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THE INITIAL AND RECOVERY HEAT PRODUCTION OF VERTEBRATE NERVE.

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THE experiments reported in this paper constitute a redetermination of the course of the heat production accompanying activity and recovery therefrom in vertebrate nerve. During the several years that have elapsed since the classical studies of Downing, Gerard and Hill [1926] and Gerard [1927], there have been marked improvements in technique which make possible a more satisfactory experimental procedure. Using some of these newer methods Hill [1929] has recently found that the initial heat production in crustacean nerve is only about 2 p.c. of the total instead of 11 p.c. as previously reported for vertebrate nerve. Although there are satisfactory reasons for expecting a considerable difference in this ratio for the two types of nerve, it has seemed desirable to re-investigate this important question, and especially so because the present methods make possible a very much more accurate analysis than was formerly attainable.

The two important instrumental improvements which have made this work possible are a new type of thermopile and an improved moving magnet galvanometer. By abandoning the older wedge design of nerve thermopile and patterning one after the improved muscle type, Downing and Hill [1929] have obtained marked advantages for "(*a*) it gives about twice the E.M.F. per 1° C., (*b*) it loses heat appreciably less rapidly, and (*c*) it has a more constant 'zero.'" All of these features are of vital importance in an accurate determination of the recovery heat. By increasing the zero stability random errors are appreciably minimized. When one considers that the heat production following a brief period of excitation continues for some 10 minutes, during which time the galvanometer deflection must be continuously and accurately determined, the importance of this factor becomes strikingly apparent. The slower loss of heat retards the return of the galvanometer to its zero position, thus making possible a more accurate control warming curve and final analysis. And, finally, by increasing the total thermo-electromotive force, it has been possible to discard thermal amplification without increasing the galvanometer sensitivity to a value that involves appreciable unsteadiness.

The sensitivity required is nevertheless great, and the absence of unsteadiness is due to the remarkable improvements made by Downing in the construction of his moving magnet galvanometers. The instrument used in the present study had a sensitivity of about 4×10^{-12} amp., a period of 3 to 5 seconds and a zero stability of approximately ± 2 mm. in the absence of violent magnetic disturbances in the immediate neighbourhood. Using such a galvanometer and thermopile it has been possible to work without the system of thermal amplification previously employed, and thus to avoid the time lag inherent in that method. With the thermopile directly connected to the galvanometer in these experiments 9 seconds' stimulation has given deflections of as much as 125 mm.

In order to have deflections of such a size and to obtain a more slowly falling control curve six or eight pairs of sciatic nerves from three or four large Hungarian frogs (*R. catesbiana*) were used for each experiment. After being carefully dissected out they were placed in a phosphate Ringer's fluid for about an hour. Following this they were taken out, the solution was carefully removed from their surface by blotting on a filter paper, and they were then placed on the thermopile side by side, with the central ends hanging over the upper stimulating electrodes. The thermopile was then inserted into a glass cover, on the inside of which were placed a few pieces of filter paper moistened with Ringer's fluid. A slow stream of oxygen was passed through the vessel, and the whole immersed in a constant temperature bath.

Several hours were then allowed for temperature equilibration in the thermopile vessel before starting the experiments. These were usually carried out between the hours of midnight and four in the morning and, whenever possible, on Saturday and Sunday nights, at which times magnetic disturbances from moving motors in the neighbourhood were at a minimum. Stimuli that were somewhat supra-maximal were delivered to the nerves from a Harvard induction coil giving 140 make-and-break shocks per second. In each experiment a test was made to ascertain that the stray heat from the stimulating current was not responsible for more than about 1 p.c. of the observed deflection. The duration of the stimuli was accurately regulated to either 9, 12, or 15 seconds, and the galvanometer deflection was observed every 3 seconds throughout the first 2 minutes and then every half-minute for the following 12 to 15 minutes. From six to ten such complete series of readings were made on each set of nerves, the deflections at each time of observation were averaged and the results plotted to give such a deflection-time curve as is represented in Fig. 1. In most of the experiments there was a certain amount of zero drift that was not easily avoided. This was allowed for by drawing a straight line—shown as a broken line in the figure—from the origin to the curve at the farther end.

