The Anti-ferment Reaction in Tropistic Movements of Plants [1]

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I.

TO determine whether or no an external stimulus has been perceived by a plant we have generally only one method: namely, to observe whether or not a movement follows the stimulus. The positive observation of a distinct movement is a proof that the stimulus has been perceived. When no distinct movement is to be found, it is possible, either that the stimulus has been perceived, but that a movement for some reason could not be carried out, or that the external stimulus was not perceived.

Therefore in such experiments negative results cannot be utilized. Positive results, moreover, depend exclusively upon observations made on the motor zone. Where perception of stimulus and reaction take place in separate zones of the organ, as in the geotropic perception of the root-tip, discovered by the Darwins, father and son, and in the geotropic curvature in the motor zone of the root, no processes can be detected in the sensory zone by the general method of investigation above mentioned.

My wish to gain some knowledge of what goes on in the sensitive root-tip was realized in 1897, after much fruitless endeavour (1).

A number of root-tips of *Vicia Faba major*, half of which had been geotropically stimulated, while the remainder were unstimulated, were treated in the following manner. Thick longitudinal sections were prepared and boiled in an ammoniacal solution of silver nitrate: they all gave a strong reduction. But when the specimens, carefully squeezed on the slide with the cover-glass, were held towards the light, it was clear that the stimulated tips were always darker than the unstimulated ones. This difference was already distinct long before the first beginning of the geotropic curvature. Another result was found in the fact that unstimulated root-tips placed in a water-emulsion of alcoholic guaiacum solution were coloured blue before the stimulated root-tips; in the same manner the colour tests with alkalin solution of α-naphthol + p-phenylen diamin, or with reduced indigo, were retarded to a remarkable degree. I succeeded therefore at this time in demonstrating the action of an oxydase in the three tests mentioned. But I could not decide whether the decrease in oxydasic effects (after a geotropic stimulation) is caused by a quantitative

[1] Read before the Botanical Section of the British Association, Cambridge, August, 1904.

alteration in the amount of the enzyme present, or by some retardation of the oxydasic effects. The substance reducing nitrate of silver, which increases in amount in stimulated roots, was recognized to be a phenol acid, but could not be more closely identified. Analogous changes were also found in the tip of the cotyledon of *Avena* after geotropic stimulation. These results, though of obvious significance, may for the moment be passed over.

To place my results on an unassailable basis a quantitative method was necessary, and in my search for such a method I was for some time unsuccessful. In the year 1900 it was observed in my laboratory that roots of *Lupinus albus*, being in an asphyxiated state or in chloroform narcosis, showed numerous spherical crystals which could be recognized as those of tyrosin (2). I immediately saw that this precipitate of tyrosin did not occur in the root-tip or in the youngest parts of the growing region, just in the parts where I had observed the strongest reduction of silver. It seemed possible that the reducing substance and the tyrosin crystals were genetically connected. To test this Lupine roots were treated with chloroform water until they were rich in tyrosin crystals, when they were further digested at 28° Cels. in a chloroform atmosphere. At first the cells containing tyrosin did not show any silver reduction; but after a few days the crystals of tyrosin disappeared and the cell-content reduced ammoniacal solution of silver very strongly. Roots of *Lupinus* were then finely ground and treated with chloroform water at 28° until the Millon reaction could not be observed. I then added a little pure tyrosin from Merck, Darmstadt. This also disappeared, as could be seen by the Millon test becoming gradually weaker and finally imperceptible. But the mixture was getting an exceedingly strong power of reducing silver; so that the suggestion that a silver-reducing substance is produced from tyrosin was certainly confirmed. It was also shown that this decomposition of tyrosin is connected with enzyme action.

