Activation Heat in Frog Sartorius Muscle

C. L. GIBBS, N. V. RICCHIUTI, and W. F. H. M. MOMMAERTS

From the Department of Medicine, Los Angeles County Heart Association Cardiovascular Research Laboratory, University of California at Los Angeles

ABSTRACT Upon excitation of a muscle with two stimuli and variation of the interval between them up to the end of the period of full mechanical fusion, an increment of the isometric heat over that found in a single twitch is obtained. This is a good approximation to the activation heat, directly at 0°C, or after certain corrections which become important at higher temperature. The activation heat so found is independent of the muscle length and nearly independent of temperature. It is increased by nitrate and caffeine.

INTRODUCTION

From earlier investigations on the production of heat in muscles not performing external work, there has emerged the concept of an activation heat (e.g. Hill 1949, 1950 *a*, *b*, 1958) as that part of the isometric initial heat which is not correlated with measurable mechanical activity, and which represents "a triggered reaction setting the muscle in a state in which it can shorten and do work" (Hill, 1949). In the quoted investigations, Hill showed that this heat production commences during latency relaxation and thus antecedes tension development; gradually the activation heat has become operationally defined as the heat production which remains after work and tension development are eliminated, regardless of muscle length. It is thus distinct from the other components of isometric twitch heat which is now recognized (Hill, 1964 *c*) to include heat caused by internal work performance and heat associated with the persistance of tension during relaxation.

This paper will deal with a different approach to the measurement of activation heat in a muscle twitch, already outlined in a preliminary publication (Gibbs and Ricchiuti, 1965). The method consists of measuring the isometric initial heat resulting from the contractions caused by giving the muscle a conditioning stimulus followed by a test stimulus after a variable interval τ. Over a certain range of τ within the interval of complete mechanical fusion, the values of the heat so measured rise to a plateau; when spaced beyond the range of fusion, eventually the heat will be that of two separate twitches. It was suggested (Gibbs and Ricchiuti, 1965) that the occurrence of

a plateau in the curve indicated complete repetition of the activation cycle, but without repetition of the associated mechanical side effects, and thus the additional heat indicated by this plateau was taken to be the activation heat. The aim of the present paper is twofold: to further investigate the foundations and the sources of error of the method; and second, to apply it to see what factors may alter the activation heat so determined. Increased temperature, caffeine, and nitrate-Ringer's were the factors studied with respect to their influence upon the activation heat vs. stimulus interval (AHSI) curve.

METHODS

The experiments were performed on the sartorius muscle of *Rana pipiens*. The physiological solution used is described by Aubert (1956) and contains NaCl 95 mM; KCl 2.5 mM; $CaCl_2$ 1.0 mM; $MgSO_4$ 1.0 mM; $NaHPO_4 \cdot 2H_2O$ 1.7 mM; NaH_2PO_4 0.6 mM; $NaHCO_3$ 20 mM. The solution was aerated with 98% O_2 and 2% CO_2 and the pH was about 7.2. All experiments were run in the presence of *d*-tubocurarine hydrochloride at a concentration of 0.05 to 0.1 mg/ml of physiological solution. The nitrate solution was made by replacing the NaCl with an equivalent amount of $NaNO_3$. In some experiments solutions were made up by mixing proportions of the normal and nitrate solutions. Hypertonic Ringer's was made by adding sucrose to normal Ringer's solution, assuming 220 mM sucrose to be isotonic. The values for the tension per cross-sectional area, (Pl_o/M), exerted by our muscles were at first lower than those reported by A. V. Hill. In the *Rana pipiens* available to us, however, part of the sartorius muscle seems to fuse with the muscle below it, the adductor longus, before connecting to the pelvic bone. In dissecting out the muscles we were often losing up to one-third of the fibers at least as regards their ability to transmit tension to the attachment. This loss could be prevented by careful dissection and raised our Pl_o/M values from an average value below 1200 g/cm² to an average about 1500 g/cm². The muscles used in the present experiment had lengths ranging from 28 to 35 mm and weights ranging from 76 to 240 mg a pair. The experiments were run with the muscle length between l_o and $l_o + 2$ mm, except when the measurement required otherwise. Tension was recorded with a capacitance transducer described by Schilling (1960). The total compliance of the transducer, stainless steel rod, and cotton ties was 2.2×10^{-4} cm/g weight.

The heat measurements were done with a thermopile and photoelectrically amplified galvanometer system along the lines indicated by Hill (1965). The galvanometer was a Kipp A81 (Kipp & Sons, Delft, The Netherlands) with an undamped full period of 0.008 sec, an internal resistance of 20 ohms, an external resistance of 80 ohms, and a sensitivity for 1 mm deflection at 1 m of 100 nanoamp. Negative feedback was applied to achieve 90% response time of 5 msec. The thermopile was made up of 44 silver-constantan junctions of which 29 were active and 15 protective, with a total length of 14 mm and a thickness of 40 μ. Its sensitivity was 732 μv per degree. The muscle-thermopile system possessed an exponential heat loss with a constant, dependent on the muscle weight of 3 to 6% per second at 0°; this heat loss was electrically corrected by an integrating circuit. The required calibrations were

repeated for every relevant change in conditions such as muscle length or temperature. These various technical aspects will be described in full by one of us (N.V.R.) in a later paper.

In the present investigation, readings were taken after the initial rapid rise in temperature had taken place, and before the recovery heat became evident. At room temperature, this usually meant 2 to 5 sec after stimulation, at 0° after 3 sec or later.

The sartorii were mounted one on each side of the pile, with the pelvic bone in a clamp and the tibial ends tied to a thin, stainless steel rod leading to the isometric transducer. During an experimental series, the muscles were frequently reimmersed in the oxygenated bathing solution for interspaced recovery periods; measurements were then resumed several minutes after draining the muscles and pile. They were similarly reimmersed and drained at the termination of the experiments, the muscles cut free and weighed, first with the adhering fluid, then after gentle blotting; the difference averaged 8% of the weight.

