

wind. The evaporative cooling power of the wind may, therefore, have had an effect.

The retardation of growth cannot be attributed to the shaking produced by the motor, because seeds sheltered from the wind, but submitted to the shaking, grew as well as the control seeds.

The conclusion is reached that the stunting effect produced by wind is not only due to a less favourable wetting, but to greater cooling. The growing point may be robbed by wind of heat which is produced in the cellular growth processes—heat which facilitates growth.

I am much indebted to Mr. R. H. Davis, of Messrs. Siebe Gorman, Ltd., who gave me facilities for carrying on this research at a time when the National Institute of Medical Research was being used as a War hospital.

Reflex Times in the South African Clawed Frog.

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This investigation was undertaken with the object of determining the times of certain reflexes in a species of frog which has not, so far as I know, already been used for the purpose, viz., the South African clawed frog or toad (*Xenopus laevis*, or an allied species), and by analysing these times to obtain a measure of the delay in transmission of the reflex impulses in the spinal cord, or "synapse time." Spinal and decerebrate animals have been used as well as the intact frog. Einthoven's string galvanometer was employed to indicate the beginning of activity in the limb muscles.

It seemed desirable to eliminate, as far as possible, everything that would tend to introduce doubt into the interpretation of the records, even at the cost of making the experiments more troublesome to carry out. For this reason no strychnine or other drug was used. The spinal and decerebrate preparations were kept alive before experiment until the wounds were entirely healed and the frogs appeared to be in thoroughly good condition. Records were then taken from day to day, and the influence of temperature and of the period of survival after operation was studied. Any operative

procedure at the time of experiment was rendered unnecessary by employing the mechanical stimulus of a tap by a slight hammer to the toes, or a squeeze of the toes, and the electrical changes in the limb muscles were recorded by means of the wicks of non-polarisable electrodes, moistened with salt solution and impregnated with kaolin, bound round the limb over the unbroken skin. The wicks were applied at knee and ankle, or thigh and ankle, the skin area covered by each wick being about 1.5 cm. broad. The advantage of this method of leading off is that there is no injury done to the preparation, which can be experimented with for months. A disadvantage is that one cannot record in this way the pure response of a single muscle nor control exactly the points led off from, and the form of the electrical response is found accordingly to be rather variable. The application of a mechanical stimulus to the toes instead of electrical stimulation of a nerve, besides being more "natural," is advantageous from its avoiding injury to the preparation, and also by avoiding the difficulties which arise in recording and interpreting the electrical response when the stimulus is also electrical. Precautions will prevent an induction shock given to a dissected-out nerve from affecting the galvanometer, but it is often difficult to get rid of some slight action upon the string, and the absence of this complication makes me more confident in interpreting the curves. There is also no danger of the stimulus spreading further than is desired. The disadvantage of the use of a tap or squeeze is that a direct stimulation of muscle or nerve might perhaps be caused, whose electrical expression would be visible on the record and be mistaken for a reflex effect. This is a real difficulty in the case of homonymous stimulation, and the attempt has been made to avoid it by stimulating the toes alone and not the rest of the foot. The difficulty does not arise in the case of the heteronymous reflex where the electrodes are applied to the limb opposite to the one stimulated. Here there is no appearance on the records of anything which could be the expression of a direct excitation of the foot.

The photographic records of the galvanometer movements are made upon plates falling behind the slit of Cremer's apparatus at a rate of about 1 metre per second. The shadow of the string of a small string galvanometer with electromagnet is also thrown on the slit and signals the moment of stimulation. A tuning-fork, vibrating at the rate of 200 D.V. per second in front of the slit, throws its shadow on the plate and serves as time marker. The vertical lines showing the direction of the slit are produced by the teeth of an episkotister. In some cases the episkotister has not been used. The curves are measured by the aid of an enlarging camera. The intervals of time are given in thousandths of a second (σ), fractions are also given.

The frog is suspended in a jacket with the head bandaged as a precaution

against any stimulation of the acoustic nerves when the mechanism for giving the stimulus is set in action.

Two plates of vulcanite, hinged below with lead to form a V, and held on an adjustable stand, are brought up to the foot to be stimulated so that the toes lie in the opening of the V, and the vulcanite plates are closed on them without pressure. Two forms of stimulation are employed. One is innocuous and consists of a tap from a light hammer, held on a separate stand and attached to a spring. The head of the hammer, which bears a piece of platinum foil, is arranged to strike the vulcanite plate lying against the frog's toes on the release of an electromagnet by the plate-holder as it travels. On the outer surface of the vulcanite is a piece of platinum foil, and the circuit through the signal galvanometer is closed when the hammer-head comes in contact with this.

The other form of stimulus may be termed nocuous, since after several applications it will damage the foot. In this form the hammer is replaced by a spring-vice held on a separate stand, which, on the release of the electromagnet, forcibly compresses the vulcanite arrangement in which the toes lie. This stimulation has been chiefly used in order to obtain the heteronymous reflex in the decerebrate frog.

By the use of these arrangements a mechanical stimulus, weak or strong, can be given without moving the foot perceptibly. The hammer-tap is itself of the nature of a slight sudden squeeze, since the foot is not displaced to one side by the blow, but held against the vulcanite plate. The strong squeeze is given in such a way that the foot remains stationary. Thus, there is no change in the position of the wick on stimulation, and consequently no alterations of resistance from this cause in the galvanometer circuit, nor is there any stretching of the muscles.

The method employed in the experiments is to attach the wicks of the electrodes to one leg and to lead off from it during the course of the experiments. The toes on the same side as the electrodes are first stimulated, then the toes on the opposite side, and so the homonymous and heteronymous reflexes are recorded alternately. Dummy electrode wicks are attached to the opposite leg, to keep the conditions uniform on the two sides.

It is not proposed in this communication to compare in any detailed way the results obtained in the clawed frog with the findings in *Rana* or in the mammal. I have not at present access to all the literature on the subject.

It is not easy to compare the reflexes obtained from a preparation studied when it is thoroughly "normal" with those observed after the administration of drugs or previous cooling to a low temperature, or which have been subjected to dissection immediately before experiment; nor is it easy to

compare the results of mechanical stimulation with those recorded when the stimulus is electrical and may be suspected of spreading, as Miss Buchanan (1) points out, even so far as to affect nerve-fibres in connection with the motor cells or nerve of the opposite side.

A considerable number of observers have employed strychnine to raise the excitability of the cord; indeed, in the case of Wundt's (2) work the doubt has been expressed as to whether he ever obtained the heteronymous reflex in an undrugged preparation; but the action of strychnine on the neural mechanisms is so profound that responses and latencies observed under its influence must be studied apart from those of the undrugged preparation.

The range of temperature at which this investigation has been carried out is higher than that generally employed with the frog, and the shortness of the periods of delay obtained are to be explained to some extent on this account.

The Spinal Frog.

The spinal cord was cut aseptically and the wound allowed to heal thoroughly. The reflexes were recorded at intervals while the frog survived. The room temperature was noted, and observations at higher temperatures were also made by keeping the frog during experiment in an incubator which could be regulated to different temperatures. The frog's temperature was then noted from a thermometer in the mouth.

The reflex times are found to vary a good deal in the same frog and between different frogs, and it is advisable to make a considerable number of observations and to average the results.

The factors which may be expected to influence the length of the reflex time are the temperature, the length of the period of survival after operation, and the length of the conducting nervous path, that is to say, the size of the animal. Its age will doubtless also have an influence and can be estimated from the size. Frogs were chosen of about the same size. They were allowed to live as long as possible, and *post mortem* the nerves were dissected out and measured. Towards the end of the period of survival the reflex times appear to lengthen somewhat.

The response in the spinal frog to a tap on the toes is a sudden sharp flexion of the same leg and extension of the opposite leg. There is also adduction of the limbs. The reflex is of a jerky character.

The chief object of the investigation in the spinal preparation was to study the time difference between the onset of homonymous and heteronymous reflex activity of the hind limbs. In the spinal mammal it has been shown that this interval is very brief, amounting only to about 0.001 second. In the spinal frog I have found this difference to amount sometimes to as much

as eight thousandths of a second (8σ), that is to say, the crossed is longer than the same-side time by this interval, but sometimes there is a reversal and the crossed time is shorter than the same-side time. In other cases no appreciable difference of time is found between the two reflexes.

