

THE TIME COURSE OF EVOLUTION OF OXIDATIVE RECOVERY HEAT OF FROG'S MUSCLE

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IN an earlier paper [D. K. Hill, 1940] a method was described for determining the time course of oxygen consumption of stimulated frog's muscle at 0° C. Comparison was made with the time course of oxidative recovery heat production under the same conditions and the results given in this paper were there quoted.

In the past there have been investigations at 0° C. of the initial heat production and of the negative phase in the anaerobic delayed heat, but never of the oxidative delayed heat. The reason is that the latter is evolved very slowly at such a low temperature and the thermopile-galvanometer system previously available did not possess the necessary combination of sensitivity and stability. The difficulty has now been overcome. The method and technique are due to A. V. Hill and have already been described in detail [A. V. Hill, 1937, 1938, 1939]. Accurate analysis of the galvanometer record over the complete period of recovery is greatly simplified by virtue of a certain property of the modern thermopiles. It is that the rate of fall of temperature at the hot junctions is almost exactly proportional to the temperature difference between the hot and the cold junctions: this means that a control heating curve has a logarithmic form. The application of this property in the analysis has been outlined by A. V. Hill [1939]. It will be seen that the oxidative delayed heat at 0° C. is not complete for about 30 min. and analysis for a period as long as this by the old method would be very laborious. By the new method the analysis can be completed in less than an hour.

METHOD

The method has been described by A. V. Hill [1939]. The following points require mention:

(1) The solution for preliminary soaking of the muscles must have the same composition as that used in the experiments on oxygen consumption to ensure comparison under identical conditions. It consists of Ringer's solution containing 10 mg. P/100 c.c. in the form of phosphates at pH 7.2.

(2) The adequacy of supply of oxygen to the innermost layers of the muscle must be considered. The thickness of muscle lying on the thermopile is never more than 1 mm. The resting rate of oxygen consumption at 0° C. is 10^{-4} c.c./c.c. \times min. Krogh's diffusion constant may be taken as 1.1×10^{-5} at 0° C. The concentration of oxygen at a depth of 1 mm. in the muscle is (by calculation) 29.5 mm.³/c.c. [see A. V. Hill, 1928]. With a 12 sec. tetanus the total quantity of oxygen required for complete recovery is 26 mm.³/c.c. of muscle. There is thus adequate supply. In two experiments the durations of tetanus were 18 and 24 sec. respectively and there is thus some possibility of lack of oxygen in the innermost layers of muscle. However, by reference to the previous paper [D. K. Hill, 1940] it will be seen that the oxygen consumption occurs so slowly after activity as to allow ample time for replenishment by diffusion.

(3) The duration of tetanus was varied from 2 to 24 sec. for different experiments. It will be seen that the time course of recovery heat production is nearly independent of stimulus duration over this range so that for purposes of comparison with the time course of oxygen consumption the duration need not be exactly specified.

RESULTS

The time course of delayed heat production at pH 7.2

The results are given in Table I. The total quantity of delayed heat (anaerobic plus oxidative) is given at one minute intervals up to 30 min. for ten different experiments. The figures for the last six experiments have been added together. The average duration of tetanus for this group is 11 sec. which is approximately the duration of stimulus employed in the oxygen consumption experiments. In order to make a comparison of the time courses of oxidative delayed heat and oxygen consumption the former has to be obtained by subtracting from the total delayed heat that part of the delayed heat which is still present in the absence of oxygen. It was shown by Hartree [1932] that this is a justifiable pro-

