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THE NEGATIVE DELAYED HEAT PRODUCTION IN STIMULATED MUSCLE

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In a paper on the delayed ('recovery') heat production of frog sartorius muscles, Hartree (1932*a*) described experiments at 0° C in nitrogen which appeared to show an absorption of heat lasting for about 40 sec after tetanic contractions of 1–5 sec duration. This 'negative' heat production amounted, on the average, to 3 % of the initial heat, varying from 1 to 5 %. Six years earlier Furusawa & Hartree (1926), working with frog muscles in nitrogen at room temperature, had reported the frequent observation of early negative delayed heat after a short stimulus (0·05 sec or less); it amounted to 2–4 % of the initial heat. After a longer stimulus (0·3 sec) it was absent. They were inclined to regard it as an error due to irregular distribution of the heat production, though they had taken precautions to avoid such effects by using a special thermopile making contact with both faces of the muscle. In view of the results of the present paper it seems likely that the effect they observed was genuine: but their doubt about its reality is shown by the fact that they did not mention it in the summary of their paper. Two years later Hartree & Hill (1928, p. 210) reported 'in our experience there is never any negative heat'. Its possibility was in their minds, because Nachmansohn (1928) had just discovered that up to 30 % of the phosphagen split during a tetanus was resynthesized in about 20 sec at room temperature in the absence of oxygen: and he claimed that no lactic acid was formed at the same time, a result shown 3 years later, by the work of Lundsgaard (1931) and of Lehnartz (1931), to be wrong. Had his result about lactic acid been correct there might have been a large negative heat: though it is not evident then where the free energy could have come from to drive the resynthesis of the phosphagen.

Because of the special interest of his result, and because of previous doubts and failures with negative heat, Hartree examined the conditions of his experiments very critically. The negative heat was small and could possibly have been due to several errors that might have affected his measurements. He was able to discard any possible errors which could be

attributed to irregularities, if they occurred, in the distribution of heat within the muscles; the time course of the negative heat was much too long to be explained in this way. He was inclined to believe that it might be due to an error in his 'heating control' curves; this could be caused by a small change of weight of the muscle on the thermopile between the times (1) when the records of heat production were made on the living muscle and (2) when those of artificial electrical heating were made on the dead muscle after it had been killed by chloroform vapour. If the weight had been increased (e.g. by the absorption of water vapour due to increased osmotic pressure, or by slight shortening and thickening) the muscles would cool more slowly after electrical heating: then the analysis of the heat production in the living muscle would show an apparent absorption of heat.

Hartree was the more inclined to accept this explanation of the effect observed, because he found it to occur equally in muscles adequately poisoned with iodoacetate, in which lactic acid is not produced. This seemed to eliminate the possibility that the apparent negative heat could be due to the restoration of creatine phosphate by means of the free energy of lactic acid formation. He was not really satisfied, however, with dismissing, as a technical error, an effect he had observed so regularly and he returned to the subject in a later paper (Hartree, 1932*b*), employing a new method of making his heating control curves. Instead of killing the muscles with chloroform and using an alternating current of low frequency for heating, he adopted a valve oscillator providing an alternating current of frequency high enough (100 kc/s) not to stimulate. With this the heating control could be made on the living muscles at any time during a series of records of the heat production due to stimulation. His previous observations were entirely confirmed. In nitrogen at 0° C, after a 1 sec stimulus, there was negative heat production completed in 15–50 sec and varying from 1 to 8 % of the initial heat. After a long stimulus (e.g. of 10 sec) negative heat was not found: it was masked, Hartree surmised, by the onset of the usual positive delayed heat.

Two years later Bugnard (1934), using an extremely accurate method with single twitches at room temperature of a muscle in nitrogen, obtained clear evidence of negative delayed heat. It amounted to about 4 % of the initial heat and appeared in the first 15–20 sec after the shock. In oxygen also Bugnard found signs, in the first 10–15 sec, of negative delayed heat. Both in oxygen and in nitrogen the negative heat was followed by the usual positive heat, which presumably cut it short. After single twitches the positive delayed heat, whether aerobic or anaerobic, comes on very slowly compared with that after even a short tetanus. That probably is why Bugnard was able to detect the negative heat at room temperature.

The only other published reference to negative delayed heat is that by

D. K. Hill (1940). But his observations were only incidental in an investigation of the time course of the oxygen consumption, of the recovery heat and of the pH changes in muscles stimulated at 0° C. His results added nothing material to Hartree's.

The importance of Hartree's and Bugnard's results, if confirmed, in connexion with the thermodynamics of the chemical processes known, or supposed, to follow contraction made it desirable to examine the negative heat again; and in particular to find out whether this negative heat was uniquely characteristic of 0° C and lack of oxygen, or whether it occurred also, as Bugnard's results suggested, in the presence of O_2 and at higher temperatures. Greater precision is possible today than with the thermopiles available to Hartree in 1932, particularly because of the valuable property of the present ones that the rate of heat loss of muscles lying on them is exponential: which means that the course of the delayed heat production can be calculated from the records, over long times, with greater accuracy and far less labour than earlier.

The new examination of the negative delayed heat not only has confirmed its existence at 0° C in the absence of oxygen, and the general description of it by Hartree, but has shown (1) that it occurs also at room temperature (15–20° C), and (2) that it is apparent at both temperatures in oxygen as well as in nitrogen; though in oxygen it is cut short and partly masked by the usual recovery heat. It is a general phenomenon, therefore, and must be taken into account in any discussion of the chemical events that occur after contraction and relaxation.

