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THE ANALYSIS OF THE DELAYED HEAT PRODUCTION OF MUSCLE.

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IN earlier experiments on the analysis of the recovery heat [*e.g.* Hartree and Hill, 1922, 1924; Hartree and Liljestrand, 1926] the time of stimulus had to be short, 0·5 sec. or less, on account of the methods then used. The thermopile was purposely a slowly acting one, because the effect of the recovery heat on the galvanometer deflection was assumed to be nearly enough determined simply by subtracting from the "live" curve a "heating control" curve of the same maximum. It was supposed that the initial heat was so concentrated in time that the control curve gave a close enough determination of its effect at later times: this could be the case only if the heat was correctly "integrated," *i.e.* if the maximum deflection was independent of the distribution of the initial heat. Even if the defect in integration was only a small fraction, such a fraction of the control curve, especially when its height was still large, might be a serious part of the difference between "live" and "control."

The error can be avoided to some extent by taking the time of heating for the control curve about the same as the time of stimulus, but since the initial heat is by no means uniformly distributed during the time of stimulus, unknown errors are likely to arise for long stimuli. Further, since the galvanometer reached its maximum deflection in about 5 sec. and the "live" and "control" curves were then made equal in height and subtracted, no information could possibly be derived of the recovery heat during the first 5 sec. after the stimulus, and the next one or two steps of 5 sec. in the analysis were open to doubt. It had to be assumed, moreover, that any possible recovery before 5 sec. did not affect the maximum. Thus the curves showing the rate of recovery heat were not well determined near the start and not determined at all before 5 sec. These objections, it is admitted, would have little effect on the subsequent course of the analysis.

It is not possible by resources at present available to push much further the analysis of the initial heat [Hartree, 1931]. It was desirable, however, to use these resources in order to make a more accurate analysis of the course of the recovery heat, and in particular (1) to avoid the possible occurrence of the errors mentioned above, (2) to find the recovery heat starting immediately after the stimulus, and (3) to examine the conditions for any duration of stimulus.

PROCEDURE.

In recent experiments the following method was used. A fairly rapid thermopile, constructed by Mr A. C. Downing (constantan-iron couples, bakelite insulation, brass frame, glass cover), was connected with the very rapid moving magnet galvanometer described in the previous paper [Hartree, 1931]. Photographic records were taken on a quickly running drum so that the initial parts of the "live" curve could be analysed with sufficient accuracy. The heating control curves were usually for 0·1 sec., and the analysis of the initial heat was carried out with intervals of 0·1 sec. for short stimuli (up to 0·5 sec.), increasing to 0·4 sec. for long stimuli (up to 5 sec.). The photographs were continued on the drum for 12 to 15 revolutions (32–40 sec.), when successive rings came so close that there might be confusion if they were continued: after that the light was allowed to fall on the paper only for a fraction of a second, at intervals of 4 sec., up to 80 sec. or longer.

The analysis of the initial heat was usually carried to 4 sec. for a short stimulus and to 8 sec. for a long one.

From the results the heat, over the period for which the analysis was carried out, was distributed into equivalent amounts at equal intervals, usually 0·4 sec. The deflections due to these amounts at these times were then calculated for subsequent times from the control curve, which must therefore be determined at every 0·4 sec. over the whole time for which the recovery is to be calculated. The sum of such deflections at the subsequent times evidently gives the part of the observed deflection in the "live" curve which is due to the initial heat, or rather to the heat which has been included in the initial analysis, whatever its nature may be. (It will be seen later that a considerable part of the heat soon after the end of the long stimulus is not what is usually called initial heat.) This sum at subsequent times is then deducted from the live curve at the corresponding times, the difference being the deflection due to the heat which has occurred after the period of the analysis.

There is no chance of an error arising from a false maximum, as in the original method referred to above. With the thermopile used the deflection fell off rather quickly after its maximum. So far was it from "integrating" the heat from a long stimulus that the true heat was actually 25–30 p.c. more than that calculated simply from the maximum deflection. By the method described, however, the whole heat is properly taken into account[1].

Having obtained the deflections due to the heat occurring after the above analysis, these are in turn analysed to give the time course of the subsequent heat. The heat rate, except quite soon after a long stimulus, will now be very small compared with the initial heat rate, so it will be sufficient to analyse it in much longer time intervals: a suitable interval to use is 4 sec. Consequently the above calculation for the deflection due to the initial heat at subsequent times, and the measurements of the deflections from the live curve, are only required at every 4 sec.

