The Mechanochemistry of Cardiac Muscle

I. *The isometric contraction*

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ABSTRACT The utilization of creatine phosphate (CP) and adenosine triphosphate (ATP) was studied in the iodoacetate (IAA) and nitrogen (N_2)-treated cat papillary muscle. Under these conditions the net production of ATP does not occur, and the net utilization of ATP is reflected in a fall in CP concentration. The rate of energy utilization of the IAA-N_2-treated cat papillary muscle resting without tension was 0.68 μmole CP/g/min. This rate was increased to 1.07 μmole/g/min when muscles were passively stretched with 2 g of tension. In a series of isometrically contracting muscles CP utilization was found to be proportional to the number of activations and the summated contractile element work. These rates of CP utilization were 0.083 μmole/g/activation and 0.0059 μmole/g-cm of work. The calculated mechanochemical coupling efficiency was 33%.

INTRODUCTION

While the thermodynamics of skeletal muscle have been investigated with heat measurements for half a century (1), it is only in the past decade that information on the chemical energetics of skeletal muscle has been obtained (2–6). The first extension of heat measurements to cardiac muscle in vitro has been made in the past year by Ricchiuti and Gibbs (7). No studies of chemical energetics in cardiac muscle have been reported.

The object of the present study was to analyze the chemical energetics of cardiac muscle during isometric contraction. The right ventricular papillary muscle of the cat was used because it was a muscle sample of convenient size with fibers arranged in parallel. The utilization of high energy phosphate stores was measured under conditions in which no resynthesis of these stores could take place. To prevent the aerobic synthesis of ATP, muscles were maintained in an oxygen-free environment, and to prevent ATP synthesis by anaerobic glycolysis, muscles were treated with iodoacetic acid (IAA).

The IAA-nitrogen–treated frog sartorius muscle has been studied by Carlson and Siger (8) and Padieu and Mommaerts (9) who have shown that

IAA concentrations sufficient to inhibit anaerobic glycolysis completely do not inhibit the in vivo activity of creatine phosphokinase:

$$\text{Creatine phosphate (CP)} + \text{adenosine diphosphate} \xrightleftharpoons{\text{creatine phosphokinase}} \text{ATP} + \text{creatine}$$

Thus, the IAA-nitrogen–treated muscle provides a model in which the utilization of ATP, the immediate energy source for muscle contraction (10, 11), is reflected in a fall in CP concentration.

In the present study, the energy utilization of the isometric contraction has been evaluated along with the energy utilization of resting metabolism and the effect of resting length on this utilization.

METHODS

Right ventricular papillary muscles which were obtained from adult mongrel cats (2–4 kg), anesthetized by the intraperitoneal injection of pentobarbital (25 mg/kg), were placed in a myograph. The base of each muscle was held in a plastic clip while the chorda tendinea was securely fastened to a force transducer by a short length of 4-0 braided silk suture. A sintered glass gas dispersion tube was used for aeration of the bath, and a thermistor probe monitored bath temperature. Above the plastic clip was a force transducer[1] controlled in its vertical direction by a micrometer.

The muscle bath had a capacity of 100 ml and was kept at constant temperature by a surrounding water jacket. The muscle bath also contained an entrance and a drain in its bottom to replace solutions which flowed by gravity from a heat-controlled storage tank in which they were preequilibrated with the desired gas mixture. All experiments were carried out at 26°C.

The bathing medium for all muscles was a modified Krebs solution (pH 7.4) containing:

Na^+	145.8 mM	Cl^-	128.4
K^+	3.6	$H_2PO_4^-$	1.2
Ca^{++}	2.5	$SO_4^=$	1.2
Mg^{++}	1.2	$H_2CO_3^-$	24.8
		Glucose	5.6

Muscles were stimulated[2] directly by platinum wires on the inner surfaces of the attachment clip. Square wave pulses of 3 msec duration at a rate of 12/min with a voltage 10% above threshold were employed.

Blockade of Energy Production

Glycolysis was inhibited in all muscles by exposure to 5×10^{-4} M IAA for 30 min. To determine the adequacy of blockade, some of these muscles were sliced and incu-

[1] Sanborn Company, Waltham, Mass., model FTA 100.

[2] American Electronic Laboratories, Philadelphia, Pa., model 104A.

bated with glucose-6-^{14}C for 1 hr at 38°C following which the evolution of $^{14}CO_2$ was measured. The incubation method of Bloom et al. (12) was employed except that hyamine was used to absorb the evolved CO_2.

