AN ANALYSIS OF THE INITIAL HEAT PRODUCTION IN THE VOLUNTARY MUSCLE OF THE TORTOISE.

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THE chief difficulty in the accurate analysis of the heat production of an isolated voluntary muscle is the extreme quickness with which events occur. It is possible, to some degree, to overcome this difficulty by improvements in the instruments used for recording the rise of temperature, as discussed in a recent paper(1). A simpler method is, if possible, to utilise the muscles of an animal whose movements are naturally slower than those of the frog, the chief animal hitherto employed in such investigations. The slowest moving of all animals readily available in this country is the tortoise, and the following investigation has been concerned with the *biceps cruris* of that animal.

The speed of contraction in the muscles of frogs in good condition is always approximately the same. In tortoises, however, the speed varies largely from one individual to another. Possibly these differences may be connected with the hibernating habits of this animal: possibly they may be due to the great variations in age of the individuals employed. In the slowest twitch hitherto encountered relaxation was not complete for 30 or more seconds: 10 seconds at least were required for the tension to fall to half its maximum value. This was at 13° C.: at 0° C. the contraction would have occurred several times slower still. The animal from which this particular record was obtained was in excellent condition. Perhaps because of the very slowness of its individual muscle twitch it was able to maintain a contraction with great economy. The *biceps cruris* used throughout this investigation (see Bojanus(2)), in the ordinary size of tortoise obtainable in this country, is usually about 5 cm. long and averages in weight 400 mgrm. It is of two to three times the cross-sectional area of a single frog's sartorius and very uniform throughout its length. This uniformity of section is essential for experiments in which a control deflection must be made by electrical heating of the dead muscle, since otherwise the latter will not be uniformly

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heated by the warming current. A single muscle only has been employed, and not a double one as in the case of the frog's sartorius preparation. The rise of temperature in a contraction of this muscle is not as great, under similar circumstances, as in the much more quickly responding frog's muscle. Ample sensitivity, however, is available in the myothermic instruments, and the rather smaller amount of heat is no very great disadvantage (see, however, below). Altogether the muscle is admirably suited for myothermic experiments. In most cases the quickness of response was much greater than in the one described above, but still much less than in that of a frog's muscle. In general we may say that what happens to a frog's muscle in 1 second occurs in a tortoise's muscle in a time of the order of 15 seconds. This enormously simplifies and improves the accuracy of the analysis.

With the apparatus already described (1) experiments were made to determine the course of the evolution of heat at three different temperatures, 5°, 10° and 15° C. It was found impossible to perform satisfactory experiments at 0° C. Eight reliable analyses were obtained at 5° C., with times of stimulation 0, 0, 0, 0, 0·1, 0·1, 0·2, 0·4 second respectively, 0 standing for a single shock. Seven reliable analyses were obtained at 10° C. with times of stimulation 0, 0, 0, 0, 0·05, 0·1 and 0·2 second; eight at 15° C. with times 0, 0, 0, 0, 0, 0·04, 0·05 and 0·10 second. The course of the evolution of heat is shown in Fig. 1 as the average of the several experiments performed at each temperature. For comparison the isometric tension curves at the three temperatures also are shown, averaged for the several experiments at each temperature by adding all the tensions (using percentage of maximum in each case) in the several curves at each quarter of a second and re-plotting. The experiments were all performed between March and May; this may be of importance since the condition of tortoises varies considerably during the year.

The heat rate curves of Fig. 1 are plotted to a scale in which the *total* initial heat production (*i.e.* the total production of heat during the contraction and relaxation as distinguished from the "recovery heat production" following relaxation) is denoted by unity. The initial heat rate is very high and lasts only for a very short time. The tension curves shown by broken lines are plotted to an arbitrary scale with unity as a maximum.

The most striking fact which emerges from these analyses is the way in which the heat is split up into two completely distinct phases. There is a large initial outburst of heat occurring in the first 0·2 second or less after the stimulus, followed by what appears in the analysis to be a gap, during which practically no heat appears at all, and then followed by

