Capillary Ultrastructure and the Blood-Brain Barrier in Human Malignant Brain Tumors*

DON M. LONG, M.D., PH.D.
Department of Neurosurgery, University of Minnesota, Minneapolis, Minnesota

It is well known that certain dyes administered intravenously do not stain brain parenchyma, though they pass freely out of the vascular system in other regions of the body. This observation has given rise to the concept of a blood-brain barrier, and this barrier phenomenon has been explored extensively for a variety of substances. It is also well known that the barrier mechanism is defective in and around human malignant brain tumors. This fact has been utilized clinically in brain scanning with radioactive substances and in the localization of brain tumors at surgery. Previous attempts to localize the blood-brain barrier and its defects failed to delineate a specific, anatomical substrate. Recently, however, using uranyl acetate en bloc immersion, and peroxidase or lanthanum tracer techniques, Reese and Karnovsky, and Karnovsky have demonstrated definite anatomical and functional differences between endothelial cell junctions within the brain and those outside the nervous system. In tissues other than brain the apparently tight junctions between capillary endothelial cells are, in fact, open channels that allow the passage of the tracer materials into the basement membrane and subsequently into the extracellular space. In the brain, these apposing capillary endothelial membranes are fused into pentalaminar junctions which are impermeable to the tracers utilized and analogously to protein.

It is no longer possible to think of the blood-brain barrier as a unitary phenomenon, and there seems to be no need to invoke a single anatomical substrate. Rather, the barrier phenomenon can be considered to be a complex system of multiple active and passive transport mechanisms and active and passive exclusion mechanisms subserving the metabolic needs of the brain and protecting it from unwanted or unneeded materials. Steinwall and Klatzo have demonstrated that it is possible to study the barrier phenomenon differentially and have also shown that it is possible to have varying degrees of injury to the various systems which make up the barrier. The vital dyes and radioactive substances which are commonly utilized for brain tumor localization bind avidly with serum protein, notably albumin. The apparent blood-brain barrier to these substances to a large extent reflects the impermeability of brain capillaries to protein and, similarly, the derangements in the blood-brain barrier as measured by these dyes or radioactive substances indicate an increased vascular permeability to the protein-tracer complex. Therefore, assessment of capillary ultrastructure in human malignant brain tumors in which the blood-brain barrier was known to be permeable to these tracers was undertaken to provide anatomical localization of the mechanisms responsible for the abnormality of the protein barrier within these tumors.

Method

To obtain information on the configuration of the normal human cerebral capillary, samples of normal brain were removed from six patients during the course of hypophysectomy or the surgical approach to an anterior communicating artery aneurysm. Nineteen examples of human malignant brain tumors were also studied (13 glioblastomas, 1 metastatic carcinoma, 1 malignant meningioma, and 1 dural sarcoma). All of the operations were performed under general anesthesia utilizing halothane pentothal-flaxedil and assisted respiration. All of the patients with brain tumors were given 5 cc of a 20% solution of sodium fluorescein intravenously.

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immediately after the induction of anesthesia. Tissue samples were usually obtained 30 to 60 minutes after the fluorescein had been administered. All tissues were removed as atraumatically as possible and either immersed directly in 1% osmium tetroxide or the aldehyde fixative suggested by Karnovsky. Prior to fixation all tumor tissue was exposed to an ultraviolet lamp and all tumor samples were noted to fluoresce brightly. In addition, samples of edematous brain near, but separate from, the main tumor mass were obtained and processed in the same manner.

All tissue immersed directly in osmium was kept in this solution for 1 hour and then processed by our usual technique for embedding in epon. The aldehyde-fixed tissue was kept in the fixative for periods varying from 3 to 24 hours, then washed in buffer, and half of each sample was immersed in a 2% solution of uranyl acetate in 0.2 maleate buffer at pH 5.2 for 2 hours. Tissue samples were then osmicated for 1 hour, and again one half of the samples were treated with a 1% osmium solution containing a 2% suspension of neutralized lanthanum nitrate. Following these procedures the tissue was processed in the standard manner and embedded in epon. Following the preparation of thin sections for electron microscopy, the tissue that had not been treated with uranyl acetate was stained with a lead citrate, except for the lanthanum-treated material which was left unstained to facilitate identification of the tracer material.

A portion of each sample was processed for light and fluorescence microscopy and examined to verify tumor histology. The passage of the fluorescent tracer as ascertained by fluorescence microscopy was also studied. All of the thin sections were examined in an EMU 3 F electron microscope except for some of the examples of capillary endothelium which were photographed at high magnification with a Phillips 300 electron microscope. In the photographs of the sections (Figs. 1–17), the following abbreviations are used:

A = astrocyte processes
BM = basement membrane
CO = collagen
EC = extracellular space
EN = endothelial cell cytoplasm
G = glycogen
L = lumen
M = swollen mitochondria
MV = multivesicular body
P = pericyte cytoplasm
T = tumor cell
V = vesicle.

Normal Capillary Ultrastructure

The morphology of normal central nervous system capillaries as visualized with standard electron microscopic techniques is well accepted and needs no more than a summary here. The vascular lumen is surrounded by a continuous layer of endothelial cell cytoplasm in which an occasional nucleus can be seen (Fig. 1). The individual endothelial cells are apposed by short junctions which appear to be closed. Utilizing the uranyl acetate en bloc treatment method to delineate cell membrane structure, one sees that these junctions are indeed tight and appear identical to the pentalaminar junctions described by Reese and Karnovsky in the mouse (Fig. 2 inset). The endothelial cell cytoplasm is bounded by a basement membrane of homogenous structure and rather uniform electron density. The central portion of the basement membrane is slightly less dense in the human as in other species. Within the basement membrane occasional pericytes are found. Both the capillary endothelial cells and the pericytes have the usual complement of subcellular organelles. However, vesicles, presumably pinocytic in nature, are less common in cerebral capillaries than in blood vessels elsewhere in the body. Closely applied to the basement membrane and completely investing the capillary are a series of astrocytic processes. The astrocyte cell processes are closely apposed but not in contact with each other, and an extracellular space between processes appears to exist. The pericapillary foot processes of astrocytes in the human commonly contain glycogen, but are not otherwise unique.

Brain Tumor Capillary Ultrastructure

The capillary ultrastructure of the brain tumors studied can be best considered as a continuum ranging from vessels that appear very similar to normal capillaries through all