It was hoped that the difference of time would be suitable for an investigation of the influence of temperature upon the delay at a synapse, on the assumption that an additional synapse is interposed in the path of the heteronymous reflex, but it is difficult to explain on this assumption the complete disappearance of the interval in some experiments or the occasional reversal. On the other hand a difference of 8σ is quite consistent with the view that the heteronymous path involves more synapses than the homonymous and indeed is most easily explained in this way. One is inclined to contemplate the possibility that as the conditions change, the path taken through the spinal cord in the heteronymous reflex may alter.

The following results were obtained by the use of the innocuous stimulus. The times are given in thousandths of a second (σ) and the temperature in degrees Centigrade (see Table on p. 36).

The time difference between the reflexes on the two sides, when present, is small. In the above experiments, which I have grouped together, the longest time difference, 4.1σ occurred at the lowest temperature used, 14° , but the differences generally do not seem to have a simple relation to the temperature; at any rate, from the data at present available such is not evident. It may be mentioned that frog B, which gave the difference of 4.1σ , died three days later (forty-five days after operation) so that presumably at the time of experiment it was not in the best condition, and this may have had an influence upon the time difference.

I do not think that the above experiments taken by themselves would justify us in assuming that in the spinal frog at the temperatures employed, and with the frogs in good condition, the intraspinal path of the heteronymous reflex involves a larger number of synapses than that of the homonymous reflex. The fact that the former may sometimes have a shorter time than the latter, which seems also to have been observed by Rosenthal (1), tells against such a view. It would rather appear that we are dealing with mechanisms involving the same number of neurones on the two sides. Even if they are similarly constituted as regards number of synapses one could hardly expect that the mechanisms would work absolutely synchronously. The fact that the homonymous time is usually slightly the shorter may possibly be due to the motor neurones responding rather more readily to stimuli reaching them through afferent nerves from the same side of the body. Doubtless the greater number of the reflex flexions of a leg will, in the ordinary course of

Frog.	No. of experiment.	Days after operation.	T.	Homonymous reflex time.	Heteronymous reflex time.	Difference obtained by subtracting average homonymous from average heteronymous time.
A	1	13	20 (room)	12·0	17·0	0
				16·4	12·8	
				15·6	13·5	
				15·8	15·9	
				15·3	15·9	
B	2	40	20 (gullet)	13·9	13·9	1·3
				14·5	16·4	
				13·5	15·6	
	3	34	28 (room)	14·0	15·5	1·7
				11·7	13·1	
				11·0	13·2	
	4	35	27 (room)	11·1	14·8	1·6
				11·8	12·8	
				12·0	12·0	
	5	35	23 (room)	11·4	10·7	- 2·8
				14·0	10·2	
				14·4	10·7	
	6	34	21 (room)	20·8	20·4	1·1
				16·6	18·0	
				15·8	18·2	
	7	36	20 (room)	18·1	22·1	3·0
				17·0	20·0	
				17·1	19·3	
				18·0	21·6	
				18·0	18·0	
	8	42	14 (gullet)	16·5	21·7	4·1
				24·0	26·4	
				22·1	26·4	
C	9	14	30 (room)	19·3	25·0	- 0·1
				10·8	11·0	
				9·9	9·7	
	10	13	28 (room)	10·1	10·0	1·5
				10·8	13·6	
				10·4	12·4	
	11	14	20·5 (room)	11·1	10·9	1·3
				11·5	13·8	
				12·2	12·8	
	12	13	19	—	12·8	2·8
				10·5	13·8	
				11·8	14·0	
				12·3	15·1	

Average of above heteronymous reflexes (41 in number) 15·7 σ Average of above homonymous reflexes (40 in number) 14·3 σ Average difference 1·4 σ

events, be aroused by sensory stimuli playing upon the same limb. This may have led to slightly greater facility and shorter delay in transmission at the same-side synapse.

The actual time of the reflex varies, of course, with temperature, size, and condition of animal.

The temperature in the room in the Physiology Department where the frogs are experimented upon rises in Summer as high as 26° C. If we select a reflex time recorded at a room temperature of 23° C. from a frog in good condition, as for example frog B, Experiment 5, first response, thirty-five days after operation, we find an interval of 11·4 σ for the total reflex time. The length of nerve from toes to cord and from cord to thigh was found *post mortem* in this frog to be 16 cm. The rate of conduction in the nerve of *Xenopus* at 23° C. was determined in the usual manner by electrical stimulation at two points, with the electrical change in the muscle as indicator, and was found to be 37 metres per second. The latency of the muscular response to nerve stimulation close to the muscle was found to be 2·4 σ . If we allow 1 σ for the latency of sensory nerve endings we may suggest the following analysis:—

Total reflex time	11·4 σ
Nerve conduction	4·3
Muscle latency	2·4
Latency of sensory endings.....	1·0
	<hr/>
Synapse time	3·7 σ

The conclusion to which one is led by these results is that, at the higher range of temperatures, and when the frog is in good condition, the homonymous and heteronymous reflexes each involve a path through the spinal cord which contains one synapse or set of synapses having a delay of 3·7 σ . The reflex in the spinal frog is of the nature of a jerk, and we may compare this synapse delay of 3·7 σ in *Xenopus* with the delay of about 2 σ found for the knee jerk in the mammal (3), (4).

There is however evidence that at lower temperatures, or when the frog is not in good condition, the short path to the crossed motor neurones, which we have described as containing one synapse, is not open. I have grouped together the remainder of the experiments performed on the spinal frog as examples of this. The figures are as in Table on p. 38.

In this second group of experiments there is a considerable time elapsing between the beginning of activity in the two limbs. The extra delay of the crossed reflex amounts to about 8 σ , which suggests that a heteronymous path is being traversed here which contains more synapses than the homonymous path. We have seen reason to conclude that the delay at one synapse is almost 4 σ , and we have to consider whether this difference of

Frog.	No. of experiment.	Days after operation.	T.	Homonymous reflex time.	Heteronymous reflex time.	Difference obtained by subtracting average homonymous from average heteronymous time.
C	13	17	16.5 (room)	15.0 15.2 12.9 11.8 13.5 13.7 15.5 15.6 14.7	23.1 21.3 26.5 22.8 17.6 — — — —	8
D	14	3 (after cord section) 123 (after decerebration)	19 (room)	14.4 14.4 21.5 15.4 —	18.1 22.5 21.1 26.6 32.6	
E	15	20 (after cord section) 141 (after decerebration)	30 (room)	8.4	16.2	7.8
A	16	2	15.5 (room)	18.9 19.1 18.9 16.2 17.0 18.3 13.2 19.0 19.7	23.6 22.2 21.8 28.8 26.5 37.0 22.1 18.2 —	7.2
B	17	1	15.5 (room)	12.2 12.7 15.5 18.4 17.8 —	25.8 24.8 29.0 23.8 22.2 17.4	

Average of above heteronymous reflexes (25 in number) 23.7 σ
 Average of above homonymous reflexes (28 in number) 15.7

Average difference 8.0 σ

about 8 σ can be accounted for by the interposition in these cases of one extra synapse in the crossed path, whose delay has been increased to as much as double, owing to lower temperature or less satisfactory general condition of the preparation. The average homonymous time is practically the same in the two groups of experiments.

I find it difficult to believe in so great a lengthening of the delay at one synapse, and think it more probable that two extra synapses have been

interposed in the crossed path in this group of experiments, and conclude that the heteronymous path employed here contains two synapses more than the homonymous, or three synapses in all.

It seems to depend upon the general condition of the frog as to whether the short path is pervious or not. Thus in Experiments 16 and 17, where the observations were made on the second and first days respectively after section of the cord, and where the frogs may not entirely have recovered from the hæmorrhage and other immediate effects of the operation, and where the wounds were not yet healed, the short path is apparently impervious. In Experiments 14 and 15, where the frogs had been decerebrated and preserved in the laboratory for several months before the cord was cut, and where they were emaciated, with flabby muscles, the long path appears to have been used. Even the high temperature used in Experiment 15 does not diminish the extra delay of the crossed reflex, although it reduces the time of each reflex.

