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ENERGY BALANCE IN FROG SARTORIUS MUSCLE DURING AN ISOMETRIC TETANUS AT 20° C

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SUMMARY

1. Changes in the concentrations of ATP, ADP, AMP, IMP, creatine and phosphorylcreatine (PC) have been measured in frog sartorius muscles after different periods of isometric stimulation at 20° C. The heat production was measured in parallel experiments with a thermopile of the Hill-Downing type.

2. Muscles were either in O_2 and unpoisoned or in N_2 and poisoned with iodoacetic acid to prevent aerobic and glycolytic recovery processes.

3. Poisoning did not appear to alter the heat production of these muscles and had little effect on the tension for up to 8 sec tetanus.

4. The break-down of high-energy phosphates ($\sim$ P) during contraction was faster in the poisoned muscles. Normal muscles were thus able to resynthesize high energy phosphates during the contraction. The resynthesis began at its maximum rate; part of it was probably due to glycolytic activity.

5. During the first 2 sec of contraction (poisoned muscles), the only net reaction was an hydrolysis of PC, with an apparent enthalpy change of $-8{\cdot}3$ kcal/mole. During longer contractions, the PC hydrolysis was accompanied by a net ATP hydrolysis and appearance of AMP and IMP.

6. For the first 2 sec of contraction in the poisoned muscles, the observed heat agreed with that expected from the observed chemical changes multiplied by their molar enthalpy changes. After 2 sec, the observed heat was greater than that expected. At 12 sec this excess was about 74 mcal/g. Possible explanations for this discrepancy are discussed.

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INTRODUCTION

The validity of the Lohmann hypothesis, discussed in the preceding paper (Canfield & Maréchal, 1973), regarding the position of ATP in the chemical events of contraction, does not establish the uniqueness of that position. There may be some other, unknown, source of chemical energy contributing to the total energy utilized during contraction. The way to investigate this possibility is to compare the chemical energy mobilized with the physical energy (heat, Q, and work, W) released by the muscle. Elementary thermodynamics shows that, if ΔH_{ATP} is the molar enthalpy change of ATP hydrolysis and ΔH_{PC} the molar enthalpy change of phosphorylcreatine (PC) hydrolysis to free creatine (C_F) then

$$W+Q = -n_{ATP}\,\Delta H_{ATP} - n_{PC}\,\Delta H_{PC}, \quad (1)$$

where n_{ATP} and n_{PC} are the numbers of moles hydrolysed. For brief isometric contractions $n_{ATP} = 0$ and $W \simeq 0$; the apparent molar enthalpy change for PC hydrolysis *in vivo* is then equal to Q/n_{PC} (Wilkie, 1961).

The value of this ratio (Q/n_{PC}) varies according to the authors from $-7{\cdot}5$ kcal/mole (Maréchal, 1964) to -11 kcal/mole (Wilkie, 1968); see also Woledge (1971) for a complete discussion of the reasons for such a range of values). As these figures are close to the *in vitro* value of ΔH_{PC}, it has been concluded that no other major energy yielding reaction occurs during contraction.

More recent work has cast doubt upon the adequacy of this scheme (for a review, see Maréchal, 1972). Gilbert, Kretzschmar, Wilkie & Woledge (1971) found that, early in an isometric tetanus at 0° C, more heat was produced than could be accounted for by the observed PC hydrolysis. The present work also describes a large imbalance between heat production and chemical break-down during an isometric tetanus at 20° C. A short preliminary communication has been given (Canfield, Lebacq & Maréchal, 1971).

METHODS

Experimental design

The object of the experiments was to draw up a balance sheet between the heat produced during a tetanus, and the corresponding chemical changes. Because many seasonal, batch and procedural effects may affect the results, it was necessary to measure all the required data simultaneously. However, heat and chemistry could not be evaluated on the same muscle pairs, because it is technically impossible to rapidly freeze a muscle pair mounted on a thermopile. Therefore, heat production and chemical changes were measured in two series of experiments which were performed concurrently.

It was thought essential to evaluate the effect of the duration of the tetanus on the thermodynamic balance. The heat production could easily be measured as a function

of the tetanus duration on one muscle pair; but the estimation of the time course of the chemical changes required the destruction of a number of pairs, as each one provided only one result for a preset duration of tetanus. As rather long tetani at 20° C were investigated, it was also necessary to check whether glycolytic and/or oxidative reactions had occurred. This check was made by comparing the results from muscles stimulated in O_2 with those obtained from muscles poisoned with iodoacetate (IAA) and stimulated in N_2.

Two experimental designs were used. Each one was specifically adapted to the requirements of each of the two series of experiments, and these are briefly described below.

Design of the series of chemical experiments. We required to estimate the changes in nucleotides and PC content of muscles stimulated isometrically for 0, 0·5, 1, 2, 4, 8 and 12 sec either in O_2 or in N_2 (after iodoacetate poisoning).

