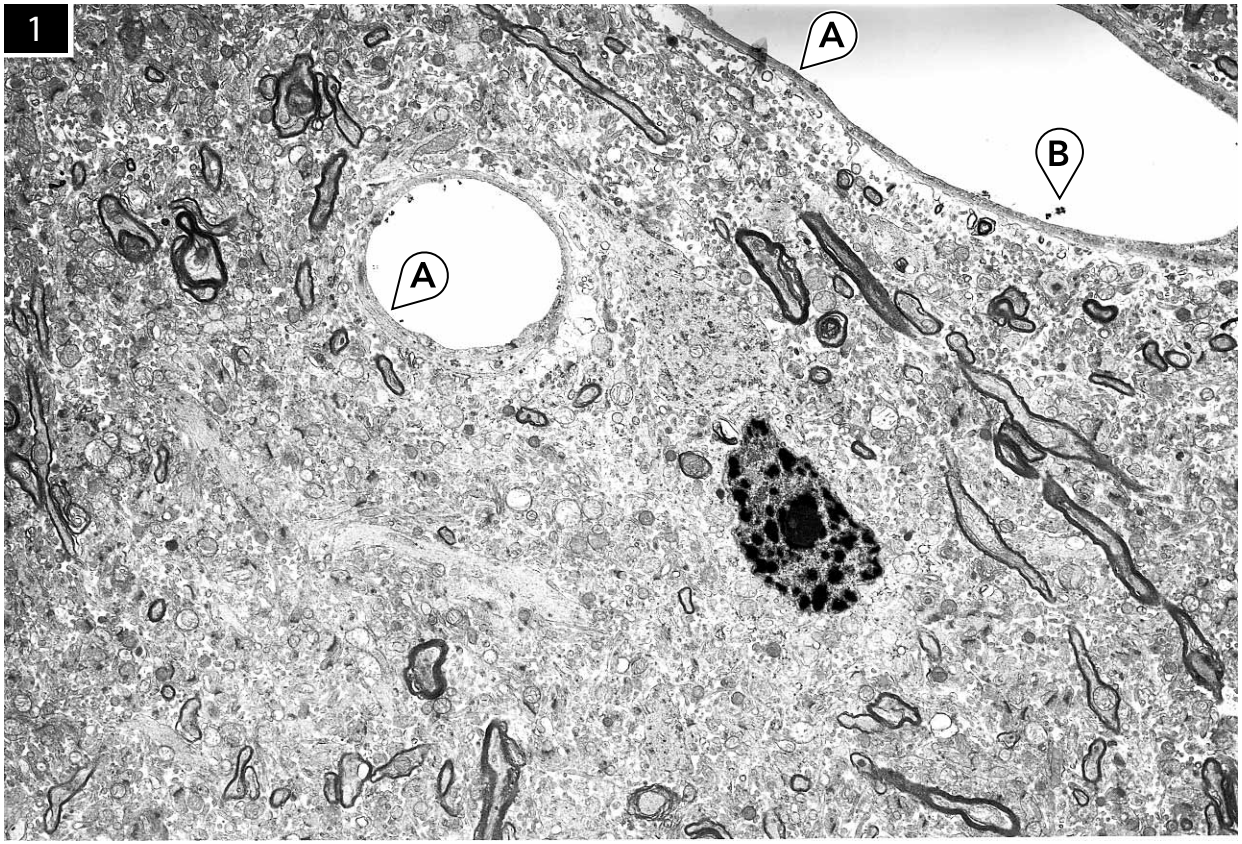
Comparison of Canine Brain Cryoprotection using Differering Glycerol-Based Protocols

In June 1995 a paper evaluating the cryoprotection of canine brains was published on the CryoNet news group (archives of which are available at www.cryonet.org).

The paper by Michael Darwin, Sandra Russell, Larry Wood, Candy Wood, and Steven B. Harris MD was subsequently summarized and excerpted in *CryoCare Report* issue number 4, dated July 1995. Electron micrographs which provided the primary data for the paper were included with explanatory captions and overlays identifying features of interest.

The captioned micrographs are reproduced here in two versions: 72 dpi for the web, and 200 dpi for laser printing. The higher-res images reveal considerably more detail.

Note that the versions published in *CryoCare Report* were scaled and cropped to fit the magazine layout. The versions here are reproduced at a uniform scale without any cropping, providing the closest possible fidelity to the original electron micrographs, which were supplied as photographic prints.



