STUDIES ON NERVE METABOLISM. I. The influence of oxygen lack on heat production and action current.

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In previous papers (1, 2, 3) it has been shown that activity of nerve is associated with a definite heat production, and the absolute value for a single impulse obtained. This heat appears in two phases, an initial very short and intense one associated with the actual conduction and immediate restitutive processes, and a delayed feeble but very prolonged one associated with "recovery" processes. In the case of muscle, which shows two similar phases, the delayed heat is almost all oxidative, since it disappears in oxygen lack, whereas the initial heat is non-oxidative, being independent of oxygen supply. It was expected that a similar mechanism would be found in nerve, possibly based, as in muscle, on a glycogen-lactic acid system. The present research represents an attempt to expose the nerve mechanism; and the results obtained do not support this hypothesis, but do not positively eliminate it. The problem is being followed further by chemical methods.

Especially interesting is the evidence that for minutes after activity a nerve has not returned to rest—not only does extra heat production last long after conduction is over, but potential or permeability changes may also considerably outlast the transmitted impulse (see Levin (4) and Verzar (5)). The dependence of these electrical changes on the presence of oxygen has been much studied but the results can hardly yet be given a sound interpretation.

Method.

These experiments were carried out during the same period as those already reported, and the two galvanometer arrangements described (1) and (2) were both used. The general arrangement of the electric circuits was also as described.

In a large number of experiments the action potential as well as the heat was recorded. The former was led off by electrodes (3) and (4) from

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the uninjured side and crushed end respectively to a moving coil galvanometer. The electrodes were ordinary silver ones and easily polarisable, but this did not matter, for in series with the galvanometer in the "circuit" was a 10 mf. condenser. Any change in E.M.F. would tend to charge (or discharge) the condenser and in so doing produce a pulse of current through the galvanometer which would respond with a ballistic throw. The large capacity of the condenser used made its time of charge and discharge relatively long so that very rapid changes of E.M.F. were averaged; and it also insured a relatively large current pulse when being charged by a low E.M.F. By tetanisation of the nerve, a regular series of action potentials was produced and the condenser charged to the average potential. A quantity of current proportional to the change of potential surged through the galvanometer and gave a ballistic throw that directly measured the average potential developed. When tetanisation was stopped the potential would return to zero (or rather to the original injury potential) and the condenser discharge through the galvanometer, giving a deflection in the opposite direction. Except for minute leaks in the condenser the total current flow was nil, and the galvanometer zero steady, as the slow changes of injury potential would not be perceptible. The galvanometer deflections during charge and discharge of the condenser must be equal and opposite if the system has returned to its initial state. As a matter of fact under ordinary conditions the throw when stimulation was stopped was one-third to one-half again as great as that when it was begun. This may have been partly due to a gradual increase of action potential from one impulse to the next so that the condenser continued to charge for a time after the main surge of current, whereas the discharge of all the accumulated potential did occur in one surge. Another factor may have contributed to this effect, however, for after the negative variation ends the nerve does not return to zero potential but rather "overshoots" to a positive variation. The effect is absent when a nerve has begun to fail in nitrogen, charge and discharge becoming equal.

The galvanometer used, a high resistance, high sensitivity (1 mm. = 2×10^{-10} amp.) Cambridge moving coil instrument, had a very slow period (8 sec. one way) and was therefore able easily to sum the short currents produced. Deflections of about 100 mm. were obtained at 3 metres distance.

Only spring and autumn Dutch *esculenta* frogs were used, the sciatic nerves prepared as before. Their arrangement on the thermopile, however, was different, in that the groove containing the "hot" junctions

was not filled with nerves flush to the level but only lined with a layer of them one deep, and each nerve in contact laterally with its neighbours. This gave, in effect, a plate of nerve tissue lying on the thermopile and made possible more certain control of the gas diffusion factor in oxygen deprivation experiments. In one experiment the nerves were laid on the thermopile in the usual manner and then completely covered, except at the end for stimulating, with a layer of white vaseline over 1 mm. thick, to delay gas diffusion—this procedure had no effect on the results. In many of the experiments, to ensure true monophasic action currents, the entire bundle of nerves was tightly ligatured between the thermopile and electrode 4.

The special Harvard coil giving 140 make and 140 break shocks a second was used throughout, and stimuli were usually of 10 secs. duration. Stimulation was always repeated at regular intervals of 1, 2, 5 or 10 min. to avoid irregular "fatigue" effects. The arrangement of the nerves and electrodes in the chamber was such that the stimulated end was in the same gas as the remainder of the nerve. It was necessary therefore to be certain that any fall in observed heat production was not due to the stimulus becoming submaximal as excitability diminished. In several experiments the maximal stimulus at different stages of asphyxia was roughly determined and then an appropriate maximal stimulus used throughout.

The temperature in all experiments was about 15° C.

