

# Energy Liberation and Chemical Change in Frog Skeletal Muscle during Single Isometric Tetanic Contractions

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**ABSTRACT** Recent data obtained from *Rana temporaria* sartorius muscles during an isometric tetanus indicate that the time-course of phosphocreatine (PC) splitting cannot account for the total energy (heat + work) liberation (Gilbert et al. 1971. *J. Physiol. (Lond.)*. **218**:163). As this conclusion is important to an understanding of the chemical energetics of contraction, similar experiments were performed on unpoisoned, oxygenated *Rana pipiens* sartorius muscles. The muscles were tetanized (isometrically) at 0°C for 0.6, 1, or 5 s; metabolism was rapidly arrested by freezing the muscles with a specially designed hammer apparatus, and the frozen muscles were chemically analyzed. Comparable myothermal measurements were made on frogs from the same batch. Results of these experiments indicate: (a) The energy liberation parallels the PC and ATP breakdown with a proportionality constant of  $-10.7$  kcal/mol; (b) comparably designed experiments with sartorius muscles of *R. temporaria* revealed that the ratio of energy liberation to PC splitting was significantly greater than that observed in *R. pipiens* sartorius muscles; (c) there is no systematic difference between experiments in which metabolism was arrested by the hammer apparatus and others using a conventional immersion technique.

## INTRODUCTION

Biochemists and physiologists have long been attempting to account for the time-course of energy liberation by contracting skeletal muscle in terms of known chemical reactions. Gradually the idea emerged that the heat + work liberated by a muscle could be accounted for by an ATP breakdown which was rapidly restored via creatine phosphate (PC) splitting (e.g., Carlson et al., 1963, 1967; Marechal and Mommaerts, 1963; Wilkie, 1968; Mommaerts, 1969). This concept depended in part upon the assumption that only one net reaction was occurring in the contracting muscle (which seemed reasonable for iodoacetate-poisoned muscles in nitrogen). Since the ratio of heat + work

to the observed breakdown of PC was a constant of approximately  $-11$  kcal/mol, similar to that anticipated from calorimetric measurements (Meyerhof and Schulz, 1935), it seemed likely that the *major* reactions occurring in the muscle were represented.

This conclusion was based primarily on experiments in which total energy production and PC splitting were compared *after* a complete contraction-relaxation cycle or after many such cycles. However, as a muscle *presumably* returns to its initial (precontraction) state after a contraction-relaxation cycle, it may be that *during* the cycle rapid, reversible exothermic or endothermic reactions occur which exhibit no net energy liberation at the completion of the cycle. Consequently, Wilkie (1968) has cautioned that the results of the previously mentioned experiments do not imply that from instant to instant during a single cycle all the liberated energy is derived from PC splitting. In fact, results of recent experiments (Gilbert et al., 1971, 1972) in which the contraction phase of an isometric tetanus was investigated, have been interpreted as indicating that the time-course of PC splitting cannot account for the total energy production.

Gilbert et al. (1971) utilized liquid  $N_2$ -cooled hammers to flatten muscles on freezing. This approach resulted in a faster muscle freezing time than with the conventional immersion technique (the rate of cooling of a muscle occurs approximately in inverse proportion to the square of its thickness) and thus presumably leads to a sharper resolution of the chemical events. Using this device to arrest metabolism, Gilbert et al. (1971) found that: (a) their results were compatible with the interpretation that PC was split at a constant rate from the beginning of stimulation despite the fact that energy was liberated as a nonlinear function of time; (b) since the PC splitting rate was not proportional to the energy liberation rate, the ratio of heat + work to PC split was not independent of time and ranged from greater than  $-30$  kcal/mol to approximately  $-15$  kcal/mol (if PC splitting were the only energetically important reaction occurring during contraction, the energy liberation per mole of PC split would be expected to be constant and have a magnitude of  $-8$  to  $-11$  kcal/mol); (c) there was a higher PC/ $C_T$  (where  $C_T$  is total creatine) ratio in muscles frozen by the hammer technique than that observed using the immersion method. This suggests that the immersion technique leads to a spurious breakdown of PC. These observations challenge the validity of previous measurements in which the immersion technique was employed, raise the possibility that there are other reactions occurring in contracting muscle (aside from ATP and PC splitting), and render unlikely any theory of muscle contraction which assumes that PC splitting is proportional to the energy liberation.

Because of the importance of these observations we felt that it was necessary to repeat these experiments using a hammer-freezing device and compare the

results obtained to the immersion technique. The results of our experiments indicate: (a) In *R. pipiens* sartorius muscles, the total energy production parallels the PC and ATP splitting resulting in a constant ratio of  $-10.7$  kcal/mol; (b) in *R. temporaria* sartorius muscles, the ratio of energy liberation to PC splitting is significantly greater than observed in *R. pipiens* sartorius muscles; (c) there is no systematic difference between experiments performed with the hammer apparatus and the immersion technique.

## METHODS

### *General*

Frogs, *R. pipiens* of both sexes, were obtained from the Steinhilber Co. (Oshkosh, Wis.) and *R. temporaria* of both sexes, from L. Haig Ltd. (Surrey, England). The frogs were kept in moist tanks at  $6^{\circ}\text{C}$  which were flushed several times each day with cold water. All animals were maintained in this environment for at least 2 wk before being used in experiments. On the day before an experiment the animals were killed by decapitation, pithed, and the sartorius muscles dissected. The distal tendons were affixed to 5-0 silk, the in vivo rest length of the muscle ( $l_0$ , defined as the muscle length when the legs are at right angles to body) measured, and the muscles removed from the body still attached to the pelvic bone. Muscles were aerated overnight in 95%  $\text{O}_2$ , 5%  $\text{CO}_2$  at  $4^{\circ}\text{C}$  in a Ringer solution which contained (mM): 95.0 NaCl, 20  $\text{NaHCO}_3$ , 2.5 KCl, 1.0  $\text{MgCl}_2$ , 1.0  $\text{CaCl}_2$ , at a pH of 7.0. Early on the day of chemical experiments the pelvic bones of the muscle pairs were split under a dissecting microscope. At this point the muscles were also examined under the dissecting microscope and any muscle pairs exhibiting damaged fibers were rejected. The muscles were then returned to the  $4^{\circ}\text{C}$  Ringer solution and allowed to recover for 3–4 h. Although this was the usual procedure, muscles were often dissected on the same day of experimentation with no apparent difference in the results.

