Metabolic Studies on Cardiac Tissue Obtained by Needle Biopsy in the Intact Unanesthetized Dog

By Gus G. CasteI, M.D., And Julian B. Marsh, M.D.

This paper describes the use of needle biopsy of the heart as a method of obtaining specimens of cardiac muscle for biochemical study in the intact unanesthetized dog. The procedure is simple and may be repeated many times. Studies indicate that tissue obtained by this method resembles conventional heart slices in its behavior in vitro. Included are data on respiration and the effect of digitalis on cardiac glycogen stores.

INFORMATION concerning the intermediary metabolism of the heart has been derived mainly from studies of slices and homogenates of cardiac muscle. These experiments require sacrifice of the experimental animal, impeding repeated observations in the same animal. The only approach to the study of cardiac metabolism that allows serial measurements in the intact animal in a normal state is that of coronary sinus catheterization. This method is useful in studies of the over-all metabolism of the heart related to the utilization of various metabolites. However, it reflects gross changes and little information can be obtained concerning underlying chemical events. For this purpose one must obtain a sample of cardiac tissue for direct analysis. The desirability of repeatedly obtaining cardiac muscle in the dog for chemical analysis free from the effects of anesthesia and surgery has led to the use of needle biopsy of the heart. This was made possible by combining two previously described technics; fixation of the heart to the chest wall rendering the apex easily available for puncture as employed by Gregg and colleagues,1 and cardiac puncture with methods commonly used in needle biopsy of the liver.2

The purpose of this communication is to describe the relatively simple technics involved and to report some of the preliminary chemical studies on cardiac tissue obtained by use of this method.

CHEMICAL METHODS

Glycogen was determined by the method of Good, Kramer and Somogyi3 as modified by Stadie, Haugard, and Marsh,4 using the Nelson technic for glucose analysis.5 Oxygen uptake was measured in Warburg vessels with an effective volume of 2 ml., made by filling the main compartment of 15 ml. vessels with paraffin. Nitrogen was determined by the method of Hiller, Plazin and Van Slyke.6

The composition of the medium during the measurement of oxygen uptake was as follows: NaCl, 0.1 M; NaH2PO₄, 0.017 M; NaH₂PO₄, 0.017 M; pH 6.8. In the experiments concerned with the synthesis of glycogen in vitro, the medium was 0.3 M glucose and 0.0003 M phosphate, pH 7.0.

TECHNICS OF CARDIAC BIOPSY

A preparatory operation serves to position the heart for future biopsy. In this procedure mongrel dogs, weighing 10 to 15 Kg., are anesthetized with intravenous Nembutal, 30 mg. per kilogram of body weight. Under sterile conditions and employing artificial positive pressure respiration, the chest is entered through an incision in the fifth left intercostal space. A 3 to 4 cm. portion of the costal cartilage of the sixth rib is isolated by blunt dissection and resected from its sternal juncture. Adequate exposure of the heart is obtained by use of a self-retaining rib retractor. The pericardium is incised widely, freeing the heart and allowing the apex of the left ventricle to be brought up into the bed of the resected sixth costal cartilage. The cardiac apex is firmly anchored to the tissues of this area with

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three sutures of fine catgut. Penicillin, 300,000 units, is instilled into the chest cavity and a rubber catheter inserted. The chest wall is closed in layers with catgut and the skin with silk. Care is taken to cover the apex of the heart only with subcutaneous tissue and skin. The chest is evacuated of air and the catheter removed. Penicillin is administered for three days after operation.

