

THE RELATION BETWEEN TOTAL AND INITIAL HEAT IN SINGLE MUSCLE TWITCHES.

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(*Received July 30, 1934.*)

FOR a muscle giving a series of twitches A. V. Hill [1928b] found a mean value of 2.07 for the ratio (total heat in oxygen): (total heat in nitrogen). The latter includes the delayed anaerobic heat [see Cattell and Hartree, 1932], and if this were 8 p.c. of the whole the ratio (total heat in oxygen): (initial heat) would be 2.24. It was interesting, therefore, to determine the latter ratio directly in a series of twitches, and at the suggestion of Prof. A. V. Hill this has been done for the case of a muscle twitching at regular intervals during the maintenance of a steady state of activity. The same method was applied for the case of a muscle in nitrogen, though here, strictly speaking, a "steady" state is not possible.

Principle of the method. If to a muscle on a thermopile is applied a regular series of shocks the heat deflection occurs on a rising base line owing to the accumulation of the recovery heat due to previous contractions. After a certain time a steady state is reached and successive deflections become identical, starting from the same point and running over the same course. Some time is required for the attainment of the steady state, which occurs only when recovery from the earliest twitches of the series has been completed. When once the steady state has set in, between each pair of the regular series of twitches is produced a total amount of heat equal to the recovery heat from either of the said twitches, so that between one shock and the next the total heat set free is equal to the total heat of one of the twitches. Although during the steady state the recovery heat for each twitch does not occur in the interval between that twitch and the next, the recovery heat in the interval must be equal to the full recovery heat of one twitch. If, therefore, we can be sure that the steady state is fully attained, there is no need

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to follow the heat production for a long time in order to obtain the recovery heat of one contraction. The state being steady, the area of the deflection-time curve above the interpolated base line gives, in each interval, the value of the total heat (initial + recovery) for a twitch. Fig. 1 illustrates the procedure. So long as a steady series of twitches is being maintained it can be applied equally to a muscle in nitrogen.

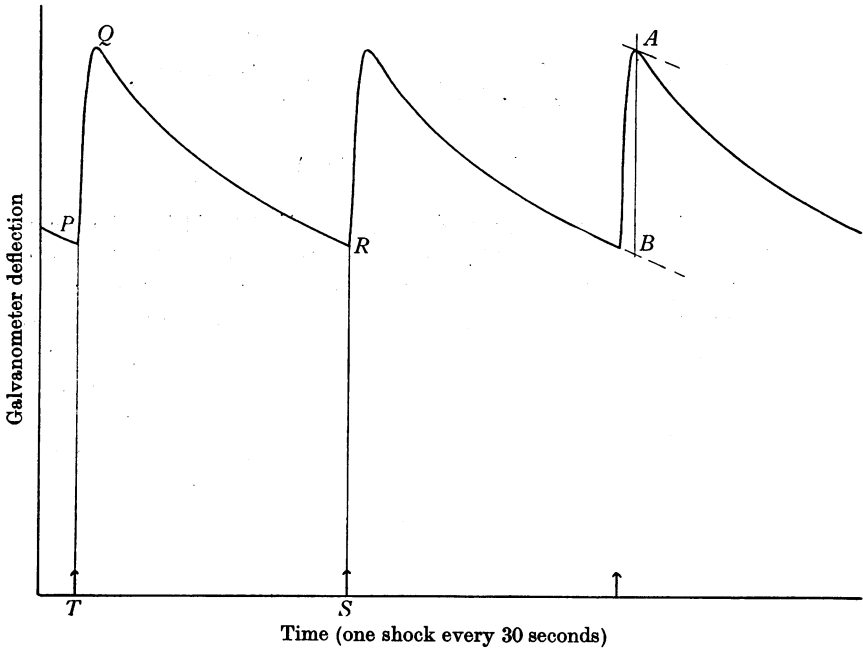


Fig. 1. Muscle heat production. Curve of galvanometer deflection reproduced from photographic record when the muscle had reached a steady state of activity. The horizontal axis is the initial base line and the deflections for successive twitches lie between two parallels to this line. In the interval between two successive shocks the area between the curve and the base line gives the total heat corresponding to one twitch. The initial heat is given by the maximum deflection of the curve measured from a sloping base line extrapolated beyond the response from the previous twitch. For example (in different twitches), the initial heat is given by the length AB , the total heat by the area $PQRST$.