Preparation of oxygen low medium. As ordinarily used, the nerve thermopile is covered with a large test tube, moist with Ringer and closed with a rubber stopper through which glass tubes pass. Some of these carry the necessary wires, others permit gases to be passed in and out of the enclosed space. The whole is immersed in stirred water in a vacuum flask. The space about the thermopile, within the test tube, contains air or oxygen and measures about 200 cc. To obtain an oxygen free medium, the ideal procedure would be to fill this with well boiled saline which could be displaced with pure nitrogen, but this is impossible for several reasons. In each experiment it is desirable to make observations both in the presence and absence of oxygen, and to get comparable results the thermal characteristics of the medium must remain the same; so that both must be performed in a gaseous medium. (A liquid would conduct a great deal of the heat produced away from the junctions, so much decreasing the sensitivity; and it would also surely lead to electrical leaks.) To displace the air first with boiled saline and this with nitrogen would produce great temperature changes and prohibit observations for

several hours, by which time the nerve would no longer respond to stimulation. The method actually adopted therefore involved displacement of the air or oxygen by gases.

Two gas conduit tubes were carried through the stopper into the thermopile chamber, one ending just within the stopper and the other reaching almost to the floor of the test tube. These were connected externally through three way stop-cocks so that each tube could communicate with the outside air (when serving as outlet) or with a source of gas under pressure (when serving as inlet). The nerves were set up in air and observations taken; then hydrogen was admitted at the top, slowly at first to mimimise mechanical mixing and later rapidly to sweep out remaining traces of the original gas, the tube reaching to the bottom serving as exit.

After 10 min. of continued passage of hydrogen, the cocks were reversed and nitrogen admitted at the bottom while the hydrogen was blown out at the top, the stream being passed fairly slowly for 5 to 10 min. The hydrogen would displace air most effectively because their different densities tend to prevent mixing; but it was necessary to have nitrogen as the final medium because the thermal conductivity of hydrogen is much greater than that of air and deflections obtained in it would not be comparable to those obtained in air. This procedure never gave an oxygen free medium, but in several experiments analysis showed less than 0·2 p.c. oxygen present in the gas. It was found that five or ten times this amount of oxygen made no difference in the results, so it may be safely assumed that in the complete absence of it the results would have been the same. Following the passage of these gases, the heat equilibration was soon re-established so that reliable readings could be obtained in 10 to 20 minutes.

The gases used were themselves purified of oxygen. The hydrogen was taken directly from a commercial cylinder and bubbled through alkaline pyrogallol or hydrosulphite and washed in alkali. This was sufficient because the commercial gas has a very low oxygen content, and the whole was subsequently replaced by nitrogen. Commercial nitrogen contains 2 p.c. or more of oxygen which could not be removed by bubbling through absorbing solutions. An airtight system of glass tubes and large reservoirs therefore was constructed so that nitrogen from a cylinder might bubble through several pyrogallol chambers and also remain for several days standing over a surface of the same solution. The nitrogen finally obtained in this way contained 0·1 p.c. oxygen or less, as determined by Haldane's method, and gave clear cut results when used.

To eliminate, however, the possibility that failure of the nerve in this gas might be due to something formed from the pyrogallol (*e.g.* carbon monoxide) rather than to oxygen lack, the whole system was later charged with alkaline hydrosulphite solution in place of the pyrogallol. The nitrogen obtained then showed no oxygen at all on analysis and the nerves behaved just as before.

In one experiment, to eliminate all possibility of direct oxidative processes due to the traces of oxygen always present some hydrogen cyanide gas was passed into the chamber instead of the other gases. This was generated by dropping sulphuric acid on solid sodium cyanide in a test tube and led into the chamber as usual. The outlet tube dipped into strong alkali to absorb any cyanide leaving the chamber. The results were again like those in nitrogen.

Results.

(*a*) *Initial heat.* For a 10 sec. stimulation of nerves, the maximum deflection of the galvanometer gives the total initial heat directly, so a curve connecting successive maxima obtained by regular repeated stimulation indicates directly any changes in the initial heat. Fig. 1 contains

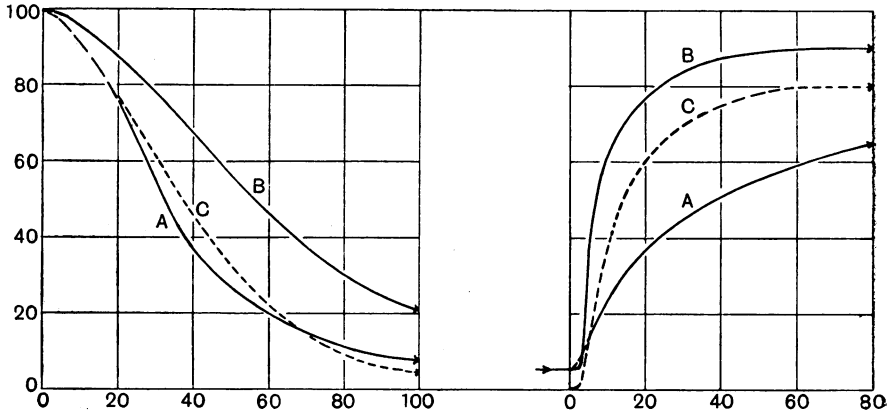

Fig. 1. Failure of (*A*) initial heat and (*B*) action potential [(*C*) = action potential squared] in nitrogen, and return in oxygen. Vertically, maximum deflection of galvanometer in p.c. of the initial value: horizontally, time in nitrogen or in oxygen following nitrogen. The dashed portion of (*A*) is interpolated.

such curves showing the failure of initial heat when a nerve is deprived of oxygen and its return when oxygen is re-admitted. The curves pass through mean points obtained by averaging observations from over ten individual experiments, all of which gave consistent results. The first

portion of each curve (dashed) is interpolated, since no observations can be made at once after changing the gases.

