AN EXAMINATION OF ABSOLUTE VALUES
IN MYOTHERMIC MEASUREMENTS

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The impulse to the investigation described in the following pages arose from the recent discovery of a serious discrepancy between the absolute values, obtained by current methods, of the heat produced in isometric contractions of frog sartorii and those reported in several papers published between 1921 and 1932 (Hartree & Hill, 1921, 1922, 1928 a; Hartree 1932). The older values were all considerably greater than the recent ones. The evidence for the discrepancy is discussed in connexion with Figs. 1 and 2 below.

Myothermic experiments are of two general types: (a) those in which absolute values are not required, the heat being compared with itself under different conditions, for example, at various temperatures, with different durations and frequencies of stimulus, before and during partial fatigue and in the several phases of contraction, maintenance, relaxation and recovery; and (b) those requiring accurate calibration, in which the heat is being compared in absolute units with mechanical work (positive or negative), with mechanical tension, with chemical and osmotic changes and with O_2 consumption. In (a) an error of calibration is usually unimportant; in (b) it can be serious.

The existence of so large a discrepancy led first to a critical examination of the methods now used. Apart from the finding that in some minor respects their accuracy could be somewhat improved, they survived the test. The frogs were the same (English *Rana temporaria*) and the conditions (temperature, etc.) were similar: only different instruments and methods were used. The absolute values, therefore, of the heat reported in the older papers referred to were, without doubt, considerably and consistently too great. This could only be due to an error of calibration. In five other papers, however, published during the same period (Fenn, 1923, 1924; Wyman, 1926; Hartree & Hill, 1928 b, c), for which similar methods and instruments were used, there was no direct evidence of an error of calibration, either for or against. In these papers, work (W) and heat (H) were compared under various conditions of shortening or stretch. It seemed unlikely that an error of calibration which gave heat values 50 % or more

too great when isometric contractions had previously been recorded would be absent when muscles had been allowed to shorten, or had been stretched, during contraction. In fact, in all these papers, indirect evidence suggests that the same error was present: the 'mechanical efficiency', $W/(H+W)$, was always considerably less than was found later by Hill (1939), and this is what would happen were H too large. Moreover, Fenn's (1924) conclusions on the absorption of work by muscles would have been greatly strengthened, had he obtained smaller values of H: for then $(H\text{-}W)$ would have been less, possibly much less as was found by Hill & Howarth (1959) thirty-five years later.

Another striking discordance exists between previous results and later ones in respect of the 'isometric heat coefficient' (Pl_0/H) in a single twitch. The older values were much smaller (Hill, 1913, 3–6; Hartree & Hill, 1921, mean 5·5; Hill, 1928c, mean 6·4; Rosenberg, 1934, mean 8·2; Hill, 1958, 10·0). In the older experiments this must have been due in part to the value of H being too great: but another reason was the greater compliance of the tension recorder and its connexion to the muscles. This is discussed below.

Once an error of heat calibration had been detected in one particular method, it was essential that the accuracy of results obtained by later methods should be scrutinized. Many of the investigations made between 1928 and 1937 were obtained with methods and instruments described by Hill (1928a): with these no evidence was found in the published records of any consistent error of calibration comparable in size with that detected with the earlier methods. Since, however, suspicion existed a number of the more important results were critically re-examined, and in some cases correction has been found necessary. During this re-examination some new and more accurate results have been obtained on the relation between heat and tension in isometric contractions of various durations, and these have been included here.

Before 1937 calibration was carried out electrically, separately in every experiment, by liberating a known amount of heat in the muscles and comparing this with the galvanometer deflexion obtained. In that year, however, the new very thin thermopiles were introduced by Mr A. C. Downing (Hill, 1937). Their heat capacity was so small that a new and more convenient method of calibration became possible. These are the instruments used in all recent experiments and the accuracy of their calibration has been tested anew by several methods as described in the next section.

METHODS

Calibration: present method

The present method of calibration, by which the heat production, in millicalories, is obtained from the recorded deflexion expressed in microvolts, has been used since the new thin thermopiles came into use in 1937. It was described rather shortly in two earlier papers (Hill, 1937, p. 125, 1938, p. 142) but further experience has led to various modifications and improvements which may justify the more detailed description that follows. The method involves the following quantities or processes:

(a) the sensitivity of the thermopile, expressed as $\mu V/1^\circ$ C;

(b) the heat capacity of the thermopile, expressed as its equivalent half thickness, i.e. the thickness of muscle which would have the same heat capacity as the half thickness of the thermopile;

(c) the heat capacity of the muscles, including the thin layer of Ringer's solution adhering to them;

(d) the usual allowance for heat loss;

(e) the analysis to allow for lag in thermopile and galvanometer.

(a) *The sensitivity of the thermopile.* This can be determined by three separate methods the results of which agree:

(1) By calculation from the known number of couples and the measured $\mu V/1^\circ$ C of one couple made from the same wires as the thermopile. For the test couple the wires should be treated (e.g. rolled and annealed) in the same way as those used in constructing the thermo-pile. The sensitivity of a couple varies with the temperature at which it is measured; see below.

(2) By transferring the thermopile rapidly between two baths at known temperatures and recording photographically the deflexion of a quick galvanometer connected to it. The hot junctions warm up very rapidly, the cold junctions only slowly since they are well shielded; if necessary the recorded deflexion can be extrapolated back to the moment at which the transfer was made.

(3) By liberating a known amount of heat by a condenser discharge in a pair of artificial 'muscles' lying on the thermopile. The discharge is made between two electrodes situated a few millimetres above and below the thermopile. Each 'muscle' used in the present tests was a single layer of filter paper impregnated with agar jelly made up with salt solution. It stuck closely to the thermopile. The 'muscles' must be of uniform section, otherwise the rise of temperature produced by the condenser discharge will not be uniformly distributed along them. The deflexion following the discharge is recorded, and a small allowance made for heat loss. When enough records have been made the 'muscles' are removed and cut off at previously marked points corresponding to the positions of the electrodes. The central portion is weighed, then dried and weighed again. From the weights the heat capacity can be calculated. By dividing the energy in the condenser discharge by the heat capacity the rise of temperature is obtained: this is compared with the deflexion expressed in microvolts and so the sensitivity is obtained.

Method (3) is similar to the method of calibration used before 1937, but with the pre-caution that 'muscles' of uniform section were used and the weight of the part heated could be measured more accurately. In the former method the calibration was made with the actual muscles used in an experiment.

The results of the three methods of obtaining the sensitivity of the thermopile were compared in the case of the two thermopiles D_1 and D_4 used in the experiments later described. The new thermopile D_4 (36 couples), constructed by Mr A. C. Downing, is of particular interest because it was made with chromel and constantan wires giving a high thermo-

electromotive force (thermo-e.m.f.) per 1° C and a low rate of heat loss ('chromel' is 10 % chromium, 90 % nickel). A single couple was tested with one junction at 0° C and the other at various temperatures x between 0 and 40° C: the thermo-e.m.f. $E(\mu V)$ followed the formula:

$$E = 57 \cdot 71x + 0 \cdot 0685x^2. \tag{1}$$

From this the sensitivity dE/dx ($\mu V/1$° C) is obtained at any temperature:

$$dE/dx = 57 \cdot 71 + 0 \cdot 137x. \tag{2}$$

Transferring the thermopile suddenly (method (2)) from a bath at 0° C to one at 20° C gave $E/x = 2140 \,\mu V$, while formula (1), for 36 couples, gives $E/x = 2127$. The agreement is good.

