The Effect of Calcium Depletion upon the Tension-Independent Component of Cardiac Heat Production

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ABSTRACT Rabbit papillary muscle has been exposed to calcium concentrations ranging from 2.5 mM to zero. Its mechanical and electrical activity has been monitored and its heat production measured using a myothermic technique. Calcium depletion decreased the magnitude of the tension-independent heat per contraction from a mean of 0.45 mcal/g muscle to 0.31 mcal/g muscle at room temperature (18° to 22°C). Calcium-chelating agents did not abolish action potential conduction under the experimental conditions used but they further reduced the magnitude of the tension-independent heat. Raising the temperature from room level to 32°C decreased the tension-independent heat from a mean of 0.52 to a mean of 0.24 mcal/g muscle. Calcium depletion at 32°C further decreased this heat and it was calculated that the energy now liberated in activating the muscle was about 2% of the total energy normally liberated in the working heart. The results are interpreted in terms of current biochemical and myothermic data.

INTRODUCTION

Cardiac heat production can be divided into several phases. In an isometric response there is a phase of tension-independent and a phase of tension-dependent heat. In an isotonic contraction there is additional heat liberated, above that required in an equivalent isometric contraction and this heat is assumed to be associated with shortening (Gibbs, Mommaerts, and Ricchiuti, 1967).

In both skeletal and cardiac muscle there seems to be a tension-independent heat component (Hill, 1949; Aubert, 1956; Gibbs, Ricchiuti, and Mommaerts, 1966; Gibbs et al., 1967). Biochemically such a component has been demonstrated in skeletal muscle (Sandberg and Carlson, 1966) and in cardiac muscle (Pool and Sonnenblick, 1967). Hill's experiments with skeletal muscle showed that this heat, the activation heat, was liberated early in the contraction cycle and this is probably also the case in cardiac muscle (Gibbs, 1967 *a*).

Davies, Kushmerick, and Larson (1967) have recently shown that in muscles poisoned with 2,4-dinitrofluorobenzene (DNFB) the ATP utilization upon stimulation is insufficient to account for the magnitude of skeletal muscle activation heat even if all its free energy were degraded. Similarly Jöbsis and Duffield (1967), monitoring oxidative metabolism during the recovery period after the contractile event, could find no evidence of a recovery component that could be related in magnitude to the activation heat.

The present experiments were undertaken because Klocke, Braunwald, and Ross (1966) have reported that electromechanical dissociation achieved by lowering the calcium concentration reduced the oxygen cost of electrical activation of the whole heart to less than 1 % of the total oxygen consumption of the normally working heart. Their results are not compatible with the existence of a tension-independent heat component unless this component is calcium-dependent or has no biochemical equivalent.

Experimentally we found it difficult to abolish mechanical activity at 18° to 22°C even in calcium-free solutions containing chelating agents. For this reason certain of the calcium depletion experiments were run at 32°C where rapid and virtually complete electromechanical dissociation could be obtained.

METHODS

Papillary muscles were obtained from the right ventricles of rabbits. In some experiments pieces of right ventricular wall were also excised and mounted in a bath so that electrophysiological records could be obtained from the same heart. The papillary muscles ranged in length from 4.9 to 9.8 mm under resting tensions of 0.5–1.0 g. They possessed a mean weight of 5.8 mg and ranged from 1.6 to 12.5 mg. 32 animals were used in the present study. The experiments were usually carried out at temperatures ranging from 18° to 22°C but some experiments were run at 32°C.

The muscles were normally bathed in Krebs-Henseleit solution aerated with 95% oxygen and 5% CO_2. In certain experiments a phosphate-buffered solution described by Lee, Richman, and Visscher (1966) was used. It contained NaCl 145.5, KH_2PO_4 0.15, Na_2HPO_4 2.05, KCl 4.8, $CaCl_2$ 1.8, and glucose 5.56 mmoles/liter. To produce a nominally calcium-free solution calcium chloride was omitted. Sodium citrate or the disodium salt of ethylenediaminetetraacetate (EDTA) was added to the calcium-free solution to produce lower calcium concentrations. Sodium citrate concentrations ranged from 25 μmoles to 0.5 mmole and the EDTA concentrations ranged from 10 to 100 μmoles.

When changes in calcium levels were made the muscles were exposed to the new solutions for at least 20 min before heat and mechanical measurements were made. Exposure time to solutions containing chelating agents was normally kept as short as possible (30–60 min) but several experiments were undertaken in which bathing times between 1–2 hr were used with moderate citrate concentrations (50–250 μmoles). At 32°C, 10–15 min at moderate levels of chelating agents was generally sufficient to abolish tension development. At all temperatures the muscles were con-

tinually stimulated during the exposure periods. When heat and tension measurements were made the stimulus rate was 15–20 beats per min at room temperature and 60 beats per min at 32°C. The tension-independent heat values quoted in this paper refer to the heat liberated in a single contraction. In actual practice this was the average heat output per beat in 15–60 consecutive contractions.

