Total Energy Production and Phosphocreatine Hydrolysis in the Isotonic Twitch

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ABSTRACT Using frog's sartorius muscles we have found no correlation between phosphocreatine hydrolysis and shortening under conditions (iodoacetate poisoning and anoxia) where this reaction was the only expected source of energy. Phosphocreatine hydrolysis did, however, show a constant term corresponding to the activation heat of A.V. Hill, and a linear term with work. It was concluded that shortening heat comes from some other chemical reaction, or else Hill's equation ($E = A + W + ax$) fails to describe correctly the energy output in a complete cycle of contraction and relaxation. To decide between these possibilities direct measurements of heat and work during a complete cycle were made. Also, experiments were performed in which heat, work, and phosphocreatine breakdown were measured simultaneously on the same muscles. The total energy output in a complete twitch could be most simply represented by a fixed "activation" heat, plus the work. There was no term corresponding to the shortening heat. Hill's equation must, therefore, be held as invalid for the complete isotonic twitch. A value of 9.8 ± 0.5 (SE) kcal/mole was obtained for the *in vivo* heat of hydrolysis of phosphocreatine. This quantity showed no significant dependence on load, and it is in good agreement with the value obtained from thermochemical data. It is concluded that phosphocreatine hydrolysis and its associated buffer reactions can account quantitatively for the total energy output of isometric and isotonic twitches.

INTRODUCTION

Under conditions in which no energy can be drived from oxidative processes or from glycolysis, it appears from a growing body of evidence that the hydrolysis of phosphocreatine (PC) is the only net chemical reaction. If this is the case, the amount of PC hydrolysis should always be proportional to the total output of energy (heat + work).

This has been demonstrated to be so in a series of isometric twitches, where it is known (Hill, 1928; Hartree, 1931) that the total heat output is proportional to the total tension developed, *i.e.*, the sum of the individual peak twitch tensions. Lundsgård (1930) showed that in such series of twitches the PC breakdown is also proportional to the total tension developed. This result was confirmed by Carlson and Siger (1960). They also showed that when PC broke down, orthophosphate and creatine (C) appeared in equivalent amounts, and that there was no net hydrolysis of adenosine triphosphate (ATP). This supports the hypothesis that PC hydrolysis and the resulting buffer reactions are the only net reactions occurring.

However, in isotonic twitches, where mechanical work is performed, the equivalence between energy output and chemical change remains to be established. In this case it should be possible to calculate the total energy output from Hill's (1949 *c*) equation:

$$E = A + W + aX$$

where E = total energy liberated; A = activation heat, a constant amount probably independent of the length of the muscle (Hill, 1949 *a*, p. 210); w = work performed; X = shortening; a = a constant.

In a series of isotonic twitches where the work done is varied both by changing the load and by varying the number of twitches, Hill's equation predicts that the energy output should depend both on the work done and on the load (because the load determines the amount of shortening). However, Carlson and Siger's results (1957, 1958) strongly suggested that there was no correlation between PC splitting and shortening.

To secure this point, a further extensive series of experiments was conducted by one of the authors (F. D. C., 1958–1960) in which the number of twitches was kept fixed at twenty and the dependence of PC splitting on shortening, work, and load was examined. The results of this study, presented in brief by Carlson (1961) are given in detail later in this paper. They showed that the amount of PC split in an isotonic twitch was different from that split in an isometric twitch. The difference was proportional to the work. This meant that the PC split corresponded to the first two terms only in Hill's equation—there was no splitting of PC equivalent to the heat of shortening.

Mommaerts (1960) and Mommaerts and Seraydarian (1960) have reported finding in a tetanus definite amounts of PC splitting associated with activation, work, and shortening in agreement with Hill's equation. Recently, however, Mommaerts, Seraydarian, and Marechal (1962) have published the results of a statistical analysis of their earlier data. They found a significant correlation between PC splitting and work, but the correlation between PC splitting and shortening was inconclusive.

There is, it appears, no reported evidence in the literature which convincingly demonstrates a dependence of PC splitting on shortening. We thus have no reason to doubt our own results which show that there is *no* dependence of PC splitting on shortening, or if there is it is only a small fraction of what is to be expected from Hill's equation. It was concluded, therefore, that the shortening heat comes either from some other chemical reaction, independent of PC splitting, or else Hill's equation fails to describe correctly the energy output in a complete cycle of contraction and relaxation. To decide between these alternatives it has been necessary to make direct measurements of heat and work during a full cycle of contraction and relaxation under exactly the same conditions as those employed in the chemical studies. To consolidate the conclusions still further, some experiments have been performed in which heat, work, and PC breakdown were measured simultaneously on the same muscles.

Arrangements were made to do this at University College, London. Publication of the chemical studies mentioned above was delayed until they could be presented together with the results of the direct thermal measurements.

In what follows we present, in order, the detailed results of the 1958–1960 investigation of the dependence of PC splitting on load, work, and shortening; the determination of the total energy produced in a full cycle of contraction and relaxation using the myothermic techniques of Hill; and the estimation of the *in vivo* heat of hydrolysis of PC from combined chemical and thermal studies conducted on the same muscles.

Our results show that Hill's equation is not valid for the full cycle of contraction and relaxation in a twitch. The total energy production actually seems to consist of only two terms, a constant plus a term proportional to, or equal to, the work. Using this relationship, there is good agreement between total energy production and the observed PC splitting.

MATERIALS AND METHODS

(a) *Phosphocreatine Breakdown in Isotonic Twitches*

PREPARATION OF MUSCELS As in our previous studies (Carlson and Siger, 1960) the sartorius muscles from *R. pipiens* were used throughout. Paired muscles were carefully dissected, freed of extraneous tissue, and left attached to the pelvic bone. Parasitized or damaged muscles were rejected at this point. A platinum S hook was bound to the tibial tendon as close to the body of the muscle as possible. The muscles were stored overnight at 4–6°C in 80 ml of aerated Ringer's solution. Prior to use the muscles were again examined for damaged fibres. Usually the muscles showed no signs of damage but any that showed twenty or more injured fibres were rejected.

The Ringer's solution was the same as that used previously. Muscles were first treated with 0.5 mM iodoacetate (IAA) for 25 to 30 minutes at 20°C and then mounted

in the moist chamber where they were flushed for 20 minutes at 1–2°C with cold Ringer's solution that had been equilibrated with nitrogen.

The pelvic bone, which was uppermost, was clamped in an isometric lever and the S hook of one of the muscles was attached to an isotonic lever beneath. One stimulating electrode, the cathode, was placed 1.5 to 2.0 cm below the pelvic tendon, and the S hook served as the other electrode. The other muscle was pulled to one side and hung over an insulated lucite support so that there was no possibility of stimulation by leakage currents from the stimulating electrodes. Direct electrical stimulation consisting of a 2.0 msec. shock of twice maximal strength was delivered every 3 seconds. Each muscle took its turn in the recording position, the isometric series being made first.

EXPERIMENTAL PROCEDURE In these studies the control muscle was not a resting muscle but one that had performed twenty isometric twitches. This was to be compared with the experimental muscle which performed twenty isotonic twitches. This procedure of using an isometric control made the most efficient use of the paired muscles in determining the difference between the amount of PC split in an isotonic contraction and that split in an isometric contraction. Had a resting muscle been used as control an isometric series would have been necessary and the determination of the difference between the isotonic and isometric cases would have required a test for the significance of the difference between the means of two populations instead of a test for a significant difference from zero for the mean of a single population.

In addition to having each muscle perform twenty isotonic or isometric twitches, both muscles were made to perform three isometric twitches after the series. These three isometric twitches were compared to see whether both mucsles were capable of developing the same amount of tension after treatment with IAA and after roughly equivalent amounts of contractile activity. The difference in tension between the last of the test twitches was determined for each pair and expressed per micromole of total creatine. Those pairs in which this difference was greater than three times the standard deviation for all pairs, at the same load, were rejected. In this way, paired muscles which were widely different in their functional ability were eliminated. In all, eleven experiments were rejected out of 97.

Following its prescribed series of contractions each muscle was cut free of the lever and pelvic bone, dried rapidly on a cold cellulose tissue, and plunged into petroleum ether cooled with solid CO_2 to about -70°C. It was then weighed rapidly, extracted with perchloric acid, and analyzed for PC and C as previously described by Carlson and Siger (1960). The difference between the PC content of the isotonic and isometric members of a pair represents the increase or decrease in PC split in twenty isotonic twitches under the load used compared to that split in twenty isometric twitches. The three test isometric twitches given to both muscles were presumed to make no net contribution to this difference.

An initial load of 5 gm was used in all cases (except for the 2 gm load series where an initial load of 2 gm was used) to set the stop on the lever and thus define the initial length of the muscle. The load on the lever was increased (after loaded) to give the desired isotonic load, P. In *R. pipiens* of the size used here a 2 gm and a 5 gm initial load would have corresponded to initial lengths of $l_0 + 2$ mm and $l_0 + 4$ mm respec-

tively. For these muscles l_0 had a value between 37 and 40 mm. Tension and shortening were recorded on a Sanborn recorder. The load P was expressed as a fraction, P/P_{0t}, where P_{0t} is the peak twitch tension, obtained on the isometric member of the pair.