It is impossible to analyse the "subsequent" heat directly by the control curve for 0·1 sec. heating, since this curve will lose a considerable part of its maximum deflection in 4 sec. There are two possible methods of procedure: (*a*) to make separate 4 sec. heating curves and to use them to analyse the subsequent heat in 4 sec. steps: the heat units, however, for the "initial" and the "subsequent" analyses will now be different and the "subsequent" analysis can only be expressed in terms of the initial heat (as is required) by making a still further analysis, namely, of the 4 sec. heating curve by the 0·1 sec. control: the further analysis could of course be avoided if the ratio of the heats given to produce the two control curves were accurately known; (*b*) a much better method is to "build up" a 4 sec. heating curve from the 0·1 sec. heating curve: this is very easily done since the ordinates of the latter must be found in any case at short intervals of time, as they are required for the initial analysis and for the calculation of the effect of the initial heat at subsequent times, as described above.

Besides saving time in taking and measuring further records, method (*b*) has the great advantage that the "built up" 4 sec. heating curve can be made for exactly the same heat as was used for the 0·1 sec. control, and the analysis of the subsequent part by this control will therefore be in the same units as those in which the initial heat is expressed.

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[1] The rapid heat loss inevitably leads to the subsequent deflection being considerably smaller than for the slow-acting thermopile, with some loss of accuracy on account of this smallness. The method, therefore, is hardly suitable for investigating the total recovery heat.

RESULTS.

The general form of the results of the original experiments was confirmed, the longer stimulus giving a larger and earlier maximum rate of recovery, stimuli of 4 or 5 sec. showing a comparatively enormous heat rate quite soon after relaxation. This rate, however, usually fell rapidly for about 15 sec. after which there was a distinct rise to a maximum at 25 to 30 sec., and then there was a gradual fall in the rate, as shown in Fig. 1.

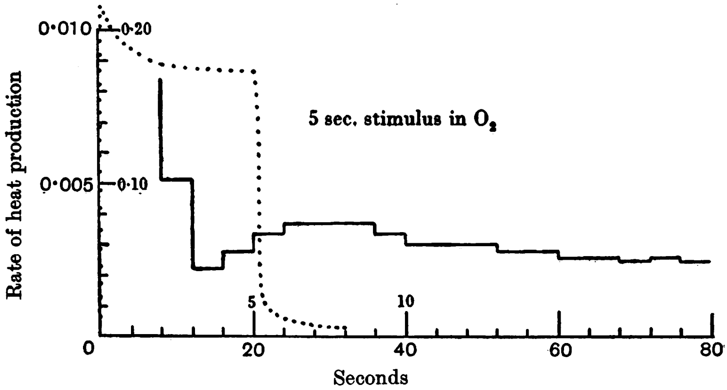

Fig. 1. Rate of heat production for a 5 sec. stimulus in oxygen at 17° C. The dotted line, with inner scale, gives the result of the initial analysis up to 8 sec., showing the characteristic high rate soon after a long stimulus. The tension fell only slightly during the stimulus; at 0·4 sec. after the stimulus it had fallen to a very small value, at 0·6 sec. it was inappreciable.

The full line, with outer scale, gives the result of the subsequent analysis in 4 sec. steps up to 80 sec.; this is the continuation of the dotted curve on 20 times the scale, showing a distinct maximum at about 30 sec. due to oxygen recovery, whereas the earlier quickly falling part is almost entirely due to anaerobic delayed heat.

The unit for the vertical scale is the initial heat per sec.

Such a result has been observed before [Hartree and Hill, 1928, Fig. 3, p. 213], but its significance was not then understood. It is now found that if a similar long stimulus be given in nitrogen (freed from oxygen by passing over heated copper) the part of the delayed heat curve soon after relaxation is practically unaltered, and there is no subsequent rise, in fact there is no observable heat at all after 15–20 sec. It is clear, therefore, that the heat soon after relaxation is nearly entirely due to the same cause as the anaerobic delayed heat, so no proper investigation of the heat in oxidative recovery can be made without first determining the anaerobic delayed heat for various times of stimulus and deducting this from the total heat observed after relaxation.

