612.745.3

A REVISED ANALYSIS OF THE INITIAL HEAT PRODUCTION OF MUSCLE.

By W. HARTREE.

(*From the Physiological Laboratory, Cambridge.*)

WHEN a frog's sartorius muscle is mounted on a thermopile in the usual way, with a considerable length of the narrow part of it beyond the end of the thermopile, it can be seen on stimulation that there is an appreciable motion of muscle on to the thermopile due to the extension of the thinner part by the contraction of the thicker. This may happen although the ends of the muscle may be quite rigidly fixed. It is possible that this movement may have affected previous heat analyses to some extent, so revised analyses have been made, mounting the muscle in the reverse direction. The lower electrode is placed quite close to the end of the thermopile, and with the new arrangement the thinner end of the muscle is taken only 1 or 2 mm. beyond this electrode and is held firmly by the tendon and tied round fairly tightly at the electrode. Thus, during contraction, the motion of muscle on to the thermopile at this end is very small. At the other end of the thermopile the motion of the muscle on stimulation is negligible. Thus, with this method of mounting the muscle there can be very little change during contraction in the amount of muscle on the thermopile: its shape certainly alters perceptibly but this is unavoidable, and it can only be hoped that this change in shape is not sufficient to have such an effect on the flow of heat from muscle to thermopile as to upset analyses made by means of control heating curves taken when the muscle is in its resting shape.

RESULTS.

A. *Twitches.*

The results of the analyses in several experiments on single twitches at 0° C. were at least fairly uniform. Two curves are given in Figs. 1 and 2, these being somewhat similar to Fig. 5 in a previous paper [Hartree, 1931]. Since no cases like the anomalous figures with multiple "humps," or drawn out initial outbursts (Figs. 6 and 7 in that paper), were found,

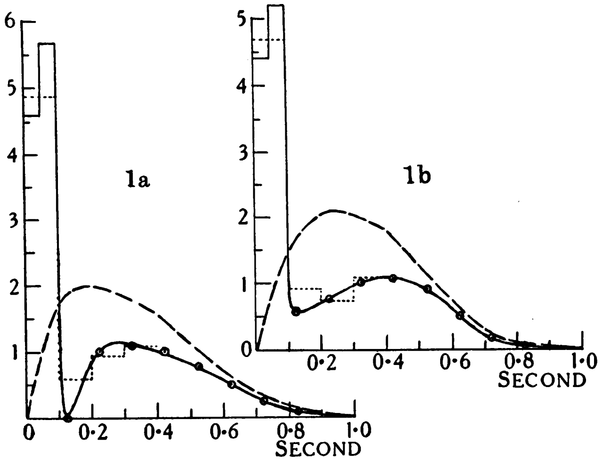

Fig. 1.

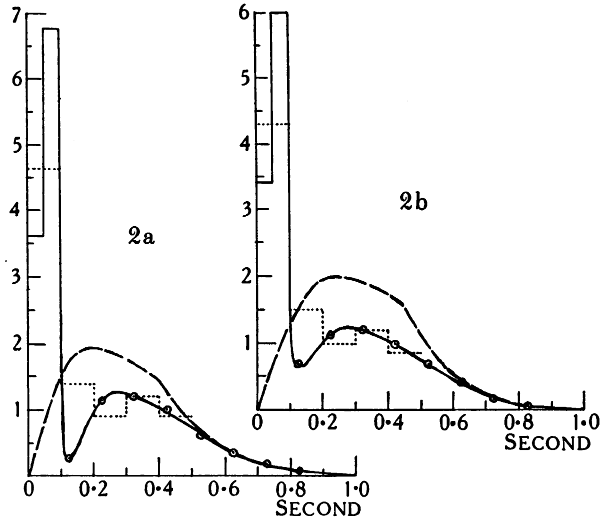

Fig. 2.

Figs. 1, 2. Single isometric twitches of frog's sartorius (English *R. temp.*) at 0° C. Full lines: results of heat analyses using control with 0·05 sec. heating and starting with two steps of 0·05 sec. Dotted lines: results of analyses of the same curves using control with 0·1 sec. heating (for comparison see text). Broken lines for tension curves: these show in every case a distinct "shoulder" between 0·4 and 0·45 sec., presumably due to friction between muscles and upper electrode. The tension curve ends very closely with the heat curve, between 1·0 and 1·2 sec., but cannot be shown clearly beyond 1 sec. Fig. 1 refers to one pair of muscles and Fig. 2 to another pair, these two experiments being chosen to show the greatest observed difference before 0·1 sec. Cases *a* refer to early stimuli and cases *b* to later and somewhat stronger stimuli.

The unit for the vertical scale is the total initial heat per sec. for each case.

it must be admitted that the peculiarities there shown were probably due to motion of the muscle on, or on to, the thermopile affecting the flow of heat from one to the other.