### *Myothermal Measurements*

Myothermal measurements from the same batch of frogs for comparison with the chemical data were generally performed within a week of or during the course of the chemical experiments. The general procedures outlined by Homsher et al. (1972) and Homsher and Rall (1973) were employed. The thermopiles used were E-1 and E-4 (having active regions of 9.5 mm and 12.8 or 18.4 mm, respectively). Muscles were mounted on the thermopile, attached to the tension transducer, and allowed to equilibrate for 10–40 min. The solution was then drained and the heat loss correction factor determined by either radio frequency heating of the muscle or by heating the muscle thermopile system through a heating wire. (A  $12.5\text{-}\mu\text{m}$  diameter Nichrome wire insulated with Isonel [California Fine Wire Co., Culver City, Calif.], was bonded to both surfaces of E-4.) The muscles were then stimulated and heat recorded. In some experiments on oxygenated unpoisoned muscles (tetanized for 1 or 5 s) the muscles were given two to four single twitches to align them on the thermopile and to obtain the heat loss factor. Subsequent experiments were performed to test the effect of this previous activity on the tetanic heat output. In muscles subjected to three

twitches the tetanic heat production expressed relative to the tension developed per cross-sectional area was greater, but not significantly different, in the muscles with no activity (the  $\Delta$  [heat/tension], millicalories per gram per kilogram per square centimeter,  $N = 12$ , at 1 s was  $10 \pm 5\%$  and at 5 s was  $2 \pm 5\%$ ). Because of the uncertainty associated with previous activity, muscles stimulated in oxygen for 0.3 s, in 1-fluoro-2,4-dinitrobenzene (FDNB) for 0.4 s or in oxygen for 4.8 s (*R. temporaria* sartorius muscles) were not previously activated. In eight experiments on *R. pipiens* sartorius, after resting the muscles for 20–30 min, a measurement of the work dissipated in stretching the series elastic component was performed using a controlled release against an ergometer (Homsher and Rall, 1973) moving at a velocity of 20 cm/s. Muscles were then exposed to a Ringer solution containing 10 mM procaine to block electrical activity and the stimulus heat was recorded. Finally, the muscles were removed from the thermopile and their drained and blotted weight determined, they were frozen in a liquid  $N_2$ -isopentane slush, and their free and total creatines were measured.

In the analysis of the myothermal data, heat recordings of tetani longer than 1 s were not corrected for conduction of heat from the muscle to the thermopile whereas those of 0.5 s were increased by 5% (Hill, 1965, p. 314). The stimulus heat was subtracted from the heat records (this was less than 5.5% in the worst case, a 5-s tetanus), and the remainder was added to the stored series elastic work as estimated in *R. pipiens* from our measurements and in *R. temporaria* from values published by Hill (1970). The calculated thermoelastic heat (a thermoelastic heat coefficient of 0.01 was assumed as suggested by Gilbert et al. [1971]) was next added to obtain the total energy (in millicalories per gram). The total energy output was then normalized by dividing by the total creatine (in micromoles per gram). Since an accurate determination of absolute energy liberation is important in these studies, we have reevaluated the accuracy of the standard techniques for determining muscle energy liberation used in this work.

**ABSOLUTE ENERGY CALIBRATION.** Routinely the microvolt output of a thermopile was converted to millicalories utilizing the thermopile temperature sensitivity and the mass of the muscle and adhering Ringer solution as described by Hill and Woledge (1962). The accuracy of this standard technique was checked by heating the muscle-thermopile system with an independent energy-liberating source in a manner similar to that employed by Wilkie (1968). E-4 has an insulated Nichrome wire bonded to its surface permitting heating of the muscles while observing the thermopile output. To test the accuracy of the nichrome wire calibration unit, a heater element, into which known amounts of energy were liberated, was mounted on each surface of E-4. This heater element was constructed of a slab of silver ( $0.125 \text{ mm} \times 3 \text{ mm} \times 19 \text{ mm}$ ) about which a  $25\text{-}\mu\text{m}$  (diameter) insulated constantan wire was wound, and the element was embedded in epoxy yielding an artificial "muscle." By passing a known current through the constantan wire, an energy sensitivity of the artificial muscle-thermopile system of  $1.676 \pm 0.043 \times 10^{-2} \text{ mcal}/\mu\dot{V}$  ( $N = 5$ ) was measured. When the artificial "muscles" were heated by passing current through the nichrome wire, the energy sensitivity of the artificial muscle-thermopile system was  $1.660 \pm 0.018 \times 10^{-2} \text{ mcal}/\mu V$  ( $N = 5$ ). These results indicate that the nichrome wire can be used to accurately calibrate the thermopile output.

A comparison was next made of the energy sensitivity of the muscle-thermopile system as determined by the standard technique and the direct heating of the muscle-thermopile system via the nichrome wire. Pairs of semitendinosus muscles were mounted on E-4 and the energy sensitivity (in millicalories per microvolts) was determined by the two techniques. In five pairs of muscles the ratio of the energy sensitivity obtained by the standard technique to that observed by direct heating was  $0.980 \pm 0.027$ . The agreement obtained using these two techniques verifies that the accuracy of the standard calibration technique and confirms similar conclusions by Aubert (1956) and Hill and Woledge (1962).

**LONGITUDINAL THERMAL INEQUALITIES** Total heat production was estimated from a measurement of the lower (pelvic) 26–70% of the muscle length and the question could be raised as to whether thermal inequalities along the length of the muscle could cause a gross under- or overestimation of muscle heat output. Evidence from the literature suggests that this is unlikely since: (a) Hill and Howarth (1957) found, when recording from various parts of a muscle, that in a twitch the heat production during contraction was virtually superimposable (inequalities appeared only during relaxation), and (b) Aubert and Lebacqz (1971), in recording the heat production from the pelvic and tibial portions of sartorius muscles stimulated tetanically at  $l_0$ , found that during the contraction the pelvic portion produced 9% more heat (not enough experiments were performed to establish statistical significance). To secure this point seven pairs of sartorius muscles were mounted on thermopiles E-1 and E-4 and during 2 s isometric tetani heat was measured at the pelvic or tibial ends. The heat production recorded at the pelvic end of the muscle at 500 ms, at 2 s, and at the end of relaxation was greater than that recorded at the tibial end by  $4.4 \pm 1.0\%$ ,  $4.1 \pm 1.8\%$ , and  $4.5 \pm 2.3\%$ , respectively. Our results differ significantly from those of Aubert and Lebacqz (1971) in that the difference between pelvic and tibial heat production did not change during relaxation. Taken together these results indicate that, if anything, our measurements slightly overestimate the heat production by a contracting muscle.

**HEAT LOSS AND HEAT TRANSPORT IN RESTING AND CONTRACTING MUSCLE** The correction for an exponential heat loss and heat conduction from the muscle to the thermopile are determined on resting muscles and applied to heat production of active muscles. Thus it is tacitly assumed that the heat loss coefficient and heat transport properties of contracting muscles are the same as resting muscles. However, Carlson et al. (1972) have concluded that there is considerable intracellular motion in the isometrically contracting muscle. This idea raised the possibility that the muscle may no longer be considered as an unstirred aqueous layer and that the heat loss coefficient and conduction of heat from the muscle to the thermopile may differ in the contracting and resting muscle. However, in experiments in which the heat loss coefficient and the rate of heat conduction from the muscle to the thermopile were measured in both resting and isometrically contracting muscles no change was observed (see Appendix).

**POSSIBLE SOURCES OF ERROR IN THE ESTIMATION OF TOTAL ENERGY DURING MUSCULAR CONTRACTION** In the estimation of total energy liberation from the myothermal recordings, corrections were made for the following: (a) heat loss during the contraction, (b) stimulus heat (c) conduction of heat from the muscle to the thermopile (only in contractions of 600 ms), (d) series elastic work, (e) thermoelastic heat. Fig.

