ON THE RECOVERY OF MUSCLE AND NERVE AFTER THE PASSAGE OF A PROPAGATED DISTURBANCE.

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CONTENTS.

IN 1909 I drew attention to the fact that when two adequate stimuli are sent into the sartorius muscle of the frog at an interval little greater than the refractory period, the electric response to the second stimulus appears only after abnormal delay[1]. Since that time I have extended my observations to other tissues. There have also been published papers by Samojloff[2] and by Gotch[3], dealing with similar phenomena.

I. *The electric response of the gastrocnemius muscle of the frog to two stimuli applied to the sciatic nerve.*

Samojloff[4] points out that he had already described in 1908[5] a similar delay in the electric response of the gastrocnemius muscle to a

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[1] This *Journal*, XXXIX. p. 331. 1909.

[2] *Zentralbl. f. Physiol.* XXIV. p. 45. 1910.

[3] This *Journal*, XL. p. 250. 1910.

[4] *Loc. cit.* p. 46.

[5] *Arch. f. (Anat. u.) Physiol.* Suppl. p. 1. 1908.

second stimulus applied to the sciatic nerve. He states[1] however that he took as the time of appearance of the second electric response the point at which the curve of the second response begins actually to turn upwards away from the abscissa. He now observes that in his records, obtained with a string galvanometer, the response produced by the two stimuli diverges from the response to one stimulus alone at a point considerably earlier than the beginning of the actual upward inclination of the second response. On taking the point where the combined curve first diverges from the single curve as the true beginning of the second response he comes to a conclusion opposed to that which he had previously expressed. He finds in fact that the response to the second stimulus takes place on the whole without abnormal delay.

It is essential, if this matter is to be cleared up, that we should have a definite understanding of what is meant by the beginning of the response to the second stimulus. For my own part I have always taken the beginning of the second response to be the point at which the response to the two stimuli first diverges visibly from the response produced by the earlier stimulus acting alone. Or, if I may use Samojloff's terminology, I have measured from the beginning of the deformation of the first curve by the second response. All the measurements which I have published relate to this point. I cannot see that the point previously used by Samojloff, namely that at which the second curve begins actually to be inclined upwards, has any useful significance. The location of that point is determined by the degree of downward inclination of the first curve from which the second is diverging, and by the magnitude of the second response. The use of such a point for measurement of the beginning of the second response is reduced to an absurdity by the fact that one not infrequently obtains a response to two stimuli in which the second response appears in the corrected electrometer curve as a mere decrease in the downward sweep of the first response, and fails to turn upwards (away from the abscissa) at all. In Exp. 1 (Figs. 1 and 2) there are shown two curves corrected by analysis from capillary electrometer records. The first of these (Fig. 1) is the monophasic response of the sartorius muscle of the frog to one stimulus only, the second (Fig. 2) the response to two stimuli at an interval of ·0108 sec. In Fig. 2 there is a well-marked second response, which never leads to an upward inclination of the curve. These curves demonstrate the absurdity of measuring the beginning of a second response from the first upward inclination of the second part of

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[1] *Zentralbl. f. Physiol.* xxiv. p. 52. 1910.

the curve. We may assume therefore that Samojloff's more recent conclusion, based on the other method of measurement, represents the more correct interpretation of his curves. In fact the upshot of his work is to show that in the muscle excited from its motor nerve there is no such delay in the electric response to a second stimulus as I have shown to occur in the sartorius excited directly.

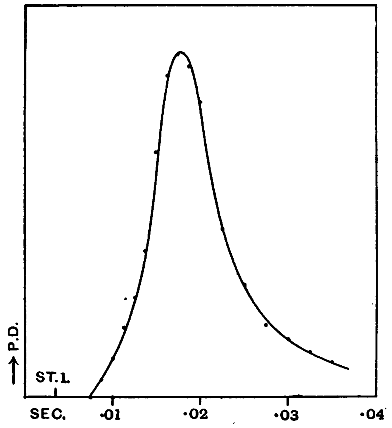

Fig. 1.

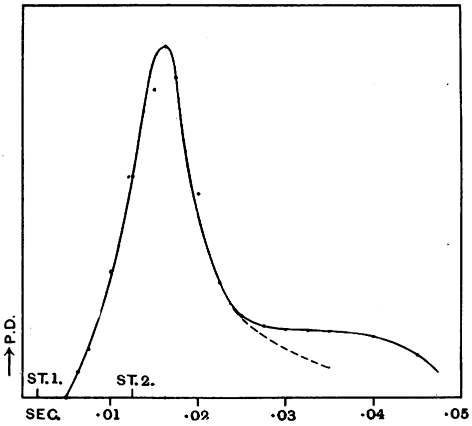

Fig. 2.

The records on which Samojloff's conclusions are based were obtained with a string galvanometer. He speaks of using the string galvanometer in order to "verify his results with a more perfect instrument than the capillary electrometer[1]." In my own experience the string galvanometer though admirably adapted to the recording of such slow changes as occur in cardiac and unstriated muscle, has so much lag that it cannot follow the rapid changes in striated muscle with sufficient exactness to be able to dispense with the necessity of correction. And here it shows itself as vastly inferior to the capillary electrometer; for it is not susceptible of that exact method of correction which gives to the capillary electrometer its peculiar value. On these grounds I thought it worth while to repeat the observations of Samojloff, using as he did the sciatic-gastrocnemius preparation of the frog, and recording the electric responses with the capillary electrometer.

Method. The details of the capillary electrometer, recording apparatus, muscle-chamber, and comparator for the analysis of records, have been described in a previous paper[2]. The gastrocnemius was led

[1] *Loc. cit.* p. 50.

[2] This *Journal*, xxxix. p. 210. 1909.

off by moist threads attached to its middle and its tendinous end. The cathodes of the coreless induction coils were applied to the nerve at a point about one centimetre distant from the muscle. A series of photographic records was made with two stimuli sent into the nerve at gradually increasing intervals. From time to time during the experiment the response to the first stimulus alone was recorded for comparison with the combined curves. The photographic records were subsequently analysed and plotted with time as abscissæ and P.D. as ordinates. The analysed response to two stimuli was then so superposed upon the response to one stimulus alone that the point of divergence of the combined from the single curve could be measured.

I reproduce here a few of the plotted curves from one of these experiments.

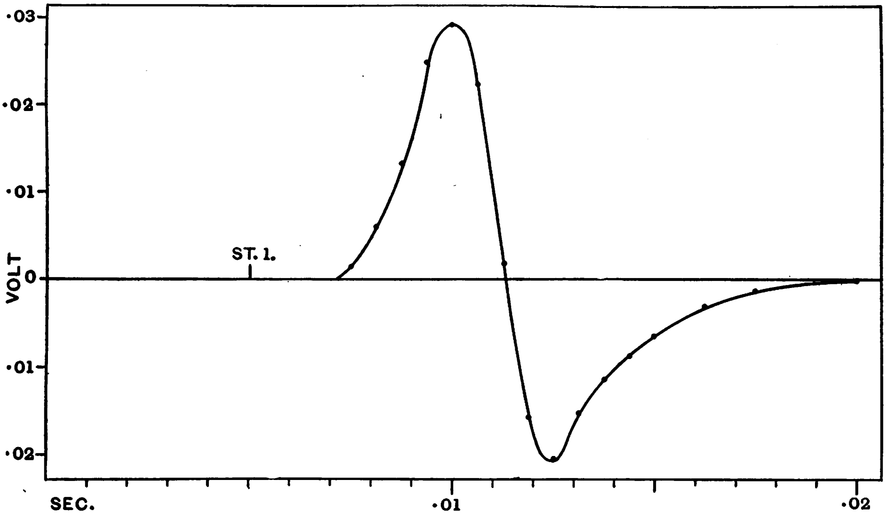

Fig. 3.

Fig. 3 shows the diphasic response of the gastrocnemius to one stimulus sent into the sciatic nerve. When two stimuli were sent in at an interval of ·001 sec. there was no difference in the response, the second stimulus falling within the refractory period. The same occurred when the interval was ·002 sec. An interval of ·003 sec. between the stimuli gave the result plotted in Fig. 4. In this figure the response to the two stimuli is shown as a full line[1], while the response to a single

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[1] The first part of the first response is also shown as a broken line in Figs. 4 and 5, because this part of the curve was transferred from Fig. 3, no new analysis being made.

stimulus is continued as a broken line, its course being found by superposing Fig. 3 upon Fig. 4. The point of divergence of the combined from the single curve is marked by a broken line let fall upon the abscissa. This line marks then the beginning of the second response.

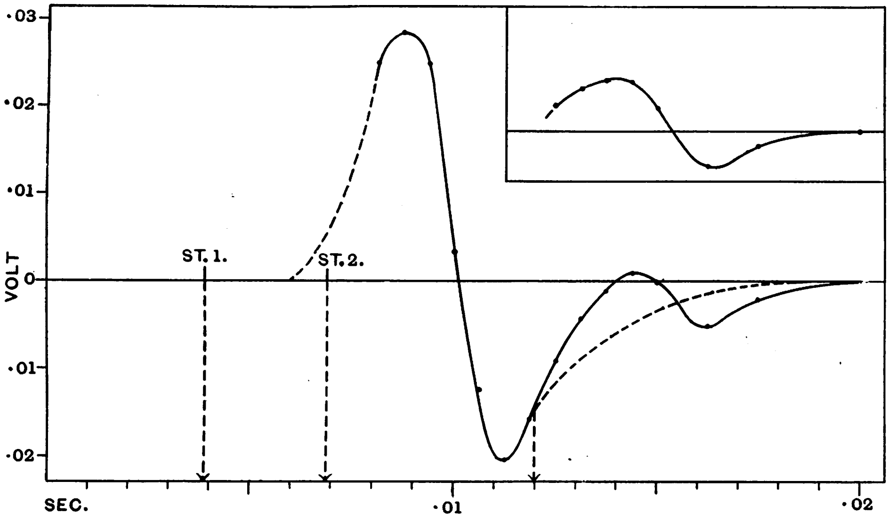

Fig. 4.

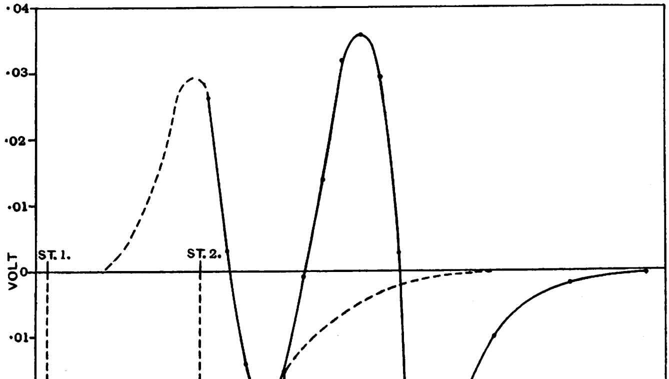

Fig. 5.

