Geotropism and Phototropism in the absence of free oxygen

by

U. P. VAN AMEIJDEN.

INTRODUCTION.

The reason which led me to begin this investigation was the fact that the problem of geotropism and phototropism in the absence of free oxygen has so far been very little considered in connection with the more recent investigations on gravitational and light stimulis. In the literature Correns' view is still accepted who claims to have shown, that seedlings are able to execute geotropic curvatures in the presence of the minutest traces of oxygen, whereas a higher oxygen-content the air is required for phototropic movements. Considering the state of knowledge at that time, we can understand how Correns arrived at this conclusion.

Later Àrpád Paál published a paper on this same subject. Although he already took into account factors which were still quite unknown at the time of Correns' investigation, yet his conceptions with regard to the problem in question, are not wholly satisfactory.

Thus there existed every reason for me to take up this question up again and now on entirely different lines. In the first place I have taken care not to place experimental material under reduced pressure, but to leave them always under the pressure of one atmosphere, so that only the partial pressure of the oxygen was reduced. This was carried out in a thermostat specially constructed for my investigation in which the free oxygen could be replaced by nitrogen by gradual diffusion.

CHAPTER I.

Review of the Literature.

The first investigations on the question whether free oxygen is necessary for the appearance of a phototropic curvature, date from Payer[1]. He worked with seedlings of *Lepidium sativum*, and found, that the medium in which the experiments were carried out, cannot inhibit a reaction, but can diminish the curvature. Thus he saw very distinct curvatures occur under water, and further in an atmosphere of nitrogen or hydrogen.

Von Mohl[2] accepted the accuracy of Payer's investigations. He himself carried out no experiments in this direction, but only used Payer's results in order to controvert Dutrochet's[3] theory of light on a certain point.

In 1878 the question of the dependence of heliotropic processes on the presence of free oxygen was taken up again by Wiesner[4]. He worked with seedlings of

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[1] T. Payer. Mémoire sur la tendance des tiges vers la lumière. Comptes rendus 1842. T. XV, p. 1194.

[2] H. von Mohl. Grundzüge der Anatomie und Physiologie der vegetabilischen Zelle (Abdruck aus R. Wagner's Handwörterbuch der Physiologie 1851, p. 297).

[3] M. Dutrochet. Rapport sur un mémoire de M. Payer, intitulé Mémoire sur la tendance des racines a fuir la lumière. Annales des sciences naturelles, 1844, 3e serie, T. II, p. 96.

[4] T. Wiesner. Die heliotropischen Erscheinungen im Pflanzenreiche, eine physiologische Monographie 1878, p. 58.

Phaseolus multiflorus, Vicia sativa, Lepidium sativum and *Sinapis alba* — and so arranged the containing vessels that their open ends dipped into potash solution. After 36 to 48 hours the potash reached its highest level so that all the oxygen had been absorbed. Up to this time the whole apparatus had stood in the dark, but then the seedlings were illuminated unilaterally. There was still no trace of curvature to be seen even after several hours. If he allowed air to enter, then the commencement of the curvature was already visible after one hour. He therefore concluded, that no heliotropic phenomena are observable without free oxygen.

Wortmann[1]) in his investigation on intramolecular respiration made the statement that all irritabelity is lost in an oxygen-free atmosphere, but that even in the case of plants which have for weeks been deprived of oxygen, irritabelity returns on renewed access of oxygen. A very small portion of his investigation is concerned with the geotropic sensitiveness of seedlings. He placed the germinating seeds of *Vicia Faba, Phaseolus vulgaris* and *Phaseolus multiflorus* in absorption tubes of 100 c.c. capacity of which the openings were in contact with mercury covered with a layer of water. The seeds remained for several days in this enclosed space till a distinct increase in volume had taken place. Then all the oxygen had been used up. Roots germinating in a horizontal position now showed no geotropic curvature. He got the same negative results with parts of the stem of *Paeonia peregrina.* Wortmann did not understand why the intramolecular respiration is not a source of energy to the plant capable of furnishing the forces which bring about growth.

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[1]) J. Wortmann. Ueber die Beziehungen der intramolecularen zur normalen Athmung der Pflanzen. Arbeiten des Bot. Inst. in Würzburg II. 1880, p. 509.

Kraus[1]) in an appendix to his paper on the distribution of water in plants, deals with the behaviour of geotropically irritable organs in a CO_2- or H-atmosphere.

He used for his experiments flowering shoots of *Taraxacum, Ranunculus, Anthriscus silvestris* etc., which he placed horizontally in a vessel, through which a constant current of CO_2 or H was passed. Six hours later no trace of erection was visible, but, if the stems were afterwards brought into the air, they were, after 5 hours, curved upwards. Irritability is therefore lost in an oxygen-free atmosphere, but returns in ordinary air, a proof therefore, that the plants merely pass into a condition of rigor („asphyxie", „starre").

An entirely new method of studying the influence of oxygen on geotropic processes was adopted by Wortmann[2]). He used seedlings of *Phaseolus multiflorus, Helianthus annuus* and *Lepidium sativum* and placed them under the bell-jar of an airpump. In a partial vacuum, a slight curvature, which had previously been induced in ordinary air, was continued, but only for a short time and no further continuation of the curvature could be obtained even after admitting atmospheric air. But if air was admitted while a slight curvature was still taking place, the movement again became more vigorous and could even proceed as far as in ordinary air. He remarks, that the same phenomenon had already been observed by Wieler[3]) for growth; since geotropism is a growth

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[1]) G. Kraus. Ueber die Wasservertheilung in der Pflanze IV. Die Acidität des Zellsaftes. Abhandlungen der Naturforschenden Ges. zu Halle, Bd. XVI, 1884, p. 199.

[2]) J. Wortmann. Studien über geotropische Nachwirkungserscheinungen. Bot. Zeitg. 1884, p. 705.

[3]) A. Wieler. Die Beeinflussung des Wachsens durch verminderte Partiärpressung des Sauerstoffs. Unters. aus dem Bot. Inst. zu Tübingen I, 1883, p. 189.

movement, it is also intelligible that the after-effect of the geotropic stimulus can still occur so long as slight growth is still possible. He also carried out experiments in a hydrogen atmosphere. Shoots, which had been kept in this for some time in a horizontal position, showed when brought into the air, a further growth in length but no trace of a geotropic after-effect.

Correns[1]) has written a long paper on the influence of oxygen on all kinds of stimulation-phenomena, including the movements due to gravitational- and light-stimuli. He also followed the method of placing the experimental material under reduced pressure or in a vacuum. In order to expel the oxygen completely, after having exhausted he let in hydrogen, then pumped the vessel emply again and repeated this several times. He worked with seedlings of *Helianthus, Vicia Faba, Lepidium sativum* and *Sinapis alba*. The various types of stimulation-movements require various concentrations of oxygen. The reason for this lies partly in the different oxygen requirements of various species of plants, independently of the character of the stimulation-movements, and partly in the conditions which are characteristic of certain stimulation-movements. He first discusses the geotropic experiments. The vessel in which the seedlings were placed was exhausted in the way described and then placed in a horizontal position. The plants were examined after 6 to 12 hours. They all behaved in the same way, that is to say, so long as growth could be observed, the geotropic curvature still took place. After exhausting and letting in hydrogen five times, Correns still obtained perceptible curvatures, for example, in *Helianthus* on the other hand *Sinapis alba* only reacted with an oxygen-content of 4 % to 5 %

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[1]) C. Correns. Ueber die Abhängigkeit der Reizerscheinungen höherer Pflanzen von der Gegenwart freien Sauerstoffes. Flora 75, 1892, p. 87.

of the normal-atmosphere. When, a geotropic curvature, however small, occurred in the vessel, it continued when, on return to atmospheric air, the seedling was placed in a vertical position. But if absolutely no reaction had taken place, in the first instance, there was no after-effect whatsoever.

He concluded from all these experiments that oxygen is necessary to the carrying out of a geotropic process. He also placed seedlings for 1 to 2 hours horizontally in the vessel, pumped it emply, filled it with hydrogen and led a current of hydrogen through it. Afterwards the seedlings were placed in the dark in an upright position. Even when the material had in this way been in contact with hydrogen for several hours an after-effect could still be observed, but the longer time hydrogen had been passed the smaller the 'after-effect was found to be. From this experiment he draws the conclusion that the after-effect had been only interrupted by the deprivation of oxygen, but not destroyed.

Correns saw even less occurrence of movements in his heliotropic experiments without oxygen. Illumination took place by turning the uncovered side of the vessel towards the day-light. In order to get a heliotropic reaction about 1 % of the original quantity of oxygen must be present, and in the case of *Sinapis* seedlings even 6 %, at least to get a curvature visible to the naked eye.

The geotropic curvatures are therefore carried out by the same plants with smaller quantities of oxygen than the phototropic ones.

He also carried out experiments in which he allowed both stimuli to act simultaneously on the same material, and in an atmosphere, containing so much oxygen that growth and geotropic curvatures could take place, but in which the seedlings were not able to give a heliotropic reaction. Seedlings, which were placed horizontally in the vessel and parallel to the window, showed a very clear geotropic curvature without exhibiting the smallest heliotropic reaction towards the side of the light.

He further carried out experiments in order to investigate whether at that oxygen-content, in which both growth and geotropic curvatures are still possible, a heliotropic stimulus can be perceived and whether a stimulus perceived in air progresses at this oxygen-content. In order to answer the first question, the required atmosphere was obtained by partial exhaustion of the vessel and admission of hydrogen, and the seedlings were exposed for $1^1/_2$ to 2 hours to a unilateral illumination of a gas-flame, without a curvature appearing.

Then the gaseous mixture was replaced by ordinary air. There was no trace of any after-effect. In order to decide the second question the seedlings were first illuminated from $1^1/_2$ to 2 hours by the same source of light which caused an obvious curvature. The oxygen was then diminished to 4 %. No further after-effect took place, but the curvature was neutralized by the counter-effect of geotropism.

He finds it very remarkable that the after-effect remains absent in an atmosphere in which the oxygen-content still allows the geotropic-reaction, because this shows that the way in which the heliotropic curvature is executed is different from that of the geotropic, although both are phenomena of growth. It may certainly be assumed that the actual growth is in both cases the same and further that the heliotropic after-effect has certainly been induced. Hence between the establishment of the disposition to the after-effect and its mechanical execution there must necessarily intervene a process that is specially influenced by oxygen.

In his general results Correns says that he recognizes in all stimulation-movements a difference between perception and reaction and that the absence of an after-effect under normal conditions when the material is stimulated in vacuum may not be regarded as a proof that the stimulus has not been perceived; there are indeed three possibilities:

1. That neither can a stimulus be perceived nor a movement be induced in a vacuum.
2. The stimulus is perceived, but the reaction cannot take place, or the disposition towards it cannot arise.
3. The stimulus cannot be perceived, although the reaction can take place. Correns admits that he has not been sufficiently successful in determining the effect of oxygen on perception and reaction separately, so that he cannot decide between the above three cases.

I have discussed in detail Correns' paper because it is by far the most important on this subject; but nevertheless in some respects his methods of investigation and his conclusions are open to criticism. The method of exhausting seems to me to be distinctly objectionable, because various changes of condition may occur in the plant which are wrongly ascribed to the absence of oxygen, but which really have quite another cause. The comparisons which Correns draws between geotropism and phototropism depend on the one hand, on experiments in which the seedlings were stimulated from 6 to 12 hours in a horizontal position, on the other hand they were continuously illuminated unilaterally by day-light; he does not state how long this illumination lasted. It seems to me by no means impossible that with a shorter period of geotropic stimulation, as for example, if the geotropic reaction was observed after 2 hours, he might not have obtained any curvature at the pressure indicated by him, whilst with a greater quantity of oxygen after 2 hours a distinct reaction might be obtained. For the time, in which, under normal conditions the reaction begins to be visible is much less than 2 hours. It is evident from the following figures which I found that in different plants the geotropic and phototropic stimuli often have widely differing values for definite strengths of curvature: when seedlings of *Avena* are geotropically stimulated, by being placed in a horizontal position for 15 minutes, then the maximal strength of curvature amounts to 2 m.m. A maximal phototropic reaction of 2 m.m. is obtained by stimulation with 40 M.C.S.[1]) seedlings of *Sinapis* must be stimulated for 10 minutes in order to get a maximal geotropic reaction of 3 m.m., whilst for a phototropic reaction of only 2 m.m. a stimulation with 400 M.C.S. is necessary. If one takes into consideration that the experiments with *Avena* took place at a temperature of 20° C and those with *Sinapis* at 23° C, then according to the law of Van 't Hoff the geotropic sensitiveness of *Avena* and *Sinapis* are more or less equal: phototropically, however, *Avena* is much more sensitive than *Sinapis*. From this it is sufficiently evident that one must be careful in making comparisons between geotropic and phototropic reactions, particularly when working with a stimulus of unknown strength. In my own experiments I therefore followed a different method to that of Correns, as will be seen below.