The course of the heat production during a twitch occurs in two phases, as has always been found before. If we regard the mechanical response as due to the formation and decay of some substance, or to the development and disappearance of some physico-chemical state, it is natural to associate one phase of the heat with the formation or development, the other with the decay or disappearance. Regarding the matter in another way, the first phase is waste heat in the development of tension, the second is potential energy degraded as the contraction disappears. It is possible, however (though see the discussion of Fig. 3 below), that the "dip" at 0·1 sec. may possibly be due either (*a*) to motion of the muscle during the rise of tension, or (*b*) to part of the muscle being less active than the rest.

Every precaution was taken to reduce the effect of motion of the muscle, and in the experiments referred to it is believed that the change in the amount of muscle on the thermopile during contraction was negligible; there is still the possibility, however, of an effect due to the change of shape, and this cannot be avoided.

As regards (*b*) it was noticed that for a single twitch the maximum tension seemed to increase continually as the strength of the stimulus was increased; thus, although care was taken to give a fairly strong stimulus it was always possible that there was some small region of inactivity in the muscle, and if the less active part were not in contact with the thermopile the result of such inactivity would necessarily show as a fall in the heat rate, *i.e.* of heat reaching the thermopile, quite soon after the stimulus. This possibility is discussed in another connection in a recent paper [Hartree, 1932]. It seems rather unlikely, however, that the inactive region would occur so regularly in the same position relative to the thermopile as always to give an artificial appearance of a separation between the contraction and relaxation phases of the heat production: though it must be admitted that the same side of the dissected muscle was always outside.

Without further improvement in technique, however, by which change of shape is altogether prohibited, or its possible effect annulled, it does not seem possible to obtain more decisive results.

A few details of the analyses should be mentioned. In every case control heating curves were taken by passing a high-frequency current through the living muscle for 0·05 sec.: this did not stimulate it. When, as in Fig. 2, the heat produced during the first 0·1 sec. is far from uniform, it is necessary, in order to avoid an early large remainder in the analysis, to start with two steps of 0·05 sec., but there is no advantage in continuing with steps of less than 0·1 sec. From the above control it is easy to construct one for 0·1 sec. heating, and the result of using this is shown by the dotted line; it is clear that the use of such a control in cases in which the heat rate is varying rapidly does not give a good estimate of the heat occurring in each 0·1 sec., and the estimate of that appearing between 0·1 and 0·2 sec. is so wrong as to be very deceptive.

It is obvious that, when using the 0·1 sec. control, any heat really occurring at 0·1 sec. will be considered as half from 0 to 0·1 and half from 0·1 to 0·2 sec. Similarly any heat really occurring uniformly between 0·05 and 0·1 sec. will be considered as ¾ from 0 to 0·1 and ¼ from 0·1 to 0·2. In Fig. 2 there is large heat from 0·05 to 0·1 sec. (not necessarily uniform), consequently the analysis by 0·1 sec. heating control will necessarily show about a quarter of this between 0·1 and 0·2, although in fact there may be no real heat in that interval. A further necessary consequence of taking too much heat in the interval 0·1 to 0·2 is that (to keep the remainders small) too little must be taken in the next step 0·2 to 0·3 sec., etc., the dotted line "oscillating" on each side of the more correct full line, the results being indistinguishable after 0·4 sec. It happens that the dotted lines avoid the "dip" at 0·1 sec. and so perhaps give a more reasonable looking result than the full line, but there is no doubt that the full line gives the best result possible from the observations.

The records for the twitch of Fig. 2*a* and the corresponding heating control are given for comparison with the cases which follow.

Sec.	0	·05	0·1		0·2		0·3		0·4		0·5		0·6		0·7		0·8	
0·05 sec. control	0	5	50	152	289	434	564	680	776	848	901	939	963	980	991	995	998	999
Fig. 2*a*	0		10		106		263		428		578		709		813		890	
Heat	0·180	·34	0·03		0·115		0·12		0·10		0·06		0·035		0·022			

Sec.	0·9		1·0		1·1		1·2		1·3		1·4		1·5		1·6
0·05 sec. control	1000	999	998	997	995	994	992	990	988	986	984	982	980	978	976
Fig. 2*a*	943		975		988		996		999		1000		999		998
Heat	0·01 approx.														

Details of the thermopile are given in the appendix. A Downing moving magnet galvanometer was used, sensitivity 1 mm. at 1 m. $= 6{\cdot}5 \times 10^{-9}$ amp., critically damped, total period undamped 0·81 sec., resistance 12½ ohms.

