An Automatic Method for the Investigation of Velocity of Transmission of Excitation in Mimosa

J. C. Bose

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III. *An Automatic Method for the Investigation of Velocity of Transmission of Excitation in* Mimosa.

By Prof. J. C. BOSE, *M.A., D.Sc., C.S.I., Presidency College, Calcutta.*

Communicated by Prof. S. H. VINES, *F.R.S.*

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INTRODUCTORY.

The question whether the transmitted effect in *Mimosa* is due to a hydro-mechanical or excitatory impulse is held to be of much interest in Plant Physiology. I shall therefore deal with the subject in some detail and adduce results of additional investigations that I have carried out recently. The general belief that the transmitted impulse in the plant is hydro-mechanical has been largely based on two well-known experiments of PFEFFER[*] and HABERLANDT.[†] In the former of these the effect of strong stimulus was found to travel over chloroformed parts of the stem. PFEFFER assumed that the conductivity of this portion must have been abolished, since chloroform is known to abolish motile excitability. In the experiment of HABERLANDT, an intervening tissue was killed by scalding; in spite of this, stimulus was found to be transmitted across the scalded area.

From these two experiments it was inferred that the impulse which was transmitted could not have been of a true excitatory nature. It was held, on the contrary, that the strong stimulus had given rise to a variation, whether of increase or diminution, of hydrostatic pressure. This variation of pressure, it was assumed, had been hydro-mechanically transmitted, and, on reaching the distant pulvinus, had inflicted on it a

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[*] "Ueb. Fortpflanzung des Reizes bei Mimosa," 'Prings. Jahrb.,' 1873–4, vol. 9, p. 308. Also 'Pflanzenphysiologie,' 1904, vol. 2, p. 473.

[†] 'Reizleitendes Gewebesystem der Sinnpflanze,' 1890. (See also MACDOUGAL, 'Bot. Gaz.,' 1896.)

[A comprehensive survey of the whole subject is FITTING's "Reizleitungsvorgänge bei den Pflanzen," p. 124, in 'Ergebnisse der Physiologie,' 1907.]

blow which had proved as effective as if a mechanical stimulus had been applied locally. It is thus held that in *Mimosa* there is a *mere transmission of stimulus but no transmission of excitation.*

As regards PFEFFER's experiment it was assumed that the conducting power was arrested under chloroform. It has, however, been pointed out by VINES* that, though a narcotised pulvinus certainly loses its motile excitability, it does not necessarily follow that its conductivity likewise is completely abolished. Instances are known, in fact, to physiologists in which a tissue whose excitability has been abolished will still persist in maintaining its conducting power. This fact may be demonstrated in the case of plants by taking a specimen of *Biophytum* and applying a strong stimulus to an old leaf, the motility of whose leaflets has been abolished on account of age. Though its own leaflets do not afford any motile indication, the excitation is found to be *conducted* through the petiole of the old leaf so as to induce the fall of the leaflets in a neighbouring young leaf.

It is again extremely doubtful whether, in the particular experiment with *Mimosa*, the conducting tissue in the interior could have been effectively narcotised by the external application of the anæsthetic. The task would almost be as difficult as narcotising a nerve-trunk lying between muscles, by the application of chloroform on the skin outside. In the case of a plant it is conceivable that, after a very long application, a small quantity of narcotic may, by absorption, get access to the internal conducting tissue; but narcotisation under these circumstances can only be partial. In such a case, the transmitted effect of a feeble or a moderate stimulus will alone be arrested; but the block will fail to arrest the transmitted effect of intense stimulation. These considerations will probably explain PFEFFER's observation that, while the effect of strong injury-stimulus was always transmitted across the narcotised area, a moderate mechanical stimulus was but occasionally transmitted.

In HABERLANDT's experiment the conducting tissue was supposed to have been killed by scalding. Assuming this to have been the case, it might be supposed that under an exceptionally strong stimulus a hydrostatic disturbance had been transmitted through the dead tissue and caused stimulation of the distant leaf, as a mechanical blow, *de novo*. But excitatory transmission in a plant is usually accomplished by a stimulus which is feeble. Grave doubt may again be entertained as to whether the tissue had really been killed. In my own experience, I find it extremely difficult to be sure of killing the interior of a tissue by scalding the outside. This derives additional support from certain experiments of KÜHNE on conduction of excitation in a nerve, the specimen employed being the sartorius of a frog.

"The delicate nerve, which enters the middle of the sartorius by one side, divides within the muscle, so that the single fibres that constitute the bifurcation branch many times dichotomously. When KÜHNE threw the broad upper end of the muscle into heat rigor by dipping it into warm oil, the half which remained normal twitched

* 'Lectures on Physiology of Plants,' 1886, p. 585.

on cutting the rigored portion with scissors, showing that excitable nerve fibre could still be mechanically excited between the rigored and dead muscle fibres, and thus carrying the excitation centripetally into branches which divide above the rigored portion of the muscle."*

In this experiment we have an instance of transmission of excitation through heat-rigored animal tissue, parallel to HABERLANDT's experiment on transmission through scalded plant tissue. In both these cases it is evident that the scalded tissues, though under heat rigor, were not really killed; and that any induced block or abolition of conductivity (caused by heat rigor, electrotonus, and so on) is, after all, relative. There may thus be an effective physiological block for normal intensities of stimulation, which would, however, fail under abnormal intensities of stimulus such as that of a burn or of a cut. In KÜHNE's experiment, the intense excitation of scissors-cut failed to be arrested, though the conductivity of the nerve had been depressed under heat rigor. Similar considerations will explain how the intense excitation caused by a burn or a cut may be transmitted through the narcotised or scalded areas in *Mimosa*.

The experiment of KÜHNE shows, further, that conductivity may persist even after the abolition of motile excitability. The rigored muscle is seen to have lost its motility, though the embedded nerve retained a certain amount of conductivity for the excessively strong stimulus of a cut.

In turning our attention to KÜHNE's experiment, we realise the error involved in ignoring the factor of intensity of stimulus in the matter of the effectiveness of a given block to the transmission of excitation. The necessity of discarding crude and drastic methods of stimulation in researches on variation of conductivity will now have become obvious. The object of our inquiry is not to find whether a mechanical disturbance caused by some violent blow is transmitted to a distance, but the determination of propagation of physiological change under normal modes of stimulation. By employing stimulus of graduated intensity it should be easy to determine the character of a given impulse by observing the effects of various physiological depressors in modifying the power of conduction.

In order to bring the question—whether in a plant there is true transmission of excitation, or mere passage of a mechanical disturbance—to a satisfactory issue, it is clear that we ought to proceed in the following ways. First, we have to inquire whether it is not possible to find modes of excitation for the plant which would be purely physiological. Transmitted effect in such a case would be due to propagation of excitatory protoplasmic change. Second, crucial tests ought to be devised as between the transmission of protoplasmic reaction and of mere mechanical disturbance. Thus, it is obvious that if we subject a specimen to conditions which are known to affect physiological activity, then it would be inevitable that the rate of transmission should be correspondingly affected, if it is a transmission of true

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* BIEDERMANN, 'Electro-physiology,' vol. 2, p. 57 (Macmillan).

excitation. If, on the other hand, it were a question of the transmission of a mere physical disturbance, the physiological variation of the tissue could not have any effect on the rate. And lastly, we could subject the question to the final test of the physiological block, which would arrest an excitatory impulse but could have no effect on the passage of a hydro-mechanical disturbance.

I have, in my work on Plant Response, adduced considerations which appeared to show that there is in plants a transmission of true excitation. The results which were then put forward were more or less qualitative, and based on personal observation. I have since then felt the importance of attacking the problem by new methods of inquiry. The research resolves itself ultimately into quantitative measurements of velocity of transmission, and its changes under definite physiological variations. It is essential then that we should be able to make quantitative determinations of the highest accuracy of the velocity of transmission of the excitatory impulse. And in order to eliminate the errors that might be inherent in personal observations, it is desirable that all the data for this determination should be furnished automatically in records made by the plant itself. Successive records, moreover, should enable us to determine with equal accuracy not only the normal velocity but also its variation under given conditions.

In order to obtain the time relations in the responsive reaction of *Mimosa*, it is thus necessary to devise means for obtaining automatic records. The phytographic records would then, in the case of plants, supply us with all the information that myograms afford us in the case of animal tissues. The experimental difficulties which the plant offers are, however, very great. In the case of muscle-contraction, the pull exerted is considerable, and the friction offered by the recording surface constitutes no essential difficulty, though even here the time relations of the curve are, I have reason to think, rendered somewhat unreliable by this friction. In the case of plants, however, the pull exerted by the motile organ is relatively feeble, and in the movement of the leaflet of *Desmodium*, for instance, a weight so small as four-hundredths of a gramme is enough to arrest the pulsating leaflets. When employing the very lightest lever, the extremely minute friction offered by the smoked glass surface of the recording plate is sufficient in this case to cause complete cessation of the record. Even in the leaf of *Mimosa*, the friction offered is enough to introduce errors into the amplitude and time relations of the curve.

I have recently been able to overcome these difficulties by the successful device of the Resonant Recorder, which measures accurately time-intervals shorter even than one two-hundredth part of a second. This instrument has made it possible to carry out several new lines of investigation which could have not been otherwise accomplished. As regards the experimental determination of the velocity of transmission, the process has been rendered perfectly automatic. It should be remembered that, for the accurate determination of the velocity, it is also necessary to know the exact value of the latent period of the responding pulvinus.

I propose to describe the present research under five principal headings—

I. The Resonant Recorder for obtaining automatic records giving time relations of mechanical response of *Mimosa*.

II. Determination of the *latent period* of *Mimosa*.
   1. Determination under normal conditions.
   2. Effect of intensity of stimulus on the latent period.
   3. Effect of fatigue.
   4. Effect of temperature.

III. Determination of the velocity of transmission of excitation in *Mimosa*.
   1. The direct method.
   2. The differential method.
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IV. Experiments in confirmation of the excitatory character of effect transmitted.
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   2. Paralysis of conductivity and restoration under tetanising electric shock.
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   4. The effect of localised application of poison in inducing block of conduction.

I.—THE RESONANT RECORDER.