Now since the year 1891 a substance has been known arising from tyrosin in human and animal metabolism, and possessing an active power of reducing silver. This substance was prepared by Wolkow and Baumann (3), and must be regarded according to these authors as the next higher homologous acid to gentisinic acid. This substance $C_8H_8O_4$ received in the nomenclature of Tiemann the name of 'homogentisinic acid':


Gentisinic acid:
$C_7H_6O_4$
COOH
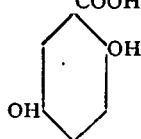
Homogentisinic acid:
$C_8H_8O_4$
$CH_2 \cdot COOH$
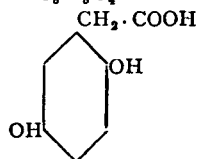
Enzymes decomposing tyrosin with formation of homogentisinic acid have been frequently prepared in recent times from animal and vegetable tissues. Such a tyrosinase Bertrand (4) discovered in Hymenomycetes (*Russula*), in the tubers of *Dahlia,* in the root of beet. Gonnermann (5) showed lately, in the case of the tyrosinase of the beet-root, that the substance produced in the fermentative alteration of tyrosin is identical with homogentisinic acid.

The effect of tyrosinase on tyrosin is oxydation accompanied by the formation of ammonia and carbon dioxide. The formation of homogentisinic acid from tyrosin is expressed by the equation :

$$C_9H_{11}NO_3 + O_3 = C_8H_8O_4 + NH_3 + CO_2$$
Tyrosin   Homogentisinic acid
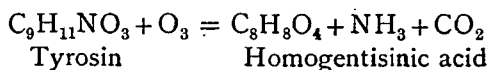
There remains the question, whether the enzyme called tyrosinase is a distinct undivisible substance or a mixture of an oxydizing and desamidizing enzyme.

An important fact established in my laboratory is the occurrence of an oxydizing enzyme in root-tips, which changes homogentisinic acid into a substance not able to reduce silver nitrate. The homogentisinic acid, therefore, cannot be regarded as a final product of metabolism from the tyrosin.

The result of our experiments shows, therefore, that a short time after the beginning of the geotropic induction, there appears a retardation of the normal destruction of tyrosin, to be recognized by an accumulation of homogentisinic acid. We have in the following report to confirm it more exactly and to describe the methods of investigation.

II.

The substance reducing $AgNO_3$ can easily be extracted from the ground root-tips by treating them with alcohol of 96 per cent. After evaporating the alcoholic solution and dissolving the remainder in water a brown solution is obtained, weakly acid to litmus, nearly free from sugar and reducing ammoniacal solution of $AgNO_3$ very strongly. The solution, even when weakly acid or neutral, assumes a dark colour on being allowed to remain in contact with air. Crystals I could never obtain from such extracts. The reactions of those solutions were nearly the same as those described by Baumann for solutions of pure homogentisinic acid. Treated with alkali the solution changes to a reddish brown or a dark brown according to the concentration ; ammoniacal solution of $AgNO_3$ is immediately reduced on warming ; cold Fehling's solution is not reduced, but is feebly reduced on boiling. Iron chloride gives a green colour, iron vitriol a blue-violet. Millon's reagent gives a yellow colour ; acetate of lead gives a precipitate. To identify homogentisinic acid (which is difficult

to crystallize) Baumann recommends the preparation of the easily crystallizable ethylester of the acid, which gives most of the tests in the same manner as does the free acid. For this purpose gaseous hydrochloric acid was passed in large excess in an alcoholic solution of the root-tip substance well cooled with snow. After twenty-four hours water was added, the fluid made alkaline with Na_2CO_3, and shaken with ether. The ether having been evaporated, there remained a syrupy mass containing embedded crystals. These crystals could finally be obtained quite pure by recrystallizing from hot water and washing with alcohol. The solubility and shape of crystals as well as the melting-point (120°) agreed exactly with the properties of the ethylester of homogentisinic acid. The reducing substance of the root-tips is therefore undoubtedly to be regarded as homogentisinic acid. Homogentisinic acid gives a reddish colour with hydrogen-peroxide, and I suppose that the red colour produced in the tissue of *Faba* roots by hydrogen peroxide, as described by Pfeffer, is connected with the presence of homogentisinic acid. Wolkow and Baumann elaborated an excellent method for the quantitative estimation of homogentisinic acid, particularly in urine. This method can be made use of for our purposes without any important modifications. Baumann titrated with decinormal solution of silver nitrate, and determined with weighed quantities of absolutely pure homogentisinic acid how much ammoniacal silver solution is needed to oxidize 1 g. homogentisinic acid. According to this estimation 1 g. of homogentisinic acid corresponds fairly closely to 2·60–2·65 g. silver ; 1 cc. decinormal solution of $AgNO_3$ therefore corresponds to 4·1 to 4·2 mg. of pure anhydrous homogentisinic acid.

