

THE ANAEROBIC DELAYED HEAT PRODUCTION IN MUSCLE. BY K. FURUSAWA AND W. HARTREE¹.

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DURING the past nine months we have carried out over 100 experiments in order to verify the existence and to determine the amount of the anaerobic delayed heat occurring in isolated frog's muscle after stimulation. Hartree and Hill⁽¹⁾ concluded that this is about 0.25 of the total initial heat, but in view of the importance of this quantity it seemed desirable to remove any possible doubt concerning it. The method employed was the same as that described in the above paper, but in all the later experiments a special thermopile with two faces was used which will be described later.

The Ringer's solution which filled the chamber initially had been carefully boiled to exclude oxygen, and the nitrogen employed was freed from oxygen as completely as possible. The quality of the nitrogen was frequently tested. On a few occasions it was found to contain about 0.1 p.c. of oxygen, but usually it contained about 0.05 p.c. or less.

In several cases we found that when such purified nitrogen was passed through the muscle chamber for an hour or so after the Ringer's solution had been blown out, the muscle became slowly inexcitable, as Bayer⁽²⁾ and Fröhlich⁽³⁾ have shown in the case of nerve. In such cases a complete recovery could be brought about by soaking the muscle for a short time in aerated Ringer's solution. A certain minimum, therefore, of oxygen (less than 0.1 p.c.) is necessary to keep the muscle excitable. This state of inexcitability evidently started on the side of the muscle exposed to the nitrogen, producing peculiar results which should be mentioned. When the outside of the muscle is inactive, the temperature of that part will not rise immediately on stimulation, so part of the heat produced in the inside (and near the thermopile) must flow to the outside soon after the stimulus. The thermopile, therefore, will be affected in exactly the same way as if there were an absorption of heat by the muscle following shortly after the initial rise of temperature. In other words, there will be an apparent negative heat production soon after the stimulus. This was verified long ago by

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scorching the surface of the muscle with a hot wire; records taken after this showed an apparent large negative heat soon after the contraction. Of course if this negative heat be large, or if it occur early, it may affect the maximum deflection itself (especially when using a slowly acting thermopile), and since the delayed heat is determined by the difference of the "live" curve and the "control" curve when these are reduced to the same maximum—which would be correct if the muscle were uniformly heated when it contracted—it can be seen that if the live curve has a false maximum the effect on the above difference may be relatively large. Apart from this possible error due to a false maximum, the state of inexcitability spreading inwards from the exposed surface is sometimes well shown by the increasing "negative heat" after successive stimuli.

On the other hand, in many experiments we observed that there was a large early positive difference between the live curve and the control curve, as observed by Hartree and Hill (1), p. 133). This was specially evident when using longer stimuli (0.3 sec. or more) as was done in that case—in fact we have several experiments in which this happened, and yet when a short stimulus (0.05 sec. or less) was given, the early apparent *negative* heat was very evident. These irregularities can possibly all be explained by supposing that the heat is not produced uniformly across the section of the muscle on stimulation, due possibly either to the over-stimulation of some of the fibres or to the inactivity of others. In general it is probable that the inside of the muscle will remain in better condition than the surface, which may have been damaged during preparation or affected by contact with the thermopile.

During the course of our experiments Prof. Otto Meyerhof suggested that we should make some observations on the effect of very strong stimuli on the delayed anaerobic heat. A preliminary statement of our results was published in Pflüger's *Archiv*(4). There we showed that after a strong faradic stimulus (containing five to eight times as much energy as a just maximal stimulus) there occurs an anaerobic delayed heat amounting to 20–60 p.c. of the initial heat.

Subsequently we have made several experiments with an extra strong stimulus of short duration (0.05 sec.). The energy of this stimulus was several times greater than that in the initial response of the muscle. In this case, following the initial heat production there is a relatively enormous outburst of delayed heat, the total extra heat produced during a period of 2 or 3 minutes after the stimulus being well in excess of the initial heat itself. This delayed heat, which cannot be regarded as

associated with a normal muscular contraction, but rather as due to injury produced by the excessive stimulation, is obviously to be associated with the delayed lactic acid formation found by Embden⁽⁵⁾ and discussed by Meyerhof and Lohmann⁽⁶⁾.

Such "electrocution" of the muscle is of little interest in connection with normal muscular contraction, but it is obvious that measures must be taken to avoid its effect when employing direct stimulation of muscles, and the question necessarily arises whether those fibres of a muscle which are immediately in contact with electrodes do not themselves receive excessive stimuli even when, for the muscle as a whole, the stimulus is only just maximal.

Returning now to the case of ordinary stimuli, when there is apparent negative heat soon after the stimulus its amount invariably increases with successive stimuli of the same duration, and in general the size of the curve of delayed heat rate falls slightly for successive stimuli, the first curve being often abnormally high, due possibly to traces of oxygen.