In diagram (fig. 1) the time differences in all the experiments are arranged in order of magnitude, and a step-like appearance is shown.

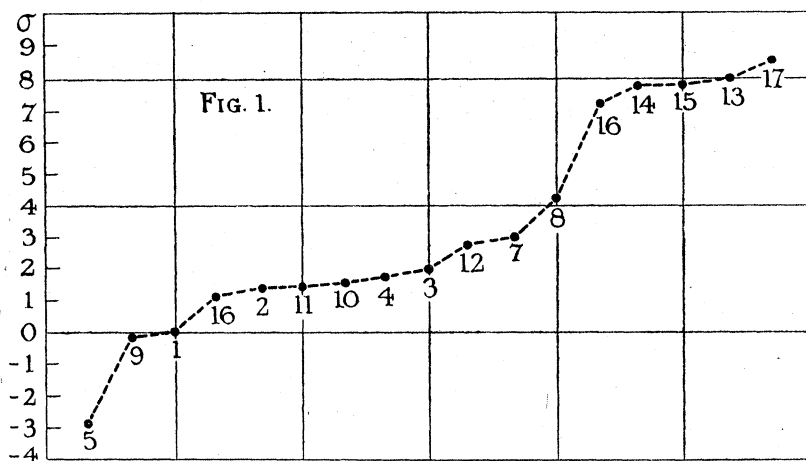


FIG. 1.—Diagram giving in order of magnitude the time differences obtained by subtracting the homonymous reflex times in the hind-limb of the spinal frog from the heteronymous reflex times. The numbers are the numbers of the experiments and the time differences are plotted in thousandths of a second (σ). The diagram exhibits a step-like character. A negative difference means that the heteronymous reflex time is shorter than the homonymous.

The Form of the Electrical Response in the Spinal Frog.

Figs. 2 and 3 represent forms of variation recorded on homonymous stimulation of the toes, fig. 4 on heteronymous stimulation. The electrical variation at its commencement resembles that of a twitch, but the curve

shows, after the initial deflections, other oscillations which are absent from the record of a twitch: thus, for example, in fig. 4 there is a series of three

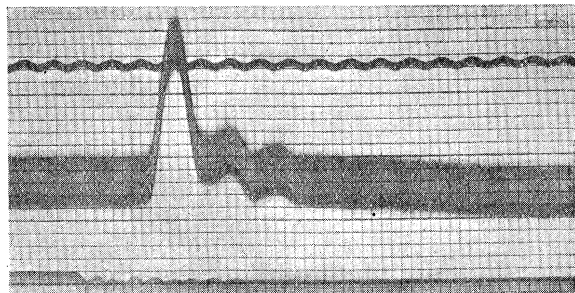


FIG. 2.

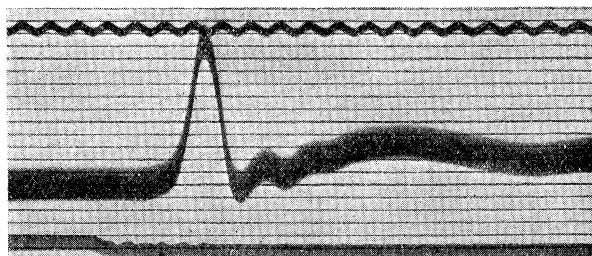


FIG. 3.

FIGS. 2 and 3.—Electrical variations evoked reflexly from the muscles of the hind-limb of the spinal frog by mechanical stimulation (hammer tap) of the same-side toes. The wicks of non-polarisable electrodes are applied over the skin at knee and ankle. The upper line is the shadow of a tuning-fork vibrating at the rate of 200 D.V. per second. The middle line is the shadow of the string of the galvanometer. The lower line is the signal, giving the moment of tapping the toes. The curves are to be read from left to right. All are somewhat reduced in size.

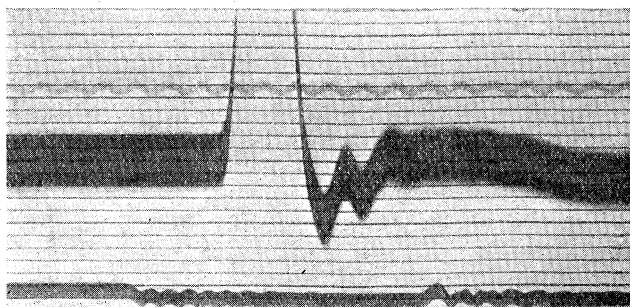


FIG. 4.—Electrical variation evoked reflexly from the muscles of the hind-limb of the spinal frog by tapping the heteronymous toes. Time marker and signal as in figs. 2 and 3.

small oscillations occurring at a rate of about 216 per second. This record was made at a temperature of 18° C. Some curves show a double summit, as though one twitch were followed closely by another. These resemble some of the curves figured by Miss Buchanan (1). Sometimes the records are complicated in such a way as to suggest that the reflex is composed of a series of twitches. Occasionally, there is visible in the records of the homonymous effect a small initial deflection, following the stimulus after an interval of a few σ . It was at first thought that this might be some fortuitous effect upon the string due to the mechanism of stimulation, which involves the release of an electromagnet, but precautions do not prevent its occasional appearance, and it must be attributed to some form of direct stimulation of the foot by the hammer tap. Its very short latency is consistent with this.

The twitch-like nature of the response is what one would expect from the jerky movement of the muscles in the spinal preparation. The succeeding series of small rapid oscillations indicates that there is also an element of tonic contraction involved.

The Decerebrate Frog.

The cerebral hemispheres were removed aseptically and the wounds allowed to heal. The frogs were preserved until they died, usually several months, and the reflex times were tested at intervals. The wicks of the non-polarisable electrodes were applied over the skin, either at thigh and ankle or knee and ankle. The reflex movement elicited by stimulating the toes in the decerebrate animal has not the twitch-like character which it assumes in the spinal frog. The reflexes are not obtained with the same certainty as in the spinal frog. The heteronymous reflex sometimes fails even when the stimulus used is the nocuous form of a strong squeeze. In other cases the reflex is delayed very considerably. Under these circumstances, it seemed desirable to make a number of alternated observations of crossed and same-side reflex activity at intervals of a day or two, and to average the results, in order to obtain a measure of the time difference between the commencement of the two reflexes, rather than to rely upon the differences found in individual pairs of responses.

With regard to the delayed heteronymous responses, such times as the following have been recorded: 43.1 σ at 22°, thirty-eight days after decerebration; 45.4 σ at 26°, twenty-nine days after decerebration; and 55.2 σ at 19.6°, ninety days after decerebration. Such long delays have not been found in spinal preparations, but in the intact frog I have found periods of 52 σ , 57.5 σ , and 59 σ , and also some of a still greater duration, viz., 82.5 σ ,

and 92.5 σ . Waller (5) records delays for the crossed reflex in *Rana* as long as 228 σ .

