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THE METABOLISM OF PHOSPHOCREATINE DURING AN ISOMETRIC TETANUS IN THE FROG SARTORIUS MUSCLE

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SUMMARY

1. A study was made of the phosphocreatine breakdown in anaerobic iodoacetate-poisoned muscle associated with one or several isometric tetani at 0°.

2. The rate of phosphocreatine breakdown in the course of tetanization, apart from the earliest moments, was 0.28 μmole/g/sec. This was constant for up to 60 sec until the near-exhaustion of all phosphocreatine, although the tension decreased by 0.89 % per sec, and was not affected by dividing the period of stimulation into several separate tetani.

3. This rate of phosphocreatine breakdown is consistent with known values of the maintenance heat if the apparent enthalpy change for phosphocreatine hydrolysis and associated ionization changes amounts to −10000 cal/mole.

4. An extra-metabolism was found which was proportional to the number of tetani and not to their durations, and which averaged 0.33 μmole/g/tetanus.

5. A small part of this extra-metabolism is due to elastic work and internal shortening during tension generation, and a larger part may represent the residual activation heat during relaxation. The remainder appears insufficient to account for the "labile part" of the maintenance heat, at the present state of knowledge. However, several aspects of this problem require further examination.

INTRODUCTION

While it is axiomatic that the energy liberated in active muscle originates in chemical reactions, it remains necessary to establish the identity of these, and to study the quantitative connections between them and the amount of energy that is released. In this paper, we shall report on such studies with respect to the biochemical changes accompanying an isometric tetanus in the frog sartorius, over a time interval in which the economy of tension maintenance changes.

Two qualifications define the position of this investigation in relation to certain problematic points. First, while a splitting of ATP is regarded as the primary link between activity and metabolism, no cases have so far been reported in which, in physiologically stimulated muscles and without the use of additional inhibitors, this

Abbreviation: PC, phosphocreatine.

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reaction could be separated from the rapid transphosphorylation from PC. Hence, we shall discuss the problem entirely in terms of PC splitting, the more so since, if a comparison with myothermic measurements is to be made, it is the enthalpy change of the overall reaction which is relevant, regardless of its intermediary reaction steps. Secondly, while it has been impossible to detect a phosphagen splitting in certain turtle muscles[1], thus raising the question of a separately definable exergonic process within the contractile structure itself, no such uncertainty afflicts the investigation of the frog sartorius. Here, from the first moments of stimulation on, creatine and phosphate are formed in equivalent amounts[2], so that the overall reaction of PC splitting may be expected to dominate the events throughout a tetanus.

The aims of this work were derived from myothermal and mechanical investigations which show that in the early part of an isometric tetanus the rate of maintenance heat production drops from a high initial to a low terminal rate. This was first observed by HARTREE AND HILL[3], elaborated upon in several papers from the HILL school[4], and finally specified by AUBERT[5], in terms of an initial "labile" heat of maintenance which declines exponentially with a time constant of the order of 1 sec at 0°, and which is superimposed upon a "stable" heat of maintenance. If no other reactions occur, this change in power ought to be relatable to conjugate quantities in PC breakdown. This is the specific question studied in this investigation.

METHODOLOGY

The muscle preparation

Batches of male *Rana pipiens* were kept in the cold for several weeks, avoiding the use of summer frogs. Before dissection, each animal was injected in the ventral lymph sac with 3 mg of D-tubocurarine, pithed 30 min later, and left for a further 30 min in the cold. This procedure seemed to reduce the variation between the two sartorius muscles of the same frog (see Table I, *Variance within pairs*). The muscles

TABLE I

VARIABILITY OF THE CREATINE CONTENTS OF MUSCLES THROUGHOUT THIS INVESTIGATION

The given number of muscle pairs used in each set of experiments is distributed in balanced incomplete blocks; the variance between pairs and within pairs with the number of degree of freedom given (d*f*) was computed according to FISHER AND YATES[10].

Date	*Number of muscle pairs*	*Variance between pairs*	*df*	*Variance within pairs*	*df*
		Non curarized			
10/60	6	3.99	5	15.15	5
		Curarized			
11/60	7	3.08	7	1.05	7
12/60	14	16.26	32		
1/61	23	28.12	19	6.14	16
4/61	13	14.16	8	1.43	9
5/61	5	0.96	2	1.78	3
5/61	28	2.01	38		
6/61	33	12.89	48		
6/61	5	20.23	12		
6/61	15	6.42	24		

were then carefully dissected, being left attached pairwise to the pelvic bone, and aerated with O_2–CO_2 (95:5) in Ringer solution (95 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 20 mM $NaHCO_3$), containing 3 mg D-tubocurarine in 250 ml, at 3–4° for 1–3 h. They were then treated for 1–3 h in a medium of the same composition containing also 0.4 m*M* iodoacetate and 0.4 m*M* lactate. After blotting with tissue paper, they were mounted on the stimulation assembly of the rapid-freezing apparatus[6], and kept in humidified N_2–CO_2 (95:5) for 10 min at 0–2° prior to the experiment, which consisted of tetanic stimulation with 5-msec square pulses from a constant current device, just above threshold, at a frequency of 10/sec.