The first and second stimuli are marked in a similar way. Fig. 5 shows a similar treatment of the response to two stimuli at an interval of ·005 sec. The second response in this case is very much larger than with the interval ·003 sec. The series was continued to considerably greater intervals between stimuli than those shown in Figs. 4 and 5. For the rest of the curves I shall restrict myself to giving a table of the time of occurrence of the second stimulus and of commencement of the second response for each observation[1].

Exp. 2. Sciatic-gastrocnemius preparation. Frog. Temp. = 17·5° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
B	·001 sec.	No second response	—
C	·002	No second response	—
D	·003	·0081 sec.	·0051 sec.
E	·0037	·0077	·0040
F	·0050	0077	·0027
G	·0065	·0089	·0023
H	·0147	·0172	·0025

The last column in this table shows the delay of the second response. With the shortest interval between stimuli which gives a second response (Obs. *D*) the delay is practically twice as long as it is when the second stimulus falls considerably later.

This experiment indicates that in the gastrocnemius muscle excited from its motor nerve there is a marked abnormal delay in the response to the second of two stimuli sent in at a short interval of time. But there is an obvious source of error in the observations. The time of commencement of the second response is taken from the first measurable divergence of the combined from the single curve. The electric response always rises with a gradual sweep from zero, so that it does not reach a measurable height instantaneously with its beginning. The smaller the response the longer will be the interval between its actual beginning and the moment when it attains a measurable height. Consequently a small response will not be detected so soon after its actual beginning as will a larger one. It appears from all my observations that the response is smaller the earlier the stimulus by which it is provoked. Is it not possible therefore that the responses to early stimuli appear to be delayed only because they are small and in consequence are detected a considerable time after their actual beginning?

In this connexion the small inset in Fig. 4 should be examined. It shows the second response alone, obtained by subtracting the ordinates

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[1] For the analysis of these curves see the Appendix at the end of this paper.

of the single from the combined curve. Measurement shows that the time from commencement to summit of the first phase of this response is about ·0021 sec., whereas that of the first phase of the first response is nearly ·0029 sec. Gotch[1] has demonstrated that the time relations of a small electric response are normally identical with those of a larger one. May not the difference observed here be due to the early part of the second response having escaped detection? Perhaps a more correct rendering of the second response would be obtained by prolonging its time of rise to ·0029 sec.

Considerations such as these make it very probable that there is some error in estimating the time of commencement of the small second response. The question to which we need an answer is whether the error is accountable for the whole of the apparent abnormal delay which my observations show. It seems possible to compensate fully for possible error by making use of the fact that the time relations of large and small responses are alike, and consequently assuming that the second response always begins at a fixed time before the attainment of its maximum P.D.[2]. If we take Exp. 2, and suppose the second responses to begin always at ·0029 sec. before their maxima, as does the large first response, then we get the following values for the times of occurrence of the second response.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
B	·001 sec.	No second response	—
C	·002	No second response	—
D	·003	·0074 sec.	·0044 sec.
E	·0037	·0076	·0039
F	·0050	·0074	·0024
G	·0065	·0091	·0026
H	·0147	·0169	·0022

The last column shows that the general relation of the delay to the time of occurrence of the second stimulus is not materially different from that obtained before. The delay for the earliest second stimulus

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[1] This *Journal*, XXVIII. p. 402. 1902.

[2] Gotch has observed (This *Journal*, XL. p. 261, 1910) that in some cases his earlier second responses in nerve show actually a longer interval than the first response between their commencement and their moment of maximum P.D. This, as Gotch points out, is a sign of slowed propagation under the electrode which lies near to the seat of stimulation. But, as he shows, there is no slowed propagation in the region between the electrodes. We need not therefore concern ourselves in these experiments with the possibility that the rise of the first phase of the second response may be longer than normal, since our observations are made at a point far more remote from the seat of stimulation than the position of even the more remote of the electrodes in Gotch's experiments.

is still twice as great as that for the latest. Very similar results are obtained if we assume the time from the commencement of the second response to its point of equipotentiality to be constant in every case.

In order to present in a graphic form the results of the whole of this experiment I have constructed in Fig. 6 a diagram giving the relation between the time of occurrence of the second stimulus and the time of commencement of the second response. In this diagram abscissæ represent time, measured from the occurrence of the first stimulus as zero. Each observation is plotted along a horizontal line, the first stimulus being plotted on the zero line Stim. 1, the second stimulus on the line Stim. 2, and the beginning of the second electric response on the line Resp. 2. The successive observations are plotted at such heights above the abscissa that the second stimuli of all observations fall upon the straight line Stim. 2. If then the second stimulus always produces its response with the same delay, the line Resp. 2 will be a straight line running parallel to Stim. 2 throughout its course. But as a fact the line Resp. 2 runs parallel with Stim. 2 only when the interval between the stimuli is large. When the second stimulus occurs soon after the refractory period, as in observations *D* and *E*, the line Resp. 2 bends away from Stim. 2, showing an increased delay in the appearance of the second response.

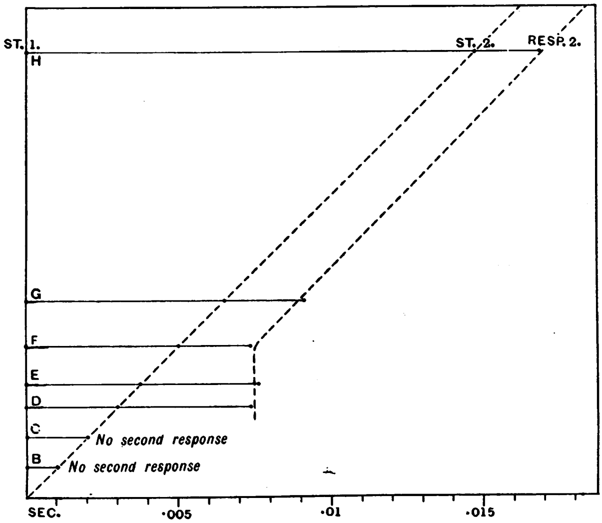

Fig. 6.

I was not completely satisfied with the several experiments which I made by the method described above, because of the difficulty of superposing the combined curves upon the single curves with sufficient accuracy. I thought greater accuracy could perhaps be obtained by a modification of the method of superposed photographs used by Samojloff[1]. In my experiments the object was to compare the response to the first stimulus acting alone with the response to the two stimuli acting in succession. I have therefore modified Samojloff's procedure, exposing first with the first stimulus alone, and then, without disturbing any adjustment, exposing the same plate with both the first and second stimuli in action. At the second of these exposures the image of the meniscus starts by tracing over its previous course in answer to the first stimulus, and continues to do so until the second response begins, when it diverges. The first response appears strongly imprinted on the plate, and the point of divergence of the fainter second response can be determined with precision. I have found no difficulty in making the plate travel with sufficiently nearly the same velocity in the successive exposures, but I have taken the precaution of inscribing the tuning fork record at both exposures in every case, so that any error may be detected.

The particular interest of the experiments which were made by this method of double exposure lies in their demonstration of an extremely small and very much delayed second response resulting from the application of the second stimulus at the shortest intervals after the first. For stimuli following one another at intervals of ·003 sec. or more these experiments simply reproduced the phenomena already observed by the other method. It was with intervals as short as ·001 to ·002 sec. that the new method detected extremely small responses which might have been overlooked in the earlier experiments. Of course the exact

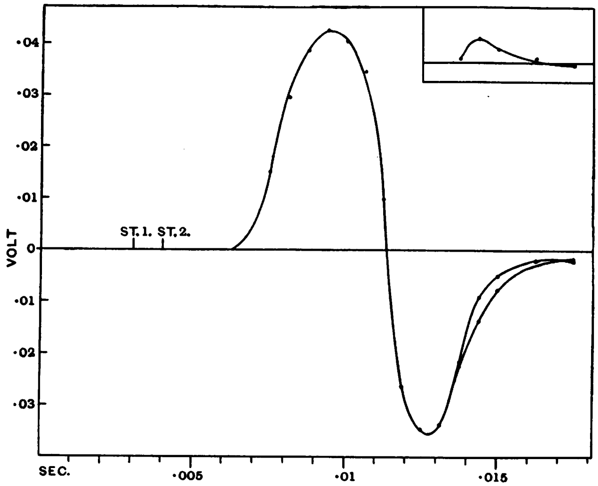

Fig. 7.

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[1] *Arch. f. (Anat. u.) Physiol.* Suppl. p. 4. 1908.

point of commencement of these smallest responses was more difficult than ever to determine with precision. They are so small and diverge so gradually from the single response that I have been forced to use the moment of their maximum P.D. as a basis for measuring the time of their beginning. Whatever the method of measurement used there seems to be no escape from the conclusion that they are even more delayed than were the most delayed second responses found in my previous experiments. In Figs. 7 and 8 there are reproduced two of the curves showing these very small second responses. Fig. 9 shows the larger second response produced by a rather later second stimulus. In each case the second response is plotted by itself on a horizontal base-line in the small inset. In the table below (Exp. 3) there are given the data from a whole series of observations made with stimuli at varying intervals.

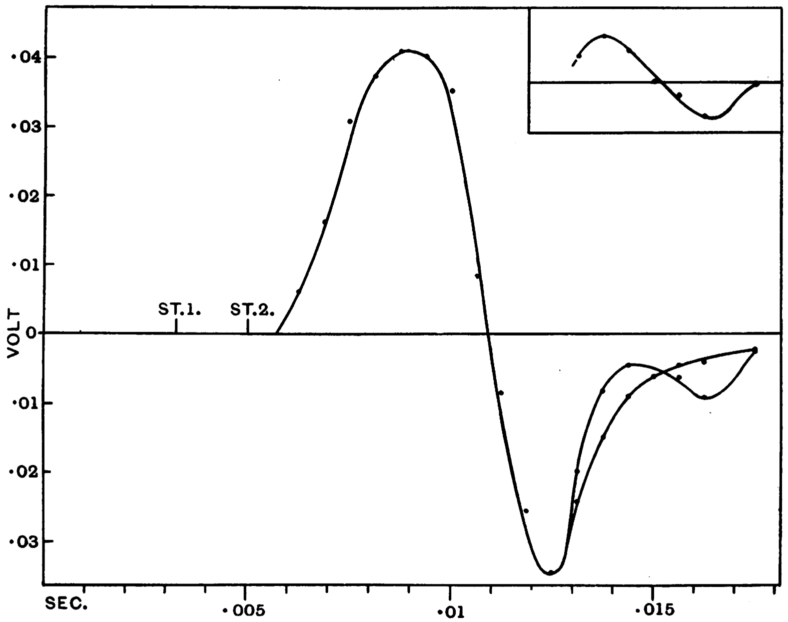

Fig. 8.

