THE RECOVERY PROCESS OF EXCITABLE TISSUES.

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CONTENTS

In 1912 Keith Lucas and I(1) investigated the course of recovery in a nerve fibre after the passage of an impulse and we were able to map out the return of the two main functions of conductivity and excitability. We found that the nerve returned gradually to normal during the relative refractory period and then passed through a stage in which its excitability and its power of conduction were increased above their normal value. We called this stage of recovery the "supernormal period," and we showed that its existence might be of great importance in the mechanism of the central nervous system, since it might account for the type of nervous summation in which a series of impulses succeed in passing a region of imperfect conduction whereas a single impulse fails to do so. The supernormal rise of excitability was present in all our experiments, but it was never very great and it showed considerable variation from one preparation to another. We did not seek the cause of these variations since we were concerned with the mechanism of summation and not with the nature of the recovery process.

The present investigation is an attempt to carry the analysis a stage further, to discover what factors account for the observed variations in the supernormal phase and if possible to suggest some explanation of its existence. I was all the more anxious to undertake it because in the course of some later work I had observed much greater variations in the supernormal excitability than those mentioned in our paper. In fresh preparations the rise of excitability was often absent, or at any rate too small to detect, whilst in preparations which had been kept in Ringer's fluid for twelve hours or more the supernormal excitability was sometimes as great as 115 p.c. of the resting value.

Moreover the existence of the supernormal phase of recovery was itself a puzzle. It seemed that the recovery process overshot the mark before the tissue settled down to its resting condition and such a proceeding did not suggest any simple process such as the diffusion away of the products of activity or the re-establishment of equilibrium by a chemical reaction. It seemed possible that a knowledge of the factors which determined this apparent overthrow would go some way towards elucidating the whole mechanism of recovery.

At the beginning of the investigation it was thought that the variations in the form of the recovery curve might be due to variations in the concentration of calcium ions with which the tissue was in equilibrium. Lucas had found a progressive change in the excitability constants of certain tissues and he had shown that this was due to a lack of equilibrium between the concentration of calcium in the tissues and that in the surrounding fluid. The progressive change in the recovery curve in some preparations suggested the same cause. It was however very soon clear that the concentration of calcium ions had little or nothing to do with the supernormal phase and that the only important factor was the concentration of that ion which has already shown itself to be of paramount importance in so many biological processes, namely the hydrogen ion. How much the form of the recovery curve depends on the $H^{\cdot}$ ion concentration will appear in the following sections.

I. The influence of $H^{\cdot}$ ion concentration on the recovery process.

(a) *The recovery of excitability in nerve.*

Method. In the following experiments the nerve of a sciatic gastrocnemius preparation of the frog was perfused[1] with a buffered Ringer's

[1] It is perhaps incorrect to talk of perfusing a nerve when the fluid surrounds it, but no other word will make it clear that the fluid is continually renewed.

fluid of known $H^{\cdot}$ ion concentration and the course of recovery was mapped out by the method already described by Lucas and Adrian(1). The muscle nerve preparation was set up in the vulcanite chamber shown in Fig. 1. The muscle and the distal part of the nerve lie in a bath of neutral Ringer's fluid and the proximal part of the nerve is bathed by a slow stream of Ringer's fluid of required P_H from one of three Mariotte bottles connected to the inflow tube. The nerve lies in a series of chambers which are completely filled with the perfusing fluid. The chambers are closed above by a glass plate and the point of exit

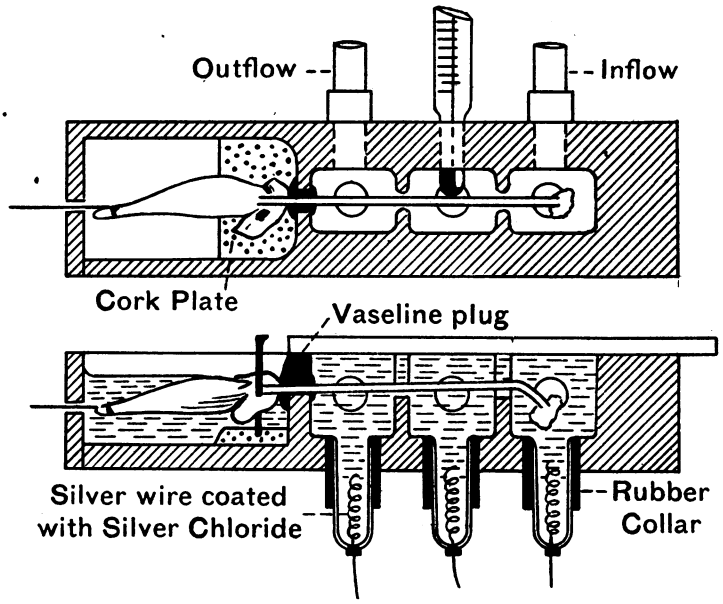


Fig. 1. Chamber with non-polarisable electrodes for perfusing nerve with fluids of different $H^{\cdot}$ ion concentration.

of the nerve is sealed with a plug of vaseline so that the perfusing fluid shall not come into contact with the nerve ending or the muscle.

The stimulating current is led into the chamber by a series of non-polarisable electrodes of the type devised by Lapicque(2). Each electrode consists of a small glass tube filled with Ringer's fluid and containing a spiral of fine silver wire sealed in with wax. Before the electrode is used, a thin coating of silver chloride is deposited electrolytically on the wire by connecting it to the positive pole of a two volt accumulator. The negative pole is connected to a platinum cathode dipping into the fluid in the chamber. The current is passed for two minutes and the fluid

is changed before the preparation is set up. These electrodes show no polarisation after several days' work and it is a simple matter to renew the coating of silver chloride from time to time. They have the advantage of a low resistance and it is never necessary to dismantle them for cleaning or recharging. The current is led through the fluid from these electrodes and takes effect on the nerve at one of the slots where the density of current flow is suddenly increased.

The fluid used was that employed by Mines for work on the heart (3). Besides the ordinary ingredients of Ringer's fluid it contains small quantities of boric acid and sodium acetate to act as buffers. Two stock solutions are made up, one strongly acid and the other strongly alkaline, and these are mixed in different proportions to give a fluid of the required P_H. The fluid is easily made up and its only drawback is that it is most sensitive to additions of acid or alkali about the region of neutrality. The P_H of the fluid was determined by the colorimetric method using the standard solutions of Clark and Lubs (4). During the course of an experiment samples of the fluid were taken from the Mariotte bottle and from the outflow tube of the chamber. The P_H of the two samples showed no difference unless the rate of perfusion was extremely slow.

The recovery of excitability is mapped out by stimulating the nerve with the break shocks of two induction coils which have their primary circuits connected to the keys of a Lucas pendulum. By altering the distance between the two keys the time interval between the stimuli can be accurately adjusted over a wide range (from about ·0001 sec. to ·1 sec.). The second stimulus is sent in at different intervals after the first and for each interval its strength is so adjusted that it is just able to set up a second impulse in the nerve and cause a summated contraction in the muscle instead of a single twitch. The strength of stimulus needed to set up an impulse at any stage of recovery varies inversely as the excitability of the nerve at that time. Thus the return of excitability can be mapped out by determining the strength and time of occurrence of the second stimulus which will just suffice to give a summated contraction.

