

Activation Heat in Various Cross-Striated Muscles of *Rana esculenta*: Microcalorimetric Measurements

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Abstract. Activation heat (AH) is an important component in energy balance of muscle contraction. It represents energy dissipation of biochemical processes enabling muscle in rest to perform contraction. According to our measurements on intact muscles, AH is 20% of initial heat production in twitch, and 10% in tetanus. Significantly different AH values have been obtained for muscles of parallel and not parallel fibre architecture.

Key words: Activation heat — Cross striated muscles — Frog

Introduction

Since 1950s, electron microscopy, X-ray and biochemical studies have been extending our understanding of muscle heat production.

Originally, activation heat (AH) was defined (Hill 1949) as "... the heat which a muscle would give out in a single twitch as a consequence of passing through a single cycle of activity if shortening, internal as well as external, of its contractile component could be completely obviated...", and also as representing the heat produced by a "...triggered reaction setting the muscle in a state in which it can shorten and do work". According to Hill AH cannot be measured directly. He determined it by subtracting heat of shortening from actual heat production. The magnitude of AH varied between 10–80% of initial heat production as estimated by different experimental methods (Hill 1949; 1950; 1958; Gibbs et al. 1966; Jöbsis 1968; Jöbsis and O'Connor, 1966).

According to Woledge (1971) all these investigations went away from the original idea as they did not eliminate heat produced by the actomyosin system.

According to the recent concept, AH is related to the Ca^{2+} cycle during muscle contraction. This heat is the sum of thermal effects of calcium liberation from sarcoplasmic reticulum, of its movement among the myofibrillar binding sites, and of its repumping to the storage sites. This transport process is ATP-dependent. To measure the heat produced by calcium movement the energy consumption by

cross-bridges has to be minimized. This can be done, according to Homsher et al. (1972) and Smith (1972), by stretching muscles up to the minimum filament overlap when the active muscle force development is negligible (Δ -state). AH measured according to this procedure was found to be 20–30% of the initial heat.

The maintenance heat in tetanus is strongly dependent on the type of fibre in the same sort of muscle (Elzinga et al. 1984), the tetanic force development not reflecting ATP splitting.

In the present study a different heat measurement procedure was employed: AH was investigated during twitch and tetanus contractions in different cross-striated muscles of frog.

Materials and Methods

Whole intact sartorius muscle, semimembranosus muscle and gastrocnemius muscle of *Rana esculenta* were used. M. sartorius and m. semimembranosus have fibers directed in parallel. Fibers are running more randomly in m. gastrocnemius, with numerous interposed tendons. The muscles were stretched up to the " Δ -state", with excitation-contraction coupling being maintained and thick-thin filament overlapping being nearly zero. Two criteria are generally accepted as the main check of filament overlapping in whole muscle:

- a. resting length, with isometric tension reaching its maximum value, and
- b. stretched state beyond delta state, in this case active tension can be neglected (minimum overlap), and there is a linear fall of tension between the two states.

The weight of muscles used for experiments ranged between 0.8–1.0 g for gastrocnemius, 0.7–0.9 g for semimembranosus, and between 0.3–0.4 g for sartorius. The tendons of gastrocnemius and semimembranosus muscles frequently broke during a twitch and tetanus when the muscles were stretched beyond $1.3 l_0$. Square-wave pulses of 30 ms duration and 6 V amplitude were used to elicit twitches, and a train of square-wave pulses of 66 Hz and 6 V amplitude lasting for 2 seconds were used to induce tetanic contracture. The heat production by the resting length (l_0) muscle was used as reference. The resting length (l_0) is defined by the in situ length of the respective muscle. It is by approx. 10% greater than the excessed length ($0.9 l_0$) at which maximum force is produced (i.e. $1.3 l_0$ is equal to $1.45 \times (0.9 l_0)$!). The heat flux was measured by a microcalorimeter developed in our laboratory (Lőrinczi and Futó, 1974) and based on heat conduction. The muscles were kept in normal Ringer solution (115 mmol/l NaCl, 2.5 mmol/l KCl, 1.8 mmol/l CaCl_2 , 3 mmol/l Na_2HPO_4 and KH_2PO_4) at pH 7.0 until the experiment. The experiments were done at room temperature (20 °C).