Mechanical arrangements

The muscles in the apparatus were attached to cold-stretched steel wires by means of small loops of cotton string. This introduced some compliance, permitting perhaps a few tenths of a millimetre shortening, which could have been reduced by tighter connecting, but not without considerable manipulation of the muscles which we preferred to avoid. The steel wires themselves were considered inextensible (12 μ per 100 g load). Unless otherwise stated, the muscles were at standard length l_0. Tension was measured by means of the isometric capacitance gages of this laboratory[7], and was recorded via a tuning circuit with a Sanborn recorder with rectilinear coordinates. The system had a linear response, and was occasionally calibrated with weights.

Biochemical methods

These were the same as in the preceding paper of this series[6]. Free and total creatine were measured in all experiments. As the mean total creatine did not change, the hydrolysis of PC was simply measured by the increase in free creatine. ATP was measured by the luciferin–luciferase method[8], and the hexose mono- and diphosphates by a fluorometric procedure[9].

Design of experiments

The muscles were stimulated tetanically for various durations, either with one tetanus, or with several tetani in sequence. Thus a number of different treatments had to be compared. The variability between muscles from different frogs is much greater than the variability between the two muscles of the same frogs. By the technique of balanced incomplete blocks, it is possible to take advantage of the smaller variance of the muscles within the pair, even when the number of treatments is larger than two[10].

Each pair of muscles was regarded as a block of 2 plots. With more than 2 treatments to be studied simultaneously, the v treatments were combined two-by-two in b balanced blocks. The number of replications r was equal to $v - 1$ or a multiple of this. The variances between blocks and within blocks, which are the variances between different animals and that between muscles of the same pair, were computed with the standard techniques of the analysis of balanced incomplete blocks. The means were corrected, and the standard deviations given below are those of these corrected means. Table I gives the residual variances per plot of the free creatine, that between animals being greater than within a pair. The variances of the total creatine were similar. They have been grouped in Table I according to the experimental batches during the duration of this work. The differences may reflect seasonal changes

in variability, but may also be coincidental due to characteristics of the batches of animals purchased or to differences in their treatment.

RESULTS

Description of the tetanus

During a given tetanus, tension decayed slowly during 60–80 sec, after which it dropped abruptly. If we define by T the tension of the muscle, the force at the time t is given, with good approximation, by

$$T = T_0(1 - \beta t) \quad (1)$$

where β, the decay constant of tension, was 0.0089/sec, meaning a 0.89 % tension reduction per second of stimulation (see Table VI); this value holds only for the first phase of the tetanus. In many experiments, the muscles received a number of tetani; in those cases, the tension decayed as if the stimulation had not been interrupted. The abrupt decay which occurred during the second phase was correlated with an approaching exhaustion of the PC, as will be shown below.

Phosphocreatine metabolism

Of crucial importance for the experimental design are the questions whether all observed PC breakdown is connected with contractile activity, and whether all of it takes place during activity. With respect to the former problem, we refer to a previous paper[6], in which evidence is given that, with the treatment of the muscles employed here, all or nearly all the PC breakdown is assigned to the rephosphorylation of ATP used in contraction, only negligible amounts being utilized in the phosphofructokinase reaction. The second question was experimentally investigated as follows.

Groups of 12 muscles were tetanized for various lengths of time, and were then left for 1, 10, 100 and 1000 sec after a termination of stimulation. In Table II, the results are segregated according to the time of rest between stimulation and freezing, but are pooled with respect to the duration of activity (there being no interaction between the duration of the tetanus and the interval of subsequent rest). It is seen that there was no indication of a post-stimulative breakdown; in fact, such delayed PC metabolism is statistically excluded on the scale of magnitudes considered here. There is no information whether after a twitch of brief tetanus some retarded breakdown may not occur within a second*. This negative finding cannot be explained by a compensation of further breakdown of PC by its anaerobic resynthesis, since this was excluded by iodoacetate**.

Certain other metabolic studies will be mentioned briefly. Occasional determinations of lactate gave amounts of the order of 0.03 μmole/g, illustrating the effective-

* If in other experiments a post-stimulative breakdown of PC were to be encountered, it would stand to reason that this might be ascribed to occurrence of the phospho-fructokinase reaction; in the present work there was no accumulation of hexose phosphates (see below).

** We are aware that, even in iodoacetate-poisoned muscles, transient positive or negative phases of recovery heat have been detected[11]. We believe that those might be due to reactions of NAD^+ or NADH with glycolytic intermediates. As far as we can see, this could only be a formation of phosphoglycerol from triose phosphates, or a formation of pyruvate from lactate, or a formation of phosphogluconate from glucose phosphate. None of these reactions are phosphorylative, and anyway the available amounts of the reactants are too small to be of significance on the scale considered.

ness of the suppression of glycolysis by iodoacetate and otherwise. The ATP levels averaged 2.35 ± 0.18 ($n = 24$) μmoles/g, with no systematic differences connected with activity. Two determinations of hexose monophosphates and of hexose diphosphates plus triose phosphates[9] indicated no changes (Table III), in keeping with our present knowledge of the absence of esterification[6,9] under these circumstances. This point is of significance, as discussed above, since phosphorylation of hexose monophosphates would entail a utilization of PC linked with contractility. A small amount of such utilization might be concealed by a balance of formation and phosphorylation of hexose monophosphates, or of formation of hexose diphosphates and reduction of triose phosphates. However, the findings excluding a post-stimulative breakdown of PC limit these possibilities to very small quantities.