Inhibition of aerobic metabolism was achieved by exposing the muscles to Krebs solution previously equilibrated with 95% N_2, 5% CO_2. Determinations of the oxygen tension of this solution with a polarographic electrode[3] showed that oxygen tension always fell to less than 1 mm Hg within 20 sec.

Freezing

At the end of each experiment the muscle was rapidly frozen by quickly removing the muscle bath and replacing it with a beaker of 2-methylbutane (isopentane) previously cooled in liquid nitrogen to −150 to −160°C. This procedure froze the muscle without further contraction.

Protocol

The initial portion of the protocol was similar for all muscles in the study. Each muscle was stimulated to contract isometrically for 30 min with a resting tension of less than 0.1 g. Its length-tension curve was determined, and the muscle was stimulated to contract at the top of this curve for an additional 30 min. Then stimulation was terminated and IAA added to the bath to a final concentration of 5×10^{-4} M. After the muscle had remained in this solution for 30 min without tension, the oxygenated, IAA-treated Krebs solution was abruptly drained from the bath which was flushed with 95% N_2, 5% CO_2 and refilled with Krebs solution previously equilibrated with this gas mixture. Subsequently, three separate protocols were followed:

a. RESTING ENERGY UTILIZATION

Muscles rested without tension and were frozen either immediately after the change to a nitrogenated solution or after 3, 7, or 10 min periods of nitrogenation.

b. EFFECT OF STRETCH

Muscles rested in the nitrogenated solution for 3 min without tension. Then they were stretched passively with 1 or 2 g of tension while continuing to rest for an additional 4 min prior to freezing.

c. ISOMETRIC CONTRACTION STUDIES

Muscles rested in the nitrogenated solution for 3 min without tension. Then they were stretched to a tension equivalent to the top of their previously determined length-tension curves and stimulated to contract isometrically 11 to 59 times prior to freezing.

Chemical Analyses

The frozen muscles were stored in liquid nitrogen. At the time of analysis each muscle was reduced to powder with a stainless steel pestle in a small stainless steel test tube

[3] Instrumentation Laboratory, Inc., Boston, Mass., model 113.

held in an aluminum block at less than $-50°C$. The same tube was then transferred to another aluminum block at 0°C and 0.4 ml of 0.3 M perchloric acid was added with continued grinding. The mixture was allowed to thaw and extract for 10 min at 0°C. In the same tube, the mixture was centrifuged at 0°C at $25{,}000 \times g$ for 10 min. The supernatant was removed, diluted with 3 volumes of distilled water, and maintained at 0°C for determination of CP, inorganic phosphate (Pi), ATP, and creatine.

Total creatine was determined by the α-naphthol-diacetyl method (13, 14) after liberation from CP by a 9 min acid hydrolysis at 65°C in the presence of 0.13 N HCl. An appropriate standard was carried through the procedure with each sample. The optical density of the final reaction mixture was read in a spectrophotometer[4] at 530 mμ. CP and Pi concentrations were determined by the Furchgott and de Gubareff (15) modification of the Fiske and SubbaRow (16) technique. The optical density was read at 660 mμ in a spectrophotometer.[4] A 60 min reading following acid hydrolysis of CP represented the total of Pi plus CP. Appropriate standard solutions of Pi and CP were assayed simultaneously.

ATP was assayed by a modification of the firefly luminescence technique of Strehler and McElroy (17) using firefly lantern extract.[5] Duplicate determinations of peak luminescence were read on a photomultiplier microphotometer[6] following the addition of 0.1 ml extract to 0.1 ml distilled water and 1.0 ml dilute (5 mg/ml) firefly lantern extract. An appropriate ATP standard was measured simultaneously in duplicate.

Weighing the muscles in the frozen state was found to be inaccurate because of their small size (5–15 mg), while thawing the muscles for weighing led to changes in the concentration of high energy phosphate compounds. Therefore, an alternative method of determining weight was employed. As each papillary muscle was removed from a heart, two samples of the right ventricular (RV) wall were obtained, blotted, and weighed. The total creatine concentration was determined in both samples and the average concentration used to predict the weight of the papillary muscle once its total creatine *content* was determined. To test the accuracy of this method 15 other papillary muscles were carried through the experimental protocol, but rather than being frozen, they were blotted and assayed for total creatine. The mean difference of these muscles from their corresponding RV samples was -0.04 μmole/g with a standard deviation of the difference between them of ± 1.17 μmole/g. The mean creatine concentration of the RV samples was 15.8 μmole/g, while the standard deviation of paired RV samples was ± 1.11 μmole/g. Muscles weighing less than 5 mg were discarded.