This early delayed heat represents presumably the excess of the heat due to delayed lactic acid formation over that absorbed in delayed phosphagen resynthesis [Lehnartz, 1931; Lundsgaard, 1931; Meyerhof and Schulz, 1931], and this theory of its origin is borne out by the fact that Meyerhof and Schulz found that the lactic acid formed during and immediately after the tetanus, as also the phosphagen resynthesized during the same period, is the same in a muscle saturated with oxygen as in one which is entirely oxygen-free. We are justified, therefore, in assuming the same source for the early delayed heat in both cases.

The anaerobic delayed heat. There was some trouble in making a sufficiently good determination of the anaerobic delayed heat because it was soon evident that it was less, and over earlier, for successive stimuli, and with long stimuli generally much less for the later ones as has been noted elsewhere [Cattell and Hartree, 1932]. For example (16. x. 31) several stimuli of 1 sec. gave

No. 1. A.D.H. = 11 p.c. over in 40 sec.
No. 3. A.D.H. = 4 p.c. over in 20 sec.
No. 8. A.D.H. = 2 p.c. over in 4 sec.

Also (16. ix. 31) three 4 sec. stimuli, after others, gave

No. 3. A.D.H. = 9 p.c. over in 20 sec.
No. 4. A.D.H. = 7 p.c. over in 16 sec.
No. 5. A.D.H. = 3 p.c. over in 12 sec.

Thus it was necessary to have an early stimulus in several different experiments to obtain consistent results. Fig. 2 shows the mean results for an early stimulus from twelve experiments. In all these the total anaerobic delayed heat (after completing the diagram for the shorter stimuli)[1] was about 8 p.c. of the initial heat. No greater anaerobic delayed heat than 12 p.c. was obtained.

There is some evidence that about 2 p.c. of the initial heat occurring in a few seconds after the tension has fallen to its original value may be due to the relaxation from internal stresses which do not contribute directly to the tension, as such heat is frequently observed after shorter stimuli when both the anaerobic delayed heat and the oxygen recovery heat are probably at a very small rate and cannot account for it; and further, this 2 p.c. occurs at a falling rate before the subsequent rising rate associated with the anaerobic delayed heat or the recovery heat.

[1] For stimulus 0·8 sec. the anaerobic delayed heat seems to be practically complete in about 50 sec. and for stimulus 0·4 sec. in about 80 sec.

After a long stimulus, the tension when the stimulus is over does not usually fall quickly to its original value but may be quite appreciable for a few seconds: such tension is small and the heat due to relaxation from it is not likely to reach 1 p.c. (it is probably considerably less): the above considerations, however, suggest that the anaerobic delayed heat has, if anything, been over-estimated and that after comparatively few long stimuli its total amount is inappreciable. The possibility, however, must not be overlooked that for the subsequent stimuli the anaerobic delayed heat may have become so early in its appearance that it cannot be separated from the initial heat.

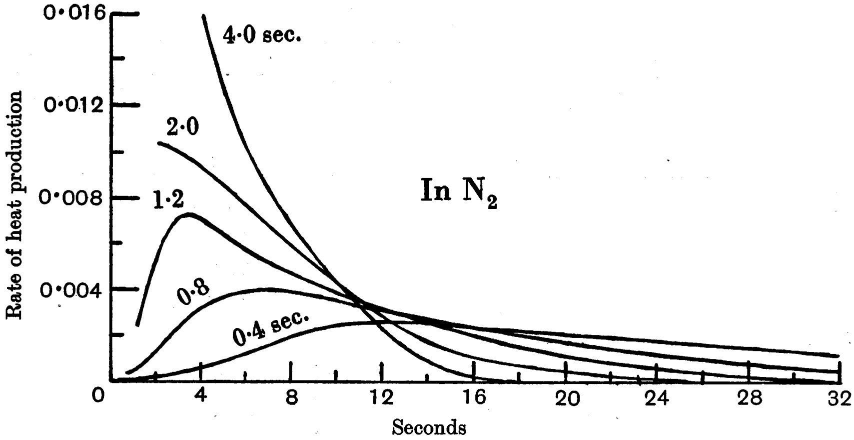

Fig. 2. Curves showing the mean relative heat rate for early stimuli of 0·4, 0·8, 1·2, 2·0 and 4·0 sec. at 17° C. in N_2, from the results of twelve experiments. The curve for 0·8 sec. stimulus goes on for about 50 sec., that for 0·4 sec. stimulus for about 80 sec. The total heat (area) for each curve is about 8 p.c., except for the 4 sec. stimulus in which it is less, as a considerable amount of the heat came before the end of the stimulus. The curves, especially before 4 sec., are approximate; the results of any one experiment sometimes differed considerably from them. The unit for the vertical scale is the initial heat per sec. for each curve.