METHODS

Electrical heating controls. Measurement of the delayed heat production of muscle always requires the use of a 'control' curve made by electrical heating of the muscles lying on the thermopile exactly as they were for the records of the heat production due to stimulation. The only purpose today of these heating control curves is to provide the constant needed for an accurate allowance for heat loss: the rest of the analysis (i.e. the allowance for time lag in thermopile and galvanometer) is carried out by other means (Hill, 1949*b*). The fall of temperature, after the initial heat production, is rapid, 2·5 %/sec in the larger muscles used, 5 % in the smaller ones. The former would reduce the initial rise of temperature within 1 min to 22 %, the latter to 4·6 %; and there is no exact method of allowing for such heat loss except by making a heating control curve in every experiment. Before 1938 the allowance was very laborious, involving successive subtractions, as in the older form of analysis (see Hartree, 1931, Appendix II), over the whole period (minutes) in which the delayed heat appeared.

With most of the thermopiles constructed since 1938, however, this labour is avoided by the fact that the fall of temperature of the muscles on them is accurately exponential (Hill, 1939). The rate of heat loss is embodied in a single time constant, derived from the heating control, which can be applied to any heat record from start to finish. When the records of the heat produced by the stimulated muscles have been measured and tabulated, the rest of the operation, using this time constant, can be carried out with an adding machine. The final result is a set of numbers from which the true curve of heat production can be plotted.

In order to ensure that the fall of temperature due to heat loss is accurately exponential the following precautions are needed:

(1) The behaviour of the thermopile must be carefully examined: if the fall of temperature is not accurately enough exponential the thermopile should not be used for measuring delayed heat.

(2) The part of the muscle lying on the thermopile should be as uniform as possible; if the cross-section varied, the heating current would produce differences of temperature along the muscles.

(3) Special heating electrodes should be provided, lying between the muscles in the plane of the thermopile, several millimetres beyond its ends. The reasons for this are (*a*) that at the heating electrodes themselves the current lines are concentrated, so the rise of temperature there is greater than in the middle region of constant current density; and (*b*) in the regions of muscle beyond the electrodes no heat is produced by the warming current, though it is by stimulation. If the heating electrodes were too close to the active thermocouples these effects could produce serious errors.

(4) The muscles should be mounted under a small tension at a length about equal to that (l_0) in the body. They behave most regularly at this length, and preliminary stimuli should be given to make them settle down to a constant position. They must be firmly fixed at each end, so as to avoid movement during contraction. In the present experiments strong threads from the tibial tendons were tied round a fixed cross-bar just above them, so that the least possible shortening could occur.

(5) A frequency of 50 kc/s is high enough to avoid stimulation by the heating current, but not so high as to cause capacitative leaks and other troubles. It was obtained from an ordinary commercial oscillator.

Thermopile. A drawing of the thermopile chiefly employed in the present work was given by Abbott, Aubert & Hill (1951, Fig. 1). The central stimulating electrode was not used, it was insulated. The two dummy thermocouples at each end, and the two stimulating electrodes, help to side-track any disturbances that might be conducted from irregular temperature changes at and beyond the heating electrodes. The best published drawing of a thermopile of the present type is in Fig. 2 of a paper by Hill (1938). One can see there how heating electrodes can be fixed a few millimetres beyond the stimulating electrodes (*n*) at each end: the clamp and the muscles then have to be displaced a few millimetres to the left.

Galvanometer. The galvanometer used had a period of 22 msec and its deflexions were amplified photo-electrically and displayed on a cathode-ray tube (Hill, 1948). A fairly large amount of heat was produced by the muscles, from 7 to 40×10^{-3} cal/g according to the temperature and the duration of stimulus; so considerable negative feed-back could be applied, increasing the speed of the galvanometer and stabilizing it. The galvanometer, indeed, was so fast that for the purposes of the work described here no allowance for lag was needed. To get greater long-term stability the 12 V lamp was used with 9 V, and the heaters of the head amplifier each with 4·5 V: the voltage was maintained constant by occasional adjustment with a rheostat.

Recording. The amplified deflexion of the galvanometer was displayed on the screen of a cathode-ray tube whose beam was modulated to give spots at intervals of 0·02–1·0 sec (as required). During heat production successive sweeps fell clear of each other, and so could all be recorded photographically upon a single piece of stationary sensitive paper 10·8 cm × 8·3 cm in size. This is convenient for measurement and storage, and the deflexion of any spot up to 75 mm was measured to 0·1 mm. The end of the stimulus was indicated by diminished brightness of the spot from this moment, and the base line was drawn by a single sweep immediately before the record was taken.

Muscles. A pair of sartorius muscles was used, from frog (*Rana temporaria*) or toad (*Bufo bufo*). Rather large muscles, up to 300 mg the pair, are better than smaller ones, because

their temperature falls more slowly, so the allowance for heat loss is relatively less; though muscles of 150 mg the pair give quite satisfactory results.

Stimulation was maximal, by means of short condenser discharges (about 0·2 msec) of frequency high enough to give a just complete tetanus.

The Ringer's solution used contained (mM) NaCl 115·5, KCl 2·5 and CaCl 1·8. It was buffered at pH 7·0 by phosphate 3·0 mM.

Thermostat. The thermopile, with its cover on, was sunk deep in a large double-walled vacuum flask (capacity 6 l.), filled either with water or with a mixture of water and ice, which was kept vigorously stirred by bubbling.

Nitrogen. No special precautions were necessary to ensure freedom from oxygen, since oxygen-free nitrogen is now supplied commercially. Usually the muscles, mounted on the thermopile, were kept for some time in oxygen-free Ringer's solution, with nitrogen bubbling through it, before the fluid was replaced by nitrogen.

Analysis of heat records. No analysis was usually needed, except the allowance for heat loss, at any time later than 1–2 sec after the end of relaxation. By then the thermopile has reached the temperature of the muscles. For an accurate determination of the early heat production, particularly at higher temperatures, an analysis is required to allow for delay in the transfer of heat from muscle to thermopile. When this was necessary it was carried out by the method of 'factors' described in earlier papers (Hill, 1949*a*, *b*).