Exp. 3. Sciatic-gastrocnemius preparation. Frog. Temp. = 17·5° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response	Time second stimulus to second response
A	·0010 sec.	·0081 sec.	·0071 sec.
B	·0017	·0074	·0057
C	·0025	·0064	·0039
D	·0038	·0066	·0028
E	·0051	·0081	·0030
F	·0072	·0092	·0020

The times of beginning of the second responses are estimated on the assumption that the response begins always at a fixed time (·0031 sec.) before it attains its maximum P.D. Full allowance has therefore been made in these figures for the difficulty of detecting the first appearance of a very small response. Even so the delay in the response to the earliest second stimulus is many times as great as the normal. Fig. 10 shows the experiment graphically in the same way as Exp. 2 is shown in Fig. 6. It will be seen that the line Resp. 2 is by no means parallel to Stim. 2 throughout its course. It is nearly parallel for the longer intervals between stimuli, but

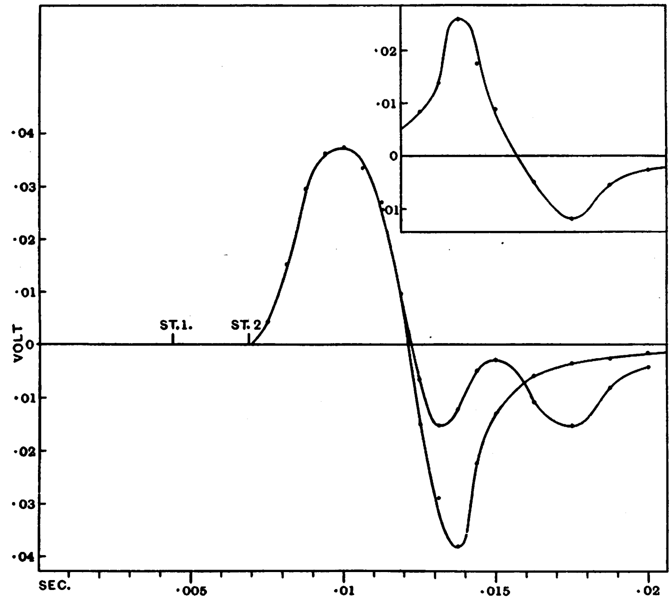

Fig. 9.

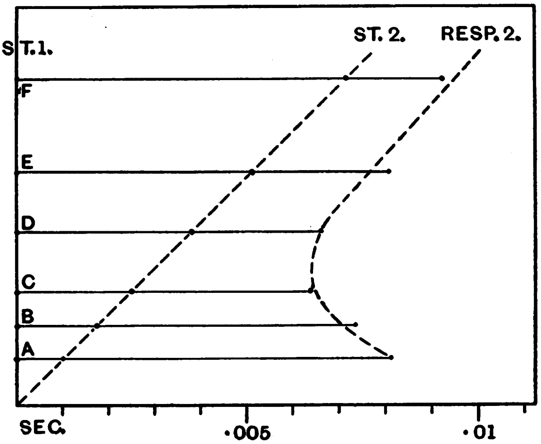

Fig. 10.

for the shorter intervals it diverges very widely, indicating a delay increasing rapidly with the earlier incidence of the second stimulus.

This experiment confirms the result obtained in Exp. 2. The analysed electrometer records show that in the gastrocnemius muscle excited from the sciatic nerve the response to a second stimulus falling soon after the end of the refractory period exhibits a delay which may be many times as great as the normal delay. This conclusion is in direct opposition to that expressed by Samojloff. To account for the difference between the results obtained by Samojloff and myself I can only suggest that possibly the uncorrected string-galvanometer records do not give so true a picture of the changes of P.D. as the corrected electrometer records.

II. *The electric response of the sartorius muscle to two stimuli applied directly to the muscle-fibres.*

The observations on the response of muscle to a second stimulus which I published in a previous paper[1] were based on estimations of the time at which the second response first reaches a measurable magnitude. In the preceding section of the present paper I have given reasons for supposing that such a method of measurement may involve serious error. It is probable therefore that my former observations overstated the abnormal delay which occurs in the response of muscle to a second stimulus falling just after the end of the refractory period.

It should be possible to correct such error by treating my former curves in the manner described in the preceding section, namely, by taking as the basis of measurement the point of maximum P.D. of the second responses, and assuming that they began at a constant time before that point. Unfortunately in the series of curves which I published the response to the earliest second stimulus (Fig. 3, p. 334) is a small curve of almost flat summit, which does not allow more than a very rough determination of the point of maximum P.D. As nearly as I can measure the curves the times of commencement of the second responses are the following.

Curve	Time first stimulus to second stimulus	Time first stimulus to beginning of second response on assumption that response begins ·006 sec. before its maximum P.D.	Time second stimulus to beginning of second response
3	·007 sec.	[·014]	[·007]
4	·0095	·0155	·006
5	·0120	·017	·005
6	·0172	·021	·0038

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[1] This *Journal*, XXXIX. p. 331. 1909.

These figures indicate that there is a large increase of the delay (last column) as the incidence of the second stimulus becomes earlier. The measurement of Curve 3 is however too uncertain. I decided therefore to make fresh experiments on the sartorius muscle, giving particular care to the precise determination of the point of maximum P.D. in the second response.

The method of observation was identical with that used in the earlier experiments[1]. The muscle was kept at a constant temperature in the special chamber described, was excited at its pelvic nerve-free end, and led off by moist threads attached to its middle and tibial end. The results of two experiments are given below. In each case the measurement actually made was from the incidence of the first stimulus to the summit of the second response[2]. From this value the interval between the first stimulus and the beginning of the second response was obtained by subtracting the observed interval between the beginning of a normal single response and its moment of maximum P.D. The interval subtracted in Exp. 4 was ·0050 sec. In Exp. 5, which was made at a temperature 10° C. higher, the interval subtracted was ·0035 sec.

EXP. 4. Sartorius of frog. Direct stimulation. Temp. = 8° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0028 sec.	No second response	—
B	·0052	·0187 sec.	·0135 sec.
C	·0075	·0183	·0108
D	·0100	·0185	·0085
E	·0125	·0198	·0073

EXP. 5. Sartorius of frog. Direct stimulation. Temp. = 18° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0018 sec.	·0107 sec.	·0089 sec.
B	·0030	·0110	·0080
C	·0039	·0110	·0071
D	·0046	·0105	·0059
E	·0073	·0115	·0042

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[1] This *Journal*, XXXIX. p. 207. 1909.

[2] Such a correction for possible error seems hardly necessary in this case, since the second responses were all fairly large, and consequently the results agree fairly closely whether the time of beginning of the second response is obtained by this method or by direct measurement of the first measurable divergence of the curves. For example the direct method gives for Exp. 4 *B* and *C* the intervals ·0170 sec. and ·0175 sec., while the indirect method gives ·0187 sec. and ·0183 sec. In Exp. 5 the times ·0107 and ·0110 found by the indirect method become ·0120 and ·0122 by the direct method. With either method the general relations remain essentially the same.

The change of temperature between the two experiments makes a large difference in the refractory period of the muscle. In Exp. 4 the second stimulus at ·0028 sec. is not effective; in Exp. 5 at 18° C. the second stimulus at ·0018 sec. is effective. But at both temperatures the phenomenon of the increased delay with earlier incidence of the second stimulus is essentially the same. As the last columns of the tables show, the delay is practically doubled within the range of each experiment. In Fig. 11 the relation between the time of occurrence of the second stimulus and the time of commencement of the second response is shown for Exps. 4 and 5.

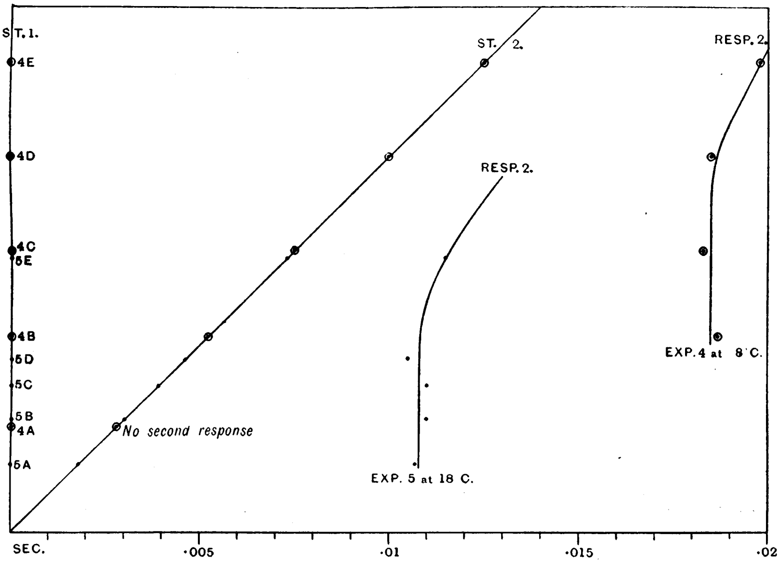

Fig. 11.

These experiments confirm the conclusion which I reached in my previous paper, that in the sartorius muscle excited directly the response to a second stimulus falling soon after the end of the refractory period occurs after abnormally long delay.

III. *The electric response of cardiac muscle to two stimuli.*

The problem of the delay in the response to a second stimulus is far more easily attacked in the case of cardiac than of skeletal muscle. In the latter the earliest possible second response occurs at a time when the first response has not yet subsided, so that we are faced with the difficulty of analysing the complex response to two stimuli into its components and determining the exact point at which the second response begins to diverge from the first. In cardiac muscle the earliest second response does not occur until the first has completely subsided. Both first and second responses spring therefore from a horizontal base-line, and can be compared on equal terms. Also in cardiac muscle the second response is not appreciably altered in size when it occurs soon after a previous one; by this fact a source of error is removed which was of serious magnitude in the measurements made on skeletal muscle. These circumstances, coupled with the slow time rate of the electric changes in cardiac muscle, render unnecessary the correction of the capillary electrometer curves when the only object is to determine the interval between the occurrence of a stimulus and the beginning of the consequent electric response.