B. *Tetanus.*

A number of experiments were made using the Moll microgalvanometer constructed by Kipp. Although this galvanometer is not sufficiently sensitive to give good sized records for a single twitch, it can be used with excellent results for a tetanus. At 0° C. a tetanus of 0·5 to 1·0 sec. would give a deflection of about 50 mm., and at room temperature a tetanus of 0·2 to 0·3 sec. a deflection of about 100 mm. The mirror of the galvanometer is comparatively large, so good photographic records can be taken at a distance of 4 m. and the spot is always very steady. The action of the galvanometer is so rapid (total period 0·193 sec.), and the thermopile is so quick, that definite analyses can be made using steps of only 0·05 sec. This is necessary for experiments at room temperature (see below), but the galvanometer was also used for a few cases at 0°C., the

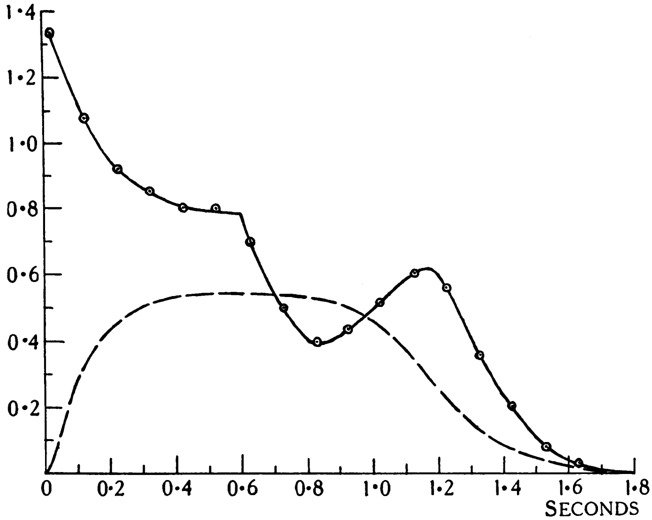

Fig. 3. Full line: result of heat analysis for 0·6 sec. tetanus at 0° C., employing the **Moll** galvanometer. Broken line for tension. The curves appear to end together. The numbers on the vertical scale must be divided by 1·02 to express heat rates in terms of the total initial heat per second.

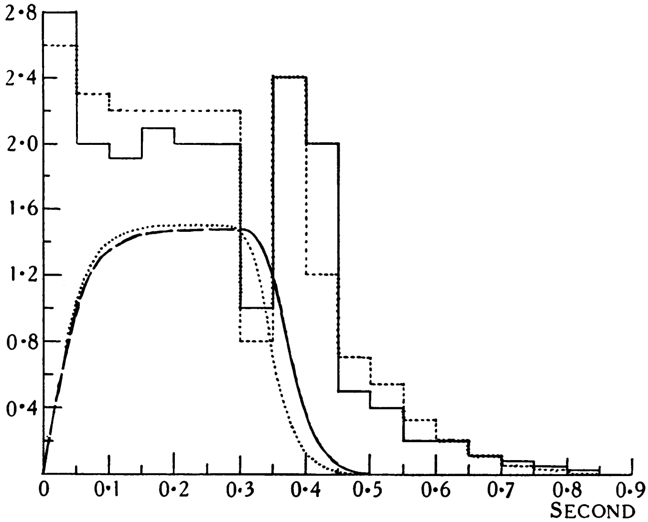

Fig. 4. Results of heat analysis for stimulus of 0·3 sec. approximately, at 17° C. Full line: result of heat analysis using **Moll** galvanometer and steps of 0·05 sec. Broken line for tension. Dotted lines for an experiment on another day, with stimulus about 0·28 sec., showing very similar results. In each case the heat production lasts much longer than the tension, so that only the early part of the heat production after the stimulus can be associated with the fall of tension energy. The vertical scale gives the heat rate in terms of the total initial heat per second.

result being always very "smooth," as in Fig. 3, of which the observed curves were as follows:

Sec.	0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0	1·1	1·2	1·3	1·4	1·5	1·6	1·7	1·8
0·05 sec. control	0	320	833	952	981	993	999	1000	999	998	995	991	987	983	978	973	968	963	957
0·6 sec. stimulus	0	16	73	125	169	213	253	292	324	349	372	393	421	448	472	488	496	499	500
Heat ...	0·067	0·054	0·046	0·043	0·040	0·040	0·035	0·025	0·020	0·022	0·026	0·030	0·028	0·018	0·010	0·004	0·002		

Details of the thermopile are given in the Appendix. The Moll microgalvanometer had a sensitivity of 1 mm. at 1 m. $= 4 \times 10^{-8}$ amp.; it was critically damped and had a resistance of 21 ohms.

It is clear, in this case at least, that the sudden drop in the rate of heat production at the end of a stimulus cannot be due to motion of the muscle, since in Fig. 3 the tension did not begin to fall appreciably for some time afterwards; indeed the rate of heat production had reached a minimum before any movement could have occurred. This is pertinent to the question, discussed above for the case of single twitches, of whether the break in the heat production just after 0·1 sec. might be due to movement.