Ten days after the preparatory operation, the heart is sufficiently adherent to the chest wall to permit needle biopsy. This is performed with the dog at rest lying on its right side and loosely tied to a cushioned board. The left chest is cleansed with alcohol in the region of the apical impulse, but otherwise asepsis is not employed. The skin and subcutaneous tissue overlying the vigorous apex beat are infiltrated with 1 per cent procaine, and a small incision is made in the skin with a sharp pointed knife. The outer cannula of a "Vim"-Silverman biopsy needle is then inserted through the tissues down to but not into the heart (fig. 1A). This is held stationary while the inner or split cannula is inserted into the myocardium (fig. 1B). The outer cannula is then advanced over the prongs of the inner cannula and the entire instrument rotated to cut the specimen (fig. 1C). The instrument is withdrawn and firm pressure applied over the wound to control bleeding. The procedure is repeated until the desired amount of cardiac tissue is obtained. Frequently, the inner or split cannula is inadvertently directed through the thin wall of the apical portion of the left ventricle into the ventricular cavity and negligible amounts of tissue are obtained. Therefore, several attempts may be required to gain access to the myocardium of the left ventricle or interventricular septum. It is essential that both needles have sharp points and keen cutting edges for optimum results.

The specimens of cardiac muscle so obtained are blotted free of gross blood, rapidly weighed on a microtorsion balance, and the chemical analyses performed.

RESULTS AND DISCUSSION

Over 65 dogs have been surgically prepared for needle biopsy of the heart. There have been no complications or fatalities resulting from this simple procedure. During a period of one year, each animal has been subjected to at least five (one-third of the dogs have had as many as 8 to 10) biopsy procedures, each procedure involving three to five individual cardiac punctures. A recovery period of several days is usually allowed to elapse before the biopsy procedure is repeated, but in many dogs they have been performed as often as four hours apart. No infection, prolonged bleeding, or other complications has occurred in a group of 50 normal dogs subjected to repeated biopsies in the resting state. One fatality occurred early in the course of developing the biopsy technic and was due to advancement of the outer cannula deep into the myocardium resulting in puncture of the left main stem coronary artery. It is essential that the outer cannula be inserted only to a depth sufficient to cover the tip of the prongs of the inner or split needle. In a more recent and separate study to...
be reported elsewhere, three additional fatalities have occurred in a group of 15 dogs. These experiments were performed on normal dogs immediately following a period of vigorous muscular exercise on a treadmill and on dogs with surgically produced large arteriovenous fistulas. The animals exhibited markedly increased cardiac activity and death was due to bleeding into the chest cavity.

The average specimen of cardiac tissue weighs approximately 15 mg. This is usually in the form of an intact plug that is approximately 2 cm. in length and 0.5 to 0.75 mm. in diameter. The degree of fibrosis surrounding the cardiac apex increases following each procedure rendering subsequent biopsies some-what more difficult. This ultimately limits the number of successful biopsies in a single dog. As the needle may be passed through this area of fibrosis, a small piece of fibrous tissue is sometimes attached to the end of the specimen of cardiac muscle. This is easily distinguished from muscle by gross inspection. In addition to the gross apical fibrosis, histologic sections show several fibrous tracts in the myocardium of the left ventricle and interventricular septum that appear to follow the course taken by the needle during cardiac biopsy. These well circumscribed fibrous tracts are approximately 0.5 to 1.0 mm. in diameter and are surrounded by muscle of normal appearance.

A complete study of the effects of cardiac biopsy upon the electrocardiogram has not been performed. However, tracings employing the three standard limb leads have been taken from eight dogs before and two weeks following fixation of the heart to the chest wall. These show only the expected alterations due to change in position of the heart. Additional records taken from 1 to 12 days after cardiac biopsy reveal occasional and varying T wave and S-T segment changes without significant alterations in the QRS complex. No records have been taken during the biopsy procedure but occasional runs of premature beats frequently occur upon insertion of the needle into the heart. These subside upon withdrawal of the instrument.