A few later papers have appeared on this subject. Czapek[2]) in his paper on geotropism, published 1895, devoted a chapter to the influence of the withdrawal of oxygen on geotropic phenomena. From the observation bij Wortmann[3]) and by Correns[4]) that seedlings, which had been stimulated geotropically for 6 hours in

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[1]) Metre-candlepower-seconds.

[2]) F. Czapek. Untersuchungen über Geotropismus. Jahrb. f. wissensch. Bot. 1895, Bd. XXVII, p. 274.

[3]) Wortmann. l.c., p. 711.

[4]) Correns. l.c., p. 132.

hydrogen, showed no after-effect, we may not conclude, that no perception is possible in an atmosphere free from oxygen, or that the „disposition to curve" cannot arise. Czapek also repeated Correns' experiments in hydrogen, but found that a sojourn of more than 6 hours was harmful. Now the method which he adopted, was based on an investigation by Chudiakow [1]) who had found that in an oxygen-free atmosphere seedlings perish the sooner, the higher the temperature is. Czapek experimented with lupin seedlings which he placed vertically in the receiver of an air-pump. He exhausted to 1 m.m. of mercury and then admitted a current of hydrogen. He repeated these two operations 6 to 8 times. Then the vessel was placed horizontally in powdered ice. In this way the roots remained 24 hours at a temperature of from 0° to 2° C. After this interval the roots looked perfectly normal. They were then placed on the clinostat and after 4—6 hours a distinct geotropic after-effect had made its appearance. Although in the vessel longitudinal growth did not take place, geotropic induction is evidently still possible, provided that care is taken to exclude harmful factors.

In 1912 Árpád Paál [2]) published a paper, which likewise only dealt with geotropism. The method which he adopted for studying the influence of reduced pressure on the geotropic-stimulation process, consisted in the determination of reaction- and presentation-times. The radicles of *Phaeseolus vulgaris*, with which he worked, were placed horizontally under the bell-jar of an air-pump, and he observed at different pressures of air, how long it required before the majority of the roots showed a curvature. The result of these experiments was that the

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[1]) N. v. Chudiakow. Beiträge zur Kenntniss der intramolekularen Athmung. Landwirtsch. Jahrb. 1894, Bd. 23, p. 333.

[2]) Árpád Paál. Analyse des geotropischen Reizvorgangs mittels Luftverdünnung. Jahrb. f. wisschensch. Bot. 1912, Bd. L, p. 1.

reaction-time was lengthened as the air-pressure was reduced. Then he investigated separately the perception and reaction. As measure of the influence of pressure on the sensory phase he used the presentation-time. The roots were placed horizontally in air of known pressure for a time and then transferred to the clinostat. If, after an interval, 50 % of the roots showed a curvature, they were assumed to have been stimulated during the presentation-time. These experiments showed that the presentation-time is prolonged when the pressure of air is reduced; this indeed is already marked at 0.74 of an atmosphere, then it decreases gradically to 0.21 of an atmosphere, below which the decrease is very rapid. The experiments further showed that under these conditions also the reaction-time is prolonged. A continued stay under reduced pressure lengthened the reaction-time more than the presentation-time. Therefore the cause of the delayed reaction cannot be wholly due to the lengthening of the sensory phase, but also of the motor phase.

He finally carried out experiments by exposing to normal pressure and allowing the reaction to take place under reduced pressure. Here also a lengthening of the reaction-time took place.

Finally Kenkel[1]) mentions experiments on the influence of reduced air-pressure on geotropism and phototropism. He followed exactly the same method as Correns[2]) and like him found for various plants, that with a definite oxygen-content, at which the geotropic reaction can still take place, the heliotropic no longer occurs, or is at least diminished.

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[1]) J. Kenkel. Ueber den Einflusz der Wasserinjektion auf Geotropismus und Heliotropismus. Inaugural-Dissertation, Münster 1913.

[2]) Correns. l.c., p. 138.

CHAPTER II.

Material. General arrangement of the Experiments.

Material.

Seedlings of *Avena sativa* were chosen as experimental objects, because numerous recentinvestigations have shown them to be a very suitable material for geotropic and phototropic experiments, and further seedlings of *Sinapis alba*, for reasons which will be given later. The oat-grains were peeled before use. For the rest the seeds were treated alike, i. c. they were first placed on damp filter-paper to germinate and later transplanted into boxes with soil. This method is described in detailed by Rutgers[1]. The oat-grains had to lie for 2 days on the filter-paper and had then germinated sufficiently to be planted. This time varied somewhat for the seeds of *Sinapis* being between four and six days. The culture-boxes were of zinc, and measured 10 × 3 × 3 c.m. Nine seeds were planted in each vessel. They were placed diagonally for the photropic experiments in order to avoid mutual shading when illuminated. The oats were of one pure strain "Svalöfs Goldregnshavre". The seeds of *Sinapis* were ordinary commercial seeds. Very many of them

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[1] A. A. L. Rutgers. De invloed der temperatuur op den praesentatietijd bij de geotropie. Dissertatie 1910, p. 41.

appeared to be incapable of germination by the method described, whilst the rest showed great differences in germination so that seeds of equal development had to be selected most carefully for each vessel.

Cultivation took place in the laboratory glass-house, in which the temperature was not constant (about 20°) and generally ranged over a few degrees. After the seeds had been planted in the vessels, the latter were placed in a box, covered with black cloth, so that they were completely in the dark. These boxes were regularly ventilated for otherwise the seedlings did not grow quite perpendicularly in consequence of the impure air.

This precaution was also adopted for the same reasons by Miss De Vries[1]. After these four days the length of the coleoptiles of *Avena* reached $1^1/_2$ to 2 c.m., whilst the hypocotyls of *Sinapis* seedlings attained a length of about $1^1/_2$ to 3 c.m. Oblique growths and too long or too short seedlings, were removed before the boxes were used for experiment.

The Thermostat.

The thermostat necessary for this investigation was made according to my specifications by Mr. Roelink, attendant at the Botanical Laboratory. It consisted of an outer and inner box (Pl. XV, XVI and XVII). The outer box had a height of 300 m.m., a breadth of 295 m.m., and a depth of 145 m.m. For the inner one these dimensions were respectively 240, 220 and 65 m.m. The two bottoms and back walls were of copper, the fronts and the right and left side walls of glass. The lid of the inner box was also of copper, whilst the outer box was open on top.

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[1] Marie S. de Vries. Der Einfluss der Temperatur auf den Phototropismus. Recueil des Trav. botan. Néerland. 1914, Vol. XI, p. 200.

The dimensions of the thermostat were chosen as small as possible in order to expedite the replacement of the air in the inner box by nitrogen. In the centre of the back walls there was an opening through which the axte (Pl. XVI, *a*) of the clinostat passed, contained in an oil-packing (Pl. XVI, *b*) so that the wall was completely air-tight and the axte could turn without appreciable friction. To the end of the clinostat-axte in the thermostat a clamp (Pl. XV and XVI, *c*) could be screwed for the purpose of attacking the vessels containing the seedlings. Since it was necessary before measuring the curvatures to take the seedlings out of the thermostat, it was desirable so to construct the fastening that this could be done as quickly as possible. For this reason a screw (Pl. XV and XVI, *d*) was attacked to each top corner of the inner box, in such a way that the lid, provided on the under side with ad indiarubber plate, could still just be placed free on the inner-box. Two springs (Pl. XV, *e*) were now placed through the screws, and pressed the lid on the box so as to make it air-tight, after a nut (Pl. XV and XVI, *f*) had been screwed down on each of the screws. In the space between the inner and outer box, two heating-lamps (Pl. XV and XVI, *g*) were placed, and in addition, a mercury thermoregulator (*h*) and two stirring-arrangements (*i*), which were driven by an electric-motor, and finally, there was a thermometer. For the rest, this space was completely filled with water. In order to open the thermostat, each time enough water was syphoned out to make the level descend just below the lid. Through the lid two tubes passed, of which one (*k*) was bent at a right-angle just under the lid, and ended blindly, but was provided with many small holes. This sewed for the admission of nitrogen. The other tube (*l*) reached almost to the bottom of the inner-box, and served as exit of nitrogen.

The temperature.

The temperature was always kept constant in all the experiments. This was ensured by joining the thermoregulator with an accumulator and a relay in an electric-circuit, so that the current of the heating lamps was immediately interrupted when the temperature had sufficiently risen [1]. In addition a second relay was included, in order to prevent discharge of the accumulator as much as possible.

The source of light.

A simple electric carbon filament lamp served as the source of light for the phototropic experiments and its luminosity was determined by Weber's [2] photometer and as a distance of one metre, was found to equal 5 M.C. (metre candles). For this determination I placed the lamp in the thermostat in the position which the boxes with the seedlings occupied during the experiments. The space between the inner and outer case was filled with water and I placed the photometer at a distance of 1 metre from the lamp. Thus in determining the luminosity, the absorption of light by the glass-walls and layer of water was taken into account. The lamp was so arranged in the experiments that it was at a distance of exactly one metre from the middle of a box of seedlings, which was placed in the thermostat, in such a way that illumination took place through the side-walls of the thermostat.

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[1] For a detailed description of this arrangement as well as of the thermoregulator, see Rutgers, l.c. pp. 46 and 48.

[2] Prof. Snellen was kind enough to place the photometre at my disposal for this purpose.

Arrangement of experimental objects.

The boxes containing the seedlings of *Avena* were, immediately after planting, packed in absorbent gauze in order to prevent the soil from falling out when rotating on the clinostat. The boxes were fastened into the clamp arranged for this purpose, in such a way that the seedlings were perpendicular to the horizontal axte of the clinostat, by which arrangement the geotropic and phototropic stimuli were so applied that all curvatures occurred in one and the same plane, namely in that of rotation. In short experiments — such in which the seedlings had only a short fore-period in nitrogen — the seedlings were rotated on the clinostat before stimulation. This could be done with two boxes at a time, fastened into the clamp with their bottom-walls against each other.

Since on account of the cotyles dicotyledonous seedlings cannot grow through absorbent gauze, the boxes of *Sinapis* were not rotated on the clinostat, but were simply placed in a vertical position after being stimulated. Of course these experiments could never be carried out with two boxes at a time.

The Nitrogen.

As I needed large quantities of nitrogen, I obtained it from the metal cylinders of commerce. These were found, however, to contain a fairly considerable proportion of oxygen, which I estimated with a phosphorus pipette [1]). As an average of several determinations I found 3.3, 4.3 and 4.7 $^0/_0$ oxygen in the three cylinders which I successively used. In order to absorb the oxygen, the nitrogen was

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[1]) Prof. van Romburgh was kind enough to allow me the loan of this apparatus.

passed through an alkaline pyrogallol solution, as described by Weyl and Gott[1]). They found that most oxygen was absorbed when 0.25 gram pyrogallol was dissolved in 10 c.c. NaOH of specific gravity 1.03. In the nitrogen purified in this way the seedlings showed however very clear signs of poisoning. The apices became strikingly brown and all sorts of curvatures appeared. Since CO is formed by the passage of oxygen through pyrogallol, it is to this that the poisoning may be attributed. That this was indeed so, became evident when the gas was passed through the tube of a combustion furnace, in which there was a small quantity of CuO, as well as reduced copper which served to fix any oxygen which might have escaped absorption. This CuO oxidized the CO to CO_2. Gas treated by this method no longer gave the slightest indications of poisoning.

The dark room.

The dark room in which I performed the first part of my experiments, was situated in the centre of the Botanical Laboratory. In this room there was an air-shaft, by means of which, fresh outside air could always be sucked through the thermostat, in those experiments, in which this was necessary. Afterwards in consequence of structural changes in the laboratory, this room was no longer at my disposal and I continued the experiments in a small separate building in the Garden consisting of two small rooms, which had each a separate door to the garden. One of these was made into a dark room in which, by a very simple arrangement the outside air could be directly sucked

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[1]) Th. Weyl and A. Gott. Ueber die Absorption von Sauerstoff durch Pyrogallol und Phloroglucin in alkalischer Lösung. Berichte der Deut. Chem. Gesellsch. 1881, 14e Jahrg., II.

through the thermostat, whilst the other room contained the nitrogen-cylinder, the two wash bottles and the combustion furnace.

