Botany. — *"A method of recording growth under various external influences"*. By V. J. KONINGSBERGER. (Communicated by Prof. F. A. F. C. WENT).

(Communicated at the meeting of November 26, 1921).

Introduction. Since the researches of BLAAUW [1]) the problem of the influence of "stimulus" on growth has called the attention of the investigators. Hitherto BLAAUW's experiments have only been affirmed and extended by others on the base of the "photo-growth-reactions". BLAAUW's own method was always used i.e. determining growth by means of a horizontal microscope in weak red light, other external conditions being kept as constant as possible. That even this method is not quite perfect has been shown lately by Miss CL. ZOLLIKOFER [2]), who found that weak red light too exerts its influence upon growth.

Moreover the method of microscopical measurement would fail entirely for research concerning the influence of gravity upon growth as, e.g., with this method, accurate definition of growth on a clinostat is impossible.

Preliminary experiments, done with the same apparatus with which Miss ZOLLIKOFER has worked (magnifying power 90 $\times$) made it clear, that even errors, due to physical causes (vibrations, etc.) are not excluded. Since irregular values in measuring growth of an Avena-coleoptile were obtained, the latter was replaced by a micrometer-slide. Having focussed the microscope at a fixed line on this micrometer, the position of this line was determined every three minutes. Instead of an unvariable position, the following variations were found during half an hour:

$+12$; $+8$; -15; ± 0; $+3$; -10; $+7$; $+15$; -12; $+7\,\mu$.

A small horizontal adjustment of the microscope, necessary at this enlargement at the slightest nutation of the seedling, led to errors varying from $+52$ to -68 μ.

The impossibility of measuring growth on a clinostat and in full darkness with this set of apparatus is clear. Moreover the larger

[1]) A. H. BLAAUW. Zeitschr. f. Bot. 1914, **6.**, id. 1915, **7.**, Meded. v. d. Landb. Hoogeschool, Wageningen, 1918, **15.**

[2]) CLARA ZOLLIKOFER. Proc. Kon. Akad. v. Wet. Amst. Vol. XXIII. 1920, N⁰. **4.**

or smaller, but inevitable, observational errors must be added to the occurrence of physical disturbances.

These considerations have given rise to the planning of an autographical method.

In literature only one apparatus, used for these purposes, occurs; namely that of Bose and Das [1]), who transmit growth on a smoked plate, moved on by clockwork, by means of a lever-system at a magnification of 1000 to 10000 times. This crescograph can only record growth for a very short time, but the authors are not concerned with this factor, as the growth is so highly magnified. That observation-time is, however, of great importance may follow from the fact that the changes in velocity of growth, caused e.g. by light, are extended over a very long region of time. Further the test-plant is fixed on the elaborate lever-system by means of a thread, whereby the chance of physical disturbances is enlarged.

Moreover, this apparatus too is not adapted for use on the clinostat. So another kind of auxanometer was constructed in order to evade the errors mentioned above.

The principle of the apparatus. The growing test-plant closes a very weak electric circuit, by means of a refined contact, mounted on a micrometer-screw. This screw has a pitch of 0,5 m.M.; at its end is fixed a cogwheel with 100 teeth. The weak current, closed by the plant goes through a relais of high sensibility, which closes a stronger circuit. This stronger current moves on the cogwheel one, two or more teeth by means of an electro-magnet.

The screw is turned in this way $^1/_{100}$, $^1/_{50}$, etc. around its axis, the contact thus is raised resp. 5, 10 or more μ. The plant has to grow 5, 10 or more μ before it closes the circuit again.

Meanwhile a recording-pen on a rolling carriage-frame is moved on with a velocity of 1 m.M. a second by an electric clockwork (connected with a second-pendulum) along a non-moving recording-drum. The pen thus writes a straight line. At the instant of contact-making by the plant, the pen leaps back and arriving on its starting-point the drum is turned over a distance of 1,5 m.M. and directly the pen begins to write again with the same velocity. In this manner a series of lines will arise, while the length of each of them, measured in m.M. gives the time, in seconds, needed by the plant for growing 5, 10 or more μ. The curve, connecting the tops of these lines reproduces the course of growth. The machine records

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[1]) Sir J. C. Bose and G. Das. Proc. of the Royal Soc. of London, Series B, Vol. XC, 1919.

how long a time is wanted for a certain increase, in contrast with the "microscopical" method, where direct growth in a certain lapse of time is measured.