In these experiments the strength of the stimulus was adjusted by varying the resistance in the primary circuit of the coil. As the coils had no iron core the current induced in the secondary would be directly proportional to that in the primary and therefore inversely proportional to the total resistance in the primary circuit. This was controlled by inserting a dial resistance box reading from one to one thousand ohms

in series with the primary of the coil and a two volt accumulator. The secondary coil was shifted until the break shock would just excite when the resistance in the primary was about 100 ohms; the secondary was not moved after the preliminary setting and the strength of the shock was altered by adding or subtracting resistances. This method of determining the strength of the stimulus has proved far more rapid and

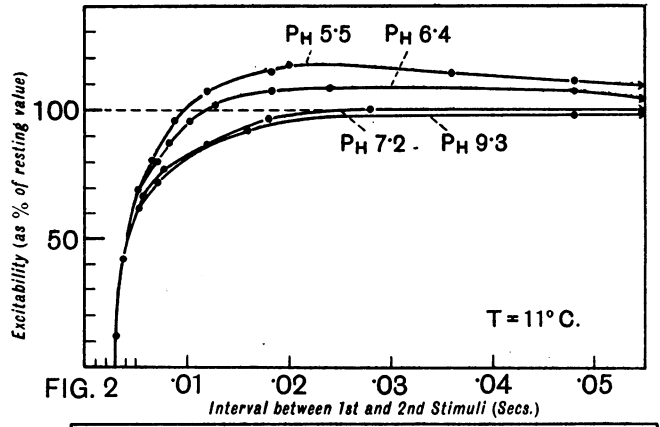


FIG. 2

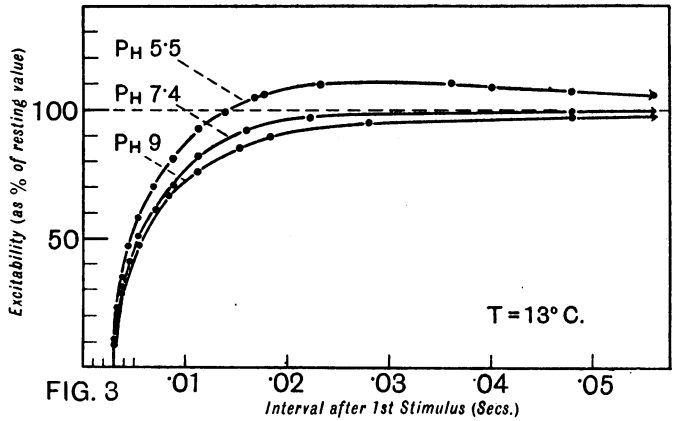


FIG. 3

Figs. 2 and 3. Recovery of excitability in nerve perfused with fluids of different P_H.

more accurate than the usual method of shifting the secondary coil and working out the strength from a calibration curve. As a rule the secondary circuits were connected so that the two stimuli fell at different points on the nerve, but the exact arrangement made no difference to the result.

The most important outcome of these experiments can be seen at once from Figs. 2 and 3. These show the return of excitability in two prepara-

tions perfused with solutions of different $H^{\cdot}$ ion concentration. The nerve was perfused for half an hour with each solution before the recovery curve was determined and the normal or resting excitability was measured afresh for each solution. The normal excitability has a different value for fluids of different P_H (the exact significance of these differences will be dealt with later), but in the figures the curves have been drawn so that the normal excitability in each case is given the value 100. The mean error in each determination is about $\pm$ 1 p.c.

In Fig. 2 it will be seen that as long as the perfusing fluid is on the alkaline side of neutrality (*i.e.* with a P_H greater than 7) the recovery curve shows no supernormal phase. The excitability returns to its resting value and stays there. The return to normal takes place more slowly in the more alkaline fluid (P_H 9·3) and recovery is not complete until ·06 sec. has elapsed after the first stimulus. In the solution which is nearly neutral (P_H 7·2) the recovery is complete in ·03 sec. but there is no sign of a supernormal rise.

When the perfusing fluid is on the acid side of neutrality the recovery curve shows an obvious supernormal phase. In the fluid of P_H 6·4 the maximum excitability is 109 p.c. of the resting value, in the more acid fluid (P_H 5·5) it rises to 117 p.c. The initial return to normal is much more rapid than it is in the fluid of P_H 7·2, but the whole recovery process is not complete in ·06 sec.

Fig. 3 shows the same effect with fluids of P_H 9, 7·4 and 5·5, and an identical result has been obtained in all the other preparations which have been tested. Altogether the experiment has been repeated on 23 different nerves and from these 56 complete curves have been determined in fluids ranging from P_H 3·8 to P_H 10·7.

Certain minor points deserve mention. In the first place it is difficult to estimate the exact $H^{\cdot}$ ion concentration at which the supernormal phase begins to appear. It is invariably present after perfusion for half an hour with a fluid of P_H less than 7 and invariably absent with a fluid of P_H greater than 7·4, but in between these limits the type of curve for a given P_H depends to a considerable extent on the past history of the preparation. If it has been recently perfused with an acid fluid it usually retains the acid type of recovery curve (*i.e.* the curve with a supernormal phase) even after perfusion for half an hour or more with a fluid of P_H 7·4. If it has been in alkali it may retain the alkaline type of curve after perfusion with a fluid of P_H 7, although the alkaline type of curve is changed more easily than the acid. Thus the nerve which is perfused with a fluid near the neutral region tends to retain the form of

recovery curve which it had before, and for this reason it is impossible to specify any single $H^{\cdot}$ ion concentration which can be regarded as the equilibrium condition for the appearance or non-appearance of the supernormal phase in the curve. The existence of this neutral zone within which either type of curve may persist for an indefinite time suggests that the change in the nerve fibre brought about by an altered $H^{\cdot}$ ion concentration exhibits something akin to hysteresis. Even with fluids which are well outside the neutral zone the nerve does not respond immediately to a change in P_H. After it has been in a fluid of P_H 6 it may take 10 or 15 minutes perfusion with P_H 8·5 before the supernormal phase disappears. This is not due to any failure to bring about a rapid change in the P_H of the fluid in the perfusing chamber, for the analysis of samples of the fluid leaving the chamber shows that the change may be complete in less than a minute. No doubt the delay is due in part to the slow alteration in the fluid which immediately surrounds each nerve fibre, but it is evidently related also to the phenomena of hysteresis within the neutral zone.

Another point of some interest is that there is no evidence that the supernormal phase of the curve appears only between certain limits of $H^{\cdot}$ ion concentration. A fluid of P_H 3 usually suspends conduction altogether in an hour or more, but up to this concentration the supernormal rise of excitability becomes greater and greater as the acidity increases. Thus in one experiment the perfusing fluid was changed eight times at intervals of about three-quarters of an hour and nine recovery curves were determined. The relation of the supernormal rise of excitability to the P_H of the perfusing fluid is shown below. Evidently the more acid the perfusing fluid the greater is the increase of excitability during the supernormal phase.

Sciatic nerve of frog. Temperature 11° C.

P_H of fluid	Maximum excitability (resting excitability = 100)	Time
(7·1)	(100)	11.15 a.m.
(8·3)	(100)	12 noon
(9·5)	(100)	12.30 p.m.
(7·0)	(100)	1.5
6·4	107	2.0
5·2	115	2.45
(9·5)	(100)	3.30
5·2	112	4.30
4·0	121	5.15

The curves which showed no supernormal rise are placed in brackets.

So far then it has been shown that a nerve in equilibrium with a fluid more alkaline than P_H 7·4 does not pass through any supernormal

phase in its recovery after the passage of an impulse. A nerve in equilibrium with a fluid on the acid side of P_H 7 passes through the supernormal phase and this becomes more and more pronounced as the acidity rises. These facts make it easy to explain the variations which Lucas and I observed. In our original investigation we used "neutral Ringer," *i.e.* Ringer's fluid without buffers, and the P_H of this is usually in the neighbourhood of 6·5. Consequently we obtained the supernormal rise in every case. In some later experiments I used Ringer containing small traces of alkali and in these I was unable to detect the supernormal phase. It is interesting to note that in the nerves supplying the claw muscles of Astacus, Lucas (5) found the supernormal excitability as great as 120 p.c., and in these nerves there was a rapid progressive change which led to the failure first of single stimuli and then of all kinds of stimulation. Such a change might well be due to the development of acid in the tissues and this would evidently account for the great supernormal rise.

(*b*) *The recovery of conductivity in nerve.*

Keith Lucas and I found that the supernormal phase of recovery involved an increase of conductivity above the resting value as well as an increase of excitability. An impulse set up during this phase could travel further through a region of decrement than an impulse set up in resting nerve, and for this reason a series of impulses falling at the right intervals might succeed in passing a nerve ending or a synapse when a single impulse would fail. From the point of view of the theory of nervous summation we regarded the increased conductivity during the supernormal phase as a great deal more important than the increased excitability, in fact we dealt with the latter only because it confirmed our view of the existence of a supernormal phase in recovery. Thus it becomes a question of some interest to decide whether the $H^{\cdot}$ ion concentration has the same influence on the recovery of conductivity as it does on that of excitability.