1. BioPreservation protocol.

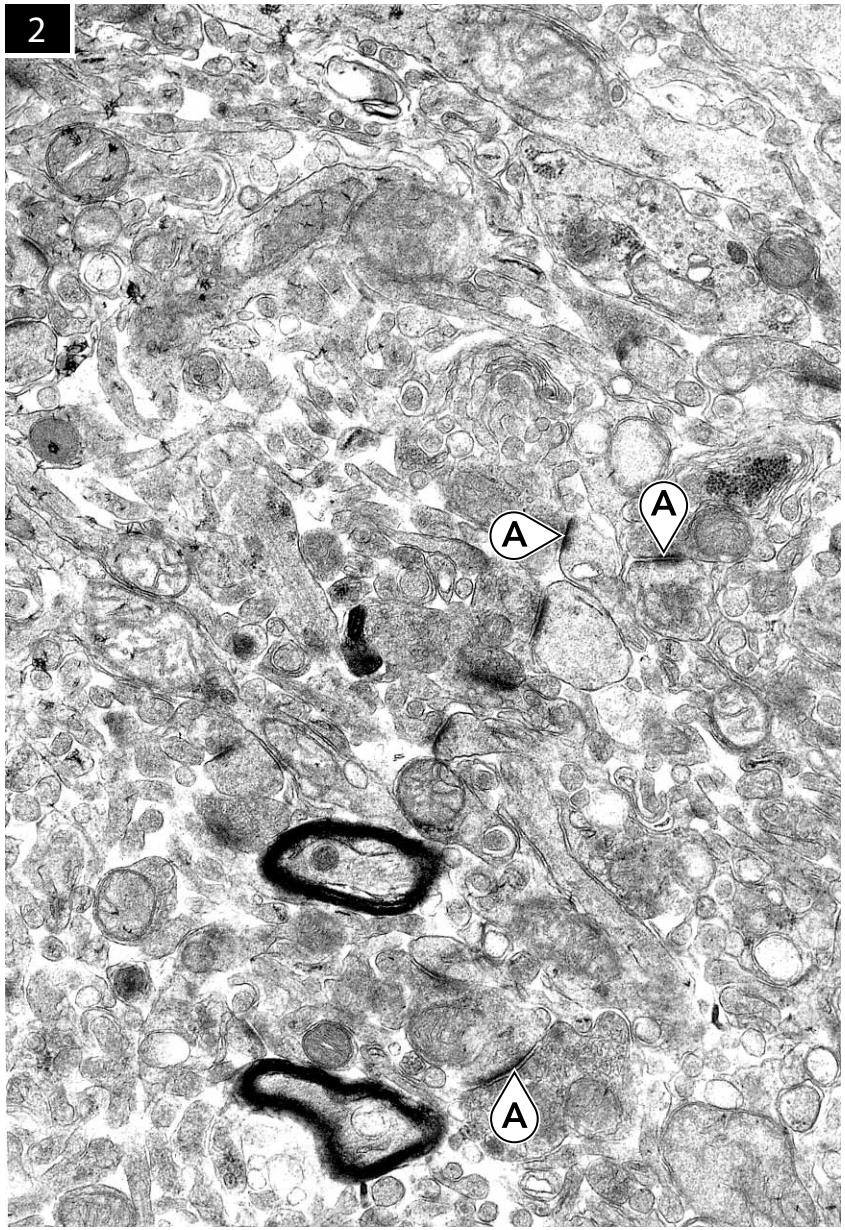
Typical appearance of gray matter at 6700x magnification. Note intact capillary endothelial cells (A) and particles of carbon (B) in the capillary lumens. The overall appearance of the neuropil and of the axons and neurons is excellent.

The electron micrographs on these pages are from samples treated in three different ways.

BioPreservation protocol. The canine brain was glycerolized to 7.4M, frozen to –90˚C, main-tained at this temperature for one year, then thawed and reperfused with glycerol containing fixative solution. The initial phase of this proce-dure is identical to that used by BioPreservation on human patients.

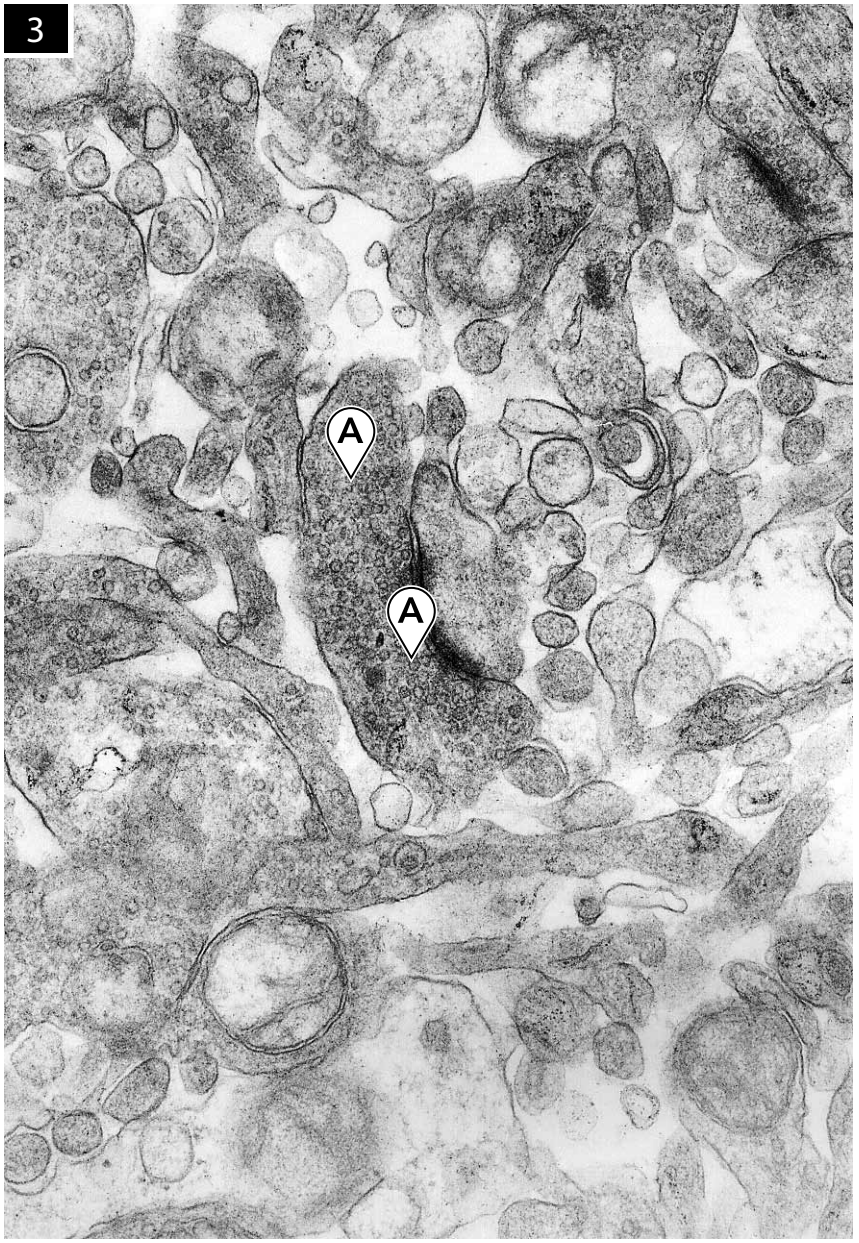
Simplified protocol. The brain was glycerolized to a lower level (4M) at a faster rate (700 mM/minute) before being frozen to –77˚C for one week, thawed and reperfused with fixative. This simplified perfusion is NOT used by Bio-Preservation on its human patients but is similar to practices which were typical in cryonics up to the 1980s. Similar protocol is still preferred today by some cryonicists who advocate a policy of limited medical intervention.