When this apparatus was practically constructed, my attention was called to a paper by BOVIE[1]), who has already put into practise the same device for an auxanometer. His method has several disadvantages. In the first place the linkage by means of an invar-thread is detrimental to the plant and in the secoud place the connection between plant and apparatus is by no means a rigid one. The chief disadvantage, however, lies in the fact that the plant itself closes the circuit, which effects the upward movement of the contact. This current, activating an electro-magnet, must be of a rather high voltage. Therefore it, inevitably, will emit sparks at the opening of the circuit. These sparks will burn the contact-metals, causing an inconstancy in the place of contact. BOVIE's auxanometer records on a drum, revolving at a velocity of **1** m. M. a minute. At each contact a pen makes a check on the drum. The distance between two checks thus corresponds to the time, wanted for a certain growth. Apart from the tedious counting of checks and measuring their distances, the slow movement of the drum leads to high errors in taxation.

Moreover, BOVIE didn't design his machine for our purposes; it meant to be a precision-machine for demonstration. Some years afterwards he describes[2]) a simplification of his apparatus, whereby, however, it has lost much of its accuracy.

To obtain a high grade of accuracy, many obstacles had to be overcome. The whole apparatus has been constructed by Mr. P. A. DE BOUTER, mechanic at the Botanical Laboratory of the University of Utrecht, from whose knowledge of engines the writer owes many ideas. The writer wants to render him his best thanks for the constant energy, with which he carried out his work.

The Auxanometer is mounted on a working-axis (**1**) (See fig. 1), 18 c.M. in length, that may be fixed on a clinostat-table (**2**). In order to revolve the plant vertically in regard to its revolving-axis, a side-axis (**3**) may be fixed on the working-axis (1). The test-plant, growing in a little pot of zinc (3 c.M. high and 4 c.M. diameter) may be fixed on a movable little table (**4**) by means of a handle (**6**) of the cover.

This handle (**6**) is fixed by a single screw (**7**), so that there is an unmovable connection between plant, pot and table.

[1]) W. T. BOVIE. Bot. Gazette, 1912. **53**.

[2]) W. T. BOVIE. Am. Journal of Bot. 1915. **2**.

This table (**4**) may be fixed on the auxanometer by the upward movement of handle (**5**). There is an opening in the cover for the plant and on that cover three little mirrors have been fixed for