To test this point a nerve was first of all perfused for half an hour with a fluid of known P_H, and a length of about 2 cm. between the electrodes and the muscle was then bathed in a fluid of the same P_H containing in addition 6 p.c. of alcohol. This concentration was great enough to suspend conduction in about half an hour and towards the end of this period the proximal part of the nerve was stimulated alternately with single maximal break shocks and with groups of two shocks separated by an interval of ·024 sec. (this interval corresponds with the maximum rise of excitability in a nerve perfused with an acid fluid at 11° C.).

It was found that if the narcotising fluid was on the alkaline side of neutrality, conduction failed at the same moment for the single and the double stimuli alike. If the fluid was acid there was, as a rule, a period of about 20 seconds within which the two stimuli produced a contraction in the muscle whereas the single stimulus had no effect. It is by no means easy to catch the nerve at the exact moment when conduction fails for a single impulse and therefore in two out of six "acid" experiments the difference between single and double stimuli was not observed. However in four of them the double stimuli continued to affect the muscle after the single stimulus had failed; on the other hand in all the "alkaline" experiments the double and single stimuli failed at the same moment.

In a second series of experiments the use of alcohol as a narcotic was dispensed with and the impaired conduction was brought about by treating a length of nerve with strongly acid or strongly alkaline fluids. A fluid of P_H 2·8 caused failure of conduction for a single stimulus in 83 minutes but the double stimuli still produced a contraction in the muscle for two minutes longer. In another experiment with a Ringer of P_H 2·1 the single stimulus failed after 39 minutes and the double stimuli succeeded for another 50 seconds. On the other hand a fluid of P_H 11·8 caused failure in 23 minutes for single and double stimuli alike and one of P_H 11·4 had the same effect after 63 minutes. In the two "alkaline" experiments the interval between the two stimuli was varied from ·024 to ·08 sec. but none of these intervals led to summation.

These experiments show that in a nerve which has been perfused with an acid fluid it is possible to impair the conduction in such a way that a single impulse fails to reach the muscle, although two impulses may succeed if the second travels in tissue which is in the supernormal phase of recovery. When the nerve is in an alkaline fluid no such effect is observed. Thus the "acid" nerve shows a phase of increased conductivity as well as a phase of increased excitability, whereas both phenomena are absent in the alkaline nerve.

(*c*) *The recovery process in cardiac muscle.*

At an early stage in the investigation it was thought desirable to extend these results to other excitable tissues besides the sciatic nerve of the frog. Skeletal muscle has the disadvantage that the contractile change outlasts the whole of the recovery process and therefore a stimulus falling during the recovery from a previous disturbance will find the muscle still contracted. The change in shape may cause apparent changes in excitability which will mask the true recovery phenomena. Cardiac

muscle is more easily dealt with, because in it the contraction is usually over before recovery is complete and therefore it is possible to measure the excitability in the later stages of recovery without the same liability to error.

The only published determinations of the recovery of excitability in the heart are those of Trendelenburg(6). In his experiments the circulation was intact and the heart was beating spontaneously; the curves show a gradual return to normal excitability without a supernormal phase.

I have repeated these measurements in hearts exposed to fluids of different P_H, paying special attention to the later stages of recovery. For the accurate measurement of this part of the curve it is essential that the heart should not beat spontaneously, as a fresh beat may arrive from the sinus before recovery is complete. It should be possible to overcome this by using a preparation of the isolated ventricle, but in practice it was found difficult to keep such a preparation from beating by itself, or to perfuse it adequately if it did not beat. In the method finally adopted the frog's heart was perfused for half an hour with a fluid of known P_H through the inferior vena cava and at the end of this period a Stannius ligature was applied. This put an end to the perfusion, and the determinations on the quiescent ventricle were carried out as rapidly as possible to minimise the effect of any slight alteration which might take place in the reaction of the fluid remaining in the ventricle. As a matter of fact the response to fluids of different P_H was so constant that it is very unlikely that the condition of the ventricle can have changed rapidly after the ligature was applied. As in the case of nerve fibre, the change brought about by an altered $H^{\cdot}$ ion concentration proceeds very slowly and may not be complete after perfusion for a quarter of an hour; thus a slight change in the P_H of the fluid remaining in the ventricle would be unlikely to have any effect for several minutes at least.

The ventricle was stimulated by break induction shocks from two coils with their primary circuits connected to a rotating contact breaker. Non-polarisable electrodes were used and these were applied on either side of the ventricle midway between base and apex. In estimating the normal, or resting excitability it is important to allow the heart to remain at rest for at least 30 seconds, since the whole recovery process may not be complete for 15 seconds or more. The probable error in each determination is greater than in the case of nerve as the threshold is more variable. The error in most of the determinations is about $\pm$ 2 p.c.

The effect of fluids of different P_H may be seen from Fig. 4. This gives the recovery curves from four different hearts perfused with Ringer of P_H 6·2, 6·4, 9 and 9·2. The time scale of Fig. 4 is 200 times as long as that in Figs. 2 and 3, but in other respects the curves for cardiac muscle and for nerve resemble one another very closely. The two hearts which were perfused with fluids of P_H 6·2 and 6·4 show a well-marked supernormal phase and the hearts perfused with P_H 9 and P_H 9·2 show none. The neutral region appears to be slightly more on the alkaline side than it is in the nerve fibre since in two experiments the supernormal phase was just perceptible after perfusion with fluids of P_H 7·6 and 7·8, though it was invariably absent when the P_H was greater than 8. It

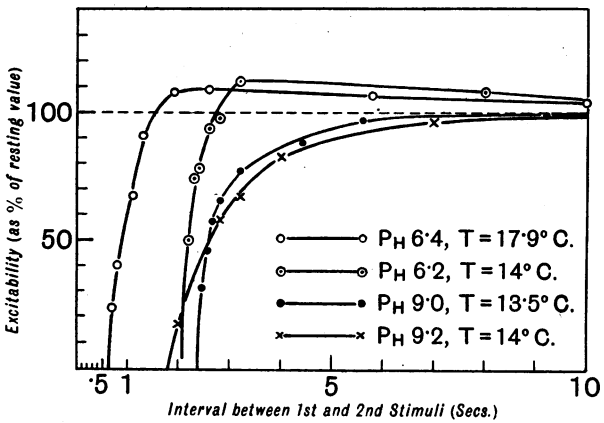


Fig. 4. Recovery of excitability in cardiac muscle.

is impossible to test the effect of fluids more acid than P_H 6 as the ventricle becomes completely inexcitable after half an hour's perfusion.

So far then the results confirm those obtained from the nerve fibre, but the most interesting part of these experiments is that which deals with the recovery of the contractile power in the heart as distinct from its electrical excitability. It has been known since the pioneer work of Bowditch(7) that if the quiescent ventricle is excited by two stimuli separated by a short interval the second contraction may be greater than the first. If the second stimulus falls during the relative refractory period the second contraction is smaller, but as soon as this period is over the second contraction may rise to a greater height and the effect is present with intervals as long as 15 seconds between the first and second contraction. The close agreement between the time relations of this effect and the time relations of the supernormal excitability in

the heart suggests that the two may have a common origin, and an inspection of the graphic records obtained after perfusion with different fluids shows that they are certainly very closely related.

The heights of contraction in response to the two stimuli were measured in 18 hearts. Figs. 5 and 6 show the actual tracings obtained in two experiments and Fig. 8 gives the results of four others. The effect of an alteration in the P_H of the perfusing fluid can be seen at once by

Fig. 5.