The Arrangement (Pl. XVII).

The nitrogen first passed through the wash bottles with pyrogallol and the furnace, after which the exit tube entered the dark room through an opening in the wall. The gas was first cooled by passing through a leaden spiral, placed in a vessel of water and then passed through the thermostat. The waste-pipe ended under water and served as a control against leakage, because in that case bubbling stopped at once. The nitrogen-cylinder was always opened so far that the bubbles of gas succeeded one an other at a constant rate.

In those experiments in which fresh air was drawn through the apparatus, the tube which led into the thermostat was connected to a tube through which the outer air could enter. Further the exit tube was attached to an aspirator.

CHAPTER III.

Experiments in Air.

The experiments described in this chapter, were performed in order to obtain a measure of the deviations which occur, if during a definite period the ordinary atmosphere was replaced by nitrogen. These experiments are of four kinds:

1. Geotropic experiments with seedlings of *Avena sativa*.
2. Phototropic experiments with seedlings of *Avena sativa*.
3. Geotropic experiments with seedlings of *Sinapis alba*.
4. Phototropic experiments with seedlings of *Sinapis alba*,

In all four cases the same method was followed in principles.

The boxes with the seedlings remained for half an hour in the thermostat before stimulation. Two boxes of *Avena* were always arranged together and fastened in the clamp as described on p. 165. During the fore-period of half an hour they were rotated on the axte of the clinostat. This was of course also done after stimulation. In the experiments with seedlings of *Sinapis* only one box at a time was placed in the thermostat, because these, as stated (p. 165) could not be rotated on the clinostat.

A stimulus of definite strength was applied in each set of experiments as follows:

set 1: 900 mg.-secs. i.e. the seedlings remained for 15 minutes in a horizontal position;
set 2: 40 M.C.-secs. i.e. the seedlings were exposed for 8 seconds to the action of a lamp of 5 candle power at a distance of 1 metre;
set 3: 600 mg.-secs. i.e. the seedlings remained for 10 minutes in a horizontal position;
set 4: 400 M.C.-secs. i.e. an exposure of 80 seconds to the lamp of 5 candle power at a distance of 1 m.

The duration of the stimulation was determined accurately by means of a stop-watch.

After this I traced the extent of the maximal curvatures which correspond to these quantities of energy, and the length of the periods which elapse between the beginning of stimulation and the time at which the maximal curvature was reached, i.e. the reaction times. For Arisz[1]' experiments have shown that to each quantity of energy there belongs a maximal curvature of definite extent and a definite curvature time. The extent of the curvature was expressed by the perpendicular deviation of the apex from the vertical, measured over the centre of the coleoptile. This measurement was made by placing a strip of paper marked in millimetres behind the coleoptile. A few preparatory experiments showed after about what interval of time the maximal curvature became visible. In order to determine this curvature-time accurately, the boxes containing seedlings were taken out of the thermostat after different intervals and the curvatures were measured. I tried to narrow the limits defining the time at which maximal curvature appeared; Thus I first measured the

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[1] W. H. Arisz. Untersuchungen über den Phototropismus. Recueil des Trav. Botan. Néerland., 1915, Vol. XII. p. 44.

curvatures after 40, then after 90 minutes. Afterwards I compared with one another the results obtained after 50 and 80 minutes, and so on. Therefore Table I does not give the sequence in which the experiments were done.

A. *Geotropic experiments with seedlings of Avena.*

TABLE 1.

Strength of stimulus 900 mg.-secs. Temperature 20° C.

Time elapsed since beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.								
40	5	1	½	½	½	½				
	5	1½	1½	1½	1	1				
	9	1½	1	1	1	1	½	½	½	0
	7	1	1	1	1	1	½	½		
	7	1	1	½	½	½	½	½		
	8	1	1	½	½	½	½	½	½	
50	8	2	1½	1	1	1	1	1	½	
	8	2	2	1½	1½	1½	1	1	½	
	6	2	1½	1½	1½	1	1			
	6	1	1	1	½	½	½			
	9	1½	1½	1	1	1	1	1	½	½
	6	1	1	½	½	½	½			
	5	1½	½	½	½	0				
	7	1½	1½	1	1	1	1	½		
	7	1½	1½	1½	1	1	1	1		
60	8	2	1½	1½	1½	1½	1½	1½	1½	
	7	2	2	1½	1½	1½	½	½		
	6	2½	2½	2	2	1½	1½			
	9	2	2	2	2	1½	1½	1	1	½
	7	2½	2½	2	2	1½	1	1		
	8	3	2½	1	1	1	1	1	1	
	6	3	2½	1½	1	1	0			
	6	3	2	1	1	0	0			

Time elapsed since beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.
65	7	2 2 2 1½ 1½ 1 1
	5	2 1½ 1½ 1½ 1
	6	3 2½ 1½ 1 1 1
	5	2½ 2 1½ 1½ ½
	7	2½ 2 2 1 1 1 ½
	6	3 1½ 1½ 1½ 1 0
	7	2½ 2½ 1½ 1½ 1 ½ 0
	8	2 2 2 1½ 1½ 1 1 0
	6	3 2½ 2 2 1½ 1
	6	2 2 2 1½ 1½ 1
	6	2½ 2 2 1½ 1 1
	8	2½ 2 2 2 1½ 1½ 1 1
	9	2½ 2 2 1½ 1 1 1 ½ ½
	8	2 2 2 2 1½ 1 1 1
	6	2 2 2 1 1 ½
	8	3 2½ 2 2 2 1½ 1½ 1
	9	2 2 1½ 1½ 1½ 1 ½ 0 0
	7	2 2 1½ 1½ 1½ 1½ 0
	6	2½ 2 2 1½ 1½ 1
70	8	3 2 2 2 2 2 1½ 1½
	8	2½ 2 1½ 1½ 1 1 1 ½
	9	2 2 2 1½ 1 1 1 1 ½
	8	2 2 1½ 1½ 1 1 1 1
	6	2 2 1½ 1½ 1½ 1
	8	3 2½ 2 2 1½ 1 1 0
	6	2½ 2 1½ 1½ 1½ 0
	7	2½ 2 1½ 1½ 1 ½ 0
	6	2 2 1½ 1 1 ½
	5	1½ 1 ½ ½ 0
	5	3 2 1 1 0
	4	1½ 1½ 1 ½
	6	1½ 1½ 1 1 1 1

Time elapsed since beginning of stimulation in minuten	Number of seedlings	Extent of the curvatures in m.m.
	6	2 1½ 1 1 1 ½
	5	1½ 1 1 1 1
	6	2 2 1½ 1 ½ ½
	7	3 1½ ½ ½ ½ 0 0
	6	3 2 1½ 1 1 1
	6	2 2 1½ 1 1 1
	6	1½ 1½ 1 1 1 ½
	6	2 2 2 2 1½ ½
	4	2½ 1 ½ ½
	5	2 2 1½ 1 1
	7	2 2 1½ 1½ 1½ 1 0
80	7	2 1½ 1 1 1 ½ ½
	5	1½ ½ ½ ½ 0
	6	1½ 1½ 1 1 ½ 0
	6	1½ ½ ½ ½ 0 0
	5	1½ 1½ 1 1 ½
	6	1½ 1 ½ ½ ½ 0
	6	2 2 1 1 ½ ½
	8	2 1½ 1 1 1 ½ ½ ½
90	6	2 1½ 1 0 0 0
	7	1½ 1 ½ ½ 0 0 0
	7	2 1 1 ½ ½ 0 0
	8	1 1 1 1 1 ½ 0 0
	8	2 1½ 1 1 1 ½ ½ 0

It is at once evident from the above table that 40 minutes after the beginning of the stimulation the maximal curvature has not yet been reached and that after 90 minutes it was already falling off. The same applies to the periods of 50 and 80 minutes. The observations after 60 and 70 minutes are summarized in the following table.

TABLE 2.

Number of plants observed after 70 minutes 111 and after 60 minutes 114.

Extent of curvature in m.m.	Number of plants		Percentage	
	70 min.	60 min.	70 min.	60 min.
3	4	6	3.5	5
$2^1/_2$	4	12	3.5	10
2	21	24	19	20
$1^1/_2$	25	32	22.5	29
1	34	28	30.5	25
$^1/_2$	15	6	13.5	5
0	8	6	7	5

A consideration of table 2 suggests that for both observation times the percentage of curvatures of 2 m.m. is rather high, that after 60 minutes the magnitude of the curvatures is still increasing but that after 70 minutes it is already falling off again. If this be so, the extent of curvature after 65 minutes should exceed those after 60 and 70 minutes. For this reason determinations were made after 65 minutes. The results are summarized from table 1, as follows:

TABLE 3.

The total number of plants was 112.

Extent of curvature in m.m.	Number of plants	Percentage
3	2	2
$2^1/_2$	6	5
2	39	35
$1^1/_2$	28	25
1	28	25
$^1/_2$	6	5
0	3	3

We see indeed that the extent of the curvature is considerably greater than after 60 or after 70 minutes, while from the high percentage of curvatures of 2 m.m. we may draw the conclusion that the maximal reaction is 2 m.m. This occurs after 65 minutes, so that for the maximal curvature of 2 m.m. the reaction time amounts to 65 minutes.

B. *Phototropic experiments with Avena seedlings.*

The strength of stimulus selected was 40 metre-candle-power-seconds (M.C.S.). As was stated above I had to illuminate the seedlings with my lamp for 8 seconds. After the exposure, they were again rotated on the clinostat axte and after various intervals from the beginning of stimulation the extent of curvature was determined in the same manner as under A.

TABLE 4.

Strength of stimulus 40 M.C.S. Temperature 20° C.

Time elapsed since beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.								
60	9	1½	1	1	1	1	1	1	½	0
	9	1	1	1	1	1	½	½	0	0
	8	2	2	1½	1	1	½	½	0	
	6	1½	1	1	½	½	0			
	5	½	½	½	0	0				
	5	1	½	½	½	0				
65	7	2	2	1½	1½	1	1	1		
	7	1½	1½	1	1	1	1	½		
	8	2	2	1½	1	1	1	½	½	
	8	2	2	1½	1½	1	1	0	0	
	8	1½	1	1	½	½	½	0	0	
	7	1½	1½	1	1	½	0	0		

Time elapsed since beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.								
70	6	3	$2^1/_2$	2	2	$1^1/_2$	$1^1/_2$			
	7	2	2	2	$1^1/_2$	$1^1/_2$	1	1		
	7	3	2	2	2	$1^1/_2$	1	$^1/_2$		
	9	3	2	2	$1^1/_2$	1	1	$^1/_2$	0	0
	7	2	$1^1/_2$	1	1	1	1	0		
	7	$2^1/_2$	2	2	$1^1/_2$	1	1	1		
	9	3	2	2	2	1	1	1	$^1/_2$	$^1/_2$
	7	$2^1/_2$	2	$1^1/_2$	$1^1/_2$	1	1	0		
	6	$2^1/_2$	$1^1/_2$	$^1/_2$	$^1/_2$	0	0			
	7	2	2	$1^1/_2$	$^1/_2$	$^1/_2$	0	0		
	8	2	2	2	2	$1^1/_2$	1	1	0	
	9	$2^1/_2$	2	2	$1^1/_2$	$1^1/_2$	1	1	1	$^1/_2$
	8	2	2	1	1	1	$^1/_2$	$^1/_2$	0	
	7	2	2	$1^1/_2$	$1^1/_2$	1	$^1/_2$	$^1/_2$		
	5	3	$2^1/_2$	2	$1^1/_2$	1				
	9	2	2	$1^1/_2$	$1^1/_2$	1	1	1	0	0
	7	3	2	$1^1/_2$	$1^1/_2$	$1^1/_2$	1	$^1/_2$		
	8	2	2	2	$1^1/_2$	$1^1/_2$	1	1	1	
75	6	2	2	2	$1^1/_2$	1	1			
	7	3	$2^1/_2$	2	2	1	1	$^1/_2$		
	6	$2^1/_2$	2	2	2	$^1/_2$	0			
	6	$2^1/_2$	2	2	1	1	$^1/_2$			
	8	2	2	2	2	$1^1/_2$	$1^1/_2$	$1^1/_2$	1	
	5	$2^1/_2$	2	2	$1^1/_2$	$^1/_2$				
	9	2	2	2	2	$^1/_2$	$^1/_2$	$^1/_2$	$^1/_2$	0
	7	$2^1/_2$	2	2	2	$1^1/_2$	1	0		
	6	2	2	1	1	$^1/_2$	0			
	6	2	2	2	$1^1/_2$	1	1			
	4	2	2	$1^1/_2$	$1^1/_2$					
	6	2	2	$1^1/_2$	$1^1/_2$	1	$^1/_2$			
	8	3	2	2	2	2	$1^1/_2$	1	$^1/_2$	
	7	$2^1/_2$	2	$1^1/_2$	1	1	1	$^1/_2$		