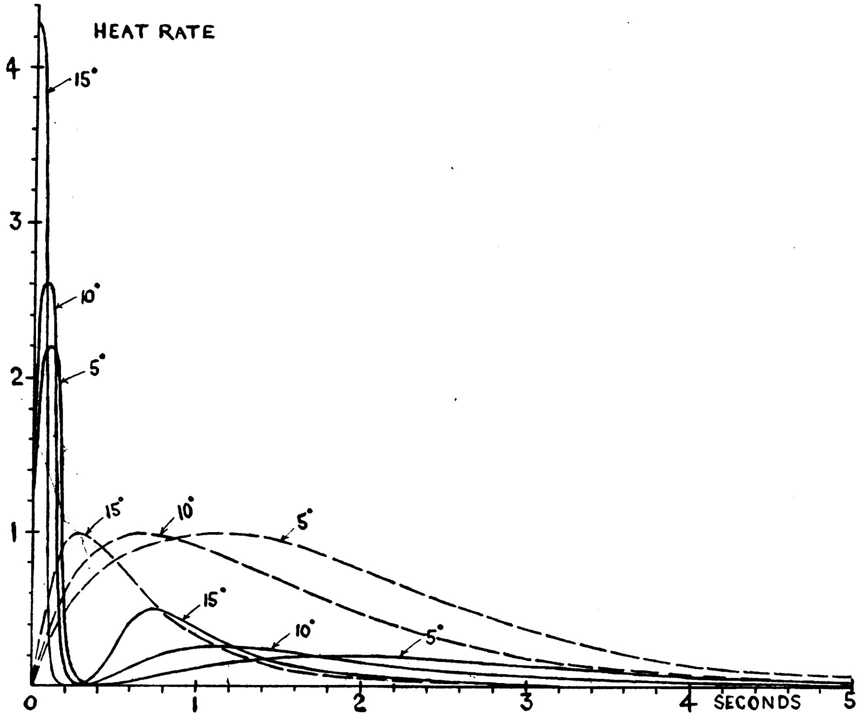

Fig. 1. Full line: analysis of heat production in twitch, or very short tetanus, for comparison with broken line, mechanical response. Horizontally: time in seconds. Vertically: rate of heat production, or tension in isometric contraction. N.B. The unit of heat-rate is the total initial heat per sec. The unit of tension is arbitrary in each case.

a prolonged evolution of heat which continues as long as any tension is exerted by the muscle. In the analysis there is a sharp distinction between the two separate stages of the heat production, and it is possible to divide up the total heat in the initial stages of contraction quite definitely into its two separate parts. At 15° C. the initial outburst of heat averages, in 14 separate sets of observations (including the eight most reliable experiments referred to above), for the case of a single shock or of a stimulus lasting not more than 0·2 second, about 49 p.c. of the total initial heat. The probable error of this mean value, calculated in the usual way, is about 2 p.c. At 10° C. a similar series of ten sets of observations gave an initial outburst equal to 44 p.c. of the total initial heat, again with a probable error of the mean of about 2 p.c. At 5° C.

a similar series of ten sets of observations, with stimuli of not more than 0·4 second duration, gave an average value for the initial outburst equal to 39 p.c. of the total initial heat, this time with a probable error of the mean of 2·5 p.c. It may be significant that the outburst of heat is rather a smaller fraction of the total initial heat at the lower temperature. This may, however, be due to chance, and with the number of experiments performed and their probable errors it is not possible to make any final statement on that point. It would seem certain, however, that in the contraction of a tortoise's muscle, in response to a single shock or to a very short tetanus, there is an initial outburst of heat completed in the first 0·1 or 0·2 second, which is about half the total initial heat production and occurs more rapidly at a higher temperature.

If the stimulus be of longer duration this initial outburst of heat is less clearly defined, and naturally forms a smaller fraction of the total initial heat. In many cases an analysis was carried out also of the heat production during a prolonged tetanic stimulus. Such analyses, however, led to no results of striking interest and need not be discussed here. It is sufficient to say that during the prolonged maintenance of tension there is a prolonged production of heat, and that during relaxation there is, as was to be expected, a continuous liberation of heat gradually dying away, as in the relaxation after a shorter stimulus.

After the initial outburst of heat is over the slower heat production, which previously was associated with relaxation (3), gradually works up to a maximum which occurs at each temperature at the moment when the tension is falling most rapidly. It then slowly decays as relaxation proceeds. In a general way, after relaxation has well commenced, the falling off of the tension runs parallel to the falling off of the rate of heat production.

It is tempting to discuss the chemical meaning of these two separate phases of the contraction. One would naturally tend to associate the sudden initial outburst of heat with the sudden formation of lactic acid from its precursor immediately following the application of a stimulus. At 15° C. at least a large part of this initial outburst occurs before the tension has risen appreciably. If we can imagine the reaction liberating lactic acid to be able to occur only during a short interval following a stimulus, for example, during a change of permeability associated with the presence of the action current, then we can understand the suddenness of this initial outburst of heat. Expressing the matter in terms of lactic acid: after the acid has been produced it must do two things, firstly react with the substance of the muscle, for example with Garner's liquid crystals(4), so as to cause the mechanical response, and then secondly be neutralised by the alkalies of the muscle substance. The process of reacting with the receptive material of the fibres or fibrils may be accompanied by a heat production which one would naturally place in the initial outburst. The neutralisation of the acid would inevitably be associated with a production of heat, and this, together with the dissipation of the mechanical energy in relaxation, we appear to find in the second phase shown in Fig. 1.

In order to present the evidence more clearly and to give some idea of the magnitude of the errors attached to experiments and analyses such as these, an experiment is described in detail in the Appendix. In the individual experiments the same type of result is found as in the mean curve obtained by averaging a number of them. The errors attached to these observations are, as a matter of fact, rather larger than had been hoped. This is due in part to the fact that in the contractions provoked by short stimuli or by single shocks the heat production is less than in a frog's muscle, being only a few thousandths of 1° C., so that the instruments must be made more sensitive, less quickly reacting, and therefore more subject to error.