Method (3), with a condenser discharge applied to artificial 'muscles', gave at 0° C 2050, 2080 and 2025 $\mu V/1$° C in three experiments, mean 2051: formula (2) at 0° C with 36 couples gives 2077 $\mu V/1$° C. Again the agreement is good.

The same tests made with thermopile D_1 (manganin and constantan wires) gave similar results.

It is clear, therefore, that the sensitivity of the whole thermopile can be accurately calculated from the number of junctions and the measured thermo-e.m.f./1° C of the metals used.

(b) *The heat capacity of the thermopile.* The method of determining the equivalent half-thickness b was described by Hill (1949b, p. 233). This number is important because it is used when analysing for thermopile lag, and this process automatically allows for the heat capacity of the thermopile. If no analysis is done the thermal equivalent of the 'drained' muscles (see below) must be increased by a small fraction equal to the ratio of the equivalent thickness of the thermopile to the total average thickness of the two muscles calculated from their weight, length, width and density. The equivalent half-thickness of thermopile D_1 is about 17 μ, of thermopile D_4 about 45 μ: these are fairly accurately known, and are quite small compared with the thickness of each of the muscles of a pair, generally 600–700 μ.

(c) *The heat capacity of the muscles.* According to Hill's measurements (1931), if u is the dry weight (% solid) of a muscle its specific heat at room temperature (17° C) is $1 - 0 \cdot 68u/100$; at 0° C 1 % should be added.

In calculating the heat the weight of the muscles must include that of the Ringer's fluid adhering to them, as it was during contraction. The fluid in the muscle-thermopile chamber was always lowered several minutes before a contraction in which the heat was to be measured; and the draining of the muscles could be aided by hanging a small soft cotton wick from the pelvic bone. When the muscles are finally to be taken out for weighing, the cover of the chamber should be removed and the thermopile held vertical in a clamp, not laid flat on a bench: the latter position may allow fluid to creep back. The two muscles should then be cut off at their tendons and immediately weighed: this gives the 'drained weight'. They should then be placed between strips of filter paper, gently pressed to remove adhering fluid, and weighed again: this gives the 'true weight'. In muscles of the size used (generally 200–300 mg the pair), and with thermopiles D_1 and D_4, the drained weight was on the average about 6 % greater than the true weight.

When a pair of muscles contracts, the heat produced is shared between true muscle, adhering fluid and thermopile. Allowance for the heat capacity of the thermopile is referred to above. Disregarding this for the moment, the heat produced is equal to the rise of temperature observed multiplied by the thermal equivalent of the muscles. The thermal equivalent is the product of the drained weight and the specific heat: the specific heat should be calculated from the dry weight (solid) as a percentage of the drained weight. This gives the heat in absolute units, usually expressed as millicalories. When the heat in mcal/g is required, the heat should be divided by the true weight of the muscles.

Until recently the procedure was less exact. When the muscles were to be weighed they were cut out (with the thermopile lying flat on the bench) and gently drawn across a dry,

smooth, non-absorbent surface to remove excess of fluid. This procedure must have given about the same result as that for the drained weight described above. But owing to a persistent oversight the muscles were not then 'blotted' between filter papers, to give the true weight. The heat expressed in millicalories was probably nearly accurate, but expressed in mcal/g muscle it must have been consistently rather too small. With muscles between 200 and 300 mg the pair the error would have been about 6 %; with smaller muscles it would have been greater.

(d) *Heat loss.* The allowance for this is easy and exact, and has been referred to in two recent papers (Hill, 1961 a, b).

(e) *Analysis.* The allowance for delay due to heat flow into the thermopile was made in the usual way by the method of factors (Hill, 1949 a, b). Usually no allowance was necessary for lag in the galvanometer, since the quantities of heat were large and considerable negative feed-back to the galvanometer made it very rapid.

Calibration: former methods

The method of calibration always used between 1913 and 1937 was, after every experiment to liberate a known amount of heat in the muscles by passing an electric current through them between electrodes in the plane of the thermopile above and below its ends. This allowed the deflexion of the galvanometer to be expressed directly in heat units, it avoided the necessity of knowing the heat capacity of the thermopile, and it took account automatically of any adhering fluid. It even allowed for heat loss, if the maximum deflexion of the galvanometer was reached in about the same time for calibration as for muscle heat production. The electrical measurements themselves were accurate enough, but various difficulties and possible sources of error existed. The first difficulty was that the muscles were not really of uniform cross-section except in the central region, and if the parts beyond the ends of the thermopile were thinner an undue proportion of the heat was liberated in them. The next difficulty was that the muscles had to be made inexcitable, and this could lead to changes of shape or even of weight. In all the earlier experiments until 1928 the muscles were made inexcitable by killing them with chloroform vapour, and Hartree continued to use this method up to 1932. Other workers from 1928 onwards employed 'electrocution', i.e. prolonged overstimulation, to render the muscles inexcitable. The use of chloroform puts the muscles into rigor, which tends to make the central portion thicker at the expense of the ends. This would exaggerate the non-uniformity of the muscles and cause the rise of temperature of their middle part on the thermopile to be too small. Another possible source of error is that the current lines in the immediate neighbourhood of the electrodes are concentrated, so an undue part of the electrical energy may be turned into heat there. A further possible source of error is polarization at the electrodes: though this can be largely avoided by using a single condenser discharge, with a high voltage, as the source of electrical energy.

The effect of all these errors would always have been in the same direction, namely, to give too small a rise of temperature in the region of the muscles on the thermopile. This would result in a smaller deflexion for a given electrical heat production, and so lead to too high an estimate of the heat produced when a muscle contracted. The existence of these possible sources of error was realized but it seems certain now that in the earlier experiments, and in all of Hartree's up to and including 1932, their effect was underestimated. Nevertheless, the older method of calibration, if applied with sufficient precautions (Rosenberg, 1934), was able to give at least approximately correct results. His average heat in a single isometric twitch was about 3·5 mcal/g, which today would be regarded as normal for muscles in good condition and moderately stretched.

Unfortunately none of the old equipment now exists, so it is impossible to examine the cause of the error experimentally.

The heat produced in an isometric contraction in relation to the duration of stimulus
and the tension developed and maintained

In order to allow a valid comparison with the older results, about thirty-five experiments were made on the heat produced in isometric contractions, with various durations of stimulus, and at lengths close to the standard length l_0. The muscles used were the sartorii of English frogs, *Rana temporaria*, and the Ringer's solution in which they were soaked, before and between recordings of heat production, contained (mM): NaCl 115·5, KCl 2·5, CaCl$_2$ 1·8, phosphate 3 or 4; pH 7·0 or 7·2. All records were made with the muscles in O$_2$. With the longer stimuli (4 or 5 sec at 17° C) the recovery process had already begun by the time the initial heat was complete, but its rate then was still so small that no significant amount of it can have been included in the initial heat as measured.