Osmotic effect on base line. When a muscle is stimulated in the absence of oxygen the break-down products of activity accumulate in it and its vapour pressure falls: so water distils over to it from elsewhere and causes, by condensation, a continuing production of heat (Hill & Kupalov, 1930). The base line, therefore, shifts in a positive direction. If not allowed for this can cause a substantial error when the delayed anaerobic heat is measured over a long period. The effect, however, was small in the times involved in the present experiments, and would be in the positive, not the negative, direction.

RESULTS

At 0° C

In Fig. 1 are two curves of deflexion representing the heat produced, during and after contraction at 0° C, by a pair of toad sartorii. Details are given in the legend. The records were continued to show the early stages of the recovery heat, aerobic or anaerobic. Curve *A* is for the muscles in O_2, curve *B* for the same muscles in N_2 57 min after O_2 had been removed. This was the first stimulus in N_2. The base lines are different, and are a long way below the figure. The deflexions plotted are millimetres as recorded, but corrected for heat loss. The sensitivities are stated in the legend. Only the last stage of the initial upward deflexion is shown in either case. In toad muscles at 0° C relaxation is not complete until 2 sec after the last shock, and the deflexions were within 0·4 mm of their maxima by 1 sec later.

After the maxima the corrected deflexions fall, reaching minima in about 37 sec in O_2, in about 57 sec in N_2 (reckoning from the beginning of stimulation). These times are so long that the fall could not be due to inequalities, within the muscles, in the spatial distribution of the heat produced during contraction and relaxation. Equalization of temperature

would not take more than about 2 sec, if such inequalities occurred (see below). In both cases the fall was clearly cut short by later positive heat production, in O_2 (*A*) by the usual oxidative recovery heat, in N_2 (*B*) by the smaller and slower delayed anaerobic heat. The negative heat in O_2 was 5 % of the initial heat, in N_2 6·4 % of the initial heat. The experiment was continued with later stimuli in N_2; all the results in N_2 are given in Table 1.

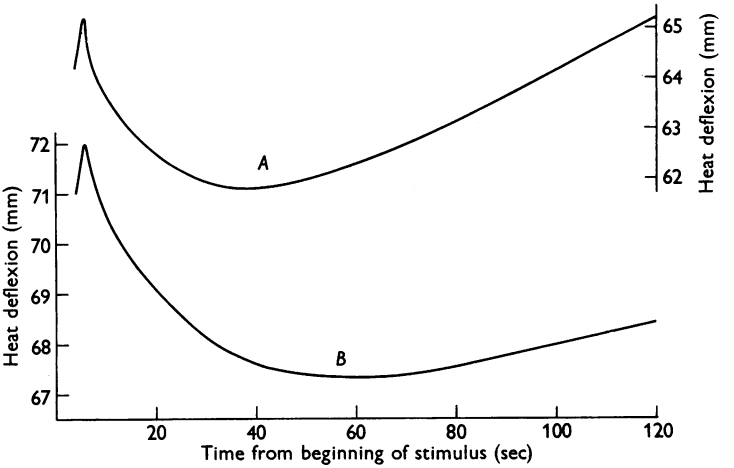

Fig. 1. Final stage of initial heat production at 0° C, followed by negative heat. Toad sartorii, in O_2 (*A*) and in N_2 57 min (*B*). 3 sec tetanus, 10 shocks/sec.

A. Scale of deflexion on right, 1 mm = $0{\cdot}16 \times 10^{-3}$ cal/g. Initial heat to max. = $10{\cdot}4 \times 10^{-3}$ cal/g muscle: negative heat to min. = $0{\cdot}53 \times 10^{-3}$ cal/g muscle.

B. Scale of deflexion on left, 1 mm = $0{\cdot}148 \times 10^{-3}$ cal/g. Initial heat to max. = $10{\cdot}65 \times 10^{-3}$ cal/g muscle: negative heat to min. = $0{\cdot}68 \times 10^{-3}$ cal/g muscle.

TABLE 1. Negative delayed heat at 0° C after various periods without oxygen

Time without O_2 (min)	57	65	84	100	110
Stimulus duration (sec)	3	3	3	8	8
Initial heat (10^{-3} cal/g muscle)	10·65	9·74	9·52	17·6	16·8
Negative heat (10^{-3} cal/g muscle)	0·68	0·67	0·65	0·81	0·73
Negative heat (% of initial)	6·4	6·9	6·8	4·6	4·4

A similar experiment was made (in oxygen only) at 0° C on another pair of toad sartorii, with different durations of stimulus. All showed negative heat production after the initial maximum, but this was cut short by the oxidative recovery heat more and more as the duration of stimulus was increased. See Table 2. The time to the minimum is reckoned from the start of the stimulus. If it were reckoned from the end of the stimulus there would be a substantial decrease with longer stimuli. It has long been known (since Hartree & Hill, 1922; see also Hartree, 1932*a*) that the recovery heat production is relatively more rapid after a greater initial

heat production; so the negative heat is cut off sooner by the positive heat after a longer stimulus.

