ON THE SUMMATION OF CONTRACTIONS.

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THE classical experiments of Bowditch (1871) showed that the force of contraction of ventricular muscle is independent of the strength of the induction shock which provokes the contraction. Keith Lucas (1905) gave evidence that the same holds good for the single fibres of skeletal muscle and in 1909 he showed that it is also true when the induction shocks are applied to the nerve, so that the muscle is excited by nerve impulses.

The experiments of Symes and Veley (1911) on the treatment of nerve fibres with local anæsthetics showed that as the conductivity of the nerve was depressed the fall in response of the muscle to excitations applied to the nerve above the block, was in a series of very well defined steps. The number of different heights to which the muscle would contract was limited.

In a number of experiments made in 1908 I obtained results pointing in the same direction; since they were obtained by quite different methods I think it of interest to describe them briefly before proceeding to the main thesis of the present paper.

Experiments on the excitation of the intra-muscular nerves of the sartorius with finely graded stimuli.

The sartorius of the frog was dissected out and the pelvic attachment secured to a glass rod by means of a hook transfixing the two acetabular cavities. A thread attached to the tibial tendon connected it to the short arm of a light lever arranged to write with as little friction as possible on a smoked drum. The sartorius was surrounded with fluid in an apparatus which differed only slightly from a modification of Lucas' fluid electrodes which I have described before (Mines, 1908, Fig. 9, p. 423). The inner tube of this apparatus was cut off

short and not tapered. On the end of the inner tube a thin disc of celluloid was cemented with wax. In this disc a rectangular slot was cut (about 5 × 1·5 mm.), through which the sartorius passed. The glass rod holding the sartorius was fixed to a rigid clamp; the rest of the apparatus, bearing the non-polarisable electrodes and the slotted disc, by means of a rack and pinion could be raised and lowered around the muscle without disturbing it, so that various portions of the sartorius from the pelvic to the tibial end could be brought within the slot at will. In this way very accurate localisation of the stimulus was possible, since the change in current density at the slot was very abrupt.

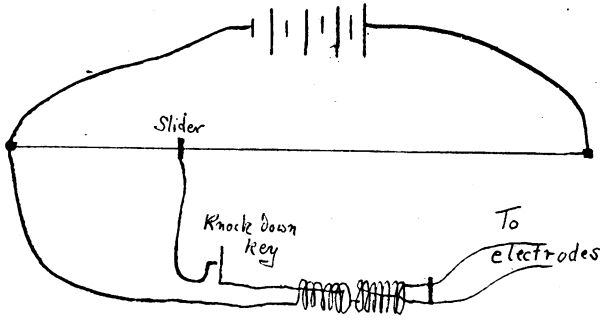


Fig. 1. Described in text.

As stimuli I employed break induction shocks from a coreless induction coil; stimuli towards which the nerve fibres of the sartorius are much more sensitive than the muscle fibres. In a coreless coil the strength of the secondary currents is directly proportional to the strength of the primary currents inducing them over a wide range. I found it easy to get a fine gradation of stimuli by using a metre bridge in the primary circuit, by means of which different fractions of the battery current were led off. Four or five Daniell cells were used to keep up the potential difference between the ends of the potentiometer wire. Since the internal resistance of the battery was high as compared with that of the primary of the inductorium, it is evident that with the arrangement of apparatus shown in Fig. 1 the increase in current strength produced by shifting the slider from left to right was very gradual. Trials with a delicate microampèremeter showed that the increase was very smooth.

The fluid with which the electrodes were filled was a Ringer's solution containing NaCl ·7 %, KCl 0·025 %, $CaCl_2$ 0·04 % in distilled water.

The difference in excitability of muscle and of intramuscular nerves towards the type of stimulus which is here employed is marked. An instance is quoted.

Region of muscle in slot	Position of slider for minimal twitch
Pelvic end	Off scale (greater than 100)
1 mm. from pelvic end	,, ,,
2 ,, ,, ...	,, ,,
3 ,, ,, ...	,, ,,
4 ,, ,, ...	,, ,,
5 ,, ,, ...	,, ,,
6 ,, ,, ...	89
7 ,, ,, ...	72
8 ,, ,, ...	41
9 ,, ,, ...	52
10 ,, ,, ...	47
11 ,, ,, ...	54
12 ,, ,, ...	27
13 ,, ,, ...	30
14 ,, ,, ...	31
15 ,, ,, ...	24
16 ,, ,, ...	28
17 ,, ,, ...	23
18 ,, ,, ...	29
19 ,, ,, ...	31
20 ,, ,, ...	25
21 ,, ,, ...	42
22 ,, ,, ...	Off scale (greater than 100)
23 ,, ,, ...	,, ,,
24 ,, ,, ...	,, ,,
25 ,, ,, ...	,, ,,
26 ,, ,, (Tibial end) ...	,, ,,

The intramuscular nerves were stained by May's method, the preparation projected with a camera lucida and drawn at a magnification of 10 diameters. Nerve fibres appeared at a distance of 6 mm. from the pelvic end and continued as far as 21 mm. from the pelvic end. The region of nerve entry was 15 mm. from the pelvic end.

In Fig. 2 is shown a tracing obtained later by stimulating the various regions of the same muscle with induction shocks, the potentiometer being kept at 90 throughout. Beneath the tracing is a copy of the drawing made from the preparation, reduced so as to correspond to the tracing. At the time when the tracing was taken the excitability of the muscle fibres had increased, so that a few were excited directly in the tibial region.

Stimulation in the neural region with a series of induction shocks of gradually increasing strength led to the following results. The minimal response was generally of considerable size. It varied in different cases, but the contractions always appeared and disappeared abruptly. In making the experiments the stimulus first used was always sub-minimal. The stimuli were then sent in at regular intervals (about 18 per minute)

the slider of the potentiometer being shifted 0·5 or 1·0 mm. after each shock. Fig. 3 gives a typical instance of the curves obtained. Perhaps the most striking feature is that shown through the small irregularities. When the stimulus is very near a threshold value, the response is sometimes of one height and sometimes of another, but it always falls into line with one or other of the steps. These steps showed considerable

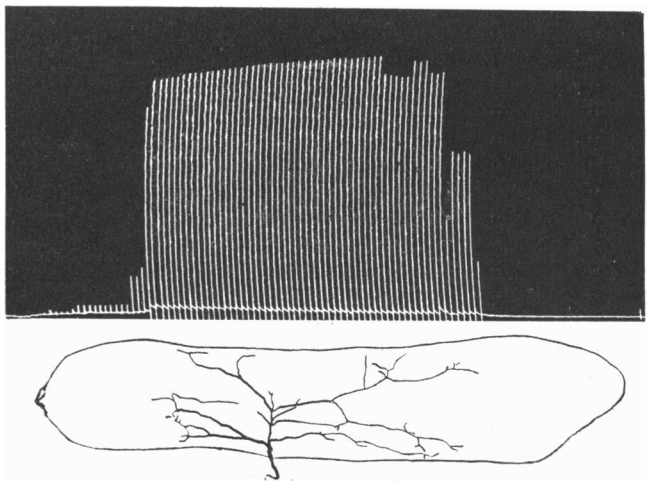


Fig. 2. Described in text.