TABLE II

INDEPENDENCE OF PHOSPHOCREATINE BREAKDOWN OF THE TIME INTERVAL AFTER THE TERMINATION OF ACTIVITY

S.D., standard deviation of the means on the same row; n, number of muscles which received the same treatment.

Time between end of tetanus and freezing (sec)	*Free creatine (μmole/g)*	*Total creatine (μmole/g)*
1	14.1	30.7
10	13.5	28.6
100	14.6	29.4
1000	14.1	28.7
S.D.	0.72	1.71
n	9	6

TABLE III

ABSENCE OF ESTERIFICATION DURING BRIEF TETANIC ACTIVITY

Type of stimulation	*Controls*	*1 tetanus of 6 sec*	*6 tetani of 1 sec*
Hexose monophosphates (μmole/g)	0.19; 0.34	0.37; 0.27	0.17; 0.31
Hexose diphosphates (μmole/g)	0.42; 0.65	0.84; 0.56	0.46; 0.89

Stimulation parameters

A series of experiments dealt with the effect of stimulation frequency, comparing stimulation at 5, 10 and 20/sec. There was a trend towards increased metabolism with increased frequency (Table IV), significant as to the 5 *versus* 20/sec comparison, not significant between 10 and 20/sec. This is related to the incomplete fusion and lower average tension observed at the lower frequency. The present work was done with a stimulation frequency of 10, while 20 has been used in some other investigations from this laboratory[2,6].

Orientating experiments were undertaken as to the effect of length upon maintenance metabolism. At 0.6, 0.8, 1.0 and 1.2 times the standard length the rate of PC breakdown was determined up to a 48-sec interval. No differences were obtained that were correlated with the length, but the experiments were not regarded as final.

Phosphocreatine breakdown during one tetanus

In a major experimental series, comparisons were made of the free creatine contents after various lengths of stimulation, from 0 to 120 sec, applied as one uninterrupted tetanus (Table V). The total PC breakdown was computed by subtracting the free creatine of the stimulated muscle, and the results are plotted in Fig. 1. Within reasonable limits, identical slopes for the time curves were found for all subgroups, spanning both relatively short and long tetani, no account being taken

TABLE IV

PHOSPHOCREATINE BREAKDOWN DURING A TETANUS AT DIFFERENT FREQUENCIES OF STIMULATION

Each number is the mean of five experiments.

Frequency (cycles/sec)	*Rate of PC breakdown (μmole/g/sec)*
5	0.17
10	0.21
20	0.28

TABLE V

FREE CREATINE CONTENTS *(F)* IN MUSCLES TETANIZED FOR VARIOUS LENGTHS OF TIME *(θ)*

Muscles tetanized with one single tetanus of various duration θ; n is the number of muscles which received the same treatment; F, mean free creatine; T, mean total creatine, both in μmoles/g; S, tension time in kg/cm²/sec.

Expts.	*No.*	*θ (sec)*	*0*	*3*	*6*	*9*	*12*	*18*	*24*	*36*	*50*	*60*	*120*
	1	F	9.6	10.9	10.7	12.4							
69–82		S	0	5.5	9.0	15.4							
		T	33.8	34.3	32.1	34.4							
		n	6	6	6	6							
	2	F	9.0		11.2		12.9	15.0					
83–88		S	0		11.8		25.6	35.2					
		T	24.9		28.5		23.9	25.6					
		n	3		3		3	3					
	3	F	9.1		12.0		14.0		16.2				
97–125		S	0		12.0		22.5		40.9				
		T	30.7		28.6		29.4		28.7				
		n	12		12		12		12				
	4	F	9.1						16.2		24.3		33.3
111–126		S	0						40.9		87.6		138.2
		T	30.7						28.7		29.3		27.8
		n	12						12		4		4
	5	F	10.3							20.3		25.9	
127–163		S	0							61.2		102.3	
		T	31.2							29.6		29.3	
		n	7							7		7	
	6	F	10.5								21.2		
162–181		S	0								75.2		
		T	25.2								27.1		
		n	4								4		

of the fall of tension during the contraction. The aggregate average was 0.28 μmole PC split/g and/sec (Table VI). If a correction for tension is applied, this rate increases slightly to 0.32 μmole/g/sec (see DISCUSSION). While the data are compatible with a constant slope, a curvilinear time-dependency is not explicitly excluded. A slight decrease in PC splitting rate may well occur after 40–60 sec tetanization, and a temporary increase in PC splitting occurs at the beginning of the tetanus, as demonstrated below. Such a temporary increase might be suspected from the fact that the best fitting line has an intercept of 0.38 μmole/g.