It would have been inefficient (in a statistical sense) to use a design where one muscle of the pair was always left at rest (control muscle) whilst the other was tetanized for one of the durations required (stimulated muscle). We preferred to use a form of balanced, incomplete block design, described fully by Li (1964). In this type of design, the various treatments are combined two by two in all possible ways. In our case, this gave the following pairs (duration in sec): (0, 0·5); (0, 1); (0, 2); (0, 4); (0, 8); (0, 12); (0·5, 1); (0·5, 2); (0·5, 4); (0·5, 8); (0·5, 12); (1, 2); (1, 4); etc. The number of different pairs (21) was much too large to be handled in a single day. We therefore arranged the various treatments into two groups. The first had the four treatments: 0, 0·5, 1 and 2 sec (subscripted 2). The second had treatments: 0, 4, 8 and 12 sec (subscripted 12). Each group was either poisoned (P) or unpoisoned (O). There were thus four groups: O_2, O_{12}, P_2 and P_{12}. Each group now required only six muscle pairs for the combination of treatments. This was a convenient size and two such groups could be handled each week. The composition of the O_2 group, for example, was: six pairs tetanized according to the scheme: (0, 0·5); (0, 1); (0, 2); (0·5, 1); (0·5, 2); (1, 2). Each treatment was thus repeated three times in a group. This was too few and so each group was repeated three times giving a total of nine repetitions of each tetanus duration. In any particular group, the treatment pairs were allotted at random to the muscle pairs, and then each treatment was allotted at random to a muscle. A table of random numbers was used for this purpose.

The balanced, incomplete block design does not specify the order in which the groups should be performed experimentally. They were arranged as follows:

$$P_{12}, O_2, O_2, P_{12}, O_{12}; \quad O_{12}, P_2, P_{12}, O_2, O_{12}, P_2.$$

The basic principle which was used in setting up that order was to balance the groups as far as possible. This is apparent if we consider the interval between the two consecutive O_{12} groups as a centre, from which the consecutive groups are numbered. The sum of the order is a constant for the various types of groups. For example, groups of the P type have a sum of -11 before this centre, and $+11$ after it; the O series have sums of -10 and $+10$; the 2 series have -12 and $+12$; the 12 series have sums of -9 and $+9$. This design improves the comparison between P and O, but impairs somewhat the homogeneity of the groups O_2, P_2, O_{12} and P_{12}.

Each group (O_2, O_{12}, P_2, P_{12}) was analysed separately according to Li (1964), where each muscle pair forms one experimental block. First one computes a quantity, Q_j, for each treatment. This measures the effect of that treatment. Suppose we have four treatments a, b, c, d (for example, the PC of muscles of the O_2 group) Q_a is the sum of the *differences* obtained from the muscle pairs: $(a-b)$; $(a-c)$ and $(a-d)$; Q_b is the sum of $(b-a)$, $(b-c)$ and $(b-d)$; Q_c and Q_d are computed in the same manner.

The data reported in the Results are the means corrected for any bias introduced by

any inequalities between the various muscle pairs. These means, ($\bar{X}_j$) were computed from

$$\bar{X}_j = \bar{X} + Q_j/t\lambda,$$

where $\bar{X}_j$ is the corrected mean for treatment j; $\bar{X}$ is the general mean (of all treatments in that group combined); t is the number of treatments ($t = 4$ in our experiments) and λ is the number of muscle pairs which received the same treatment pair ($\lambda = 3$ in our experiments). A useful property of the symmetrical pair design is that the standard error is the same for all the corrected means. It was computed from a simple variance analysis. The error variance, s^2 is

$$s_2 = \frac{\Sigma(\Delta i)^2 - \Sigma(Q_j)^2/t\lambda}{(rt - b - t + 1)},$$

where $\Sigma(\Delta i)^2$ is the sum of squares of the *differences* between paired muscles; r is the number of replications of any *one* treatment in a group ($r = 9$ in our experiments) and b is the number of muscle pairs in a group ($b = 18$ in our experiments). The standard error of a mean, (s_j), is not equal to $\sqrt{(s^2/r)}$ because the design is not orthogonal; it may be shown (Li, 1964) to be equal to

$$s_j = \sqrt{\frac{s^2k(t-1)}{rt(k-1)}},$$

where k is the number of treatments per block ($k = 2$ with paired muscles). The results obtained in the P series are reported and discussed in more detail in the preceding paper (Canfield & Maréchal, 1973).

Design of the myothermal experiments. The muscles used for the myothermal experiments were dissected at the same time as those used for the chemical experiments. They were poisoned with iodoacetate, if the corresponding muscles for the chemical experiment belonged to a group of the P series. They were tetanized on the thermopile for 12 sec (either in O_2 or in N_2 if they had been poisoned). We planned to measure the heat production of three muscle pairs for each group of the P and the O series. This plan was not fulfilled and only eight satisfactory records were obtained for each series. As it was essential that each muscle contracted only once, muscle pairs were rejected if technical failures had prevented a satisfactory first tetanus being obtained. Priority in the use of the remaining dissected muscle pairs was always given to the chemical experiments in order to preserve the balance of their statistical design. As a consequence, fewer muscle pairs remained for the myothermal experiments than was originally intended. It was not advisable to perform separate myothermal experiments at a later date because there would have been no sound basis on which to compare these with the earlier chemical results.

The heat records, corrected for heat losses, were analysed in periods of 1 sec except for the first such period which was divided into two halves. The mean heat production and its standard deviation was calculated for the eight records at 0·5, 1, 2, 4, 8 and 12 sec of tetanus for both the O and P series of experiments.