1 illustrates the magnitude of these corrections on a typical myothermic recording. It should be noted that a correction of about 15% was made at each point of the record. This correction was primarily composed early in the tetanus of the internal work and thermoelastic heat and late in the tetanus of the heat loss. In considering the accuracy of the total energy estimate the following points are relevant. First, the heat loss characteristics of our thermopiles were strictly exponential, the heat loss coefficient was independent of whether the muscle was relaxed or contracting, and the heat loss coefficient, as well as the stimulus heat, were measured in each experiment. Second,

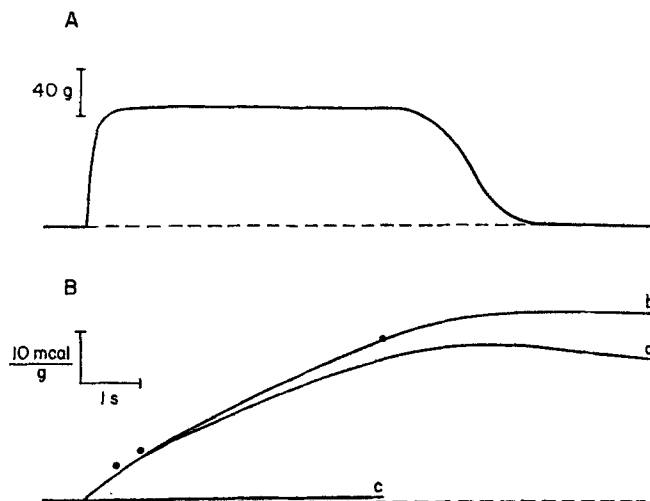


FIGURE 1. Tension generation and uncorrected and corrected myothermic recordings in a 5-s isometric tetanus at 0°C. (A) This tension recording has been taken from a pair of *R. temporaria* sartorius muscles. (B) Myothermic traces: (a) uncorrected heat production; (b) corrected for an exponential heat loss only (time constant = 25 s); (c) stimulus heat production recorded after poisoning the muscle pair with procaine. The dots (•) represent the final corrected energy liberation at 0.6, 1, and 5 s. The final values are the observed uncorrected deflections to which corrections have been made for: heat loss, stimulus heat, conduction of heat from the muscle to the thermopile, series elastic work, and thermoelastic heat (see text).

the correction for heat conduction from the muscle to the thermopile was small (5%) in our shortest contraction (0.6 s) and became insignificant as the tetanus duration was lengthened (1 and 5 s). This correction was independent of whether the muscle was at rest or contracting. Third, the internal work was measured in the eight pairs of muscles utilized for the 0.6-s isometric tetanus in this study (Table II, row F). The percentage of the total energy attributable to the internal work was greatest in this case. From these measurements it was found that, for any given tension per cross-sectional area, the mean of the internal work per gram had a standard error of less than 5% of the mean and was within 10% of values previously reported by Hill (1970, p. 82). Furthermore, since the internal work was maximally 7% of the total energy, any error involved in extrapolating the internal work from our measurements

on *R. pipiens* and Hill's values for *R. temporaria* should result in changes in the total energy of less than  $\pm 1\%$ . Fourth, the thermoelastic heat correction ( $\Delta Q = R\Delta R l_o$ ) was the parameter most subject to systematic error. The experimentally measured values of  $R$  range from 0.007 (Woledge, 1963) to 0.014 (Woledge, 1961), and an intermediate value of 0.010 was thus assumed. Since the thermoelastic heat correction ranged from 3% of the total energy (at 5 s) to 9% (at 0.6 s) even if the assumed value of  $R$  was  $\pm 50\%$  in error, the net effect would result in an error in the total energy estimate ranging from  $\pm 1.5\%$  to  $\pm 4.5\%$ . Taken together, the maximum accumulative effect of any errors in the above corrections is likely to be, on the average, less than 5% of the total energy at 0.6 s and to decrease as the duration of the tetanus is lengthened.

#### *Chemical Measurements*

The hammer device which was used to rapidly freeze muscles was similar in design to that of Kretzschmar and Wilkie (1969) but with several modifications. First the pelvic bones were fixed in stainless steel bone clamps and the muscles were vertically mounted side by side each in contact with a platinum wire stimulating grid (23 mm long consisting of three cathodes and four anodes). A thermostated temperature control unit (Electronic Cryo-Bath, Cole-Palmer Instrument Co., Chicago, Ill.) containing Ringer through which 95% O<sub>2</sub>, 5% CO<sub>2</sub> was constantly bubbled, was then raised up about the muscles. A thermistor bead (model 3248, VECO, Springfield, N.J.) was mounted on the muscle holder and allowed the ambient temperature about the muscles to be constantly monitored. Knowledge of the ambient temperature in the muscle chamber at the time of stimulation is extremely important as the following example illustrates. The  $Q_{10}$  for the maintenance heat ( $h_b/P_o l_o$ ) is about 3.1 and the  $Q_{10}$  for the tetanus tension ( $P_o$ ) is about 1.2 (C. J. C. Kean and E. Homsher, unpublished results for *R. pipiens* semitendinosus muscles). Consequently the rate of heat production, and hence presumably the rate of PC splitting, will increase by about 14% between 0 and 1°C. The Ringer fluid was drained by gravity away from the muscles after 5 min at 0°C and precooled gas allowed to enter the chamber. The muscle chamber remained at 0°C for at least 1 min before the temperature began to drift slowly upward. After approximately 15 s in the gas the experimental muscle was stimulated (stimulus parameters were: frequency of 10–15 Hz, 3-ms duration, 18-V amplitude controlled by a Devices stimulator and Digitimer [Devices Instruments Limited, Herts, England]). Concurrent with the stimulation an electronically controlled sequence began which caused the incubation chamber to move down from the muscles and the copper hammer heads (which had been precooled to  $-196^\circ\text{C}$  in liquid N<sub>2</sub>) to begin moving toward the muscles. With this arrangement the muscles were exposed to room temperature for approximately 500 ms before they were flattened. The hammer heads were attached by 54-cm long metal arms to a gear arrangement which controlled the velocity at which the heads converged and assured that the hammer heads would meet in the plane of the muscles. Also attached to these arms was a device which caused the stimulating electrodes to revolve out of the muscle plane  $\sim 150$  ms before the hammers flattened the muscles. (A disadvantage of this arrangement is that the muscle cannot be stimulated up to the instant that it is frozen). The muscles were removed while still encased in the hammer heads and placed

directly into liquid  $N_2$ . Under liquid  $N_2$ , the flattened muscle was removed and placed into a precooled stainless steel cartridge of the type previously described (Seraydarian et al., 1961). The advantage of this method is that the muscles are never exposed to room temperature after smashing and thus any spurious chemical change in the flattened muscles as a result of thawing is avoided. The muscles were then extracted and analyzed for free creatine ( $C_F$ ), total creatine ( $C_T$ ), ATP, ADP, AMP, and inorganic phosphate ( $P_i$ ) as previously reported (Homsher et al., 1972; Mommaerts and Wallner, 1967). Since the muscles were not weighed, all chemical values were referred to the muscle total creatine content.