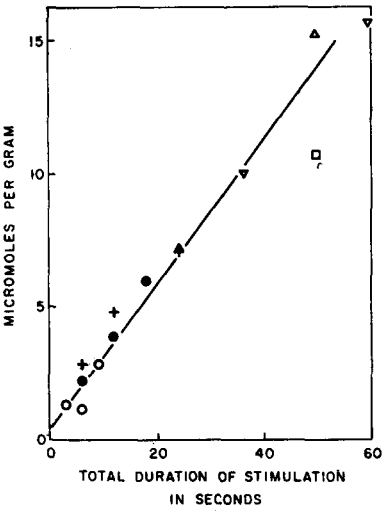

Fig. 1. Hydrolysis of phosphocreatine during a single tetanus, calculated as the difference between the mean control, free creatine, and the mean stimulated free creatine; 6 sets of experiments, detailed data in Tables V and VI; ○, corresponds to set 1; ●, to set 2; +, to set 3; △, to set 4; ▽, to set 5; □, to set 6. The line is the mean weighted slope for all the experiments, uncorrected for tension decay.

TABLE VI

EVALUATION OF RESULTS IN TABLE V

P_0 is the mean maximum stress, and β the tension decay time constant. The slopes of the free creatine *vs.* duration of stimulation are given uncorrected, and corrected for tension decay. S.D. are the standard deviations of these slopes and of the free creatine values given in Table V.

No.	*n*	P_0 *(kg/cm²)*	β *(per cent/sec)*	*μmole/g/sec*		*S.D. of slopes*	*S.E. of means*
				Slopes	*Corrected slopes*		
1	6	1.70	1.0	0.27	0.28	0.09	0.58
2	3	2.12	1.0	0.33	0.35	0.02	0.95
3	12	1.96	0.8	0.29	0.32	0.02	0.41
4	4	2.14	1.0	0.30	0.37	—	—
5	7	2.10	1.1	0.26	0.34	0.03	1.31
6	4	1.94	0.4	0.22	0.27	—	—
Weighted means		1.96	0.89	0.28	0.32		

The extra-metabolism

While the findings in the preceding sections essentially apply to the steady maintenance metabolism in prolonged activity, we shall now turn to the changes associated with the primary activation and the relaxation representing the extra-metabolism. These could not be investigated in the same manner, the extra-meta-

bolism in one tetanus, although suggested by Fig. 1, being too small to be reliably detected. Instead, the information was obtained by comparing single with multiple tetani, covering the same duration or range of durations. Various experimental series were performed along different patterns, to be presented seriatim.

TABLE VII

CREATINE PHOSPHATE BREAKDOWN IN 1 SINGLE TETANUS AND IN 6 TETANI OF THE SAME TOTAL DURATION

S.D., standard deviation of the means; n, number of muscles which received the same treatment.

Free creatine of a stimulated muscle (μmoles/g)		S.D.	n
1 tetanus of 6 sec	*6 tetani of 1 sec*		
11.5	14.1	0.98	17

The simplest type of experiment is that given in Table VII comparing 1 tetanus of θ sec with n tetani of θ/n sec, the difference representing $(n - 1)$ times the extra-metabolism in the interval θ/n, diminished by the labile metabolism, if any, in the remaining $(n - 1)$ θ/n sec. In the given example, this extra-metabolism amounted to 0.52 μmole/tetanus (the highest result obtained). In another experiment (Fig. 2) 12 tetani were given 0.2, 0.4, 0.6, 0.8 and 1.0 sec duration, and compared with the control analysis of the same block (means of 5), from which there is drawn a line at a slope of 0.28 (the previously established value for PC/θ). The experimental points

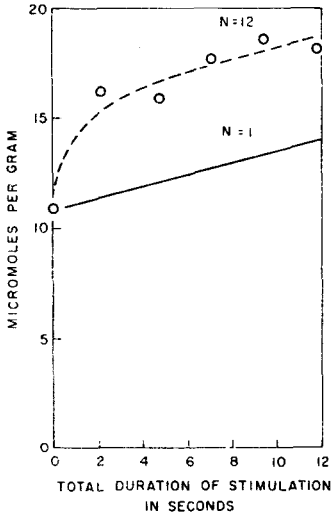

Fig. 2. Time course of PC breakdown, as indicated by the free creatine content, in 1 single tetanus of the indicated duration (N = 1), and for 12 tetani of the indicated total duration.