In carrying out investigations on the velocity of transmission of excitation we apply a stimulus on the petiole of *Mimosa* at a known distance from the responding pulvinus. We have then to find the time-interval between the incidence of stimulus and the initiation of the responsive movement. In order to secure these data it will be necessary to make a graphic record of the responsive movement of the leaf of *Mimosa*. This is done by means of a writing lever, which, deflected by the pull of the falling leaf, traces on a writing surface, moving at a known rate, the concomitant

curve.   In fig. 1 is given a diagrammatic representation of such a recorder.   An axis, supported on a frictionless jewelled bearing, carries two arms of a horizontal lever,
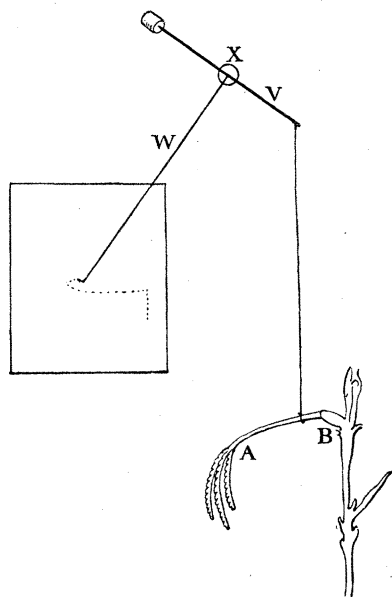

Fig. 1.—Diagrammatic representation of a response recorder.

and a thin vertical wire with a fine bent tip, to serve as the writer.   A point of the petiole of the responding leaf is attached to one arm of the lever, the other having on it a small weight to act as a counterpoise. Suppose an instantaneous stimulus, say an induction shock, applied on the petiole at A.   The excitation will after a time be propagated from A to the responding pulvinus at B.   After a short latent period the leaf will undergo an excitatory fall, pulling down with it the attached arm of the lever.   The vertical writer W is then moved, say, to the left; and the finely pointed bent end of the writer, pressing lightly against the smoked surface of a glass plate, which is allowed to fall at a uniform rate by means of a clockwork, traces a curve giving the time relations of the mechanical response.

I have already referred to the error introduced in the time relations of the response-curve by the friction offered by the recording surface.   As long as I employed the ordinary method of continuous contact of the writing-point with the glass surface, it was impossible to overcome this particular difficulty.   It occurred to me at last that the problem might find a solution, if I could succeed in making an intermittent, instead of a continuous writing-contact.   In the determination of the latent period, and accurate determination of the velocity of transmission of excitation, we require time measurements of the order of one-hundredth of a second.   It will be shown that these measurements can be carried out with great precision, by means of the intermittent dots themselves, when the periodicity of their recurrence is rendered perfectly constant.   For such purposes, then, we require a frequency of intermittent contacts amounting to something like one hundred times per second.

The advantage of this intermittence may be understood from a concrete example. It is to be remembered that the writing-point, under the action of the responsive fall, moves parallel to the surface of the recording plate.   If now, by means of some mechanism, the writing-point be made to vibrate to and fro, say ten times in a second, at right angles to the plate, this will in no way affect the record, beyond the fact that, instead of a continuous, a dotted line will be traced.   The record will not now labour under the defects inseparable from the friction of continuous contact. Instead of this we shall have the vibrating writer, tapping a record, which is practically free from friction.   For it will be understood that, as in our concrete example, a recording-point which is vibrating ten times in a second will execute

one entire to-and-fro movement in one-tenth of a second.   The duration of contact, at the extreme forward end of the swing, will represent only a small fraction, say one-fifth, of the entire period of one vibration.   Hence, after each contact, lasting only one-fiftieth of a second, the recording-point is absolutely free to take up the movement impressed upon it by the moving leaf.   In a record lasting for one second the sum of the intermittent contacts will then amount to one-fifth, and the period of entire freedom, four-fifths of a second.   We can thus see the theoretical advantages of an intermittent over a continuous contact.   What has been said of the writer vibrating ten times in a second holds good equally in those cases where the vibration-frequency is much higher.

Theoretically it is possible to make the recording writer (made of fine steel wire) vibrate by placing a rectangular electromagnet behind it, and completing the circuit in an intermittent manner.   Serious difficulty is, however, met with on account of the disturbing action of lateral pull exerted by the edge of the magnet.

It is therefore absolutely necessary so to arrange matters that the electromagnet shall be without laterality.   This condition I was able to fulfil by making the pole of the electromagnet in the form of either a cylinder or a ring.   The axis, from which the writing index is suspended, is supported accurately perpendicular to the plane of the circular section of the magnetic pole at its centre.   Everything is thus made symmetrical, and as there is no laterality, there can be no tendency whatsoever for the index to execute its to-and-fro vibrations in any other direction than that which is perpendicular to the plane of the terminal pole of the magnet.   As this plane may be adjusted parallel to the glass recording surface, the tapping movement of the writing index can be made to take place perpendicularly to the recording surface.

Coercer and Vibrator.—Next, in order to overcome the difficulty of the irregular timing of those electrical impulses which are to maintain the recording index or writer in a state of periodic vibration, I devised the Resonant Recorder.   If we know the natural frequency of vibration of the recording index, and if by means of some mechanism we can send periodic currents of exactly the same frequency through the electromagnet, then the intermittent magnetic pulls will exactly synchronise with the natural swings of the writing index.   Owing to this perfect tuning, the index will now resonate, breaking out into a persistent and regular vibration of large amplitude.   In practice, all that is necessary in order to secure this, is to take a long steel reed, which, in the course of its regular vibration, will periodically interrupt the electromagnet circuit of the vibrator coil.   The reed itself is maintained in a state of persistent vibration by the usual electromagnetic arrangement.   I shall for the sake of convenience refer to this reed interrupter as the coercer ; and the writing index simply as the vibrating recorder or vibrator (fig. 2).   The reed is at first purposely selected of too great a length, so that the natural frequency of the coercer shall be slower than that of the vibrator.   One end of the reed is clamped, and by

shifting the clamping point the vibrating length of the reed is continuously shortened. This has the effect of gradually raising the vibration frequency of the interrupting reed. A time soon comes when the frequency of the coercer is exactly the same as that of the vibrator. The latter, which has been hitherto more or less inert, now suddenly breaks out, as foreseen, into very regular and sustained vibrations of large amplitude. For some purposes, it is important that the vibration frequency of the recording index should have a definite value. The various frequencies most suitable for these researches were 10, 20, 50, 100, and 200 vibrations per second. The exciting reed was previously calibrated, by means of frequency meters, or standard tuning-forks, to give these values. Then, with great expenditure of time and patience, different vibrating recorders, having various standardised frequencies, were constructed.

For periodic interruption, the coercing and vibrating coils may be put in series, but I find it is much easier to obtain a persistent vibration when the coercer coil is placed in a multiple arc with the vibrator coil. An electromotive force of 4 volts should be sufficient for the purpose of maintaining a steady vibration of both the coercer and the vibrator.

Having thus secured the requisite perfection of the resonating writer, it is only necessary to refer briefly to the complete apparatus for obtaining records of responses in *Mimosa* and other sensitive plants. For this purpose we require a slide carrier to hold the recording
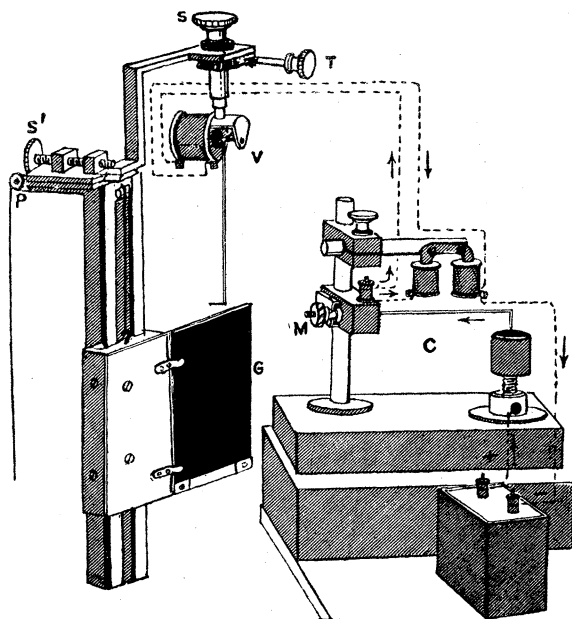

Fig. 2.—Upper part of resonant recorder (from a photograph). Thread from clock (not shown) passes over pulley P, letting down recording plate. S', screw for adjustment of distance of writing-point from recording plate. S, screw for vertical adjustment. T, tangent screw for exact adjustment of plane of movement of recorder, parallel to writing-surface. V, axis of writer supported perpendicularly at centre of circular end of magnet. C, coercer. M, micrometer screw for adjustment of length of coercer.

plate, and this has to be dropped at a definite speed, without jar; we also require the clockwork by which it is to be actuated. It is necessary, again, that the movement of the writer should be absolutely parallel to the recording surface, and that its tip or contact point should be capable of delicate adjustment as regards distance. It should be possible, moreover, to bring this writing-point to any position on the recording surface that may be required. The devices by means of which all these conditions have been met will be found illustrated in fig. 2.

II.—Determination of the Latent Period of Leaf of *Mimosa pudica*.

When the motile pulvinus of *Mimosa* is subjected to an exciting shock, a short time elapses between the incidence of this shock and the initiation of the responsive movement.   There are several difficulties met with in the accurate determination of the latent period of tissues in general.   In the corresponding case of animal tissues, as the muscle-record and the time-record are separate, a certain error is liable to be introduced in inferring the time-value of any point on the muscle curve, especially when the total time to be measured is very small.   There is again the error due to the inertia of the recording lever.

In the apparatus which I have employed for the determination of the latent period of *Mimosa* these sources of error have been reduced to a minimum.   In the first place, the curve of response or phytogram is at the same time a chronogram.   The uncertainty which might arise from an inference based on a neighbouring time-record is thus eliminated.   The error due to the inertia of the recording apparatus is reduced to a minimum by making the writing-lever excessively light.   In the muscle recorder the weight of the recording lever is about 3·5 grm.   The lever which I employ weighs only 0·04 grm.   The recording part of my apparatus is thus about ninety times lighter than that used for muscle record.

The accuracy of the time-record, when made by the response recorder itself, may be gauged from records giving simultaneous tracings of the exciting standard tuning-fork (100 d.v.) and the resonant vibration in the recorder induced by it.   This latter had been previously tuned to give exactly 100 double vibrations in a second.   A light aluminium stylus attached to the tuning-fork traced a sinuous line on a falling plate of smoked glass.   The tip of the vibrating recorder was so adjusted as to make successive dots during its vibration, simultaneously with the tuning-fork tracings. It will be seen from the record (fig. 3) that, corresponding to the crest of each tuning-fork wave and slightly to its right, we have a dot.   The record given represents a period of fourteen one-hundredths of a second, there being fourteen crests made by the tuning-fork time-marker, and

Fig. 3.—Simultaneous record of vibrating recorder and exciting tuning-fork of 100 d.v. per second.

exactly coincident with these are the fourteen dots made by the vibrating recorder. The interval between any two dots therefore is an accurate measurement of one-hundredth part of a second.