Calculations

Calculations of contractile element work (CEW) were made from the formula $CEW = P/k$ where P equals the summated total active tension (g/mm^2) generated by each muscle during the experimental period, and k is the series elastic constant previously

[4] Beckman Instruments, Inc., Fullerton, Calif. model DU 2.

[5] Sigma Chemical Company, St. Louis, Mo.

[6] American Instrument Company, Silver Spring, Md.

derived (18) from the relation of the modulus of elasticity of the series elastic (dp/dl) to load, P: $dp/dl = k \cdot P$.

Statistical tests of significance were performed by the paired t test and the t test of the difference between group means when appropriate (19).

The multiple regression analysis (20, 21) of the data from the isometrically contracting muscles was performed with the aid of a Honeywell 800 digital computer. The input to the computer program was purposely large with the desire to include any possible factor which could have influenced the results significantly. The dependent variables included the concentrations of ATP, CP, Pi, ATP plus CP, and a number of combinations of these factors. The independent variables consisted in part of the number of stimuli, the contractile element work, the normalized total tension generated, the mean resting tension, the peak active tension achieved, length, cross-sectional area, and total creatine concentration. An independent variable was selected by the computer if the probability that it made no contribution to the regression equation was less than 0.05.

RESULTS

1. *The Effect of IAA Treatment*

To determine whether the concentration of IAA and the time of exposure were adequate to inhibit glycolysis, six muscles (1.0–2.0 mm^2 cross-sectional area) were treated with 5×10^{-4} M IAA for 30 min. Then they were sliced and incubated with ^{14}C-glucose, and the evolution of $^{14}CO_2$ measured. Six normal muscles evolved $^{14}CO_2$ at a rate corresponding to the metabolism of the terminal carbon of 30.0 μg glucose/g muscle/hr. In the IAA-treated muscles, however, $^{14}CO_2$ evolution was completely inhibited. Previous experiments in which muscles were treated for only 15 min with IAA showed variable small amounts of metabolic conversion ranging from 0–10 μg glucose/g muscle/hr.

To determine whether CP stores were affected by IAA treatment, nine IAA-treated muscles were compared with five normal muscles carried through the routine protocol to the time of nitrogenation. There was no significant difference between the groups (9.89 $\pm$ 0.60 vs. 9.93 $\pm$ 0.82 μmoles/g).

Fig. 1 shows the tracing obtained from a muscle which was stimulated until rigor occurred. Its pattern of contractile force was typical of all the isometrically contracting muscles that were frozen at various points prior to rigor. The initial force treppe was similar to that seen in normal muscles not treated with IAA. All muscles stimulated more than 25 to 30 times showed a subsequent fall in generated force, but all were frozen before force fell to less than 25 % of peak force and before rigor occurred.

The mechanical performance of the isometrically contracting muscles could be evaluated from the maximal tension generated. The mean maximal active tension generated by these muscles was 3.6 $\pm$ 0.2 g/mm^2. This was

slightly less than the mean maximal active tension (5.1 ± 0.3 g/mm²) generated by these same muscles at the end of the equilibration period.

2. *Resting Energy Utilization*

Nine muscles were frozen at the onset of nitrogenation. After 3, 7, and 10 min periods of nitrogenation at rest, additional groups of 9, 9, and 13 muscles each were frozen. These four groups of muscles (Fig. 2) had CP concentrations of 9.89 ± 0.60 (SE), 8.31 ± 0.47, 5.59 ± 0.36, and 3.88 ± 0.63 μmoles/g respectively. Corresponding ATP concentrations were 6.60 ± 0.56, 5.22 ± 0.24, 5.13 ± 0.21, and 4.94 ± 0.40 μmoles/g.

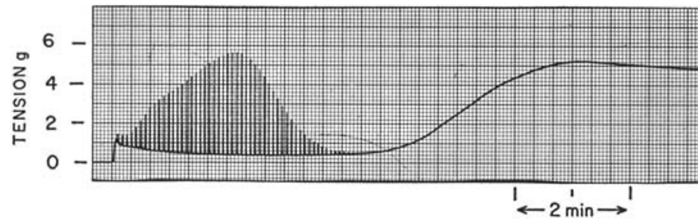

FIGURE 1. Original record of force generated by an IAA-N_2-treated papillary muscle. Stimulation was continued until rigor occurred.