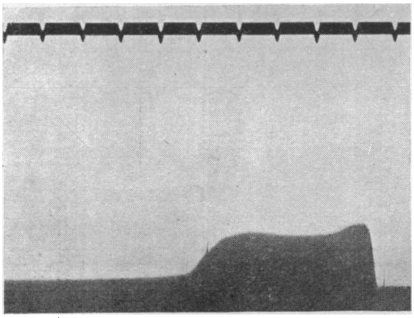

Fig. 12. **Read from right to left.**

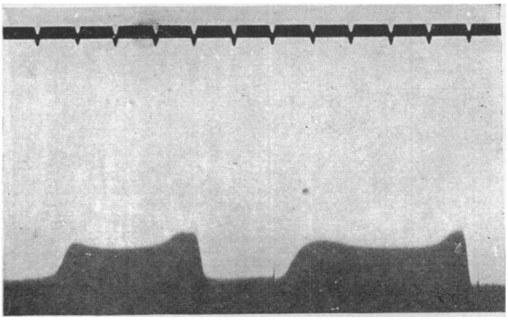

Fig. 13. **Read from right to left.**

My experiments were made on the ventricular muscle of the frog. Two methods were used. The first of these consisted in stopping the heart by the Stannius ligature, setting it up in the muscle chamber with electrodes applied to one uninjured and one injured point on the ventricle and recording with the capillary electrometer the monophasic responses to two single-induction shocks sent in at varying intervals. The cathodes of the induction coils were applied either to the auricle or to a point between the base of the ventricle and the proximal leading-off electrode. The injury was always under the leading-off electrode most distant from the base of the ventricle.

This method was sufficient to determine without any doubt that the delay of the electric response to a second stimulus occurring soon after the end of the refractory period is many times as great as the normal delay. Exp. 6 (Figs. 12 and 13) illustrates this point. In these records the time marker shows fifths of seconds, and the moment of stimulation is marked by an escape of the current from the induction coil. In Fig. 12 the second stimulus occurs just before the end of the refractory period, and there is no second response. In Fig. 13 the second stimulus is just after the end of the refractory period, and provokes a second response after a delay many times as long as the delay between the first stimulus and first response.

The second method was to use the intact beating heart, and to send in stimuli at varying intervals after the electric response accompanying a spontaneous beat. This method was adopted because the Stannius preparation is apt to begin beating when stimuli have been sent into it frequently. The second method is therefore better adapted for obtaining a long series of observations with stimuli sent in at widely different intervals after a response. In this way I was able to map out a curve relating the delay of the second response to the time of occurrence of the stimulus. The two tables given below show the results obtained. For convenience of reference the observations are given in a definite order, those with the earliest stimuli coming first. In the actual work the observations were made at random in no such order, so that there cannot be any question of the longer delay observed with some stimuli being due to a progressive change in the tissue. The lettering of the observations shows the actual order in which they were made, *A* being the first in each experiment.

Exp. 7. Ventricle of frog. Stimulus near base of ventricle. Temp. = 15·5° C.

Obs.	Time from beginning of first response to stimulus	Time from beginning of first response to beginning of second response	Time from stimulus to second response
I	·44 sec.	No second response	—
M	·50	1·44 sec.	·94 sec.
L	·62	1·45	·83
F	·68	1·48	·80
K	·73	1·49	·76
G	·73	1·50	·77
E	·86	1·57	·71
D	·87	1·59	·72
B	1·10	1·73	·63
A	1·13	1·75	·62
H	1·20	1·82	·62
C	1·24	(1·60)	(·36)
J	1·46	2·02	·56

In these observations the delay (last column) increases with the earlier incidence of the stimulus until it is nearly doubled. The two stimuli which fall nearest to the end of the refractory period give responses beginning at nearly the same interval after the beginning of the first response (1·44 sec. and 1·45 sec. in the third column). There is one divergent observation, namely obs. *C*, which gives a delay quite out of agreement with the rest; for this I have no explanation to offer. During this experiment the heart was beating at a rate which gave 2·88 sec. between the beginnings of two spontaneous electric responses.

Exp. 8. Ventricle of frog. Stimulus in auricle. Temp. = 15° C.

Obs.	Time from beginning of first response to stimulus	Time from beginning of first response to beginning of second response	Time from stimulus to second response
M	·46 sec.	No second response	—
N	·58	1·49 sec.	·91 sec.
L	·70	1·48	·78
I	·91	1·59	·68
J	·92	1·59	·67
K	·98	1·64	·66
Q	1·00	1·63	·63
F	1·07	1·68	·61
R	1·11	1·73	·62
E	1·23	1·83	·60
C	1·27	1·91	·64
G	1·29	1·87	·58
D	1·41	2·02	·61
B	1·41	2·02	·61
A	1·45	2·07	·62
P	1·46	2·03	·57
H	1·58	2·14	·56
O	1·67	2·22	·55

Exp. 8 reproduces very closely the facts already seen in Exp. 7. In the latter the delay of the second response ranges from ·56 sec. to ·94 sec.; in the former it ranges from ·55 sec. to ·91 sec. The observations from Exp. 8 are shown graphically in Fig. 14. This diagram is constructed in the same way as Figs. 6, 10 and 11 to show the relation between the time of incidence of the stimulus and the delay of the consequent response. The only difference is that in Fig. 14 the zero line from which times are counted is the beginning of the first response, and not the first stimulus, since the first response was always a spontaneous one. The figure shows that when the stimulus falls between 1·0 and 1·5 sec. after the beginning of the first response the delay of the second response is practically constant at ·6 sec. With earlier stimuli the delay becomes longer. For stimuli between ·5 sec. and ·7 sec. after the beginning of the first response the delay increases so rapidly that the second response occurs at an almost constant time. Earlier stimuli than these fall within the refractory period and produce no second response. During this experiment the spontaneous responses were following one another at an interval of 2·4 sec.

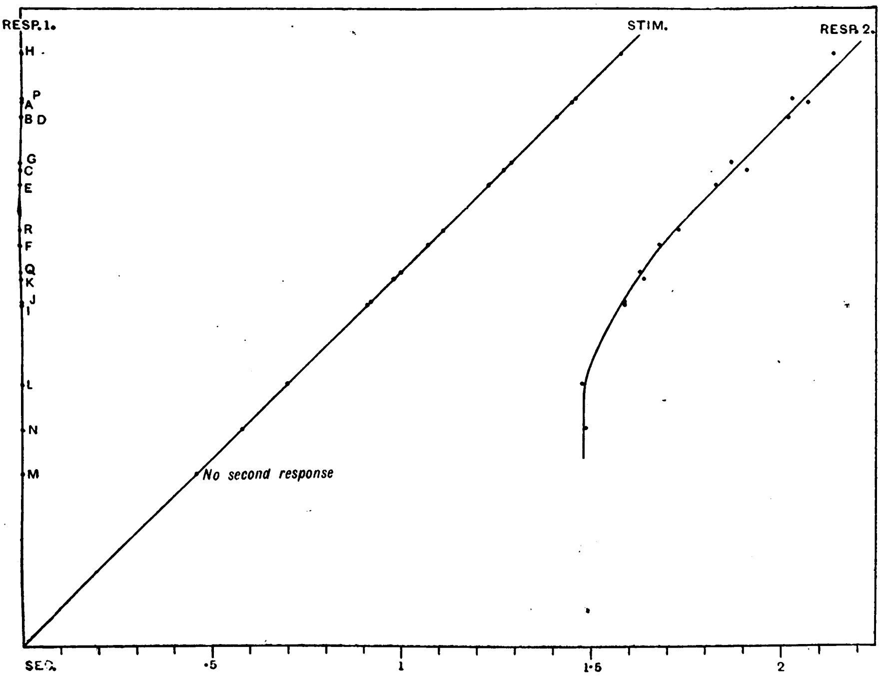

Fig. 14.

We have in these experiments on cardiac muscle a proof of the existence of the increased delay of a second response based on observations free from any difficulty of measurement and demanding no minute analysis of the photographic curves. The agreement of these results with those of the more difficult observations on the rapid tissues is a valuable confirmation. It leads to the conclusion that an abnormally long delay in the response to stimuli falling just after the refractory period is a phenomenon common to different excitable tissues.

IV. *Discussion of the phenomenon of the delayed second response.*

The observations which I have made on skeletal muscle excited directly and on cardiac muscle show in common not only a delay of the second response increasing with the earlier incidence of the second stimulus, but also the phenomenon which I previously described as the "irresponsive period[1]." In other words there is a range of time, succeeding the end of the refractory period, within which the second stimulus may be moved without any alteration of the time of occurrence of the consequent second electric response. This is indicated in the diagrams which I have published in this paper by the line Resp. 2 becoming vertical (Figs. 11 and 14). There is however, as the result of the more extended range of my more recent experiments, a modification to be made in my original conception of this phenomenon. The limited number of intervals between stimuli which I used in my earlier experiments led me to state that second responses which began later than the fixed moment of commencement of the earliest second responses occurred with a delay equal to that of the first response. It can now be seen from the curves published in this paper that such responses still continue to show a considerable increased delay, which disappears only gradually as the second stimuli fall later. This is in complete agreement with the phenomena which Gotch has found in nerve. The correction is one of great significance, as will presently be shown.

In the more complex cases with which I have dealt, namely the excitation of nerve and the recording of the consequent electric response in the innervated muscle, it appears that a new phenomenon must be recognised; the electric response becomes still later when the second stimulus is brought very near to the first (see Fig. 10). This suggests an important difference associated with the passage of the propagated disturbance through successive tissues having unlike time relations. It may be a profitable subject of investigation when the more simpler

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[1] This name is not satisfactory, because it does not indicate the essential feature of the phenomenon, that it is a question of the delay of responses to a fixed moment, not of their complete failure as in the refractory period. For this reason I have not used the expression in the present paper. At the same time it is obviously inconvenient to refer constantly to "the period to the end of which the responses to the earlier second stimuli are delayed." Gotch (This *Journal*, XL. p. 272, 1910) uses the expression "period of modification" for the whole time during which the second response is delayed more than the first; but this includes a time longer than the period to which I am referring. It will perhaps be better to adopt no special terms for these phenomena until further experiment has thrown more light on their relations.

cases, where only one kind of tissue is concerned, are better understood. At present I shall confine my discussion to the single tissues.

In addition to those observations on the sartorius muscle and the ventricle which I have described there is the long series of measurements made by Gotch[1] on the sciatic nerve of the frog. He sums his observations up in the following words: "There is some evidence that at low temperatures (3° C.) second nerve responses commence at the same delayed moment, although the second stimulus is varied as regards its moment of application. This corresponds with the results obtained by Keith Lucas in the sartorius muscle when directly excited by two stimuli at 12° C. Since however at temperatures above 3° C. the moment of commencement of the second response of nerve varies with the time of the application of the second stimulus, the conclusions arrived at by Keith Lucas as regards a fixed terminal limit of an 'irresponsive period' are not applicable to nerve." Now my recent observations on cardiac and skeletal muscle show that the occurrence of the second responses at the same delayed moment is only to be expected when the second stimuli fall quite soon after the end of the refractory period. And (as may be seen from Fig. 11) the greatest interval at which the second stimulus may follow the first while still producing its response at a constant moment becomes smaller as the temperature is raised. We ought therefore to enquire, before postulating a real difference between muscle and nerve in this matter, whether Gotch's observations have always explored the region within which the occurrence of second responses at the same delayed moment is to be expected.

Gotch's experiments at each temperature consist in most cases of several groups of two or three observations derived from different preparations. It is clear I think that we shall not be justified in considering together measurements made on different preparations; we cannot rely on the identity of two nerves within the narrow limits of time measurement demanded by these experiments. The observations made on each preparation must stand alone.

