Influence of Temperature on Development of Rigor Mortis in Dog Hearts

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The effects of temperature on the development of rigor mortis in cardiac muscle have never been investigated systematically. Brecher and Kissen found that pressure-volume characteristics of non-beating, submerged dog ventricles remained constant for at least 30 minutes following circulatory arrest at 35°C. Using similar methods, Griggs et al. found this period to be approximately 80 minutes at 36°C and 300 minutes at 5°C. These and other related studies of rigor mortis were not primarily directed at the effects of varying temperature under controlled conditions. We have therefore adapted the technique of Brecher and Kissen to permit systematic investigation of the effects of temperature on the onset and development of rigor mortis in dog hearts. This study provides a background for future investigations in which the time limits for development of rigor mortis must be known.

Methods

The principle of the method is to establish an "elastic equilibrium" of the ventricles by submerging the heart in Ringer's solution. The state of elastic equilibrium of a ventricle is characterized by zero transmural pressure. Under this condition there is always a certain volume of fluid in the freshly excised ventricle. Upon reducing the intraventricular volume, the transmural pressure becomes negative and upon fluid addition it becomes positive. This method lends itself to pressure-volume studies in which any stretch of the ventricular walls can be avoided. Since such stretch might affect the development of rigor mortis, half of the experiments were done with negative transmural pressures only and compared with the others in which both negative and positive transmural pressures were produced.

Fifty-five mongrel dogs of various ages were used, 37 for studies on left ventricles, 18 for studies on right ventricles. Preceding these experiments, most of the animals had been employed in other studies which did not involve the cardiovascular system. The animals were anesthetized intravenously with sodium pentobarbital (30 mg/kg of body weight). Six minutes after the injection they were rapidly exsanguinated from a femoral artery. Four minutes after the beginning of exsanguination the removal of the heart from the pericardial sac was completed. The aorta and pulmonary artery were severed 5 cm from their origin and the systemic and pulmonary veins were divided at their entrance into the atria. The hearts were then rinsed in Ringer's solution and the aorta and pulmonary artery stumps were ligated. The experimental arrangement is shown in figure 1.

A Tygon cannula (inner diameter 10 mm) was placed in the atrioventricular orifice and secured by a ligature around the atrioventricular groove. The hearts were submerged in Ringer's solution in a constant temperature bath. The bath could be adjusted to temperatures between 1°C and 50°C (± 0.25). The composition of the buffered Ringer's solution used was NaCl 0.9 g, KCl 0.03 g, CaCl₂ anhyd. 0.026 g, 3\( \sqrt{3} \)√ HCO₃ 0.055 g, water to 100 ml. The ventricular cannula was connected via three meters of Tygon tubing (inner diameter 9 mm, walls 2.5 mm), to a burette, graduated in 0.2 ml. Half of the Tygon tubing was immersed in the bath to minimize temperature fluctuations in the heart when fluid was added. A side tube with a stopcock permitted equalization of the fluid levels in the bath and burette. Operations were completed within four minutes after removal of the heart.

The pressure-volume relationships were investigated in 29 experiments in which the intraventricular pressures covered only a range below atmospheric pressure. The pressure, and thereby the volume within the ventricle, was reduced stepwise by lowering the burette. The pressure differences between the surface of the bath and meniscus of the burette (i.e., transmural ventricular pressure) were read in millimeters of water. Volume increases in the burette represented corresponding volume decreases in the ventricle. A steady state,
generally reached in less than 15 seconds, was established before each reading. The lowest intraventricular pressure applied was 30 mm Hg subatmospheric. In 26 experiments the pressure-volume relationships were determined with positive transmural pressures up to 50 mm Hg. In these experiments volume reductions in the burette represented volume increases in ventricle. In all experiments three series of control measurements were made in rapid succession immediately after immersion of the heart. Thereafter each series of readings was repeated every five minutes for experiments above 34°C, every 15 minutes for experiments between 26°C and 34°C, every 30 minutes for experiments between 11°C and 25°C, and every 60 minutes for experiments between 1°C and 10°C. Between each series of readings the stopcock of the side tube was opened to avoid undue stretching of the ventricular walls.