The absence of prolonged periods of delay in the spinal frog, and their presence in the decerebrate and intact animals, suggest that in these cases

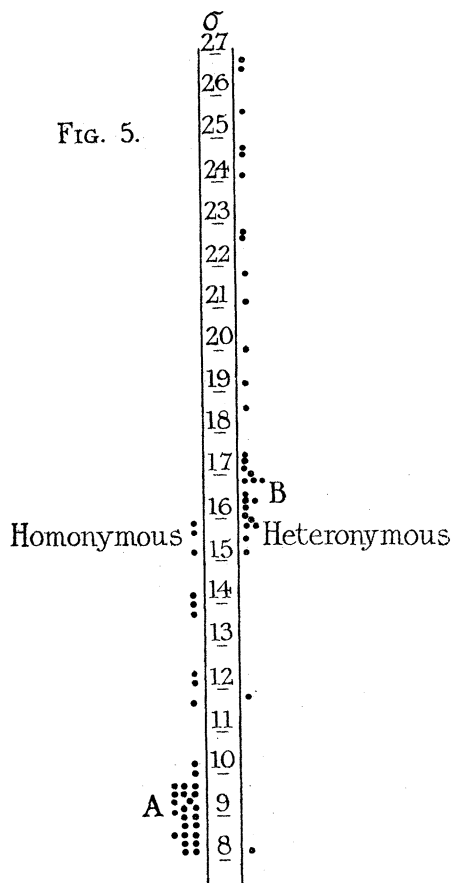


FIG. 5.—Diagram giving the reflex times obtained from the hind-limb of the decerebrate frog on homonymous and heteronymous stimulation of the toes. The times are plotted in thousandths of a second (σ). The diagram shows two groups of reflex times, a group, A, of homonymous reflexes averaging 9 σ and a group, B, of heteronymous reflexes averaging 16.4 σ .

the simple spinal reflex has failed, and that we are dealing with responses which involve the lower part of the encephalon. In favour of this view is the fact that in some records we find, in addition to the deflection which obviously represents the spinal crossed reflex, a second deflection, appearing much later in the curve, which seems to be due to a second outflow of impulses from the centres. Thus, in frog E, fifty-two days after decerebration, we have at 23° a deflection occurring 15.8 σ after stimulation, and a second 44 σ after stimulation, and in the same frog, eighty days after operation at 21.5°, a deflection at 15.3 σ and a later one at 41.2 σ . If this view is correct, then these delayed responses should be placed in a class by themselves, and not averaged along with the other responses.

The following are the average times in thousandths of a second of the heteronymous reflex which have been obtained from decerebrate frogs by the use of the nocuous stimulus at an average room temperature of 21.6° C.: frog A, 19.4 σ ; frog B, 15.5 σ ; frog C, 22 σ ; frog D, 16.8 σ ; frog E, 17.1 σ ; frog F, 16.2 σ ; and frog G, 17 σ . The individual times

are arranged in diagram (fig. 5) in order of magnitude.

The average of all these crossed reflex times—thirty-five in number—is 18.5 σ . Two of them, both recorded from frog E, are exceptional. In the

one case, the deflection appears 8.3σ after stimulation; in the other case, the onset of the deflection occurs 11.8σ after stimulation, and is followed by a large second deflection 4σ later, *i.e.*, 15.8σ after the stimulus. This time— 15.8σ —agrees with other crossed reflex times from the same and other decerebrate frogs, but 11.8σ is exceptionally short for a crossed reflex, and agrees rather with the homonymous reflex times. There have been a number of other cases recorded where the commencement of the deflection shows a double character, a second deflection following the first by about 4σ . Caution must be observed in the interpretation of such curves recorded by means of electrodes on the skin, where one has not point leads on individual muscles. It is easy to understand how complicated curves can be obtained from a single muscle when an electrode is not applied exactly to the point where the motor nerve enters the muscle. In the cases mentioned, however, the form of the curve, taken together with the unusually short delay, rather suggests that, for some reason, the impulse, in passing from one side of the cord to the other, has here taken a short path which is not usually open in the decerebrate frog, and that another impulse, traversing a path longer by the addition of one synapse, has later caused a second discharge from the motor neurones. It may be that the short path used here in the decerebrate preparation, as a rare exception, may be the same path as is usually followed in the spinal preparation, where, as we have seen, the heteronymous delays are, with frogs in good condition, similar to the homonymous.

If we attempt to analyse the average delay of the heteronymous reflex in the decerebrate frog, assuming the delay at a synapse to be the same as in the spinal frog, the following may be suggested:—

Average delay of heteronymous reflex (35 records)	18.5σ
Conduction in nerve (16 cm. at 35.5 metres per sec., 21.6° C.) ...	4.5
Muscle latency	2.4
Latency of sensory endings.....	0.5
Delay at three synapses (3.7σ each)	11.1
	<hr/>
	18.5σ

It is doubtful whether any allowance need be made for the latency of sensory endings. With the strong squeeze it is probable that the afferent nerve fibres may themselves be stimulated.

Homonymous Responses in the Decerebrate Frog.

The times of the homonymous responses determined in the decerebrate frog are arranged in order of magnitude in diagram (fig. 5). The average

delay of all the responses, thirty-three in number, is 10.7σ . The average temperature is 21°C .

It will be seen that a number of records have been obtained of what appear to be reflex effects from the same-side limb, which have a very short delay. It is evident from diagram (fig. 5) that there is a group of same-side responses averaging 9σ , and a group of crossed responses averaging 16.4σ . If these homonymous responses are in reality reflex then the time difference between homonymous and heteronymous reflexes is 7.4σ . This is just double the figure which we have found in the spinal frog to represent the delay at one synapse, and we would conclude from this that the path of the impulse across the spinal cord involves two synapses more than the homonymous path, or three synapses in all, with a latency of 3.7σ at each synapse.

As regards the brief delay, it is certainly surprising to find such short reflex times as 9σ . At first one was inclined to think that they must be due to direct stimulation as opposed to reflex action, and the strong squeeze is undoubtedly capable of causing direct stimulation at times, but the latency with direct stimulation is much shorter than 9σ . The following experiments show clearly the difference in latency between the muscular response to stimulation of a motor nerve in these decerebrate frogs and the group of responses which we are considering. The sciatic nerve was isolated at the back of the thigh, without being cut, and small vulcanite rods were placed above and below the nerve. The nerve was then mechanically stimulated by a light hammer tap on the upper rod, the signal circuit being closed at the same moment. The wicks of the non-polarisable electrodes were attached round uninjured skin, one below the knee, the other at the ankle. The latency of the muscular activity at 20°C . was found to be 3.2σ , after deducting 0.7σ for nerve conduction at the rate of 34 metres per second through 2.3 cm. of nerve intervening between the point stimulated and the gastrocnemius muscle. In another decerebrate frog, twenty-one days after operation, the latent period of the muscle was found by the same method to be 4σ at 17°C . With electrical stimulation of the nerve by an induction shock the latent period has been found to be as short as 2.40σ at 22°C .

Since it is found in this way that the latent period of the muscles of the decerebrate frog is so short, it is difficult to entertain the view that delays of over 8σ , obtained by squeezing the toes in lively decerebrate preparations in good condition, with the circulation intact and no operative interference at the time of experiment, can be other than reflex effects. Under the conditions of experiment one would not expect the muscle latency to be lengthened to such an extent, and with the data at present available we must regard this delay of 9σ at 21° as a reflex time, which includes the delay at one synapse.

It must be borne in view that the decerebrate preparation suspended as in the experiments is not very quiescent. Alternating with periods of quiescence are periods of violent "struggling" movements. Presumably afferent impulses initiated by the stretching are being received by the centres from the sensory receptors of the limbs which are hanging extended under the influence of gravitation, and these impulses cause the motor cells to discharge at intervals. There will thus be periods of gradual increase in the excitability of the motor centres ending in discharge. Perhaps the shortest times recorded are due to mechanical stimuli which chance to be imparted at a time when the motor centres are nearing their maximal excitability, and are to be regarded as examples of what may be termed "hair-trigger" action.

The Reflex Nexus between Forelimb and Hindlimb.

Some experiments were performed in which the reflex activity of the hind-limb was recorded in response to a mechanical stimulus applied in the one case to the hand of the same side, and in the other to the hand of the opposite side. The electrodes were applied at knee and ankle and the hand was subjected to the strong squeeze. On stimulation of the hand of the same side the following delays were obtained from three decerebrate frogs at room temperature of 22° C.:—(1) 16.4 σ , sixty-eight days after operation; (2) 16.9 σ , seventy-six days after operation; (3) 16.7 σ , thirty-eight days after operation; (4) 23.9 σ , thirty-eight days after operation; and (5) 11.8 σ , forty-eight days after operation, giving an average delay of 17.1 σ . On stimulating the hand of the opposite side the following delays were recorded from two frogs at room temperature of 22° C.:—(1) 17.5 σ , forty-eight days after operation and (2) 15 σ , thirty-eight days after operation.

From these figures one cannot assume a difference in time between the cases where impulse descends on the same side of the cord from the hand stimulate, and where it has to cross in descending to the opposite side. Further, the times recorded agree well with those obtained from a hind-limb on stimulating the opposite foot. The length of path to be ascribed to nerve conduction from hand to spinal centre is found on dissection to be shorter than that from foot to spinal centre by about 5 cm., so that we deduct 1.4 σ from the average time of the latter reflex in order to compare with the former, and obtain the same figure.

