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Technic for Preparation of Glycerol-extracted Whole Frog Hearts for Study of Myocardial Fiber Responses.* (21952)

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Since the demonstration by Szent-Györgyi that glycerinated skeletal muscle fibers contract on exposure to adenosine triphosphate, interest has developed in this preparation as a model for the study of muscle contraction (1). Attention has been directed largely to glycerinated psoas muscle fibers because of their length and relative absence of connective tissue(2-4). Glycerol-treated cardiac muscle fibers are more difficult to handle and have not been extensively studied. However, Taeschler and Bing(5) were able to prepare and observe glycerol-extracted trabecular fibers of dog hearts. The purpose of this communication is to describe a glycerinated frog-heart preparation which is suitable for the study of contraction-relaxation cycles.

Method. The frog heart has no coronary circulation, so that tissue nutrition depends upon the diffusion of solutes through the endocardium. The quantity of diffusion is enhanced by numerous fissures in the ventricular walls(6). Therefore, thorough glycerine extraction of the whole heart should be achieved by perfusion and immersion in a glycerol solution. After extraction, ventricular perfusion with suitable solutes should produce contraction of the muscle fibers. Large bullfrogs (*Rana catesbiana*) are employed. For the preparation of glycerinated hearts, the central nervous system is destroyed and the thorax opened ventrally. The pericardium is excised and the heart is dissected from the mediastinum, care being taken to avoid injury to the ventricle. The fresh heart is placed in Ringer's fluid at room temperature and allowed to beat for several minutes until largely cleared of blood. The heart is then tied to an applicator stick by a ligature tightly drawn around the aortae and upper portion of the atria. A small wire hook is inserted through the ventricular muscle near the apex; the free end of the hook is tied to the stick so as to apply a slight stretch to the ventricle. A needle introduced through the atrium permits the infusion of 50% glycerol into the heart until it is moderately distended. This procedure removes all blood, and the tissue becomes translucent. The mounted specimen is then placed in a capped test tube containing 50% glycerol and is stored at −30 to −10°C for 48 to 72 hours. A thin coat of ice may form at the glycerol-air interface; the tissue should be kept below this level.

Results. After extraction for 2 to 3 days, contraction and relaxation of the glycerinated hearts may be produced by using the perfu-

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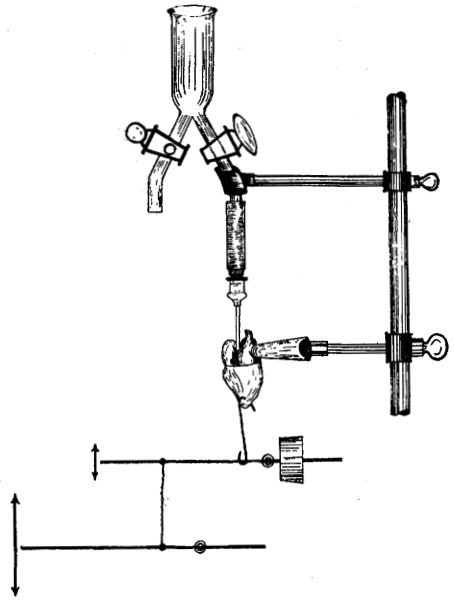

FIG. 1. Diagrammatic sketch of perfusion apparatus with glycerinated frog heart in place. Rate of perfusion of "saline" may be regulated from 30 ml funnel by the stopcock. The counterweighted compound lever system has a net wt of about 800 mg; the system is readily moved by the ventricular muscle fibers.

sion procedure. For this purpose a simple apparatus is employed (Fig. 1). The stick is cut above and below the atrial ligature and the tie holding the hook is severed. The heart is suspended in the apparatus by a pinch clamp applied to the atrium adjacent to the AV groove. The lower end of the hook engages a compound lever system the movements of which are plotted on BMR paper mounted on a kymograph. Writing levers or smoked drums may be used if desired. The heart is perfused by means of a 15-gauge needle inserted through the atrium into the ventricular cavity; the fluid escapes from the ventricle through the small orifice made by the hook. Thorough perfusion of the entire ventricle must be obtained in order to induce maximum contractions. We have found that adequate distention and uniform perfusion of the specimen is assured when the needle is placed through the atrium medial to the bulbus cordis, and a few millimeters into the ventricular chamber. Moderate distention of the tissue probably enhances diffusion of solutes around muscle fibers, and facilitates contraction by stretch of the fibrils(5). The glycerinated myocardium is friable and caution must be observed to prevent tearing of the tissue.