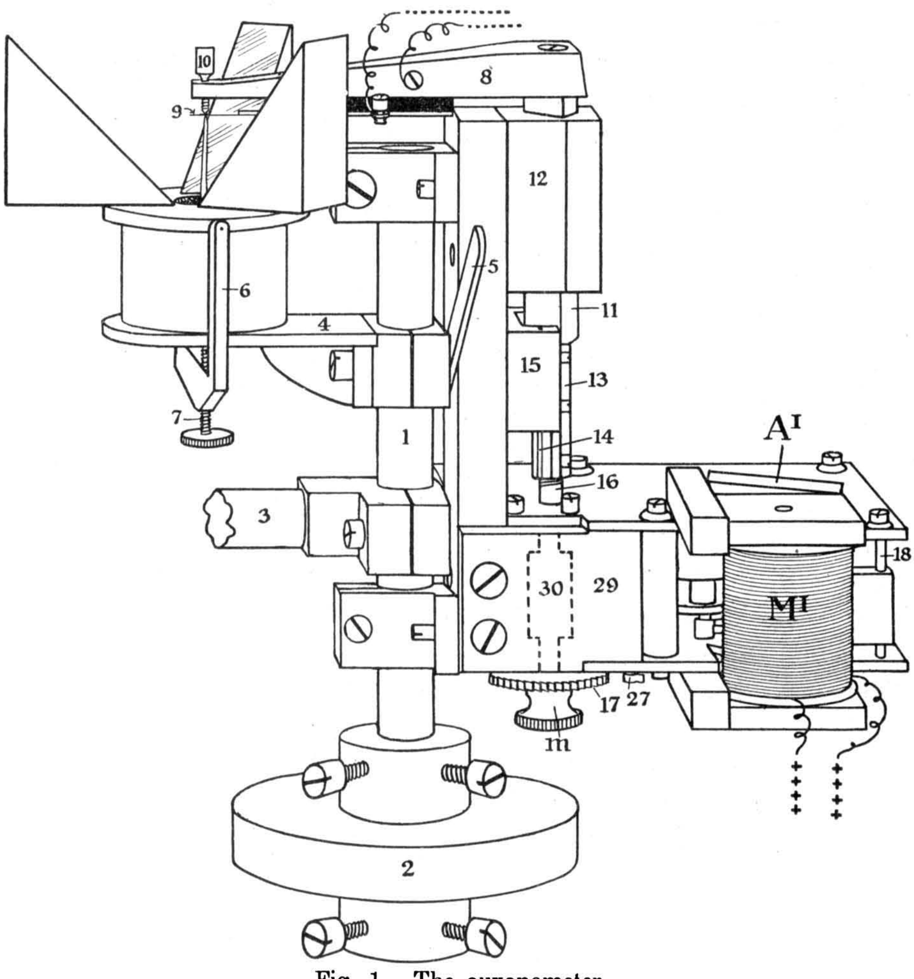

Fig. 1. The auxanometer.

exposition to light. The plant is drawn up exactly under the centre of the contact-device (**9**). On the brass piece (**8**) a small plate of brass has been mounted, isolated by ebonite, at which has been soldered a thin platinum strip (**9**). On this platinum strip lies a minute piece of polished gold, just opposite to a screw (**10**) in the brass piece (8) in the end of which has been soldered a fine point of platinum. This apparatus has been carefully made. It is an imperative condition that the platinum strip should yield very lightly to pressure and yet be elastic. If the screw (**10**) is at the minimum distance from the golden plate, without making contact, a weight of 2 mgr. on the platinum strip is sufficient (in inverse position) to close the circuit. Furthermore the experiment must be stopped, as soon as the plant makes nutations; or grows in a wrong way. Therefore the platinum strip is narrow and allows but an excursion of the plant-top, less than **1** m.M.

The brass piece (**8**) has been mounted on a hexagonal brass prism (**11**) that runs true up and down in a well-fraised closed bearing (**12**). This prism (**11**) is linked by means of an internal strong spiral spring with a split-nut (**13**). In order to get a straight up and down movement this nut (**13**) runs by means of side-wings (**14**) in slits (**15**). The precision-micrometer-screw (**16**) runs in the nut (**13**). In the direction of the screw is saved a cylinder of larger diameter (**30**). Both screw-axis and cylinder (**30**) fit in a block (**29**). The turning of the screw-axis will raise split-nut (**13**), prism (**11**) and the brass piece with contact-device (**8**). At the lower end of the screw-axis under the block (**29**) a cogwheel has been mounted with 100 teeth (**17**) at such a rate that the contact is raised, when the wheel is turned in the direction of the sharper edges of the teeth.

In the beginning stage of the work, an energic electro-magnet

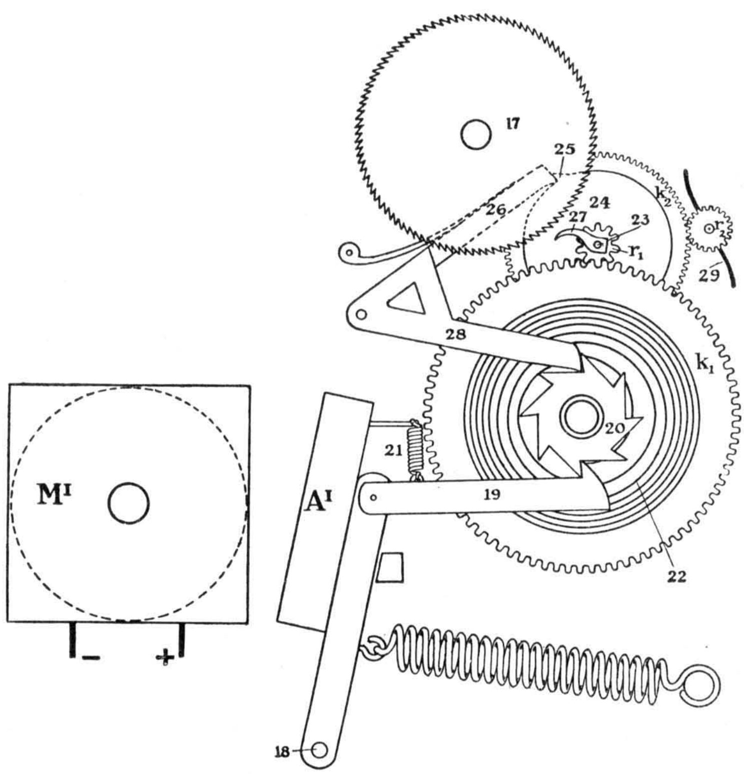

Fig. 2. Explanation in the text.