In determining homogentisinic acid in root-tips I proceeded as follows. From 100 radicles the apical 2 mm. are quickly cut off and immediately ground as finely as possible in a small mortar with 5 cc. distilled water and 2 g. of the purest glass-powder. The contents of the mortar are washed with a small quantity of water into a measuring-flask containing 25 cc. The flask is then filled up to the mark and the contents shaken and filtered. The entire homogentisinic acid of the root-tips is now in the filtrate. Ten cc. of the filtrate are transferred by means of a pipette to a flask. Then 10 cc. of dilute ammonia[1] are added, and immediately afterwards a small quantity of $\frac{n}{10}$ silver solution from the Burette. Generally 1 cc. $\frac{n}{10}$ silver nitrate can be added at once without risk of excess. The mixture is now boiled. To facilitate the filtration of the silver precipitate the flask is allowed to cool for five minutes, then five drops of moderately concentrated calcium chloride solution are added, and ten drops of ammonia carbonate. The contents of the flask are then shaken and filtered.

[1] Commercial concentrated ammonia of sp. gr. 0·900 diluted in the proportion 1·10.

The filter-paper should be as small as possible, and the fluid should drain off thoroughly. Now ammonia and 0·3 cc. of silver solution are again added to the filtrate. If any reduction occurs, $CaCl_2$ and $(NH_4)_2CO_3$ are again added and the solution filtered, and this process may be repeated three or four times. If at last no distinct precipitation of silver occurs, it is necessary to see whether a precipitate of silver chloride appears on adding HCl so as to give a distinct acid reaction. If a white cloud appears there must have been a slight excess of silver solution. In this case 10 cc. of the original filtrate are measured off and treated as before, except that the silver solution is diminished by 0·1 to 0·2 cc. If no excess of $AgNO_3$ can now be detected, the right value must lie between the two results of titration. Since the limits of error cannot be more in this method than $\pm$0·2 cc., the homogentisinic acid can be estimated within $\pm$1 mg. The colour and the quantity of the precipitate in the reduction tests of the extracts prepared from root-tips can be exactly distinguished even if the tests differ in concentration so little that the difference corresponds to 0·3 cc. $\frac{n}{10}$ silver solution; usually the limit of error is found to be about $\pm$0·2 cc. $\frac{n}{10}$ silver nitrate.

In such experiments 5·0 cc. of $\frac{n}{10}$ silver nitrate are usually required for 100 root-tips. Of course by far the greater part of the silver solution consumed is not reduced by the homogentisinic acid, but by precipitation of proteids and other substances. Therefore the coefficient of conversion calculated by Baumann (4·1 mg. homogent. acid = 1 cc. $\frac{n}{10}$ $AgNO_3$) cannot be applied here. It is, therefore, necessary to determine by weighing the share in the entire silver consumption taken by homogentisinic acid in the before mentioned experiments. First of all it had to be made out whether the whole of the reducing substance could be obtained in solution by alcoholic extraction of the dried root-tips. A hundred root-tips were dried in Hofmeister's weighing-glasses and weighed, then powdered with the glasses in a mortar, extracted with absolute alcohol, then the remainder after drying and weighing was extracted with distilled water. The alcoholic and the watery extract were titrated with $\frac{n}{10}$ $AgNO_3$. Two such tests, together with a control experiment without root-tips, gave the following results :—

	I.	II.	Control.
The weight of the glass	1·5634 g.	1·6240 g.	1·0315 g.
The bowls with 100 root-tips	1·5843 g.	1·6421 g.	1·0315 g.
The weight of the root-tips	0·0209 g.	0·0181 g.	—

	I.	II.	Control.
Weight of alcoholic extract	0·0189 g.	0·0188 g.	0·0154 g.
Thereof soluble in water	0·0148 g.	0·0146 g.	0·0102 g.
Titration with $AgNO_3$	0·75 cc.	0·75 cc.	no reduction.
Residue after extraction with alcohol .	2·0327 g.	2·0725 g.	1·4802 g.
The part soluble in water titrated with			
$\frac{n}{10} Ag NO_3$	0·50 cc.	0·50 cc.	no reduction.
Insoluble in water	2·0031 g.	2·0442 g.	1·4564 g.
Insoluble in alcohol and soluble in water	0·0296 g.	0·0283 g.	0·238 g.