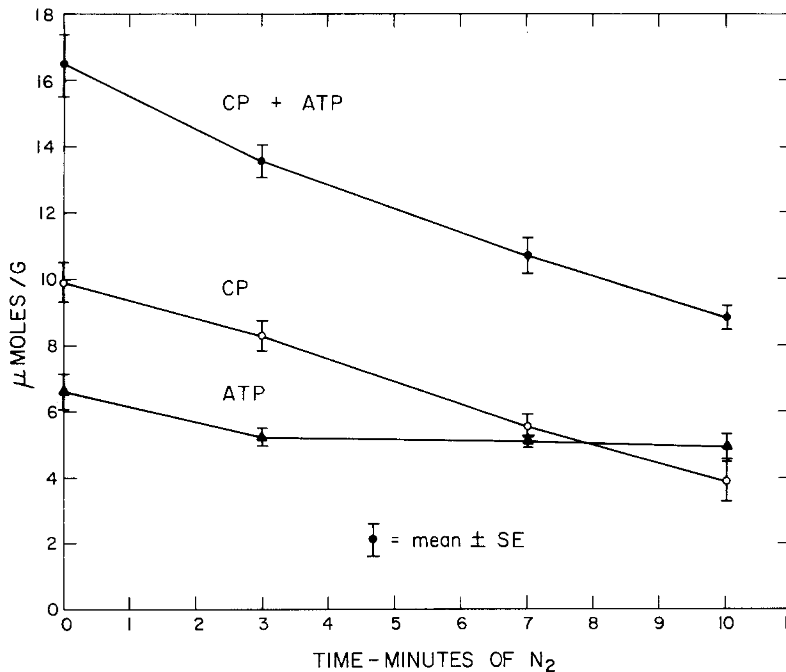

FIGURE 2. The diminution of CP, ATP, and the sum of CP + ATP with time in IAA-N_2-treated muscles resting without passive tension (±SE).

The difference in CP concentration between each of the above groups was significant ($p < 0.05$), while ATP was not significantly changed ($p > 0.5$), except in the first 3 min ($p < 0.05$). The rate of resting CP utilization was 0.68 μmole/g/min. The correspondence of this rate to the rate of utilization of CP + ATP (0.70 μmole/g/min) indicates that the total utilization of high energy phosphates was represented by that of CP.

3. *The Effect of Stretch on Resting Energy Utilization*

In 20 muscles, the effect of an increase in resting tension (and/or length) on CP utilization was determined (Fig. 3). As resting tension was increased, the

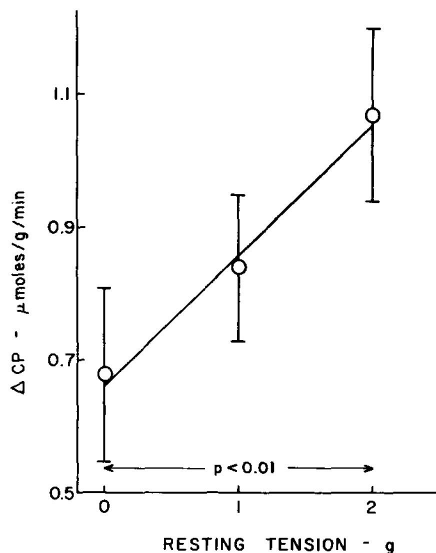

FIGURE 3. The resting rate of CP utilization in IAA-N_2-treated muscles stretched with 0, 1, and 2 g of passive tension (±SD).

rate of CP utilization increased from 0.68 μmole/g/min at zero tension, to 0.84 with 1 g tension, and to 1.07 with 2 g. The change in the resting rate of CP utilization from zero tension to 2 g of tension was highly significant ($p < 0.01$). The mean concentration of CP in the group stretched with 1 g of tension lay between those of the other two groups although its difference from them was not significant ($0.2 > p > 0.1$). The same data also were normalized by dividing the applied tension (1 or 2 g) by the cross-sectional area of each muscle. These normalized tensions (g/mm^2) were subjected to a linear regression analysis. The correlation coefficient was −0.56 ($p < 0.01$) and the change in rate of resting CP utilization by this method was. 0.27 μmole/g muscle/g resting tension/min.