Heat measurements

The methods used to measure heat production and tension have been described elsewhere (Aubert, 1956; Aubert & Lebacq, 1971). The thermopile used in our experiments was made of two sets of constantan–nichrome couples which were connected in parallel in all experiments. Each set of couples was 9 mm long and the total length of the pile was 25 mm including three stimulating electrodes and four protecting couples.

The thermopile was calibrated in two different ways: (i) a single couple was

calibrated and its sensitivity was found to be 44·07 μV/° C, with a negligible temperature coefficient. For eighteen couples, the expected sensitivity was thus 793·3 μV/° C; (ii) the thermopile was rapidly transferred from water in equilibrium with ice into a bath of water maintained at a higher temperature and its output signal was recorded. This was performed separately for the two sets of couples. A sensitivity of 802 μV/° C was found, in good agreement with the expected value.

Each pair of muscles was tetanized only once as in the chemical experiments. After stimulation the muscles were drained, weighed, frozen in liquid N_2 and extracted in the same way as the muscles used for chemical estimations. These extracts were used for measurement of total creatine content. All the results are expressed per μmole of total creatine content of the muscle (C_T).

Temperature control

Although the muscles for heat measurements were processed simultaneously with those for chemical measurements, they were mounted on a different apparatus. It was important to ensure that the temperature of the muscles was the same in both cases. Two controls were used to check this.

1. The thermopile was immersed in a water-bath which was kept at 20° C ± 0·1. The muscles used for chemistry were surrounded by a water jacket into which water was pumped from a storage tank; the temperature of the water in the tank was also kept at 20° C ± 0·1. Temperatures were checked every 5–10 min for all muscles.

2. The temperature *of the muscle* on the thermopile was the same as that of the surrounding water-bath, as a state of thermal equilibrium had to be established before any measurements could be performed. This was not necessarily true for the muscles which were on the fast freezing apparatus. In control experiments, a thermocouple was inserted into a muscle hung on the freezing apparatus and submitted to the ordinary routine for the chemical experiments. At the time of stimulation, the temperature of the muscle was 20 ± 0·3° C. We feel confident, therefore, that the temperature of the muscles used for chemical experiments was, to within 0·4 C, the same as that of the muscles used for the heat measurements.

General and chemical procedures

These were identical with those described in the preceding paper (Canfield & Maréchal, 1973) and only a brief summary is included here.

The muscles were poisoned with iodoacetic acid (0·4 mM for 30 min at 20° C) if required, mounted either on the quick freezing apparatus (Maréchal, 1964) or on the thermopile and maintained, in oxygen or in nitrogen if they had been poisoned, at 20° C (± 0·3° C) for 10 min before stimulation at 50/sec. The muscles used for chemical determinations were frozen on the last shock of the tetanus by rapid immersion in isopentane cooled to − 160° C with liquid nitrogen and then extracted with perchloric acid. Creatine and phosphorylcreatine were measured by the alpha naphthol-diacetyl method. ATP, ADP, AMP and IMP were separated by thin layer chromatography (cellulose–PEI) and quantitatively estimated on the plate by reflectance measurements at 260 nm.

RESULTS

(*a*) *Tension, heat and chemical changes during a tetanus*

Figs. 1 and 2 summarize the results. The number of experiments used to obtain the means for each point is about the same for heat data (Fig. 1, $n = 8$) and for chemical data (Fig. 2, $n = 9$).

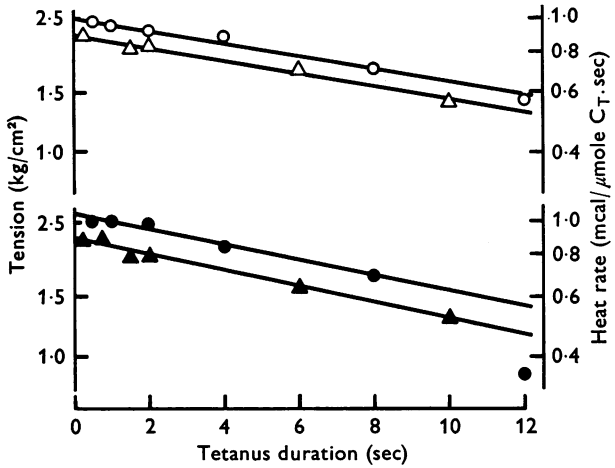

Fig. 1. The exponential decrease of heat-rate (triangles; mcal/μmole C_T.sec) and isometric tension (circles) with duration of tetanus for poisoned (filled symbols) and unpoisoned (open symbols) muscles (means of eight experiments). The tension at 12 sec in the poisoned muscles was not included in calculating the regression line. The coefficient of variation changes somewhat with the duration of the tetanus; it is about 14% for the heat rate and 11% for the tension. Both heat and tension are plotted on logarithmic scales.

