

elevation and depression of the centre of gravity of each atmospheric column. Herein may lie the reason why lateral expansive and contractive currents are found to exist.

A reference made by Dr. Andrews, in the course of his inaugural Address to the British Association at the recent Glasgow Meeting, to the work of General Menabrea, appears to give weight to this conjecture. He says—"His great work on the determination of the pressures and tensions in an elastic system is of too abstruse a character to be discussed in this address; but the principle it contains may be briefly stated in the following words:—When any elastic system places itself in equilibrium under the action of external forces, the work developed by the internal forces is a minimum."

December 14, 1876.

Dr. J. DALTON HOOKER, C.B., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read:—

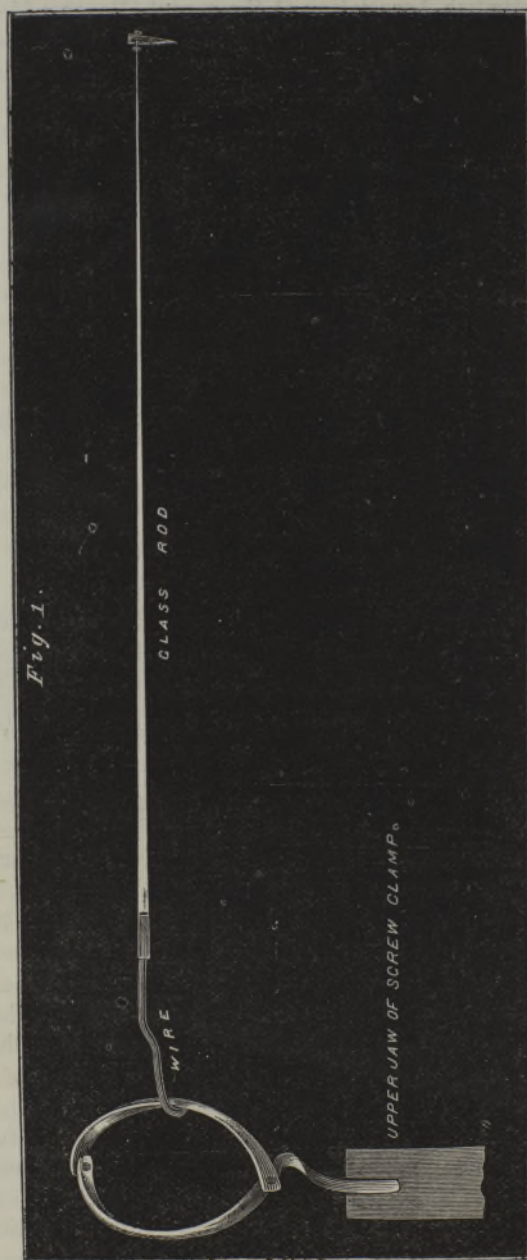
- I. "On the Mechanical Effects and on the Electrical Disturbance consequent on Excitation of the Leaf of *Dionæa muscipula*." By J. BURDON-SANDERSON, M.D., F.R.S., Professor of Physiology in University College, and F. J. M. PAGE, B.Sc., F.C.S. Received November 23, 1876.

Part I.—MECHANICAL EFFECTS.

The mechanism by which the leaf of *Dionæa* closes after mechanical excitation has been already studied by Mr. Darwin and many other naturalists. It was, however, necessary, in order to connect the electrical phenomena which form the principal subject of this paper with this mechanism, to study the successive changes of form which the leaf undergoes in the act of closing. The investigations we have made relating to this subject have brought to our knowledge facts which have an important bearing on the general question of the nature of the excito-contractile process in plants and animals.

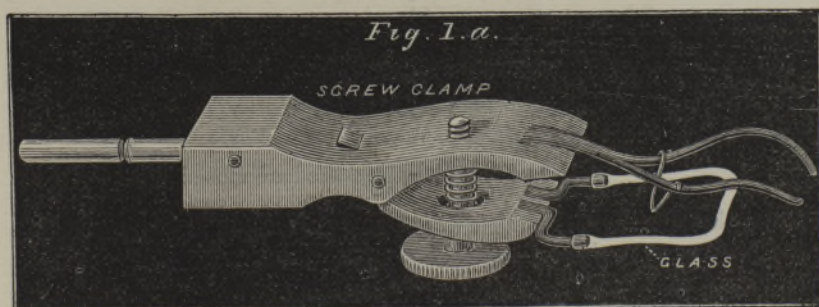
The smooth green outer surface of a leaf of *Dionæa* in full vigour is concave, and the marginal hairs are thrown back so that they are nearly in the same plane with the lobe from the edge of which they spring. If one of the sensitive hairs of a leaf in this condition is carelessly touched the leaf usually closes. If, however, a hair is touched

very cautiously, with the aid of a camel-hair pencil, it can be predicted with certainty that no visible effect will be produced; and a similar gentle contact may be repeated several times before the leaf begins to answer to the irritation by any movement. Sooner or later,



however, the marginal hairs bend inwards, and if the leaf is carefully watched it may be observed that each touch is followed by a slight approach of the lobes to each other. If the observation is continued, it is seen that each approach exceeds its predecessor in extent, until at last the lobes suddenly come together in the manner which is, by this time, familiar to every one.

It being our primary purpose to determine the time which elapses between each several touch and the immediately resulting jerk-like approach, and to measure the extent of such approach, so as to learn how much it contributes to the final result, we made it our first business to devise some method of measurement by which we could verify the conclusions to which we had come from rough observation, namely, that each several excitation of the leaf is attended by some mechanical effect. With this view, we constructed an instrument of the form shown in figs. 1 & 1a. It consists of a hinged screw-clamp by which the leaf is held, as between the thumb and fore finger. On the lower jaw of the clamp, the one corresponding to the thumb, which is made of glass, the midrib of the leaf rests by its under surface. The upper jaw consists of an arch of copper wire, of which the curvature corresponds to that of the margins of a lobe of the leaf. The two jaws meet each other in such a way that when they are brought together by the screw the two ends of the arch are in apposition with the ends of the midrib close to its upper surface. Each end is pierced by a pin: the points of these pins are directed towards each other, so that together they serve as an axis of rotation for a second similar arch of which the curvature is made a little larger than the other, so that it may comprise it. From the middle of the second arch a wire springs at right angles, to which a light glass lever is attached. By means of this lever it can be rotated outwards on its axis, and thus made to diverge from its fellow at any desired angle.



Clamp ordinarily used for holding a leaf during prolonged electrical exploration.