TABLE I. pH 7.2

	Time (min.)																								Duration of stimulus sec.										
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		24	25	26	27	28	29	30			
I	0	2	11	23	36	46	53	60	66	71	75	80	83	87	89	91	93	94	95	97	97	98	99	99	99	99	100	100	100	100	100	100	100	2	
II	0	4	14	22	30	38	45	51	57	62	67	71	75	79	83	85	88	90	91	93	95	96	97	98	99	99	100	100	100	100	100	100	100	3	
III	0	9	19	28	35	43	49	55	60	65	70	74	78	81	83	86	88	90	91	93	94	95	96	97	98	98	99	99	100	100	100	100	100	2	
IV	0	6	16	25	32	40	46	53	58	63	68	72	76	79	81	84	86	88	89	91	93	94	95	96	97	98	98	99	99	100	100	100	100	2	
V	0	5	13	22	30	36	43	48	54	59	63	67	70	74	76	79	82	85	86	87	89	91	92	93	94	95	96	97	98	98	99	99	100	3	
VI	0	5	13	22	30	36	43	48	54	59	63	67	70	74	76	79	82	85	86	87	89	91	92	93	94	95	96	97	98	98	99	99	100	6	
VII	0	5	13	22	30	36	43	48	54	59	63	67	70	74	76	79	82	85	86	87	89	91	92	93	94	95	96	97	98	98	99	99	100	12	
VIII	0	8	17	25	33	40	46	52	56	61	65	69	72	75	77	80	83	85	86	87	89	91	92	93	94	95	96	97	98	98	99	99	100	18	
IX	0	9	21	31	40	47	54	59	64	69	72	75	78	81	83	85	87	89	91	92	93	94	95	96	97	98	98	99	99	100	100	100	100	3	
X	0	8	18	26	35	42	47	53	59	63	67	70	73	76	79	81	83	85	87	89	90	91	92	93	94	95	96	97	98	99	100	100	100	24	
Sum V-X	0	40	95	148	198	237	276	308	341	370	393	415	430	454	467	483	499	514	522	528	539	549	555	561	566	572	578	583	589	590	596	596	596	For initial heat = 100	
AN.D.H.	0	3	6	8	10	11	12	12	13	14	15	15	15	15	16	16	17	17	17	17	17	18	18	18	18	18	19	19	19	19	19	19	19	19	For initial heat = 100
O ₂ .D.H.	0	4	10	17	23	29	34	39	44	48	51	54	57	61	62	65	66	69	70	71	72	74	75	76	75	76	77	78	79	79	80	80	80	80	To maximum 100
O ₂ .D.H.	0	5	13	21	29	36	43	49	55	59	64	68	71	76	78	81	83	86	88	89	90	93	94	95	94	95	96	98	99	99	100	100	100	100	To maximum 100

AN.D.H. = Anaerobic delayed heat. O₂.D.H. = Oxidative delayed heat.

TABLE II. pH 6

	Time (min.)																								Duration of stimulus										
	1	2	3	4	5	6	7	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40		42	44	46	48	50	50	50	6		
I	1	6	11	17	22	28	33	37	46	54	61	68	74	78	83	87	89	92	93	95	96	97	98	98	99	99	99	100	100	100	100	100	100	6	
II	0	6	11	16	21	26	30	35	43	50	56	63	68	73	78	81	84	87	90	92	94	96	97	98	99	99	100	100	100	100	100	100	100	12	
AN.D.H.	-2	-1	2	2	2	3	3	3	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	12
O ₂ .D.H.	0	7	9	14	19	23	27	32	40	46	52	59	64	68	73	76	79	82	85	87	89	91	92	93	94	94	95	95	95	95	95	95	95	95	12
Same to max. 100	0	7	9	15	20	24	28	34	42	49	55	62	67	72	77	80	83	86	89	92	94	96	97	98	99	99	100	100	100	100	100	100	100	100	12

AN.D.H. = Anaerobic delayed heat. O₂.D.H. = Oxidative delayed heat.

cedure: he found that the early stages of the delayed heat at 17° C. are identical in the presence of oxygen and in the earliest runs of a series in the absence of oxygen. Included in Table I, therefore, is a column giving the figures for the earliest runs of a series in nitrogen. The figures are expressed as percentage of the initial heat. The sum of the six delayed heat experiments is brought to a final maximum of 100. The total delayed heat is very nearly equal to the initial heat [A. V. Hill, 1939] so the latter may be taken as 100 and the figures for the anaerobic

