ELECTRON MICROSCOPY OF THE BRAIN IN EXPERIMENTAL EDEMA*

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It is a well known fact that the brain increases in volume as a consequence of damage caused by trauma or other procedures. Cerebral edema, the term often applied to this condition, suggests by analogy with edema in other locations that the swelling is caused by accumulation of fluid in the extracellular spaces of the brain. This concept has received support from data showing that the chloride or inulin space, presumably representing the extracellular fluid compartment, is increased in brain-swelling.\textsuperscript{8,11,18} However, recent studies with the electron microscope on the structure of the central nervous system have indicated that there is an exceedingly small interstitial space between the cells and their closely packed meshwork of processes.\textsuperscript{9} Electron micrographs have an advantage over other means of studying the structure of the central nervous system, in that not only is increased resolution possible but, also, neurons and glial cells of all types are visible in a single preparation. For these reasons, it now seems feasible to examine the brain in experimental edema in order to determine the site of accumulation of fluid. Specifically, the present investigation was designed to discover whether the additional fluid associated with brain-swelling was located extracellularly or whether it was accumulated in one or more of the brain's intracellular compartments.

MATERIAL AND METHODS

Swelling of the brain was produced in anesthetized rabbits. In 5 rabbits the edema occurred during ether anesthesia following craniotomy and opening of the dura mater.

Increase in volume of the brain as evidenced by protrusion above the skull occurred in these rabbits within 30 minutes to 1 hour following craniotomy. This was not considered to be a uniform manner in which to produce edema. The mechanism of the swelling was unknown but was believed to be a combination of anoxia and possibly of trauma. These 5 rabbits were included in the present study because the occurrence of swelling in their brains is similar to that which may occur inadvertently during craniotomy in surgical patients. In another group of 5 rabbits anesthetized with Nembutal injected intramuscularly, edema was produced by the injection of distilled water into the inferior vena cava. Gross swelling of the brain occurred with administration of 125 cc. at 5 to 10 cc. per minute, and could be increased by larger amounts.

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Control material was provided by examination of cortical specimens obtained from a number of rabbits (approximately 25) of different ages, in which the brain was not grossly swollen.

The edematous brains were removed. A coronal section was made at about the level of the amygdala. Small blocks were removed from the cortex and underlying white matter along the midpoint of the convexity of the hemisphere. They were immediately placed in Dalton's osmic acid fixative. The tissue-blocks were fixed for 1 hour, dehydrated in graded ethanol solutions (10 to 100 per cent), infiltrated in a mixture of butyl and methyl methacrylates, and finally in methacrylate to which was added a catalyst, benzoyl peroxide. The tissue was embedded in partially polymerized plastic and hardening was completed at 60°C. Sections for phase and light microscopy were cut at 1 to 2 microns on glass knives in a Porter-Blum microtome. Adjacent thin sections for electron microscopy were mounted on collodion-covered copper grids. They were examined in a RCA electron microscope (models EMU-2E or 3C) without removing the plastic. Original micrographs were made at 1000 to 8000 diameters and subsequently enlarged photographically.

OBSERVATIONS

Normal. Cerebral cortex from normal rabbits is a complexly organized tissue composed of neurons, glial cells, their intertwined cellular processes, and blood vessels. The distinguishing characteristics of the various cell types comprising nervous tissue have been described previously. The cells and their processes are tightly packed together so that the extracellular space is reduced to only the narrow region between two contiguous cell membranes—of the order of 100 to 200 Ångström units in width. In their most delicate extensions, astrocytic processes are distinguished from neural with difficulty. Oligodendroglial ramifications, on the contrary, are readily identified by their relatively large size and pale cytoplasm (Fig. 1). Glial processes surround cortical capillaries. No Virchow-Robin space is present between the adjacent neuropil and the capillary basement membrane. Rather, fascicles of astrocytic processes and oligodendroglial expansions closely encompass the capillary (Fig. 2). On the other hand, small arteries and veins often possess two basement membranes, separated from one another by a connective-tissue layer. About these vessels the glia abut upon the outer basement membrane which is separated from the outer surface of the wall of the vessel by a space containing collagen fibrils. Occasional neurons and neural processes may be closely applied to the outer surface of capillaries as well as the more usual glial investment.

Experimental. The changes associated with cerebral edema were the same in both of the methods of production. The oligodendroglial cells were prominent because of the increase in quantity of their cytoplasm. The mitochondria and ergastoplasm of these cells, although present in the same amount, appeared scant and widely scattered because of the increased cytoplasmic volume. In the neuropil, the oligodendroglial processes were larger than usual and even indented axons or compressed adjacent astrocytic processes. The most impressive change associated with edema was in the pericapillary
Fig. 1. Electron micrograph of an oligodendroglial cell (O) forming a satellite cell to three neurons (N). The cytoplasm of the oligodendroglial cell is pale but contains mitochondria (arrow) and scant ergastoplasm. The neural cytoplasm is more dense than that of the oligodendroglia because of the closely arranged and densely granular ergastoplasm. Normal adult rabbit cortex. Approximately 8,000X.

