THE HEAT-PRODUCTION OF SURVIVING AMPHIBIAN MUSCLES, DURING REST, ACTIVITY, AND RIGOR.

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In a recent paper[1] I showed that it is possible to estimate the heat produced by isolated surviving frogs' muscles, (*a*) during rest and (*b*) during heat- or chloroform-rigor. These experiments have led to a more complete investigation of the energy exchanges of muscles under various conditions. The direction taken by this investigation has been determined largely by ideas derived from Fletcher's work[2] on the CO_2 liberation, and from the work of Fletcher and Hopkins[3] on the lactic acid formation of surviving muscles. A quantitative comparison[4] of the heat produced during the various stages of what is really the dissolution or gradual death of a muscle, with the CO_2 set free during these stages, is as Frank[5] points out a matter of obvious interest in discussing the internal nature of the muscular machine. A further comparison of the heat with the lactic acid formed is of equal importance. It is on lines such as these that we may best hope to separate, from among the various survival processes, those which exist during normal life and those which occur only during dissolution and death.

The muscle is undoubtedly a chemical machine: by no stretch of the imagination can it be supposed to be a heat-engine. Later in the paper[6] is discussed how the developments of physical chemistry, making it possible to predict "free energy" from certain constants in a

[1] A. V. Hill. This *Journal*, XLIII. p. 280. 1911.

[2] W. M. Fletcher. *Ibid.* XXIII. p. 10. 1898: XXVIII. pp. 354 and 474. 1902.

[3] Fletcher and Hopkins. *Ibid.* XXXV. p. 247. 1907.

[4] Blix (*Scand. Arch. f. Physiol.* XII. p. 94. 1902) claims to have established the heat-production of resting muscle. Experience with his instrument makes me, however, very doubtful of the validity of his proof. At any rate he failed entirely to obtain quantitative results.

[5] O. Frank. *Ergebn. d. Physiol.* III. (2), p. 390 &c. 1904.

[6] p. 507.

chemical reaction, may help us to develop the theory of muscular contraction upon rational lines. The present investigation is an attempt to follow the disintegrative processes of a muscle cut off from its circulation from the point of view of the total change of energy in these processes. As regards a comparison of the energy with the chemical products of these reactions I have had many opportunities of discussing the problems involved with Dr Fletcher: to this fact I owe much, both in information and ideas.

The heat-production of an isolated muscle under various conditions has however an interest of its own apart from a comparison with the simultaneous formation of chemical substances, in particular of lactic acid and CO_2. It can be used as an independent means of investigation, based upon the fact that a rise of temperature in the muscles communicates itself, with no appreciable delay, to the recording apparatus: whereas (*a*) as Fletcher has shown the CO_2 once formed takes some hours to diffuse out of the muscle and (*b*) the present method of lactic acid estimation is so elaborate that many experiments cannot be done by it. The heat-production is likely, therefore, to be of greater use than the formation of chemical products, in helping us to follow the time-relations of certain muscular processes.

PART I. THE HEAT-PRODUCTION OF RESTING MUSCLES.

The method of estimation was that of the micro-calorimeter described recently[1]. Owing however to the extreme smallness of the quantities of heat to be estimated—leading in some cases to a rise of temperature of not more than ·01° C. per hour—I have, for reasons given below, adopted somewhat more elaborate precautions to secure equality of temperature outside the two flasks. The differential method is of course based on the supposition that the two flasks are subjected to identical external temperature conditions, so that the physical loss of heat from one flask is balanced by an equivalent loss of heat from the other. Naturally I imagined that if the two flasks were standing side by side on a table, in a cellar of very uniform temperature, such conditions would be fulfilled. For all ordinary experiments, such as I have described hitherto, this is in fact the case. But in the estimation of the very minute quantities of heat given out by resting surviving muscles in their later stages it was found that there were sometimes certain irregularities which could be accounted for only by the

[1] *Op. cit.* Throughout this paper calorie denotes gram-calorie.

supposition that the two flasks were being subjected to external temperatures differing by as much as 0·1° C., or even more. This might occasionally lead to an error of 50 % in the very small quantities of heat being estimated, and had obviously to be avoided as much as possible.

The difference of temperature at two points on the table might be due to either or both of two factors:—(*a*) inequality in draughts of air, or in exposure to radiation: (*b*) heat-flow along the table from the inside to the outside of the building. Whichever be the case the error was to some extent avoided by the following device:

The flasks were kept inside a large tin box, which was sufficient to hold four of them, and which stood, insulated from the table, upon four corks at its corners. Since the box was a good conductor, differences of temperature along it tended to be equalised by conduction: and moreover the flasks were protected more effectively from radiation and draughts. The system was not completely satisfactory, but at any rate was better than before. Without very serious complications it is difficult indeed to see how one can get a more complete equality of temperature outside the two flasks. The limits of error in the experiments are given below. In any one experiment during the later stages of survival it will be seen that there is the possibility of very considerable errors. But for many months experiments have been made in the hope of reducing these errors: and the accumulated results of all these experiments, *in none of which the errors are, a priori, more likely to be positive than negative*, must present a picture of the resting heat-production which is very close to the truth. In fact to aim at a higher accuracy than the mean of (say) all the experiments made in the neighbourhood of 16° C., would be useless because of the inconstancy and variability of the material under investigation, viz. frogs' muscles.

The experiments have all been made upon *Rana temporaria*; in those given in Table I, only arms and legs, or legs alone, have been used. Skinning has usually been avoided for several reasons: (*a*) ease and quickness of manipulation: skinning 100 gr. of frogs' legs takes some time, and it is often desirable to make the first observation as soon as possible after death; (*b*) to avoid injury as much as possible; (*c*) to avoid, to a certain extent, exposure of the muscles to the Ringer's solution. Needless to say the muscles have never been separated from the bones. The results are expressed in gr. calories per hour, per c.c. of tissue, unskinned. To reduce these values to those per c.c. of tissue skinned, it is necessary to multiply by a factor which is approximately 1·2. In Table II are given results obtained by the use of whole frogs, with their brains and cords destroyed immediately before the experiment.

The use of whole frogs has, though in an even higher degree, all the advantages, (*a*) (*b*) and (*c*), given above: it has however the disadvantage that one does not know exactly with what one is dealing: the survival processes in intestine, liver and heart all add their quota to the heat produced by the surviving muscles. The possibility of bacterial decomposition in the gut leading to survival heat-changes can be neglected, for the guts of laboratory frogs are almost always practically empty; in any case however the muscles provide the main portion of the active living constituents of an animal: and the exact quantitative and qualitative similarity of the curves of heat-production, for the whole pithed animal and for the legs only, shows that we may safely use the whole animal for the experiments.

The experiments were made (*a*) during the very hot summer of 1911 and (*b*) during the months January to May of 1912. The more careful and elaborate experiments are the later ones: some of the earlier experiments however are included here, because although the frogs were in a very different state (owing to the hot weather) the same general type of results was obtained. In the later experiments stirring before each reading (to get the mean temperature of each flask) has been done with thin pieces of stick, passing through the cotton-wool stopper, and left resting in the flasks throughout the experiment. This I believe to be simpler, less liable to error, and more efficient than the bubbling of air previously advocated. With a little stirring a perfectly constant reading is obtained, so that further stirring causes no change of reading to within $\frac{1}{300}$° C. The actual galvanometer readings were made at short intervals during the first few hours, and at longer intervals later. Curves were drawn through the observed points plotted on squared paper, and were then corrected for heat-loss[1]: from the curves so corrected the heat-production during the various stages of survival was read off. It is impossible to give full details of all the experiments quoted. For those however who wish to see the actual details of a typical experiment, Exp. I, Table I and Exp. I, Table II are given in full. It should be noted that, when the contrary is not definitely stated, the Ringer or salt solution used is the ordinary laboratory solution which contains a certain amount of dissolved oxygen, say from 0·2 to 0·6 c.c. %. This is important in view of the effects of O_2 seen in Table III and described later.

[1] Cf. the description of method, A. V. Hill, *op. cit.* The constants of heat-loss of the flasks were very carefully redetermined for these experiments, and found to be practically unchanged after nine months' use of the flasks.

TABLE I. *Heat-production of resting isolated frogs' muscles.*

Exp. I. Feb. 1912. Eight frogs killed, 97 c.c. arms and legs cut off as rapidly as possible, measured and put in flask No. 1 at 13·5° C. in Ringer's solution: total 250 c.c. In control-flask No. 5 285 c.c. water. Coefficient of heat-loss $k = ·0406$ per hour. Stirred with thin sticks before each reading. The whole was very well shaken and stirred before the readings began: the initial reading is therefore a correct[1] one, and the amount of oxygen[2] dissolved in the 250 − 97, *i.e.* 153 c.c. of Ringer, must have been about 1·1 c.c. The time is given in hours from the time of death of the frogs, and the reading in scale divisions: 100 s.d. = ·324° C.

Time	·3	·35	·4	·5	·7	1·0	1·3	1·9	2·5	3	4·6	5·5	6·7
Reading	−118	−113	−110	−108	−103	−97	−90	−76	−74	−70	−58	−49	−44

Time	8·1	22·7	23·9	26·9	28·3	31·3	33·4	47·7	49	51·1	53·2	74	102
Reading	−36	+37	+43	59	68	77	92	128	135	140	143	234	415

At the end of the experiment the muscles were decomposing strongly: that the later large rise of temperature is due to this, is seen from the fact that after placing at 102 hours a little chloroform into the flask, the reading fell in 24 hours to 220. Judging by other experiments the chloroform does not immediately check all the processes set up by bacteria: but neglecting the first few hours after the introduction of chloroform, it is seen that there is practically no heat-production after cessation of the bacterial action. Autolytic changes are not stopped by chloroform, so these cannot be responsible for the heat-production in the later stages given in the Table.

From the observations given above, and the curve of heat-production corrected for heat-loss, the following numbers are obtained and shown in Fig. 1. The rate of heat-production is calculated in calories per hour, per c.c. of tissue, during the periods named. Time in hours, measured from death of animals.

Period beginning at ...	·3	·55	·8	1·0	1·5	2	3	4	6	8
ending at ...	·55	·8	1·0	1·5	2·0	3	4	6	8	13
Rate of heat-production	·36	·20	·15	·12	·08	·07	·05	·04	·035	·038

Period beginning at ...	13	23	33	43	50	60	70	80	90
ending at ...	23	33	43	53	60	70	80	90	100
Rate of heat-production	·049	·07	·07	·07	·08	·11	·14	·17	·20

The rate of heat-production is possibly subject to an error of ± ·016 cal. per c.c. per hour. The same error, whatever it be, must however affect every estimation alike, and in the same direction.

In the following experiments (II—VI) the possible error was ± ·02 cal. per c.c. per hour.

Exp. II. Similar Exp.: legs and arms at 13° C. in NaCl ·7 %. Feb. 1912. Heat-production in gr. cal. per c.c. of tissue per hour, during the periods named. Time in hours.

Period beginning at ...	0·5	1·0	2	3	4	6	9	12	20	30
ending at ...	1·0	2·0	3	4	6	9	12	20	30	80
Rate of heat-production	·16	·09	·056	·052	·050	·040	·043	·046	·050	·053

[1] If shaking is not carried out carefully the mixture of muscles and Ringer may take some time to settle down to a uniform temperature.

[2] The solubility of O_2 in water at 13·5° is about 3·4 c.c. %: the dilute O_2 of the air is therefore soluble to the extent of about 0·7 c.c. %.

Exp. III. Similar Exp.: at 18° C. in Ringer. Feb. 1912.

Period beginning at ...	·3	·8	1·3	2·0	5	9
ending at ...	·8	1·3	2·0	5·0	9	32
Rate of heat-production	·29	·20	·16	·06	·06	·08

Exp. IV. Frogs' legs at 15° C. in *boiled* Ringer (no O_2 present). Apr. 1912.

Period beginning at ...	1	2	4	8	20	30	40
ending at ...	2	4	8	20	30	40	45
Rate of heat-production	·054	·051	·045	·040	·033	·039	·058

Exp. V. Frogs' legs at 22° C. in Ringer. Aug. 1911.

Period beginning at ...	·5	1·0	2·0	3	4	6
ending at ...	1·0	2·0	3·0	4	6	10
Rate of heat-production	·30	·26	·19	·17	·15	·13

Exp. VI. Frogs' legs at 16·5° C. in NaCl ·7 %. Aug. 1911.

Period beginning at ...	4	5	6	8	10	13	22
ending at ...	5	6	8	10	13	22	28
Rate of heat-production	·07	·04	·035	·033	·033	·033	·033

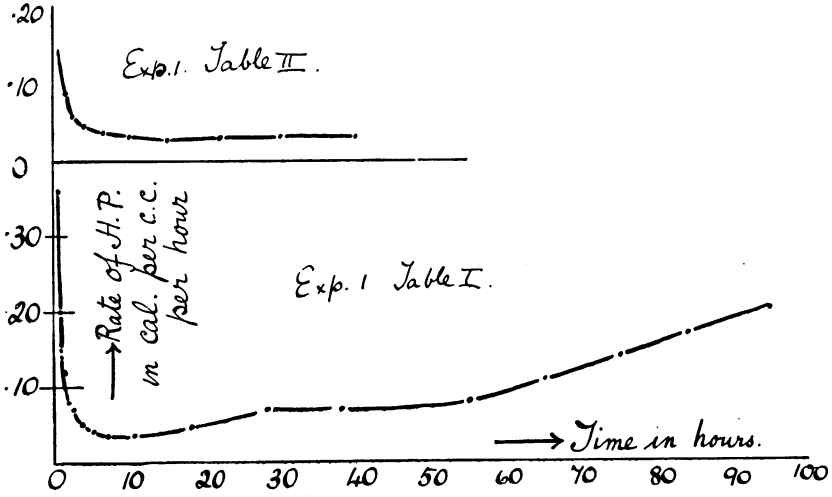

Fig. 1. Course of survival heat-production, in actual experiments.

TABLE II. *Heat-production of resting pithed frogs.*

Exp. I. May 1912. Eight frogs killed, 160 c.c. frogs + 170 c.c. *boiled* Ringer in flask No. 1 at 15° C. In control flask No. 5 400 c.c. water. Coeff. of heat-loss, $k = ·0293$ per hour. Time in hours from death: readings in s.d. 100 s.d. = ·306° C.