In this experiment only 2·25 cc. of silver solution were consumed for the substances soluble in water and alcohol. By far the larger quantity of the reducing substances was soluble in absolute alcohol, as shown by the high titration value of the alcohol extract, a smaller quantity of them was insoluble in alcohol and only soluble in water. This last portion, reducing Fehling's solution and giving the well-known test with phenyl-hydrazine, must contain principally sugar. The fitness of the Baumann method for investigating the processes of metabolism in geotropically stimulated roots was still to be tested by investigating the increased values obtained by silver titration in the case of alcoholic and watery extracts of geotropically stimulated roots in comparison with unstimulated specimens.

Two sets of unstimulated roots and two sets of roots stimulated by thirty minutes in a horizontal position were used for analysis. A control experiment was included, and the arrangement was otherwise as in the above described instance :—

	Not stimulated.		Stimulated.		Control.
I. The glasses	1·0468 g.	1·4197 g.	1·3377 g.	1·0858 g.	1·3925 g.
II. Glasses with root-tips .	1·0678 g.	1·4461 g.	1·3603 g.	1·1113 g.	1·3925 g.
III. The tips alone . . .	0·0210 g.	0·0264 g.	0·0226 g.	0·0255 g.	,,
IV. Alcoholic extract soluble					
in water	0·0082 g.	0·0093 g.	0·0087 g.	0·0108 g.	0·0053 g.
V. Alcoholic residue soluble					
in water	0·0182 g.	0·0214 g.	0·0172 g.	0·0190 g.	0·0131 g.
Titration of IV.	0·75 cc.	0·75 cc.	1·00 cc.	1·00 cc.	$\frac{n}{10} AgNO_3$
Titration of V	0·50 cc.	0·50 cc.	0·50 cc.	0·50 cc.	,,

The same test was carried out by rubbing up the fresh root-tips directly with glass-powder and absolute alcohol; the subsequent proceedings were the same.

	Not stimulated.		Stimulated.		Control.
I. Alcoholic extract soluble					
in water	0·0081 g.	0·0084 g.	0·0093 g.	0·0087 g.	0·0048 g.
II. Alcoholic residue soluble					
in water	0·0211 g.	0·221 g.	0·0223 g.	0·240 g.	0·0170 g.

	Not stimulated.	Stimulated.	Control.
Titration of I	0·75 cc. 0·75 cc.	1·00 cc. 1·00 cc.	$\frac{n}{10}$ AgNO$_3$
Titration of II	0·75 cc. 0·75 cc.	0·75 cc. 0·75 cc.	,,

The increasing of the amount of AgNO$_3$ in the titrations after geotropic stimulation therefore depends only on the portion soluble in alcohol, and the substances in the root-tips which are not soluble in alcohol do not participate in augmenting the reducing power.

Therefore the view is a very probable one, that silver-reducing substances other than homogentisinic acid take no share in the alterations of metabolism caused by geotropism. The amorphous yellow residue of the alcohol extract certainly cannot be regarded as pure homogentisinic acid, and may probably contain other distinct silver-reducing substances. But so far as reliance can be placed on calculations from the small amounts available, the reducing power of the residue from the alcoholic extract does not differ much from the results obtained by Baumann for pure specimens of homogentisinic acid.

If the amounts obtained from the control experiments are subtracted from the average amounts for the portion of alcoholic extract soluble in water, the average weight of this portion can be calculated as 3·87 mg. for 100 root-tips; the average value of titration with $\frac{n}{10}$ AgNO$_3$ as 0·875 cc. Therefore 1 cc. silver solution can be taken as equivalent to 4·478 mg. 'homogentisinic acid.' Baumann gives 4·1 to 4·2 mg. for pure homogentisinic acid.

Homogentisinic acid was also prepared from the upper parts of seedling roots by converting the tyrosin contained in them by means of the root enzyme (tyrosinase) by autolysis in chloroform water, and by extracting the product with alcohol. Ten cc. of the alcoholic extract, being a solution of crude homogentisinic acid, reduced 6·3 cc. of $\frac{n}{10}$ AgNO$_3$ and gave 26·4 mg. dried residue; 20 cc. of the same solution reduced 12·4 cc. silver nitrate and gave 51·1 mg. residue. It follows from this result that 1 cc. of the silver solution is equal to 4·1 mg. dried residue, i. e. crude homogentisinic acid.