Mechanical Measurements For heat and tension recording the papillary muscles were tied at either end with braided noncapillary silk (Ethicon 50) and the silk at the ventricular end of the muscle was clamped 2 mm from the muscle. The free tendinous end of the papillary muscle was connected to a stainless steel wire. The tension transducer was a Sanborn differential transformer type (Model FTA-100).

Heat Measurements Two thermopiles were used in the present series of experiments. Both had silver-constantan junctions and were made using the method devised by Mr. N. V. Ricchiuti and described elsewhere (Ricchiuti and Mommaerts, 1965). The thermopiles had 94 and 101 active junctions and an output of 2.71 and 3.48 mv/°C, respectively. Both piles were used with an Astrodata 120 nanovolt amplifier (Astrodata Inc., Anaheim., Calif.) whose frequency response was reduced to 20 Hz by a filter network. The average heat loss of the muscle thermopile system was 10.8%/sec. The heat loss was practically exponential and could be corrected for electrically.

In certain experiments it was decided to operate at 32°C. At this temperature base line stability was not as good as at 20°C but reasonably accurate heat measurements could be made about 1–1½ hr after the temperature change was made. There was always, however, some slow base line drift because of temperature equilibration effects. The drift occurred in a constant direction and its rate declined with time.

Calibration The calibration procedure has been described previously (Gibbs et al., 1967). Additional safeguards had to be employed in the present experiments to correct for the heat artifact produced by the stimulus current. After exposure to calcium-free solutions there is a progressive increase in the excitation threshold. It was standard practice to employ a stimulating voltage between 5 and 7 v. This voltage was never changed and normally required a stimulus duration of about 0.5 msec to activate the muscle at 20°C. Upon prolonged calcium depletion the stimulus duration had to be progressively raised and might reach 5–10 msec. At such levels the heat from the stimulus is often greater than the muscle heat. It is therefore critical to have an accurate evaluation of this artifact. It is not possible to rely upon measurements of the artifact made later on electrocuted muscles or muscles rendered inexcitable with KCl because calcium depletion produces large fluctuations in the interelectrode resistance. These fluctuations occurred during the exposure period and, more disturbingly, could still be detected for some time after return to normal solution. To correct for this artifact two procedures were adopted. The stimulus current, which depended upon the interelectrode resistance of the muscle and was normally about 10KΩ, was determined by measuring the voltage drop across a 10 ohm resistance in series with the stimulating electrode. The stimulus voltage was measured with the muscle on the thermopile. The stimulus duration could also be accurately determined and from these three measurements the heating effect of the stimulus could be calculated. These measurements were made before and after each reading in the

calcium-free solutions and they were also made after return to normal solution. The other correction method used was to obtain a maximal mechanical response at a certain stimulus duration and then to record the heat produced. If the stimulus duration was now increased, generally doubled, the mechanical response did not alter but extra heat was produced. The extra heat measured in the second series of contractions can be ascribed to the stimulus artifact. There was good agreement between the two methods. At 32°C the stimulus duration was normally only about 0.1 msec and the much briefer exposure times did not produce large increases in duration (less than 0.2 msec).

Electrical Recording The action potential produced by skeletal 'twitch' muscles and cardiac muscle, in response to an adequate stimulus, is generally regarded as the event triggering the normal contractile response. In the present experiments energy liberation following the uncoupling of the excitation-contraction sequence was studied. It was necessary to ensure that following calcium removal, over a period equivalent to that during which heat measurements were being made, cardiac tissue was still capable of conducting an action potential.

Pieces of right ventricle were pinned out in a wax-based bath, endocardial surface uppermost, sandwiched between two circular Ag-AgCl electrodes. Stimuli were applied to the tissue from a Tektronix 161 pulse generator, via an RF isolation unit (Bioelectric Instruments (ISA100)). Maximum voltage pulses were generally used: their width was reduced until a just threshold condition was attained and was adjusted when necessary during the experiments. Transmembrane potentials were recorded using KCl-filled glass microelectrodes (20–40 MΩ) coupled to the input of a high impedance unit gain "negative capacity" preamplifier (Picometric amplifier, Instrumentation Laboratories, Inc., Boston, Mass.) via an Ag-AgCl electrode. A reference Ag-AgCl electrode was placed in the bathing solution.

Statistical Design and Analysis A randomized complete-block design was used (Steel and Torrie, 1960, p. 132) so that variability attributable to the papillary muscle preparations could be excluded from the experimental error when treatment differences were examined. Comparisons between treatments, based on single degrees of freedom, were made as described by Steel and Torrie (1960, p. 213). The data are presented in the tables, with the upper half of each table showing the treatment means while the lower half shows the analysis of variance.