Starting with the lower temperatures we have at 3° C. five observations all made on the same preparation.

Group A. Temp. = 3° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
967	·0110 sec.	·0150 sec.
966	·0120	·0150
965	·0130	·0155
964	·0140	·0160
963	·0150	·0165

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[1] This *Journal*, XL. p. 253. 1910.

This is the case to which Gotch refers as agreeing with my observations on muscle. The second response occurs at ·0150 sec. with stimuli ·0110 and ·0120 sec. apart. It should be noticed that Gotch gives the refractory period of this preparation as ·0075 sec., so that the constant time of the second response may have occurred with second stimuli over the whole range from ·0075 to ·0120 sec.

At 4° C. there are observations from three preparations.

Group B. Temp. = 4° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1071	·0100 sec.	·0120 sec.
1072	·0125	·0135
997	·0080	·0135
996	·0100	·0135
969	·0090	·0135
970	·0100	·0135

The refractory period is ·007 sec. In observations 1071 and 1072 the second stimuli follow the first at too great an interval to show second responses commencing at the same delayed moment. Both the other preparations, with stimuli nearer to the refractory period, do give second responses occurring at a fixed time.

At 4½° C. the results are derived from four preparations as follows.

Group C. Temp. = 4½° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1064	·0100 sec.	·0125 sec.
1063	·0120	·0135
1014	·0070	·0110
1015	·0080	·0110
1016	·0100	·0125
1125	·0080	·0120
1137	·0080	·0110

The single observations are of no use for our purpose, except in showing that measurements made on different tissues with the same interval between stimuli do not agree. The observations 1064 and 1063 are made with stimuli too far apart. The group of three observations made with second stimuli closer to the end of the refractory period (given as ·007 sec.) brings out the fixed time of occurrence of the second responses again quite definitely; stimuli at ·0070 sec. and ·0080 sec. both have their responses at ·0110 sec.

At 5° C. the observations are made with different distances between electrodes and point of stimulation, and no indication is given of the preparations used, so that the results cannot be used here. At 8° C. there are observations from three preparations.

Group E. Temp. = 8° C.

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
1001	·0045 sec.	·0075 sec.
1000	·0065	·0088
999	·0080	·0100
1022	·0050	·0080
1021	·0060	·0080
1011	·0040	·0070

The observations 1001 to 999 do not show the occurrence of second responses at a fixed time; the observations 1021 and 1022 do show it. Neither of these sets is carried right down to the end of the refractory period, which is given as ·0035 sec.

Finally, at 12° C. there is a group of three observations all made on the same preparation and not showing second responses at the same delayed moment though carried to within ·0007 sec. of the end of the refractory period.

Group F. Temp. = 12° C.

Time first stimulus to second stimulus	Time first stimulus to second response
·0027 sec.	·0040 sec.
·0035	·0046
·0045	·0055

We find, then, on considering together only observations made on the same tissue, the occurrence of responses at the same delayed moment at 3°, at 4° and at 4·5° C. whenever the stimuli are brought near enough to the end of the refractory period. There are observations showing that the range of time after the end of the refractory period within which the second stimulus may be moved, without movement of the second response, is as great as ·0045 sec. at 3° C., ·003 sec. at 4° C. and ·001 sec. at 4·5° C. It is also demonstrated that the range is not greater than ·0055 sec. at 3° C., ·0055 sec. at 4° C. and ·003 sec. at 4·5° C. Clearly the range is becoming rapidly shorter as the temperature rises. At 8° C. one preparation shows the phenomenon occurring with stimuli up to ·0025 sec. after the end of the refractory period; the other preparation shows that at ·003 sec. after the end of the refractory period, the range within which the phenomenon is observed has not been reached, but for want of earlier stimuli gives no indication whether the stimulus at ·001 sec. after the refractory period does or does not fall within that range. At 12° C. it is shown that a stimulus ·0015 sec. after the end of the refractory period does not fall within the required range, there is no indication whether the stimulus ·0007 sec. after the end of the refractory period does or does not.

The conclusion which I would draw from these observations is that in nerve the occurrence of a response at the same delayed moment to stimuli sent in at varying short intervals after the end of the refractory period has been demonstrated at temperatures from 3° C. to 8° C.; that the range of time after the end of the refractory period within which stimuli must fall if their responses are to occur at the same delayed moment becomes rapidly shorter with rise of temperature; that at 12° C. there is no evidence whether such a range exists at all, but if it does it is certainly shorter than ·0015 sec. In these facts I cannot find reason for supposing the phenomena in nerve to differ in any way, save only in absolute time values, from those which I have described as occurring in skeletal and cardiac muscle.

As to the meaning of the delay of the second response to a fixed moment, it is obviously very tempting to accept Gotch's illuminating suggestion that the second response is delayed because it is propagated along the muscle or nerve more slowly than the first. This not only fits in with the observation of Engelmann[1] that in the ureter a second wave can be seen to progress at a rate decreasing with the time interval since the passage of a previous wave, but it offers a simple account of the fixed time of occurrence of responses to stimuli sent in at different times. If the first propagated disturbance sets up in the tissue some modification which persists at each point for a definite time after the disturbance has passed and is of such a nature as to slow the propagation of a subsequent disturbance, then it is obvious that the second disturbance will be slowed in its propagation if it finds the modification still persisting at any part of the tissue which it enters; but as soon as, in consequence of such slowing, it comes to lag so far behind the earlier disturbance as to find each part of the tissue on which it enters just recovered from the modification, then it will no longer be slowed, but will follow the former disturbance at an equal pace. The result of such a mechanism would be that the second disturbance, provided only that it set out before the modification due to the first had

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[1] *Arch. f. d. ges. Physiol.* II. p. 268. 1869.

subsided, would come to follow the first at a fixed interval equal to the duration of the modification which the first disturbance had set up.

If this were all, the matter would be simple. But in this scheme no account is given of the fact clearly established by the experiments of Gotch as well as by my own, that second responses which, owing to their stimuli falling too late, begin later than the fixed limit are nevertheless slightly delayed as compared with responses occurring in a tissue not recently excited. Such a result could not arise in the simple way which I have suggested above. It might however result from some local modification of the tissue at the seat of the first stimulus lasting slightly longer than the supposed modification due to the propagated disturbance. In this way Gotch's suggestion that the delay is partly due to a local factor resulting from the passage of the exciting current, and partly the consequence of a modification set up by the propagated disturbance, might account for all the observed facts. This tempting hypothesis is at once ruled out by the occurrence of an increased delay in responses falling later than the earliest limit in cardiac muscle. The first response in my experiments on cardiac muscle was a spontaneous one, so that there cannot have been any local modification set up by an exciting current. All the phenomena of delay observed in the experiments on cardiac muscle must have their origin in modification of the tissue by a previous propagated disturbance only.

This preliminary survey of the causation of the increased delay forced me to recognise that the first necessary step was a close investigation of the factors concerned, and particularly a determination of the relative importance of such modifications as may be set up by the stimulus itself and by the consequent propagated disturbance. The following section of this paper is an account of the experiments which I have made upon this question.

V. *Investigation of the factors concerned in the increased delay of the second response.*

We have here two separate problems to solve. There is the question which of the changes produced in a tissue by the first stimulus is the condition delaying the second response. Is it the local effect of the exciting current at the seat of excitation, or is it the consequent propagated disturbance? And there is the problem how the delay is effected. Is it by a slower development of the second local process of excitation, or by a slower propagation of the second disturbance?

In his paper on the delay of the electrical response of nerve to a second stimulus Gotch has dealt with the first of these problems. He reaches the conclusion that there are two factors concerned, namely "stimulation fatigue," or the local impairment of the tissue by the electric current at the actual seat of stimulation, and "functional fatigue," or the impairment of the tissue over the region which has previously been traversed by a propagated disturbance. Gotch brings quite definite evidence of the reality of the second of these factors, when he shows that local cooling or warming of portions of a nerve may alter the amount of the increased delay, although the locality exposed to the alteration of temperature is not the seat of the exciting stimulus. This observation leaves no room for doubt that the delay is in part at least caused by some change brought about by the actual passage of the propagated disturbance alone the nerve.

The evidence as to the importance of the other factor, namely, stimulation fatigue, does not appear to me to be equally convincing. The experiments which are held to indicate that stimulation fatigue is partly responsible for the delay in the response to a second stimulus are of two kinds. First, it is shown that if an interrupted current is applied to a nerve for five minutes, and the response to a single stimulus is taken a few seconds after the cessation of the interrupted current, that response occurs after abnormal delay[1]. If however the stimuli used for provoking the test responses are not applied at the seat of prolonged stimulation, then the increased delay is comparatively slight[2].

This experiment affords clear evidence that stimulation fatigue can be induced by prolonged stimulation; but it does not appear to touch the problem with which we are now concerned. We have to deal with a modification produced by one single stimulus and lasting in the case of nerve for a time of the order of a hundredth of a second. It may be that the stimulation fatigue set up by a single stimulus is of negligible magnitude, and it may be that the functional fatigue lasting for a hundredth of a second after a single stimulus is of such considerable magnitude as to outweigh entirely the stimulation fatigue during that time. The fact that at an interval of several seconds after a stimulation of several minutes the stimulation fatigue is proved to be the more important tells us nothing of the relative importance of the two fatigues at the time with which we are concerned; and it is just their relative

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[1] *Loc. cit.* p. 263, Fig. 6.

[2] *Loc. cit.* p. 265, Fig. 8.

importance which we need to know. The increased delay which we observe must be determined by that fatigue which is most important during the actual time of the delay. In fact the question is whether in observing the increased delay we are obtaining a measure of the duration of stimulation fatigue or of functional fatigue.

The second line of evidence adduced to show the importance of stimulation fatigue appears to be of far greater value. Gotch states "A further confirmation of this is shown by some of my records with an inadequate first stimulus, these show increased delay in the response to the second and only adequate exciting agent[1]." If a single stimulus so weak as to set up no propagated disturbance still causes stimulation fatigue enough to effect a considerable increased delay, a fortiori the stimulation fatigue of a stimulus of adequate strength will be of real importance. Unfortunately Gotch has given us no further details of this interesting line of experiment.

In view of these considerations I was unable to regard the relative importance of "stimulation fatigue" and "functional fatigue" in causing the increased delay as being sufficiently closely determined. I have attempted to devise experiments by which some further light might be thrown upon the problem in the case at least of the response of muscle to a second stimulus.

