Supplement to NATURE

No. 3106

MAY 11, 1929

The Maintenance of Life and Irritability in Isolated Animal Tissues.¹

By Prof. A. V. HILL, F.R.S.

NOT infrequently one hears the view expressed that physiologists are too ready to work with isolated tissues, not willing enough to study the intact and living animal. The isolated organ is said to be 'abnormal', its behaviour too remote from that of its previous self, in its usual environment, to throw much light upon the normal processes of life. There is indeed a danger that those who work under artificially simplified conditions may, in their enthusiasm, extend their results too far: the greater danger—I speak with feeling—is that their friends, and the daily Press, may do so for them. If you describe how a nerve-fibre maintains the electrical potential difference at its boundary by an active process involving the consumption of oxygen, you may find your name in all the newspapers, and be invited to America to raise people from the dead: if you prove that chemical disintegration sets in, in a stimulated muscle deprived of oxygen, you may be charged with attempting to create a living cell (and indeed a living 'soul') in the laboratory.

Realising this danger, however, appreciating that only after hard and critical thinking may the results of laboratory work under simplified conditions be applied to the graver practical problems of life, we may—in fact, we must—go forward in the confident belief that only by investigating phenomena under such simplified conditions can we really hope to understand them.

Here, to the scientific mind, is in fact a definite and presumably soluble problem, that provided by 100 mgm. or so of isolated muscle or nerve, capable of responding in a regular and reproducible manner to certain treatment: academic—certainly: so was the study of the conduction of electricity through gases—until it led to X-rays and amplifying valves: abnormal—if you like: but still a fact, and one that presumably can be explained. The irritability, the responsiveness, of this little bit of surviving tissue can be maintained, under conditions which we are beginning slowly to understand,

for considerable periods: and during all this time we can study the processes of life, in abnormal form if you wish, but still as phenomena, as facts, under conditions which allow us to apply the methods of physics and chemistry as we could never hope to do in the normal intact animal.

ENERGY EXCHANGES IN NERVE.

The isolated nerve of a frog, placed in an appropriate salt solution containing oxygen, will live, or at any rate continue to function, for days. We can detect its activity most readily by leaving it connected to a muscle, which will twitch when we stimulate the nerve. A better method, since it involves the properties of the nerve alone, is to record the 'action current', which passes for a few thousandths of a second between electrodes placed upon its surface. Another method, but more difficult to apply, is to measure the heat produced by the nerve when stimulated.

For long periods the surviving nerve will show all the outward and visible signs of a response to stimulation. During prolonged survival at rest it consumes oxygen and gives out carbon dioxide: at 20° C. about half a cubic millimetre per gram per minute, more at a high temperature, less at a low. During maximal activity, due to continual stimulation, its metabolism is doubled, its oxygen consumption at 20° C. is about one cubic millimetre per gram per minute. It gives out corresponding heat. Of this heat, only about one-tenth appears during the passage of the impulse—the rest comes off slowly, during the following fifteen minutes: clearly it is related to some recovery process, by which the nerve is 'recharged', by which its potential energy, so to speak, is restored.

The fact that extra oxygen is used as the result of activity is, in a sense, easy to understand. Break-down has occurred, free energy has been liberated, and if the process is to be reversed, oxidation is necessary to supply the free energy required in the re-synthesis. The oxygen consumption at rest is much more difficult to comprehend. Why should

¹ From the Ludwig Mond Lecture delivered at the University of Manchester on Mar. 6.

an isolated tissue, doing nothing at all except continuing to exist—that is, continuing *to be ready* to respond to a stimulus—require what is in fact a considerable amount of oxygen, three-quarters of its own volume per day at 20° C., three to four times its own volume at human body-temperature? Energy, we may say, is required to maintain the organisation. In what manner, however, is the energy being applied? What will happen if the supply of oxygen be stopped?

