AN INQUIRY INTO SOME CHEMICAL FACTORS OF FATIGUE.

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THE seat of ordinary fatigue was placed by Mosso[1] in the central nervous system, for he found that a muscle, which could no longer be made voluntarily to contract, would do so on electrical stimulation of its nerve. Later investigations by Joteyko[2], however, have shown that though these nerve centres are not unfatigable, their resistance to fatigue is incomparably greater than is the case with the nerve endings.

Thus, there is a return to the older view that fatigue is peripheral, and that normal fatigue is rather a condition in which the stimuli sent out from the central nervous system, in all probability of undiminished intensity, tend to be blocked in the passage from nerve to muscle.

It is well known that certain chemical substances may cause fatigue in a nerve-muscle preparation, either by preventing the nerve impulse from reaching the muscle, a condition generally spoken of as fatigue of the nerve-ending, or by lowering the irritability of the muscle itself. The present research was carried out at the suggestion of Prof. Gotch, to whom I am indebted for much kindly help and advice, to determine further the mode of action of some of these substances.

Method. The experiments were carried out on *Rana temporaria.* The brain was first destroyed by pithing, the spinal cord being left intact. The preparation was then treated in such a way as to leave only the spinal column, back muscles, kidneys, aorta, and abdominal vein, the last being preserved with a strip of muscle on either side. The right iliac artery was now ligatured, a cannula inserted into the aorta, and the blood completely washed out of the left leg before coagulation could take place. During the breeding season the blood of male frogs coagulated so readily as to make perfusion impossible.

The preparation was fixed to the board by pins at the knee and ankle joints, and along the spinal column. A small "window" was next cut in the skin at the ankle and a thread attached to the achilles tendon.

The perfusion height was about 35 cm., and when boiled liquids were employed they were placed in flasks to avoid oxygenation.

The muscle was excited directly by needles, one placed in the region of the tendon achilles, and the other at the knee joint. Indirect stimuli were given from the cord, one needle being inserted into the spinal canal, and the other into the bone at the lower end. The current was obtained from two Daniell's cells, the secondary coils being at 0 for direct stimulation, and just supra-maximal for indirect, in the latter case the coils had no iron cores. Current reversers were placed in each circuit so that the place of stimulation was sometimes the upper and sometimes the lower end of the muscle or cord respectively. A commutator was also placed in the primary circuit, so as to send the whole of the current either to the "direct" coils, or to the "indirect," but not both simultaneously, to avoid errors due to current leakage.

The muscle was unsupported, and carried a weight of 20 grms. Break induction shocks only, at intervals of some two seconds, were used for excitation purposes, the making ones being automatically cut out by a rotating commutator.

Note. By normal saline is meant 0·6 % sodium chloride in tap water. The use of this was controlled throughout by test perfusions varying from 2 to 72 hours. In all cases both responses were found vigorous at the end of the perfusion. The substances, whose action was to be tested, were dissolved in 0·6 % sodium chloride, and then the requisite quantity of these "stock" solutions added to 0·6 % NaCl or Ringer, unless otherwise mentioned, the composition of the "Ringer" being 0·6 % NaCl, 0·025 % KCl, 0·025 % $CaCl_2$. The water used for making up these solutions was the ordinary distilled water of the laboratory, which was redistilled and condensed over glass. The action of the perfusing solutions was tested on frog's blood corpuscles to avoid errors of an osmotic type.

The action of lactic acid and lactates. The first experiments on the physiological action of lactic acid, and other substances associated with muscular fatigue, appear to have been performed by Ranke[3]. He found that the acid in small quantities increased the excitability of muscle, as estimated by the distance of the secondary coil from the primary for obtaining an induction shock which would elicit a response;

in greater concentrations it abolished the power of responding, and recovery of the muscle in these cases was found to be facilitated by the subsequent injection of weak alkalies, those actually employed being sodium carbonate and kreatinin. Later researches by the same author led him to the conclusion that his fatigue substances acted in virtue of their acidity.

More recently, Lee[4] has investigated their action and extended it to some of the lactates. Like Ranke he regards their action as being of two opposite kinds; in small quantities an augmentation of the muscle activity; in medium or large quantities, or prolonged action, a depression of activity, fatigue. The effects occurred in both curarised and non-curarised muscles, and hence their action was asserted to be on the muscle protoplasm itself.

The present experiments have been performed by perfusing different concentrations of lactic acid in Ringer's solution. As shown in Fig. 1, with small or medium concentrations of the acid (0·02—0·15 %), the series of muscular responses recorded on a slow-moving surface resembled those of an ordinary fatigue curve obtained by stimulating, now the nerve, and now the muscle, with a series of single shocks. After the primary period of augmentation the indirect response gradually diminished, and at the point of its disappearance the direct response was still vigorous. Wave-like variations were present in the later stages. On replacing the lactic acid perfusing fluid with one of normal saline alone, there was no immediate restoration of function. Instead, the muscle became œdematous, and the capability for response underwent still further diminution. With continued perfusion of normal saline this œdema gradually disappeared, and corresponding with its disappearance there was a return of response.

The magnitude of the œdema, and the length of time it lasted, and also the extent of the further diminution of muscular response, were all roughly proportional to the concentration of the acid employed.