4. *The Energy Utilization of the Isometric Contraction*

40 muscles (Table I) were stimulated to contract isometrically 11 to 59 times. The CP concentration of each of these muscles is shown in Fig. 4 as a function

TABLE I

Experiment No.	Length	Cross-sectional area	Total creatine	CP	ATP	Pi	No. of Acti-vations	Contractile element work
	mm	*mm²*	*μ moles/g*	*μ moles/g*	*μ moles/g*	*μ moles/g*		*g-cm/g*
54	8.0	1.79	15.2	3.69	5.44	6.25	28	339.0
75	5.2	2.02	18.6	3.86	4.86	10.30	36	496.0
83	4.0	1.95	14.1	3.05	3.63	4.32	41	212.0
97	5.3	1.62	16.4	2.41	3.06	10.30.	30	459.0
100	5.1	1.17	15.2	3.07	4.01	7.10	33	302.0
104	7.0	1.08	15.4	1.87	2.32	6.84	39	298.0
135	6.0	1.59	17.6	2.49	3.06	5.82	49	262.0
142	7.0	1.66	14.4	3.56	5.09	6.74	34	332.0
157	7.0	1.35	18.0	3.33	5.08	6.60	42	494.0
161	7.0	1.37	11.3	2.46	3.15	5.06	36	264.0
164	4.7	1.79	17.2	3.37	4.85	6.22	35	358.0
167	5.8	2.26	18.6	5.04	5.05	5.60	34	285.0
174	5.5	2.34	16.7	4.96	4.91	4.73	11	33.4
182	6.1	1.50	14.1	7.96	4.02	3.27	13	45.2
185	7.0	1.51	16.8	9.12	3.34	3.26	11	44.2
192	6.8	2.35	16.2	9.24	4.49	2.65	11	68.6
200	6.0	1.83	17.2	7.46	3.79	5.25	13	41.7
203	7.2	0.89	20.1	10.10	6.44	6.74	17	97.4
524	4.5	0.81	14.4	4.05	3.94	7.36	20	178.0
527	6.0	1.75	14.0	6.03	3.54	5.16	20	179.0
613	4.2	2.07	13.5	6.56	5.90	2.68	25	57.1
629	6.1	2.82	13.6	5.48	3.73	4.18	20	71.9
749	5.8	1.94	16.4	3.05	3.19	5.40	53	353.0
755	7.0	1.43	16.5	0.82	3.66	4.66	38	430.0
762	5.1	1.93	17.9	2.76	4.61	6.89	44	264.0
770	6.8	1.10	14.8	0.96	4.30	6.84	46	222.0
782	6.2	1.65	15.4	1.54	3.90	6.75	47	507.0
786	9.0	2.18	16.0	2.50	3.54	6.44	50	246.0
790	6.1	1.36	16.8	4.16	3.75	4.88	59	416.0
795	8.8	1.14	19.0	0.74	4.75	8.11	53	616.0
803	6.3	1.50	17.1	4.21	5.00	6.32	52	419.0
841	4.1	1.84	19.8	1.70	5.19	9.26	41	309.0
845	4.1	2.13	15.6	2.37	4.94	6.78	35	152.0
886	6.2	1.02	11.4	0.98	3.48	6.77	40	456.0
935	6.4	1.71	18.6	3.69	3.98	4.81	47	445.0
942	5.9	1.48	14.0	3.10	5.84	4.89	52	378.0
955	7.0	1.60	12.8	2.81	3.52	5.01	40	268.0
965	6.8	1.98	14.8	4.00	5.59	6.22	26	239.0
972	8.0	1.20	14.2	3.02	5.83	4.93	38	481.0
980	6.1	1.81	17.8	4.48	6.86	5.98	49	212.0

of the calculated contractile element work performed. These data also were subjected to computer analysis. Of all the independent variables tested there was none except the total creatine concentration which fulfilled the significance criterion for predicting ATP concentration. By a separate analysis which did not exclude variables falling outside the 5% confidence limits, it was possible

to predict ATP concentration, but the maximum value for the multiple correlation coefficient was 0.402, implying that the ability to predict the ATP concentration from the values of the independent variables was poor.

The CP concentration, however, could be predicted with considerable accuracy. With CP as the dependent variable, three independent variables were selected as significant predictors: the total creatine concentration (C); the number of times the muscle was activated (A); and the contractile element work (CEW) as shown in equation (1).