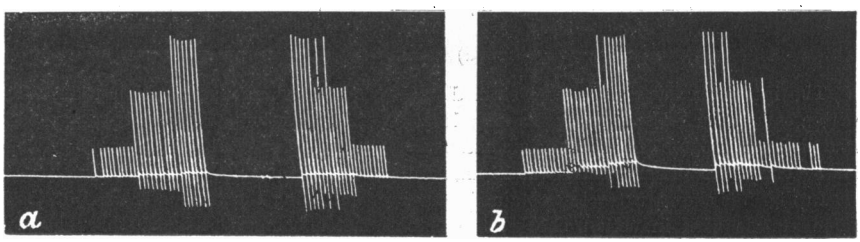


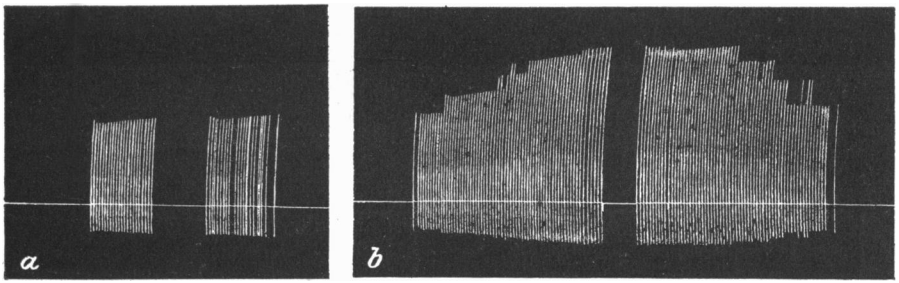
Fig. 3. Described in text.

regularity and persistence. Thus in Fig. 3 (*a*) was obtained an hour and a half before (*b*), care being taken of course not to shift the electrodes. A region of the muscle 5 mm. from the pelvic end was in the slot[1]. In the first curve, stimulations were started with the

[1] This was within the neural region in this muscle. See Mines, This *Journal*, xxxvii. p. 431 for further figures of the frog's sartorius showing the variability in extent of the non-neural region at the pelvic end of the muscle.

potentiometer slider at 55, the first response appeared at 68, another step appeared at 79 and a third step at 89: stimulations were continued up to 95. In the descending series, steps appeared at 87, 82 and 73. (The number given as the step number is always the slider position for the first of the greater contractions in the ascending series or the last of the greater contractions in the descending series.)

In the curve 3 (*b*) the steps were at 67, 79 and 88 in the ascending series and at 89, 82 and 70 in the descending series. The same steps could be made out in later tests.



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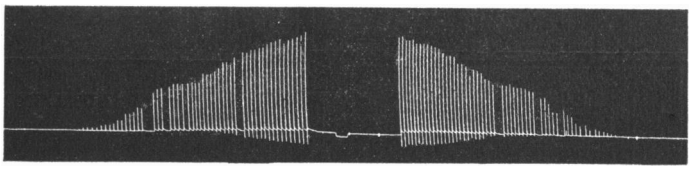

Fig. 5. Described in text.

It was noticed in many cases that the number of steps was greater when the region stimulated was well within the neural region than just at its borders. Fig. 4 shows two curves obtained by exactly similar treatment of the same muscle, except that in (*a*) the region in the slot was 10 mm. from the pelvic end, while in (*b*) the region stimulated was 11 mm. from the pelvic end.

It will be noticed that there is some progressive alteration in the height of the responses on the same "step," but this is seen both with the increasing and decreasing series of stimuli and depends therefore on the altered condition of the muscle as a result of previous stimulation: the most usual form of this change is the Treppe. If the strength of the induction shocks is increased considerably, it is possible to excite the

muscle fibres directly, in which case the step effect is scarcely to be seen, probably because the fibres are so numerous. Fig. 5 shows a tracing taken by stimulation of the pelvic end of the muscle in this way. When the neural region of the muscle was in the slot, excitation was almost entirely indirect with the strengths of current usually employed. This is shown further by the action of curare. Fig. 6 shows the responses to the same series of stimulations before and two minutes

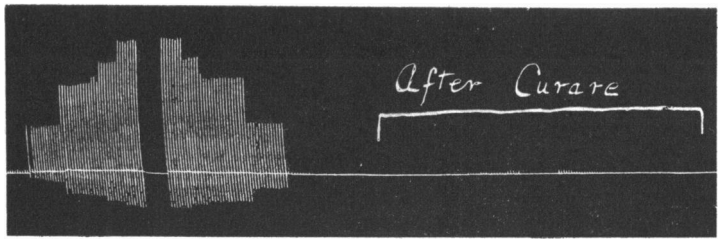

Fig. 6. Described in text.

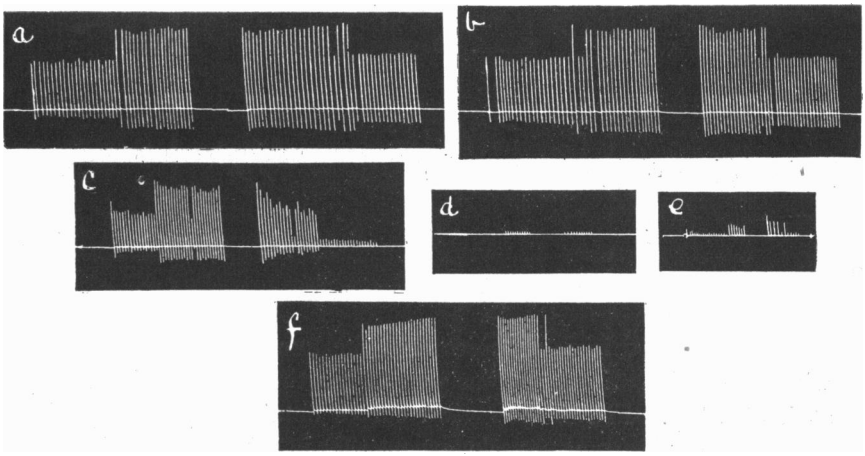

Fig. 7. Described in text.

after the substitution of ·01 % curare in Ringer for the plain Ringer's solution. With the pattern of electrodes used the change of solution is effected with great ease. With more dilute solutions of curare the size of the responses belonging to the different steps is progressively reduced, but the existence of the steps may still be recognised as occurring at about the same current strength.

Thus in Fig. 7 the stimuli were applied at a region 9 mm. from the pelvic end of the muscle. (*a*) was taken at 12.0 for the ascending and 12.10 for the descending series. (*b*) was taken at 12.20 and 12.25, the muscle remaining in Ringer's solution. The step occurred in (*a*) at 70 and at 65: in (*b*) at 73 and 70. At 12.28 a solution containing ·0001 % curare was substituted. At 12.30 and 12.33 the records shown in (*c*) were made, with steps at 66 and 70. During this series there is a marked falling off in the responses. At 12.38 and 12.41 the records shown in (*d*) were taken. Although the responses are in this series exceedingly small, there is a perfectly obvious step at 71 and at 69, clearly representing the step seen in the other tracings. (*e*) was taken at about 1.10, six minutes after removing the curare solution. It shows steps at 70 and 70 with some increase in the size of the responses, while (*f*) was taken an hour and a half later: it shows complete recovery, the steps are at 66 in the ascending and 73 in the descending series.