The instrument is used as follows:—A leaf having been placed in position, that is with its midrib resting on the glass support, the two arches are brought down by the screw until they all but touch the trough of the leaf at opposite ends. This done, the arches are made to diverge

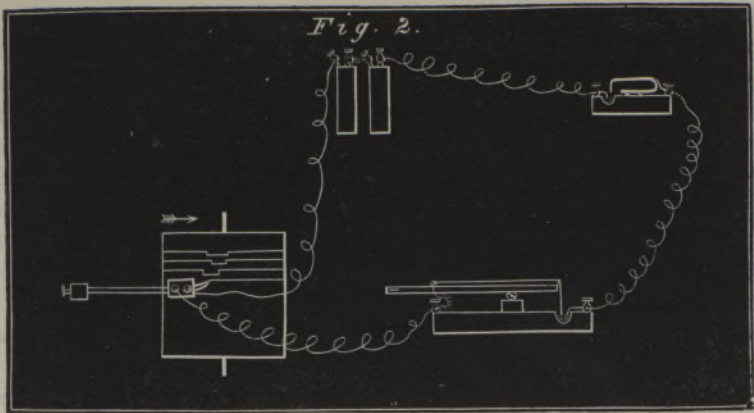
until both are in contact with the internal surface of each lobe close to its border. All that remains is to fix a graduated arc against the lever, to select a sensitive hair for excitation, carefully to touch it at regular intervals, and to record the movements of the lever. Each approach of the leaf is thus indicated in magnified proportion by the end of the lever. The following Table gives the results of an observation made in this way.

TABLE I.

Showing the result of mechanical successive excitations of the hair γ , at intervals of two minutes, continued until the leaf closed.

Number of excitations.	Angular measurement of effect.	Time in seconds which elapsed between contact and the first perceptible approach.
1 to 7	0	∞
8	0	∞
9	0	∞
10	$\frac{1}{4}$	15.5
11	$\frac{1}{4}$	10.8
12	$\frac{1}{2}$	7.3
13	1	5.8
14	$1\frac{1}{2}$	5.0
15	$1\frac{3}{4}$	4.5
16	$2\frac{1}{2}$	5.4
17	3	4.5
18	2	7.6
19	$3\frac{1}{4}$	3.8
20	$3\frac{3}{4}$	3.7
21	$4\frac{3}{4}$	3.3
22	$5\frac{1}{2}$	4.0
23	7	2.7
24	$8\frac{1}{2}$	2.5
25	8	Not observed.
26	10	2.2
27	At the 27th excitation the leaf closed.	

From this experiment, which was repeated several times and always gave similar results, it was learnt:—(1) that the first half-dozen excitations were absolutely without mechanical effect; (2) that the first effectual excitation was followed by so slight a movement that if it had not been enlarged by the lever it would have been imperceptible; and (3) that after this each successive approach of the lobes, in most cases, exceeded its predecessor. The numbers recorded in the third column relate to the time of interval between excitation and effect, and were obtained by the following method.



The apparatus (fig. 2) employed for this purpose consists of:—1, a recording-cylinder; 2, an electro-magnetic chronograph made for us in Paris by M. Verdin, the perfection of which we owe to the kindness of our friend Prof. Marey; 3, signalling-keys, of which one closes, the other opens, the signal-circuit—that of a battery of two Daniells. The cylinder, of which the surface is blackened in the usual way, revolves with great regularity five times per minute, and has a circumference of half a meter, so that the rate of horizontal movement of its surface is two and a half meters per minute (4.166 centims. per second); consequently a hundredth of a second (0.4166 millim.) is readily measurable.

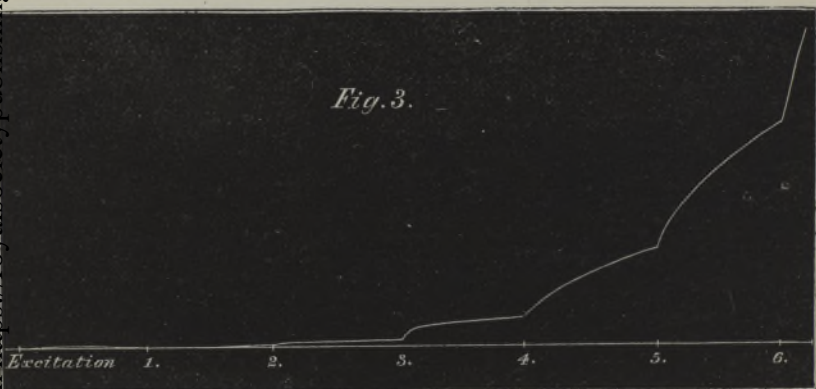
In the present experiment it was necessary that the touching of the sensitive hairs should be accomplished with great gentleness, care, and exactitude, and always in the same manner. The touch in each experiment was made by one observer at an expected signal from the other, the arrangement being that A should count aloud 1, 2, 3, 4, 5, that B should touch at the moment 5 is said, and that at the same moment A should close the signal-circuit, having his eye on the lever, and being ready to break the circuit at the first perceptible movement. Considering that the periods to be measured were of several seconds' duration, this method was quite accurate enough for the purpose.

The time-measurements, as will be seen at a glance, stand in a remarkable relation to the mechanical effects, showing that the delay between excitation and effect diminishes as the extent of the effect increases, both facts having the same meaning—namely, that in the plant, as in certain cases well known to the animal physiologist, inadequate excitations when repeated exercise their influence by what has been termed summation, *i. e.* that when any number of such stimulations, say *a, b, c, d, e, &c.*, follow each other in succession, the effect of each is prepared for and aided by its predecessors: so that although, as in the present instance, *a, b, c, d*, may seem to produce no effect whatever, each of them really produces a change in the excited structure, and each contributes, when

summed with its predecessors and successors, to the bringing about of the visible effect which follows *e*. During the remainder of the process the operation of the same law shows itself in the gradual augmentation of the increments, the last contraction, that by which the leaf closes, being the result of the summation of the excitation which immediately preceded it with all the previous excitations. Our conception of the nature of the process may be otherwise expressed by saying, that under the influence of successive excitations the latent excitability of the leaf gradually increases; for whereas before it either made no response or postponed its response indefinitely, it now answers to the same stimulus by visible motion of which the promptitude and the extent increase together.

In one of our experiments we arranged our apparatus in such a manner as to obtain a graphic record of the successive approaches of lobe to lobe by which closure is ushered in; a reduced, but otherwise accurate, copy of this record is given in fig. 3. It shows a fact which we had already ascer-

Fig. 3.



tained by observation, namely, that in each approach the rate of motion augments rapidly at the beginning, and then very slowly subsides. It was for this reason that we allowed two minutes to elapse between each excitation and its successor; for if the interval were less, the effect of the excitation began before that of the previous one had ceased. In the experiments represented graphically the excitations were repeated every minute, so that the lever was still rising at the moment that each new ascent commenced.

It appeared important to ascertain whether, after the leaf is closed, it still continues to make mechanical efforts. We had already observed that a leaf which is repeatedly excited after closure seems to be clenched with greater and greater force, and we thought it probable that mechanical work would continue to be done by a leaf after closure if it had the opportunity. To test this, all that was necessary was to attach weights to our lever sufficient to keep the lobes expanded. The result of a single

experiment was quite conclusive. Each time, without exception, that the leaf was excited the weight (one gramme at a distance of 10 centims.) was slightly lifted, the extent of movement of the lever varying from one to three degrees.