Since in these anaerobic experiments we have observed many cases of apparent early negative heat and also of apparent early positive heat occurring after contraction is over, it was inevitable in a long series of experiments that there would be several cases in which the live curve and the control curve were practically identical for some time (half a minute or more) from the start. If any other state of affairs is taken as being due to experimental error, these cases are entitled to the greatest weight, although even then it might have happened only because the positive and the negative errors were initially equal. In many cases there was only a small early negative heat or a small early positive heat above that due to a smooth curve starting from time zero. In such cases an attempt was made to correct the result. Early positive heat was estimated by analysis of the early part of the curve, and this was deducted from the observed difference in the areas of live and control curves. Similarly, if there was apparent early negative heat, this was estimated by analysis and added to the observed difference in the areas of live and control curves. Such corrections, however, were made only when they were not more than 2 or 3 p.c.; in exceptional cases they might be as much as 8 p.c. (For numerical results see later.)

We are admittedly attempting to find the minimum value of the anaerobic delayed heat. For this reason about a quarter of the experiments which gave a delayed heat of between 20 and 30 p.c. of the initial heat (as found by Hartree and Hill) have been discarded,

although the nitrogen used in these experiments was of good quality and there was no special reason to suspect the results. In a further quarter of the experiments the early negative or extra-positive heat was too large to be taken into account with any accuracy. Even so, however, we have 40 reliable experiments on which to base our results.

Owing to the occurrence, especially in the purest nitrogen, of large apparent negative heat, which, as explained above, was probably due to the inexcitability of the outside of the muscle on the ordinary thermopile, Prof. A. V. Hill suggested a new type of thermopile, which was made by Mr Downing of University College, London, and consists of two parallel thermopile plates joined in series, with the muscle lying between them so that only its edges are exposed. Since a single sartorius muscle was rather small and thin to warm both faces of this thermopile, a semi-membranosus muscle was used in all the experiments with it. The thinness of the insulation and the good contact of the muscle with this type of thermopile caused the heat to be transferred very quickly to the hot junctions, with the result that the maximum galvanometer deflection was reached in about 3 seconds, even with a large muscle weighing 0.3 gm. or more. Moreover, loss of heat from hot to cold junctions was rapid. These facts would have rendered technically very difficult an actual *analysis* of the heat rate throughout the record, and none was actually made, only the total area of the deflection-time curve being determined in order to ascertain the total delayed heat.

This thermopile did not entirely eliminate the negative heat—in fact in all the cases in which a short stimulus (0.03–0.05 sec.) was used it showed 2–4 p.c. early negative heat; with a longer stimulus (0.3 sec.) the same muscle never showed any early negative heat, though usually there was some early extra positive heat in these cases. In all the experiments made with this thermopile the nitrogen had about 0.3 p.c. of oxygen present in it, and in only one or two cases was there obviously increasing negative heat for successive stimuli. The very small surface of the muscle exposed cannot have allowed any perceptible amount of oxygen to pass in, probably only sufficient to maintain the excitability.

The numerical results from 40 fairly reliable experiments, all at 18° C. in nitrogen, including those using both types of thermopile and employing various times of stimulus from 0.03 to 0.3 sec., were as follows. In 12 experiments with no correction for early irregularities the mean total delayed heat was 12 p.c. (one result over 13 and one under 10); in 12 experiments with not more than 3 p.c. of early positive heat the mean total delayed heat after deduction of this early positive heat was

11½ p.c. (two results over 14 and three under 10); in 16 experiments with not more than 3 p.c. of early apparent negative heat the mean total delayed heat after addition on this early negative heat was 12½ p.c. (three results over 14 and three under 10).

The minimum value, therefore, of the anaerobic delayed heat now appears to be reduced to 12 p.c., with the reservation that this result is obtained by excluding a considerable number of experiments, apparently reliable, which gave a much greater result, and a few which gave a slightly less result; taking all the experiments into account the mean is roughly 15 p.c., but, as Prof. Hill suggests, it is quite possible that the anaerobic delayed heat is not a constant fraction of the initial heat, but depends upon conditions. At first sight it might be supposed that this number, which was about 50 p.c. according to Hartree and Hill in 1922(7) without using specially prepared nitrogen, and about 25 p.c. according to their re-determinations in 1923(1), should in reality be zero. Progressive improvements in the technique seemed always to reduce the figure for the anaerobic delayed heat. We cannot agree, however, with such a conclusion. Our observations seem definitely to prove the existence of some such delayed heat, since in all cases, even in the purest nitrogen, *the galvanometer deflection after a stimulus persists much longer than in the case of the control curve*. No initial irregularities of heat production between different points of the muscle could possibly have any effect on the deflection after the time taken by the control curve to return to zero. Deflection after that time must mean heat production going on after the contraction is over, and the amount of that deflection is much larger than can be accounted for by any possible experimental error. At 18° C. the deflection is observable in the curves of the live muscle up to 11 or 12 minutes, whereas in the control curve it has reached zero in about half that time. The maximum deflection was always about 500 mm.; readings were made to the nearest half-millimetre and are certainly correct to the nearest 1 mm. The greatest difference between the live and the control deflections, in the cases giving the smaller total of delayed heat (10–15 p.c.), was usually from 6 to 8 mm. and 2 to 4 mm.; deflection persists in the live curve at a time when the control curve has returned completely to zero.