When high concentrations of the acids were used (0·25 %) there was a rapid and complete disappearance of the indirect response, followed at a short interval by that of the direct. Perfusion of normal saline now gave an immediate and apparently complete recovery of the direct response, but none of the indirect. In a very short time, however, the perfusion of normal saline being continued, the direct response began to decrease in magnitude, whether the excitation was continuous or not, until in from 4 to 6 hours it became imperceptible. At this stage the œdema of the muscles was very marked, and in some cases the

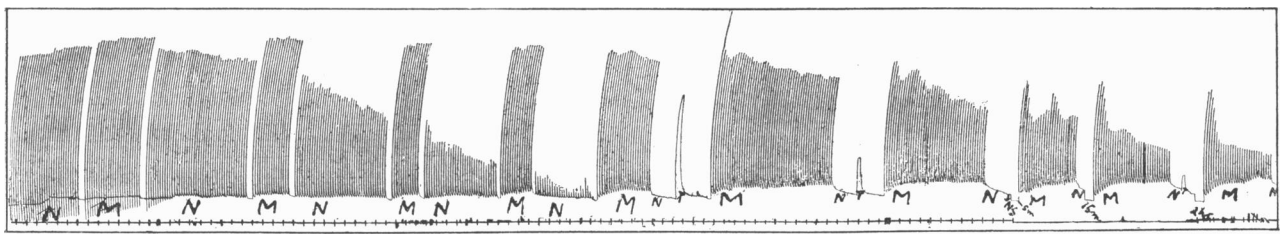

Fig. 1. Perfusion of 0·017 % lactic acid in boiled Ringer's solution up to point *NS*. Response of gastrocnemius to groups of induction shocks applied directly and indirectly. *N*=indirect excitation. *M*=direct excitation. *T*=a series of make and break shocks in quick succession (tetanus). Perfusion of normal saline was commenced at point *NS*. 5 m., 15 m. and ½ hr. represent intervals of 5 minutes, 15 minutes and ½ hour respectively. Time marked every 10 seconds.

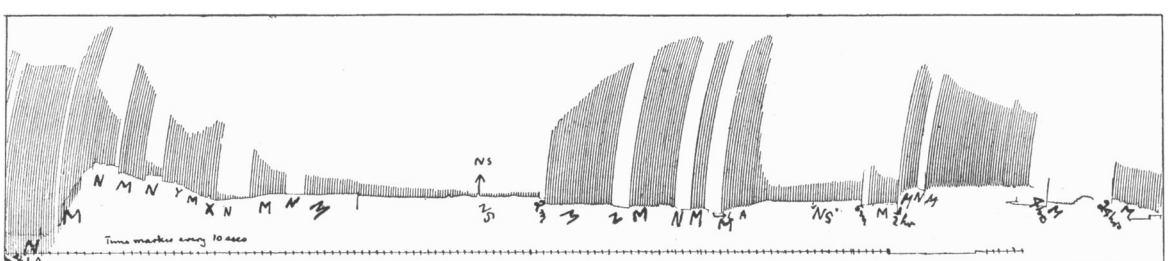

Fig. 2. Perfusion of 0·25 % lactic acid in 0·6 % NaCl. *M*=direct excitation. *N*=indirect excitation. Between *Y* and *X* the perfusion was stopped resulting in some degree of spontaneous recovery. *LA* marks commencement of perfusion of the acid. *NS* marks commencement of perfusion of normal saline. At 2 m., 5 m. and ½ hr. there were intervals of 2 minutes, 5 minutes and ½ hour respectively. 4 hrs. and 24 hrs. represent times that had elapsed since the commencement of the experiment.

perfusion was stopped here: the muscles were dissected out, weighed and their weights compared with those of the opposite control legs. There was a distinct increase in weight amounting to 30 %.

In those cases where the perfusion of saline was continued further (50—90 hours), some recovery both of the direct and indirect responses was noted, but with marked differences in their extent. The former would attain a value comparable with its original magnitude, but the latter in the most favourable cases would never attain more than half that of the other, the direct; usually it was much less as may be seen from Fig. 3.

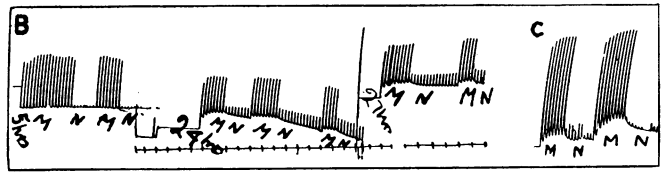

Fig. 3. *B* and *C*=stages in the recovery of a muscle after a second perfusion of 0·25 % lactic acid in Ringer. The times marked on tracings represent times that have elapsed since experiment began. *M*=direct excitation. *N*=indirect excitation. Time marked every 10 seconds. In *C* the tall and the short are the responses given to the direct and indirect excitations respectively after 56 hours' perfusion with normal saline.

Although the magnitude of response returned to its original value almost immediately on washing out the lactic acid solution with normal saline, the effects noted with further perfusion seemed to show that the recovery of the muscle itself was not complete. This could be further demonstrated by perfusing with lactic acid a second time immediately after the first recovery. The rate of failure was found to be so greatly accelerated as to be almost immediate, and the subsequent apparent recovery with saline was both retarded and incomplete. Œdema effects then occurred as before. The general results of perfusion with these high concentrations are shown in Fig. 2.

Fibrillary contractions were seen when medium and high concentrations of lactic acid were used, and there was also a distinct shortening of the muscle, which latter also occurred during the period of œdema.

The results noted were unaffected by oxygenating the perfusing liquid containing the acid, by first boiling and then passing carbon dioxide through, or by the presence or absence of calcium salts.

It was also found possible to obtain a vigorous indirect response from a muscle the nerve of which had been placed for 48 hours in lactic acid of the highest concentration (0·25 %) used in these experiments.

Sodium lactate had no observable effect on the direct response at low and medium concentrations (0·08—0·04 %), but caused the indirect to diminish gradually. This latter depressant effect occurred in a somewhat anomalous manner. The diminution could be observed throughout, but it was in great part one of diminution to excitation by regular single break shocks; on throwing in the make shock as well as the break, the muscle would respond to these make shocks to the same extent as it did before to the break, but the response now to the break was greatly increased, and reached a value comparable to that of the direct, at any rate, in the earlier stages. After perfusion had been in progress for some 30 minutes or so, this second response became smaller, while that of the make was zero, and also that to the simple break shocks. It must be noted, however, that perfusion of 0·6 % sodium chloride alone, or of a high concentration (1—2 %) of this salt in Ringer, was found to give a similar result.