pulled on this cogwheel by means of an armature with a pawl. A spring drew back this armature just as far that, with the next closing of the circuit, the cogwheel should go on one, two or more teeth. This simple method, however, was not reliable, as the wheel sometimes turned too far, after the shocklike movement.

This error could only be eliminated by a rather complicated mechanism, ingeniously devised by Mr. DE BOUTER. Each time, when a current passes through the coil of electro-magnet (M^I) the armature (A^I) is attracted. This armature turns around an axis (**18**). (See fig. 2). On the armature is fixed a lever with a tooth (**19**), pressed against cogwheel (**20**) by means of spring (**21**). On the axis of cogwheel (**20**) is fixed a spiral-spring (**22**) which is wound up, when the armature (A^I) is attracted. This spring tries to relax itself on a little clockwork, consisting of some toothed wheels (k_1, r_1, k_2 and r_2). On the axis (**23**) of toothed wheel (k_2), however, is fixed an escapement-wheel (**24**) with a single tooth (**25**), which is held up by an escapement-lever (**26**). On the same axis (**23**) is fixed a little bolt (**27**) which, when revolving, should catch a tooth of cogwheel (**17**) of the auxanometer.

The escapement-lever (**26**) is one with the lever (**28**) that prevents the direct relaxing of spring (**22**). When the armature (A^I) is attracted and spring (**22**) is wound up, the lever (**28**) makes way, slipping over a tooth of cogwheel (**20**). In the mean time, the escapement-lever (**26**) makes way and relaxes the tooth of escapement-wheel (**24**), at the moment when the lever (**28**) slips over the top of the tooth. The escapement-wheel (**24**) makes one revolution, bolt (**27**) too, implicating in its revolution cogwheel (**17**) over a certain distance. By means of changing the length of bolt (**27**), one can adjust very accurately the number of teeth that cogwheel (**17**) shall turn. Two teeth ($= 10\,\mu$) proved to be the most practical arrangement.

The gradual movement of the cogwheel (**17**) warrants a high accuracy. The proportions of the teeth-number on the wheels (k_1) and (r_1) is chosen in such a way, that the clockwork is relaxed exactly as much as it is wound by the attraction of the armature (A^I). ($k_1 : r_1 = 8 : 1$). The relaxation-velocity of the clockwork is moderated by a fan (**29**).

The auxanometer described is capable of recording a total increment of the test-plant of 3,5 c.M. This is a considerable amount for seedlings. After each experiment, the contact-device is put down into its lowest position by turning the nut (m). The machine has been tested by the micrometer-screw of a Zeiss I A microscope. It has been placed in a room for constant temperature and shortly will be mounted on the axis of a VAN HARREVELD's clinostat. The electric connections will be secured, in that case, by sliding-contacts on the clinostat-axis.

As BOVIE (l.c.) has already mentioned, it is a great advantage that the test-plant may be as far as desirable from the recording apparatus. In our case, the latter has been placed in quite another part of the building.

The relais. As remarked above, a weak current is closed by the plant. In order to eliminate sparking at the interruption of the current, a condenser or a resistance (parallel to the contact) could be inserted into the curcuit. In the latter case, the resistance ought to be less than the atmospheric resistance, but large enough to prevent the relais to react. It can, however, be eliminated more safely by using a current of very low voltage, led through a fit relais. This relais has been found in the form of a galvanometer, with two coils of 4000 windings each. On the mirror of this galvanometer an iron electrode (**31**) (see fig. 3) is fixed, on which two platinum tips have been soldered. When the mirror (with the electrode) turns, these platinum tips are moved into two small cups, filled with mercury (**32**), whereby a second circuit is closed.