Time elapsed since beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.								
	8	2	2	2	2	2	1	1	0	
	7	3	3	3	2	1	1	½		
	4	2	2	1½	1					
80	8	3	2½	2½	2½	2½	2½	1	½	
	8	2	2	2	2	2	1½	1½	½	
	7	3	2	1½	1½	1	1	1		
	6	2½	2	2	1½	1	½			
	9	3	2	2	2	1½	1	½	½	0
	8	2½	2	2	1½	1½	1	½	½	
	7	2	2	1½	1	1	1	½		
	9	2½	2	1½	1	1	½	½	½	0
	8	2	2	1	1	1	1	0	0	
	8	1½	1½	1	1	1	1	0	0	
	8	2	2	1½	1½	1	1	1	0	
	9	2	2	1½	1½	1½	1½	1½	0	0
	9	2½	2	2	2	1½	1	1	0	0
	6	2	1½	1	1	1	½			
	7	2	2	2	1½	1	½	0		
90	9	2	1	½	½	½	0	0	0	0
	5	2½	2	1½	1½	½				
	8	1½	1	½	½	0	0	0	0	
	8	1	1	1	1	½	½	0	0	

A consideration of the numbers of this table shows at once that after 60 and 65 minutes the maximal curvature has not been reached and that after 90 minutes it is already falling off again. The observations after 70 and 80 minutes are collected in a separate table as follows:

TABLE 5.

Number of plants observed after 70 minutes 133, after 80 minutes 117.

Extent of curvature in m.m.	Number of plants		Percentages	
	70 min.	80 min.	70 min.	80 min.
3	6	3	4.5	2.5
2½	6	9	4.5	7.5
2	36	29	27	25
1½	25	22	19	19
1	34	29	26	25
½	14	13	10	11
0	12	12	9	10

For the same reason, why in table 1 the observations were made after 65 minutes, they were made in this case after 75 minutes. The result is here quite simular, as is seen from table 6.

TABLE 6.

Number of plants observed after 75 minutes 110.

Extent of curvature in m.m.	Number of plants	Percentage
3	5	4.5
2½	6	5.5
2	45	41.5
1½	14	12.5
1	22	20
½	13	11.5
0	5	4.5

A comparison of tables 5 and 6 makes it quite evident, on account of the high percentage of curvatures of 2 m.m. in table 6, that the reaction time at a strength of 40 M.C.S. is 75 minutes and that the maximal curvature is 2 m.m.

C. *Geotropic experiments with Sinapis seedlings.*

Sinapis seedlings grown in the dark show a curve in the hypocotyl, so that the upper part of this organ, with the two cotyledons, is at right angles to the lower portion. The cotyledons do not spread out (fig. 1). The germinated seeds were always planted in such a way that all plants had the bent portion pointing in the same direction. In order to render good measurements, the curvature was always induced in such a manner, that they became opposed to the natural curve of the hypocotyl.

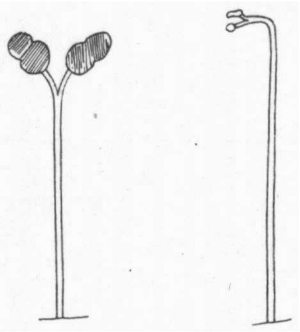

Fig. 1.

A striking feature of the experiments was the large number of plants which did not curve. I have therefore introduced into the tables a new column, for the plants which did not curve, which I have left out of account in my determination of the maximal reaction. A few preliminary experiments with a strength of stimulus of 900 m.g.-secs. showed, that this gave with *Sinapis* a much stronger curvature than with *Avena*, so that I chose a duration of the stimulus of 10 minutes, which consequently corresponds to a strength of stimulus of 600 m.g.-secs. As I did the experiments on *Sinapis* in the summer months, it was impossible to keep the temperature constant at 20° C, so that for all these experiments I chose a temperature 3 degrees higher.

TABLE 7.

Strength of stimulus 600 m.g.-secs. Temperature 23° C.

Time elapsed since beginning of stimulation in minutes	Number of seedlings curved	Number of seedlings not curved	Extent of curvatures in m.m.
40	4	2	4 3 2½ 2½
	5	0	5 4 3 3 2½
	7	2	3 3 3 2 2 2 1
	5	3	3 3 2½ 2 2
	6	2	2 2 2 2 1½ 1
	5	4	2 2 1½ 1½ 1½
	4	2	3 2 2 1
	4	1	3 2½ 2½ 1
	5	1	4 3 3 3 2½
	4	1	3 3 2 1½
	4	0	4 2½ 2 2
	5	2	2½ 2½ 2½ 2 2
	3	3	3 2½ 1
	4	3	2½ 2½ 1½ 1
	5	1	4 3 2 2 2
	4	2	3 2½ 2 1
45	4	2	3 2½ 2½ 2
	6	2	4 3 3 3 3 2½
	6	2	3 3 3 2½ 2 2
	4	1	2½ 1½ 1½ 1½
	4	0	3 3 2 1½
	4	2	3 3 3 2
	5	3	4 3 3 2 1½
	4	2	2½ 1½ 1½ 1
	4	1	3 2½ 2 2
	5	3	4 4 3 2 2
	4	0	3 3 2 1½
	3	3	3 3 1½
	7	1	4 3 3 3 2½ 2½ 1

Time elapsed since beginning of stimulation in minutes	Number of seedlings curved	Number of seedlings not curved	Extent of curvatures in m.m.
	5	1	3 3 $2^{1}/_{2}$ $2^{1}/_{2}$ 2
	5	4	5 3 3 $2^{1}/_{2}$ $1^{1}/_{2}$
	4	0	3 3 $2^{1}/_{2}$ 2
	4	2	3 $2^{1}/_{2}$ $1^{1}/_{2}$ $1^{1}/_{2}$
	7	1	3 3 $2^{1}/_{2}$ $2^{1}/_{2}$ $2^{1}/_{2}$ 2 $1^{1}/_{2}$
	4	3	3 $2^{1}/_{2}$ 2 $1^{1}/_{2}$
	3	3	3 2 1
	6	0	3 3 3 3 2 2
50	4	2	5 4 $2^{1}/_{2}$ 2
	6	2	3 3 3 $2^{1}/_{2}$ $2^{1}/_{2}$ 2
	4	3	3 $2^{1}/_{2}$ $2^{1}/_{2}$ 2
	6	2	4 3 2 2 2 1
	4	2	4 3 3 2
	4	1	3 3 2 2
	4	3	3 $2^{1}/_{2}$ $1^{1}/_{2}$ $1^{1}/_{2}$
	5	1	3 $2^{1}/_{2}$ $2^{1}/_{2}$ 2 2
	4	2	3 $2^{1}/_{2}$ 2 $1^{1}/_{2}$
	4	2	3 $2^{1}/_{2}$ $1^{1}/_{2}$ 1
	4	0	4 $2^{1}/_{2}$ $1^{1}/_{2}$ $1^{1}/_{2}$
	4	2	3 3 $1^{1}/_{2}$ 1
	4	3	3 $2^{1}/_{2}$ $2^{1}/_{2}$ 2
	7	0	3 3 3 3 $2^{1}/_{2}$ $2^{1}/_{2}$ $2^{1}/_{2}$
	5	0	3 3 2 $1^{1}/_{2}$ 1
	7	0	3 3 $2^{1}/_{2}$ 2 $1^{1}/_{2}$ $1^{1}/_{2}$ 1
	4	1	4 3 $2^{1}/_{2}$ $1^{1}/_{2}$
	5	1	3 2 2 $1^{1}/_{2}$ $1^{1}/_{2}$
	4	0	4 3 3 $2^{1}/_{2}$
60	6	2	2 2 $1^{1}/_{2}$ 1 $^{1}/_{2}$
	4	2	2 2 $1^{1}/_{2}$ 1
	4	2	3 2 2 $^{1}/_{2}$
	3	2	2 $^{1}/_{2}$ $^{1}/_{2}$
	5	2	3 2 2 1 1

The time in which the maximal curvature is brought about, follows at once from tables of the curvatures after 40, 50 and 45 minutes.

TABLE 8.

Number of plants observed after 40 minutes 74, after 50 minutes 95.

Extent of curvature in m.m.	Number of plants		Percentage	
	40 min.	50 min.	40 min.	50 min.
5	1	1	1.5	1
4	5	6	7	6.5
3	18	31	25	32
2½	15	19	20	20
2	22	19	30	20
1½	6	13	8	13.5
1	7	6	9	6.5

TABLE 9.

Number of plants observed after 45 minutes 98.

Extent of curvature in m.m.	Number of plants	Percentage
5	1	1
4	5	5
3	39	40
2½	18	18.5
2	18	18.5
1½	14	14.5
1	3	3

Since the percentage of seedlings which after 45 minutes show a curvature of 3 m.m., is considerably higher than after 40, or after 50 minutes, it follows that in this case the reaction time is 45 minutes and the maximal curvature 3 m.m.

D. *Phototropic experiments with Sinapis seedlings.*

So far we have seen, that in *Avena* at a temperature of 20° there is required for a geotropic curvature of 2 m.m. a stimulus of 900 m.g.-secs. and for an equally strong phototropic curvature a stimulus of 40 M.C.S., further that in *Sinapis* at 23° a geotropic reaction of 3 m.m. is brought about by a stimulus of 600 m.g.-secs. We might therefore expect that for a phototropic curvature of 3 m.m. in *Sinapis* a stimulus of less than 40 M.C.S. would be required. I began therefore with a quantity of light of 25 M.C.S. but even after two hours I could not see the slightest reaction. Then I tried with 40, 60 M.C.S. but always with the same negative result. Finally I stimulated with 400 M.C.S. and in that case observed a distinct curvature after one hour.

TABLE 10.

Strength of stimulus 400 M.C.S. Temperature 23° C.