Another reason for regarding this anaerobic delayed heat as due to causes within the muscle, is the fact that at 0° C. its rate is considerably diminished (see Appendix), which would not be the case were it due to purely physical causes of any kind.

As to the origin of this anaerobic delayed heat we have no very

definite suggestions to offer. It is conceivable that an ordinary maximal stimulus may be supermaximal for the fibres in immediate contact with the electrodes and that these fibres then exhibit the delayed heat production shown above to result from excessive stimulation. It would seem unlikely, however, that a sufficient number of fibres should be so over-stimulated as to produce the amount of extra heat actually found. It would appear more probable that there is a genuine delayed anaerobic heat of normal physiological origin, for which some explanation must be found in the mechanism of the muscle. It is now commonly believed that a hexose diphosphoric ester is the immediate precursor of the lactic acid liberated during stimulation, and it would seem possible that the re-formation of this compound, at the expense of phosphate and glycogen, occurring only slowly in the almost complete absence of oxygen, may be the origin of this delayed anaerobic heat.

If the number 12 p.c. for the delayed anaerobic heat be now accepted as of genuine physiological origin, and the revised value of 390 calories taken for the heat per gram of lactic acid liberated during contraction (Meyerhof⁽⁸⁾), then the "balance sheet" of Hartree and Hill ⁽¹⁾, p. 135) must be revised as follows:

	Relative	Absolute: calories
Total anaerobic	1.12	390
Initial	1.00	348
Delayed anaerobic	0.12	42
Delayed oxidative	1.50	522
Total oxidative	2.50	870

Employing Slater's latest value⁽⁹⁾ for the heat of combustion of 1 grm. of dissolved glycogen, namely 3836 calories, this result implies that only $\frac{870}{3836} = \frac{1}{4.4}$ of each gram of lactic acid liberated during contraction is actually oxidised, or in other words, 4.4 molecules of lactic acid are removed in recovery for each one oxidised.

If the number now found, namely 12 p.c., for the delayed anaerobic heat be still attributed to error, a conclusion which we regard as improbable, then the balance sheet is as follows:

	Relative	Absolute: calories
Total initial	1.00	390
Delayed oxidative	1.50	585
Total oxidative	2.50	975

This implies that $\frac{3836}{975} = 3.94$ or approximately 4 molecules of lactic acid are removed in recovery for each one oxidised. Either of these

values, 4.4 or 4.0, is in close agreement with Meyerhof's results in the extensive series of researches he has carried out on the subject.

APPENDIX.

Several analyses were made, using the slow-acting thermopile, of the rate of delayed heat production at 18° C. The results usually showed a maximum rate of about 0.0006 initial heat per second, as previously found (1, p. 133), but this maximum occurred earlier than before (at about 80 or 90 seconds after the stimulus) and the fall after the maximum was more rapid, making the area of the curve only about half that previously obtained.

In addition a few experiments were carried out at 0° C.; in this case the maximum rate of delayed heat production was rather less than half that at 18° C. and the maximum probably did not occur before 3 minutes after the stimulus; in such cases it is impossible to estimate the total delayed heat but the form of the curve for the first 5 or 10 minutes showed that the total delayed heat was not going to be reduced much, if at all, by lowering the temperature.

A further set of experiments was made when the muscle was kept in hydrogen; these are not so reliable as those in nitrogen; this is partly due to the high conducting power of the hydrogen making the deflection curves have an earlier maximum and a much smaller area (for the same maximum), and partly because there was a greater proportion of these experiments in which there was apparently a large early negative heat, and this may have been due to some specific effect of the hydrogen; the results of a few good experiments seemed to show about the same total delayed heat as in nitrogen, but the maximum rate was usually smaller, and at a longer time after the stimulus, than when nitrogen was used.

Lastly, we tried eliminating the oxygen by keeping the muscle in a partial vacuum, exhausting the muscle chamber until the Ringer's solution boiled at room temperature. In this case, however, we obtained no reliable results, probably because relatively large temperature changes were brought about by rapid evaporation from the surface of the muscle after stimulation and, further, the difficulties referred to in the case of the hydrogen experiments were exaggerated.

SUMMARY.

1. The delayed anaerobic heat production in muscle has been re-investigated. A new double-faced thermopile designed to lie in contact with the muscle on both sides was used in the later experiments.

2. The minimum value of the total delayed anaerobic heat is shown to be about 12 p.c. of the initial heat. It is probable that this heat has a physiological origin in the normal mechanism of the muscle and is not to be attributed to any constant source of error.

3. Accepting this value it can be calculated that 4.4 molecules of lactic acid are removed in recovery for each one oxidised.

4. An excessive stimulus, directly applied, may cause a large delayed anaerobic heat attributable rather to injury by the stimulus than to normal physiological processes.

5. The delayed lactic acid production found by Embden in muscles directly stimulated is probably to be associated with the delayed heat found under such conditions of excessive stimulation.

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