As regards the interval between excitation and the resulting movement, our observations show that it varies from $2\frac{1}{4}$ seconds (the shortest observed after numerous excitations) to 10 seconds. The last estimate, however, is probably exaggerated; for in the early stages, when the motion is of extremely small extent, it is not possible to determine exactly when it commences. In the later stages this source of inaccuracy does not exist, so that we may confidently take two seconds as the inner limit of the period in question.

Part II.—ELECTRICAL DISTURBANCE OF THE NORMAL LEAF.

Section 1.—*Electrical Condition of the Leaf in the unexcited State.*

The electrical condition of the leaf of *Dionæa* in the unexcited state has very recently been made the subject of a minute investigation by Prof. Munk, of Berlin. He has found (1) that if we conceive the external surface of the leaf divided into strips by parallel lines crossing the midrib nearly at right angles, and coinciding in their direction with the veining, the external surface of each lobe is negative to the midrib; (2) that in comparing different points of the midrib with each other there is one, of which the position is two thirds of the distance from the near to the far ends of the midrib, which is positive to the rest. He has further (3) stated that the potential of any point on the internal surface of the lobe is exactly equal to that of the corresponding and opposite point on the external surface.

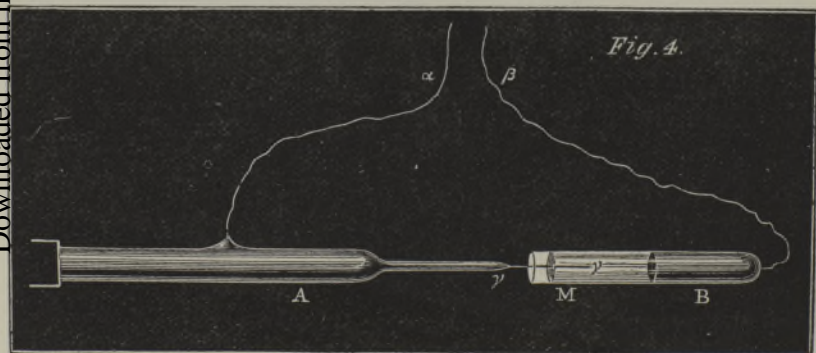
These three statements may be generalized as follows:—On the external surface of the leaf three lines may be distinguished—one which may be called the positive line, which corresponds to the midrib; and two others, the lines of greatest negativity, which lie between midrib and margin and are nearly parallel to the first. Although it is not the special purpose of this paper to investigate this part of the subject, we may state generally that we have found the first two propositions above enumerated to be true in all normal leaves with the following exceptions, namely:—first, that although the central part of the midrib is positive to either end (more positive to the near than to the far end), the position of the point of greatest positivity is not so definite as Dr. Munk states, but differs in different leaves; secondly, that the different points in his isoelectrical negative line are never found to be absolutely identical. As regards the third proposition we are compelled to say, generally, that it is without foundation. We have found that so far from its being generally true that opposite and corresponding points of the two surfaces exhibit the same potential, such identity occurs so rarely and exceptionally, that it may be regarded as abnormal. In a future paper

we propose to discuss the electrical condition of the leaf of *Dionæa* when unexcited as compared with that of other non-excitables leaves. For our present purpose it is sufficient to state as the general result of our observations on this subject, first, that the part of the midrib which lies nearest the two central sensitive hairs is positive to every other part of the external surface of the leaf, but has usually the same potential as the petiole and other inactive parts of the plant; and secondly, that the external surface, so long as the leaf is in vigour, is *always positive to the internal surface*. These two statements, and particularly the second, may be accepted with confidence; but with reference to the first it must be borne in mind that, inasmuch as unexplained differences of potential often present themselves between symmetrical points of opposite lobes, even in leaves which appear to be in a normal state, the determination of the difference between any point and the midrib must necessarily be a matter of great difficulty.

Section 2.—*Method.*

The method employed in the present research differs from that generally used in previous investigations relating to animal or plant electricity in two important particulars, viz.:—first, in the adoption of the electrometer as a means of investigating the electrical changes; and secondly, in the substitution of a constant for a variable potential.

The electrometer used is that of Lippmann. We became acquainted with this instrument through the kindness of Prof. Marey, who had already adopted it in physiological investigations relating to animal electricity. We append the following description of the instrument, referring the reader for further information to the original paper of the author*.



The instrument consists of two glass tubes, A and B, fig. 4, of which the former is drawn out into a capillary point γ , the lumen of which is about $\frac{1}{100}$ millim. At the end opposite the capillary, A communicates

* "Beziehungen zwischen den capillaren und electrischen Erscheinungen von G. Lippmann," Poggendorff's Annalen, 1873, Bd. 149.

with a *cul-de-sac* of thick india-rubber (not shown in the diagram), the cavity of which, as well as that of the tube itself, is, with the exception of a small bubble of air, filled with mercury. The *cul-de-sac* can be compressed by a screw-clamp, so that the mercury in A can be subjected to the pressure required in order to force it through the capillary, or to any less pressure. The tube B also contains mercury at its closed end: the remainder of its cavity is occupied by dilute sulphuric acid, into which the end of the capillary γ is plunged. Each of the two tubes A and B has a platinum wire fused into it, by which the two masses of mercury can be severally brought into connexion with any two surfaces of which it may be desired to compare the electrical condition.

The instrument is prepared for use by first increasing the pressure in A until mercury escapes from γ , and then diminishing it, until it stands at a point previously fixed upon (which may be called the zero point), *care being taken that the wires α and β are in contact with each other*. If, instead of touching each other, any electromotive arrangement or structure is interposed between them, as (*e. g.*) a muscle-cylinder, the mercurial meniscus in the capillary alters its position, retreating from γ if the surface with which it is connected is negative to the other, and *vice versa*. The difference of tension thus indicated may be measured, either by increasing or diminishing the pressure in A, so as to bring back the meniscus to its zero position, and measuring the change of *pressure* required for the purpose ("compensation pressure"), or by measuring the *distance* of the point at which the mercurial column in the capillary stands when the terminals are in contact with each other from that at which it stands when the electromotive structure is interposed. For physiological purposes the latter plan is the most convenient. In order to carry it out, the capillary must be mounted on the stage of a suitable microscope, and furnished with micrometrical arrangements capable of measuring accurately to a hundredth of a millimeter.

The electrical values to be assigned to the measurements so obtained must be learned in respect of each instrument by a preliminary process of empirical graduation.