(b) *Energy Production in Isotonic Twitches*

Experimental conditions were made as similar as possible to those used in the chemical studies. *R. pipiens* was used, but the small number of animals available to us in London limited the number of experiments. Since the primary object of these experiments was to test the validity of Hill's equation for the full cycle of contraction and relaxation, there was no need in this particular set of experiments to poison the muscles with IAA and nitrogen. The muscles, after dissection and overnight storage as described above, were exposed to oxygenated Ringer's solution throughout the heat determination experiments.

The total heat measured after a series of twenty twitches which were applied at 3 second intervals under aerobic conditions contains a small contribution from the recovery heat of the earlier twitches in the series. Even if each twitch made an equal contribution to the rate of recovery heat production, the total amount of recovery heat would be only 4 per cent of the total initial heat. In fact, the recovery heat is almost certainly less than this, so we have made no correction for it in our results.

Since we were interested in measuring the total heat evolved during the minute occupied by the set of twenty twitches, it was not necessary to use the rapid recording technique developed by Hill (1937, 1938, 1949 *d*). In fact, the technique of integrating the heat production in a series of twitches over a period of a minute or so, gains other advantages at the expense of temporal resolution. The major advantage of this technique is that the thermal gradients within the muscle attenuate with time and as a result of the muscle's motion over the thermopile. Consequently, a more accurate estimate of the total heat is likely to be obtained.

The thermopile (D4, 36 junctions of chromel-constantan, 42.8 ohms, equivalent half thickness 45 μ, sensitivity 2077 μv/°C at 0°C for thirty-six junctions) was used with twenty-two junctions active and fourteen as a protection; the active region started 4 mm and ended 17 mm from the pelvic bone. Protecting junctions minimize troublesome temperature gradients which might otherwise develop along the length of the muscle and give rise to errors in the heat measurements when shortening occurs. The thermopile was connected directly to a Zernicke galvanometer (Zc, 2.6 second period, slightly underdamped, sensitivity 1.46×10^{-9} amp/mm at 2 m) and the deflection read every 5 seconds. It was not necessary to correct for the time lag due to heat conduction in the thermopile, or for the lag of the galvanometer response. All the readings were corrected for heat loss. Fortunately, thermopile D4 has an accurate exponential heat loss characteristic, so the simple procedure described by Hill (1939) could be followed. The pair of muscles was mounted, one on each side of the thermopile, with the pelvic bone clamped and the tibial tendons tied to the chain connecting to the isotonic lever. Each set of twitches was preceded by 15 second control heating with 100 kc/second current. From the decline of the resulting galvanometer deflection, the exponential decay constant for the loss of heat from the

muscle-thermopile system was determined. For each muscle pair this constant varied only slightly throughout the experiment, despite repeated filling and emptying of the thermopile chamber with Ringer's solution. It is important to determine this constant accurately, because even with a pair of muscles (decay constant 2 to 3 per cent/second) the calculated correction comprises about 60 per cent of the estimated heat production.

ABSOLUTE CALIBRATION Methods of calibration have been reviewed by Aubert (1956, p. 30), and recently Hill and Woledge (1962) have emphasized the difficulties in absolute calibration. They recommend that the thermopile should be calibrated to read temperature. The main uncertainty in measuring heat then arises from the determination of the thermal capacity of muscle and thermopile with which the temperature rise must be multiplied in order to obtain the total amount of heat produced. This uncertainty arises from the fact that the weight of the muscle itself is unsure due to variations in the amount of extracellular fluid weighed with it. We have, therefore, weighed the muscles just as they were removed from the thermopile, where they had been allowed to drain, with no blotting at all.

An experiment was done to determine whether the heat production during isotonic contraction and relaxation differed widely in the two halves (pelvic and tibial) of the muscle. This was done by connecting the two halves of the thermopile in series opposition so that the temperature difference between them was recorded. It was found that the temperature difference between the two halves was less than 2 per cent of the temperature rise in both muscles. Inequalities in strength along the length of the muscle which might have resulted in a non-uniform distribution of heat do not, therefore, present a major problem in experiments of this type.

STIMULATION The stimulating electrodes were located 3 mm beyond the ends of the thermopile. The muscles were mounted so that one stimulating electrode was about 1 mm from the pelvic bone. This put the other electrode 26 mm away from the pelvic bone, which in *R. pipiens* of the size used, would place it 10 to 15 mm from the tibial end, in the vicinity of the entrance of the nerve into the muscle. This electrode configuration is similar to that used by Hill (1949 *a*, *b*, *c*). The muscle was stimulated by supramaximal condenser discharges (time constant 0.4 msec.) once every 3 seconds. The shocks alternated in direction.

In some experiments a multielectrode array was used to determine what effect, if any, the configuration of the stimulating electrodes had on heat production. The details of the electrode geometry are, in these cases, given with the results.

MECHANICAL MEASUREMENTS Afterloaded isotonic contractions were performed against a lever coupled through a capacitance transducer to a Sanborn type 320 recorder. During relaxation the load fell and stretched the muscle. The kinetic energy of the system was always negligible, so no net work was performed by the muscle. The heat measured thus represents the total energy output in the complete cycle of contraction and relaxation. Nevertheless, we wanted to know how much of the total energy had once existed as work, and how much shortening had occurred. The external work was easily calculated from the record of shortening, but in order to calculate the internal work it was necessary to know the series compliance of the

muscle and the connection between the muscle and the transducer. The former was measured and calculated by the quick-release technique of Jewell and Wilkie (1958). For a single sartorius of *R. pipiens* (l_0 = 40 mm, weight = 130 mg) the compliance was approximately linear and equal to 6.4×10^{-4} cm/gm weight. For a pair of such muscles joined to a glass connection 49 cm long, the combined compliance was 7×10^{-4} cm/gm weight.

(c) *Measurement of Energy Production and PC Breakdown on the Same Muscles*

EXPERIMENTAL PROCEDURE In order to do this some modifications had to be made in the experimental myothermic technique. The muscles were poisoned with IAA, and since only one member of the pair was to be stimulated, the other serving as control, the pelvic clamp was modified so that one muscle was held out of contact with the stimulating electrodes and thermopile junctions, but at about the same length as its stimulated partner. The use of only one muscle on the thermopile has the drawback that the heat loss increases to about 4 per cent/second. In consequence the final value of the heat consists of relatively more "correction" and less "deflection."

After setting up the muscles on the thermopile they were left for 35 minutes in Ringer's solution bubbled with nitrogen, in order to achieve anaerobic conditions and attain thermal equilibrium with the thermostat. At the end of this period the Ringer's solution was allowed to run out in such a way that it was replaced by nitrogen that was also in equilibrium with Ringer's solution in the thermostat.

A heating control was then made in order to determine the heat loss coefficient, then the muscle was stimulated as required for the particular experiment. The galvanometer deflection was read every 5 seconds and the mechanical events were recorded. After the last stimulus, galvanometer readings were continued for about 30 seconds, so as to provide an estimate of the rate of "recovery" heat production. The thermostat and thermopile cover were quickly removed and the experimental muscle cut off close to the pelvic bone and frozen in solid CO_2–petroleum ether. About 30 seconds elapsed between the last heat measurement and the freezing of the muscle, and of this about 10 seconds was spent in air at room temperature. The heat produced during the 30 seconds was estimated from the previously determined recovery heat rate. The control muscle was frozen about 10 seconds after the experimental.

RESULTS

(a) *Phosphocreatine Splitting in the Isotonic Twitch*

Table I presents the data obtained on 86 pairs of muscles, from which the results presented in this section were derived. A statistical summary of these data is given in Tables II and III. The probability values given in these tables were obtained on the hypothesis that the observed difference in PC content between isometric and isotonic members of a pair was zero. Clearly there is a dependence of PC splitting on load. At low loads there is no significant difference between the amount of PC split in an isotonic twitch and that

TABLE I

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
				2 Gm Isotonic Load				
281 T	0.1564	*27.1*	1.27	55.6			4.36	0.628
281 M	0.1560	1487.5		56.2	64.9	0.031	4.06	0.596
282 T	0.0889	*13.8*	0.65	34.6			2.91	0.618
282 M	0.0901	847.5		34.6	41.1	0.049	2.81	0.612
283 T	0.0924	*19.8*	0.93	34.6			3.04	0.520
283 M	0.0920	849.7		34.6	38.9	0.051	3.08	0.578
284 T	0.1291	*21.1*	0.99	46.7			3.90	0.667
284 M	0.1367	1142.2		43.3	51.1	0.039	3.76	0.686
285 T	0.1129	*24.9*	1.17	35.6			3.60	0.533
285 M	0.1186	1037.8		42.2	48.9	0.041	3.94	0.574
286 T	0.1166	*19.4*	0.91	40.0			3.84	0.620
286 M	0.1167	891.1		37.8	42.2	0.047	3.41	0.633
287 T	0.1168	*18.1*	0.85	35.3			3.07	0.534
287 M	0.1280	1108.6		45.7	50.3	0.040	3.58	0.575
288 T	0.1886	*26.2*	1.23	62.2			5.33	0.604
288 M	0.1786	1538.2		62.2	71.1	0.028	4.72	0.576
289 T	0.1218	*14.3*	0.67	32.9			3.43	0.618
289 M	0.1139	988.9		40.0	46.7	0.043	4.08	0.603
290 T	0.1160	*19.6*	0.92	32.0			2.57	0.665
290 M	0.1200	875.4		36.6	38.9	0.051	2.93	0.717
291 T	0.1799	*22.2*	1.04	46.7			5.24	0.674
291 M	0.1680	1155.5		46.7	64.4	0.031	5.17	0.648
292 T	0.1716	*24.7*	1.16	60.5			5.22	0.623
292 M	0.1714	1301.6		52.0	60.5	0.033	5.06	0.603
293 T	0.1052	*20.7*	0.97	28.1			3.40	0.429
293 M	0.1004	854.0		34.6	41.1	0.049	3.56	0.497
294 T	0.1369	*23.5*	1.10	43.4			3.36	0.515
294 M	0.1316	1046.9		43.4	48.0	0.042	4.86	0.494