The experiments consisted of measuring the total initial heat after activating the muscles with a double stimulus administered with a variable interval between them, and plotting the so determined heats as a function of the stimulus interval. Usually, measurements were commenced at the longest interval, continued sequentially till the shortest interval, and then continued in the reverse order. Sometimes, the opposite sequence was used The plotted data all are the averages of the values obtained in such a symmetrical sequence.

Unless otherwise indicated the temperature was 0°C, which was maintained by immersing the thermopile chamber in a well stirred mixture of ice and water contained in a large stainless steel Dewar vessel. Other temperatures, mostly 10–23°C, were maintained with good stability by filling the same vessel with water of the desired temperature. If temperature drifts occurred, they were too slow to disturb the measurements.

Stimulation was administered through platinum electrodes situated in the plane of the thermopile about 1 mm beyond either end. At first, square pulses were administered, 50 to 100% above the voltage required for maximal stimulation. The pulse duration was 3 msec at 0°, or 0.5 to 1.0 msec at room temperature. Later, condenser discharges (0.05 μf, 30 to 50 v) were used instead, to avoid, at room temperature, any possibility of break-excitation at the cessation of a square wave stimulus. The thermal equivalent of the stimulation was determined, in those cases in which it was not negligible, after the end of an experimental series by killing the muscles with 100 mM KCl and then applying the same impulse as was used in the experiments. The stimulus heat was substracted from the experimental results, giving a correction of 3% or less of the isometric twitch heat.

RESULTS

Foundations of the Double Twitch Method While some of the results will be given in a descriptive way, most will be used toward the establishment of a method for estimating the activation heat. The basis of this application will first be set forth.

As indicated in the Introduction, the unit experiment consists of measuring the total initial heat resulting from activating the muscle pair with a test stimulus administered after a selected interval from a preceding conditioning stimulus. Experimental records are reproduced in Fig. 1, whereas Fig. 2 shows the heat values plotted as a function of the stimulus interval. It is seen that the total heat, above the base line determined by the isometric heat in a single contraction (shown by the dotted line), rises in two stages. We propose that the first stage, from the origin to a brief plateau, represents the activation heat in response to the test stimulus, increasing as the lengthening interval

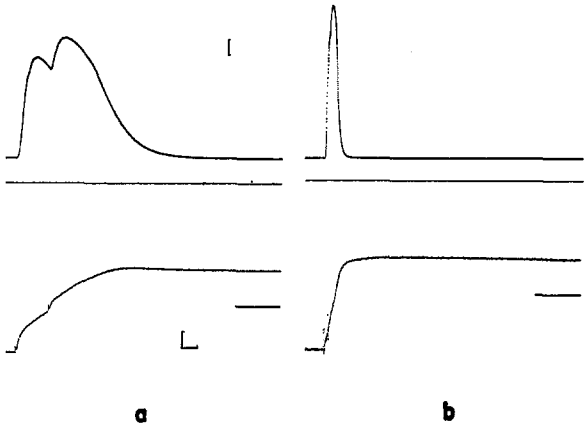

FIGURE 1. The tension and heat production records in response to two stimuli obtained at 0°C (*a*) and at 16°C (*b*). The top traces are the tension records and the calibration is 10 g per division; the bottom traces are the heat records: the calibration is 1.0 mcal/g muscle. The time scale is 250 msec/division. The horizontal lines in the heat records show the amount of heat produced in a single contraction. The interval between stimuli is 63 msec in (*b*) and 500 msec in (*a*). The muscle was 3.3 cm in length and had a blotted weight of 122 mg.

allows a more and more complete occurrence of a full activation cycle. The second stage of heat production commences when the interval is increased until the mechanical record shows that there is no longer complete fusion, from which moment the heat rises to a final plateau which approaches a level twice the value of isometric twitch heat. At this stimulus interval the mechanical record shows two separate twitches. Thus, as part or all of the mechanical response is repeated, the heat due to this cause is also added to the total heat.

According to Hill (1964 *c*) the energy output of a muscle in a twitch is:

$$E = A + \alpha x + W + h \qquad (1)$$

in which A is the activation heat proper (Hill, 1949), αx the shortening heat in which the coefficient α is now known to be dependent on the load or tension

(Hill, 1964 *a*), W the work done, and h an extra term related to the presence of tension (Hill, 1964 *b*). The heat output in an isometric twitch is then:

$$H = A + \alpha X_i + W_i + h \tag{2}$$

in which X_i is the internal shortening when the contractile component stretches the internal and external compliances, and W_i the work performed in this deformation.

In a double twitch we obtain therefore:

$$H + \Delta H = A + \Delta A + \alpha (X_i + \Delta X_i) + W_i + \Delta W_i + h + \Delta h \tag{3}$$

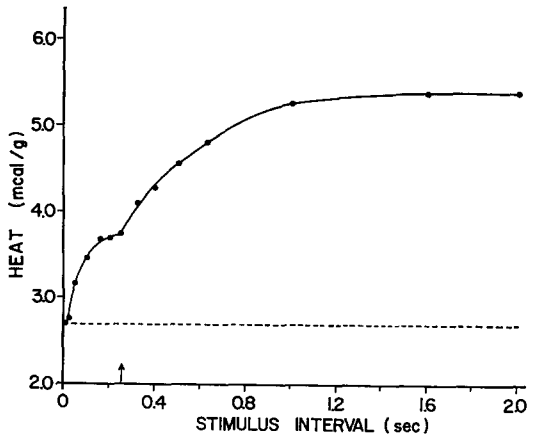

FIGURE 2. The total heat production at 0°C in response to two stimuli in a pair of frog's sartorii, blotted weight 115 mg and length 3.4 cm. The abscissa is the interval between the two stimuli and the dotted line represents the heat produced in a single twitch, which was 2.7 mcal/g. The arrow indicates the mechanical fusion interval.

in which the Δ terms denote the increments of the respective quantities in response to the second stimulus, stipulating that α may not have strictly the same value in the case when peak tension is increased notably. Thus, subtracting (2) from (3) one obtains:

$$\Delta H = \Delta A + \alpha \cdot \Delta X_i + \Delta W_i + \Delta h \tag{4}$$

In the ideal case, approximated in most of the experiments, we assume that (*a*) there is no tension increase with the test stimulus, or if there is it has been eliminated by the correction described below, thus $\alpha \cdot \Delta X_i$, ΔW_i, and Δh become zero, (*b*) ΔH measured at the end of the plateau equals ΔA, and if activation heat production is nearly complete at this time, then ΔA equals A.