No perfusion or freezing. To provide control data, these samples were taken from anesthe-tized dogs that were perfused with fixative, not glycerol, and were not cooled at all.



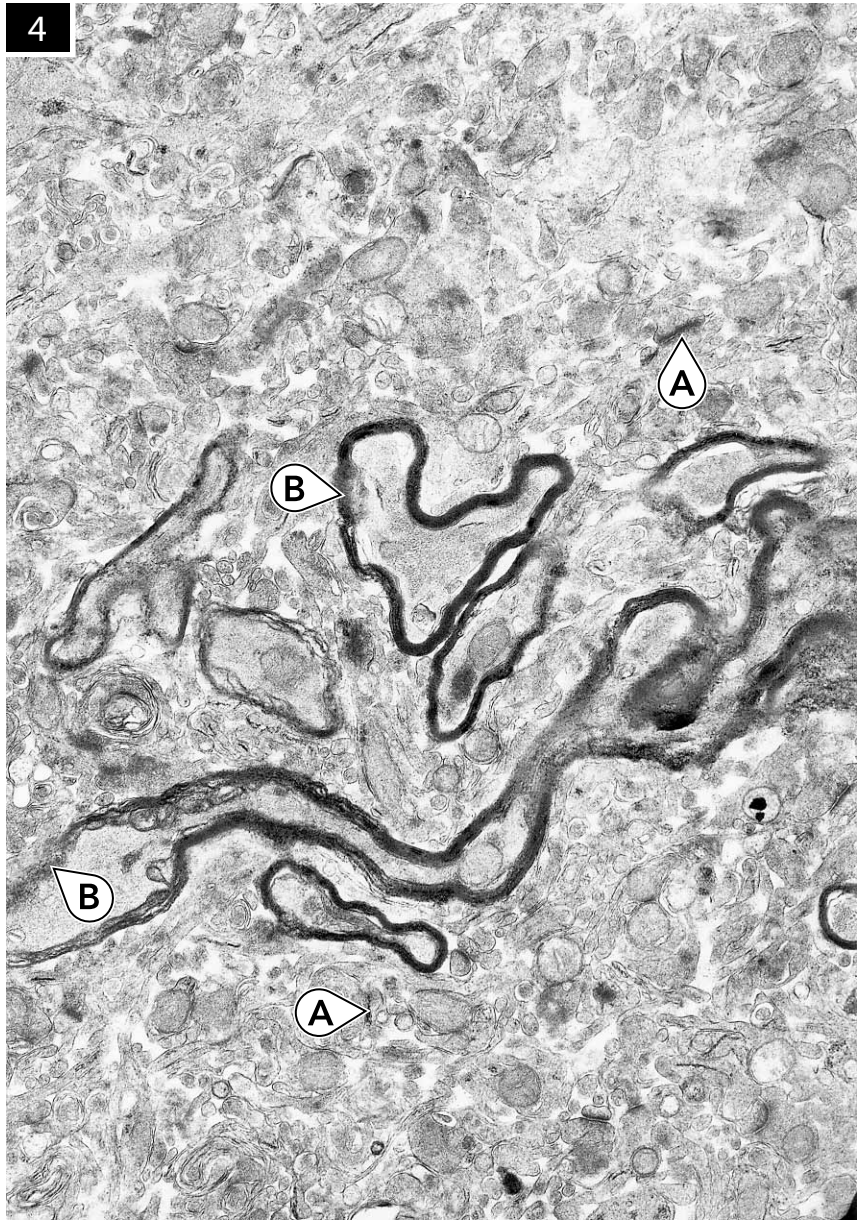
2. BioPreservation protocol.

Neuropil in gray matter from the hippocampus at 35,500x magnification. Architecture of intracellular components, synapses (A), and neuronal membrane integrity are excellent.



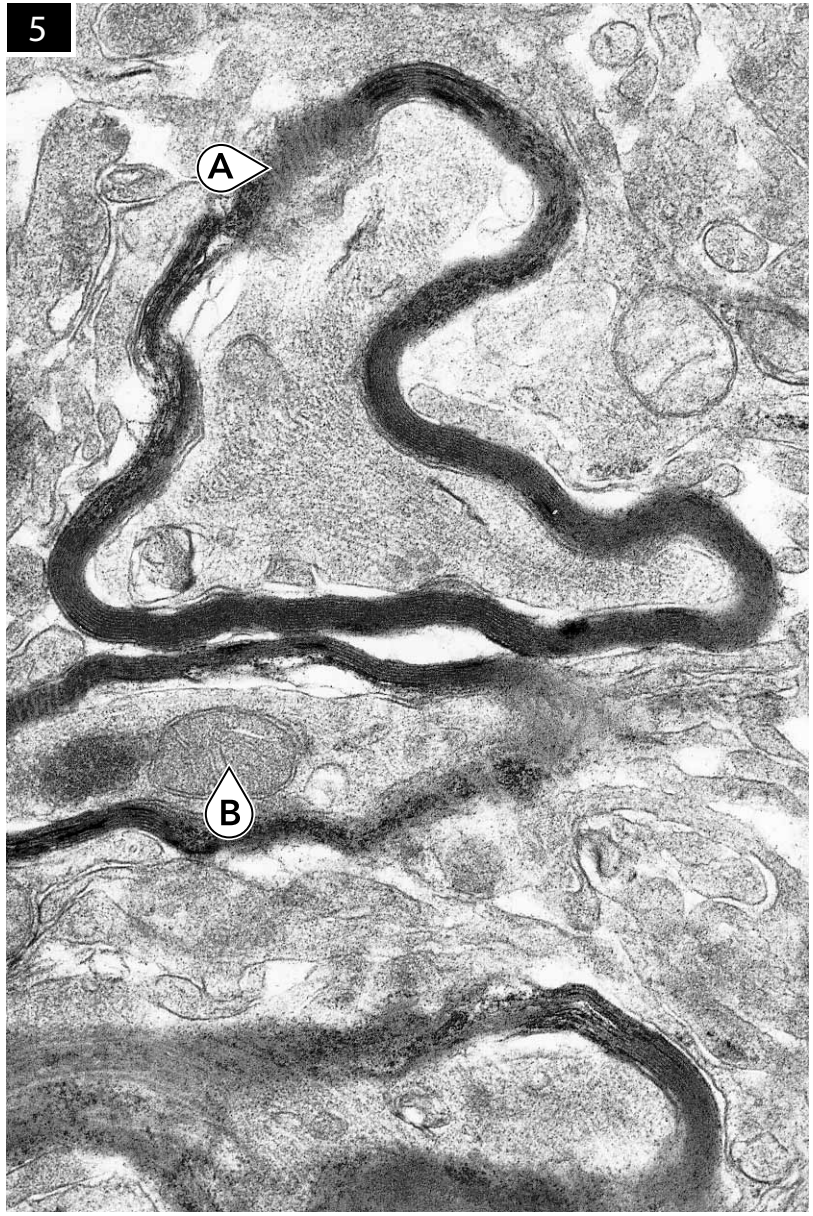
3. BioPreservation protocol.