Time elapsed since beginning of stimulation in minutes	Number of seedlings curved	Number of seedlings not curved	Extent of curvature in m.m.
	5	3	2 1½ 1½ 1½ 1
	3	3	2 1 1
	3	2	1½ 1½ 1
	4	4	2 1½ 1 1
	5	3	1½ 1½ 1 1 1
	4	2	1½ 1½ 1 1

Time elapsed since beginning of stimulation in minutes	Number of seedlings curved	Number of seedlings not curved	Extent of curvature in m.m.						
	4	2	2	2	$1^1/_2$	1			
	4	2	$2^1/_2$	2	$1^1/_2$	1			
	3	3	$1^1/_2$	$1^1/_2$	1				
	5	1	$1^1/_2$	$1^1/_2$	1	1	1		
	4	5	$2^1/_2$	$1^1/_2$	1	1			
	4	3	3	2	$1^1/_2$	1			
	4	4	3	2	$1^1/_2$	$1^1/_2$			
60	6	1	2	2	$1^1/_2$	$1^1/_2$	1	1	
	3	2	3	2	$1^1/_2$				
	3	2	2	$1^1/_2$	1				
	4	0	$2^1/_2$	2	2	1			
	5	2	2	2	2	$1^1/_2$	1		
	4	1	$2^1/_2$	$2^1/_2$	$2^1/_2$	2			
	4	2	2	2	1	1			
	4	2	3	$2^1/_2$	2	2			
	7	2	3	2	$1^1/_2$	$1^1/_2$	$1^1/_2$	1	1
	5	2	2	2	2	$1^1/_2$	1		
	5	3	2	2	2	$1^1/_2$	$1^1/_2$		
	5	1	2	$1^1/_2$	$1^1/_2$	1	1		
65	4	1	$1^1/_2$	$1^1/_2$	1	1			
	4	2	3	3	$1^1/_2$	1			
	5	0	$2^1/_2$	$1^1/_2$	$1^1/_2$	$1^1/_2$	1		
	6	3	2	2	2	$1^1/_2$	1	1	
	5	1	2	2	$1^1/_2$	1	1		
	6	3	3	$2^1/_2$	2	2	$1^1/_2$	$1^1/_2$	
	4	2	$1^1/_2$	$1^1/_2$	1	1			
	3	3	$1^1/_2$	1	1				
	4	3	2	$1^1/_2$	1	1			
	4	0	2	2	2	2			
	4	3	2	2	2	$1^1/_2$			
	6	2	2	2	2	$1^1/_2$	$1^1/_2$	1	
	4	2	2	2	$1^1/_2$	1			

Time elapsed since beginning of stimulation in minutes	Number of seedlings curved	Number of seedlings not curved	Extent of curvature in m.m.
70	3	3	$1^1/_2$ $1^1/_2$ 1
	3	2	2 1 1
	5	2	3 $1^1/_2$ $1^1/_2$ 1 1
	3	2	2 1 1
	4	2	$1^1/_2$ 1 1 1
	5	1	2 $1^1/_2$ $1^1/_2$ $1^1/_2$ $1^1/_2$
	4	2	$2^1/_2$ 2 $1^1/_2$ $1^1/_2$
	6	3	3 $2^1/_2$ $2^1/_2$ 2 $1^1/_2$ $1^1/_2$
	3	2	$1^1/_2$ $1^1/_2$ 1
	5	0	3 $2^1/_2$ $1^1/_2$ $1^1/_2$ 1
	5	1	$1^1/_2$ $1^1/_2$ $1^1/_2$ $1^1/_2$ 1
	6	2	2 $1^1/_2$ $1^1/_2$ $1^1/_2$ 1 1

The observations for 55, 65 and 60 minutes are summarized below as before.

TABLE 11.

Number of plants observed after 55 minutes 52, after 65 minutes 59.

Extent of curvature in m.m.	Number of plants		Percentage	
	55 min.	65 min.	55 min.	65 min.
3	2	3	4	5
$2^1/_2$	2	2	4	3.5
2	8	20	15	37
$1^1/_2$	20	18	38.5	31.5
1	20	16	38.5	27

TABLE 12.

Number of plants observed after 60 minutes 55.

Extent of curvature in m.m.	Number of plants	Percentage
3	3	5.5
$2^{1}/_{2}$	5	9
2	22	40
$1^{1}/_{2}$	13	23.5
1	12	22

A comparison of these two tables, together with the high percentage of curvatures of 2 m.m. after an observation time of 60 minutes, shows at once that with a stimulus of 400 M.C.S. the maximal reaction for *Sinapis* seedlings is 2 m.m. and that it reached after 60 minutes.

If we compare the results obtained with *Avena* and with *Sinapis*, the much greater variability of the curvatures of the latter plant is at once evident and further the large number of non-reacting seedlings. As against 289 *Sinapis* seedlings which gave a geotropic reaction, there were 102 which did not curve, i.e. of the 391 objects used 102, or 26.1 $^{0}/_{0}$, did not react. In the phototropic experiments 104 of the 322 seedlings remained straight, i.e. 32.3 $^{0}/_{0}$. These figures show that we must not be too ready to draw conclusions from the non-curvature of a few seedlings.

CHAPTER IV.

Effect of the complete withdrawal of free oxygen on the perception.

In order to find out whether oxygen is necessary for the perception of a stimulus, I first left the seedlings for some time in the thermostat through which a continuous current of nitrogen was passed. After this they were stimulated in nitrogen whilst the nitrogen current was at once replaced by air, drawn through the thermostat by means of an aspirator. The seedlings were therefore in ordinary air during the reaction-time.

An examination of the gas, that flows out of the thermostat, showed me, that after $1^1/_2$ to 2 hours all the original air had been expelled. When, for instance, a fore-period of 3 hours in nitrogen is spoken of in the tables, there 3 hours are reckoned from the beginning of the passing through of the current, so that during the first 2 hours the seedlings were not yet in an atmosphere of pure nitrogen.

A. *Geotropic experiments with Avena.*

The seedlings of *Avena* were placed vertically in the thermostat [1]), whilst for a time nitrogen was passed through, then they were for 15 minutes geotropically stimulated

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[1]) For this reason henceforward the experiments were carried out with one box at a time. A second box was indeed fastened in the clamp, but this served exclusively to balance the other in order to secure the uniform rotation of the clinostat during the reaction-time.

and afterwards whilst air was drawn through they were rotated on the clinostat. The extent of the curvature was then always measured after 65 minutes, namely, the reaction-time in air. These measurements were made in red light, because I afterwards placed the objects again for some time on the clinostat in order to find out whether, in those cases, in which the reaction after 65 minutes was weaker than it would have been in ordinary air, there might possibly be an increase again afterwards. This possibility was in no case realised, so that, by perception in nitrogen no lengthening of the reaction-time was caused.

TABLE 13.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.
Reaction-time 65 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.							
2 hours	7	2½	2	2	1½	1½	1	0	
	5	3	2	1	1	1			
	5	2	2	2	1	1			
	6	2½	1½	1½	1½	1½	½		
	4	2	1½	1½	1				
	7	3	2½	2	1½	1	1	½	
	6	2	2	2	1½	1½	½		
	6	2	2	1½	1	1	0		
	8	2	2	2	2	1½	1½	1	0
3 hours	6	2	2	1½	1½	1	1		
	6	2	1½	1½	1	½	½		
	7	2	2	1½	1½	1	1	½	
	7	2	2	2	1½	1½	1½	1½	
	4	2	1½	1½	0				
	7	2½	2	1½	1½	1½	1½	1	
	7	3	2	2	1½	1½	1½	1½	
	8	2½	2	2	2	1½	½	½	½

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.
	7	2 2 2 1 ½ 0 0
	6	2½ 2 2 2 1½ 1½
	9	2½ 2 2 2 2 1½ 1½ 1 1
	8	2 2 2 1½ 1½ 1½ 1 ½
	5	2 2 1½ 1 ½
	9	3 3 2½ 2 2 1½ 1½ 1½ 1
	7	2½ 2 2 2 1½ 1 1
5 hours	5	1 1 ½ 0 0
	7	1 1 1 ½ 0 0 0
	5	1 ½ ½ ½ 0
	4	½ ½ 0 0
6 hours	8	all without curvature
	5	" " "
	7	" " "

It follows therefore from this table that a fore-period of 2 or 3 hours has not the least effect, as directly appears when, for instance, the results of observations with a fore-period of 3 hours are collected in a table, as follows:

TABLE 14.

Number of plants observed 103.

Extent of the curvatures in m.m.	Number of plants	Percentage
3	3	3
2½	6	6
2	35	34
1½	33	32
1	14	13.5
½	9	8.5
0	3	3

If the percentage-figures are compared with those of table 3, the agreement is very striking, for both tables show a maximal curvature of 2 m.m. When there is a fore-period of 5 hours in nitrogen a great weakening of the reaction is traceable, whilst there is absolutely no reaction when the fore-period in nitrogen amounts to 6 hours. (Pl. XVIII, fig. 1).

B. *Phototropic experiments with Avena.*

Everything that has been said of the course of the geotropic experiments, applies also mutatis mutandis to these, with the difference naturally, that the stimulus used was 40 M.C.S. and that the extent of curvature was measured after 75 minutes. Here also the curvature-process was followed out for a longer time, but no increase in the reaction was ever observed.

TABLE 15.

Strength of stimulus 40 M.C.S. Temperature 20° C.
Reaction-time 75 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of curvatures in m.m.							
3 hours	6	2	2	$1^{1}/_{2}$	$1^{1}/_{2}$	1	1		
	7	$2^{1}/_{2}$	2	2	2	$1^{1}/_{2}$	1	$^{1}/_{2}$	
	5	2	2	$1^{1}/_{2}$	$1^{1}/_{2}$	$^{1}/_{2}$			
	5	2	2	1	1	1			
	6	3	2	2	2	1	0		
	4	2	2	$1^{1}/_{2}$	1				
	8	$2^{1}/_{2}$	$2^{1}/_{2}$	2	2	$1^{1}/_{2}$	1	$^{1}/_{2}$	$^{1}/_{2}$
	5	2	2	$1^{1}/_{2}$	$^{1}/_{2}$	0			
	6	2	2	2	$1^{1}/_{2}$	1	1		
	6	2	2	2	$1^{1}/_{2}$	$^{1}/_{2}$	$^{1}/_{2}$		

Fore-period in nitrogen	Number of seedlings	Extent of curvatures in m.m.
	8	3 2 2 $1^{1}/_{2}$ $1^{1}/_{2}$ 1 1 0
	4	2 $1^{1}/_{2}$ $1^{1}/_{2}$ 1
6 hours	5	$1^{1}/_{2}$ 1 $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$
	8	$1^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ 0 0 0 0
	7	1 1 $^{1}/_{2}$ $^{1}/_{2}$ 0 0 0
	4	$^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$
	6	1 $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ 0 0
	5	$1^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ 0
	7	1 1 $^{1}/_{2}$ $^{1}/_{2}$ $^{1}/_{2}$ 0 0
8 hours	6	all without curvature
	6	„ „ „
	8	„ „ „

Even without collectury the results in a table, it is at once seen that a fore-period of 3 hours has as little effect as in the geotropic experiments. If the fore-period lasts 6 hours then the influence is undoubtedly noticeable, because the reaction is considerably weaker, whilst it remains entirely absent when the seedlings before stimulation have been for 8 hours in a nitrogen atmosphere.

From all these experiments it follows therefore that the seedlings must be deprived of oxygen for a considerable time in order that a reaction may be completely inhibited. There are clearly two possible explanations of this:

1. It is possible that the objects of experiment in consequence of intramolecular respiration have sufficient energy at this disposal to enable them, after a long time, albeit to a lesser degree, to perceive a stimulus and execute a reaction, when after stimulation they have again been supplied with atmospheric air.

2. The material has only apparently been deprived of air for all these hours, since it is quite possible that besides in the intercellular cavities of the cells there still remains after a long time a certain quantity of air in the cavity between the coleoptile and the first leaf. Consequently oxygen-respiration continues at first in sufficient degree to explain the occurrence of the reaction. In order to decide between these two possibilities I have carried out the same experiments with seedlings of *Sinapis alba*, because in them the cavities under the cotyledons are absent.

C. *Geotropic experiments with Sinapis.*

The experiments were therefore repeated in the same manner, namely, a fore-period in nitrogen, afterwards geotropic stimulation also in nitrogen, by placing the seedlings for 10 minutes in a horizontal position. Then atmospheric air was passed through in order that a possible reaction might take place.

TABLE 16.

Strength of stimulus 600 m.g.-secs. Temperature 23° C.
Reaction-time 45 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.						
1 hour	5	4	$2^1/_2$	$1^1/_2$	1	0		
	5	5	3	3	$2^1/_2$	$2^1/_2$		
	6	3	3	3	$2^1/_2$	1	0	
	6	3	3	$2^1/_2$	2	2	0	
	4	3	2	2	0			
	7	3	3	2	$1^1/_2$	$1^1/_2$	0	0
3 hours	4	$1^1/_2$	$1^1/_2$	1	0			
	4	$2^1/_2$	2	0	0			

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.
4 hours	4	3 1 1 1
	6	$1^1/_2$ $1^1/_2$ 1 1 0 0
	6	$2^1/_2$ 2 $1^1/_2$ 1 1 0
	5	2 2 1 1 0
	5	1 0 0 0 0
	6	all without curvature
	7	" " "
	4	" " "
	5	" " "

A fore-period of 1 hour has no effect, as might be expected, because there was as yet no pure nitrogen in the thermostat. With a fore-period of 3 to 4 hours a weakening of the reaction is clearly seen, whilst further the table shows that seedlings which have been for 4 hours without oxygen, remain upright, notwithstanding stimulation. (Pl. XVIII, fig. 2).

D. *Phototropic experiments with Sinapis.*

TABLE 17.