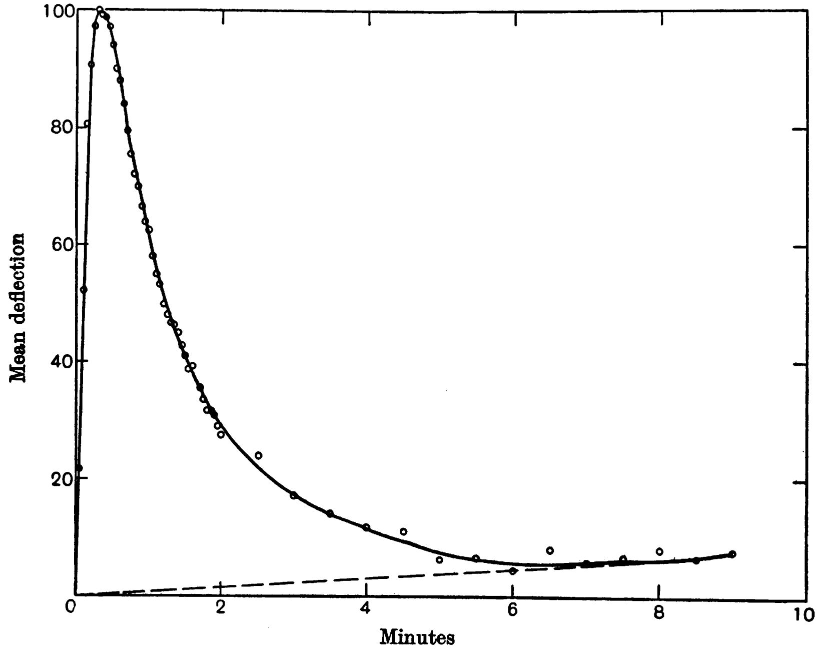

Fig. 1. Galvanometer deflection resulting from a 9-second stimulus. The broken line is drawn to the far end of curve to correct for zero drift. Mean of seven sets of readings. 24·5° C. 20. vii. 29.

In order to analyse this corrected curve for the actual course of the heat production, a control curve was determined at the end of each experiment in the usual manner. Current from the inductorium was passed through the entire lengths of the killed nerves for 3 seconds and the galvanometer deflections were observed at 3-second intervals. The time to maximum was also determined, for the accuracy of the early part of the control curve is the most essential to a correct analysis. Ten such controls were usually taken, and the average results plotted as in Fig. 2.

As the result of a 3-second heating of the nerves, the galvanometer reaches its maximum in about 5 seconds from the start and does not return to its zero position in less than 1½ to 2 minutes, thus giving a curve which makes possible a fairly accurate analysis. In the case of those analyses employing a time unit longer than 3 seconds, a control heating curve for a corresponding time is required. Thus, in the analysis shown in Fig. 4, 15-second units have been employed, necessitating a control curve resulting from a 15-second heating. This was constructed by combining five 3-second controls assumed to start at 0, 3, 6, 9 and 12 seconds. The use of this method saves the labour of experimentally determining controls for the various time units of analysis employed.

