Method for Study of Contraction of Isolated Heart Muscle Under Various Physical Conditions

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It is general knowledge that the strength with which heart muscle contracts may be influenced greatly by physical factors as well as by chemical agents. Thus, when isolated heart muscle is used to study the inotropic actions of substances, pains are usually taken to control the temperature, the diastolic fiber length, and the frequency of contraction of the muscle. These factors are most conveniently controlled by fixing them at some arbitrarily chosen level—a practice which facilitates rapid screening of compounds with relatively simple methods, but which may lead to oversight of any influence that changes in the physical state of the muscle might have upon drug effect. If mechanisms of action are shared at some point by certain of the physical and chemical determinants of contractility, there may be interactions between the determinants. An example of such interaction is already available in the demonstration that the cardiac glycosides markedly alter the relationship between stimulus frequency and the contractile force of the isolated ventricle of the frog heart. Drugs might also be capable of displacing the diastolic pressure-volume curve, of altering the relationship between fiber length and the strength of contraction, or of changing the influence of temperature upon contractility. Furthermore, it is conceivable that substances might affect differently the work performed by the muscle when shortening is permitted and the maximum force developed when it is not. Apart from their theoretical interest, actions of the types just mentioned would be of great practical importance to anyone testing substances for inotropic activity: the apparent effects upon myocardial contractility of agents having any of the properties discussed would depend upon the physical conditions under which the test was carried out.

Although certain aspects of the problem have been investigated, no comprehensive analysis has yet been made of the influence of physical factors upon the actions of substances affecting myocardial contractility. Undoubtedly, one reason for this has been the lack of a suitable preparation. To explore the possibilities outlined above, one must be able to vary and measure not only the concentrations of substances in the solution bathing the muscle, but also the temperature, the interval between contractions, the tension in the muscle, the length of the muscle, and the resistance to shortening. A preparation developed for the purpose is described in this paper, and a few observations are included as examples of its application.

Methods

Preparation

Any cardiac chamber of convenient size can be used if its walls are thin enough to permit oxygenation without coronary perfusion. The apparatus was constructed to accommodate rabbit atria, but with suitable changes in cannula size it has been used with the atria of puppies, cats, guinea pigs, rats, chickens, and turtles, and with the ventricles of turtles and large frogs. In general, preparations of the left atria of rabbits have proved the most satisfactory.

The left atrial preparation is set up as follows. A cannula (fig. 1, A) is passed through the atrio-ventricular valve from the ventricle and tied into the left atrium with a ligature which excludes the pulmonary veins. A mixture of 95 per cent oxygen...
and 5 per cent carbon dioxide under moderate pressure (6 to 8 cm. H₂O) is admitted to the cannula. Adjacent tissue is then dissected away, leaving a thin-walled muscular sac filled with gas. A stream of bicarbonate-buffered physiological solution (for composition, see below) is kept dripping over the atrium while it is being prepared. The cannula bearing the atrium is mounted in chamber B, which is then filled with the physiological solution. Chamber B communicates through a low-resistance valve (C) with a volume-sensing chamber (D). By virtue of a groove around the plug of valve C, chamber B is in communication with an unbonded strain-gauge pressure transducer (E) whatever the position of the valve. The solution in chamber B is kept separate from that filling the valve, pressure transducer, and volume-sensing chamber by means of a thin, flaccid rubber membrane. The whole assembly is immersed in a water bath to control temperature, which is measured with a small thermometer inside chamber B. The cannula is connected by large-bore tubing and a large stopcock to either of two 22.5-liter pressure bottles fitted with water manometers. The pressure applied to the cannula is recorded using a Statham P-23 AA strain-gauge pressure transducer. All major parts of the apparatus are made of acrylic plastic (Plexiglas) except the valve plug, which is polytetrafluoroethylene (Teflon), and the chamber walls (unshaded in the diagram), which are glass. Metal parts (shown in solid black) other than the stimulating electrodes are stainless steel. Cannulae are made either of glass or of acrylic plastic. The joint between the cannula and the bottom of chamber B is ground to standard taper and sealed with stopcock grease. The cannula has a blunt tip pierced with many small holes, and is circled by two platinum stimulating electrodes. One, just below the tip, serves both as an internal electrode and as a ridge beneath which the atrium can be tied; the second, below the first, contacts the fluid bathing the outside of the atrium. The atrium, otherwise quiescent, is stimulated with square-waves of 5- or 10-msec. duration and slightly greater than threshold voltage.