For longer stimuli the initial rate is very high, which fully accounts for the distinctive "tail pieces" in all the heat diagrams after such stimuli. For a 4 sec. stimulus the maximum rate probably occurs before the end of the stimulus though it cannot be separately observed, but even after this the rate is actually five times the maximum possible rate due to oxygen recovery (see later), although the whole anaerobic delayed heat is only about 8 p.c. of the whole oxygen-recovery heat. After a stimulus of this length the anaerobic delayed heat is complete in under 20 sec., which agrees with the fact that many observers have found the phosphagen resynthesis and the delayed lactic acid formation to be complete in a comparatively short time.

Fig. 2 shows the relative rates of anaerobic delayed heat, *i.e.* the ordinates of the curve for each stimulus are fractions of the initial heat for that stimulus. If these rates be required in absolute units they must be multiplied in each case by the initial heat.

A calibration was made in several of the experiments for the anaerobic delayed heat, and also in several for the oxygen-recovery heat (considered later); the mean values of the initial heat for various times of stimulus, from a single twitch up to 4 sec., were plotted against the time of stimulus: from the curve the initial heats for the times of stimulus used in Fig. 2 were read off and these are entered in Table I, together with the maximum rate of anaerobic delayed heat.

TABLE I. Maximum rates of anaerobic delayed heat for different initial heats.

Time of stimulus (sec.)	0·4	0·8	1·2	2·0	4·0
Initial heat (10^{-2} cal. per g.)	3·3	5·0	6·9	11·1	19
Maximum relative rate	0·0025	0·004	0·007	0·010	0·020*
Maximum absolute rate (10^{-4} cal. per g. per sec.)	0·8	2·0	4·8	11	38
Approximate time to maximum rate (sec.) ...	14	7	3	2	—

* The figure 0·020 is an estimate, since the maximum rate for a 4 sec. stimulus probably occurred before 4 sec.

From the numbers it may be seen that the maximum absolute rate of anaerobic delayed heat is closely proportional to the square of the initial heat preceding it.

The oxygen-recovery heat. Having determined the curves of anaerobic delayed heat rate for early stimuli of various durations these were subtracted from the curves for corresponding stimuli when the muscle was in oxygen in order to get the oxygen-recovery heat alone. It seems reasonable to subtract such anaerobic delayed heat curves from the mean curves for various times of stimulus using all the observations in oxygen, on the supposition that when the muscle was allowed to recover the anaerobic delayed heat effect would always be the same as for an early stimulus in nitrogen, which corresponds to the case of the fresh muscle.

The mean curves for the relative rates of oxygen-recovery heat are shown in Fig. 3 and the absolute rates in Fig. 4. For even the longest stimulus used, namely 4 sec., the maximum rate does not occur before 20 sec. The relative rate is considerably less for the longest stimulus, and it seems that in this case the absolute rate is approaching its maximum possible value (see Fig. 5).

Shorter stimuli were also given in oxygen with a view to finding the recovery rate for a single twitch. In this case the maximum relative

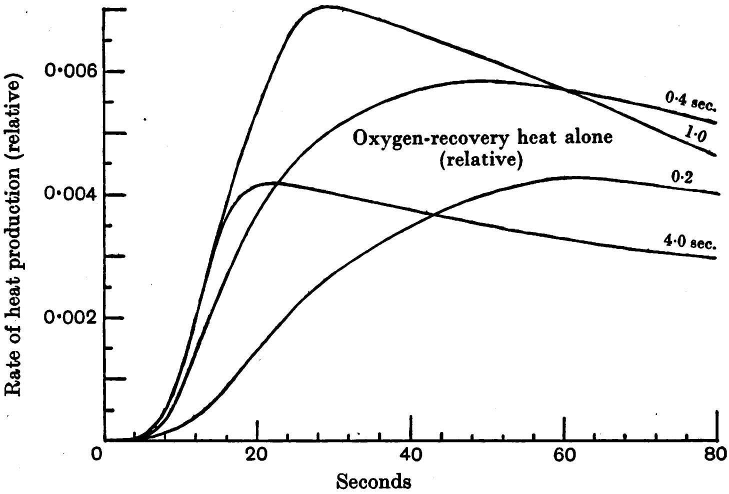

Fig. 3. Curves showing the mean relative rates of oxygen-recovery heat for stimuli 0·2, 0·4, 1·0 and 4·0 sec. at 17° C., from the results of sixteen experiments. The means of three or four analyses for each time of stimulus (in several different experiments) were taken and the anaerobic heat, deduced from Fig. 2, subtracted in each case. The unit for the vertical scale is the initial heat per sec. for each case.