Strength of stimulus 400 M.C.S. Temperature 23° C.
Reaction-time 60 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.
1 hour	6	2 2 1 1 0 0
	5	2 $1^1/_2$ 1 0 0
	7	2 2 2 $1^1/_2$ 1 0 0
	4	$2^1/_2$ 2 2 1
	6	3 $2^1/_2$ 2 2 0 0

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.						
3 hours	6	2	$1^1/_2$	$1^1/_2$	1	1	0	
	5	$1^1/_2$	$1^1/_2$	$1^1/_2$	1	0		
	6	2	$1^1/_2$	1	1	0	0	
	6	2	2	1	1	1	0	
	5	$1^1/_2$	$1^1/_2$	1	0	0		
	7	2	$1^1/_2$	$1^1/_2$	1	1	0	0
4 hours	4	$1^1/_2$	0	0	0			
	5	1	0	0	0	0		
	4	1	0	0	0			
	4	all without curvature						
	4	,, ,, ,,						
	5	,, ,, ,,						
	7	,, ,, ,,						

In this case also after a fore-period of 3 hours in nitrogen a weakened reaction is seen to take place, whilst it further follows from the table, that seedlings, which have passed 4 hours in a space without oxygen are no longer able to react to the stimulus.

A comparison of the results of the experiments with *Avena* and with *Sinapis* shows that, in the case of the latter the reaction is indeed abolished sooner, but the differences are too slight to allow of the deduction that the seedlings of *Avena* react so much longer in consequence of the oxygen present in the space under the coleoptile. If this were the case, then the difference between the two would have to be greater and the irritability in *Sinapis* would have to disappear much earlier. I think I may therefore conclude that in both cases it is due to intramolecular respiration that a stimulus can yet have an effect after the seedlings have passed a somewhat long fore-period in nitrogen.

These experiments therefore all have the same result. We find that when the seedlings have been deprived of oxygen for a sufficiently long time, a stimulus applied in nitrogen cannot find expression, although immediately after stimulation, the nitrogen was replaced by atmospheric air.

May we then at once conclude, that the stimulus is not perceived? I imagine not, for it is always quite possible that in all the cases described above, the stimulus was really perceived, but that the processes in the plant which cause the reaction had already been affected in advance by the prolonged absence of oxygen to such an extent that no curvatures took place. In order to settle this point, I did experiments in which the seedlings were given as long a fore-period in nitrogen, as in those cases described above in which a reaction no longer occured, but now the stimulus was not applied until the nitrogen had been replaced by air, so that therefore stimulation took place in air. The passing through of air was of course continued during the reaction-time.

E. *Geotropic experiments with Avena.*

A fore-period of 6 hours in nitrogen. Afterwards for ¼ hour geotropic stimulation in air. (Pl. XVIII, fig. 3).

TABLE 18.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.
Reaction-time 65 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.							
6 hours	8	2	1½	1	1	1	1	½	½
	7	1½	1½	1	1	1	1	½	
	7	2	1	1	1	1	1	½	
	8	1½	1½	1½	1	1	1	½	½

If this table is compared with the results obtained after a fore-period in oxygen of 6 hours, which are collected in table 13, then the difference can only be explained by the fact that in the earlier experiments the stimulus was given in the absence of and in the later ones in the presence of oxygen. We have now therefore become entitled to the conclusion that the seedlings can no longer perceive a geotropic stimulus when they have been for a long time deprived of oxygen.

We should note, however, that the curvature of table 18 are smaller than those wich arise under normal conditions. This suggests, that, as a result of continued want of oxygen, the seedlings undergo a harmful influence, which is still felt when normal conditions are re-established.

F. *Phototropic experiments with Avena.*

Fore-period of 8 hours in nitrogen. Afterwards phototropic stimulation in air. (Pl. XVIII, fig. 4).

TABLE 19.

Strength of stimulus 40 M.C.S. Temperature 20° C.
Reaction-time 75 minutes.

Fore-period in nitrogen	Number of seedlings	Extent of the curvatures in m.m.
8 hours	9	$1^1/_2$ $1^1/_2$ 1 1 $^1/_2$ $^1/_2$ $^1/_2$ $^1/_2$ 0
	8	$1^1/_2$ $1^1/_2$ 1 1 $^1/_2$ $^1/_2$ $^1/_2$ 0
	6	1 1 $^1/_2$ $^1/_2$ $^1/_2$ $^1/_2$
	8	$1^1/_2$ $1^1/_2$ 1 1 1 $^1/_2$ $^1/_2$ 0

The result is exactly the same as in the geotropic experiments and it follows in the same way that the seedlings, in consequence of the lengthy absence of oxygen, are unable to perceive a phototropic stimulus.

A stimulus in ordinary air, after a long fore-period in nitrogen, is indeed perceived, the plant seems however to have lost a part of its power of perception, as is shown by the curvatures being smaller than when the whole process takes place under normal conditions.

CHAPTER V.

Effect of the complete withdrawal of free oxygen on the reaction.

The influence of oxygen on the perception was determined, by stimulation in nitrogen, after a long fore-period in the same gas, and allowing the reaction to take place in atmospheric air. This suggests a consideration of the influence of oxygen on the reaction-process in the reverse direction, by allowing the seedlings to perceive in ordinary air and to rotate them on the clinostat in nitrogen during the reaction-time. But this method is quite impracticable, because the replacement of atmospheric air by nitrogen requires $1^1/_2$ to 2 hours and it is therefore impossible to place the material in nitrogen at once after perception.

I had therefore to proceed in a wholly different manner. I began by giving the seedlings a fore-period of 3 hours in nitrogen, then stimulated them in nitrogen and left them in it for the whole of the reaction-time. The difference between these experiments and those under A, B, C and D of chapter IV lies therefore in the fact that there a normal reaction occurred in atmospheric air, after the fore-period in nitrogen had lasted three hours, and the stimulus had been applied in this gas, whilst in these experiments the whole stimulation-process took place in nitrogen.

If there is an unequivocal difference between the two sets of experiments, then it can only be attributed to the influence on the reaction of the deprivation of oxygen.

The strength of the stimulus was of course the same in both series of experiments.

I left a few boxes of seedlings in a nitrogen atmosphere in the clinostat for a considerable time after stimulation and in this way followed the course of the process for a somewhat longer time than the normal reaction-time.

I carried out these experiments in order to discover whether there was perhaps in this case, in consequence of the absence of oxygen, a lengthening of the reaction-time. In no case, however, was a curvature found. In a second series of experiments, I always stopped the supply of nitrogen after the end of the reaction-time, that is to say, after the period that was found, in the previous chapters to be the reaction-time. Then ordinary air was sucked through the thermostat for some time. In that case after a certain time a curvature took place, albeit a weak one.

A. *Geotropic experiments with Avena.*

Fore-period of 3 hours in nitrogen. Then stimulation in nitrogen, followed by a prolonged-period, also in nitrogen.

TABLE 20.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.

Fore-period in nitrogen.	Time elapsed since the beginning of stimulation in minutes.	Number of seedlings	Extent of the curvatures in m.m.
3 hours	65	7	all without curvatures
	100		„ „ „
	125		„ „ „
	150		„ „ „
	65	8	all without curvatures
	100		„ „ „

Fore-period in nitrogen	Time elapsed since the beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.
	130		all without curvatures
	65	8	7 plants without curvatures, 1 with asymmetric apex.
	100		”
	130		”
	65	7	all without curvatures

This table shows that when a geotropic stimulus is perceived, the latter does not express itself in the absence of oxygen.

B. *Geotropic experiments with Avena.*

Fore-period of 3 hours in nitrogen. Stimulation in this gas; 65 minutes after the commencement of stimulation the nitrogen replaced by atmospheric air (Pl. XVIII, fig. 5).

TABLE 21.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since the beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.								
3 hours	65	9	½	0	0	0	0	0	0	0	0
	125		1	½	0	0	½	½	0	½	1
	65	8	0	0	0	0	0	0	0	0	
	90		0	0	½	½	½	½	0	½	
	120		½	½	½	1	½	1½	0	½	
	65	9	0	0	0	0	0	0	0	0	0
	120		½	½	½	½	½	½	½	1	½
	65	7	½	0	0	0	0	0	0		
	125		1½	0	½	½	1	0	1½		
	65	9	0	0	0	0	0	0	0	0	0
	105		1	0	0	½	1	1	1	½	½

If this table is compared with the foregoing one, the curvatures which have occured must be attributed to the oxygen, which after 65 minutes began to replace the nitrogen. These experiments are therefore another proof that perception of the stimulus has taken place, but that without oxygen it cannot express itself in a reaction.

That the curvatures remain fairly weak in this case (they never became stronger even with a longer observation time), is due to the fact that the stimulus is already falling off. From this it directly follows that we cannot speak of the lengthening of the reaction-time in consequence of deprivation of oxygen, because in that case the maximal reaction after stimulation with 900 m.g.-secs. i.e. a curvature of 2 m.m., would have to occur later than under normal conditions.

Of all this there is no question at all. What we see is that the reaction cannot occur at all without oxygen, whilst when the nitrogen is replaced by oxygen at a stage when the stimulus is not yet wholly exhausted, the reaction can still occur to a slighter degree. An experiment completely in agreement with this explanation is one in which there was a departure from the experiments described above in so far as that I admitted the oxygen 45 minutes after the beginning of stimulation.

TABLE 22.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since the beginning of stimulation in nitrogen	Number of seedlings	Extent of the curvatures in m.m.								
3 hours	65	9	1	1	1	1	1/2	1/2	1/2	0	0
	80		1	1	1	1 1/2	1/2	1	1/2	1/2	1/2
	100		1	1	1	1 1/2	1/2	1 1/2	1/2	1/2	1/2
	120		1 1/2	1 1/2	1	1 1/2	1	1 1/2	1	1/2	1/2

The earlier therefore that the perceived stimulus is enabled to find expression, the greater the reaction becomes. Nevertheless in this experiment the exhaustion phenomenon was also well marked, so that sufficient proof has been given that the reaction-time is not lengthened in consequence of the absence of oxygen, but that a seedling of *Avena* cannot furnish a geotropic reaction in an atmosphere without oxygen, although it can do so again as soon as normal conditions are restored. The extent of the curvature depends in that case on the greater or lesser extent to which the stimulus is already falling off.

C. *Phototropic experiments with Avena.*

Fore-period of 3 hours in nitrogen. Stimulation in this gas followed by a long period, also in nitrogen.

TABLE 23.

Strength of stimulus 40 M.C.S. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since the beginning of stimulation in nitrogen	Number of seedlings	Extent of the curvatures in m.m.
3 hours	75	7	all without curvatures
	100		" " "
	140		" " "
	170		" " "
	75	8	all without curvatures
	105		" " "
	130		" " "
	75	8	all without curvatures
	100		" " "
	135		" " "
	75	9	all without curvatures

This table therefore shows that a phototropic stimulus which has been perceived cannot give a reaction in the absence of oxygen.

D. *Phototropic experiments with Avena.*

Fore-period of 3 hours in nitrogen. Stimulation in this gas; nitrogen replaced by atmospheric air 75 minutes after beginning of stimulation, i.e. after the reaction-time of a maximal phototropic curvature of 2 m.m. under normal conditions (Pl. XIX, fig. 1).

TABLE 24.

Strength of stimulus 40 M.C.S. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since the beginning of stimulation in minutes	Number of seedlings	Extent of the curvatures in m.m.							
3 hours	75	8	0	0	0	0	0	0	0	0
	140		$^1/_2$	1	$^1/_2$	$^1/_2$	1	$^1/_2$	0	0
	75	8	0	0	0	0	0	0	0	0
	135		0	0	$^1/_2$	1	1	0	1	$^1/_2$
	75	6	0	0	0	0	0	0		
	135		$^1/_2$	$^1/_2$	1	0	1	1		
	75	8	0	0	0	0	0	$^1/_2$	0	0
	135		$^1/_2$	0	0	$^1/_2$	$^1/_2$	$1^1/_2$	$^1/_2$	1
	75	7	0	0	0	0	0	0	0	
	130		1	$^1/_2$	$^1/_2$	$^1/_2$	$^1/_2$	0	$^1/_2$	

The results of the phototropic experiments therefore completely correspond *mutatis mutandis* with those of the geotropic, so that here also it is seen that a perceived phototropic stimulus cannot find expression without the presence of oxygen, but does so immediately on the restoration of normal conditions. Since the stimulus is already falling off, a weaker reaction takes place in that case, so that there is no reason to conclude that the reaction-time is lengthened in consequence of oxygen-deprivation.

CHAPTER VI.

Effect of an Atmosphere with low oxygen-content.