Although calculation had shown (see also below, p. 187) that any inequalities of temperature produced in a muscle during contraction and relaxation would be evened out by conduction within 1–2 sec, it was conceivable that some unknown factor had been neglected in the calculation. A few experiments, therefore, were made with single muscles, in which after a few records had been obtained the muscle was reversed on the thermopile and the records repeated. When a pair of muscles is used, the outer surface, on which the epimysium lies, is bound to be in contact with the thermopile. It was possible that the inner fibres, usually outside on the thermopile, might somehow behave differently from the outer fibres,

TABLE 2. Negative delayed heat in O_2 at 0° C with various durations of stimulus

Duration of stimulus (sec)	1	2	3	4	8
Initial heat (10^{-3} cal/g muscle)	6·5	9·0	11·1	12·9	19·8
Negative heat (10^{-3} cal/g muscle)	0·32	0·39	0·38	0·25	0·14
Negative heat (% of initial)	4·9	4·3	3·4	1·9	0·7
Time to minimum (sec)	23	25	24	23	23

normally in contact with the thermopile. The experiment shown in Fig. 2 was performed in O_2 at 0° C, on a single large sartorius of a frog with 1·2 sec tetanus. First, two records were made with the muscle arranged in the usual way: then a heating control was recorded. Next the thermopile was taken out of the thermostat and the muscle was reversed; when temperature equilibrium had been restored two more records were made, and finally another heating control.

The two records of the heat production with the epimysium inwards were averaged and corrected for heat loss. So were the two records of the heat production with the epimysium outwards. The results, in this experiment, were in fact nearly the same with the muscle one way round and the other: they were finally averaged and are shown in Fig. 2. Curve *A* gives the whole deflexion, from the start to 25 sec; curve *B* gives the later part of the deflexion, on ten times the scale, but at the same times. The negative heat occurred as usual; the minimum was at about 18 sec, after which the oxidative recovery heat began. The negative heat was $0{\cdot}37 \times 10^{-3}$ cal/g muscle, about 5·8 % of the initial heat.

In some experiments at 0° C in O_2 the minimum was considerably earlier, and the negative heat was correspondingly less; in these the recovery heat had evidently begun sooner, and had partly masked the negative heat. In one such experiment the negative heat in N_2 was several times as great as in O_2, because the positive delayed heat came on much more slowly in N_2 than in O_2.

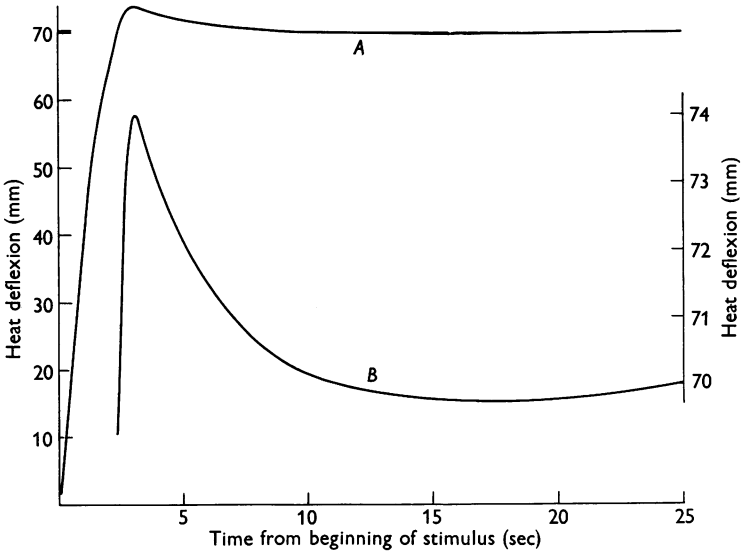

Fig. 2. Initial heat production at 0° C followed by negative heat. Single sartorius of frog in O_2. The deflexion, corrected for heat loss only, is the mean of 4 records, 2 with the outer surface of the muscle in contact with the thermopile, 2 with the inner surface. 1·2 sec tetanus. *A* shows the whole deflexion from the start to 25 sec, *B* shows the later portion only, on 10 times the scale; times are the same for both. Scale of deflexion, *A* on the left, *B* on the right: 1 mm = $0{\cdot}086 \times 10^{-3}$ cal/g muscle. Initial heat to max. = $6{\cdot}4 \times 10^{-3}$ cal/g muscle: negative heat to min. = $0{\cdot}37 \times 10^{-3}$ cal/g muscle.

At room temperature

In eight experiments with muscles in O_2 at temperatures from 16 to 20° C, with short stimuli, the negative heat was clearly present; but (reckoned as a fraction of the initial heat) it was smaller than at 0° C and was evidently being cut short by the earlier development of the positive recovery heat. In other experiments there was little sign of negative heat and with longer stimuli (1·2 sec and above) it never appeared. But the initial heat, with a 1·5 sec stimulus at 18° C, is rather large, usually about 50×10^{-3} cal/g in frog muscles, about 30×10^{-3} cal/g in toad muscles: so the positive recovery heat, the early rate of which increases more than in proportion to the initial heat, becomes too strong to allow the negative heat to appear. In twelve contractions with stimuli from 0·2 to 1·0 sec the negative heat varied from 0·1 to $1{\cdot}4 \times 10^{-3}$ cal/g muscle, from 0·9 to 3·8 % of the initial heat: it was complete (or cut short by the recovery heat) in times, reckoned from the last shock, of 1·8 sec for the longer stimuli to 9·0 sec for the shorter ones.

Figure 3 shows the negative delayed heat in two of these experiments, *A* in frog muscles at 16·7° C, *B* in toad muscles at 16·8° C. Details are given in the legend. In each experiment the last shock was marked on the record and the deflexion was analysed in units of 0·04 sec. In *A* the negative heat was 3·8 % of the initial heat, in *B* 3·5 %. In the same experiment as *A* a later contraction with a 1 sec stimulus gave negative heat $1{\cdot}4 \times 10^{-3}$ cal/g muscle (3·7 %), but two contractions with 2 and 4 sec stimuli gave practically none.