These approximations appear to be adequate when there is a plateau as in Fig. 2. However, there are exceptions; in one batch of frogs only half the animals gave this result, and in all about one-fifth of the frogs we examined did not show a well defined plateau (Fig. 3). This often correlates with a low twitch to tetanic tension ratio.

When working at 0°C, the twitch:tetanus tension ratio is usually about 0.8 and the increase in tension at the plateau interval was often below 10%.

If it becomes 10% or more, as is especially the case at higher temperatures, the approximations lose validity. A modified experimental approach will be described which may extend the method to such cases (*The Influence of the Conditioning Stimulation Pattern*), but this has not yet been developed.

Instead, it is possible to apply a correction for the effect of additional tension development. This is often superfluous at 0°C but essential under

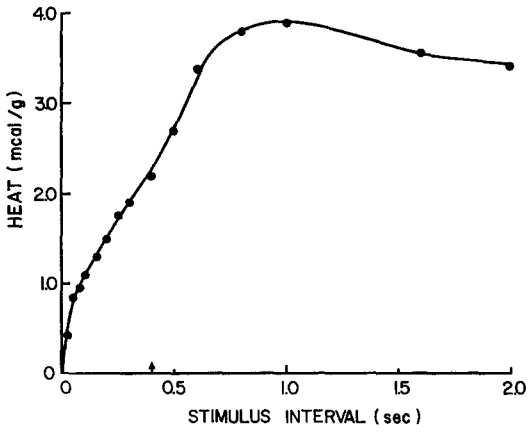

FIGURE 3. The same plot as in Fig. 2, except that the base line heat in response to one stimulus is not shown. The mechanical fusion interval is marked by the arrow. Note the absence of a plateau and the phase of supernormal heat production at stimulus intervals in the region of 1 sec. The muscles had a blotted weight of 196 mg and a length of 3.3 cm. The temperature was 0°C.

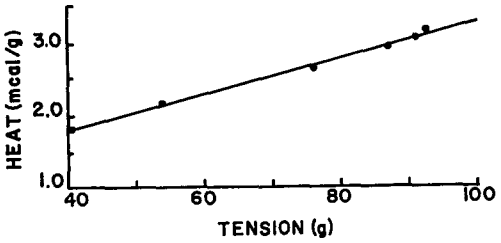

FIGURE 4. The relationship at 0°C between the tension developed in a twitch and the heat produced, in a muscle that had a blotted weight of 188 mg and a length of 3.2 cm. Each point is the average of 3 determinations at the following muscle lengths, reading from the left, $l_o - 8$, $l_o - 6$, $l_o - 4$, l_o, $l_o + 2$ mm.

other circumstances. The correction involves the determination of the relationship between heat and tension in a twitch. Tension was varied in the present paper by altering the muscle length as shown in Fig. 4. This relationship is often, but not always rectilinear. The curve was determined for each muscle pair when the AHSI curve was to be corrected and used as follows (Fig. 4): assume that the second stimulus, occurring at about the fusion interval, increases the summated tension to a value 5 g above that in the single twitch; from the plot it can then be read that this tension corresponds to 0.12 mcal per g of heat. The quantity so determined for *each* of the different stimulus intervals is subtracted from the total heat values at those intervals

to give the corrected AHSI curve. At 0°C, the correction is often negligible, but at room temperature where the tension increment is large, the correction becomes essential (see Fig. 9).

The method of estimating the increment in tension, above the tension in a single twitch, is shown in Fig. 5. It is this tension increment, used in conjunction with a heat tension plot (Fig. 4), that determines the heat correction. These corrections often allow the determination of an AHSI curve even in those muscles which do not show a plateau (Fig. 3); in about half these cases, the correction lead to the appearance of a well defined plateau to the AHSI curve; in the others, the deviations must have been too large, or further complicated by additional factors.

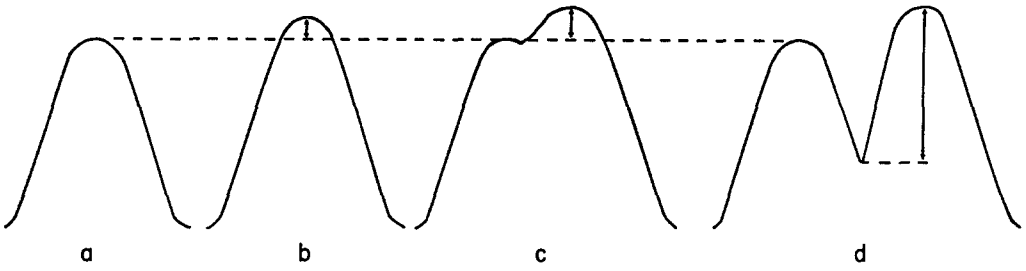

FIGURE 5. Schematic drawings showing the tension record in response to a single stimulus (*a*) and in response to two stimuli *b*, *c*, *d*. In (*b*) the stimulus interval is less than the mechanical fusion interval, in (*c*) the stimulus interval is nearly equal to the fusion interval, and in (*d*) the stimulus interval is in excess of the fusion interval. The arrows indicate the measured tension increment used in making heat corrections.

Comparison with Hill's Methods Hill (1949) made the first estimate of the activation heat by stimulating a muscle after it had been maximally shortened by previous stimuli. This method was applied to all muscles and gave the same results as the double twitch procedure. In those muscles which do not show a plateau even after the tension correction, Hill's method is still applicable and was used by us. Hill (1950 *b*) has also stretched muscles until they could no longer develop active tension at which time activation heat can be determined. We found this method to be less reliable in comparative studies because of the muscle damage that frequently occurs. Hill (1958) has also used another method which is described later.