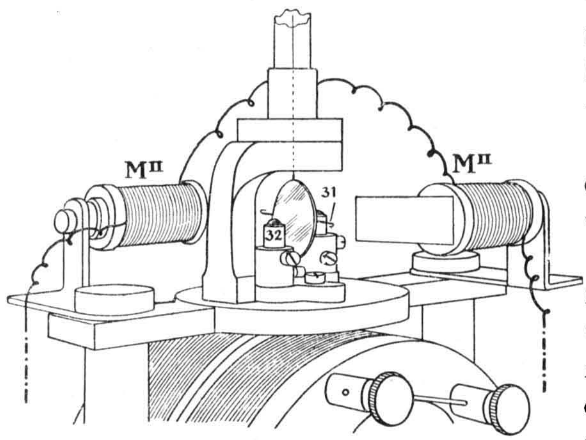

Fig. 3. The galvanometer, transformed into a relais.

This galvanometer, transformed into a relais, has the following advantages:

First. It has an extraordinary high sensitiveness.

Second. As there is no iron pith, in contrast with other kinds of relais, the self of the current is so small, that at the opening no spark will occur.

Third. The turning of the mirror takes a rather long time ; the relais having a great inertia. Short current-pulses, as will result from vibrations, do not possess enough turning-power, to make the electrode (**31**) reach the mercury (**32**). In order to obtain this result, the circuit is to be closed at least for $^1/_5$ second.

In this simple method the influence of vibrations is efficiently eliminated.

The mirror gives a full excursion at a current, obtained from an accumulator (I) (see fig. 4) and diminished by a resistance (II) of several hundreds of Ohms. The intensity of the current is about 1 milliampère. This current, which affects the galvanometer (III) (.................................) closes a second circuit (-------------) derived from the same accu. This circuit passes through a second relais (IV). There a third circuit (·—·—·—·—·—·—·—·—) is closed. The current for this circuit (and for others, that will be mentioned below) is derived from the central-net. The voltage (220 volts direct current) is diminished by resistance-lamps. These lamps pass ± 0,6 amp.

This current has three things to do:

First. It affects a third relais (V) which closes the circuit (+++++++++++++++) activating the auxanometer-magnet (M^{I}). In this way, the contact-device is raised.

Second. When the platinum-tips of the galvanometer penetrate into the mercury, a rather large power is required to turn the mirror back, as the surface-tension of the mercury is considerable. The terrestrial magnetism doesn't generate enough power, to warrant this safely. Therefore two little magnets (M^{II}) have been placed perpendiculary to the iron rod (**31**). When the current affects these magnets, the electrode will be pulled energetically out from the mercury; the swinging movement is to be damped by strips of paper, glued on the magnet-piths.

Third. It has to activate magnet (M^{III}) of a turn-over switch (VII) which is an essential part of the:

Recording-Apparatus (see fig. 4 and 5). By means of a simple clockwork the pen, mounted on a carriage-frame (**34**), is drawn along the paper. Two electro-magnets (M^{V} and M^{VI}) regulate this movement. The carriage (**34**) is connected, by means of silk-threads, at one end with a wooden cylinder (**33**), at the other end with a weight (**35**). The cylinder (**33**) is mounted on the same axis as the cogwheel (**36**), which turns to the right, when the metal device (**37**), with a pawl (**38**), moves down.

Every second a circuit (••••••••••••••••••••••••) is closed by a second-pendulum (IX) which affects a relais (VI). This relais activates by circuit (·+·+·+·+·+·+·+·+·+·) magnet (M^{V}) which attracts the device (**37**) as its armature (**40**) is mounted on this device.

With device (**37**) pawl (**38**) is pulled down and the latter involves cogwheel (**36**) in its movement; i.e. cogwheel (**36**) is turned one tooth and cylinder (**33**) winds up the silk-thread; the carriage (**34**) advances 1 m.M. A spiral-spring (**42**) pulls back the device (**37**) till it is arrested by a metal block (**43**), just when pawl (**38**) catches the next tooth of cogwheel (**36**). A contra-pawl (**41**) prevents the rolling back of the carriage during this moment; this pawl (**41**)