T = isotonic
M = isometric

TABLE I—*continued*

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
				2 Gm Isotonic Load—continued				
295 T	0.0937	*18.5*	0.87	25.2			2.96	0.564
295 M	0.1002	857.1		34.3	41.2	0.049	3.44	0.573
296 T	0.1242	*20.2*	0.95	40.0			4.14	0.553
296 M	0.1160	1037.7		42.2	46.7	0.43	4.27	0.562
297 T	0.1787	*20.4*	0.96	53.3			5.43	0.584
297 M	0.1658	1286.7		51.1	60.0	0.33	5.56	0.610
298 T	0.1217	*18.1*	0.85	37.8			4.18	0.596
298 M	0.1146	891.1		37.8	40.0	0.050	4.06	0.562
299 T	0.1242	*16.2*	0.76	32.0			3.83	0.650
299 M	0.1205	860.0		36.7	40.0	0.050	3.96	0.634
300 T	0.1103	*20.4*	0.96	34.3			3.49	0.610
300 M	0.0938	745.5		30.9	32.8	0.060	3.05	0.639
301 T	0.897	*22.4*	1.05	30.9			2.66	0.560
301 M	0.0993	748.6		30.5	34.3	0.058	2.72	0.621
302 T	0.0936	*17.7*	0.83	27.3			2.83	0.555
302 M	0.816	739.1		30.5	34.3	0.058	2.65	0.540
303 T	0.0754	*17.7*	0.83	25.5			2.45	0.584
303 M	0.861	836.2		34.3	38.1	0.052	2.69	0.617
				5 Gm Isotonic Load				
258 T	0.1678	*19.5*	2.29	46.7			4.39	0.581
258 M	0.1573	1150.9		46.7	51.1	0.098	4.35	0.669
260 T	0.1218	*16.0*	1.88	—			3.43	0.682
260 M	0.1287	876.4		35.6	40.0	0.125	3.23	0.724
261 T	0.1028	*15.0*	1.76	44.0			2.96	0.557
261 M	0.1107	1150.0		47.5	52.5	0.095	3.23	0.632
262 T	0.1177	*20.1*	2.36	37.1			3.49	0.473
262 M	0.1124	943.3		40.0	43.3	0.115	3.51	0.610
263 T	0.1020	*17.0*	1.99	40.0			3.60	0.514
263 M	0.0971	874.7		36.5	40.0	0.125	3.05	0.574

TABLE I—*continued*

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
				5 Gm Isotonic Load—continued				
264 T	0.1264	*16.2*	1.90	38.3			3.61	0.557
264 M	0.1206	1083.4		45.2	48.7	0.103	3.47	0.674
265 T	0.1120	*18.8*	2.21	41.2			2.71	0.568
265 M	0.1166	958.1		40.0	45.5	0.110	3.17	0.688
266 T	0.1321	*22.2*	2.60	41.8			3.88	0.534
266 M	0.1359	1030.8		41.8	47.3	0.106	3.89	0.645
267 T	0.1040	*16.8*	1.97	34.5			3.26	0.583
267 M	0.1015	1096.3		43.6	50.9	0.098	3.31	0.616
268 T	0.1487	*21.1*	2.48	43.6			4.58	0.552
268 M	0.1518	1061.7		43.6	49.1	0.102	4.18	0.608
269 T	0.1833	*21.0*	2.46	63.1			5.44	0.619
269 M	0.1789	1421.7		58.2	65.5	0.076	4.95	0.648
				10 Gm Isotonic Load				
319 T	0.1116	*9.9*	2.32	—			4.13	0.496
319 M	0.1016	571.4		—	29.7	0.337	3.91	0.563
320 T	0.1050	*10.4*	2.43	—			3.49	0.511
320 M	0.0996	674.3		—	34.3	0.292	3.45	0.611
321 T	0.1194	*7.8*	1.83	—			4.52	0.572
321 M	0.0900	682.3		—	35.3	0.283	3.32	0.622
322 T	0.0752	*2.5*	0.58	14.7			1.94	0.691
322 M	0.0778	381.0		14.7	19.0	0.526	1.65	0.718
323 T	0.1335	*15.0*	3.53	37.9			3.92	0.535
323 M	0.1239	1031.6		42.1	46.3	0.216	3.39	0.676
324 T	0.1357	*15.3*	3.59	58.4			4.67	0.439
324 M	0.1466	1128.4		46.3	50.5	0.198	4.98	0.556
325 T	0.1328	*12.2*	2.85	33.7			3.93	0.517
325 M	0.1419	1213.6		49.2	55.4	0.181	4.77	0.579
326 T	0.1202	*7.2*	1.68	26.7			3.65	0.410
326 M	0.0838	504.4		20.0	22.2	0.450	2.99	0.492

TABLE I—*continued*

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
			10 Gm Isotonic Load—continued					
331 T	0.1458	*10.7*	2.51	27.5			3.99	0.266
331 M	0.1303	1217.3		24.7	28.3	0.177	3.75	0.525
332 T	0.1024	*10.9*	2.57	30.6			3.16	0.481
332 M	0.1061	960.0		40.0	42.7	0.234	3.06	0.603
333 T	0.1027	*9.2*	2.15	22.9			3.50	0.488
333 M	0.0972	646.9		25.1	29.7	0.337	3.30	0.518
334 T	0.1203	*11.5*	2.69	35.3			3.94	0.544
334 M	0.1282	1157.6		51.8	54.1	0.185	4.10	0.612
338 T	0.0926	*7.6*	1.77	23.3			3.14	0.605
338 M	0.0875	629.2		26.0	28.1	0.356	3.08	0.679
344 T	0.0798	*5.0*	1.16	22.9			2.99	0.488
344 M	0.0771	526.7		22.9	23.0	0.435	3.54	0.605
368 T	0.1300	*10.6*	2.49	37.9			4.20	0.640
368 M	0.1346	1117.9		46.3	50.5	0.198	4.20	0.726
369 T	0.1099	*10.1*	2.38	33.3			3.71	0.615
369 M	0.1152	1026.7		42.2	46.7	0.214	3.95	0.719
370 T	0.1382	*10.8*	2.54	37.9			4.01	0.663
370 M	0.1288	1004.2		40.0	46.4	0.216	3.38	0.728
371 T	0.1344	*12.4*	2.90	36.8			3.67	0.738
371 M	0.1380	1141.6		45.4	51.9	0.193	4.08	0.724
372 T	0.1230	*11.6*	2.72	34.3			4.46	0.594
372 M	0.1253	950.9		38.9	43.4	0.230	4.58	0.666
374 T	0.1183	*11.6*	2.71	—			4.39	0.562
374 M	0.1280	902.2		37.9	42.2	0.237	4.29	0.667
382 T	0.0988	*11.8*	2.78	30.6			3.26	0.525
382 M	0.0940	823.5		35.3	37.7	0.265	3.20	0.622
387 T	0.1155	*7.8*	1.82	25.9			3.03	0.541
387 M	0.1126	847.0		35.3	27.4	0.365	3.60	0.580