**Cardiac Glycogen Studies.** The glycogen content of cardiac biopsies was determined in the 24-hour fasted dog with an open chest at the time of the preparatory operation. The dogs were selected at random without regard to age, sex, or weight. When the dog had recovered, 10 days to two weeks later, the biopsy was repeated with the dog at rest in the unanesthetized, nondigitalized state. It can be seen from table 1 that there was no significant difference in the cardiac glycogen content in the open chest, unanesthetized state. Attempts to demonstrate a net synthesis of glycogen from glucose in vitro by these biopsy specimens were unsuccessful. This failure may be attributed to the high initial glycogen content of the tissue (39 μM per gram), since Stadie, Haugaard and Perlmutter

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### Table 1: Glycogen Content of Biopsy Specimens from Normal Dog Heart

<table>
<thead>
<tr>
<th>State of Dog</th>
<th>Number of Observations</th>
<th>Glycogen Content (as μM Glucose/Gm. wet weight)</th>
<th>Glycogen Synthesis in vitro (as μM Glucose/Gm. in 90 min. at 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open chest anesthetized</td>
<td>18</td>
<td>35.7 ± 2.60</td>
<td>+0.6†</td>
</tr>
<tr>
<td>Intact unanesthetized</td>
<td>26</td>
<td>38.7 ± 2.03</td>
<td>-2.6†</td>
</tr>
<tr>
<td>Intact unanesthetized 5 hrs.</td>
<td>18</td>
<td>55.8* ± 4.06</td>
<td>+2.7†</td>
</tr>
<tr>
<td>after Lanatoside-C (.05 to .06 mg/Kg.)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different (t = 3.7, P < .001) from the mean glycogen content in the intact, unanesthetized, nondigitalized group.
† Not significantly different from zero.

### Table 2: Oxygen Uptake and Nitrogen Content of Biopsy Specimens from Normal Dog Heart

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Oxygen Uptake as μM per Gm. (wet weight) per hour</th>
<th>Nitrogen Content as % of N per Gm. (wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>35.8</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>35.4</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>30.7</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>30.7</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>33.8</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>27.8</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>28.5</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. 29.8 ± 2.27 32.7 ± 1.38
found that little or no synthesis occurred in rat heart slices when the initial glycogen content exceeded 25 μM (as glucose) per gram.

Administration of an average therapeutic intravenous dose of 0.05 mg. per kilogram of body weight of lanatoside-C (Cedilanid) to our dogs resulted in a significant increase in glycogen content after four to five hours (table 1). The literature contains many conflicting reports concerning the effects of toxic and therapeutic doses of various digitalis preparations on the cardiac glycogen of the rat, rabbit, and dog. Although the effect appears clear in our experiments, we are unwilling to draw any definite conclusions concerning its significance in the energetics of cardiac contraction. Lanatoside-C was without effect in the synthesis of glycogen from glucose in vitro (table 1).

Oxygen Uptake and Nitrogen Content. The oxygen uptake of heart biopsy tissue was found to average 30 μM per gram (wet weight) per hour (table 2). This compares favorably with values of 40 to 50 μM per gram per hour which we have observed with 0.5 mm. slices of dog heart (left ventricle) measured under the same in vitro conditions. Respiration of biopsy tissue was linear for at least two hours at 37 C. The nitrogen content averaged 36.6 mg. nitrogen per gram (wet weight).

These data indicate that biopsies of dog heart muscle obtained by the present technic resemble conventional heart slices in their behavior in vitro. The usefulness of this technic of obtaining heart muscle for chemical studies apparently depends on the availability of microanalytic technics for analysis of the small amounts of tissue obtained. Although repeated biopsies may be performed, it is impractical to obtain more than 75 mg. at a time.

SUMMARY

1. For the purpose of repeatedly obtaining samples of cardiac muscle for metabolic studies in intact, unanesthetized dogs, a needle biopsy technic has been devised.

2. Employing this technic, the mean glycogen content of cardiac muscle biopsy specimens was 35.7 ± 2.60 μM per gram (as glucose). In the anesthetized dog, the mean was 38.7 ± 3.03 μM per gram. Administration of lanatoside-C to the intact animal significantly increased the glycogen content to 55.8 ± 4.66 μM per gram.

3. The average oxygen uptake of the biopsy tissue was 30 μM per gram (wet weight) per hour. The mean nitrogen content was 32.6 mg. nitrogen per gram (wet weight).

4. The advantages and limitations of this technic in the study of cardiac metabolism in the dog are discussed.

REFERENCES

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