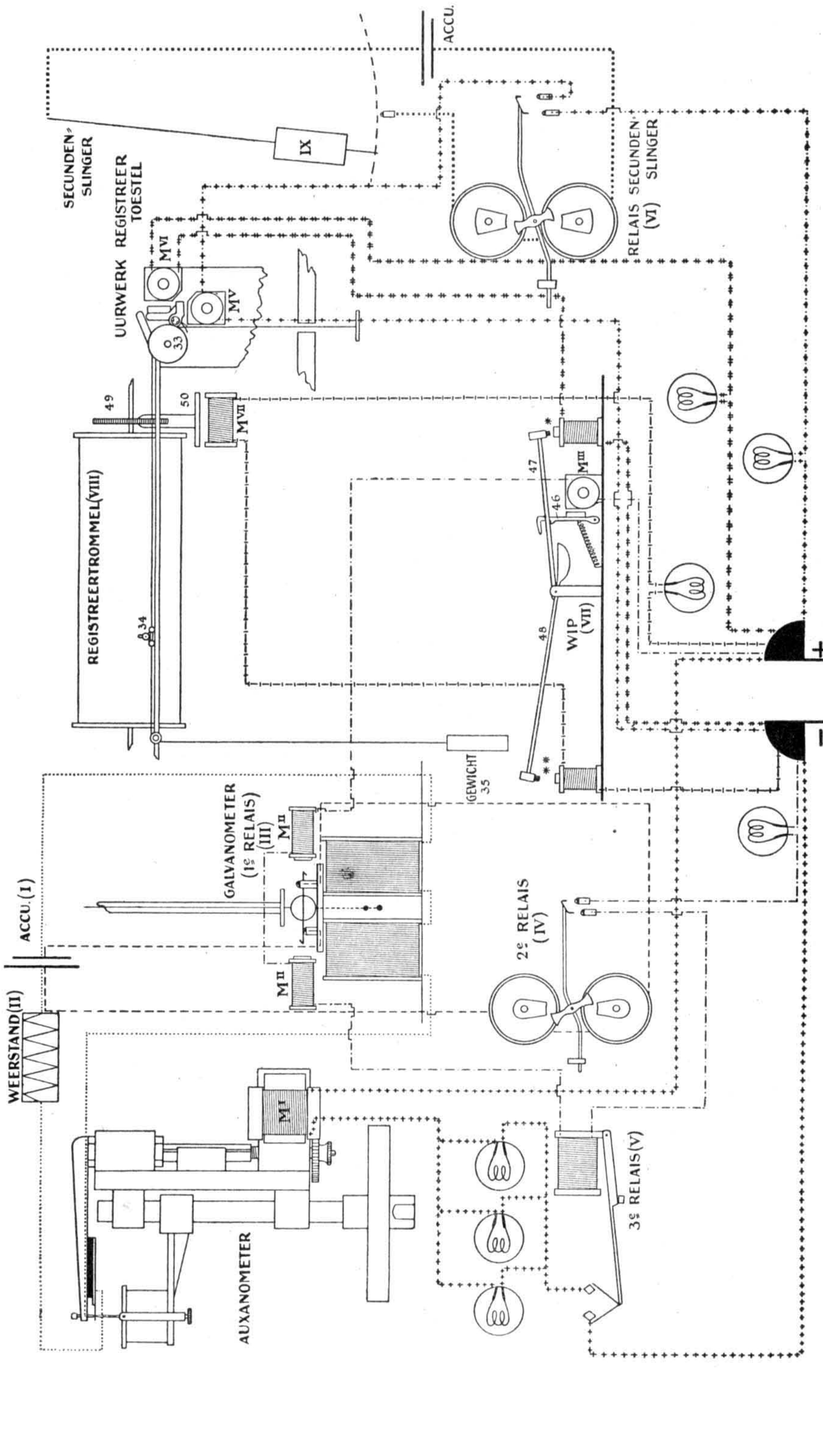

Fig. 4. Explanation in the text.