Time	·4	·7	·9	1·0	1·1	1·3	2·5	3·6	4·9	6·8
Reading	−69	−64	−60	−57	−56	−54	−39	−29	−20	−7
Time	8·0	10·3	20·5	22·9	25·4	28·9	34·4	44·7	47·7	
Reading	−1	+10	+46	53	62	72	83	100	106	

At the end of the experiment the feet of two of the frogs were still excitable: but there was an obvious smell of decomposition. From the observations a corrected curve of heat-production was made, and the following numbers were obtained and are shown in Fig. 1. H.-P. in calories per c.c. of tissue per hour, during the periods named. Time in hours, measured from death of animals.

Period beginning at ...	1	2	3	5	8	12	18	26	34
ending at ...	2	3	5	8	12	18	26	34	46
Rate of heat-production	·09	·06	·047	·039	·032	·029	·030	·031	·030

Possible error, ± ·007 cal. per c.c. per hour.

In Exps. II to IV the possible error was not > ± ·02 cal. per c.c. per hour.

Exp. II. Whole frogs, unskinned, at 16·5° C. in Ringer. Feb. 1912.

Period beginning at ...	·3	1·0	2	3	10	15	20
ending at ...	1·0	2·0	3	10	15	20	25
Rate of heat-production	·23	·15	·08	·05	·055	·065	·07

Exp. III. Whole frogs, unskinned, at 12·5° C. in NaCl solution. Feb. 1912.

Period beginning at ...	·5	1	2	3	4	6	12	20	40
ending at ...	1·0	2	3	4	6	12	20	40	60
Rate of heat-production	·10	·07	·055	·037	·034	·030	·021	·025	·031

Exp. IV. Whole frogs, unskinned, at 21° C. in Ringer. Aug. 1911.

Period beginning at ...	·5	1·0	2	4	6	8
ending at ...	1·0	2·0	4	6	8	10
Rate of heat-production	·19	·14	·12	·10	·08	·06

From these experiments it is seen that the heat-production of isolated resting muscles follows a change represented graphically in Fig. 1. The same relations are shown by the heat-production of pithed frogs. The heat-production of the normal resting live frog, as was shown in a previous paper[1], is about ·32 cal. per c.c. per hour at 16° C. This value is strikingly the same as values obtained for the initial heat-evolution in Table I exps. I, III, V, Table II exp. II, and Table III exps. II, III and VIII. The production of heat therefore by the resting surviving muscle starts at a high value which is apparently about the same as that for the normal live resting frog. It is impossible, as a matter of fact, to estimate the rate of heat-production immediately after death; the method requires at least 15 minutes before an observation can be made. If however one exterpolates the curve back, one finds a value for the initial survival evolution of heat which is, in any case, not less than that shown by normal living frogs. The rate of heat-production falls thereafter for several hours, along a more or less "exponential" curve, until at 16° C. it reaches a value of about ·05 cal. per gr. of tissue per hour, both for frogs' legs or for whole frogs. At

[1] Cf. A. V. Hill. This *Journal*, XLIII. p. 390. 1911.

this value it may remain constant for many hours, without any very obvious variation, until finally decomposition sets in, followed by an increasing rate of heat-production. Before proceeding to discuss the bearing of these facts, it may be well to refute certain objections that may be made against them.

In the first place a year's experience with the method has shown me that for these experiments the apparatus is working somewhere near the limit of its power. The greatest liability to error arises from the impossibility of securing exactly similar temperatures outside the two flasks: but it must be remembered that, from several control experiments in which the final equilibrium difference of temperature between two flasks (*both* filled with water) has been estimated, it is possible to put a superior limit to the errors which may arise in this way: and further the errors cannot always be positive, or always negative, for each experiment has been made in a different position upon the table. At least 60 experiments altogether have been made, of which the 10 given above are typical: the same results qualitatively and quantitatively have been seen in all, so there is no possibility of any considerable error of this type affecting the general result. From the best of these 60 experiments I have calculated below an average value for the heat-production of "surviving" frogs' muscles: but it will be seen from the facts connected with the presence or absence of oxygen, which are given in Table III, that it is of no value, obtaining very exact results in any but the late periods of survival change, because the numbers obtained depend chiefly upon the amount of oxygen present. The average values given below are as accurate as it is worth our while to make them.

The next criticism is as follows. Possibly the peculiar conditions of the experiment have rendered the balancing of the flasks against one another no longer exact. The difference in the specific heats of water and muscle might have had this effect, were not this difference too small. It may be urged that the differential method applies only when exactly similar substances are in either flask. In these experiments a mixture of solids and liquid is in one flask, and water alone in the other. The solid substance, viz. frogs' muscles, may hinder convection currents and mixing of the fluid, and so prevent the equalisation of temperature: this might appreciably change the constant k of heat-loss in the flask considered. The flasks might no longer be properly balanced, and the method faulty. The one with muscles in it might always lose or gain heat slower than the one with water only. As a matter of fact this is

not the case. If it were, the errors would add on to the observed heat-production when the temperature of the calorimeters is above that of the room: and subtract from it when the temperature is below that of the room. The universal concordance observed between the experiments made above room temperature and those made below room temperature proves that such errors must be very small. Further, muscles left in chloroform-water after death have never shown any positive or negative evolution of heat. No appreciable proportion of the total observed heat-production can therefore be due to an error arising from incomplete balancing of the coefficients of heat-loss k of the two flasks: whether any such lack of adjustment be supposed due to the presence of solid substances, to inequality of specific heats, or to faulty calibration of the flasks.

A third objection is that possibly the initial high rate of heat-production is due to the rigor and death of injured muscle fibres. The experiments upon whole frogs were designed originally to test this, and the complete equivalence observed shows that, whatever else the survival heat-production may be, it cannot be due to death changes set up by mechanical injury of the muscles.

By taking the average of 23 of the best experiments made, the following results were obtained: the average temperature was 15·5° C., and in order to compare the results directly with Fletcher's results for the CO_2-evolution, the numbers are all given multiplied by 1·2, the factor necessary to reduce the heat-production observed per c.c. of animal unskinned to the heat-production per c.c. of animal skinned.

Rate of heat-production of resting frogs' muscles, reckoned in gr.-calories per hour per c.c. of skinned animal, during the periods named, at 15·5° C. Time in hours, reckoned from death of animal. Results plotted graphically in Fig. 2.

Period beginning at ...	·3	·5	1·0	2	3	5	10	20	30	40
ending at ...	·5	1·0	2·0	3	5	10	20	30	40	50
Rate of heat-production	·31	·14	·11	·078	·065	·050	·045	·046	·049	·060

Let us proceed now to a comparison of these facts with Fletcher's results on the liberation of CO_2. In the first place there is obviously a great similarity between the curve in Fig. 2, and the curves given by Fletcher[1]. There is the same initial fall from a high value, and the same attainment of a low constant value (named by Fletcher the "plateau" of CO_2-liberation) lasting for long periods, in fact at 15·5° C. from the 5th to the 40th hour; and finally there is the same rise when decomposition comes on. The second and third stages are obviously

[1] See especially Fletcher. This *Journal*, XXIII. pp. 27, 29, 63. 1898.

analogous in the two cases: the CO_2 and heat-liberations run parallel, at any rate after the 5th hour. As Fletcher[1] says, the "normal curve of CO_2 discharge may be divided into three stages: the first stage extends over five hours and is irregularly declining. The second stage, the 'plateau,' is many hours longer and shows a very slowly declining rate of discharge": and again[2] "the sudden and final change of direction of the curve, showing a rapidly increasing rate of CO_2-discharge, is invariably found to be accompanied by a distinctly putrefactive smell."

Of these three stages the third and last, that of bacterial invasion, need not be further discussed. It is an accident in survival life which has only the historical interest of playing a large and unrecognised part in the "muscle respiration" studied in the earlier work of Hermann and others.

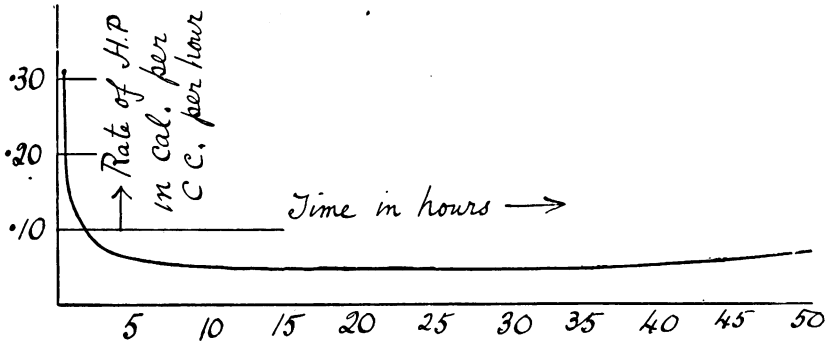

Fig. 2. Course of survival heat-production: mean of 23 experiments at average temperature of 15·5° C.

Of the survival respiration proper, the second stage is that of the long "plateau" of CO_2 and heat-production steadily maintained for many hours, culminating in the mechanical changes of rigor. As Fletcher[3] says "the maintained output of CO_2 marked the progress of a continuous chemical change, which only at a late stage induced the opacity and stiffness of rigor." The CO_2-output was maintained under anærobic conditions, it increased in air, and might be trebled in an atmosphere of oxygen, but in the atmosphere of oxygen the onset of rigor was prevented. The conclusion that the increase of CO_2 in the presence of abundant oxygen was the mark of the oxidative removal of a product causing rigor if unremoved was confirmed by Fletcher and

[1] *Op. cit.* p. 62.

[2] *Op. cit.* p. 28. This fact is also exactly what I have observed with respect to the evolution of heat.

[3] This *Journal*, XXVIII. p. 476. 1902.

Hopkins in their work on the lactic acid of muscle. The striking parallelism between the "plateau" of CO_2-output and the linear rate of lactic acid and heat-production (under anærobic conditions) points very strongly to their being due to one and the same process. This will be discussed below.

With regard to the first stage of survival respiration, lasting for about five hours, during which the CO_2 and heat outputs decline rapidly at first and then more slowly to reach their steady constant values, the interpretation is more difficult. Fletcher originally suggested the hypothesis that this stage of the CO_2- discharge was due to three factors. "The bulk of the discharge is due to destructive processes going on within the muscle independently of the surrounding medium": that is to say the events causing the later CO_2 "plateau" were supposed to be present also during the first stage, in addition to the other processes yielding the extra CO_2. In the next place he believed that "part of the earliest discharge is of CO_2 already existing as such within the muscle, and which escapes with the rest by diffusion outwards." Since such diffusion can cause no appreciable heat changes, and since the same fall is observed in the heat-production as in the CO_2-output, this factor cannot, as Fletcher believed, explain the general initial form of the curve, and it is probably of no importance. Finally he suggested that "the remaining part of the discharge is due to a process in which oxygen is absorbed from the air and CO_2 produced, and which is to be taken as an imperfect continuation of part of the normal process of respiration during somatic life." And in a later paper[1] he showed that the muscle could avail itself from the earliest periods onwards of a supply of oxygen in the atmosphere, producing a much larger yield of CO_2. It is this last factor, in a modified form, which I believe to be one of the chief causes of the phenomena observed. It should be noted that these hypotheses, and others which were considered and rejected, were put forward before the days when the brilliant work of Fletcher and Hopkins on the lactic acid formation of surviving muscles had made so many factors clear.

It will be shown below that in all probability the long continued "plateaus" of CO_2- and of heat-production are due to one and the same factor, viz. the chemical breakdowns leading in the absence of oxygen to the liberation of lactic acid. We know however that these breakdowns do not occur in the presence of sufficient oxygen[2], and Dr Fletcher agrees

[1] This *Journal*, xxviii. p. 358. 1902.

[2] Fletcher and Hopkins. This *Journal*, xxxv. p. 282. 1907.

with me in believing that probably these processes are not at work, at any rate fully, until the first stage of declining CO_2- and heat-evolution is over. No large part of the CO_2- and heat-liberation of the first stage is due to the processes which are known to occur in the later stages. Thus of Fletcher's three factors, the first cannot be of serious account. Of the second we may safely say that the very high initial rate of heat-production must go, during the first half-hour or hour after death, with a high but falling rate of oxidative breakdown, the CO_2 of which is no longer eliminated by the blood stream. Hence, lagging awhile after the heat-production, is a high initial rate of CO_2-elimination due to the excessive amounts of CO_2 collecting in the tissues.

Frogs can live an hour or more in oxygen-free water, and their tissues must therefore contain some amount of dissolved or stored oxygen. After cessation of the circulation, the muscles continue for awhile their normal oxidative life-processes, using the oxygen still present in the tissues. The time relations of Fletcher's curves seem to me perfectly to allow this extension of his view: it avoids also the difficulty of explaining how the CO_2 was initially at so high a concentration immediately after death. To some extent the rate of oxidation in the tissue would be expected to depend upon the concentration of the oxygen remaining there. Thus, as time goes on, the oxygen becomes exhausted, and the rate of oxidation (and therewith of heat- and CO_2-liberation) falls along an exponential curve.

Of Fletcher's third factor, "the remaining part of the discharge being due to a process in which oxygen is absorbed from the air and CO_2 set free," the experiments in Table III afford decisive evidence: undoubtedly the presence of oxygen can increase the rate of heat-production, and therefore of CO_2-liberation, so it seems to me that the whole description of the initial high value of the heat-production, falling rapidly to a much lower constant value, can be summed up as follows. The muscle continues, for about five hours at 15·5° C., to carry on the normal oxidative processes of life but at a declining rate, using oxygen already present in the muscles or oxygen which has diffused in from outside: and the initial rate of oxidation, immediately after death, is the same as—a mere continuation of—that of life. The gradually declining nature of the evolution of heat is due both (*a*) to the gradual exhaustion of the oxygen and (*b*) to the gradual accumulation of waste-products, other than CO_2, owing to cessation of the circulation: possibly also (*c*) to exhaustion of oxidisable material.

Let us now turn to the further experimental evidence at hand. It was noticeable in the first place that the rate of heat-production in the initial stages was much more variable, from experiment to experiment, than that occurring in the later stages: even though the percentage error of observation is much greater in these later stages. This suggested that some arbitrary unknown factor was at work, such as the amount of oxygen dissolved in the water in which the frogs' muscles lay. The apparatus is not suitable for experiments on the evolution of heat by muscles hanging in oxygen gas: in fact I doubt if any such apparatus can at present be made. It is necessary to have the muscles lying in salt solution in the flasks, in order both to calculate or balance correctly the loss of heat by the flask, and also to get an exact reading of the mean temperature inside. The experiments were therefore made as follows, and in all cases except Exp. I upon whole frogs unskinned. Ringer's solution, or water, was boiled and cooled, so as to boil off the oxygen; and then one experiment was made with frogs in the oxygen-free water, and another experiment with frogs in the same water after shaking some time with oxygen. Water at 16° C. does not dissolve much oxygen, only about 3·2 c.c. %, but quite enough to show the characteristic effects given in Table III. It will be noticed that the initial evolution of heat is very largely increased by the presence of oxygen in the water. Further the experiments are the most accurate hitherto made, and one can be quite confident of the genuineness of the phenomena seen. Exp. I in the Table shows the same effect of oxygen, but was done in a different way.