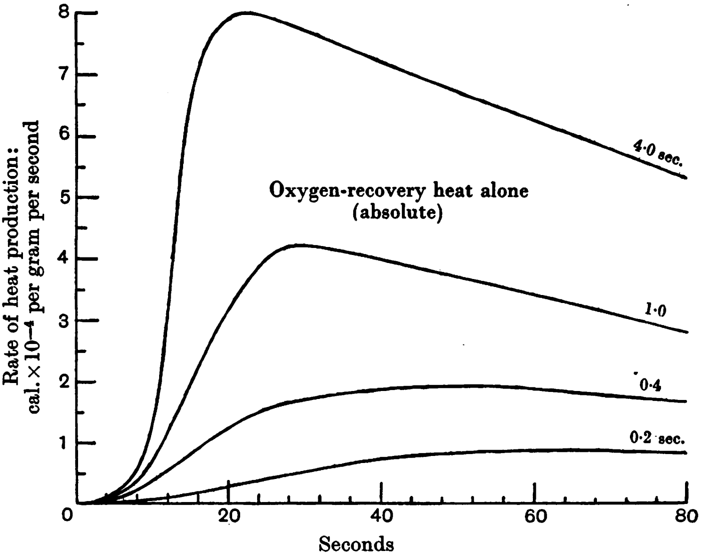

Fig. 4. Curves showing the mean absolute rates of oxygen-recovery heat for stimuli 0·2, 0·4, 1·0 and 4·0 sec. at 17° C. These are the same curves as in Fig. 3, but each multiplied by the corresponding initial heat.

rate of total recovery (including the anaerobic delayed heat) was about 0·002 of the initial heat per sec., giving an absolute maximum rate of about 10^{-5} cal. per g. per sec., and this occurred at about 80 sec. In this case of course the galvanometer had to be made much more sensitive than when dealing with the longer stimuli, so the small differences (between the live and the control curve at some time after the stimulus) on which the method depends, were not so reliable: it was therefore considered impossible to make reliable determinations of the anaerobic delayed heat in such a case, and it can only be stated that after a single shock the maximum oxygen-recovery heat rate is somewhat less than 10^{-5} cal. per g. per sec. and that this does not occur before 1½, or possibly

TABLE II. Maximum rates of oxygen-recovery heat for different initial heats.

Time of stimulation (sec.)	0·2	0·4	1·0	2·4	4·0
Initial heat (10^{-2} cal. per g.)	2·1	3·3	5·9	12	19
Maximum relative rate	0·0043*	0·0058	0·0070	0·0062	0·0042
Maximum absolute rate (10^{-4} cal. per g. per sec.)	0·9	1·9	4·1	7·4	8·0
Approximate time to maximum (sec.)	65	50	30	—	22

* The anaerobic delayed heat was not observed for stimuli less than 0·4 sec., so this number which required an extrapolation is doubtful, as are the further numbers in the same column.

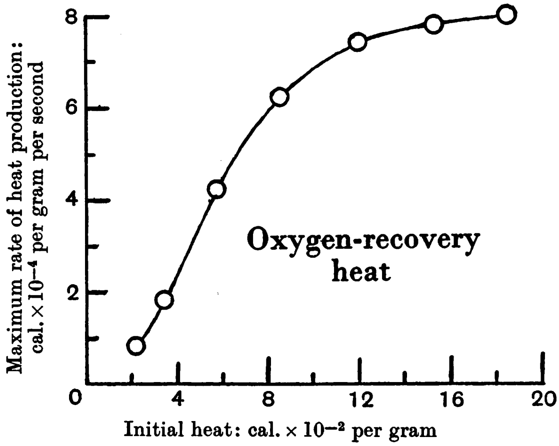

Fig. 5. Showing the maximum absolute rates of oxygen-recovery heat at 17° C., after different initial heats for times of stimuli 0·2, 0·4, 1·0, 1·6, 2·4, 3·2 and 4·0 sec. The maximum possible rate of oxygen-recovery heat at 17° C. cannot apparently be much greater than 8 × 10^{-4} cal. per g. per sec. and this is practically reached after a stimulus of 4 sec. The plotted points are the means from about forty results in sixteen different experiments.

