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THE HEAT OF SHORTENING DURING REPEATED TETANIC CONTRACTIONS OF MUSCLE TREATED WITH 1-FLUORO-2,4-DINITROBENZENE

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SUMMARY

1. The effect of repeated stimulation and of FDNB on the heat of shortening has been studied in sartorius muscles of *Rana temporaria* at 0° C.

2. The muscles were stimulated at 15 min intervals during 1·2 sec. During the plateau of tetanic activity, they were allowed to shorten 4·3 mm at constant velocity. After eight such contractions, they were poisoned with 0·4 mM-FDNB and the series continued.

3. From tetanus to tetanus, the tension during shortening decreased faster than the isometric tension; FDNB enhanced this effect. Despite a progressive decrease in the initial heat production, the heat of shortening did not change significantly in the normal muscle; immediately after poisoning it became larger, but from then on, it declined rapidly from tetanus to tetanus, faster than the over-all heat production.

4. The first tetanus after FDNB is thus especially favourable when looking for a chemical counterpart of heat of shortening. On the other hand, the postulated development of a friction in the intoxicated muscle cannot explain the changes in mechanical and thermal properties of the muscle. This point is discussed.

INTRODUCTION

In a previous paper (Aubert & Lebacq, 1971) it has been shown that when a tetanus with shortening is compared with an isometric tetanus, the excess of heat accompanying the change of length (i.e. the heat of shortening) persisted for its main part up to the end of relaxation. Therefore, it could not be interpreted as a reversible exchange of entropy and should not escape biochemical analysis: this interpretation had been proposed to account for the lack of chemical source of energy accounting for heat of

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shortening (see Mommaerts, 1969, and Woledge, 1971, for an extensive discussion).

But the most extensive search for the chemical counterpart of heat of shortening (Kushmerick, Larson & Davies, 1969) was partly made on muscles poisoned with 1-fluoro-2,4-dinitrobenzene (FDNB), which inhibits the resynthesis of degraded ATP by the exchange of energy-rich phosphate between phosphorylcreatine and ADP. Now, FDNB has been shown to affect the initial heat production, especially when stimulation is repeated (Aubert, 1964); for this reason, it was important to look into the effect of FDNB on the heat of shortening, so as to make sure that the shortening heat can be seen in such poisoned muscle, in conditions in which it is definitely present in normal muscle.

An additional reason for this enquiry is that FDNB also modifies the mechanical properties of shortening muscle: Dumoulin & Maréchal (1970) have shown that when muscles are stimulated repeatedly, the tension during shortening declines from tetanus to tetanus more rapidly than the isometric tension, this effect being greatly enhanced after poisoning with FDNB. According to these authors, the progressive development of a frictional force could account for the observed changes. Now, as the work against a frictional force is finally degraded as heat, the heat produced during shortening could be expected to increase from tetanus to tetanus, especially after poisoning with FDNB. It was thus decided to measure the effect of both repetition of stimulation and poisoning with FDNB on the heat of shortening.

METHODS

The experimental method has been described in a previous paper (Aubert & Lebacq, 1971). The following points are to be noted: pairs of sartorius muscles of male *Rana temporaria* have been used at 0° C. The average weight of a pair was 241 mg and the average P_0 (isometric tension at the standard length l_0) was 2·42 kg/cm^2.

The muscles were stimulated by condenser discharges at a frequency of 20 c/s during 1·2 sec. After 0·4 sec of stimulation at the length l_0, the muscles were released over 4·27 mm in 0·2 sec at a constant velocity controlled by a Levin–Wyman ergometer. After the movement was over, the stimulation was continued long enough to allow the tension to reach a steady level at the new shorter length.

Experimental design. In the experiments previously reported, a symmetrical plan had been adopted in which tetani with shortening were alternating with isometric tetani at both extreme lengths of the movement. In FDNB-treated muscles such a plan usually proved inadequate because of the rapid change of state of the muscle; therefore a different design was adopted dispensing altogether with isometric contractions. After thermal equilibration in moistened oxygen and before poisoning with FDNB, the muscles were stimulated eight times at 15 min intervals. Between the fourth and the fifth contractions, they were rinsed in oxygenated Ringer solution. At the end of this first series of eight contractions, the muscles were rinsed again for

30 min and then poisoned for 40 min with 0·4 mM-FDNB. A new series of contractions was then started, again at 15 min intervals; eight contractions could usually be recorded before obvious rigor started to develop.