To determine the initial heat the sudden deflection following a shock is measured from the extrapolated falling line of the previous deflection. The maximum deflection corresponding to the initial heat occurs (Fig. 1) at the point where the direction of the line traced out by the galvanometer is parallel to the extrapolated line of the previous twitch at the same moment. The deflection due to the initial heat being very rapid, the

extrapolation is quite short, and on photographic records the maximum deflection can be quite easily read. The initial heat is obtained from the record as a distance. The total heat is obtained as an area. It is necessary, therefore, to make a "control" by which heat measured in the two different ways can be compared. The comparison is made by heating the muscle electrically for a time short compared with that necessary for the maximum deflection to be reached. We have then two measures of the same heat: (i) the maximum deflection and (ii) the area of the deflection-time curve. The heat in the "control" is the same when measured in either way, so that a direct comparison is available and can be expressed for any given muscle in such a form as this: 1 mm. of maximum deflection represents the same heat as x mm. \times sec. of deflection-time area. The control must be made separately in each experiment to determine x .

Experimental details. Usually a pair of sartorius muscles of an English frog *Rana temporaria* was employed at temperatures between 17 and 24° C. A few experiments made with Hungarian frogs *R. esculenta* kept some time in the laboratory gave identical results. The weight of a pair of muscles between the calibrating electrodes was about 120 mg. Large muscles were avoided since, into these, the diffusion of oxygen is slower and a steady state would not be obtainable at so high a level of activity. Twitches were isometric but not recorded.

A constantan-iron thermopile was used connected to a rapid moving coil galvanometer (Kipp Zb). One muscle lay on each face of the thermopile. The thermostat was that described by Feng and Hill [1933] for neurothermic experiments. With this the base line for the muscle at rest was perfectly stable, the thermopile being shielded from every kind of external disturbance.

The galvanometer deflection was registered photographically in a Boullitte camera. An adjustable potential divider allowed the spot to be adjusted as required for recording. The records, which were of about 60 mm. amplitude for each twitch, were readable to 0.1 mm.

Condenser discharges were employed as stimuli, 6 volts and 0.07 μ F being sufficient in general to give a maximal response. The cathode was in the lower half of the muscle. The series of shocks was given by hand by pressing a Morse key at regular intervals. The frequency was chosen so as to allow a sufficient supply of oxygen by diffusion. According to Hill [1928c, p. 49] for a muscle with one face only exposed to oxygen and of thickness not exceeding 1 mm., a frequency of two shocks per minute is perfectly compatible with a good supply of oxygen. This was the frequency generally adopted and there was every reason to suppose that a

genuinely steady state of activity was obtained. In certain experiments with very thin muscles a frequency of three shocks per minute was employed.

For the instantaneous "control" heating, an oscillatory current of high frequency (300,000 per sec.) was used, the arrangement being similar to that described by Hartree [1932*b*]. The passage of the current was of short duration determined by the opening of a short-circuit key by a revolving arm and the closure of the key by a spring as soon as the arm had gone by. Since a high frequency current, not stimulating the muscle, was used for controls, these could be made at any convenient time during an experiment.

The muscles were mounted upon the thermopile surrounded by Ringer's solution of pH 7.2 and placed in the thermostat for $2\frac{1}{4}$ – $2\frac{3}{4}$ hours with oxygen bubbling so as to obtain as perfect a thermal and osmotic equilibrium as possible before the experiment proper began. By this time also the excitability of the muscle in its new environment should become constant. The Ringer's solution was then withdrawn and the muscle placed in an atmosphere either of oxygen or of nitrogen. In the latter case traces of oxygen were removed by passing the gas over heated copper. The thermostat was then sealed up by closing its brass taps.