The purpose of smashing muscles with this device is to decrease the freezing time and thus to sharpen the temporal resolution of the chemical analysis. To measure the cooling time of smashed muscles, a 25- $\mu\text{m}$  copper-constantan thermocouple was placed between a pair of sartorius muscles (*R. pipiens*). Since we were not able to smash a pair of muscles reliably to the smashed thickness of a single muscle, we extrapolated our paired muscle-freezing times to that for a single muscle by assuming that the time for a muscle to freeze is inversely proportional to the square of its thickness (Carslaw and Jaeger, 1959, p. 283). A sample group of single muscles smashed under normal conditions had an average thickness of  $243 \pm 10 \mu\text{m}$  ( $N = 9$ ) with a range from 200 to 300  $\mu\text{m}$ . Smashed pairs of muscles ( $N = 6$ ) were 300–500  $\mu\text{m}$  thick, and based on their times to complete freezing (156–420 ms), it was estimated that a 243- $\mu\text{m}$  muscle would be completely frozen in  $88 \pm 4$  ms. This value is similar to the 80 ms reported by Gilbert et al. (1971), is significantly less than the 200–300-ms values predicted for the immersion technique (Mommaerts and Schilling, 1964), and is representative of the average situation in our experiments. Fig. 2 shows a typical

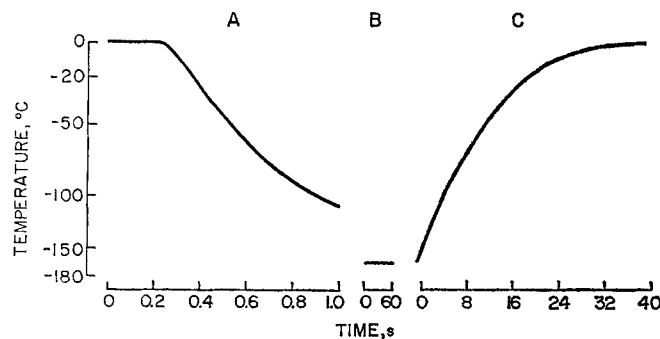


FIGURE 2. Typical cooling and warming curves for a pair of smashed sartorius muscles. A 1-mil copper constantan thermocouple was placed between the muscle pair and then the muscles were mounted and incubated at  $0^\circ\text{C}$ . In A, the pair was smashed by liquid  $N_2$ -cooled hammers at time zero resulting in complete freezing in approximately 240 ms (smashed muscle pair thickness was 425  $\mu\text{m}$ ). In B, the muscles remained enclosed in the hammer heads near the temperature of liquid  $N_2$  for 1 min. In C, the hammers were quickly removed and the warming of the center of the pair monitored. The center temperature of the muscles was back to nearly  $0^\circ\text{C}$  in 40 s. To extrapolate to an average single muscle thickness of 243  $\mu\text{m}$  the time on the abscissa is multiplied by  $(243)^2/(425)^2$  or 0.33. This results in an estimate of the freezing time of 78 ms and the warming time to near  $0^\circ\text{C}$  of 13 s.



cooling curve for a pair of smashed muscles and also emphasizes the speed at which the same pair warms on exposure to the ambient temperature. Details of the immersion apparatus and the arrest of metabolism by immersion in liquid N<sub>2</sub>-isopentane were similar to that previously reported (Mommaerts and Schilling, 1964; Homsher et al., 1972).

In the experiments in which muscles were poisoned with FDNB, a muscle pair was exposed to a Ringer solution containing 0.38 mM FDNB at 0°C, gassed with 98% N<sub>2</sub>, 2% CO<sub>2</sub> for 35 min (Infante and Davies, 1962). The muscles were then mounted in the hammer apparatus and equilibrated for an additional 10 min before stimulation.

In all, 120 pairs of sartorius muscles were frozen and analyzed. Of this number, five pairs were rejected on the criterion that their average change in PC or P<sub>i</sub> was more than three standard deviations away from the mean of their group. The significance of the differences between sample means was tested with the *t* test. A 5% level of significance ( $P < 0.05$ ) was utilized throughout.

## RESULTS

### *A Comparison of the Arrest of Metabolism by the Immersion and Hammer Technique in Resting Muscles*

Gilbert et al. (1971) found that the ratio of PC/C<sub>T</sub> in muscles frozen using the hammer technique was significantly greater than in muscles frozen by immersion in a liquid N<sub>2</sub>-isopentane mixture. These results indicate that freezing by immersion results in an additional splitting of PC. This possibility was tested by dissecting 16 pairs of *R. pipiens* sartorius muscles, 8 of which were frozen using the hammer technique, 8 of which were frozen using the immersion technique. All muscles were handled in an identical fashion. Since none of the muscles was stimulated, this procedure also permitted a test of the assumption that unstimulated paired muscles have the same PC/C<sub>T</sub>, P<sub>i</sub>/C<sub>T</sub>, ATP/C<sub>T</sub>, and AMP/C<sub>T</sub> ratios. Accordingly at the time of freezing, muscles were randomly designed as a control or experimental muscle. The results of these experiments are shown in Table I and reveal that with the exception of the ATP/C<sub>T</sub> ratio, there is no significant difference between the chemistry of resting muscles frozen by the immersion or the hammer method. Whereas the ratio of ATP/C<sub>T</sub> is significantly less using the immersion technique, no significant concomitant changes in P<sub>i</sub>/C<sub>T</sub> or ADP/C<sub>T</sub> were found, as might be expected if the immersion method were causing a spurious splitting of ATP. Though our PC/C<sub>T</sub> values are significantly less than those reported by Gilbert et al. (1971) of 0.88–0.90 employing a hammer apparatus, they are similar to the results of Curtin and Woledge (1974) of 0.806 also utilizing the hammer technique. It is further noteworthy that the ratio of PC/C<sub>T</sub> found using the hammer technique compares well with those generally observed using the immersion method (Carlson and Siger, 1960; Marechal, 1964; Sandberg and Carlson, 1966; Dydyńska and Wilkie, 1966; Wilkie, 1968; Homsher et al.,

TABLE I  
COMPARISON BETWEEN PAIRED SARTORIUS MUSCLES OF *R. PIPIENS* AT REST  
IN OXYGENATED RINGER AT 0°C FROZEN BY THE IMMERSION OR  
SMASHER METHOD

	N	Content*		N	Δ Content, E-C	
		Immersion	Smasher		Immersion	Smasher
PC/C <sub>T</sub>	16	0.798 ± 0.005 †, §	0.797 ± 0.009	8	-0.0043 ± 0.0036	-0.0026 ± 0.0037
P <sub>i</sub> /C <sub>T</sub>	16	0.096 ± 0.006	0.107 ± 0.008	8	-0.0094 ± 0.0086	+0.0105 ± 0.0065
ATP/C <sub>T</sub>	16	0.124 ± 0.004	0.136 ± 0.004	8	+0.0071 ± 0.0035	-0.0074 ± 0.0040
ADP/C <sub>T</sub>	8	0.0125 ± 0.0002	0.0125 ± 0.0005	4	-0.0005 ± 0.0007	0.0000 ± 0.0008
AMP/C <sub>T</sub>	8	0.0021 ± 0.0002	0.0019 ± 0.0002	4	+0.0005 ± 0.0003	-0.0003 ± 0.0004

\* Total creatine for this batch of frogs as determined from sartorius muscles frozen by immersion is  $33.38 \pm 0.48 \mu\text{mol/g}$  (N = 16).

† Mean ± SEM.

§ Results expressed as  $\mu\text{mol}/\mu\text{mol}$ .

||  $P < 0.05$ .

1972). Similarly a comparison of the differences between paired muscles shows there is no significant difference between resting muscles of a given pair, supporting the assumption on which the remaining experiments are premised.

