**Myocardial Biopsy in Dogs**

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**SUMMARY**

Full-thickness biopsies of ventricular dog myocardium, beating and in arrest, have been studied by conventional fixation (immersion in fixative solution), by the use of cryostat sections thawed on slides, and by freeze drying of tissue blocks. Three types of artifacts were found and could be related (1) to cutting into the beating heart, (2) to thawing of frozen sections on slides, and (3) to the failure of fixative solutions to immobilize still reactive myocardium immediately. The method described yields a biopsy specimen of sufficient diameter to delineate the cutting artifacts. For histological preparation freeze drying appears to be the method of choice.

**Additional Indexing Words:**
Histology artifacts

**VARIous biopsy procedures** have been developed for the heart during the last 6 to 10 years. At present, biopsies at open chest operations, percutaneous or open chest needle biopsies, catheter biopctomes, and power drills are used.\(^1\)\(^–\)\(^9\) In general, reports stress the feasibility of obtaining a suitable piece of tissue and the absence of complications during and following the procedure rather than the results of the pathological examination.

This report will deal with microscopic and submicroscopic changes that occur when one takes biopsies from the ventricles of beating and arrested hearts and with microscopic changes that are related to the effects of fixatives and cryostat sectioning of excitable heart-muscle. It also presents a biopsy procedure and a method for pathological preparations which make the artifacts of cutting recognizable as such in microscopic and electron-microscopic sections. Our light microscopic preparation avoids the artifacts of fixation as well as the thawing of routine cryostat sections.

The present study is based on 98 full-thickness biopsies taken from 41 dog hearts.

**Methods**

**Experimental Animals**

Pointers and mongrel dogs weighing 11.5 to 21.5 kg were used for experiments using extracorporeal circulation and periods of anoxic arrest as well as arrest of the heart by intracoronary artery injection of acetylcholine.

![Figure 1](image)

**Biopsy tool.** (a) Syringe; (b) plastic and metal tube; (c) cork borer with inside cutting edge.


Figure 2

A pie-shaped section of the myocardial biopsy, x 75. The area of tissue, outlined in black, was the section for electron microscopy. The electron micrographs of figures 5 and 6 were derived from the encircled area in the upper portion of the outlined area; the one in figure 7 was taken from the encircled area in the lower portion. (A) Peripheral edge of biopsy; (B) edge resulting from the longitudinal division of the biopsy before fixation; (C) edge resulting from division of the semicircular slices into pie-shaped pieces when unpolymerized Epon.

Biopsy Tool and Procedure

The procedure is an open chest operation in a heparinized and anesthetized dog. The biopsy tool (fig. 1) is a modified cork borer of stainless steel with an inside cutting edge. The borer has an inside diameter of 0.5 cm and slides over a metal tube. This tube is connected with a syringe to apply mild suction on the epicardium to hold the borer in place for cutting. The specimen is aspirated and then removed from the tube by emptying the syringe. The biopsy site is sutured following the biopsy. As many as three biopsies have been obtained from the same left ventricle without major difficulties manifested in physiological studies of the cardiac function.

Tissue Preparation

The tissue cylinder was either divided lengthwise for electron microscopy and freeze drying or used entirely for histological preparation by freeze drying. For light microscopy the tissue was cut into blocks of about 0.3-cm thickness parallel to the epicardial surface. Within 30 seconds after removal from the beating or arrested heart, the blocks were frozen in isopentane cooled with liquid nitrogen. The frozen blocks were transferred to a plastic tube to be stored and transported under liquid nitrogen in the canisters of a Linde LD-10 liquified gas container.