The last question is very readily answered by experiment. The air around the nerve is replaced by pure nitrogen, and from the known diffusion constant of oxygen and the known oxygen consumption of the nerve, we can calculate that in a very few minutes not a trace of molecular oxygen is left. A stimulus is applied at intervals, and the action current, or the heat, is used as a sign of activity. At first no particular change occurs: the nerve responds as before. Long after *all* the molecular oxygen is gone, action current and heat-production remain almost unaltered. Even the recovery heat, which surely is of oxidative origin, is unaffected. Presumably there is some source of intra-molecular oxygen, or some store of hydrogen-acceptor, which, for a time, can supply the energy required for recharge. Gradually, however, a change comes on: action current and heat diminish, and in two hours after the oxygen was removed they disappear together.

The nerve, however, is not dead: let oxygen in and it revives: its return is gradual, much slower than the inward diffusion of the gas—the oxygen clearly has some duty to perform, some debt to pay, before the situation is cleared up. The nerve asphyxiates much quicker a second time if its exposure to oxygen be cut short. Indeed, by the admission of oxygen alone, complete recovery from asphyxia is not possible: however long be the exposure to oxygen, subsequent asphyxia (as Gottschalk showed) is quicker than it was originally. Washing the asphyxiated nerve with *oxygen-free* salt solution restores it temporarily. Complete restoration, however, is attained only if washing be combined with oxygen. Then the nerve returns triumphantly to its full initial activity, apparently unaffected by the intervening period of asphyxia. It seems as though, in the absence of oxygen, two things have happened: (a) some metaphorical accumulators have run down and need recharging—a process which requires oxygen; and (b) certain abnormal substances have appeared, which cannot be removed by oxygen, but will diffuse away into surrounding salt solution.

POTENTIAL DIFFERENCES IN NERVE.

Many attempts have been made in recent years, before the latest and most successful ones, to measure the gaseous exchanges of isolated nerve. Actually in the refined methods employed by Meyerhof and by Warburg, modifications of those of Barcroft, a means has long been available of making these important measurements. When Downing and I succeeded at last in measuring the heat-production of nerve, it seemed to us, and to Gerard who had joined us, that corresponding determinations of oxygen consumption should be carried out. I wrote, therefore, to Meyerhof and asked him if Gerard could come to make these with him. Meyerhof waited a day to reply: his answer was, as I expected, "Of course, let Gerard come"; but also, as I had not expected: "es ist ausserordentlich leicht, I did it yesterday on the receipt of your letter". So Gerard went and made the experiments in Berlin. At the same time Fenn was doing the same thing in his laboratory at Rochester, New York. The oxygen consumed at rest, the oxygen needed for activity, and finally, the oxygen required for recovery from asphyxia, were all measured and are now tabulated for those who need to use them for their calculations.

I mention these measurements partly for their own sake—as the happy ending to a long series of persistent attempts—but more particularly for a curious by-product which, like many by-products, is likely to prove more important than the original object. An American worker, some years before, attempting to measure the carbon dioxide production of nerve, had employed a very convenient object, the limb nerve of the spider crab. Reading his paper I noticed that, whereas he had stimulated the nerve for long periods, he gave no evidence that the nerve had really responded at all to his stimuli. Knowing from experience of medical practical classes how often nerves do not respond to the best-intentioned stimuli, I thought I had better try for myself. So, being at Plymouth, where there are much bigger and better spider crabs than in America, I tried, and by good fortune a whole beautiful new field of work appeared.

The experiment was a simple one. *A* and *B* are two non-polarisable electrodes placed upon the nerve, which for the sake of the argument we take as a single nerve fibre. *A* is at an uninjured point, *B* at the cut and injured end. *A* and *B* are connected to a galvanometer. A difference of potential exists between *A* and *B*, the so-called injury potential, which produces what is called the 'demarca-

tion current' when it is allowed to flow through the galvanometer. *A* is positive to *B* in the external circuit. When we apply an induction shock to the nerve at a distant point *C*, the potential difference between *A* and *B* momentarily falls as the impulse passes *A*: the current through the galvanometer diminishes: we witness what is called the 'negative variation of the injury current'. If we apply a succession of induction shocks at *C*, each produces its effect at *A* as its corresponding impulse goes by, and if the galvanometer be a relatively slow one, these effects are summed up, and as we continue stimulating, the galvanometer returns

FIG. 1.

towards its zero, deflecting again when the series of stimuli ends. Such, at any rate, is what happens in a frog's nerve. The fact has been known for two generations: it is demonstrated to students: but curiously enough—so far as I am aware—nobody had ever tried to show it in a crab's nerve. In that tissue, if stimulation be continued long enough—for a minute or two—the galvanometer does indeed move backwards during the stimulus towards its zero, recording the usual 'negative variation', but it does not return outwards again when stimulation ends.