After the introduction of the gas it was necessary to wait until a steady position of the spot was obtained. A short record of the base line was made and the adjusting potential divider was read. Stimulation then began with a series of shocks of the chosen frequency until a steady state of heat production was obtained. This state was marked by the fact that successive deflections took place between the same limits, that is were inscribed between two lines parallel to the base line on the record. Usually with muscles in oxygen 13–18 min. were required for the steady state to be reached; with muscles in nitrogen 4–5 min., less owing to the absence of the recovery heat.

Records were made for 2 or 3 min., that is for five or six successive twitches. Usually to bring the spot into the camera it was necessary to adjust the potential divider, which was then read at the position required for the record. A calibration of the potential divider in terms of deflection on the record was made later. In this way the distance of the record from the original base line could be accurately determined.

The area of a record above the base line was calculated from its successive ordinates at suitable intervals. The mean was taken for several successive twitches.

A typical experiment. Date, March 23, 1933. Two shocks per minute, 6 volts, $0.07 \mu\text{F}$. Steady state in oxygen reached after 15 min. stimulation (30 shocks). Record began after 20 min.

Mean height of record above initial base line 125.4 mm . Total heat per twitch therefore $125.4 \times 30 = 3762 \text{ mm.} \times \text{sec.}$, the interval between shocks being 30 sec.

From control heating, 1 mm . maximum deflection is equivalent to $33.4 \text{ mm.} \times \text{sec.}$ deflection time.

The average maximum deflection per twitch was 56.6 mm . The initial heat, therefore, corresponds to $56.6 \times 33.4 = 1890 \text{ mm.} \times \text{sec.}$

Ratio of total heat to initial heat therefore $3762/1890 = 1.99$.

When an experiment is prolonged it may happen that the base line changes somewhat. Account can be taken of this if it be assumed that the displacement occurs continuously. After the end of stimulation the muscle is left at rest for a time sufficient for recovery. A new base line is then recorded. Any difference might be due partly to a change of metabolism, partly to a change of osmotic pressure. The true base line at any moment can then be interpolated with sufficient accuracy between the initial and the final reading. When a muscle is in good condition the change of base line is generally quite unimportant for the case of oxygen, but owing to the osmotic changes it is more obvious for the case of nitrogen.

RESULTS.

For regular stimulation in oxygen at a frequency of two shocks per minute, after the establishment of a steady state, the value of the ratio (total heat): (initial heat) was found to be 1.97 as the mean of seventeen determinations. The values found lay within quite a narrow range, namely from 1.86 to 2.12. When determinations were made at two or more frequencies, the values found for the ratio were very close: for example, two determinations at a frequency of one shock per minute gave a mean of 1.92; four determinations at a frequency of three shocks per minute have a mean of 1.97; four determinations at a frequency of four per minute gave a mean of 1.94. The ratio is not modified when the steady state is maintained for a long time, provided that the muscle remains in good condition, giving constant deflections.

When the muscle is kept in nitrogen the value of the ratio is about 1.04 at a frequency of about 2 shocks per minute. (Six determinations, extreme values 1.01 and 1.06.)

Observations on single twitches and short tetani. For comparison with the results described, experiments were made on the ratio of total to initial heat for the case of single contractions, either single twitches or short tetani. In this case the area of the galvanometer deflection-time curve for a given maximum was compared for the two cases, (i) contrac-

tion, (ii) heating control, the controls being made as described earlier. The great accuracy of the thermostat made it quite easy to measure the total heat for a single twitch. The time necessary for complete return to the base line was rarely more than 15 min., which is about the same as that required in the previous experiments for the attainment of the steady state. For twitches in nitrogen the deflection was followed for $7\frac{1}{2}$ min., when the deflection was constant again. In a few experiments for comparison, tetani as well as single twitches were examined.