The specimens were dried in a modified Cold Hand Tissue Freeze Dryer\* for 2 days at \(-30\) C in a vacuum of \(1 \times 10^{-3}\) mm Hg. For portions of the experiments, the vacuum was maintained with a Leybold high vacuum pump 2D and a Leybold oil diffusion pump DO-30. In later experiments the drying chamber was pumped down with the help of the mechanical and diffusion pumps. Then the vacuum was maintained by cryosorption pumping using Linde Molecular Sieve 5A cooled with liquid nitrogen. The vacuum was measured with thermocouple gauges

\*Canal Industrial Corporation, Bethesda, Maryland.
(type 501*) and an ionization gauge (type 518*) with the help of the thermocouple-ionization control (type 710*). After the tissue was dry, it was embedded in paraffin (M.P. +58°C) without breaking the vacuum. The blocks were cut on a Leitz base sledge microtome (type 1300) at 5 and 7 μ. The sections were mounted dry on glass slides with or (in case of certain enzyme procedures) without egg albumen. Deparaffinization was accomplished by petroleum ether or xylene. Other frozen blocks were cut in a Labtek-cryostat at 7 μ and thawed on dry slides for enzyme procedures conventionally said to required cryostat sections.10,11

A few biopsy specimens were simply immersed in 10% buffered Formalin after removal from the heart and processed through the usual paraffin-embedding procedure.

In preparation for electron microscopy, the cylindrical myocardial specimen (0.5 cm in diameter) was divided longitudinally. One of these hemicylindrical pieces of tissue was treated for freeze drying as described above; the other was immersed immediately in cold buffered glutaraldehyde solution for 2 hours. It was transferred to cold buffer solution and allowed to remain there overnight. Thereafter, this hemicylindrical myocardial specimen was divided into 1-mm slices parallel to the epicardial surface. These slices then were post-fixed in 1% phosphate-buffered osmium tetroxide solution for 2 hours, dehydrated in ethanol and placed in Epon 812. While in Epon, each of these slices was divided into three pie-shaped pieces (fig. 2) and subsequently embedded in Epon 812. The area outlined in black (fig. 2) was sectioned for electron microscopy.

After the experiment, the entire heart was fixed in 10% Formalin by controlled perfusion.
fixation: coronary perfusion and immersion in the fixative.12

Results

Macroscopic Examination
The specimen taken from the beating heart was roughly cylindrical in the outer third to half of the left ventricular wall. It was somewhat irregular for the rest of the core. The endocardium was sometimes absent. If the core was removed from the arrested left ventricle, a cylinder of 0.4 to 0.5 cm in diameter with epicardium and endocardium was obtained. No gross differences could be observed in the biopsy specimen from the beating and arrested right ventricular conus.

Microscopic Examination: Cutting Artifacts
All specimens were cut parallel to the epicardial surface in order to obtain longitudinal sections of parallel myocardial fibers. Cross sections of the tissue cylinders from the beating heart showed a peripheral circumferential zone to a depth of 0.5 mm with considerable distortion of architecture. Hypercontraction bands, vacuolization, and disruption of myofibers and myofilaments were found as well as separation of muscle fibers from each other. There was occasionally extravasation of blood cells, but this was not widespread. The border between distorted and well-preserved tissue was a rather sharp one if the section was truly parallel to the epicardial surface (fig. 3). Even at higher magnifications the cutting artifact could be clearly delineated.

Figure 4

Biopsy (MAc2) from acetylcholine-arrested heart, preparation as in figure 3, × 450. (a) Zone of damage due to tool; (c) normal myocardium. Note the higher glycogen contents than in figure 3 due to immediate arrest.
Figure 5

An electron micrograph depicting the peripheral edge of the specimen in the left portion of the field. Note the intracellular damage which is continuous with that of figure 6; $\times 7,800$. 

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Figure 6

An electron micrograph showing the intracellular damage continuous with that in figure 5. In the right portion of the field a resemblance of normal myocardium is evident; × 7,800.
Figure 7

An electron micrograph showing the absence of intracellular damage when tissue is divided while in unpolymerized Epon; × 7,500.
No such zone of distorted architecture was found in specimens from arrested hearts. It was also absent along the cut made by the knife in order to bisect the no longer contracting specimen for EM and freeze drying. The only alteration in these circumstances was a narrow outer zone a few microns deep with frayed and squashed ends of muscle fibers (fig. 4).

Electron Microscopy

In figure 2, a photomicrograph of a pie-shaped section of Epon-embedded tissue is shown. The surface edge marked A is the peripheral surface of the biopsy; B is the surface edge resulting from dividing the biopsy longitudinally; C is the surface edge resulting from the division of the hemicircular slices into pie-shaped pieces. The electron micrographs (figs. 5 and 6) show tissue within the encircled area of the surface edge marked A and the one in figure 7 shows the tissue within the encircled area of the surface edge marked C.