The absolute amount of homogentisinic acid contained in root-tips can also be calculated from the results before mentioned. Since the average weight of dry substance of 100 root-tips of *Lupinus albus* according to ten estimations can be taken as 22·7 mg., the 3·87 mg. homogentisinic acid contained in the dry substance equal 17 percentage of the dry substance. After a geotropic stimulation this amount is increased by one-quarter and rises above 20 percentage of the dry substance.

By means of the method described the increase of silver-reducing

power after geotropic stimulation can easily be shown in all root-tips, hypocotyls, epicotyls, and, speaking generally, in all organs of plants hitherto investigated by me (7). The increase in homogentisinic acid always appears much earlier than the first traces of geotropic curvature, and the maximum is observed after about thirty minutes in roots kept at 18° to 20° C. The same phenomenon is also generally to be found in phototropic and hydrotropic movements (8); it has not in fact been absent in any tropistic movement investigated up to the present time. When the roots have finished their geotropic curvature, the difference in the amount of homogentisinic acid in comparison with vertical roots is nearly imperceptible. The course of the process may be illustrated by the following figures, giving the result of the titration of 10 cc. of filtrate (entire filtrate 25 cc.) obtained from 100 ground-up roots of *Lupinus albus* which had been previously stimulated:

Duration of geotropic stimulation. Minutes.	$\frac{n}{10}$ AgNO$_3$ used for 10 cc. of filtrate. in 100 stimulated roots.	in 100 control roots.	Difference.
5	2·1 cc.	2·0 cc.	0·1 cc.
10	2·0 ,,	2·0 ,,	0·0 ,,
15	2·0 ,,	1·9 ,,	0·1 ,,
20	2·2 ,,	2·1 ,,	0·1 ,,
25	2·2 ,,	1·9 ,,	0·3 ,,
30	2·4 ,,	2·1 ,,	0·3 ,,
45	2·3 ,,	2·0 ,,	0·3 ,,
60	2·15 ,, Curvature distinct .	1·9 ,,	0·25 ,,
90	2·15 ,,	2·0 ,,	0·15 ,,
120	2·25 ,, Curvature finished .	2·15 ,,	0·1 ,,
180	1·90 ,,	1·90 ,,	0·0 ,,

The observed differences are certainly above the limits of error of the method. But they are, however, so small, that they can only be regarded as just certain differences, since the quantities are very small and the error of the method rises to 10 per cent. of the values obtained. It became, therefore, a matter of importance to obtain control of a method giving a much closer insight into the metabolism of stimulation than could be got from the direct estimation of homogentisinic acid.

III.

The inhibition of oxidase action which I found accompanying an accumulation of homogentisinic acid must be assumed (in accordance with my present experience) to be in causal connexion with the increased values obtained by silver-titration.

It is easily demonstrated that the ground-up root-tips kept for some

time in chloroform-water lose the power of reducing silver nitrate ; even small amounts of homogentisinic acid intentionally added to the preparation cannot be detected after some time. This is an enzyme action : preventable by boiling the mixture, and to be observed even in filtrates from a Chamberland filter. The alcohol precipitate of this filtrate has the power of oxydizing homogentisinic acid in the same degree. We have here an enzyme (oxidase) capable of acting on homogentisinic acid, p-phenyl diamin + α-naphthol, guaiacum, &c. It is clear that either the action of oxidase is somehow inhibited during geotropic stimulation, so that the homogentisinic acid, which is otherwise further acted on, disappears more slowly than under ordinary circumstances, and accumulates to a certain degree : or there must be temporarily a diminution in production of oxidase by the root-tip.

If the latter were the case, it might be supposed that a mixture of an equal number of stimulated and non-stimulated root-tips would show the homogentisinic acid disappearing at a rate halfway between preparations derived entirely from stimulated and unstimulated roots. But the experiments I made show distinctly that such a mixed preparation does not differ in its behaviour from tests prepared entirely from stimulated roots. Therefore the existence of a specific retarding substance seems to be more probable.