For single shocks in oxygen the mean value of the ratio (total heat): (initial heat) was 1.69 (15 determinations, extreme values 1.47 and 1.92). The measurements were made either on fresh muscles by recording the first twitches of all, or (after complete recovery) on muscles which had been used for an experiment of the kind described earlier. In three experiments the ratio (total heat) : (initial heat) was determined for 1 sec. tetani, the mean being 2.45.

For single twitches of muscles in nitrogen the ratio (total heat): (initial heat) had a mean value of 1.03 (26 determinations, limits 0.94 and 1.09). In a certain number of experiments the muscles had already been used for previous determinations in oxygen; this seemed to make no difference to the ratio.

The course of the delayed heat production. In a certain number of experiments on isolated twitches in nitrogen the ratio (total heat): (initial heat) had values lower than unity. This indicated a negative delayed heat production. When the phenomenon appeared the difference between the ordinates at the same moment of the deflection curve, for the twitch and the control respectively, when reduced to the same maximum, was generally negative till about 45 sec. after the shock. An analysis of the curve showed that the negative heat, when it occurred, appeared during the first 20 sec. It was generally followed by a positive production of heat lasting for 2 or 3 min. The relative magnitude of the negative and the positive heat determined whether the ratio (total heat): (initial heat) had a value greater or less than 1.

In some experiments on isolated twitches in oxygen there was the same evidence, during the 10 or 15 sec. following the shock, of a small quantity of negative heat. Four experiments were made to determine the time course of the heat more accurately. The records were made on a more rapidly moving surface and analysed in intervals of 0.4 sec. up to 10 sec., for the same muscle contracting, first in oxygen and later in nitrogen. The initial heat and the early stages of the delayed heat being thus known, an analysis of the further delayed heat in nitrogen was

carried out up to 68 sec. in intervals of 4 sec. The results obtained were subtracted from the figures found for the total heat in oxygen by a similar analysis. The difference gives the time course of the production of heat in purely oxidative recovery.

The results obtained were variable from one muscle to another and do not allow any very clear conclusion. The mean of four experiments gave a negative production of heat in nitrogen equal to about 3 p.c. of the initial heat; the negative heat occurred during the first 16 sec. following the stimulus; then a positive anaerobic delayed heat appeared during the interval from 16 to 68 sec., totalling about 4 p.c. of the initial heat. The oxidative recovery heat is very variable from one experiment to another in the moment of its appearance. The results of these analyses, and of others made under conditions allowing solely a detection of the total delayed heat (oxidative and non-oxidative), seem to show that the total delayed heat after a single twitch is clearly apparent only after a rather long time—15–30 sec.—from the stimulus. Hartree [1932*a*] working with tetani of various durations found that both in oxygen and in nitrogen the delayed heat had a slower onset and a lower maximum rate the shorter the duration of the tetanus. The present experiments fit into Hartree's scheme, showing that the delayed heat after a single twitch starts up more slowly and has a still lower maximum rate than even the shortest tetanus.

In order to confirm the reality of the negative heat and to avoid the possible objection of a layer of non-responding damaged muscle at the surface distant from the thermopile the experiment performed by Hartree [1932*a*, p. 284] was repeated: a strip of filter paper moistened with Ringer's solution was laid on the external face of each of the muscles to represent an irresponsive layer. In agreement with Hartree and with calculations by Hill [1931, Appendix III] I found an apparent production of negative heat ending within 3 sec. after the twitch; with a pair of sartorii weighing 112 mg. and two strips of moist filter paper weighing 44 mg. (an irresponsive layer equal to 28 p.c. of the whole) I found an appearance, during the third second, of a block of negative heat equivalent to 14 p.c. of the initial heat. This is much earlier than the negative heat found in the experiments proper and cannot explain it. Presumably, therefore, that negative heat had a genuine physiological origin.

DISCUSSION.