*Time-Course of PC Splitting and Energy Liberation in Isometric Tetanic Contractions of R. pipiens Sartorius Muscles*

In these experiments (see Table II) muscles were tetanized at  $l_0$  for various lengths of time and frozen. In the first series of experiments muscles were frozen at 1 or 5 s after the beginning of stimulation. Muscles were also frozen by the immersion method for comparison with results obtained using the hammer technique. The results of these experiments show several features: (a) The amount of PC breakdown as estimated from the increase in  $C_F$  agrees well with that estimated from the increase in  $P_i$ . (b) The rate of PC splitting is not a linear function of time, i.e., PC is split during the first second of an isometric contraction at a rate which is about three times greater than the succeeding 4 s. This is not in agreement with the interpretation of Gilbert et al. (1971) who suggested that in *R. temporaria* sartorius muscles PC was split as a linear function of time. (c) When muscles were frozen using the immersion technique at 5 s, the amount of PC broken splitting was not significantly different from the results obtained using the hammer device. This result indicates that for a 5-s isometric tetanus the immersion technique does not give spuriously high  $\Delta C_F/C_T$  ratios due to a slower freezing time. To test for the variability among batches of frogs, further isometric measurements were performed on two additional batches of frogs. In one case (lines D and E of Table II) muscles were again frozen at 1 or 5 s after beginning the tetanus and in a second case (line F of Table II) muscles were frozen at 600 ms after beginning a 300-ms tetanus. The results were not significantly different from the earlier experi-

TABLE II  
ISOMETRIC TETANIC CONTRACTIONS FROM *R. PIPIENS SARTORIUS* MUSCLES  
UNPOISONED IN OXYGEN

Experimental conditions*				Batch of frogs‡	Content control N	Δ Content, E-C			Total energy liberation			
Stimulus duration	Time at freezing	Type of arrest†	N			PC/C <sub>T</sub>	ΔC <sub>F</sub> /C <sub>T</sub>	ΔP <sub>i</sub> /C <sub>T</sub>	ΔATP/C <sub>T</sub>	Heat + work/C <sub>T</sub>	Heat + work/ΔC <sub>F</sub>	
<i>s</i>	<i>s</i>				<i>μmol/μmol</i>	<i>μmol/μmol</i>		<i>mcal/μmol kcal/mol</i>				
(A)	5.0	5.0	I	Dec.	9	0.787 ±0.008	+0.056 ±0.004	+0.061 ±0.005	-0.0002 ±0.0011	5	0.508 ±0.043	-9.1±1.0
(B)	4.68	4.9	S	Dec.	17	0.723 ±0.007	+0.056 ±0.003	+0.054 ±0.002	+0.001 ±0.001	5	0.508 ±0.043	-9.1±0.9
(C)	0.82	1.0	S	Dec.	12	0.746 ±0.011	+0.023 ±0.004	+0.018 ±0.003	-0.001 ±0.001	5	0.201 ±0.016	-8.7±1.7
(D)	4.70	5.0	S	Feb.	13	0.843 ±0.008	+0.053 ±0.005	+0.056 ±0.004	+0.001 ±0.001	8	0.543 ±0.020	-10.2±1.0
(E)	0.75	1.0	S	Feb.	17	0.787 ±0.006	+0.0154 ±0.0025	+0.0142 ±0.0017	-0.002 ±0.002	8	0.208 ±0.007	-13.5±2.2
(F)	0.28	0.6	S	April	5	0.727 ±0.012	+0.012 ±0.002	+0.0104 ±0.0013	-0.0014 ±0.0029	8	0.144 ±0.009	-12.0±2.1

\* All experiments performed in 95% O<sub>2</sub>, 5% CO<sub>2</sub> Ringer at 0°C with muscles stimulated at 10–15 Hz.

† I, freezing by immersion in isopentane-liquid nitrogen slush S, freezing by smashing with hammers.

‡ Batch parameters: (Dec) C<sub>T</sub>/g = 28.70 ± 0.55 μmol/g (N = 30), P<sub>o<sub>2</sub></sub>/M = 2.02 ± 0.04 kg/cm<sup>2</sup> (N = 15); (Feb.) C<sub>T</sub>/g = 32.25 ± 0.63 μmol/g (N = 16), P<sub>o<sub>2</sub></sub>/M = 1.95 ± 0.07 kg/cm<sup>2</sup> (N = 8); (April) C<sub>T</sub>/g = 37.42 ± 0.60 μmol/g (N = 16), P<sub>o<sub>2</sub></sub>/M = 2.11 ± 0.05 kg/cm<sup>2</sup> (N = 8).

|| Mean ± SEM.

ments (lines A–C of Table II) and further showed that as early as 600 ms there is a statistically significant PC splitting.

For myothermal counterparts to these chemical experiments (Lines A–E of Table II) pairs of muscles from these batches of frogs were tetanized for 5 s. The heat production was read at 1 and 5 s. The results of the total energy measurements (after correction for the internal work, thermoelastic heat, and stimulus heat) are given in Table II. The myothermal equivalent of the experiment listed in line F of Table II was a 300-ms tetanus with the heat production read at 600 ms, and the total energy production by those muscles (after the appropriate corrections) is also given in Table II. These experiments agree with the biochemical data in that there is an initial high rate of energy liberation during the first second followed by a much slower energy production during the succeeding 4 s. Like the biochemical data there is no significant effect of the batch of frogs on the total energy produced.

The myothermal and biochemical results were used to calculate the ratio of heat + work liberated to PC split and these values are also given in Table II. From these ratios, none of which is significantly different from the other, there does not appear to be a significant change in the ratio of energy liberation to PC splitting with duration of the tetanus as was the case for the data of Gilbert

et al. (1971). Secondly the overall average ratio of heat + work to  $\Delta C_F$  is  $-10.7 \pm 0.6$  kcal/mol which is somewhat greater than the in vitro value for PC splitting of  $-8.1$  kcal/mol suggested by Woledge (1972). Finally, unlike Gilbert et al. (1971) we observe no evidence in the tetanic contractions of an ATP synthesis (Table II).

*PC Splitting and Energy Liberation in R. temporaria Sartorius Muscles in a 5-s Isometric Tetanus*

Since our results of chemical and total energy liberation differed from those obtained by Gilbert et al. (1971), we performed a similar experiment on the sartorius muscles of *R. temporaria*. Muscles were dissected and handled in a manner identical to *R. pipiens* muscles. The muscles, unpoisoned and oxygenated, were stimulated for 4.8 s at 0°C and frozen using the hammer device at 5 s. For the myothermal experiments the muscles were stimulated for 4.8 s and the heat read at 5 s. The results of these experiments are given in Table III. Several features are apparent. First, the control PC/ $C_T$  ratio is similar to those observed with *R. pipiens* but is significantly less than those reported by Gilbert et al. (1971). Second, the amount of PC split (as estimated by the increase in  $C_F$  or  $P_i$ ) is not significantly different from that observed in *R. pipiens*. Furthermore, the amount of PC split in 5 s is almost identical to that predicted from the data of Gilbert et al. (1971) (see their text fig. 1). Third, the amount of energy liberated in the tetanus is significantly greater in *R. temporaria* than in *R. pipiens* (more than 50% greater) and agrees with data given by Gilbert et al. (1971) (see their text fig. 1). Finally, the ratio of heat + work to  $\Delta C_F$  in *R. temporaria* sartorius muscles is  $-17.8 \pm 2.0$  kcal/mol, which is significantly different from the *R. pipiens* value but is in agreement with the data of Gilbert et al. (1971) (see their text fig. 1 and table 5). The difference between the present work and that of Gilbert et al. (1971) appears to be due to an energetic difference in the species of frogs.