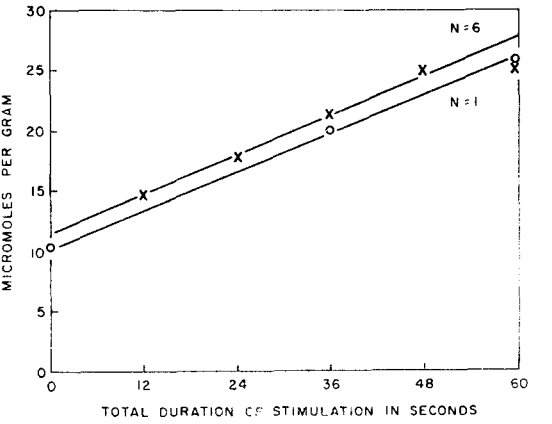

Fig. 3. Time course of PC breakdown, as indicated by the free creatine content, in 1 and in 6 tetani of varying total duration. Each point is the mean free creatine of seven muscles, and has a standard deviation of the mean of 1.31. Two sets received one single tetanus and five sets 6 tetani, respectively. The two lines are statistically parallel and the calculated slope is 0.27 ± 0.03 μmole/g/sec. (Note that the regression analysis for the N = 1 line alone gives 0.26 ± 0.03, see Table VI, line 5.)

themselves approached this same slope, but indicated an extra metabolism associated with 12 tetanizations of 4.0 μmoles, or 0.33 μmole/tetanus. In a third series (Fig. 3) there were the following comparisons: a single tetanus of 36 or 60 sec, a control, and 6 tetani of 2, 4, 6, 8, or 10 sec (means of 7). In the last case, there was fargoing depletion of the PC, and we will disregard the point*. The slopes of the line were identical, 0.27 ± 0.03 μmole/g/sec, while the difference in the intercepts indicated an extra-metabolism of 0.25 μmole/tetanus.

The last experimental series is that given in Table VIII in which, at a total interval of tetanization of 6 sec, a considerable range of individual tetanus durations was covered. The maximal stress was identical in each of the variations, except that it was slightly lower in the briefest tetani, 0.2 and perhaps 0.4 sec, in which there was insufficient time to allow complete manifestation of the active state. The stress-time values likewise decrease for the shorter tetani on account of the greater share of the ascending phase of the contraction. The PC hydrolysis is greater for the larger number of shorter tetani, but the extra-metabolism per tetanus is seen to decrease sharply when the duration of the tetanus becomes very short.

TABLE VIII

PHOSPHOCREATINE BREAKDOWN DURING 6 SEC OF TETANIZATION, DELIVERED AS INDICATED ON LINES 3 AND 4

Type / *No. of muscles*	*o* / 8	*a* / 8	*b* / 8	*c* / 8	*d* / 8	S.D.
Total duration of stimulation (sec)	6	6	6	6	6	
Duration of one tetanus (sec)	6	2	1	0.4	0.2	
Number of tetani	1	3	6	15	30	
Free creatine (μmole/g)	10.7	11.2	13.4	13.3	14.0	0.91
Total creatine (μmole/g)	32.4	31.5	31.5	30.0	32.0	0.98
Maximal stress (kg.cm^{-2})	1.95	2.01	1.98	1.83	1.71	0.07
Stress-time (kg cm^{-2}.sec).	12.3	10.7	10.2	8.3	6.4	0.73
Extra-metabolism $E_n - E_1$	—	0.5	2.7	2.6	3.3	
Extra-metabolism per tetanus	—	0.25	0.54	0.22	0.12	

DISCUSSION

The stable metabolism

Time course and amount: Associated with each contraction of muscle, there occurs a breakdown of PC which progresses with increasing duration of stimulation, provided that no resynthesis occurs as in the case in the anaerobic iodoacetate-poisoned preparation. We conclude that this breakdown occurred, in our experiments, during contraction itself, as no changes in PC content were noticed from 1 to 1000 sec after stimulation. This seems in contradiction with the result of LUNDSGAARD[12], who reported a further breakdown of 1 μmole/g in 30 sec after a 5-sec tetanization, but as his work was done at 17°, and hexosephosphate formation occurred, we may ascribe his finding to the phosphohexokinase reaction. A prolonged alkalization after contraction has been detected by DUBUISSON[13] and DISTECHE[14], which would most readily be explained as indicating PC hydrolysis.

* This would mean that the single tetanus point at 60 sec is also questioned, although it happens to be in accordance with the line as drawn. However, one might accept that this point is correct and is just on the limit of exhaustion; whereas in the multiple tetanus series exhaustion is reached earlier, on account of the additional demands of the extra-metabolism.

The rate of PC hydrolysis during a contraction at 0°, apart from the initial phase, was approximately constant up to about 60 sec, and amounted to 0.28 μmole/g/sec. This value was obtained from the slope of the hydrolyzed PC plotted against the duration of stimulation given as one single tetanus, and it was thus independent of any extra-metabolism that might have occurred during the tension development, or the relaxation. LUNDSGAARD[12] gives data from which the rate of PC hydrolysis can be deduced. He states that at 4°, the isometric coefficient for tetanus defined as

$$K_Z = \frac{\text{Tension in grams} \times \text{length in centimetres} \times \text{seconds of tetanization}}{\text{PC hydrolyzed in grams orthophosphate}} \quad (2)$$

is equal to $21 \cdot 10^6$. With a tension of 1100 g (for frog gastrocnemius) and a length of 3.3 cm, one calculates a rate of PC hydrolysis of 1.7 μmoles/g/sec, a value obviously much higher than the one reported here. The greater part of his work was done at 17°, and K_Z for this temperature is given $15.2 \cdot 10^6$, or 2.2 μmoles/g/sec. The Q_{10} of the maintenance heat rate is given by FENG[15] and AUBERT[5] as 3, and as there is surely a close correspondence between this maintenance heat and the stable PC metabolism, we can accept a similar Q_{10} for the latter. The rate of PC breakdown would then be reduced some 8 times when the muscle is cooled from 17° to 0°, and would equal 0.28 μmole/g/sec, identical with our value.