2 min. after the stimulus. This time is longer than that given above, as the effect of deducting the anaerobic delayed heat from the total recovery is to make the maximum of the resulting curve (the oxygen-recovery) come at a later time than that of the original curve.

The maximum rate of oxygen-recovery heat production shown in Fig. 5 approaches asymptotically a value of about 8×10^{-4} cal. per g. per sec., for the temperature of the present experiments (17° to 17½° C.). There is a limit, it seems, in the rate of oxidative heat production, set presumably by the maximum possible activity of the enzymes or other factors concerned in oxidation. The limit is not set by the amount of oxygen available: a fully recovered muscle at 20° C. in oxygen should contain [Hill, 1928 *b*, p. 47] about 90 p.c. of the full amount its water would dissolve at 1 atmosphere partial pressure, say, about 0·022 c.c. per g. This would allow about 0·1 cal. of energy to be liberated per g., taking no account of further oxygen diffusing in: of the 0·1 cal. about one-half (0·05 cal.) presumably would appear as heat, the other half would be absorbed in the endothermic processes of recovery. According to Fig. 4 (4 sec. curve) not more than about one-fifth of this 0·05 cal. is liberated up to the time that the maximum is obtained, so that presumably nearly all the oxygen is still available at that time.

Discussion.

All the experiments were carried out on a pair of sartorii of *R. temp.*, and in most cases the muscles were small or very small: for a constant length of 21½ mm. between the stimulating electrodes, the weight of the pair of muscles was always between 30 and 70 mg. (except for one or two cases in which a single twitch was used when they were somewhat larger). The average thickness of each muscle was 0·4 mm. estimated from the weight, length and mean breadth. The small size of the muscles ensured rapid distribution of heat and rapid diffusion of oxygen from outside. The temperature was always between 17° and 17½° C.

In nearly all the experiments on anaerobic delayed heat there was an apparent small heat production at the end of a considerable time; in fact there was frequently a very small rise in the galvanometer deflection after about 60 sec. There was of course always the possibility that such a result might be due to a temperature error in the course of the record, but on several occasions the spot was observed for a considerable time after the muscle was dead or had not been stimulated for a long interval, and there was no observable motion. The apparent small heat production referred to is no doubt a vapour-pressure effect that has been observed in other cases [Hill and Kupalov, 1930], so this final part of the calculated heat has never been included in the results, but the heat rate has been smoothed off at the end on the supposition that such final parts are due to this technical error. The times, therefore, referred to above in which the anaerobic delayed heat is stated to be complete, must be accepted with some reserve since they depend on an estimate of the final error.

The form of the curves in Figs. 3 and 4 for the true oxygen-recovery heat rate makes it clear that the recovery process does not immediately start off at its maximum value but passes through a complicated curve, of the meaning of which we have at present little idea. When more is known about the chemical events occurring in recovery they will presumably fit into the outline shown in these figures. That the anaerobic delayed heat occurs so rapidly after the longer stimuli and so relatively slowly after the shorter ones also has no explanation at present, but it obviously has an important bearing upon observations made by chemical methods on the processes occurring after activity. It is satisfactory (*a*) that the duration of the anaerobic delayed heat appears to be about the same as that of the time during which lactic acid formation occurs and phosphagen restoration is effected, and (*b*) that the heat which we have called the anaerobic delayed heat occurs also after stimulation in oxygen and so conforms to the chemical facts now known about lactic acid and phosphagen. That it was present in both cases had in fact been seen before [Hartree and Hill, 1928] and had been assumed whenever anaerobic delayed heat was subtracted from oxygen recovery heat in finding the ratio of recovery heat to initial heat.