Let me pass for a moment to a fact recently established by Furusawa. If a crab's nerve be kept in air in a moist chamber, the difference of potential between *A* and *B* is maintained for long periods. If, however, the air be replaced by pure nitrogen, the difference of potential between *A* and *B* gradually diminishes. On introducing air again it rises to its original value, not immediately but in an hour or so. Clearly, oxygen is being used to maintain a potential difference somewhere within the nerve. Where can this potential difference be other than at the surface of the fibre itself? At the cut end the electrode is in contact with the naked protoplasm of the cell: at the uninjured point the electrode is in contact with the outside of the membrane surrounding the tissue. We must imagine that the injury potential is really the difference of potential across the membrane of the nerve fibre, the contents of the fibre between *B* and *A* simply acting as a continuation of electrode

B. Apparently, then, Furusawa's observation shows that the potential difference existing across the membrane bounding the nerve is maintained by oxidation, and gradually 'runs down' if oxidation be prohibited.

Picture the passage of the impulse along the nerve as being momentarily accompanied by a breakdown, or maybe a short-circuit, of the membrane, perhaps by a change of permeability allowing local currents to run and so to propagate the impulse. Thinking of the membrane as similar to an accumulator of small capacity, a series of such momentary short-circuits might 'depolarise' it, so that by stimulation we might effect a decrease in the observed injury potential. Now Levin found, and Furusawa has confirmed the fact, that a prolonged stimulus applied at *C* causes a return of the galvanometer towards its zero, which is not followed by a deflection outwards again when the stimulus ends. Furusawa, moreover, has proved that if local fatigue at the point of stimulation be avoided by employing, in rapid succession, a series of such points, the nerve can be *completely depolarised by activity*. The 'accumulator' which lies along the bounding membrane of the nerve fibre can, it seems, be caused to run down completely by prolonged activity. Let us now withdraw the stimulus and wait. If the nerve be in air, the potential difference between *A* and *B* gradually rises again until finally it attains its original value. This is the recovery process to which I referred earlier. If, however, the nerve be in nitrogen, it remains depolarised and further activity is impossible. The potential difference existing across the boundary of the living cell is not only maintained but also restored by an active process of oxidation.

OSMOTIC DIFFERENCES IN THE EGG.

In a recent paper from Holland by J. Straub, an investigation has been described of the difference of salt concentration, and of freezing-point, between the white and the yolk of an egg. It appears that, in the living fresh egg, there is an appreciably higher concentration of potassium, sodium, chlorine, and lactate ions in the yolk, and an excess lowering of freezing-point of about 0.15° C. In preserved eggs this difference is much less. The membrane surrounding the yolk is apparently freely permeable to water, even in its live condition, and the difference of freezing-point on the two sides is much too large to be accounted for by any such effect as that of the Donnan equilibrium. A difference of freezing-point of 0.15° would correspond to a difference of pressure of 1.8 atmospheres, and it is inconceivable

that a thin membrane so extensive as that surrounding the yolk could possibly stand a pressure such as this.

It is difficult to resist the conclusion that the existence side-by-side of white and yolk cannot be regarded as a thermodynamic equilibrium, and Straub suggests that the difference of concentration on the two sides of the membrane is maintained by an active process of oxidation. It is known that oxidation occurs in the living egg, and, according to a rough calculation, the amount of energy supplied thereby is ample to account for any osmotic work that would have to be performed to maintain, against diffusion, the observed concentration differences. It is suggested that the observed inequalities in concentration and in freezing-point must be due to some active life process, and the author discusses an electrical scheme for the employment of the energy obtained by oxidation in the egg. He supposes that the membrane acts as a galvanic oxidation element for glucose, and that the difference of potential so set up across the membrane results in the transfer, against diffusion, of the various positive ions in question. Such a galvanic battery existing across the membrane, together with differences in permeability, might be a sufficient explanation of the inequalities observed. A large number of physiological phenomena would be more intelligible were we able to suppose that oxidation at the surface of a cell is largely employed in maintaining the osmotic and other differences that exist between the outside and the interior.