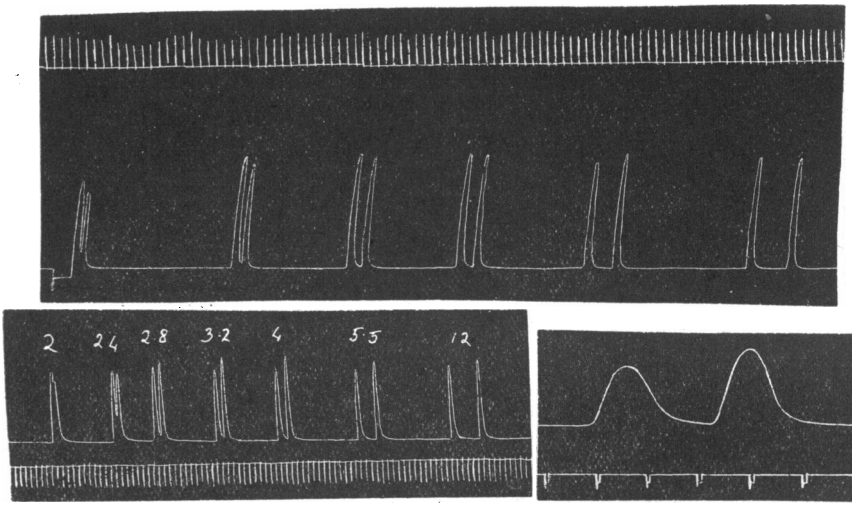


Fig. 6. Fig. 7.

Fig. 5. Recovery of contractile power in heart perfused with fluid of P_H 8·8 showing absence of supernormal phase. Time marker beats every two seconds.

Fig. 6. Heart perfused with fluid of P_H 6·6; supernormal phase present. Time marker beats every two seconds.

Fig. 7. Heart perfused with fluid of P_H 6·4. Time marker beating seconds.

(Read from left to right.)

comparing Fig. 5 with Fig. 6. In Fig. 5 the heart had been perfused for half an hour with a fluid of P_H 8·8 before the Stannius ligature was applied: the second contraction is never greater than the first except in the case of the fifth group of contractions where the height of the first is abnormally small. The interval between the two varies from 2–12 seconds (and intervals up to 20 seconds were tried although these are not recorded in the figure). In Fig. 6 the heart was perfused with a fluid of P_H 6·6 and the second contraction is evidently larger than the

first when the interval between them is greater than 2·8 seconds. The numbers placed over each group of contractions give the interval in seconds between the two stimuli. Fig. 7 (from another heart) shows the form of the two contractions more clearly as the recording surface is moving at a greater rate. Fig. 8 gives a summary of the results in four other hearts: the curves are obtained by comparing the heights of the first and second contraction and giving the first contraction the value 100. The abscissae give the intervals between the first and second contraction and not the intervals between the two stimuli.

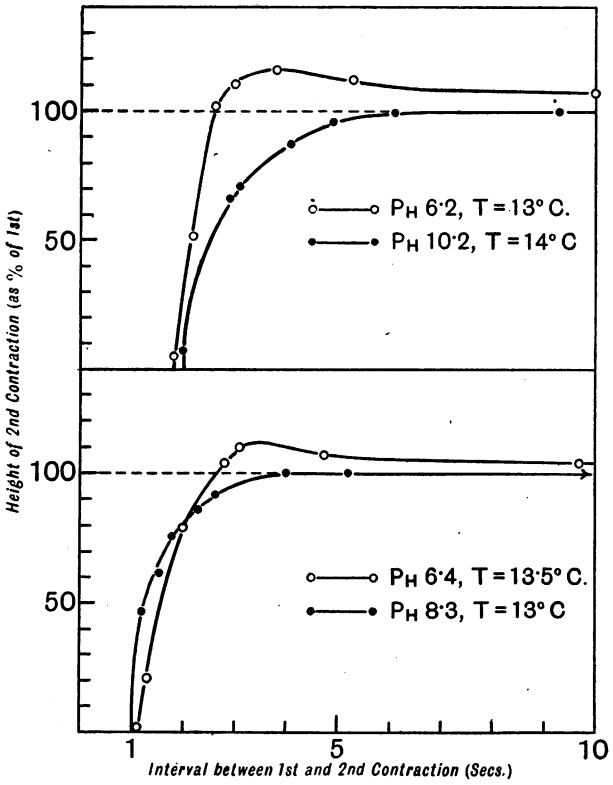


Fig. 8. Recovery of contractile power in hearts perfused with fluids of different P_H.

In the early stages of recovery the second contraction begins before the first has subsided. In this case the height of the second contraction is measured from the point at which it begins to diverge from the first contraction. Thus in the second group in Fig. 6 the height of the second contraction would be taken as 50 p.c. of the first although the summits of both contractions are on a level.

The four curves are in every way comparable with those expressing the return of excitability in the heart or in the sciatic nerve. When the heart had been perfused with a fluid of P_H 8·3 or P_H 10·2 the recovery of contractile power showed no supernormal phase, when the fluid was of P_H 6·2 or P_H 6·4 the supernormal phase was well marked. A similar result was found in the remaining eleven experiments. Evidently the increased contractile power described by Bowditch and others is one expression of the supernormal phase of recovery and like the other phenomena of the supernormal phase it occurs only when the tissue is in equilibrium with an acid solution.

Owing to the method of experiment it was impossible to determine the recovery curves of the same heart in equilibrium with more than one fluid and for this reason it is difficult to say whether the supernormal rise increases with the acidity of the perfusing fluid, as it does in the case of a nerve fibre. After perfusion with a fluid of P_H 5·8 the contractions become so small and so sluggish that it is difficult to make out whether the supernormal rise exists or not. Certainly it is more easily detected when the P_H of the fluid is in the neighbourhood of 6·3, but this is very nearly the limiting acidity with which the ventricular muscle can be in equilibrium as a more acid fluid causes a progressive change leading ultimately to complete inexcitability.

Dale and Thacker(8) have shown that the optimal $H^{\cdot}$ ion concentration varies for the different chambers of the heart and is more acid for the auricle than for the ventricle. I have some rather indirect evidence to show that the critical $H^{\cdot}$ ion concentration for the appearance of the supernormal phase varies in the same way. In several experiments the recovery of excitability and of contractile power were measured at the same time. As a rule they agreed closely, but in one experiment the second contraction was greater than the first although the excitability did not rise above the resting value. It was found that the stimulating electrodes had slipped down to the auriculo-ventricular junction, and it was therefore possible that the excitability which was measured was that of the auricle and not of the ventricle. When the electrodes were replaced on the sides of the ventricle the recovery of excitability gave the usual supernormal phase. The heart had been perfused with a fluid of P_H 6·8 and this might well have been on the acid side for the ventricle and on the alkaline side for the auricle. The experiment was not repeated and as it stands it can only be regarded as suggesting a possible difference in the critical concentrations for ventricle and auricle.

(*d*) *The staircase effect in skeletal muscle.*

The staircase effect or "Treppe" in skeletal muscle is generally regarded as having the same mechanism as the Bowditch effect in the heart, although it persists for a very much longer time in skeletal muscle in spite of the shorter duration of the refractory period. If the two phenomena are related we should expect to find the staircase present only when the muscle is perfused with an acid fluid. To test this point the frog's sartorius muscle was set up in a perfusion chamber and stimulated by break induction shocks recurring at intervals varying from one to three seconds. The rate of stimulation was controlled by a rotating contact breaker and the stimuli took effect on the nerve-free pelvic end of the muscle.

In the first series of experiments the contractions were recorded by a very light tension lever with a small moment of inertia. Under these conditions the staircase effect was never observed at all although the muscle had been perfused for four hours with fluids varying from P_H 10 to P_H 4. With repeated stimulation the contractions diminished in height, but after a rest of from 1–10 minutes renewed stimulation did not give rise to the staircase phenomenon and the muscle could be fatigued to complete exhaustion without showing any signs of it.

The staircase however did appear when the tension lever was discarded and an isotonic lever used in its place, and the effect was more easily produced when the moment of inertia was increased by moving the weight further from the axis of the lever. The staircase then appeared when stimulation was renewed after a short pause. Its appearance bore no relation to the P_H of the perfusing fluid between the limits of P_H 10 and P_H 4 and it seemed to depend entirely on the state of fatigue of the preparation.