The mode of procedure for obtaining the latent period is, first, to make the recording writer vibrate at its own definite frequency, say of 100 times per second. The recording plate is now released, and presently, when its motion has become uniform, we cause an instantaneous stimulation of the pulvinus.   There should be
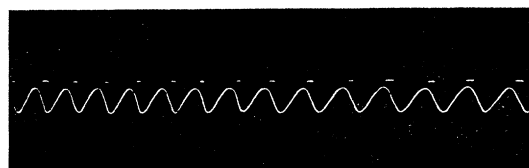
a mark made on the recording plate corresponding exactly to the moment of stimulation. The horizontal record, consisting of a series of dotted points representing one-hundredth of a second, is suddenly deflected upwards, on the initiation of the responsive fall of the leaf. The number of dots intervening between the mark of stimulation and the sudden flexure of the response-curve gives us the value of the latent period for the specimen.

Method of Instantaneous Stimulation.

We have next to consider the practicability of devising, for the excitation of the plant tissue, certain modes of instantaneous stimulation, the intensity of which can either be maintained constant, or varied in a perfectly known manner. I have been able to devise several methods by which this can be accomplished, one of the most practical being that due to induction shock from a coil.

We may in this manner apply a single break induction shock for the purpose of stimulation. This instantaneous stimulation cannot be effected by hand at any exact predetermined point on the record. This must be done automatically by the falling recording plate itself. We cannot again give an instantaneous break-shock without previously completing the primary circuit of the induction coil, which would cause a disturbing make-shock. In order to avoid this, the secondary electrodes, during make, must be short-circuited by means of a thick conducting wire, so that the secondary shock may be practically diverted from the plant through the path of least resistance, which is the conducting wire. All these requirements are provided for, in practice, by the special mechanical devices of the apparatus.

Device for Automatic Stimulation.

The essential parts of the automatic arrangement by which a break-shock is given at a predetermined point on the recording plate are shown in the accompanying illustration (fig. 4).

The recording plate is allowed to drop by pressing the handle H. The winding disc W is attached to the revolving axis of a phonographic motor. The disc is wound in a right-handed direction, and at the same time winds the spring of the phonographic motor. The circumference of the disc is the same as the length of the recording plate. One complete turn pulls the recording plate up to its highest position. A projecting catch below the disc is caught by a pin attached to the spring-handle H, when a complete turn has been made. The recording plate is thus held arrested at its highest position. When desired, a pressure on the handle H releases the disc; the axis of the motor begins to unwind, and the plate is allowed to fall. The motor is fully wound at the beginning, and the partial unwinding during one revolution is exactly compensated before the next observation, by the winding necessary to pull up the plate. Owing to the constancy of this winding, the rate of fall in successive experiments is

kept the same. The pressure on the handle H, which releases the plate, also causes " make " of the current in the primary coil.

In the circuit of the primary coil there is included a contact-breaking device. This consists of a long strip of ebonite, fixed along one edge of the recording plate carrier. On the lower end of the ebonite, a conducting strip of platinum is sunk in, and provided with a binding screw. In front of this slides a rod with contact point tipped with platinum. This can be adjusted up or down by means of a fine micrometer screw, A. When the recording plate is released, carrying with it the conducting
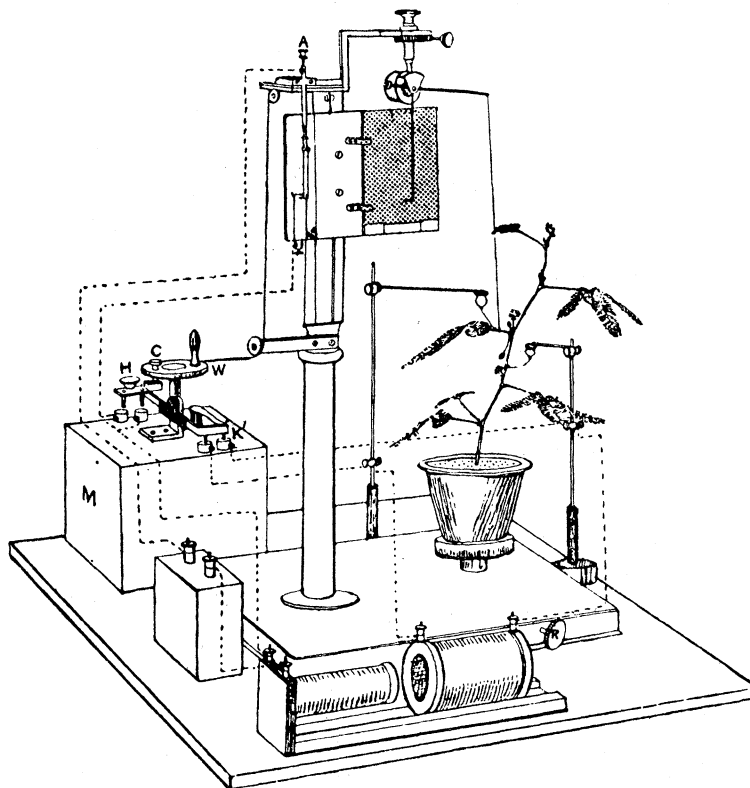

Fig. 4.—Apparatus for determination of latent period of *Mimosa*. M, spring motor. W, winding disc. C, projecting catch. H, release handle, pressure on which also completes primary circuit of induction coil. K′, short circuit key. The automatic break consists of contact rod adjusted by micrometer screw A.

strip, the primary circuit is broken as soon as the line of junction between platinum and ebonite is reached. This sudden interruption of the primary current gives rise in the secondary coil to an instantaneous break-shock, which passes through the plant. In order that shocks in successive experiments shall always be given at the same definite predetermined position in the fall of the plate, the following device is adopted. The recording plate slides up and down a vertical support of triangular section. A movable peg fixed in the support holds it temporarily at a certain selected point, which is chosen as that at which, during the descent of the plate, the shock is to be given automatically to the plant. For the purpose of adjustment a galvanometer is

interposed in the primary circuit.   As long as the point of the contact-rod is in touch with the conducting strip, so long will there be a deflection in the galvanometer.   By means of its screw adjustment, the contact rod is gradually raised till the line of junction between platinum and ebonite is exactly reached ; the deflection in the galvanometer will now cease suddenly.   In this way the point of interruption or " break " is determined with precision.   By pulling the thread in connection with one arm of the recording lever, we now trace a slightly curved line on the smoked plate. This indicates the exact position, in succeeding records, of the moment of application of stimulus.   After making this mark on the plate, the peg is removed.   It is easy to see that in successive experiments stimulation will occur at that definite moment which corresponds to this marked line of stimulation.

K′ represents a key-device by which the make-shock is prevented from exciting the plant.   One end of a lever carries a bent metal rod of U-shape which is kept down, dipping into cups of mercury, by means of a spring.   During the depressed position of this key, the secondary coil is short-circuited.   When the handle H is slightly pressed, there is a " make " of the primary current.   But the make-shock is short-circuited, as K′ is still in the depressed position.   Further pressure of the handle H lifts K′ up, removing the short circuit of the secondary coil.   When the break-shock is given by the contact-breaker of the falling slide, there is no short circuit to divert the shock, which now passes through the plant and excites it.

The sequence of these events, then, is as follows :—

By turning the disc W the recording plate is lifted and held arrested in the up position.   Pressure upon the handle H releases the plate-carrier, which now begins to fall.   At the same time the primary circuit is completed and a make-shock is induced in the secondary.   But this make-shock is diverted by the short-circuit key K′, which is still in the depressed position.   Further pressure on the handle H removes the short circuit by lifting the ends of K′.   All this takes place during one pressure of the handle, which is being held down.   During the descent of the plate, stimulation due to instantaneous break-shock takes place at the definite moment corresponding to the stimulation-mark.

Electric connections are appropriately made with the plant by means of threads moistened with dilute saline solution.   One electrode of the secondary coil is thus connected with the stem of the specimen.   The moistened thread in connection with the other electrode is lightly wound round the pulvinus.   The connections are so made that the current of the break-shock enters by the stem and leaves by the pulvinus, the latter being thus the cathode.   The reason of this is, as will be shown later, that excitation is initiated at the cathode.   In making electrolytic contact directly with the pulvinus, its excitability may sometimes be found diminished by absorption of water.   This difficulty, I find, may be obviated by addition of glycerine to the electrolyte.   We may again apply the exciting electrodes one immediately above and the other below the pulvinus, so that the responding organ is included in the path of the shock.

Short-lived alternating shocks of moderate intensity are found to cause simultaneous excitation throughout the included tract. Hence this mode becomes equivalent to direct stimulation.

1. *Determination of the Latent Period.*

I now give the record (fig. 5) of an experiment carried out in summer, for the purpose of determining the latent period in a specimen of *Mimosa*. Stimulus was
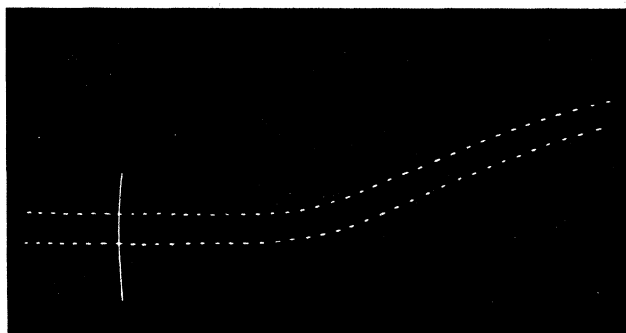

FIG. 5.—Two successive records exhibiting identity of latent period. Recorder 100 d.v. per second. The vertical line indicates the application of the stimulus.

applied at the point marked by the vertical line, and the upper of the two records was the first taken. The vibrating recorder employed had been tuned to exactly one hundred vibrations per second; successive dots therefore represent intervals of 0·01 sec. It will be seen that the responsive movement begins to occur between the tenth and eleventh dots and very near the latter. There are thus 10·9 spaces, each of the value of 0·01 sec. The latent period is therefore 0·109 sec. In order to test to what extent successive experiments might give concordant results, I took a second record with the same specimen, which appears in the record as the lower of the two, having given it an interval of rest of 20 minutes after the taking of the first record. It will be seen that the second record is essentially a replica of the first, thus demonstrating that, with proper precautions, successive experiments on the value of the latent period will give results which are of very great constancy.