RESULTS

1. The tension

The tension curve of a single contraction is represented in Fig. 1 (inset), where the tension has nearly reached its maximum before the shortening starts; the muscle is then released at a rather high velocity (2·14 cm/sec) and the tension drops accordingly; at the end of the movement the tension redevelops to its new steady value before relaxation occurs. Dumoulin & Maréchal (1970) have extensively reported on the effects of the repetition of stimulation and of intoxication with FDNB on both the isometric tension and on the force–velocity relationship. The present experiments confirm the earlier observations: from tetanus to tetanus in the unpoisoned muscle the tension during the shortening decreases faster than the isometric tension, so that the ratio 'tension during shortening/isometric tension' also decreases (by about 1% per tetanus); this tendency is enhanced in the FDNB-treated muscle, in which the ratio decreases by 5–10% per tetanus.

Oddly enough, the kinetics of the relaxation is not much altered by FDNB in our conditions. If anything, the tension decay tends to become faster as the active tension is reduced.

2. The heat production and the heat of shortening

Fig. 1 shows in a condensed state the analysis of the heat produced during every tetanus of a complete experiment. As the tension diagram in inset shows, each contraction–relaxation cycle can be divided in a series of 0·4 sec periods: during the first one (marked *A*), before the shortening, the muscle is isometric at the initial longer length, while the tension rises towards its maximum; the second 0·4 sec period (marked *B*) includes the shortening itself and the subsequent redevelopment of tension to about 95% of its maximum level at the new length. Considering the heat produced during this whole block largely avoids the necessity of correcting for an eventual thermoelastic effect of the change of tension (Woledge, 1961). During the third period (*C*) the muscle is isometric again, but at the shorter length; relaxation occurs at this new length during the fourth and the fifth periods (marked *D* and *E*).

Accordingly, the total heat produced during each tetanus has been divided between these five blocks (*A–E*), and is represented by a series of five points on the same vertical line in Fig. 1. The meaning of the symbols is given in the inset. The abscissa of the main Figure gives the serial number

of the tetanus in the experiment: tetani 1–8 before poisoning with FDNB; tetani 9–16 after poisoning (see Methods).

Before intoxication (tetani 1–8) the heat production decreased slowly and regularly from tetanus to tetanus in every block, except between tetani 4 and 5 when the muscle had been rinsed in Ringer solution. This shows the small effect of the rinsing as such. For each tetanus the diagram provides a

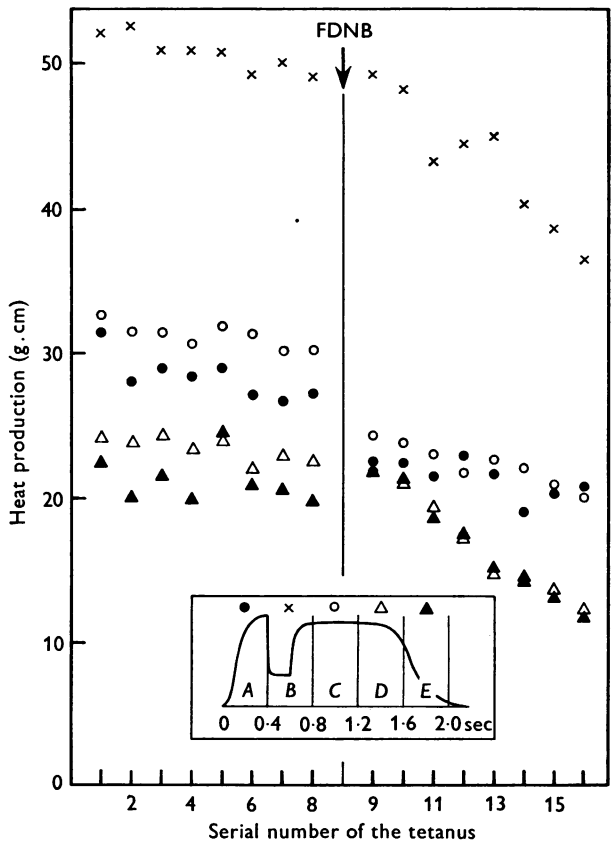

Fig. 1. Heat production during 1·2 sec tetani divided in blocks of 0·4 sec, as a function of the serial number of the tetanus. The vertical bar between tetani nos. 8 and 9 indicates the time when the poisoning occurred. In the inset: tension diagram of a single tetanus, divided in 0·4 sec periods so as to give the signification of the symbols. The length change occurs during the first half of the second period. (Expt. 3: P_0 = 196 g-force; l_0 = 3·2 cm; muscle weight: 268 mg.)

rough estimate of the heat of shortening, i.e. on each vertical line the difference of level between the cross (which corresponds to the 0·4 sec period including the shortening) and the two circles (which give an average

isometric reference). It is seen that this difference remains fairly constant from tetanus 1 to tetanus 8.