The majority of the results given in Figs. 1 and 2 below were obtained by methods described in recent papers (Hill, 1961 *a*, *b*): each observation was of the heat produced in a single contraction. There is a difficulty, however, in obtaining accurately by this method the relation between heat production and duration of stimulus: in a series of several contractions of the same muscles with different durations of stimulus the condition of the muscles tends to depreciate. The alternative method, therefore, was also used, in which the heat was recorded in a single long contraction of fresh muscles (4 or 5 sec at 17° C, 15 sec at 0° C) and the record was fully analysed, a simultaneous record being made also of the tension. By this means the total heat produced up to any time throughout a contraction could be obtained. Moreover, the rate of heat production at any moment could be measured and related to the tension at that moment. This made it possible to calculate what the heat production would have been in a contraction in which, after reaching its maximum, the tension remained constant throughout, and did not fall off as almost always happens with long stimuli.

Of course the total heat produced in a contraction with a stimulus of duration x is not the same as the heat produced up to time x: the former includes the heat produced after the last shock, the latter does not. But the heat produced after the last shock of a stimulus was examined in detail in a recent paper (Hill, 1961 *b*) and found to be, on the average, about 7 mcal/g at 17° C and about 3 mcal/g at 0° C. So the heat produced by a stimulus of duration x sec can be obtained from the heat *at* time x sec during a continuing contraction by adding 7 mcal/g at 17° C (see Fig. 3 there), or at 0° C 3 mcal/g. The procedure is not entirely accurate but any possible error is less than would probably occur, owing to progressive change in the condition of the muscles, when several successive stimuli of different durations were applied. This expectation is confirmed by the fact that, in Fig. 1 below, the great majority of the spots, representing individual contractions, are below the curve which was obtained by the method just described.

The isometric heat coefficient (Pl_0/H) in a twitch

A few experiments were made in order to obtain quantitative information on two points: (*a*) the expectation that when the compliance of the tension recording system was reduced to a minimum the value of Pl_0/H in a twitch would have the same high value (about 10) at 17° C as it was found previously to have at 0° C (Hill, 1958); and (*b*) the extent to which the value of Pl_0/H is reduced at 17° C by introducing a known extra compliance into the connexion of the muscles to the tension recorder. For (*a*), the muscles were joined by a strong wire to an R.C.A. mechano-electronic transducer (5734); for (*b*) a length of strong 'button-thread' of measured (non-linear) compliance was placed in series with the wire to the transducer. The heat was read in the usual way, with a small correction for heat loss.

*The heat produced in an isometric contraction in relation to the
duration of stimulus*

In Fig. 1 the filled circles represent the total heat produced in 40 iso-
metric contractions of frog sartorii, for various durations of stimulus, in
13 experiments from October to December 1960 and in May 1961. The frogs
used were in good condition, and the sartorii were rather large ones, usually
between 200 and 300 mg the pair. No significant difference was noted
between the different seasons. The temperature was near 17° C. The results
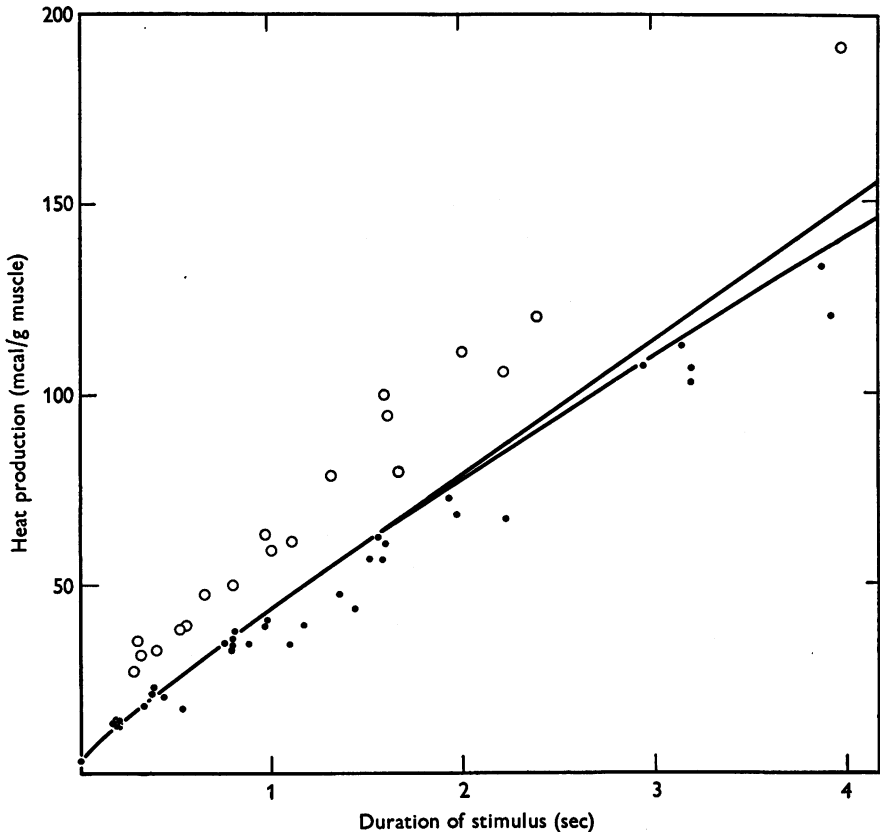

Fig. 1. Initial heat production of frog sartorii at 17° C against duration of stimulus.
●, forty individual results obtained in thirteen experiments; of these, the results of
four twitches are shown by a single spot on the vertical axis, and there is a cluster of
six points near 0·2 sec. Lower curve, mean of five experiments each with a single long
stimulus, analysed as described in the text. Upper curve, the same experiments,
but the results of each were calculated for a contraction in which the tension was
fully maintained throughout. O, individual results reported in papers published
between 1921 and 1932.

were all 'reduced' to 17° C, in order to make them directly comparable, by applying a temperature coefficient $Q_{10} = 3\cdot1$ to the durations of the stimuli. Thus, for example, the heat produced by a 2 sec tetanus at 18° C was plotted not against 2 sec, but against $2 \times 3\cdot1^{0\cdot1} = 2\cdot24$ sec; conversely the heat obtained from a 1·2 sec stimulus at 16·3° C was plotted against $1\cdot2 \times 3\cdot1^{-0\cdot07} = 1\cdot11$ sec. For reasons given above (p. 315), 6% was added to all the heats observed. The mean value (3·5 mcal/g) of the heat with single twitches, in four experiments, is shown by the filled circle on the vertical axis.

The value $Q_{10} = 3\cdot1$ is probably rather too small; strictly speaking it is applicable to the case of H/Pl_0, not of H/M (heat per gram of muscle). But the temperature differences from 17° C were not large and no significant errors can have been caused.