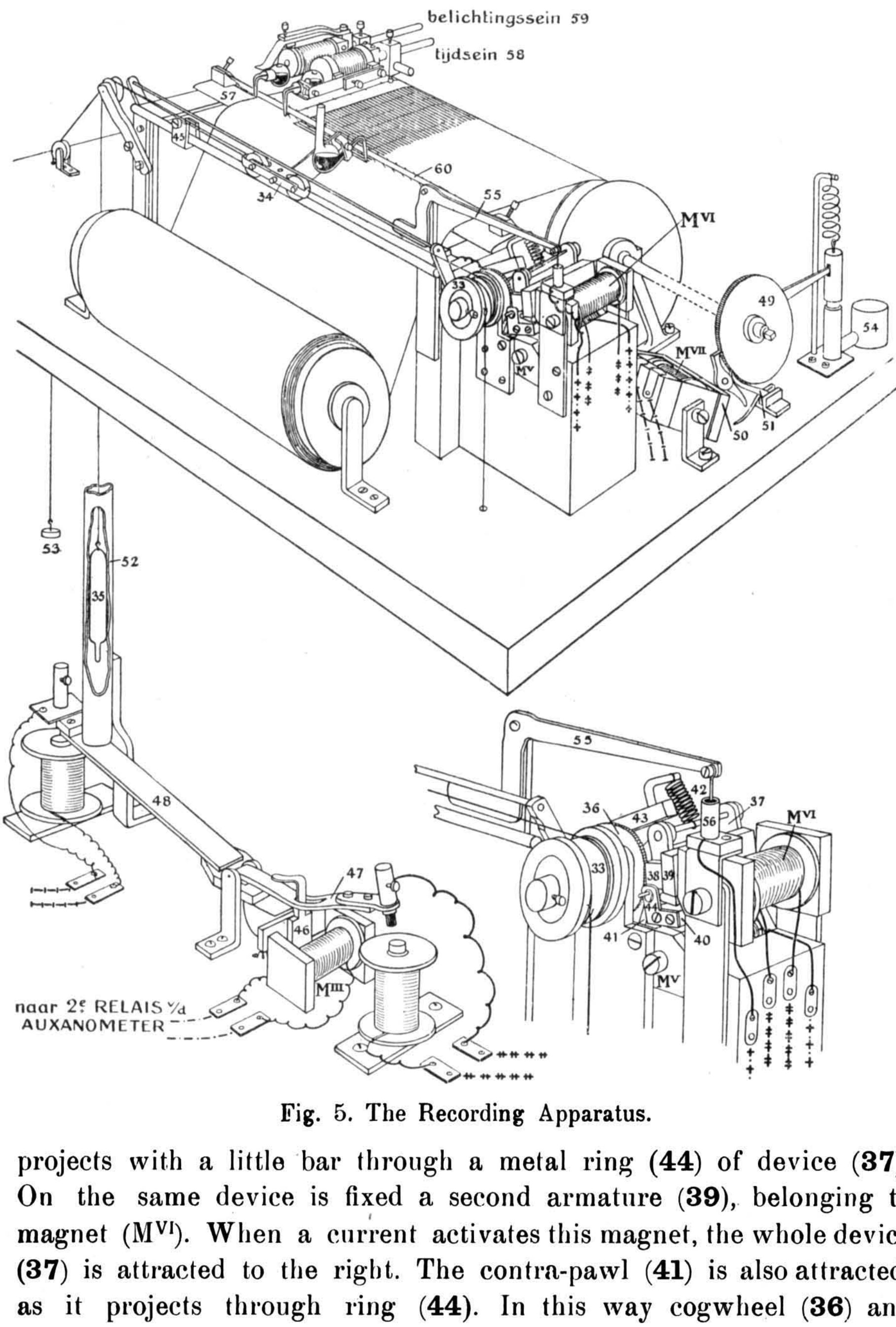

Fig. 5. The Recording Apparatus.

projects with a little bar through a metal ring (**44**) of device (**37**). On the same device is fixed a second armature (**39**), belonging to magnet (M^{VI}). When a current activates this magnet, the whole device (**37**) is attracted to the right. The contra-pawl (**41**) is also attracted, as it projects through ring (**44**). In this way cogwheel (**36**) and cylinder (**33**) come free and carriage (**34**) rolls back, pulled by the falling weight (**35**).

The magnet (M^{VI}) will be activated by the switch, for the circuit closed by the second relais (IV) passes through the coil of magnet (M^{III}). The armature (**46**) of this magnet is attracted and the support of lever (**47**) is taken away. This lever closes at *) the curcuit (-+-+-+-+-+-+-+-+-+-+-+-), which affects magnet (M^{VI}).

When weight (**35**) has fallen down straight (in a metal tube (**52**) it presses down lever (**48**), that lifts by its shorter arm lever (**47**) and opens the circuit of magnet (M^{VI}). Instantaneously the device (**37**) is relaxed from magnet (M^{VI}) by a spring (invisible on fig. 5) and the second-pendulum begins again to draw up the carriage.