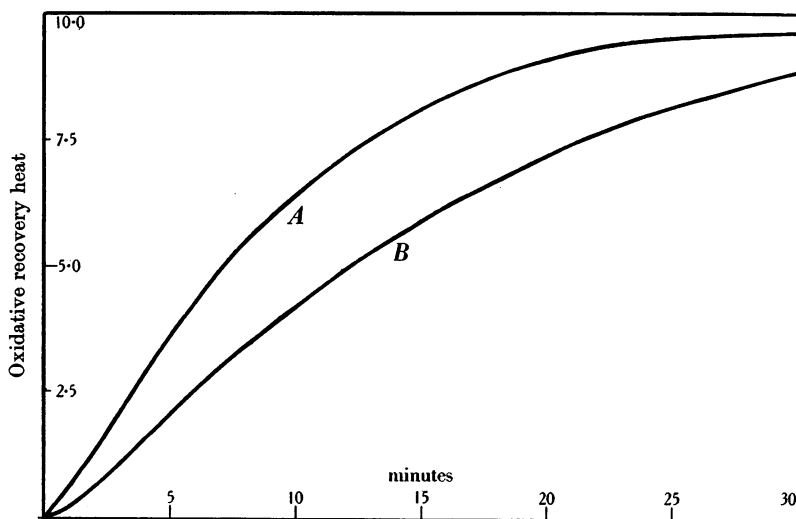


Fig. 1. Oxidative recovery heat following a 12 sec. tetanus at 0° C. A, pH 7.2; B, pH 6.2.

delayed heat at the corresponding times subtracted. The result gives the time course of the oxidative delayed heat (see Fig. 1). The determination of the anaerobic delayed heat is the subject of a later paper.

The time course of delayed heat production at pH 6

Oxygen consumption following a tetanus is considerably slowed by lowering the pH of the medium used for the preliminary soaking [D. K. Hill, 1940]. It is desirable to determine the time course of oxidative delayed heat production of muscle at 0° C. and pH 6 for comparison with the time course of oxygen consumption under these conditions.

The solution for preliminary soaking was made identical with that used in the oxygen experiments, namely Ringer's solution containing 25 mg. P/100 c.c. in the form of phosphates at pH 6. The results are shown in Table II. Each series is the mean of three runs taken with one

pair of muscles. At this *pH* the time course of total heat production varies with the duration of tetanus to a greater extent than at *pH* 7.2. For comparison with the time course of oxygen consumption, therefore, the duration of tetanus must be specified: it should be 12 sec. The time course of *oxidative* delayed heat for this duration of tetanus is derived, in the same way as at *pH* 7.2, by subtraction of anaerobic heat from total heat (Table II and Fig. 1).

DISCUSSION

This research was done for the specific purpose of obtaining a comparison of recovery heat and oxygen consumption under identical conditions and for that reason is deficient in one respect: the variation of the time course of heat production with duration of tetanus has not been fully explored. The results in Table I do not demonstrate clearly the trend of variation with change in stimulus duration and experiments should be done using much shorter and much longer durations. The observed changes in time course of oxygen consumption with the longer stimuli is consistent with Hartree's results and the existence of a maximum possible rate of oxygen consumption is also to be correlated with Hartree's discovery of a maximum possible rate of oxidative recovery heat production. It is probable that corresponding changes would be found in the recovery heat production at 0° C.

At present the time course of oxygen consumption cannot be investigated accurately with stimuli of less than about 5 sec. so that comparison with very short durations of tetanus (up to 1 sec.) is impossible. It would nevertheless be worth while exploring these lower reaches especially for the purpose of investigating further a point mentioned by Hartree, namely that with short durations of stimulus the times to maximum of the rates of production of anaerobic and oxidative heat vary in a similar manner as the duration of stimulus is changed. This acquires special significance in view of the conclusions of the next paper where it is shown that the early rapid phase of production of the anaerobic delayed heat must be attributed to the breakdown and not to the re-synthesis of phosphocreatine. It would seem probable that the breakdown products of phosphocreatine are responsible for the initiation of the oxidative recovery processes in muscle.

SUMMARY

1. Owing to improvements in technique it is possible to determine the time course of oxidative recovery heat production of frog's muscle at 0° C.

2. Following a 12 sec. tetanus this heat production is half complete in 7 min. at pH 7.2 and in 12 min. at pH 6.

3. At pH 7.2 the duration of tetanus was varied from 2 to 24 sec. This is not a sufficiently wide range to bring out clearly the dependence upon stimulus duration and further experiments are required using shorter and longer durations.

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