My object was to devise an experiment by which it should be possible to differentiate directly between the delay caused by the passage of the exciting current as such, and the delay caused by the consequent occurrence of a propagated disturbance. For this purpose it was necessary to compare the effect of an exciting current of adequate strength, which nevertheless failed to set up any propagated disturbance, with that of the same current when it did set up a propagated disturbance. This result was obtained by the use of a stimulus falling within the refractory period. The experiments were made in the following way. In the first observation the plate was exposed to the response of a sartorius excited by a single stimulus, which I will call *A*. At the second exposure of the same plate the stimulus *A* was followed by a stimulus *C*, which fell shortly after the end of the refractory period. This observation gave then an ordinary first response with a delayed and modified second response superposed. The second observation consisted in a first exposure with stimulus *A* as above, and a second exposure with the stimuli *A* and *C* and also a stimulus *B* so timed as to fall within the refractory period of *A*. The third observation was made

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[1] *Loc. cit.* p. 266.

with stimulus *B* alone, to show that it was adequate to produce a full response when acting alone. The fourth observation was made with *A* alone at the first exposure, and *A* followed by *B* at the second exposure, to show that *B* did actually fall in the refractory period of *A*. If now the modification of the response to stimulus *C* observed in the first observation were due in part or wholly to the fact that *A* had a local effect on the muscle apart from the propagated disturbance which it set up, then in observation 2 there ought to be greater modification of the response to *C*, since not only was *C* preceded by two currents instead of one, but the second of these, namely *B*, occurred at a shorter interval before *C* than did *A* in the first observation. If, on the other hand, the modification of the response to *C* were due entirely to the fact that *A* set up a propagated disturbance, and not at all to any local effect which it had on the muscle, then the response to *C* ought to be identical in the first observation and the second, that is whether the current *B* was sent in or not, since *B* did not set up any propagated disturbance.

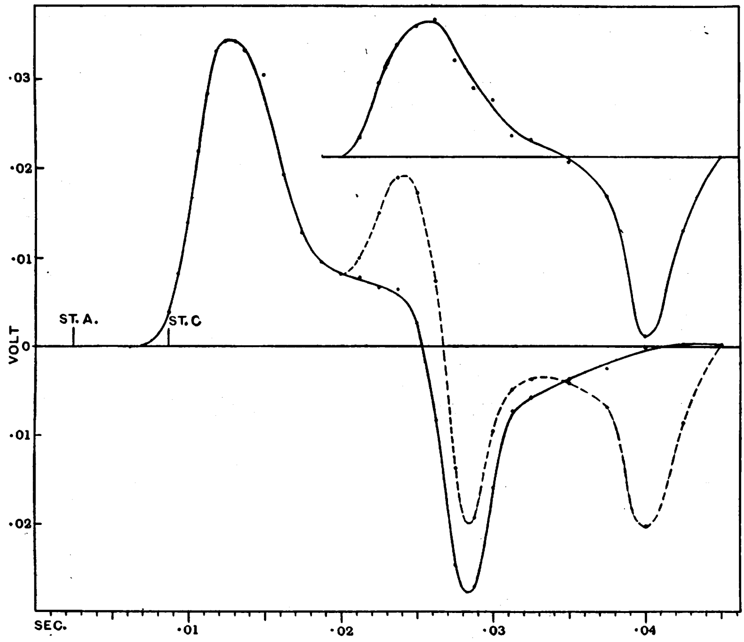

Fig. 15.

The result of this experiment is that the response to stimulus *C* is not materially altered by the interposition of the stimulus *B* between

A and *C*, provided that *B* falls in the refractory period of *A*. I give in Figs. 15 and 16 the analysed curves from two such observations (Exp. 9). I have chosen this experiment out of all that I made because it is the only one in which even a very slight difference between the second responses in the two observations could be made out on superposing the photographic curves. The response to *A* is shown as a full line, that to *C* diverges as a broken line. The complicated path which the latter follows proves, on subtraction of the ordinates as shown in the curves plotted on a higher level, to be nothing but a simple diphasic curve. It will be observed in Fig. 15 that the stimulus *C* has been so timed as to produce a response with considerable increased delay. The summit of the first response occurs about ·011 sec. after stimulus *A*, that of the second response about ·017 sec. after stimulus *C*. If we suppose the time from beginning to summit of a response to be about ·006 sec. (which appears from Fig. 15 to be the approximate value) then the delay of the response to *A* is approximately ·005 sec.; that of the response to *C* ·011 sec. Clearly the stimulus *C* falls well within the range of time which gives a delayed and modified response. Within this range, as

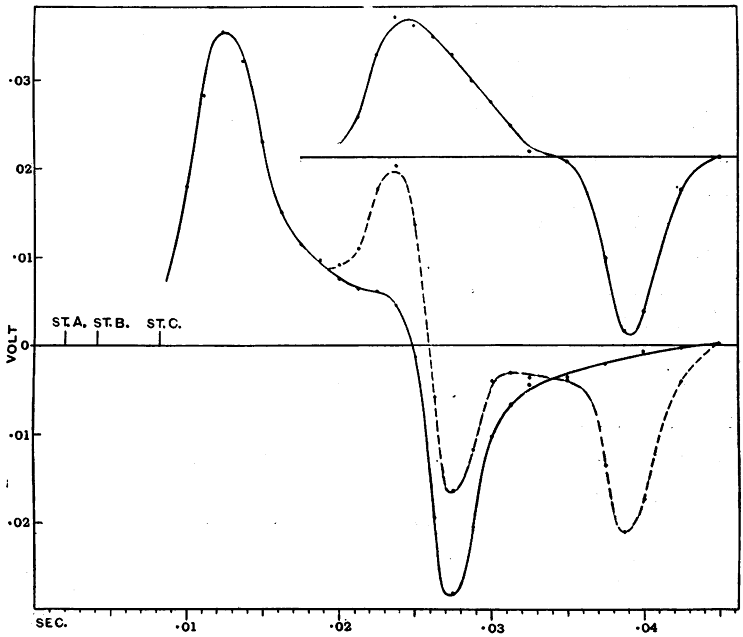

Fig. 16.

may be seen from other curves published in this paper, a shifting of the second stimulus may alter the time of occurrence of the second response very little, but will alter very largely the size (degree of modification) of the second response. If then the interpolated stimulus *B* has any effect we should expect it to produce rather an increased modification than an altered delay of the second response. As a fact it appears to produce neither. The second response in Fig. 16 agrees closely with that in Fig. 15 both in size and in time relations. The one slight difference between the curves (which I mentioned above as being visible when the photographic curves are superposed) is that in Fig. 15 the downward sweep of the first phase of the second response (see the curve plotted above the main curve) appears to be rather more convex towards the abscissa than that in Fig. 16. As I have said, other experiments did not show even this minute difference as a result of interpolating the stimulus *B*. The experiments made ranged over temperatures from 8·5° C. to 17·5° C.; that plotted in Figs. 15 and 16 was made at 8·5° C.

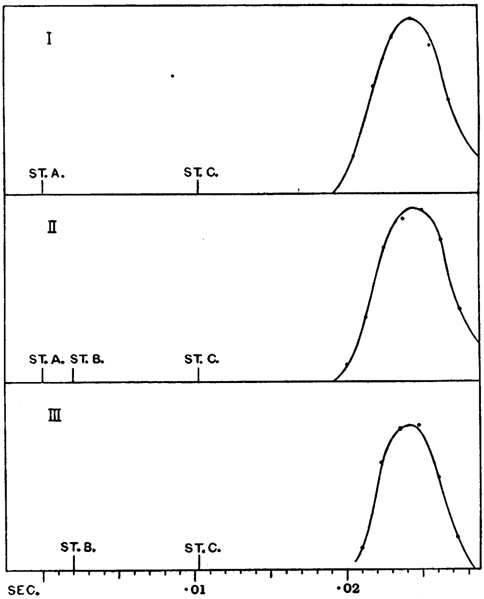

Fig. 17.

So far it has been shown that the greater proximity of the stimulus *B* to the stimulus *C* produces no measurable increased modification in the response to *C*, provided that *B* sets up no propagated disturbance. It remains, in order to complete the evidence, to show that if a stimulus which does set up a propagated disturbance is brought as near to *C* as *B* is, then the modification of the response to *C* will be actually increased by a measurable amount. This was tested in Exp. 10, the results of which are plotted in Fig. 17. This figure shows only the second responses in each case, obtained by subtracting the ordinates of the single response from those of the combined curve. The procedure of this experiment was as follows. In the first observation (marked I in the figure) the first exposure was with stimulus A, the second with A followed by C at an interval of ·0102 sec. In the second observation the first exposure was with stimulus A, the second with A followed by B at ·0020 sec. and C at ·0102 sec. In this case B fell within the refractory period of A, and the delayed response to C was practically identical with that in observation I. A third observation was then made, the first exposure with B alone, the second with B followed by C at the same interval as before, namely, ·0082 sec. (*i.e.* ·0102 – ·0020 sec.) This observation showed the effect of bringing the stimulus which set up a propagated disturbance to the same distance from C as was the stimulus B in the second observation. The result was a marked increase in the modification of the response to C. The E.M.F. reached in the first phase of the response to C was reduced in the ratio 46 : 39[1].

I conclude from these experiments that in the sartorius muscle the factor of importance in causing the modification of a second response is the setting up by the first stimulus of a propagated disturbance, and not in any measurable degree the local effect of the actual passage of the electric current at the seat of stimulation. In other words these experiments show that the effect of the stimulation fatigue produced by a single stimulus of maximal strength is not measurable as increased modification of the subsequent response.

I have attempted to control this result by an entirely different method of experiment. If the delay of the response to a second stimulus is due entirely to the occurrence of a previous propagated disturbance, and not to a local effect at the seat of previous stimulation, then the delay ought to be the same whether the second stimulus is sent in at the same point as the first stimulus, or at any other part of the tissue which is passed over by the propagated disturbance. My experiments upon this point have been made with the sciatic-gastrocnemius preparation of the frog excited from its motor nerve. The sartorius muscle, owing to its complex composition, does not lend itself to stimulation with like stimuli at two different points. The difficulty of this line of experiment is that the interval between the beginning of the first propagated disturbance at the seat of the second stimulus and the

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[1] If the modification of the second response in Exp. 9 had been due to the "stimulation fatigue" set up by the previous stimulus, we should have expected the interpolated stimulus B to reduce the second response by an amount considerably greater than this change of 46 : 39 observed in Exp. 10. For in Exp. 10 the interpolated stimulus B was only 20 % nearer to C than was the original first stimulus A; whereas in Exp. 9 the stimulus B was 34 % nearer to C than was the first stimulus A.

occurrence of the second stimulus will differ according as the second stimulus is or is not sent in at the same point as the first. If the second stimulus is sent in nearer the muscle than the first, then at the seat of the second stimulus the propagated disturbance will begin later (by the time of conduction between the two seats of stimulation) than it began at the seat of the first stimulus. Consequently the interval between the first and second stimuli will with separate points of stimulation be in effect less than when the stimuli fall on the same spot. This means that unless the time of conduction between the points of stimulation is known it is not possible by a single pair of observations to determine whether the delay of the response to the second stimulus is the same whether the stimuli fall on the same or on different parts of the nerve; for it will not be possible to ensure that the second stimuli shall in the two cases catch the first propagated disturbance in the same stage of its development. I thought the best way of getting round this difficulty would be to repeat each kind of observation with a large number of time intervals between the stimuli. If this is done it becomes possible to plot out, as was done in Figs. 6 and 10 above, the time of occurrence of the second response over a wide range of intervals between stimuli for both kinds of observation. The results of such an experiment are tabulated below (Exp. 11). Four observations were made with the second stimulus at the same point as the first, four with the second stimulus at a point on the nerve 11 mm. nearer to the muscle. The anode was in every case at the same point, namely 5 mm. further from the muscle than the seat of the first stimulus. The times of occurrence of the second response are given twice over for every observation made. In Table *A* the beginning of the second response is measured directly from the first apparent divergence of the combined from the single curve. In Table *B* the point actually measured is the time of maximum P.D. of the second response, and the time of beginning of the response is found by subtracting ·0022 sec. which is the observed interval between the beginning and culmination of a normal response.