When high concentrations of sodium lactate were employed, both responses rapidly became smaller, and also the time of the whole process of contraction became increased. Complete recovery took place on subsequent perfusion of normal saline.

The above results were such as were obtained with the chemically pure sodium lactate of Merck. Experiments were performed with two other specimens of the 'pure' commercial sodium lactate. Their action was found to be rather that of a poison. Both responses gradually failed and the whole time of contraction also increased. No recovery took place when normal saline was substituted for the lactate, except where a high concentration had been employed, and in those cases the actual amount of recovery was small.

Potassium lactate. Perfusion with this salt gave a primary augmentation of both direct and indirect responses followed by depression, this latter affecting the indirect much more than the direct. The lower concentrations employed would do away with the former while leaving the latter comparatively unaffected. As the concentration was increased, the direct became more and more influenced until it, too, would rapidly vanish. Perfusion of normal saline was then followed by a rapid return of the direct but little or none of the indirect; the type of tracing obtained at this stage was very much like those obtained under similar conditions with lactic acid. No œdema was observed, however, and

complete recovery of the indirect response occurred in about an hour or so.

Ammonium lactate also influenced the indirect response more than the direct, there being, at low or moderate concentrations, little or no observable effect on the latter beyond wave-like variations when the former had vanished. In one experiment, a stray bubble of air in the cannula accidentally stopped perfusion. This occurred at a stage when the indirect response had been reduced to about one-fifth of its original value. A spontaneous recovery then began, which only ceased when the cause of obstruction had been removed and perfusion had been re-established. Artificial blocking of the perfusion by placing a clip on the rubber tubing connecting the cannula with the reservoir of perfusion liquid gave similar results, both as regards the effects of stopping the perfusion and its re-establishment. The conclusion was drawn that the muscle contained within itself the means for disposing of ammonium lactate in such quantities as had been perfused.

With high concentrations of the salt both responses rapidly disappeared, and also rapidly returned on subsequent perfusion of saline though with some lag of the indirect. In general, the muscle made a good recovery from ammonium lactate.

Calcium lactate gave some slight primary augmentation, but at medium and low concentrations there was very little other effect observable. After one hour's perfusion of 0·1—0·4 % of the salt in Ringer solution, the direct response was apparently unaffected, and only a very small decrease noted in the indirect. Both responses disappeared when high concentrations of the salts were employed (up to 3 %). Subsequent recovery with saline was good, though that of the indirect took distinctly longer than was the case with the direct response.

It will be noted that the action found for each lactate at low and medium concentrations, by which is meant for the lactates those concentrations at which there was apparently no osmotic factor, bears a marked resemblance to that found for the positive ion by other observers, except in the case of the sodium ion. This last is not usually regarded as actively affecting the indirect response, though there is evidence available which suggests that such may be the case. Thus Locke[5] found a muscle placed in 0·6 % sodium chloride quickly lost its excitability to indirect shocks, whereas the direct excitability persisted for some 24 hours. The addition of 0·2 % calcium chloride restored the indirect response in five minutes; moreover, perfusion

of 0·6% sodium chloride has been shown by Harvey Cushing[6] to result in a rapid failure of the indirect response followed by an even quicker recovery on subsequent perfusion of a physiologically balanced solution. Lithium, ammonium and potassium have also been shown by Overton to act on the indirect response in what he terms a 'curarising' manner, and as sodium is a member of the same chemical family it does not seem improbable that it should possess the similar property of actively abolishing the response from the nerve. The results given above with respect to the perfusion of high concentrations of sodium chloride seem also to support such a view.

The lactate ion, however, appears to possess only slight fatiguing properties, if any, on the direct response, though it has a much more marked effect on the indirect. Thus all the lactates give some decrease in the indirect response on perfusion, though this effect was somewhat masked with the calcium salt. There is also the lag of recovery of the indirect when high concentrations of the salts were used, and such lag was found to be distinctly greater than was the case with perfusion of the corresponding chlorides. To examine these points further a solution was made up containing the same weights of sodium, potassium, and calcium as are present in an ordinary Ringer's solution, but with lactates substituted for chlorides. This solution was then perfused. At first the muscles of the experimental leg entered into a state of fibrillary contraction, which later subsided. No other effect was noted for some time save a depression in the amount of the indirect response. At the end of two hours the direct preserved its original height, but the total duration of each twitch (contraction and relaxation) had been so greatly increased that it was possible to produce a condition of tetanus with stimuli at half-second intervals. The indirect response had by this time disappeared or nearly so; later the direct response also decreased in height, and at the end of some 8 to 10 hours had vanished, but the muscles still gave a slight response to a tetanic series of strong shocks. The conclusion was then drawn that the lactates had a fatiguing action on the direct response, though very small, and a more marked effect on the indirect response. The properties of such a perfusing fluid are receiving further attention, since considering the position of lactic acid in metabolism, it is conceivable that the inorganic lactates may have an energy value.