I demonstrated (9) that a mixture of eighty unstimulated root-tips and twenty stimulated ones gives the same rate of disappearance of reducing power as a preparation of stimulated root-tips. Further, it was shown that even four stimulated root-tips added to ninety-six normal root-tips gave a distinct retardation of the disappearance of the reducing power. The hindering effect therefore is an exceedingly strong one. Finally, it was discovered that the inhibitory substance can be destroyed by boiling, and can be isolated by filtration through a Chamberland filter, and precipitation by alcohol. The substance therefore has the properties of an enzyme, and may be characterized as an anti-oxydase, on account of its effect being contrary to that of the oxydase of the root-tips. This anti-oxydase has the property of other anti-enzymes, namely, that it is a strictly specific effect. In this way the anti-enzyme of geotropically stimulated hypocotyls of *Lupinus* has an effect on the oxydizing enzyme of a *Lupinus* root-tip, but not on the root-tip enzyme of *Zea Mays* or *Cucurbita*. Between the oxydase and anti-oxydase of nearly related plants, therefore, a mutual action occurs, but not between enzyme and anti-enzyme of plants widely separated in regard to affinities (9). From these experiments on anti-enzymes it may be shown that the anti-enzyme produced in phototropic stimulation does not differ in any way from the geotropic anti-enzyme. It was established that the anti-oxydase is destroyed at 62° C., while the oxydase of the root-tip loses its power at 63 to 64° C. In a similar manner the anti-toxines lose their power at a lower temperature than the toxines,

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and it is possible to prepare by means of a certain temperature an effective specimen of toxin, from a mixture of toxin and anti-toxin, by destroying the anti-toxin. In the same way, heating for one hour to 62° entirely annuls the retardation in the disappearance of homogentisinic acid in a preparation of stimulated root-tips ; so that the reaction becomes identical with that observed in unstimulated root-tips.

This result is interesting in still another point. The experiments show that preparations of unstimulated root-tips lose their oxydasic effects at a certain temperature without the occurrence of any acceleration of the oxydation of homogentisinic acid. Therefore it may be supposed that the anti-oxydase does not exist in unstimulated roots, but it is formed only by tropistic stimulation.

Thus the interpretation of the process of metabolism in geotropic stimulation discovered by me is more exactly defined. It is essentially a retardation in the metabolism, in other words, in the decomposition of tyrosin. The tyrosin is, by tyrosinase, converted into homogentisinic acid, as occurs normally, but the further oxydation of the homogentisinic acid by the oxydase is inhibited by the production of an anti-oxydase, rendering the oxydase partially inefficient and causing by this means an accumulation of homogentisinic acid.

This process may, for convenience sake, be called the ' Anti-ferment Reaction.'

The exact investigation of the retardation of oxydation by the tropistic anti-enzyme is precisely suited to the application of chemical methods to the movement of plants, because the differences between stimulated and unstimulated organs can be magnified at will by the addition of homogentisinic acid, and can thus be completely removed from the doubts and uncertainties which interfere with the results of the *direct* titration method described in the earlier part of this paper. We thus obtain the very delicate and trustworthy method of chemically investigating the phenomena of irritability, which I proceed to describe.

The root-tips to be analysed are ground as quickly as possible with glass-powder and water (10 cc.), and are thus converted to a thin homogeneous emulsion or mash. The mash is washed without loss into an Erlenmeyer flask of $\frac{1}{8}$ litre capacity, and 50 cc. of standard aqueous solution of homogentisinic acid are then added. The titration of the acid used in my experiments gave 10 cc. as about equivalent to 2.3 cc. $\frac{n}{10}$ AgNO$_3$.

Finally, 5 cc. chloroform are added. The mixture is allowed to settle for ten to fifteen minutes, then 5 cc. are taken to determine the initial reducing power. The specimens now remain uncovered in an incubator (28 to 30°), and are shaken several times every day. At intervals of five days 5 cc. are taken to determine the reducing power. In such a way the decrease

in reducing power may be observed during a sufficiently long time. Or more homogentisinic acid may be introduced at first to extend the time of observation still more.

When so much homogentisinic acid is added, the slight error caused by the simultaneous presence of other reducing substances in the mixture, such as sugar, can be still more neglected. Since, after some time, no reduction at all can be observed in the specimens, even these substances must be destroyed by enzymes.

