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 cells, 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 2½–4 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 chromo-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 H₂O with respect to the interaural line. In the 2nd group, normal cats were perfused under an elevated pressure of 10–15 cm H₂O. In the 3rd group, acute hydrocephalic cats, 7 days after intracisternal injection of kaolin, were perfused at pressures which varied between 12–25 cm H₂O. In the fourth group, chronic hydrocephalic cats, approximately 2 months after kaolin, were perfused at pressures which ranged from 10 to 20 cm H₂O. 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