The ratio (heat in oxygen) : (heat in nitrogen) for a series of muscle twitches [Hill, 1928 *a*] is about 2·07. If H_i be the initial heat, H_o the oxygen-recovery heat, H_a the anaerobic delayed heat, then the heat in oxygen is $(H_i + H_o + H_a)$ and the heat in nitrogen $(H_i + H_a)$. Hence $H_o/(H_i + H_a) = 1{\cdot}07$. In previous investigations [Hartree and Hill, 1922, 1924; Hartree and Liljestrand, 1926] the ratio of total delayed heat to initial heat $(H_o + H_a)/H_i$ has been determined by methods less exact indeed than those adopted in the present investigation but still probably good enough for total heats. Now if the results of the present experiments on tetani can be applied to series of twitches, $H_a = 0{\cdot}08\ H_i$. Hence $(H_o + H_a)/H_i = 0{\cdot}08 + H_o/H_i = 0{\cdot}08 + 1{\cdot}08 \times 1{\cdot}07 = 1{\cdot}24$. This is not far from the average of previous estimations.

An apparent negative delayed heat.

Since the anaerobic delayed heat at 17° C. after a long stimulus is of such high initial rate it seemed possible that determinations of it might be made at 0° C. In five experiments on English and four on Dutch frogs at 0° C. in nitrogen, stimuli of 1–5 sec. were given. In every case with 1 sec. stimulus the analysis, carried out as explained above, gave apparent negative heat after relaxation; the average amount was –3 p.c., lasting for about 40 sec., and there was a distinct tendency for this negative heat to become numerically greater after successive stimuli, the variation being usually from about –1 p.c. for fresh muscle to about –5 p.c. after a few stimuli of 1 sec., and to a somewhat greater negative number when a 1 sec. stimulus was given after a longer stimulus (4 or 5 sec.).

If real, this delayed negative heat in the absence of oxygen would be very interesting: it might be a sign of the endothermic restoration of phosphagen outstripping the exothermic one of lactic acid formation: there is no *a priori* reason why at some stage in this double process the total energy (as distinct from the free energy) should not be negative. The negative heat, however, was very small and it was necessary to see whether it could be explained as a technical error of some kind.

If the outer layers of the muscles (*i.e.* those further from the face of the thermopile) were less active than the rest, their rise of temperature, after a stimulus, would be less than that of the rest, so they would take heat from the inner part and this would appear in the analysis as delayed negative heat. The redistribution of heat in such a case of non-uniform contraction was investigated mathematically by Hill [1931, Appendix II]: it appeared that the process was so rapid that it would not affect the analysis after a very few seconds, and so could not be the cause of the effect observed.

It was safer, however, to examine the matter experimentally as well, which was done as follows. Each muscle of a pair was covered with a layer of absorbent paper to provide an "inactive layer" which would be heated when the controls were taken; the muscles were then given a short stimulus (0·2 sec. at 17° C.) and the heat was analysed in the usual way.

When thick blotting paper was used weighing (wet) 1·4 times as much as the muscles, the apparent negative heat was about –60 p.c. of the initial heat, and it was inappreciable within 3 sec. after relaxation; when thin cigarette paper was used weighing (wet) 0·15 × (weight of muscles), the apparent negative heat was about –18 p.c. of the initial heat and it was inappreciable within $1\frac{1}{4}$ sec. after relaxation. The equalization of temperature was so rapid that the experiments had to be made at a high temperature, and even then some of the negative heat overlapped the relaxation heat; an approximate allowance for this was made when deducing the above numbers.

Since the apparent negative heat was at its greatest rate within a few tenths of a second after the stimulus, and inappreciable after the short times given above, it was clear that inactivity of the outer layers could not account for the long-continued negative heat observed at 0° C.

A more probable explanation is as follows. Suppose that, when the control curve was taken, the muscles (owing to shortening or swelling in killing[1]) were of greater weight than when the live curve was taken. The heavier muscles would lose temperature less

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[1] The muscles were always killed with chloroform vapour, but this was always so weak that, apart from one or two exceptional cases, there was no sign of contracture and no change in the appearance of the muscle. The chloroform vapour was usually removed from the thermopile chamber before the control curves were taken.

rapidly than the lighter ones, so the control curve taken on the former would be too high at all times after the maximum and, as the analysis of the initial heat would be hardly affected, the calculated allowances for the effect at all subsequent times of the initial heat would be too great, so that the result of subtracting them from the live curve would be negative.

To test this, curves were taken consequent on heating (for 0·1 sec.) a pair of strips of thick blotting paper (wetted with Ringer's solution) and also for the same blotting paper covered with a layer of thinner absorbent paper. When the latter was filter paper, giving an increase of weight of 39 p.c., the apparent negative heat, in an analysis of the first curve by the second, was about -25 p.c. of the initial heat and lasted about 110 sec.; when the thinner paper was cigarette paper, giving an increase of weight of 12½ p.c., the corresponding negative heat was about -8 p.c. of the initial heat, and lasted for about 70 sec.

