et al.*, 1976; Gopinath *et al.*, 1979). Normally, the interependymal surface made up of tight junction is shown on the ciliated ependyma where fluid as well as protein, lipid and other electrolytes egress into the subependymal space in case of increased IVP (Thomson and Manganiello, 1947; Oksche, 1956; Lue *et al.*, 1970; Page, 1975; Torvik *et al.*, 1976). Moreover, the wider interstitial space of white matter (500-1000Å) than that of gray matter (200-500Å), permits easier subependymal fluid collection (Dobbing, 1963; Lee, 1971), thereby the flattening of the overlying ependymal cell produces the regional variation of the ependymal cell. The present ulutrastructural changes based on the SEM didn't prove the hypothesis that the SECs have morphological changes commensurate with deranged function, i.e., fluid movement, renewal of debris as well as secretion and absorption of CSF. Our previous report revealed no definite SECs, even though RBCs and their debris appeared to be caught in the array of cilia around the aqueduct of Sylvius (Kim *et al.*, 1981). The SECs were found in the control and experimental specimens of the whole cat ventricular system from frontal horn to the aqueduct of Sylvius, while this study revealed that a relatively great number of SECs could be found as macrophages without difinite confirmation. Prior to this study, we expected in case of autogenous blood infusion, there might be SECs as a specific from of macrophage which is scattered and round cells on the ventricular surface. We speculated that they might function as scavenger cells in the shape of a multi-blebbed form for RBC ingestion (Chamberlain, 1974). Our results showed that the longer the duration of blood infusion lasted, the more significant the changes of the ependymal morphology were without special reference to the maximal 3cc of blood infused.

We supposed the greater part of RBCs and other elements of blood were caught in and/or around the caudal third ventricle and/or the aqueduct of Sylvius in the acute phase of hydrocephalus which was also suggested by Dohrmann (1971). Additionally, it was also suggested that fibrosis and siderosis of arachnoid villi, basal adhesive arachnoiditis and a severe fibrous cuff around the superior cervical cord and medulla resulted from organization of entrapped clotted blood, RBCs and cell debris obstructing these channels of CSF resorption. Therefore, they resulted in excessive accumulation of CSF after intraventricular hematoma with significant scar requiring more than weeks to develop hydrocephalus (Ojemann and New 1963; Dohrmann, 1971; Butler *et al.*, 1972; Blay-lock and Kempe, 1978; Vassilouthis and Richardson, 1979; Kim *et al.*, 1981; Choi *et al.*, 1982).

The RBCs which escape through the wall of the fourth ventricular formaina into the cisterna magna or basal communicating pathway to the superior sagittal sinus may play somewhat an inhibitory role on normal CSF circulation to pass the foramina of the fourth ventricle (Bagley, 1982a, b; Ellington and Margolis, 1969; Torvik and stenwig, 1977; McKenna and Chairetakis, 1980). Thus, the combined insults may work on the deranged CSF circulation due to surface alterations lining the foramina of the fourth ventricle, resulting in the development of the acute or chronic phase of hydrocephalus.

According to a literature review, the experimental hydrocephalus have two types of ultrasturactural changes due to the primarily mechanical effect on the ependymal surface and secondarily on the permeability alteration of the subependymal glial sheath (Burr and McCarthy, 1900; Foltz and Ward, 1956; Bering and Sato, 1963; Ojemann and New, 1963; Lux *et al.*, 1970; Sahar *et al.*, 1972; Page, 1975; Torvik *et al.*, 1976; Torvik and Stenwig, 1977; Blaylock and Kempe, 1978; Vassilouthis and Richardson, 1979; Kim *et al.*, 1981; Choi *et al.*, 1982).

The gross surface changes of the wall of the whole ventricular system revealed that pial cells of the subependymal glial sheath would respond extremely rapidly to a stressful situation such as in the case of an IVP increase and during a decompensated process of hydrocephalus. Within 1 hour following complete obstruction of the ventricular system, there was suggestive evidence that new ependymal cells and fibrous astorocytes were apparently being produced and synechiae were beginning to form (Dehrmann, 1971; Hochwald *et al.*, 1973). That is to say, if the ventricular volume exceeded by far the normal size at any stage of development, the ventricular sulcation resulted from active growth of ependymal cells and not from the passitve narrowing of the ventricular lumen which was also confirmed by our previous report on the aqueduct of Sylvius (Kim *et al.*, 1981).

Milhorat *et al.*, (1970b) reported that the first dilated portion of the ventricular system was the lateral ventricle (frontal horn, occipital horn and body portion in that order of severity) after complete obstruction of the cisterna magna. These findings showed that maximal change in the subependymal glial sheath developed 3 to 6 hours later. Thereafter, edematous change became opparent for 48 hours by slight ventricular expansion manifested with flattening of ependymal cells and a frank tear at the weakest point in the ventricular surface. The dilatation of the third ventricle began to occur after 3 to 6 hours. However, the dilatation of the aqueduct of Sylvius was not obvious until 24 hours later.