TABLE III. *Effects of oxygen on the heat-production of surviving muscles.*

Exp. I. This experiment is of a different kind from the rest in the Table.

Five frogs killed, skinned, and left in oxygen 14 hours. Then their heat-production estimated immediately in the calorimeter at 16° C. Time in hours from death. H.-P. in cal. per c.c. of skinned animal per hour.

Period beginning at	14	15	16	17	18	20	22
ending at	15	16	17	18	20	22	40
Rate of H.-P. ...	·19	·10	·08	·06	·05	·04	·06

There is, for three hours, a much higher rate of H.-P. than occurs normally in muscles 14 to 17 hours after death.

Exps. II, III, IV and V. Made on the same day with the same batch of frogs. The possible error was ±·01 cal. per c.c. in every reading in each Exp.

Exps. II and III. In Ringer's solution saturated with oxygen, at 15·6° C. and 16° C. respectively.

Period beginning at ...	·3	·6	1	2	4	6	10	20	30	40
ending at ...	·6	1·0	2	4	6	10	20	30	40	45
Rate of H.-P., Exp. II	·40	·19	·10	·065	·045	·037	·035	·032	·037	·045
Rate of H.-P., Exp. III	·60	·3	·20	·08	·06	·049	·049	·048	—	—

Exps. IV and V. In O_2-free Ringer's solution at 15·9° C. and 15·8° C.

Period beginning at ...	·3	·6	1·0	2	3	5	10	20	30	40
ending at ...	·6	1·0	2·0	3	5	10	20	30	40	45
Rate of H.-P., Exp. IV	·12	·08	·05	·042	·037	·035	·038	·033	·035	·043
Rate of H.-P., Exp. V	·15	·13	·07	·053	·046	·038	·036	·035	·041	·051

Exps. VI and VII. Frogs kept (alive) in oxygen two hours before experiment began. Exp. VI in water saturated with O_2 at 16·5° C.: Exp. VII in O_2-free water at 16·5° C.

Period beginning at ...	1·0	2	3	5	10	15	20	25
ending at ...	2·0	3	5	10	15	20	25	30
Rate of H.-P., Exp. VI	·12	·10	·08	·065	·05	·043	·045	·048
Rate of H.-P., Exp. VII	·057	·044	·032	·032	·031	·031	·034	·038

Exps. VIII and IX. Exp. VIII in distilled water saturated with O_2 at 14·2° C. Exp. IX. in O_2-free distilled water.

Period beginning at ...	·5	1·0	2	4	10	20
ending at ...	1·0	2·0	4	10	20	30
Rate of H.-P., Exp. VIII	·20	·075	·03	·015	·021	·027
Rate of H.-P., Exp. IX ...	·12	·05	·02	·016	·016	·017

Here from Exp. I, we see that immediately after removal from O_2 there is a much higher rate of H.-P. than normally occurs in muscles 14 hours after death: it is only after removal from the O_2 that the second stage comes on, although in the absence of O_2 it would have been present ten hours previously. The oxygen in fact, which is known to prevent the liberation of lactic acid[1], and the onset of rigor[2], and nearly to treble the rate of CO_2-output, keeps up the respiratory oxidative activity of the surviving muscle to a high level, and even after removal from the oxygen, the rate of H.-P. only falls slowly, *i.e.* in three or four hours, to the normal rate for the second stage of survival. With regard to the rest of Table III, it will be noticed that the presence of oxygen increases very largely the heat-production during the first few hours after death: but that in the later stages there is little or no noticeable difference. By the time the second stage comes on, the oxygen is practically exhausted however, as is shown by the following calculation.

In the oxidation of carbohydrate 1 c.c. of O_2 produces 5·4 calories: 150 c.c. of water at 16° C. holds some 4·8 c.c. of O_2 after shaking with O_2: this quantity of O_2 is equivalent therefore to 26 calories. With 100 c.c. of frogs' muscle in 150 c.c. of water this yields an extra heat-production of ·26 calorie per gram of muscle: in Table III, Exps. II to V, just these quantities of material were used, and a short calculation shows that in the period ·3 hour to 5·0 hours the muscles in the oxygenated Ringer produced just about ·30 calorie per c.c. of muscle, more than the muscles in O_2-free Ringer. The equivalence between this ·30 and the ·26 is striking.

[1] Fletcher and Hopkins. *Op. cit.* p. 284.

[2] Fletcher. This *Journal*, xxviii. p. 479. 1902.

There can therefore be no appreciable proportion of the initial oxygen left after five hours: if there were, Exp. I would show that the rate of heat-production would continue greater than normal until this oxygen had vanished. This is entirely in accordance also with the experiments of Fletcher and Hopkins[1], who showed that at any stage before rigor is complete, the presence of oxygen appreciably retards the formation of lactic acid.

Oxygen therefore can be absorbed through the skin, or directly by the exposed muscles, and used in every stage to maintain the activity of the muscles and to raise the rate of CO_2-evolution, and the rate of heat-production. Even in the absence of external oxygen there is already sufficient dissolved, or combined with hæmoglobin in the tissues, to allow the muscles to keep up a higher, though gradually declining, chemical activity.

The most interesting fact, therefore, about the first stage in survival life, is that the metabolism depends on the presence of O_2 without which the muscles shortly relapse into the later stage of lactic-acid production, which ends in rigor-mortis. With sufficient oxygen the muscles would probably maintain a metabolism as high as during life, either until their stores of oxidisable material were exhausted, or until waste products which could not escape were in sufficient concentration to destroy the muscle mechanism. This shows the necessity of providing an oxygen atmosphere in physiological experiments upon surviving muscles. It is impossible otherwise to prevent the onset of the pathological conditions arising from insufficient oxygen.

We now come to the second stage of survival heat-production, the long continued "plateau" lasting until the death of the muscle. It is of great interest to compare this, not only with Fletcher's "plateau" of CO_2-liberation, but also with the linear rate of production of lactic acid, in muscles left without oxygen[2]. This triple similarity of CO_2, lactic acid and heat-liberation, in conjunction with the experiments upon the heat-production of rigor in the next section of this paper, has convinced me that these three processes really represent three sides of the same reaction. This reaction is the liberation, in the absence of oxygen, of free lactic acid from some precursor with the evolution of heat: we have *e.g.*

$$AHL \longrightarrow A + HL + Heat,$$

where A is some at present unknown chemical body, and HL is lactic acid. The lactic acid, as soon as it is formed, combines with the sodium

[1] *Op. cit.* [2] Fletcher and Hopkins. This *Journal*, xxxv. p. 272. 1907.

bicarbonate of the tissues to form sodium lactate and CO_2: so that CO_2 is liberated at a rate proportional to the production of lactic acid. According to this scheme we have,

$$NaHCO_3 + HL \longrightarrow NaL + H_2O + CO_2,$$

so that one molecule of lactic acid liberates one molecule of CO_2. Now according to Fletcher and Hopkins[1], there is a possible production of lactic acid up to about ·27 grm. % (·36 % Zn-lactate) in frogs' muscles during the months of March, April and May. The muscles in the experiments which I have carried out at 16° C. have usually been inexcitable in 36 hours after death: so that there must have been a lactic-acid production of about ·000075 gr. per gr. of muscle per hour. This is equivalent in the above reaction to an amount ·018 c.c. of CO_2 per grm. of muscle per hour. In Fletcher's original paper the weights of the frogs' legs used were not recorded. In order therefore to obtain a comparison I have assumed an average weight, viz. 12 gr. for each pair of legs. This number cannot be far wrong, and with it the following results were obtained.

TABLE IV. *After Fletcher. CO_2-liberation in c.c. per gr. of frogs' legs per hour, during the periods named.*

Exp. I. Fletcher, This *Journal*, xxiii. p. 98. 1898. At 15° C.

Period beginning at...	·5	1·1	4	7	9·2	11	27
ending at ...	1·0	1·7	5·2	7·6	10·3	23	27·6
Rate of CO_2 liberation	·043	·030	·022	·028	·027	·020	·017

Exp. 54. Fletcher, *Ibid.* p. 99. In nitrogen at 15—17° C.

Period beginning at...	·5	1·8	3·8	5·3	11·1
ending at ...	·8	2·2	4·3	5·8	11·6
Rate of CO_2-liberation	·037	·022	·018	·013	·013

It is seen that the production of CO_2 in the later stages is strikingly equivalent to the amount of lactic acid that must have been formed. Where, from above, we should expect ·018 c.c. of CO_2 per gr. per hour we find ·013 to ·020. On the other hand, if we suppose the CO_2 in the later stages to originate from oxidative processes, then supposing the oxidation to be that of carbohydrate the CO_2 formed must be calculable directly from the heat-production. From the average heat-production at 15·5° C. calculated above[2], assuming that the oxidation of 1 gr. carbohydrate gives 4000 calories and therefore that 1 calorie ≡ ·185 c.c. of CO_2, we can obtain the following table. The CO_2 is reckoned per c.c.[3] of animal per hour, at 15·5° C.

[1] *Op. cit.* p. 266. [2] p. 474. [3] At normal temp. and pressure.

Period beginning at ...	·3	·5	1·0	2	3	5	10	20	30	40
ending at ...	·5	1·0	2·0	3	5	10	20	30	40	50
Rate of CO_2-production	·057	·026	·020	·0144	·012	·0093	·0083	·0085	·0091	·0111

It is seen that the numbers obtained in the later stages are decidedly smaller than those observed by Fletcher: this is even more definitely the case, because undoubtedly the lactic-acid formation liberates a considerable quantity of heat (see the next part of this paper, on the heat-formation in rigor) and this must be subtracted from the observed heat-production if we are to reckon how much heat is due to the supposed oxidative processes. Probably we ought to reduce the quantity of heat to at least a half by this subtraction, so that the above values of the CO_2 calculated from the heat are also to be reduced by a half. This makes the numbers so small that under no conditions can they be supposed to correspond to Fletcher's observations. The CO_2 in fact, in the "plateau" stage, is not due mainly to oxidative processes, for there is too little heat to correspond to such oxidations. The equivalence observed between the lactic acid and CO_2 formations, in conjunction with many other facts, gives us very good grounds for believing that the chief part of the CO_2 comes from the reaction between lactic acid and the sodium bicarbonate of the tissues. On the other hand, during the first hour or two, there is a very close coincidence between Fletcher's CO_2 observations and the values calculated from the heat, on the supposition that both heat and CO_2 are due to the oxidation of carbohydrate. This further strengthens the view that the first stage is characterised by still surviving oxidative processes.

In further confirmation of these conclusions is the fact that Fletcher found[1] that in the first stage of the CO_2 output about a fifth part of it can be abolished by the removal of oxygen from the atmosphere, and this "is probably due to a respiration of the muscle substance continuing that of normal life, but disappearing gradually as the changes occur which accompany loss of irritability and inaugurate rigor." Even without a contemporary supply of oxygen from outside the earlier processes in survival life are oxidations: the later are, as Fletcher suggested, the same as lead finally to the phenomena of rigor. Further evidence for this view is given in the next part of this paper. In any case one thing is certain: the known liberation of lactic acid in survival processes not only may but must drive off CO_2 from its combination with sodium: and the amount of CO_2 so driven off must in any case be a large fraction of the total CO_2-evolution. In

[1] This *Journal*, XXVIII. p. 355. 1902.

this connection a further point arises: may not the heat given out in the reaction,

$$NaHCO_3 + HL \longrightarrow NaL + H_2O + CO_2 \text{ (dissolved)}^1$$

i.e. the heat of neutralisation of lactic acid with sodium bicarbonate, be sufficient to account for all the heat-production of surviving muscles? This question is discussed in the next section of this paper. It will be seen that, although such a heat of neutralisation must exist, it is insufficient to account for more than about ·002 calorie per grm. of tissue per hour[2]. This is only about 4 % of the quantity observed, so it may be entirely neglected. We are therefore reduced to the assumption that the heat-production is due to the reaction which liberates lactic acid: by this method we can calculate the total energy of the lactic acid precursor. This calculation can be dealt with better after a discussion of the experiments on the heat-production of rigor.

Finally we must treat shortly of the question as to whether autolytic or other unknown breakdown processes occurring in the muscle can be responsible for the evolution of heat and CO_2. In the first place autolysis does not occur to any degree at such a low temperature as 15·5° C.: and secondly in several experiments in which muscles were left for three or four days in chloroform water,—after undergoing chloroform-rigor—there was never observed any positive or negative heat change. Chloroform water does not inhibit autolytic phenomena; so we may conclude that there is no appreciable heat-production due to autolysis. With regard to other possible reactions, we know as yet of none such, and until they are discovered we may argue on the assumption that the lactic acid formation is the chief characteristic of survival life.

PART II. THE HEAT-PRODUCTION OF RIGOR.

(i) *Heat-rigor.*

An investigation of the heat produced during the onset of rigor was made many years ago, both by Fick and Dybkowsky[3] and by Schiffer[4]. In view of the experiences of later observers in this type

[1] In the calorimeters the CO_2 will not come out of solution appreciably: if it does heat is absorbed.

[2] The heat of neutralisation of 1 gr. of lactic acid with $NaHCO_3$ is about 27 calories: the production of ·000075 gr. of acid liberates therefore about ·002 cal.

[3] *Vierteljahresschr. d. naturf. Gesellsch. in Zürich.* 1867.

[4] *Du Bois-Reymond's Archiv*, p. 442. 1868.

of work the methods are of very doubtful validity: certainly however they established the bare fact that there is an evolution of heat during the onset of rigor. Fick and Dybkowsky first plunged a thermometer into a collection of muscles, which they then warmed very slowly on a water-bath. As the temperature rose the muscles became stiff, and after awhile were at a higher temperature than the bath. This proved that there is a liberation of heat; but the absence of any heat-insulating apparatus made them lose about 97 % of the heat produced, so that their estimations are many times too small. Later they used a thermopile, one set of junctions of which was covered with a fresh muscle, the other with one already stiff. This they warmed in an incubator, and during the moments when the muscle began to go stiff always found a positive deflection of the galvanometer. According to their observations, the heat-production occurred only during the actual contraction and stiffening at the onset of rigor: this is not in accordance with my experiments, which have shown that the *gradual* production of lactic acid leading finally to rigor, is responsible for the evolution of heat, and I believe the explanation of the difference to be that it was impossible by their method to warm the muscles uniformly throughout. The movements of the muscle during shortening brought the thermopile junctions into closer contact with warmer parts of the muscle, and hence the only observation made was made during the shortening. The numbers they obtained by the first method for the rise of temperature were: (*a*) frogs' muscles: not greater than ·03° C., (*b*) rabbits' muscles: ·23° C., results which are very much too small.