A synapse in gray matter from the hippocampus at 40,200x magnification. The presynaptic junction contains small packets of neurotransmitter (A) visible as granules. Note the overall crisp appearance of both the synaptic membranes and adjacent structures of the neuropil. This degree of preservation at the synaptic level was uniformly observed in all samples examined.



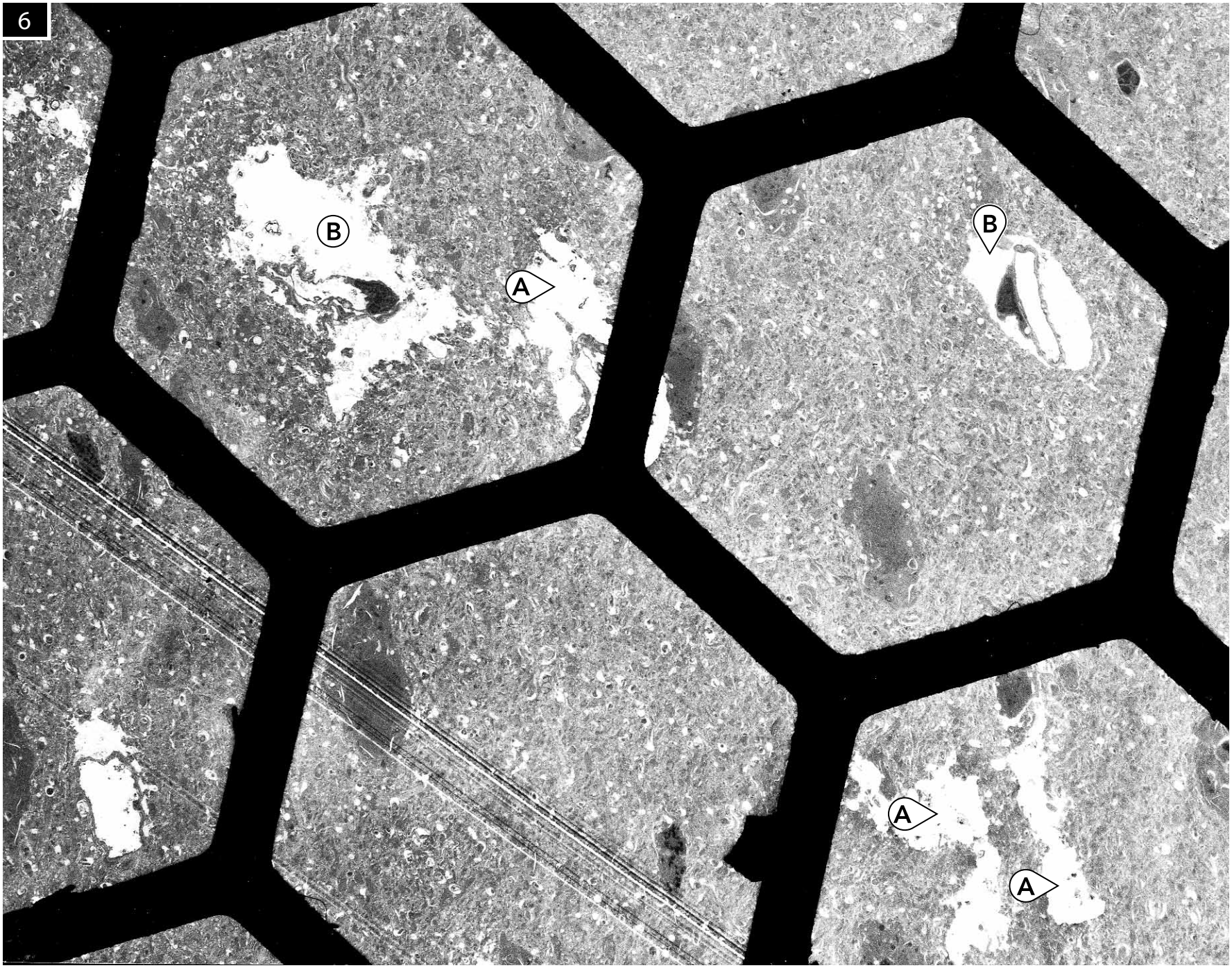
4. BioPreservation protocol.

Gray matter from the hippocampus at 6700x magnification showing two of many synapses (A) and some defects in myelin (B). The axoplasm is intact, with good internal structure and overall high-quality appearance of the neuropil (weave of brain connections) by comparison with similar samples using the simplified protocol. The heavy, wiggly lines across the center of the picture are myelinated axons.