In each of five special experiments, made at 17° C in November 1961 on the muscles of similar good frogs, a single long stimulus was applied and the heat and tension were recorded throughout, as described on p. 316. The heat was analysed from beginning to end, and for the calibration all the precautions referred to on p. 314 were taken. The maximum tension had an average value $Pl_0/M = 2610$. The lower of the two curves represents the mean of the five experiments. The upper curve is referred to later.

The 19 open circles represent the results recorded in four earlier papers (Hartree & Hill, 1921, 1922, 1928a; Hartree, 1932). The observations were all made between 15 and 20° C, and the results were reduced to 17° C in the way described above. The discrepancy between the earlier measurements and the present ones is obvious: there is no overlap, and the older observations are 40–60% too high.

Figure 2 is similar to Fig. 1 but for 0°C. Here, however, the thirty-three individual observations made between October 1960 and May 1961 could not be shown separately because of overlap, so their mean values only are given. Each mean is represented by a filled circle, with a figure indicating the number included. The average value (3·7 mcal/g) of the heat in single twitches, in five experiments, is shown by the filled circle on the vertical axis. The lower curve was obtained by the calculation explained above for a single long tetanus; the mean value of the maximum tension was $Pl_0/M = 1960$. The open circles represent the results given in two earlier papers (Hartree & Hill, 1921, 1922). Again there is a large discrepancy between the old results and the recent ones.

Most of the filled circles in Figs. 1 and 2 are rather below the lower curves, particularly for longer times. The reason for this is that the measurements were made during the course of other experiments, usually several on a single pair of muscles. The curves, however, were obtained with completely fresh muscles in a single long contraction.

What is rather striking about the older results, shown by open circles in Figs. 1 and 2, is their comparatively good agreement among themselves. The divergence from the present results is so large and consistent that it had to be accepted as a fact. It was impossible to believe that the muscles in recent experiments were not in as good condition as they were in the past, for present knowledge of how to keep isolated muscles functioning well is better than it was 30–40 years ago. One possibility was that in the earlier experiments the contractions were less rigidly isometric than they were in the later ones. This, because of the heat of shortening and the greater amount of work done against external compliance, might have led to the production of extra heat; but the amount of such extra heat could not have been more than a small fraction of the excess observed. The only
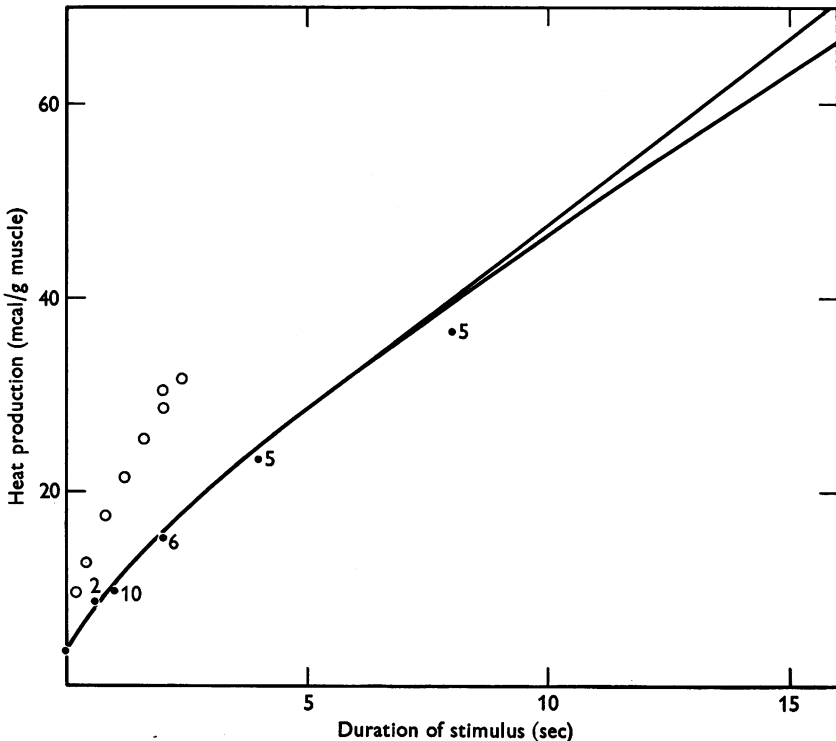

Fig. 2. Initial heat production of frog sartorii at 0° C against duration of stimulus. ●, thirty-three individual results obtained in thirteen experiments. It was impossible to show all the results separately because of overlap, so the mean values only are given, each with a figure indicating the number in the mean. The mean for five twitches is shown by a spot on the vertical axis. Lower curve, mean of three experiments each with a single long stimulus, analysed as described in the text. Upper curve, the same experiments, but the results of each were calculated for a contraction in which the tension was fully maintained throughout. ○, individual results reported in papers published in 1921 and 1922.

possible conclusion was that a serious and persistent error of calibration occurred in the earlier experiments: possible causes of this were considered above (p. 315).

In the experiments with continuous stimulation the rate of heat production and the tension were both available at all times, and Table 1 gives, for 17° C and for 0° C, the values of $(dH/dt)/Pl_0$. dH/dt is the rate of heat production and P is the tension, both at time t sec from the beginning of the stimulus. If H is expressed in gram-centimetres, P in grams and l_0 in centimetres $(dH/dt)/Pl_0$ has the dimensions sec^{-1}. As shown in Table 1, the

TABLE 1. Rate of heat production during continuous isometric stimulation in relation to the tension maintained

dH/dt is the rate of heat production and P is the tension, both at time t reckoned from the beginning of the stimulus; l_0 is the standard length of the muscles and M is their mass.

(A) at 17° C, mean of five experiments. The average value of the maximum tension was $Pl_0/M = 2610$, and of the tension at 4 sec $Pl_0/M = 2120$

t(sec)	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0
$(dH/dt)/Pl_0$	0·591	0·590	0·582	0·576	0·576	0·561	0·560	0·563

(B) at 0° C, mean of three experiments. The average value of the maximum tension soon after the start was $Pl_0/M = 1960$, and of the tension at 14 sec $Pl_0/M = 1660$.

t(sec)	2	4	6	8	10	12	14
$(dH/dt)/Pl_0$	0·105	0·088	0·084	0·083	0·083	0·083	0·083

Note. The final steady values of $(dH/dt)/Pl_0$ at 17°, say 0·561, and at 0° C, 0·083, are in the ratio of 6·76:1. This corresponds to a temperature coefficient $Q_{10} = 3·08$, which is very close to Feng's 3·1.

tension fell substantially during these long contractions, yet the values of $(dH/dt)/Pl_0$ became steady. One purpose in making these measurements was to compare their results with those given by Feng (1931). According to Feng (his p. 526) $(dH/dt)/Pl_0$ has a Q_{10} of 3·1 (which we have confirmed, see Table 1) and he smoothed and classified his final mean values at different temperatures: his value at 0° C was 0·094, at 17° it was 0·645. The steady value reached in Table 1 at 0° C was 0·083, at 17° C about 0·561. Feng's values are about 14 % greater than ours, but have the same temperature coefficient.