ENERGY EXCHANGES IN MUSCLE.

In isolated muscle it has long been known that oxygen is necessary for the preservation of the excitable state. A muscle left at rest in oxygenated salt solution maintains its condition for long periods if it be thin enough for the relatively slow process of diffusion to supply it adequately with that gas. A resting muscle uses oxygen continuously at a rate depending on the temperature: at 20° C. this is about 0.7 cubic millimetre per gram per minute: probably this process of oxidation at rest supplies the energy necessary in order to maintain the complex dynamic equilibrium of the living material in a steady state. The known diffusion constant of oxygen through muscle, as found by Krogh, and the rate of its consumption, allow us to calculate that a thin sartorius muscle of a frog can easily remain in a steady state in oxygen just so long as combustible material is available. The isolated muscle at 20° C. uses about its own volume of oxygen per day: and if it contains 1 per cent of

glycogen this form of fuel alone should be sufficient, at that rate of oxidation, for some eight days. Dissected aseptically and suspended at rest in a suitable salt solution a frog's sartorius will, in fact, if supplied with oxygen, function for a week or more.

Deprived of oxygen, such a muscle produces lactic acid from its glycogen, the glycogen breaking down in this case about five times as fast as it did in oxygen. If the lactic acid be able to diffuse away, as is the case when the muscle is suspended in oxygen-free salt solution, the muscle lasts for a day or two, responding to a test stimulus at any time within that period: finally, however, its excitability vanishes with the disappearance of its store of carbohydrate. It may remain longer if glucose be included in the salt solution. Apparently, if oxygen be not available, the breakdown of carbohydrate into lactic acid can replace the oxidation of carbohydrate as a source of energy. Presumably, therefore, in the absence of oxygen, the formation of lactic acid is the process which supplies the free energy by which the dynamic equilibrium is maintained, by which, so to speak, the accumulators are kept charged.

The term 'free energy', used in this connexion, should be understood in its strict thermodynamic sense. It is interesting and important, as Burk has recently done, to calculate the free energy of the reaction by which, in living tissue, glycogen is broken down into sodium lactate. We consider the whole process, dissolved glycogen transformed in the buffered alkaline medium of the muscle into dissolved sodium lactate. Owing to the difference in chemical structure between the lactic acid and the glucose molecule, namely, in the ratio of the number of carbon atoms to the number of carbonyl oxygens, there is a considerable amount of free energy available in this breakdown, about 400 calories per gram of glycogen transformed. For this reason, presumably, Nature has selected this particular reaction as the means of providing, in the absence of oxygen, the free energy required, either for doing work, or for maintaining (against diffusion and similar irreversible processes) the osmotic and other differences existing during life at the boundaries and other interfaces of the cell. The free energy of the oxidation of glycogen is of course greater than that of its splitting to lactic acid: it is about 4000 calories per gram. If oxygen be absent a given process requires, let us say, 1 gram of glycogen to be broken down to lactic acid, yielding 400 calories of free energy: the same process, if oxygen be present, requires only one-fifth of a gram of glycogen to be oxidised, yielding 800 calories of free

energy: 400 of the latter are wasted, apparently, in the recovery process.

If, as I said, lactic acid is able to diffuse away, the muscle can continue to function until all its carbohydrate store is spent. If, however, it be suspended in nitrogen and not in salt solution, its lactic acid cannot escape and the end comes on much earlier. At 20° C. in nitrogen a muscle produces about four millionths of its weight of lactic acid every minute: about 0.3 per cent in 12 hours. At this stage the increase in hydrogen ion concentration due to the accumulating acid renders the muscle completely inexcitable: in less than 24 hours it attains the so-called lactic acid maximum and passes into *rigor mortis*.