Thus in its reaction to different concentrations of $H^{\cdot}$ ions the staircase of skeletal muscle behaves quite differently to the summation effect in the heart. This is very easily understood if we take into account Fröhlich's work on the cause of the staircase (9). He finds that it depends entirely on the slower time relations of the contractile response induced by fatigue. The first stimulus after a pause finds the muscle rested, and the contraction and relaxation are rapid; after a few stimuli the onset of fatigue prolongs the rate of contraction and relaxation without, at first, diminishing the force of contraction. With a heavy recording lever this prolongation by itself may be enough to cause an apparent increase in the height of the contraction, and the experiments recorded above

suggest that the whole effect often depends on the inertia of the recording apparatus. However Fröhlich shows that the increased duration of the contraction may lead to an actual increase in the tension exerted by the whole muscle, because the longer the duration the more chance is there that all parts of the muscle will be fully contracted at the same moment. Fröhlich's records show clearly that in skeletal muscle the increased height of contraction at the end of a "staircase" is associated with a slower development and subsidence of the contractile process. Thus the whole effect is nothing more than a preliminary stage of fatigue and it is not surprising that the $H^{\cdot}$ ion concentration of the perfusing fluid should make very little difference to its onset except in so far as it may hasten or retard fatigue.

On the other hand in the heart the increased height of a contraction occurring in the supernormal phase of recovery is certainly not due to fatigue. Fig. 6 shows that there is no appreciable difference in the duration of the first and second of a pair of contractions, though the height of the second may be very much greater than that of the first. This is confirmed by records of the contractions made on a rapidly moving drum. Fig. 7 shows such a record from a heart which had been perfused for half an hour with a fluid of P_H 6·4. The time marker signals every second. The second contraction is 130 p.c. as high as the first, but the duration of the two contractions is exactly the same.

Thus Fröhlich's explanation will not cover the case of the supernormal contractions of cardiac muscle, though it is evidently true for the "Treppe" in skeletal muscle. In fact the two effects are essentially different, as indeed their time relations would lead one to suppose.

II. The nature of the supernormal phase.

It has been shown that in a tissue which is in equilibrium with an acid perfusing fluid the three main functions of excitability, conductivity and contraction are all increased above their normal resting value at a certain stage in the recovery from a previous disturbance. The discussion which follows is concerned mainly with the increased excitability because this function is more easily investigated than conduction or contraction. Also the experimental results are more easily interpreted because the excitation is an immediate consequence of the external stimulus, whereas many processes may intervene between the stimulus and the contractile response.

(*a*) *Factors accounting for the increased excitability.*

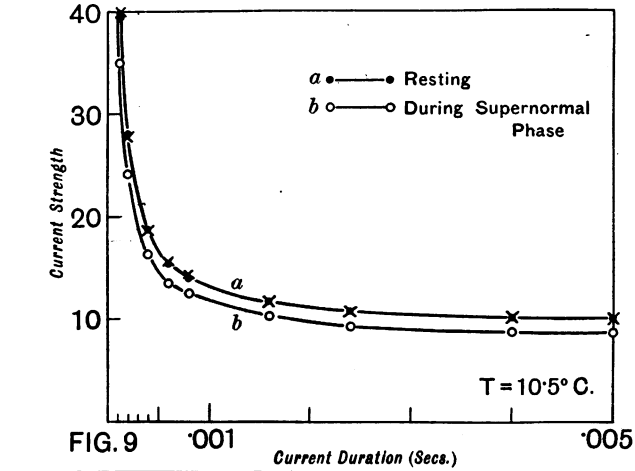
Within recent years the mechanism of electrical excitation has been worked out by Nernst, Lapicque, Lucas, Hill and others, and it is generally agreed that the necessary condition for excitation is that the current should produce a certain heaping up of ions at some membrane in the tissue within a certain time. With brief currents such as the break shocks of a coreless induction coil the time limit need not be considered, since it will always outlast the stimulating current. Thus the success or failure of such a current will depend on two factors (*a*) the ease with which the ions can be moved up to the membrane, and (*b*) the concentration which must be produced at the membrane. The factor (*a*) will depend on the mobility of the ions concerned; the concentration brought about by the current will tend to dissipate itself by backward diffusion, and if the mobility of the ions is increased this escape by backward diffusion will outweigh the greater rapidity with which the ions will move up to the membrane under the influence of the electric field.

Keith Lucas(10) showed that it is possible to estimate the share of either of these factors in causing a given alteration of excitability by mapping out the curve relating current duration to current strength required to excite. If the shape of the curve is not altered and the only effect of the change has been to increase by a constant percentage the current strength corresponding to each duration, then the change must be due entirely to an increase in the concentration of ions required at the membrane and the mobility of the ions has not been affected. If the shape of the curve changes, then the mobility of the ions must have altered, and the extent of this alteration may be calculated from the constants of the curve according to Hill's equation(11).

Thus at the outset we may enquire whether the increased excitability during the supernormal phase is due to a decrease in the mobility of the effective ions or to a decrease in the concentration required at the membrane. To do this we must map out the strength-duration curves for currents sent into the resting nerve and for currents sent in during the supernormal phase of recovery. Fortunately the excitability of the nerve remains approximately constant for about ·005 sec. during the height of the supernormal phase, and the complete strength-duration curve can be mapped out without using durations greater than this.

Fig. 9 shows the result of an experiment carried out in this way. The nerve was perfused for two hours with a fluid of P_H 6, and the recovery

curve was then determined. At an interval of ·0216 sec. after the first stimulus the excitability had risen to 115 p.c. of its resting value, and it remained at this level for ·017 sec. The strength-duration curve was then determined (*a*) for the resting nerve, and (*b*) with currents which began



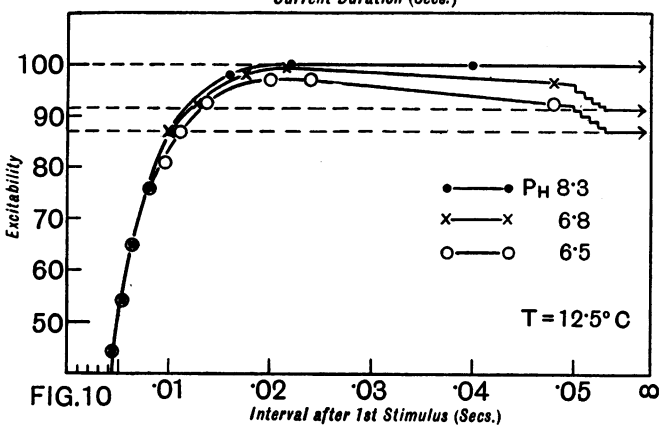


Fig. 9. Relation between strength and duration of current required to excite (*a*) during rest and (*b*) during supernormal phase of recovery.

Fig. 10. Recovery of excitability in different fluids. Not corrected for change of threshold.

·024 sec. after a single maximal stimulus. These currents would fall on tissue which was at the height of the supernormal phase. The two strength-duration curves are shown in the figure: the upper curve (*a*) is that for the resting nerve and the actual measurements are shown by the small

dots on the curve, the minimal current required to excite being given the value 10; the lower curve (*b*) is that determined during the supernormal phase, and the minimal current has the corresponding value of 8·7, as the excitability was increased to 115 p.c. The crosses on the upper curve represent the current-strengths of the lower curve multiplied by $\frac{115}{100}$. They agree so closely with the points determined for the resting nerve that there can be no doubt that the shape of the two curves is identical.

Thus the increased excitability during the supernormal phase must be due to a decrease in the concentration of ions necessary for excitation to occur and not to any alteration in the mobility of the ions concerned.