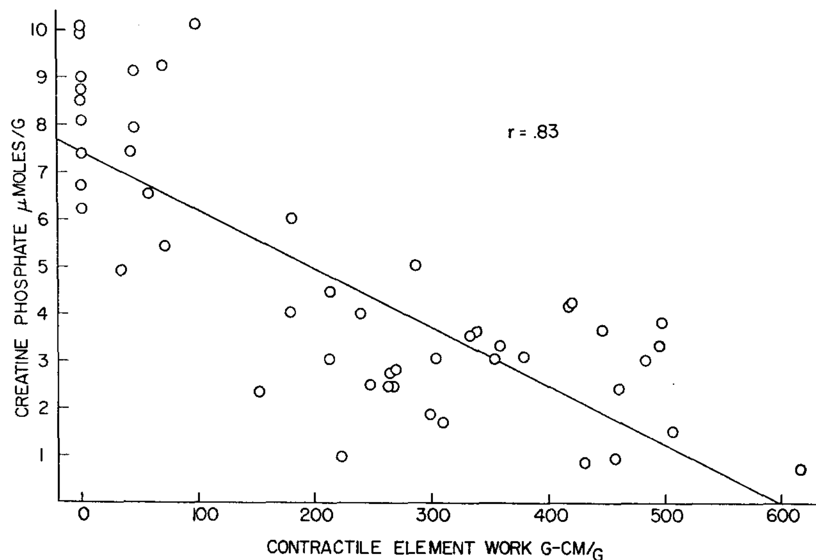

FIGURE 4. Creatine phosphate concentration as a function of contractile element work for each muscle. No independent variables held constant.

$$CP = 3.635 + (0.3068 \pm 0.0998)\,C - (0.0834 \pm 0.0196)A - (0.0059 \pm 0.0020)\,CEW \quad (1)$$

The mutliple correlation coefficient of this equation is 0.878, while the standard error of the estimate is 1.363. The p value for each of the coefficients is <0.01 signifying the probability that its value is zero.

Because the total creatine concentration remains constant throughout each experiment (see Methods), the mean value for total creatine in the study (15.8 μmoles/g) can be used if the equation is to predict the absolute CP concentration. To predict the change in CP, the creatine term can be eliminated and the total expression reduced to the form of equation (2).

$$\Delta CP = (0.0834 \pm 0.0196)A + (0.0059 \pm 0.0020)\,CEW \quad (2)$$

Expressed in this form the dependence of CP utilization on each term is independent of the other term. Because the stimulation rate was constant, the utilization of CP during activation cannot be separated from resting utilization. Both these factors are included in the A term and can be estimated separately only by subtracting the previously determined value for the resting utilization of CP (see Discussion).

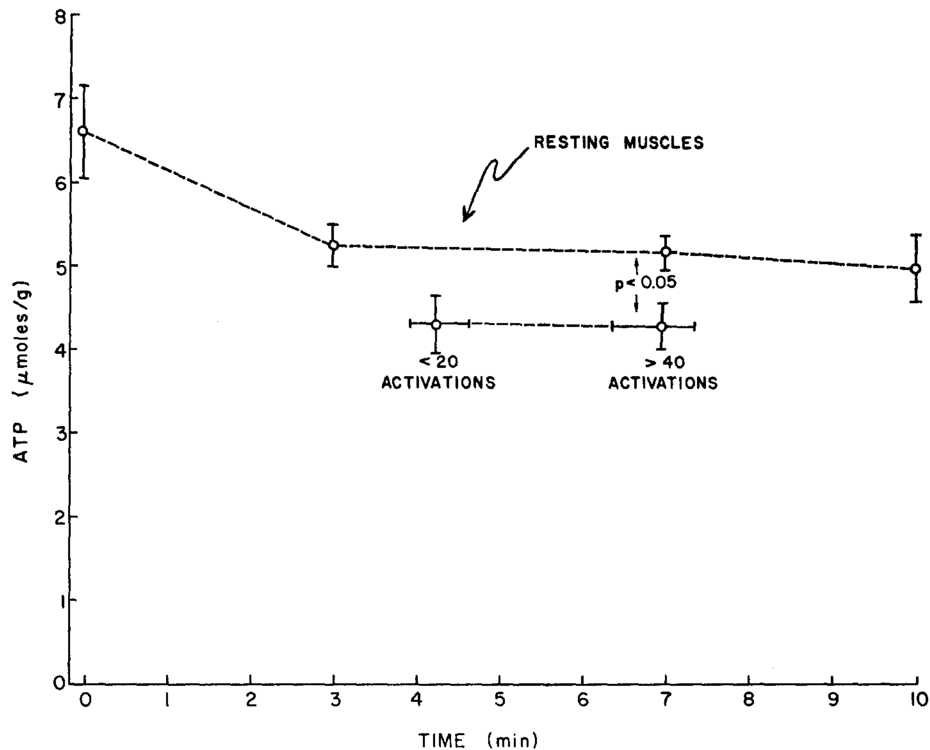

FIGURE 5. A comparison of ATP concentrations between resting muscles and isometrically contracting muscles (see text). Vertical bars = ±SE; horizontal bars = range.