As already mentioned, each of the lactates at high concentrations had an apparent fatiguing action in that the responses rapidly disappeared and subsequently recovered on perfusion of saline. Examination

of such solutions showed that they were hypertonic. Accordingly, experiments were performed in order to ascertain what possible effects were produced by these means, and the method adopted was the perfusion of concentrated solutions of the chlorides of sodium, potassium, and calcium, the actual strengths employed varying from 1 to 5 % in distilled water. A hypertonic solution of sodium chloride gradually abolished both responses with the more marked effect on the indirect as mentioned before. As the direct response diminished so the total time of contraction increased, the curves resembling those of a fatigued muscle. Some shortening of the muscle also occurred. Recovery took place on subsequent perfusion of saline. Potassium chloride rapidly abolished both responses, which just as rapidly recovered on subsequent saline perfusion, provided this had taken place immediately after or before the failure of response was complete. If, however, the hypertonic solution was allowed to perfuse for some time after this complete failure had occurred, the subsequent recovery with normal saline was delayed and also there was a marked lag in that of the indirect response. The action on the indirect response, however, was never found to be so great as that which occurred with corresponding concentrations of the lactate. Other effects of these high concentrations of potassium chloride will be described later. Calcium chloride also rapidly abolished both responses. There was a very slight shortening of the muscle at first which was followed by an elongation. The recovery with saline was good, and, though a delay was present in the indirect response, it was not so prolonged as with the lactate. No signs of a 'drawing out' of the contractile process were observed with calcium or potassium. The results thus obtained showed that the sodium and potassium ion, when combined with another innocuous ion, could give effects simulating fatigue provided suitable concentrations were employed, those actually required being a hypertonic one of the sodium salt and a fairly low concentration of the potassium one; so that statements regarding the fatiguing action of salts of these metals must be accepted with some caution, unless the actual concentrations employed are also given.

All the lactates perfused readily, the rate being from two to four times as great as that of a solution of normal saline at the same height, suggesting that the lactates may have a specific action in dilating the arterioles of a muscle.

As regards the action of lactic acid, an examination of my records shows that it has the augmenting and fatiguing properties discovered by Ranke[3]. The effects noted, however, on subsequent perfusion of

saline seem to show that in addition an internal (intrafusal), and an external (circulatory) action must be assumed. Both of these produce a fatiguing effect, but that due to the intrafusal cause is much longer lasting; for whereas the circulatory may be got rid of by washing out the muscle, removal of the intrafusal would appear to depend on some form of oxidation such as that proved to occur by Hopkins and Fletcher[7]. It may be fairly assumed that this internal lactic acid must be the greatest at the time of commencement of perfusing normal saline. In spite of this, the muscle response makes a full recovery at first, while the subsequent diminution that occurs varies with the extent of the œdema. Thus it appears that the œdema, qua œdema, is the cause of this subsequent diminution, possibly as suggested by Ranke[3], acting as a diluent of the contracting materials; though there is no doubt that lactic acid is the cause of the œdema. The dependence of the contractile process upon a sufficient increase of osmotic pressure in the muscle fibre has been pointed out by Macdonald[8], and the presence of this œdema would undoubtedly cause a lessening in the extent of the osmotic pressure by dilution, and so make the contraction less efficient.

There is also the possibility that intrafusal lactic acid of itself is not fatiguing. On chemical grounds a fatigue substance should represent an end product of the chemical action leading to contraction. The high energy value of lactic acid seems to negative such a conclusion, and the acid maximum of tetanus is much less than the acid maximum of extra-physiological processes, making it possible that though the process of contraction has been brought to a close by fatigue, those leading to the production of lactic acid have not. But it should be remembered in this connection, that the method used by Hopkins and Fletcher[7] to fatigue a muscle is not a complete one; for it has been shown by Joteyko[2] that when a muscle no longer replies to induced currents, it is possible by means of a galvanic current to produce a fresh series of long-continued contractions, which also undergoes a fatigue process, and it is probable that the acid maximum of fatigue can be brought nearer to the other maxima.

Comparing the results obtained with lactic acid and the lactates, it can be seen that most of the effects produced by the former are due to the action of the hydrogen ion. The lactate part has comparatively little action, even on the more susceptible indirect response, but the subsequent œdema after perfusion with saline and also the harmful effect on the indirect response would appear to be wholly due to the

hydrogen ion. Vernon[9] has shown in another connection, that the action of lactic acid was in accordance with the extent of its ionisation.

Potassium chloride. Potassium salts have been shown by Overton[10] to abolish the indirect response of muscle. This action can be shown by perfusing a comparatively weak concentration (0·05 %) of potassium chloride in Ringer. The indirect response could be abolished when the direct was still at its full strength. On perfusion with normal saline recovery was complete and fairly quick. Both responses were affected on increasing the amount of potassium salts, but the indirect much more than the direct as was also shown by the lag of recovery. After several successive perfusions of potassium, followed by washings out with normal saline, this lag of recovery of the indirect was so increased as to leave the direct response at its original value when the indirect had only recovered to a very slight extent.

A still further increase in the amount of potassium accelerates the rate of failure of both responses, and also diminishes the amount of lag in the recovery of the indirect response on a first washing out with normal saline, although successive perfusions will make the lag manifest. Fibrillary contractions occurred in the muscles when these increased amounts of potassium were perfused.

It was noted in addition that potassium chloride had a primary augmenting action on both responses, thus confirming Mines[11] in the case of the direct, and that the above results were not affected by the presence of the calcium salts in the Ringer solution, as also shown by Overton[10], by lack of oxygen, or by excess of carbon dioxide.

It was also considered of interest to examine the character of the contractions given by a muscle as it gradually failed under the action of potassium chloride. This was done on a fast moving recording surface. The total time of the whole process was found to be practically unaltered, the muscle curves merely becoming less steep, and developing a slight plateau on the top. The greatest concentration of potassium chloride used in any of the above experiments was 0·225 % KCl (0·2 % added to 'Ringer').

In the next group of experiments high concentrations of potassium chloride were perfused (1·5 to 5 % in distilled water). Such concentrations were shown by Grützner[12] to produce a slow and long continued contraction in muscle, his results being confirmed by Zenneck[13] and Zoetout[14]. The last found the contraction to persist for times varying from 20 to 100 minutes.