The explanation of these observations is not difficult. There can be little doubt that each step is the result of the excitation of a fresh nerve fibre or of nerve fibres, thus bringing a new group of muscle fibres into play. It would seem from the large size of the steps usually found that the excitation of a nerve fibre usually brings in all the muscle fibres supplied by it. But in the action of curare, the block is established in one neuro-muscular junction before another, and thus the excitation of a nerve fibre will affect progressively fewer and fewer of the muscle fibres which it innervates. The excitability of the nerve fibre itself is unaffected, and thus if any neuro-muscular synapses remain unblocked, the steps appear at the same current strengths as before. The curare in the strength used in these experiments (·0001 %) was found not to reduce the excitability of the muscle fibres towards the form of stimulus employed.

It may be noted that these experiments suggest either that in the excitation of a nerve fibre by single induction shocks there is no gradation in response to graded stimuli, or else that the smallest excitation which can be provoked is sufficient to excite any neuro-muscular synapse which is capable of being excited at all by a single impulse.

Experiments on the excitation of nerve fibres with graded stimuli show that there is no graded response of the muscle fibres attached to each nerve fibre; but unless it is shown that by such graded stimulation of nerve fibres nerve impulses of graded strength are provoked, it

cannot be maintained that muscle fibres show no gradation in their response to graded nerve impulses. We have at present no conclusive evidence that the strength of an impulse in a single nerve fibre varies at all in strength with the strength of the stimulus used to excite it. Moreover, the recent observations of Adrian (1912) show that in a given region of a nerve fibre the strength of the disturbance is unaffected by that of the conducted disturbance which evokes it. In other words, regarding the nerve impulse in the region of the nerve fibre immediately preceding that region under observation as "the stimulus" for the latter region, the all or none principle is true for this region.

On the relation of tetanic contraction to the response to a single stimulus.

It has been proved by Keith Lucas that the response of a muscle fibre to a single induction shock is independent of the strength of the induction shock, provided it is strong enough to elicit any response at all. The difference in behaviour of masses of skeletal muscle and of heart muscle in response to graded series of induction shocks depends on the fact that excitation spreads from one fibre to another in the myocardium but not in the skeletal muscle.

It is of course fully recognised that the extent of the contraction in a muscle fibre depends on the condition of the fibre at the time. Bowditch himself noted this, and drew special attention to the fact that the contraction of heart muscle in response to a single induction shock of whatever strength was influenced by the previous activity of the muscle, and by the lapse of time since such activity. It is familiar that the extent to which heart muscle contracts may be increased enormously by stimulation of the sympathetic nerves and that the size of the "maximal response" of skeletal muscle to a single induction shock is increased on repeated stimulation (time being allowed for complete relaxation), showing the same staircase effect as does heart muscle.

In spite of these facts, the attempt has been made to explain the difference in the magnitude of a single response of skeletal muscle and the tetanic response, by purely mechanical factors. Thus it is commonly said that when a second stimulus is thrown into skeletal muscle very shortly after the first, and before the contraction due to the first is completed, the second response is higher because it starts from a new base line. Similarly, the hypothesis has been maintained, and has been expressed very clearly quite recently by Starling (1912), that the difference in shortening in a single twitch and in tetanus does not

depend on any difference in the degree of tension developed in the two cases, but on the fact that in the single twitch the tension lasts for so short a time that it cannot produce its maximal mechanical effect, owing to the lag of the lever system used to record the contraction.

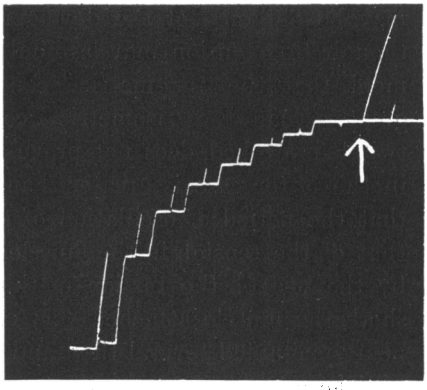

Fig. 8. Sartorius of frog. After-loading experiment. At the arrow a tetanising current was used. Further description in text.

The experimental basis for this hypothesis is the well-known after-loading experiment of von Frey. It is said that by after-loading the muscle, a response to a single induction shock may produce as great shortening as does a response to a tetanising current. Those who have attempted to repeat this as a class experiment will know that it almost invariably fails, that is to say, the response to the tetanising current is greater than that to the single stimulus, however late the after-loading.

In the figure reproduced by Starling (*loc. cit.* p. 227) the curve called a "tetanus" is obviously an incomplete tetanus. Had the stimuli been thrown in more frequently, the height of the response would have been increased. Fig. 8 shows a repetition of the after-loading experiment in which, when the maximal height of the after-loaded twitch was obtained (single maximal induction shocks being used as stimuli), a tetanic series of shocks was thrown in. The result is a further marked contraction, rising actually higher above the new base line than did the first twitch above the original base line. The shortening is about 50 % in excess of the greatest shortening of the after-loaded muscle in response to a single shock. It is interesting to note that very shortly after the tetanus the single shock could raise the lever a little higher than before.

It may of course reasonably be objected to this criticism that after-loading removes only a portion of the resistance to the shortening of the muscle, as the result of the tension set up. It is necessary, therefore, to test experimentally whether the maximum tension set up in the muscle fibres in response to a series of stimuli is or is not greater than that set up in response to a single maximal stimulus.

If a muscle is placed under tension, and connected to a stiff but light and very short spring to which a small mirror is attached, records may be obtained of the development of tension in the muscle, even though this tension may last only a very short time. The mere statement that the "iso-metric" contraction of muscle when tetanised is greater than the "isometric" contraction when a single "maximal" twitch is evoked, does not prove that the maximum tension developed in the latter case is greater than that in the former, unless it is shown that the period of oscillation of the "isometric" lever is shorter than that of the tension in the muscle. This condition is realised with ease by the use of the torsion method; the lever being made extremely short, the muscle attached very close to the axis, and the movements being magnified several thousand times by an optical lever.

The contractile stress of a single twitch lasts in the frog's muscle for not less than ·01″ at room temperature.

The period of oscillation of the lever employed in the experiments about to be described was less than ·0015″, and the muscles were under considerable tension throughout, so that there was no time lost in "taking up slack."

The tension set up as a result of tetanising the muscles, either by direct or indirect excitation, was always greater than the maximal tension developed in a single twitch.

Illustrations are given in the following experiments:

Indirect stimulation. Gastrocnemius-sciatic preparation of frog. Nerve on electrodes. Calibrated coil. Two Daniells in series in primary.

Deflection at break	68	at make	69

Increased the strength of current *10 times.*

Deflection at break	70	at make	68

It is evident that these stimuli must be regarded as maximal.

With same arrangement a rapid series of stimuli was sent in, tetanising the muscle. Deflection 156.

Other experiments gave similar results[1].

Direct stimulation. A photographic record of the tension changes in the sartorius was taken. See Fig. 9. The record reads from right to left. The first two twitches are to make and break shocks with a current strength we may call 1. The second pair of responses are to currents 5 times as strong, the third pair to current strength 10, and then follows a tetanus. Finally another pair of single responses.