The concentrations of ATP in the resting muscles and in the isometrically contracting muscles are compared in Fig. 5. There was no significant ($p >$ 0.05) change in ATP concentration in the resting muscles after the first 3 min. To determine whether a change in ATP concentration, not detected by computer analysis, occurred in the contracting muscles, those which contracted less than 20 times were contrasted as a group with those which contracted more than 40 times. The ATP concentrations in the two groups were 4.32 ± 0.35 and 4.36 ± 0.26 μmoles/g respectively, values which were not significantly different. However, the muscles which were stimulated to contract

had significantly lower concentrations of ATP than the resting muscles, even when broken down into comparable groups. Those muscles stimulated less than 20 times had a mean ATP concentration significantly lower than the muscles which rested 3 min (4.32 ± 0.35 vs. 5.22 ± 0.24 μmoles/g, $p < 0.05$), and those muscles stimulated more than 40 times had an ATP concentration significantly lower than those which rested for 7 min (4.36 ± 0.26 vs. 5.13 ± 0.21, $p < 0.05$). This confirms the inability of the computer analysis to detect a change in ATP concentration related to time, activation, or contractile element work, but seems to indicate a significant difference between ATP concentrations in the resting and contracting muscles.

DISCUSSION

The present results permit a quantification of the chemical energy utilization of cardiac muscle in the isometric contraction. In addition, the resting rate of chemical energy utilization has been determined and this rate has been shown to increase when resting tension is increased in the unstimulated muscle.

Certain assumptions are implicit in this study: (*a*) that net ATP production has been completely inhibited; (*b*) that there is an available finite energy store which may be utilized throughout the experiment; and (*c*) that the performance and efficiency of the IAA-treated muscle are similar to those of a normal muscle.

Inhibition of energy production by IAA and N_2 has already been demonstrated in other studies on both skeletal and cardiac muscle (8, 9, 22, 23). These have shown that concentrations and durations of IAA exposure similar to those used in this study have been adequate to block glycolysis completely while not inhibiting creatine phosphokinase activity (as the latter enzyme is protected from inhibition by substrate). In addition, phosphoglyceraldehyde dehydrogenase is the most sensitive of the enzymes of intermediary metabolism to inhibition by IAA (24). At the onset of the experimental period, energy stores in the IAA muscles were identical with those in control muscles. The preservation of these stores and their lack of preservation following N_2 indicate the continuing integrity of oxidative phosphorylation during IAA treatment. Further, the failure of the IAA-treated muscles to produce $^{14}CO_2$ from glucose-6-^{14}C in the presence of intact oxidative phosphorylation is evidence for inhibition of anaerobic glycolysis.

The mechanical performance of the muscles was well maintained as judged by the peak tension of 3.6 ± 0.2 g/mm^2 reached after IAA-N_2 treatment as compared to that of 5.1 ± 0.3 g/mm^2 just before IAA. It is not known whether this somewhat lower peak tension represented an inability to achieve normal tension or was secondary to the preceding rest period. The IAA-N_2–treated muscles developed a peak tension after about 25 contractions equivalent to 71 % of their pre-IAA tension, while normal nontreated muscles which had

not been activated for 30 min developed a peak tension of 77 % of their pre-resting tension in 25 contractions.