We conclude that the reflex path from fore-limb to hind-limb, either homonymous or heteronymous, contains the same number of synapses as the path from foot to opposite hind-limb, viz.: three synapses with a delay at each of about 3.7 σ .

We may compare this path with that for the scratch reflex in the spinal

mammal as given by Sherrington (6). This author regards the reflex path when the skin of the shoulder is stimulated as consisting of three neurones, and as entering the grey matter twice, that is to say, it is a disynaptic arc. But Sherrington states that this does not mean that there are necessarily only two synapses, but that there may be other synapses due to the interposition of one or more short intraspinal neurones. The diagram given by this author would be consistent with the results here obtained for the flexion reflex in the frog on stimulation of the hand, if we assume that a short intraspinal neurone occurs between the long descending propriospinal neurone and the motor neurone, and that this intraspinal neurone connects not only with the motor neurone on one side of the cord but, by a branch, with that of the other side also. The chain would thus consist of four neurones with three synapses.

A number of delayed responses were obtained from the hind-limb on hand stimulation, similar in time to those recorded from stimulation of the foot. Thus homonymous hand stimulation gave a delay of 42.5σ , and heteronymous stimulation delays of 57.5σ , 70σ , and 72σ .

In one experiment where the stimulation was given to the homonymous hand a reflex from the leg was recorded 16.9σ after stimulation and another deflection followed 58σ after stimulation. Experiments such as these suggest that the delayed reflexes involve higher centres than those of the spinal cord, and that the path through these centres in the lower part of the encephalon involves a large number of synapses. In a double response the segmental spinal centre has discharged with its brief delay, while the afferent impulse has also ascended to the brain and has led to a second reflex with a long delay. If we assume the same delay at a synapse in the encephalon as we have seen reason to believe affects the spinal synapse, then in the experiment last mentioned the long path through the brain would contain ten or eleven more synapses than the spinal path.

We have assumed that the heteronymous hind-limb path, and also the fore-limb to hind-limb path, contain three synapses, and we have been led to this conclusion by using for comparison the reflex in the spinal frog with its single synapse and the group of decerebrate responses marked Group A on diagram (fig. 5), which we assume to be reflex in nature and to involve one synapse. The possibility, however, is not excluded that homonymous reflexes in the decerebrate preparation may, in response to some forms of stimulation, employ a path involving two synapses. It must be borne in mind that the stimulus used to evoke reflexes in the decerebrate frog has been the powerful squeeze. This is desirable in the case of the crossed reflex, because it requires a strong mechanical stimulus to elicit the crossed reflex with fair regularity, but the homonymous reflex is more easily obtained. The light

hammer-tap or innocuous stimulus is sufficient to produce it, and there is some evidence that the central delay is then longer than with the stronger form of stimulus. My observations have not yet extended far in this direction, as attention was concentrated in the case of the decerebrate frog on the effort to obtain crossed reflexes with constancy, and to compare their time with the homonymous reflexes elicited by the same form of stimulation. This necessitates a strong squeeze. It would be advisable in future work to compare the crossed reflex, evoked as here described, with the same-side response to the innocuous stimulus. Even with the data before us, it may be possible to recognise a form of homonymous reflex whose path involves two synapses. If we disregard Group A in diagram (fig. 5) altogether for the present, and average the remainder of the homonymous reflexes, we have 14.4σ for the homonymous reflex as against 18.5σ for the average of all the heteronymous reflexes, and 14.4σ on analysis suggests delay at two synapses, and the extra delay of the crossed reflex would then be attributable to the interposition of one additional synapse. This would accord with previous work on *Rana*. Thus Wundt (2) finds the extra time of the crossed reflex to be 4σ , and Miss Buchanan (1) assumes that one extra synapse is interposed in the path of the crossed reflex, whose delay is normally between 10σ and 20σ , but may be reduced under the influence of strychnine to 4σ . This author, however, assumes that there is only one synapse in the path of the same-side reflex with a delay of between 10σ and 20σ . We may compare the delay at two synapses in the clawed frog, say 7.4σ , with the synapse delay in the mammalian homonymous reflex, which has been shown to be 4.3σ (3), or, according to Forbes and Gregg (7), even less. These authors find the reduced reflex time in the flexion reflex of the cat to lie in general between 3σ and 5σ .

Form of the Response in the Decerebrate Frog.

Waves of different rates are observed in the electrical responses recorded. Sometimes the record shows the presence of waves occurring before the stimulus has been given, and these are presumably the electrical expression of the tonic activity of the limb muscles, and when they are very distinct indicate a condition of heightened tonus. Fig. 6 is an example. Here the waves occur at the rate of 54 per second, at a room temperature of 19°C . Other rates recorded are 35 at 16° , 54 and 55.5 at 21.3° , 49 at 22° , and 53 at 25° . These waves are of rather a higher rate than those described by Piper (8) in *Rana*, doubtless owing, to some extent, to the higher summer temperatures in South Africa. Piper states that on throwing the quadriceps femoris of the frog reflexly into contraction, one obtains an inconstant rhythm,

but at summer temperatures the rhythm is one of at least 30 per second. This author considers that these waves are of central origin.

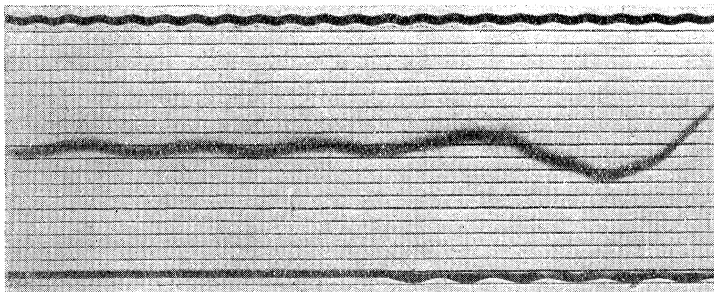


FIG. 6.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the homonymous toes. The curve shows waves occurring before stimulation at a rate of 54 per second. Room temperature, 19° C. Time marker and signal as in figs. 2 and 3.

The reflex responses to stimulation exhibit, in addition to waves of similar rate to the foregoing, other waves of greater rate, which occur especially at the commencement of the electrical charge. Fig. 7 is an example of this. The initial waves have here a rate of 206 per second, at a room temperature of 20°. Other rates observed are 277 at 21·8°, 145 at 23°, 137 at 16°, and 128 at 21°. We are reminded of the two varieties of oscillations described by Miss Buchanan (9) in strychnine tetanus as waves and wavelets, but the rapid initial oscillations shown here are of much greater rate than these wavelets, which occur at a rate of 40 to 100 per second, and which Miss Buchanan regards as of peripheral muscular origin. A muscular rhythm so high as 277 per second has, so far as I know, never been suggested, even for the muscles of warm-blooded animals, and we must look for some explanation other than peripheral muscular rhythm. The wicks of the electrodes are placed in these experiments round the thigh and knee, so that the action currents of various muscles which take part in the flexion reflex can show themselves in the electrical response.