Stimulus Intervals beyond Fusion At stimulus intervals beyond the mechanical fusion interval there is additional heat as described, and eventually we obtain double the amount of heat in a single twitch. In some trials, we have attempted to apply the heat-tension correction to this phase as well, as is shown in Fig. 6; the tension increment was measured as in Fig. 5*d*. It might be expected that from the plateau onward this correction would keep the

results at the same level. This occurs occasionally, as shown in Fig. 6 (bottom), but in general there is a subsequent rise in the corrected curve (top). This rise appears not to be caused by a passing increase in activation heat, but by an error inherent in the correction procedure when applied over this interval range (see Discussion).

Uncoupling of Active Tension In order to measure the magnitude of the activation heat beyond the fusion interval, and as further substantiation of the work in general, we have eliminated active tension development by a method used by Howarth (1958) and Hill (1958), by immersing the muscle in

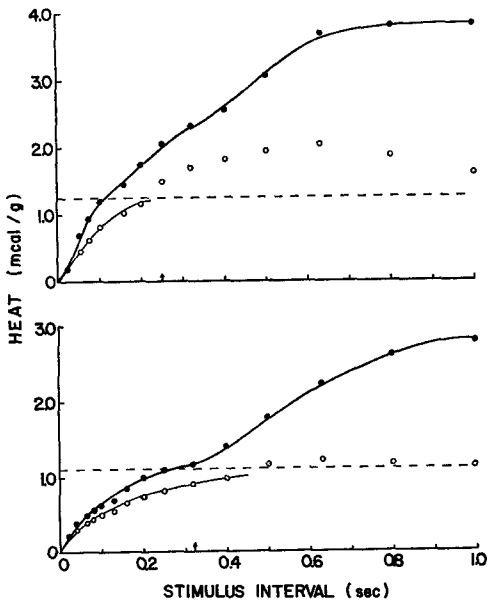

FIGURE 6. The same plot as in Fig. 3 (Solid circles) for two different muscles. The open circles show the tension-corrected records. The temperature was 0°C. The dotted horizontal line shows the magnitude of activation heat obtained by shortening the muscle. (See text.) Top, blotted muscle weight 210 mg; length 3.4 cm. Bottom, Blotted muscle weight 112 mg; length 3.1 cm.

a Ringer solution made hypertonic by the addition of 440 mM sucrose. After soaking the muscles in this medium for 15 to 60 min, no twitch tension is produced, but activation heat is maintained. Thus, the double stimulus experiment can be carried out in the absence of tension (sometimes the second stimulus may produce up to 3 g of tension), and the AHSI curve now assumes the simple form shown in Fig. 7. In comparison with a normal muscle, it is found that a heat production equal to that at the plateau is now found to be independent of the stimulus interval beyond the fusion period. The sucrose heat records have been corrected for the change in muscle mass that occurs after soaking in hypertonic Ringer's. In the illustration shown, the value of activation heat measured either by Hill's method (dashed line) or by the two twitch method (open circles) in normal Ringer's is in good agreement with the value found in hypertonic Ringer's (solid horizontal line). However, in

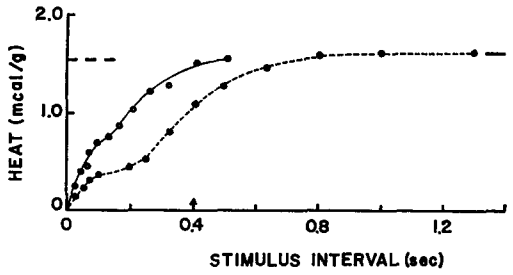

FIGURE 7. The tension-corrected AHSI curve (open circles) obtained at 0°C in normal Ringer's. The AHSI curve in hypertonic Ringer's solution in the absence of tension (solid circles). The dotted horizontal line denotes the value of activation heat in normal Ringer's obtained by shortening the muscle as far as possible. The solid horizontal line shows the amount of activation heat in response to a single stimulus in hypertonic Ringer's. The muscle had a blotted weight of 180 mg and a length of 3.4 cm.

the majority of the muscles we examined activation heat was 10 to 20% less in hypertonic Ringer's than in normal Ringer's whether measured by the two twitch method or by shortening the muscle down (Hill, 1949). The change in the shape of the AHSI curve in sucrose may be due to altered diffusion times.

The Effect of Muscle Length The double twitch method permits us to study the effect of the muscle length upon the activation heat. We used the correction method described previously to correct for any tension increments at the various muscle lengths. We refer to Fig. 8 for typical results, for lengths

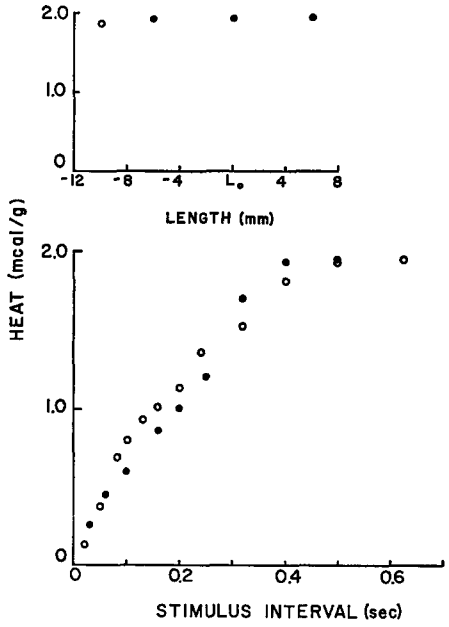

FIGURE 8. The effect of muscle length on activation heat. Top, solid circles represent the values of activation heat at l_o + 6 mm, l_o, and l_o − 6 mm determined by the two twitch method. The open circle shows the value obtained by shortening the muscle until it could no longer develop twitch tension (Hill, 1949). This occurred at l_o − 10 mm. Bottom, The tension-corrected AHSI curves obtained at l_o − 6 mm (open circles) and at l_o + 6 mm (solid circles). Note that the final value of activation heat is the same. The temperature was 0°C and the blotted muscle weight was 185 mg, length 3.3 cm.

both below and above l_o. While the time course of the AHSI curves differs slightly at different lengths, the final quantities are identical.