In order to investigate the geotropic and phototropic stimulation-process in an atmosphere with low oxygen-content, I passed the nitrogen from the cylinder directly through the thermostat without allowing it first to pass through the wash-bottles with pyrogallol and the tube with glowing copper.

I investigated the influence of such a low oxygen-content on perception only.

The seedlings consequently underwent a fore-period of several hours in the atmosphere in question: here stimulation also subsequently took place, whilst immediately after this ordinary air was sucked through the thermostat. Since it is evident from a former chapter, that there is no where any question of a lengthening of the reaction-time, but only of a weakening of the reaction, I always measured in this case also the curvatures after the times, which are determined in Chapter III as reaction-times.

A. *Geotropic experiments with Avena.*

(Pl. XIX, fig. 2, fore-period of 8 hours, fig. 3, fore-period of 24 hours).

TABLE 25.

Strength of stimulus 900 m.g.-secs. Temperature 20° C.
Reaction-time 65 minutes.

Fore-period in $4.3\,^0/_0$ oxygen	Number of seedlings	Extent of the curvatures in m.m.
6 hours	9	$2^1/_2$ 2 2 2 2 $1^1/_2$ $1^1/_2$ $1^1/_2$ 1
8	9	3 $2^1/_2$ 2 2 2 2 $1^1/_2$ $1^1/_2$ $1^1/_2$
24	8	2 $1^1/_2$ 1 1 1 1 $^1/_2$ $^1/_2$

It is sufficiently clear from this table that the seedlings in a comparatively low oxygen-content remained for a long time in a condition to perceive a geotropic stimulus in a normal manner. When, however, they remain for 24 hours in an atmosphere of an oxygen-content of $4.3\,^0/_0$, an influence is distinctly recognisable which expresses itself by a weaker reaction, of which the cause can only be that the seedlings on account of the deficiency of oxygen were no longer able to perceive the stimulus normally.

B. *Phototropic experiments with Avena.*

(Pl. XIX, fig. 4, fore-period of 10 hours, fig. 5 fore-period of 24 hours).

TABLE 26.

Strength of stimulus 40 M.C.S. Temperature 20° C.
Reaction-time 75 minutes.

Fore-period in $4.7\,^0/_0$ oxygen	Number of seedlings	Extent of the curvatures in m.m.
10 hours	9	$2^1/_2$ 2 2 2 2 $1^1/_2$ $1^1/_2$ $1^1/_2$ 0
24	9	2 $1^1/_2$ $1^1/_2$ $1^1/_2$ 1 1 1 0 0

From this also it is evident that a fore-period of 10 hours in 4.7 $^0/_0$ oxygen has not the least influence on perception. After a fore-period of 24 hours the seedlings curve less strongly, so that in the long run they are evidently unable to perceive a phototropic stimulus normally in this atmosphere. The results are therefore identical with those of the phototropic experiments.

The object with which I had begun these experiments was as follows: to determine that oxygen-content of air at which, for geotropism as well as for phototropism the seedlings could still carry out the normal stimulation-process.

Since, however, in order to feel the retarding influence of an oxygen-content of 4—5 $^0/_0$ the seedlings must already undergo in it a fore-period of 24 hours, I have not continued these experiments. It was naturally superfluous to experiment with an atmosphere of smaller oxygen-content than 4—5 $^0/_0$, because the plants would undoubtedly be affected adversely by a fore-period in it of some duration. With regard to an atmosphere with a higher oxygen-content, it is very difficult to decide whether this has a harmful effect or not. It is quite possible that the seedlings in, for example, 8 $^0/_0$ oxygen still give a normal reaction after 24 hours but not after 48 hours. For we can only postulate a weakening of the power of perception in a given atmosphere if the seedlings have remained in it long enough before the application of the stimulus. Such experiments are impossible on account of the rapid growth of the experimental material.

CHAPTER VII.

Discussion of Results.

If the results here obtained are compared with those of Correns, they are in so far alike that he also says that for the occurrence of stimulus-movements oxygen is necessary.

Further he has published statements dealing with the oxygen-content at which the stimulation-process can still just take place. Since he finds different percentages of oxygen for the perception of the geotropic and phototropic stimulus, he concludes that geotropic curvatures can occur with smaller quantities of oxygen than phototropic ones. I cannot, with the results obtained in this investigation confirm this conclusion, on the contrary, the phenomena in the geotropic and phototropic stimulation-processes are in all respects pretty much alike. Although I have done very few experiments with seedlings in atmospheres of low oxygen-content, these experiments, however small in number, sufficiently point to a like behaviour. The reason for Correns' conclusion lies in the fact that he always worked with continued stimulations and consequently with curvatures that were not comparable, whilst moreover in that case the plants experienced a change of sensibility during the experiment. For this reason I worked with an equal maximal curvature of constant extent, both in the geotropic and in the phototropic experiments.

As Correns himself admits, he did not succeed in studying separately the influence of oxygen on perception and reaction. On this point, however, Árpád Paál was successful, but he only performed geotropic experiments.

In order to investigate how the sensory phase is dependent on the oxygen content, he determined the presentation-times, by which, in accordance with the old conception, he understood the shortest possible times during which the material must be stimulated in order to see with the naked eye as after-effect a curvature in at least 50 % of the seedlings. He found that on diminishing oxygen-content the presentation-time became longer. This is readely harmonized with my results. I always worked with the same strength of stimulus, but I have shown that when the seedlings have for long enough time been deprived wholly or partly of oxygen and perception takes place, a weaker curvature results. Now if I had applied a weaker stimulus, which did not give, as in my case, a maximal curvature of 2 m.m., but a reaction which was just visible, various seedlings would then have remained straight, anyhow to the naked eye. In order in this same atmosphere to obtain a curvature in a greater number, I should have had to strengthen the stimulus, and this therefore entirely agrees with Árpád Paál's contention.

Further there exists an apparent disagreement between my conclusion that the reaction-time was not shortened by withdrawal of oxygen and that of Árpád Paál that on diminution of the oxygen-content the reaction-time becomes longer. I have always taken as reaction-time Arisz's conception of curvature-time [1], namely, the time which elapses from the beginning of stimulation till definite maximal reaction is reached, whilst Árpád Paál employs the old conception and takes the time which elapses

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[1] Arisz, l. c. p. 60.

between the beginning of stimulation and the point at which the curvature first becomes visible. Since I have found that when the reaction takes place in the absence of oxygen, whilst the stimulus is normally perceived, a weaker curvature arises, it follows directly that the first beginning of the curvature takes place later. And this is, what Árpád Paál regards as a lengthening of the reaction-time. In this respect also therefore my results are not in conflict with his.

In most investigations, in which the influence of oxygen-withdrawal was always determined by pumping out the air, it is mentioned that the plants in a definite oxygen-content are still able to execute a geotropic or phototropic stimulation-process. When, however, still more oxygen is withdrawn, they lose this power. I cannot accept this opinion and the cause must, I think, be sought for exclusively in the method by which all these investigations were carried out, namely in the partial or total vacuum, in which the experimental material was placed. My experiments, following an entirely different method, also led to an entirely different result. In the first place my seedlings during the experiments were always under a pressure of one atmosphere, so that any possible influence of a vacuum was wholly excluded. The material which was kept in an atmosphere completely free from oxygen, preserved in it for some time the power to perceive a stimulus of 900 m.g.-secs. or of 40 M.C.S., which power, however, was entirely lost after some hours.

In the gas-mixtures with low oxygen-content, which I investigated, stimuli of this strength were still normally perceived, even when the seedlings had been for 10 hours in this atmosphere after the application of the stimulus. Yet this amount of oxygen was found to be insufficient for the seedlings in the long run, at least perception was considerably weakened after a fore-period of 24 hours.

This fore-period of the material in a given atmosphere, plays therefore an important rôle in determining whether perception is still possible in this atmosphere or not. Without further data no limits of oxygen-withdrawal can be fixed at which perception still is, and is no longer possible.

Adopting the method of exhausting in a short time one can easely investigate the influence of oxygen withdrawal on perception and reaction separately; in the first case by applying the stimulus in and vacuum and allowing the reaction to take place in ordinary air, in the other case, by stimulating in air and letting the reaction take place in a vacuum. Although I found it impossible to carry out this method of investigation, because in my experiments it took $1^1/_2$ to 2 hours for the air in the thermostat to be replaced by nitrogen, I nevertheless consider I have determined separately the influence of oxygen-withdrawal on the perception and on the reaction.

Closely connected with this investigation are the papers dealing with the influence of withdrawal of oxygen on growth [1]), because the geotropic and phototropic stimulation movements are both growth-movements. Now the opinions as to how growth in seedlings is influenced by an oxygen-free environment are greatly at variance with each other. Wortmann, Correns, Wieler and Shull state that without the least trace of oxygen no growth

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[1]) Wortmann. l.c., p. 509.

Correns. l.c., p. 139.

Wieler, l.c., p. 189.

A. J. Nabokich. Ueber die Wachstumsreize. Beih. z. Bot. Centralbl. XXVI, 1, 1910, p. 7.

E. Lehmann. Zur Kenntnis des anaeroben Wachstums höherer Pflanzen. Jahrb. f. wiss. Botanik. Bd. 42, 1911. p. 61.

Ch. A. Shull. The oxygen minimum and the germination of Xanthium seeds. The Botanical Gazette LII, 1911, pag. 453.

can take place, Nabokich and Lehmann on the other hand hold the opinion that this is quite possible, Lehmann says, for example, that seedlings of *Helianthus* continued to grow for 24 hours and longer without oxygen. Afterwards they gradually stopped and when air was introduced, became again normal. Nevertheless he asserts explicitly that all plants are not capable of growth without oxygen.

These investigations are therefore partly in agreement with my results. Even although it were to be shown that no growth can take place in an atmosphere completely free from oxygen, my results would not necessarily be in contradiction therewith, because it might quite well be possible that the seedlings which had lost their power of growth through deprivation of oxygen, had again recovered it in consequence of a geotropic or phototropic stimulus.

Summary.

All experiments were carried out with a stimulus of definite, known intensity.

In order to determine the influence of oxygen withdrawal, no complete or partial vacuum was used, but the air was always replaced by nitrogen by means of gradual diffusion. Thus the seedlings always remained under a pressure of one atmosphere.

When seedlings, which have been long enough removed from the influence of oxygen, are stimulated geotropically or phototropically, likewise in the absence of oxygen and are then at once placed in atmospheric air, they are unable to execute a reaction.

If the seedlings are given a similar fore-period in an oxygen-free atmosphere, but if the stimulus is administered in air, in which the plants are also left subsequently, a reaction does occur.

In an oxygen-free environment the perception of a stimulus cannot therefore take place, provided that the condition of a sufficiently long fore-period has been satisfied.

If, after perception of a geotropic or phototropic stimulus, the seedlings are left in the oxygen-free atmosphere, they do not react, so that the presence of oxygen is also necessary for the occurrence of the reaction.

In an atmosphere with low oxygen-content the seedlings remain for a long time able to perceive normally, but a prolonged stay in such an atmosphere weakens the power of perception.

There are no indications that, on complete or partial withdrawal of oxygen, the reaction of seedlings to a geotropic stimulus differs from their reaction to a phototropic one.

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EXPLANATION OF PLATE XVIII.

Fig. 1. Fore-period of 6 hours in nitrogen. Geotropic stimulation in nitrogen. Afterwards in air during the reaction-time.

Fig. 2. Seedlings of *Sinapis*. Fore-period of 4 hours in nitrogen. Geotropic stimulation in nitrogen. Afterwards in air during the reaction-time.

Fig. 3. Fore-period of 6 hours in nitrogen. Geotropic stimulation in air.

Fig. 4. Fore-period of 8 hours in nitrogen. Phototropic stimulation in air.

Fig. 5. Fore-period of 3 hours in nitrogen. Geotropic stimulation in this gas. 65 minutes after the beginning of stimulation nitrogen replaced by air. Then in air for a further 55 minutes.

EXPLANATION OF PLATE XIX.

Fig. 1. Fore-period of 3 hours in nitrogen, in which phototropic stimulation. 75 minutes after the beginning of stimulation nitrogen replaced by air. Then in air for a further 65 minutes.

Fig. 2. Fore-period of 8 hours in 4.3 % oxygen, in which geotropic stimulation. Then in air during the reaction-time.

Fig. 3. Fore-period of 24 hours in 4.3 % oxygen, in which geotropic stimulation. Then in air during the reaction-time.