RESULTS

The Effect of Lowered Calcium Levels upon the Tension-Independent Heat

It has been shown (Gibbs, 1967 *b*) that reducing the external calcium level from 5.0–0.6 mM produces little change in the total amount of heat produced for a given level of tension development. There was some evidence that the tension-independent heat component decreased by about 10 % at the lower calcium levels.

In the present experiments the normal calcium level was 2.5 mM and the muscle was exposed to physiological solutions containing 1.25, 0.6, and 0

mM calcium. The periods of exposure to the altered calcium levels were 40 min at the 1.25 and 0.6 mM level and 20 min at the zero level. The experimental sequence of altered calcium concentrations went 2.5, 1.25, 0.6, 0, and 2.5 mM.

Even when the calcium was omitted from the bathing solution it was not possible to completely abolish mechanical activity which generally decreased to about 25 % of its original value in 2.5 mM calcium. In order to abolish

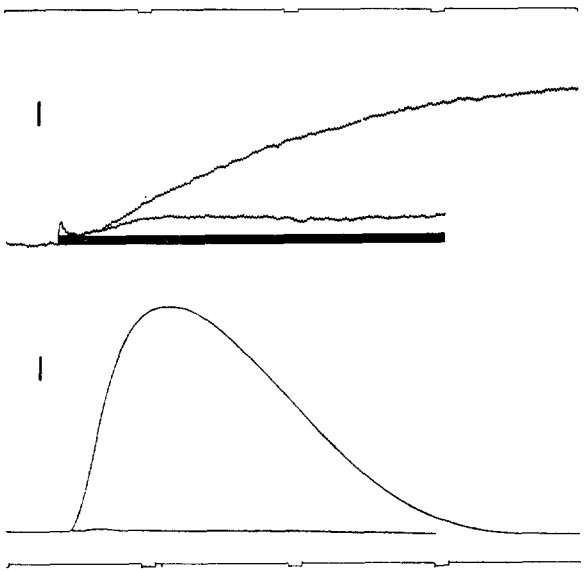

FIGURE 1. The heat (top) and tension (bottom) traces when the muscle was activated at two different muscle lengths. The smaller heat output, the tension-independent heat, occurred when the muscle was 5.2 mm long. The larger heat output and the normal tension response were obtained with the muscle at a length of 7.4 mm. The records are not retouched but have been superimposed at the time of the stimulus artifact. The muscle weighed 8.9 mg. The horizontal bar on the heat trace indicates the heat due to the stimulus. The time calibration marks are 1 sec apart. Tension calibration (bottom trace) = 0.5 g. Heat calibration (top trace) = 0.49 mcal/g muscle.

tension at the normal calcium concentration the muscle was allowed to shorten until it reached a length at which it could not develop tension. When the muscle was now stimulated, no tension developed but heat was still liberated. This method was first used by Hill (1949). The tension-independent heat was always measured at this muscle length in every calcium solution. At the lower calcium levels a much smaller decrease in muscle length would obliterate active tension development. A comparison of the heat produced at both muscle lengths showed no detectable difference. In Fig. 1 two experimental records have been superimposed to show the heat and mechanical

outputs of a muscle at two different lengths. The traces are a good indication of the relative magnitudes of the tension-independent and tension-dependent heats.

Minimal changes in the excitation threshold were produced by the experimental procedures indicated above. The rising phase of action potentials recorded from rabbit ventricle was depressed but there was no evidence of conduction failure. Upon return to normal solution the action potentials rapidly resumed their usual shape. Tension development was normal within 10–15 min.

TABLE I
EFFECT OF ALTERED CALCIUM LEVELS
UPON TENSION-INDEPENDENT HEAT

	Calcium concentration				
	2.5 mM	1.25 mM	0.6 mM	0	2.5 mM‡
Tension-independent heat, *mcal/g**	0.45	0.41	0.37	0.31	0.48

Analysis of variance

Source of variation	DF	MS	F	*P*
Between Ca levels (*T*)	4	438	50.5	<0.001
*T*2.5 vs. *T*1.25	1	68.5	7.90	<0.01
*T*2.5 vs. *T*0	1	980	113.0	<0.001
*T*2.5 vs. *T*2.5‡	1	36.5	4.21	<0.05
Between preparations (*P*)	9	988	113.9	<0.001
P×*T* (error)	36	8.67		

* Treatment means of 10 experiments.
‡ Refers to recovery after low calcium experiments.

The experimental results are given in Table I. It can be seen that there was a gradual decrease in the magnitude of the tension-independent heat as the external calcium concentration was lowered. There was a significant difference between the control, 2.5 mM level, and all other levels. This could be demonstrated even when the control was compared to the 1.25 mM level ($P < 0.01$). Interestingly there appeared to be a greater than expected increase in the magnitude of the tension-independent heat upon return to the 2.5 mM calcium level ($P < 0.05$).