The Effect of Temperature The obvious influence of temperature is well illustrated in Fig. 9; as the temperature rises, the plateau of the AHSI curve is reached at shorter stimulus intervals, the temperature coefficient is considerable. As noticed before, it becomes essential at elevated temperatures to

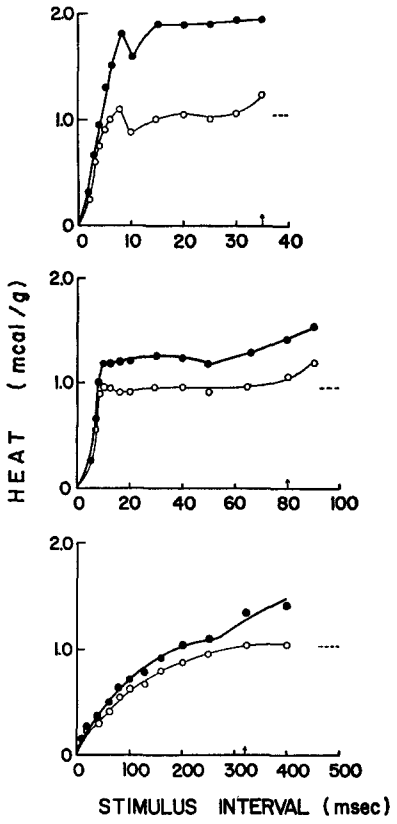

FIGURE 9. The effect of temperature on the AHSI curve. Top, 19°C; middle, 10°C; bottom, 0°C. The open circles show the tension-corrected records. Note that the heat tension correction increases as the temperature increases. The dotted lines represent the value of activation heat measured by the shortening method. Note the pronounced dip in the AHSI curve at 19°C. The blotted muscle weight was 160 mg and the muscle length was 3.2 cm.

apply the tension correction, as the twitch:tetanus tension ratio often becomes appreciably smaller than at 0°C. We made heat:tension plots at all temperatures used for each muscle examined.

The effect of temperature upon the absolute magnitude of the activation heat is somewhat variable. In most cases, there is no sizable change. Table I lists the results obtained both with the present procedure and with the Hill procedure of stimulating a maximally shortened muscle. While there is some variability, perhaps due to the limited accuracy of the correction, it is evident that there is no systematic temperature effect upon magnitude as there is upon the time course.

Another feature of the results at temperatures above 0°C is the appearance of a dip in the AHSI curve. This is often observable in the tension record as well. This effect on tension of two closely spaced stimuli has been described by Brown and Sichel (1940) and was taken to be an additional indication of the "alpha process." The latter name is given to the phenomenon of increased rate of tension development and peak tension seen if a muscle is exposed to several atmospheres of pressure for a very short time at the beginning of a

TABLE I

Experiment No.	Date	Activation heat		Temperature	Twitch tension	Tetanic tension
		mcal/g		*°C*	*g*	*g*
1	29 Oct. 64	0.8		7	64	
		0.85		13	61	
		0.8	0.9*	16	42	
2	1 Nov. 64	1.05	1.15*	0	56	74
		1.15		7.5	62	94
		1.3		15	67	102
		1.3	1.2*	21.5	58	102
3	18 Nov. 64	0.9		0	65	80
		0.9		9	47	110
		0.9	1.0*	18	30	122
4	20 Nov. 64	0.85		0	58	81
		0.9		9	62	95
		1.0	1.0*	18	45	92
5	11 Dec. 64	1.0		0	70	
		0.95	1.0*	10	46	
		1.1		19	32	
6	3 Feb. 65	1.55	1.6*	0	103	
		1.5		5	100	
		1.5	1.5*	18	74	

* Values obtained by shortening the muscle length until no tension developed (Hill, 1949).

contraction. Brown and Sichel reported that the effect was much more pronounced at elevated temperatures. In those instances, where the dip in the AHSI curve was not observed, it may have been absent, or it may have been missed if the narrow interval range over which it occurs was not used.

The Influence of the Conditioning Stimulation Pattern We have also studied the effect of giving several conditioning stimuli instead of one prior to the test stimulus. As shown in Fig. 10 this causes a significant change in the AHSI curve. Full activation heat is now produced at stimulus intervals greater than in the case of the single conditioning stimulus. It should be emphasized, however, that the final activation heat magnitude is the same in both cases.

These experiments might suggest an alternative method for eliminating the effects of increased tension developed by the test contraction, and so obviate

the need for the tension correction in those cases in which this is now used. We wish to leave this possibility open for future investigation, but must indicate its disadvantage: when the heat produced in the conditioning contraction is increased by multiple stimulation, this will necessitate working at a lower sensitivity setting; the heat resulting from the test contraction becomes a smaller fraction of the total measurement and therefore is obtained with less accuracy. Also, the effect of possible changes in the economy of the muscle will need investigation. The present method assumes the activation heat to be independent of the preceding stimulus, and nothing has indicated otherwise. With several conditioning stimuli, however, this may need further inquiry.

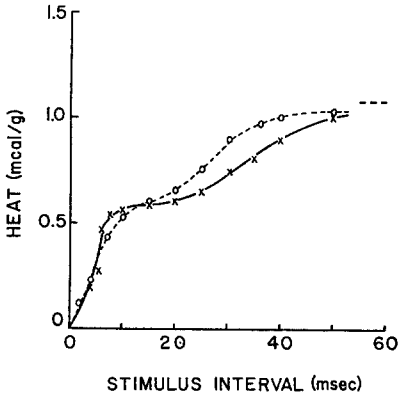

FIGURE 10. This figure illustrates the change in shape of the tension-corrected AHSI curve caused by giving two conditioning stimuli, separated by 10 msec (×) and one conditioning stimulus (open circles). The ultimate level is the same in both curves. Temperature was 18°C. The dotted line is activation heat measured by the shortening method. The blotted muscle weight was 76 mg and the muscle was 3.4 cm in length.