The same processes can be observed to occur more rapidly when activity due to stimulation is substituted for resting survival. A muscle subjected to a series of maximal induction shocks in nitrogen fails when it has given some 400 twitches, when its lactic acid concentration has reached about 0.25 per cent. Suspended in oxygen-free salt solution and stimulated with a frequency low enough to allow its lactic acid to escape by diffusion, it can give several times as many twitches (as my friend Kupalov has recently shown), and will continue until practically all its carbohydrate has been broken down; suspended in oxygen, or in oxygenated salt solution, it can give several thousand twitches and maintain its activity until all its carbohydrate is oxidised. Moreover, as is now well known, if it be fatigued in nitrogen, and then allowed to recover in oxygen, its lactic acid vanishes and four-fifths of the corresponding amount of glycogen reappears. The free energy required for the reaction

Lactate → glycogen

is provided by the oxidation of a fraction (about one-fifth) of the lactic acid. How this synthesis occurs, and what the chemical nature of its mechanism is, are not known: but it undoubtedly does occur, and not only in muscle but also in practically every organ and tissue examined.

The work of Warburg on the metabolism of tumour and other tissue is a highly important product of these studies originally conducted on muscle and earlier, in another form, by Pasteur on yeast. It seems that nearly every kind of animal tissue employs the lactic acid breakdown, when deprived of oxygen, as the source of the free energy required for the maintenance—against irreversible processes—of its steady living state: and that certain types of tissue, particularly those found in malignant growths, actually prefer the lactic acid mechanism and may be relatively incapable of employing that of oxidation.

There is no reason to believe that when oxygen is present the processes at work are other than the sum, or the resultant, of these two. The free energy of the lactic acid breakdown is apparently the source of the mechanical energy liberated by muscle, alike in the presence and in the absence of oxygen. In the former case, however, a slow recovery process ensues, in which—perhaps under the action of a galvanic combustion element, as suggested by Straub for the case of the hen's egg—the lactic acid formed in the initial process is re-formed into its precursor glycogen. Moreover, the processes of resting survival and of activity are so similar that there appear to be strong grounds for supposing that, at rest also, the primary mechanism in which free energy is liberated (to counteract irreversible processes which lead finally to chaos and death) is that of lactic acid formation from carbohydrate.

It is not a wild extrapolation from this, and from the work of Warburg, to conclude that the same is true in all animal tissues. The living cell is a complex organised system of enzymes, interfaces, potential and osmotic differences, chemical substances: infinitely improbable in the thermodynamic sense, and yet existing in a steady state so long as free energy is available to maintain the organisation. The free energy of the carbohydrate-lactic acid breakdown is apparently the *sine qua non* of this maintenance, the common factor in the organisation of living animal cells. The primary function of oxidation is the reversal of this breakdown.

REVERSIBLE INEXCITABILITY IN MUSCLE.

All who have worked with isolated muscles have found—alas, too often—that these may 'die' without apparent cause and spoil their experiments. They do it more at some seasons than at others, often for weeks on end: some muscles are worse in this respect than others: and if we have not attributed it to the machinations of the devil (physiology leads many of us to a belief in that gentleman) we have been fain to call it 'fatigue', thereby expressing our ignorance of the whole matter. If we knew that a muscle survived better in oxygenated salt solution than in oxygen gas, we talked about the removal of 'fatigue products' in the former, even though the muscle—being supplied with oxygen and at rest—was never fatigued at all. It took two chemists, Dulière and Horton, to detect what physiologists should have recognised long ago, that a state of reversible inexcitability sets in spontaneously in isolated dissected muscles. It is true that some years ago, in the biochemical laboratory at Cambridge, it was shown that the legs of frogs, kept

at a low temperature in oxygen, gradually in the course of a week or so lose their irritability, which can be restored by soaking in salt solution. It is not certain, however, that this is the same phenomenon, and in any case Dulière and Horton have demonstrated it in much more striking and—one might almost say—provoking form.

A sartorius muscle is dissected with the utmost care from a frog and suspended in moist oxygen, or nitrogen, or air: silver or platinum electrodes are brought in contact with it and at intervals its response is tested. At first stimulation leads to an active contraction; as time goes on, however, the muscle apparently dies: it is not fatigued, it has been quite infrequently stimulated, and in oxygen or air there should not be, and in fact there is not, any accumulation of lactic acid. In an hour or two the muscle is apparently dead, it responds not at all to the strongest stimulus, though if it be taken out and tested chemically it is found to show all the chemical characteristics of resting muscle.