Twenty minutes after the displacement of oxygen is begun (five minutes after it is finished) the heat has fallen to three-fourths of its initial value. It continues to fall rapidly at first and then more slowly, to 7 p.c. of its initial value in 100 min. and to zero in about three hours. If oxygen is then admitted, heat production on stimulation is resumed within a few minutes and increases for an hour or two. The final equilibrium value attained is occasionally just as high as the original one, but usually is less, about 20 p.c. less on the average.

In two experiments the nerves were asphyxiated and allowed to recover twice. In one, during the first asphyxiation they were tetanised for 30 sec. every min., and during the second asphyxiation for 10 sec. every 10 min. In the other experiment this order was reversed. It was hoped to show in this manner that, when more active, nerve heat failed in nitrogen in a shorter time than when almost resting. There was no clear cut difference in either case, though probably the frequent stimulation did hasten complete failure of response.

The rate of recovery of heat production after oxygen was re-admitted into the chamber seemed definitely to vary with the time the nerves had been in nitrogen. If the nerves were kept in nitrogen two or three hours after heat production had completely failed, recovery in oxygen was slower than if oxygen was admitted shortly after failure was complete. Due possibly to the time factor, recovery was much more rapid when oxygen was admitted before complete failure than when admitted after it. No measurable recovery followed the admission of small amounts of air until the gas contained 2 to 3 p.c. of oxygen. (One experiment.)

(*b*) *Recovery heat.* This is determined from the total area of the galvanometer deflection-time curve. In a normal production of heat the ratio of the total area (in mm.–sec.) to the maximum deflection is constant at about 190. As the time in nitrogen increases the maximum deflection steadily falls and the area also falls, but the ratio remains unchanged. The general shape of the curves in air or nitrogen also remains the same (Fig. 2), though careful recording shows a slight delay in the nitrogen curves reaching the maximum deflection (Fig. 3). The small difference in maxima remains after oxygen is re-admitted in cases where the nerve heat does not return to its full original value, and is due to failure of the deeper fibres next to the junctions to produce heat. The heat produced by other fibres must therefore be conducted through them and is so delayed. This does not modify the general fact that the total

heat remains in quantity and time distribution in essentially constant relation to the initial heat. The recovery heat, then, fails in lack of oxygen,

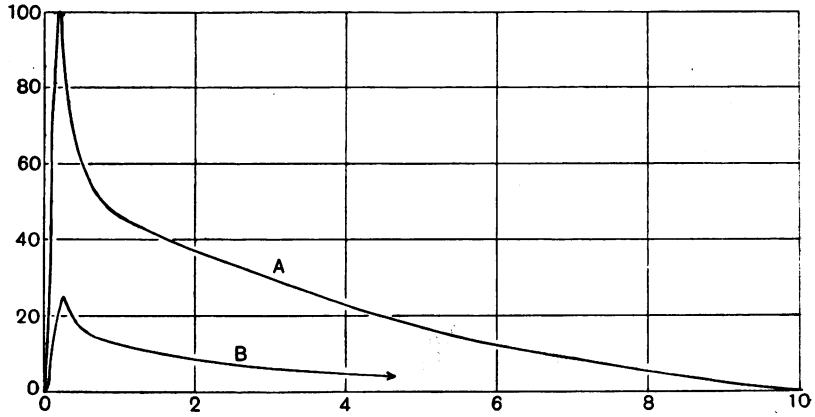

Fig. 2. Vertically, deflection of galvanometer in arbitrary units: horizontally, time in minutes. Curves for 10 sec. stimulation (*A*) in oxygen, (*B*) after 1 hour in nitrogen. The general shape of the curves, corresponding to heat production, is the same in both.

but only in so far as the initial heat that it follows is failing. This statement may not be exact in its comprehensiveness, for the error in taking

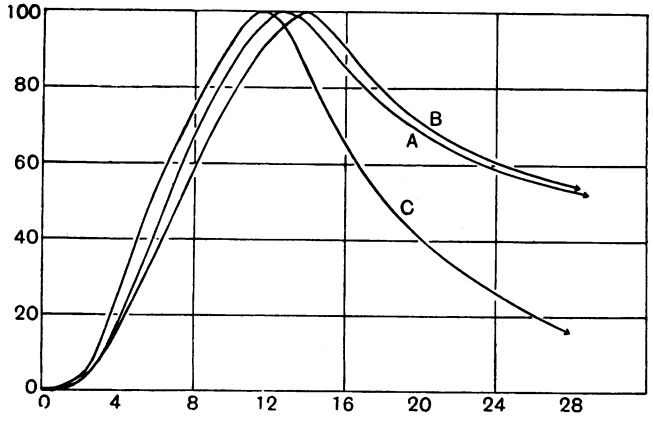

Fig. 3. Vertically, deflection of galvanometer in p.c. of maximum: horizontally, time in seconds. Curves for 10 sec. stimulation (*A*) in oxygen, (*B*) after 1 hour in nitrogen; or (*C*) for 10 sec. warming with alternating current through the length of the nerves after they had become entirely inactive in nitrogen.