TABLE I—*continued*

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
				15 Gm Isotonic Load				
339 T	0.0925	*4.4*	1.55	28.9			3.13	0.514
339 M	0.0869	662.2		22.2	31.1	0.482	3.26	0.549
342 T	0.0978	*5.8*	2.02	28.0			3.55	0.507
342 M	0.1017	684.0		24.6	30.0	0.500	3.60	0.528
343 T	0.1076	*4.5*	1.57	24.0			3.07	0.609
343 M	0.0883	572.0		26.0	26.0	0.576	2.94	0.629
				20 Gm Isotonic Load				
336 T	0.1099	*5.6*	2.64	37.9			4.16	0.550
336 M	0.1199	951.6		37.9	44.2	0.452	3.97	0.577
337 T	0.0983	*3.8*	1.76	28.9			3.70	0.581
337 M	0.1023	689.9		29.5	31.6	0.633	3.63	0.595
340 T	0.1140	*5.5*	2.57	31.1			3.57	0.515
340 M	0.1158	782.2		31.1	35.6	0.562	3.56	0.593
345 T	0.1126	*2.7*	1.29	26.0			4.02	0.669
345 M	0.1148	768.4		31.6	35.8	0.558	4.01	0.681
349 T	0.1558	*6.3*	2.97	39.0			5.82	0.576
349 M	0.1700	1231.5		46.3	52.6	0.380	5.15	0.613
355 T	0.1436	*5.2*	2.46	35.8			4.07	0.575
355 M	0.1334	880.0		33.7	42.1	0.475	4.26	0.701
359 T	0.1410	*5.2*	2.43	40.0			4.62	0.616
359 M	0.1400	987.3		42.1	44.2	0.452	4.77	0.664
360 T	0.1358	*6.3*	2.95	37.9			4.43	0.589
360 M	0.1351	1010.5		42.1	48.4	0.414	4.37	0.650
365 T	0.1380	*4.6*	2.14	37.9			4.57	0.711
365 M	0.1273	1101.0		44.2	50.5	0.407	4.00	0.755
366 T	0.1292	*4.5*	2.13	41.1			4.29	0.683
366 M	0.1241	1037.8		43.2	47.6	0.420	4.05	0.716
367 T	0.1232	*4.7*	2.21	38.9			3.74	0.639
367 M	0.1168	998.9		41.1	45.4	0.440	3.78	0.735
377 T	0.1101	*4.8*	2.28	30.6			4.28	0.458
377 M	0.1296	828.2		32.9	37.6	0.531	4.50	0.544

TABLE I—*concluded*

Experiment	Blotted weight	*Total shortening, cm* or Tension, gm	Total work	Tension in last twitch	Peak tension P_{0t}	Fractional load P/P_{0t}	Total creatine C_T	PC/C_T
	gm		*mcal*	*gm*	*gm*		*μmoles*	*mole/mole*
			20 Gm Isotonic Load—continued					
379 T	0.1054	*6.5*	3.07	32.9			3.94	0.637
379 M	0.1084	774.1		30.6	35.3	0.566	3.75	0.693
381 T	0.1108	*4.3*	2.00	28.2			3.61	0.415
381 M	0.1081	816.5		32.9	37.6	0.531	3.30	0.567
383 T	0.1524	*9.2*	4.32	40.0			4.68	0.545
383 M	0.1522	1134.1		47.7	51.8	0.386	4.44	0.655
385 T	0.0921	*4.0*	1.90	32.0			2.91	0.632
385 M	0.0956	781.2		32.9	35.3	0.566	3.05	0.705
			30 Gm Isotonic Load					
347 T	0.1914	*1.5*	1.03	38.8			6.88	0.660
347 M	0.2080	1403.6		55.8	65.5	0.458	7.76	0.651
353 T	0.1570	*3.1*	2.20	42.0			4.89	0.673
353 M	0.1534	1200.0		50.0	54.0	0.555	4.88	0.672
354 T	0.1480	*3.1*	2.20	46.3			4.61	0.665
354 M	0.1376	1072.0		44.0	50.0	0.600	4.61	0.642
356 T	0.1783	*3.7*	2.57	50.5			6.14	0.707
356 M	0.1725	1311.5		54.7	59.0	0.508	5.36	0.683
357 T	0.1684	*2.8*	1.98	50.5			5.59	0.639
357 M	0.1818	1204.2		50.5	54.7	0.548	5.98	0.689
358 T	0.1323	*2.6*	1.80	42.1			3.87	0.656
358 M	0.1456	1149.4		46.3	52.6	0.570	4.30	0.695
361 T	0.1672	*3.6*	2.55	—			5.16	0.692
361 M	0.1718	1301.0		52.6	61.1	0.491	5.19	0.694
373 T	0.1575	*3.9*	2.74	47.1			5.38	0.650
373 M	0.1563	1315.3		54.1	61.2	0.490	5.40	0.683
375 T	0.1774	*4.4*	3.07	48.9			6.40	0.530
375 M	0.1622	1122.2		44.4	51.1	0.586	5.77	0.572
376 T	0.1979	*6.9*	4.83	40.0			6.71	0.608
376 M	0.1898	1553.3		53.3	64.4	0.465	5.13	0.641
378 T	0.1855	*4.8*	3.37	44.4			4.94	0.567
378 M	0.1872	1102.2		46.7	51.1	0.586	5.15	0.615

TABLE II
STATISTICAL SUMMARY OF RESULTS

Isotonic load	Fractional load P/P_{0t}	Total creatine	PC/C_t after 23 isometric twitches	$\Delta(PC/C_t)$ (isometric-isotonic) equivalent to 20 twitches	Work in 20 isotonic twitches mcal/ μmole total creatine
gm wt.		*μmole/gm*			
2.0	0.045	30.8	0.60	−0.009	0.27
	SE 0.006	SE 0.5	SE 0.03	SE 0.007	SE 0.01
	$N = 23$	$N = 46$	$N = 23$	$N = 23$	$N = 23$
				$P = 0.11$	
5.0	0.105	28.8	0.64	−0.080	0.59
	SE 0.004	SE 0.5	SE 0.05	SE 0.01	SE 0.03
	$N = 11$	$N = 22$	$N = 11$	$N = 11$	$N = 11$
				$P = 0.005$	
10.0	0.28	32.6	0.63	−0.090	0.63
	SE 0.02	SE 0.7	SE 0.02	SE 0.01	SE 0.03
	$N = 22$	$N = 44$	$N = 22$	$N = 22$	$N = 22$
				$P = 0.0005$	
20.0	0.48	33.0	0.65	−0.061	0.61
	SE 0.02	SE 0.5	SE 0.02	SE 0.008	SE 0.04
	$N = 16$	$N = 32$	$N = 16$	$N = 16$	$N = 16$
				$P = 0.005$	
15.0	0.52	34.2	0.57	−0.025	0.49
	SE 0.04	SE 1.3	SE 0.03	SE 0.015	SE 0.04
	$N = 3$	$N = 6$	$N = 3$	$N = 3$	$N = 3$
				$P = 0.11$	
30.0	0.53	32.2	0.66	−0.020	0.45
	SE 0.02	SE 0.6	SE 0.01	SE 0.03	SE 0.05
	$N = 11$	$N = 22$	$N = 11$	$N = 11$	$N = 11$
				$P = 0.27$	

N = number of observations.

split in an isometric twitch. At moderate loads there is definitely more PC split in the isotonic twitch, but as the load increases further the amount of PC split falls and approaches that split in the isometric case.

DEPENDENCE ON WORK AND SHORTENING A multiple regression analysis was performed on the data to determine whether or not the observed differences in PC content, ΔPC, could be described by a relationship with the same form as Hill's equation, that is:

$$\Delta(PC/C_t) = k_0 + k_1 w + k_2 x \qquad (1)$$

where Δ (PC/C_t) is the observed difference expressed as a fraction of the total creatine C_t to eliminate differences due to size; k_0 is a constant; k_1 the

coefficient of regression of the change in PC content on work; k_2 the coefficient of regression of the change in PC content on shortening; w the work per micromole of total creatine content; and x the shortening per micromole total creatine content. Since we are examining the difference in PC content between an isometric and an isotonic twitch, it might be expected that the constant, k_0, would be zero. This would be so only if there were no work done in an isometric twitch, which is not the case. It is to be expected, therefore, that k_0 will be different from zero and have a magnitude given by $-k_1$ times the amount of work done internally in an isometric twitch.

TABLE III

Fractional load P/P_{0t}	PC split per twitch $(\Delta PC)_1$	External work per twitch $(w_e)_1$	Internal work per twitch $(w_i)_1$
	μmole/gm	*mcal/gm*	*mcal/gm*
0.045	0.30±0.02	0.41±0.02	0.00
0.105	0.40±0.02	0.96±0.06	0.00
0.28	0.43±0.02	1.04±0.08	0.01
0.48	0.39±0.02	1.03±0.08	0.05
0.52	0.33±0.03	0.84±0.08	0.03
0.53	0.31±0.04	0.72±0.09	0.12
1.00	0.29±0.01	0	0.31

The following relationship was obtained for the difference in PC content between twenty isotonic twitches and twenty isometric twitches:

$$\Delta(PC/C_t) = 0.050 - 0.177w - 0.0030x \qquad (2)$$

The regression coefficient, $k_0 = 0.050$, has a standard error of $\pm$ 0.0043 and a $P < 0.0005$, making it significantly different from zero. The regression coefficient, $k_1 = -0.177$, has a standard error of $\pm$ 0.024 and a $P < 0.0005$, making it highly significant. The regression coefficient $k_2 = 0.0030$ has a standard error of $\pm$ 0.0022 and a $P > 0.05$, making it *not* significantly different from zero.

Using the value of 0.31 mcal per gm of muscle for the internal work done in a single isometric twitch (see Table III), and the mean total creatine content, 31.7 μmole/gm, the total internal work done in twenty isometric twitches is estimated to be 0.20 mcal/μmole. On multiplication by $-k_1 = +0.177$ this gives a value of 0.035 for k_0 which compares favorably with the value of 0.050 obtained from the multiple regression analysis.

