Visualization of the Cerebrospinal Fluid Drainage into the Galen's Vein*

Paulo H. HASHIMOTO, Takahiro GOTOW, Takao ICHIMURA, Toshio NAKATANI, Nobuo TAKASU, Ryuhei KODAKA, Satoru SUMITANI and Takaichi FUKUDA

Department of Anatomy (Prof. P. H. HASHIMOTO), Osaka University Medical School, Osaka, Japan

Received December 24, 1984

Summary. Arachnid granulations are not always present in lower mammals and primate newborns. In order to visualize the route for the cerebrospinal fluid (CSF) to drain into the venous system, horseradish peroxidase (HRP) was injected into the lateral ventricle or cisterna cerebellomedullaris of the mouse and rat.

From 30 to 60 min after the commencing of a slow infusion for 15–30 min of 0.05–0.1 ml solution containing 10–20 mg HRP, the mouse, whose skull had been exposed, was dropped into cold acetone at dry ice temperature; other animals were fixed by perfusion with aldehyde solution. The frozen head was dissected in a cryostat kept at -18°C to remove the skull, but leave the dura mater and the falx cerebri. The brain with meninges was cut into 30–45 μm sagittal sections in the cryostat, and processed for peroxidase reaction. The perfusion-fixed brains were used for scanning electron microscopy and for electron microscope observation of the tracer.

The reaction product was found within fenestrated venous capillaries of the choroid plexus. The route for the HRP in the CSF to drain into the sinus rectus via the vena chorioidea and vena cerebri magna was directly visualized in the mouse.

Since Weed (1923) proposed the arachnoid granulations or villi as the site of absorption of cerebrospinal fluid (CSF) into the venous system, it has been believed that the CSF drains into the superior sagittal sinus through them (Levine et al., 1982). This view has been applied even to lower mammals (Mann et al., 1979), although the arachnoid granulations appear later than does the CSF, both ontogenetically and phylogenetically (Osaka et al., 1980). This discrepancy has not yet been explained satisfactorily even though Butler et al. (1983) recently showed some transcellular channels in the sinus endothelium covering "arachnoid villi within dural wall" in the rat.

In a previous report, we showed electron microscopic evidence that sites of CSF absorption in rats and monkeys are those devoid of the blood-brain barrier, i.e., the circumventricular organs (Hashimoto et al., 1982). In the present paper, we will show pictures indicating the direct influx of peroxidase-stained CSF into the internal cerebral vein through choroidal venules.

*Supported in part by Grant No. 59225012 from the Ministry of Education.
Fig. 1. Low power SEM view of the left half of a mid-sagittally bisected mouse brain. At the upper left, the straight sinus (R) merges with the superior sagittal sinus (S) to form the confluens sinum (O). Throughout the superior sagittal sinus, which has been pulled up during the drying process, no arachnoid villus is detected. The straight sinus directly continues to the great cerebral vein (M) and bifurcates to make the basal vein (B) and the internal cerebral vein (I) which receives the posterior vein of the corpus callosum (C) and the venules from the choroid plexus (ch). The splenium of the corpus callosum (sp) as well as the posterior (p) and the habenular commissures (h), and the pineal recess (r) of the 3rd ventricle (3) form landmarks. PA pia-arachnoid of the longitudinal cerebral fissure. × 48

Fig. 2. Closer view of the framed area in Figure 1. Ependyma (Ep) of the third ventricle, choroid plexus (ch) and endothelium (En) of the internal cerebral vein revealing an opening of a venule from the choroid plexus (arrow) are clearly distinguishable from each other by their surface specializations. PA pia-arachnoid of the area dentata. × 310
MATERIALS AND METHODS

Ten healthy adult ddY mice weighing about 40 g and 3 Wistar rats (150-200 g) were used. Under chloral hydrate anesthesia (0.35 mg/g body weight), 10-20 mg horseradish peroxidase (HRP), Sigma type II, dissolved in 0.05-0.1 ml water for mice, or 20 mg type VI HRP in 0.1 ml water for rats was injected very slowly into the lateral ventricle or the cisterna cerebellomedullaris, the procedure taking 15-30 min. From 30 to 60 min after the onset of the slow infusion, the mouse, whose skull had been exposed, was dropped into cold acetone at dry ice temperature. The frozen head was dissected in a cryostat kept at -18°C to remove the skull but not the dura mater nor the falx cerebri. The brain with all meninges intact was cut into 30-45 μm sagittal sections in the cryostat. They were retrieved and adhered to slides with a brush and finger heat in the cryostat, air-dried in room temperature, fixed in a half strength KARNOVSKY (1965)'s formaldehyde-glutaraldehyde fixative in 0.1 M cacodylate buffer for 1 hr, washed in 0.1 M sodium cacodylate buffer, pH 7.4 for 3-4 hr, and incubated in GRAHAM and KARNOVSKY (1936)'s medium containing 50 mg 3, 3′-diaminobenzidine tetrahydrochloride in 100 ml of 0.05 M Tris-HCl buffer, pH 7.6, initially without H₂O₂ for 10 min at 4°C, then with H₂O₂ for 15 min at 20°C. The specimens were washed in cold distilled water, post-stained with cresylechtviolett, dehydrated, and mounted in balsam. The HRP infused rat brains were fixed by perfusion through the aorta with 1/4 strength Karnovsky’s aldehyde mixture in 0.1 M cacodylate buffer and processed for electron microscopic detection of HRP as previously described in detail (GOTOW and HASHIMOTO, 1979; HASHIMOTO and HAMA, 1968).