The Effect of Nitrate Ions These experiments were carried out in Ringer's solutions in which 70 to 100% of the NaCl had been replaced by $NaNO_3$. In most cases, measurements were done in chloride and nitrate alternately and the effects were completely reversible.

The presence of nitrate increases the activation heat at all temperatures (Table II), but there are certain changes in the AHSI curves. If these were evaluated only up to the end of the fusion interval in chloride, they would lead us to believe that there was no increase in activation heat, since the value of activation heat up to there is often unaltered. But if the complete course of the curve (Fig. 11) is taken into account, and the plateau is read at the interval of fusion in nitrate and not in chloride, the activation heat is clearly enhanced. Nitrate is known to prolong the active state duration (Hill and Macpherson, 1954; Ritchie, 1954; Kahn and Sandow, 1955) and the finding that the interval of mechanical fusion is prolonged is in line with this. The increase of the activation heat was also found by the other methods. It is to be noticed, however, that the initial part of the AHSI curve often has a similar time course to that obtained in ordinary Ringer's, only the latter part

leads to additional heat. At 0°C, the mean values were 1.2 mcal/g in normal Ringer's, 1.6 mcal/g (7 muscles) in nitrate medium; at room temperature (16–23°) these means were 1.3 and 1.8 mcal/g (12 muscles).

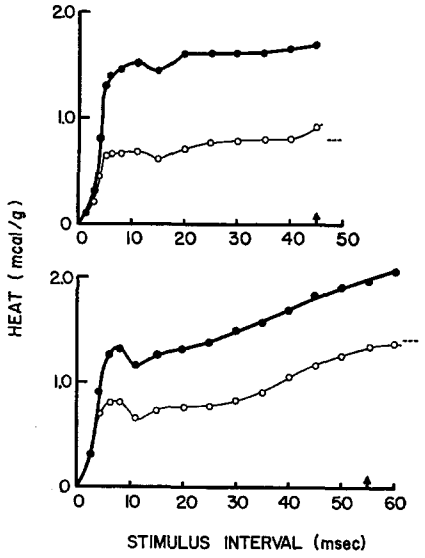

FIGURE 11. The AHSI curve in normal Ringer's (top) and after a 45 min soak in 100% nitrate Ringer's (bottom). The open circles are the tension-corrected records. The dotted lines and the arrows have their usual significance. The blotted muscle weight was 166 mg, length 3.3 cm, and temperature 17°C. Note the increase in the mechanical fusion frequency.

TABLE II

Experiment No.	Date	Solution	Activation heat		Twitch heat	Twitch tension	Temperature
				mcal/g	*mcal/g*	*g*	*°C*
1	9 Jan. 65	Normal	1.15	1.1*	2.8	60	0
		90% NO_3	1.4	1.35*	3.3	52	
2	12 Jan. 65	Normal	1.6	—	3.1	84	0
		80% NO_3	2.3	2.4*	3.6	92	
3	14 Dec. 64	Normal	1.0	1.05*	2.5	50	0
		100% NO_3	1.65	1.65*	3.5	48	
4	6 Jan. 65	Normal	1.1	1.1*	1.8	27	21
		100% NO_3	1.7	1.7*	4.05	70	
5	10 Dec. 64	Normal	1.0	1.0*	1.65	30	17
		100% NO_3	1.7	1.75*	3.9	76	
6	1 Nov. 64	Normal	1.3	1.35*	4.25	58	21.5
		80% NO_3	1.65	—	4.65	60	

* Values obtained by shortening the muscle length until no tension developed (Hill, 1949).

The Effects of Caffeine This drug was used in concentrations of 1 to 1.5 mM; at 2 mM there was evidence of contracture as manifested by an increased resting heat rate and a small increase in resting tension. The effects of caffeine are not completely reversible; thus, it was not possible to repeat ordinary experiments as controls afterwards. After an ordinary series in Ringer's

solution the muscles were soaked for 30 to 60 min in the caffeine-containing medium and the experiments were repeated.

The results are similar to those in nitrate Ringer's, as the activation heat increases in magnitude (see Fig. 12). This change is progressive, being greater after 60 than after 30 min exposure to caffeine. The mean values at 0°C are

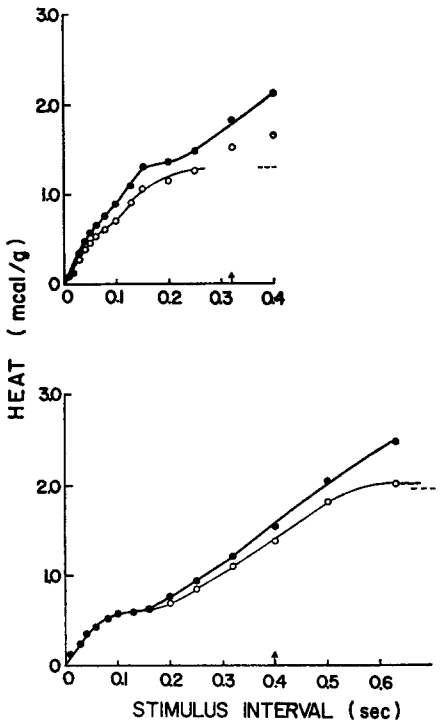

FIGURE 12. The AHSI curve in normal Ringer's (top) and after a 30 min soak in Ringer's solution containing 1.0 mM caffeine (bottom). The open circles are the tension-corrected records. The dotted lines and the arrows have their usual significance. Note the pronounced plateau in the AHSI curve in caffeine. Temperature 0°C; muscle length 3.4 cm; muscle blotted weight 187 mg.

2.0 mcal/g in caffeine as compared to 1.4 mcal/g (6 muscles) in normal Ringer's, and 1.7 as compared to a normal of 1.2 mcal/g (11 muscles) at room temperature.