Each capillary used as an electrometer must be graduated. The graduation is effected by Poggendorff's method of compensation, with the aid of Mr. Latimer Clarke's potentiometer. As a standard cell we have used a silver-chloride element, kindly given us for the purpose by Mr. De La Rue. The coil of our potentiometer consists of 60 turns of platinum wire of 0.26 millim. in thickness. The operation of graduating is rapidly performed; consequently it can be repeated frequently to ensure accuracy. The reasons why for our purpose we preferred the electrometer to the galvanometer in the present investigation are easily made clear. In all investigations relating to the electrical phenomena of plants and animals the object in view is to ascertain in what way any changes in the electrical condition

of a living part are correlated with other vital phenomena; and for this purpose, what is required is always to measure the electromotive force between two points differing in function (in the physiological sense). By the electrometer this measurement is made directly. In employing the galvanometer a result is obtained which acquires no absolute value until another investigation of very great difficulty has been gone virtually through—that of measuring the electrical resistance of the tissues which intervene between the two points investigated. In addition to this obvious reason for preferring the measurement of tension difference to the measurement of current, there are other reasons why the capillary electrometer above described is specially adapted to the purposes of the physiologist. One of the principal is that its indications are sensibly instantaneous, on which account it is admirably suited for the investigation of electrical changes of extremely short duration, and further that it is portable and little liable to be injured by being moved from place to place. A third advantage that it possesses is that it can be made and graduated by the investigator himself. When in addition it is further remembered that for every measurement made with the galvanometer at least ten can be made in the same time with the electrometer with greater accuracy, it does not appear unreasonable to anticipate that the latter will in future be much used for physiological investigations.

The second respect in which our method differs from those previously employed is purely physiological. It had long been known as regards the living animal body that the only tissues which are electromotive are the nervous and muscular. All others behave, so far as has been ascertained, as ordinary moist conductors. Investigated electroscopically they exhibit, so long as they are in the living state, no variation of potential. In the plant it is the same. An ordinary stem of a herbaceous plant has the same potential as the soil in which it grows, or exhibits such trifling variations that it may be said to be constant. In most of the innumerable researches which have been made in the domain of animal electricity since the early discoveries of Du Bois-Reymond, the method has been adopted of comparing the electrical state of the part to be investigated not with some other part of the organism outside of the area of electrical change, and therefore possessing in relation to such change a constant potential, but with some other part of the electrically active organ itself. Thus, in the case of muscle, the cut surface has been compared with the natural surface, the tendon with the muscular surface, &c. These considerations led us to begin our investigation by comparing those parts of the leaf of *Dionæa* which appear to be the seat of electrical change, not with other parts of the same organ, but with the earth or with some other part of the same plant which we had previously ascertained to be electrically indifferent and constant, *i. e.* free from electrical vicissitudes. The way in which this was carried out will be explained in the next section.

The greater number of the observations were made at Kew during the month of August of this year, the plants being obtained from the hot-houses of the Royal Gardens, through the kindness of the Director, and brought to a conservatory in the house in which one of us resided several days before they were used. The parts to be compared were connected with the electrometer by means of electrodes of the same construction as those which we always employ for electro-physiological purposes. Each consists of a U-tube supported by a convenient holder, and half filled with saturated solution of zinc sulphate. Into one arm is plunged a zinc rod, of which the immersed end is amalgamated; into the other a glass tube filled with kaolin made into a paste with 0.75 per cent. solution of chloride of sodium. The upper end of this mass of clay forms a cushion, which can be moulded to any required shape. Electrodes of this form are convenient to work with, and possess manifold practical advantages, the greatest of all being (1) the facility with which they can be renewed in case they are found (by testing with the galvanometer) to exhibit polarity, and (2) the facility with which they can be brought into any desired position.

The arrangement most usually adopted is as follows:—The pot containing the plant had been previously kept plunged in water. Three electrodes are used; by one of them (called the fixed electrode) the damp surface of the pot is connected with a gas-pipe. The other two (called movable electrodes) are in contact with any two surfaces of the leaf which it is desired to investigate. By means of a switch either can be brought into connexion with the larger mercurial surface of the electrometer. The capillary surface is connected directly with the earth. In many of the experiments the capillary surface was connected directly with the fixed electrode.

Section 3.—*General Characters of the Electrical Disturbance.*

When the capillary mercurial surface of the electrometer is connected by the fixed electrode with the surface of the pot or of the petiole, and the movable one is in contact with any part of the surface of the leaf, whether inside or out, the effect of touching a sensitive hair is (with certain exceptions to be mentioned hereafter) to produce a transitory advance of the mercurial column in the capillary tube towards its orifice. Such a movement will hereafter be referred to as a negative excursion. Its extent may be readily measured with the aid of the vertical hair-line which crosses the field of the microscope, and the result recorded in a number which may express either the change of position of the mercurial meniscus in millimeterets, or the change of potential as compared with some unit of electromotive force.

Considering that the value of the direct measurement varies according to the part of the capillary measured, it is clearly more convenient to

write all results in terms of the electromotive force of a standard cell, of which the letter d is understood to denote the hundredth part.

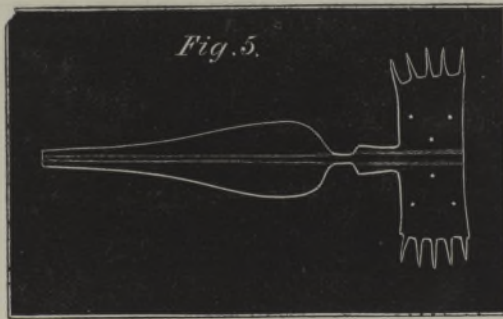
When the whole of the outer surface of the leaf is covered with a mass of clay moistened with salt-solution, and the mass is brought into contact with the movable electrode, the fixed contact being, as before, with pot or petiole, the effect of mechanical excitation is to produce a *negative* excursion, indicating a change of potential at the movable contact of from $3\cdot5d$ to $5\cdot0d$: when a similar plug is applied to the internal surface, so as to cover the whole of it, the result is the same, but the extent of the excursion is somewhat less. Hence it may be generally stated that the electrical disturbance (which, as will be afterwards shown, lasts for about a second) consists in this, that the surface of the leaf becomes more negative as compared with any other surface of which the potential is constant, and that, on the external surface, the change is greater than on the internal.

The electrical disturbance is strictly limited to the surface of the leaf. If, the fixed electrode being in contact with the petiole, the movable one is brought into contact first with the midrib at its middle and then in succession with points nearer and nearer to the petiole until at last the line is crossed which divides the petiole from the isthmus or bridge by which it is united with the leaf, and the leaf is excited after each change of contact, it is found that each excitation is followed by a negative excursion *so long as the contact is on the leaf side of the line referred to*, but that as soon as that line is passed no sign of electrical disturbance manifests itself. Hence if two points of contact, a and b , are selected on opposite sides of the limiting line, and the movable electrode so shifted alternately from one to the other, an excursion of $1d$ or $2d$ is observed in the one case, while in the other the mercurial column remains absolutely motionless. We shall hereafter see that the fact that the bridge or isthmus is electromotive is of importance to the understanding of certain phenomena.