I have been struck, in studying the oscillations of the higher rate which appear in the reflex response of the decerebrate frog, with the similarity of the intervals between the deflections and the period which we have seen reason to believe represents the delay at a synapse. Thus, in Experiment fig. 7, the intervals between the rapid deflections at the beginning of the curve are $4\cdot3\sigma$ and 4σ . In another experiment the interval is $3\cdot6\sigma$, while the average of the periods in the other cases above mentioned is $7\cdot3\sigma$, the double of the delay at a synapse. If this is more than a coincidence, it

would suggest that these initial deflections of rapid rate are central in origin, and either that they represent the muscular responses to successive discharges

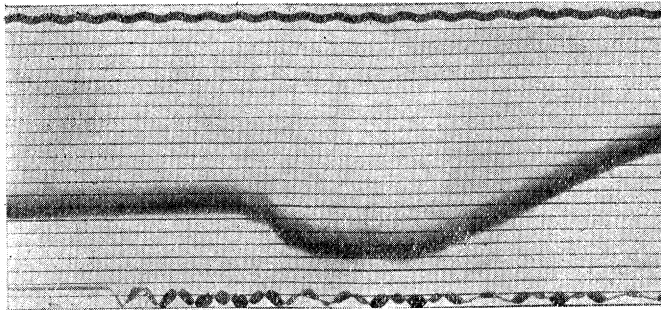


FIG. 7.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the heteronymous toes. The response, at its commencement, shows waves at a rate of 206 per second. Room temperature, 20° C. Time marker and signal as in figs. 2 and 3.

from motor neurones, or that one group after another of motor neurones has been thrown into activity, the delay between the excitations of the groups indicating that one synapse or two synapses respectively have to be traversed in the course of this irradiation. As Sherrington (6) points out, grading of intensity of reflex contraction within one and the same muscle group, and even within one and the same individual muscle, seems not only possible but probable, by the numerical implication of more or fewer motor cells, and it seems not improbable that the rapid succession of deflections described above is evidence of the successive implication of neurones in the spinal centres.

Conclusions.

The reflex times of the homonymous and heteronymous reflexes in the hind limbs of the spinal clawed frog have been measured at temperatures ranging from 14° C. to 30° C. The average heteronymous times (sixty-six observations) is 18.7σ (0.0187 second). The average homonymous (sixty-eight observations) is 14.9σ . That is to say, the crossed reflex time is longer than the same-side reflex time by 3.8σ .

It is suggested that the experiments can be divided into two classes, the chief determining factor being the general condition or "fitness" of the spinal preparation. In the first class, where the preparation is normal and the temperature fairly high, the average heteronymous time is 15.7σ and the average homonymous 14.3σ . Since the difference between the crossed and same-side reflex times is here very brief, viz., 1.4σ , and as in some

experiments the crossed reflex time is the shorter, it is concluded that the intraspinal paths of the two reflexes are similarly constituted as regards number of synapses, that is to say, each path normally involves one synapse.

In the second class, where the frog is not in the best condition, either on account of a recent operation or from long survival since operation, the short path is not open for the crossed reflex, and a longer path, containing two additional synapses, or three synapses in all, is employed. The average heteronymous time is here 23.7σ , and the average homonymous 15.7σ , giving an excess delay for the crossed reflex of 8σ .

The delay at a synapse in the spinal cord is found to be about 3.7σ . This is considered to be referable to a single synapse, but it is not intended to exclude the possibility that it represents the delay at a set of synapses.

The electrical variation recorded from the limb muscles is similar to that of a twitch followed by a series of rapid oscillations.

In the decerebrate and in the intact frog, a group of delayed crossed responses from the hind limb are described, having delays ranging from 43σ to 92σ . It is suggested that these involve the action of the lower parts of the brain.

Spinal reflexes are recorded from the decerebrate frog, the average time of the heteronymous reflex being 18.5σ , and of the homonymous 10.7σ , giving an excess delay for the crossed reflex of 7.8σ . There is evidence that in some same-side reflexes the delay is longer than the average mentioned, being 14.4σ , and the excess delay of the crossed reflex is then about 4σ .

It is considered that, normally, with a strong mechanical stimulus, the intraspinal path of the homonymous reflex in the decerebrate frog involves one synapse (although in some cases it may involve two), while the heteronymous path contains three synapses.

The path followed in the reflex activity of the hind limb, evoked by stimulation of the fore limb, both homonymous and heteronymous, contains three synapses.

The electrical variation recorded from the hind limb muscles of the decerebrate frog shows a series of oscillations at its commencement, at rates reaching 277 per second. It is suggested that these are the expression of irradiation within the spinal centres.

I have to express my indebtedness to Dr. C. Lawrence Herman for his kindness in preparing a considerable number of frogs by decerebration and cord section for use in the investigation, and to my students, Mr. C. A. Oosthuizen and Mr. H. Zwarenstein, for assistance from time to time in the experiments.

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Studies of Photo-synthesis in Marine Algæ.—1. Fixation of Carbon and Nitrogen from Inorganic Sources in Sea Water.
2. Increase of Alkalinity of Sea Water as a Measure of Photo-synthesis.

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The series of experiments recorded in this communication were carried out at Port Erin; the subsequent analyses for amounts of nitrogen fixed were made at the temporary laboratory of the Department of Applied Physiology, M.R.C., at the Lister Institute.

The results of the series confirm and amplify those obtained with fresh-water algæ,* which showed a convincing uptake of nitrogen from the air, but on account of the change of the medium of growth from fresh to sea water, there are several important modifications in the medium itself as well as in the growing algæ, which appear to us to possess considerable importance in the annual life of the sea, and in the inductance at certain definite periods of the year of increased processes of cell-division and reproduction of species, and possibly in guiding the development of variations in species, and the

* Moore and Webster, 'Roy. Soc. Proc.,' B, vol. 91, p. 201 (1920).

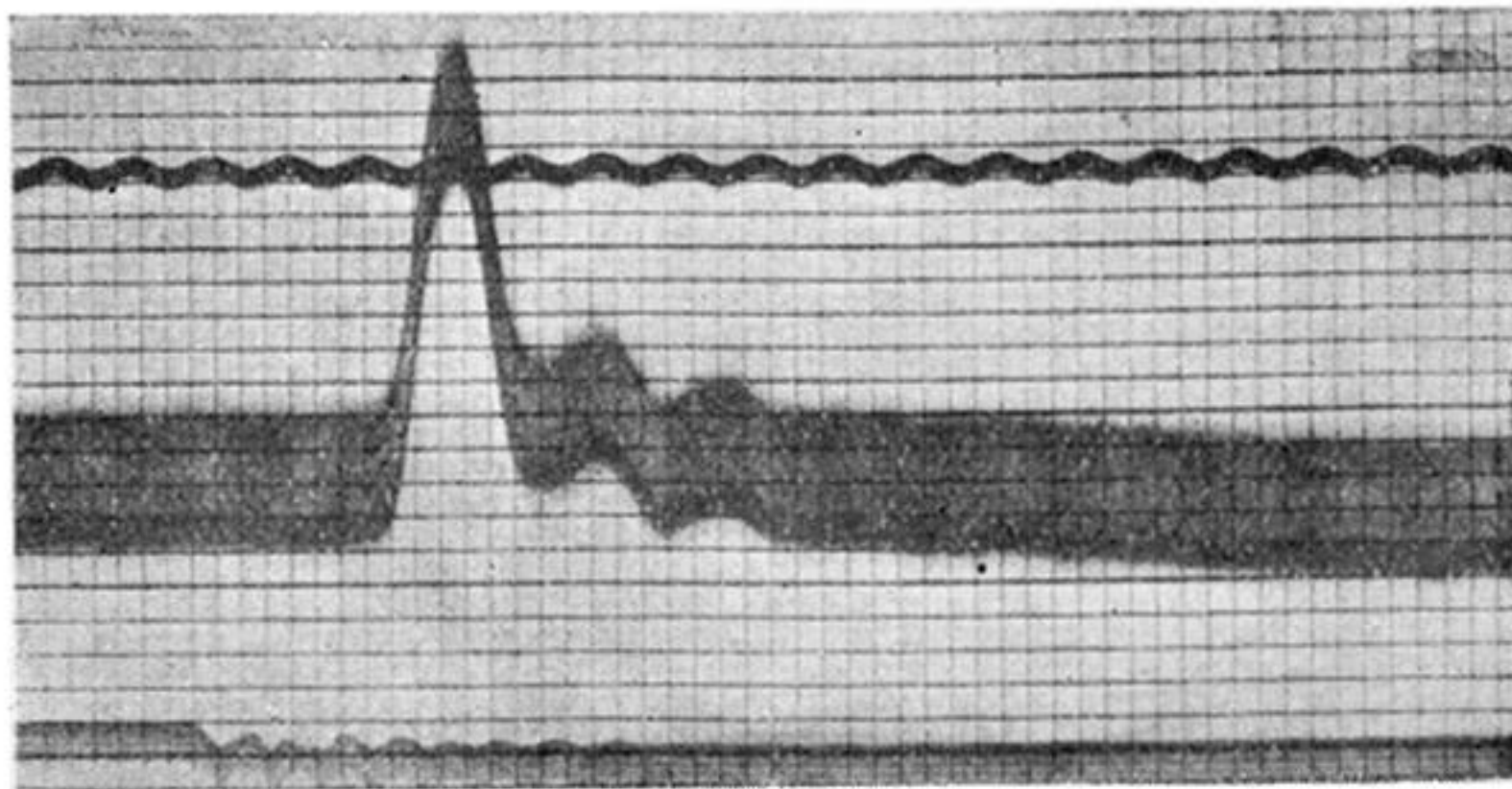


FIG. 2.