For the determination of the latent period in plants, this accuracy of an order higher than hundredths of a second is more than ample. But this limit is easily exceeded. As an example of this, I give a record (fig. 6) made with a different recorder, whose frequency was an octave higher than the last, namely, 200 double vibrations in the second. The successive dots are therefore in this case 0·02 sec. apart. Wider spacings of dots have been secured by photographic enlargement to three times the original record. It is not difficult to measure, say, one-fifth of the distance between two successive dots, themselves representing an interval of 0·02 sec. In other words, the calculation can be carried into thousandths of a second. In the present case there are 15·2 spaces between application of

stimulus and initiation of response.   The latent period of the specimen is therefore 0·076 sec.
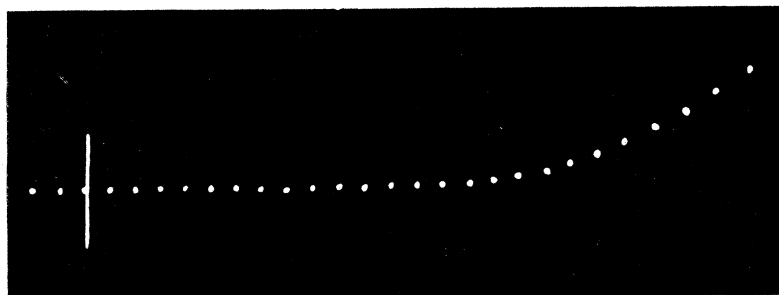

FIG. 6.—Record of L of *Mimosa* with a 200 d.v. recorder.   Time interval between successive dots is here 0·005 sec.

I have been able, moreover, to construct a vibrating recorder whose frequency is 500 times per second, a fact which enables us to make an easy determination of time-intervals less than 0·001 sec.   These recorders, owing to their excessive lightness, have the additional advantage of having a very small moment of inertia.   It is obvious, therefore, that the employment of such recorders would not only bear favourable comparison with those at present used in animal physiology, but would also have the advantage of reducing the error due to inertia to the minimum possible, and of making the record itself its own chronogram.

It has been said that owing to the extreme lightness of the vibrating recorder, the slight error usually due to instrumental inertia is here negligible.   To what extent this is true may be judged by taking records from the same plant with two separate recorders of different sizes and comparing the results.   If the factor of inertia were prominent, then two such determinations of an identical latent period would give results varying somewhat from each other.   I therefore took two different records from the same specimen, using the same stimulus, but varying the mode of record.   That is to say, the vibrator used in one case had been tuned to 50 vibrations per second, the length of the recorder being 12 cm.   The speed of the recording plate was in this case relatively slow.   The result is seen in fig. 7.   The other record in fig. 8 was taken immediately afterwards from the same
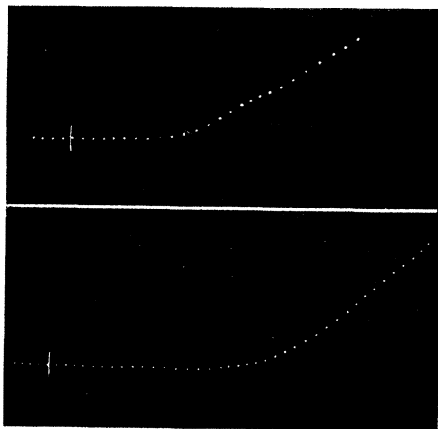
FIG. 7.

FIG. 8.

FIGS. 7 and 8.—Two successive records taken with same leaf; upper with 50 d.v. recorder and slow moving plate; lower with 100 d.v. recorder and fast moving plate.   Latent period in either case is 0·165 sec., proving that the value of L is unaffected by any peculiarity of the recorder.

specimen with a vibrator tuned to 100 double vibrations per second, the recording plate moving at a faster rate. The length of the recorder in this case was 9 cm. It will be seen from fig. 7 that the time interval in the first case is represented by 8·5 spaces each representing 0·02 sec. ; this therefore gives the latent period L to be 0·17 sec. This, it should be mentioned, was an autumn specimen, in which the latent period is somewhat longer than in summer. In the second record, fig. 8, under its different speed, and with the shorter recorder giving 100 vibrations per second, we find the intervening spaces to be 17. This gives the latent period as again 0·17 sec. The identity of these values shows that the inertia of the recorder has but little effect on the results obtained.

The latent period in any given specimen of the pulvinus of *Mimosa* is, as we have seen, under uniform condition extremely constant. It differs, however, in different specimens and from season to season.

With *Mimosa pudica*, I have carried out more than a hundred different determinations, and give below a tabular statement of seventy determinations of those values which occurred most frequently amongst these. Specimens giving a latent period shorter than 0·08 sec. or longer than 0·12 sec. in summer may be regarded as rather exceptional.

TABLE I.—Values of Latent Period L of Leaf of *Mimosa* in numbers of Different Specimens.

Number of specimens.	Value of L.
	sec.
10	0·08
9	0·09
26	0·10
10	0·11
15	0·12

The shortest latent period that I have come across is 0·06 sec., obtained in summer and when the temperature was specially high. The longest in summer was 0·14 sec. Most specimens have a latent period not appreciably differing from 0·1 sec. This may be regarded as approximately the average value for summer. In winter and with sluggish specimens, the latent period may be prolonged to a value of something like twice as much, that is to say, 0·2 sec., more or less.

I will next describe different experiments carried out for the purpose of observing the effect of varying external conditions—such as the intensity of stimulus, fatigue, and temperature—on the latent period. The mode of procedure adopted was first to take a record giving the latent period under standard conditions, and then to make further records under conditions similar in all respects to the first, except in regard to the one special factor whose influence was to be determined.

2. *Effect of Intensity of Stimulus.*

The series of experiments to be described below have in each case been repeated at least twelve times, with results that were invariably concordant. I content myself, however, with giving two records in each case, obtained from different specimens. In order to test still further the reliability of these results I was careful, with each pair of figures given for comparison, to employ two different recorders, the vibration frequency of the first being 100, and of the second 50 per second.

In fig. 9, with vibrating recorder of 100, are given two records testing the effect of intensity of stimulus on the latent period. The lower of the two was obtained with the minimum stimulus of unit intensity. The unit chosen here, though arbitrary, has nevertheless a more or less definite significance. By unit intensity is here meant that intensity which, passed through the arms of a human subject, causes just perceptible sensation. The latent period in this experiment is seen to be 0·155 sec. In the upper of the two records we have the result of the maximal stimulus of 5 units. The latent period is now found to be reduced to 0·1 sec.
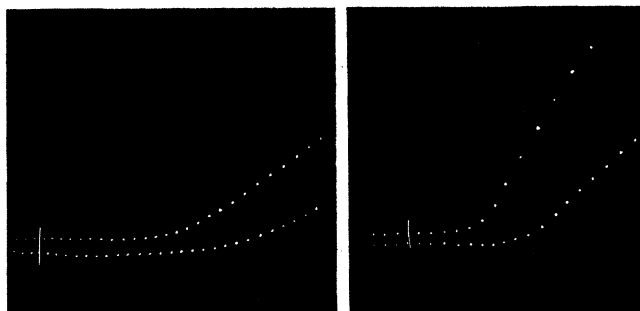

FIG. 9.   FIG. 10.

FIGS. 9 and 10.—Effect of intensity of stimulus on latent period. The upper record in each is that due to the stronger stimulus.

In fig. 10, with vibrating recorder of 50 and taking a different specimen, we find a very similar result. The lower of the two records, with the minimal stimulus of 1, shows a latent period of 0·14 sec. The upper, with stimulus 2, which in this individual case was maximal, shows a latent period of 0·09 sec.

It is interesting to note alike in figs. 9 and 10 the great vigour of the responsive movement under higher intensity of stimulus, as seen in the abruptness of the rise of the curve and the wider spacing of the successive dots.

I give a table (p. 79) showing the effect of intensity of stimulus on the latent period.

It is thus seen that with the increase of the intensity of stimulus there is a corresponding reduction of the latent period. But it would appear from further experiments that a limit is soon reached, when the stimulus begins to be maximal: further increase of the intensity of stimulation above this point has little or no effect

in reducing the latent period. When the conditions are optimal, the length of the latent period is but slightly affected by the intensity of the stimulus.

TABLE II.—Effect of Intensity of Stimulus on Latent Period.

Number.	Intensity of stimulus.	Latent period.
		sec.
1	1	0·155
	5	0·10
2	1	0·14
	2	0·09
3	1	0·15
	4	0·11

3. *Effect of Fatigue.*

We have seen that the successive values of the latent period become constant, provided a resting interval be allowed for complete protoplasmic recovery. The period required for full recovery I find to be about 20 to 25 minutes in summer, more or less. If this resting-interval be shortened, the effect of fatigue is seen in the prolongation of the latent period; if this shortening be carried too far, then the motile excitability is temporarily abolished.

I give below a tabular statement of the results of experiments on the effect of fatigue :—

TABLE III.—Effect of Fatigue on the Latent Period.

Number.	L in fresh specimen.	L when fatigued.
	sec.	sec.
1	0·11	0·16
2	0·10	0·14
3	0·10	0·22
4	0·11	0·17
5	0·8	0·13
6	0·11	0·15

4. *Effect of Temperature.*

The effect of rise of temperature is to induce diminution of the latent period. This will be seen in the following table :—

TABLE IV.—Effect of Temperature on the Latent Period.

Number.	Temperature.	Latent period.
	°	sec.
1	23	0·165
	28	0·125
	33	0·065
2	24	0·140
	29	0·102
	33	0·070
3	20	0·190
	25	0·10
	31	0·08

III.—Determination of Velocity of Transmission of Excitation in *Mimosa* and its Variations.

Before entering into the details of the experiments, the preliminary questions may be briefly considered : With what degree of accuracy is it possible to determine the normal velocity of transmission ?   And how far may we depend on the constancy of this velocity, in successive experiments, under normal conditions ?—with reference to which some misgivings might naturally arise ; for the possible disturbing factors will in all probability prove to be numerous.   It was only after a long course of investigations, some of which will be presently described, that I was able to analyse and provide against all such sources of variation.   But even after this I was by no means prepared for the very great consistency of the results which it has been my good fortune to obtain.   For successive determinations with the same specimen, of the periods required for the transmission of excitation through a given length of conducting tissue, did not differ from each other by so much as one-twentieth of a second, and were often actually identical.