In the experiments from which the lower lines of Figs. 1 and 2 were constructed the mean tension fell off as contraction continued, at 17° C by about 19 %, at 0° C by about 15 %. This fall varied from one experiment to another and it was desirable to know what the relation would be, between heat and duration of stimulus, in a contraction in which the tension was fully maintained throughout. The fact that $(dH/dt)/Pl_0$ varies rather little, during a single contraction, makes the calculation easy. To start it, the observed value of H/Pl_0 is taken at a moment soon after the

maximum tension is reached; then, assuming Pl_0 to remain constant, later values of H/Pl_0 are obtained at the desired intervals by simple numerical integration, using the observed value of $(dH/dt)/Pl_0$ appropriate to each interval. This gives the relation between H/Pl_0 and the duration of stimulus throughout a contraction in which the tension was fully maintained: then, to get the relation between H/M and duration of stimulus, the numbers have all to be multiplied by Pl_0/M.

In Figs. 1 and 2 the upper lines, diverging gradually from the lower lines, represent the relation between heat and duration of stimulus, calculated as just described for contractions in which the tension was assumed to be fully maintained throughout. At $17°$ C the relation is linear after about 2·0 sec, at $0°$ C after about 6 sec. The upper curve in Fig. 1 is for $Pl_0/M = 2610$; for any other value of Pl_0/M the ordinates should be multiplied, or reduced, in proportion. The upper curve in Fig. 2 is for $Pl_0/M = 1960$.

The results just described allowed Fig. 3 to be calculated, in which H/Pl_0 is shown against duration of stimulus. Curve A is for $17°$ C, curve B for $0°$ C, and curve C (with one modification, see below) is taken from Fig. 1 of Feng's paper (1931). In order to show Curve A in the same figure as curves B and C, the durations of stimulus at $17°$ C were all multiplied by 6·82 which is $3·1^{1·7}$. In Feng's paper a generalized relation was proposed between H/Pl and duration of stimulus, applicable to all temperatures. Assuming a temperature coefficient $Q_{10} = 3·1$ for the duration of stimulus, the value of H/Pl for any duration and any temperature could be read off from a single curve. Feng took P as 'the mean tension developed and maintained'. His elegant generalization would agree strictly with our results if our two curves A and B, for $17°$ C and $0°$ C, coincided. The difference between them is small, though probably genuine; but many more experiments would be needed to establish it with certainty.

Feng's final values of $(dH/dt)/Pl$ are about 14 % greater than ours, which can probably be attributed to an error of calibration. It is not far from the 10 % excess calculated below (p. 328) from results obtained by other authors using the same instruments. The modification referred to above was, before plotting them as curve C, to divide all Feng's values of H/Pl by 1·14, in order to make the final slope the same as that of curves A and B. A more important difference then appeared between Feng's results and ours, namely that his curve is substantially higher: no modification of scale could make his curve and ours coincide. What the cause of the difference can be is not clear, though it may have been due to the rather peculiar method by which Feng's measurements of the heat were made (see p. 329 below). The present methods are considerably more accurate than those available to Feng in 1931, and we have no doubt that our results are the more correct. Feng's results have been used by various

authors, e.g. Lundsgaard (1934) and Aubert (1956a, p. 160). It would be easy from curves A and B of Fig. 3 to read off more accurate values.

Two points should be noted: (a) our results were obtained at the standard length l_0 of the muscles; and (b) they refer to the sartorii of *Rana temporaria*. As regards (a), Feng worked at a length which he did not define, though it was probably rather greater than l_0. At lengths much less than l_0 the value of H/Pl_0 for a given duration of stimulus may be considerably greater than at l_0; at lengths greater than l_0 the value of H/Pl_0 is about the same
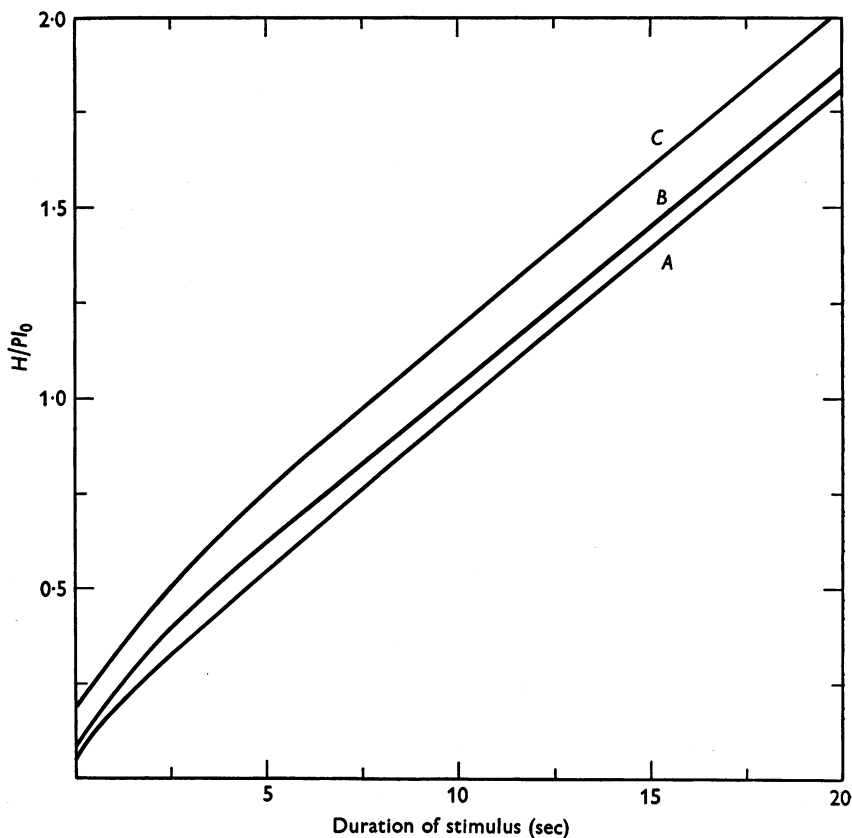

Fig. 3. H/Pl_0 plotted against duration of stimulus 'reduced' to $0°$ C. Curve A contains the values of H/Pl_0 obtained at $17°$ C plotted against 6·82 times the durations of stimulus: this corresponds to a Q_{10} of 3·1. Curve B gives the values of H/Pl_0 obtained at $0°$ C plotted against the actual durations. Curve C is reproduced from the curve in Fig. 1 of Feng's paper, but with the ordinates (H/Pl) divided by 1·14. Note. At 'reduced' times of less (say) than 1·2 sec the tension has not reached its final level. In calculating H/Pl_0 for a short contraction P is not the maximum tension actually reached but the maximum tension that would have been reached if the stimulus had been continued. The latter is a measure of the intensity of the contraction, the former is not.

as at l_0 (Aubert, 1956a, pp. 168, 172, etc.). As regards (b), other muscles, or muscles from other species of animals, may have different intrinsic speeds, and the rate constants (i.e. the slopes of the curves of Fig. 3) may be different. Almost certainly, however, the same general relations exist: indeed, it may be necessary only to change the scale of time in order to make curves *A* and *B* of Fig. 3 applicable to any muscles.