DISCUSSION

The results depend upon the changes in total heat production that occur when the interval between two stimuli is varied from zero to just past the mechanical fusion interval. The findings are interpreted in terms of the various components of twitch heat, as set forth by Hill (1964 *c*), but can be explained in any scheme in which there is a tension-independent and a tension-dependent component or components. The experiments provide a method for measuring activation heat. This gives the same result as other methods previously employed by Hill, but offers the advantage of being applicable at any length, and without the need for altering the osmotic environment of the tissue. The

experiments are technically simple, since they require no resolution of the heat production as a function of time, the total heat being read several seconds after the last stimulus. Indeed the experiments do not seem to differ from those by Hartree and Hill (1921) when they were examining a different problem.

As mentioned in the preliminary presentation of the fundamentals of the method, it is assumed that there is no tension increase caused by the second stimulus, or that this has been eliminated by the tension correction. In reality, the neglected quantities may not be completely zero, and ΔA would then not equal A, but the occurrence of a plateau in the AHSI curve indicates that such errors as may occur are either negligible or zero. When no plateau is seen, the approach cannot be used at all, because we do not know which condition has failed. But when the conditions are fulfilled, the method gives results which are in excellent accord with the shortening method of Hill (1949). The correction procedure is essential at higher temperatures, where, without it, no valid results would be obtainable.

The approximations break down when the quantities related to internal work, shortening, and feedback heat in equation (4) become sizable; that is when the test stimulus causes a significant increase in tension. A correction based upon measurement of the increase of isometric heat with tension was found to eliminate this satisfactorily when used up to the end of the interval of mechanical fusion; thereafter, it is usually unsuccessful. Hartree and Hill (1921) have noted that in this range of stimulus intervals there is often but not always a supernormal amount of heat liberated, and always a supernormal tension phase (Fig. 6 of their paper); the two supernormal phases do not have to coincide. Clearly there are unknown phenomena here, and neither our direct correction method nor that used in Fig. 6 of Hartree and Hill, nor other efforts we have made, have been successful. One possible explanation is suggested by the results of Ritchie (1954), who showed that when the tension exerted by the muscle is still high, the active state can have declined to a low value. Thus for a critical range of stimulus intervals after the tension peak in response to the first stimulus, the second stimulus will arrive when the muscle tension is still high, because of the series compliance of the muscle, but when the active state may be low. The test stimulus will therefore produce only a small increment in tension and hence in ΔX and ΔW_i but it will cause a large change in the active state intensity and hence in h. A correction based solely on tension change would then be inadequate.

It is not advisable, as we did in our preliminary paper (Gibbs and Ricchiuti, 1965), to express the activation heat as a fraction, 0.39 ± 0.03 (SE), of the isometric twitch heat; the latter is greatly dependent on various factors and its variations would affect the percentage ratio independently of true changes in A. Instead, we now express the activation heat in absolute

figures. It amounts to 1.2 ± 0.3 (SD) mcal per g at 0°C (mean of 36 muscle pairs) out of a total isometric heat of 3.0 ± 0.5 (SD) mcal/g at l_o; in the temperature range between 15 and 22°C, these figures are 1.2 ± 0.2 (22 muscle pairs) out of 2.7 ± 0.9 mcal/g. It can be seen that at 0°C, activation heat represents 0.4 of the isometric twitch heat, while at room temperature it represents 0.45 of the twitch heat. In both cases, however, it had the same absolute value; *i.e.*, 1.2 mcal/g muscle. These figures apply to winter frogs.

The effects of temperature are of considerable interest. While there is a large effect upon the time relations, the magnitude of the activation heat is practically unaffected. The relative effects of nitrate and of caffeine are also independent of temperature. This finding must be distinguished from the sizable increase of the maintenance heat rate with temperature, for which Hartree and Hill (1921) and later authors (Feng, 1931; Aubert, 1956) reported a Q_{10} of 2.8 or more. Maintenance heat in a tetanic contraction is the summated effects of the heat of activation resulting from successive stimuli (Hill, 1949). The high temperature coefficients, found by the above authors, are not in disagreement with our finding that activation heat is independent of temperature. The high temperature coefficients result primarily from the effects of temperature upon the mechanical fusion interval which is also apparent in our results.

It seems appropriate to attempt some interpretation of the course of the AHSI curve. Two points must be kept in mind (*a*) except at 0°C, the shape of the AHSI curve is noticeably changed by the tension correction employed; (*b*) if the AHSI curve has not reached a plateau before the fusion interval, then we cannot follow its subsequent time course, although we may know the ultimate level from other measurements of activation heat.

One possible explanation is that the AHSI curve reflects the decay of the active state. As the active state from the first stimulus progressively declines, then more energy will be needed to reinstate it to full intensity, the later the second stimulus will occur. It must be remembered, however, that activation heat starts being liberated before there is any sign of contractile activity (Hill, 1950 *a*). Thus there is little reason to expect that the AHSI curve should exactly correspond to the mechanically measured active state curve. For example the level of free intracellular calcium rather than the degree of completion of the actin-myosin interaction could be the factor that controls the amount of activation heat produced. The time course alone of the AHSI curve clearly shows that it is not an exact counterpart of the mechanical active state decay as presently measured. In some other respects it corresponds closely to known properties of the active state. Ritchie and Wilkie (1955) have shown that prior stimulation will lengthen the active state duration and the results in Fig. 10 show a slower decay and a pronounced plateau in the middle of the AHSI curve. The effects of nitrate and caffeine are interesting.

There is a pronounced slowing of the decay, and a plateau midway through the AHSI curve becomes very pronounced both at 0°C and at room temperature. Now many investigators have shown that these agents increase active state duration (Kahn and Sandow, 1955; Hill and Macpherson, 1954; Ritchie, 1954), but these authors also reported that active state intensity was not increased. We have found, however, that activation heat is increased about 40%. At 0°C we did not see much evidence of increased tension and occasionally we even recorded a slight fall in twitch tension in nitrate or caffeine. Recently, however, Sandow and Preiser (1964) have reported that if active state intensity is measured by determining the velocity of shortening of the contractile component, both nitrate ions and caffeine do markedly intensify the active state during the early part of a twitch. Thus tetanic tension may not always be a true reflection of the maximum capability of the contractile mechanism of a muscle. This point has been discussed by Jewell and Wilkie (1958); Pringle (1960); Sandow (1961); and Pennycuick (1964).