Schiffer, working more carefully on much the same lines, found for the stiffening of fish-muscles a rise of temperature of 1° C., which he compared with the rise of temperature of 0·5° C. in clotting blood. With the fish he found that the heat was produced before the stiffening began: with the mammal that there was a continuous evolution of heat during the whole period until stiffness came on. In opposition to Fick and Dybkowsky he concluded that the heat-production is not due to the change in the condition of aggregation of the proteins, accompanying rigor, but to the chemical processes preceding it. This conclusion, as a matter of fact, is exactly the same as the one to which we are led by the experimental evidence given below: we know more definitely now what those chemical processes are.

Burridge and Scott[1] have pointed out that in heat-rigor at 37° C. two stages of contraction occur, one half of the total shortening occurring rather rapidly at first, and

[1] *Proc. Physiol. Soc.* Feb. 17, 1912. This *Journal*, XLIV. p. iii. 1912.

the other half more slowly and beginning only after the first stage is almost complete. At 42° C. these stages are fused together and only one contraction is seen. This I have been able to confirm. At lower temperatures such as 36° C. the onset of heat-rigor is very slow and regular: the shortening may not be complete for many minutes. On the other hand at higher temperatures such as 42° C. and over, the shortening ensues and the rigor is complete almost immediately on raising the temperature of the muscle. Thus at 36° C., the shortening does not occur suddenly after several minutes exposure to the temperature, but continues from the very beginning. The same seems to be true of the evolution of heat, both in heat- and chloroform-rigor. The gradual shortening is presumably due to the action of the chemical substances (lactic acid especially) which, at lower temperatures, are liberated gradually in the muscle: and the heat-production arises from the reaction liberating these bodies.

The method I have adopted for estimating the heat produced in heat-rigor is again that of the micro-calorimeter. Hot water is poured into one flask with its temperature so adjusted that when a measured quantity of cold muscles is thrown into it, the final temperature shall be approximately the same as that of the other hot water in the ordinary control-flask.

The muscles are prepared and their temperature taken, water at about 36° C. is poured into the control flask, hot water adjusted to the calculated temperature is poured into the experimental flask, and then when all is ready the muscles are thrown suddenly into the hot water: the experimental flask is then stirred vigorously for about two minutes, until the whole of its contents are at one uniform temperature throughout. Readings of the rise of temperature are then made with the thermocouple and galvanometer as before. The temperature is usually adjusted to be the very lowest possible temperature which will cause the complete development of rigor within about one hour; for thereby the processes leading to rigor occur slowly, and no great error is caused by the impossibility of making readings in the first three or four minutes. It is in fact absolutely impossible to obtain reliable readings within the first few minutes: the muscles, however, well stirred, require some little time in order to settle down uniformly to the same temperature as the water and the walls of the flask. As mixing goes on, the readings decrease rapidly at first, owing to cooling of the hot water by the cold muscles: when however there is a uniform temperature throughout the flask the readings increase slowly, because of the gradual liberation of heat by the muscles. I have generally aimed at securing a temperature of about 35·5° C. in the experimental flask, in which case rigor is usually complete in from 40 to 50 minutes. If the temperature is a degree or so higher there is a considerable error due to the unknown quantity of heat liberated in the first few minutes;

if the temperature is from ·5 to 1° C. lower the rigor comes on so slowly that the method is rendered invalid for the following reason:

It is impossible to balance exactly the constants k of heat-loss of the two flasks: the error caused by the slight inequality is proportional to the difference of temperature between the inside and the outside of the flasks. For long periods therefore one tries to work at or near room-temperature: with a difference of temperature as great as 20° C. the errors become very large if we extend the observations beyond an hour.

At the end, in all the following experiments, the muscles have been perfectly stiff, exhibiting all the phenomena of rigor.

I have found by experience, and possibly the preceding description will show, that these experiments are exceedingly difficult to make: they require great quickness and care in manipulation, and continual arithmetical calculations during their course, in order to secure that the final temperature of the mixture should be about 35·5° C. when cold muscles at one temperature plus hot water at another are poured into a flask at still another. Further the quantities to be observed are exceedingly small if one requires any high degree of accuracy. It would be tedious to use the method for an extended series of experiments: for the further development of the subject therefore I adopted the use of chloroform-rigor at ordinary room temperature. In Table V however are given a few of the experiments done in connection with heat-rigor, showing as they do fairly accurately the actual value of the heat-production, and proving quite definitely that the heat-production precedes the actual final onset of rigidity. The effects of fatigue and injury on the production of heat also are described.

TABLE V. *The heat-production of heat-rigor.*

EXP. I. Ten small summer frogs = 92 c.c. pithed and used whole for the experiment: the frogs were tipped into 158 c.c. of hot water, so that in five minutes the temperature was 35·1° C. 100 s.d. = ·149° C. Time in minutes, readings in scale divisions. The results are plotted in Fig. 3.

Time	5	6	8	10	12	14	16	19
Reading	− 258	− 251	− 242	− 228	− 216	− 207	− 196	− 184
Time	22	25	31	35	39	57	67	76
Reading	− 167	− 152	− 122	− 111	− 104	− 100	− 98	− 97

The heat-production in heat-rigor of whole unskinned frogs is therefore ·69 cal. per c.c.

EXPS. II to V. Fresh summer frogs, pithed, whole, unskinned, put into rigor at 34·8° C., 35° C., 36° C., 36·1° C., respectively. Heat-production per c.c. of frog: 0·59 cal., 0·74 cal., 0·62 cal., and 0·5 cal. respectively.

EXPS. VI and VII. (VI) Fresh autumn frogs, pithed, whole: (VII) Fresh autumn frogs, legs only. Put into rigor at 35·5° C. Heat-production per c.c. of tissue: 1·1 cal. and 1·0 cal. respectively.

Exps. VIII to X. Autumn frogs, damaged by mechanical injury and left some hours after death. Heat-production of rigor per c.c. of tissue: 0·58 cal., 0·65 cal., and 0·6 cal. respectively.

Exps. XI to XIV. Whole pithed frogs, fatigued by severe electrical stimulation for an hour or more. Heat-production of rigor per c.c. of tissue: ·44 cal. (only 20 min. stimulation), ·3 cal. (legs only), ·18 cal., ·09 cal. respectively.

Experiments similar to those of Table V but much more trustworthy in nature, have been done by the use of chloroform-rigor instead of heat-rigor. These are given in the next section of this paper, so that

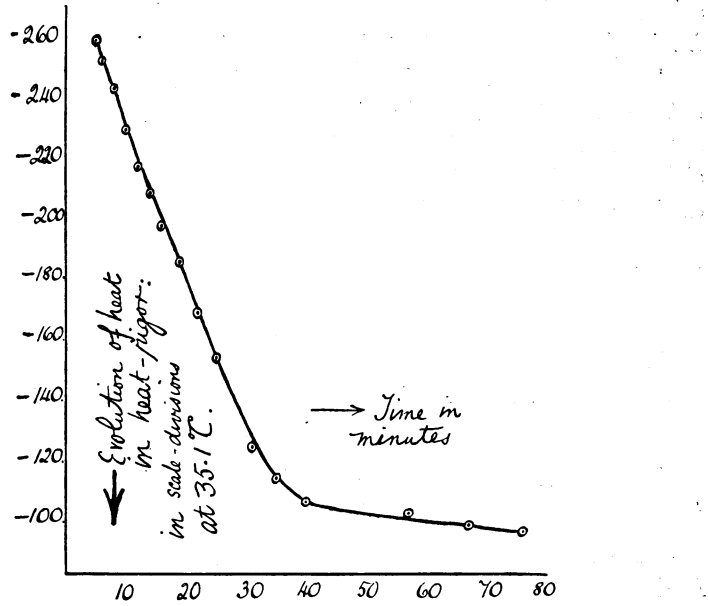

Fig. 3. Evolution of heat in heat-rigor at 35·1° C.: readings (negative) in scale-divisions: Exp. I, Table V. Note that the heat is given off gradually, and that the rate of heat-production, and therefore presumably of lactic acid liberation, is linear.

the full discussion is reserved till then. From these experiments however we see that:

(*a*) The heat-production of heat-rigor is not an accompaniment of the final stage, the coagulation and stiffening, but starts (see Fig. 3) from the moment when the temperature of the muscles is raised. It is due to the chemical processes preceding and causing the shortening, as Schiffer believed. These are presumably the same as are seen in the second stage of survival life without oxygen, viz. the liberation of lactic acid from some precursor. This process is, as we should expect, quickened many times by the rise of 20° C.

(*b*) In summer frogs the value of the heat-production in rigor per c.c. of frog unskinned, is about 0·7 cal.: for autumn (October) frogs it is about 1·1 cal.

(*c*) Mechanical injury and survival after death cause a lessening of these values. Cf. *e.g.* Exps. VIII to X with Exps. VI and VII.

(*d*) Fatigue due to previous stimulation causes a lowering of the value of the heat-production in rigor, inasmuch as tetanus causes a production of lactic acid in the muscle. If the heat is set free by the production of lactic acid from some precursor, then the more the lactic acid previously produced the less should be the heat set free in rigor, *i.e.* in the breaking off of the rest of the lactic acid.

The very low values however recorded in Exps. XI to XIV should not be altogether trusted: rigor is more quickly set up at 35·5° C. in a muscle already fatigued, and probably in these experiments appreciable quantities of heat have been set free in the first five minutes during which they could not be recorded.

All these facts are entirely in accordance with the belief that the heat is liberated by the production of lactic acid from its precursors. We have mentioned before the possibility that some, if not all, of the heat might be due to the neutralisation of the lactic acid by the sodium bicarbonate of the tissues. Fletcher and Hopkins[1] have shown that under all conditions rigor is accompanied by a liberation of lactic acid: there must therefore be some heat due to the combination of the lactic acid with the soda. How large is this production of heat? According to the *Chemiker Kalendar*,

$$Na_2CO_3 + 2HL \longrightarrow 2NaL + H_2O + CO_2 \text{ (dissolved)} + 6500 \text{ calories.}$$

And

$$2NaHCO_3 + 2HL \longrightarrow 2NaL + 2H_2O + 2CO_2 \text{ (dissolved)} + 4800 \text{ calories.}$$

From these one may deduce that the combination of 1 gr. of lactic acid with Na_2CO_3 liberates 36·1 calories, and with $NaHCO_3$ 26·7 calories. As however the conditions of concentration and the like were not stated, and as I could find no references to the original determinations, I repeated the experiments under conditions bearing directly upon the problem in hand.

The percentage of lactic acid in the rigid muscle is from ·27 to ·4 %. Small accurately weighed quantities of pure dry Na_2CO_3 were added to lactic acid of about this strength in a Dewar flask: the rise of temperature on stirring was noted with a Beckmann thermometer. The heat of solution of the Na_2CO_3 was estimated similarly in the same flask by adding Na_2CO_3 to water instead of to lactic acid solution. The difference gave the heat

[1] *Op. cit.* pp. 265, 266.

of combination of lactic acid and sodium carbonate at concentrations which are the same as those existing in the tissues. The mean of five exps., all within about 10 % of one another, was 37·8 calories for the neutralisation of 1 gram of lactic acid: this is quite sufficiently close to the value 36·1 derived above. Thus the complete saturation of ·3 % lactic acid by sodium carbonate would lead to a rise of temperature of not more than about ·1° C.

Seeing that the alkali is almost certainly $NaHCO_3$ and not Na_2CO_3, because of the excess of free CO_2 in the tissue, the rise of temperature which can be due to the neutralisation of the lactic acid in a muscle cannot be much greater than about ·07° C. There is, moreover, insufficient $NaHCO_3$ to saturate all the lactic acid formed up to ·3 %: the possible rise of temperature must, therefore, be still further reduced, say to ·05° C. Thus of the ·6 to 1·0 cal. per gram produced in heat-rigor, not more than ·05 cal. is due to the neutralisation of lactic acid with carbonate. The simultaneous liberation of CO_2 from the $NaHCO_3$ is however quite sufficient to explain Fletcher's results on the evolution of CO_2 following heat-rigor. He found[1] that heat-rigor is followed by "a large outburst of CO_2 amounting to about 30 % by volume (of muscle substance)": the lactic acid (·3 %) formed, if it were completely neutralised, could account for about 74 % by volume, and must account for a large fraction of this. Thus the reaction liberating lactic acid is accompanied by an evolution of heat, and the CO_2-production of rigor is due to the combination of the acid with carbonates.

(ii) *The heat-production of chloroform-rigor.*

The fact that the rigor induced by chloroform in frogs' muscles is accompanied by a development of the same quantity of lactic acid as is heat-rigor was shown by Fletcher and Hopkins[2]: and that the yield of CO_2 from an excised frog's muscle is largely increased by the same agent was shown by Fletcher[3]. This makes it very probable that the processes culminating in rigor (1) under the action of a high temperature and (2) under the action of chloroform are the same. This view is supported strongly, as is shown below, by the identity of the values of the heat-production in the two cases, and also by the fact that anærobic survival and fatigue reduce the liberation of heat in both.

Since the experiments upon chloroform-rigor are relatively easy, and with care the results of considerable accuracy, the heat-production of

[1] This *Journal*, XXIII. p. 93. 1898.
[2] *Ibid.* XXXV. p. 266. 1907.
[3] *Ibid.* XXIII. p. 50. 1898.

chloroform-rigor is a very valuable tool in pushing further back our knowledge of the chemical processes going on in a muscle. The method adopted is as follows. Using the micro-calorimeter, the muscles (whether skinned or unskinned[1]) are placed in one flask in Ringer, and water at slightly higher temperature (say 0·2° C.) in the control flask. When all has been well shaken up, so that muscles, Ringer and the inside of the flask are all at identically the same temperature, the thermocouple is introduced and some 3 or 4 c.c. of chloroform is let in from a pipette. The muscles are then stirred vigorously for 20 seconds, the flask is stoppered with a cotton-wool plug, and the first reading is taken. Shortly the readings begin to increase, owing to the action of the chloroform, and observations are made at intervals for one-half to three-quarters of an hour until finally a constant reading is again obtained for several minutes. Rigor is then complete. The time required for this process depends upon the temperature, upon the presence or absence of the skin, and upon the size of the frogs: at 15° C. for ordinary frogs' legs, skinned, it is about 30 to 40 minutes. The time between the introduction of the chloroform and the taking of the first reading is so short—in contrast with the corresponding stage in the heat-rigor experiments—that no appreciable change can have taken place in it. There is usually no appreciable change until 1·5 minutes after taking the first reading. If care is taken that the control flask is initially about 0·2° C. above the temperature of the muscles, the final reading is about zero and is rapidly reached, so that no correction need be applied for the heat lost by conduction during the experiment. For this purpose as for many others, the differential micro-calorimeter is far simpler to use than one might expect from reading the general description: with a little ingenuity it is often possible, in short experiments, to avoid any allowance for heat-loss.