Section 4.—*On the relative Intensity of the Excitatory Electrical Disturbance at different parts of the Surface of the Leaf.*

We have already seen that the area of disturbance does not exceed that of the leaf itself. In our endeavour to limit that area further, or at all events to determine the position of the *centre of greatest intensity*, we have allowed ourselves to be guided by the consideration that as the disturbance itself must be regarded as correlative with the property possessed by the leaf of contracting when excited, that centre is likely to have its seat in the excito-contractile part of the organ. Now there can be little doubt that the excito-contractile property is localized in that part of the internal surface on which the sensitive hairs are planted, and further that the tissue which takes an active part in the changes of form by which that property manifests itself is the parenchyma, of which an

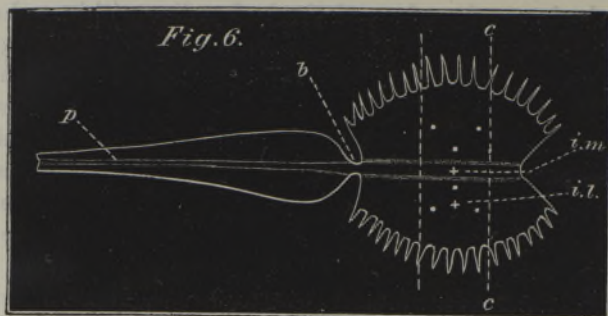
abundant stratum stretches from one lobe to the other across the midrib. This being so, we have a region roughly mapped out for us comprising so much of the internal surface of the leaf as lies within and around the sensitive hairs on either side along with the whole of the space which lies between these parts. If, now, we proceed to contemplate the region thus indicated, in relation to its structure, and limit it by two parallels crossing the midrib and following the direction of the veining, we have the whole leaf divided into three structurally analogous parts, of which the middle part may, with reference to the excitatory changes of form, and therefore, it may be presumed, with reference to the excitatory changes of potential, be regarded as representative of the other two. That we are justified in thus regarding the leaf is rendered strikingly evident by the result of an experiment in which a leaf is mutilated by first cutting away entirely the further third in the line *c* (fig. 6), and then removing all of the nearer zone, with the exception of the midrib, so that the leaf is reduced to the form exhibited in fig. 5. In such a



mutilated leaf the electrical phenomena of the whole leaf are unaltered, there being at first no difference between it and the entire leaf, either as regards the potentials of the surfaces or the changes which are produced by excitation.

It having, on these grounds, been determined that we should direct our attention to the middle third of the leaf, the next step was to fix on contact points for electrical investigation. On the internal surface of each lobe we selected the point (evidently the most important) which is equidistant from the three sensitive hairs, on the external surface the point opposite and corresponding to the first, and similarly two points on the external and internal surface respectively of the midrib, situated in the line of junction of the before-mentioned points on the lobes. We now proceed to state what results are obtained when these several points are investigated, it being understood that each of them exhibits a negative variation when it is compared with some other part of the plant lying outside of the area of excitation. We shall employ the following abbreviations—viz. the letters *l* and *m* to denote the points already indicated

on the lobes and midrib respectively; *el* for the external surface (fig. 6) and *il* for the internal surface of each lobe; *em* the external surface, and *im*



the internal surface of the midrib, and *p* for the petiole. We shall further use the capital letter *P* for potential, and the capital letter *V* for variation of potential, *emP* for the potentials of the outer surface of the midrib, and *emP* - *pP* for the difference of potential between that surface and the surface of the petiole, in other words, for the result of an observation made with the fixed contact at the petiole and the movable contact at *em*.

a. *Mechanical Excitation.*—In four leaves (*a, b, c, d*) observed in succession, the potentials and variations of the external surfaces of the midrib and lobe were severally, in hundredths of the standard cell, as follows:—

<i>emP</i> - <i>pP</i>	0	0	0	0
<i>elP</i> - <i>pP</i>	1.6	0	0	1.6
<i>emV</i>	-5.0	-6.5	-4.2	-4.5
<i>elV</i>	-2.0	-6.5	-4.0	-4.0

The observations given, although taken in succession, exhibit great differences of result. They serve to exemplify the general statement that, as regards the external surface, the variation is greatest near the midrib.

When the variation is compared on opposite and corresponding points, it is almost always found that the external variation exceeds the internal. Thus in nine observations the *elV* and *ilV* were respectively -6.5 (*elV*) and -2.0 (*ilV*), -4.5 and 0, -6.5 and -1.2, -3.5 and -2.0, -3.5 and -1.8, -6.5 and -3.0, -4.5 and -2.2, -5.5 and -2.5, -4.5 and -2.5. The following were the actual results obtained in two of these cases in which successions of alternate measurements were made:—

Leaf *a* (12 measurements) *ilP* - *pP* = -1.2, *elP* - *pP* = 0.

elV = -3.6, -4.0, -4.2, -4.0, -4.0, -4.5.

ilV = -1.5, -1.7, -1.6, -1.8, -2.2, -2.2,

Leaf *b* (20 measurements) *elP* - *pP* = -1.0 - 1.5, *ilP* - *pP* = -3.5.

elV = -5.0, -6.2, -6.5, -5.5, -5.1, -5.0, -4.8, -4.9, -4.9, -4.9.

ilV = -2.0, -2.0, -2.0, -3.0, -3.0, -2.1, -2.5, -2.5, -2.5, -2.4.

Similarly in a vigorous leaf specially investigated with reference to the internal and external surfaces of the midrib, the results were as follows:—

$$imP - pP = -3.0, emP - pP = 0, imV = -3.0 \text{ to } -3.5, emV = -5.5.$$

In at least a dozen excitable leaves which we have investigated with reference to the conditions of the external and internal surfaces, we have met with no exception to the statement made above as to the predominance of the external variation. But in two leaves which, though apparently healthy, failed to close when excited, the external variation not only did not exceed the internal, but was actually positive, ranging from $0.5d$ to $1.0d$, the internal variation being normal, *i. e.* negative, and ranging from -0.5 to -1.2 . In both instances the potential of the outer surface was unusually negative, both as compared with the petiole ($elP - pP = -4.5d$) and as compared with the opposite internal surface ($ilP - pP = -3.5d$).

These were the only instances in which the external surface exhibited a positive variation. Other leaves, however, were observed in which the same condition presented itself on the internal surface, and was again associated with an extremely negative potential. Thus in leaf 51, in which the internal variation was $+1.2d$ and the external $-3.5d$, the difference of potential was $3.8d$; and in leaf 52, in which the internal variation was $+6.0d$ and the external $-3.5d$, the difference of potential was $4.5d$.

Before stating in what way we think that these apparent anomalies may be explained, we must give an account of a phenomenon of the same kind but of much more frequent occurrence. In perfectly fresh leaves, *i. e.* in leaves which have not yet been touched, it sometimes happens that when a single hair is excited at regular intervals, the first few excursions obtained with the movable contact on the internal surface of the lobe differ from the succeeding ones in being preceded by what may be provisionally called a preexcursion in the opposite direction. Thus in leaf 22 the first effect was a mere shudder of the mercurial column, followed by a very small negative excursion. The second was larger ($ilV = -1.2d$) preceded by a very perceptible movement of the mercury in the opposite direction. The next two were still larger, but the preexcursion was still perceptible. After this the excursions were purely negative. Three other instances were met with of the same kind.