After a 60-sec tetanus, the PC content was drastically reduced. In the control muscles, the total creatine was between 25 and 34 μmoles/g and the free creatine between 9 and 11 μmoles/g. The PC before any stimulation was therefore between 14 and 25 μmoles/g. There was very little PC left after a 60-sec tetanus, which used up to 17.5 μmoles/g. The PC hydrolysis rate seems independent of the PC concentration. This can be easily interpreted if the PC hydrolysis is coupled with a resynthesis of ADP to ATP, which reaction is not impeded by the iodoacetic acid *in vivo*[16,17], and limited at the ATP breakdown rather than at the transphosphorylation step. The K_m of this reaction is such that any ADP formed during the contraction would be converted to ATP, until the PC concentration has become very small[16]. This will occur at 0° after 60 sec tetanization, and we have indeed frequently observed then a rather sharp drop of the tension. With a Q_{10} of 3, a 60-sec tetanus at 0° would be equivalent to a 6-sec tetanus at 20°. MARÉCHAL AND AUBERT[18] have observed in a normal muscle that at this temperature the tension is well maintained for 4–8 sec, and then drops suddenly to low values; a short interruption of the stimulation restores the tension back to its value before the sharp drop. This fact can be explained if there is a fargoing exhaustion of the PC reserves during the tetanus; a short rest however would allow enough ATP or PC resynthesis to bring about a rapid restoration of the tension.

Connection with tension: In order to compare chemical and mechanical events, we must equate the corresponding energies over the time interval of tetanization:

$$\Delta\text{PC} = \frac{Tl\theta}{K} \quad (3)$$

where ΔPC is the phosphagen split, T the tension of the muscle, l its length, and θ the total duration of stimulation. K is the isometric coefficient for tetanus. If the tension is time dependent:

$$\Delta\text{PC} = \frac{l}{K}\int_0^\theta T\,dt \quad (4)$$

As a good approximation, the tension is described by:

$$T = T_0 (1 - \beta t) \tag{1}$$

where T_0 is the tension at the beginning of the tetanus, and β a decay constant equal to 0.89 %/sec. The tension decay is the same whether the muscle be stimulated by one long tetanus, or several short ones, provided that the total duration of stimulation, θ, is constant. Introducing (1) into (4):

$$\Delta PC = \frac{T_0 l \theta}{K}\left(1 - \frac{\beta}{2}\theta\right) = \frac{T_0 l \theta'}{K} \tag{5}$$

where θ' is the reduced duration of stimulation, *i.e.* the duration of stimulation that would have given the same stress time, had the tension not decayed. The rate of PC hydrolysis corrected for tension decay is the slope of the free creatine experimental values *vs.* this corrected time (given in Table VI under *Corrected slopes*). The mean rate of PC hydrolysis is raised from 0.28 μmole/g/sec to 0.32 μmole/g/sec; the effect of this correction is small, and may often be neglected.

The coefficient K is estimated as $22.8 \cdot 10^3$, based upon the values: tension, 2000 g; $l = 3.2$ cm, $\Delta PC = 0.28$ μmole/g/sec. K must be multiplied by 10^4 in order to be in the same units as the K_z of LUNDSGAARD. At the end of a 60-sec tetanus, K is reduced to $11.4 \cdot 10^3$, because the tension has decayed 50 %, although the rate of PC hydrolysis has not changed. LUNDSGAARD had already noticed a similar trend in his experiments, which is now fully demonstrated. The economy of the muscle, as measured by K, decreases during the stimulation, an effect which is opposite to fatigue, where the economy increases[19].

Comparison with heat experiments: The rate of heat production during the maintenance of an isometric tetanus is described by AUBERT[5] as:

$$h = h_A e^{-\alpha t} + h_B \tag{6}$$

AUBERT has divided it into two parts: a labile maintenance heat, $h_A e^{-\alpha t}$ which decreases exponentially with a time constant of about 1 sec at 0° toward a stable maintenance heat rate, h_B. MARÉCHAL[20] showed that this stable maintenance heat rate decreases very slowly during 60 sec, much less than the tension, at least at 0°. During this long tetanus, the tension decreased at the same rate as in the experiments reported in this paper. The parallelism between maintenance heat rate and PC hydrolysis is striking.