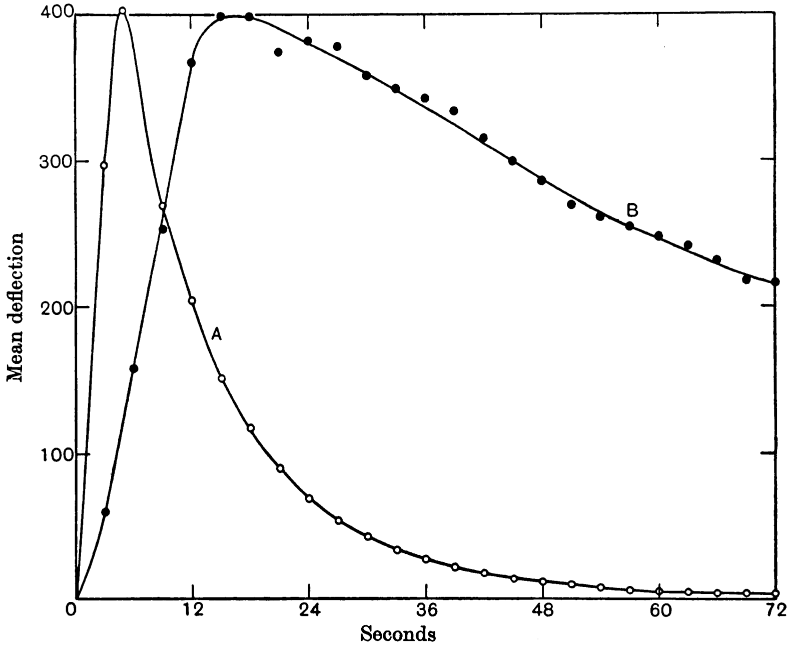

Fig. 2. A. Deflection-time curve resulting from a 3-second control heating. Mean of twelve series of readings. B. Early portion of deflection-time curve due to 12-second stimulus of same nerves. Mean of eleven series. 24·2° C. 24. vii. 29.

The method of analysis closely follows that employed by Hill [1929]. From the smooth curve drawn through the mean of the observed galvanometer deflections for the stimulated nerves are taken values at intervals corresponding to the time unit of analysis. These values are then analysed for the actual course of the live heat production by the use of the control heating deflections at corresponding times. Thus the analysis represented in Fig. 3 has been obtained as follows from the curves of Fig. 2.

Time in seconds ...	0	3	6	9	12	15	18	21	24	27	30	33
Control heat deflection	0	297	380	269	204	150	117	90	69	54	43	33
Live heat deflection ...	0	60	157	253	367	400	399	392	382	372	360	350
0–3 sec. Subtract 60/297 (0·20) of control ...	0	60	77	54	41	30	24	18	14	11	9	7
Remainders	0	0	80	199	326	370	375	374	368	361	351	343
3–6 sec. Subtract 80/297 (0·27) of control ...	0	0	80	102	72	55	41	32	24	19	15	12
Remainders	0	0	0	97	254	315	334	342	344	342	336	331
6–9 sec. Subtract 97/297 (0·33) of control ...	0	0	0	97	124	88	67	49	38	29	23	18
Remainders	0	0	0	0	130	227	267	293	306	313	313	313
9–12 sec. Subtract 130/297 (0·43) of control	0	0	0	0	130	166	118	89	66	51	39	30
Remainders	0	0	0	0	0	61	149	204	240	262	274	283
etc.												

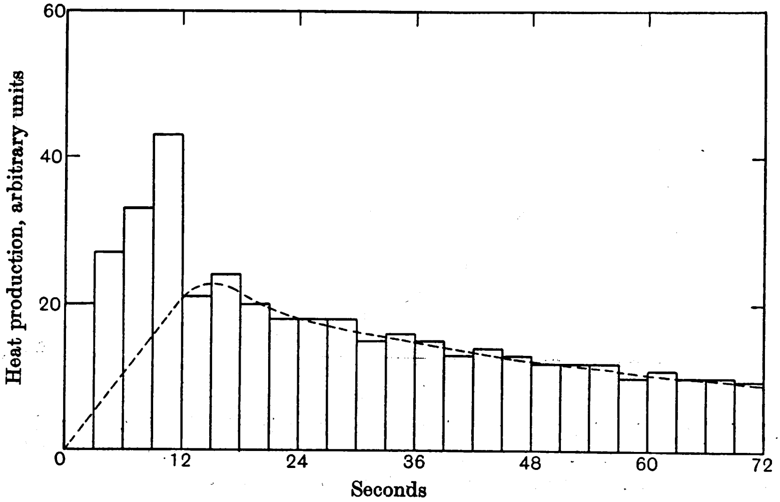

Fig. 3. Analysis of curve B of Fig. 2 in 3-second units to show course of heat production during and immediately after the 12-second stimulus. Broken line indicates rate of recovery heat production. Heat produced in each 3 seconds represented as a block for that interval. 24·2° C. 24. vii. 29.