That any part of the surface of the leaf should exhibit a positive excursion seems, at first sight, inconsistent with the statement which has been made that the variation both of the external and internal surface of the leaf is negative. That it is not so, may, however, be readily understood from the following considerations.

We have already seen that the petiole or other part of the plant with which the fixed electrode is directly or indirectly in contact is electrically indifferent, or, in other words, serves the purpose of an ordinary moist conductor, just as if it were part of the clay plug by which the contact

is made. By means of the petiole, the whole plant and the electrode which is applied to it and the capillary tube are in contact with an electromotive part of the leaf, namely, the "bridge" already mentioned. The bridge, as we have seen, is within the area of electrical disturbance, and participates in the general negative variation; but by reason of its position, *i. e.* of the fact that it is virtually in contact with the fixed electrode, and through it with the capillary mercurial surface, its action on the electrometer, is opposed to that of the surface to which the movable electrode is applied. Supposing the variation at the two contacts to be simultaneous and to have the same sign, they must counteract each other. In general the variation at the surface of the leaf far exceeds that at the bridge, so that the sign of the excursion is purely negative; but whenever the disturbance in the neighbourhood of the bridge happens to exceed that which occurs at the movable contact in intensity, the excursion becomes partially, or entirely, positive. That this is so may be easily shown by the following experiment:—If in a leaf of which the internal lobe-variation is negative, the fixed contact is shifted from the petiole to the end of the midrib next the bridge, it is observed either that the excursion diminishes or becomes at once positive, or that there is a short negative excursion followed by a longer positive one, this signifying that although at first the lobe-variation has the better, it yields immediately after to the more intense disturbance at the midrib.

The principle thus illustrated applies not merely to the particular case now under consideration, but to every case in which the difference of potential is measured between any part of the surface of the leaf and any part of the surface of the plant assumed not to be electromotive. In every such case it must be assumed that the difference measured represents not the whole of the electromotive force exerted at the surface of the leaf, but only so much of it as is in excess of the electromotive force exerted at the end of the midrib next the bridge.

We propose to apply the term "interference excursion" to the effect observed whenever, as in the experiment referred to at the close of the last Section, contact is made with both electrodes *within the area of disturbance*; so that the electrical state of the surface is compared, not with another surface of which the potential is constant, but with a surface which is itself a seat of change.

The characters of such excursions are much more complicated than those we have hitherto considered—a fact which can be readily understood when it is borne in mind that, as will be shown in a future section, the electrical disturbance, although it exhibits the same general character everywhere, does not either begin or attain its maximum at the same moment in different parts of its area. To a great extent the characters of interference excursions may be understood, if along with the want of synchronism the differences of intensity of the changes going on at the two contacts during the period of disturbance be taken into account;

but it will be readily understood that, in some instances, the conditions are so complicated that we can scarcely hope to explain their operation satisfactorily or completely*.

b. *Electrical Stimulation*.—In the experiments of which the results were communicated to the Royal Society in 1873 it was found that the passage of induction-shocks in rapid succession (faradization) through the tissue of the leaf in the immediate neighbourhood of the external surface of a lobe produced effects which resembled those which follow mechanical excitation. It also appeared that this mode of excitation was exhausting; for although a leaf could be excited at 2 minutes' intervals for an hour or two without any appreciable impairment of its excitability, it soon ceased to respond when excited very frequently.

During the last summer we have confirmed these observations, using not only faradization but single induction-shocks. For both purposes we have found it convenient to employ as exciting electrodes steel needles sheathed in glass and bound together so that their exposed points were about a millimeter apart. These points were thrust through the epidermis of the leaf either on the internal or external surface.

For the production of single closing shocks the primary circuit of Du Bois's induction-apparatus (that of a single Daniell) was closed at regular intervals by a Donder's fall-apparatus (a modification of Pflüger's), the opening shocks being suppressed by a suitable contrivance. The experiment was begun with a fresh leaf and with the secondary coil at a distance of from 15 to 20 centims. The electrometer indicating that there was no effect, the distance was gradually shortened until the electrical disturbance took place. The excitation was then repeated at one minute intervals until the leaf ceased to respond. It was always found that it was necessary to push up the secondary coil to within a couple of inches of the primary—in fact, to use induction-currents of such strength as would be sufficient to awaken reflex action from the cutaneous surface of the frog. There was an appreciable delay between the passage of the induction-shock and the electrical disturbance which resulted from it, a delay of which the duration varied according to conditions to be discussed in the next section.

When a leaf is excited at intervals of a minute, or oftener, by closing shocks which are of just sufficient intensity to produce a response, it invariably happens that after a time the effect ceases, *i. e.* that the mercurial column remains motionless. The effects can, however, be reproduced either by shifting the needle-points to a new spot or by diminishing the distance of the secondary coil. The same result can be attained without interfering either with the coil or the electrodes, by allowing the leaf to rest for a longer interval.

* Compare Du Bois-Reymond, "Ueber die neg. Schwankung des Muskel-Stromes" (*Arch. f. Anat. u. Physiol.* 1873, p. 549). In this paper is described an analogous effect (*doppelsinnige Schwankung*) observed in the gastrocnemius and triceps of the frog.

We next proceed to compare the effects of single shocks with that of faradization. The difference is striking. If the same experimental method is employed as before, with the exception that each excitation consists of the passage through the tissue not of a single induction-shock, but of a rapid succession of such shocks in alternately opposite directions, it is found that an effect is obtained with currents of relatively small intensity, so that if the experiment is begun with the coil at 20 centims. distance, and pushed up a centimeter at a time, the leaf begins to respond after one or two approximations.

This observation appeared to us to indicate plainly that, with relation to stimuli, the excitability of the leaf resembles that of the terminal organs of the higher animals, with reference to which it has been lately ascertained that they can be so excited as to awaken reflexes by relatively feeble electrical stimuli, if applied at very short intervals and repeatedly. We therefore proceeded to make experiments in which each excitation consisted of a definite number of induction-shocks in rapid succession. The results were in accordance with our expectations. It having been ascertained that with the electrodes in a certain position a single instantaneous contact had no effect when the secondary coil was at 7 centims., but was followed by an excursion when it was pushed into 6.5 centims., it was set once more at 7 centims. Again a single contact was without result; two such contacts at $\frac{1}{5}$ -second intervals were also futile; but after 3 contacts there was an excursion. This experiment having been several times repeated, the coil was pushed back to 8 centims., and successive experiments were made with series of 2, 3, and 4 contacts at $\frac{1}{5}$ intervals, which were in each case without effect. After 5 contacts, however, there was an excursion. On now interposing the vibrator of the induction-apparatus, of which the rate of vibration was 30 per second, it was found that an excursion was readily produced at a distance of 11 centims., thus showing that the feebler the excitation the more frequently must it be repeated in order that a result may follow.