Other mouse brains without HRP infusion were fixed by perfusion through the aorta with 1/4 strength Karnovsky’s aldehyde mixture in 0.1 M phosphate buffer and prepared for scanning electron microscopy. Those with all meninges intact were bisected mid-sagittally, immersed in the same fixative for additional hours, then immersed in a solution containing 2% sucrose, 2% sodium glutamate and 2% glycine for 2 hr at room temperature, followed by an overnight immersion in 1% tannic acid in a refrigerator (MURAKAMI, 1974). They were washed in distilled water for 1 1/2 hr, post-fixed in 1% OsO₄ for 2 hr, all washed in distilled water at 4°C, dehydrated in ascending concentrations of ethanol, critical point-dried with CO₂, ion-sputter coated with platinum, and examined in a Hitachi S-800 scanning electron microscope (SEM) equipped with a field emission gun. Three control mouse brains with all meninges intact were subjected to an immersion fixation with 10% neutral formol, embedded in Histosec (Merk), cut in frontal, sagittal and horizontal planes, respectively, and stained with hematoxylin and eosin for light microscopic observation.

RESULTS

Observation under the scanning electron microscope of the superior sagittal sinus of adult mice revealed no formation similar to arachnoid granulation, nor any villus on its endothelial surface. A low power view (Fig. 1) followed by a higher power examination (Fig. 2) allowed us to distinguish the system of Galen’s vein from the pia-arachnoid space, and the ventricular lumen including its recess, by the fine structure of endothelium, of connective tissue elements, and of the ciliated ependyma.

When HRP was injected in the lateral ventricle, the whole brain was more or less
Fig. 3. A mid-sagittal cryostat section of a mouse brain which has received an intraventricular infusion for 30 min of 20 mg HRP into the left lateral ventricle, and then freeze-fixed by dry ice-acetone after 30 min interval. Not only the whole brain together with the ventricular system and the subarachnoid space, but also the sinus rectus and the sinus sagittalis superior (S) merging into the confluens sinuum (O) are stained by peroxidase reaction. p Posterior commissure, sp splenium of the corpus callosum, 3 third ventricle. x 20

Fig. 4. Enlarged part of a section adjacent to that shown in Figure 3. The reaction product for HRP is positive in the vena cerebri interna (I) and the vena cerebri magna (M), as well as in the third ventricle (3) and in the connective tissue of pia-arachnoid surrounding the choroid plexus. h Habenular commissure, p posterior commissure, r pineal recess, sp splenium of corpus callosum. x 44
Fig. 5. Mid-sagittal section of a mouse brain which has received an intracisternal infusion for 15 min of 10 mg HRP into the cisterna cerebellomedullaris, and frozen by dry ice-acetone after a 15 min interval. The subarachnoid space and a limited depth of the brain from the pial surface as well as the internal cerebral vein, the straight sinus, and a part of the superior sagittal sinus are stained by HRP. The subfornical organ (f) is stained and observed to continue to the choroid plexus (ch) which is also stained. No reaction product is observed in the third ventricle (3). p Posterior commissure, sp splenium of the corpus callosum. × 16