In my experiments when the perfusion pressure was at its usual height (about 30 cm.) the contraction of the arteries[14] and muscles in the thigh region was sufficient to prevent for a time the access of fluid to the lower parts, but when some slight relaxation had occurred, the liquid apparently passed through slowly to the gastrocnemius, which then entered into contraction in a series of steps due presumably to the different times at which different sets of fibres supplied by each artery became affected. By raising the vessel containing the perfusing liquid to a height of some 70 cm., and also by suitably pinching the rubber tubing connecting this to the cannula, it was possible to perfuse, or rather inject, all parts of the leg practically simultaneously. Under these conditions the muscle rapidly shortened, remained in the fully contracted state a short time (15 to 30 secs.), and then gradually relaxed, returning in 5 mins. to about half its original length. Probably then the very long time of contraction noted in some of Zoetout's experiments is due to the use of baths, for then the fibres in the centre of the muscle would not become affected until diffusion had taken place from the outside. The height of the contraction was always greater than that produced by a single maximal induction shock, and for these and other reasons, I regard it as tetanic in character.

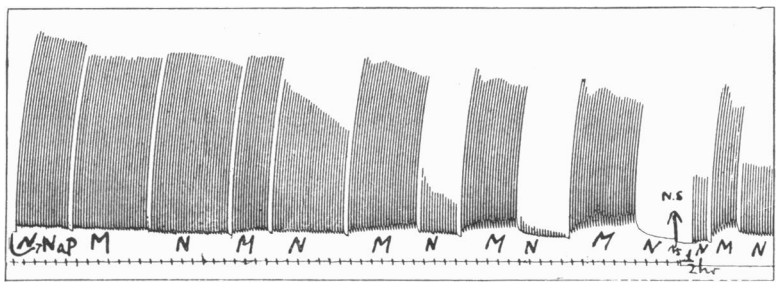

Fig. 4. Effect of perfusion of 0·04 % acid sodium phosphate in 0·06 % sodium chloride. *M* = direct excitation. *N* = indirect excitation. At *NS* perfusion of normal saline was begun, and the amount of recovery after ½ hour is also shown.

Acid phosphates. A solution of acid sodium phosphate in 0·6 % NaCl was associated with a rapid failure of the indirect response, this falling to zero while the direct was still intact. The phosphate was employed at various contractions, and in all cases the direct was still vigorous at the time the indirect had vanished. The amount of primary augmentation given by acid sodium phosphate, if any at all, is exceedingly small.

Recovery of the indirect response by perfusion of normal saline was complete at all concentrations, though it took some three or four hours.

Addition of an acid phosphate to normal saline caused the formation of a precipitate which could, however, be dissolved by passing CO_2 through the liquid. Such a solution had a less marked effect on the indirect response, but the failure of this before the direct was still present. In this connection, the remarks regarding the effect of perfusing a liquid with insufficient calcium, as mentioned under the lactates, is of importance.

I found that when a small quantity of potassium chloride was added to the acid sodium phosphate solution, a distinct augmenting action was seen both on the direct and indirect responses. There were marked

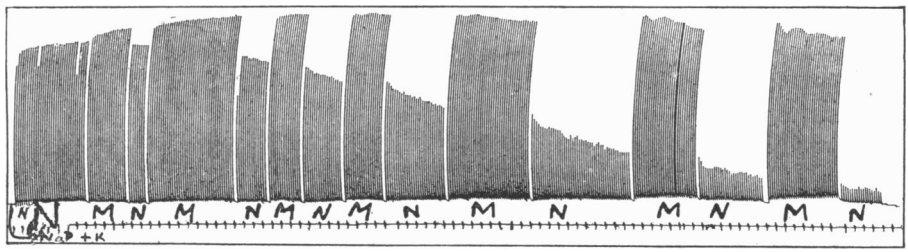

Fig. 5. Same strength of acid sodium phosphate as employed in above expt. (Fig. 4) but with the addition of 0·04 % potassium chloride. M = direct excitation. N = indirect excitation.

differences as regards the length of time during which this augmentation persisted. That of the indirect was much briefer in duration, and depression occurred while the direct still increased its vigour. The nature of the action may be understood by reference to Fig. 5. There is a complete though somewhat delayed recovery of both responses to perfusion by normal saline.

Acid potassium phosphate acted similarly to the sodium compound when potassium salts are also present in small amounts. Recovery was complete. In regard to this Overton[10] has also found that acid phosphate is without permanent injurious effect on the nerve endings.

Wave-like variations were observed in the fatigue curves obtained with acid phosphates, with a solution of carbon dioxide in Ringer, with ammonium lactate and as mentioned above with lactic acid. The

physical conditions of the production of these waves have been investigated by Symons[15], who also noted an earlier production with lactic acid and acid potassium phosphate. Though the circumstances of excitation throughout were similar, I have not noted the production of these waves when neutral salts, such as potassium chloride, sodium lactate, etc., were perfused. All the substances with which they were produced, with the exception of ammonium lactate, contain the hydrogen ion, leading to the conclusion that the stage at which these waves may be produced is one at which a free acid is in process of neutralisation by ammonia.

Potassium chloride and an acid phosphate. As already mentioned, the addition of a potassium salt to an acid phosphate caused an augmentation of both responses, that of the indirect being much shorter in duration and changing to depression while the augmentation of the direct is still in progress. Under varying proportions of these two substances together it is found that the greater the proportion of the potassium to the acid phosphate, the quicker both direct and indirect responses disappeared, and also the quicker the recovery on perfusion of normal saline.

Lactic acid and acid phosphate. Mixtures of these in 0·6 NaCl or Ringer have a depressing action on the indirect response which vanishes long before the direct. Wave-like variations of extent of response are usually present in the later stages. Recovery on perfusion with normal saline is slow but complete. When calcium salts are present (Ringer) the indirect response takes longer to disappear, but otherwise the results are the same.

A solution of carbon-dioxide in 'Ringer,' with or without lack of oxygen, was found to do away with the indirect response at a time when the direct was apparently unaffected. Wave-like variations of response were noted in the tracings obtained.