These facts afford the key to the understanding of the phenomena when the leaf is excited at short intervals by faradization, the excitation being continued each time until an excursion is produced. When this plan is adopted we have the opportunity of observing the combined influence of summation and of gradually increasing exhaustion. At first the leaf responds after eight or ten excitations, and a series of results present themselves quite analogous to those related in the preceding paragraph; but as the tissue immediately surrounding the electrodes loses its excitability, the number of excitations required to awaken it to action rapidly increases, the effect being postponed for longer and longer periods until it finally fails to occur.

Thus, in eleven successive series of excitations at one-minute intervals,

the number of double shocks which preceded the excursion increased as follows:—

18, 18, 20, 23, 21, 24, 24, 27, 33, 32, 74.

After the latter no further effect was producible.

The result may be thus interpreted. Each excursion is an effect to which each of the induction-shocks which precedes it contributes. At first the summation is rapid; for the tissue traversed by the currents is still prompt to enter into that molecular change of which the excursion is the visible sign. As this promptitude to change or, as it might be called, explosiveness, diminishes, the number of individual stimuli required in order to bring about the discharge becomes rapidly larger and larger, until at last the result is indefinitely postponed.

When a leaf is excited at regular intervals by single shocks of such intensity as to be just beyond the limit of adequacy, so that the slightest diminution would render them futile, it is sometimes observed that the effects become rhythmical. Thus in a series of 54 successive excitations at half-minute intervals, we obtained the following results:—Excitations 1, 2, 3, and 4 were effectual; but of the sixteen excitations following, every other was futile, the alternate ones being followed by excursions; then followed during 8 minutes a series of futile excitations, after which the leaf was allowed to rest for 2 minutes. On resuming, the alternate rhythm again appeared for six excitations, then becoming modified so that an excursion followed every fourth instead of every third excitation, a state of things which continued for a quarter of an hour. In other instances the same tendency showed itself, but less distinctly, the usual result being (as has been already stated) that no further effect was produced so long as the same spot was acted upon by currents of the same intensities.

The fact that by changing the seat of insertion of the needle-points the excursions could at any time be reproduced we regard as of importance, as showing that the excitability of the plant is a property possessed, so to speak, independently by the protoplasm of every cell in the excitable area. When, after repeated excitations at any particular point, effects cease to manifest themselves, their absence denotes, not that the whole leaf is exhausted (for if it were so, change of insertion would not renew them), but merely that the excitability of the tissue in the immediate contact with the needle-points has been blunted.

Section 5.—*The Electrical Disturbance considered in Relation to the Time which it occupies.*

It has already been stated that the change of form consequent on excitation does not begin until electrical disturbance is entirely over. In other words, the latter occupies a period during which, while no visible changes are taking place, molecular changes must certainly be in progress in the excited part—a period which, with reference to muscle, Du Bois-

Reymond has fitly termed the "period of latent excitation." In the plant the time occupied by this preparatory and invisible change is, as might be expected, many times as great as it is in animal muscle; and in consequence of this greater prolongation the electrical phenomena, the only ones with which we are as yet acquainted, which accompany it can be studied with much greater completeness. In muscle the electrical disturbance begins, according to the researches of Bernstein, confirmed by Du Bois-Reymond, about 0''·005 after electrical excitation of the nerve*. In the plant this period, which may be called the period of electrical delay, is always of perceptible duration, and may last over a second. To measure it we have employed the apparatus previously described as used for investigating the time of commencement of the first mechanical effects, with the exception that the signalling-key is so modified that the same act which closes the signal-circuit excites the leaf. This is effected by fixing to the spring of the closing-key a lead wire which carries at its end a fine camel-hair pencil, so that when the spring is depressed contact is made, and the hairs of the leaf are touched at the same moment.

The time which intervenes between excitation and the beginning of the electrical disturbance varies in different leaves according to their vigour, but is very much affected by variations of temperature. In summer weather and with normal leaves the variation on the external surface at the midrib, or at the outer surface of the lobe, when the sensitive hairs on the same side are touched, is found to begin about one eighth of a second after excitation. When the opposite hairs are excited, the period is increased to a quarter of a second. In six fairly normal leaves under various conditions, in which the delay was measured with the fixed contact on the petiole, and the movable one on the outer surface of one lobe, the results given in the following Table were obtained.

TABLE II.

No. of Leaf.	Date.	Mean temperature of day.	Hairs of same side excited.		Hairs of opposite side excited.	
			Mean delay in seconds.	Number of observations.	Mean delay in seconds.	Number of observations.
<i>a</i>	Aug. 10	64·8 F.	0·13	4	0·24	3
<i>b</i>	" 11	62·4	0·09	4	0·23	2
<i>c</i>	" 15	76·2	0·17	4	0·23	3
<i>d</i>	" "	76·2	0·34	4	0·51	3
<i>e</i>	" 21	64·7	0·23	14	0·56	6
<i>f</i>	Oct. 23		0·43	3	0·65	3

In six other leaves in which the delay was measured at the outer surface of the midrib, the mean results were as follows:—

* Du Bois-Reymond, *loc. cit.* p. 575.

TABLE III.

No. of Leaf.	Date.	Mean temperature of day.	Mean delay in seconds.	Number of observations.
<i>g</i>	Aug. 21	64.7 F.	0.24	5
<i>h</i>	" "	64.7	0.26	13
<i>i</i>	Oct. 11	57.7	0.23	3
<i>k</i>	" 19	56.4	0.23	3
<i>l</i>	" 21	46.4	0.48	6
<i>m</i>	" 22		0.33	2

Combining the numbers contained in the two Tables, we have 0.295 as the mean delay at midrib, 0.231 as the mean delay at the outer surface of the lobe when the seat of excitation is close to the contact at which its effect is observed, and lastly 0.403 as the delay when the electrical disturbance has to make its way through the midrib from the opposite side of the leaf; so that 0.17, or one sixth of a second, is the time required for the transmission of the effects from one side to the other. All the observations on which these numbers are founded were made by mechanical stimulation. In a few experiments we substituted stimulation by single induction-shocks, modifying our apparatus by introducing the electro-magnetic chronograph into the primary circuit, so that the moment of its closure was recorded on the cylinder. The results we obtained were remarkably uniform, and confirmed those already recorded. Thus in four observations in which the needle-points were inserted into the outer surface of one lobe close to the movable contact, the intervals were respectively 0.25, 0.25, 0.28, 0.25. When they were inserted into the opposite lobe of the same leaf they were 0.42, 0.49, 0.52, 0.48, 0.56, 0.45, 0.54, 0.52, 0.50, 0.46, 0.55. The mean gives 0.18 as the time which the variation takes in order to cross from one side to the other. If we assume the distance thus traversed by what we may call the wave of negative variation to be 8 millims., more or less, we have the rate of propagation about 4.4 centims. per second, that is 600 times as slow as in nerve. This estimate is, no doubt, too low; for some of the observations were made in cool weather, and we now know that the process is much affected by temperature. If we take as our basis, observations made under the most favourable circumstances in this respect, we have for the outside of the leaf close to the seat of excitation 0.13, and for the opposite side 0.24, which gives 0.11 instead of 0.18 as the time required for transmission. In one instance, indeed, we observed by repeated careful measurements as short a period as 0.06 under normal circumstances. In the experiments to be mentioned in the next section, when the leaves were artificially warmed, the delay was similarly abbreviated. This subject requires further investigation*.