The procedure has been to assume that in each time interval an amount of heat is produced equal to such a fraction of that required to give the control deflection as is represented by the ratio of the live heat deflection at that time to the control deflection at the end of the first time unit. In order to determine the ordinates of the curve representing the remaining heat production, the original deflection curve is reduced by subtracting the proper fraction of the control heating deflection curve. The values of the several ratios represent in arbitrary units the heat production in each time interval and are plotted as blocks in Fig. 3.

As in the above example the analysis of the first few minutes has been carried out by units which are less than the duration of the period of stimulation in order to show the course of the heat production during and immediately after the stimulus. The figure clearly shows the progressive increase in rate of heat production during the stimulation period and the sharp drop immediately following the end of stimulation. The initial rise is obviously due to the fact that the second unit contains recovery heat resulting from the activity of the first 3 seconds as well as its own initial heat, the third unit contains recovery heat from the activity of both the first and second 3 seconds, and so on. The rapid drop following the end of stimulation comes as a result of the cessation of initial heat production.

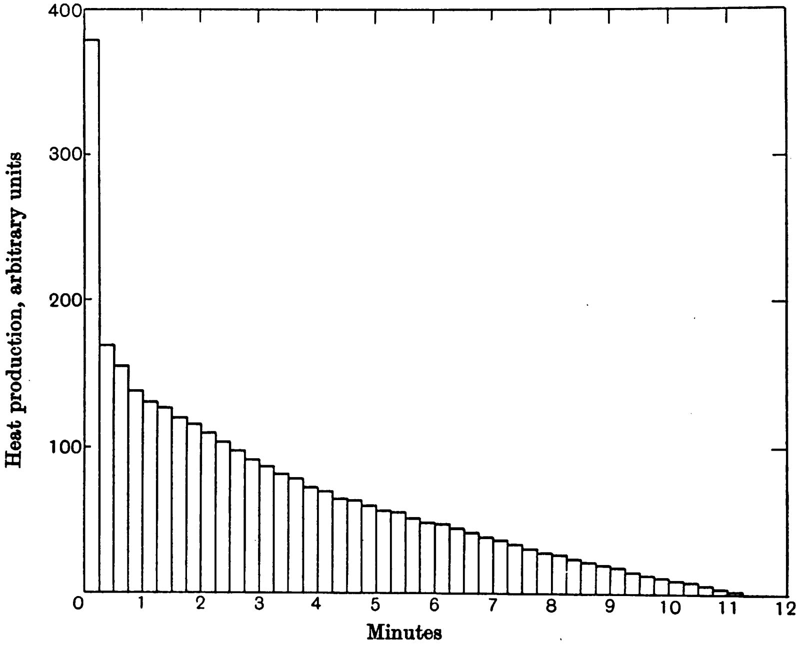

Fig. 4. Analysis in 15-second intervals of the rate of heat production resulting from a 15-second stimulus. Heat produced in each 15-second period shown as a block for that interval. First block contains both initial and recovery heat; others recovery only. 19° C. 30. vii. 29.

The subsequent course of the recovery phase is better shown by such an analysis as that represented in Fig. 4, which is carried through for the entire period of heat production. In order to reduce the labour of computation these complete analyses have been made in longer intervals, which were usually equal to the duration of the stimulus. The extreme regularity of the analysis given in this figure is characteristic of all the series, and constitutes good evidence for the accuracy of the experiments and the satisfactory nature of the method of analysis. It will be seen that the rate of recovery heat production is a maximum at the end of stimulation and then gradually decreases, more and more slowly, for a period of some 7 to 11 minutes.