As the drum is a non-moving one, a second line would be drawn on the same spot as the first. Therefore the fallen weight closes a circuit at **) (-ı-ı-ı-ı-ı-ı-ı-ı-ı-), which affects magnet (M^{VII}).

Armature (**50**) is attracted and a pawl with it. The pawl pulls on cogwheel (49) that is mounted on the axis of the drum (VIII).

The latter is turned 1,5 m.M. The new line thus will be drawn 1,5 m.M. from the preceeding one. As the drum has a great inertia and the system was moved on some times more than agreed with one tooth of the cogwheel (**49**), the movement is moderated by an oil-pump (**54**), whereas a contra-pawl (**51**) prevents the turning back of the drum. When weight (**35**) rises again, the circuit is opened, as lever (**48**) is lifted by a weight on its shorter arm.

In this way a registration has been obtained, at which the length of each line expresses in m.M. the time in seconds which the plant needs for an increase of 10 μ. As the lines are drawn 1,5 m.M. apart, 15 c.M. of the paper corresponds to 1 m.M. growth (100 lines).

Several precautions have been arranged to secure a safe record. In the first place, the record has to be stopped automatically, when the plant grows in a wrong way, i.e. when it ceases to make contact. In that case, the carriage (**34**) is drawn up entirely and pulls at the end on lever (**55**). The current, which affects magnet (M^{V}), passes through this lever (omitted in fig. 4) and a small cup (**56**) with mercury. When the lever is lifted out of the mercury by the pressing carriage, the circuit is opened.

To obtain a record over a long lapse of time, the use of several yards of paper is necessary. These have been rolled on a second drum and laid over a metal plate (**57**). So the pen has to write on the same level, while the drum (VIII) increases in bulk.

In order to get a straight abscissa the carriage has to stop always at the same starting-point. This is obtained by a metal block (**45**) and a small plate, connected with cylinder (**33**). The under-side of the table stops this plate, when the carriage reaches block (**45**). A second weight (**53**) has to pull the carriage, when the power of weight (**35**) is broken on lever (**48**).

To prevent the tedious burning of the contacts on the switch

(VII) the principle of coil-contacts has been put into practise; the self of the current blowing away the opening-spark.

The space-marking on the paper is arranged, by using the fact that the ink (a solution of eosin in water and some glycerin) remains wet for some time. On a metal strip (**60**) stiff bristles have been glued, at distances of 5 mM., which drag over the wet ink and make checks in the lines.

An electro-time-sign (**58**) draws a straight line parallel to the abscissa of the record and makes checks e.g. every 10 minutes. As the lines are drawn 1,5 mM. apart, the distance in mM. between two time-checks (devided by 1,5) gives the increase in 10 minutes. As it may happen that a check falls just before the carriage will be driven back, and the next check just after this moment, the maximum error that may occur will be 20 μ. In this way a simple method has been found, to compare the results with those of other investigators.

Lastly a second time-sign (**58**) can be put into action from the experiment-room. One can, by pressing a bell-push, put a point on the paper at the moment, when light is dosed or when the auxanometer has been put on the clinostat, etc.

Next figure 6, a little reduced in size, gives the record of 1 mM.

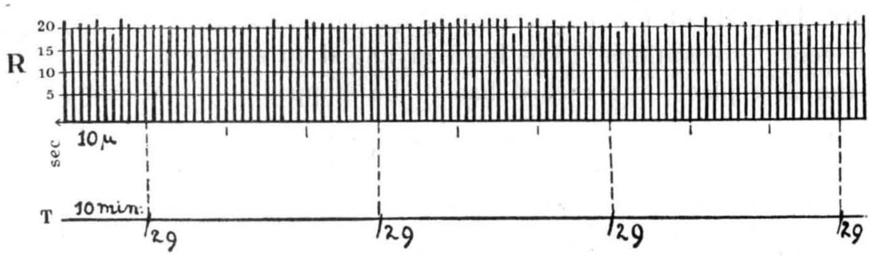

Fig. 6. Record of 1 mM. growth. Each line at *R* gives the time needed for 10 μ growth; T = time-line; every 10 minutes the increase is constantly 290 μ.

growth, belonging to a paper of 4,05 M. in length, containing the record of 27 mM. growth, in the dark.

Utrecht, November 1921. *Botanical Laboratory.*