Fig. 4. Fore-period of 10 hours in 4.7 % oxygen, in which phototropic stimulation. Then in air during the reaction-time.

Fig. 5. Fore-period of 24 hours in 4.7 % oxygen, in which phototropic stimulation. Then in air during the reaction-time.

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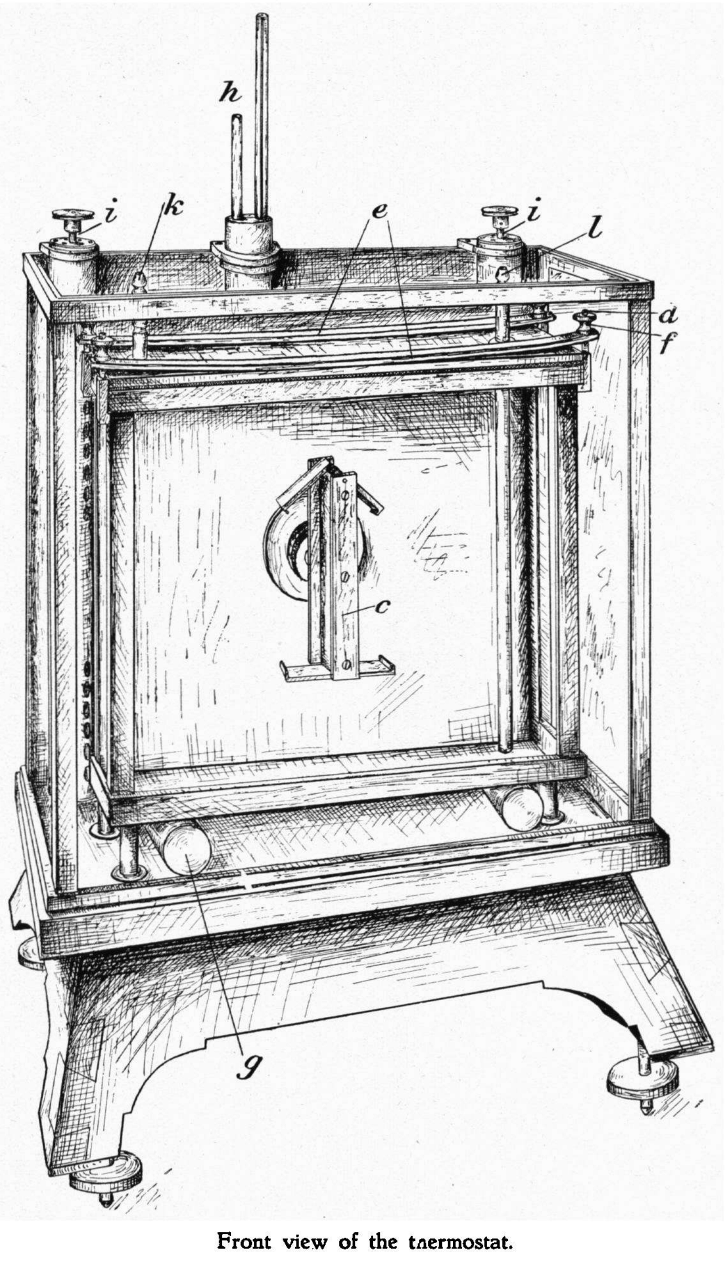
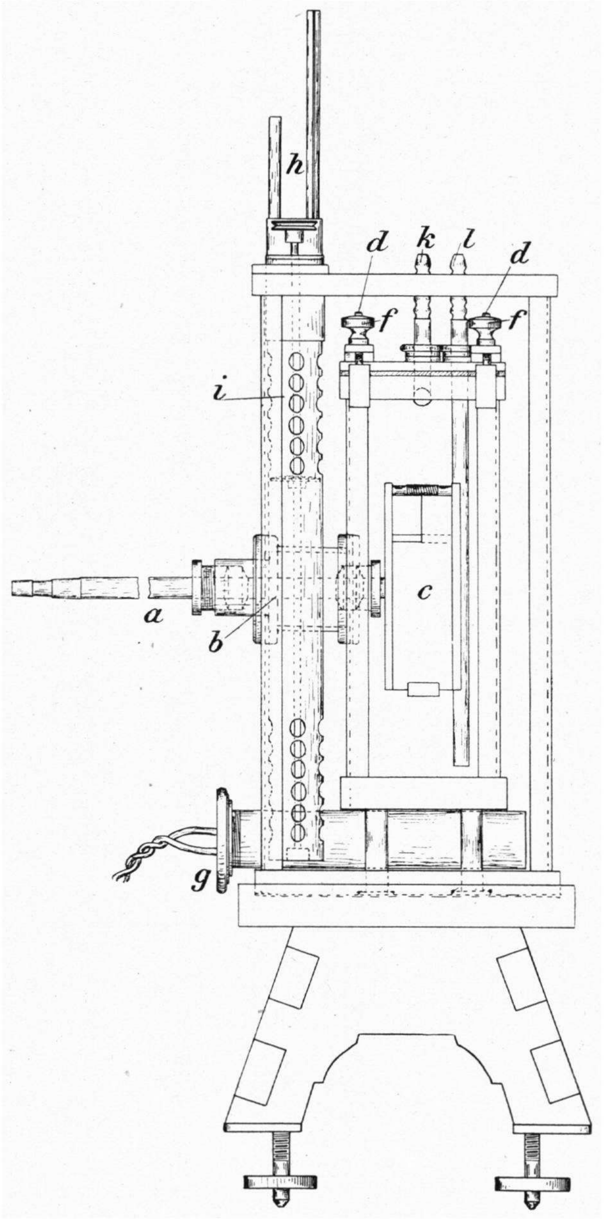
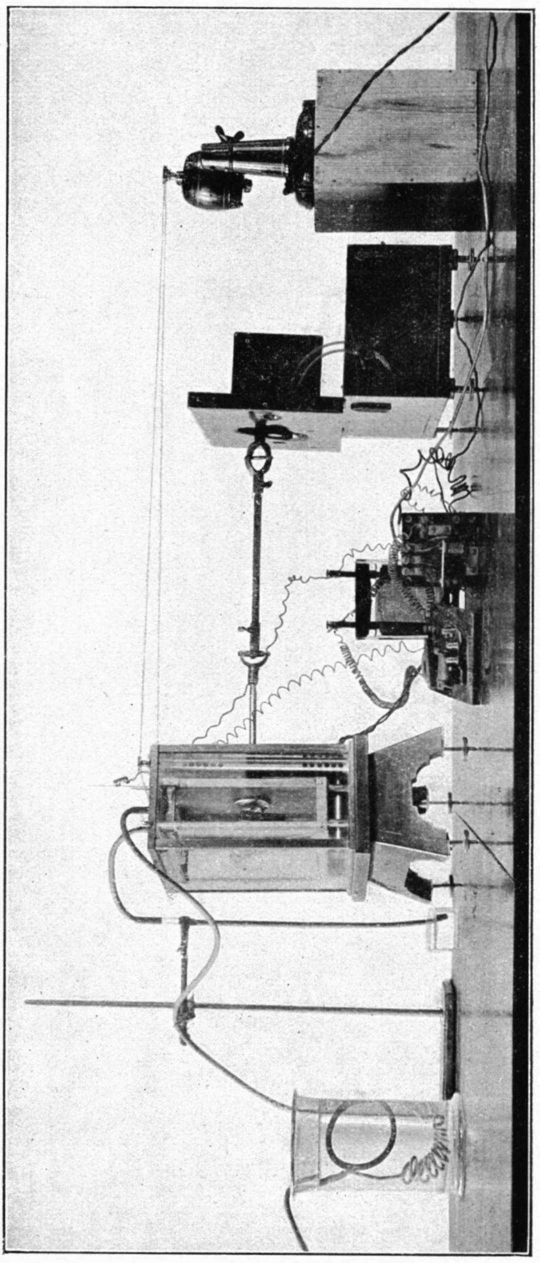
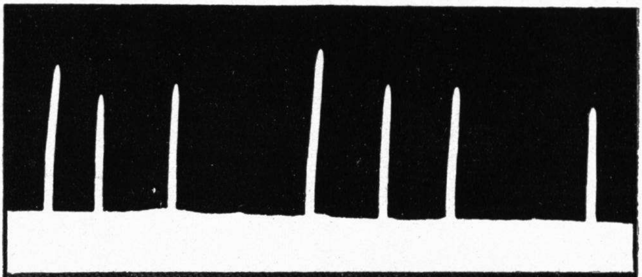


Front view of the thermostat.



Lateral view of the thermostat.





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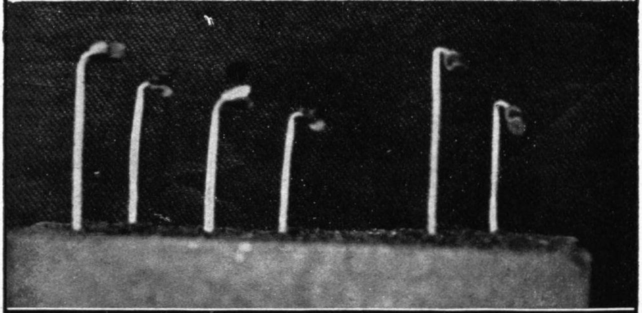

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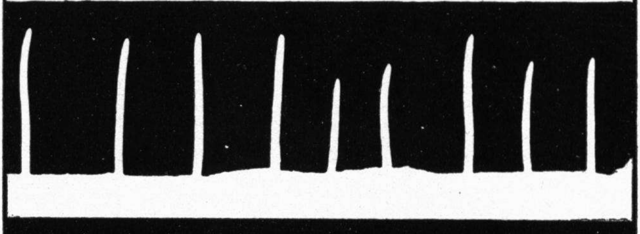

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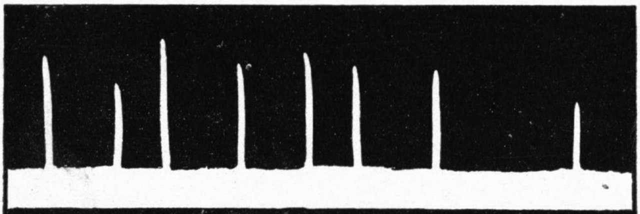

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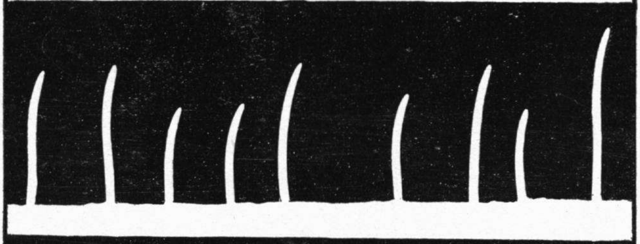

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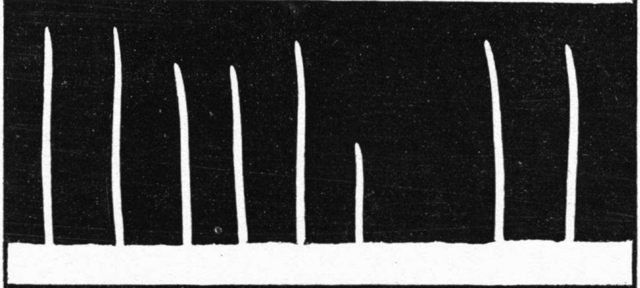

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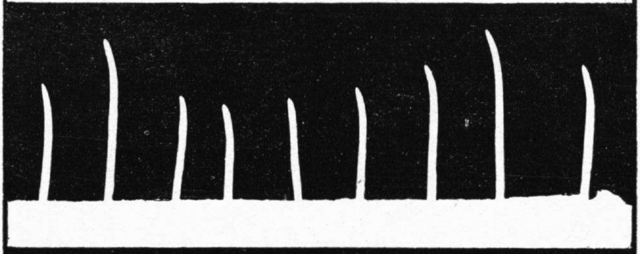

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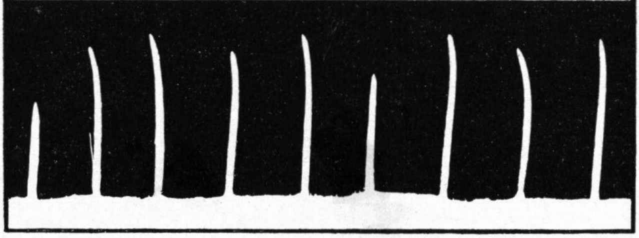

fig. 5