The volume of fluid in chamber D is measured with an alternating-current Wheatstone bridge circuit incorporating two stainless-steel plates partially immersed in an electrolyte solution (see fig. 1). Making the plates diverge as they enter the solution makes the changes in bridge output very nearly directly proportional to the changes in the volume of fluid in chamber D. The required angle between the plates, a function of the dimensions of the electrode assembly and of the conductivity of the electrolyte solution, is readily determined empirically. A Sanborn model 150-1100 AS carrier preamplifier is used both to excite the bridge with alternating current and to amplify and demodulate the bridge output. Static pressure and volume measurements are precise to within 2 mm. H₂O and 0.02 ml., respectively. The compliance of the fluid-filled portions of the apparatus is less than 0.01 ml./500 cm. H₂O. Because of the low compliance of the system, volume changes in chamber D accurately reflect changes in atrial volume under all conditions. The accuracy with which dynamic measurements of volume changes may be made is limited by the amount of distortion produced in the air-liquid interface of chamber D by jets of fluid from the orifice of valve C. To estimate the extent to which such distortion might interfere with dynamic measurements of atrial volume, a sinusoidal volume fluctuation (amplitude 2 ml. peak-to-peak, frequency variable) was imposed upon the system through the orifice normally occupied by cannula A. Distortion was negligible at frequencies up to 5.5 cycles/sec., at which it became 5 per cent of the peak-to-peak amplitude; higher frequencies were associated with a great deal of distortion. The peak rate of flow at 5.5 cycles/sec. (approximately 35 ml./sec.) is about twice the maximum encountered in most experiments with rabbit atria at 37 C. Valve C was made short, with as large a bore as possible, to minimize the resistance to contraction offered by friction and the inertia of the fluid in the system.
Care was taken in the design to minimize distortion of the pressure record. The pressure transducer (E) is a Statham model P23D, and the channel from the manometer to chamber B is short, rigid, and free of constrictions as possible. Although the frequency response of the system has not been measured, it probably is considerably better than that determined\textsuperscript{2} for the same transducer recording through a 22-cm. PE 50 catheter (flat to 30 cycles/sec). Chamber D has a large (12 cm.\textsuperscript{2}) cross-sectional area, so that changes in atrial volume do not appreciably change the hydrostatic pressure opposing atrial distention.

Records of atrial volume, of distending pressure, of the pressure in chamber B, and of the potential difference between the stimulating electrodes are made with a Sanborn model 150-104 direct-writing oscillograph, equipped with three model 150-1100 AS carrier preamplifiers and one model 150-1600 ECG-general purpose preamplifier.

The physiological salt solution used has the following composition before equilibration with CO\textsubscript{2}: Na\textsuperscript{+}, 140 mEq./L.; K\textsuperscript{+}, 5 mEq./L.; Ca\textsuperscript{2+}, 4.5 mEq./L.; Mg\textsuperscript{2+}, 2 mEq./L.; Cl\textsuperscript{-}, 98.5 mEq./L.; SO\textsubscript{4}\textsuperscript{2-}, 2 mEq./L.; H\textsubscript{2}CO\textsubscript{3} and HCO\textsubscript{3}\textsuperscript{-}, 24 mM; H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, and HPO\textsubscript{4}\textsuperscript{2-}, 1 mM; glucose, 10mM; L-glutamate, 5 mM; pyruvate, 5 mM; fumarate, 5 mM; insulin (Lilly, crystalline zinc), 5 units/L. The solution is equilibrated with 95 percent O\textsubscript{2} and 5 percent CO\textsubscript{2}; pH after equilibration is approximately 7.4.