The duration of this period of recovery heat production is, of course, dependent upon the temperature. Although no specific study of this point was made, a number of the experiments were made by chance at quite different temperatures and, therefore, give some information regarding the influence of this factor. The interval has been determined that elapsed between the stimulus and the time when the rate of heat production had fallen to about 1 p.c. of the maximum rate of recovery heat production. In three experiments at a temperature of 19° C. this interval was 11·0, 11·0 and 10·7 minutes, whereas in other experiments at a temperature of between 24 and 25° C., the intervals were 6·0, 9·0 and 8·2 minutes. The mean for 19° C. is, therefore, 10·9 minutes and for 24–25° C. is 7·7 minutes.

The sharp division between initial and recovery heat production is strikingly apparent in both Fig. 3 and Fig. 4. In order to determine the relative amounts of heat to be allotted to each of the two phases, the following procedure was employed. The total area of all the blocks for the entire period of heat production has been determined and represents the total heat. Such analyses as that represented in Fig. 3 indicate that the increase in rate of recovery heat production during the period of stimulation is presumably linear with time. In those analyses in which the unit of analysis is equal to the period of stimulation one can, therefore, take half the second block as the recovery heat evolved during the period of stimulation. Subtracting this from the area of the first block gives the initial heat. Obviously the difference between the latter and the total heat gives the heat associated with the recovery process. The ratio of the initial heat to the total heat and to the recovery heat is given for each experiment in the following table.

The value of 8·9 p.c. for the ratio of initial to total heat given by these experiments is to be compared with the earlier value of "about 11 p.c."

Date	Temperature in ° C.	Duration of stimulus in seconds	Initial heat in p.c. of recovery	Initial heat in p.c. of total
13. vii. 29	—	9	8·1	7·5
17. vii. 29	24·0	9	10·2	9·2
20. vii. 29	24·5	9	9·4	8·5
24. vii. 29	24·2	12	9·5	8·7
28. vii. 29	19·0	12	9·5	8·6
29. vii. 29	19·0	12	11·2	10·0
30. vii. 29	19·0	15	10·9	9·8
		Mean	9·8	8·9

reported by Downing, Gerard and Hill [1926] and by Gerard [1927]. Considering the fact that the earlier work was done with less satisfactory apparatus and the value was obtained by comparing the area of the control curve with the nerve heat deflection curve instead of by the more elaborate analyses carried out in the present experiments, the agreement is good. Compared, however, with the value of 2·25 p.c. for crustacean nerve, the divergence is still large. Probable reasons for this difference have been suggested in the paper by Hill [1929].

The earlier value of 11 p.c. for the ratio of initial to recovery heat did not exclude the possibility of a lactic acid mechanism in the process of nervous conduction. This view, which was strengthened by the knowledge of the ready formation of lactic acid in the central nervous system, would have been possible "if one assumed that the whole of the lactic acid formed initially were oxidized in recovery, in which case the ratio would be 385/3836 = 10 p.c." As regards crustacean nerve such a possibility was definitely ruled out by Hill's finding that the ratio of initial to recovery heat was 2·25 p.c. The present work makes the conclusion definite for vertebrate nerve as well, and would seem finally to rule out the possibility of a lactic acid mechanism.

SUMMARY.

1. Recent improvements in thermopile and galvanometer design have been employed in a determination of the course of the heat production of frog's nerve. Many of the essential findings of the earlier workers have been in large part confirmed.

2. An analysis of the course of the heat production resulting from a 9- to 15-second stimulus shows a progressive increase in the rate of heat production during the period of stimulation. This is the sum of the heat associated with the conduction process and that accompanying the recovery therefrom.

3. The recovery heat production is at a maximum rate immediately

following the end of stimulation, and continues for some minutes at a decreasing rate.

4. The duration of this recovery phase is largely dependent upon the temperature. At 19° C. it was about 11 minutes and at 24° to 25° C. about 7¾ minutes.

5. From seven experiments comprising about seventy individual determinations the initial heat production has been found to be 8·9 p.c. of the total. This is compared with the earlier determinations of 11 p.c. for frog's nerve and Hill's recent value of 2·25 p.c. for crustacean nerve.

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