Chelating Agents and Tension-Independent Heat Production

Even with calcium omitted from the physiological solutions, it was not possible to completely abolish tension development. In an endeavor to remove any calcium ions still present in the bathing solution, either because of chemical

impurities or because of efflux from the muscle, chelating agents were used. At first we used solutions containing either 10 μM EDTA or 25 μM sodium citrate but these concentrations were later increased to 100 and 250 μM, respectively. EDTA was used as the chelating agent in about 25 % of the experiments and sodium citrate was used in the remainder. Before exposure to the solution containing a chelating agent the muscle normally had a 20 min equilibration period in a zero calcium solution. Even with this pretreatment it usually took 20–60 min to reduce active tension development below 20 % of normal (2.5 mM level). Complete arrest of tension development could eventually be achieved by allowing even longer exposure periods or by increasing the chelating concentrations to about 1.0 mM. With this treatment there is, however, evidence of conduction failure and upon return to normal solution complete tension recovery is rare and very slow. We found as Lee, Richman, and Visscher (1966) have reported that complete tension recovery from EDTA is unusual.

We preferred therefore to use lower concentrations of citrate or EDTA when concurrent electrophysiological studies had demonstrated that action potential conduction was present. The action potentials themselves were noticeably altered showing a decreased rate of depolarization, alterations in duration, and often a failure to overshoot (see Fig. 2 *b*). There was some evidence of membrane depolarization. In Fig. 2 we have deliberately chosen to show records from the two preparations in which depolarization was most noticeable. Even in these two muscles, however, the depolarization levels at the time the heat records were obtained were much less. When the results from all the cells were examined we could not show a significant difference ($P > 0.1$, t test) between the magnitude of the membrane potentials obtained in normal and zero calcium, when these were measured over the normal experimental times.

The effect of calcium depletion upon the stimulus threshold was quite marked and to achieve the maximum tension output the stimulus duration had to be progressively increased after prolonged exposure. The duration often had to be increased up to 10 times normal, presenting stimulus artifact problems, which have been discussed previously (see Methods). In two preparations in which these long durations had to be employed there was evidence that action potential propagation had failed. The procedure was to establish threshold conditions with the recording electrode in a cell near the upper stimulating electrode (see Methods). The preparation was then probed for quiescent fibers; in only the two preparations mentioned above were the fibers not all excited, and then it was only near the cut edges that stimulation was not adequate. This effect was seen only after exposure times in excess of 60 min and tension recovery in these preparations was both slow and incomplete upon return to normal solution. Two other muscles were encoun-

tered in which there were only small changes in the stimulus duration, less than double, after exposure to 250 μM citrate for periods in excess of 1 hr. Electrophysiological studies on one of the muscles showed less action potential distortion than normal (see Fig. 2 *a*). Tension recovery upon return to normal solution was complete within 10 min. The tension-independent heat still decreased noticeably in these preparations (see Fig. 3). The majority of papillary muscles, however, showed behavior between the two extremes. In Table II data are given showing the effects of these chelating agents upon

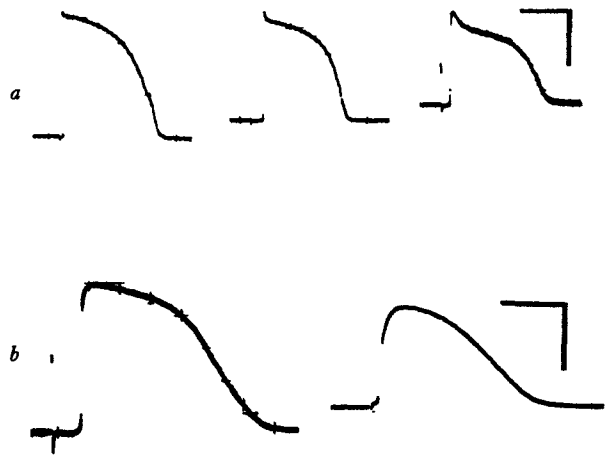

FIGURE 2 *a*. (top traces). Left, typical action potential in 1.25 mM Ca^{++}. Center, action potential after 1 hr in 250 μM citrate-Krebs. Right, action potential after 1½ hr in citrate-Krebs. Notice that in this preparation a rapid rate of rise is maintained in spite of membrane depolarization. Temperature was 20.1°C. *b* (bottom traces). Left, action potential in 2.5 mM Ca^{++}. Right, action potential after 1 hr in 250 μM citrate-Krebs. Note the more usual decrease in the rate of depolarization. Calibration, vertical bars = 40 mv; horizontal bars = 200 msec (top traces), 100 msec (bottom). The records in the calcium-free solution were taken from cells that showed the lowest resting potential during the recording period. Temperature was 19.3°C.

heat and tension production. The data from both the citrate and EDTA treatments have been pooled.