Use of the Preparation

If chamber D is filled to a level slightly above the atrium, the atrium may be collapsed gently and uniformly, whatever the distending pressure, by applying the same pressure to the surface of the liquid in chamber D. With the atrium collapsed, the Wheatstone bridge is balanced and the volume recorder is calibrated by injecting known volumes into chamber D. When chamber D has again been opened to the atmosphere, various pressures may be applied to the cannula, and the corresponding atrial diastolic and stroke volumes recorded. At any point, valve C may be closed, preventing the atrium from changing its volume. Contractions then produce a fall in pressure in chamber B. Contractions occurring when valve C is open are opposed by a constant pressure (except for the effects of inertia and friction), and are therefore termed isobaric; those occurring when the valve is closed are isochoric (Gr. isos + chora, space). Isobaric and isochoric contractions are, respectively, the three-dimensional counterparts of isotonic and isometric contractions. With the simple means of switching from isotonic to isometric recording afforded by this method, one may examine virtually simultaneously the effects of changing distending pressure upon diastolic volume, the amplitude of isobaric contraction, and the pressure generated by isochoric contraction. Because of the inertia of the fluid displaced, each isobaric contraction is followed by a series of oscillations in atrial volume. If the frequency of contraction exceeds two or three beats per second when the distending pressure is low, these oscillations may persist throughout diastole, preventing accurate determination of diastolic and stroke volumes. If the oscillations are somewhat damped by partly closing valve C, this difficulty may in some degree be overcome, for diastolic volumes can be measured at high frequencies of contraction. The amplitude of isobaric contraction cannot be measured in this way, however, because the smaller the valve orifice, the greater is the departure from truly isobaric contraction. "Auxotonic" contractions (see Paton\textsuperscript{3}) may be achieved by limiting the airspace in chamber D.

Results

The relations among many of the variables that can be studied with the preparation just described are conveniently expressed in terms of three types of curves: (1) diastolic volume vs. distending pressure (diastolic pressure-volume curves); (2) stroke work of isobaric contraction vs. diastolic volume (isobaric Starling curves); (3) pressure generated by isochoric contraction vs. diastolic volume (isochoric Starling curves). Examples of these are presented as illustrations of the use of the method. Atrial volumes and distending pressures are corrected, respectively, for the volume of the tip of the cannula and for the height of the fluid in chamber D above the point at which the atrium is tied to the cannula. Stroke work is expressed as the product of stroke volume (in ml.) and distending pressure (in mm. H\textsubscript{2}O). Neglect of the kinetic factor is proper, since energy spent in acceleration of fluid early in contraction reappears as potential energy during deceleration.

Resting and Diastolic Pressure-Volume Curves

Attention to the phenomena of creep and hysteresis is essential to the reproducible determination of pressure-volume curves. When the pressure distending an atrium is suddenly raised or lowered, there is an immediate rapid adjustment in volume, followed by further slow change in the same direction (slow yielding or creep) which may amount to as much as 20 percent of the total volume change. The
relative magnitudes of the rapid and slow volume changes and the time course of the creep vary with the amount and direction of the pressure change and the range over which it is made. Creep is usually perceptible for three to five minutes after a rise in distending pressure, but may be significant even longer under some conditions.