The utilization of energy by the isometrically contracting muscles can be described in terms of a resting energy, an activation energy, a work energy, and an efficiency ($W/\Delta H$ since the entropy term is not known). In Table II, some of the data from this study are compared with similar data from heat studies at 20°C on cardiac muscle by Ricchiuti and Gibbs (7). The resting rate of CP utilization in the present study varied from 0.68 μmoles/g/min at zero tension to 1.07 μmoles/g/min with 2 g of passive tension. Ricchiuti and Gibbs reported a value equivalent to 2.87 μmoles/g/min[7] in muscles with

TABLE II

	Present study	Ricchiuti and Gibbs
Resting energy, *mcal/g/min*	6.8 → 10.7	28.7
Activation energy, *mcal/g/c*	0.83	0.63
Work energy, *mcal/g/c*	1.2	1.24
Σ Activation + work energy, *mcal/g/c*	2.03	1.87
Contractile element work, *mcal/g/c*	0.47	0.45
Efficiency $\frac{CEW}{\Sigma\ AE + WE}$	23%	24%
Efficiency $\frac{CEW}{(\Sigma\ AE + WE) - RE}$	33%	24%
PL/H	7.9	5.2
Twitch tension	5 g/mm²	4.7 g/mm²

1 mole CP = 10 kcal.
CEW = Contractile Element Work; *AE* = Activation Energy; *WE* = Work Energy; *RE* = Resting Energy; *c* = contraction.

1.0 g of resting tension. The latter value, however, must include heat lost in the process of ATP generation. In the present study energy production cannot occur, and therefore a minimum reduction of resting heat production on the order of 50 % would be anticipated. With this in mind, the values are quite comparable, although they were working at a lower temperature.

The activation energy has been taken from the regression coefficient of the number of activations of the isometrically contracting muscles and is 0.083 μmole CP/g/activation. The physiologic meaning of this term is open to question since it accounts for all time-related processes including resting energy utilization and may exclude factors which are dependent on the contractile element work. The extent to which the rate of basal energy utilization may change in the repeatedly activated muscle is unknown, but if the resting energy utilization per beat at the mean resting tension is subtracted from the

[7] Assuming 1 mole CP yields 10 kcal.

activation energy, the revised activation energy would be 0.021 μmole CP/g/activation. Such a small activation would be consonant with the failure to find significant activation heat in skeletal muscle by Cain et al. (4), but would be somewhat smaller than the activation heat of skeletal muscle found by Hill and others (equivalent to 0.075–0.200 μmole CP/g) (25, 26).

The energy utilization associated with contractile element work is shown in Table II, calculated from the computer prediction for an arbitrary isometric contraction of 5 g/mm^2. The energy utilization of 0.12 μmole CP/g is compared to that of Ricchiuti and Gibbs in which the heat equivalent of 0.14 μmole CP/g was used in an isometric contraction of approximately 4.7 g/mm^2.

Based on the above figures, the mechanochemical coupling efficiency can be calculated. If the resting energy utilization is subtracted from the sum of work plus activation energy, which is implicit in heat methods, the efficiency is 33 %. This compares closely to the efficiency of frog sartorius reported in similar studies by Cain et al. of 39 %, but is somewhat higher than that of Ricchiuti and Gibbs, owing largely to the higher nonbasal activation energy in the muscles of their preparations. A value for PL/H (P = nonnormalized tension; L = length at L_{max} ; H = sum of activation and work energy less resting energy) which relates force generated to energy liberated is 7.9 compared to Hill's value of 10.0 for frog skeletal muscle (27).

The finding that the rate of resting chemical energy utilization increases as the passive tension is increased is an extension to cardiac energetics of the Feng effect (28). A similar effect in oxygen consumption studies with the cat papillary muscle has been reported by Cranefield and Greenspan (29). However, these authors interpreted their results as evidence for inadequate diffusion of oxygen to a central anoxic core in muscles greater than 0.64 mm in diameter (0.31 mm^2 cross-sectional area) at 35°C and that the effect of stretch was to expose the previously anoxic core to oxygen. They also suggested that this limited the usefulness of papillary muscle preparations. The present data, however, are not subject to this limitation as values were obtained in the absence of oxygen. Feng, in his original study, also showed that the stretch reponse was present in a nitrogen atmosphere. In addition, the muscles used in the present study, many of which were two to three times the critical diameter stated by Cranefield and Greenspan, were capable of maintaining undiminished high energy phosphate stores at a time when they were being treated with IAA. Under these conditions, when anaerobic metabolism is being eliminated, the muscles must depend upon oxygen supply to maintain their high energy phosphate stores. Thus, these data do not support the conclusion that oxygen supply is a limiting factor in the average size cat papillary muscle (diameter = 1.2 mm).

In the present study we have examined the two most closely related (by present techniques) links of mechanochemical coupling, the high energy phos-

phate stores and mechanical performance. Whether the efficiency of this mechanochemical linkage in heart muscle is fixed, or whether it can be changed by altering the contractile state of heart muscle remains to be determined.

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