TABLE III  
FIVE-SECOND ISOMETRIC TETANIC CONTRACTIONS OF *R. TEMPORARIA*  
SARTORIUS MUSCLES UNPOISONED IN OXYGEN\*

N‡	$\Delta$ Content, E-C				Total energy liberation		
	Content control PC/ $C_T$	$\Delta C_F/C_T$	$\Delta P_i/C_T$	$\Delta ATP/C_T$	N	Heat + work/ $C_T$	Heat + work/ $\Delta C_F$
	$\mu\text{mol}/\mu\text{mol}$		$\mu\text{mol}/\mu\text{mol}$			$\text{mcal}/\mu\text{mol}$	$\text{kcal}/\text{mol}$
13	0.780 $\pm 0.010$ §	+0.047 $\pm 0.005$	+0.049 $\pm 0.004$	+0.0009 $\pm 0.0010$ (N=10)	8	0.838 $\pm 0.025$	-17.8 $\pm$ 2.0

\* All experiments performed in 95% O<sub>2</sub>, 5% CO<sub>2</sub> Ringer at 0°C with muscles stimulated at 15 Hz for 4.8 s and frozen at 5.0 s by the smasher method.

‡ Batch parameters:  $C_T/g = 33.91 \pm 0.62 \mu\text{mol}/g$  (N = 16),  $P_{o_2}/M = 2.4 \pm 0.1 \text{ kg}/\text{cm}^2$  (N = 8).

§ Mean  $\pm$  SEM.

*ATP Splitting and Energy Liberation in a Short Isometric Tetanus*

Since ATP is thought to be the primary source of energy for contraction, an experiment was performed, using the hammer apparatus, in which FDNB-poisoned sartorius muscles of *R. pipiens* were tetanically stimulated for 0.4 s and then frozen at 0.54 s. Similar experiments were performed on FDNB-poisoned muscles on thermopiles to estimate the total energy liberation. The results of these experiments are shown in Table IV. The results indicate that there were significant decreases in ATP and significant increases in  $P_i$ , ADP, and AMP. The effectiveness of the poisoning is exemplified by the observation that there is no significant breakdown of PC ( $\Delta C_F/C_T$  is not different from

TABLE IV  
ISOMETRIC TETANIC CONTRACTIONS OF *R. PIPPIENS* SARTORIUS MUSCLES  
POISONED IN 1-FLUORO-2,4-DINITROBENZENE (FDNB) PLUS NITROGEN

Experimental conditions* †			$\Delta$ Content, E-C							Total energy liberation		
Stimulus duration	Time at freezing	Type of arrest‡	N	Content control PC/C <sub>T</sub>	$\Delta C_F/C_T$	$\Delta P_i/C_T$	$\Delta ATP/C_T$	$\Delta ADP/C_T$	$\Delta AMP/C_T$	N	Heat + work/C <sub>T</sub>	Heat + work/ $\Delta P_i$
<i>s</i>				$\mu\text{mol}/\mu\text{mol}$	$\mu\text{mol}/\mu\text{mol}$						$\text{mcal}/\mu\text{mol}$	$\text{kcal}/\text{mol}$
(A) 0.41	0.54	S	13	0.813 +0.007	+0.0014 $\pm 0.0019$	+0.0115 $\pm 0.0036$	-0.0045 $\pm 0.0011$	+0.0032 $\pm 0.0014$	+0.0017 $\pm 0.0004$	8	0.125 $\pm 0.005$	-10.9 $\pm 3.4$

\* All experiments performed in 0.38 mM FDNB and 95% N<sub>2</sub>, 5% CO<sub>2</sub> at 0°C.

† Batch parameters: C<sub>T</sub>/g = 35.23 ± 1.04 μmol/g (N = 26), P<sub>o</sub>l<sub>o</sub>/M = 1.48 kg/cm<sup>2</sup> (N = 17).

‡ S, freezing by smashing with hammers.

|| Mean ± SEM.

zero). The estimated amount of ATP split (based on the increase in  $P_i$ ) was about 0.4 μmol/g which is in good agreement with similar measurements by Mommaerts and Wallner (1967) who observed  $\Delta P_i = +0.36$  μmol/g in a 0.2-s isometric tetanus frozen at 0.6 s. The observed ratio of heat + work to  $\Delta P_i$  of  $-10.9 \pm 3.3$  kcal/mol agrees with the results of Homsher et al. (1972) of  $-9.9 \pm 1.1$  kcal/mol and with the calorimetric data and calculations of Woledge (1972) of  $-9.8$  to  $-11$  kcal/mol.

## DISCUSSION

*Arrest of Metabolism: Comparison of Hammer and Immersion Techniques*

As a partial explanation for the differences between their results and those of others Gilbert et al. (1971) found that resting muscles frozen by immersion had a significantly lower PC/C<sub>T</sub> ratio and, under the light microscope, displayed much smaller sarcomere spacings (as small as 1 μm) than those frozen using the hammer apparatus. These results strongly suggest that the slower freezing time associated with the immersion method results in muscle con-

traction with an accompanying splitting of PC. We found no significant difference in the PC/C<sub>T</sub> ratio of muscles frozen using the two techniques (Table I). In fact, in sartorius muscles of *R. temporaria* the PC/C<sub>T</sub> ratio can be the same as that observed in *R. pipiens* (Tables I and III) so the dissimilarity is not one attributable to the different species. Additionally, our results are not significantly different from those of Curtin and Woledge (1974), who also employed a hammer apparatus, from *R. temporaria* sartorius muscles. Though apparently not affected by freezing technique, the PC/C<sub>T</sub> ratio is one parameter that often shows significant variability (Table II). These general conclusions can also be extended to stimulated muscles. In a 5-s isometric tetanus the PC split was the same irrespective of freezing technique (Table II).

A partial explanation for the results of Gilbert et al. (1971) may reside in the fact that with the immersion technique as developed by Mommaerts (Mommaerts and Schilling, 1964) and Davies (Cain and Davies, 1964) the muscles are held stationary and the coolant mechanically propelled about the muscles resulting in uniform and reproducible freezing. In the immersion experiments of Gilbert et al. (1971), the muscles were manually submerged in a liquid N<sub>2</sub>-isopentane mixture. This adaptation of the immersion technique may have resulted in a slower cooling with a spurious breakdown of PC and shortening of some sarcomeres. In our hands the immersion method yields the same results as smashing with liquid N<sub>2</sub>-cooled hammers.

The only demonstrable difference between the hammer and immersion techniques is the slower cooling time of the latter (muscle completely frozen in 200–300 ms [Mommaerts and Schilling, 1964; Cain and Davies, 1964] compared to approximately 80–100 ms [Gilbert et al., 1971; our own measurements]). Under what circumstances might the slower cooling time of the immersion method become detrimental? This is a difficult question to assess since it is not apparent how cold is cold enough to stop all relevant reactions. Surely the disparity in cooling times is not relevant in a 5-s tetanus of frog muscle at 0°C although this result might have been expected. However, this question must still be considered pertinent for shorter duration contractions at 0°C and for contractions performed at higher temperatures.