In Table VI are given experiments dealing with the course of the evolution of heat under the action of chloroform.

TABLE VI. *The heat-production of chloroform rigor.*

N.B.—In these experiments it is advisable to cut the legs or the whole frogs into portions at the joints, in order that when rigor comes on the limbs, which will then be stiff and straight, shall not be sticking out of the Ringer's solution. If this latter is allowed the readings obtained are variable and inexact, because the heat given out by the limbs no longer under the water is not communicated quickly to the water. Of course

[1] Skinning increases somewhat the velocity with which chloroform induces rigor: the skin compared with the muscles is relatively impermeable to chloroform. The increased velocity due to skinning is an advantage, both for accuracy and the saving of time.

the cutting is done only just before introducing the chloroform, so that the results are not vitiated by the possibility of much heat being set free by the injury.

Exp. I. Autumn frogs, 41 c.c., quite fresh, skinned. Chloroform-rigor at 17·6° C. in 209 c.c. Ringer. 100 s.d. = ·328° C. = 2·16 calories per c.c. of frog.

Time: mins.	1	2	3	4	5	6	7·5	9	11	14·5	19	22·5	27	32·5	41	47
Reading: s.d.	0	0	2	6	9	13	15	18	22	26	31	33	36	39	38·5	38·5

The results are plotted in Fig. 4. The heat-production amounted to ·83 cal. per c.c. of frog.

Exp. II. Autumn frogs, fatigued, 65 c.c. skinned. In chloroform-rigor at 17·6° C. 100 s.d. = 1·36 cal. per c.c. of frog. Results shown in Fig. 4.

Time: mins.	1·3	2·0	2·5	3·5	5	7	9	12	21	28	41	54	76
Reading: s.d.	−26	−22	−18	−15	−11	−7	−3	0	+7	13	15	17	17

Total H.-P. = 0·58 cal. per c.c. of frog.

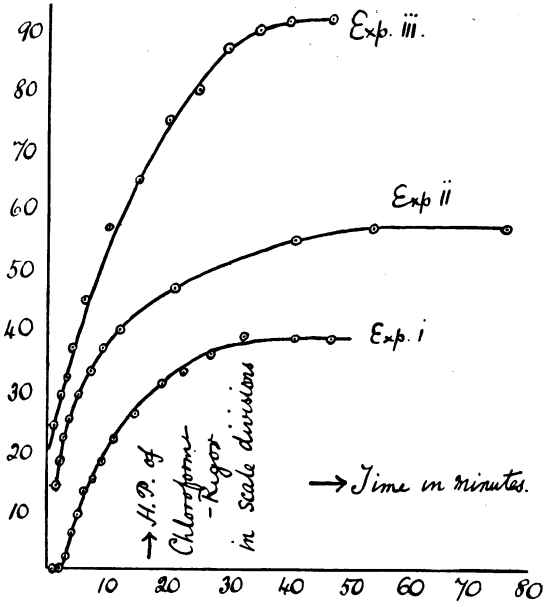

Fig. 4. Evolution of heat in chloroform-rigor: readings in scale-divisions: Exps. I, II and III, Table VI.

Exp. III. Winter frogs, 60 c.c. quite fresh, skinned. Chloroform-rigor at 17° C. 100 s.d. = 1·19 cal. per c.c. of frog. Results in Fig. 4.

Time: mins.	1	2	3	4	6	10	15	20	25	30	35	40	47
Reading: s.d.	−36	−31	−28	−23	−15	−3	+5	15	20	27	30	31·5	32

Total H.-P. = 0·81 cal. per c.c.

Exp. IV. Winter frogs, severely stimulated 1·2 hours, chloroform rigor at 17° C.

Time: mins.	1	2	3	11	16	18	22	27	31	33	36	38
Reading: s.d.	−9	−8	−6	+13	+16	18	20	23	26	27	27	27

Total H.-P. = 0·41 cal. per c.c.

Exp. V. Winter frogs, severely stimulated, skinned and left in oxygen to recover. Chloroform-rigor at 17·2° C.

Time: mins.	1	2	3	4	11	27	38	48
Reading: s.d.	−64	−63	−59	−54	−27	−5	−1	0

Total H.-P. = 0·77 cal. per c.c.

Exp. VI. Winter frogs, fresh, skinned. Chloroform-rigor at 21° C. Results in Fig. 5.

Time: mins.	1	2	3	4·5	6	8	10·5	12·5	19	21·5	27·5	30	35
Reading: s.d.	−91	−88	−81	−71	−64	−55	−50	−45	−36	−33	−27	−27	−26

Total H.-P. = 0·77 cal. per c.c.

Exp. VII. Winter frogs, fresh, skinned. Chloroform-rigor at 10·5° C. Readings in s.d. corrected for heat loss. (The experiment was so prolonged that a small correction was necessary.) Results in Fig. 5.

Time: mins.	1·0	5	10	15	20	30	40	50	60	80	100
Reading: s.d.	−10	+1	+14	24	32	41	49	55	59	63·5	64·5

Total H.-P. = 0·75 cal. per c.c.

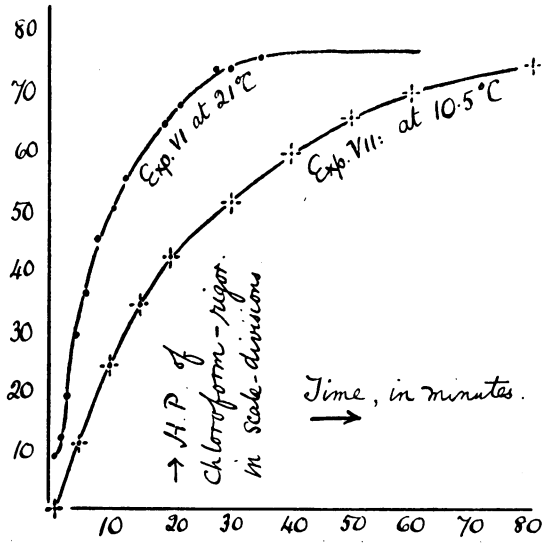

Fig. 5. Evolution of heat in chloroform-rigor: to show effects of temperature. Exp. VI at 21° C. rises much more rapidly than Exp. VII at 10·5° C.

In the above Table are given the time-relations of the production of heat by chloroform acting upon muscle. The absolute values will be discussed later. Curves of the evolution of heat are given in Figs. 4 and 5. There are two possibilities with regard to the prolonged time taken by chloroform to act upon muscle: these are: (*a*) the chloroform takes some time to penetrate into the muscle: or (*b*) the time is taken by the chemical reactions set up by the chloroform in the muscle substance.

In either case we should expect a curve of the type shown in the figures, a type which is generally referred to as "exponential." To compare the velocity of the reaction it is best with these curves to calculate the time required to reach some fixed fraction of their total rise: two-thirds of their total rise is about the best fraction to take. The times taken in Exps. I to VII for the evolutions of heat to reach two-thirds of their full values are, respectively, 14·5, 12·5, 16, 15, 16, 12, and 29 minutes. Thus the velocity of the action of chloroform is appreciably the same, except in Exp. VII where the temperature was some 7° C. lower than in the other experiments. This constancy is observed in the three separate cases of frog's muscles (*a*) fresh, (*b*) fatigued, and (*c*) fatigued and restored with oxygen. With variation of temperature, however, the velocity of the reactions causing the heat-production is very largely affected. It is increased about 2·5 times for a rise of 10° C. The chloroform must take some time to diffuse into the muscle: if the rate of heat-production is governed by the rate at which the chloroform diffuses in, then the high temperature coefficient would suggest that the diffusion depends rather on the power of chloroform to combine with living tissues (and hence to penetrate them) than upon purely physical factors.

That the heat-production is not itself a purely physical or chemical consequence of the absorption of chloroform by, or the combination of chloroform with, constituents of the muscle, is shown by the fact that the heat is evolved *only* when chloroform is brought in contact with *living* muscles. With dead muscles absolutely no evolution of heat is observed.

We shall proceed next to a discussion of the actual quantitative results obtained. Experiments have been made upon frogs' muscles from freshly killed frogs, both in autumn, winter, spring and early summer. Additional experiments were made upon the heat produced by the action of chloroform upon (*a*) worms and (*b*) frogs' guts. The results are given in Table VII. In this and subsequent tables some experiments were made upon legs only, others upon whole frogs: some upon skinned and others upon unskinned animals. The numbers obtained per c.c. of unskinned animal are afterwards "reduced" to values per c.c. of skinned animal, by multiplying by 1·2[1].

Taking an average of all the experiments in Table VII, it is seen that in freshly killed frogs the development of chloroform-rigor leads to

[1] Obtained by measuring the volume of frogs before and after skinning. It is assumed that no heat is given out in the action of chloroform on the skin.

an evolution of heat of about 0·85 cal. per c.c. of frog. This is practically the same as the average of all the values recorded in Table V for the heat-production of heat-rigor, which, reckoned per c.c. of whole skinned animal, is about 0·89 cal. The coincidence of these values, together with the fact that both may be reduced by fatigue and survival after death (cf. Table V with Tables IX and X), suggests very strongly that we are dealing with exactly the same reactions in either case. The chemical processes set up in the muscle by a temperature of 36° C., which must be largely the same processes as occur more slowly at 20° C., are the same as the processes induced in a muscle by chloroform. All these end in the production of the same quantity of lactic acid and heat, and in the same condition of rigor.

TABLE VII. *Absolute values of the heat-production of fresh frogs' muscles undergoing chloroform-rigor.*

(i) Autumn frogs (Nov.) freshly killed. H.-P. per c.c. skinned:
  (*a*) Whole animals: 0·83 cal. (17·6° C.).
  (*b*) Legs only: 1·1 cal.

(ii) Winter frogs (Feb.) freshly killed. H.-P. per c.c. skinned:
  (*a*) Whole animals: ·81 cal. (17° C.), ·77 cal. (21° C.), ·75 cal. (10·5° C.).
  (*b*) Legs only: 1·0 cal. (11° C.), ·75 cal. (12° C.).

(iii) Spring frogs (May) freshly killed. H.-P. per c.c. skinned:
  Whole animals: 1·0 cal. (18° C.), ·6 cal. (15° C.).

(iv) Frogs' guts, three hours after death. H.-P. per c.c. 0·42 cal.: 0·39 cal.

(v) Worms. H.-P. per c.c. = 0·46 cal.

It is noticeable that the results given in Table VII are somewhat variable: this may be caused in small part by experimental error, but in the main the variations are too large to be due to this cause. They must be due to the variable conditions of the frogs used. Fletcher and Hopkins[1] showed that at different times of the year, and with different batches of frogs (in different states of health and nutrition), the quantities of lactic acid obtained by rigor might also be very different. If the evolution of heat is due to the reaction which liberates lactic acid, then if the lactic acid maximum is variable for different batches of frogs the total evolution of heat also should be variable.

It is of interest to record also that chloroform produces a noticeable, though smaller, evolution of heat in the reactions it induces in worms

[1] *Op. cit.* p. 266.

or in frogs' guts: presumably therefore it acts upon unstriped muscles in the same way as upon striped.

There is one more point to be noted, in connection with Tables VI and VII: the total heat-production in rigor seems not to depend upon the temperature—at any rate between 10° C. and 21° C.—of the muscles during the onset of rigor. The absolute maximum of heat-production is independent of the temperature.

Let us turn next to the effects of leaving muscles for long periods after death, and to the effects of fatigue, in lowering the amount of heat evolved during chloroform rigor. These effects are exactly analogous to the production of lactic acid observed by Fletcher and Hopkins to take place in muscles under the same conditions.

TABLE VIII. *Effects of long periods of survival in reducing the heat-production of chloroform-rigor.*

The following experiments were made upon frogs' legs, or legs and arms only, at various times between Oct. and Feb. Heat-production of chloroform-rigor reckoned in calories per c.c. of tissue, skinned. Survival periods in hours. During these periods of survival the legs, or the intact pithed frogs, were left unskinned in air or in Ringer's solution (not O_2-free) at the temperature named. Of about 30 exps. made 22 are given below.

Number of exp.	1	2	3	4	5	6	7	8	9	10	11
Period of survival	0	0	0	2·5	3	4	4·5	6	6	6	11
Temp. of survival	—	—	—	11	16·5	16	16	16	15	10·5	15
H.-P. of rigor	1·0	·75	1·1	1·0	·95	·70	·90	·92	1·1	·82	·48

Number of exp.	12	13	14	15	16	17	18	19	20	21	22
Period of survival	11	12	16	22	23	24	24	25	31	46	53
Temp. of survival	15	12	12·5	10·5	16	15	15	12	15	11	15
H.-P. of rigor	·70	·70	·67	·62	·72	·49	·40	·70	·20	·52	0

From Table VIII we see that, in muscles left in air or in Ringer's solution, the heat-production of chloroform-rigor remains appreciably constant up to about the 6th hour: after that it falls slowly and continuously for about two days, until it reaches zero, when the muscles have become completely inexcitable. The very variable results obtained are in part more apparent than real. The experiments were at various temperatures, and only experiments at the same temperature are directly comparable. The rate of lactic acid production is much less at lower temperatures. Further, owing to scarcity of frogs, after the hot summer of 1911, it was impossible to make very many experiments on the same batch of frogs: for each of these 22 experiments required about 10 frogs. Hence the experiments were made at various times in

the autumn and winter upon many different lots of frogs. This in part explains the variable results obtained: the animals used were in various conditions of health and nutrition. Further, I was at the time unaware of the great effect on survival life exercised by atmospheric oxygen. Apparently even traces[1] of oxygen can be absorbed by surviving muscles, and used by them to hinder the liberation of lactic acid and the onset of the processes leading to rigor. In nearly all these experiments a certain variable amount of oxygen could come in contact with the muscles during the survival periods. This accounts in part for the variable results obtained: it also explains why the heat-production of rigor falls so slowly with time. The constancy of the H.-P. of rigor during the first six hours is entirely in accordance with the results described in the first part of this paper. The large, but gradually falling survival heat-production during this period was credited to the oxidative processes which continue until all the oxygen is exhausted. So long as there is still oxygen present there is little or no formation of lactic acid, so that the amount of lactic acid (and therefore of heat) actually liberated by the action of chloroform, at any moment within the period, remains constant. After this however the H.-P. of rigor begins to fall. According to the hypothesis put forward in this paper, the heat-production in the later stages of survival life is due to the slow development of the same processes as occur more rapidly in the onset of chloroform- or heat-rigor. If this is so, then the decrease of the H.-P. of rigor during any period should be equal to the total heat liberated by similar surviving muscles during the same period. If, for a given batch of muscles, H_t be the heat-production of rigor at time t then the total liberation of heat by similar surviving muscles during any time $(t_1 - t_2)$ should be $H_{t1} - H_{t2}$. Now, as a matter of fact, this is not so far from being the case even in these experiments. We know that for muscles at 15° C. after about the 6th hour there is a steady rate of survival heat-production of about ·045 cal. per c.c. per hour. Supposing that the H.-P. of rigor at the end of the 6th hour is about 1·0 cal., and that this diminishes at the rate of about ·045 cal. per hour, we should obtain the following calculated results. The observed results given for comparison are those only which are directly comparable, viz. those in which survival life went on at or near 15° C.