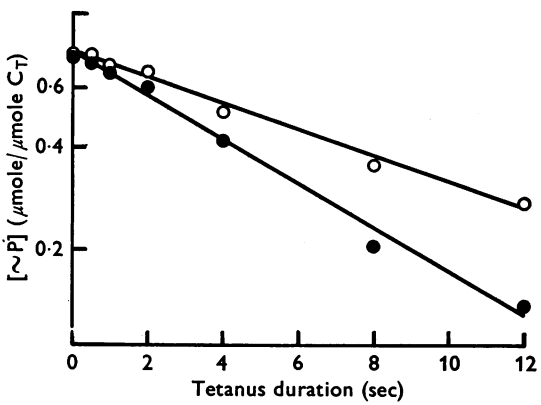

Fig. 2. The exponential decrease in ~P content (μmole/μmole C_T) on a *logarithmic* scale with tetanus duration in poisoned (filled symbols) and unpoisoned (open symbols) muscles. The S.E. of the means (n = 9) is the same for all the means; 0·025 (unpoisoned muscles), 0·026 (poisoned muscles). This is a property of the statistical designed used (see Methods). The tension developed by these muscles was practically the same as that shown in Fig. 1 for the muscles used in the myothermic experiments.

The rate of heat production decreases exponentially with tetanus duration; the time constant is about 17 sec, and it is not modified if the muscle is tetanized in nitrogen after poisoning with iodoacetate, in agreement with the findings of Hartree (1931) and Fisher (1931).

The tension also decreases during the tetanus; its time course is not really exponential, but falls according to an S-shaped curve. This is very clearly seen in poisoned muscles, where the tension falls abruptly after 8 sec of tetanus (see also fig. 1 of Canfield & Maréchal, 1973). However, the tension decay during the tetanus may be approximated by an exponential with a time constant of about 17 sec for both muscles in O_2 and in IAA–N_2 (up to the rapid fall after 8 sec of tetanus). This means that the ratio of the rate of heat production ($\dot{Q}$) over the tension maintained (P) times length (l_0) of the muscle is a constant. This ratio is easily computed with the results of our experiments. At the start of the tetanus, the heat rate is 0·9 mcal/μmole C_T.sec, and the tension is 2·5 kg/cm^2 (muscles in O_2). As there are 33·9 μmoles C_T/g, the heat rate per gram is 30·5 mcal/g.sec or 1300 g.cm/g.sec. This value divided by 2500 g/cm^2 equals 0·52 sec^{-1} (neglecting the density of muscle). It is lower than the values reported by Feng (1931) and by Aubert (1956) for muscles tetanized at the same temperature (20° C); these authors found that $\dot{Q}/Pl_0$ was equal to 0·90 sec^{-1}. Maréchal & Aubert (1958) found that, in muscles in O_2, the ratio $\dot{Q}/Pl_0$ was 0·70 sec^{-1} in the first tetanus but decreased on successive tetani to 0·35 sec^{-1} in the fourth one (at 18° C). It seems probable that the ratio $\dot{Q}/Pl_0$ may change somewhat according to conditions. It must be remembered that the muscles used in these experiments were dissected the previous evening, whereas the authors previously quoted made their measurements a few hours after the dissection. This may explain the apparently low value found in our experiments for $\dot{Q}/Pl_0$. However, a larger heat production would increase the imbalance described later in this paper between heat production observed and heat expected from the chemical reactions.

Fig. 2 summarizes the chemical data. For each tetanus duration, nine muscles have been analysed. The total high energy phosphate content of the muscle, designated total ~ P, decreases exponentially with the duration of tetanus. *The time constant is however completely different from that of the heat rate or the tension* (Fig. 1): it is about 9 sec for muscles in O_2, and about 5·7 sec for muscles in IAA–N_2. This difference is not explained by a corresponding difference in the time course of tension in the chemical experiments. On the contrary, this time course is the same (within 10 %) as that shown in Fig. 1 for the muscles used for myothermal experiments.

(*b*) *Recovery metabolism in unpoisoned muscles*

The implication of Fig. 2 is that recovery metabolism occurs in the unpoisoned muscles during contraction. Fig. 3 shows the total ~ P hydrolysed during the contraction for the two groups of muscles. The difference between the two curves is probably due to ~ P synthesis by recovery processes; it is also plotted (curve *A*). The rate of recovery

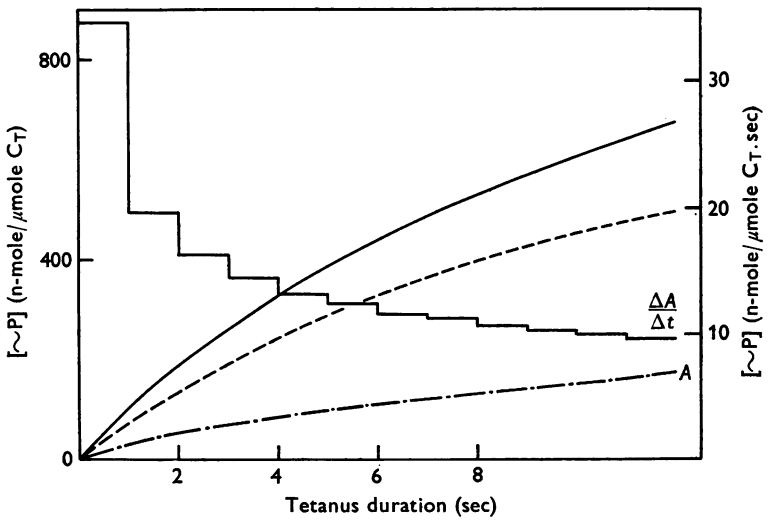

Fig. 3. The total ~ P hydrolysed (n-mole/μmole C_T) during the tetanus by the poisoned (continuous line) and unpoisoned (dashed line) muscles. The difference between the two curves, postulated as ~ P synthesis due to recovery, is also plotted (curve *A*). The rate of this recovery ($\Delta A/\Delta t$) is plotted on a different scale (right hand side: n-mole/μmole C_T.sec).