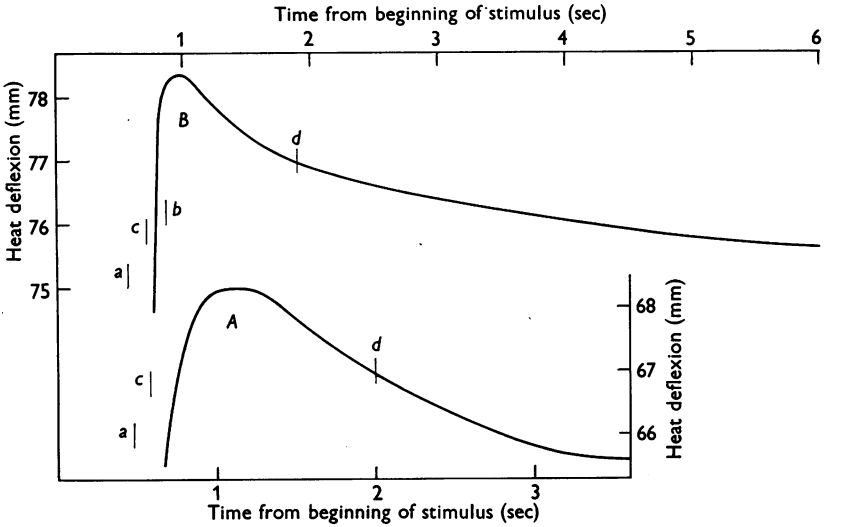

Fig. 3. Final stage of initial heat production in O_2 at room temperature, followed by negative heat.

A: frog sartorii, 16·7° C, isometric tetanus, 25 shocks, last shock (*a*) at 0·48 sec. Peak of relaxation heat (*c*) at 0·58 sec. Negative heat half complete (*d*) at 2·0 sec. Time marking below: scale of analysed deflexion on right, 1 mm = $0{\cdot}382 \times 10^{-3}$ cal/g muscle. Initial heat to max. = $26{\cdot}1 \times 10^{-3}$ cal/g muscle: negative heat to min. = $1{\cdot}03 \times 10^{-3}$ cal/g muscle.

B: toad sartorii, 16·8° C, isometric tetanus, 15 shocks, last shock (*a*) at 0·56 sec. Tension had fallen to zero (*b*) by 0·86 sec. Peak of relaxation heat (*c*) at 0·71 sec. Negative heat half complete (*d*) at 1·89 sec. Time marking above: scale of analysed deflexion on left, 1 mm = $0{\cdot}137 \times 10^{-3}$ cal/g muscle. Initial heat to max. = $10{\cdot}7 \times 10^{-3}$ cal/g muscle: negative heat to min. = $0{\cdot}37 \times 10^{-3}$ cal/g muscle.

In the past, when negative deflexions were observed after the initial maximum one was inclined to attribute them to inequalities, within the muscles, of the spatial distribution of the heat produced during contraction and relaxation. That such irregularities can occur was clearly shown by Hill & Howarth (1957), particularly during relaxation under large isotonic loads: and they are very evident in records of the heat production in isometric contractions with submaximal stimuli. Relaxation can be very

sudden, and if in one part of a muscle it began slightly earlier than in another the latter would be left bearing the tension, and a disproportionate amount of the elastic energy, built up during contraction, might appear as heat in that part. The fibres near the outer (skin) surface of the frog sartorius are smaller than those elsewhere (Hill, 1949*b*, Pl. 8, fig. 1, and p. 234) and if they relaxed later than the rest they would get an undue proportion of the relaxation heat. The possible effect on heat records of such an uneven distribution of heat in a muscle at the end of contraction and relaxation

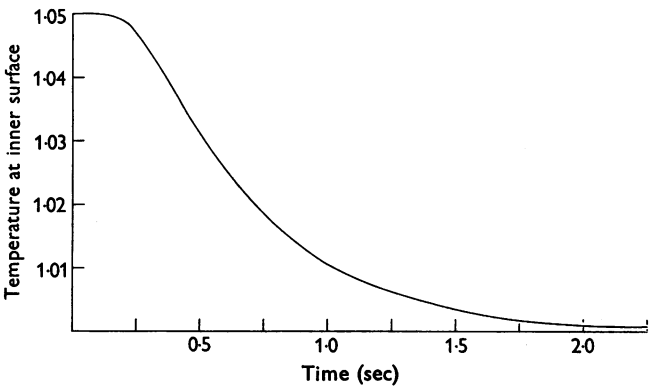

Fig. 4. To show the calculated fall of temperature of the inner surface of a muscle 0·742 mm thick after instantaneous heating of the inner 0·707 mm only, the outer 0·035 mm not being heated, except by later conduction of heat from the inner region. Initial temperature of the inner surface 1·05 (arbitrary scale): final temperature throughout 1·00.

was discussed quantitatively by Hill (1938, p. 149 and Fig. 4). The maximum delayed effect on a record would occur if a thin layer in the region of the muscle furthest from the thermopile produced no heat at all, and Fig. 4 in that paper gave a curve calculated for the hypothetical case of a muscle in which only the inner 10/11 of its cross-section produced heat. In a muscle 1·1 mm thick there would then be a 9·1 % fall of temperature at the surface of the thermopile, completed in a few seconds.

At 0° C negative heat production certainly occurs after contraction: but at higher temperatures it was necessary to look critically at the possibility that its appearance could be due to an error of the kind just described. For this purpose the curve of Fig. 4 was calculated for the case of a pair of muscles each 0·742 mm thick, which was about the thickness of the muscles used for the experiment of Fig. 3*A*. The constant k for heat conduction in muscle was taken as $1{\cdot}25 \times 10^{-3}$ (Hill, 1949*b*, p. 231). Figure 4 represents an extreme example of uneven distribution, namely that of muscles in which an outermost layer, only 5 % the thickness of the rest, failed to

produce any heat. If the middle 5 % of the same muscles produced no heat, the subsequent fall at the surface would be about four times as quick.