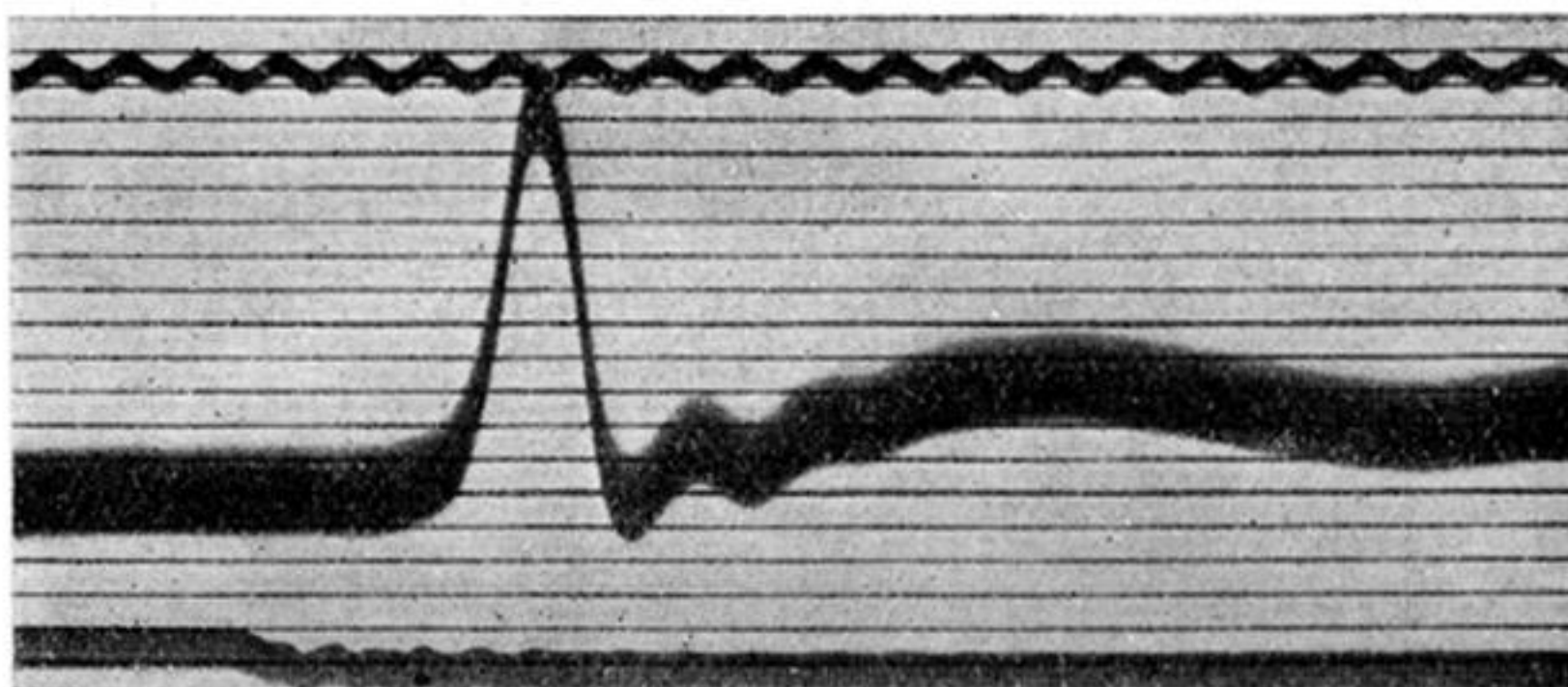


FIG. 3.

FIGS. 2 and 3.—Electrical variations evoked reflexly from the muscles of the hind-limb of the spinal frog by mechanical stimulation (hammer tap) of the same-side toes. The wicks of non-polarisable electrodes are applied over the skin at knee and ankle. The upper line is the shadow of a tuning-fork vibrating at the rate of 200 D.V. per second. The middle line is the shadow of the string of the galvanometer. The lower line is the signal, giving the moment of tapping the toes. The curves are to be read from left to right. All are somewhat reduced in size.

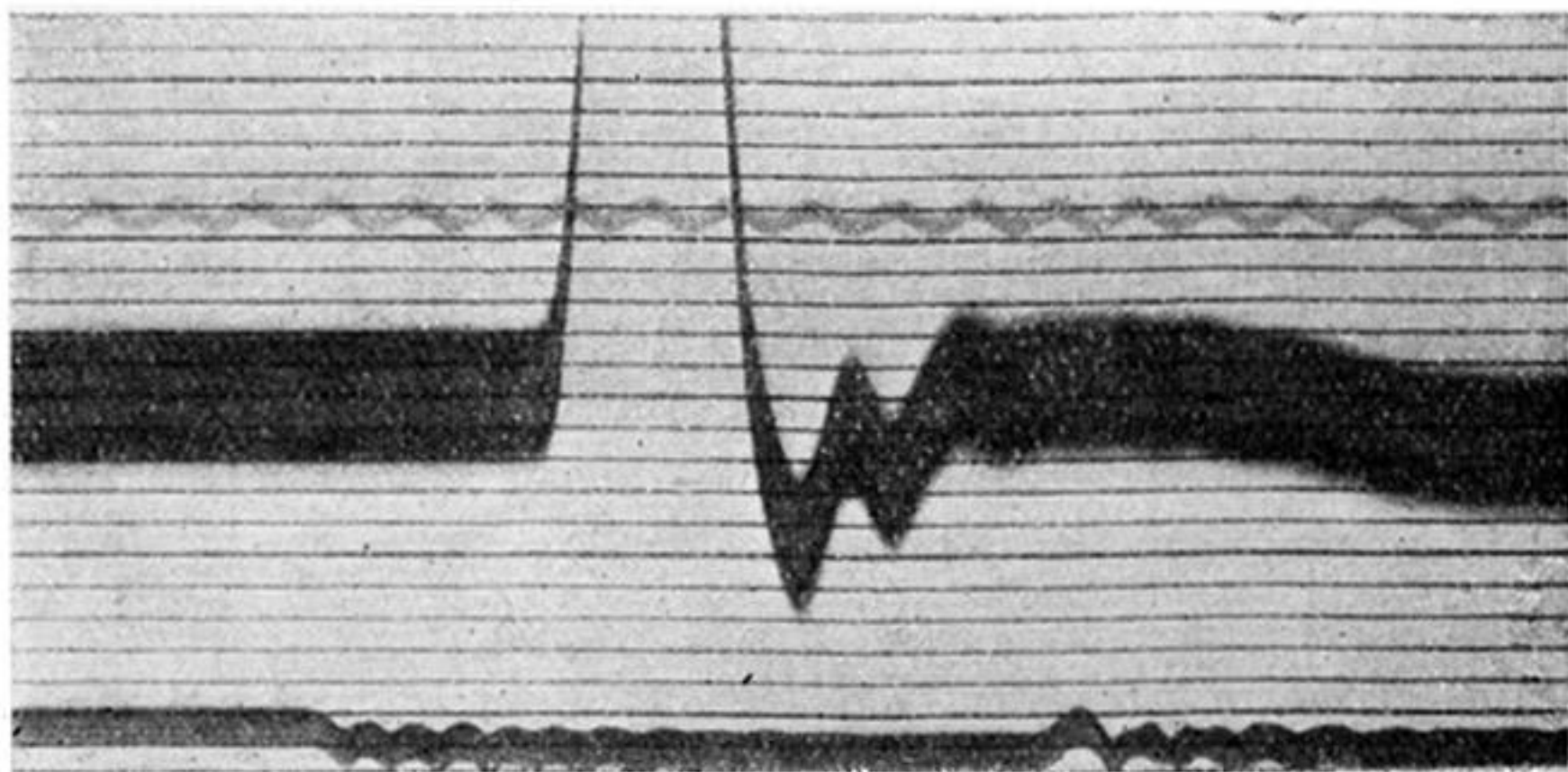


FIG. 4.—Electrical variation evoked reflexly from the muscles of the hind-limb of the spinal frog by tapping the heteronymous toes. Time marker and signal as in figs. 2 and 3.