total heat curves in nitrogen is considerable (due to drifts, the continually changing state of the nerve, and the relatively few observations that can

be made and averaged in each experiment). It is impossible to exclude discrepancies of, say, 25 p.c. in the ratio of initial to total heat in nitrogen, but if such a change in the ratio exists it is certainly an increase; *i.e.* there may be somewhat more delayed heat in proportion to initial in nitrogen than in air. This is suggested by the results of prolonged stimulation.

When the nerves are tetanised in air or nitrogen for 5 to 10 minutes, or warmed after becoming inactive in nitrogen by passing a weak alternating current through their length for a similar time, heat curves like those shown in Fig. 4 are obtained. The control warming curve has reached

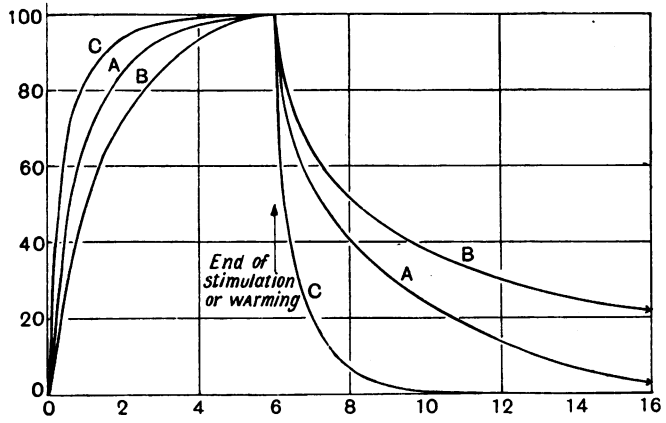

Fig. 4. Vertically, deflection of galvanometer in p.c. of maximum: horizontally, time in minutes. Curves for continued stimulation for 6 min. (*A*) in oxygen, (*B*) after 1 hour in nitrogen; or (*C*) for equivalent warming of the totally asphyxiated nerve.

90 p.c. of its maximum deflection in one and a half minutes after warming starts and correspondingly lost 90 p.c. of its deflection one and a half minutes after warming ends. For stimulation in oxygen the deflection is only 78 p.c. complete in one and a half minutes after beginning stimulation, which means that relatively more heat is being produced after this time than in the control. The latter represents heat production at a constant rate, so that the nerve must be giving heat at an increasing rate; in other words, the delayed portions of the nerve heat for each impulse continue to sum (until at the end of delayed heat production from the first impulses, in about 10 minutes, the rate of heat production reaches a maximum). Also because of the delayed heat, the fall after stimulation ends is less rapid than the rise, only 55 p.c. of the deflection being lost in one and a half minutes. In the case of nitrogen, the

same effect is seen more exaggeratedly, so that if the above interpretation is correct the delayed heat is relatively more important here; that is, the initial heat has been more cut down in nitrogen than has the delayed heat, or the delayed heat has been prolonged in time. It may be noted that the delay in rise and fall of these curves could not be explained by inactivity of the deeper fibres, for the delay so introduced is only about one second. Also any falling off of heat as stimulation continued, due to fatigue, would tend to hasten the maximum deflection rather than delay it, so the difference between the curves obtained in air and nitrogen could not be due to greater fatigability in the latter. The possibility of some heat being conducted down from the stimulating electrode region is, however, very difficult to exclude in such prolonged stimulations; and if it occurred it would have a relatively greater effect in nitrogen because the live heat is less, and the effect would be to delay the maximum. It is a just possible alternate explanation of these curves.

The two experiments with vaseline and cyanide have been mentioned. In both of these the total heat curves retained their normal shape and area-maximum ratio. The fall of maximum deflection with time was also in general similar to that in nitrogen alone, though it was striking that under vaseline the nerves lasted considerably longer than without it.

(*c*) *Resting heat production.* All the observations on nerve heat production so far given apply to the excess heat production of activity over that of rest. The latter cannot well be measured by these methods, for it would reveal itself only as a constant potential from the thermopile. If the system settled down to true zero, so that with a dead nerve, say, the potential always was zero, it would be simple to obtain the resting heat production from the potential when the nerve was living. The random errors are too great and equilibrium too imperfect to justify this ordinarily. When, however, a nerve has come to equilibrium in air, is then allowed to reach a steady state in nitrogen after excitability is entirely lost, and finally returns to the original equilibrium in air, it may be assumed that the difference between the two equilibria represents a change in resting heat production. If, further, it is assumed that in nitrogen all resting metabolism is stopped (an assumption approximately valid only after some hours in nitrogen, for at first metabolism surely continues, but at a diminishing rate), a rough estimate of the resting heat production in oxygen may be made. This comes out at somewhat under one-third of the excess heat due to continued activity or at a rate of $2{\cdot}0 \times 10^{-5}$ cal. per sec. The value cannot be regarded as exact, but is of interest for comparison with the resting gas exchange of nerve. This

has been measured recently under conditions nearly identical with those of these heat experiments (6), and the resting O_2 consumption is 16 c.mm. per gram per hour, corresponding to $2{\cdot}3 \times 10^{-5}$ cal. per sec.