Fig. 6. Enlarged part of the section next to that of Figure 5. The posterior vein of the corpus callosum (c) and the superior anastomotic vein (a) are HRP positive and drain into the superior sagittal sinus. Close observation of the content of the internal cerebral vein (I) reveals negatively stained red cells and positively counterstained leucocyte nuclei, indicative of a true blood vessel. White lines on the choroid plexus (arrows) indicate negative images of the ventricular space lined by the choroid epithelium (see also Fig. 1). r Pineal recess, h habenular commissure, ch choroid plexus, f subfornical organ, sp splenium of the corpus callosum, 3 ventriculus tertius. × 46
stained with this enzyme in 1 hr (Fig. 3). This heterogeneous marker protein of 40 k dalton was shown to drain into the straight sinus via the internal cerebral vein as well as into the superior sagittal sinus. The perivascular connective tissue of the choroid plexus demonstrated the heaviest staining (Fig. 4). When HRP was injected into the cisterna cerebellomedullaris, the pia-arachnoid connective tissue of the whole brain, plus a limited depth of the brain from the pial surface, were intensely stained. The system of Galen's vein was found to be filled with the reaction product (Fig. 5). The subfornical organ, together with the choroid plexus, was also stained to a moderate density. No reaction product was observed in the ventricular lumen even in the spaces between choroid epithelial layers (Fig. 5, 6). This indicates that the drainage of CSF into choroidal venules is not through the choroid epithelium as indicated by van Deurs et al. (1978), but rather through the basal connective tissue of the pia-arachnoid. The posterior vein of the corpus callosum and the superior anastomotic vein which drain into the superior sagittal sinus were also strongly reactive (Fig. 6), indicating that the source of HRP in the superior sagittal sinus, at least in part, is the HRP in the internal cerebral vein. Close observation of vascular contents of the internal cerebral vein revealed a negative image of red cells made by the reaction product, and a positive image of polymorphic as well as round nuclei of leucocytes stained with the basic dye indicating that the lumen was filled with blood.

Electron microscopic observation of the choroid plexus after intraventricular HRP

![Fig. 7. Transmission electron micrograph of the choroid plexus of the third ventricle from a rat which has received an intraventricular infusion for 15 min of 20 mg HRP into the left lateral ventricle, and then fixed by the aldehyde perfusion after a 15 min interval. Thin sections were slightly stained only with uranyl acetate. The tracer stains connective tissue elements and a basal infolding (×) surrounded by basal lamina, then invades the intercellular space between choroid epithelial cells (ch), but is dammed by a tight junction at the apical end (arrow). Some HRP deposits are detectable in the fenestrated capillary (asterisk). ×8,300]
infusion revealed influx of the tracer into the fenestrated capillary through the connective tissue of the pia-arachnoid (Fig. 7, 8).

DISCUSSION

Until now, it has been the general concept that the CSF is produced in the choroid plexus to be pumped out into the lateral, third, and fourth ventricle, flow out to the subarachnoid space through apertures of Magendie and of Luschka, run to the vertex, and be absorbed into the superior sagittal sinus through the arachnoid villi, even though the latter are distinct as granulations only in higher mammals, especially in old ones. This paper has presented evidence to indicate that the CSF drains into the Galen’s vein (vena cerebri interna and vena cerebri magna) through venules of the choroid plexus via fenestrated venous capillaries of the choroid plexus as well as of other circumventricular organs. This new concept seems more acceptable as it is a general rule of an endocrine gland or any other epithelial tissue to obtain raw material and oxygen from the arterial capillary in connective tissues, and to excrete the end-product and carbon dioxide into the venous capillary through tissue fluid surrounding the glandular tissue. The choroid plexus does not necessarily seem to be an exception.

The second point of discussion is on the discrepancy between the occurrence of the CSF and of the arachnoid granulations. The former appears early in development phylogenetically and ontogenetically (OSAKA et al., 1980), whereas the latter appear later and increase in number and size in accordance with the increasing cranial volume and aging. They are more suitably regarded as a tool to prevent the central nervous tissue from compression caused by a sudden rise of the intracranial pressure. They are most likely a bulbous safety valve for the CSF circulation.
Reviewing previous literature, Weed (1935) never examined the direct CSF absorption from the arachnoid granulation, neither did Welch and Friedman (1960), who introduced the word “valve” for this small organ. Shaibo and Maxwell (1968) denied any direct connection between subarachnoid CSF in the villi and venous blood in the sinus. Alksne and Lovings (1972) stated that CSF absorption requires metabolic activity of arachnoid villi endothelial cells instead of a passive procedure as suggested in the past. Tripathi (1974) showed large openings on the endothelium of the arachnoid villus. However, an opening of this kind was demonstrated as a result of high CSF pressure (Levine et al., 1982). Mann et al. (1978) reported a CSF outflow resistance progressively decreasing in the rat, dog, and man. This seems to be dependent on the number and size of arachnoid villi as a shock absorbing balloon. They also tested clearance of inulin and polystyrene beads from the CSF to the venous system in the rat (Mann et al., 1979). Since they collected venous blood from the confluens sinuum where the Galen’s vein opens, it is apparent that their claim of “rat arachnoid villi” does not exclude the route from the choroidal veins.