Atrial pressure-volume curves exhibit hysteresis—the volume contained by an atrium at a given distending pressure is greater if that pressure is approached from above than if from below (see fig. 2). The changes manifest in the hysteresis loop of moderate distention are largely reversible: when the atrium is collapsed after having been distended for some time, the pressure-volume characteristics (as judged by redistention) reapproach their original state with a time course much like that of the slow volume change following distention. Preparations subjected to high distending pressures undergo an irreversible shift of the pressure-volume curve in the direction of greater volume (see fig. 2). There is probably no clear-cut pressure threshold above which this process occurs, for a slight progressive shift of the same sort is observed during experiments in which only moderate distending pressures are employed. However, the shift is usually so slight that successive pressure-volume curves are virtually identical unless the distending pressure considerably exceeds the level associated with maximal contractility (see below). Because of the phenomena of slow yielding and hysteresis, pressure-volume curves are comparable only if measurements are made in the same sequence and at comparable times after pressure changes.

Whereas the diastolic pressure-volume curve of an electrically driven atrium contracting isobarically is not appreciably different from the resting pressure-volume curve, the diastolic pressure-volume relationship is changed somewhat by vigorous isochoric contractions. If an atrium is caused to beat isochorically after the phase of slow yielding to distention has run its course, further shift in the diastolic pressure-volume relationship occurs in the direction of diminished resistance to distention.

**Starling Curves**

Figure 3 is an example of paired isochoric and isobaric Starling curves obtained from the left atrium of a rabbit. Such paired curves are drawn from measurements made concurrently and are comparable even in the absence of a steady state. The reproducibility of the Starling curves and the effects of overdistention are illustrated.

If carried to high enough volumes, the Starling curves of isolated rabbit atria always exhibit a descending limb—that is, contractility declines at very high volumes. This is true for both isobaric and isochoric curves. In the range of volumes greater than that associated with maximal contractility, the diastolic pressure-volume curve is exceedingly steep, however, and extreme distending pressures are required to produce a prominent descending limb. The application of such pressures always produces irreversible shifts in the diastolic pressure-volume curve, and usually decreases the maximum pressure (or work) that the atrium is capable of generating. Because of variation in the size of preparations, it is pointless to compare the volumes at
which contractility is greatest in a series of atria. The distending pressure offers a basis for comparison: in nineteen rabbit-atrium preparations, the mean distending pressure at which the isochoric Starling curve reached its maximum was 100 (SD 15) mm. H₂O. In a given preparation, the isobaric Starling curve reaches its peak at approximately the same volume as the corresponding isochoric curve, although minor differences occur under various conditions.

Discussion
The reasons for a number of the details of the method are not immediately apparent, and deserve mention. Gas is used to fill the atrium primarily because aqueous solutions under pressure transude rapidly through the walls of the preparation, precluding accurate measurement and control of volume. Surface tension at the interface between the distending gas and the interstitial fluid of the tissue prevents leakage of gas bubbles. The gas-filled preparation has other advantages: by changing the pressure of the distending gas, one can put varying loads upon the preparation without appreciably changing the inertia of the system; resistance to flow through the cannula and connecting tubing is low; it is not necessary to oxygenate or recirculate the medium once it is in chamber B. The use of gaseous oxygen rather than a physiological salt solution to fill the atrium has the effect of lengthening the diffusion path from the body of the solution to some parts of the tissue, and it may reasonably be asked how this will affect the work capacity of the preparation. To determine the distances involved, histological sections were made of a number of left-atrium preparations from large (3 Kg.) rabbits. In many places the atria are only a few cells thick, in most areas the tissue is less than 0.2 mm. thick, and the thickest portions are thinner than 1 mm. Since a pO₂ of about one atmosphere is maintained at the inner surface of the tissue, oxygenation should be at least as good as in conventional preparations of isolated heart muscle, although the adequacy of the oxygen supply to all parts of most of the latter is questionable. There is more likely to be serious restriction of the exchange of ions and nongaseous metabolites between the liquid medium and the pectinate muscles which bridge the interior of the atrial appendix. Accumulation of large amounts of carbon dioxide in the preparation, either as a metabolic product or as a result of concentration of the carbon dioxide in the gas phase through utilization of oxygen, has been ruled out by determining that the pH of the solution in the chamber is stable within 0.1 pH unit over periods of several hours.
The unconventional physiological salt solution used was adopted in an effort to obtain maximal stability of the preparation by facilitating the metabolic production of energy. Fundamentally, the solution is that described by Krebs. Insulin was added because it has been found to increase the uptake and utilization of glucose by isolated heart muscle. The solution was adopted on theoretical grounds, and no carefully controlled experiments have been carried out to compare the performance of preparations kept in it with that of preparations maintained in conventional Krebs-Henseleit solution.