The fact that k_2 is not significantly different from zero prompts the conclusion that there is no correlation between PC splitting and shortening. Such a conclusion is not justified solely on the basis of a lack of statistical significance for it could be the result of excessive scatter in the data and not a lack of

correlation with shortening. k_2 is not only not significantly different from zero, but so small that even for the lightest loads, where the shortening is greatest and the work least, it makes a negligible contribution to the change in $\Delta(PC/C_t)$. This justifies the conclusion that there is no dependence of PC splitting on shortening in the isotonic twitch. For example, in the 2.0 gm load series the average shortening was 5.7 cm/μmole and the average work was 0.27 mcal/μmole. Using k_2 and k_1 given above these values give -0.017 for the change in PC/C_t due to shortening, and -0.048 for the change due to work. The change in PC/C_t corresponding to the activation term for twenty twitches can be obtained from the figure of -0.009 obtained by Carlson and Siger (1960) for the change in PC/C_t in one isometric twitch, subtracting the k_0 term given above. The answer is -0.130. The total change in PC/C_t, 0.195, is the sum of the three terms. Clearly the shortening term contributes less than 10 per cent of the total change in PC content under the very conditions where it would be expected to make its maximum contribution. We conclude therefore that the dependence of PC splitting on shortening in the isotonic twitch is less than one-sixth the value expected from Hill's (1949 *a*, *c*, 1953 *a*) results, and may therefore be considered as essentially non-existent. There is, however, a constant term corresponding to the activation heat, and a highly significant dependence on work. Even the internal work performed in an isometric twitch is accompanied by PC splitting in approximately the amounts to be expected.

The above results, together with the value obtained by Carlson and Siger (1960) for the amount of PC split in an isometric twitch can be combined to show how the total PC splitting depends on work in one twitch. This has been done after omitting from the regression analysis the k_2 term which has been shown to be small and not significant, and redoing the analysis to obtain slightly revised values for k_0 and k_1. The term k_0 for one twitch was obtained by dividing by twenty the term obtained for a series of twenty twitches. k_1 requires no such reduction. The units were then transformed to micromoles per gram by multiplying by 31.7 μmoles/gm, the mean value of the total creatine content of all the muscles used in this study. The change in PC content and the work are now expressed in micromoles per gram and millicalories per gram respectively. To obtain an expression for the total change in PC content in an isotonic twitch, the value of -0.286 μmole/gm obtained by Carlson and Siger (1960) for the PC split in an isometric twitch was added to the expression for the difference in PC content. The result obtained is:

$$(\Delta PC)_1 = -0.235 - 0.166w \qquad (3)$$

Where $(\Delta PC)_1$ is the amount of PC split in a twitch in micromoles per gram; and w is the work done in a twitch in millicalories per gram. The standard

errors of the coefficients -0.235 and -0.166 are $\pm$ 0.013 and $\pm$ 0.023 respectively. Both coefficients are highly significant with $P < 0.0005$. The solid line in Fig. 1 is a plot of the regression line given by equation (3) above. The data given in Table I, reduced to single twitches and expressed in appropriate units, are also shown in Fig. 1.

An incidental result of these experiments is the confirmation of the value of 0.286 μmole/gm previously found by Carlson and Siger (1960) for the PC

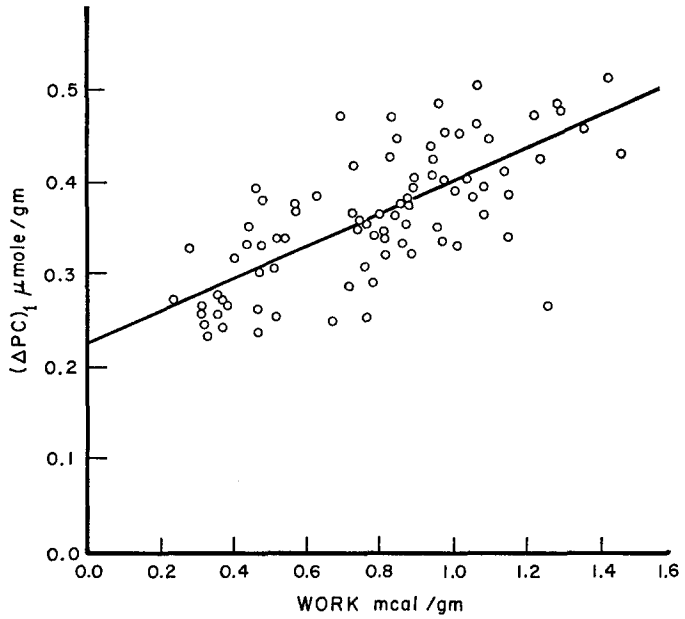

FIGURE 1. Dependence of PC splitting on work done in an isotonic twitch. Ordinate, change in PC in a twitch in micromoles per gram. Abscissae, work done in a twitch in millicalories per gram. Solid line, plot of the regression equation $(\Delta\ PC)_1 = -0.235 - 0.166w$. Open circles, experimental data given in Table I reduced to single twitches.

split in a single isometric twitch. Had the muscles used in the present study contained the same amount of PC in the resting state and split the same amount in a single isometric twitch then the isometric controls, after twenty-three twitches, should on the average have had a value of 0.64 for (PC/C_t). The values actually obtained and given in Table II range from 0.57 to 0.66 and are not significantly different from the expected value.

(b) *Energy Production in Isotonic Twitches*

Table IV contains all the basic data obtained on the five pairs of muscles (*R. pipiens*) used in this study. In this and in the combined heat and chemical study reported below we were restricted by the small number of frogs avail-

able to us; the first set of twenty twitches was always under isometric conditions, so that the peak twitch tension, P_{0t}, could be determined and the appropriate loads calculated, so that the *fractional* loads, P/P_{0t} would be

TABLE IV

HEAT, WORK AND SHORTENING IN ISOTONIC TWITCHES

Tabulated values are for twenty twitches, expressed per gram blotted weight. Wet weight appoximately 1.15 times as great.

Blotted weight of pair	P/P$_{0t}$	H/weight	W_e/weight	X/weight	W_i/weight
gm		*mcal/gm*	*mcal/gm*	*cm/gm*	*mcal/gm*
0.203	0.054	63.7	14.3	114.1	0.01
0.203	0.095	73.2	16.9	102.9	0.04
0.203	0.270	88.6	30.0	64.0	0.30
0.203	0.460	88.6	30.2	37.9	0.88
0.203	0.595	82.4	24.3	23.6	1.48
0.203	1.000	67.0	—	—	4.18
0.296	0.045	75.5	10.8	92.4	0.01
0.296	0.091	90.6	20.0	85.1	0.05
0.296	0.273	113.1	32.7	46.5	0.47
0.296	0.455	115.9	33.6	28.6	1.31
0.296	0.636	97.1	24.7	15.0	2.57
0.296	1.000	86.0	—	—	6.35
0.197	0.045	73.4	12.1	128.8	0.01
0.197	0.091	78.9	20.5	109.2	0.05
0.197	0.271	112.0	41.4	73.6	0.45
0.197	0.452	102.5	36.1	38.5	1.26
0.197	0.622	86.6	27.0	20.9	2.38
0.197	1.000	73.4	—	—	6.16
0.239	0.047	57.7	11.1	111.7	0.01
0.239	0.094	69.0	20.3	101.8	0.05
0.239	0.275	85.4	37.2	63.5	0.41
0.239	0.472	85.5	35.5	36.0	1.20
0.239	0.650	73.8	31.6	22.8	2.27
0.239	1.000	54.2	—	—	5.37
0.186	0.052	57.7	11.0	141.4	0.01
0.186	0.104	68.1	20.2	130.0	0.04
0.186	0.314	79.4	30.5	65.0	0.33
0.186	0.520	80.8	29.2	37.7	0.91
0.186	0.723	57.6	9.5	8.8	1.77
0.186	1.000	54.2	—	—	3.38

virtually the same for each of the five muscles, namely 0.05, 0.1, 0.25, 0.45, 0.65. The five loads were presented to the five muscles in a Latin square array to eliminate any possible effect due to the order of loading.