The phenomenon is not due to oxygen as such, or to the absence of oxygen: it cannot be attributed to surrounding the isolated muscle by a gas, since it happens also in liquid paraffin and mercury. I called it 'reversible'. Immerse the muscle, when it has become completely non-excitability, in salt solution, and its excitability returns, rapidly at first, more gradually later, following approximately a course we should expect were the return of excitability due to the outward diffusion of something present in the muscle. Any reasonable salt solution will cause a return of the excitability: Ringer's solution, sodium chloride,—anything, in fact, which does not itself lead directly to inexcitability in the muscle.

It would be easy, so far, to imagine that some product of activity in muscle, gradually accumulating, produces a toxic effect which leads to the inexcitable state. This simple suggestion, however, is not sufficient. If we soak a muscle, for three hours after removal from the animal, in salt solution, and then suspend it in a gas, it will remain excitable indefinitely. If we wash it for three hours after it has become inexcitable, its returned excitability will remain indefinitely, that is, until its carbohydrate reserves are used up or bacterial invasion sets in. If, however, we wash it for a shorter time, say for half an hour, after it has become inexcitable, its excitability indeed returns, but when we place it again in a gas, it becomes inexcitable once more. Not until the sum of the times of immersion has attained a certain value does the muscle become permanently excitable in the present sense. If any product of metabolism

has a toxic effect, why should further metabolism after a 3-hour preliminary soaking in Ringer's solution now have no result? Are we dealing here with the same curious phenomenon as Furusawa found in the case of a crab's nerve? Moreover, why do the muscles of a frog's leg, allowed to remain *in situ* after the death of the animal and the removal of the skin, retain their excitability for hours, while a companion muscle dissected out and, to all intents and purposes, uninjured, becomes inexcitable in an hour? The muscle is not dead, for it shows a normal resting metabolism and can be revived by washing with salt solution: it may then live for days, which is a sign that any injury due to dissection is of negligible importance. Is the effect of a subtle physical nature, due to contact of the living tissue with a medium of unusual dielectric constant? Or is it to be attributed to the production of some substance, in or between the cells, which can be washed away by contact of the muscle with salt water? The phenomenon is an easy and dramatic one to witness, once one realises its existence, but a difficult one to explain. The realisation, however, that it occurs has greatly simplified experiments with isolated muscles, for we know now that by a preliminary period of washing we can prevent an occurrence which has spoilt so many experiments.

There is one possible explanation. If a Ringer's solution be prepared containing four times the usual concentration of potassium chloride, a muscle immersed in it slowly becomes inexcitable. One which has become spontaneously inexcitable by standing in oxygen shows no return of excitability when immersed in this solution. We might have expected a temporary return. A muscle consists of fibres and interspaces, mainly fibres. In the inside of the fibres there is a very high concentration of potassium, in the interspaces a low one. Excitability may depend—among other things—upon a normal concentration ratio of potassium across the boundary of the cell. Experiments have shown that when muscles are perfused, potassium tends to leak into the perfusing fluid. If it leaked into the interspaces between the fibres—not much would be required—it might gradually produce the same state of inexcitability as we can cause by the artificial application of a high potassium concentration. The chief difficulty in this explanation, which has much else to commend it, is that one cannot see why a few hours' immersion in salt solution should prevent any further egress of potassium. In any case it seems that the phenomenon is of a physical or of a physico-chemical nature, and has no connexion with the oxidative mechanism of the cell.

ANAEROBIC DISINTEGRATION IN MUSCLE.

