Rapid Freezing and Frozen Sectioning as a Means Of Preserving Brain Vessel Morphometric Characteristics

MICHAEL NOEL HART, M.D., AND KAREN E. HANSEN, B.S.

SUMMARY A study was performed for the purpose of determining whether ordinary frozen sections of brain could preserve in vivo parenchymal arteriole morphometric characteristics. The results showed that flash-freezing followed by frozen sectioning is just as reliable a means of preserving brain arteriole morphometric characteristics as the time consuming process of flash-freezing followed by freeze substitution.

STUDIES OF THE PHYSIOLOGIC PROPERTIES of blood vessels such as blood flow and resistance would ideally be combined with morphometric studies of the vessels' caliber if such studies were possible. Thus, form could readily be reconciled with function. However, it is very difficult to "capture" or fix a vessel in vitro in the exact morphologic state that it held in vivo.

In previous publications we have shown the inadequacy of formaldehyde fixation as a means of preserving basilar artery morphometry and meningeal vessel morphometry. In contrast, rapid freezing followed by freeze substitution is an excellent method of preserving meningeal vessel morphometry. Freeze substitution, however, is tedious and time consuming and only a few vessels can be analyzed from each animal.

Consequently we undertook the following study to determine if rapid freezing followed by ordinary frozen sectioning is as efficacious and reliable as freeze substitution for morphometric preservation.

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Methods

Eight male Sprague-Dawley rats weighing 265-283 gm were killed by intraperitoneal injection of an overdose of barbiturate. A 1 cm cylinder of frontal lobe was removed and flash-frozen for 60 seconds by immersion in isopentane (2-methyl-butane) cooled to -160°C in liquid nitrogen. Ponten et al. have shown that the outer 2 mm of the brain is frozen within 10 seconds by this procedure. The frozen brain was then mounted on a frozen section chuck with ordinary mounting media. The gyral surface was oriented upwards and was parallel to the surface of the chuck (figure 1). The specimen was placed in a cryostat at -20°C and after reaching temperature equilibrium, frozen sections of 6 μm thickness were cut parallel to the surface of the brain in order to cross-section the penetrating arterioles. The last section was taken not deeper than 1 mm from the brain surface and was air-dried, fixed for 30 seconds in 10% sodium acetate buffered formaldehyde and stained with hematoxylin and eosin (H & E). The remainder of the block was then immersed in acetone containing 1% osmium tetroxide for 2 weeks at -70°C. Following freeze substitution with osmium the tissue block was dehydrated by conventional means, embedded in paraffin and a single surface section of 6 μm thickness was taken and stained with H & E. By this method the external diameters (exclusive of adventitia) of each arteriole fixed by frozen section were compared to the same diameter of the same arterioles fixed by freeze substitution. Thus, the measurements represented sites on each vessel no more than 12 μm apart. Only arterioles cut perfectly in cross-section were measured. Measurements were made with a light microscope at 400 x using a ruled eyepiece calibrated with a stage micrometer. We had previously shown that freeze substitution followed by paraffin block sectioning preserved in vivo vessel morphometry. It was now reasoned that if measurements of the portions of arterioles prepared by rapid freezing and frozen sectioning were the same or nearly the same as those measurements from the freeze substituted portion of the same arterioles, then it could be concluded that frozen sectioning is an adequate means of vessel morphometric preservation.

Results

The results are shown in figure 2. Diameters of freeze-substituted arterioles on the y axis are compared to diameters of the same vessels prepared by frozen section on the x axis. There is excellent predictability of results with the slope approaching 45° and a correlation coefficient of 0.993. The range in the percent change in vessel diameter was from -14% to 11% but only 3 of 31 vessels in the frozen section group differed by more than 10% from the freeze substitution measurements.

Discussion

This study shows that rapid freezing of brain results in excellent preservation of in vivo brain parenchymal arteriole caliber. Although it has been previously shown that rapid freezing followed by freeze substitution also preserves vessel morphometry, the procedure is time consuming and relatively few vessels can be studied. Utilizing ordinary frozen sections, however, many vessels can be studied from one experimental animal and in vivo morphometry reflecting the precise physiologic state of the vessel at the time of fixation can be preserved.

The procedure outlined in this paper utilized pieces of brain removed from the experimental animal before freezing. This was done simply for purposes of comparison of the two methods. In utilizing this procedure to perform morphometric studies of brain vessels under various physiologic conditions it would be advisable to freeze the desired portion of the brain before removal in order to capture the in vivo morphometric state of the vessel.

One of the most important potential benefits of this
procedure is that it provides the means to study the morphometric characteristics of brain parenchymal vessels. Pial vessels have been systematically studied under numerous conditions because of their accessibility via cranial windows. There has not been a ready means to study the morphometric characteristics of parenchymal vessels for the purpose of comparison with pial vessels.

IN ACUTE CEREBROVASCULAR DISEASE (CVD) both necrosis and reversible changes in neurons and other cells occur. The damaged tissue releases only small amounts of substances to the blood because of the specific qualities of the blood-brain barrier, but larger quantities are more easily released into the cerebrospinal fluid (CSF). It has thus been difficult to show any characteristic serum pattern of a biochemical marker in acute CVD, and there is no clinically useful method such as the aminotransferase pattern in acute myocardial infarction. In theory, spinal fluid contains many potential markers of tissue damage in acute CVD. Most studied in CSF are enzymes like aspartate-aminotransferase (ASAT), alanineaminotransferase (ALT), lactic dehydrogenase, creatine phosphokinase, aldolase,[4] adenylyl kinase[5-6] and biogenic amine metabolites[7] or markers for altered energy metabolism.[8-10] Although some of these markers show increased concentrations in some patients with large cerebral lesions, there are so far no conclusive results which establish their usefulness for quantitatively estimating the degree of brain damage. Furthermore, these markers lack brain specificity. Spectrophotometric analysis of blood debris, such as haemoglobin and its breakdown products, has been used to differentiate haemorrhagic from non-haemorrhagic cerebrovascular lesions.[11-12] However, this spectrophotometric method is sometimes of questionable value, as contamination of blood in lumbar puncture is a problem in routine practice, and early assessment as a means of preserving meningeal vessel morphology. Brain Res 260: 178-180, 1983


**References**


**Brain and Plasma Proteins in Spinal Fluid as Markers for Brain Damage and Severity of Stroke**

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I. KARLSSON, M.D.,‡ AND B. WINBLAD, M.D.*

**SUMMARY** Forty well-defined acute stroke patients were investigated for some cerebro-spinal fluid (CSF) markers of cerebral damage. Myelin-basic protein (MBP), tau-fraction, albumin, IgG and transferrin were analyzed on two early occasions after onset of clinical symptoms. Patients with transitory ischaemic attack (TIA) had normal values for MBP both at first and second lumbar puncture. Patients with cerebral infarction and haemorrhage had mean MBP concentrations higher than normal at both lumbar punctures. In cerebral infarction there was a significant increase in MBP from the first to the second lumbar puncture. Patients with intracerebral haemorhage showed the highest mean MBP values and MBP was markedly elevated already at the first lumbar puncture, suggesting different mechanisms of destruction of nervous tissue in cerebral infarction and bleeding. The amount of MBP was also significantly correlated to the visibility of the cerebral lesion at CT-scan and to the short-term outcome of the patients. The tau-fraction, indicating damage to grey matter, was higher than normal in the majority of patients with cerebral infarction and TIA. The concentration of MBP increases with the extent of brain lesion and a high value indicates a poor short-term prognosis for the patient. This study shows that the brain specific MBP in CSF is a useful marker of cerebral damage in acute cerebrovascular disease.

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