($\Delta A/\Delta t$) begins at a maximum in the first second of contraction (Fig. 3), declines rapidly at first and then much more slowly from 4–12 sec. Lactate concentrations were not measured in these muscles. In a separate series of experiments performed under identical conditions, it was found that lactate concentration increased from $0{\cdot}53 \pm 0{\cdot}05$ μmole/g for resting muscles to $2{\cdot}15 \pm 0{\cdot}23$ μmole/g for muscles tetanized isometrically for 12 sec ($n = 17$). Isolated muscle appears to be able to resynthesize ATP during a maintained isometric contraction at 20° C, partly from glycolysis. For this reason, the energy balance-sheet has only been drawn up in the case of poisoned muscles in which recovery metabolism was inhibited.

(*c*) *The heat expected from the reactions occurring during an isometric tetanus of an iodoacetate poisoned muscle in nitrogen*

The energy balance sheet for these muscles is reported in Table 1, for an isometric tetanus of 12 sec duration. Lines 1–3 in Table 1 are not the original data but have been calculated in accordance with the equilibrium hypothesis described in detail in the preceding paper (Canfield & Maréchal,

TABLE 1. Energy balance-sheet in a 12 sec isometric tetanus at 20° C for muscles in N_2 poisoned with iodoacetate (total creatine: 33·9 μmole/g) (see text for comments)

	Duration of tetanus (sec)						
	0	2	4	6	8	10	12
Substrates (n-mole/μmole total creatine)							
1 PC	666	481	347	250	181	131	95
2 ATP	86	83·4	79·8	68·0	55·6	44·5	33·1
3 AMP	0·1	0·4	1·3	2·6	5·4	9·2	14·4
4 IMP	0	0·8	2·2	5·0	10·1	19·7	37·7
5 ~ P hydrolysed*	0	188·7	328·7	441·5	530·9	605·3	676·0
Heat (μcal/μmole total creatine)							
6 Heat expected from ~ P hydrolysis	0	2075	3620	4860	5845	6660	7445
7 Heat expected from Lohmann reaction	0	−370	−638	−832	−970	−1070	−1142
8 Heat expected from myokinase reaction	0	1·1	3·5	7·5	15·5	28·8	52·1
9 Heat expected from IMP deaminase	0	2·3	6·7	14·9	30·3	59·1	113·0
10 Total heat expected from chemical reactions	0	1709	2992	4050	4921	5678	6468
11 Heat measured with thermopile	0	1801	3460	4950	6320	7550	8650
12 Ratio $\frac{\text{expected heat}}{\text{observed heat}}$	0	0·95	0·86	0·82	0·78	0·75	0·75

* The ~ P hydrolysed was obtained by subtracting the substrates at a time t sec from the substrate concentrations of the unstimulated muscle (t = 0 sec). It was computed as the *decrease* in PC and ATP to which was added the *increase* in AMP and IMP (see text: Results, section *c*).

1973). (The validity of this procedure when applied to energy balance is dealt with in the Discussion.) It is shown, in that paper, that the concentrations of PC, ATP, ADP and AMP remain in an equilibrium set by two reactions:

$$\text{the Lohmann reaction:} \quad \text{ADP} + \text{PC} \rightleftharpoons \text{ATP} + \text{C}_\text{F}, \tag{2}$$

$$\text{and the myokinase reaction:} \quad \text{ATP} + \text{AMP} \rightleftharpoons 2\text{ADP}, \tag{3}$$

throughout a 12 sec tetanus. The values in Table 1 were computed from these two equations, using $K_2 = 22$ and $K_3 = 0{\cdot}44$, and the exponential time course of PC break-down (fig. 2 in Canfield & Maréchal, 1973). The values obtained from this theoretical treatment are in very good agreement with those obtained experimentally (see fig. 3 of Canfield & Maréchal, 1973).

The advantage of this theoretical treatment is that it improves the accuracy of the observations. The reason for this is that the value of any substrate, ATP for example, at a duration of tetanus, t sec, is determined, not only by the observed mean value of ATP at t sec, but also by the mean values of PC, ADP and AMP at t sec in accordance with eqns. (2) and (3). The values of all four substrates at t sec are further determined by all the values at both earlier and later times than t because of the relation between PC break-down and duration of tetanus. In this way the value of the ATP at t sec, in our example, is contributed to by the whole 'weight' of the experiment and not simply by the nine values observed for ATP at t sec taken in isolation.