Considering contractions in the presence of oxygen, the different values of the ratio (total heat) : (initial heat), for different types and durations of stimulus, diminish regularly as one passes from tetani,

through series of shocks more or less frequent, to single twitches. For tetani the accepted value is 2.5 on English frogs' muscles [Hartree and Hill, 1922, 1924]. For a rapid series of twitches (60–80 in 2 min.) Hill [1928*b*] found a value of 2.07 for the ratio (total heat in oxygen):(total heat in nitrogen) which, allowing for 8 p.c. delayed anaerobic heat in the denominator, gives a value of about 2.24 for the ratio (total heat in oxygen):(initial heat). Cattell [1933, 1934], in experiments completed while these were being made, found with *Rana pipiens* a ratio of 2.05 for single twitches, 2.5 for 1 sec. tetani. Cattell and Shorr [1932] found for mammalian muscle (fine bands of the dog's scalenus) a ratio of 3.5–4.5 for a tetanus of 1 sec., 2.5 for a single shock. Cattell and Lundsgaard [1933], studying the ratio T/H during the course of a series of isometric twitches, found that the "efficiency" of the contraction was greater in a shorter series. The present experiments fit well into this succession, by showing that in a series of twitches much slower than that by Hill [1928*b*] the ratio is as low as 1.97, falling to 1.69 for isolated twitches. The value of the ratio obtained during the course of the present research for a 1 sec. tetanus agrees completely with its predecessors. The value 1.69 for isolated twitches is lower than that by Cattell [1934], but the difference may perhaps be attributed to the fact that the present experiments and his were on different species of frogs.

For twitches in nitrogen the delayed heat is very small. In a series of twitches at low frequency it is 4 p.c. of the initial heat: in an isolated twitch it is 3 p.c. This result agrees with that of Cattell and Hartree [1932] and of Hartree [1932*a*], who showed that the delayed anaerobic heat may vary from 20 to 8 p.c. of the initial heat, according to the duration of the stimulus, falling to still lower values for the case of very few twitches.

In addition to the quantitative changes shown by the ratio (total heat):(initial heat) as one passes from tetani to twitches, the experiments here reported confirm the fact that the time relations of the delayed heat are altered also. The delayed anaerobic heat is much later in its appearance after a single shock. It occurs only after an interval during which there is no heat at all or perhaps a negative heat. This is in agreement with Hartree's results [1932*a*], who has shown that the delayed anaerobic heat occurs earlier and more quickly the greater the degree of the stimulus. Hartree [1932*b*] also has shown the existence of a negative delayed heat after a short tetanus at 0° C. In a twitch the oxygen recovery heat also is delayed and slowed as compared with the case of a tetanus [see Hartree, 1932*a*, Fig. 3].

It seems, therefore, that the processes of muscular recovery differ in extent, and in their relation to the time, for a longer tetanus, for a shorter tetanus, for a series of shocks at greater or less frequency, and for a single twitch. For a single twitch, or for a series of twitches at low frequency, the production of heat is the only sign of the metabolic activity of the muscle which can be studied, chemical investigations in this case of the processes accompanying (or following) muscular activity being difficult, or impossible, owing to the smallness of the quantities involved and the rapidity of the changes. The measurement of the heat production shows that it is not certain that what happens in a single twitch is identical with what happens in a tetanus. During prolonged activity, successive twitches find the muscle in a chemical state different from that of a muscle at rest and it is not surprising that, even if the initial process is the same, the phenomena of recovery are different in the two cases. Cattell and Lundsgaard [1933] and Cattell [1934] have discussed the possibility of a decrease of "efficiency" of the chemical processes accompanying muscular contraction, as one goes from a single twitch to a tetanus. The increase of the ratio (total heat) : (initial heat) with the extent of activity can be expressed as a decrease of "efficiency" with increased extent of contraction.