Fig. 2. This is an electron micrograph of a tangential section through a capillary in normal adult rabbit brain. A red blood cell is present in the capillary lumen. The entire circumference of the vessel is covered by glial processes. The pale processes (OL) are oligodendroglial and the closely packed more dense expansions (A) are astrocytic. Myelinated axons (AX) are evident in the neuropil. The compact arrangement of the cell processes and the lack of an interstitial space is well demonstrated. Approximately 6,500X.
region, where the oligodendroglial processes were massively increased in size (Fig. 3). That they were oligodendroglial expansions, and not a perivascular space was obvious because scattered mitochondria and ergastoplasm were still present. Distended oligodendroglial processes compressed the interspersed astrocytes until only narrow tenuous strands remained. These compressed astrocytic fibers represented the spider web-like strands which have been seen by light microscopy extending across the prominent “perivascular space” around capillaries in cerebral edema, for the plasma membranes separating two oligodendroglial processes are below the level of resolution. Thus, the compartmentalization of the space about vessels was not recognized by light microscopy. The degree of enlargement of the perivascular

Fig. 3. This is an electron micrograph of a capillary in edematous rabbit brain. It is surrounded by distended oligodendroglial (OL) processes containing mitochondria or a remnant of ergastoplasm. Separating the oligodendroglial expansions are the compressed astrocytic feet (arrows). At the left is an unmyelinated axon. Its fibrils are evident at the lower left. Approximately 6,500X.
oligodendroglial processes was extraordinary; some small capillaries actually were dwarfed by the surrounding glia (Fig. 4). Neurons, astrocytes, microglia and axons appeared relatively normal except for focal compression by the expanded oligodendroglia.

**DISCUSSION**

It is of questionable validity to differentiate between brain swelling and cerebral edema. The consensus is that they represent variable degrees of the same process, and as such it now appears best to consider them synonymous, rather than to continue an artificial distinction. Severity of the pathologic process should be related to degree of alteration rather than by application of different names.
Weed,\textsuperscript{19,20} while studying formation and circulation of the cerebrospinal fluid, experimentally produced cerebral edema by intravenous injection of distilled water. He noted distention of the neuroglial network without compression of the "perivascular spaces" even when the sulci had been obliterated. Indeed, he pointed out that the "perivascular spaces" actually were enlarged. Penfield and Cone\textsuperscript{12,13} and Cone\textsuperscript{2} used the same method of producing cerebral edema. They associated cellular changes, called by them acute swelling of the oligodendroglia, with edema whether it was naturally occurring or caused by experimental means. However, they were uncertain that acute swelling of the oligodendroglia was the primary change in edema. Perret and Kernohan,\textsuperscript{14} among others,\textsuperscript{2,6,7,12,13,16–18} examined human brains with clinical cerebral edema and pointed out the massive increase in oligodendroglial cytoplasm evident, especially in regions where these cells were arranged in rows.

Investigators utilizing light microscopic technics have demonstrated three distinct alterations in cerebral swelling: 1) expanded perivascular spaces, 2) swelling of oligodendroglial cells, and 3) a sieve-like appearance of the white matter and of the neuropil. By electron microscopy it is evident that, in cerebral edema, the fluid increase is entirely within cellular compartments, and that these cellular compartments may be equated with the cytoplasm of oligodendroglial cells or their processes. Indeed, the sieve-like appearance of the neuropil is caused by dilatation of oligodendroglial processes, rather than by an increase in interstitial space.

Transport of fluid, oxygen, glucose, and other substances to the neuron is of necessity through a second cell, since only rarely do the neurons or neural processes directly make contact with the wall of the capillary, and since there is not an interstitial space of sufficient size to allow for simple diffusion. The massive increase in oligodendroglial volume in experimental edema suggests that this cell may be intimately associated with transport of intracerebral fluid. Edström,\textsuperscript{5} in an excellent review of the problem of the blood-brain barrier, offers the same concept as does Zülch,\textsuperscript{24} that the barrier is actually the interpolation of a glial cell between the capillary and the nerve cell. Thus, not diffusion, but transport across cell membranes is associated with the apparent decreased permeability of cerebral capillaries as compared with those in other than neural locations where capillaries are surrounded by an interstitial space into which there is diffusion. This concept proposed by Zülch and Edström and that of Dempsey and Wislocki\textsuperscript{4} may be equated, since all have proposed that the glial or cellular envelopment of the cerebral capillary is the morphological counterpart of the blood-brain barrier, and that the absence of such an investment characterizes those regions lacking such a barrier.

Naturally occurring edema, and edema caused by anoxia, trauma or injection of hypotonic solutions, results in swelling of oligodendroglial cytoplasm and cytoplasmic expansions. These expansions contribute to the glial investment of the capillaries. Distention of oligodendroglial pericapillary
processes leads to detachment or at least decreased capillary-surface contact with astrocytes. It is not surprising then to realize that cerebral edema at least partially destroys the blood-brain barrier. The loss of blood-brain barrier with edema has been demonstrated in attempts to use radioactive fluorescein-tagged albumin as a means of detection and localization of brain tumors. The rationale of this method depends on the lack of a hematocerebralic barrier within the tumor, but the degree of localization is obscured by a similar loss of the barrier in areas of edema.

Often cerebral edema is localized rather than diffuse. This is true in the marginal zone about tumors of the brain, at the edges of infarcts, and experimentally in the zone of trauma following localized application of cold as well as with other localized insults to the brain. The focal nature of many forms of experimental or naturally occurring swelling of brain tissue adds support to the concept of intracellular swelling. If the accumulation of fluid were within an extracellular space, localization of the fluid would be impossible, since this space would be continuous throughout the cerebrum. Thus the increased fluid would spread, rather than remaining as a focal zone of swelling. On the contrary, accumulation of fluid within a cellular space is consonant with a localized edematous response to a focal zone of natural or experimental cerebral damage.

SUMMARY

Electron microscopy of experimentally produced swelling of the rabbit brain showed the increased fluid to be contained within oligodendroglial cytoplasm. The cytoplasm of the oligodendroglia and of their processes was expanded. No increased interstitial space was present in edema. The sieve-like appearance of the neuropil was caused by swelling of oligodendroglial processes. The massive perivascular spaces associated with edema actually were dilated oligodendroglial processes, not distended Virchow-Robin spaces.

REFERENCES