Period of survival ...	6	11	23	24	31	53
Calculated H.-P. of rigor	1	·78	·23	·20	0	0
Observed H.-P. of rigor	·94*	·59	·72	·44	·2	0

* Mean of three observations.

[1] Cf. Fletcher and Hopkins. *Op. cit.* p. 272 (top).

The H.-P. of rigor diminishes therefore at a rate which is of the same order of quantities as the already determined rate of survival heat-production. Seeing the many causes of variation which have beset the experiments in Table VIII these results are all we can expect. The fact that in nearly all the experiments oxygen was not cut off from the muscles, would cause the processes leading to rigor not to develop quite so rapidly as under quite anærobic conditions. Further the value assumed above for the rate of survival H.-P., viz. ·045 cal. per c.c. per hour, is possibly slightly too large owing to the inclusion in it of the heat due to a certain degree of still continuing oxidation. It was seen to be desirable however, when the point at issue had been made clearer by other experiments, to compare the rate at which the H.-P. of rigor diminished under absolutely anærobic conditions, with the rate of survival heat-production. The following experiments were therefore performed.

TABLE IX. *Decrease of H.-P. of rigor under completely anærobic conditions.*

The following Exps. were made on the same day on the same lot of frogs. Relatively large quantities of frogs were used, in order to ensure accuracy.

Exp. I. Spring frogs, fresh, skinned. H.-P. of rigor (estimated at 18° C.) = 1·03 cal. per c.c. of frog.

Exp. II. Same frogs, kept in a closed vessel 14 hours after death, in O_2-free water at 16° C. H.-P. of rigor (estimated at 18° C.) = ·50 cal. per c.c. of frog.

Exp. III. Same frogs, kept in closed vessel 23 hours after death, in O_2-free water at 16° C. H.-P. of rigor (estimated at 18° C.) = ·00 cal.

Thus the rate at which the H.-P. of rigor (assumed to be the same at 6 hours as at 0 hours survival) diminishes with time between the 6th and 14th hours is ·53/8 = ·066 cal. per c.c. per hour: and between the 14th and the 23rd hours it is not less than ·50/9 = ·056 cal. per c.c. per hour. These two quantities are strikingly the same as the rate of survival H.-P. at 16° C., which is about ·053 cal. per c.c. per hour. Thus we may say there are good grounds for believing that, if all action of oxygen is eliminated, the rate of survival heat-production is the same as the rate at which the H.-P. of rigor diminishes with time. The heat-production of surviving muscles in the second stage of survival is due to the same processes as lead, under the action of chloroform or heat, to the development of rigor.

We shall discuss next the effects of fatigue. Shortly after death the frogs or the frogs' legs used were stimulated by strong induction shocks

for periods varying from ·4 to 1·7 hours. The current passed through a "chain" of legs, or of whole frogs, connected together in series by pieces of copper wire. After skinning the muscles were used for the chloroform-rigor experiments. Either whole frogs (marked in Table X with an asterisk) or legs alone were used. Generally a few short pauses were given during the stimulation.

TABLE X. *The effects of fatigue in lessening the development of heat in chloroform-rigor.*

The Exps. were made at various times from October to March, upon freshly killed animals. H.-P. in calories per c.c. of animal, or tissue.

Number of exp.	1	2	3	4	5*	6	7	8	9	10*	11*	12*	13*
Duration of tetanus	·4	·5	·5	·7	·7	·9	1·0	1·0	1·0	1·0	1·3	1·5	1·7
Total H.-P. of rigor	·72	·68	·36	·4	·70	·87	·53	·4	·5	·32	·41	·35	·33

The variability of the results shown after shorter stimulation is due partly to the variability of the frogs used, and partly to differences in the strength of the stimulating current.

From Table X we see that severe and continued stimulation leads to a large fall in the total heat-production of the processes leading to rigor under the action of chloroform. Fletcher and Hopkins[1] showed that fatigue is always accompanied by a liberation of lactic acid. In the fatigued muscle therefore there is less lactic acid to be set free by the action of the chloroform, and consequently less heat. This further substantiates the view that the production of heat is due to the process liberating lactic acid. There is a further fact to be noticed. If surviving muscles are left long enough before being treated with chloroform, no heat may be set free—the full amount of lactic acid has been produced. On the other hand, so far as I have seen, no amount of excitation will ever reduce the H.-P. of chloroform-rigor to less than about one-half of its full value. In the last two experiments performed, one hour stimulation reduced the H.-P. of rigor from ·77 cal. to ·32 cal. while further stimulation for ·7 hour (of other members of the same batch of muscles) had absolutely no effect—the value was ·33 cal. after the extra stimulation. In Exps. 8 to 13 in the Table the results given are uniformly about 0·4 cal. per c.c. This is in striking agreement with the observation of Fletcher and Hopkins[2], that whether stimulation is (within limits) short or long, by the nerves or direct, the production of acid by fatigue can never be made to exceed certain limits, about 50 % of the maximum. Out of a possible production[3] of ·32 to ·54 % in rigor "the highest yield (in

[1] *Op. cit.* p. 279 &c. [2] *Op. cit.* p. 280. [3] Reckoned as Zn-lactate.

fatigue) was ·28 %, the lowest ·147 %, and 13 of the determinations lay between the limits ·18 to ·25 %."

The hypothesis that the lactic acid liberation is what accounts for the evolution of heat is thus confirmed, not only qualitatively but quantitatively.

The last, and perhaps the most striking evidence is afforded by observations on the effects of oxygen. It was known (*a*) that oxygen prevented the liberation of lactic acid during long survival periods[1], and (*b*) that oxygen removed, or reinstated in its previous position, the lactic acid which had been liberated by fatigue[2]. Exactly analogous phenomena I have found to be true for the H.-P. of rigor. In surviving muscles left exposed to pure oxygen for long periods, the H.-P. of rigor does not diminish: in fatigued muscles, exposure to pure oxygen for 8–10 hours restores the H.-P. of rigor to its previous high value. The evidence for this is given in Table XI.

TABLE XI. *The effects of oxygen in maintaining, or restoring to its original value the development of heat in rigor.*

(i) *Survival.* The muscles (frogs' legs skinned) were left in oxygen for various periods, given below in hours, and finally sent into rigor. Except in the second exp. the original value of the H.-P. of rigor was not taken: it may however be assumed that it is about ·95 cal. per c.c., the average value for frogs' legs.

Period of survival in O_2	7·5	16	17	20
H.-P. of rigor, beginning	[·95]	·70	[·95]	[·95]
H.-P. of rigor, at end	1·0	·70	·8	1·1

Had it not been for the O_2 the value in the fourth exp. would have been about zero. See Table IX.

(ii) *Fatigue.* The fatigued muscles (legs only, except when marked with an asterisk) were skinned, and hung in pure oxygen in a bottle, usually all night for a period of about 15 hours. The temperature in the bottle was from 11° C. to 16° C.

Number of exp.	1	2	3	4	5*	6*	7*
H.-P. of rigor: fresh muscles	[·95]	[·95]	[·95]	[·95]	·81	·81	·77
H.-P. of rigor: fatigued ...	·68	·36	·4	·36	·35	·41	·32
H.-P. of rigor: restored in O_2	1·4	·57	·92	·54	·69	·77	·69

From these experiments we see that oxygen possesses the power (*a*) in surviving muscles of maintaining at its original value and (*b*) in fatigued muscles of restoring to its original value the total H.-P. of rigor. This is again exactly analogous to the phenomenon described by Fletcher and Hopkins[3] who showed that oxygen would, in some way, get rid of

[1] Fletcher and Hopkins. *Op. cit.* p. 269. [2] *Ibid.* p. 284.
[3] *Op. cit.* p. 282.

the free lactic acid in fatigued muscle, but that nevertheless the total store of lactic acid available on the production of heat-rigor is unaltered thereby.

As a method of demonstrating this phenomenon, viz. the recovery from fatigue on leaving the muscles in oxygen, this estimation of the heat-production of chloroform rigor is far simpler to perform than the estimation of the lactic acid formed. For further investigation on these lines, or for class demonstrations, it ought to provide a valuable method.

(iii) *The heat produced by the action of free alkali on muscle.*

Fairly dilute alkali, say 1 % NaOH, renders a muscle inexcitable in a very short time. Under its action a certain amount of heat is evolved, more than under the action of chloroform. The experiments are done in exactly the same way as those with chloroform: instead of the latter a little strong NaOH (2 or 3 c.c.) is introduced and the heat-production after the first half minute observed. If we assume that the soda first liberates the lactic acid and then combines both with it and with the free CO_2 in the tissues, the phenomena are very simply correlated with those already observed.

TABLE XII. *The H.-P. of alkali acting on muscles.*

The Exps. I *a*, I *b* and I *c* were made upon muscles of the same batch: similarly for (*b*), (*c*) and (*d*) respectively.

Exps. I: *a*, *b*, *c* and *d*. Alkali acting direct on muscles. H.-P. in cal. per c.c. of tissue, skinned. Cf. with Exps. II, *a*, *b*, *c* and *d*, and Exps. III, *a*, *b*, *c* and *d* respectively.

Exps. II: *a*, *b*, *c* and *d*. Chloroform acting on similar muscles.

Exps. III: *b*, *c* and *d*. Alkali acting on muscles, first put into rigor with chloroform.

Number of exp.	*a*	*b*	*c*	*d*
H.-P. I	1·1	1·5	1·7	1·3
H.-P. II	·8	1·1	1·0	·4
H.-P. III	—	·26	·8	·7

Exp. IV. Alkali acting on muscles already put into heat-rigor.

(*a*) ·7 cal. (*b*) 1·4, 1·4. (*c*) 1·3, rigor at 38° C. (*d*) 1·0 cal., heat-rigor at 55° C. (*e*) ·7, heat-rigor at 75° C.

From these experiments we see that the following relation is approximately true: (H.-P. under action of alkali direct) = (H.-P. of chloroform-rigor) + (H.-P. under action of alkali of muscle already rigid after chloroform). The values of the left hand side of the equation in

Exps. (*b*), (*c*) and (*d*) are 1·5, 1·7 and 1·3 respectively and the values of the right hand side 1·4, 1·8, 1·1 respectively. The strong alkali seems to cause the same breakdowns in muscle as does chloroform, and then to combine with the products formed. If ·4% lactic acid in muscle were to combine with free alkali, there should be produced approximately ·6 cal. per c.c. of tissue. The heat produced by the combination of CO_2 with alkali also is considerable.

Further with reference to Exps. IV, it is known[1] that the production of heat-rigor (or rather of muscle coagulation) at higher temperatures is not accompanied by so great a liberation of lactic acid as at low temperatures: the lactic acid is, in some manner, "fixed." This may explain why the heat produced by alkali acting on muscles put into heat-rigor at 55° C. and at 75° C., is less than that for muscles put into heat-rigor at 38 to 40° C.

These experiments were not carried any further, because it was obvious that a very large proportion of the heat might come from the combination of the alkali with the more or less unknown amount of CO_2 dissolved in the tissues. So far as they go however they confirm the previous observations.

Discussion of results.

One conclusion seems inevitable from all the experiments given above: viz. that both in the later stages of anærobic survival life, and also in chloroform- and heat-rigor the production of heat and the liberation of lactic acid are due to one and the same chemical reaction. For under all conditions they seem to vary together. The further development of the subject rests on more hypothetical grounds. As suggested above the estimation of the heat-production of rigor may give us some evidence as to the nature of the chemical body whose breakdown liberates lactic acid and heat. To take a definite case, let us suppose that lactic acid originates from dextrose. So far as we know there are no products of the changes leading to rigor other than lactic acid: hence it is natural to assume that 1 gr. of dextrose breaks down into 1 gr. of lactic acid according to the scheme,

$$C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3.$$

Now the heat of combustion of dextrose is known: from Landolt and Börstein we find three estimations by different observers, viz. 3692, 3742, and 3762 gr. calories per gr. and in the *Chemiker*

[1] Fletcher and Hopkins. *Op. cit.* p. 267.

Kalendar the value 3765. In the latter we find also the heat of combustion of lactic acid, viz. 3658 cal. per gr. The difference between these two must be the heat given out in the suggested transformation of dextrose into lactic acid. Assuming the value for lactic acid to be correct, and the *largest* value for the dextrose, we find that the heat of transformation of 1 gr. of dextrose into lactic acid is only 107 calories: assuming the smallest value for the dextrose it would be only 34 calories. Now never more than 0·4 % lactic acid seems to be liberated in muscle, *i.e.* ·004 gr. per gr.: assuming again this *maximum* value we find that the liberation of heat in muscle if supposed due to the transformation of dextrose into lactic acid, cannot be greater than the value ·004 × 107, *i.e.* ·43 cal. per gr. of muscle. But in whole frogs' legs I have found often a production of heat of 1·0 cal. per c.c.: this is lowered considerably by the presence of bones: for muscle alone it must be at least 1·3 cal. per gr. This value is three times as great as the maximum value calculated above, and at least ten times as great as the minimum value. If therefore (i) we are right in assuming that the formation of lactic acid is the main reaction occurring in the processes leading to rigor, and if (ii) our thermal data (heats of combustion) are correct, then the liberation of lactic acid cannot be due to the breakdown of simple dextrose. It must come from a body of noticeably more energy than dextrose, so that it has probably to be built up into this precursor by the living tissue, under the influence of oxygen. This precursor is possibly the characteristic substance which determines the muscular contraction. Undoubtedly it is very closely linked with it.