These muscles also produce IMP by the deaminase reaction:

$$\mathrm{AMP} + \mathrm{H^+} \rightarrow \mathrm{IMP} + \mathrm{NH_4^+}. \qquad (4)$$

The data reported in Table 1, line 4, were obtained by geometric interpolation from measurements of IMP made for tetani of 0·5, 1, 2, 4, 8 and 12 sec (detailed data in Canfield & Maréchal, 1973).

The cumulated amount of $\sim$ P hydrolysed during the tetanus (line 5) was computed as the *decrease* in PC and ATP to which was added the *increase* in AMP and IMP

$$(\Delta \sim \mathrm{P} = -\Delta\mathrm{PC} - \Delta\mathrm{ATP} + \Delta\mathrm{AMP} + \Delta\mathrm{IMP} = n_1).$$

Note that this computation does not involve ADP and so this was not included in Table 1. The heat produced by each reaction was computed, the molar enthalpy changes needed for these calculations being taken from Woledge (1971).

The heat expected from $\sim$ P hydrolysis (ΔH_1) is that for ATP hydrolysis, -11 kcal/mole, because presumably only ATP reacts with the contractile proteins (or the calcium pump). If n_1 is the amount of $\sim$ P split for a tetanus of up to 12 sec duration, the heat expected from this reaction is simply $-n_1 \Delta H_1$; it is reported in line 6 of Table 1.

The heat expected from the Lohmann reaction (eqn. (2)) is $+2$ kcal/mole. If n_2 is the amount of PC split, the heat produced is $-n_2 \Delta H_2$; it is reported in line 7 of Table 1. This reaction is only slightly endothermic, but, as n_2 is large, the heat absorbed by this reaction decreases the total heat production by more than 15%.

The heat expected from the myokinase reaction (eqn. (3)) is $\Delta H_3 = -1$ kcal/mole. The amount of AMP produced equals the *increase* in AMP, (n_3), *plus* the amount of IMP which appears in the muscle, (n_4). The heat expected from this reaction is therefore $-(n_3+n_4)\,\Delta H_3$. It is reported in line 8 of Table 1. The heat contributed by this reaction is negligible (less than 1% of the total heat) both because its ΔH is small, and the amount of AMP produced is small.

The heat expected from AMP deamination (eqn. (4)) is $\Delta H_4 = -3$ kcal/mole. The amount of IMP produced is n_4 (line 4, Table 1), and the heat expected from this reaction is $-n_4\,\Delta H_4$; it is reported in line 9 of Table 1. This heat is twice that produced by the myokinase reaction, but it is still only a small part of the total heat production.

The total heat production (Q_e) expected from chemical reactions is simply the sum:

$$Q_e = -n_1\Delta H_1 - n_2\Delta H_2 - (n_3+n_4)\Delta H_3 - n_4\Delta H_4. \quad (5)$$

It is reported in line 10 of Table 1.

(d) Energy balance in an iodoacetate poisoned muscle tetanized isometrically for 12 sec

The heat produced by an iodoacetate poisoned muscle is reported in Table 1, line 11, for an isometric tetanus of 12 sec (obtained from Fig. 1). It should be compared with the heat expected from reactions 1–4, which is reported in line 10 of the same table. The ratio of the two estimates of heat production is reported in the last line of the table.

During the first 2 sec of the tetanus, the heat produced is slightly larger than that computed. For such a short tetanus, the only important chemical change is a break-down of phosphorylcreatine; the changes in the concentrations of the nucleotides are very small. If they are neglected altogether, this small discrepancy may be explained by an underestimate of the enthalpy change for PC break-down used in our calculation: it equals the sum $-\Delta H_1 - \Delta H_2 = -9$ kcal/mole. An *in vivo* estimate of this value may be made from the ratio of the initial rate of heat production (0·88 mcal/μmole C_T.sec) to the initial rate of PC break-down (0·106 μmole PC/μmole C_T.sec) obtained from a regression analysis of the experimental data): it equals $-8{\cdot}3$ kcal/mole, slightly less (as expected) than the theoretical -9 kcal/mole which was used for the calculations.

In a longer tetanus, dephosphorylation of ATP to ADP and AMP and deamination of AMP to IMP occur to a considerable extent. The heat expected from these reactions is, however, much smaller than the heat produced by the muscle. The discrepancy increases with the duration of the tetanus and becomes so large that, after 12 sec, 25% of the heat measured by the thermopile is not accounted for by the chemical reactions

which have been measured. It then amounts to 2·18 mcal/μmole C_T, or about 74 mcal/g muscle.

DISCUSSION

We have found that the heat produced during an isometric tetanus (12 sec, 20° C) is much larger than the heat expected from the PC and ATP break-down under conditions where glycolysis is blocked by iodoacetate and aerobic metabolism is inhibited by lack of oxygen. Such a finding may have an obvious importance, as it may indicate unaccounted (or unknown) sources of energy for muscular contraction. However, one must first assess the significance of this imbalance, before any far reaching conclusion is considered.

(*a*) *Experimental errors*

A first explanation of the imbalance is that it arose from experimental errors of which there are several varieties. Absolute calibrations for creatine and nucleotides were all checked with internal standards. The mean total creatine content was 33·9 μmoles/g; this value is on the high side of those found in the literature. Nevertheless, our creatine determinations would need to be too low by 20% in order to account for the discrepancy. It seems impossible that such a large error occurred in the absolute calibration of the chemical measurements. The absolute calibration of the thermopile has been checked by two different methods (see Methods). The muscles used for chemistry were at the same temperature (within 0·4° C) as those used for heat experiments.