Fig. 2 shows the combined results from all five muscles. The mean heat

output per twitch (solid circles), and the work, internal and external (open circles), are shown as a function of the load. Subtraction of the total work from the total heat yields the points indicated by crosses. A straight line can be passed through these points at 2.95 mcal/gm to within the accuracy that

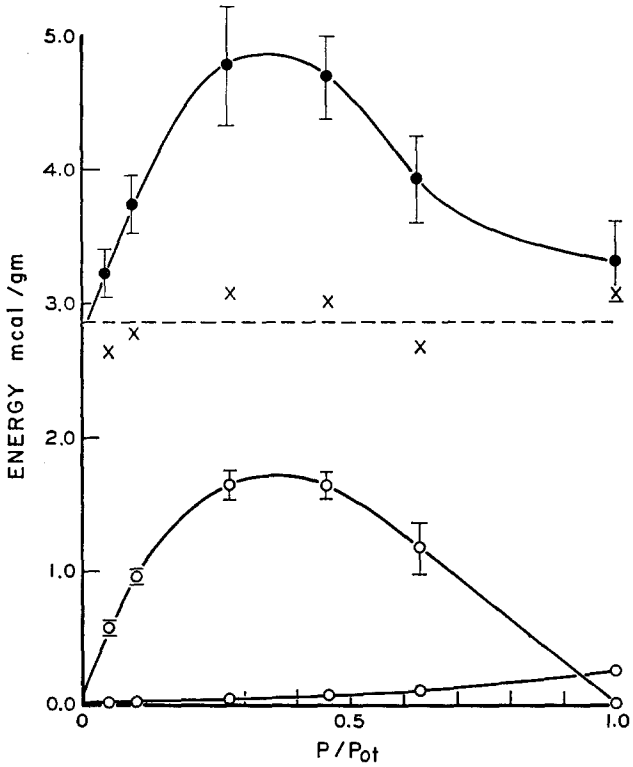

FIGURE 2. The variation of energy output with load in afterloaded isotonic twitches; the load was allowed to fall during relaxation. Abscissae, load P as a fraction of the peak isometric twitch tension P_{0t}. Ordinates, energy output in millicalories per gram and twitch (mean of 100 twitches, twenty by each of five muscles; ± 1 standard error plotted as a vertical bar). Upper curve, solid circles, total heat. Lower curves, open circles, external and internal work. Crosses, total heat minus total work. They lie near to the horizontal interrupted line drawn at 2.95 mcal/gm. Paired sartorii of *R. pipiens*, 0°C, L_0 37 to 40 mm, all contractions started at $L_0 + 2$ mm.

the data allow. It appears from this graphical analysis that the energy output can be most simply represented by a fixed activation heat plus the work, *viz.*

$$E = A + (\text{total work}) \tag{4}$$

The same result can be obtained by a somewhat different analysis. In Fig. 3 heat is plotted against work. The open circles are the experimental values from which the variation between muscles has been removed as follows: the

curves from individual muscles were plotted as in Fig. 2; clearly, they could be more nearly superimposed by shifting them vertically. This was done in such a way that the mean heat produced by each individual muscle became equal to the mean heat for all muscles.

The solid line is a plot of the regression equation derived from these points

$$h = 2.95 + 0.971w \tag{5}$$

Heat, h, and work, w, are expressed per gram of muscle. Both coefficients are highly significant, with standard errors $\pm$ 0.07 and $\pm$ 0.081 respectively. By

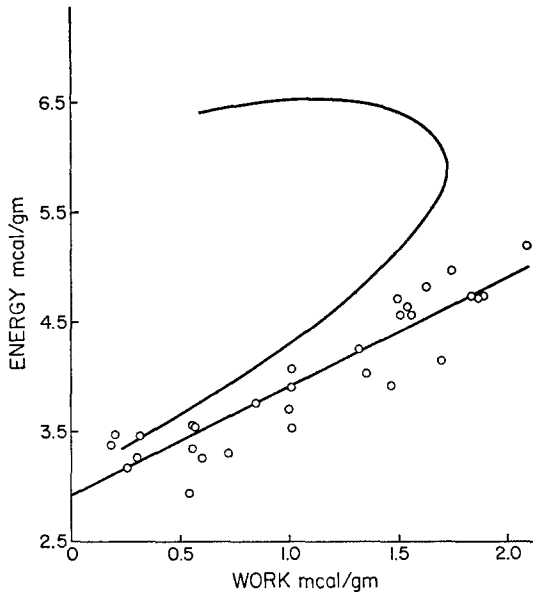

FIGURE 3. Ordinates, total heat, millicalories per gram and twitch. Abscissae, total work, millicalories per gram and twitch.

the "run" test (Crow, Davis, and Maxfield, 1960) the hypothesis of linearity could not be rejected. This is another way of demonstrating that the total energy consists of two terms only, an activation heat of about 2.95 mcal/gm, and the work. There is no term corresponding to the shortening heat of Hill (1949 *a*, *c*). Had such a term been present the pattern of heat production would have been, as shown by the upper curve in Fig. 3, very much different from what was observed.

In Fig. 4 a direct comparison is made of Hill's equation (upper curve), the simpler equation (4) without any shortening term (lower curve), and the experimental points. Clearly the points fit equation (4) and do not fit Hill's equation. The conclusion that the total energy consists of two terms only, a

constant activation heat and the work, must be regarded as a first approximation. The five isometric points in Fig. 3 are segregated above, or to the left of, the regression line. This could indicate the existence of a systematic error

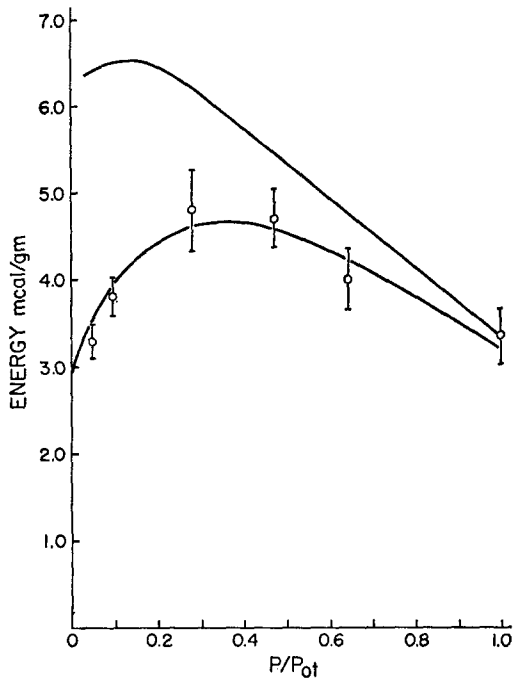

FIGURE 4. Ordinates and abscissae as in Fig. 2. Upper curve, Total energy as predicted from Hill's equation:

$$E = A + W + ax$$

A assumed to be 2.95 mcal/gm; a assumed to be 2.22 mcal/cm in 1 gm muscle, corresponding to 380 gm cm/gm as given by Hill (1949 *a*); shortening and work are experimental values from Fig. 2. Lower curve, total energy predicted from:

$$E = A + W$$

Points, experimental values, as in Fig. 2.

either from an underestimation of the internal work or from the fact that the isometric determinations were always made before the isotonic ones. If these points should, in fact, lie further to the right, it would follow that the slope of the regression line should be greater than one. Actually a slope of 1.14 is obtained if these points are omitted. Further work is needed to resolve this point. However, this uncertainty does not alter the fact that no term corresponding to a shortening heat can be found in our results.

HEAT PRODUCTION WITH MULTIPOINT STIMULATION When the muscle is stimulated by electrodes near the ends of the thermopile, as in most of our experiments, a wave of excitation passes over it (see Fischer, 1926). This might be associated with a certain amount of internal shortening and of internal work that was not accounted for in our calculation from the series elasticity and the tension. In order to assess whether or not this leads to an appreciable error, we performed an experiment in which the conduction time within the muscle was substantially reduced. For this purpose the thermopile was equipped with four additional stimulating electrodes, two on each side, which rested lightly on the surfaces of the muscles that were not in

TABLE V

THE EFFECT OF ELECTRODE CONFIGURATION ON ENERGY OUTPUT

Pair of sartorii from *R. temporaria*, wet weight 150 mg; 0°C; all contractions started at L_0 = 31 mm. End to end (E) or multipoint (M) stimulation, for details see text.

		All these values are the mean per contraction	
		tension, gm/wt.	*heat, mcal*
10 isometric twitches	M	56.8	0.393
10 isometric twitches	E	49.0	0.328
10 isometric twitches	M	47.6	0.322
10 isometric twitches	E	48.5	0.344
3 isometric 1 sec. tetani	E	61.9	0.91
3 isometric 1 sec. tetani	M	58.8	0.90
3 isometric 1 sec. tetani	E	55.7	0.86
		External work, mcal	
10 isotonic twitches, load 2 gm wt.	M	0.0291	0.329
10 isotonic twitches, load 2 gm wt.	E	0.0293	0.320
10 isotonic twitches, load 2 gm wt.	M	0.0295	0.329
10 isotonic twitches, load 2 gm wt.	E	0.0291	0.333

contact with the thermopile. They were spaced 7 and 16 mm from the pelvic electrode and adjusted under a microscope so that they just made contact with the muscle. Electrical continuity was confirmed after the thermopile had been set up by stimulating through each wire in turn. The experiment consisted in stimulating the muscle either from end to end (E in Table V) or with the four electrodes alternating in polarity (M).

The result, as shown in Table V, is that there is no consistent difference between the two modes of stimulation in isometric twitches, isometric tetani, or isotonic twitches under a small load. In the first set of contractions the heat was greater than in the others, but so was the total tension; after this the muscle behaved very consistently for the rest of the experiment. We conclude

that the internal work resulting from the wave must be small. Incidentally, this table confirms on *R. temporaria* that the energy output is roughly the same in an isometric twitch and in an isotonic twitch under a small load.