For the purpose of these experiments I used by preference the petiole of *Mimosa*, for the reason that in this the conducting strands situated in the fibro-vascular tissue would be more continuous and evenly distributed than in a branching specimen.   In order to determine the velocity of transmission, the stimulus of induction shock is applied to the petiole at a distance d from the responding pulvinus (*cf.* fig. 1).   Let us suppose t to be the true time for transmission of excitation to the motile organ ; the initiation of the responsive movement will however be further delayed by the latent period of the pulvinus L.   The total time-interval T observed to elapse between the application of stimulus and the initiation of response will therefore be the true time t plus the latent period L.   To obtain the true time of transmission, we have to subtract the latent period L from the observed interval T, thus $t = T - L$.   The velocity of transmission is then found by dividing the

distance by the true time. The necessary data are therefore the distance between the stimulated point and the pulvinus, the observed time-interval between the application of stimulus and the initiation of response, and the latent period of the individual pulvinus.

In making these determinations the apparatus employed is the same as that for the determination of the latent period. As in these experiments we have to measure time which may be several seconds in duration, the recording plate is made to travel at the relatively slow rate of 2 cm. in a second or thereabouts. The vibrating recorder is selected according to the degree of accuracy that is required. For our present purpose a time measurement accurate to one-tenth or one-twentieth of a second is ample.

We first obtain a series of records of indirect stimulation. The two electrodes, E and E', in connection with the exciting secondary coil are applied about 10 mm. apart, the proximal electrode E being at a distance d from the pulvinus. The recording plate during the course of its descent completes the primary circuit of the induction coil for a definite length of time, which is about one-twentieth of a second. This gives rise to a definite number of alternating shocks to the plant. The stimulus is always applied at a definite instant in the descent of the plate : hence successive records on the same plate always commence on the same level, the vertical line in the record indicating the moment of the application of stimulus. After taking one or more records of the effect of indirect stimulation, an additional record is taken of the effect of direct stimulation of the pulvinus. This gives the latent period L of the particular specimen.

Before proceeding further, I must point out the necessity of special precautions for the perfect insulation of the electrodes in connection with the secondary coil. If one of these should happen to touch the table, then, even with connections made for indirect stimulation, a portion of the current would pass through the flower pot holding the plant, and the pulvinus would be directly stimulated by this escaping current or current of leakage. In my own case it was some time before I discovered that certain anomalous results were to be traced to this particular source of disturbance, at first little suspected. To overcome this difficulty, the flower pot should be placed on a block of insulating ebonite, the electrodes also being carefully insulated on ebonite rods.

1. *The Direct Method.*

I will now proceed to give the actual records obtained with the arrangements just described. In the experiment I am about to describe the specimen of *Mimosa* was very vigorous. The distance at which the stimulus was applied was 30 mm. from the responding pulvinus, and the intensity of stimulus was 3 units. The frequency of the vibrating recorder was 10 per second : hence the distance between any two successive dots in the record represents a time-interval of one-tenth of a second, and

from the record itself it will not be found difficult to estimate intervals of even one-fifth that amount. The lowest of th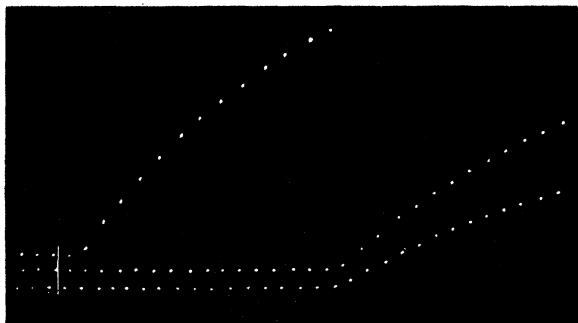e three records in fig. 11 represents the results of the first experiment. It will be seen that the interval between the stimulus and the beginning of response is 16·2 spaces, each of the value of 0·1. Therefore, the total time, T, is 1·62 sec. After an interval of 15 minutes—which I find to be the time required in summer for complete recovery of conductivity—a second record was taken, under the same conditions, on the same plate. It will be seen that the time-interval in this case is the same as before, namely, 1·62 sec. The third record was taken with direct stimulation, and from this it

FIG. 11.—Determination of velocity of transmission in *Mimosa*. The two lower records are in response to stimulus applied at a distance of 30 mm.: the upper record, in response to direct stimulation. Recorder, 10 d.v.

will be seen that the latent period is 0·12 sec. Thus the true velocity of transmission as given by both these experiments is identical, namely—

$$T = (1\!\cdot\!62 - 0\!\cdot\!12) \text{ sec.} = 1\!\cdot\!5 \text{ sec.} \qquad V = d/t = 30/1\!\cdot\!5 = 20 \text{ mm. per sec.}$$

2. *The Differential Method.*

In order to put the constancy of these results to a still more rigorous test, I next modified the experiment in the following way, employing the *Differential Method.* The stimulus was first applied at a distance d from the responding pulvinus, and the total time, T, was found from this record. In the next experiment the distance of the point of stimulation was reduced to d_1, and its corresponding total time, T_1, found in the usual manner. And, lastly, a record was taken under direct stimulation. This furnished the value of the latent period L.

It will be seen that we have here three different sets of data for the determination of the absolute value of the velocity of transmission. In two of these we derive the velocity, in the usual manner, from the distance, the total time, and the latent period. In the third, knowledge of L is not required. For it will be seen that the difference in the times, $T - T_1$, of transmission observed in the first two cases represents the time taken by the excitation to travel the difference between the two distances, $d - d_1$. Hence, three separate determinations, V_1, V_2, and V_3, are obtained with the same specimen, viz.—

$$V_1 = \frac{d}{T-L}\,; \qquad V_2 = \frac{d_1}{T_1-L}\,; \qquad V_3 = \frac{d-d_1}{T-T_1}.$$

The rigour of this test of constancy will be gauged by the extent to which the different determinations of V_1, V_2, and V_3 are consistent with each other.

In an experiment carried out in this way, the intensity of stimulus applied was 3 units, and the vibrating recorder had a vibration frequency of 10 per second. In the first experiment the point of application of stimulus was at a distance of 30 mm.; the total time was found to be 1·9 sec. In the next experiment the distance was reduced to half, that is to say, 15 mm., and the total time was found to be 1 sec. And lastly, the latent period was determined under direct stimulation at 0·08 sec. (fig. 12). Thus—

$$V_1 = \frac{30}{1·9 - 0·08} = 16·4 \text{ mm. per sec.} \qquad V_2 = \frac{15}{1 - 0·08} = 16·3 \text{ mm. per sec.}$$

$$V_3 = \frac{15}{1·9 - 1} = 16·6 \text{ mm. per sec.}$$

The three results thus obtained from independent data are here seen to be extremely consistent. They bear very emphatic testimony, not only to the accuracy of the method, but also to the constancy of the velocity in a given specimen under unvarying external conditions.
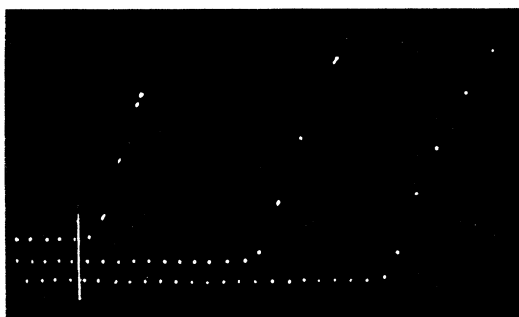

FIG. 12.—Determination of velocity by differential method. The three records, from below upwards, are in response to stimuli applied at distances of 30 mm., 15 mm., and directly. Recorder, 10 d.v.
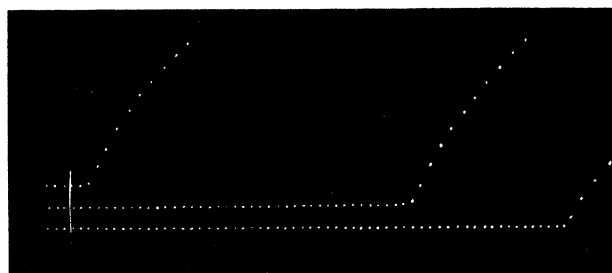

FIG. 13.—Differential method. Uniform stimuli applied at distances of 30 mm., 20 mm., and directly. Recorder, 20 d.v.

It has been said that the accuracy of these time measurements can be pushed to almost any extent. In order to demonstrate this fact, and also to exhibit the high mutual consistency of various determinations, I will here give another set of records from a different specimen (fig. 13), from which the velocity of transmission is to be determined by the *Differential Method*. In this case a new recorder was taken, with a vibration frequency of 20 times per second. Hence the distance between any two successive dots represents a time-interval of one-twentieth of a second. The stimulus intensity was again 3. The lowest record gives us the result obtained when the point of application of stimulus was 30 mm. away from the responding pulvinus. The total time, T, is here seen to be 2·9 sec. The next record gives us the result when the point of stimulation was at a distance of 20 mm. and the total time, T_1, is

1·985 sec. The third and highest of these records gives the record of direct stimulation, the latent period being shown as 0·085 sec. Thus—

$$V_1 = \frac{30}{2 \cdot 9 - 0 \cdot 085} = 10 \cdot 7 \text{ mm. per sec.} \qquad V_2 = \frac{20}{1 \cdot 985 - 0 \cdot 085} = 10 \cdot 53 \text{ mm. per sec.}$$

$$V_3 = \frac{10}{2 \cdot 9 - 1 \cdot 985} = 10 \cdot 9 \text{ mm. per sec.}$$

It is thus seen that, taking the precautions described, successive determinations of the velocity of transmission may be arrived at which are of great constancy. It may be said of the velocity of transmission in the petiole of *Mimosa* that it is constant with a given specimen, but undergoes some variation with different individuals. It is also subject to modifications induced by season. In winter the velocity is much reduced. The highest velocity which I have obtained with summer specimens of the petiole of *Mimosa* is 30 mm. per sec. The lowest, in sluggish specimens, may be as little as 4 mm. per sec.

Having now obtained means for the accurate determination of the velocity of transmission, I will next proceed to describe the effects of various agencies in inducing changes in the normal rate under standard conditions. And first we have the important question as to whether the velocity is in any degree dependent on the intensity of stimulus.

In answer to this it may be said, in anticipation, that the effects are, to some extent, modifiable in a definite way by the condition of the conducting tissue. If the specimen happen to be in a sluggish condition, then increasing intensity of stimulus will be found to be attended by increasing velocity of transmission. Again, a moderately strong intensity of stimulus is often found to leave, as an after-effect, increased conducting power. That is to say, to a tissue which has been sluggish, stimulation itself imparts a higher conductivity.