A comparison between maintenance heat and the PC metabolism could yield an apparent enthalpy for PC hydrolysis and associated changes during contraction. This calculation is subject to two main errors. The first refers to the different physiological materials subjected to the experiments, as the PC metabolism was measured on American *Rana pipiens*, and the thermal metabolism on European *Rana temporaria*; one cannot assess the importance of the species difference, but we will suppose that this is not great, compared to the other factor. Second, the PC metabolism was measured on iodoacetate-poisoned muscle. The iodoacetate might affect the maintenance heat, and careful comparative experiments are needed. MARÉCHAL[20], has found that this maintenance heat is markedly affected by the iodoacetate. From 3.9 mcal/g/sec in an intact muscle, it decreases to 2.9 mcal/g/sec in a poisoned one. Comparison of this last value with the PC rate of hydrolysis (0.28 μmole/g/sec) gives an apparent enthalpy change for PC hydrolysis *in vivo* of −10 kcal/mole, which is

closely comparable to the estimation of CARLSON AND SIGER[21], —9.6–11.5 kcal/mole*.

The increasing economy of muscle during tetanus: an interpretation: What can be the meaning of the decrease in economy that occurs during a tetanus, or in other words, how can we conceive that the tension drops while the metabolism associated with it (PC hydrolysis or maintenance heat) does not?

We see two types of interpretation of this phenomenon, the one assuming a mechano-chemical uncoupling, the other based on a mechanical feature. According to the first of these explanations, there would occur some form of loss of energy transfer, by a lesser stoichiometric coupling so that some ATP would be hydrolyzed extraneously, or would otherwise fail to be utilized in the contraction process. This is a definite possibility, *e.g.* it is known that depolarization of the muscle membrane by high-potassium solutions elicits a greatly increased metabolism without necessary accompaniment of contraction; since Ca^{2+} is necessary for this effect[22], it has been concluded that this metabolic activation makes use of elements of the normal excitation–contraction coupling mechanism, but that with the abnormal membrane condition this mechanism is interrupted. One might well assume that some aspect of the mechano–chemical coupling alters during the phase of decreasing economy under discussion.

The mechanical interpretation is based upon the finding[23], that in a series of isometric tetani, there occurs a shift of the length–tension relation toward greater lengths. Similarly, MARÉCHAL[24,25], has shown that in fatigue after isometric tetanization under certain conditions, the tension is very much increased by a slight lengthening. Both observations could be interpreted as due to an extension of the series elastic component. Now AUBERT[5] has shown that the maintenance heat decreases much less than does the tension when a muscle is stimulated isometrically at shorter length. A quantitative consideration of this contingency[20] shows that this mechanical hypothesis could account for about half of the decrease in economy observed in the course of a long tetanus.

The extra-metabolism

Definition and magnitude: When a muscle receives several short tetani, the PC hydrolyzed is greater than in the control muscle which is given one tetanus of the same total duration. The difference is defined as the extra-metabolism. The weighted mean for all our determinations was 0.33 μmole/tetanus, but the span was rather large, from 0.11 to 0.54 μmole/g/tetanus, depending on the experimental conditions.

NACHMANSOHN[26] reported experiments in which he compared 2 or 3 tetani with a single tetanus of the same, or usually somewhat greater, total duration, and found that in the former case more PC was broken down. His conclusion[26] that "evidently the development of tension is connected with a greater splitting of phosphagen than the maintenance of tension" foreshadows our present inquiry, but he drew no analogy between his results and those of HARTREE AND HILL[3]. The difference, of the order of 1 μmole/g PC, would be a measure of the extra-metabolism at 17° on the frog gastrocnemius. We have extended the investigation on the extra-metabolism of the

* In a preceding paper[6], we have reported a substantially lower value for the apparent enthalpy of PC *in vivo*: 7600 cal/mole. This, however, is much less accurate than the present one, because it was estimated on a tetanus of 400 msec duration. But the active state does not stop abruptly after the last stimulus, but continues during an unknown time; this fact was not considered at the time, and means that the value of 7600 cal was too small.

sartorius muscle at 0° which allows accurate comparisons with the myothermic experiments of HILL AND AUBERT.

Theoretical evaluation of the extra-metabolism: Before attempting a correlation with the labile maintenance heat as defined by AUBERT[5], we must first inquire whether the extra-metabolism encountered in the investigation of several tetani can be due to other events either in the beginning of tetanization or in relaxation.

During the rise of tension, the contractile component shortens and mechanical work is being stored in the elastic component and in the apparatus, which can be estimated as 0.4 (see ref. 27) and 0.3 mcal/g respectively; since work performance is accompanied by an increment in metabolism[6], this accounts for additional PC breakdown, which does not appear as heat in the sense of the FENN effect[4], and which is not reversed when in relaxation this work is returned to the contractile structure, since it is then known to appear as heat. The heat of shortening during this initial period might amount to another 0.2 mcal/g, but its coverage by extra-metabolism is problematical[6] and this small quantity will be ignored.