(*d*) *Action potential.* The average action potential falls with time in nitrogen, though less rapidly than does heat, and returns in oxygen much more rapidly (Fig. 1). Since heat directly measures energy, and the action potential does not, a direct comparison of them is of limited value. The true energy equivalent of the action currents cannot be obtained (except by integration from the exact form of the action potential curve obtained under the given conditions); but since energy varies as the square of potential, this gives a better series of values for comparison with the heat. The dashed lines in Fig. 1 represent the square of the action potential which more nearly, but still far from, parallel the heat curves. The average action potential may be reduced by short circuiting through inactive fibres or increased by prolongation of the action potential in each fibre, so that the galvanometer records a varying fraction of the true potential changes as asphyxia progresses. The disagreement with the heat values is, therefore, not very significant.

In the first experiments it was found regularly that the action potential increased markedly during the first few minutes in nitrogen. This was finally traced to the fact that prior to admitting the nitrogen the nerves were being stimulated every 5 or 10 min. only, whereas after admitting it tetanisation was repeated every minute to obtain a good series of readings during the initial part of the fall. The effect is entirely independent of the presence of oxygen and was eliminated by stimulating at the same time intervals before and after displacing the oxygen. Fig. 5 shows the effect of frequent or infrequent tetanisation in air—the total action potential regularly shows a "treppe" with frequently repeated stimulation. This may be due to increased height of the maximum potential developed or to a longer duration of potential following each impulse or, of course, to both. The method used does not permit discrimination between them, but other workers have found evidence of both effects. Davis and Brunswick (7), using a string galvanometer ballistically, have observed a rise in total action current from one impulse to the next when tetanisation is begun, and Prof. Gasser has informed me that the oscillograph shows a definite rise in the maximum potential through the first few impulses of a tetanisation. On the other hand, Tigerstedt (8), Borruttau (9), and other workers have found that the descending limb of a single action current becomes definitely prolonged and the maximum lower under conditions which might be regarded as

leading to fatigue or injury—continued stimulation, asphyxia, cold, etc. It is difficult, therefore, to know how to interpret this effect; as a "fatigue" setting in very early or as a "facilitation." The behaviour of heat production with different intervals of tetanisation gives a clue to this,

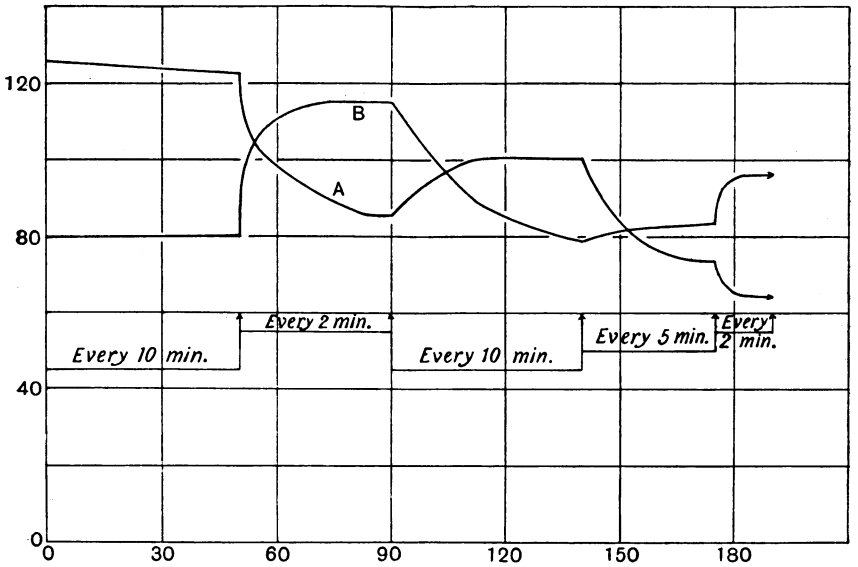

Fig. 5. Vertically, maximum deflection of galvanometer in arbitrary units: horizontally, time in minutes. (*A*) initial heat and (*B*) total action potential produced by 20 sec. stimulations at 2, 5 or 10 min. intervals in oxygen. The heat for each stimulus is less and the total action potential more for short intervals between stimulation periods than for long ones. The steady fall of heat with time, shown by the general slope of the line, is not usual.

though the results have not been entirely uniform. Fig. 5 shows the results of an experiment which represents the course of heat production in most cases, and shows a clear cut falling off with frequent stimulation, but in a few experiments this effect has been absent for no discovered reason. Field and Brücke(10) have found that the absolutely refractory period of nerve may be increased ten times during ten minutes' continued tetanisation; and observations on the excess oxygen consumption of a nerve stimulated at different intervals point in the same direction(6). The conclusion seems justified, therefore, that for minutes after a tetanisation lasting a number of seconds a nerve is less able to liberate energy than before; in other words, it shows definite "fatigue" after short activity. This is also in accord with the existence of the prolonged recovery heat lasting for ten minutes.