A solution of 0.15 M KCl and 0.02 M $MgCl_2$ (or other molarities as desired) in glass-distilled water serves as the basic perfusate. This solution is similar to that described by Bozler(4), and is designated as "saline." The suspended heart is irrigated with the "saline" at room temperature for several minutes before the administration of ATP. The di-sodium salt of adenosine triphosphate (Sigma) prepared in "saline" is administered through the perfusion funnel. Concentrations ranging between 0.05% and 4.0% ATP have been used. However, reproducible maximal contractions more often occur with 0.5 to 1.0% ATP solutions. Because of the acidity of ATP, it is our custom to adjust the ATP-saline solution to physiological pH range. The pH adjustment may be carried out with N/10 KOH immediately before use.

More than 130 glycerinated frog heart preparations have been studied using this technic. Marked contraction develops within 1 minute from the beginning of ATP perfusion and peak tension occurs within 3 minutes in most instances. We have repeatedly noted

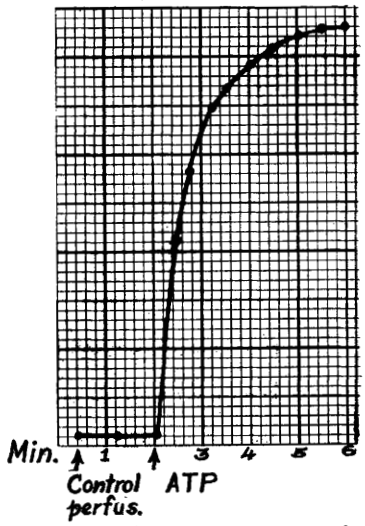

FIG. 2. Example of contraction-curve of a glycerinated whole frog heart. Basic perfusate of KCl .003 M and $MgCl_2$ .0004 M. 1% ATP in basic perfusate added at indicated point.

that ATP contractions of the preparations are enhanced by permitting the specimens to come nearly to room temperature before they are detached from the applicator sticks and set up for perfusion.

Comment. This model system may be of value in *qualitative* studies of contraction and relaxation phenomena in glycerinated cardiac muscle. Observations concerning mechanisms of relaxation are in progress. The rapidity of the diffusion of solutes around the muscle cells is confirmed by the rapidity of ATP response. The ease of operation of the model system and the simplicity of preparation commend this technic for experimental observations of myocardial contractile proteins.

Summary. A method is described for the study of contraction-relaxation phenomena in glycerol-extracted cardiac muscle. Excised hearts of bullfrogs are mounted and extracted in 50% glycerine; subsequently, perfusion with electrolyte solutions and ATP may be carried out, and the contraction recorded by a mechanical system. The ease of preparation and the dependability of the muscle model may prove to be of value in the study of glycerol-extracted heart muscle.

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Effect of Lipemia and Heparin on Free Fatty Acid Concentration of Serum in Humans.* (21953)

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The discovery that the *in vitro* clearing of lipemia by plasma obtained after injection of heparin involves lipolysis of triglyceride with release of free fatty acid(1-5) has directed attention to the possibility that free fatty acid might be released *in vivo* by the action of post-heparin clearing factor. Furthermore, the possibility that clearing factor may participate in normal fat metabolism and transport, even when heparin has not been injected (6-8), suggests that free fatty acid may play a physiological role.

It was shown(9) that in rats the free fatty acid concentration of the serum rose when heparin was injected or when alimentary lipemia was present. The present study extends these observations to human subjects in whom lipemia was induced both by the alimentary route and by intravenous injection of fat emulsion and in whom heparin was injected intravenously both in the fasting state and during alimentary lipemia.

Subjects, materials and methods. Ten healthy young adult males, conscientious objectors assigned to the Metabolic Research Division of this laboratory, served as subjects. Heparin (Testagar, 10 mg/ml) was injected intravenously at a dose of 0.5 mg/kg body weight. For the production of alimentary lipemia commercial cottonseed oil (Wesson) was used. The fat emulsion used for intravenous injection (Lipomul, Upjohn) had the following composition: cottonseed oil 15%, dextrose 4.2%, soybean phosphatide 1%, and polyethoxy-propylene oxide (Pluronic F-68, Wyandotte) 0.5%. *Blood lipid analyses.* Venous blood samples were taken from the antecubital vein in chilled syringes and placed immediately in an ice water bath.

* The opinions expressed in this paper are those of the authors and do not necessarily represent those of any governmental agency.

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