The application of Fig. 4 to the curve of heat production in Fig. 3*A* is made as follows. The heat most likely to be unevenly distributed is that which occurs very suddenly in relaxation; and anyhow this is the latest contribution to the heat, so its uneven distribution would have the greatest effect later. The maximum rate of relaxation heat occurred at about 0·58 sec. If none of this heat appeared in the outermost 5 % of the muscle, the temperature of the inner surface would fall (according to Fig. 4) half way to its final value in a further 0·64 sec, i.e. in 1·22 sec from the start. In Fig. 3*A* the deflexion was still near its maximum at this time; it fell half way to its minimum only 0·8 sec later. In spite, therefore, of the extreme assumption made in the calculation, the fall of the deflexion after the maximum cannot be attributed to an uneven distribution of the heat.

The quantitative argument applies with even greater force in the case of muscles thinner than those of Fig. 3*A*. The time scale of the curve of Fig. 4 depends on the square of the thickness of the muscles, so for muscles of thickness a mm the times should be reduced in the ratio $(a/0{\cdot}742)^2$. In the experiment of Fig. 3*B* the muscles were each 0·55 mm thick, so the times should be reduced in the ratio $(0{\cdot}55/0{\cdot}742)^2 = 0{\cdot}55$. The curve then of Fig. 4 would fall to one half in 0·35 sec. The negative heat in this contraction had scarcely begun at 0·35 sec after the peak of the relaxation heat; it reached half its full value only in about 1·18 sec from then, instead of 0·35 sec. Again, therefore, the time relations of the negative heat are much too long to be explained by an uneven spatial distribution of the relaxation heat.

It seems safe, therefore, to conclude that the early negative heat observed at higher temperatures is a genuine phenomenon, as it obviously is at 0° C. It is more difficult to observe with certainty at higher temperatures and it is cut short more quickly by ensuing positive heat production, particularly in the presence of oxygen. But it clearly is part of a general phenomenon, not characteristic only of 0° C and lack of O_2.

The effect of iodoacetate on the negative delayed heat

In order to confirm, by present methods, Hartree's results (1932*a*) on the continuing presence of the negative delayed heat after poisoning with iodoacetate (IAA), the experiments of Table 3 were made at 0° C in N_2 on toad sartorii. In each experiment the muscles, mounted on the thermopile, were given a preliminary soaking in ordinary oxygenated Ringer's solution at room temperature. Then at time zero the solution was changed for one containing iodoacetate (neutralized to pH 7), through which oxygen bubbled. At about 60 min the thermopile chamber was placed in the

thermostat at 0° C, at 110 min nitrogen was bubbled through the solution, at 140 min the solution was replaced by nitrogen and at 180 min the first stimulus was applied and a record made of the heat production. After that successive records were taken at intervals of 10–15 min. Finally, the temperature was raised to that of the room and a succession of shocks was applied in order to verify that the characteristic contracture of IAA poisoning set in.

The only analysis necessary for the records was to make the allowance for heat loss. When this had been done the corrected deflexion was found to remain steady for a second or two after the muscle had relaxed; then it began to decrease, reaching a minimum in about a minute, after which it started to increase slowly.

TABLE 3. The effect of iodoacetate on the negative heat of muscles at 0° C in N_2

Expt. no.	1			2			3			4		
Stimulus (sec)	3			5			3			3		
IAA concentration	1/25,000			1/25,000			1/15,000			1/15,000		
Number in series	1	4	6	1	4	5	2	5	7	1	2	9
Initial heat (10^{-3} cal/g muscle)	8·6	8·3	7·7	13·7	12·3	12·1	8·8	7·9	6·0	6·3	7·2	5·9
Negative delayed heat (%)	7·9	11·4	9·2	7·2	3·6	4·0	4·4	4·6	3·9	4·0	4·0	4·7
Complete in (sec)	60	70	50	70	60	60	65	45	50	55	60	60

Table 3 shows that the negative delayed heat appeared in every contraction; in experiment 1 it was rather large. This confirms Hartree's conclusions, though the quantity of negative delayed heat observed in the present experiments is somewhat greater than he found.

In every one of the twelve contractions reported in Table 3 the negative deflexion was followed by a slow positive one, which was already obvious at 80–90 sec. If this later positive deflexion in the poisoned muscles were really due to the same cause as the usual positive anaerobic delayed heat, it would be impossible to explain the latter in the accepted way as the net thermal effect of the resynthesis of creatine phosphate with free energy supplied by the production of lactic acid. One was reminded, however, of the statement by Cattell, Feng, Hartree, Hill & Parkinson (1931, p. 298), on the anaerobic delayed heat of muscles poisoned with iodoacetate and stimulated at room temperature, that 'spontaneous heat production occurred in nitrogen too early to allow a reliable end to the heat record, even after the first stimulus'. Hartree also had found (1932*a*, p. 285), at 0° C, that 'for later stimuli there was a conspicuous progressive change due to subsequent positive heat occurring earlier and at a higher rate'. Moreover, Lundsgaard (1934) found that in muscles poisoned with iodoacetate at 0–2° C creatine phosphate continued to be split for some time after a 25 sec tetanus. A few experiments were made at room temperature

in the same way as those reported above for 0° C. At 13·3° C with 1/15,000 iodoacetate no negative delayed heat was found: the heat deflexion due to a stimulus was unsteady and there was a large positive shift of base line which must have been due to a permanent increase in the rate of heat production. In two experiments at 12·6 and 13·1° C with 1/25,000 iodoacetate there was clear evidence of negative delayed heat production, but it was obviously cut short by a large ensuing positive one: and after later contractions the delayed heat production was positive and persisting. In one experiment at 14° C with 1/30,000 iodoacetate there was a large positive shift of the base line, even after the first stimulus.