*Time-Course of PC Splitting and Total Energy Liberation in Contracting Frog Skeletal Muscle*

Our major finding is displayed in Fig. 3 where the time-course of total energy liberation and PC splitting in an isometric tetanus is plotted as a function of tetanus duration. Specifically, throughout the contraction phase of an isometric tetanus of *R. pipiens* sartorius muscle PC is split in a nonlinear fashion with respect to time (the average rate of ATP and PC splitting is 0.75 μmol/g · s during the first 600 ms, 0.58 μmol/g · s for the first second, and 0.27 μmol/g · s between 1 and 5 s) but in direct proportion to the liberated energy. The energy

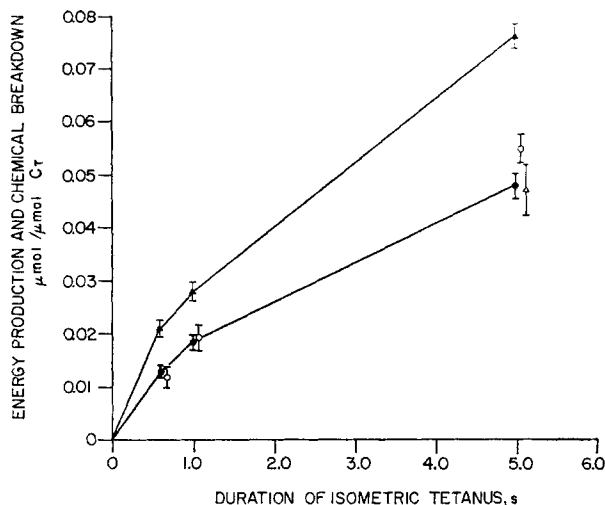


FIGURE 3. The time-course of PC splitting and total energy liberation in isometric tetanic contractions of frog sartorius muscle. For *R. pipiens*:  $\circ$  indicates PC split (as measured by  $\Delta C_F/C_T$ ,  $\mu\text{mol}/\mu\text{mol}$ ) as a function of time and  $\bullet$  represents accompanying energy liberation converted to equivalent chemical units ( $\mu\text{mol}/\mu\text{mol } C_T$ ) by dividing by 11 kcal/mol. The solid line connects the  $\bullet$  points. The superimposition of the chemistry and energy data suggests that they are related by a proportionality constant of about  $-11$  kcal/mol. For *R. temporaria*:  $\triangle$  represents PC split in a 5-s isometric tetanus whereas  $\blacktriangle$  is the accompanying energy liberation at 0.6, 1, and 5 s divided by 11 kcal/mol. A solid line connects the  $\blacktriangle$  points. Deviations from each symbol represent  $\pm 1$  SEM. Data have been taken from Tables II and III.

output and PC split are related by a proportionality constant which does not differ from  $-11$  kcal/mol at any point in time. The quantitative relationship between energy output and chemical change has been emphasized in Fig. 3 by converting the energy production to chemical quantities by dividing each value by 11 kcal/mol. The superimposition of the chemistry and energy values verifies the above conclusion. In experiments in which PC splitting was measured as a function of isometric tetanus duration (from 3 to 120 s), Marechal and Mommaerts (1963) concluded that PC was split at a constant rate of  $0.28$ – $0.32$   $\mu\text{mol}/\text{g}\cdot\text{s}$  (in good agreement with our value between 1 and 5 s) and that short duration tetani had a greater *average* rate of PC splitting. In isometric tetani lasting between 0.56 to 1.5 s, Infante et al. (1964) found an average rate of ATP splitting of  $0.72$   $\mu\text{mol}/\text{g}\cdot\text{s}$  which is again in good agreement with our results. Finally, from the data of Kushmerick et al. (1969) it was found that the rate of ATP splitting during the first 180 ms of an isometric tetanus was  $1.2$   $\mu\text{mol}/\text{g}\cdot\text{s}$ . Whereas this result would appear to be significantly greater than that predicted by our data, it can be in part attributed to uncertain temperature control in their experiments. Thus our measurements of ATP and PC splitting by muscles of *R. pipiens* are similar to others present in

the literature and, when coupled with our parallel myothermic data suggest that the time-course of the energy liberated by isometrically contracting muscles may be attributed to PC splitting and its side reactions.

The results of Gilbert et al. (1971) obtained from *R. temporaria sartorius* muscles differ in several aspects from our own. They concluded that in an isometric contraction, PC is split from the start of stimulation at a constant rate and thus not in proportion to the nonlinear energy liberation. (Actually at 0.5 s they did not observe a significant breakdown of PC. Rather than suggest that a muscle can contract for 500 ms without splitting PC they suggest that this value is probably a representation of a point just beyond the upper limit of sensitivity of their technique.) Their initial findings have been confirmed by Gilbert et al. (1972). Gilbert et al. (1971) suggest that their results are compatible with the notion that the unexplained early energy liberation corresponds to the labile heat (Aubert, 1956) plus the heat and work produced during the first second of an isometric tetanus. Also, after the first 2 s of a tetanus the rate of PC splitting was related to the energy produced by a proportionality constant  $-11$  kcal/mol. However, Chaplain and Frommelt (1972) using *R. temporaria sartorius* muscles found a ratio of  $-11.5$  kcal/mol from 0.6 to 2.6 s of an isometric tetanus. Their results limit any imbalance to the first 0.6 s whereas those of Gilbert et al. (1971) exhibit an imbalance throughout the first 2 s.

Since our results seemed different from those of Gilbert et al. (1971), and since different preparations were employed, we felt compelled to examine *R. temporaria sartorius* muscles. In a 5-s tetanus, our heat measurements suggested that  $0.076 \mu\text{mol PC}/\mu\text{mol C}_T$  would be split if energy production and PC splitting were related by  $-11$  kcal/mol. Interpolation of the chemical results of Gilbert et al. (1971) predicts a splitting of  $0.043 \mu\text{mol PC}/\mu\text{mol C}_T$  and a heat + work to PC splitting ratio of  $-19.5$  kcal/mol. Experimentally we observed  $0.047 \pm 0.005 \mu\text{mol PC}$  split per micromole  $C_T$  and calculated a heat + work to PC splitting ratio of  $-17.8 \pm 2.0$  kcal/mol (Table III and Fig. 3). Thus results are consistent with the data of Gilbert et al. (1971). However, our results on *R. temporaria* would also be consistent with a ratio of heat + work to PC split of  $-18$  kcal/mol throughout the contraction phase of an isometric tetanus. We appear to be dealing with an energetic difference in the species of frogs. However, after 2 s of an isometric tetanus in *R. pipiens* and apparently in *R. temporaria sartorius* muscles the energy liberation and chemical breakdown are related by a proportionality constant of  $-11$  kcal/mol. But whereas in *R. pipiens sartorius* muscle the first 2 s are also ascribable to a proportionality constant of  $-11$  kcal/mol, in *R. temporaria* most of the early energy liberation cannot be attributed to any known chemical reaction and certainly not PC splitting. Are there any differences in the labile heat production of sartorius muscles from these two species that might explain the dis-



parity observed in the initial 2 s of contraction? There is no significant difference in the magnitude of the labile heat though the rate of its production is slightly greater in English frogs (C. J. C. Kean and E. Homsher, unpublished results). The only notable difference is that the stable maintenance heat production is 50% greater in *R. temporaria* sartorius muscles (C. J. C. Kean and E. Homsher, unpublished results). Since our own chemical and myothermic techniques give results on *R. temporaria* sartorius muscles which are in accord with the predictions of Gilbert et al. (1971), we have no reason to doubt the validity of their short tetanic contraction data or the validity of our own. The difference between *R. pipiens* and *R. temporaria* at early times in an isometric tetanus appears to be real and remains unexplained.