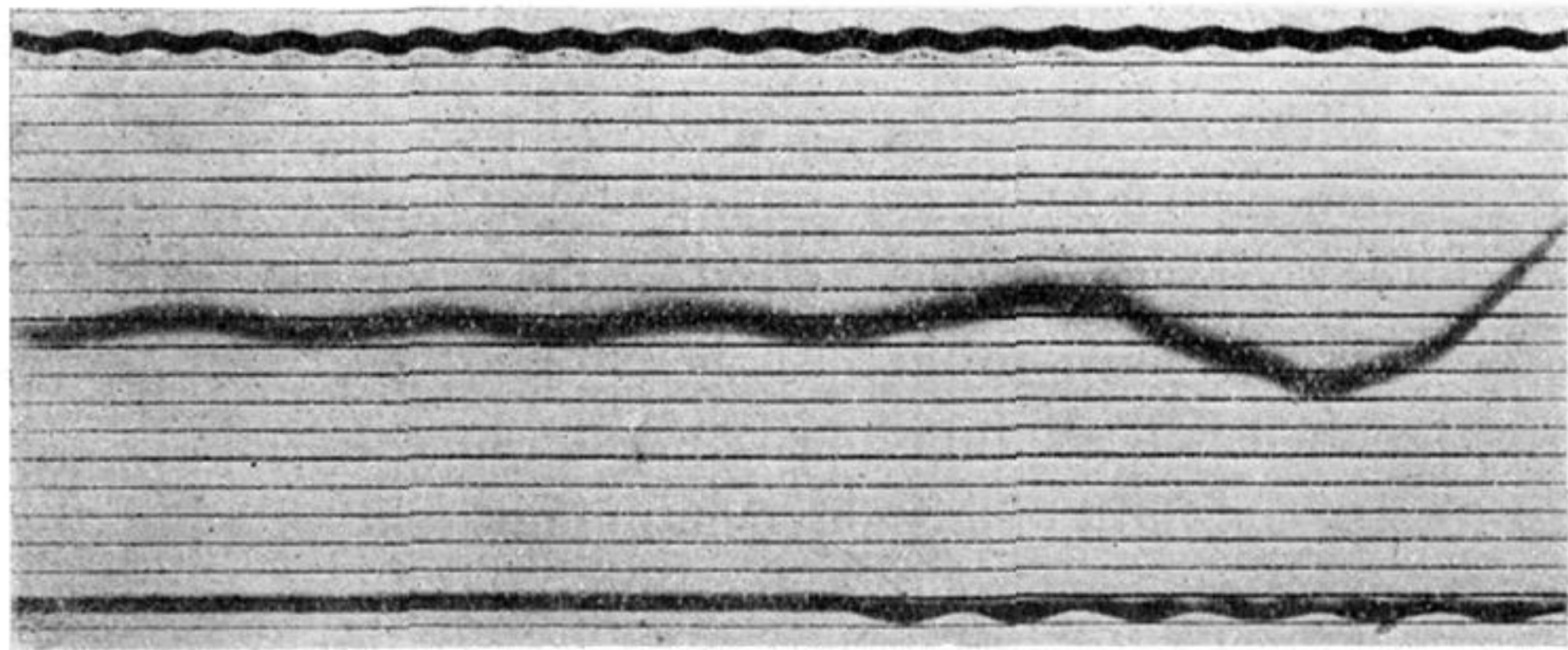


FIG. 6.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the homonymous toes. The curve shows waves occurring before stimulation at a rate of 54 per second. Room temperature. 19° C. Time marker and signal as in figs. 2 and 3.

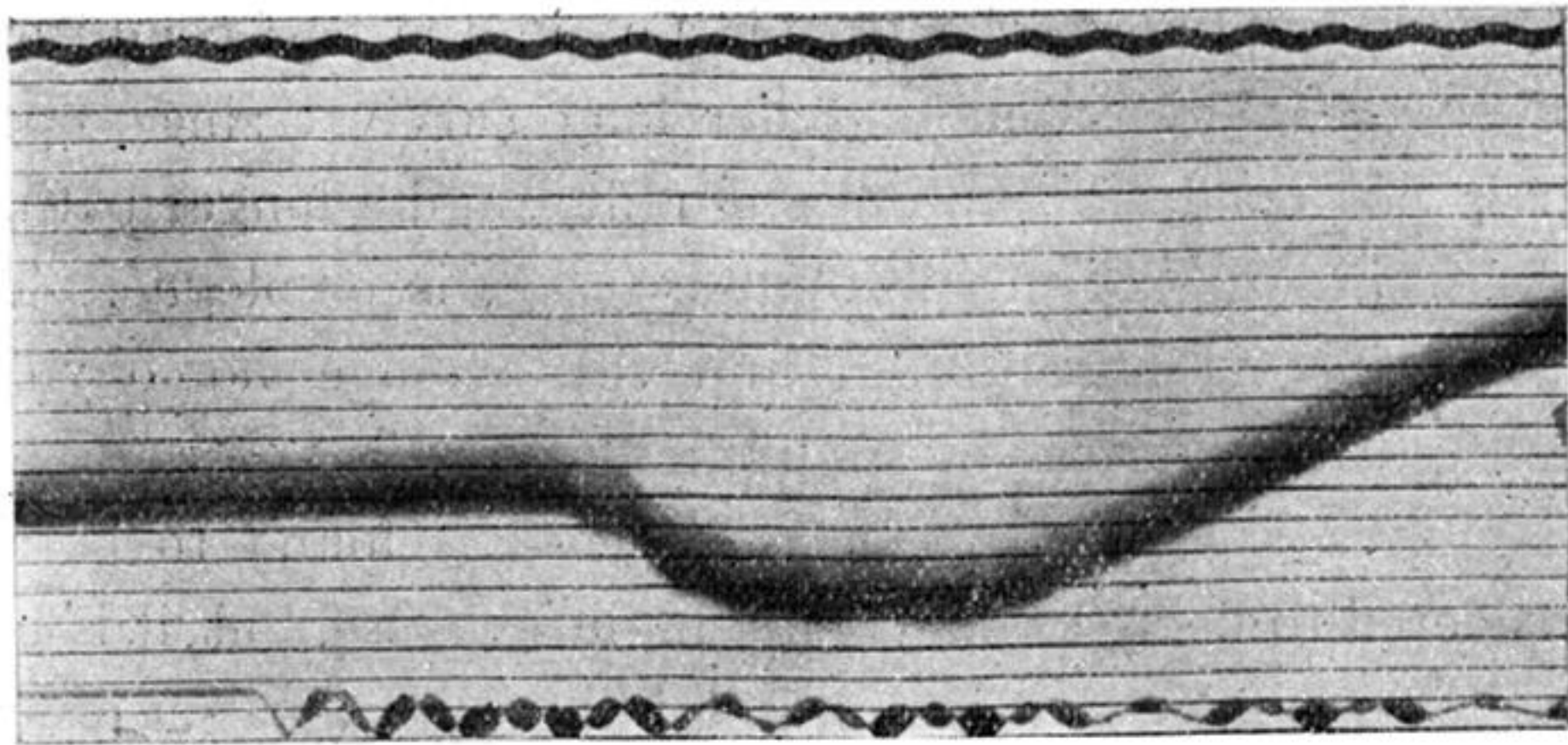


FIG. 7.—Electrical variation evoked reflexly from the muscles of the hind-limb of the decerebrate frog by mechanical stimulation of the heteronymous toes. The response, at its commencement, shows waves at a rate of 206 per second. Room temperature, 20° C. Time marker and signal as in figs. 2 and 3.