In each case the negative rate fell rapidly from an early high value so that about one-third of the whole negative heat occurred in the first 4 sec. Except for this, the distribution was somewhat similar to that of the negative heat observed at 0° C., but a close comparison cannot well be made, since at 0° C. the end of the relaxation heat may overlap the beginning of the negative heat and the usual anaerobic delayed heat may overlap the end of it. It must be remarked, however, that in many cases after a 1 sec. stimulus at 0° C. there was a very perfect "wash out" at the end of the initial analysis, so that there was no observable heat of any kind between 3 and 4 sec.; in this interval the relaxation heat can hardly have had an appreciable effect (unless, as has been suggested elsewhere, there is a small amount of relaxation heat after the tension has fallen to its original value) and the negative heat, if due to a difference of weight as discussed above, would then be at about its greatest rate, so it could hardly escape detection.

Another possible objection to this explanation of the negative heat lies in the fact that the negative heat for stimuli at 0° C., invariably increased (numerically) for successive stimuli of the same duration, so that it would be necessary to assume that the usual anaerobic delayed heat masked it less and less for successive contractions; but it has been noted above that at 17° C. the anaerobic delayed heat does diminish in this way.

In several cases of longer stimulus (4 or 5 sec.) at 0° C. the negative heat, though present in every case, was always less (when reckoned as a fraction of the initial heat) than for a short stimulus: this may have been due to the earlier occurrence of the anaerobic delayed heat, which might be expected after a longer stimulus.

In five experiments at 0° C. the muscle was poisoned with iodoacetic acid (1 hour at room temperature and ¾ hour at 0° C. in 1/25,000 iodoacetic acid, which gave a good contracture after repeated stimulation in a muscle separately tested). For an early stimulus of 1 sec. at 0° C., the apparent negative heat was very uniform, -2 to -3 p.c.; for later stimuli there was a conspicuous progressive change due to subsequent positive heat occurring earlier and at a higher rate. The fact that iodoacetic acid neither abolishes nor increases the negative heat, suggests that this heat is not due to a balance between phosphagen restoration and lactic acid formation, for in the poisoned muscle the latter does not occur.

On the balance of evidence it seems most likely that the negative heat is due to a small increase of weight between "live" and "control." It is hoped to test this by a method (used by Dr E. Bozler for the snail's retractor) employing a high-frequency current which will allow "controls" to be taken, mixed with "live" records, on the living muscle. If the negative delayed heat prove to be an error due to change in weight of muscle, it must be remarked that all published determinations (including the present) of the anaerobic delayed heat are probably slightly too small. The oxygen-recovery curves, however, would be unaffected, since they were obtained by subtraction.

SUMMARY.

1. A detailed analysis has been made of the delayed heat production occurring after a muscular contraction.

2. In a fresh muscle deprived of oxygen this delayed heat amounts to about 8 p.c. of the initial heat; it occurs earlier and at a greater rate, and ends sooner, after longer stimuli. After a 4 sec. tetanus at 17° C. it is complete in about 20 sec. In successive stimuli the amount decreases, or perhaps occurs earlier so that it cannot be so completely separated from the initial heat.

3. In a muscle in oxygen the delayed heat occurs in two phases: (*A*) the anaerobic delayed heat, which appears exactly as in a muscle without oxygen: (*B*) the true oxygen-recovery heat. In the present experiments (*B*) has been separately examined by subtracting (*A*) from the total in oxygen.

4. The true oxygen-recovery heat rate rises to a maximum and then slowly declines. The maximum is earlier and higher the greater the initial heat. The greatest rate of oxygen-recovery heat production approaches the value (at 17° C.) 8×10^{-4} cal. per g. of muscle per sec. This maximum is determined by a limit, not in the rate of supply of oxygen, but in the rate of chemical reaction.

5. The anaerobic delayed heat represents a balance between the endothermic resynthesis of phosphagen and the exothermic formation of lactic acid. These processes occur equally in the presence or the absence of oxygen, and are complete in a time about equal to that of the heat after the longer stimuli.

6. The oxygen-recovery heat represents a balance between exothermic oxidation and endothermic lactic acid removal and phosphagen resynthesis.

7. The observation of an apparent negative delayed heat is discussed.

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