Results and Discussion

Fig. 1 shows heat flow curves for single isometric twitches of m.gastrocnemius, m.sartorius and m.semimembranosus at resting (l_0) and stretched lengths ($1.3 l_0$). The absolute values of heat production are summarized in Table 1. According to our experiments, AH is 20% of total heat production in a single twitch, and there is a significant difference between AH produced by muscles of parallel fibre

architecture and that produced by muscles with non parallel fibres. The active force exerted by stretched (30% over the resting length) muscles was below the

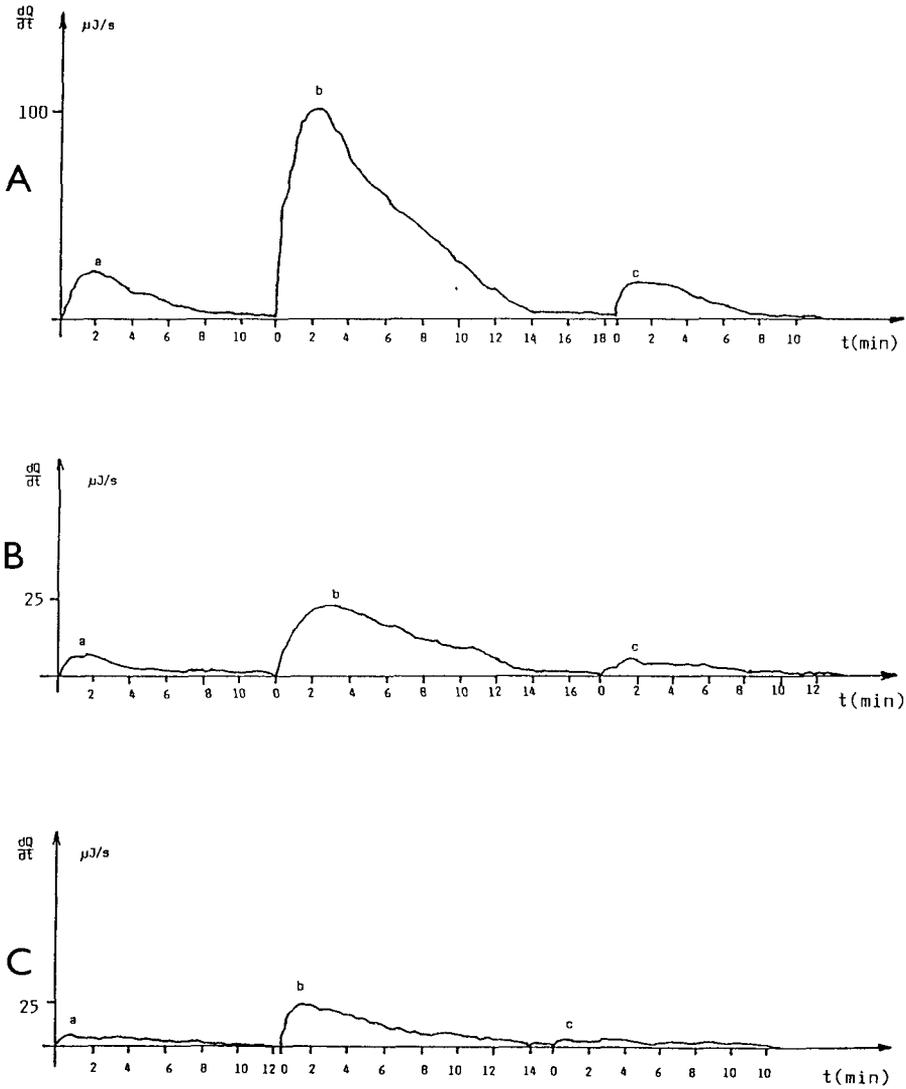


Figure 1. The heat flow curves of heat production during a single isometric twitch of m.gastrocnemius (A), m.semimembranosus (B) and m.sartorius (C) of *Rana esculenta* (a, c: at $1.3 l_0$, b: at l_0 , where l_0 = the resting length of muscle). Direct electrical stimulation with square wave pulse of 6 V and 30 ms duration.