After the cessation of stimulation, there is, apart from quantities of heat of mechanical origin, a declining residual maintenance heat closely equalling the activation heat of a twitch[27], which has been experimentally estimated at 1.8 mcal/g. Thus, there may be up to 2.5 mcal/g to be accounted for from chemical sources, each time a contraction is initiated and terminated, which both qualitatively and quantitatively is reminiscent of the activation heat in a single twitch[28]. This quantity will be denoted as R, and the extra-metabolism in n contractions of θ/n duration as compared to 1 contraction of time θ equals:

$$H_n - H_1 = (n - 1)R \tag{7}$$

as far as due to the indicated circumstances.

The second possible source of extra-metabolism is the labile part of the maintenance heat[5]:

$$H_n = H_A + H_B = \int_0^\theta h_A e^{-\alpha t}\,dt + \int_0^\theta h_B dt \tag{8}$$

where H_A and H_B are the total labile and stable maintenance heats for a tetanus of duration θ. The difference in maintenance heat between 1 tetanus of θ and n tetani of θ/n sec is:

$$H_n - H_1 = \frac{(n-1)h_A}{\alpha} - \frac{h_A}{\alpha}\left(ne^{\frac{-\alpha\theta}{n}} - e^{-\alpha\theta}\right) \tag{9}$$

The total extra-metabolism, due to both causes combined is:

$$H_n - H_1 = (n-1)\left(R + \frac{h_A}{\alpha}\right) - \frac{h_A}{\alpha}\left(ne^{\frac{-\alpha\theta}{n}} - e^{-\alpha\theta}\right) \tag{10}$$

Interpretation of the experimental data: In Table IX are given the extra-metabolism values measured in 8 different sets of experiments as the difference in free creatine between muscle stimulated by n tetani of θ/n sec and by 1 tetanus of θ sec. These experimental results are compared to theoretical values obtained with the use of Eqns. 7 and 10, and with an apparent enthalpy change for PC hydrolysis of 10 kcal/mole, taking R as 2.5 mcal/g, α as 1 sec, h_A/α as 4.7 mcal/g. This latter value,

TABLE IX

COMPARISON BETWEEN FOUND AND CALCULATED VALUES OF THE EXTRA-METABOLISM IN THE INITIAL PHASE OF TETANIZATION

Expt.	*Duration of one tetanus (sec)*	*Number of tetani*	*Extra-metabolism (μmole/g)*		
			Found	*Eqn. 7*	*Eqn. 10*
1. Fig. 3	0.2–1	12	4.3	2.7	5.8
2. Table VIII*d*	0.2	30	(3.3)	(7.3)	(25.3)
3. Table VIII*c*	0.4	15	2.6	3.2	5.4
4. Table VIII*a*	1.0	6	2.7	1.3	2.6
5. Table VII	1.0	6	2.6	1.3	2.6
6. Table VIII*b*	2.0	3	0.5	0.5	1.2
7.	2.0	6	1.3	1.3	3.2
8.	8.0	6	1.3	1.3	3.2
Mean (No. 2 excluded)			2.2	1.7	3.4

taken from AUBERT[5] (Table XII), applies to normal muscle and is probably a lowest estimate, as the total labile heat tends to increase when the muscle is poisoned with iodoacetate[20].

The examination of this table leaves little doubt that application of the combined Eqn. 10 leads to an estimation for the extra-metabolism which is in excess over that found experimentally. On the other hand, application of Eqn. 7 alone does not appear to account for the experimental results completely. One would conclude that there is metabolic coverage for the quantities envisaged in Eqn. 7, but that those additionally provided for by Eqn. 9 and which represent the labile part of the maintenance heat, are only partly accounted for by our work. While it is possible that experimental error is responsible for this, and also that the quantity R may have been overestimated, it must nevertheless be considered at the present stage that the discrepancy is real.

Two main interpretations are suggested for this metabolic deficit. The first is that the estimate of the labile metabolism H_A is exaggerated. Indeed, the assumption is implied in Eqns. 9 and 10 that the labile part of the maintenance metabolism does not change in a rapid sequence of tetani. This is not explicitly known at present; it may not be the case, since after-effects from the previous contraction may improve the economy during the initial phase of a new contraction. The second is that the labile heat is indicative of a transitional phase towards the final steady state, during which certain shifts take place. This is not a shift from primary ATP breakdown to steady state PC breakdown, since no such temporal separation has been encountered[2,29], and since this would not be expected to yield more heat. It might be a shift from one to another type of ionization or neutralization reaction, or it could be a structural change with thermal effects of its own*.

* With respect to the latter contingency, we propose that the increase in economy during this phase of maintenance be regarded as a partial approximation toward the locking of the contractile structure by a "catch" mechanism, as is considered to occur more prominently in invertebrate smooth tonus muscles. In these, the phenomenon is currently ascribed to a crystallization of paramyosin[30,31]. In view of the similarities in composition between this protein and vertebrate tropomyosin[31–33], we consider the hypothesis that tropomyosin might function by partial locking of the contractile structure in the course of a maintained contraction.

Thus, there is a good possibility that upon further examination our figures would be found to account for all phases of the maintenance heat if properly evaluated; if not, however, the possibility of an additional energy source may have to be investigated, the more so since at the present state of knowledge there are muscles in which the energetics may not at all be balanced by PC breakdown[1].

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