I come lastly to the most difficult problem of all: the cause of a phenomenon which I discovered in 1927 and about which I speculated last spring, perhaps rather rashly, in the *Proceedings of the Royal Society*. I say rashly, partly because my paper was the innocent cause of much excitement in the autumn, when the public Press discussed—in the 'silly season'—the 'mystery of life': partly because the explanation I originally gave may not be sufficient. The facts, however, seem certain, and are these. By improvements in technique, the rate of resting heat-production of a muscle can be measured, in oxygen or in nitrogen. The muscle lies in nitrogen upon the warm junctions of a thermopile, in a thermostat maintained at a constant temperature. The rate of the resting heat-production at 18° C. is (say) 60 gm. cm. per gram per minute: about $1\frac{1}{2}$ thousandths of a calorie. The muscle has previously been washed for some time in salt solution, so that it does not now become spontaneously inexcitable in the manner described by Dulière and Horton. It is stimulated and gives a series of twitches: the heat due to activity is registered by the galvanometer attached to the thermopile: stimulation ends: the galvanometer returns: we should expect it to return, gradually, of course, to its original position, the muscle to revert to its original heat-rate. Nothing of the kind. I may illustrate what happens by a typical experiment.

Time : A.M. or							
P.M.	11.56	11.59	12.1	12.2	12.3½	12.5	12.6
Galvanometer							
deflection : mm.	22	23½	23½	22½	22½	24	23

The muscle was then stimulated by single shocks to fatigue: the galvanometer deflected, and, when the stimulation ended, returned to rest once more.

Time P.M.	12.20	12.23	12.25
Galvanometer de-			
flection : mm.	130	130	130

The resting heat-rate in this case has been increased between five and six times.

The phenomenon has never failed to appear, and it occurs always in the same quantitative form. The quotient

$$\frac{\text{increment in heat-rate per minute}}{\text{total heat by stimulation}}$$

is always (in the frog's sartorius at 17° C.) of the order of 0.0075. The high heat-rate induced by stimulation is permanent so long as the muscle remains in nitrogen. It may attain 800 gm. cm. per gram per minute after severe fatigue—more than 1 calorie per gram per hour. It may remain at this level, so long as the muscle is kept in nitrogen,

for 24 or even 48 hours, in which time the total heat liberated may be many times as great as can possibly be accounted for by the breakdown of *all* the available carbohydrate into lactic acid. Since, apart from carbohydrate, there is very little except the actual protein of the muscle which we can imagine to break down with such an evolution of heat, we are forced to conclude that the process of anaerobic activity (or its products) has somehow induced the degradation of the muscle protoplasm itself to bodies containing less total energy.

If, when the muscle has been fatigued in nitrogen, and its resting heat-rate is high, we admit oxygen, a recovery process sets in as usual, with a considerable evolution of heat. The lactic acid is removed and the muscle is restored to its previous resting condition. This occupies about an hour. If, now, we replace the oxygen by a stream of pure nitrogen, within half an hour diffusion on one hand, and the resting metabolism of the muscle on the other, have removed the last traces of oxygen from the interior of the muscle substance, and the resting heat-rate in nitrogen can again be measured. Instead of the high value found after stimulation, we now observe a low value of the same order of size as before it. The breakdown processes produced by anaerobic activity have been cut short and the muscle has regained its previous steady state. The same treatment can then be applied once more. If the muscle be stimulated to fatigue again its resting heat-rate rises: if oxygen be again admitted recovery ensues, and finally a low value of the resting heat-rate appears as before.

There seem to be two alternatives: either (1) the provision of energy by oxidation has restored to their normal state the membranes, interfaces, or agents, which in ordinary life hold apart the unstable reacting substances present in the living cell, which prevent—as I said in my paper—the organised system of the living cell from becoming a biochemical chaos; or (2) in the presence of oxygen some substances have been removed, perhaps by simple oxidation, perhaps by restoration to a precursor, which, if they be allowed to remain, assist as catalysts, or in some other capacity, in the anaerobic disintegration of the living material. One thing seems certain—the high heat-rate is a sign of some kind of irreversible breakdown or disintegration: if—as Kupalov has shown—it be allowed to continue for a few hours, no subsequent restoration of the muscle to its normal excitability is possible, either in oxygen or in oxygenated salt solution.