The maximum tension which a muscle fibre is capable of exerting is never developed (except perhaps under the action of such a drug as

[1] The experiments described in this section were demonstrated to the Physiological Society at the meeting on November 23rd, 1912.

veratrin) as the result of a single induction shock or of the nervous impulse elicited by the application of an induction shock to a motor nerve.

It is familiar that if a series of maximal stimuli are thrown into a muscle at intervals not too great, yet sufficient to allow of complete relaxation of the muscle between whiles, that the successive responses increase in height—this is the well-known staircase effect or Treppe. Moreover, under the same conditions, the sooner the second contraction comes after the first, the greater will be its increment of shortening. If, now the second contraction comes still nearer the first, so that the mechanical movements actually overlap, there is no reason whatever to doubt that this increase will be maintained.

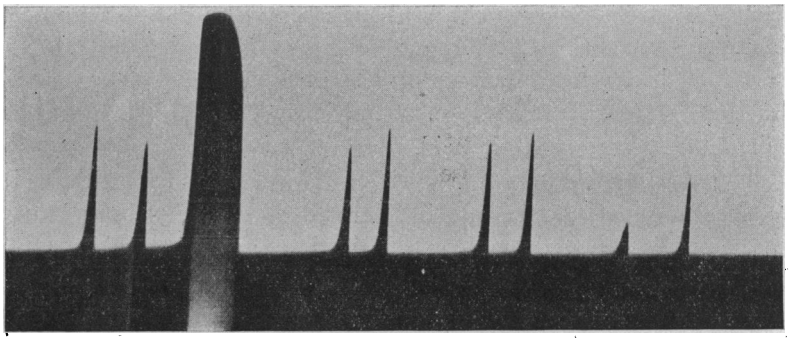

Fig. 9. Described in text.

There is not the smallest reason to suppose that the subsidence of the mechanical act of contraction is essential for the development of an increased response to the second stimulus. But it is known that there is a close relation between the duration of the electric disturbance in muscle and the refractory period. There is reason to believe that the occurrence of the action current in muscle signifies the occurrence of the propagated excitatory disturbance which precedes contraction. We know that a muscle can give separate electric responses to stimuli of much higher frequency than is needed for complete fusion of the mechanical responses.

Thus a muscle or nerve-muscle preparation from the frog will give electric responses succeeding one another at the rate of 50 per second if stimuli are thrown in at this rate. The contraction, however, is absolutely smooth—no sign whatever of the appearance and disappearance of tension during the tetanus is to be obtained. If there existed

rhythmic changes in the tension, there would be no mechanical obstacle in the way of recording them by means of a tension lever.

A further point of great importance in this connexion is that the tension set up in response to a more rapid series of stimuli is greater than the tension set up in response to a series of stimuli of frequency just sufficient to give complete fusion. This is inexplicable on the von Frey hypothesis.

On the relation between excitation and contraction in muscle.

Recent work emphasises the distinction between the propagated excitatory disturbance in muscle and the act of shortening which usually follows it. It is unnecessary to recapitulate the evidence for the dissociation of these processes in various instances: a critical account of the matter has been given recently by Keith Lucas in his Croonian Lecture (1912). It has long been thought that the act of shortening is due to the action of certain electrolytes on a "contractile structure." The electrolyte of whose liberation we have the most indisputable proof is lactic acid.

The supremely important work of Hopkins and Fletcher (1907) has taught us how lactic acid is liberated as the result of stimulating muscle and is removed by oxygen. We have no direct evidence of the time relations existing between the liberation of lactic acid and the mechanical act of shortening, but there is some indirect evidence that the liberation of acid precedes the shortening. For we know that the electric response of muscle when stimulated is very large, and that the difference of potential between an excited and a non-excited region is like that which exists between an injured (and therefore lactic acid rich) region and an uninjured region. Further we know that currents resembling injury currents both in magnitude and in direction can be obtained by the application of very dilute acids not only to uninjured muscle but also to artificial systems of membranes. The idea that the electric currents set up in living tissues are due to concentration cells of a special type complicated by membranes exhibiting differential permeability (diosmotic cells Brunnings) is very well founded. It is an experimental fact that in artificial membrane cells, differences of potential are most readily set up by the use of acid or alkaline solutions, owing to the remarkable properties of the H and OH ions. With these facts it is tempting to make the inference that the liberation of lactic acid is largely the cause of the action current observed and that therefore it does not begin later. If this is the case, the liberation of lactic

acid definitely precedes the mechanical act of shortening. If it precedes the act of shortening, it is not futile to discuss whether the liberation of lactic acid may not cause the shortening. The suggestion that the muscle machine which produces mechanical energy from chemical reactions with remarkable economy, does so through changes of surface energy, is supported by very many theoretical (Fitzgerald, 1878) and experimental considerations. Both the conceptions of alteration in curvature of surfaces and the imbibition hypotheses come under this heading, and need not for our present purpose be differentiated. The point is that the application of acid to a colloidal system of particular disposition of parts may give rise to a large production of mechanical energy. This may be demonstrated experimentally without difficulty. The experiments of Fischer and Strietmann (1912) give a very practical expression to such ideas. They show that thin strands of catgut immersed in solutions of dilute acid undergo quick and extensive shortening, a process which is completely reversible when the acid is removed and may be repeated an indefinite number of times.

Now it is well known that skeletal muscle undergoes a very similar shortening when it is treated with dilute acid solutions and that in both cases the shortening is antagonised by the action of various salts. Moreover this shortening in muscle as a result of the application of acid may be produced when the muscle is in such a condition that it cannot be excited by the strongest induction shocks—that is to say when its excitatory mechanism is apparently out of gear. Another point of similarity between the muscle and the catgut thread (not noted by Fischer and Strietmann) lies in the fact that both of them undergo strong shortening as a result of the application of alkaline solutions. This effect also is reversible.

The tension set up in muscle by the application of acid to its surface, even in the case of a thin muscle, is far inferior to that produced by tetanising the fresh muscle, though the contraction caused by the application of acid may cause as great a shortening in a lightly loaded muscle as does tetanus. It is certain that in order to get the maximal mechanical energy from a membrane system by the application of acid to it, what will be required will be local, relatively high concentrations, rather than a uniform distribution of acid through the system. And the particular regions in which the concentrations of acid are required to produce the effect at its greatest will probably be pretty closely limited. It is no strain on our conception of the complexity of the muscle mechanism to suppose that the liberation of lactic acid occurs

in certain regions of the muscle and not in others. It may be that the regions where the acid is liberated are present in numbers in every cubic micro-millimetre of every muscle fibre. As Keith Lucas (1912), has well said, "we need, perhaps, to look very far into the future before we shall see the present histology of the cell amplified by that new histology whose duty it will be to locate within the cell surfaces of physico-chemical importance."