In view of these results at room temperature it seems rather likely that a similar permanent change in the resting heat rate took place at 0° C, too small easily to detect and occurring stepwise after each contraction. This does not at all invalidate the conclusion that a negative delayed heat production occurs after contraction in muscles poisoned with iodoacetate; indeed the reverse, for a small permanent increment in heat rate following each stimulus could only tend to mask the negative delayed heat, which may really have been greater than calculated. But the effect deprives us of any direct answer to the question of whether positive delayed anaerobic heat of the usual kind can occur in a poisoned muscle.

DISCUSSION

The negative delayed heat is rather small, though—as seen above—easily measurable. At 0° C, where it is not so quickly cut off by the positive delayed heat, it may reach a value of nearly 10^{-3} cal/g, though usually it is less. This, however, may not be a true measure of the extent of the chemical reactions involved. It is possible that the observed heat absorption is the result of a coupled reaction in which the process

$$A \rightarrow B$$

occurring during contraction, with heat production h_1, is reversed by the free energy supplied by another reaction occurring later,

$$C \rightarrow D$$

the heat production of which is h_2. The net heat absorption in the two reactions together would be $(h_1 - h_2)$. If this were small compared with h_1 or h_2 the extent of the chemical reactions involved might be considerably greater than one might infer from the magnitude of the difference $(h_1 - h_2)$. In looking, therefore, for a chemical explanation of the negative delayed heat it would be reasonable to consider processes which separately could provide heat up to, say, 5×10^{-3} cal/g.

If this heat came from the splitting of phosphate from some known, or unknown, organic precursor with a heat production of 5000 cal/mole, the

amount of phosphate produced would be 1 μmole/g muscle, and this should be detectable by present chemical methods. That at least gives the order of size of the changes to be sought, namely fractions or small multiples of 1 μmole/g muscle.

All recent chemical evidence is against the possibility that at the end of a short contraction in a normal muscle any net break-down of adenosin triphosphate (ATP) has occurred (Fleckenstein, Janke, Davies & Krebs, 1954; Mommaerts, 1954, 1955; Fleckenstein & Janke, 1957); while the experiments of Carlson & Siger (1960), with frog sartorii poisoned with 0·5 mM (1/11,000) IAA at 1–2° C, gave convincing evidence that no reduction of ATP had occurred when the muscles were frozen about 30 sec after the end of a short series of twitches and then analysed. Moreover, the experiments of Fleckenstein, Gerlach, Janke & Marmier (1960) suggested that 'the increased O^{18} incorporation, at least into ATP and creatine phosphate, was more correlated with metabolic reactions in recovery... than with the contraction process itself'. The possibility also that creatine phosphate continues to be split after a contraction is eliminated by such experiments as those of Nachmansohn (1928), of Meyerhof & Nachmansohn (1930) and of Lehnartz (1931), who showed, on the contrary, that a considerable resynthesis occurs in 20 or 30 sec at room temperature. The delayed creatine phosphate break-down found by Lundsgaard (1934) was in muscles poisoned by iodoacetate: no evidence exists that it occurs in normal muscles. Finally, present thermochemical data suggest that in living muscle the heat of the reaction CrP + ADP → Cr + ATP is positive and fairly large, not negative. According to Carlson & Siger (1960) the 'physiological heat of hydrolysis' of CrP in frog muscle at 1–2° C lies between 9·6 and 11·5 kcal/mole: while the calculations of Bernhard (1956), based partly on the heat measurements of Podolsky & Morales (1956), gave 3·6 kcal/mole as the total heat produced in mammalian muscle by the splitting of ATP to adenosine diphosphate (ADP) at 37° C. Admittedly the conditions were very different in these two cases, but it is hard to believe that the physiological heat of splitting ATP in frog muscle at 0° C could be three times as great as in mammalian muscle at 37° C.

Altogether, therefore, it is impossible to attribute the negative delayed heat to a delayed synthesis of ATP by means of the free energy supplied by creatine phosphate hydrolysis. The appearance equally of the negative delayed heat in muscles poisoned with iodoacetate shows that it cannot be due to the resynthesis of creatine phosphate either by means of the free energy supplied by lactic acid formation, or in the reaction suggested by Meyerhof & Schulz (1935),

phosphopyruvic acid + creatine → creatine phosphate + pyruvic acid,

of which the heat of reaction is negative (about -3000 cal/mole). The possibility exists that phosphopyruvic acid might appear in poisoned muscles by some process outside the normal chain of anaerobic breakdown of carbohydrate, but even so there is no chemical evidence that creatine phosphate is restored in such muscles in the absence of O_2—rather the contrary, as Lundsgaard (1934) found.

It seems, therefore, that none of the processes at present known in the chemistry of muscle can be invoked to explain the delayed negative heat. Fleckenstein *et al.* (1954) produced evidence that 'inorganic phosphate was liberated during contraction from an unidentified precursor', and this might conceivably provide the key to the riddle. That unknown substances *are* liberated in muscles stimulated to fatigue was pointed out long ago by Hill & Kupalov (1930): they found that only 80 % of the fall of vapour pressure caused by stimulation to fatigue could be accounted for by known chemical bodies produced. This conclusion was confirmed by Meyerhof (1930), and by Meyerhof & Grollman (1931), measuring the depression of freezing point; also by Hill & Parkinson (1931) with muscles poisoned with iodoacetate; and it was emphasized again by Hill (1950). Nothing has been discovered in the last 30 years to fill this admitted gap in our knowledge of the chemistry of muscle. Perhaps the difficulty of explaining the negative delayed heat may provoke further experiments to fill it.