(c) *Combined Chemical and Thermal Studies*

A comparison between the chemical studies and heat studies described above in sections a and b is presented in Fig. 5 where it was assumed that the heat of hydrolysis of PC obtained in the latest work (9.83 kcal/mole, see Table VI), also applied to the muscles used in the earlier chemical studies. The open circles are the heats obtained on this assumption. The solid circles are the

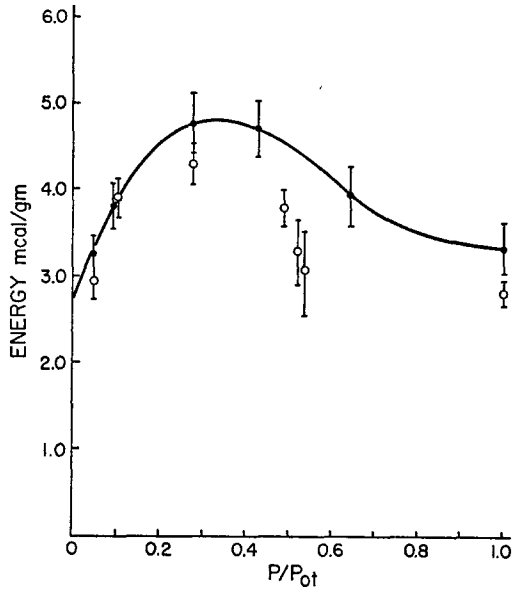

FIGURE 5. Dependence on load of total measured heat, solid circles; and heat calculated from PC splitting, open circles, using a heat of hydrolysis of 9.83 kcal/mole.

actual values of the heat produced, obtained in 1961–1962 on *R. pipiens* in London. The agreement between the two curves is good, and suggests that PC splitting is the energy-yielding reaction for contraction, and that the *in vivo* heat of hydrolysis of PC is about 10 kcal./mole.

The three chemical points around $P/P_{0t} = 0.5$ fall below the heat curve. This may be due to the fact that they were obtained on IAA-poisoned muscles whereas the heat data were obtained on normal muscles. The poisoned muscles, when heavily loaded, produce relatively less work towards the end of a series of twitches. Thus the effective value of P/P_{0t} may actbally be greater than the initial value which is plotted in the graph.

To further secure this point we conducted determinations as the available

supply of *R. pipens* would allow, on the *in vivo* heat of PC hydrolysis, for different isotonic loads. The initial heat production and PC splitting were measured on the same poisoned muscle. The relatively small amount of anaerobic recovery heat was not included because we have evidence that it does not come from PC splitting. The results of these experiments are given in Table VI.

It is evident that the ratio between energy output and PC splitting remains constant despite wide variations in both the work and the shortening. We conclude, therefore, that PC splitting and the consequent buffer reactions can account quantitatively for the energy output in isometric and isotonic twitches.

TABLE VI

"IN VIVO" HEAT OF HYDROLYSIS OF PHOSPHOCREATINE

Phosphocreatine splitting and energy production measured on the same muscles. *R. pipiens*, 0°C, poisoned with IAA and nitrogen.

Type of contraction	No. of experiments	ΔH
		kcal/mole
30 isotonic twitches, 2 gm initial load; 0 afterload; large shortening, small work	4	9.16±0.30
25 isotonic twitches, 2 gm initial load; 15 to 35 gm afterload; moderate shortening, large work	7	10.43±0.98
30 isometric twitches; small shortening, small work	6	9.58±0.67
Combined results	17	9.83±0.47

(d) *A Summary of Chemical Results and Heat Results*

In their essential features the chemical and heat production studies described above are in good qualitative and quantitative agreement with one another. Both show a linear dependence of total energy, or total PC hydrolysis, on work with no significant dependence on shortening. Both show a constant term corresponding to the activation heat, and both show the same characteristic dependence of total heat (PC hydrolysis) on fractional load. There is, however, a discrepancy between the slope of the dependence of PC splitting on work, obtained from the early studies, and the expected value for this quantity obtained from the more recent heat production and chemical studies. These latter studies have shown that to a good approximation the total heat in an isotonic cycle of contraction and relaxation can be expressed as the sum of two terms: the activation heat (a constant), and a term equal to the work. This last term presumably arises from the quantitative conversion of work into heat when the load falls during relaxation. In addition, the combined heat and chemical studies are consistent with the conclusion that irrespective of the load, 9.8 ± 0.5 kcal of heat are produced for each

mole of PC hydrolyzed under *in vivo* conditions during a twitch. These two findings lead to the expectation that 9.8 kcal of work should be obtained for each mole of PC hydrolyzed in the muscle. The early chemical studies (see Fig. 1) give a value of 0.171 μmole/mcal for the PC split per unit of work. The reciprocal of this quantity, 5.9 kcal/mole, is the amount of work perfomed per mole of PC split. It is substantially less than the expected amount, 9.8 kcal/mole.

In an effort to resolve this discrepancy we have examined our data for systematic errors in the determination of the chemical, mechanical, and thermal quantities, and for systematic differences in the treatment or performance of the muscles used in the early studies and those used in the more recent studies. In neither the early studies nor in the more recent ones can we find any grounds for suspecting a systematic error in the determination of the work of sufficient magnitude to explain a significant part of the discrepancy. Neither is there any evidence of a systematic difference in the determination of the PC and C contents of the muscles. Both sets of muscles show essentially the same total C contents. This would not have been so if there had been a substantial systematic error in the determination of C or PC in one and not the other set of muscles.

The thermopile used in the heat determinations was calibrated to read temperature as recommended by Hill and Woledge (1962). This procedure does not, unfortunately, give an absolute calibration of the heat produced. The heat capacity of the muscle and any adherent Ringer's solution must be estimated. While it is unlikely that large systematic errors are associated with the myothermic methods used here, errors as large as 5 or 10 per cent cannot be rigorously excluded.

A search for differences between the performances of the two sets of muscles (see data in Tables III and IV) shows that the maximum work performed by the IAA-poisoned muscles used in the early studies was only about 1.0 mcal/gm in a twitch, whereas both the poisoned and unpoisoned muscles used in the more recent studies gave values of 1.5 to 1.8 mcal/gm for the work. This difference in ability to do work may have been a result of the fact that the experimental procedure used in the early chemical studies involved an additional 25 to 35 minutes of exposure to IAA at a temperature intermediate between 20 and 2°C. In these studies (see Methods above) the muscle which contracted isotonically was run *after* its mate had contracted isometrically. The muscles were not rinsed after IAA treatment and before installation in the chamber, and the isotonic muscle was not flushed with IAA-free solution prior to its attachment to the lever. It remained, on this account, exposed to IAA for an additional 25 to 35 minutes. Hartree (1931) reports that muscles which have had a prolonged treatment with IAA show, in a series of isometric twitches, a decrease in T1/H, due to the fact that T falls off more rapidly

with successive twitches than does H. Presumably such muscles would show more heat produced relative to the work done with successive twitches in an isotonic series. Such a phenomenon would explain the discrepancy.

Since the muscles used in the first chemical studies gave only 5.9 kcal of work per mole of PC split instead of the 9.8 kcal of work produced by the muscles used in the heat production studies the remaining 3.9 kcal must have appeared as heat. If this were the case, the average slope of the dependence of total heat on work would have been 1.66 and not about 1.0 as found here. Fenn (1923) studied the dependence of total heat on work, just as we have, and found slopes ranging from 1.0 to 2.0 in individual muscles. If as mentioned in Results (section *b*) above the isometric points are omitted from our total heat-work plot, an average slope of 1.14 is obtained. While a slope of 1.14 is not sufficient to explain entirely the discrepancy, it is in the right direction to account for a significant fraction of it.

While the observed discrepancy may be the result of an accumulation of systematic errors, it cannot be ascribed to this cause with any high degree of certainty. As a consequence, the possibility suggested by the early chemical studies must be regarded as potentially valid. Namely, that under certain conditions (*i.e.* prolonged exposure to IAA) there may be an extra amount of heat produced which is proportional to the work. This heat is produced in addition to both the heat of activation, and the heat which appears during relaxation when the work done in contraction is dissipated as heat. If there is a production of heat proportional to the work, the slope of the dependence of total heat on work for the cyclic isotonic twitch will exceed the value of unity (0.97 to 1.14) which we have found for normal muscles. It should be noted, however, that Fenn (1923) found that apparently normal muscles gave values for this slope which ranged from 1 to 2. This result suggests that there may be additional factors (fatigue, seasonal effects, etc.) which influence this slope. From the point of view of understanding the chemistry of muscular contraction, it is important to know whether there are chemical or other factors which can determine the relative amounts of work and heat released in a twitch, and if there are, how they operate. It is expected that studies now in progress will clarify this point.

DISCUSSION

Work and Heat in Muscle Twitches It is clear that the results reported here are not in agreement with the predictions of Hill's equation. We find no counterpart of shortening heat in a whole cycle of contraction and relaxation, either in the heat production itself or reflected in the PC breakdown. Neither is there any doubt that the equation was intended to apply to the whole cycle. Hill (1949 *c*, p. 220) states "This relation is true not merely for the whole

contraction but for any part of it." Even if this statement is held to exclude relaxation, our argument is not affected, for Hill (1949 *a*, 1953 *b*) also states that during relaxation the only heat to be seen is that due to the dissipation of mechanical work and to thermoelastic effects. The heat from dissipation is held to be equal (see Hill 1953 *a*) to the work done on the muscle and is therefore included in our measured total heat. The thermoelastic heat does not contribute to the net energy production when the tension in the muscle returns to its initial value (Woledge, 1960). The disagreement cannot therefore be due to applying Hill's equation under conditions for which it should not hold.