The chelating agents were more effective than exposure to ordinary calcium-free solution in reducing the magnitude of the tension-independent heat. The decrease was highly significant ($P < 0.001$). Tension development decreased to about 20 % of the 2.5 mM level and tension recovery after exposure to the chelating agents was slower. The mean time taken for tension recovery to reach 80 % of normal was 43 min. It was a general finding that muscle heat production, for a given level of tension development, returned to normal long before the muscle was capable of developing its maximum pretreatment tension.

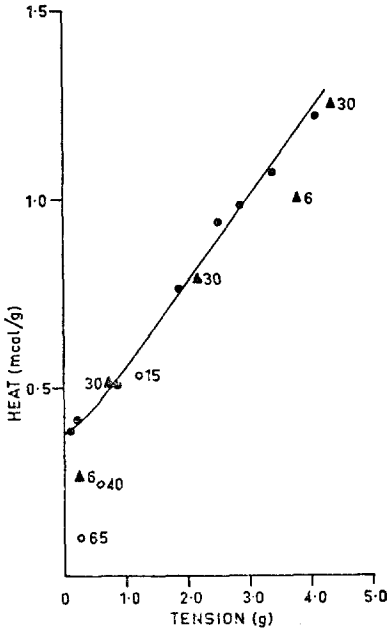

FIGURE 3. Heat vs. tension relationships: filled circles, obtained in 2.5 mM Ca^{++} at various muscle lengths; open circle, obtained in 250 μM citrate-Krebs, at a fixed length, 5.9 mm. The exposure time in minutes is shown beside the points (filled triangles) obtained upon return to 2.5 mM Ca^{++}, at different muscle lengths and at the times indicated. Bath temperature was 20.1°C and the muscle weighed 7.8 mg.

The Effect of Temperature

The experiments described in the preceding sections were carried out at 18° to 22°C. In all these experiments it was difficult to prevent some tension development even when the chelating agents were added to the solution. Because there are several reports of calcium depletion producing complete

TABLE II

EFFECT OF CHELATING AGENTS UPON TENSION AND TENSION-INDEPENDENT HEAT

	Calcium concentration				Calcium concentration		
	2.5 mM	0	2.5 mM		2.5 mM	0	2.5 mM
Tension-independent heat, *mcal/g**	0.47	0.21	0.47	Tension, *g*	2.57	0.49	2.47

Analysis of variance

	Tension-independent heat				Tension			
Source of variation	DF	MS	F	*P*	DF	MS	F	*P*
Between Ca levels (*T*)	2	6,779	43.7	<0.001	2	425,592	85.1	<0.001
*T*2.5 vs. *T*0	1	10,143	65.4	<0.001	1	628,929	125.8	<0.001
Between preparations (*P*)	30	423	2.73	<0.001	30	25,911	5.18	<0.001
P×*T* (error)	60	155			60	5,000		

* Treatment means of 31 experiments.

abolition of the mechanical response we looked for possible complicating factors in our own experiments. Other physiological solutions, both bicarbonate- and phosphate-buffered, were tried without altering the pattern of our results. We therefore decided to examine the effect of raising the temperature to 32°C. This produced certain technical difficulties but it was immediately evident that calcium depletion now produced a larger and a more rapid decline in tension. From an experimental viewpoint there was also a good side effect in that the stimulus duration at 32°C could usually be reduced to 0.1–0.2 msec and even a 30 min exposure to a solution containing citrate produced

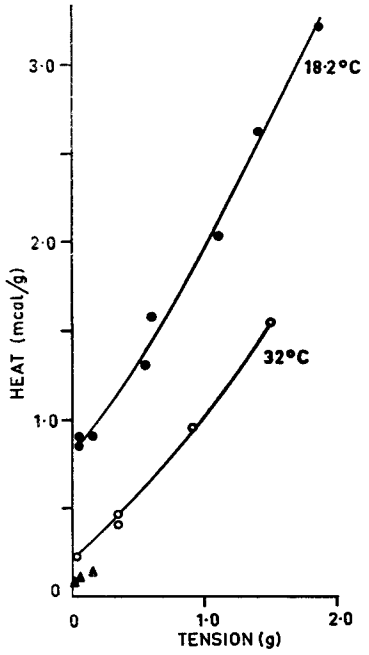

FIGURE 4. Heat vs. tension plots obtained at different muscle lengths and at the temperatures indicated. The stimulus rate was 1/sec at 32°C (open circles), and ¼ sec at 18.2°C (filled circles). The effect of 100 μM citrate-Krebs at 32°C is also shown (filled triangles), with the muscle kept at its normal length of 5.4 mm. Muscle weight was 2.7 mg.

at the most a doubling of this duration. At these durations the stimulus artifact is a much smaller source of error.