In this, as in all the following cases, the muscle was mounted in the "reverse" position as described for the case of a single twitch. Usually, after the records had been taken, the muscle was observed when stimulated; its motion past the top end of the thermopile was always negligible and that at the bottom end of the thermopile was usually of the order of 0·5 mm.

The analyses at room temperature (16°–17° C.) agreed well. Several different experiments with stimulus 0·3 sec. gave very similar results, of which two are shown together in Fig. 4. The observed curves for stimulus and control, in the case of the experiments corresponding to the full line, were:

Sec.	0	0·05	0·1	0·15	0·2	0·25	0·3	0·35	0·4	0·45	0·5	0·55	0·6	0·65	0·7	0·8	0·9	1·0	1·1	1·2
0·05 sec. control	0	60	359	670	838	914	946	964	976	985	991	995	997	999	1000	1000	999	998	996	993
0·3 sec. stimulus	0	8	57	136	224	320	417	512	595	683	778	849	896	927	946	973	988	994	998	1000
Heat ...	0·14	0·10	0·095	0·105	0·10	0·10	0·05	0·12	0·10	0·025	0·02	0·01	0·01	0·006	0·005	0·004	0·003			

Thermopile and Moll galvanometer as before.

In every case the heat went on very appreciably after the tension had fallen to zero, which must be due to genuine delayed heat, as described in a recent paper [Hartree, 1932]. It cannot be attributed to delayed appearance of heat, due to inactivity of some of the muscle, as it comes too late for that and is about the same in every case. The early part of the heat production was very regular, in fact it is remarkable how steady was the heat rate during stimulation after the first 0·05 sec.

The analyses with steps of 0·05 sec. were as good and as definite as in the previous case of a single twitch with steps of 0·1 sec. By comparing the tables above it can be seen that the 0·05 sec. control with the Moll galvanometer is now higher at 0·1 sec. than it was with the slower moving magnet galvanometer at 0·2 sec. The analysis would be even better using a control employing still shorter heating, but with the high frequency oscillator available the amount of heat, and therefore the shortness of the heating, is limited. The improvement is entirely due to the quicker galvanometer action since the same thermopile was used in every case.

The Downing moving-magnet galvanometer could not take advantage of the comparatively large amount of heat in the tetanus at a higher temperature since, if it were made very insensitive and very rapid, the damping could not be increased sufficiently. It was necessary, therefore, to increase the damping as much as possible and then to diminish the sensitivity with a resistance.

SUMMARY.

It seemed possible that the analysis of the heat production during the initial phase of muscular contraction might be influenced by slight movements or changes of shape of the muscle during contraction. A modified arrangement has made it possible to eliminate to a considerable degree the effect of such possible disturbances. When this is done the anomalous cases described in an earlier paper disappear and the heat production in a single twitch is found to occur in two phases only—contraction and relaxation. In a tetanus the same two phases occur, and so long as the stimulus is continued the rate of heat production persists at a nearly constant but slightly decreasing rate, following a more or less sharp fall soon after the beginning of the stimulus.

I am greatly indebted to Prof. A. V. Hill for his encouragement and advice.

The Moll microgalvanometer referred to in this paper was purchased with a grant from the Royal Society.

REFERENCES.

Hartree, W. (1931). *J. Physiol.* **72**, 8.
Hartree, W. (1932). *Ibid.* **75**, 284, 373.

APPENDIX.

The properties of the thermopile employed.

The instrumental factors involved in the attainment of the greatest possible sharpness of analysis have been discussed by A. V. Hill (*Adventures in Biophysics*, Philadelphia, 1931, Appendix I, p. 142).

The properties of the galvanometers used in the present investigation have been given in the text: those of the thermopile are as follows (a single instrument, constructed by Mr A. C. Downing, was used throughout):

67 flat constantan-iron couples in a distance of 14 mm., resistance 23·7 ohms, bakelite insulation.

Quickness of response. This was determined by analysing a 0·05 sec. heating record by means of the galvanometer deflection due to an impulsive current. The latter might have been obtained experimentally: it was simpler and as accurate, however, to calculate it from the equation

$$y = \alpha t e^{-\alpha t},$$

where $\alpha = 2\pi/T$, T being the complete period (= 0·193 sec.). With this the current in the thermopile, during and after 0·05 sec. heating, had the following relative values:

Sec. ...	0·025	0·05	0·075	0·10	0·125	0·150	0·175
Current	19	45	68	83	89	93	95

Sec. ...	0·200	0·225	0·250	0·275	0·300	0·325
Current	97	98	99	99½	100	100

For instantaneous heating half the maximum thermopile current would be developed in about 0·04 sec., 95 p.c. of it in about 0·14 sec. The Moll galvanometer attains 95 p.c. of its full deflection to a constant current in about 0·10 sec., so is slightly faster than the thermopile.