As for the effect of FDNB, it can be seen that it does not affect uniformly the heat production: on the one hand, the heat produced during both isometric periods of contraction (circles, blocks *A* and *C*) starts from a level which is usually lower (as in this example) and decreases slightly faster than before the intoxication; on the other hand, the heat production during both shortening (crosses, block *B*) and relaxation (triangles, blocks *D* and *E*) starts from nearly the same level as before poisoning but decreases at a much higher rate. Consequently the difference between the period of shortening and the average isometric period, which roughly corresponds to the shortening heat, is higher immediately after poisoning (tetanus 9) than before (by 25 % of the mean value in the normal muscle); moreover, it decreases in the subsequent tetani by about 6 % per tetanus.

TABLE 1. The heat of shortening and its variation in successive tetani before and after poisoning with FDNB. Heat of shortening estimated by the difference between heat produced during shortening and average isometric period (see text)

			Heat of shortening (g.cm)			
			Before FDNB		After FDNB	
Expt no.	P_0 (g)	n	Mean	Change per tetanus	First tetanus	Change per tetanus
1	163	8	19·02	+0·22±0·14	24·6	−1·54±0·39
2	218	4*	17·69	−0·31±0·61	21·7	−1·07±0·16
3	196	8	20·46	−0·44±0·37	25·9	−1·27±0·25
4	200	8	16·30	−0·16±0·26	14·8	−1·32±0·76
Mean	194		18·37	−0·17±0·28	21·8	−1·38±0·34

* In Expt 2 the length of the muscle was altered after the first series of four tetani in normal solution. Therefore the Table mentions only the results at the length finally adopted for the comparison between normal and intoxicated muscle.

A quantitative description of this effect has been attempted as follows: for each experiment, the heat of shortening has been estimated in every tetanus by calculating the difference between block *B* (including the shortening) and the mean of blocks *A* and *C* (average isometric block). The results before and after intoxication are reported in Table 1. They include for each pair of muscles one characteristic value of the shortening heat and its average change from tetanus to tetanus calculated by the method of linear regression. As the values before poisoning were fairly constant it is convenient to choose the mean value as characteristic for that period. For the poisoned muscle, however, the value given is the heat of shortening observed in the first tetanus after intoxication.

The column 'change per tetanus' shows that before intoxication the heat of shortening does not change significantly with the repetition of stimulation. As for the results after treatment with FDNB, the heat of shortening starts usually from a higher level than before poisoning (except in Expt 4), but from then on it decreases appreciably from tetanus to tetanus, by 6–8 % of the mean value before poisoning; except in Expt 4, where the scattering was rather large, this decrease is quite significant.

The evolution of the heat of shortening in successive tetani has been compared with that of the total heat. The heat of relaxation has not been considered in this comparison, for it decreases rapidly with successive tetani in the poisoned muscle (see Fig. 1); furthermore, it has been shown to be modified when a shortening has occurred during the contraction itself (Aubert & Lebacq, 1971); for these reasons, inclusion of the heat of relaxation would have obscured the comparison. The ratio (shortening heat)/(total heat of contraction) has thus been calculated for each tetanus. The results are given in Fig. 2, where the mean ratio for the four experiments is given as a function of the serial number of the tetanus. It is seen that the ratio does not change much with successive tetani in the normal muscle; but that it is appreciably increased by the poisoning; however, from then on it decreases from tetanus to tetanus, showing that the heat of shortening declines faster than the over-all heat production.

DISCUSSION

The present results show that in the same experimental conditions where a net thermal effect of the shortening has been found at the end of the whole contraction-relaxation cycle of a normal muscle (Aubert & Lebacq, 1971), FDNB-poisoned muscle still produces an excess of heat as it shortens; if anything, the heat of shortening seems to be higher immediately after the poisoning than before. When the stimulation is repeated, however, the heat of shortening decreases from tetanus to tetanus, which is not the case before poisoning of the muscle. This seems to indicate that the first tetanus after poisoning is especially favourable when looking for a chemical source of shortening heat, as in the experiments reported by Kushmerick *et al.* (1969). The discrepancy between myothermal and biochemical results is thus made more striking and accordingly, if no degradation of ATP equivalent to heat of shortening can be found in FDNB-treated muscle, other possible sources of energy accounting for the excess of heat observed must be looked for.

Table 1 and Fig. 2 show that FDNB enhances the heat of shortening both in absolute terms and relatively to the total heat production. This could be due to some hypothetical changes in the energy metabolism

induced by the drug. However, physical effects could also provide a simple explanation: poisoning with FDNB could result in the development of a friction between the actin and myosin filaments, opposing their sliding over each other. Energy spent by the muscle to overcome that friction during shortening would finally be degraded as heat, leading to an increase in the heat of shortening. According to Table 1 and Fig. 2, the postulated friction should *appear or increase* immediately after poisoning with FDNB, but from then on *decrease* from tetanus to tetanus.