3. *Effect of Increasing Intensity of Stimulus.*

Returning now to the experimental inquiry into the influence of intensity of stimulus on the velocity of transmission, I will give a set of records (fig. 14) obtained with a petiole which was slightly sluggish. The distance of the point of application of stimulus, namely 20 mm., was maintained constant, the intensity being varied in the successive experiments. The vibrating recorder had a frequency of 10 per sec. The lowest record is the result of a stimulus intensity of 0·5. The total time of transmission is seen to have been 2·1 sec. The true time is obtained by subtracting from this the latent period, whose average value we have found to be about 0·1 sec. No appreciable error will be introduced in practice, by adopting this average value for the latent period, for its variations are very slight, being of the order of hundredths of a second. The actual time here taken for transmission is thus 2 sec. with a stimulus intensity of 0·5.

The record next above gives the result when the stimulus intensity was 4, that is to say, increased to eight times its original value. The total time is now found to be decreased to 1·6 sec., the true time, after deducting the latent period, being thus 1·5 sec. The velocity under this increasing intensity is thus enhanced in the proportion of 2 : 1·5 or 33 per cent. In order to find out if there had been any after-effect of stimulation, a record was once more taken with the original feeble stimulus intensity of 0·5. It will be seen from the third record that the time now taken for the transmission of excitation was practically the same as that with the previous strong stimulus, showing that this has made the tissue more conducting, and that this property has reached a limit of uniformity. In order further to test this conclusion, a fourth record was now taken, with the second high stimulus intensity of 4. It will be seen that the time of transmission is now the same as with the feebler intensity.
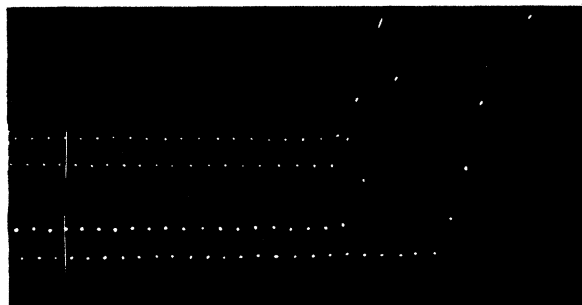

FIG. 14.—Effect of intensity of stimulus, and its after-effect on velocity. Lowest record, under stimulus 0·5; the next above, under stimulus 4. Velocity increased under stronger stimulus. Enhancement of conductivity by previous stimulation seen in upper two records, with stimulus 0·5 and 4 respectively. Velocity high and practically constant.

From these experiments it will be understood that when the tissue is in a somewhat sluggish or sub-tonic condition, the velocity of transmission is enhanced under increasing intensity of stimulation. The following table gives the results of two sets of experiments :—

TABLE V.—Effect of Intensity of Stimulus on Velocity in Sub-tonic Specimens.

Number.	Stimulus.	Velocity.
1	0·5	10·0
	4·0	13·3
2	0·5	5·9
	2·5	8·3

The fact that stimulus itself may enhance conductivity in a sub-tonic tissue may be seen in a striking manner in specimens of *Mimosa* which are in sluggish

condition.   It will there be found that the application of stimulus on the petiole will at first fail to be conducted.   If, however, we apply the same stimulus again after the usual interval of, say 15 min., the excitation, which failed in the previous case to be conducted, will now reach the pulvinus and induce the responsive fall.   Stimulus had thus imparted conductivity to the tissue.

I find again that when the plant is brought to an optimum condition, then the velocity of transmission tends to become constant even under varying intensities of stimuli.   Thus a specimen was maintained at 30° C., which is a favourable condition as regards temperature.   In measuring the velocity of transmission under the stimuli 0·5 and 4 units respectively, the result was found uniform, namely 20 mm. per sec.

4. *Effect of Temperature.*

If the phenomenon of transmission in the plant is one of protoplasmic change, then any factor that causes physiological variation must have a corresponding influence on its velocity.   One such factor of physiological variation is found in change of temperature.   If, on the other hand, the propagation had been merely of a hydrostatic blow, then a change of temperature would not have had any marked effect upon it.   Thus the accurate determination of the influence of temperature upon velocity of transmission becomes important in discriminating between the excitatory or the mechanical nature of the transmitted change.
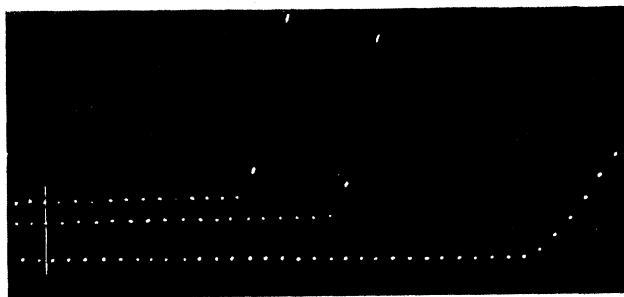

Fig. 15.—Effect of temperature in enhancing velocity of transmission.   The three records, from below upwards, are for temperatures 22° C., 28° C., and 33° C., respectively.   Recorder 10 d.v.

I will now proceed to give results as to the variation of conductivity under variation of temperature.   The plant was kept at the required temperatures in the thermal chamber, the necessary temperature being maintained by electrical appliances for heating.   In fig. 15 time records are given of the transmission of excitation at the three temperatures of 22° C., 28° C. and 31° C.

I give below in tabular form the results of this and a few out of many other experiments on the influence of temperature on velocity.   It need only be said that the effect of rising temperature was always to induce an increase in the velocity of transmission.

TABLE VI.—Effect of Temperature on Velocity of Transmission.

Number.	Temperature.	Distance.	True time.	Velocity.
	° C.	mm.	secs.	mm. per sec.
1 (Winter specimen)	22·0	10	2·93	3·6
	28·0		1·59	6·3
	31·0		1·1	9·0
2	23·0	10	1·5	6·6
	33·0		0·8	12·5
3	29·5	20	2·2	9·1
	30·5		1·7	11·8
4	27·0	10	1·0	10·0
	30·0		0·7	14·3
5	29·0	20	2·0	10·0
	32·0		1·8	11·1
	35·0		1·5	13·3

IV.—EXPERIMENTS IN CONFIRMATION OF THE EXCITATORY CHARACTER OF EFFECT TRANSMITTED.

I will now adduce various results which show that in general the initiated disturbance which undergoes transmission is not mechanical, but physiological.

1. *Polar Excitation in Plants.*

Taking the case of the nerve-and-muscle preparation in the animal, it is well known that under feeble current, excitation is initiated only at the cathode, at make; that with a current of moderate intensity, excitation takes place at cathode at make, and at anode at break. It is found that there is no excitation if the current be established or removed very gradually. The excitatory effect, generally speaking, takes place only at the moment of sudden initiation or sudden cessation of current. These characteristic phenomena of excitation are purely physiological. Effects precisely similar to these are obtained with plants. Here also feeble current gives rise to excitation at the cathode at make; with moderate current, excitation is initiated at the cathode at make, and at the anode at break. These effects take place only when the variation of current is effected suddenly.

The excitation of plants by the action of current is thus shown to be discriminative or polar, unlike the non-discriminative character of excitation by a mechanical blow.

2. *Time Difference between Excitation by Ascending and Descending Currents.*

The effects of feeble ascending and descending currents in the petiole of *Mimosa* are parallel to those in muscle and nerve preparations.   In both cases
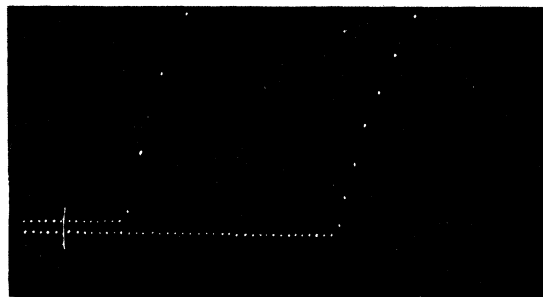

FIG. 16.—Records of responses to descending (upper record) and ascending (lower record) currents. Response earlier when current is descending, the cathode being proximal.   Recorder 20 d.v.

excitation takes place earlier when the cathode is nearer to the responding organ (fig. 16).

3. *Relative Sensitiveness of Plant and Animal to Stimulus of Electrical Current.*

The hydro-mechanical theory presupposes the occurrence of a strong mechanical disturbance to give rise to the transmitted impulse.   But initiation of excitatory impulse is found to take place under the polar action of current, in the absence of any mechanical disturbance.   This is realised when we find that an excitatory impulse is initiated and transmitted by the action of an electric current which is so feeble as not to be perceived by the very sensitive human tongue, which has always been considered a very sensitive detector of electric current.   According to LASERSTEIN the acid effect of anode is appreciated by it when the current attains a value of 6·4 micro-ampères.   But I find that the leaflet of *Biophytum sensitivum* responds to an intensity of a current which is as feeble as 0·5 micro-ampère.
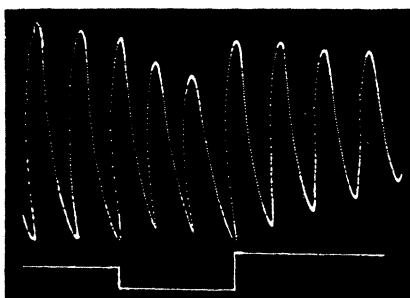

FIG. 17.—Effects of application of anode and cathode on pulsations of *Desmodium gyrans*. Signal line below the normal indicates application of anode ; line above normal, application of cathode. Note diminution of " systolic " limit under anode, and diminution of " diastolic " limit under cathode.

4. *Contrasted Effects of Anode and Cathode.*

The physiological reaction to an electrical current and the contrasted effects of anode and cathode are exhibited in the animal heart by characteristic modifications of its pulsating activity.   I find similar effects reproduced in the rhythmic tissue of *Desmodium gyrans*, the Telegraph plant.   The effect of the make of anode is to induce an expansion.   This is shown in the record (fig. 17) of pulsation of

Desmodium by a shortening of the up-stroke which indicates systolic contraction (fall of leaflet). The opposed contractile effect due to the make of cathode is seen in the shortening of the down-stroke which indicates diastolic expansion (rise of leaflet).