Potassium chloride, lactic acid, etc. These two substances together have a marked action on the indirect response which is shown by its rapid disappearance as compared with the direct, and also by its slower rate of recovery. Here, as with the acid phosphate, the rate of recovery on perfusion with normal saline depends on the magnitude of the ratio between the amount of potassium salts present and that of the lactic acid.

It should be noted that the above results were obtained when the supply of oxygen was fairly good, the perfusing solutions being shaken with air. After a perfusion which has allowed of a complete recovery,

a repetition of the whole process will bring out a marked lag of the indirect response.

If, however, the solution of potassium chloride and lactic acid be first boiled and then perfused, lag of the indirect recovery on perfusion of normal saline will immediately become apparent. The passage of CO_2 through the mixture after boiling further increases this lag. The type of tracing thus obtained is shown in Fig. 6.

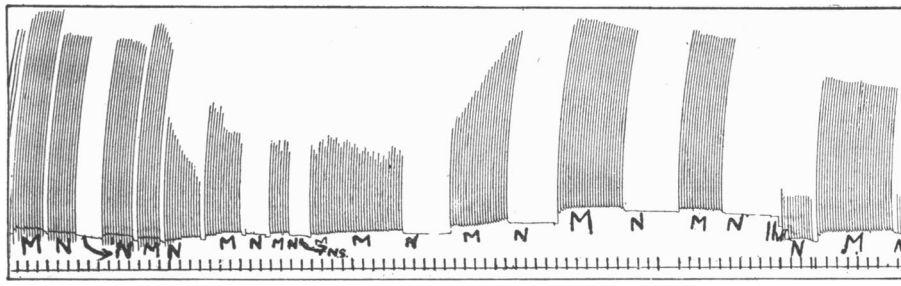

Fig. 6. The perfusing solution was 0·6 % sodium chloride, 0·22 % potassium chloride, 0·024 % calcium chloride, 0·01 % lactic acid. This was first boiled and then carbon dioxide bubbled through during the time it cooled. The first arrow marks the point where perfusion of this solution was commenced and the second arrow (*NS*) the commencement of perfusion of normal saline. *M*=direct excitation. *N*=indirect excitation. 1 hr.=interval of one hour. Time marked every 10 seconds.

With regard to such perfusing mixtures, it may be generally stated that a solution, which in presence of oxygen allows of a complete recovery of the indirect response on perfusion with normal saline, will in absence of oxygen and presence of CO_2 abolish the recovery for some time. Calcium salts and oxygen are necessary for this recovery.

The addition of an acid phosphate to the perfusing mixture assists recovery, and a solution which, as above, entirely abolished the indirect response for some time, will on adding sufficient acid phosphate allow of a complete recovery with normal saline.

Further, a solution of the three substances in which the phosphates are not present in sufficient amount, will by adding kreatinin or kreatin before boiling allow of a good recovery. Kreatin added after boiling is not so efficient as before boiling.

The amount of lactic acid required to produce these results is small, the maximum ever employed in these experiments being 0·01 grm. in 100 c.c. of liquid. It varies according to the season of the year,

the summer frog being much less resistant than the autumn and winter.

Solutions of these three substances have been obtained which immediately abolished the indirect but not the direct. The amounts, however, exhibit great variations and appear to vary with each individual and the season of the year.

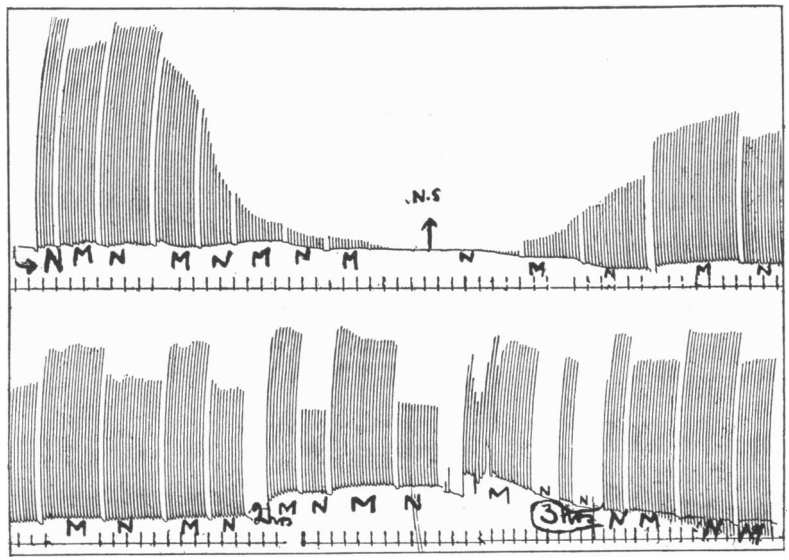

Fig. 7. The perfusing solution 0·6 % sodium chloride, 0·22 % potassium chloride, 0·01 % lactic acid, 0·023 % calcium chloride, 0·0122 % acid sodium phosphate, 0·002 gm. kreatin. Mixture was boiled and carbon dioxide passed during cooling. Lettering etc. same as before. The tracing shows after effects followed by complete recovery.

As already mentioned under lactic acid, the perfusion of saline was followed by a diminution of response with subsequent recovery. A similar action was noted when the above mixtures containing lactic acid were perfused, but in these cases it was almost confined to the indirect response, probably on account of the small quantities of acid used, which as above mentioned never exceeded 0·01 %. The recovery of the indirect might be equal to that of the direct during the early stages of the saline perfusion, but then began to fall away while the direct increased further. The effect occurred whether excitation was continuous or not, and it was not therefore due to fatigue consequent on excitation.