Another interpretation was suggested to us by the results of Hill (1932). Using frog sciatic nerves Hill examined the "recovery in a capacity to produce heat in response to a shock, as a function of the interval between shocks." He stimulated a nerve at different frequencies and then plotted the heat production per impulse against the stimulus interval. The resulting curve has a time course that resembles the AHSI curve and at 0°C it possesses a time constant of the order of 0.2 sec. Clearly this curve was not produced by the differential return of individual fibers to a state of excitability as the electrical refractory period was much less than 0.2 sec. While this recovery curve is not explained in detail, it serves to focus attention on the thermal effects of excitation rather than on those of contractile activity. Abbott, Hill, and Howarth (1958) have shown that the positive heat per impulse in the limb nerves of crab (*Maia*) amounts to about 9×10^{-3} mcals/g. The activation heat in muscle is about 1.0 mcal/g. Thus if activation heat were to be ascribed entirely to a membrane phenomenon, the muscle would have to possess a surface area about 100 times greater than nerve for a given mass of tissue. Peachey (1965) has recently estimated the total surface area of muscle including the transverse tubular system, and finds this to be about 7 times that of nerve. Inclusion of the longitudinal system would further raise this figure so that, if the magnitude of the ion fluxes per unit surface were to be similar, a comparison with nerve heat might almost be appropriate. We do not imply that K^+ and Na^+ movements occur across the internal tubular membranes, but if one were to extend the comparison to Ca movements as well, the consideration would resemble the hypothesis of Davies (1963) concerning the heat effects of the calcium movements. Although, here we suffer from a lack of information regarding the energetic quantities.

The main experimental finding is that activation heat is nearly independent

of both muscle length and temperature, but does depend on agents that prolong the mechanical active state. This knowledge will eventually be of importance in the interpretation of the molecular mechanism of activation. The temperature studies show that activation heat is relatively independent of temperature, as far as its total enthalpy effect is concerned. That length as such has no effect might well come as a surprise because one might have expected the activation heat to be connected with the tension developed which is dependent on length. Or, expressed in terms of the specific hypothesis of the sliding filament mechanism, one might have expected a relation to the regions of overlap or to the number of reactive cross-bridges that have been invoked to interpret active tension (A. F. Huxley, 1957). The feedback heat term h may explicitly depend on these factors, but the term A is clearly found not to.

This work was supported in part by research grants HE 03067 of the National Heart Institute, AFOSR 146-64 of the Air Force Office of Scientific Research, and the Muscular Dystrophy Associations of America, Inc.

Dr. Gibbs is a research fellow of the National Heart Foundation of Australia.

Received for publication 28 May 1965.

REFERENCES

ABBOTT, B. C., HILL, A. V., and HOWARTH, J. V., 1958, *Proc. Roy. Soc. London, Series B*, **148**, 149.

AUBERT, X. M., Brussels, Editions Arscia, 1956.

BROWN, D. E. S., and SICHEL, F. J. M., 1940, *Am. J. Physiol.*, **129**, 321.

DAVIES, R. E., 1963, *Nature*, **199**, 1068.

FENG, T. P., 1931, *Proc. Roy. Soc. London, Series B*, **108**, 522.

GIBBS, C. L., and RICCHIUTI, N. V., 1965, *Science*, **147**, 162.

HARTREE, W., and HILL, A. V., 1921, *J. Physiol.*, **55**, 389.

HILL, A. V., 1932, *Proc. Roy. Soc. London, Series B*, **111**, 106.

HILL, A. V., 1949, *Proc. Roy. Soc. London, Series B*, **136**, 195.

HILL, A. V., 1950*a*, *Proc. Roy. Soc. London, Series B*, **137**, 268.

HILL, A. V., 1950*b*, *Proc. Roy. Soc. London, Series B*, **137**, 330.

HILL, A. V., 1958, *Proc. Roy. Soc. London, Series B*, **148**, 397.

HILL, A. V., 1964*a*, *Proc. Roy. Soc. London, Series B*, **159**, 297.

HILL, A. V., 1964*b*, *Proc. Roy. Soc. London, Series B*, **159**, 589.

HILL, A. V., 1964*c*, *Proc. Roy. Soc. London, Series B*, **159**, 596.

HILL, A. V., Trails and Trials in Physiology, London, Edward Arnold, 1965.

HILL, A. V., and MACPHERSON, L., 1954, *Proc. Roy. Soc. London, Series B*, **143**, 81.

HOWARTH, J. V., 1958, *J. Physiol.*, **144**, 167.

HUXLEY, A. F., 1957, *Progr. Biophysics*, **7**, 255.

JEWELL, R. R., and WILKIE, D. R., 1958, *J. Physiol.*, **143**, 515.

KAHN, A. J., and SANDOW, A., 1955, *Ann. New York Acad. Sc.*, **62**, 137.

PEACHEY, L. D., 1965, *Fed. Proc.*, **24**, 1133.

PENNYCUICK, C. J., 1964, *J. Exp. Biol.*, **41**, 91.

PRINGLE, J. W. S., 1960, *Symp. Soc. Exp. Biol.*, **14**, 41.
RITCHIE, J. M., 1954, *J. Physiol.*, **126**, 155.
RITCHIE, J. M., and WILKIE, D. R., 1955, *J. Physiol.*, **130**, 488.
SANDOW, A., *in* Biophysics of Physiological and Pharmacological Actions, (A. M. Shanes, editor), 1961, Washington, D. C., American Association for the Advancement of Science, 413.
SANDOW, A., and PREISER, H., 1964, *Science*, **146**, 1470.
SCHILLING, M. O., 1960, *Rev. Scient. Instr.*, **31**, 1215.