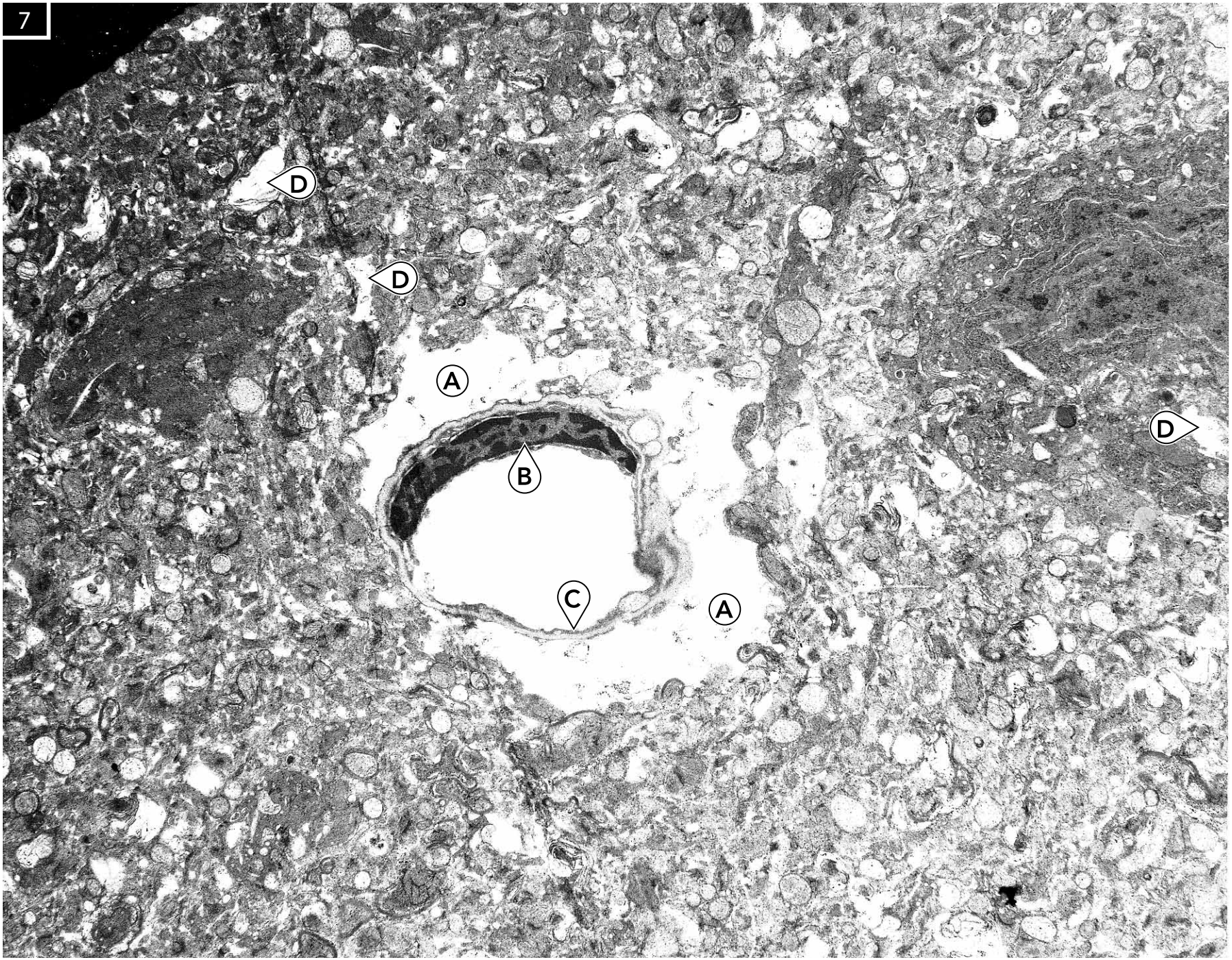
5. BioPreservation protocol.

Closeup of the same area of gray matter at 40,200x magnification. Note that while myelin is injured (A), the axoplasm within the myelin exhibits excellent structural preservation and the membranous structure of the neuropil is intact as are intracellular organelle membranes. A mitochondrion (B) is visible.



6. Simplified protocol.

Hippocampus at 2000x magnification. The black hexagons are the copper grid on which the specimen rests in the electron microscope. Note the frequency of large ice holes or "tears" (A) in the neuropil and the uniform presence of pericapillary ice holes (B).



7. Simplified protocol.

Gray matter from the hippocampus showing very poor reperfusion. A large pericapillary ice hole almost completely severs the capillary from the neuropil (A). The nucleus is denuded of cytoplasm (B) and the endothelial cell has lost its plasma membrane with only bits of cytoplasm clinging to the basement membrane (C). There are also ice holes (D) and a generalized loss of membranous structure with apparent reorganization into vacuoules. This kind of injury is typical of 4M canine brains prepared in this way.

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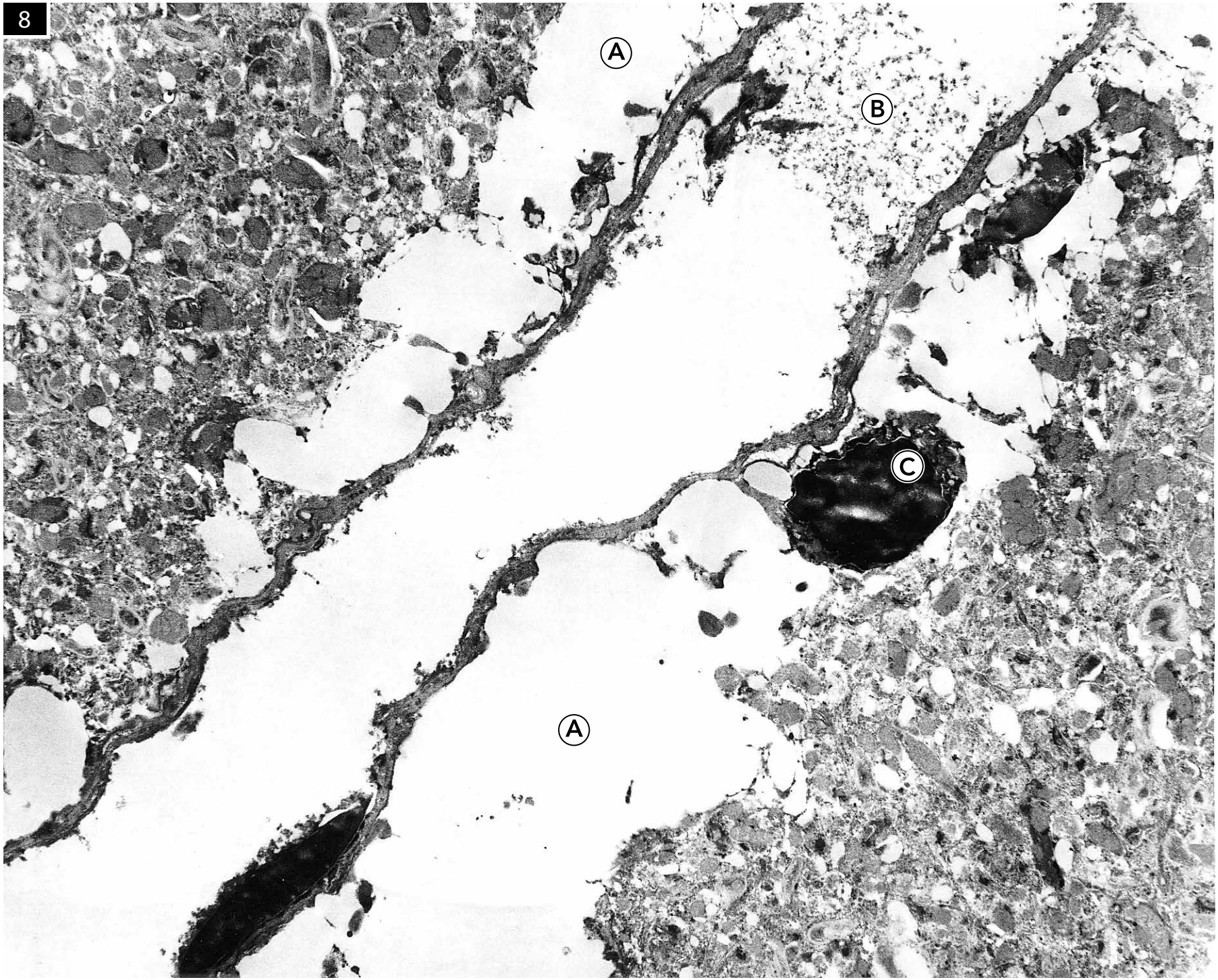