Neither are there any theoretical or experimental grounds for suspecting that our findings are the result of the particular experimental procedures used. The use of a slow galvanometer instead of a fast one would not be expected to alter the estimate of the total heat, and in fact it does not (Aubert, personal communication). Furthermore, our values for the heat produced are in good agreement with those given by Hill. Our use of a series of twenty twitches to improve the precision of the heat measurements leaves open the possibility, that had shortening heat occurred in the first one or two twitches, its effect would have been too small to be resolved. There is no direct evidence that shortening heat is unique to the first twitch or so, in a series. If this is true, the significance of shortening heat for the energetics of contraction must be trivial for its magnitude is only a small fraction of the total energy produced in the series.

In addition, our results are in good agreement with Fenn's (1923, 1924) findings obtained in experiments comparable to ours and conducted with similar techniques. The agreement becomes excellent if it is supposed that Fenn's estimates of heat were about one and a half times too great, because of the faulty techniques of calibration in use at that time (see Hill and Woledge, 1962). In qualitative terms, both Fenn's result and our result is that the energy output is greatest for a moderately heavy load, while Hill's equation predicts that energy output should be greatest for a very light load (see Fig. 4). Our result is also in good qualitative agreement with several earlier studies (Hill, 1911, 1930). The equation of Hill, however, gains no such support from these earlier works. Hill (1938, 1949 *c*) has questioned the validity of some of these studies because they were not made with protected thermopiles. However, the good agreement between Fenn's results obtained without a protected thermopile and ours obtained with one argues against this view. Furthermore, in a single experiment we determined the dependence of total heat production on load with the thermopile protected in one instance and unprotected in another. In both cases we obtained the same results, namely no dependence on shortening but only an activation heat and a term dependent upon the work.

Finally, our chemical studies show no chemical counterpart of shortening heat in PC hydrolysis under conditions where this reaction would be expected to be the only major source of energy. This result is in general agreement with the work of Rothschild (1930) on lactate production, and of Fischer (1931) on oxygen consumption. We are compelled, therefore, to accept the validity of our findings.

Significance of Shortening Heat Hill's (1938, 1949 *a*) results show that both during the active phase of a tetanus and for the first third of a twitch, more heat is produced the more the muscle is allowed to shorten. In *none* of the published data on isotonic twitches is the record of heat production continued long enough to include all of relaxation. Consequently, no direct comparison of our results for the total heat produced in a completed cycle of contraction and relaxation can be made with a continuous record of the time course of heat production throughout the cycle.

We can, however, infer the time course of heat production throughout relaxation if the validity of both Hill's data and our data is accepted. The solid curves shown in Fig. 6 are schematic representations of the inferred distribution of heat production in time for an isometric, a moderately loaded, and a lightly loaded twitch. As found by Hill, during the contraction phase more heat is produced with increased amounts of shortening. During relaxation the work done on the muscle appears as heat. If this heat, equal to the work, is subtracted from the total heat the remainder (indicated by the broken curves) approaches a constant value at the end of relaxation regardless of the amount of shortening that has occurred. This is in accord with our findings on the production of heat over the full cycle. Had the load been caught and no work done on the muscle, the production of heat would have followed the broken lines, presumably.

It cannot be, as Mommaerts *et al.* (1962) have suggested, that the increased heat due to shortening is exactly cancelled by a corresponding decrease in the heat of activation with shortening. Had this been so Hill never would have obtained the records of heat production during isotonic contractions which he has reported.

The appearance of heat proportional to shortening during the contraction phase, but not for the complete cycle would seem to imply the existence of some kind of a compensatory process. Two obvious possibilities are: (*a*) In the relaxation phase of the cycle, when the muscle lengthens, heat equal to the shortening heat is absorbed. (*b*) Heat, ordinarily produced during relaxation of the non-shortening muscle, is produced during contraction instead when the muscle shortens, and not subsequently in relaxation. The first possibility cannot stand unless it is also assumed that there is yet another process which produces heat during relaxation, since during relaxation and

contraction there is never any sign of an actual absorption of heat. This hypothetical heat-producing process must, therefore, be endowed with rather complex properties in order to insure that at no time during relaxation is there any net absorption of heat regardles of the extent or rate of lengthening of the muscle. The second possibility has the merit of greater simplicity since

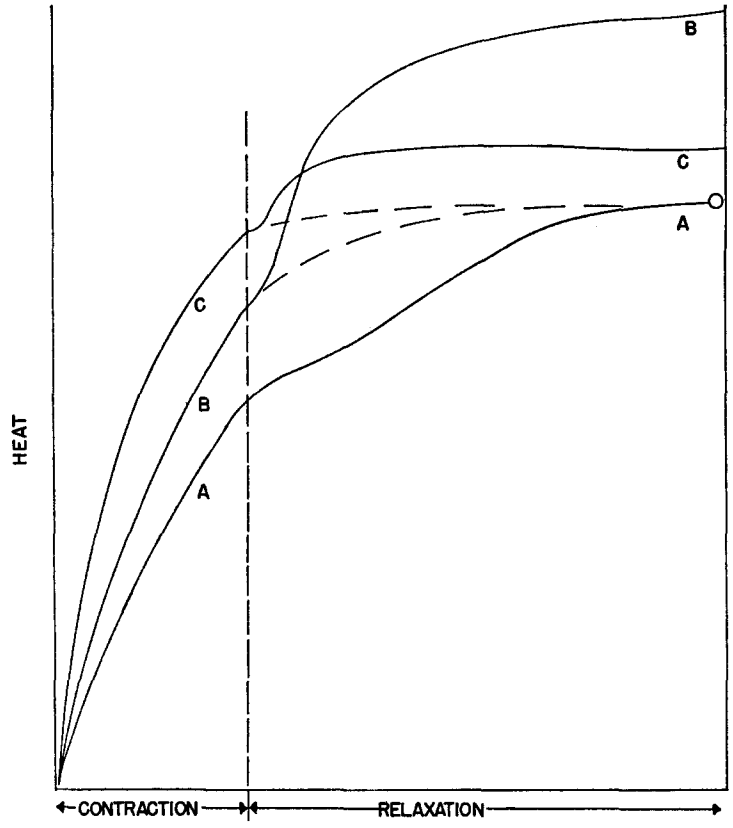

FIGURE 6. Schematic diagram of the production of heat during the full contraction and relaxation cycle of an isotonic twitch. *A-A*, isometric contraction. *B-B*, isotonic contraction with moderate load. *C-C*, isotonic contraction with light load. Open circle, O, constant activation heat obtained by subtracting the work from the total heat produced in the isotonic twitch.

it requires no *ad hoc* assumptions about processes and counterprocesses which cancel one another. It would mean that when shortening occurs in an isotonic twitch, the heat measured (exclusive of that generated by the load falling during relaxation) changes its distribution in time. With shortening, heat is produced faster but heat production stops sooner, so that there is little, if any, change in the total heat liberated.

No matter which view is taken, it is clear that the failure of Hill's equation

to apply to the full cycle of contraction and relaxation demands a revision of our concepts of the energetics of the muscle twitch. Until myothermic, or other methods, can establish with certainty the details of the production of heat throughout all phases of the twitch it will not be possible to assess what part, if any, shortening heat should play in the energetics of muscular contraction. As far as the over-all energetics of the contractile cycle are concerned, it is clear that in a cyclic isotonic twitch, shortening itself makes no net demands on the energy sources of the muscle cell. It is sufficient for the sartorius muscles of *R. temporaria* and *R. pipiens* to describe the energetics of the twitch with two terms, a nearly constant "activation" heat, and a term linear with the work. Whether or not this description applies to other muscle remains to be determined.

Hill (1938) originally described shortening heat for a tetanic contraction. It is doubtful whether in this case either, shortening heat contributes to the net energy production. We have preliminary results on 1 second tetani which show that when shortening is the greatest (load the lightest), the total energy production is, if anything, less than it is under isometric conditions.

Significance of PC Hydrolysis The finding that PC hydrolysis in an isotonic twitch shows no dependence on shortening, but contains only a constant term plus a linear term in work, gives strong support to the heat production studies reported here. Further, a close quantitative parallelism between the production of heat and the extent of PC hydrolysis during the contractile cycle has been demonstrated. This result strengthens the view that PC hydrolysis and its associated buffer reactions constitute the net energy-yielding reaction in the anaerobic, non-glycolyzing muscle. The *in vivo* heat of hydrolysis of PC has a value of 9.8 ± 0.5 kcal/mole at 0°C, and is in good agreement with the value of 9.6 calculated by Carlson and Siger (1960) from thermochemical data. This result demonstrates that PC hydrolysis is sufficient to account for the total energy produced in an isotonic twitch, and thus enables an energy balance to be made for this type of contraction. For prolonged tetani, however, we have preliminary evidence that PC hydrolysis is not the only exergonic process thus making it impossible to establish an energy balance based on this reaction alone.

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