(*b*) *Physical effects*

Another possibility is that physical effects are responsible for the excess heat. If a sudden fall in tension in actively contracting muscle is brought about by a 'quick release' a quantity of heat (thermoelastic heat) is rapidly given out (Woledge, 1961). The progressive fall of tension during the tetanus results in a continuous production of thermoelastic heat. On the other hand, during the rise of tension, an equivalent heat absorption occurs so that, before complete relaxation occurs, the net effect is a heat absorption, which reduces the total heat production. The magnitude of this effect is small (of the order of 0·5–1 mcal/g).

During contraction, the muscle performs work in stretching its series elastic element and the external recording chain. The energy for this work is yielded by an equivalent $\sim$ P break-down, is stored in the various elastic elements as long as the tension is maintained and is finally degraded as heat when the tension falls, i.e. partly during the tetanus and partly during relaxation. This would increase the 'expected' heat when the

tension rises and the observed heat when the tension falls. The total energy stored in the elastic elements of a 100 mg muscle and the recording system used here is only 0·5–1 mcal/g (Maréchal, 1964).

The heating effect of the electrical stimulation enhances the observed heat and could contribute to the discrepancy. It has been measured in muscles poisoned with IAA and stimulated repeatedly until no mechanical response to stimulation could be observed (in these conditions, a thermal response could still be partly due to activation processes). A 12 sec stimulation then led to an increase in heat production of 0·270 mcal. For a muscle of 100 mg, this is 2·7 mcal/g, obviously too small to account for the discrepancy between observed and 'expected' heat production.

(*c*) *Statistical errors*

The statistical variations between muscles are considerable and are reflected in rather high standard errors of the means computed from eight or nine experiments. The coefficient of variation for the heat data is about 14 % for the short tetani of 0·5 sec and about 11 % for the longer tetani of 12 sec ($n = 8$, heat expressed in mcal/μmole C_T). The coefficient of variation for the chemical measurements also shows variation with tetanus duration. The highest values were found for the 12 sec tetanus: 19 % for PC, 26 % for ATP, 60 % for ADP, 110 % for AMP and 42 % for IMP. Even with these errors it is still not possible to explain this discrepancy. In fact, the theoretical treatment applied to the data (see Results, section *c*) effectively reduces these errors.

(*d*) *Errors in assumed enthalpy changes*

The third uncertain factor is that of the ΔH values used to calculate the expected heat. The values accepted for the myokinase and the deaminase reactions pose no difficulty, even an error of 100 % would change Q_e by less than 3 %. If the Lohmann reaction were thermoneutral, the expected heat calculated would be about 17 % too low. Even then, the observed heat would still be larger than Q_e (by 8 % for a 12 sec tetanus). Eventually, the enthalpy change of -11 kcal/mole for $\sim$ P hydrolysis is also open to some criticism. If the true enthalpy change for PC hydrolysis is -11 kcal/mole as reported by Wilkie (1968) rather than -9 kcal/mole (as reported by Maréchal, 1964), then the enthalpy change for $\sim$ P hydrolysis might be as high as -12 kcal/mole, an increase of 18 %; the expected heat would then be 87 % of the measured heat. It seems unlikely that ΔH for $\sim$ P hydrolysis might be higher than -13 kcal/mole (see Woledge, 1971). Even if we change the ΔH's used, we still cannot account for the imbalance. Whatever change is made, the expected heat remains lower than the heat observed.

(*e*) *Compounding the errors*

One may of course combine several corrections: for example, it could be that the heat observed is 10% too high, that the Lohmann reaction is really thermoneutral, and that the PC estimates are 10% too low. This would account for the difference but we feel that such an accumulation of errors provides an improbable explanation of this large discrepancy.

An alternative approach to this problem is as follows: if *all* the muscle PC and ATP were hydrolysed with a molar enthalpy change of −11 kcal/mole for both reactions, the total heat produced would still be 3% less than that observed experimentally. This is an extreme example because the muscles had not split all the PC and ATP at the end of a 12 sec tetanus. This is shown both by the chemical analyses (see Fig. 2) and the fact that the muscles relaxed completely and were, therefore, not in rigor. Furthermore, they would contract again if stimulated.

TABLE 2. A comparison between the expected heat calculated by use of the equilibrium hypothesis and the expected heat calculated from the original experimental data (see text for discussion)

Tetanus duration (sec)	2	4	12
Expected heat (μcal/μmole C_T)			
Equilibrium hypothesis	1709	2992	6468
Original data	1596	3209	6340
Difference: (equilibrium − original) (μcal/μmole C_T)	+113	−217	+148

(*f*) *Application of the equilibrium hypothesis*

It is necessary to ask if the use of the equilibrium hypothesis to calculate lines 1–3 in Table 1 could itself be the cause of the imbalance observed between expected and recorded heat production. This question may best be answered by comparing the values of the expected heat using the hypothesis (Table 1, line 10) with the values obtained by direct calculation from the original data used to develop the hypothesis. The results of this comparison for three durations of tetanus are shown in Table 2. There is a good agreement between the two expected heat values indicating that the use of the equilibrium hypothesis to derive the substrate concentrations in Table 1 is not contributing to the observed imbalance. We believe that the use of the hypothesis to derive the substrate concentrations provides a more accurate estimate of their 'true' values than the original data for the reasons described previously (Results, section *c*), it also allows a comparison between observed and expected heat to be made at times in the tetanus for which chemical measurements are not available.