Every chemical reaction may be regarded as a balanced action: in some reactions the equilibrium may be so far in one direction or another that we are inclined to call them complete, but logically we have no right to do this. The considerations given in the Appendix to this paper, will make it clear how the efficiency of a given chemical reaction for doing mechanical work is a function, not primarily of the heat-production of that reaction, but rather of its completeness in the sense of a balanced reaction. Berthelot's principle, that the direction of a reaction is determined by its greatest heat-production, we know not to be true: every reaction goes on in the direction in which its capacity for doing external mechanical work is diminished in the highest degree, *i.e.* in the direction of the greatest diminution of the "free energy." Some reactions are known which are very complete, even though they go on with an absorption of heat: yet even though these reactions actually give out no heat, and may absorb it, their completeness makes

it possible for them to do large quantities of mechanical work. It is necessary to introduce this conception of the "free energy" here, for it is commonly stated that the liberation of lactic acid from a precursor provides insufficient energy for it to be the basis of the muscular contraction. Carbohydrate has been assumed to be this precursor. Apparently this is not the case, the liberation of energy is too large: the actual amount of energy liberated in the production of lactic acid from its precursor, whatever that may be, can be calculated approximately from the data given above. Taking an average value, ·35 %, for the lactic acid production, and—correcting roughly for the presence of bones in the muscles—taking an average value, 1·3 cal. per gr. for the production of heat in rigor, we find that the liberation of 1 gr. of lactic acid leads to an evolution of heat of 1·3/·0035 = 370 cal. This quantity is about 10 % of the total energy of the carbohydrate. According to the old fashioned method of considering merely questions of *total* energy changes in chemical reactions, this 10 % is still scarcely sufficient to account for the observed high efficiency of a frog's muscle. Whether, as a matter of fact, Fick's actual observations of the "efficiency" of a frog's muscle are justifiable, in view of the fact that both the production[1] of heat and the oxidations[2] consequent on a muscular contraction may take place some moments after the contraction is over, is to me very doubtful. A large part of the heat evolved in the processes restoring the muscle to its previous condition may be so delayed that it may be masked entirely by conduction and loss: and in order to calculate the efficiency of a process we must consider it in its entirety. Whether these observations are justifiable however, or not, is immaterial at present. There seems, from the calculations given above, to be reason to believe that the lactic acid is liberated from some precursor other than glucose: and the breakdown from this body to lactic acid may be one of those somewhat rare but by no means unknown chemical reactions which can do more mechanical work than is equivalent to their total loss of energy; by virtue of their completeness (see Appendix) they possess the power of absorbing heat from their surroundings to do this excess of work. It seems to me to be probable that the bodies liberated before and thereby causing the contraction of a muscle are afterwards, in the presence of oxygen, restored to their previous condition by the application of work and energy obtained from oxidations. At present I hold no brief for the view that lactic acid *is* responsible for the muscular contraction: but until we know exactly what the

[1] A. V. Hill. This *Journal*, XLII. p. 15. 1911.

[2] Verzár. *Ibid.* XLIV. p. 252. 1912.

precursor of lactic acid is, and what the "free[1] energy" of its breakdown into lactic acid is, we cannot adduce considerations of energy to disprove the hypothesis that the sudden liberation of lactic acid causes a contraction, whether by a change of surface tension or by some other means. This may sound strange, but it is nevertheless a fact: its justification will be found in the later development, by modern physical chemistry, of the subject of the "free energy" of chemical reactions. There are, so far as I can see, no experimental or *a priori* objections to a scheme like the following: the contraction is due to a change of surface tension set up in certain colloidal structures by the liberation of lactic acid from some precursor: this precursor has 10 % more total energy than lactic acid, but possibly 20 or 30 % more "free energy." When much work is done in a twitch, there might be for a short period a cooling of the muscle, but to the thermoelectric methods at present possible this initial cooling would be entirely masked by the rapidly ensuing processes of restoration, involving oxidations and liberations of "free energy" and heat. These oxidations might be designed to provide "free energy" and heat sufficient to replace the lactic acid in its previous condition; or they might be concerned with the oxidation of the lactic acid to provide "free energy" and heat sufficient to transform a certain amount of glucose into the lactic acid precursor, in order to replace the amount of lactic acid precursor lost during the contraction. The picture is of course incomplete, and one would naturally not care to press it: but it shows, to some degree, how these experimental and theoretical considerations of energy and "free energy" may lay open to biology certain roads and fields of work which have been hitherto unknown. Possibly the most fruitful line of research in the future would be the estimation of the total heat given out in the oxidative removal of lactic acid: this might give us some clues as to the function of oxygen in the restoration of lactic acid to its previous position of higher energy. Prof. Langley suggested this problem to me some while ago, and in the hitherto vain attempt to see a solution of the experimental difficulties the preceding work was begun. Possibly further advance will be made by the estimation of the heat produced during long periods of contraction: this is quite simply done with the micro-calorimeter. But in whatever direction advance is made, those developments of modern physical chemistry whose applications to biology are sketched in the Appendix, must be of fundamental interest and importance.

[1] *I.e.* the maximum mechanical work which the reaction can do; this may be greater or less than the heat of reaction, and bears no simple relation to it.

Conclusions.

1. It has been found possible, with the differential micro-calorimeter to estimate the heat given out by resting surviving frogs' muscles at room temperature. Immediately after death this rate of heat-production is as high as during life: but it begins to fall at once along an "exponential" curve until it reaches a constant value, which may remain constant for long periods. This latter corresponds to the constant rate of CO_2-liberation, and the constant rate of lactic acid production, described in surviving muscle by Fletcher and by Fletcher and Hopkins respectively. Finally when decomposition sets in the rate of heat-production rises again.

2. The presence of oxygen has a considerable effect upon the rate of survival heat-production: it increases it at all stages of survival life, the initial high rate of liberation of heat by isolated muscles being due simply to the presence of oxygen dissolved or combined in the tissues.

3. These and all subsequent results are explained by the assumption that the formation of lactic acid from its precursors is an exothermic reaction, and that under anærobic conditions the liberation of heat in the later stages of survival is due largely, if not entirely to this reaction. In the presence of oxygen the action is somehow inhibited, and the heat-production observed is due to oxidations. In the presence of insufficient oxygen both of these processes may go on together.

4. The liberation of CO_2 by surviving muscles is due (*a*) to the oxidations occurring so long as there is oxygen, and (*b*) in the later stages of survival simply to the turning out of CO_2 from the $NaHCO_3$ present, by the lactic acid formed.

5. The combination with alkali present in the muscles of lactic acid set free cannot account for any appreciable proportion of the total heat liberated either in survival life or in the processes leading to rigor.

6. It has been found possible to estimate the heat produced during the processes leading up to chloroform- or heat-rigor. Reckoned per gr. of frogs' legs the value is about 1·0 cal. per gr.; for muscles only it must be 1·3 cal. per gr., or more. For both forms of rigor the values are practically the same.

7. The heat-production is not due to the coagulative processes which are the final stage of rigor, but to the chemical processes which liberate lactic acid; for the heat is produced before coagulation ensues. Hence we can calculate the total energy of the lactic-acid-precursor.

8. Any factors which liberate lactic acid in the muscles, such *e.g.* as (*a*) length of survival after death, (*b*) mechanical injury, (*c*) fatigue, cause also in such muscles a fall in the value of the heat-production of rigor. Some of the heat, in fact, has already been set free by the previous liberation of some of the lactic acid.

9. The processes leading to heat-rigor are the same, only exaggerated in velocity by rise of temperature, as the processes liberating lactic acid and heat in the later stages of survival life at ordinary temperatures. Chloroform seems also merely to accelerate these processes.

10. Oxygen maintains at its initial high value the heat-production of rigor of surviving muscles, in the same way as it prevents the liberation of lactic acid during long periods of survival.

11. Oxygen restores the lowered heat-production of rigor in fatigued muscles to the initial high value usual in fresh muscles: in just the same way as it diminishes the amount of lactic acid present in fatigued muscles, without affecting the total amount given out in rigor.

12. In muscles kept anærobically the rate of diminution of the H.-P. of rigor is equal to the rate of survival heat-production.

13. The total energy of the lactic acid precursor is about 10% greater than that of lactic acid itself: the precursor is therefore presumably not glucose, since according to the thermal data available the total energy of glucose is not more than 3% greater than that of lactic acid. Lactic acid is therefore built up by the body into some, at present unknown, chemical combination of greater energy than glucose. It is suggested that this body is one containing a large store of "free energy," and that it may be able to account for the mechanical work done by a muscle simply by the process of breaking down into lactic acid. Many breakdowns are known whose "free energy" is greater than their total energy.

14. Considerations of total energy are not sufficient, as is shown in the Appendix, to disprove the assumption that muscular contraction is due to the liberation of lactic acid from a precursor. The estimation of the "free energies" of the chemical reactions possibly involved in muscular metabolism is of fundamental importance.

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APPENDIX.

The mechanism of muscular activity is not and cannot yet be made clear. Too little of the more fundamental physics of surface tension, colloidal solutions, and molecular forces is as yet known to make it even reasonable to attempt to build up an elaborate picture of the complete process. There is however even now one avenue along which physiology may progress as an exact science, viz. that of energy and thermodynamics. Unfortunately there have been, even among physiologists, many and grievous misconceptions as to the application of the laws of thermodynamics: these have been due partly to the desire to make over-hastily a complete picture of the muscular machine, partly to the completely erroneous belief that the laws of thermodynamics apply only to heat engines and not to chemical engines, and that no information can be obtained from the Second Law as to the working of a chemical machine at uniform constant temperature. The muscle fibre has been treated as a heat-engine when it is inconceivable that there are finite differences of temperature in it: to keep up a temperature difference of 100° C., as required by the observed efficiency if the muscle is a heat-engine, at two points not more than a few μ (*i.e.* 10^{-3} mm.) apart would mean an almost infinite loss of heat by conduction between the two points. A temperature gradient of (say) 30000° C. per mm. is impossible. The muscle is undoubtedly a chemical machine working at constant temperature.

Again even bacteria have been treated as heat-engines, their "efficiency" E depending on the difference of temperature $(T_1 - T_2)$ between themselves (T_1) and the medium in which they live (T_2) according to the formula

$$E = (T_1 - T_2)/T_1.$$

Arguing from this assumption the rise of body-temperature in fever[1] has been "explained" as an attempt by the body to lower the efficiency of the bacteria by increasing T_2, and consequently diminishing $(T_1 - T_2)/T_1$, T_1 being supposed limited by the fact that above a certain temperature the bacteria perish; and finally the word "work" has been used to confuse the issue as between mechanical and chemical processes by its employment in the meaningless expression "chemical work," the "chemical work" which a process can do being supposed given by the efficiency. Here again the scientific imagination which put the "chemical work" and hence the chemical activity (!) of bacteria (assumed to be heat-engines) proportional to the quantity $(T_1 - T_2)/T_1$, was not curbed by the reflection that $(T_1 - T_2)$ *must* be inconceivably small, being the difference of temperature at two points not more than 10^{-3} mm. apart. It is to be regretted that such misconceptions as these should have given the impression either (*a*) that the laws of thermodynamics do not apply to the animal body, or (*b*) that they apply only to a heat-engine which the living organism certainly is not.

The greatest successes achieved by the application of the laws of thermodynamics are in Chemistry: and these successes centre around the conception of the "free energy" of a chemical reaction. By means of changes of osmotic pressure, surface tension, volume, or vapour pressure, or by means of electrical effects set up[2], every chemical reaction in the universe which goes on spontaneously can be made to do external mechanical work. The maximum mechanical work which any given reaction can do is named the "free energy" of that reaction: and the Second Law of Thermodynamics tells us that *whatever be the mechanism of the work production* the maximum work that can be done is the same. This is of fundamental interest to the physiologist; at present we are unable to say by

[1] Simoson. *Zntrlb. d. Physiol.* XXIV. p. 702.

[2] *E.g.* the production of a galvanic current working a dynamo.

what mechanism the organism can perform mechanical work: but provided we know what chemical processes the organism carries out we can calculate in general the maximum mechanical work which these processes can yield, independent of the mechanism by which that work is produced. For most chemical reactions this calculation is at present difficult, but there is no reason to doubt that with the advance of physical chemistry it will be rendered possible for *all* chemical bodies. So that if the biological chemist can decide what reactions go on in the body in (say) the breakdown of carbohydrate, we shall be able to predict the maximum work that can be done by each of these reactions.

If, according to the scheme[1]

$$\beta B+\gamma C+\delta D+\ldots \rightleftarrows \beta' B'+\gamma' C'\times\delta' D'+\ldots$$

β molecules of B, γ molecules of C, etc. are transformed into β' of B', γ' of C' etc., then a certain amount of energy U is liberated and a certain maximum amount A of mechanical work can be obtained at constant temp. T. U is the loss of internal energy of the chemical bodies reacting, *i.e.* the "heat of reaction" when no external work is done. When external work x is done the heat of reaction must be $U-x$. A is the "free energy" of the reaction, *i.e.* the max. amount of mechanical work it can accomplish. If T is the absolute temperature the Second Law of Thermodynamics tells us that

$$\frac{\partial A}{\partial T}=\frac{A-U}{T}.$$

This is the only relation between U and A, and U and A can be equal only when $\frac{\partial A}{\partial T}=0$, *i.e.* when the max. work which the reaction can do is independent of the temperature. This relation, $\frac{\partial A}{\partial T}=0$, is in general not satisfied, so that in general A and U are different, and may be very widely different. If $\frac{\partial A}{\partial T}$ is positive, then the max. work which the reaction can do is greater than the total energy liberated in the reaction, and must be done in part at the expense of the heat of the surroundings. This type is not the most usual, but is pregnant in interest to the physiologist; several very striking examples are known, and all endothermic reactions going on spontaneously come under its head. If $\frac{\partial A}{\partial T}$ is negative, then the max. work which the reaction can do is less than the total energy liberated, and the reaction goes on always with the liberation of heat. Berthelot's principle is invalid because this case is not universal. Below we shall see how to some extent it is possible already to calculate A: and also what laws govern its behaviour. At first however it will be better to discuss more exactly what this "free energy" is, and how it affects biology.

As Nernst[2] and Boltzmann[3] have emphasised, the "struggle for existence" in organic life is not the struggle for matter in its crude state, not even for energy in any and every form, but rather for that proportion of the energy which can be turned into mechanical work, and which Helmholtz denoted by the suggestive term "free energy."