To avoid an increase in intraventricular pressure as was the case in previous reports (Butler et al., 1983; Tripathi, 1974), we performed a very slow infusion of the tracer under careful stereotactic control, allowing free drainage of the CSF through a hole. Cserr et al. (1977) have suggested that the flow of cerebral interstitial fluid drains into the fenestrated vessels of the choroid plexus and other periventricular areas. Finally, we have demonstrated an influx of CSF to the Galen’s vein which receives blood from choroid plexuses, where blood plasma effluxes from the fenestrated arterial capillary (Hashimoto and Hama, 1968), and the CSF drains into the fenestrated venous capillary (Gotow and Hashimoto 1979; Hashimoto et al., 1982; Ichimura and Hashimoto, 1982, 1984).

REFERENCES

Alksne, J. F. and E. T. Lovings: Functional ultrastructure of the arachnoid villus. Arch. Neurol. 27: 371–377 (1972).

Butler, A. B., J. D. Mann, C. J. Maffeo, R. G. Dacey, Jr., R. N. Johnson and N. H. Bass: Mechanisms of cerebrospinal fluid absorption in normal and pathologically altered arachnoid villi. In: (ed. by) J. H. Wood: Neurobiology of cerebrospinal fluid, Vol. 2. Plenum Press, New York-London, 1983 (p. 707–726).

Cserr, H. F., D. N. Cooper and T. H. Milhorat: Flow of cerebral interstitial fluid as indicated by the removal of extracellular markers from rat caudate nucleus. Exp. Eye Res. 25 (Suppl.): 461–473 (1977).

Gotow, T. and P. H. Hashimoto: Fine structure of the ependyma and intercellular junctions in the area postrema of the rat. Cell Tiss. Res. 201: 207–225 (1979).

Graham, R. C., Jr. and M. J. Karnovsky: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. Ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14: 291–302 (1966).

Hashimoto, P. H., T. Gotow, T. Ichimura and T. Arikuni: Are the arachnoid villi really the main drainage route for the cerebrospinal fluid into the blood stream? An electron microscopic study. Okajimas Fol. anat. Jpn. 58: 819–835 (1982).

Hashimoto, P. H. and K. Hama: An electron microscope study on protein uptake into brain regions devoid of the blood-brain barrier. Med. J. Osaka Univ. 18: 331–346 (1968).

Ichimura, T. and P. H. Hashimoto: Three-dimensional fine structure of elastic fibers in the peri-
vascular space of some circumventricular organs as revealed by high voltage electron microscopy. J. Ultrastr. Res. 81: 172–183 (1982).

Ichimura, T. and P. H. Hashimoto: Fine structure of basement membranes of the capillary endothelium and perivascular astrocyte in some circumventricular organs by three-dimensional SEM. J. Ultrastr. Res. 86: 220–227 (1984).

Karnovsky, M. J.: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137A–138A (1965).

Levine, J. E., J. T. Povlishock and D. P. Becker: The morphological correlates of primate cerebrospinal fluid absorption. Brain Res. 241: 31–41 (1982).

Mann, J. D., A. B. Butler, J. E. Rosenthal, C. J. Maffeo, R. N. Johnson and N. H. Bass: Regulation of intracranial pressure in rat, dog and man. Ann. Neurol. 3: 156–165 (1978).

Mann, J. D., A. B. Butler, R. N. Johnson and N. H. Bass: Clearance of macromolecular and particulate substances from the cerebrospinal fluid system of the rat. J. Neurosurg. 50: 343–348 (1979).

Murakami, T.: A revised tannin-osmium method for non-coated scanning electron microscope specimens. Arch. histol. jap. 36: 189–193 (1974).

Osaka, K., H. Handa, S. Matsumoto and M. Yasuda: Development of the cerebrospinal fluid pathway in the normal and abnormal human embryos. Child’s Brain 6: 26–38 (1980).

Shabo, A. L. and D. S. Maxwell: The morphology of the arachnoid villi. A light and electron microscopic study in the monkey. J. Neurosurg. 29: 451–463 (1968).

Tripathi, R.: Tracing the bulk outflow route of cerebrospinal fluid by transmission and scanning electron microscopy. Brain Res. 80: 503–506 (1974).

Van Deurs, B., M. Møller and O. Amtorp: Uptake of horseradish peroxidase from CSF into the choroid plexus of the rat, with special reference to transepithelial transport. Cell Tiss. Res. 187: 215–234 (1978).

Weed, L. H.: The absorption of cerebrospinal fluid into the venous system. Amer. J. Anat. 31: 191–221 (1923).

———: Forces concerned in the absorption of the cerebrospinal fluid. Amer. J. Physiol. 114: 40–45 (1935).

Welch, K. and V. Friedman: The cerebrospinal fluid valves. Brain 83: 454–469 (1960).