Table 1. Means and standard deviations of twitch heat production related to standard muscle length. First three data from ten experiments on eight muscles. The heat production is expressed in mJ/g.

Muscle length (isometric twitch)	Type of muscle		
	M.gastrocnemius	M.semimembranosus	M.sartorius
1.3 l_0	3.38 ± 0.17	3.71 ± 0.48	4.70 ± 0.62
l_0	19.79 ± 0.63	19.70 ± 1.63	20.08 ± 1.71
1.3 l_0	3.19 ± 0.14	3.52 ± 0.41	4.10 ± 0.73

Table 2. Tetanic heat production: means and standard deviations related to standard muscle length and expressed in mJ/g. First three data from eight experiments on seven muscles.

Type of muscle	Muscle length		
	1.3 l_0	l_0	1.3 l_0
M.semimembranosus	5.92 ± 3.11	46.36 ± 6.24	5.43 ± 2.94

sensitivity of the force transducer (~ 0.01 N).

In experiments performed according to the original definition of activation heat (Hill 1949; Gibbs et al. 1966; Chaplain and Pfister 1970), the magnitude of AH was about 4.2 mJ/g. Similar values were obtained with muscles stretched beyond that point, where the overlap of the filaments eliminated (Homsher et al. 1972; Smith 1972). In these cases, AH was 30% of the heat production of an isometric contraction at resting length. The considerable scattering (10–80%) of early AH data (see Introduction) can partly be explained by the different methods used. Moreover, Hill and Woledge (1962) have estimated that the results of previous experiments had been loaded by an error of 40–60% due to miscalibration. The so-called Hill's type thermopile had been used to investigate muscle heat production since early 1940s. With this kind of sensors there is a direct mechanical contact between muscle and thermoelements. As discussed in one of our papers (Lőrinczi 1982), this layout does not exclude heat production due to mechanical interaction between the thermopile and muscle; this is not the case with our method (Lőrinczi and Futó 1974; Lőrinczi 1977; 1982). The difference in activation heats (20% of initial heat measured by us, and 30% reported by Homsher et al. (1972) and Smith (1972)), can also be interpreted as being due to differences in methods; the Hill-type apparatus still allows some mechanical interaction (friction) between the muscle and the thermopile, even during isometric twitch.

Our result is in good agreement with biochemical measurements suggesting

a close relationship between Ca^{2+} release in activation-relaxation cycle and AH (Kushmerick et al. 1969) as well as with ATP free energy dissipation (e.g. Homsher and Keane 1978), being roughly 20–35% of net ATP utilization.

The differences in AH values between muscles with different fibre architecture are in good agreement with data of various authors (Homsher et al. 1972; Elzinga et al. 1984), and they are supported by investigations in chicken (Rall and Schottelms 1973) and rat (Gibbs and Gibson 1972).

Twitches and tetanic contractures were investigated in the present experiments. To induce tetanus, *m.semimembranosus* was stimulated with square-wave pulses of 10 ms duration and 6 V amplitude at 66 Hz for 2 s. AH produced at $1.3 l_0$ was about 10% of total heat production of the tetanic contracture at resting length (see Table 2).

This is explained on the assumption that the maintenance heat is not the sum of AH of the individual twitches (Abbott 1951; Abbott and Aubert 1951; Aubert 1956); and that the stable heat rate component of maintenance heat (thought to be due to ATP splitting by cross bridges) should decline as sarcomere length increases (Aubert and Gilbert 1980; Elzinga et al. 1984).

There was no technical possibility for us to check whether macroscopic stretch by 30% also caused a stretch by 30% at the sarcomere level. Therefore, it is difficult to compare our data (referring to whole muscles) with those obtained for muscle fibres: changes in sarcomere length, the tension dependence of ATP-ase activity, and Ca^{2+} binding can be extrapolated from fiber to intact muscle only with difficulty (e.g. Tanaka et al. 1979). In this study we did not investigate the temperature dependence of AH which is important for the interpretation of energetic processes, this will be the aim of future experiments.

The main aim of this study was to draw attention to critical interpretation of the results of calorimetric experiments using different methods: the existence of heat of shortening cannot be conclusively proved using a microcalorimetric method (as used by us) but the magnitude of AH thus measured is in good agreement with the results obtained using biochemical and other myothermic methods.