8. Simplified protocol.

A badly damaged capillary at 9700x magnification. Two large tears (A) are visible, with debris scattered in the capillary (B). There is a naked nucleus (C) with no nuclear membrane, from a lysed cell. Although this micrograph is of a canine brain, it shows lesions which are typical of those also seen in brains from cats and sheep subjected to the same form of sub-optimal cryoprotection.

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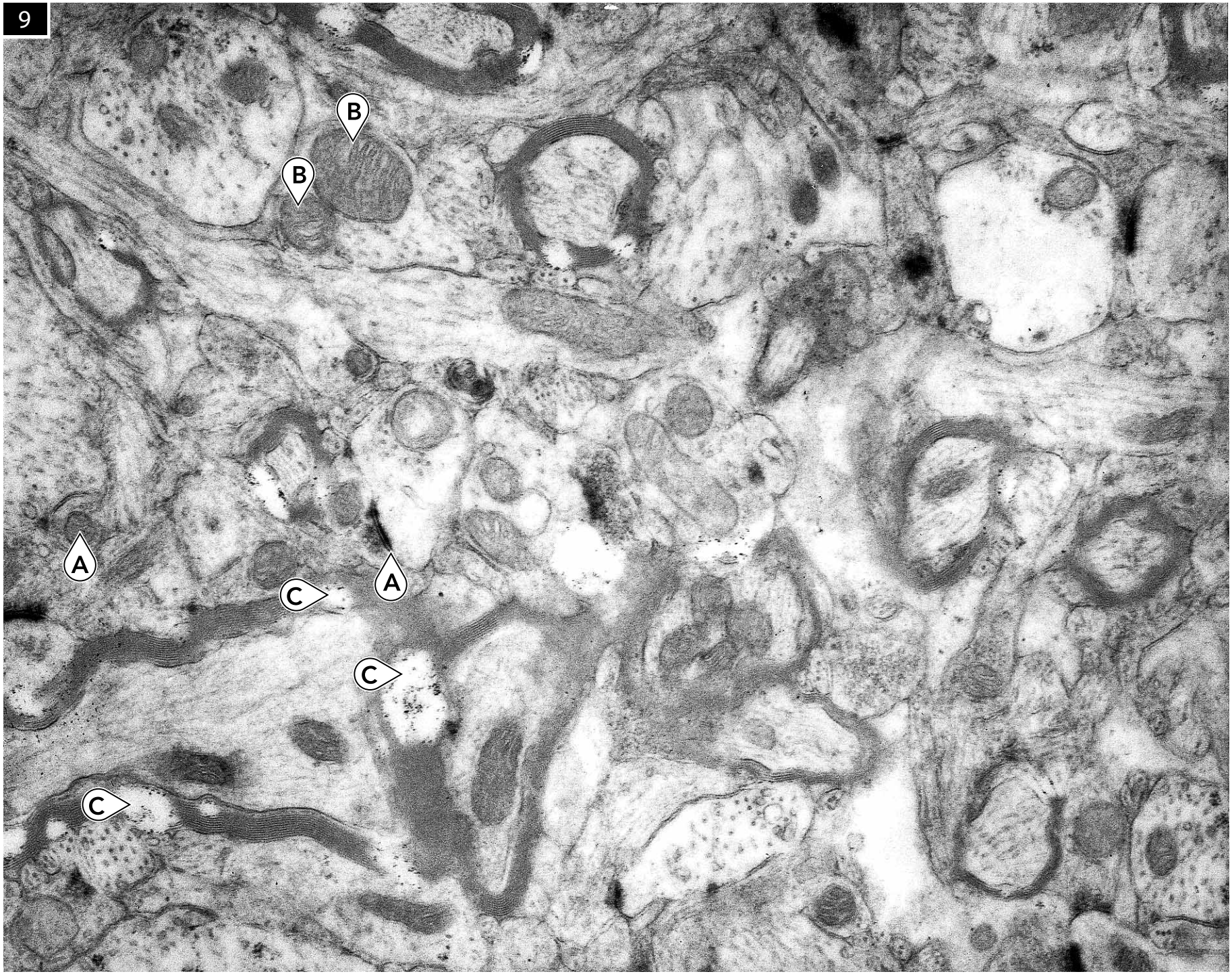

9. No perfusion or freezing.

Gray matter from the hippocampus at 40,000x magnification. Synapses are present (A) as are mitochondria (B). Interestingly, there are defects in the myelin of several axons even in this specimen from a control dog that was not frozen (C).