This expectation is borne out by the results of a large number of experiments which have been made during the last 2 years with the sartorii of toads (*Bufo bufo*). The values of the heat agree well with those for frog sartorii (Figs. 1 and 2) if they are regarded as due to a duration of stimulus equal to 0·4 times the actual duration. Thus the heat produced by toad sartorii with a 2·5 sec stimulus is about the same as that by frog sartorii with a 1·0 sec stimulus. This relation appears to hold for all temperatures and durations of stimulus.

Hartree & Hill (1921) in their Fig. 4 gave curves of H/Pl against duration of stimulus, for temperatures from 0 to 20° C. Their values are considerably greater than ours. This is probably a sign of the same error of calibration as affected other results in that period.

The effect of compliance in the recording system on the value of Pl_0/H in an isometric twitch

In considering the relation between the heat produced in an isometric twitch and the tension developed, it is necessary to multiply the tension by a length l characteristic of the muscle, otherwise the result depends on the dimensions of the muscle. For many years it was usual to choose l as the length of the muscle at which the tension developed in a twitch was a maximum (see e.g. Hill, 1928a, p. 136); it was not realized then that the length so determined depends on the compliance of the tension recorder with the thread, wire or chain connecting it to the muscle. With a nonlinear compliance the muscle has to be more and more stretched the greater the compliance is, in order to give its maximum tension in a twitch. This is not true of prolonged contractions; it depends upon the need in a twitch not to waste time in shortening during the very limited period available before the active state begins to decay. The effect of added compliance in a twitch was illustrated and discussed by Hill (1951).

Since, therefore, l defined as described above depends on the instruments as well as on the muscle, it is not properly a characteristic of the muscle alone. It is easy, however, in a frog sartorius to define a 'standard length' l_0, namely the distance between the inside ends of its tendons when the legs are laid out in line unstretched (Hill, 1952, p. 467): this length has been used in this laboratory for many years, and was referred to in earlier papers as the resting length in the body.

A value of about 10 for Pl_0/H was obtained by Hill (1958) in isometric twitches at 0° C, with the least possible compliance in the tension recorder and connexions. In the past when little attention was paid to the compliance of the tension-recording system much lower values were found at room temperature, for example, the mean in 12 experiments (Hill, 1928c, p. 164) was 6·36 (s.e. of mean 0·39). Such lower values might have been a natural property of muscle at a higher temperature, though Hartree & Hill (1921, Fig. 4) had found that Pl_0/H in a twitch is independent of temperature; or they might have been caused by an error in the heat calibration: but they could also have been due to compliance in the recording system. Experiments, therefore, were made at 17° C (a) with a strong wire connecting the muscles to an R.C.A. transducer (5734) and (b) with 30 cm of strong 'button thread' inserted between the wire and the transducer. With the wire alone the maximum value of Pl_0/H was found at a length slightly greater than l_0 and averaged about 9; with the thread in series with the wire the maximum was obtained at a length substantially greater than l_0 and averaged about 6. Against the thread the muscles had to shorten an extra 1–2 mm before the maximum tension was reached; this delayed the peak of the tension to a time when the active state was already decaying fast.

We have no doubt that if the experiments had been continued with more stringent precautions to reduce the compliance further, a rather higher average than 9 for Pl_0/H would have been obtained. The chief purpose, however, of the experiments had been fulfilled when the large effect of an added compliance had been confirmed. The length of button-thread used must have had much the same compliance as the 'thick linen thread' employed by Hill in 1928 and probably by him and others in many other investigations. Moreover, it seemed unnecessary, in view of the results of Meyerhof, Gemmill & Benetato (1933), to continue the attempt to get higher values of Pl_0/H. They had measured the total O_2 consumption of frog sartorii under extremely good isometric conditions in a series of twitches at 16° C, at a length which must have been close to l_0. Interpreting their O_2 measurements in terms of heat, their mean for Pl_0/H was 4·62. Their H, however, included the recovery heat, which on the average is about 1·2 times the initial heat. Considering initial heat only, their value would have been $2·2 \times 4·62 = 10·2$, almost identical with the values obtained at 0° C by Hill in 1958.

Rosenberg (1934), by a method of calibration which (p. 315 above) appears to have given approximately accurate results for the heat produced in a twitch, obtained values of Pl/H which averaged 8·25. He had taken special care, by using a straight wire, to avoid unnecessary compliance in the connexion to his tension lever, which was made with a strong spring. The

initial tension in his muscles was 3–4 g, which is rather more than would have been found at initial length l_0; so the value of l was perhaps 1 or 2 mm greater than l_0. The true average value, therefore, of Pl_0/H in his experiments must have been rather less than 8·0. The difference between this and 9 or 10 is probably accounted for by the compliance of his tension lever.

<div align="center">DISCUSSION</div>

<div align="center">*The relation between heat and work*</div>

The chief interest of the conclusion, that the absolute values of the heat production reported in various earlier papers were considerably too great, lies in its application to the results obtained by similar methods, during the same period, on the relation between work and heat. These can be considered under two headings, (a) the mechanical efficiency and (b) the absorption of work by muscles stretched during contraction.

(a) *The mechanical efficiency.* Fenn (1923) reported the results of a large number of experiments in which the mechanical efficiency, $W/(W+H)$, of frog sartorii was measured, when contracting isotonically or against an inertia lever. The figures in his Table 6 each represented 'the highest value which could be obtained from one pair of muscles in a large number of trials'. The averages of his highest values were, for frog and toad sartorii:

<div align="center">

isotonic, frog; 20 % (11 values; S.E. of mean 1·5),
inertia lever, frog; 23 % (17; S.E. 0·95),
isotonic, toad; 21 % (4; S.E. 1·6),
inertia lever, toad; 29 % (7; S.E. 2·6).

</div>

Three years later than Fenn, Wyman (1926) reported the results of experiments with a tortoise leg muscle (properly called the iliofibularis, Hill, 1950) from which the mechanical efficiency can be calculated. He used an earlier version of what has since been called the Levin–Wyman ergometer, which is a very efficient collector of work from contracting muscles. From the weighted means in the twelve experiments of his Table 2 the efficiency is calculated as 24·5 %.

Two years later again Hartree & Hill (1928b), using a Levin–Wyman ergometer, obtained values of the mechanical efficiency up to 26 %. In a second paper (1928c), however, they reported rather higher values, frequently up to 30 %. They had investigated very carefully the technique of getting the maximum amount of work out of contracting muscles and it is unlikely that much more could have been got. Nevertheless, Hill (1939), with the new instruments and methods for measuring heat but with the same Levin–Wyman ergometer, obtained maximum values in different experiments ranging from 35 to 43 %; with isotonic contractions the range was from 30 to 37 %. The big discrepancy between Hill's 1939

values and those in the three earlier papers cannot be explained by supposing that Hill in 1939 used more efficient methods of collecting the work. The fact that 'protected' thermopiles (Hill, 1937) had not been introduced at the time of the earlier experiments might make a small contribution to explaining the discrepancy. But the muscles were always pulled back to their original position after contracting, and the galvanometers were slow; slight inequalities of temperature, therefore, along the muscles could not have produced so large an effect.