The fluid uptake of hearts submerged in Ringer’s solution was measured in 13 additional experiments. Following gentle blotting, the heart was weighed immediately after removal from the animal and every hour thereafter. Hearts were kept at constant temperature for the following number of hours: 5°C: 70 hr; 10°C: 65 hr; 20°C: 22 hr; 30°C: 23 hr; 40°C: 19 hr; 45°C: 11 hr.

Results

Figure 2 shows the typical pressure-volume relationships of a dog heart submerged in Ringer’s solution at 35°C. This pressure-volume curve remained unchanged for 30 minutes after removal of the heart. Thereafter, there was a progressive stiffening of the ventricular walls as indicated by a decrease in volume changes in relation to the pressure changes. After two hours the pressure-volume curve became almost linear. It remained so until the beginning of autolysis, which was characterized by increased tissue fragility leading to rupture of the wall when pressure was applied. Some hysteresis is evident in all curves. At approximately 30 mm Hg subatmospheric and 50 mm Hg above atmospheric pressure respectively, the volume changes resulting from further decreases or increases in pressure were negligible. In order to prevent undue deformation of the ventricular walls, these pressure limits were not exceeded.

Figure 3 shows the pressure-volume curves from a heart at 10°C. The relationship remained unaltered between the first measurement (at four minutes) and subsequent measurements (up to three hours) after removal of the heart. The distensibility of the ventricle decreased progressively between three and thirteen hours. After thirteen hours the pressure-volume curve remained constant and S-shaped until autolysis began. This is in contrast to the almost linear relationship which was eventually attained in experiments done at temperatures above 34°C.
At all temperatures (1°C to 50°C) the pressure-volume relationships were the same before rigor started. This was deduced from the almost equal slopes at the inversion points of all curves. Thus, before the beginning of rigor, the distensibility of the heart (ΔV/ΔP) averaged 3.5 ml/mm Hg around the inversion point, irrespective of temperature.

It was often found that during the second or third series of pressure-volume measurements a larger volume could be accommodated in the ventricle, in its elastic equilibrium state, than during the first series of measurements. This phenomenon, called “primary dilatation” by Rothberger, was observed in 21 of 25 experiments on left ventricles and 11 of 18 experiments on right ventricles. In those ventricles exhibiting primary dilatation, an average of 14% more fluid could be accommodated during the second or third series of measurements than during the first.

Figure 4 shows the relationship of time and temperature to the development of rigor mortis. The criterion for the onset of rigor was the beginning of a change in the pressure-volume curve of the heart, disregarding the primary dilatation. The criterion for the steady state of rigor was the constancy of the pressure-volume relationship in three consecutive measurements after progressive stiffening had occurred. At temperatures above 40°C the onset of rigor occurred within 20 minutes after removal of the heart and the steady state of rigor was reached within one hour. As the temperature was lowered, the time for onset of the rigor and for attaining the steady state was proportionately prolonged. At 10°C the onset averaged five hours while the steady state was attained after twelve hours. Apparently there was no systematic difference between right and left ventricles regarding the development of rigor mortis.

From figure 5 it is evident that the weight gain of hearts submerged in Ringer’s solution was 5% ± 1.8 SD within five hours irrespective of the temperature of the Ringer’s solution.

Discussion

These experiments demonstrate that in the myocardium the onset of rigor mortis as well as the attainment of maximal stiffening is temperature dependent. The time-temperature relationship was found to be sufficiently reproducible to allow useful predictions of the development of rigor in the dog heart.

For some types of investigations it is important to know how much time is available

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**FIGURE 3**

Pressure-volume relationships of an excised left ventricle submerged in Ringer’s solution at 10°C, and their changes with time after excision (dog, 12.5 kg).

**FIGURE 4**

Time required for the development of rigor mortis of dog ventricles as a function of temperature.