Whether Table *A* or *B* is taken as the truer account of the times of occurrence of the second response one fact stands out clearly. When the two stimuli are sent into separate points on the nerve, as well as when they are sent in together, the delay of the second response increases in the familiar way with the earlier incidence of the second stimulus. The values obtained for the delay are not identical in the two cases, as may be seen if for example those given in Table *B* I and II are plotted together on the same scale of time. But it must be remembered that, for reasons which have been given already, the time intervals between the stimuli for observations *E* to *H* are in effect shorter than those given in the table. If we wish to represent truly the relation of the second stimulus to the previous propagated disturbance by which delay and modification are being produced, we must make allowance for the time of conduction of that first disturbance from its place of origin to the seat of the second stimulus. The length of nerve intervening was 11 mm. If we assume the rate of conduction in the nerve to have been 18 mm. per sec., the time occupied will have been ·0006 sec. For observations *E* to *H* we must therefore subtract this time from the tabulated interval between the stimuli. By this process the following values are obtained if we take the times given under Table *B*.

Exp. 11. Gastrocnemius-sciatic preparation. Frog. Temp. = 17·5° C.

A. *Time of apparent commencement of second response measured directly.*

I. With second stimulus at same point as first.

Obs.	Time first stimulus to second stimulus	Time first stimulus to beginning of second response	Time second stimulus to beginning of second response
A	·0021 sec.	·0074 sec.	·0053 sec.
B	·0030	·0075	·0045
C	·0042	·0077	·0037
D	·0067	·0095	·0028

II. With second stimulus 11 mm. nearer to muscle than first.

E	·0015	·0077	·0062
F	·0029	·0072	·0043
G	·0042	·0075	·0033
H	·0064	·0089	·0025

B. *Time of commencement of second response calculated from time of its maximum* P.D.

I. With second stimulus at same point as first.

A	·0021	·0069	·0048
B	·0030	·0070	·0040
C	·0042	·0074	·0032
D	·0067	·0093	·0026

II. With second stimulus 11 mm. nearer to muscle than first.

E	·0015	·0069	·0054
F	·0029	·0067	·0040
G	·0042	·0074	·0032
H	·0064	·0084	·0020

Obs.	Time first stimulus to second stimulus	Time first stimulus to second response
E	·0009 sec.	·0069 sec.
F	·0023	·0067
G	·0036	·0074
H	·0058	·0084

When this correction has been made the delay of the second response is found to agree in a remarkable way whether the second stimulus does or does not fall on the same part of the nerve as the first. This is made clear by Fig. 18 in which the two sets of observations are plotted side by side. The values are taken from Table *B*. The supposed time of conduction (·0006 sec.) has been subtracted from the time between stimuli in observations *E* to *H*. Observations made with stimuli at the same point are shown as dots enclosed in triangles; those with stimuli at different points as dots enclosed in circles. All the observations lie close to the broken curve. The same agreement may be seen if the values from Table *A*, which were obtained by an independent method of measurement, are treated in the same manner.

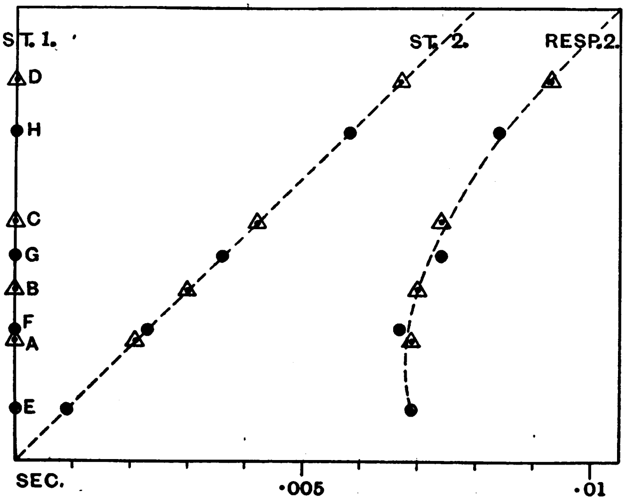

Fig. 18.

These experiments point again to the conclusion that the delay in the response to a second stimulus is due, within the limits of accuracy of the methods here used, entirely to the propagated disturbance set up by the former stimulus, and not at all to the stimulation fatigue resulting from the passage of the former exciting current.

Still one more method of experiment on this question seemed to me desirable. I have referred already to Gotch's account of experiments in which responses to an adequate stimulus showed increased delay when an inadequate stimulus had previously traversed the tissue. I attempted to repeat this observation on muscle. A photographic record was made in the following way. A sartorius muscle was connected to the capillary electrometer in the usual manner, the exciting electrodes being on the nerve-free pelvic end, and the leading-off electrodes on the middle and tibial end. At the first exposure of the photographic plate the muscle was excited with a single maximal stimulus (*B*). At the second exposure the stimulus *B* was again sent in, preceded at an interval of ·002 sec. by a stimulus (*A*) which was just subliminal. The stimulus *A* was adjusted in strength by finding the threshold for contraction of the muscle and moving the secondary coil away 1 mm. further from the primary. In the experiments made the threshold was at about 10 cm. coil distance. From calibration of the coils used it appears that a movement of 1 mm. in this region of the inductorium means a change of strength of current by approximately 5 %. The photographic record showed that the response to stimulus *B* alone and the response to stimulus *B* preceded by *A* had traced over precisely the same course. It would have been easy in these superposed records to measure a displacement as small as 0·2 mm. between the two exposures. With the speed of plate used this would mean ·0004 sec. Four observations were made by this method at 9° C., one at 14° C., one at 16·3° C., and three at 17° C. Two muscles were used for the observations. In none of the photographic records can I trace any divergence between the response to *B* alone and the response to *B* preceded by *A*. It seems clear that in muscle the response to an adequate stimulus is not delayed to a measurable degree by the passage of a previous inadequate stimulus.

The three methods of experiment which I have used lead me to the same conclusion, that the delay in the response to a second stimulus is wholly due to the propagated disturbance set up by the first stimulus, and not at all to any localized "stimulation fatigue" which may be produced. This is so whether the muscle is excited directly or through its motor nerve.

VI. *Remarks on the application of the results obtained.*

Up to this point my experiments have shown that the occurrence of a delayed response to a second stimulus is common to many tissues, and is due, in muscle at any rate, entirely to the temporary modification of the tissue by the passage of the propagated disturbance.

The value of this result seems to me to lie in its possible contribution to two of the difficult problems with which the physiology of the excitable tissues has to deal. In attempting to investigate the nature of the nervous impulse, or of the corresponding propagated disturbance in muscle, we are always hampered by the paucity of the measurable changes which that disturbance brings in its train. Up to now the change of electric potential has been the sole recognisable accompaniment of the propagated disturbance. By the delay of the response to a second stimulus we can now measure the duration of a modified state which is left in the wake of the disturbance. Some experiments made by another investigator in this laboratory and shortly to be published have given more definite quantitative evidence than existed previously to show that the refractory period of muscle and nerve is also a consequence of the propagated disturbance alone. We begin therefore to bring the propagated disturbance more closely within the grasp of quantitative experiment. The next step must obviously lie in the direction of determining more precisely the relation between those several phenomena which go hand in hand with the propagated disturbance.

The results described in this paper must also be taken into account in any consideration of the way in which the effects of successive stimuli sent into a tissue may interfere one with another. It is to the interference of successive stimuli that we must look, according to the recent hypothesis of Fröhlich, for an explanation of the phenomena of inhibition[1]. It will be necessary therefore to enquire whether that hypothesis accords completely with the facts which have here been described. If I have understood the hypothesis rightly it supposes inhibition to result from the setting up of a new refractory period by a stimulus falling within the refractory period which succeeds a previous stimulus. My experiments indicate that a stimulus falling within the refractory period of a previous one does not set up at any rate that state of modification which causes the delay of a second response, a state which we have seen to be the consequence of the propagated disturbance. We have indications that the refractory period is probably an accompaniment of the same disturbance. This is enough to suggest the need of rigid experimental proof that a stimulus falling within the refractory period of a previous stimulus does in fact set up a new refractory period of the same nature as that within which it fell. Such a proof, if it exists, must form the very foundation of the hypothesis.

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[1] *Ztschr. f. allg. Physiol.* IX. p. 55. 1909.

I might point to other ways in which the results described in the present paper bear on the question of interference between the effects of successive stimuli. But I prefer to leave the whole matter over until my own experiments have advanced a step further.

VII. SUMMARY.

Photographic records of the electric response of the gastrocnemius muscle of the frog to two stimuli sent into the sciatic nerve have been made and analysed by the usual method. The curves relating time to potential difference show that the response to a second stimulus falling soon after the end of the refractory period commences after a delay which may be many times as great as the delay of the first response. Attempts have been made to correct for the many possible sources of error involved in measurements of this kind.

Similar observations have been made on the sartorius muscle of the frog excited directly. These confirm my previous observations on the same tissue in so far as they also show the second response to a stimulus falling soon after the end of the refractory period to be greatly delayed.

On the ventricular muscle of the frog observations of a like kind have been carried out by two methods. In one method the Stannius preparation was used, and the ventricle was excited by two stimuli following one another at various intervals. In the other method single stimuli were sent into a beating ventricle at various times after a natural beat. Both these methods show that the electric response to a stimulus falling soon after the end of the refractory period is subject to great delay.

In the simpler tissues used, the directly excited sartorius and the ventricular muscle, it is found that second stimuli falling within a certain range of time after the end of the refractory period provoke responses at a fixed interval after the first stimulus. This is the phenomenon of the irresponsive period which I have previously described. But there is an important modification of my original statement of that phenomenon rendered necessary by the greater range of these later experiments. I originally stated, in consequence of using too few intervals between stimuli in my earlier experiments, that second responses beginning later than the irresponsive period exhibited no abnormal delay. I now find that such later responses are more delayed than the response to a single stimulus; their increased delay disappears only gradually as the second stimuli become later. This agrees with the recent observations of Gotch on nerve.