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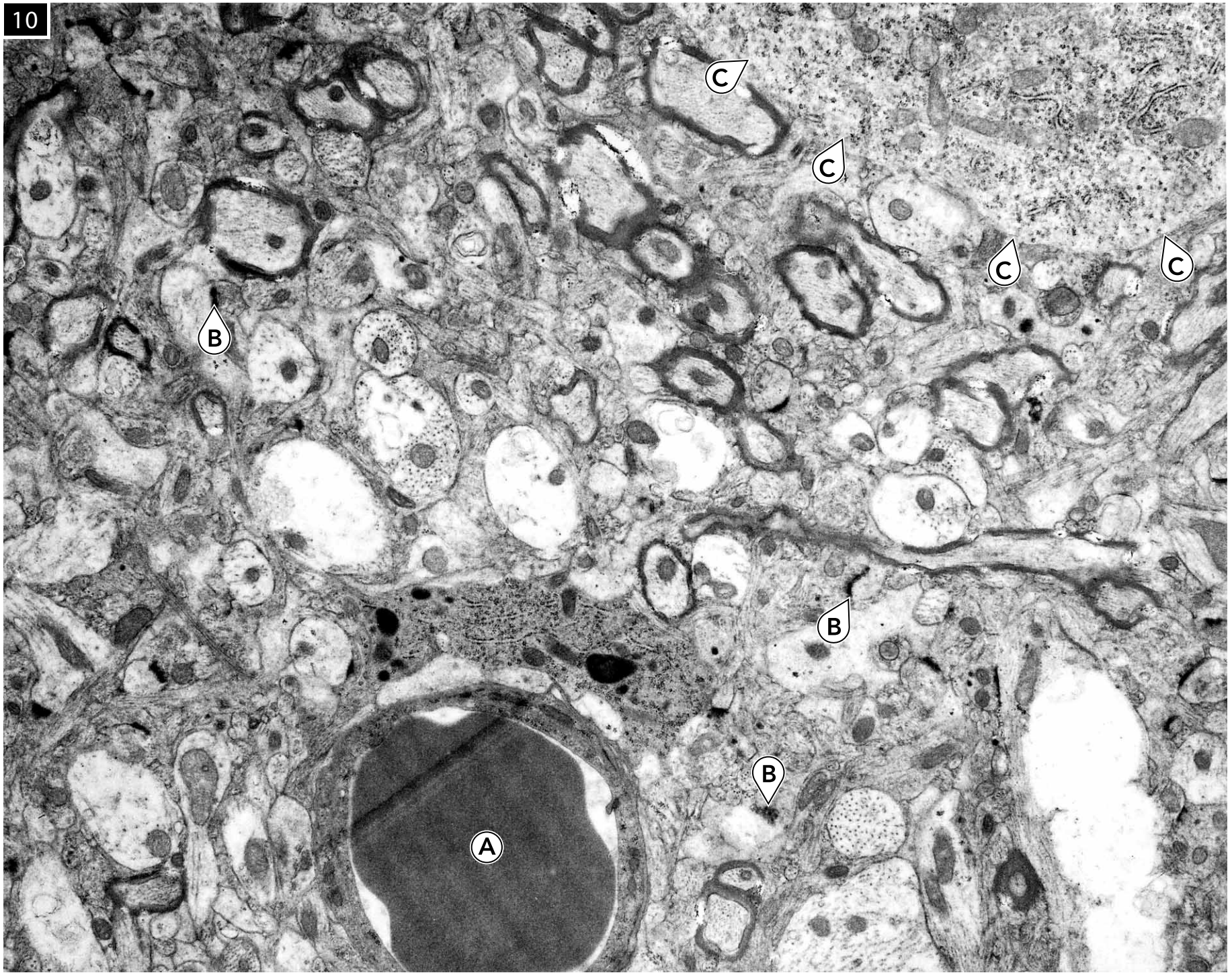
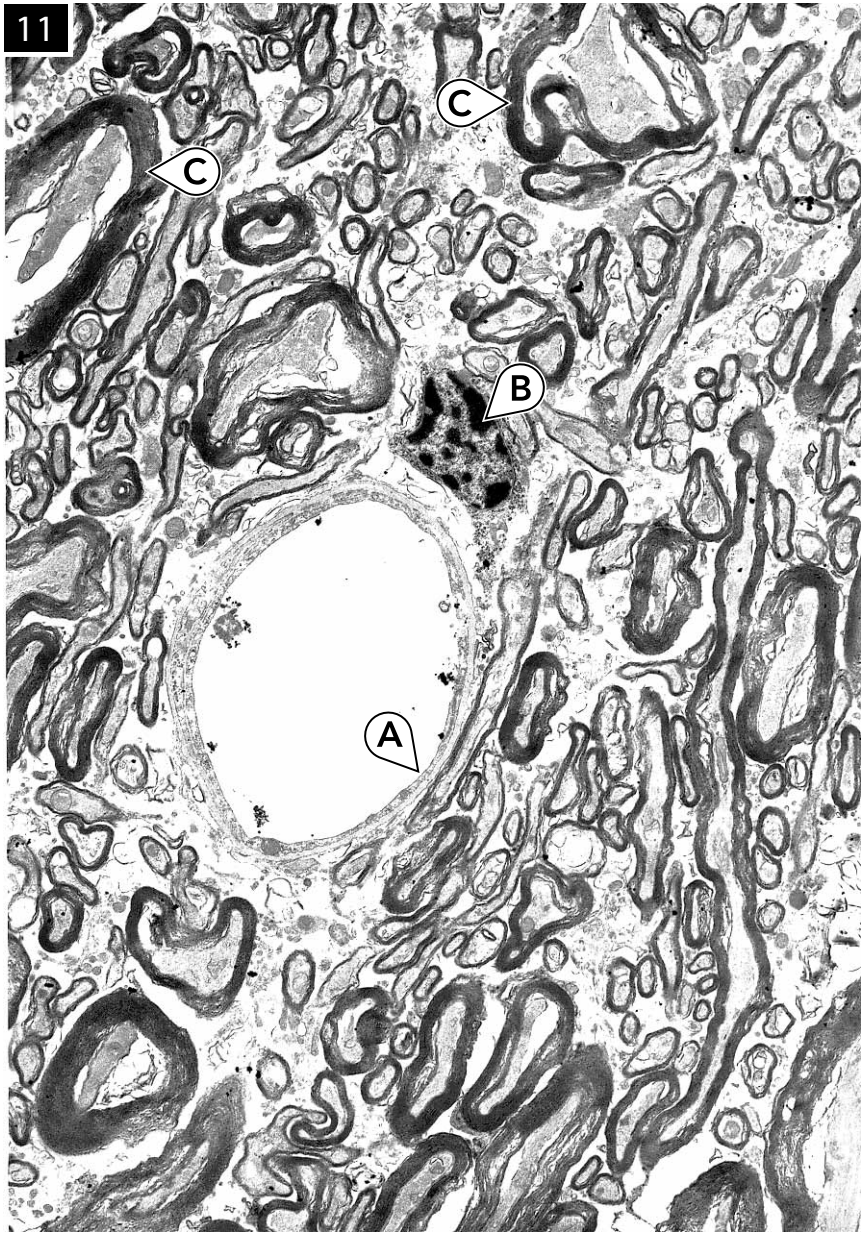