The existence of a negative heat, the smallness of the delayed anaerobic heat and the greater delay in its appearance, the decrease in the oxydative recovery and its delay, are several signs of the change in the balance of the endo- and exothermic reactions, evidence of whose existence can be provided by chemical study only after a greater degree of activity. It is not impossible, for very low degrees of activity producing no considerable want of oxygen (single twitches or very slow series of twitches), that the muscle works entirely by means of its phosphagen mechanism and that, in the presence of an adequate supply of oxygen, the resynthesis of phosphagen occurs only by a direct oxydative process. In this case one may regard the lactic acid mechanism as intervening, to aid in the resynthesis of phosphagen, only in the case of a momentarily insufficient oxygen supply or of a large phosphagen breakdown. The direct oxydative resynthesis of phosphagen might very well occur with a higher efficiency than the indirect resynthesis involving the formation of lactic acid and the ultimate resynthesis of the latter to glycogen.

Hill and his collaborators [Cattell *et al.* 1931, p. 285] have found that, for a muscle poisoned with iodoacetic acid in which contraction occurs without formation of lactic acid, the ratio (total heat) : (initial heat) after a short series of twitches is about 1.65. This figure is very

close to that found during the present work for a single twitch, namely 1.69. The agreement may well indicate that the normal muscle during a single twitch works without formation of lactic acid. It is well known moreover that the ratio of phosphate to lactic acid liberated increases when activity is prolonged. We are ignorant, and we shall remain ignorant until direct chemical determinations can be made on a much finer scale, of the value of this ratio for a single twitch; very likely it is large, perhaps even infinite [Eggleton, 1933]. In cardiac muscle, Clark and his collaborators [1932] have shown that, in the presence of oxygen, slow rhythmic activity can be maintained, for a very long time, without any evidence of lactic acid formation. That occurs only when there is a deficiency of oxygen, the decomposition of carbohydrate then furnishing the energy necessary for the resynthesis of phosphagen otherwise impossible. In the presence of oxygen the activity of cardiac muscle is fully maintained when the heart is poisoned with iodoacetic acid. So far as we know, the hydrolysis of phosphagen is then the only immediate source of energy for contraction and in the presence of oxygen the resynthesis is effected under the influence of direct oxidation. It may well be that in skeletal muscle also, during isolated twitches or twitches repeated at a very low frequency giving a steady state of activity, the phosphagen mechanism alone is called into play, the lactic acid mechanism intervening only in the case of more vigorous activity. The results of the various researches referred to, as well as of the present ones, agree with this hypothesis.

SUMMARY.

A procedure is described of measuring the ratio (total heat) : (initial heat) during the course of a series of muscle twitches at a frequency low enough to allow the establishment of a steady state. The same ratio has been measured in the case of a single isolated twitch.

At a temperature of about 20° C., for a series of twitches at low frequency, the value of the ratio (total heat) : (initial heat) is about 1.97 when the muscle contracts in oxygen, and about 1.04 when it contracts in nitrogen. For a single twitch the values are 1.69 in oxygen and 1.03 in nitrogen.

Following isolated twitches, the delayed anaerobic heat has a mean value of about 3 p.c. of the initial heat and is much more delayed in its appearance than in the case of a tetanus with a more vigorous response. There is some evidence for a phase of negative heat during the first 20 sec. after a twitch.

Following an isolated twitch in oxygen, the oxidative recovery heat appears with greater delay than in even the shortest tetanus hitherto studied. This agrees with Hartree's conclusion that the onset of recovery is more rapid the greater the magnitude of the initial breakdown.

The possibility is discussed that the normal muscle giving a single twitch, or a series of twitches at a very low frequency, contracts without the formation of lactic acid and that the latter mechanism comes in only when greater activity, or oxygen want, is imposed.

My very sincere thanks are due to Prof. A. V. Hill for his suggestion of this work and for his advice during its realization. I am much indebted to Mr J. L. Parkinson for his collaboration during the earlier experiments and for his valuable help. I am grateful to Dr McKeen Cattell for discussing with me the researches referred to.

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