But another possibility exists, of quite a different kind, which ought to be discussed. When a muscle is stimulated at any but a very high external pH, the splitting of creatine phosphate produces a rise of pH in its interior. In itself a rise of pH, leading to an increased ionization of protein and other weak acids, would cause an absorption of heat. If the rise of pH could occur later than the splitting of creatine phosphate that caused it, the positive heat of the latter would be followed by the negative heat of the former: otherwise the second would mask the first and no negative heat would appear. But the alkalization of the interior of a muscle might be expected to occur immediately after the splitting of creatine phosphate, not during the next 60 sec (cf. Fig. 1*B* for the negative delayed heat at 0° C). Nevertheless, though unlikely, it is conceivable that the creatine phosphate might be split in some highly localized region, around which a diffusion barrier of some kind might only gradually allow its products to affect the general pH. Against this must be counted the fact that the delayed negative heat appears much more quickly at a higher temperature, which is inconsistent with simple diffusion. It is a fact, however, as shown in recent experiments by Distèche (1960), using a developed form of Dubuisson's glass-electrode system, that the pH of a muscle continues to rise for some time after a tetanus or a rapid series of twitches. At a temperature near 0° C the pH of a frog sartorius in contact with the glass

electrode went on rising after contraction for times recorded up to half a minute and had not ceased then (see his figures 63, 65, 68, 69). Earlier figures in Distèche's paper showed a similar effect in tortoise muscles. It seems extremely unlikely that this continuing rise of pH could be due to a continued splitting of creatine phosphate, for there is no evidence that this ever happens in a normal muscle: and if it did it would not solve the present problem, for then there would be a production, not an absorption, of heat.

Either therefore the splitting of creatine phosphate during contraction affects the general pH of a muscle only gradually, a rather unlikely hypothesis, or some unknown chemical reaction is involved. Without more chemical evidence it is impossible to decide between these alternatives, or about the origin of the delayed negative heat.

SUMMARY

1. The early delayed absorption of heat following a short contraction, described by Hartree in 1932, has been re-examined.

2. It occurs in the presence as well as in the absence of oxygen, and at room temperature as well as at 0° C.

3. It is cut short by the ordinary positive delayed heat, aerobic or anaerobic, that follows it.

4. It may be as large as 1 mcal/g muscle after a short contraction, or 5–10 % of the initial heat.

5. In the absence of oxygen, at 0° C after a short tetanus it may continue for 50–60 sec: after a long tetanus it is masked by the anaerobic delayed heat coming on earlier. At a higher temperature it is over sooner, as it is in the presence of oxygen.

6. It occurs equally in muscles poisoned with iodoacetate.

7. It is not possible to explain it in terms of chemical reactions known as yet to take place after contraction.

8. It may be associated with the rise of pH recently observed by Distèche to occur gradually after a series of twitches or a tetanus.

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APPENDIX

BY R. C. WOLEDGE

The experiments reported in the preceding paper by Hill were made during the months of August to December. Additional evidence for the negative delayed heat has now been found in a series of experiments made, for another purpose, during May with the sartorii of spring frogs. In these

experiments records were made of the heat production in oxygen during and after tetani of various durations, at 0° C and at 17° C. At 0° C the negative delayed heat was similar to that found with autumn frogs. But, unexpectedly, the absorption of heat after a tetanus at 17° C was sometimes larger and more prolonged than with tetani of the same duration in the earlier series of experiments. There appears to be a seasonal difference in the metabolic processes of spring frogs which either increases the intensity of the negative delayed heat or slows the onset of the positive recovery heat which tends to mask it.

The results of one experiment, in which the negative heat was particularly large, are given in Table 1, which shows the amount and time course of the negative heat observed after tetani of various durations. 'Half time' means the time from the end of the tetanus to the half completion of the observed negative deflexion; 'minimum' is the time to completion of the deflexion.

TABLE 1. Negative delayed heat in O_2 at 17° C with various durations of tetanus

Tetanus duration (sec)	0·2	0·4	0·8	1·6	3·2
Initial heat (mcal/g)	13·9	19·2	32·4	53·5	97·0
Negative heat (mcal/g)	0·84	1·5	2·0	1·9	0·6
Negative heat as % of initial	6·1	7·8	6·0	3·6	0·6
Half time (sec)	2·0	1·7	1·7	1·3	1·0
Minimum (sec)	9	8	8	4	2

The thickness of each muscle in this experiment was 0·67 mm. The curve of Fig. 4 in the preceding paper can thus be applied in this case by multiplying the times by 0·75. Its application shows that any unevenly produced heat would have distributed itself within about 1 sec. The maximum rate of relaxation heat at 17° C comes about 0·1 sec after the last shock, so that any effect of its uneven distribution would be over by 1·1 sec after the last shock. In the shorter tetani the negative heat was only beginning at this time.

In another experiment (at 16·5° C) the quantities of heat absorbed after the end of the tetani were about 70 % of those in the experiment just referred to, but the records were not continued long enough to observe the end of the heat absorption. Again there was a definite negative heat after a 1·6 sec tetanus and some sign of heat absorption even after a 3·2 sec tetanus. In a third experiment the greatest negative heat was 2·1 % (after a 0·8 sec tetanus), there was a heat absorption of 0·9 % after a 1·6 sec tetanus but none after a 3·2 sec tetanus. In one further experiment there was no sign at all of the negtive heat.

The negative delayed heat at room temperature is evidently a rather variable phenomenon, as was stated in the preceding paper. But it is clear that, at room temperature, the heat absorption is sometimes particularly

evident in the muscles of spring frogs. These observations make it even more certain that the negative delayed heat really is a general phenomenon occurring both at 0° C and at room temperature, in oxygen as well as in nitrogen. Doubtless even better evidence of the effect at room temperature, were that required, could be obtained by making the experiments in nitrogen, when it would not be masked by the onset of the oxidative recovery heat.

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