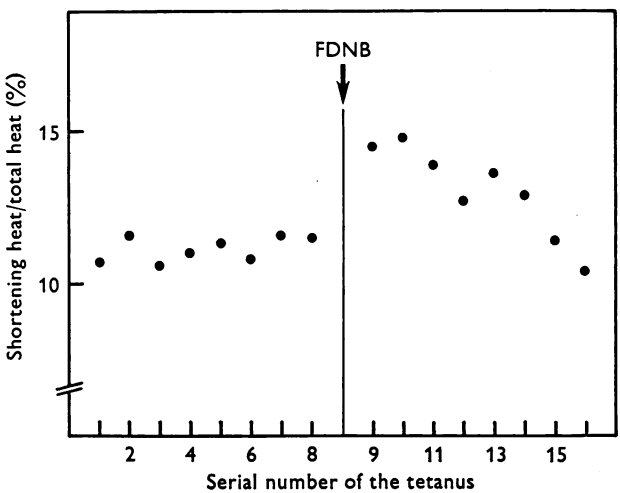

Fig. 2. The ratio (heat of shortening)/(total heat of contraction), in ordinate, as a function of the serial number of the tetanus. Same arrangement as in Fig. 1. (Note: the vertical scale is interrupted between 0 and 10%.)

In fact, a frictional force has already been postulated by Dumoulin & Maréchal (1970) in order to explain the mechanical properties of the FDNB-poisoned muscle. In their experiments, FDNB first increases the tension maintained during shortening at constant velocity; the dynamic tension then decreases with successive tetani at a higher rate than the isometric tension. In their opinion, such results could be explained if the immediate effect of FDNB were to *decrease* a pre-existent friction which would then *increase* rapidly from tetanus to tetanus. If their interpretation was correct, the heat of shortening, after an immediate depression by FDNB, should increase progressively, or, at least, decrease at a smaller rate than the overall heat production. For instance, from the data of Dumoulin & Maréchal, it can be calculated that in our experiments a supplement of about 0·8 g.cm of shortening heat should be produced after each tetanus as a result of the increased friction. As a matter of fact, Table 1 shows a reduction of about 1·4 g.cm per tetanus after the initial increase from 18·4 to 21·8.

The clear similitude of Fig. 3 of Dumoulin & Maréchal (1970) and Fig. 2 of this paper suggests that the mechanical and the thermal effects of FDNB are correlated, although they are not easily explained in terms of the concept of mechanical friction.

As for the heat of relaxation, it must be noted that the present results seem to contradict those of Aubert (1964), who observed that the relaxation heat became larger in FDNB-poisoned muscle, and was increased with each repetition of the tetanus. This increase was associated with a marked slowing of the tension decay. In our experiments the heat produced during relaxation decreased rapidly from tetanus to tetanus (see Fig. 1), with a slight quickening of the relaxation. In Aubert's experiments, however, the tetanus duration was much larger than in this case, of the order of 5–10 sec; after such long tetani, the rate of heat production during relaxation is not much changed but the relaxation phase becomes longer and longer, leading to a large excess of relaxation heat (X. Aubert, personal communication). This possibly accounts for the difference, although the exact reason is not known.

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REFERENCES

AUBERT, X. (1964). Tension and heat production of frog muscle tetanized after intoxication with 1-fluoro-2,4-dinitrobenzene (FDNB). *Pflügers Arch. ges. Physiol.* **281**, 13.

AUBERT, X. & LEBACQ, J. (1971). The heat of shortening during the plateau of tetanic contraction and at the end of relaxation. *J. Physiol.* **216**, 181–200.

DUMOULIN, C. & MARÉCHAL, G. (1970). Transient changes in the force–velocity relationship during tetanic contractions of frog sartorius muscles, normal or poisoned with 1-fluoro-2,4-dinitrobenzene. *Pflügers Arch. ges. Physiol.* **316**, 114–131.

KUSHMERICK, M. J., LARSON, R. E. & DAVIES, R. E. (1969). The chemical energetics of muscle contraction. I. Activation heat, heat of shortening and ATP utilization for contraction–relaxation processes. *Proc. R. Soc.* B **174**, 293–313.

MOMMAERTS, W. F. H. M. (1969). Energetics of muscular contraction. *Physiol. Rev.* **49**, 427–508.

WOLEDGE, R. C. (1961). The thermoelastic effect of change of tension in active muscle. *J. Physiol.* **155**, 181–208.

WOLEDGE, R. C. (1971). Heat production and chemical change in muscle. *Prog. Biophys. molec. Biol.* **22**, 37–74.