(*g*) *Time course of energy balance*

This discussion neglects, however, an important finding shown in Figs. 1 and 2. The time courses of ~ P hydrolysis and heat production are both approximated by a decreasing exponential function, *but their time constants are different*: 5·7 sec for the rate of ~ P hydrolysis, and 17 sec for the heat rate. The change in the ratio 'expected heat/observed heat' (line 12, Table 1) reflects this fact. It is impossible to manipulate the data in such a way that the two phenomena have the same time constant. The only hypothesis which could explain the discrepancy is to postulate time dependent ΔH's but such an *ad hoc* hypothesis is not acceptable. The difference in time course between ~ P break-down and heat production indicates that a new source of heat production is mobilized as the tetanus duration increases. This fact suggests that this new source may be a recovery reaction.

(*h*) *Recovery reactions*

Recovery reactions may well occur during a 12 sec tetanus at 20° C as shown by the difference in the amount of ~ P hydrolysis observed between unpoisoned muscles in O_2 and muscles poisoned with iodoacetate in N_2 (Fig. 3). Part of this recovery appears to be glycolytic (see Results, section *b*). Although this difference is large, about 25% less ~ P hydrolysed in O_2, the heat produced and tension maintained by these two groups of muscles was similar. This suggests that the metabolic cost of the tetanus was the same for both treatments but that the sources of energy used were different, there being more recovery in the unpoisoned muscles. As the coupled processes of glycolytic and oxidative recovery produce more heat per ~ P rebuilt than reactions (2 to 4), the expected heat production of the muscles in O_2 should be somewhat greater than that expected from muscles treated with iodoacetate and N_2. However, our results indicate that the heat production was about the same for both treatments. It must be remembered that 25% of the heat produced by the poisoned muscles during a 12 sec tetanus was unaccounted for by our balance sheet (Table 1, line 12). There may be a similar, but not necessarily identical, imbalance between expected and observed heat production for unpoisoned muscles in O_2. As we have insufficient information to draw up a balance sheet for these unpoisoned muscles we are unable to test this possibility. In view of these uncertainties we cannot draw any firm conclusion from the observation that the heat production of the poisoned and unpoisoned muscles is similar despite the differences in their metabolism during the tetanus.

Fig. 3 shows that glycolytic recovery may start early during a tetanus at 20° C. It is conceivable that it starts as rapidly in iodoacetate poisoned

muscle, but that it must stop at the level of triosephosphate dehydrogenase (which is the enzyme blocked by iodoacetate). These muscles do not form lactate (as was checked on a few muscles). They may form hexose monophosphate, fructose 1,6-diphosphate and triose phosphates. However, hexose monophosphate formation from glycogen is only mildly exothermic (about -3 kcal/mole; Woledge, 1971). It would require the formation of about 25 μmoles/g to account for the excess heat in a 12 sec tetanus. It seems improbable that such a large amount of hexose monophosphate is found during a tetanus. Hermans (1956) found somewhat less than 1 μmole/g in iodoacetate muscle stimulated to rigor at 17° C. Phosphorylation of fructose 6-phosphate is strongly exothermic, -10 kcal/mole (Meyerhof & Schultz, 1936). However this reaction uses ATP, this will be accounted for in our balance sheet as if the ATP had been used by the contractile proteins, with a slightly different enthalpy change (-11 kcal/mole). This reaction cannot account for the excess heat production. As for triosephosphates, their formation from fructose 1,6-diphosphate is strongly endothermic, about $+14$ kcal/mole fructose 1,6-diphosphate (Meyerhof & Schultz, 1936); obviously if this reaction occurs to any extent, it would make our estimate of the excess heat too low.

We do not know to what extent oxidative reactions can proceed in muscles poisoned with iodoacetate and in nitrogen. We have assumed that no oxidative reactions of energetic significance can occur in this condition.

(*i*) *Comparison with other energy imbalance*

According to Gilbert *et al.* (1971), more heat is produced at 0° C during an isometric tetanus than can be accounted for by an equivalent ATP or PC break-down. This energy imbalance is limited to the first few seconds of the tetanus, and points to reactions which occur early in the tetanus.

In our experiments, the imbalance increases as the tetanus lasts longer, which suggests the occurrence of a *recovery* reaction. A second difference is the size of the imbalance: it is about 10 mcal/g in the experiments of Gilbert *et al.* and more than seven times higher in our experiments.

It is our opinion that the energy imbalance found by Gilbert *et al.* and that described in this present work do not have a common origin.

(*j*) *Conclusion*

Muscles stimulated for 12 sec at 20° C produce more heat than can be accounted for by the observed hydrolysis of $\sim$ P. The origin of the excess of heat remains unknown.

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