Our results agreed with the observation of Milhorat *et al*, (1970b) because the fourth ventricle was the remotest area from the frontal horn of the lateral ventricle, as the initial point of blood infusion. The gray matter, as a buttress, appears to have the main protective effect on the fourth ventricular floor, because there were no significant changes of structural integrity of cilia and microvilli, but rather early alteration of marked permeability developed on the 7th day following intraventricular autogenous blood infusion.

It was also confirmed that the ultrastructures of the ependymal surface over the gray matter whose sequential changes due to IVP increase was well demonstrated to have three stages of alteration. In the first, the tufts of cilia became more separated from one another and, as a result, the population density of cilia became decreased. In the second, broad areas covered with microvilli appeared between tufts of cilia and their associated bare areas. The third peculiar feature was the presence of SECs on the ventricular lumen (Brightman and Palay, 1963; Leonhardt, 1968; Carpenter *et al.*, 1970; Clementi and Marini, 1972; Noack *et al.*, 1972; Westergaard, 1972; Leonhardt and Lindemann, 1973; Scott *et al.*, 1973; Page, 1975; Go *et al.*, 1976; Sutrrock, 1978; McKenna and Chairetakis, 1980). Our results showed that the wall of the fourth ventricular foramina lying, for the most part, on the gary matter manifested moderate ventricular dilatation without individual ependymal surface architectural alteration.

The SECs appeared in the control cats as well

as the experimental cats without special reference to a unit time factor or the blood volume infused. In this study, the morphological changes observed on the SEG under the TEM was more supported by that shown on the suface structure under SEM.

In the presence of hydrocephalus, the loss of lipid and protein from the SEG and particularly from the white matter, was presumed to be the major factor permitting dilatation of the ventricular system despite its volume reduction due to the glial fiber swelling (or dehylination) followed by atrophy (Fishman and Greer, 1963, Hochwald *et al.*, 1969). The volume decrease in SEG was attributed to the loss of lipid and protein associated with that of intracellular potassium (Fishman and Greer, 1963, Hochwald *et al.*, 1972). We postulated that, with the above findings, protein stagnation for ventricular elements of blood in the CSF could be a more potential factor for ventricular dilatation. The reason was the osmotic pressure gradient compensatory mechanisms may fail as an adhesion of RBC debris with the cilia and making a relative stasis of CSF through the already compromised narrow canal or foramina of the fourth ventricle. The microvilli adhered with RBC debris became incapable of regulating fluid transport between the ventricle and the surrounding brain. The impairment of normal CSF circulation i.e., CSF stasis, alternate transependymal pathway of CSF absorption is as yet would possibly participate in another contributing factor for the protein increase in the CSF containing space.

Lastly, the most significant finding we paid attention to, was the relatively constant appearance of SECs. If the infective process occured in the ependymal surface, the SECs activity as macrophages could be enhanced (Nielsen, 1940). Under antibiotic premedication, we postulated that there could be much less possibility of the ventricular ependymitis.

We assumed that multi-blebbed SECs observed following intraventricular blood infusion, was not related with RBC ingestion by SECs which was suggested by Chamberlain (174). Rather, they might be implicated in the sensor of chemical nature alteration in the CSF containing space. Nevertheless, the pathophysiological correlation between blood infusion and the appearance of SECs needs further study.

CONCLUSION

1. Under the TEM and SEM, the ultrastructural changes observed in both foramina of Magendie and Luschka were very similar.
2. There are two significant time factors for inducing the ultrastructural changes on the ventricular ependymal surface.

In the early phase (from immediate blood infusion to the end of the 7th day), a minimal to marked separation of an intercellular cleft, and intra-or intercellular vacoulation, and moderate to marked glial fiber swelling in the subependymal glial sheath were observed. These changes were attributed to the acute intraventricular pressure (IVP) increase due to direct and/or indirect insult of RBCs and their cell debris, the results of which bore a close resemblance to the acute phase of hydrocephalus.

In the late phase (28th post-infusion day to the end of the 42nd day), ependymal cells and their nuclei had moderate flattened contours under the TEM. While a marked separation of the ciliary tuft distance was more pronounced under the SEM. This might be partly attributed to an adhesive reaction of the ependymal surface structures, i.e., cilia and microvilli which were highly commensurate with the chronic phase of hydrocephalus.

3. The ultrastructural changes of the ventricular ependymal cells were presumed to be correlated

with the time factor, but not with a blood volume of less than 3cc, the maximum dose infused.

4) The supraependymal cells (SECs) seen on the ventricular surface were indicative of neuron-like structures, rather than macrophages. The above findings might explain the mechanism of secondary hydrocephalus following intraventricular hematoma.

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