* It is to be remembered that the measurements were not made in the hothouse, but in an ordinary room. At the time they were made we were not aware of the remarkable influence of temperature.

In the few cases in which we determined the moment at which the variation commenced (by mechanical stimulation) on the internal and external surface of the leaf respectively, we found that there was a difference in favour of the outside. Thus in one leaf (89) the delay at the outer surface of the lobe was 0·18, at the inner 0·30, at the outer surface of the midrib 0·34, and at the inner 0·38.

When, however, electrical stimulation is used, the difference becomes very obvious. In one of the experiments already referred to, relating to faradization with weak currents, we employed two movable electrodes, one on the external, the other on the internal surface of a lobe of a leaf, the fixed contact being on the petiole, and the needles being inserted into the opposite lobe. When by this means the excursions were taken alternately from the outer and inner surface, it became very obvious that in every instance the outside excursion began first. At the commencement of the experiment when the condition of the excited part remained fairly normal, the delays in seconds were as follows:—

Outside	0·48, 0·50, 0·52, 0·65, 0·49.
Inside	0·71, 0·61, 0·68, 0·75, 0·95.

As exhaustion came on, the difference was more and more obvious. The following observations were made at intervals of a minute:—

Outside	0·69, 0·82, 0·93, 0·78, 0·98, 2·26.
Inside	0·89, 0·99, 1·13, 1·08, 1·43, 3·57.

After which no further results were produced until the distance of the secondary coil was reduced from eight to seven centims. On then exciting every half minute the results were:—

Outside	0·56, 0·66, 1·32.
Inside	0·71, 1·43, 1·89.

After bringing the coil to six centims. we had:—

Outside	0·60, 1·08.
Inside	0·82, 2·07.

When, instead of investigating contacts comprised within the central parts of the leaf, a comparison is made between the time at which the excursion begins at the centre with that at which it becomes appreciable towards the margin of the area of excitation, delays very much greater than those we have been considering present themselves. Thus, when mechanical stimulation is used, and the two movable electrodes are placed severally on the under surface of the midrib, a little beyond the middle and on the bridge, the delays were:—

On the midrib	0·27, 0·25, 0·22, 0·26, 0·32, 0·32, 0·32, 0·25, 0·34, 0·18;
On the bridge	0·66, 0·56, 0·55, 0·52, 0·51, 0·50, 0·70, 0·74, 0·49, 0·56,—

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giving, as means, for the midrib 0·27, for the bridge 0·58. In another leaf similar comparisons were made between the excursion at the outer surface of the left lobe opposite the hairs and the bridge, with these results :—

On the outside 0·26, 0·24, 0·12, 0·18.

On the bridge 0·87, 0·65, 0·85, 0·83.

In these instances the distance traversed by the wave of negative variation was not even so great as in the cases in which its progress was investigated from one side of the leaf to the other; so that it might, at first sight, be inferred from the much greater prolongation of the delay that the rate of transmission was slower towards the root of the leaf than across it. It is possible that it is so, but it cannot be inferred from the measurements; for in the case of the observations relating to the bridge two excursions are compared of very different intensity; and when this is the case the weaker one appears to be behind the other, and is, in fact, seen last even when the two culminate simultaneously.

The time at which the mercurial column reaches its furthest point (acme of excursion), and the time at which it returns to its original position, have been severally determined in a considerable number of instances in normal leaves. The results are embodied in the following Table. All of them relate to observations in which the fixed contact was at the external surface of the leaf, viz. in four cases on the outer surface of a lobe, in four cases on the midrib. In all the cases the excitation was mechanical, and the hairs touched were in the immediate neighbourhood of the contact.

TABLE IV.

Time, in seconds, after excitation of

	No. of leaf.	Beginning of excursion.	Maximum of excursion.	End of excursion.	Number of observations.
Outer surface of lobe.	<i>a</i>	0·17	Not observed.	2·16	7
	<i>b</i>	0·12	1·06	2·01	4
	<i>c</i>	0·20	0·99	1·81	8
	Do. diff. contact.	0·22	1·44	2·69	7
Midrib.	<i>a</i>	0·27	Not observed.	1·51	7
	<i>d</i>	0·19	Do.	1·59	4
	<i>e</i>	0·17	1·34	Not observed.	3
	<i>f</i>	0·23	1·46	2·22	3
	Means	0·19	1·26	1·99	

From this Table the general conclusion may be drawn that in normal leaves, in which the excursion begins to be appreciable by the electrometer at the external surface, at about a sixth of a second after mechanical excitation, the excursion attains its maximum in one second, and that its

return occupies about the same time, so that we may regard two seconds as the duration of the whole process of latent stimulation. We have already seen that the mechanical effect of excitation, viz. the change of form of the leaf, does not begin until at least two seconds and a half after the excitation; consequently it may be concluded that in every instance the electrical effect is entirely over before the mechanical effect begins.

Section 6.—*Influence of Temperature.*

The conspicuous analogies which, throughout our investigations, have presented themselves between the phenomena of excitation in the leaf and those which occur in nerve and muscle, rendered it of great importance to ascertain whether those physical agents which are known to exercise a decisive influence on the excitation process in animal tissue have a similar influence on that in the plant. This subject we propose to investigate more fully next season. In the mean time we submit the results of two experiments, either of which would be sufficient to show that the influence of temperature on the plant corresponds completely with what is known as to its effect on the contractile tissue of animals.

In each case a leaf, which for this purpose was necessarily detached from the plant, was investigated at the ordinary temperature of a room, then placed for twenty minutes in a chamber warmed to 45 C., and then cooled by placing a block of ice in its neighbourhood. The results of the chronometrical comparison of its condition, under these circumstances, are as follows:—

TABLE V.

Time, in seconds, after excitation of

	Beginning of excursion.	Maximum of excursion.	End of excursion.	
Expt. 1.	{ Leaf at ordinary temperature, } 18°-20° C.	0·22	0·86	Not observed.
	{ After 10 minutes in chamber.....	0·17	0·51	" "
	{ After 22 minutes in chamber.....	0·12	0·42	" "
	{ Cooled 5 minutes.....	0·29	0·92	" "
Expt. 2.	{ Leaf at ordinary temperature ...	0·23	1·46	2·2
	{ After 20 minutes in chamber.....	0·11	0·79	1·37
	{ Cooled 5 minutes.....	0·44	1·48	3·1
	{ Cooled 20 minutes	0·44	1·68	2·94