The electron micrographs of figures 5 and 6 show the character of the myocardial tissue within the peripherally damaged area. The cellular damage extends from the left border of figure 5 to the right border of figure 6 where the tissue is beginning to resemble that of normal myocardium. This damage extends from the surface toward the center of the biopsy for approximately 30 to 40 \( \mu \). Within this area one can see disorganization of myofibrils, vacuolization of sarcoplasm, and disruption of mitochondria in the form of displacement of mitochondria, loss of matrix, condensation and rearrangement of cristae, and loss of cristae. Comparable damage results when the unfixed cylindrical specimen is divided longitudinally with a razor blade.
(fig. 2, surface edge B). In many of the specimens taken during this study, the damaged area was more extensive, sometimes as much as 0.5 mm. Similar results have previously been reported in abstract.13

As is evident in figure 7, there is no damage to intracellular components in the tissue along the surface edge following division of the hemicircular pieces with a razor blade while in unpolymerized Epon. The only changes resulting from this division are the separation of myocardial cells and the creation of an irregular surface edge which are probably due to movement of the cells caused by handling.

**Microscopic Examination: Artifacts of Fixation and of Thawing**

Frozen-dried sections deparaffinized in petroleum ether and thawed cryostat sections from the same biopsy were incubated for demonstration of cytochrome oxidase (Burstone method; figs. 8 and 9). These were compared with a paraffin section of Formalin-fixed tissue from the same biopsy (fig. 10). The excellent preservation of architecture of the frozen-dried tissue was readily apparent as well as the disruption of structure produced when a frozen section is thawed on a slide. Formalin fixation of the still beating or still excitable myocardium not only produced undue shrinkage of the specimen with disorientation and waviness of fibers but also histological changes which are usually described as homogenization and vacuolization. Such an appearance has sometimes been attributed to degeneration and early necrosis. None of these was apparent in our frozen-dried preparations.

**Discussion**

Biopsy of heart muscles presents a difficult problem because the technique is superim-
posed on an active mechanical state of the individual cells. The procedure produces changes at the point of impact of the instrument and for a variable distance from it. These changes are observed in all biopsies regardless of the mode of fixation. However, fixation in any penetrating fixative solution imposes a further artifact related to the failure of the fixative to reach and immobilize the still reactive myocardial elements. A third type of artifact is produced by thawing frozen sections on slides as is conventionally done when cryostat sectioning is used. These artifacts may lead to grave errors in interpretation of the microscopic appearance of the tissue. The three types of artifacts have been studied and separated by the use of conventionally fixed specimens, by cryostat sectioning, and by freezing and drying of tissue.

**Conclusions**

It is evident that (1) myocardial biopsies are feasible and (2) the complications, if any, are minor. However, to get a good histological preparation, the following conditions must be met:

1. The biopsy must have a sufficient diameter to allow delineation of the zone of core damage. This zone of core damage is shown to be related to cutting from a beating heart, and it is roughly up to 0.5 mm thick. On cross section, therefore, 1 mm of the tissue will present this change which would certainly eliminate thin needles as useful biopsy tools for this tissue. It is worth noting that the severe cutting artifacts are related only to the beating heart and not to the tool inasmuch as they are also apparent when a knife is used for epicardial specimens taken from a beating heart.

2. The histological preparation should employ frozen-dried tissue either as freeze drying of whole tissue blocks followed by paraffin

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**Figure 10**

Biopsy (M71C): Formalin-fixed, paraffin section, 7μ, H & E; × 450.
embedding or as freeze drying of cryostat sections cut at −70 C.\textsuperscript{15} Fixation of the still excitable heart muscle by immersion in fixatives is entirely out of the question since it leads to uncontrollable alterations of the structure as evidenced by figure 10. Similarly, conventional use of the cryostat (with thawing the section on the slide during pickup) yields a histologically meaningless preparation.

We have also been able to adapt enzyme procedures (supposedly requiring cryostat sections) for frozen-dried tissue with consistently good results (fig. 8). It is hoped that superior pathological methodology will do more justice to such valuable specimens from myocardial biopsies than has been the case heretofore.

References

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