The phenomena of delay in a more complex case such as that of muscle excited from its motor nerve exhibit further peculiarities for which reference must be made to the more detailed part of this paper.

Experiments have been carried out by three independent methods in order to determine whether the modification of the tissue responsible for the increased delay of a second response is caused by the direct local action of the previous stimulating current, or by the passage of the previous propagated disturbance over the tissue, or by both. The methods used are:—

(1) To determine whether a stimulus falling within the refractory period of a previous one, and therefore setting up no propagated disturbance, can alter the amount of the increased delay.

(2) To send the stimuli provoking the second response into a part of the tissue not traversed by the previous stimulating current, and to determine whether the delay is the same as when the two seats of stimulation coincide.

(3) To enquire whether a preceding stimulus of just subliminal strength produces any delay in the response to a succeeding adequate stimulus.

The experiments made in these various ways agree in showing that in the tissues used the increased delay is, within the limits of measurement available, due entirely to a modification of the tissue by the previous propagated disturbance, and not at all to any direct effect which the preceding current used for stimulation may have.

A similar conclusion may be drawn from the observed fact that in cardiac muscle all the usual features of the delayed second response are reproduced if the first response is a natural one, when of course the second stimulus falls on a tissue not previously subjected to an electric current. These results are not in conformity with the conclusion drawn by Gotch for the case of nerve, that "stimulation fatigue" (the local effect of the stimulating current) is one important factor in causing the delay.

VIII. Appendix.

In these tables the time and potential difference values are given for those analysed electrometer curves which are used in the text but not shown graphically. In order to save space the data are tabulated only for those parts of the curves which are required for determining the time of commencement or of maximum P.D. of the second response. Exps. 2, 4, and 5 are made by the method of single exposure, and require a control curve showing the response to the first stimulus alone for comparison with the combined responses which are tabulated. In Exp. 2 the control for *DEF* is *A*, which is given graphically in Fig. 3, the control for *G* and *H* is the first part of *H*. The controls for Exps. 4 and 5 are given in the tables. Exps. 3 and 11 are made by the method of superposed photography, so the analysed curves for both responses are given under every observation. The P.D. is given in arbitrary units. The number of units equal to ·01 volt is for Exp. 2, 35, Exp. 3, 27·8, Exp. 11, 22.

EXP. 2.

		P.D.	
Time from zero	E	G	H
·0033 sec.	st 1	...	...
·0070	st 2	...	...
·0009	...	st 1	..
·0074	...	st 2	...
·0040	...	...	st 1
·0187	...	...	st 2
·0081	...	-56·6	+66·3
·0087	...	-48·8	+88·8
·0094	...	-38·2	+79·2
·0100	-32·4	-19·1	+4·9
·0106	-68·6	+3·4	-48·0
·0112	-53·8	+96·9	-59·1
·0119	-35·0	+163·5	-47·1
·0125	-12·9	+208·9	-37·5
·0131	-3·5	+193·8	-25·5
·0137	+6·4	+13·9	-16·5
·0144	+11·6	-139·6	-11·8
·0150	+1·7	-150·3	-6·0
·0162	-19·5	-94·0	-3·5
·0175	-9·1	-22·4	-1·4
·0200	-1·2	-4·7	-0·5
·0212	...	...	0
·0219	...	...	+10·8
·0225	...	...	+82·4
·0231	...	...	+115·4
·0237	...	...	+156·9
·0244	...	...	+134·2
·0250	...	...	+8·6

Exp. 3.

	P.D.					
	D		E		F	
Time from zero	1st resp.	2nd resp.	1st resp.	2nd resp.	1st resp.	2nd resp.
·0030 sec.	st 1	...	...	...	...	...
·0068	...	st 2	...	...	...	...
·0012	...	...	st 1	...	...	...
·0063	...	...	...	st 2	...	...
·0007	...	...	...	...	st 1	...
·0079	...	...	...	...	...	st 2
·0081	...	...	+69·6	...	...	...
·0087	...	...	+38·2	...	-6·6	...
·0094	+88·8	...	+4·1	+5·0	-56·4	...
·0100	+69·9	...	-53·3	-27·9	-74·6	...
·0106	+25·7	...	-73·1	-27·2	-62·9	-47·8
·0112	-16·3	-11·7	-53·2	+7·8	-32·1	+5·1
·0119	-80·7	-31·1	-35·4	+52·5	-18·9	+40·3
·0125	-74·3	-3·4	-25·0	+75·6	-16·5	+99·2
·0131	-54·9	+15·9	-15·9	+77·4	-12·0	+104·1
·0137	-33·3	+29·5	-10·0	+37·6	-9·1	+96·4
·0144	-22·2	+26·3	-9·2	+12·6	-8·5	+81·4
·0150	-14·2	+10·3	...	...	...	...

Exp. 4.

	P.D.				
Time from zero	Control single response	B	C	D	E
·0030 sec.	st 1	...	...	...	...
·0012	...	st 1	...	...	...
·0064	...	st 2	...	...	...
·0037	...	...	st 1	...	...
·0112	...	...	st 2	...	...
·0030	...	...	...	st 1	...
·0130	...	...	...	st 2	...
·0040	...	...	...	...	st 1
·0165	...	...	...	...	st 2
·0175	+15·3	-0·5	+20·1	...	...
·0187	+5·6	-11·7	+9·3	...	...
·0200	-5·2	-11·1	+0·6	...	...
·0206	-9·1	...	...	...	...
·0212	-13·7	-5·6	-7·2	...	...
·0219	-16·7	...	...	...	...
·0225	-15·5	+3·9	-12·4	...	...
·0231	...	...	...	...	...
·0237	-9·3	+14·8	-3·5	+6·3	...
·0244	...	...	...	+14·9	...
·0250	-4·4	+22·1	+8·9	+27·7	+5·2
·0256	...	...	...	+33·7	+11·2
·0262	-2·1	+18·6	+21·2	+35·0	+16·7
·0269	...	...	...	+33·9	+24·2
·0275	-1·1	+10·8	+25·6	+30·4	+31·7
·0281	...	...	...	...	+34·7
·0287	...	...	+19·3	...	+37·4
·0294	...	...	...	...	+35·3
·0300	0	+4·7	+13·3	...	+30·6

Exp. 5.

	P.D.					
Time from zero	Control single response	A	B	C	D	E
0 sec.	...	st 1	...	...	...	...
·0018	...	st 2	...	...	...	...
·0015	...	...	st 1	...	...	...
·0045	...	...	st 2	...	...	...
·0020	...	...	...	st 1	...	...
·0059	...	...	...	st 2	...	...
·0015	...	...	...	...	st 1	...
·0061	...	...	...	...	st 2	...
·0010	...	...	...	...	...	st 1
·0083	...	...	...	...	...	st 2
·0075	+36·1	+48·1	...	+6·2	...	...
·0087	+15·4	+30·0	...	+30·4	...	...
·0100	+10·4	+16·3	...	+49·0	...	...
·0112	+0·1	+9·6	...	+26·4	...	...
·0119	...	+7·6	...	...	...	...
·0125	−0·4	+5·4	...	+11·8	...	...
·0131	...	+9·3	...	...	...	...
·0137	+0·9	+13·4	...	+6·4	+9·2	...
·0144	...	+16·9	...	+3·1	+16·8	+8·7
·0150	+2·7	+14·3	+7·9	+9·2	+27·6	+25·7
·0156	...	...	+11·5	+16·4	+30·3	+29·3
·0162	...	+10·4	+15·0	+25·3	+28·6	+34·5
·0169	...	...	+9·4	...	+19·5	+22·5
·0175	+2·9	+4·9	+7·5	+17·1	...	+13·3
·0187	...	...	...	+9·2	...	...
·0200	+3·6	+4·1	...	+4·6	...	...

Exp. 11. I.

	P.D.							
	A		B		C		D	
Time from zero	R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2
·0040 sec.	st 1	...	...	...	...	...	...	...
·0061	...	st 2	...	...	...	...	...	...
·0040	...	...	st 1	...	...	...	...	...
·0070	...	...	...	st 2	...	...	...	...
·0037	...	...	...	...	st 1	...	...	...
·0079	...	...	...	...	...	st 2	...	...
·0015	...	...	...	...	...	...	st 1	...
·0082	...	...	...	...	...	...	...	st 2
·0100	+119·6	...	+109·3	...	+89·6	...	−54·8	...
·0106	+35·7	...	+29·7	...	+8·6	...	−28·2	...
·0112	−78·8	...	−3·8	...	−81·9	...	−18·1	−11·9
·0119	−86·3	−37·4	−61·3	−39·2	−76·4	−53·7	−7·5	+52·6
·0125	−60·1	+43·8	−56·4	+35·0	−30·8	+54·7	−4·3	+105·9
·0131	−25·3	+94·0	−29·7	+103·3	−17·3	+113·5	−3·0	+115·9
·0137	−15·6	+90·1	−19·6	+98·6	−10·3	+111·9	−2·6	+94·0
·0144	−6·3	+19·2	−10·0	+75·7	−7·0	+88·0	...	...

Exp. 11. II.

	P.D.							
	A		B		C		D	
Time from zero	R 1	R 2	R 1	R 2	R 1	R 2	R 1	R 2
·0011 sec.	st 1	...	...	...	...	...	...	...
·0026	...	st 2	...	...	...	...	...	...
·0040	...	...	st 1	...	...	...	...	...
·0069	...	...	...	st 2	...	...	...	...
·0046	...	...	...	...	st 1	...	...	...
·0088	...	...	...	...	...	st 2	...	...
·0028	...	...	...	...	...	...	st 1	...
·0092	...	...	...	...	...	...	...	st 2
·0081	-37·5	...	...	...	...	...	...	...
·0087	-76·7	...	...	...	...	...	...	...
·0094	-63·9	+6·9	...	...	...	...	...	...
·0100	-39·6	+80·6	...	...	...	...	-53·0	...
·0106	-11·4	+91·2	+9·7	...	...	...	-89·4	...
·0112	-6·1	+10·9	-66·9	...	+104·1	...	-78·2	...
·0119	...	...	-83·5	-3·0	-52·1	...	-33·2	-28·5
·0125	-3·7	-67·4	-31·5	+71·6	-108·0	-82·7	-14·6	+36·5
·0131	...	...	-15·2	+91·5	-63·8	+15·8	-7·4	+111·5
·0137	-3·7	-25·3	-11·3	+85·3	-24·3	+113·0	-3·3	+121·8
·0144	...	...	...	...	-12·9	+115·2	-3·1	+97·7
·0150	...	...	...	...	-6·5	+81·5	...	...

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