If it is admitted as probable that the lactic acid liberated appears in certain special regions of the fibre, it follows that the immediate effects of such a liberation of acid will be imitated only very poorly, if at all, by the application of acid in low concentrations to the surface of the muscle. Moreover, the immediate effects in the muscle which depend on the localisation of certain concentrations of acid will disappear rapidly as the distribution of acid becomes equalised by diffusion. The effects accompanying the general increased acidity of the muscle, which in point of fact occurs as a result of repeated stimulation, can be imitated in detail by the application to the muscle of very dilute solutions of acid. The most notable effects produced thus are a primary increase and a secondary falling off in excitability and the onset of tonic contracture in the muscle.

These phenomena receive a general explanation along the following lines. Just as a colloidal system can retain a particular state of dispersity only within certain limits of hydrogen ion concentration of its fluid phase, just as there are limits of C_H. clearly set within which alone certain chemical compounds are stable, and just as enzymes are capable of acting only when the C_H.[1] of the solutions in which they are set to work is kept within defined values, so the activity of a living tissue (dependent as it is undoubtedly on the conditions of its colloids, on the stability of its several chemical components and on the action of ferments) can continue only within certain limits of C_H. I have already shown (Mines 1912) that the limits of C_H. within which the functions of tissues from different species of animals can continue, exhibit well-defined differences; and I have performed experiments, shortly to be published, which demonstrate that the limits differ for different tissues from one and the same animal.

The limits within which activity can continue may be fairly wide, but modification of C_H. within these limits modifies the behaviour of the tissues. From these considerations it is evident that the conception

[1] The abbreviation C_H. is used to express "hydrogen ion concentration."

of an optimal $C_{H.}$, so beautifully demonstrated for ferment actions by Sörensen (1909) can be applied also to living tissues.

In the case under discussion, the application is as follows. Since response to stimulation of skeletal muscle is impossible if the $C_{H\cdot}$ of the fluid bathing the muscle is too high or too low, there must be at least one region of $C_{H\cdot}$ between these limits, where the conditions will be most favourable for the processes concerned in response. The fresh muscle may be regarded as being just on the alkaline side of this optimum. The $C_{H\cdot}$ is just too low for the most favourable response to stimulation. The activity of the muscle, however, liberates acid (in certain special regions) and this acid being distributed through the muscle, is gradually removed in the presence of oxygen, but is not immediately removed[1]. While any of it remains, the $C_{H\cdot}$ is nearer the optimal value than it was before the acid was liberated and than it will be when the acid is removed. Consequently we have the condition familiar in the "Treppe," the condition of increased excitability and contractility which is the first result of activity of the muscle (provided this excitability is tested after the refractory period has passed). If the excitations are continued so that the optimal $C_{H\cdot}$ is exceeded, the effect is of course depression. It is to be noted that these ideas are most strongly borne out by observations on the effects of a rest during a prolonged series of excitation of muscle. In the presence of oxygen there is gradual removal of lactic acid from living muscle. If the $C_{H\cdot}$ of the muscle is at the optimal value, or a trifle lower than the optimal, the effect of removal of acid will be to reduce the response of the muscle. This is in fact observed in the early stages of such a series of contractions as we have been considering. After a pause, the next succeeding contraction is smaller than the last before the pause. If on the other hand the $C_{H\cdot}$ has reached a value greater than the optimal value, as is certainly the case in the later stages of fatigue, the effect of a rest will be to improve the condition of the muscle—still further does rest in pure oxygen improve it. These facts are entirely in accord with our conception of an optimal $C_{H\cdot}$[2]. Recognising the difference between the effects of the local and the general concentrations of acid in the muscle there is no difficulty whatever in realising how summation of contractions can occur when the muscle is so fatigued that the effects of a general increase in acidity is only to cause diminution of the response. It becomes apparent

[1] Hill has recently given evidence that the removal of lactic acid continues for over two minutes as a result of tetanus for one second.

[2] See also the following section.

also why under these circumstances fusion of contractions occurs with lower rates of stimulation. Fusion depends on the slowness of relaxation, and this in turn on the slowness of removal of local concentrations of acid—these having now to diffuse into regions themselves containing some acid. Possibly also the optimal C_H for the lactic-acid-removing mechanism has been exceeded.

Our hypothesis emerges then 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 disappear by diffusion, and the general rise in hydrogen 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 on tone.

On this hypothesis, the fact that the tension set up in a muscle fibre by single induction shocks is independent of their strength, implies not that the result of excitation in this fashion is to cause a maximal tension in the contractile mechanism in every case, but that the local concentrations of acid which are liberated under these conditions are in each case the same. By the stimulus of a single induction shock, or by the nerve impulse set up by a single induction shock, there is a certain liberation of lactic acid in the muscle. It would seem that the amount liberated by the single stimulus in a single fibre does not depend on the strength of that stimulus, provided it is effective at all. The immediate precursor of lactic acid in the muscle is uncertain; it is very probable that an immediate or remote precursor is glucose or glycogen. But it is certain that not all the glucose or glycogen present is converted into lactic acid as the result of a single stimulus. How can we account for the conversion of a certain small but definite amount on each occasion? Possibly the immediate precursor is some loosely-bound protein-carbohydrate compound, the protein forming part of the structure of the fibre and remaining unchanged after the decomposition of the carbohydrate moiety: the protein molecules exist only in relatively small numbers and therefore only a small "charge" of carbohydrate can be held ready for firing on each occasion. If one discharge follows another soon enough, the second may arrive before the local concentration of acid produced by the first has had time to diffuse away. Thus the second may sum with the first. A higher local concentration thus produced we may regard as responsible for the phenomenon of summation of

contractions. It is from this standpoint a summation of the effects of the propagated excitatory disturbance at the level of the contractile mechanism. More than one factor will be at work limiting the extent of this summation. The higher the local concentration rises the greater will be the velocity with which it begins to diffuse away. Further, a limit is set on the frequency with which the excitatory disturbance can recur. The refractory period has been shown, by the work of Keith Lucas, to be an affair of the propagated excitatory disturbance which precedes the act of shortening. It is of interest that there is some general relation between the duration of the electric response in different varieties of tissues and the duration of the refractory period (Tait, 1910).

In skeletal muscle, both are remarkably short, being completed well before the beginning of the mechanical response. In heart muscle, as is well known, the electric response is prolonged, and the long duration of the refractory period is one of the most striking properties of heart muscle. Under ordinary conditions the heart muscle is absolutely refractory to the strongest stimuli from the application of an effective stimulus to the completion of the shortening which is elicited by it. It is familiar also that after the termination of absolute refractory period the recovery of excitability is gradual, there is a more or less prolonged relative refractory period. This may of course be due to differences in the rate of recovery of the different portions of the heart muscle—the absolute refractory period lasting longer in some than in others. There is at present no conclusive evidence on this point. In any case, the prolonged refractory period in heart muscle ordinarily precludes the possibility of summation of contractions. On the views expressed above as to relations existing between the propagated excitatory disturbance and the act of shortening, it would seem possible that a reduction in the duration of the refractory period should lead to the possibility of summation of contractions in heart muscle.

On summation of contractions in cardiac muscle.

The Treppe, or beneficial effect of activity, was described in the first instance in heart muscle. Can it be regarded as due to an alteration in the general reaction of the tissue in the fashion discussed in the previous section?