It appeared to depend to some extent on the rapidity of perfusion of the washing-out solution, for a series of wave-like variations with large amplitude could be produced in the indirect response by alternately raising and lowering the perfusion pressure, the upward movement following the rise of pressure and the downward the fall. The general tendency was, however, to fall, each successive wave being shorter than the preceding one, neither was it found possible to prevent this subsequent diminution by keeping the saline perfusion at a high pressure throughout. No such diminution was ever observed with the direct response though waves of variation were usually to be noted, suggesting the presence of the hydrogen ion.

Thus we have still further evidence pointing to the fact, as mentioned before, that lactic acid in virtue of its hydrogen ion has a very marked depressing influence on the indirect response. It also appears probable that lactic acid cannot exist free and uncombined in the muscle fibre; for though it appears to be an easy matter for the acid to pass from the perfusing fluid into the muscle substance, the œdema effects already noted seem to suggest that its subsequent removal may depend wholly or in part upon oxidation, and not by diffusion which latter would be the case if it were free.

The recovery of a muscle after tetanus. Ranke[3] noticed that a frog electrically tetanised to exhaustion recovered on washing out the blood with salt solution. He did not, however, differentiate between the direct and indirect responses.

Experiments have been performed with a view to elucidating what differences might be present. It was found that muscles which had been directly tetanised until they gave no direct response rapidly recovered their power of giving such response on washing out with normal saline, but would give very little indirect response. The magnitude of the direct response underwent some diminution as perfusion progressed, and then began to increase once more; the diminution being accompanied by œdema and the increase by its disappearance. Nothing other than a partial recovery of the indirect response was ever obtained when the muscle had been tetanised to such an extent as to lose its direct response (48 hours after).

Wave-like variations of response were noted throughout the œdema period following the perfusion of saline; such waves occurred under similar conditions after lactic acid perfusion.

When, however, tetanus had only been prolonged sufficiently to produce some diminution in the amount of the indirect response

(one-third of original value) perfusion of normal saline caused an immediate and complete return of this to its former value, and spontaneous recovery was also possible at this stage. If tetanus had proceeded much further, recovery with normal saline was a very slow process occupying some hours, and was of such a character as to allow of the general statement that the greater the diminution of response by tetanus, the longer the time taken proportionally for recovery, the relation being far from a linear one. Such a tardy and a quick recovery may be noted on reference to Fig. 8. The recovery of the indirect response under these circumstances exhibited no variations whether tetanus was due to direct excitation, or to stimulation of the cord or of the nerve trunk. It was also noted that a slow recovery of the indirect response occurred always at those stages where wave-like variations showed their appearance in the curves.

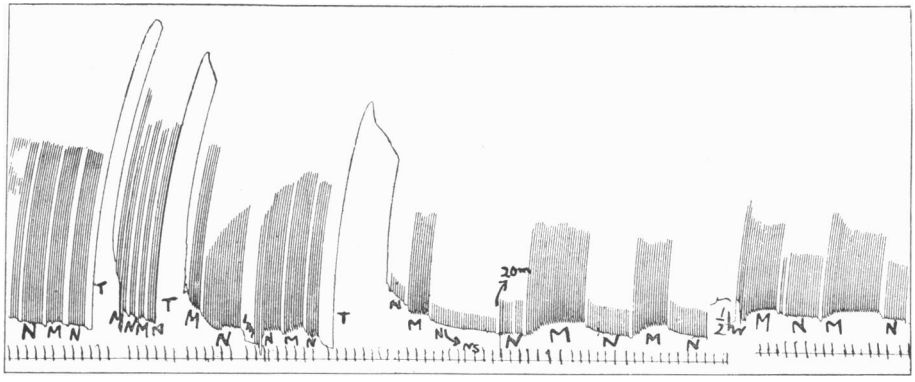

Fig. 8. Recovery of the indirect response after different periods of excitation. T = commencement of tetanus. The perfusion of normal saline was stopped immediately before tetanising and resumed immediately it was over except where otherwise stated.

Concluding Remarks.

Ability to recover is a salient feature of normal fatigue, but the rate of recovery has been found by Mosso[1] and his pupils to depend on the extent of the preceding fatigue. In the experiments of Maggiora[1] it was shown that a muscle worked at a rapid rhythm to complete exhaustion required a long time to recover completely (one to two hours).

It was also shown that the last contractions of a series were the most exhausting, since if only the first part were carried out the muscle took a much shorter time proportionately to recover. The same effect has been shown above to occur in the stages of recovery of a muscle from tetanus; for whereas when the indirect response had only slightly diminished the time taken for recovery was a matter of seconds, recovery after complete exhaustion did not occur within the remaining life of the muscle.

Turning to the results obtained with possible fatigue substances, it has been shown that a quick recovery is to be associated with potassium salts alone or in excess, while a slow and prolonged one has only been obtained as an effect of lactic acid. It has also been shown that the effect of an exhausting tetanus is almost identically the same as that found to occur when a high concentration of lactic acid has been perfused through a muscle. There is thus reason to believe that the results noted in the early stages of fatigue are mainly referable to potassium and those in the later stages to lactic acid.

The effect of potassium and lactic acid together appears to be a twofold one, first that of increasing the susceptibility of the indirect response to the action of the acid, and secondly, to increase its powers of recovery.

The actual conditions present in a muscle working at a fairly high rate are the continued liberation of high concentrations of potassium ions[15], production of lactic acid[8], together with a general lack of oxygen[16] and increase of tension of carbon dioxide. The small quantities of lactic acid required to paralyse the nerve-ending for some time under such conditions—never more than 0·01 % (in the demonstration given to the Physiological Society at Oxford in June, 1909 with the summer frog, only 0·003 %)—negative, I think, any view that this lactic acid can remain unneutralised, for Hopkins and Fletcher[7] found such amounts in "resting" muscle. In this connection attention may be drawn to the possible rôle of kreatin and kreatinin, both without effect on the muscle substance, and kreatin stated to have favourable influence on nerve ending, and they were both found capable of neutralising this lactic acid. The change from kreatin to kreatinin and vice versa is accomplished with ease under physiological conditions and the effects noted above give more than sufficient reason for the presence of a strong base in a muscle which seemed so improbable to Mellanby[16]. The total amounts of kreatin and kreatinin in a muscle are, however, little altered as a result of tetanus, a slight increase in the excised

muscle[17], and a slight decrease when the circulation is intact[18]. I found that it was possible to demonstrate small amounts of kreatinin in the perfusion liquid coming from a leg which was continually tetanised for some twenty-four hours. Mellanby[16] has also pointed out (as did Ranke[3]), that the amounts of kreatin found in muscles seemed to bear some relation to their functional activity. I would accordingly suggest that each muscle contains within itself such a quantity of kreatin as is sufficient to neutralise by the change to kreatinin the amounts of lactic acid which may be regarded as produced during its accustomed activity.