Discussion.

The results given above indicate a greater dependence on oxygen than other workers have obtained. Fillie (11), for example, found it impossible to asphyxiate nerves in saline containing between 0·1 and 0·3 mgm. of oxygen per litre (corresponding to 0·2–0·7 p.c. O_2 in a gas), whereas in these experiments heat production and action potential failed in gas containing over 1 p.c. of oxygen. It may be emphasised that no great difference in the effects of 0·2 to 1·0 p.c. oxygen was observed, though asphyxiation may have proceeded more rapidly with the lower concentrations. The availability of oxygen to the individual nerve fibres depends, of course, on its being dissolved in the tissue fluids about them, and in isolated nerve the question of oxygen diffusion must be considered.

The oxygen used by resting and active nerve respectively is 0·00027 and 0·0013 c.c. per grm. per min. (6). The amount of oxygen that could be dissolved in 1 grm. of nerve exposed to air[1] is about 0·0112 c.c. The rate of saturation of the nerves as actually used, assuming no oxygen present at the start, may be calculated from Fourier's diffusion theory. Mr F. J. W. Roughton has kindly done this for me and the results are that they would be 17 p.c. saturated in 5 sec., 60 p.c. in 30 sec., and 80 p.c. in 60 sec. If it be assumed that a balance between oxygen used and oxygen diffusing in must be established every five seconds (the nerve becoming entirely depleted in the process), the amount used by 1 grm. of resting nerve is 0·000028 c.c. and this must equal 17 p.c. of the final content of oxygen at saturation. The latter must, therefore, be 0·00017 c.c. and requires an outside oxygen pressure of $\frac{1}{65}$ that of air or 0·3 vol. p.c. of O_2 in the surrounding medium. This value (not allowing for activity) is much too low, for when the nerve has become 17 p.c. saturated with oxygen it will not be evenly distributed throughout the tissue and the deeper fibres will still not be obtaining anything like an adequate supply of the gas. Also, the nerve does not alternately use up all the dissolved oxygen and then wait for more to diffuse in, as assumed, but an equilibrium between use and diffusion is established at some constant oxygen content. This would considerably lower the rate of diffusion in, *e.g.* if the nerve remained half saturated the increment of oxygen during the first five seconds would be 8 p.c. instead of 17 p.c. of the final saturation value.

[1] Solubility of oxygen from air (at 15° C.) in 1 c.c. H_2O = 0·007 c.c., in oils about 0·038. Composition of nerve = $\frac{4}{5}$ water, $\frac{1}{6}$ lipoid, $\frac{1}{8}$ other solids. Sp. gr. = 1·06. Assuming O_2 half as soluble in the non-lipoid solids as in water, the amount in each of these would be, per grm. nerve, 0·0046, 0·0064, and 0·0008 respectively, sum = 0·011.

A more certain estimation of the diffusion factor can be made from Warburg's(12) work. He developed an equation for the equilibrium condition when a slice of tissue having one dimension definitely smaller than the others is exposed to various oxygen concentrations; and using Krogh's(13) observed values of oxygen diffusion constants for various tissues and the rate of oxygen consumption of his tissues, he was able to calculate the limiting thickness of the slice which would still permit the deepest cells to obtain oxygen. As he expressed it, $d = \sqrt{8Co\frac{D}{A}}$, where d = the thickness of the slice of tissue in cms., Co = the external oxygen concentration, D = the diffusion constant for oxygen (expressed as the number of c.c. of O_2 passing through a tissue membrane of 1 sq. cm. surface and 1 cm. thick when the difference of oxygen pressures on the two sides is 1 atmos.), and A = the oxygen consumption of the tissue in c.c. O_2 per grm. of tissue per min. This may similarly be written $C = \frac{Ad^2}{8D}$ where C is the unknown oxygen pressure necessary for a tissue of thickness d.

The nerves lying on a thermopile were laid parallel and in lateral contact, so they may be regarded as roughly forming a tissue slice with one small dimension. Eight similar nerves, cut at 2·0 cm. length, weighed on the average 0·052 grm. and, at specific gravity 1·06, occupied ·05 c.c. The width is equal to eight times the depth, so d cm. $\times$ $8d$ cm. $\times$ 2cm. = ·05 c.c. and d = 0·058 cm. In Warburgh's experiments, the tissue is exposed on both faces, whereas in these only one face is exposed to oxygen, so the effective thickness is twice as much, or d = 0·116. D, calculated from Krogh's data for fascia and corrected for temperature, is $1{\cdot}1 \times 10^{-5}$. (The true value for nerve is probably a bit higher because of the lipoids present.) A, assuming the resting oxygen consumption above, is 0·00027. C, calculated from these data, is equal to 0·04 atmos. oxygen pressure; that is, for the deepest nerve fibres to receive the oxygen necessary to maintain their resting metabolism, the surrounding gas must contain 4 p.c. oxygen. For full activity nearly 20 p.c. would be needed! The general basis for this calculation has been thoroughly confirmed by Warburg experimentally, and its validity for nerve has also been shown(6).