In connexion with the alterations in amplitude of the cardiac contractions as the result of the accumulation of metabolic products, I wish to draw attention to the explanation of certain problems which have recently arisen out of work on the action of carbonic acid on the

heart. Starling and Jerusalem (1910) showed that the output of the mammalian heart when perfused with blood was greater when the blood was brought into equilibrium with a moderate tension of CO_2 than when the CO_2 was removed as completely as possible. This confirmed the results of Henderson and others. With the heart of the frog perfused with Ringer's solution, Starling and Jerusalem could get no improvement in the output on adding even very small amounts of CO_2 to the fluid. The result they obtained was in every case depression. More recently, Ketcham, and King and Hooker (1912) have likewise failed to obtain any beneficial effects from the action of CO_2 on the hearts of warm and cold-blooded animals, and they are inclined to cast doubt on the reality of Starling and Jerusalem's results, attributing them to the effects of CO_2 on the state of dilatation of the lung vessels (Starling and Jerusalem in their work on the mammalian heart used the heart-lungs preparation).

In reality the explanation of these divergent results is extremely simple. In their experiments on the mammalian heart, Starling and Jerusalem perfused in the first instance a solution too alkaline for the proper activity of the mammalian heart. The blood without CO_2 is of hydrogen ion concentration below the optimal value. The addition of a small quantity of carbonic or of any other acid whose anion is not definitely toxic will improve the beat of a heart perfused with such a liquid, allowing its diastole to become more complete and so increasing its output. In their experiments with the frog heart and with the tortoise ventricle, Starling and Jerusalem used as the starting point not "acapnic" blood but Ringer's solution. Unfortunately they did not record the composition of the particular Ringer mixture they employed. The ordinary Ringer's solution used in most physiological laboratories contains only a very little alkali, in the form of sodium hydrogen carbonate, and is not on the alkaline side of the optimal hydrogen ion concentration for the frog's heart. The so-called "neutral Ringer," that is Ringer made up without the addition of any alkali, is in reality slightly on the acid side of absolute neutrality owing to the absorption of small amounts of CO_2 from the air: this solution is distinctly on the acid side of the optimal hydrogen ion concentration for the frog's heart. If one starts with a Ringer's solution of lower hydrogen ion concentration than the optimal value I find that the addition of a little CO_2 to the solution greatly improves it as a perfusion fluid for the frog's heart, the effects being quite similar to those shown by Starling and

Jerusalem in mammalian heart[1]. Thus Fig. 10 at the beginning of the tracing the frog's heart was perfused with "neutral Ringer" the hydrogen ion concentration of which was very roughly 10^{-6}. At the point marked, this solution was changed to one made alkaline with sodium carbonate ($C_H \cdot 10^{-10 \cdot 2}$). The height of the systole was increased, the relaxation became imperfect and so the amplitude of the beats was greatly reduced. At the next mark a small addition of CO_2 to the fluid was made by mixing 95 c.c. of the Ringer + sodium carbonate with

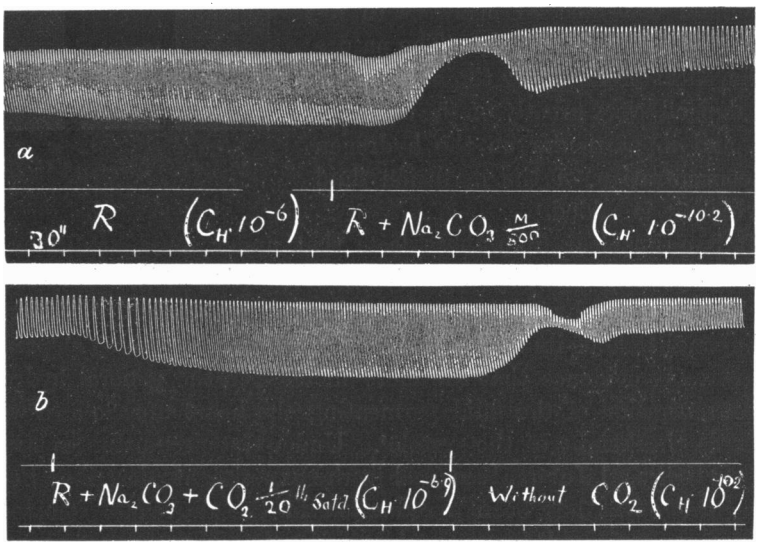

Fig. 10. Described in text.

5 c.c. of the same solution saturated with CO_2. The C_H of this mixture, as measured by the method of Sörensen, was $10^{-6 \cdot 9}$. The increase in the amplitude of the beats (the result of more complete diastole) is marked: there was also an obvious increase in the output of the heart.

[1] While this paper was in the press Prof. Starling called my attention to the fact that Göthlin (*Skand. Archiv.* XII.), who used a Ringer's solution containing more alkali than usual, obtained improvement in the amplitude of the frog's heart beat by treatment with CO_2. Prof. Starling suggests that the optimal C_H for the contraction of the cardiac muscle fibres may differ from the optimal C_H for the output, and that the difference would vary with the diastolic pressure. This is extremely probable. The optimal C_H for various processes is known to depend on other external conditions. Instances are found in the action of electrolytes on colloids, and Palitzsch and Walbum (*C. R. Lab. Carlsberg*, IX. p. 200. 1912) have just given a beautiful example of the dependence on temperature of the optimal C_H for an enzyme action.

Removal of the CO_2 led to the same condition as before. Fig. 11 is from another experiment. In the first piece of tracing Ringer with Na_2CO_3 was perfused ($C_H \cdot 10^{-10.4}$). In the second portion, the addition of a small amount of CO_2 ($\frac{1}{20}$ saturation) to the fluid had increased the C_H to $10^{-7.2}$. The third portion was taken after the removal of the CO_2, the C_H being again $10^{-10.4}$. There is no essential difference between the behaviour of the warm and cold-blooded hearts with respect to the effects of CO_2. The nature of the effect produced by the addition of CO_2 depends very greatly on the composition, and in particular on the hydrogen ion concentration, of the perfusion fluid to which it is added.

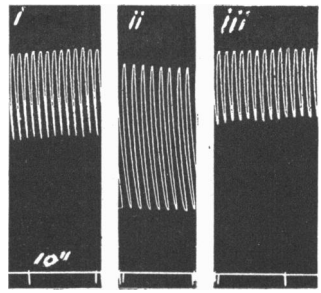

Fig. 11. Described in text.

It is quite clear from these facts that under some conditions the increase in the general hydrogen ion concentration of the heart muscle which is produced by its repeated activity may be productive of increase in the amplitude of its movements. We have not before us at present, however, any evidence that the mere increase in the general hydrogen ion concentration of the fluid permeating the heart muscle as a whole increases the height of the systole[1]. Under the circumstances studied by Starling and Jerusalem, and in the experiments described in this paper, the effect has been a reduction in the degree of shortening attained as measured from complete relaxation, though at first an increase in the difference between the diastolic and systolic conditions. The staircase effect, in which the actual shortening of the muscle increases as the result of previous activity, cannot, then, be fairly attributed to a change in the general hydrogen ion concentration throughout the muscle.

The considerations which led us to postulate the production in skeletal muscle of certain localised relatively high concentrations of lactic acid, as the culmination of the propagated excitatory disturbance, apply with equal force to heart muscle.