[1] All chemical reactions may be treated as reversible; under the requisite temp. and pressure conditions their incompleteness can be demonstrated, even though under normal conditions their state is practically complete. *E.g.* at 1000° C. the reaction

$$2CO+O_2 \rightleftarrows 2CO_2$$

is anything but complete.

[2] *Theoretische Chemie*, 6th Ed. p. 777. 1909.

[3] *Der zweite Hauptsatz der mech. Wärme.* Vortrag, Wien, Gerold, p. 21. 1886; also *Populare Schriften* No. 3. Leipzig, 1905.

Mechanical work or mechanical potential energy (including stored electric energy which is the mechanical strain energy of the dielectric) is the only form of energy which can be turned completely and instantly into any other form. Without the use of "free energy" many chemical reactions cannot go on, and many processes cannot be carried out. There is in fact another factor besides total energy which must be considered, a second quality of chemical compounds, the "free energy," which may have a very considerable influence on biological chemistry and in particular upon our notions of the isodynamic values of food-stuffs.

Let us consider a few examples of how a chemical reaction going on at constant temperature T can do work. In a Daniell cell the solution of zinc to form $ZnSO_4$, simultaneous with the deposition of copper from $CuSO_4$, produces a current which can turn a motor. The chemical reaction is

$$Zn + CuSO_4 \rightarrow Cu + ZnSO_4.$$

Similarly a concentration cell (which however scarcely involves a chemical but rather a physical reaction) can be made to do work. Again the Grove gas battery, employing the formation of water from oxygen and hydrogen to produce an E.M.F. (at 20° C.) of 1·06 volts, uses a typical chemical reaction for the production of mechanical work. Secondly there are several chemical reactions which can produce mechanical work by changes of surface tension: *e.g.* a drop of mercury in 20 % HNO_3 will behave in a very lively fashion when a crystal of potassium bichromate is added to the acid. The periodic changes of surface tension set up cause movements of the mercury which of course can be made to perform mechanical work. Thirdly an increase of volume can be set up by chemical affinities working against enormous pressures, as *e.g.* in the combination of crystals of $CuSO_4$, $4H_2O$ with an extra H_2O to form crystals of $CuSO_4$, $5H_2O$: such an increase of volume can produce large quantities of external work. Fourthly the changes of vapour pressure of a liquid after chemical combination can be used for the same purpose: *e.g.* in the case of the combination of H_2O with the $CuSO_4$, $4H_2O$, the H_2O can be allowed to evaporate and then to expand doing work at constant temperature until it is at the vapour pressure of the hydrated crystals: and then absorbed by the crystals by virtue of the chemical affinity. Finally we come to the case of osmotic pressure: the laws of osmotic pressure are so well established that whenever possible we try to calculate the free energies of chemical transformations by supposing the latter to go on in machines based on osmotic pressure. This has given a false impression that chemical machines have something in particular to do with osmotic pressure, whereas the truth is that the latter is used simply to provide a method of calculation. Whatever the mechanism of work-production be, the maximum work a chemical reaction can do is the same as that calculated by means of semi-permeable membranes: otherwise the Second Law is not obeyed. As machines for the production of work we suppose certain osmometers bounded by semi-permeable membranes, and also a given reaction mixture, *i.e.* a mixture of chemical bodies in equilibrium with one another. The duty of the reaction mixture is to transform certain chemical substances from one side of the reaction to the other. We suppose that we have certain stores of chemical bodies X kept at unit concentration, and we wish to find the maximum work which can be obtained in transforming and transferring a portion of them into certain other stores of other chemical bodies Y kept also at unit concentration. The function of the chemical affinity is the taking of dilute solutions of X and transforming them into concentrated solutions of Y: the reaction mixture being supposed dilute in X and concentrated in Y. This will be the case if the chemical reaction is a fairly complete one. We first of all take our chemical bodies X out of their stores, and allow them to expand in suitable osmometers doing external work by virtue of their osmotic pressures, until they are finally at the same concentrations as the bodies X in the reaction mixture. We next introduce them reversibly

into the reaction mixture, which then transforms them into the bodies Y. These bodies Y we then take out of the mixture reversibly and allow to expand in other osmometers, doing more external work, until they are at the same concentration as the stores of the bodies Y, into which they are then introduced. Thus the mechanical work which the chemical reaction turning 1 gr. molecule of the bodies X into 1 gr. mol. of the bodies Y can do, depends on two factors:

(*a*) It is greater, the less the osmotic pressure or concentration of the bodies X in the reaction mixture: for the less this concentration is, the greater is the degree of expansion required before introducing the bodies X into the reaction mixture.

(*b*) It is greater, the greater the osmotic pressure of the bodies Y in the reaction mixture: for the greater this osmotic pressure is, the greater is the degree of expansion required before introducing the bodies Y into their appropriate stores.

Hence to obtain much work we should use a chemical reaction which is very complete, *i.e.* one in which the equilibrium is very far in one direction. For then (i) the concentration in the reaction mixture of the initial bodies X would be exceedingly small, and much work would be done in osmotic expansions before introducing the bodies X into the reaction mixture: and (ii) the concentration of the bodies Y would be very large, so that much work might be done in expansion before introducing the bodies Y into their appropriate stores. The very complete, though still reversible, chemical reaction can produce mechanical work by virtue of the fact that it takes exceedingly dilute chemical substances, and by using the chemical affinities transforms them into other chemical substances in very concentrated solution. This transformation from very low osmotic pressure[1] to very high osmotic pressure means the storing of work: and hence we see how the "free energies" of very complete reactions are large. For doing mechanical work, the virtue of a chemical reaction lies not in its liberation of total energy, but in its completeness as a reaction: and from the Second Law we may make this statement, whatever be the mechanism by which the work is produced. The efficacy of such processes as the oxidations of fat and carbohydrate, for use by the organism, is that such reactions are in the highest degree complete, so that much mechanical work, "free energy," can be obtained from them. Only in a secondary degree does their efficacy depend on their large store of total energy, though generally to some degree, since $\frac{\partial A}{\partial T}$ is seldom large, A and U must be of the same order of size.

It can be shown that, if we have as a chemical engine the reaction mixture

$$\beta B+\gamma C+\ldots \rightleftarrows \beta' B'+\gamma' C'+\ldots$$

in which β molecules of B etc. are in equilibrium with β' of B' etc. and if K be the equilibrium constant of this reaction, viz.

$$K=\frac{[B']^{\beta'}[C']^{\gamma'}}{[B]^{\beta}[C]^{\gamma}},$$

{where $[B]$, $[C]$ etc. represent the concentrations of B, C etc. in the reaction mixture} then the maximum work which the reaction can do in transforming β molecules of B etc. into β' of B' etc. is

$$A=RT\log K.$$

R is the gas constant, viz. 1·99, and T is the absolute temperature.

Further the equation

$$\frac{\partial}{\partial T}\log K=\frac{-U}{RT^2},$$

gives the change of the equilibrium constant with temperature, where U is the heat of reaction when no work is done.

[1] We are speaking of course of "partial pressures."

Hence the greater the equilibrium constant K, i.e. the more complete the reaction, the greater will be RT log *K the free energy of the reaction.* Now most of the reactions in the body are carried out by successive stages: if K_1, K_2,... be the equilibrium constants of these successive reactions, then the total maximum work in any complete breakdown is

$$A = RT[\log K_1 + \log K_2 + \ldots].$$

The free energy A, as we have seen, is governed by the relation

$$\frac{\partial A}{\partial T} = \frac{A - U}{T}.$$

At absolute zero, when $T=0$, $A-U$ must also be zero: so that at absolute zero the free energy is equal to the total energy. At every other temperature however they are different, and may be very different: there is in fact a certain chemical reaction in an electric battery (Bugarsky's combination) in which U and A are of opposite signs. This endothermic reaction goes on with the production of a considerable E.M.F. which can be used to do external work; and in fact every endothermic reaction occurring spontaneously can be made to do work. Berthelot's principle has been replaced by the law, which is universally true, that every chemical reaction goes on the direction in which the free energy A diminishes: this quantity A is the true measure of the chemical affinity, and hence we see the importance of being able to determine A for all chemical reactions. This determination is as yet difficult: and the developments of modern physical chemistry—especially in the Nernst school—centre around the attempts to find a general method for its calculation. It would take us too far here to discuss the actual method which Nernst asserts will lead us to a complete knowledge of the affinities ("free energies") of all chemical reactions: it is based upon the assumption, which although one can see no obvious reason, for it seems to yield results in accordance with facts, that at absolute zero not only are A and U equal, as the Second Law shows, but also that they converge tangentially to their common value, *i.e.* $\frac{\partial A}{\partial T} = \frac{\partial U}{\partial T}$ at $T=0$. This assumption renders possible, or greatly simplifies, the calculation of A, and in many cases has yielded good results, whether finally it will be found adequate is a matter only for the future to decide. In any case there seems to be a large chance that in the not distant future we shall be able to determine the free energies of very many chemical transformations. For the biologist it is of fundamental interest to realise that all chemical bodies possess a property, their "free energy," which is not necessarily the same as and is often widely different from their total energy.

Speaking in terms of oxidative breakdowns, some bodies possess by virtue of the completeness of their breakdowns the possibility of doing external work, the energy for which may be taken partly from their own total energy and partly from the heat of their surroundings. They do not so much possess energy as the possibility of getting external heat turned into mechanical work: not working however as heat engines, because the reactions go on at uniform temperature.

For lack of data as to their free energy the value of food-stuffs have been based upon a comparison of their total energies, *i.e.* their heats of combustion. Many curious anomalies may be settled when we are able to determine the free energies of the oxidation of fats, carbohydrates and proteins, which free energies are very unlikely to be the same as their total energies. If it is shown, *e.g.* that carbohydrate has, calorie for calorie of total energy, a higher proportion of free energy than fat has, this would have an enormous influence on theories of nutrition. The building up of animal tissues out of amino acids and sugar, but still more the building up of plant tissues out of nitrates and CO_2, working as both processes do against very complete chemical reactions, must require a large provision of free energy: so that in processes of growth, as also of movement, an adequate

supply of free energy is essential. The body seems to obtain its energy and its free energy from oxidative breakdowns: the plant partly from sunlight and partly also from breakdowns, anærobic (as with yeast cells), or oxidative. It will be of fundamental interest to determine the free energies of carbohydrates, fats and proteins, both to compare them as food-stuffs and also to be able to determine what provision of free energy is necessary for the animal or plant in order for it to be able to build them up. Further, the transformation of soluble material from one position and concentration to another, as in the secretion of urine, also involves the utilisation of "free energy" which the body can obtain only from food-stuffs.

Apart from Nernst's recent developments of the subject there is another method by which we may progress. In many cases it is already possible to calculate the equilibrium constant K of a reaction, and hence also $RT \log K$ its free energy. The developments of ferment chemistry, especially in the case of reversible changes carried out by ferments, may make it possible to calculate directly the equilibrium constants of many breakdowns of organic material. If *e.g.* the bio-chemist can decide what chemical reactions go on in order in the process of carbohydrate breakdown, and if we can determine directly the values of K_1, K_2, K_3...for these several reactions, then it will be possible not only to give the total free energy $-RT[\log K_1 + \log K_2 + \ldots]$ but also the free energy of every stage. *At one particular stage we shall probably find that the value of* $RT \log K$ *is much greater than in the other stages*: that among all the partial breakdowns one in particular is characterised by a large liberation of free energy. *If this is so we shall have special grounds for assuming that the process of muscular contraction or of urine secretion, etc. is based upon that particular breakdown.* There cannot be many processes involved in the very short space of time necessary for the complete contraction and relaxation of an insect's wing-muscle, and presumably such a process must be based upon one particular reaction. The mechanical work of a contraction is large: and it is therefore quite probable that an analysis of the "free energies" of the several stages of breakdown of food-stuffs would give us decisive evidence as to which of these processes is involved in contraction. It will be of the highest interest to determine exactly what the free energy of the lactic acid precursor in muscle is. Possibly this "free energy" is sufficient to account entirely for the mechanical work done in a contraction. At any rate mere considerations of total energy are quite insufficient to refute the view that the contraction is due to the liberation of lactic acid from some precursor. All endothermic reactions which go on spontaneously can do mechanical work, in spite of the fact that they absorb and do not give out energy: and very many reactions can do more work than can be accounted for by their total energy. For example the galvanic combination,

Cu | Copper acetate solution | Lead acetate | Pb,

acts by turning Cu-acetate into Pb-acetate with the solution of Pb and the deposition of Cu. The total energy obtained by the transformation of 1 gram-equivalent, *i.e.* the difference in the internal energies of Cu-acetate and Pb-acetate, is 8766 calories. This means that if the process goes on, without doing external work, there is an evolution of heat of 8766 cal. per gr. equivalent. But the electric current set up can be made to do work to the extent of 10842 cal.: not only therefore has the entire energy of the transformation been used up in doing mechanical work, but the machine has managed to get work equivalent to 2076 calories more from the heat of its surroundings. This is not the only case, nor perhaps the most striking: Bugarsky's combination

$$Hg \mid HgCl \mid KCl \mid KOH \mid Hg_2O \mid Hg$$

yields an E.M.F. sufficient to do mechanical work to the extent of 7566 cal. for a transformation of 1 gr. equivalent: the chemical processes however are actually endothermic.

If the battery does *no* external work there is an absorption of heat of 3280 calories per gr. equivalent. If it *does* do max. external work there is an absorption of heat of 10846 cal. It may sound strange, but is nevertheless a fact, that this combination not only liberating no energy of itself, but even leading to an absorption of energy of 3280 cal., can nevertheless do external work to the extent of 7566 cal., thus leading altogether to an absorption of 10846 cal.

We have seen above that lactic acid is broken off from some precursor with the evolution of heat: and that the precursor is apparently not glucose. It is a compound of lactic acid of rather greater energy, at present unknown. It may be possible for the chemist to isolate it from the mixture of bodies obtained on crushing up muscles in ice-cold alcohol[1]. But until we have isolated it and determined its free energy, we dare not assert finally that the liberation of lactic acid from this precursor cannot produce enough "free energy" to account for the mechanical work known to be done by a muscle twitch.

[1] Cf. Fletcher and Hopkins. *Op. cit.* p. 252: crushing in the cold does not seem to liberate lactic acid in muscle. It is interesting to compare Hermann's views on the unstable chemical body determining contraction and the onset of rigor by its breakdown. See Hermann, *Unters. ü. d. Stoffwechsel der Muskeln*, p. 67 etc. Berlin. 1867.

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