A heat vs. tension curve was obtained at room temperature and at 32°C. The heat and tension are measured by summing both the developed tension and the heat produced in a block of 15–40 contractions and dividing the totals by the number of stimuli. Tension development is varied by changing muscle length. At room temperature the stimulus rate was 1/4 sec but at 32°C this was raised to 1/sec to increase the developed tension. This procedure also allowed us to shorten the stimulation period and hence to minimize drift errors. Typical results are shown in Fig. 4. The increase in temperature produced two major effects in the seven preparations studied: (*a*) it reduced the tension-independent heat to less than half the room tempera-

ture value, and (*b*) it decreased the developed tension. The latter effect is probably related to the stimulus rate used at 32°C as higher rates would produce greater tension.

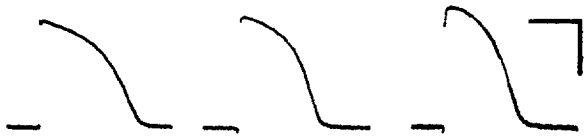

FIGURE 5. Left to right, control action potential in 2.5 mM Ca^{++}, after 90 min in 100 μM citrate-Krebs and after 120 min in citrate-Krebs. Temperature was 32°C. Calibration, vertical bar = 40 mv; horizontal bar = 200 msec.

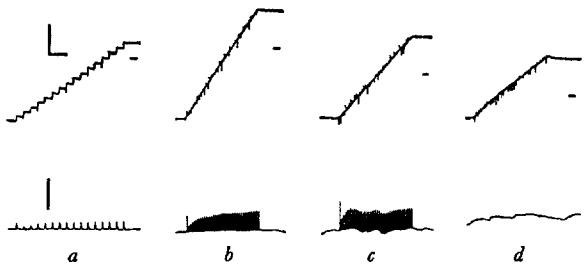

FIGURE 6. Heat and tension records obtained at 21°C (*a*) and at 32°C (*b*, *c*, and *d*). In (*a*) 16 stimuli at ¼ sec were used and in (*b*, *c*, and *d*) 42 stimuli at 1/sec. Records (*a*) and (*b*) were obtained in 2.5 mM Ca^{++}, record (*c*) in zero calcium, and record (*d*) in 200 μM citrate-Krebs. Heat traces (top) and tension records (bottom). Calibration, horizontal bar = 10 sec; vertical bar (top traces) = 3.90 mcal/g; vertical bar (bottom traces) = 0.5 g in (*a*), 0.1 g in (*b*), and 0.05 g in (*c*) and (*d*). In this experiment the stimulus duration was left unchanged at 0.4 msec. The stimulus heat artifact to be subtracted from each record is given by the interval between the top of the heat trace and the horizontal dash drawn below. Thus although there were more than twice the number of stimuli in record (*d*) than in (*a*) note that the total heat seen in (*d*) after artifact subtraction was about half that recorded in (*a*). Tension development in traces (*a*) and (*b*) has been decreased by allowing the muscle to shorten to 5.9 mm and 6.1 mm, respectively. Traces (*c*) and (*d*) were obtained at the normal muscle length of 7.1 mm, tension development being low because of the effects of calcium depletion. In the normal experiments the tension-independent heat was always measured at the same shortened length regardless of the calcium level. Calcium depletion often produced the resting tension fluctuations that can be seen in traces (*c*) and (*d*). The spikes on the cumulative heat records are stimulus artifacts; their size and direction depend on the timing of the stimulus relative to the phase of the amplifier-chopper. The muscle weighed 4.9 mg.

At 32°C exposure of the preparations to the calcium-free solution without chelating agents produced a rapid drop in tension development. In solutions containing citrate tension development was below 20 % of the 2.5 mM value within a few minutes in all preparations. Even at 20 min, however, a small amount of tension development could usually be detected at high transducer sensitivities. The mean tension developed at room temperature was 3.23 g

(stimulus rate 1/4 sec) and this dropped to 0.78 g in calcium-free solution. At 32°C and with a stimulus rate of 1/sec, the mean tension was 1.20 g and this dropped to a mean of 0.09 g within 20 min.

Electrical activity was little changed even at times greatly in excess of those at which the heat measurements were made. Membrane potentials were well maintained and there was much less action potential shape distortion (see Fig. 5). Typical experimental records, showing heat production and tension development at room temperature and at 32°C, are reproduced in Fig. 6. The results are shown in Table III.

TABLE III

EFFECT OF TEMPERATURE AND CHELATING AGENTS UPON TENSION INDEPENDENT-HEAT

	Room temperature		32°C	
	2.5 mM Ca^{++}	0 Ca^{++}	2.5 mM Ca^{++}	0 Ca^{++}
Tension-independent heat, *mcal/g**	0.52	0.31	0.24	0.10

Analysis of variance

Source of variation	DF	MS	F	*P*
Between treatments (*T*)	3	2450	23.33	<0.001
Temperature	1	4802	45.73	<0.001
Calcium concentration	1	2450	23.33	<0.001
Interaction	1	98	0.93	>0.1
Between preparations (*P*)	7	324	3.09	<0.05
T×*P* (error)	21	105		

* Treatment means of eight experiments.