Should a muscle be put to an unusual stress it will exceed the kreatinin neutralisation limit, and considerations of general metabolism suggest that in such cases ammonium lactate will be formed. This has been shown above to possess the property of blocking the impulse from the nerve into the muscle, and some evidence was given that the muscle contained within itself the means for disposing of it. There is abundant evidence available for believing that this disposal is accomplished by the formation of purins. Moreover, it is in overworked muscles that this purin formation takes place[19], the amount formed gradually decreasing on successive days as the muscles become accustomed to this extra amount of exercise. I would suggest further then that part at least of the physiological processes involved in the training of athletes is an increase in the total kreatin content of their muscles, to meet the extra production of lactic acid that occurs when the muscles become harder worked.

It has already been mentioned that the effects of fatigue are mainly referable in the early stages to potassium salts, and in the later to lactic acid. The characters of a fatigue curve taken on a fast-moving drum are well known, and an examination of these will lead to the conclusion that in the later stages the condition of the muscle is more one of inability to relax than of inability to contract. This may become so marked in the intact muscle that when greatly fatigued it cannot relax but enters in to 'contracture.'

A muscle perfused with lactic acid gives duration curves similar to the ordinary fatigue curves, and also gives a distinct diminution of height of contraction. On the other hand, potassium salts do not appear to affect the time of contraction at all. This last would appear to be probable when the work of Macdonald[20] is considered. For if a muscular contraction is the result of a sudden liberation of potassium salts formerly bound up in a colloid, the presence of free potassium salts

might reasonably be expected to limit this liberation, and impose no delay as regards the return. Lactic acid, however, does impose a delay in this return, and considering that the hydrogen and potassium ions carry like charges but at different potentials, it seems probable that in these late stages there is an adsorption compound formed between the lactic acid and the colloid with which the potassium is normally adsorbed, the hydrogen ion being one of greater potency in this connection. The œdema observed with fatigue, and lactic acid, are thus referable to free displaced potassium salts which would remain free until the lactic acid had been oxidised. The fluid from preparations which had been tetanised and perfused for some twenty-four hours was found to show a notable increase in the amount of its potassium salts, and it seems highly probable that these have their origin in the manner suggested above. Moreover, we may conclude that such potassium salts in the circulation give rise to the general fatigue, both mental and physical, which follows the performance of heavy muscular work. For Mosso[21] has demonstrated that the blood of a fatigued animal is fatiguing to another, and that the cause is not referable to such substances as lactic acid, etc., inasmuch as the blood remains alkaline; while Abelous[22] has shown that its action is more particularly on the nerve-endings.

Finally, I would suggest that the fatigue toxin and anti-toxin described by Weichardt[23] are to be regarded as having a twofold nature. There are primarily the very small amounts of free lactic acid which a muscle is able to produce under the influence of stimuli sent out from the central nervous system, which could be oxidised by the oxygen (anti-toxin) of the blood corpuscles; and secondly, some product of muscular disintegration due to autolysis of a markedly acid muscle on standing, which being of protein nature would give rise to an anti-body on injection.

SUMMARY.

1. The motor nerve-endings of a muscle were found to be more susceptible to the action of each of the possible fatigue substances examined, than was the case with any of the other elements in a muscle and nerve preparation.

2. Potassium salts have a primary augmenting action on both the direct, as shown by Mines, and indirect response of a muscle, and this augmentation increases with increase of concentration of the salts until actual excitation takes place. Solutions of pure potassium chloride

1·5 to 5% in distilled water were found capable of evoking a contraction and tetanus in a muscle independent of any other mode of excitation.

3. At low concentrations potassium salts have in addition a fatiguing action. This latter corresponded to normal physiological fatigue in affecting primarily the nerve-ending, and in its quick and easy possibility of recovery without any observable after-effect.

4. The action of potassium salts never appeared to be poisonous, for a complete recovery of a muscle and nerve was obtained after perfusion of all concentrations of the salt.

5. Evidence has been brought forward for believing that free circulating potassium salts, having their origin in working muscles, form an important factor in the general fatigue observable after heavy work.

6. The action of lactic acid corresponded closely with that due to the excessive fatigue of an overworked muscle. The nerve-endings were very markedly affected, and their subsequent recovery from the effects of the acid took some time. The effects produced are almost entirely due to the hydrogen ion.

7. Under the conditions assumed to exist in a hardworking muscle even such traces of lactic acid as have been found in 'resting' muscle, were found capable of abolishing the indirect response for some time. Hence it was considered that lactic acid must be neutralised, and that the limit of the working capacity of the intact muscle is reached at the stage when lactic acid has been produced so as to be present in the free condition beyond the merest traces. When such free lactic acid is present, the subsequent recovery of the indirect response will show a more or less marked delay, e.g. stiffness.

8. A possible rôle of kreatin is the neutralisation of lactic acid; and where this is insufficient, ammonium lactate, with subsequent formation of purins, may represent a further attempt at such neutralisation.

9. The hydrogen ion is a necessary concomitant of waves of variation in the response of a fatigued muscle.

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