All the older experiments had been made with instruments and methods of calibration similar to those which showed the discrepancies illustrated in Figs. 1 and 2 above. Let us assume, therefore, that the true heats in the earlier experiments were consistently only 0·65 of those observed. Then Fenn's results for the efficiency become respectively 27·8, 31·5, 29·1 and 38·3 %; Wyman's become 33·3 %; Hartree & Hill's become 35·1 and 39·7 %. These are near enough to the values found by Hill in 1939 to allow us to regard the disagreement as resolved.

(b) *The absorption of work by muscles stretched forcibly during contraction.* Fenn (1924), having found that the performance of mechanical work *by* contracting muscles led to the mobilization of extra energy, naturally asked whether less energy was released by muscles *on* which work was done by stretching them during contraction. Since the work done on the muscles had entirely disappeared by the time the muscles had relaxed, the energy given out by the muscles was equal to $(H - W)$, where H was the heat which was finally observed in the muscles and W was the work done on them. If the observed value of H was really considerably too great, the true value of $(H - W)$ would be less and Fenn's conclusion more decisive.

In Fenn's Table 3 (p. 386) are the results of ten experiments in which muscles were stretched by a weight released during contraction. Taking only the case of the largest weights, with which the work W done on the muscles was greatest, his recorded values of $(H - W)$, which is the energy liberated by the muscles, averaged 0·78 (0·57 to 0·95) of the heat in an isometric contraction (or in three experiments where the isometric heat was not reported in his Table, of the energy given out by the muscles when they were stretched by a small load during relaxation only). If, however, the actual heat be taken as 0·65 of the observed heat H, the average energy $(0·65\,H - W)$ becomes 0·60 (0·32 to 0·78) of the isometric heat (also reduced to 0·65 of the observed value). With Fenn's original figures the energy given out by the muscles was 22 % less than the isometric heat, with the revised figures it is 40 % less. This makes a much more striking result.

Hill & Howarth (1959) 35 years after, with the later equipment and the previous experience of Abbott, Aubert & Hill (1951) and of Abbott & Aubert (1951), found it possible to arrange the conditions of

stretching during contraction so that the net energy given out by the muscles was zero. It is impossible now to say how close Fenn came in 1924 to this ideal, but he certainly came much closer than has been realized till now.

Wyman (1926), in his Table 2, reported the results of twelve separate experiments on the tortoise iliofibularis in which the muscle was stretched by an ergometer during contraction. Referring to the average of the isometric heats in the long and the short positions as I, the weighted mean of all his values of $(H - W)/I$ was 0·92; from this it would appear that little, if any, of the work was absorbed. But if the true H were taken as 0·65 H, and the true I as 0·65 I, the mean value of $(0·65 \ H - W)/0·65 \ I$ would become 0·57. This would imply a considerable absorption of heat. It seems that Wyman also, like Fenn, was prevented by an error of calibration from obtaining a result as decisive as it ought to have been.

Methods and equipment of Hill (1928a)

The methods and equipment described by Hill (1928a) were used in a large number of investigations in many of which absolute values were required. It was very desirable, therefore, to try to find out whether errors of calibration affected these results also. The isometric heat coefficient in a twitch (Pl_0/H) might have provided an indication of such errors, if they existed. In his paper (1928c) Hill gave a mean value of Pl/H of 6·36 which is much smaller than the present accepted value (9–10): this might have been due to the value of H being too large. But it was shown above (p. 324) that the low value can be sufficiently accounted for by compliance in the tension-recording system; it provides no evidence that the values of H were too large.

A clearer test of the accuracy of the 1928 equipment is provided by another method. In a large number of experiments made with it by various authors, between 1928 and 1931, the total heat was measured in muscles stimulated to exhaustion under anaerobic conditions in a long series of twitches. In three papers by Hill (1928b, c; 1929) 9 measurements are given of the total heat under such conditions, with a mean value of 0·690 cal/g (0·53 to 0·82). In a paper by Stella (1929, Table 2) there are 5 measurements with a mean value of 0·915 cal/g (0·885 to 0·930). In a paper by Hill & Kupalov (1930) are 8 measurements with a mean value of 0·972 cal/g (0·621 to 1·165). In a paper by Hill & Parkinson (1931) the mean of 8 is 0·766 cal/g (0·554 to 0·906); and in a paper by Hukuda (1931) the mean of 10 is 1·01 cal/g (0·85 to 1·16). The average of all these 40 values is 0·917 cal/g. The variation is wide, but a large part of that must have been in the muscles used.

This mean value can be compared with results obtained by other

methods. Peters (1913) with a calorimetrical method, and a tetanic stimulation of low frequency, measured the total heat to exhaustion of frog leg muscles in the absence of oxygen. The mean of 8 values was 0·82 cal/g (0·61 to 0·94); but this should be corrected to 0·84 cal/g since he assumed too low a value of the specific heat of muscle. Meyerhof (1922, pp. 30, 31) in 10 experiments with twitches, also by a calorimetrical method with frog leg muscles, found a mean value of 0·834 cal/g (0·47 to 1·12). Hill (1955, p. 9) with twitches of frog sartorii, using the modern myothermic method, found in 5 experiments a mean value of 0·86 cal/g (0·70 to 1·00). The mean of all these is 0·840 cal/g.

The near agreement between the average (0·917) of the 40 values obtained with Hill's (1928a) method and that (0·840) of the other three investigations, does not leave any possibility of a large error of calibration in the former. The most likely conclusion is that the 1928 equipment gave heats, on the average, about 10 % too great, which agrees well enough with the 14 % excess of Feng's values of $(dH/dt)/Pl$ over ours (p. 321 above). An error of 10 % or 14 % in calibration is small compared with the error which affected the results of earlier experiments; but it is not negligible, as the following section shows.

The heat of neutralization of CO_2 by muscle

One of the more important investigations made with the 1928 instruments is that described by Stella (1929) on the heat of neutralization of CO_2 by living muscle. The results of this were discussed, and accepted, by Meyerhof (1930, p. 215) in his monograph on the chemistry of muscle: their significance was that they provided a measure of the heat of neutralization of an acid inside a living muscle cell. Stella found that the mean value of the total heat produced in frog sartorii by exposing them to an atmosphere of CO_2, at an average temperature of 19·3° C, was 0·379 cal/g.

In drawing conclusions from Stella's experiments the effect of a possible error of calibration is aggravated by the fact that a quantity about equal to half the heat observed has to be subtracted from it, so nearly doubling the result of the error. The quantity (at 19·3° C) to be deducted is 0·18 cal/g, representing the heat of solution of CO_2, less the negative heat of ionization of the part of the CO_2 combined. The difference between the observed heat and 0·18 cal/g would be reduced from 0·199 to 0·161 cal/g if there were an error of 10 % in the calibration: this would be quite serious.

In view of the importance of Stella's result it is desirable that his experiments should be repeated with methods which would give the absolute value of the heat of neutralization of CO_2 with greater certainty.