The chief advantages of the preparation lie in its stability, in the precise control and measurement of atrial volume, and in the ease of switching from isotonic to isometric contraction. From experience with several hundred preparations of each type, it may be said that the stability and useful life of gas-filled preparations are considerably better than those of comparable muscle-stripe preparations bathed on both sides by an oxygenated stream of the physiological salt solution described above. The difference between the preparations is particularly noticeable at high frequencies of stimulation. Gas-filled preparations respond with normal speed and in the expected manner to cardiac glycosides, sympathomimetic amines, and barbiturates placed in the surrounding solution.

Certain limitations are inherent in a preparation in which the volume of a cardiac chamber rather than the length of a muscle is controlled. First, since the chamber is always free to change its shape, isochoric contractions fall even farther short of being truly isometric than do contractions of length-controlled preparations in which the fibers are parallel. Second, the pressure generated by a chamber contracting at various volumes is not directly proportional to the tension developed in its walls. From the formula of Laplace, which relates tension in the walls of a hollow viscus to their curvatures and the pressure gradient across them (see Burton), it follows that a given increase in wall tension will produce less increase in pressure at high volumes than at low. The question may be asked whether the decline in pressure generated (or work done) at high fiber lengths represents a true decrease in contractility or whether it is merely a manifestation of the Laplacian relationship. Calculations based on the assumption that the atrium is a sphere indicate that the volume change alone is insufficient to account for the decrease. However, the atrium is not spherical, and calculations to take its complex shape into account would be virtually impossible. The question is better approached using length-controlled preparations: strips of rabbit atrium also develop less tension at very high than at moderate fiber lengths.

The method used to record atrial volume is simple, accurate, and reliable, and might well have a variety of other applications. It has the disadvantage that the temperature of the fluid in the volume-sensing chamber must be stable before accurate records can be made. Cooper and Kerslake have described a related method which has the advantage of not being temperature-sensitive.

The principal shortcoming of the preparation is that control of resistance to muscular contraction is compromised by the inertia of the fluid filling the recording system. In some degree, of course, this is a failing of all isotonic recording systems except the one recently devised by Lubin, in which the force required to change the velocity of the recording lever is supplied by a servo-mechanism and not by the muscle itself. When filling pressures are low and contractions vigorous, the amplitude of isobaric contraction may be limited by contact of the atrium with the tip of the cannula. Such limited contractions may cause the first few points of an isobaric Starling curve to be spuriously low. They are readily identified in high-speed tracings by flattening of the peak of the contraction curve.

Summary

Since the actions of physical and chemical influences upon myocardial contractility are not necessarily independent, study of interactions between the two may give clues to the mechanisms of action of both. A method is
described for study of the interrelations of temperature, the interval between contractions, distending pressure, volume, amplitude of contraction against constant pressure, and pressure generated by contraction at constant volume in isolated thin-walled cardiac chambers. Unusual features are the use of gas as a distending medium and a volume recorder which measures the conductance of the fluid displaced by the chamber. As illustrations of use of the method, observations on some fundamental properties of rabbit atria are presented. Hysteresis and slow yielding to stress are prominent. The passive pressure-volume relationship is not affected by contractions against constant pressure, but is changed slightly by contractions at constant volume. The Starling curve of the isolated rabbit atrium always has a descending limb at high atrial volumes. However, the distending pressures required to produce a prominent descending limb are so high that their application causes irreversible changes in the muscle.

References
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