References

- Abbott B. C. (1951): Heat production associated with the maintenance of prolonged contraction and the extra heat produced during long shortening. *J. Physiol. (London)* **112**, 438–445
- Abbott B. C., Aubert X. (1951): Changes of energy in muscle during very slow stretches. *Proc. Roy. Soc. London, Ser. B.* **139**, 104–117
- Aubert X. (1956): In: *Le Couplage Energetique de la Contraction Musculaire*. Editions Arscia. Bruxelles.
- Aubert X., Gilbert S. H. (1980): Variation in the isometric maintenance heat rate with muscle length near that of maximum tension in frog striated muscle. *J. Physiol. (London)* **303**, 1–8

- Chaplain R. A., Pfister E. (1970): The relationship between activation heat and calcium transients in frog sartorius muscle. *Experientia* **27**, 505—506
- Elzinga G., Peckham M., Woledge R. C. (1984): The sarcomere length dependence of the rate of heat production during isometric tetanic contraction of frog muscle. *J. Physiol. (London)* **357**, 495—504
- Gibbs C. L., Gibson W. R. (1972): Energy production of rat soleus muscle. *Amer. J. Physiol.* **223**, 864—871
- Gibbs C. L., Ricchiuti N. V., Mommaerts W. F. H. M. (1966): Activation heat in frog sartorius muscle. *J. Gen. Physiol.* **49**, 517—535
- Hill A. V. (1949): The heat of activation and the heat of shortening in a muscle twitch. *Proc. Roy. Soc. London, Ser. B.* **136**, 195—211
- Hill A. V. (1950): A note on the heat of activation in muscle twitch. *Proc. Roy. Soc. London, Ser. B.* **137**, 330—331
- Hill A. V. (1958): The relation between force developed and energy liberated in an isometric twitch. *Proc. Roy. Soc. London, Ser. B.* **149**, 58—62
- Hill A. V., Woledge R. C. (1962): An examination of absolute values in myothermic measurements. *J. Physiol. (London)* **162**, 311—333
- Homsher E., Kean C. J. (1978): Skeletal muscle energetics and metabolism. *Annu. Rev. Physiol.* **40**, 93—131
- Homsher E., Mommaerts W. F. H. M., Ricchiuti N. V., Wallner A. (1972): Activation heat, activation metabolism and tension-related heat in frog semitendinosus muscle. *J. Physiol. (London)* **220**, 601—625
- Jöbsis F. F. (1968): Mechanical activity of striated muscle. In: *Symp. Biol. Hung.* **8**, pp. 151—205 (Eds. E. Ernst, F. B. Straub), Akadémiai Kiadó, Budapest
- Jöbsis F. F., O'Connor M. J. (1966): Calcium release and reabsorption in the sartorius muscle of the toad. *Biochem. Biophys. Res. Commun.* **25**, 246—252
- 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 activation-relaxation processes. *Proc. Roy. Soc. London, Ser. B.* **174**, 293—313
- Lőrinczi D. (1977): The role of shortening and tension in the heat production of muscle. *Acta Biochim. Biophys. Hung.* **12**, 283—289
- Lőrinczi D. (1982): Reinvestigation of heat production of the contracting muscle by microcalorimetric method: the problem of the "heat of shortening". *Stud. Biophys.* **89**, 81—97
- Lőrinczi D., Futó Z. (1974): A new type of microcalorimeter for examination of the heat production of muscle. *Acta Biochim. Biophys. Hung.* **9**, 371—381
- Rall J. A., Schottelms B. A. (1973): Energetics of contraction in phasic and tonic skeletal muscles of chicken. *J. Gen. Physiol.* **62**, 303—323
- Smith J. C. H. (1972): Energetics of activation in frog and toad muscle. *J. Physiol. (London)* **220**, 583—599
- Tanaka H., Tanaka M., Sugi H. (1979): The effect of sarcomere length and stretching on the rate of ATP splitting in glycerinated rabbit psoas muscle fibers. *J. Biochem.* **86**, 1587—1593
- Woledge R. C. (1971): Heat production and chemical change in muscle. *Prog. Biophys. Mol. Biol.* **22**, 37—74