(*b*) *The effect of different concentrations of H· ions.*

The most striking feature of all the foregoing experiments has been the appearance of the supernormal phase when the perfusion fluid is on the acid side of neutrality and its absence when the fluid is on the alkaline side. The curves in Figs. 2 and 3 do not suggest any explanation of this effect because the curve for each fluid is drawn on the assumption that the resting excitability in that fluid has the value 100. This is legitimate enough as a means of showing the general effect of different fluids on the form of the recovery curve, but it misses one of the most important features of the experiment. The resting excitability has a different value for each fluid, and if we allow these differences to appear instead of reducing all the curves to the same threshold line the changes in the form of the recovery curve become much more significant. Fig. 10 is constructed on these lines from an experiment in which the nerve was perfused with fluids of P_H 8·3, 6·8 and 6·5. The resting excitability in the fluid of P_H 8·3 is taken as 100 and the recovery curve in this fluid is shown gradually rising to this level and remaining there. The actual determinations are marked by small dots on the curve. After half an hour in a fluid of P_H 6·8 the excitability had fallen to 91·5 p.c. of its former value. Consequently the normal is now 91·5 instead of 100, and the recovery curve is constructed on this basis, the small crosses showing the actual measurements. In the fluid of P_H 6·5 the excitability fell from 91·5 to 87 p.c. and the recovery curve is marked by the small circles.

The two "acid" curves show the usual supernormal period and the "alkaline" curve does not, but the chief point of interest lies in the fact that in the earlier stages of recovery all three curves are absolutely continuous, and that they are very nearly continuous up to the height of the

supernormal phase of recovery. Thus at ·022 sec. after the first impulse the excitability in P_H 8·3 is 99·5, that in P_H 6·8 is 99 and that in P_H 6·5 is 97. At longer intervals the excitability in the acid fluids falls off and eventually reaches the resting values of 91·5 for P_H 6·8 and 87 for P_H 6·5. Evidently in fluids of different P_H the maximum excitability at the height of the supernormal phase is much more nearly constant than the normal excitability after recovery is complete.

The same result was found in all the nerves examined. The maximum excitability in a fluid on the acid side of neutrality was always slightly less than the excitability in an alkaline fluid, but the difference was usually not greater than 5–6 p.c. whereas the difference between the resting excitabilities was often as great as 20 p.c. The result was quite independent of the order in which the different fluids were perfused, though three experiments had to be discarded because the nerve was

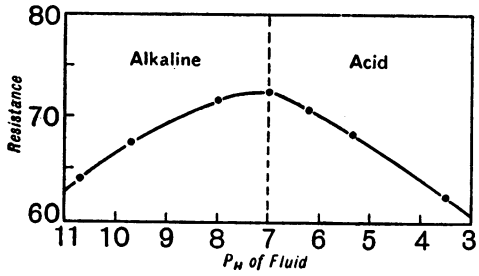


Fig. 11. Resistance of fluids of different H· ion concentration.

obviously undergoing a progressive change and the thresholds were not constant.

This result made it necessary to examine more closely the changes in excitability due to the different fluids. In the first place the arrangement of the electrodes used in the chamber (Fig. 1) makes it difficult to allow for changes in resistance in the nerve or in the perfusing fluid. The current is carried partly by the nerve and partly by the fluid surrounding it, and therefore an increase in the resistance of the fluid will cause a larger proportion of the current to pass through the nerve, and *vice versa*. To gain some idea of the magnitude of this effect the resistance of the different fluids was measured: it was found that the resistance was greatest at the neutral point (P_H 7), that it decreased when the fluid was made more acid or more alkaline and that the decrease was more rapid for increasing acidity than for increasing alkalinity. The values of the resistance in arbitrary units are shown in Fig. 11, and it will be seen that

a fluid of P_H 6 has a resistance of 70·25 and one of P_H 5 a resistance of 67·5 as against the values of 71·5 for P_H 8 and 69·5 for P_H 9. This result is only to be expected in view of the fact that the $H^{\cdot}$ and $OH^{\cdot}$ ions are more mobile than the other ions present in the fluid and that the $H^{\cdot}$ ion is more mobile than the $OH^{\cdot}$. The differences are small, but they are of such a nature that a nerve in an acid fluid will appear to have a slightly lower excitability than one in an alkaline. The resistance of the nerve shows no appreciable change after perfusion with the different fluids; it was measured with a weak alternating current led in by non-polarisable electrodes.

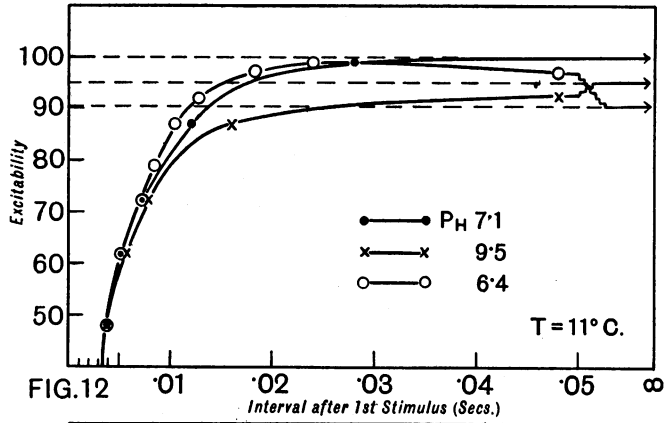
To overcome the effect of these small changes in resistance the experiment was conducted in a slightly different way. The nerve was placed on platinum electrodes immersed in the perfusing fluid and the second stimulus was a brief constant current of ·0008 second duration derived from a low resistance potentiometer wire in circuit with a four volt accumulator. With this arrangement the potential difference between the two electrodes must remain very nearly constant in spite of large changes in the resistance of the perfusing fluid, since these changes will have practically no effect on the fall of potential in the potentiometer.

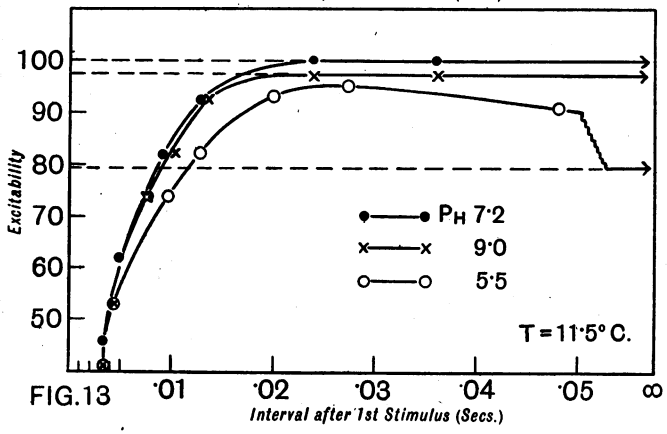
Figs. 12 and 13 show two sets of curves obtained by this method. In Fig. 12 the maximum excitability in an acid fluid is 99 p.c. of the resting value in a neutral fluid. In Fig. 13 the agreement is not so close and the supernormal excitability in P_H 5·5 is only 95 p.c. Both figures show that the resting excitability in an alkaline fluid is less than it is in a fluid nearer the neutral point. This is only true within certain limits, for a strongly alkaline fluid (P_H 11) usually causes a great initial rise of excitability followed by a sudden fall which heralds the complete failure of all stimuli. This rise is probably due to some secondary effect on the fibre, for as long as the P_H does not exceed 9·5 the excitability is generally less than it is at neutrality.

Thus there is still a slight discrepancy between the supernormal excitability in an acid fluid and the normal in a neutral fluid even when changes in resistance are taken into account. However there is another factor which has not yet been considered and this is the decrease in the mobility of the kations when the $H^{\cdot}$ ion concentration of the fluid is reduced. As stimulation takes effect at the kathode, the mobility of the kations will be an important factor in deciding whether a given current can establish the required concentration at the membrane against the opposing forces of diffusion. In a fluid on the acid side of neutrality there will be large numbers of the very mobile $H^{\cdot}$ ion, whereas

in an alkaline fluid the slower $Na^{\cdot}$ ions will take a larger share in the work. For this reason the current required to produce a given concentration of kations will be weaker when the fluid is alkaline than when it is acid.