The magnitude of the tension-independent heat changes from 0.52 mcal/g to 0.24 mcal/g when the temperature is raised ($P < 0.001$). Calcium depletion about halves these values. There was no interaction between the effects of temperature and of calcium depletion ($P > 0.1$).

DISCUSSION

The oxygen consumption of a normally beating heart at 37°C falls in the range 10–15 ml/100 g tissue/min (Rodbard, Williams, and Williams, 1959; Katz, 1963). If we take the caloric equivalent of oxygen to be 4.85 kcal/liter (Lorber, 1953), it can be calculated that the energy output for the above oxygen consumption would be about 600 mcal/g muscle/min. The dog heart rate is about 125 beats/min (Taylor, Ross, Covell, and Sonnenblick, 1967) and if the tension-independent heat has a value of 0.25 mcal/g muscle/beat it would account for about 5 % of the oxygen consumed by the heart. Calcium depletion lowers the magnitude of the tension-independent heat to

0.1 mcal/g, and hence this component would now only represent 2% of the heart oxygen consumption. Klocke et al. (1966) reported that calcium depletion of the intact dog heart reduced the oxygen cost of electrical activation to about 1% of normal. The difference between our value and theirs is not large particularly as the values for the tension-independent heat, used in the above calculations, were obtained at 32°C and evidence has been presented that this component falls as the temperature is raised. There is one cautionary statement that should be made here. In certain of our preparations examined at 32°C, there was evidence that the magnitude of the tension-independent heat increased as the stimulus rate was raised. This rate effect may to a certain extent balance out any additional temperature-induced decrease in the tension-independent heat.

Under normal conditions an electrically activated heart, when prevented from doing work or developing tension, should consume more oxygen than a quiescent heart. This point is not well-established in the literature. However, McKeever, Gregg, and Canney (1958), Kohn (1963), and Hauge and Øye (1966) have published results which support such a contention. Indeed Kohn (1963) found a mean oxygen consumption of 3.0 ml/100 g muscle in rabbit hearts beating at 120 per min but doing no external work. When these hearts were electromechanically dissociated by means of calcium-free perfusion the oxygen consumption dropped to 1.7 ml.

Mechanical Effects

In 1913 Mines showed that frog heart muscle perfused with calcium-free solution has its mechanical activity abolished while its electrical activity remains. A surprising feature of the present results was our inability to completely prevent tension development at room temperature. Langer (1965) has noted that in the perfused dog papillary muscle all the muscles he examined at 24°C maintained some ability to contract even when zero calcium perfusion had been maintained for up to 90 min. At 37°C several authors have reported complete tension abolition, generally in about 15 min. We found that calcium depletion produced a much more rapid and a much larger relative tension decrease at 32°C than at 18° to 22°C.

It is difficult to account for these effects of temperature. Lahrtz et al. (1967) have shown in guinea pig atria that cellular calcium levels depend upon two different processes: (*a*) calcium pump of the sarcoplasmic reticulum (Hasselbach and Makinose, 1961). (*b*) active transport across the cellular membrane into the extracellular space. The second process is temperature-dependent with marked inhibition occurring at 15°C. Possibly even at room temperature there is some inhibition of one or both pumps.

The tension recovery times after calcium depletion are reasonably consistent with the times it would take to replenish the phase 2, "calcium trans-

port system" (Langer, 1965). We cannot exclude the possibility that there may have been interacting biochemical factors as Zimmerman and Hülsmann (1966) have shown that calcium depletion of rat heart can cause the loss of certain enzymes from the heart when it is replaced in normal calcium solutions. This effect may account for the longer recovery times after EDTA. Tension recovery averaged 96 % (see Table II) and was 80 % complete in 43 min which would indicate that any structural damage to cell membranes was not permanent under the conditions of our experiments.

Electrical Activity

Calcium depletion of skeletal muscle has been shown to decrease the membrane resistance and the resting membrane potential and to eventually cause loss of excitability (Jenden and Reger, 1963; Edman and Grieve, 1964). In cardiac muscle Hoffman and Suckling (1956) reported that lowering calcium levels produced some membrane depolarization but no loss of activity. Our results have shown that electrical activity can be maintained in cardiac muscle exposed to calcium-free solutions and the results therefore support similar findings by Ware, Bennett, and McIntyre (1955) and Fleckenstein (1964). Unfortunately as yet there has been no detailed electrophysiological study of the effects of chelating agents upon the electrical activity of cardiac muscle. Electron microscope studies of cardiac muscle exposed to EDTA demonstrate a shrinkage of cells from the basement membranes and an increase in the distance between apposed membranes at the intercalated discs (Muir, 1967).