I shall now proceed to point out how this conception enables us to unify the phenomena of the Treppe and the summation of contractions

[1] A more complete analysis of the relations of the activity of heart muscle to hydrogen ion concentration will be attempted in a future communication. (See preliminary note in *Proc. Phys. Soc.* May, 1912. This *Journal*, XLIV. p. xxi.)

which under some conditions occurs in cardiac muscle, and to relate further experimental evidence which supports the idea.

Bowditch (1871) observed that on artificial stimulation of the quiescent ventricle, a second stimulus falling not too soon nor yet too late after the first evoked a greater contraction. Over a certain time interval the second contraction was greater as the period elapsing between the first and second contraction was reduced. Fig. 12 is plotted from one of Bowditch's experiments. Abscissæ represent the intervals between the first and second contractions, ordinates represent the heights of the second contractions. The form of the curve should be noted in detail. As the interval between first and second contractions is diminished the height of the second response increases, at first very slowly and then more rapidly. But when the interval is reduced to about five seconds, further diminution of the interval leads only to

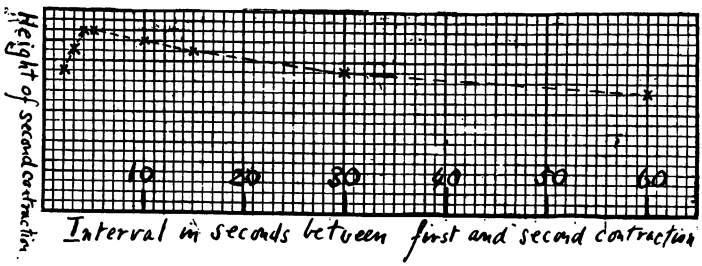

Fig. 12. Plotted from an experiment of Bowditch. Description in text.

diminution of the second response. This is due evidently to the intrusion of another factor—the refractory period. And there can be little doubt that the portion of the curve between ten seconds and five seconds is to some extent affected by the fact that the relative refractory period is not over.

When the relative refractory period is short, the curve continues to ascend (reading it from right to left) with shorter time intervals.

In a number of experiments[1] on the excised hearts of elasmobranchs I have obtained results which exemplify this point very clearly. The animals used were *Torpedo ocellata* and *Scyllium canicula*, chiefly the former. A cannula was placed in the aorta: ligatures were tied round the roots of the great veins, around the sinus venosus, or between

[1] A fuller account of this work will be given in another paper. Only the results bearing directly on the subject of this paper are mentioned here. I would take this earliest opportunity of expressing my thanks to Prof. Dohrn and his staff for their kindness in facilitating my work at the Naples Zoological Station.

auricle and ventricle, and the cavity of the heart was filled with a solution containing the necessary salts and urea (see Führer 1908) or sometimes in the case of *Torpedo* with amniotic fluid from the pregnant female. Arrangements were made for stimulating the ventricle with strong induction shocks: and in most experiments the exact moment of stimulation was signalled on the record by a spark passing from the metallic pointer to the drum. The average temperature at the time when these experiments were made was about 25° C. The preparations beat with a regular and usually slow rhythm which originated as a rule in the auricle.

It was found in a number of instances that in these preparations an extra systole of the ventricle, whether provoked by an induction shock or by a premature auricular excitation, was of greater height than a systole occurring at the normal time, and that the height of the systole was greater the shorter the interval elapsing between it and the previous systole. This held good very often for intervals much shorter than

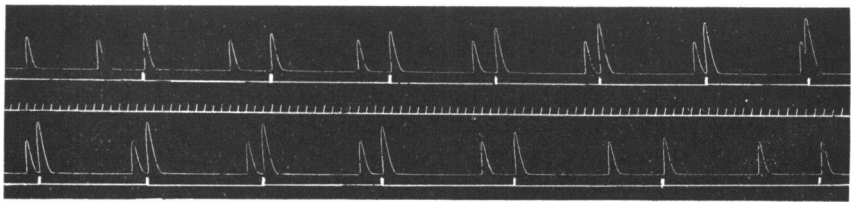

Fig. 13. *Torpedo ocellata*. Auricle-ventricle preparation. Record of ventricle. Second line of tracing taken 10 seconds after the upper line. Time marking in the middle in seconds. The preparation was beating spontaneously: the signal line shows stimulation by induction shocks.

those found by Bowditch for the frog's ventricle. It thus happened that a well-marked summation of contractions appeared very often. The height of the second response steadily increased as the interval between it and the first response diminished: there was no abrupt change in the height of the second response when it occurred before the completion of the first response. An example is given in Fig. 13. Cushny has observed similar summation of contractions in the excised mammalian ventricle, particularly when the heart muscle had been excised for some time. My experience with these elasmobranch hearts entirely coincides with Cushny's. The summation effect was rarely seen except in hearts that had been removed from the body for some time: in several cases it became much more marked after several hours. It was noticeable that in the preparations where the effect was best

marked, the refractory period was shorter than usual. In particular I observed that in a heart which at first failed to show any signs of an increased second response, the refractory period was long as compared with the refractory period in the same heart an hour and a half later, when the phenomena of increased second response and summation of contractions were well developed. In many of my experiments a stimulus arriving before the completion of systole produced a response. This shortness of the refractory period may, I think, be in some degree related to the rather high temperature at which the experiments were made. Marey (1885) found that the length of the refractory period was decreased with rise of temperature. But there was certainly in addition some progressive change in the heart muscle during the course of an experiment such as that to which I have referred, a change the relation of which to external conditions awaits more exact definition.

The fact that the summation phenomena in the heart make their appearance most clearly when the state of nutrition of the heart muscle cannot be regarded as particularly good must inevitably arouse a suspicion as to the reality of the result. May it not be that we have to do merely with a summation of subliminal stimuli in some part of the ventricular muscle which has reached a condition of impaired excitability such that a single induction shock, or a single excitatory disturbance in neighbouring portions of the muscle, is unable to arouse it to contraction, but only so to improve its excitability that a subsequent stimulus can discharge it? Such an explanation is ruled out by the following considerations. In the first place, the height of the spontaneous contractions when the heart is in the condition under discussion is not necessarily any less than that of the contractions of the freshly excised heart. Secondly, it appears that the whole of the musculature of the ventricle is affected by the refractory period which follows the first or spontaneous excitation. This is attested by such experiments as that from which Fig. 14 is taken. This figure shows the effect of throwing strong induction shocks into a ventricle at different intervals after the occurrence of an auricular excitation. The curves are arranged to show the effects of throwing in the artificial stimulus later and later after a natural excitation : they are not all in the order in which they were taken, but the whole series of tracings was obtained in the course of a few minutes. At first there are seen five spontaneous beats, no shock was sent in. In the next six contractions, a shock was thrown in, during the latent period or during the earlier part of systole, without producing any modification of the beat. The whole muscle apparently