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for making pressure-volume determinations in ventricles before changes in stiffness of the ventricular wall begin, e.g., for studies on the contribution of elastic forces to ventricular diastolic filling. The present experiments furnish necessary information concerning the period at each temperature during which such measurements can be made. There is also evidence that from 1°C to 50°C the temperature itself does not alter the pressure-volume relations before rigor begins. Based on this information it would be justified to make repeated pressure-volume determinations on dog hearts for four hours at 10°C, but only for 25 minutes at 37°C.

It must be recognized that the onset of death of an individual heart muscle fiber is indeterminable by means of presently available techniques. For lack of better criteria, the present experiments made use of overall elastic changes to define arbitrarily the onset of rigor mortis. Similar considerations pertain to the endpoint in the development of rigor mortis, i.e., the time at which no further increase in the ventricular wall stiffness occurs.

From the curves in figure 4 it can be deduced that the physicochemical processes responsible for rigor mortis are slowed by low temperatures and accelerated by high temperatures. This not only concerns the onset of stiffening but also the progression of the stiffening to its maximum, as illustrated by divergence of the broken and solid lines in figure 4. In fact, at 1°C the processes which caused stiffening never reached their maximal development, because autolysis began before the maximum was attained. This suggests that processes responsible for the onset of autolysis are not slowed at the same rate of cooling as are the rigor inducing events.

The results of the present experiments agree in principle with findings reported by Griggs et al. However, there are differences in the methods used. Griggs et al. emptied the heart, added measured volumes to the ventricle, and recorded the intraventricular pressures, which always exceeded atmospheric pressure. This would result in a stretch of the ventricular muscle that might affect the time course of rigor mortis development. This possibility was pointed out by Mosso and Pagliani in objection to Rothberger's work. Since in the present experiments the onset of rigor was the same irrespective of the ventricular volume, the stretching as such seems to have no measurable effect.

The final shape of the pressure-volume curve at maximum rigor depended upon the temperature. Only in experiments in which the hearts were exposed to temperatures above 34°C did the pressure-volume curve become approximately linear. Below this temperature, the curve remained S-shaped even until autolysis began. This difference in the final shape of the curves might be explained by the phenomenon of "heat contracture" superimposed upon rigor mortis at temperatures above 34°C, as postulated by Morita.

Among the numerous factors responsible for the physical behavior of tissues (Remington), the elasticity of a quiescent heart in Ringer's solution might be affected by fluid uptake. Since the weight gain of the heart amounted to 5% within five hours and the pressure-volume relationships did not change during this time, the effect of fluid uptake appeared to be negligible.
The phenomenon of "primary dilatation" of all types of muscle has been described by many investigators. Some of them ascribed it to an artifact resulting from stretch of the muscle. The present experiments show that deformation caused by either stretch or compression may induce primary dilatation. However, it cannot be decided from the presently available evidence whether primary dilatation is caused by a relaxation of "elastic elements" or by lengthening of "plastic elements." 

Summary

The effect of temperature (1°C to 50°C) on the onset and maximal development of rigor mortis in dog hearts was studied systematically under static conditions. Excised hearts were submerged in Ringer's solution so that the ventricle could assume an elastic equilibrium state. This was characterized by zero transmural pressure and the presence of a certain volume in the ventricle. Pressure-volume curves were obtained in 29 experiments by removal as well as by addition of fluid. The pressure-volume relations were the same at all temperatures prior to the onset of rigor mortis. The criterion for the beginning of rigor mortis was the first change in pressure-volume relations and for maximum rigor the constancy of the attained relations. The shortest interval between cessation of the circulation and the beginning of rigor mortis was 25 minutes at 37°C, and three hours at 10°C. These measurements provide data defining the time course of rigor mortis. The present experiments show that deformation caused by either stretch or compression may induce primary dilatation. However, it cannot be decided from the presently available evidence whether primary dilatation is caused by a relaxation of "elastic elements" or by lengthening of "plastic elements." 

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