5. *Multiple Excitation under Constant Current.*

According to the mechanical theory, multiple excitation can only occur under separate hydro-mechanical disturbances, caused by multiple blows. I find, however, that multiple excitations are caused in rhythmic plant tissue by the passage of a constant current. Taking a highly excitable specimen of *Biophytum*, a particular leaflet was attached to a very light recording lever. A constant current was sent for a short time through the petiole, the cathode being at a distance of 10 mm. from the leaflet. The leaflet responded to the excitation caused by cathode-make; the response here was due to the excitation transmitted through the distance of 10 mm.
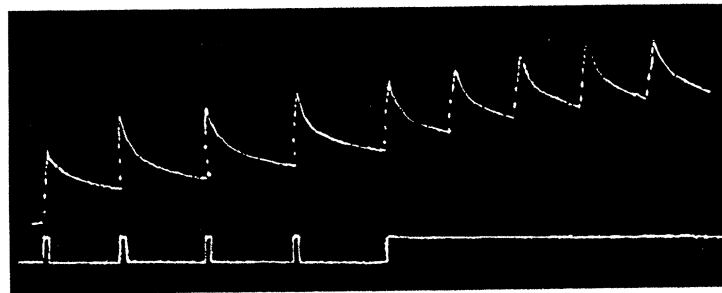

FIG. 18.—Multiple excitation in *Biophytum* under constant current. First four records are responses to individual stimuli of short-lived electrical current, applied at intervals of 3 min. The last five are multiple responses to continuous current.

The first part of fig. 18 gives four responses to four individual stimuli, applied at intervals of 3 min. After this the petiole was subjected to the action of a continuous current, represented below the record as a continuous line. It will be seen that, under the continuous current, rhythmic excitations were initiated at the cathode, which, reaching the responding leaflet, caused multiple responses. There were five such rhythmic responses in the course of 10 min., the period of each being 2 min.

6. *Effect of Temperature on Excitation by Constant Current.*

Excitation of animal nerve by induction shock is enhanced by warmth and depressed by cold. The reverse is the case, as shown by GOTCH and MACDONALD, when the stimulation is caused by constant current. The excitatory effect here is depressed by warmth and exalted by cold. These specific effects are found repeated in the conducting petiole of *Mimosa*. The excitation caused by induction current is depressed by cold and enhanced by warmth. But, as in the animal nerve, so also in the petiole of *Mimosa*, these effects are reversed under polar excitation; the excitation

is now enhanced by cold and depressed by warmth.   Minimal excitation becomes maximal under cold, and ineffective under warmth (fig. 19).
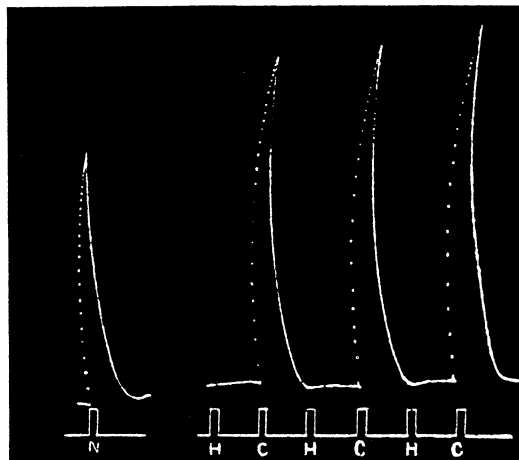

FIG. 19.—Effect of temperature on excitatory efficiency of constant current. N, normal response at 30° C.; HH, abolition of response at 36° C.; CC, enhancement of response at 10° C.   Testing stimulus of cathode-make kept constant.

V.—CRUCIAL PROOF AFFORDED BY VARIOUS PHYSIOLOGICAL BLOCKS.

1. Block of Conduction by the Action of Cold.

The object of our inquiry being the influence of cold on conductivity, special care has to be taken that the lowering of temperature does not in any way affect either
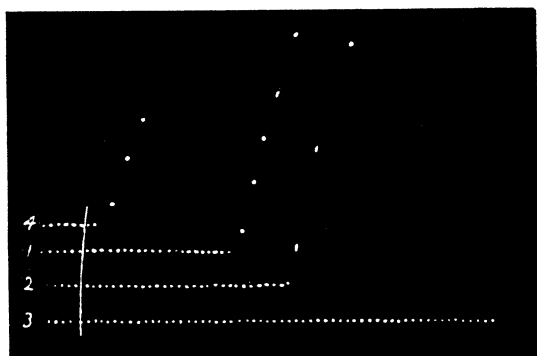

FIG. 20.—Effect of cold in inducing retardation and arrest of transmission: (1) Normal record; (2) Retardation due to slight cooling; (3) Arrest of conduction by application of ice; (4) Record of direct stimulation.

the excitability of the point of application of stimulus or the motile sensibility of the responding pulvinus.   For this reason cold is applied locally on the petiole, half way between the point of application of stimulus of induction shock and the pulvinus.   The experimental plant was highly sensitive on account of the favourable summer season. An intensity of stimulus of 0·5 unit applied at a distance of 30 mm. from the pulvinus was found to be effectively transmitted. The intensity of stimulus actually employed was two units, which was maximal.   A strip of cloth 10 mm. in breadth was wrapped round the petiole midway between the point of stimulation and the pulvinus.   This was for the purpose of local application of cold by means of cooled water or by means of small fragments of ice.

Successive records were now taken at intervals of 20 minutes, which is more than

sufficient for complete recovery from previous stimulation. In fig. 20, record (1) gives the time-interval between the application of stimulus and the response under normal conditions. There are 20·5 spaces, each space representing 0·1 sec. The latent period is 0·15 sec. The true time of transmission is thus 1·9 sec. for 30 mm.; the transmission time through 10 mm. is therefore 0·63 sec. The next record was taken when the intervening length of 10 mm. in the petiole was moderately lowered in temperature by the application of cold water. This cooling should be commenced immediately after the previous responsive fall of the leaf. This not only gives sufficient time for the localised cooling of the petiole, but also avoids the excitatory disturbance of the pulvinus caused by sudden application of cold to the petiole. During the localised cooling of the petiole, the leaf erects itself and becomes fully sensitive when the time arrives for the application of the next stimulus. Record (2) exhibits the effect of moderate cooling; the transmission period is now prolonged, the difference between the two records being a time-interval of 0·8 sec. On the assumption that the effect of cooling had remained localised, it is seen that the lowering of temperature had prolonged the period of transmission through the 10 mm. of the petiole, from 0·63 sec. to 1·43 sec. The conductivity had thus been reduced by more than half.

In the next record (3) is seen the effect of further lowering of temperature by placing small fragments of ice on the strip of cloth. The excitatory impulse initiated by the maximal stimulus of induction shock had hitherto been unfailingly transmitted. But under the action of intense cold, the impulse was arrested. In order to show that the abolition was not due to the depression of the motile excitability of the pulvinus, record (4) was taken of the effect of direct stimulation. An inspection of the record shows that the motile excitability had undergone no change. It is thus clear that the impulse initiated by the stimulus had been arrested by the physiological depression of conductivity induced by cold.

2. *Paralysis of Conductivity, and Restoration by Tetanising Shock.*

In connection with this subject, I came across the interesting phenomenon of paralysis of conductivity as an after-effect of intense cooling. After obtaining the record of the block under local application of cold, the fragments of ice were removed and the cooled portion of the petiole allowed to regain the temperature of the room, which must have been accomplished in course of 20 minutes. After this, on taking the record of the transmitted effect of stimulus, I found that the block of conduction was still persistent. The conducting power of the benumbed tissue is thus paralysed for a period which generally lasts for about 45 minutes. I have, however, discovered the very suggestive fact that the lost conducting power can be very quickly restored by subjecting the paralysed portion of the petiole to the action of tetanising electric shocks.

3. *Electrotonic Arrest of Excitatory Impulse.*

In a nerve and muscle preparation, it is well known that if we keep a constant current in an intervening tract between the point of stimulus and the responding muscle, this current will act as a block to the passage of excitation. This blocking of conductivity ceases, however, on the stoppage of the current.

I will now give a parallel experiment on a conducting petiole and responding pulvinus. The proximal of the two exciting electrodes was placed at a distance of 30 mm. from the pulvinus, the stimulation being caused, as usual, by means of
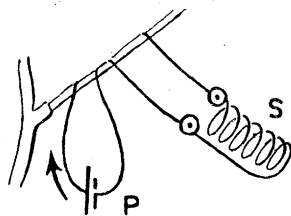

FIG. 21.—Arrangement of electro-tonic block. Polarising circuit P interposed between exciting secondary coil S, and responding pulvinus. The block is equally effective by currents flowing in ascending or descending directions.

induction shock. Half-way between the point of excitation and the pulvinus were placed two polarising electrodes, 5 mm. apart, through which a constant current could be maintained, for the purpose of serving as a block (fig. 21). The first and
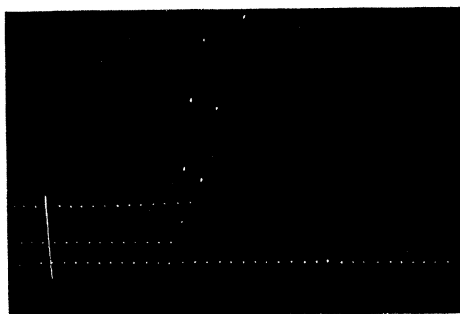

FIG. 22.

FIG. 23.

FIG. 22.—Record showing effect of electro-tonic block. Uppermost record, normal. Lowest record shows block of transmission of excitation. Middle record shows restoration of conductivity on removal of block.
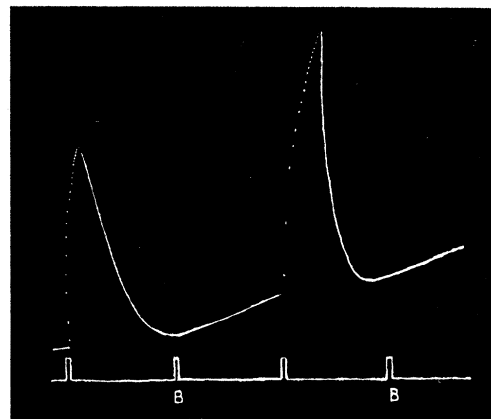
FIG. 23.—Records of transmitted excitation, with the block off and on alternately. Arrest of transmitted excitation under electro-tonic block at BB. In the first case the blocking current was in one direction; in the second case it was sent in an opposite direction.

uppermost of the three records was taken without this block (fig. 22) and with a stimulus intensity of 2. It will be seen that excitation was transmitted as usual, the velocity of transmission in this case being 29 mm. per sec. The specimen was

exceptionally vigorous and the season the height of summer, which facts account for the high velocity. The blocking current was next introduced. In order to prevent the excitation due to sudden make of current, the applied E.M.F. was gradually increased from zero to 2 volts, by means of a potential slide. During the passage of this constant current, a second stimulus was applied of the same value as before ; and it will be seen, from the lowest record, that there was no response, the transmission of excitation being effectively blocked. In order to show that this block would only persist during passage of the current, the latter was next reduced gradually to zero, by manipulation of the potential slide. This was carefully done, to avoid the excitation due to sudden cessation of current. On again repeating stimulation, the block, as is seen in the middle one of the three records, was found to be no longer operative, and response due to transmitted excitation took place as usual.