It is not surprising, in view of these figures, that the nerve heat of activity falls readily in low oxygen containing media, and only reappears with 2 or 3 p.c. oxygen in the chamber. The results of Fillie are not difficult to account for by the test used. The nerve studied ran through an asphyxiating chamber, proximal to which it was stimulated and beyond

which it ended in its muscle. The presence of a minimal muscle twitch was taken to indicate the presence of conductivity. It is obvious that some fibres about the surface of a nerve will continue to receive oxygen long after the majority have been asphyxiated, and these would give a minimal contraction. It is easy to estimate that in the nerves used here the thickness of the plate represents about 70 layers of individual fibres. The bottom layer of fibres, therefore, receives oxygen at a concentration corresponding to 0·05 vol. p.c. oxygen when the whole nerve is exposed to the critical 4 p.c. of oxygen, and at this concentration is able to function; so that fibres on the nerve surface should remain active in media containing this amount of oxygen, and probably even with considerably less. The total heat and action current from such a few fibres would, of course, be immeasurable.

The question next arises why the failure of nerve heat and action current in nitrogen? The total heat due to activity depends on three factors; the number of fibres acting, the frequency or number of impulses conducted by each, and the heat produced by each impulse. A decrease may be due to any or all of these[1].

The possibility exists that due to lack of oxygen the immediate recovery processes following conduction (during the refractory periods) are delayed so that the nerve can respond to fewer stimuli per second. Fröhlich(14) some time ago claimed that the absolutely refractory period of nerve was much prolonged in nitrogen, but Cooper(15) was unable to confirm this. Even she found, however, that in the later stages of asphyxia the least interval between two stimuli for muscular summation increased several times. This effect she believed due to conduction with a decrement in asphyxia so that the second impulse becomes extinguished if too early and therefore feeble, and she concluded that oxygen is not directly involved in the conduction of an impulse or immediately following. Later work of Kato(16) and others throws doubt on this interpretation and the ultimate conclusion is left open. It is highly probable, however, that as asphyxia proceeds the fibres cannot follow as frequent stimulation as they can in O_2.

Another possibility mentioned, that for each impulse actually transmitted the heat production is less would, of course, follow from the above when tetanising stimulation is carried out, in that each impulse falls on the nerve less recovered from the previous impulse than normally and

[1] The further analysis of these several factors in the case of heat production is very difficult though not impossible, but for action potential it is relatively simply done, and in a quantitative manner. This will be more fully gone into at another time.

so gives less energy liberation (3). There is, however, the further possibility that even for widely spaced impulses the heat due to each is less in the absence of oxygen. This would mean either (1) that all processes involved in conduction can be partially and equally inhibited—which by analogy with the action of narcotics may occur; or (2) that conduction depends on immediate processes for which oxygen is not essential and which produce but little heat, which are followed by secondary processes not essential to conduction but depending on oxygen and also producing heat. The recovery heat of nerve comes at once to mind, but it has been shown that this fails in nitrogen no faster than the initial heat associated with conduction itself; so that if such an unessential heat producing reaction dependent on oxygen does exist it must accompany conduction itself and occur within a few sigma of the explosive liberation of energy associated with conduction. It seems most unlikely that conduction should depend on some anaerobic reaction of little heat with an unessential explosive oxidation superimposed, and the whole followed by a large and prolonged anaerobic "recovery," and need not be further considered in the absence of direct evidence for it.

The third factor mentioned, that as asphyxiation proceeds more and more fibres fail entirely to conduct, undoubtedly plays a rôle, and possibly the main one, in the fall of heat. Evidence has been given that the deepest fibres are thrown out first in low concentrations of oxygen and presumably the uppermost ones fail last. This statistical falling off of heat, however, gives no clue to what happens in each fibre, and it is of basic importance in the interpretation of activity to know how the resting nerve is affected. Some suggestive evidence on this point has been given. The resting heat production of nerve presumably does not fall at once to zero in nitrogen but gradually diminishes for some time. Up to a certain stage of asphyxiation a fibre can regain full activity in oxygen; beyond this it is permanently inactive, as evidenced by the rate and completeness of recovery after different durations of asphyxia. There is no question of the nerve requiring oxygen for its basal metabolism, nor is there doubt that when the reactions of maintenance are interfered with the nerve machinery must be disrupted and conduction fail. Muscle, with a well-established anaerobic contractile mechanism, will also fail entirely to act when the medium is sufficiently depleted of oxygen (17) though with a little present it does as well as with much more (18). If this, however, be the sole cause of failure of nerve heat in absence of oxygen there is no reason to believe that conduction itself requires oxygen. To borrow Lucas' analogy, telephone service would cease if the central exchange

were filled with nitrogen in place of air, but this does not prove that oxygen is required for the passage of messages along the wires. There are many other means of stopping conduction—cold, pressure, narcotics, acids, etc.—that give a fall of conductivity similar to that in nitrogen, which may act on the conductive mechanism or on the more basic machinery of cell survival. Several investigators (15, 19) have in fact concluded that the failure of conduction in asphyxia is due solely to the accumulation of toxic metabolites, but this conclusion is open to criticism.