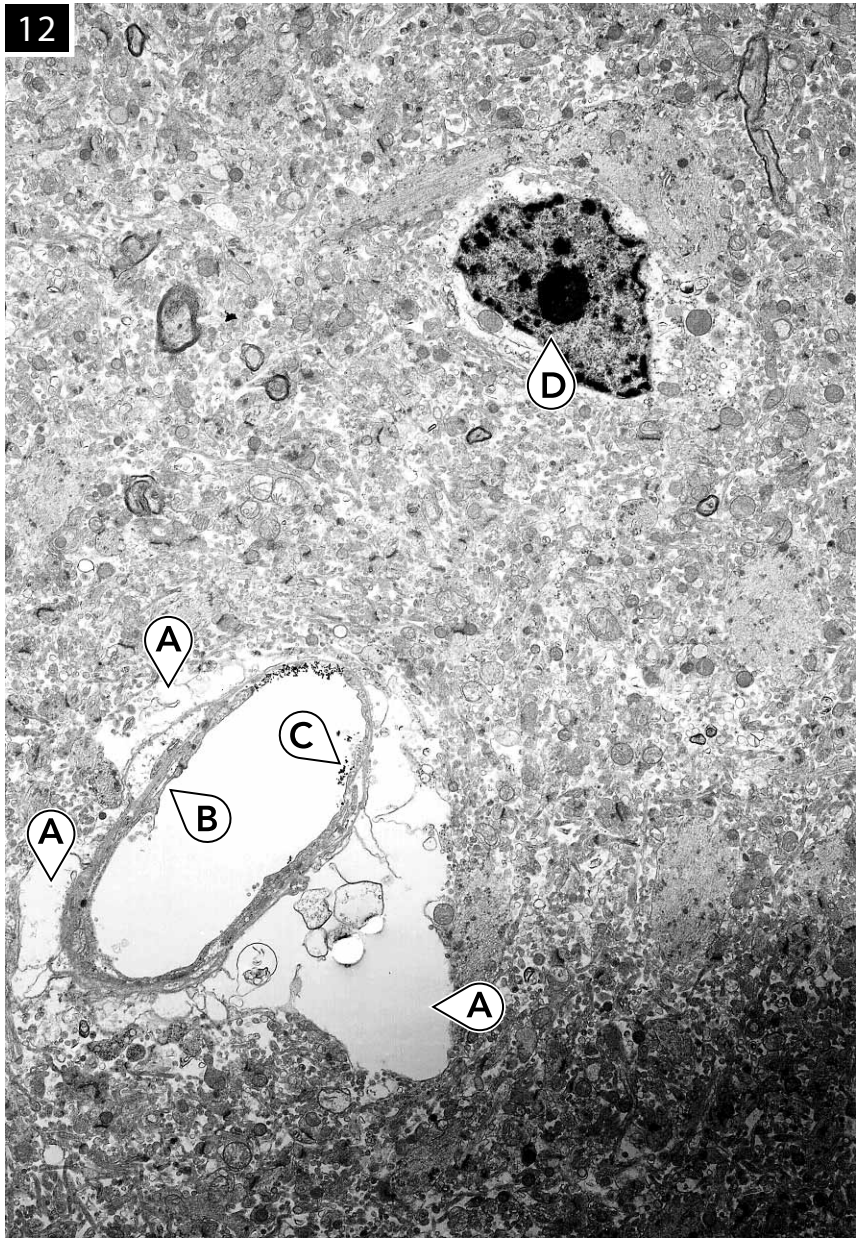
10. No perfusion or freezing.

Gray matter at 15,000x magnification. Note the red cell (A) in the capillary. Lack of dehydration is evidenced by more fine structure in the neuropil and within the axons, lack of shrinkage of the axoplasm, and a more homogeneous character of the myelin. Many synapses are present (B). The boundary of a neuron is visible (C).



11. BioPreservation protocol.

White matter from the corpus collosum at 6700x magnification. Note the excellent preservation of the capillary (A) and its endothelial cell plasma membranes. The nucleus (B) shows typical loss or reorganization of nucleoplasm; this is seen more frequently in frozen-thawed brains than in brains just perfused with glycerol and fixed without freezing. Several axons (C) exhibit typical skrinkage of axoplasm and alteration in myelin structure. The increase in free space between axons and other structures is the result of glycerol-induced dehydration.



12. Bio-Preservation Protocol.

The injury visible here was seen rarely in these samples. Note the presence of what appear to be pericapillary ice holes (A). The endothelial cell membranes of the capillary appear indistinct in several places (B) and there seem to be a few small "blebs" or vesicles of cell membrane material in the capillary lumen. The dark black specks (C) near the endothelial cells are carbon particles which were present in the fixative. Note also the peculiar pattern of injury to the cell nucleus (D) wherein it appears that nuclear material has been lost or rearranged. The nucleolus is also very shrunken.