The records in fig. 22 were taken on a fast moving plate. In the next record (fig. 23) series of response-records of transmitted excitation were taken on a slower moving plate. The testing stimulus was always the same, the difference being that the electrotonic block was off and on alternately. It will be seen that the excitation was invariably arrested whenever the block was applied at B.B., there being no such arrest after the removal of the block.

4. *Block of Conduction by the Action of Poison.*

Reference has been made as to the inconclusive character of PFEFFER'S narcotisation experiment. The ineffectiveness of the block, it was explained, might have been due to the thickness of the tissue retarding the access of the anæsthetic to the conducting elements in the interior. It occurred to me that the physiological block induced by a drug could be rendered more effective in two different ways; first, by the selection of a thin petiole in which access of the solution to the interior by absorption would be less difficult, and second, by the employment of strong toxic agents like copper sulphate or potassium cyanide solutions. The choice of a strong poison was deemed advisable because the absorption of even a small quantity might in such a case prove effective in inducing depression or abolition of the conducting power. Application of an anæsthetic, like chloroform, has the drawback that the escaping vapour renders the motile organ insensitive. There is no such disadvantage in the employment of a non-volatile poison like copper sulphate, which by local absorption would affect the conductivity of the selected portion of petiole without modifying the motile sensibility of the pulvinus. I may state here, in anticipation, that a poison like copper sulphate solution was somewhat slow in its action, whereas the effect induced by potassium cyanide solution was very rapid.

Copper Sulphate Solution.—The specimen employed for the following series of observations was the petiole of *Mimosa*. As the effect of copper sulphate solution was slow, it was very interesting to observe by means of successive records the progressive diminution of conducting power culminating in actual arrest. The

records of transmission time were taken at intervals of 20 minutes, before and after subjecting an intermediate portion of the petiole to the action of poison. Effective stimulus of induction shock was applied on the petiole, generally at a distance of 30 mm. from the responding pulvinus. The intensity of stimulus employed was maximal, being two units. The first record of the series gives the velocity of normal conduction; the second and the subsequent records exhibit progressive effect of the toxic agent. This latter was applied on a strip of cloth 10 mm. wide, wrapped round the petiole midway between the point of stimulation and the responding pulvinus. The normal record (1) in fig. 24 shows response to have taken place 27 spaces after the application of the stimulus, the interval between the successive dots being 0·1 sec. The total time was therefore 2·7 sec. Subtracting from this the latent period 0·2 sec., we obtain 2·5 sec. as the actual time for transmission through 30 mm. The time of transmission through 10 mm. was therefore 0·83 sec.
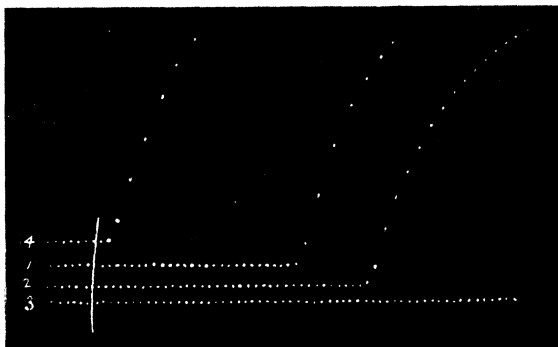

FIG. 24.—Effect of copper sulphate solution in inducing retardation and arrest of conduction. (1) Normal record; (2) Retardation caused by 20 minutes' application; (3) Arrest caused by application for 40 minutes; (4) Record of direct stimulation.

The next record (2) of the series shows the effect of application of copper sulphate solution for 20 minutes on a portion of the petiole 10 mm. in breadth. It is seen that the transmission time has been prolonged by 10 spaces, i.e. by 0·1 sec. Assuming that the effect of poison was localised, it is seen that it had during 20 minutes' application delayed transmission through 10 mm. from 0·83 to 1·83 sec. By the absorption of a small quantity of poison the conductivity had thus been reduced by more than 50 per cent.

The next record (3) of the series was taken after a further period of 20 minutes. The transmitted effect is seen to be completely blocked by the application of copper sulphate for 40 minutes. In order to show that the absence of response was due, not to the abolition of motile excitability of the pulvinus but to the local block of conductivity in the petiole, a fourth record was taken under direct stimulation, which shows that the motile excitability of the pulvinus had remained unimpaired.

Mercuric Chloride Solution.—A series of records was next taken on the effect of mercuric chloride solution. The power of conduction was found to be arrested after a period of application so short as 10 minutes. The record obtained was very similar to that given in the next figure.

Potassium Cyanide Solution.—I next took a series of records which exhibited the effect of application of a strong solution of potassium cyanide. Record (1), fig. 25, gives the normal transmission period. The next record (2) was taken as stated before, after allowing 20 minutes for recovery. The poisonous solution was, however,

applied 15 minutes after the first record, hence in the second record we see the effect of application of potassium cyanide for a period of 5 minutes only. The effect of this poison on the conductivity of petiole of *Mimosa* was so great that even with such a short application the transmission of excitation caused by maximal stimulus of two units was completely blocked. The next record (3) was taken with the secondary pushed close to the primary, the stimulus intensity being thus raised to 15 units. Even under this intense stimulation, the conduction was found to be arrested. The next record (4) was taken under direct stimulation. The response shows that the sensibility of the pulvinus had undergone no change. It is thus clear that the abolition of response
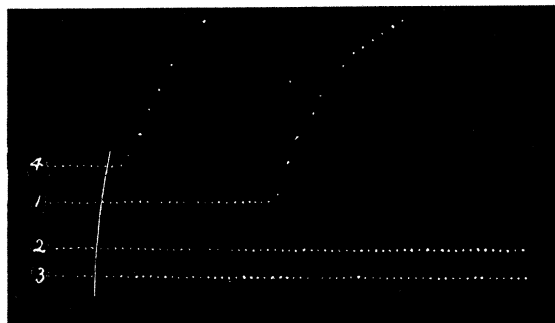

Fig. 25.—Abolition of conductivity by the action of potassium cyanide. (1) Normal record; (2) Arrest of conduction after application for 5 minutes; (3) Arrest of excitation due to strong stimulus; (4) Record of direct stimulation.

to indirect stimulation was solely due to the abolition of conductivity induced by the action of the poison.

The arrest of the transmitted impulse in *Mimosa* by the physiological block induced by cold, by electrotonus and by local application of poison, disproves the hydro-mechanical theory. The results of the various investigations described lead, on the other hand, to the conclusion that the propagated effect is one of physiological change.

VI.—General Summary.

For researches on the velocity of transmission of excitation in motile leaves, a knowledge of the latent period of the pulvinus and its variations under different conditions is necessary. It is here shown how the value of the latent period and the velocity of transmission can be obtained by the automatic records made by the plant itself.

The automatic record has been rendered possible by means of a new type of recorder, in which the writer, accurately tuned to a definite and known frequency, is maintained in resonant vibration. The vibrating writer taps a record; intervals between successive dots may thus be made to indicate time-intervals as short as 0·005 sec. The phytogram is thus its own chronogram. Error due to friction is virtually eliminated on account of intermittent contacts. As the writer can be made extremely light, error due to inertia is reduced to a minimum.

Successive determinations of the latent period under constant external conditions are found to give identical results. The latent period becomes shorter under increasing intensity of stimulus, till a limiting and constant value is reached under

maximal stimulus. The latent period is prolonged under fatigue and shortened under rising temperature. The shortest latent period obtained with the pulvinus of *Mimosa* was 0·06 sec., the average value being 0·1 sec.

The velocity of transmission of excitation has been determined by two methods— the *Direct* and the *Differential*. Successive determinations were found to give results which are practically identical. In sub-tonic specimens the velocity was found to be enhanced under increased intensity of stimulus until a limit was reached under maximal stimulus. The after-effect of previous stimulation on a sub-tonic specimen is to confer on it enhanced conducting power. In a specimen in optimum condition there is little difference in the velocity of transmission under feeble or strong stimulus.

The velocity of transmission undergoes a marked enhancement under rise of temperature. This shows that physiological changes have a modifying influence on the velocity of transmission.

The various characteristic polar effects of electric current in excitation of plants, and the arrest of excitatory impulse by various physiological blocks, afford crucial tests of the physiological character of the transmitted effect. It is shown that the passage of current as such does not, generally speaking, cause excitation ; it is the sudden commencement or cessation that is effective. The excitatory action of current is again discriminative or polar. With feeble current excitation takes place only at the cathodic point at make ; with moderate current excitatory effect is exhibited at the cathode at make, and at the anode at break. Time-records taken with ascending and descending currents show, further, that excitation takes place earlier when the cathode is proximal to the responding pulvinus than when it is distal. The intensity of current effective in causing excitatory impulse in plants is often excessively feeble. Thus a current which cannot be detected by the extremely sensitive human tongue is more than sufficient to initiate excitatory impulse in *Biophytum sensitivum*.

The physiological character of the excitation of the plant by constant current is further demonstrated by the respective reactions of anode and cathode, which are more or less antithetic. This is shown by the characteristic modifications induced in the automatic pulsations of leaflets of *Desmodium gyrans*. Under the expansive reaction of anode-make, the contractile action in the "systolic" phase is opposed, resulting in a continuous diminution of the systolic limit. The contractile effect of cathode-make is exhibited by the opposite reaction of continuous diminution of the "diastolic" limit.

Multiple-responding tissue of plant, like that in the animal, is shown to be thrown into rhythmic excitations under the continued action of a constant current.

The excitability of animal nerve to the stimulus of constant current is enhanced by cooling and depressed by warming. Precisely similar effect is shown to take place in the conducting tissue of *Mimosa*. Here the stimulus of constant current which is effective at low temperature becomes ineffective at high temperature.

The crucial test of the excitatory character of the transmitted impulse is furnished by the action of various physiological blocks which arrest the transmission of excitation.   The local application of increasing cold on the conducting petiole retards and finally arrests conduction of excitation.   As an after-effect of the application of cold, the conducting power is paralysed for a considerable length of time.   The lost conducting power may, however, be quickly restored by tetanising electric shocks.

The excitatory impulse may again be arrested by the action of electrotonic block. This arrest persists during the continuation of the blocking current, the conductivity being restored on its cessation.   Finally, the conductivity of a selected portion of a petiole may be abolished by the local application of poison.   The abolition of conducting power proceeds slowly under the action of copper sulphate solution, and quickly under potassium cyanide.

These results prove conclusively that the transmission of excitation in the plant is a process fundamentally similar to that which takes place in the animal, being, in the one case as in the other, a propagation of protoplasmic change.