The phenomena in question are so curious, and the effects so relatively large and so easily demon-

strated, that they demand an explanation. They have been tested by every means available and have withstood the attack. Were they due to a change in the hydrogen ion concentration caused by the liberation of lactic acid? A resting muscle was immersed in pure carbon dioxide and its heat-rate remained practically unaltered: the carbon dioxide must have made it as acid as extreme fatigue. Were they due to a technical error of some kind? To a reaction of some fatigue product with the metals of the thermopile? The thermopile was insulated with baked 'Elo' (an artificial resin), shellac, and paraffin wax: on top of these a piece of tin-foil: and over this a further layer of wax. The phenomenon appeared quantitatively as before. It is inconceivable that breakdown products of muscular activity can penetrate wax, tin-foil, wax, shellac, and 'Elo', one on top of the other. Was it due to injury in dissection? It was found unaltered in a frog's gastrocnemius, which can be prepared with a minimum of injury. The temperature was lowered to 0° C. The high heat-rate existing in a fatigued muscle diminished to one-sixth, which is what we should expect were it due to a chemical process occurring continuously, not at all what we should look for were a technical physical error the basis of the phenomenon.

I was inclined, when I first described the phenomenon, to the first of the two alternatives just mentioned, to the belief that oxygen restores the normal interfaces, or conditions, which prevent the organised system from becoming a biochemical chaos. During the last few months, however, I have come across another effect which inclines me a little to the second alternative—perhaps both are correct. The experiment is a simple one and the result quite certain: it ought to have been made long ago: but one only thinks of these things slowly. If a muscle showing a very high resting heat-rate induced by anaerobic stimulation be immersed for an hour or two in *oxygen-free* salt solution, its heat-rate returns to its original low level: the muscle need not even be alive: it may have been 'electrocuted' by excessive stimulation, it may have been irreversibly damaged by too long a maintenance of its high heat-rate in nitrogen. Yet, under the influence of the washing, in a time which suggests diffusion outwards of some catalysing agent, the breakdown evidenced by the previous high heat-rate is completely—or almost completely—stopped. Clearly, oxygen as such is not necessary for a reversal of the effect. Perhaps if lactic acid be not already present in excessive amount, the lactic acid breakdown can take the place of oxidation in the maintenance of the normal internal architecture of the cell: perhaps,

however, *something* is set free in the absence of oxygen, which induces—or helps to induce—the irreversible breakdown of the muscle protoplasm with a liberation of energy: a something which can be dialysed away by immersion of the muscle in salt water.

It is well known that, in man, too prolonged exposure to anoxæmia may produce harmful effects lasting for a long time or even permanently. As Haldane writes: "A short exposure even with loss of consciousness produces no serious after-symptoms: but occasionally a man's behaviour is very abnormal for a few minutes after recovery." "With severe and prolonged exposure to want of oxygen the nervous after-symptoms are of an extremely formidable nature and often end in death." "The symptoms are evidently due in the main to widespread injury to the nerve cells during the exposure." "The heart may also suffer in prolonged exposure to want of oxygen. The after-symptoms may be mainly cardiac; it may be a considerable time before the heart fully recovers." "Probably every other organ and tissue in the body feels the after-effects of severe exposure to want of oxygen. The patient often enough dies of pneumonia. Acute nephritis and gangrene of extremities have been noted." And so on. May we not be witnessing here in man the after-effects of the same partial disintegration of the living protoplasm as can so easily be demonstrated in anoxæmia in the isolated muscle?

In Warburg's work we find further evidence of a harmful effect of oxygen lack. An embryo of a chick is kept for some hours in salt solutions saturated with nitrogen: oxygen is then introduced. In the normal embryo there is practically no lactic acid formation if sufficient oxygen be present: the free energy required for continued existence is supplied by oxidation. In the embryo which has been subjected to a period of anoxæmia, however, the capacity for oxidation is found to be diminished and a large part of the energy it requires must now be derived from the lactic acid breakdown. By anoxæmia, in fact, the normal embryo has been reduced to a state in which its metabolism is similar to that which Warburg has found to characterise tumour tissue. Can it be, as Warburg's work suggests, that oxygen-lack, working upon the normal architecture and machinery of the cell, leaves behind a type of mechanism analogous to that of tumour? Dare we see in the disintegrative process set up by anaerobic activity in the isolated muscle cell an exaggerated case of the harmful effect produced in man by prolonged and severe anoxæmia, or in the chicken embryo by oxygen want? It is dangerous to speculate too far, but it is foolish not to speculate at all.