#### *Enthalpy Change for PC Splitting in Contracting Muscle*

Because myothermic measurements are inherently nonspecific, for one to conclude that the energy liberation by a contracting muscle is due primarily to PC splitting it is a necessary condition that a time-invariant ratio of heat + work to  $\Delta$  PC (with the appropriate value) be observed. (Even this observation is not a sufficient condition since enthalpy changes by other chemical and/or physical processes could be coincidentally cancelling throughout the contraction-relaxation cycle. Other techniques would be necessary to evaluate such a situation.) In the experiments described above we have shown that in the isometric tetanus the total energy liberation parallels the PC splitting with a constant ratio of  $-11$  kcal/mol. How does this value compare with the calorimetric data for the enthalpy change ( $\Delta H_{PC}$ ) for PC splitting? The predicted amount of heat liberated in muscular contraction due to PC splitting is not merely the  $\Delta H$  of the isolated reaction but must include the heat of neutralization (base is produced with PC splitting) and the heats of ionization of the intracellular buffers (Woledge, 1971). Therefore complex calorimetric studies are required to determine a  $\Delta H_{PC}$  which can be compared to results obtained from contracting muscle. Woledge (1972) has listed at least five different reactions each contributing to the total enthalpy change for PC splitting. Woledge's (1972) interim study estimates  $\Delta H_{PC}$  to be  $-8.1$  kcal/mol. This value should not be assumed to be a final determination since four factors remain to be fully quantitated. Nonetheless Woledge (1972) considers their contribution *extremely unlikely* to explain the difference between  $-8.1$  kcal/mol and  $-11$  kcal/mol. Our observed ratio of heat + work to PC split from *R. pipiens* sartorius muscles is  $-10.7$  kcal/mol and is similar to values reported by Carlson et al. (1963, 1967), Wilkie (1968), and Gilbert et al. (1971) (after the first 2 s of a contraction). The reason for this discrepancy may involve the presence of at least one additional reaction occurring in contracting muscle which is not included in the calorimetric measurements. Since the ratio of heat + work to PC split does not appear to depend on the

duration of stimulation and, hence the amount of PC split, it seems likely that if there is an additional reaction(s) it accompanies PC splitting. Since it has been shown previously (Gilbert et al., 1971) that recovery processes (glycolytic and oxidative) do not result in a detectable PC or ATP restitution in a 15-s tetanus of isolated frog muscle at 0°C, it is unlikely that recovery processes could have affected our results.

#### APPENDIX

If as Carlson et al. (1972) conclude, there is considerable motion of the contractile proteins during an isometric contraction, then it is possible that the intracellular fluid is being thereby stirred and agitated. Such a possibility may manifest itself as an increased rate of heat loss and rate of conduction of heat from the muscle to the thermopile in contracting muscles.

To determine whether the rate of heat loss is affected by contraction, the following experiment was conducted. A pair of sartorius muscles were mounted on a thermopile and the resting muscles warmed by passing a 100-KHz current through their entire length for 900 ms. As seen in Fig. 4 A, the temperature first increased by 0.482°C and, with the cessation of heating, declined exponentially with a time constant of 21.5 s. Next the muscles were tetanized for 10 s during which time the temperature increased by 0.019°C (Fig. 4 B). Finally, after the muscles were again warmed by passing the 100-KHz current for 900 ms and allowed to cool for 8 s, they were tetanized for 10 s

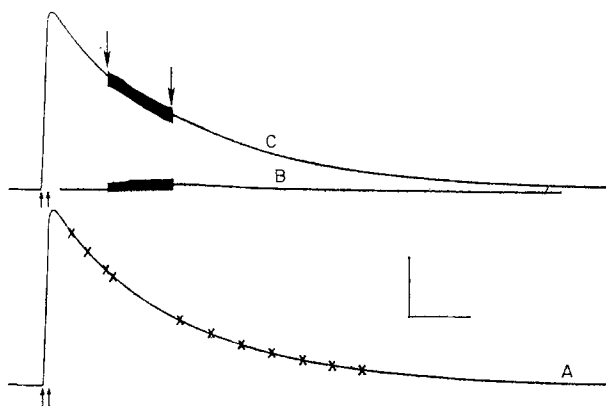


FIGURE 4. Muscle temperature during and after heating a pair of resting and contracting muscles with 100-KHz current. In curve A the temperature of a pair of resting sartorius muscles is monitored during and after 900 ms of heating (indicated by the upward pointing arrows). Curve B is the temperature change observed during and after a 10-s isometric tetanus (the thickened portion of the curve is due to stimulus artifacts and represents the duration of stimulation). Curve C results from an experiment like that generating curve A except that during the time interval indicated by the downward pointing arrows the muscles were tetanized for 10 s. The thickened portion of curve C is due to stimulus artifacts. The crosses on curve A represent the remainder when, at given points in time, the values of curve B are subtracted from curve C. The horizontal calibration bar represents 10 s and the vertical bar 0.163°C. (See text for further details.)

and the temperature recorded (Fig. 4 C). If the heat loss characteristics of the active muscle-thermopile system differed from those of the resting-muscle thermopile system subtraction of curve B from curve C would give a cooling curve that would fall either above or below curve A. The results of this subtraction, shown by the crosses in Fig. 4 A, show, however, that the two curves are superimposable. This fact indicates that there is little or no change in the heat loss coefficient between resting and contracting muscle.

If the presumed motion of the muscle proteins resulted in any substantial intracellular stirring, heat produced in the contracting muscle would be transferred to the thermopile more rapidly than in the resting muscle. The usually employed correction for conduction of heat from muscle to thermopile (Hill, 1965) would thus be overestimated and would necessitate modification of results previously obtained using this

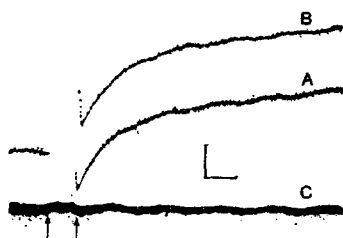


FIGURE 5. Thermopile output after heating of a resting (A) and tetanized (B) muscle pair. A pair of sartorius muscles was warmed by a 10-ms burst of 100-KHz current (see text) and the temperature change detected by the thermopile recorded on an oscilloscope. Heating of the muscles occurred during the time interval indicated by the arrows. Line C indicates the base line for the heating of the resting muscles (A) and the base line for the tetanized muscles is the horizontal line preceding the heating record of curve B. The horizontal calibration bar represents 10 ms and the vertical bar, 1.27  $m^{\circ}C$ .

correction. To test this possibility, a pair of resting sartorius muscles on a thermopile were artificially heated by a 10-ms burst of 100-KHz current. The current was passed between one electrode located on the outer surface of the muscle (near the center) and two electrodes on the inner surface (at the ends). The time-course of the rise in thermopile temperature was monitored on a storage oscilloscope (Fig. 5 A). Next the muscles were tetanized for 1 s at 10 Hz. Within 20 ms after the last stimulus, at a time before tension had begun to decline and when intracellular movement should be occurring, the muscles were again heated with a 10-ms burst of 100 KHz current and the temperature recorded (Fig. 5 B). Since the current intensity had been chosen to cause the active heat production by the muscles to be less than 6% of the artificial heating during the 100 ms following artificial heating, the temperature rise shown by curves A and B (Fig. 5) can be directly compared. It is evident that there is little or no difference in the time-course of the temperature recordings in either the resting or tetanized muscles. We conclude, therefore, that the thermal transfer properties of an active muscle are essentially identical to those of a passive muscle.

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