The fact remains, however, that during activity a nerve uses more oxygen and produces more heat and carbon dioxide than when at rest. The amount of heat produced agrees very well with the gas exchange observed on the assumption that the heat is practically all oxidative. The fall of excitability, the slowed conduction, the tendency of action potential to give equal effects on charge and discharge, and other qualitative effects of oxygen lack, seem to indicate that it enters directly into the conduction processes. On the other hand, though asphyxiation cuts out initial and recovery heat, there is no certainty that this is more than the result of the progressive failure of individual fibres due to interference with their basic metabolism.

One possible way out of this antinomy has been mentioned in a preceding paper (2), and the time course of nerve heat failure in nitrogen falls in line with this. After nitrogen has replaced air, the oxygen content of the nerves must have reached equilibrium, by diffusion or utilisation, within a few minutes. Still the falling off of activity continues more or less slowly for two or three hours before it becomes immeasurable. It is tempting to account for this by an internal reserve of oxygen or its equivalent. "Intramolecular oxygen" has often been suggested in the past and as often denied. As applied specifically to nerve, Fröhlich (14), and more extensively Gottschalk in 1914 (19), argued in favour of an oxygen reserve from his observations that after being asphyxiated in nitrogen a nerve completely regained its conductivity (measured by a muscle twitch) in 2 to 3 min. in oxygen but continued to show improved resistance to a subsequent asphyxia until it had been in oxygen for 8 min. Subsequently (1921), from a somewhat too rigorous application of the mass action law, he concluded that all oxygen stored in a nerve would necessarily diffuse out after eight minutes in nitrogen, and that, therefore, the nerve has no oxygen store but conducts by an anaerobic mechanism. It need only be pointed out here that the assumption that all physically dissolved or chemically bound oxygen would diffuse out

of the nerve on changing its environment from oxygen to nitrogen in the same time that it took to enter when the reverse change was made is not valid.

Living cells are normally poised at some characteristic oxidation-reduction level, but if kept under anaerobic conditions this level steadily falls. Cannan, Cohen and Clark (20) have shown that the electrode potential of a suspension of bacteria, yeast cells or liver, falls with time of anaerobiosis (= more reducing state) along an exponential curve; suggesting that a monomolecular reduction of some contained substance is taking place. B. E. Holmes (21) has found 50 mgms. of glutathione in 100 grm. of white matter of rabbit's brain, and Keilin (22) reports the presence of cytochrome in brain tissue. This amount of glutathione alone, if all oxidised at first (a doubtful assumption) and completely reduced by the tissue during anaerobiosis, would serve as an adequate oxidiser, or hydrogen acceptor, for 50 min. metabolism of a nerve during activity and over 3 hours during rest. Such figures serve at least to indicate how far an oxidation reserve might supply the extremely small energy requirements of active nerve. A variation in the amount of such substances from fibre to fibre, the varying oxygen available at different depths, and different metabolic rates of various fibres, could account for the gradual decline of nerve heat; though the heat per fibre also probably undergoes a fall. Similarly the delay of recovery in oxygen might be partly due to an excessive utilisation of oxygen by the outer fibres to restore their impoverished reserves, so that no oxygen would reach the deeper ones for some time.

It is worth noting finally that, so far as all evidence hitherto obtained goes, the delayed heat of nerve is dependent only on the initial breakdown; no matter how the latter is varied the recovery heat follows. This strongly confirms the previous suggestion that the delayed heat depends on a monomolecular reaction of some substance freed during the initial phase.

Summary.

1. Removal of oxygen from a nerve's environment causes:

(*a*) A progressive fall and ultimate extinction in two or three hours of the initial heat of activity. This is largely due to individual fibres becoming inactive, probably also in part to decreased heat per fibre.

(*b*) A similar fall and extinction of the delayed heat. The delayed heat continues to represent the same fraction of total heat and shows no tendency to fall more than the initial heat.

(*c*) A progressive fall of resting heat production, ending with death of fibres if sufficiently continued.

(*d*) A progressive fall and extinction of average action potential. This does not parallel the fall of heat.

2. All these return to or towards their original state when oxygen is readmitted to the system. The action potential returns much more rapidly than the heat, which requires an hour or two.

3. Practically complete asphyxiation of nerve can be obtained under the conditions of these experiments in nitrogen containing over 1 p.c. of oxygen.

4. The capacity for activity of a nerve is definitely affected by previous activity within several minutes. This appears to be a type of "fatigue."

5. The problem of oxygen diffusion into nerve and availability for its metabolism is considered.

6. The above results are difficult to reconcile with a glycogen-lactic acid system, but do not disprove an oxidative mechanism for conduction; hydrogen acceptors or other oxidising bodies in nerve may permit oxidative activity for considerable time in the absence of oxygen.

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