The heat:tension ratio in prolonged isometric tetanic contractions

It was shown above (p. 321) that Feng's (1931) generalization, that the relation between H/Pl and duration of stimulus can be described by a single curve applicable to all temperatures, is not far from accurate; and that the final slope of his curve is only about 14 % greater than in our experiments, which can probably be attributed to an error of calibration. What is not easily explained is the fact that his curve C (Fig. 3) lies so far above our curves A and B. He cannot have failed to measure his tensions accurately. If his l was rather greater than our l_0, as is likely, that would have diminished, not increased, his values of H/Pl.

Feng's method of measuring the heat may have had something to do with the discrepancy. He used 'the maximum deflexion method' (see his p. 523), each duration of stimulus giving a single reading, and every pair of muscles being subjected to several stimuli of different durations. Feng stated that by making his galvanometer and thermopile very slow he could obtain deflexions proportional to the heat production even for durations of the latter as long as 20 sec. He tested this proportionality by electrical heating of dead muscles, and found a linear relation between maximum deflexion and duration of heating, for durations up to those of the longest stimuli used. We have made a few experiments with a slow over-damped galvanometer and a thermopile with a layer of wax on its surface; and there is no doubt that up to quite long times an approximate relation of this sort exists. But it could not be exact: the maximum deflexion for short durations of heating is bound to be relatively greater than that for long ones. The method, however, which Feng used was the only practicable one at the time, unless he was prepared to make a large number of photographic records and do an enormous amount of arithmetic in analysing them numerically. It is remarkable that with it he could establish so important a generalization and obtain quantities on the whole so nearly correct.

Our present results at 0° C are in remarkable agreement with those which can be calculated from the quantities given in Table XII (p. 148) of Aubert's monograph (1956a). Aubert's muscles, like ours, were the sartorii of *Rana temporaria*. If we modify his equations (1956a, b) but use his symbols, the heat H_t up to time t in a continuing isometric contraction can be expressed as

$$\frac{H_t}{Pl_0} = \frac{h_A/\alpha}{Pl_0}(1 - e^{-\alpha t}) + \frac{h_B}{Pl_0}t \qquad (3)$$

In his Table XII are the detailed results of fifteen experiments at 0° C made

at lengths within $\pm$ 1 mm of the standard length l_0. We calculated, for these experiments, the average values of the following quantities:

$$Pl_0/M = 1670,$$

$$(h_A/\alpha)/Pl_0 = 0.153,$$

$$\alpha = 0.737,$$

$$h_B/Pl_0 = 0.0833.$$

For comparison with our results, which refer to the heat H_x given out in a contraction with a duration of stimulus x, 3 mcal/g must be added to the heat (see p. 316 above), which is 127.8 g.cm/g; and this must be divided by 1670, his mean value of Pl_0/M, the quotient being 0.077. Equation (3) then becomes

$$H_x/Pl_0 = 0.077 + 0.153\ (1 - e^{-0.737x}) + 0.0833x. \qquad (4)$$

Results calculated from this equation are given in Table 2, where they are compared with ours (Fig. 3, curve B) and Feng's (Fig. 3, curve C). The results are very close to ours and differ substantially from Feng's. They would differ more from Feng's had his heats not been divided by 1.14 for the reason given on p. 321.

TABLE 2. A comparison of the results obtained in three independent investigations of the relation between H/Pl_0 and duration of stimulus in isometric contractions of frog sartorii at $0°$ C

Durations of stimulus are given in the first row. All the other numbers are values of H/Pl_0 derived either from the present results, or from those of Aubert (1956a) and Feng (1931).

Duration (sec)	0	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0	16.0
Present paper	0.079	0.158	0.225	0.343	0.445	0.536	0.702	0.868	1.202	1.535
Aubert	0.077	0.166	0.240	0.362	0.463	0.555	0.728	0.896	1.230	1.562
Feng	0.185	0.252	0.317	0.441	0.557	0.660	0.845	1.016	1.353	1.686

Aubert's equations and his numerous experimental results give a remarkably accurate description of the course of the heat production during a continuing isometric contraction at $0°$ C; but we doubt whether his quantities h_A and α have a chemical or physical basis, and for this reason: they refer to changes which take place during the time when the tension is rising, and for a certain interval after that. But during the rise of tension a substantial amount of mechanical energy is being developed, and moreover the heat produced is greater than appears because of the negative thermoelastic effect of the rising tension (see Hill, 1961b, p. 542). Thus chemical changes are supplying total energy at a significantly greater rate than that at which they are producing detectable heat. The quantities h_A and α, therefore, would be more relevant if they referred to total energy rather than heat.

Aubert's h_B/Pl_0 is the same thing as the final slope of the curves in Fig. 3, and represents the rate of energy turnover associated with the maintenance of unit tension in unit length of muscle. In the characteristic equation, $(P+a)(v+b) = (P_0+a)b$, for the relation between the load P and the velocity of shortening v, the constant a is known to be about $P_0/4$ and b is known (at $0°$ C) to be about $(l_0/3)/\text{sec}$. If $h_B/P_0l_0 = 0.083$ (in which Aubert's results and ours agree), substituting $P_0 = 4a$, $l_0 = 3b$, we obtain $h_B = 1.00ab$; in words, the rate of energy turnover during a maintained isometric contraction is equal to the rate of heat of shortening at velocity b. This curious coincidence was noted by Hill (1939): it is perhaps worth mentioning again since a more accurate value of the maintenance heat is now available.

SUMMARY

1. Values of the heat production in isometric contractions of frog sartorii reported in various papers between 1921 and 1932 were discovered to be considerably and consistently greater than those obtained in recent experiments.

2. This led to a critical examination of present methods of calibration, which proved to be substantially accurate. The discrepancy therefore must be attributed to an error of calibration in the older methods. Possible causes of this are discussed. The method of calibration now used is described in more detail than hitherto.

3. An error of calibration is important only when heat is compared with other absolute quantities, e.g. with work, tension or chemical change. The results, therefore, of most of the investigations published during this period are not affected.

4. Several important papers, however, appeared during the period 1923–28, in which the relation between heat and mechanical work was examined, and in which the methods used were similar to those which were affected by the error referred to. Their results have been re-examined, on the assumption that the reported values of the heat were all considerably too great.

5. This has brought their conclusions into line with those obtained later by other methods. In particular, W. O. Fenn's fundamental results (1924) on the absorption of work by muscle stretched during contraction turn out to be clearer and more decisive than they appeared at the time.

6. A scrutiny of the absolute values of the heat production reported in later investigations, by means of other instruments and methods, provided no evidence that any consistent or substantial error of calibration had affected these; though it seems that the important work of Stella on the heat of neutralization of CO_2 by living muscle ought now to be repeated with a more accurate method.

7. More precise information has been obtained on the heat : tension ratio throughout a maintained isometric contraction, at 0° C and at higher temperatures.

8. With the series-compliance of the tension-recording arrangements reduced to a minimum the isometric heat coefficient (Pl_0/H) in a twitch has the same high value (about 10) at room temperature as it was previously found to have at 0° C.

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