The magnitude of this effect can be tested by constructing the curves





Figs. 12 and 13. Recovery of excitability in different fluids. Not corrected for change of threshold.

relating the strength and duration of the stimulating current in fluids of different P_H (see p. 17), since any change in the time factor of the curve must be due to an altered rate of diffusion. It was found that a change from acid to alkali did produce an appreciable shift in the curve. Fig. 14 shows two curves from a nerve perfused with fluids of P_H 9·6 and P_H 5·5. The current strength at infinite duration is given the value 10

in either case, though the threshold was in reality 36 p.c. higher in the acid fluid than in the alkaline. It will be seen that the current strength in P_H 9·6 rises at greater durations than that in P_H 5·5, and the chronaxie (*i.e.* the duration corresponding to twice the strength at infinite duration) is ·00045 sec. in the former and ·00035 sec. in the latter. A change of the same order was found in the two other preparations which were examined.

It is impossible to insist on the exact numerical values found in this experiment, because the probable error is large, but it is clear that the altered excitability in fluids of different P_H must depend on some other factor besides an alteration in the required concentration of ions, and the direction in which the curve is shifted shows that in an acid

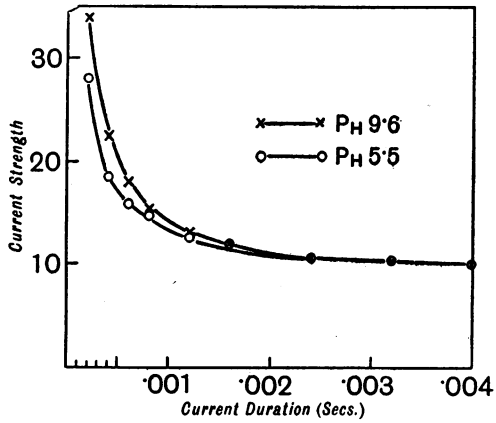


Fig. 14. Relation between strength and duration of current required to excite resting nerve in fluids of different H· ion concentration.

fluid there is an increased tendency for differences of concentration to be equalised by diffusion. This means that a part of the increased strength of current required to excite will be needed to counteract the greater rapidity of diffusion of the H· ions.

From Hill's equation it is possible to calculate the relative importance of the change in the rate of diffusion and the change in concentration required at the membrane. In this particular experiment it appears that about one-quarter of the whole change in threshold is due to the altered rate of diffusion, and three-quarters to a change in the required concentration. Now it was found that the resting excitability in an acid fluid might be 21 p.c. less than that in a neutral fluid, whereas the supernormal excitability in the acid fluid was only 5 p.c. less (Fig. 13),

It has been shown that the rate of diffusion of the ions is the same in the supernormal phase of recovery as in the resting state (Fig. 9) and therefore if we consider only the concentration of ions necessary to excite it is clear that, in the supernormal phase, this may be equal to or even less than the concentration required in a neutral fluid.

In fact if instead of the strengths of current needed to excite, we take the concentration of ions which must be brought about in the tissue, we have the result that for the resting nerve the concentration is least when the fluid is neutral and that it becomes greater when the fluid is made more acid or more alkaline, but that during the supernormal phase of recovery in an acid fluid the necessary concentration is not greater than that required at neutrality.

It would be idle to pretend that an exact agreement can be proved from the experiments submitted, but within the limits of error the agreement is certainly close enough to justify some speculation as to its cause.

Suggested explanation. It is well known from the work of Hardy (12) that a colloidal particle is most unstable in the neighbourhood of its iso-electric point, and that addition of acid or alkali makes it more stable by giving it a positive or negative charge. Let us assume that the membrane in the nerve fibre at which the concentration of ions takes effect is made up of particles which have their iso-electric point in the neighbourhood of neutrality. Let us further assume (and here we are on more debatable ground) that the passage of an impulse with its corresponding electric variation leaves the membrane with a negative charge which becomes smaller and smaller as the tissue recovers. This would mean that as recovery advanced the tissue would become more and more easy to excite, because the particles forming the membrane would become more and more unstable as their negative charge was lost. In a neutral fluid the particles would end up with no charge at all and in this condition the tissue would be most easily excited; in an alkaline fluid the particles would retain a small negative charge and consequently the excitability would never rise to the maximum value, on the other hand in a fluid on the acid side of the iso-electric point the particles on losing their negative charge would pass through a phase in which they had no charge at all and they would then take on a small positive charge from the $H^{\cdot}$ ions in the fluid. Thus the nerve recovering in an acid fluid would pass through a phase in which its excitability was equal to the maximum and would then become less excitable again as the membrane became positively charged.

An explanation on these lines would account for the appearance of the supernormal phase only when the nerve is in equilibrium with an acid fluid and for the close agreement between the excitability in the supernormal phase and the resting excitability in a neutral fluid. It would also account for the phenomena of hysteresis mentioned on p. 7, since Mines has shown that the charge conferred on a membrane by ions of different sign behaves in the same way(13).

Nevertheless it cannot be claimed that the hypothesis as it stands is free from objection. It is a far cry from a protein particle to a membrane which is probably the seat of an electrical double layer even when it is in equilibrium with a neutral fluid; again, the most vital assumption of the theory, that the excitable membrane becomes negatively charged during the passage of an impulse, is by no means certain. It is true that the passage of an impulse involves an electric variation in which the active part of the nerve becomes negative to surrounding parts as judged by electrodes placed in contact with the nerve trunk, but most of the evidence goes to show that this variation is due to the actual passage of a current in the surrounding fluid from the resting to the active part of the nerve, and it does not follow that such a current would produce a negative charge on the protein particles at the point where it re-entered the nerve. Another objection is that it is by no means certain how far the recovery of excitability after an impulse runs parallel with the subsidence of the electric response, and there are some grounds for denying any relation between the two(14).

It would however be premature to discuss these and other difficulties until further evidence is collected. The hypothesis must be regarded as nothing more than an indication for further work, and I am at present engaged on a series of experiments which may give more definite grounds for accepting or rejecting it.

III. Theories of acid production.

Although the hypothesis put forward cannot be insisted on at present, the facts on which it is based do enable us to test one particular development of the theory which regards the activity of an excitable tissue as due to the production of acid. That acid is produced is not disputed, but the point at issue is whether the production of acid precedes or follows activity. In the case of nerve there is little doubt that if acid is produced at all (and Tashiro's(15) results make this highly probable), the production cannot be a sudden event occurring at the same moment as the change which constitutes the impulse. As Bernstein(16) and

Brünings(17) have pointed out, the fact that the electric variation in nerve is not accompanied by any appreciable change of temperature makes it necessary to suppose that the current is caused not by any simultaneous chemical change, but by the liberation of free energy due to the existence of inequalities in the concentration of different ions in different regions in the fibre. In fact the nerve behaves like a concentration cell, and the production of a current without any appreciable change of temperature is comparable to the performance of work by a perfect gas expanding isothermally. This conception is greatly strengthened by the discovery by Bernstein and Tschermak(18) that the electric organ of the Torpedo shows a distinct cooling when the current it produces is led through an external circuit. The current will develop heat in passing through this circuit, but the amount of heat evolved must be exactly balanced by the cooling of the organ, since there is no change of temperature at all when the current has to travel in the fluid immediately surrounding the organ instead of passing through an external circuit. Thus the work done is derived ultimately from the warmth of the surroundings, though it involves a decrease in the free energy of the concentration cell.

After the impulse has passed, the differences of concentration which existed previously must be restored, and this process will ultimately involve a chemical change which may be associated with the production of acid. But this change may be spread over a long period, just as the recovery heat production of a muscle may continue for two or three minutes after the contraction has ceased.

Thus in nerve it seems highly probable that the passage of an impulse involves a change of physical rather than of chemical energy, and that it cannot be due to any sudden production of acid in the fibre[1].