With the thermopile employed, which reacts very quickly to a rise of temperature and loses its heat also rather quickly, the total heat, if read simply from the maximum deflection of the galvanometer, may be appreciably in error when compared with the total heat as determined by a careful analysis of the photographic record. The heat production may be so prolonged, especially in contractions at the lower temperatures or in response to the longer stimuli, that a measurable fraction of the heat liberated in the earlier stages may have been lost before the maximum deflection is attained. The error so caused may, in a single twitch at 5° C. be as much as 20 p.c. of the whole heat. Usually it is much smaller than this: in prolonged contractions, however, it may be even larger. In dealing, therefore, with tissues producing heat only slowly one must beware of accepting blindly the maximum deflection of the galvanometer as a true index of the production of heat. It is easy to test an instrument for its efficacy in integrating the heat production over a long period by passing a constant calibrating current for different periods through a dead muscle on it, and finding out the greatest period for which the deflection of the galvanometer is proportional to the time of passage of the heating current. By making a thermopile with the cold junctions far removed from the warm ones, and by means of thin wires and similar devices, it is easily possible to construct a thermopile which will go on integrating the heat production for a period of 10 or more seconds. The instrument used in this investigation did not do this, and it was always necessary, when the production of heat was not very rapid, to take account of the possibility that some of the heat may be lost before the maximum deflection of the galvanometer is reached.

At the higher temperatures the initial outburst of heat may possibly occur in a time even shorter than the 0·1 or 0·2 sec. indicated above. The analysis would, in point of fact, be satisfied by a production of heat practically instantaneous and occurring at a moment shortly after the application of the stimulus.

APPENDIX.

Exp. of 21. iv. 25. Single biceps cruris of tortoise in air, length 5·0 cm., weight 0·575 grm. Initial extension about 0·5 cm. (very small initial tension). Galvanometer curves taken when contraction strictly isometric; tension curves taken separately. Temp. 5° C. Stimulus 0·1 sec.

Control curve, with time of heating 0·04 sec., mean of four records, very nearly the same. Curve after stimulus, mean of two records, practically identical.

Time (sec.)	0	·1	·2	·3	·4	·5	·6	·7	·8	·9	1·0	1·2	1·4	1·6	1·8
Control	0	4	32	93	176	273	374	477	568	649	721	826	903	947	972
Stimulus	0	1	4	19	43	73	108	142	178	212	244	301	351	396	435
Heat	·12	·22	·01	0	0	·01	·02	—	·05	—	·03	·04	·03	·03	·02

Time (sec.)	2·0	2·2	2·4	2·6	2·8	3·0	3·2	3·6	4·0	4·4	4·8	5·2	5·6	6·0
Control	987	997	*1000*	*1000*	998	996	993	984	973	960	946	932	919	905
Stimulus	472	507	543	581	617	656	690	750	788	811	823	*830*	828	822
Heat	·07	—	·08	—	·07	—	·02	·02	·02	·01	—	—	—	—

Control curves all reduced to maximum = 1000 before mean taken (the unit being 0·1 mm. on the photographic paper, corresponding to about 5×10^{-6} degree C. in this experiment). Curves after stimulus both had a maximum = 830. Result of analysis shown in the fourth line, the interval used at the start of the analysis being 0·1 sec., later 0·2 sec. and at the end 0·4 sec. The tension in this case was very small after 6 sec., but just observable up to 8 sec. The heat produced up to 4·4 sec. is 0·87 (the unit of heat being that which, if suddenly produced, gives a control maximum = 1000; in this experiment it was $2{\cdot}5 \times 10^{-3}$ cal.). The total initial heat is about 0·90 and it will be seen that there is 0·35 (*i.e.* 40 p.c. of the initial heat) before 0·2 sec., and very little heat produced between 0·2 and 0·5 sec. Thermopile used had 64 pairs of junctions, constantan and iron, in one layer; length (along muscle) 2·0 cm., and distance between "hot" and "cold" junctions 1·0 cm. Resistance of thermopile 16·2 ohms; resistance of galvanometer 12·4 ohms. Galvanometer sensitivity 31 mm. for 10^{-6} volt, with scale at 120 cm. from mirror.

SUMMARY.

1. The skeletal muscles of the tortoise react very much more slowly than do those of the frog, a fact which simplifies and makes much more accurate an analysis of the heat production in the initial phases of contraction.

2. There is an initial outburst of heat following rapidly on the stimulus and completed long before the tension has attained its maximum.

3. Following this initial outburst there is an interval during which the tension is rising but no detectable heat production occurs.

4. During and throughout relaxation there is a production of heat, reaching its maximum rate at the moment when the tension is disappearing most rapidly.

5. Approximately half the initial heat is associated with each of these two phases.

6. The chemical basis of these results is discussed.

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