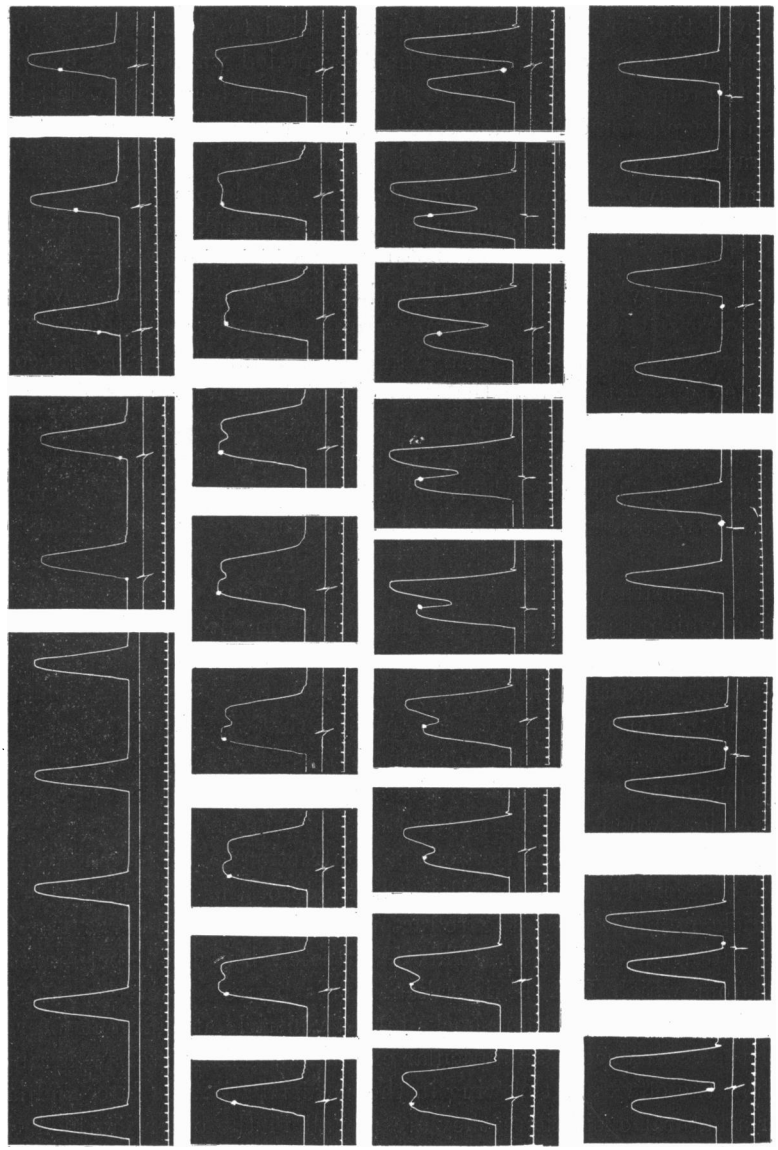

Fig. 14. *Torpedo ocellata.* Auricle-ventricle preparation; filled with Führer's solution Time tracing represents 1/5 seconds. The exact moment of stimulation is shown by the white spots on the tracing. The signal was merely a control placed in the primary circuit of the stimulating coil, and not accurately aligned. See text.

was completely refractory. The next stimulus produced a small second response: the limit of the absolute refractory period had now been reached. As the stimulus is thrown in later and later in systole the second response comes to reach the same height as the first, and shortly greatly exceeds it. Afterwards we see the effect which has already been illustrated—as the interval between the two excitations is increased, the magnitude of the second response is diminished, until, as in the last tracing, an artificial stimulus, arriving only just before a natural stimulus was due, provokes a response only just greater than the first response. If the summation were simply a summation of stimuli which taken singly were subliminal for some part of the musculature, we should expect summation irrespective of the refractory period of that part of the musculature which was in a condition to respond to a single stimulus. The only possible objection to this position would be raised by the assumption that the particular portion of the musculature incapable of responding to a single stimulus was inaccessible altogether to the direct action of induction shocks and could be excited only by the repeated excitation of neighbouring portions of the myocardium. We have no evidence for such an assumption: the size of the extra response of the ventricle was unaffected by altering the point to which the artificial stimuli were applied, and was the same if the extra excitation came over from the auricle. The simpler interpretation is to be preferred; that the whole musculature is concerned in the first contraction, but that when a second successful excitation occurs at a suitable time interval after the first, further shortening is possible. This does not conflict with the "all or none" principle any more than does the fact that the contractions of the muscle may be greatly augmented on stimulation of its sympathetic nerve supply. It merely serves to emphasise once more the fundamental similarity in the behaviour of the skeletal and the cardiac muscle fibre. In both, the greatest possible shortening of the contractile apparatus cannot as a rule be developed as the result of a single excitation; in both the response to a single induction shock is independent of the strength of that stimulus provided it is effective. In both, if a second excitation arrives sufficiently soon after the first, yet after recovery from the refractory phase, the effects of the second excitation may summate with those of the first to cause a more complete shortening of the contractile apparatus. Such summation is always elicitable from skeletal muscle; in cardiac muscle it is normally prevented by the great length of the refractory phase.

Summary of chief conclusions.

The tension developed by a skeletal muscle fibre when tetanised is greater than the maximum tension developed in response to a single induction shock or in response to the nerve impulse excited by a single induction shock. The difference ordinarily observed in the degree of shortening in the single twitch and in tetanus is not due merely to a difference in duration of the period of tension.

There are reasons for thinking that the liberation of acid in the muscle as a result of excitation precedes the act of shortening and possibly causes it. It is necessary to draw a distinction between the effects of the acid in the regions where it is liberated in the muscle fibre and its effects when it has become diffused through the muscle so as to affect the general reaction of the tissue environment. There exists an optimal hydrogen ion concentration for the chemical environment of a tissue, the value of which varies for different tissues and is affected by other constituents of the liquid. The effects of addition of acid to the immediate surroundings of a tissue will depend on whether such addition of acid brings the hydrogen ion concentration of the surroundings towards, or removes it from, the optimal value. This is well illustrated by the effects of carbonic acid on the heart. If the heart either of warm or of cold-blooded animals is perfused with a solution whose reaction lies on the alkaline side of the optimal hydrogen ion concentration, the effect of addition of carbon dioxide is to increase the amplitude of the beat and the output of the heart. Larger concentrations will reduce the output, while if the initial perfusion fluid possesses the optimal hydrogen ion concentration, or is on the acid side of it, even the smallest addition of carbonic acid will be detrimental to the activity of the heart. Changes in the hydrogen ion concentration of the liquid permeating a muscle may produce in the muscle changes of excitability, of contractility and of tone. The contraction of a muscle in response to a stimulus, such as an electric shock, is not due to a generalised alteration in the hydrogen ion concentration of the muscle: it may possibly be the result of the localised production of acid in the muscle. When a second excitation can be produced before the first localised concentration of acid has had time to diffuse away, the result may be summation of these localised concentrations and so a greater effect on the contractile mechanism. Such an effect may occur at a time when the general hydrogen ion concentration of the muscle is such

that further increase tends only to diminish the power of response of the muscle.

In heart muscle, the production of a second excitation in time for summation to occur is as a rule prevented by the very long refractory phase in this tissue. When the refractory phase becomes reduced, as in certain conditions of malnutrition, summation of the effects of propagated excitatory disturbances becomes possible, and, just as in skeletal muscle, the degree of shortening provoked by a series of stimuli is much greater than that elicited by a single stimulus.

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