In the case of muscle the reverse is generally assumed to be true and it is supposed that the contractile process is the result of a sudden production of acid in contact with the sensitive fibril mechanism. The particular form of this theory which concerns us at present is that stated by Mines(19). He says "Our hypothesis emerges in the following terms. The propagated disturbance in muscle culminates in the liberation of acid in certain localised regions of the muscle fibre. The subsequent shortening is due to the action on some colloidal system of these local concentrations of acid: the local relatively high concentrations rapidly

[1] Another argument in favour of this is the difficulty of explaining the electric variation as due to the sudden production of acid (see Lillie, *Amer. Journ. of Physiol.*, 37. p. 354. 1915).

disappear by diffusion and the general rise in $H^{\cdot}$ ion concentration (a rise which is gradually counteracted by the oxidative removal of the acid) is responsible for the more lasting effects of excitation on excitability and tone." On these lines he explains the summation of contractions and the "Treppe" of skeletal muscle, and the increased height of the second contraction in cardiac muscle. In regard to the heart he shows first of all that the force of contraction is greatest when the heart is in equilibrium with a fluid of $H^{\cdot}$ ion concentration in the neighbourhood of neutrality. He considers that the tissue of the resting heart will be on the alkaline side of this optimal value, but that the occurrence of a contraction will cause a liberation of acid which will temporarily increase the general $H^{\cdot}$ ion concentration of the tissue. As the increased $H^{\cdot}$ ion concentration is subsiding it must pass through its optimal value (near neutrality) before it returns to its resting value on the alkaline side of neutrality. Consequently a stimulus occurring at the moment when the concentration is optimal will produce a larger contraction than that set up in the resting muscle.

It is difficult to reconcile this attractive hypothesis with the behaviour of the heart described on pp. 10–13. There can be little doubt that the $H^{\cdot}$ ion concentration of the perfusing fluid bears some relation to that of the tissue when at rest, and little doubt that the force of contraction is greatest when the tissue is at or near neutrality. But on Mines's hypothesis the second contraction should be larger than the first only when the tissue passes through the optimal concentration on its way back to the resting value; this should occur when the resting tissue is alkaline but not when it is acid, for then the optimal concentration will never be reached. Thus we should expect to find the supernormal phase of recovery present when the tissue is in equilibrium with an alkaline perfusing fluid and absent when the fluid is acid. Actually we find the exact reverse of this; the supernormal phase is always present in an acid fluid and never in an alkaline. Indeed Mines's hypothesis would be in exact agreement with the limited range of facts here presented if we read $OH^{\cdot}$ ion for $H^{\cdot}$ ion and suppose that the contraction is due to a liberation of alkali instead of acid.

This proposal would be in conflict with so many well-known phenomena that it need not be seriously considered. As an alternative to Mines's hypothesis I can only offer the suggestion already put forward to account for the supernormal phase of excitability, namely that the force of contraction depends, like the excitability, on the initial degree of instability of some surface in the muscle, that this instability is greatest

at the iso-electric point and that activity causes the surface to take on a negative charge whereas an acid perfusing fluid gives it a small positive charge. Applied to explain the recovery of contractile power this hypothesis has all the defects which it has in explaining the recovery of excitability, and probably many others as well, but a discussion of these points must be left to a future occasion.

It might appear unnecessary to suppose that the increased contraction was due to any direct effect on the contractile mechanism in each element of the muscle, since it could be explained by an increase of conductivity which would bring into play parts of the muscle which were not accessible to a single stimulus. This explanation has been invoked already by Lucas and Adrian (20) to account for the increased size of the second electric response in a muscle stimulated indirectly. Here there is some justification for it because the fibres of a skeletal muscle may not all be brought into play by a single stimulus, but it is very doubtful if the same explanation could apply in the heart where the whole of the musculature responds together if it responds at all. The point need not be considered at length as it was fully dealt with by Mines in his paper.

The foregoing arguments are not intended to dispute the view that the contraction of a muscle may be due to the liberation of acid, but they do throw considerable doubt on the extension of this view to cover the favouring effects of previous activity in cardiac muscle.

IV. The mechanism of the summation of impulses.

In the course of this work certain facts have emerged which have an important bearing on the theory of nervous summation.

Lucas and I showed that the conditions necessary for summation in the peripheral nerve were as follows: in the first place the nerve or some part of it must conduct with a decrement so that a single impulse cannot pass through, secondly the nerve must exhibit a supernormal phase in recovery, and thirdly it must be stimulated by a series of impulses so timed that each falls in the supernormal period following its predecessor. Under these conditions a single impulse will have no effect on the tissue beyond the region of decrement but a series of impulses will succeed in passing through. We supposed that the same mechanism would account for the summation of impulses in a reflex arc, the region of decrement being the junctional or synaptic region between one neurone and another. To account for the great variety of response in the central nervous system we must add that the degree of decrement existing at

the synapse varies from one moment to another according to the state of fatigue of the arc, the general condition of the body, etc.

Now the facts brought forward in this paper show that no summation of this kind will be possible unless that part of the nervous arc which conducts with a decrement is in equilibrium with a surrounding fluid on the acid side of neutrality. In terms of the hypothesis on p. 24 we should say that no summation is possible unless the membranes in this part are positively charged. There is no direct proof that this condition obtains in the central nervous system, but if we consider how rapidly it is fatigued and how much it depends on its oxygen supply, it seems highly probable that the imperfect conduction at a synapse will vary with the degree of acidity of the tissue and the existence of the decrement may be due to the tissue being in equilibrium with an $H^{\cdot}$ ion concentration greater than the iso-electric value. Thus the two conditions of a decrement in conduction and a supernormal phase may be both fulfilled by the same factor, and this factor is likely to change from moment to moment according to the state of fatigue of the arc.

Evidently the nervous paths in the grey matter are much more sensitive to changes in acidity than are the peripheral nerves. It is as important for the organism that the peripheral nerves should never vary in conductivity as it is that conduction in the central nervous system should change from one minute to the next. Thus the peripheral nerves in a frog conduct without a decrement between such wide limits as P_H 10 and P_H 4, whereas the respiratory centre in a mammal responds at once to a minute change in the P_H of the fluid surrounding it.

Conclusions.

1. A nerve which is recovering from the passage of a previous impulse usually shows a supernormal phase of recovery in which its excitability and conductivity are greater than in the resting state. The supernormal phase is not always present and its extent is variable.

2. The supernormal phase depends on the $H^{\cdot}$ ion content of the fluid with which the nerve is in equilibrium; if the fluid is on the acid side of P_H 7 the supernormal phase is well marked and it increases with increasing acidity; on the alkaline side of P_H 7·4 the supernormal phase is absent. The above holds good for the recovery of conductivity as well as that of excitability.

3. In resting cardiac muscle the second of two contractions may be greater than that of the first. This increase in contractile power above its resting value is part of the supernormal phase of recovery. It is

associated with an increased excitability and it is present when the heart is perfused with an acid fluid and absent with an alkaline.

4. The staircase effect in skeletal muscle is an entirely different phenomenon. It depends, as Fröhlich has shown, on the increased duration of the contractile process in the early stages of fatigue. It is not affected by the $H^{\cdot}$ ion content of the perfusing fluid.

5. An analysis of the recovery of excitability in nerves shows that the maximum supernormal excitability in an acid fluid is very slightly less than the excitability in a neutral fluid, whereas the resting excitability is very much less.

6. If instead of the current required to excite we consider the concentration of ions which must be brought about in the tissue by this current, it appears that the minimal concentration required to excite during the supernormal period in an acid fluid is equal to the minimal concentration required in a neutral fluid, although the resting value in an acid fluid is very much greater.

7. To account for these facts it is suggested that the excitability, conductivity and contractile power are greatest when the colloidal particles which form the membranes of the tissue are initially uncharged, that a fluid on the acid side of the iso-electric point produces a small positive charge and that activity produces a negative charge. As this negative charge subsides the instability of the tissue increases and reaches its highest value when the surface is iso-electric. If the tissue is in an acid fluid the surface must pass through the iso-electric state and end up with a small positive charge, whereas in an alkaline fluid the iso-electric state is never reached and therefore the excitability never rises above its resting value. The complete discussion of this hypothesis is postponed until more data are available.

8. It is difficult to accept Mines's theory that the increased second contraction in heart muscle is due to the production of acid by the first contraction and the consequent increase in the $H^{\cdot}$ ion concentration of the tissue to its optimal value. If this were so the supernormal phase should be present in an alkaline fluid and absent in an acid, whereas the reverse is the case.

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