We do not believe that conduction failure has been a factor in our results obtained in calcium-free solutions. Even had conduction failed it was evident that the stimulating setup used with the right ventricle wall was adequate to excite all the randomly sampled ventricular fibers, except those in two preparations. In these two preparations (see Results) the quiescent cells lay outside the stimulus field and the exposure period to the chelating agents was longer than normal. Since the papillary muscles on the thermopile were activated by stimulating electrodes positioned at each end of the pile, each cell is in a more uniform stimulus field than is the case for the ventricular preparation. Some uncertainty must exist, of course, as to whether each cell has been activated in the solutions containing chelating agents. However, the decrease in the magnitude of the tension-independent heat in solutions containing 1.25 and 0.6 mM calcium argues that we are not studying a conduction failure effect. Good membrane potentials and normally shaped action potentials can be recorded for several hours in 1.25 and 0.6 mM calcium.

Indeed it has been possible to record normal action potentials from a single cell in rabbit ventricle muscle for periods of up to 2 hr in 0.6 mM calcium (Gibbs, 1963, 1964).

Tension-Independent Heat

There is at present considerable controversy over the biochemical equivalent of the activation heat (see Introduction). At the moment it is not possible to reconcile the existing biochemical and myothermic data but we would like to examine three possibilities.

1. Activation heat may be the heat released when bound calcium is liberated into the sarcoplasm. This view was put forward by Davies (1963). Now the release of bound calcium and its subsequent re-uptake during the contraction cycle have been elegantly demonstrated by Jöbsis and O'Connor (1966) and Ridgway and Ashley (1967). Such a heat output associated with calcium binding could then be the result of an entropy change consequent either upon the change in membrane potential during an action potential or the increase in intracellular sodium ion concentration (Langer, 1967; Palmer and Posey, 1967). If such a scheme operates, however, there must be a subsequent reabsorption of heat when calcium is rebound. Now myothermic studies do not at present provide any evidence of a heat absorption phase. Such a phase must either be very slow or else it must be masked by heat production from some other source, a possibility suggested by Jöbsis (personal communication). The heat output most likely to mask any subsequent reabsorption probably relates to the calcium-pumping mechanisms. The calcium pump does require ATP (Hasselbach, 1964), and depending upon the pumping efficiency and the free energy of hydrolysis under cellular conditions, such a relaxation heat would be a possibility. Sandberg and Carlson (1966) have argued that the tension-independent component that they measured biochemically in skeletal muscle tetani relates to the energy requirements of this pump.
2. Activation heat as measured myothermically may not be a distinct entity but may in fact represent internal shortening heat. Gay and Johnson (1967) have shown that in papillary muscles under zero resting tension the fibers are buckled so that when the preparation is activated they could be expected to shorten and take up the slack. These authors report that even when the resting tension was adjusted so that all fibers were straightened out, the individual sarcomeres shortened to varying degrees during an "isometric" contraction. The necessity for finding a biochemical basis for activation heat would then depend upon whether one adopted the views of Carlson, Hardy, and Wilkie (1963), Davies et al. (1967), or those of Hill (1949, 1964), Gibbs et al. (1967), and Jöbsis and Duffield (1967).
3. Activation heat is a separate identifiable heat component in the sense that Hill (1949) first used the term. If this view is correct we need to

demonstrate the breakdown of ATP or PC under conditions in which the activation heat is the sole or major heat component.

It is difficult to completely reject the first hypothesis but the results of Mommaerts and Wallner (1967) make the calcium pumping explanation unlikely unless most of the pumping occurs before the contraction peak.

The second hypothesis is undermined by experiments in which electromechanical dissociation has been produced by exposure to quite diverse agents or treatments such as D_2O, hypertonic solutions, and calcium depletion and yet heat production still occurs when the muscle is activated. Unpublished results in this laboratory with both cardiac and skeletal muscle show that the rate of heat production is normal in solutions made hypertonic with sucrose. Howarth (1958) showed that under these experimental conditions shortening velocity is greatly decreased. We would admit, however, that most estimates of activation heat in the literature are probably too high because of internal shortening heat contributing to the results.

The authors favor the third explanation. Many experimenters have now shown that enough ATP or PC is broken down to account for the heat and work output of both cardiac and skeletal muscle. The heat of hydrolysis for such splitting is in good agreement with expectations based on thermochemical data (Carlson and Siger, 1960; Wilkie, 1968). Distèche (1964) has demonstrated PC breakdown in hypertonic solutions where the mechanical response has been abolished and Mommaerts and Wallner (1967) have shown that ATP breakdown occurs during the contraction phase and that the total amount broken down is double the amount needed to account for the measured skeletal muscle activation heat.

At present it is not profitable to discuss these or other hypotheses any further. The results reported here demonstrate the involvement of calcium ions in the magnitude of the tension-independent heat component and they remove an apparent discrepancy between myothermic and oxygen consumption studies.

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