IV.

We must now pass on to a criticism of the results of our experiments. Even if between geotropically stimulated and unstimulated roots there exist constant and certain differences revealed by the anti-ferment test, we must meet the objection that the anti-ferment reaction may not be confined to tropistic stimulation, but may accompany a variety of departures from the normal condition of plant organs. Therefore it must be shown that only tropisms are able to produce the anti-ferment reaction. This can be really shown, and the following results demonstrate that neither chloroform-narcosis nor poisoning by antipyrin, acids, alkalies, nor mechanical hindering of growth by means of gypsum, nor traumatic stimulus, are by themselves able to produce anti-ferment reaction.

When chloroform or any other poison was used, the proper concentration was empirically determined in each case, namely, the strength necessary to prevent growth and curvature without permanent injury, during the period experimentally known to be sufficient for geotropism to occur. In order that the roots may be certainly influenced by the poison, they must stand in the solution during a sufficient time (one hour at $16°$ C.) in the vertical position, before they are geotropically stimulated by placing them horizontally.

Before beginning the chloroform experiments I convinced myself that the anti-ferment reaction occurs in the same degree in roots kept in damp air as in roots entirely submerged in water. The reducing power of 5 cc. of the filtrate tested in equal intervals of time was found to be—

Unstimulated :		Stimulated by placing them horizontally for thirty minutes :		
in damp air.	in water.	in damp air.	in water.	
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ AgNO$_3$	
2·5	2·5	2·8	2·7 „	„
2·1	2·1	2·6	2·4 „	„
1·7	1·6	2·3	2·1 „	„

The reducing power was, as usual, exactly equalized at the beginning of the experiments. Even between roots growing in damp sawdust and growing in water no difference was noticed. The water (from the water supply) had therefore no influence on the degree of anti-ferment reaction.

The actual curvature, however, began in roots growing in sawdust after one and a half hours, while the curvature of roots growing submerged in water, on account of hindered respiration and growth, did not appear before three hours.   I convinced myself definitely that the anti-ferment reaction is not retarded by submerging the roots in water.   With roots growing in damp air or sawdust, placing them horizontally for six minutes is sufficient to produce a distinct anti-ferment reaction, as described in the following section.   In submerged roots also the anti-ferment reaction was distinctly seen after six minutes' geotropic stimulation.   Therefore the roots sub-merged in tap-water could be considered as being normal controls for comparison with chloroformed roots.   From numerous experiments leading to the same result the following may be quoted.   For the experiments large glass vessels were used which could be easily turned through 90°, and thus the whole of the roots could be at once changed from the vertical to the horizontal position.   In each vessel the roots were fastened with a suitable apparatus.   One of these vessels was filled with chloroform-water (one part of saturated solution chloroform-water to seven parts of water), the other vessel was filled with tap-water.   All the roots remained vertical for one hour, the temperature of the fluids being 16°.   Then the apparatus were rotated so that both lots of 100 roots were placed horizontally for six minutes.   All the root-tips were then cut off and prepared for digestion.   The experiments gave the following numbers, being the reducing power of 5 cc. filtrate at intervals of five days.

Chloroform solution :		Pure water :	
roots stimulated.	unstimulated.	stimulated.	unstimulated.
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ AgNO$_3$
2·7	2·5	2·7	2·5 ,, ,,
2·4	2·0	2·4	2·1 ,, ,,
2·1	1·6	2·1	1·6 ,, ,,
1·8	1·2	1·8	1·1 ,, ,,
1·5	0·8	1·5	0·7 ,, ,,

The anti-ferment reaction is therefore connected only with geotropic induction.

Experiments arranged in the same manner with antipyrin solution 1·1000 gave analogous results.   The stimulation lasted thirty minutes.

Antipyrin :		Pure water :	
stimulated.	unstimulated.	stimulated.	unstimulated roots.
3·0	3·0	3·0	3·0 cc. $\frac{n}{10}$ AgNO$_3$
2·7	2·5	2·7	2·6 ,, ,,
2·5	2·0	2·4	2·2 ,, ,,
2·2	1·5	2·1	1·7 ,, ,,
1·9	1·0	1·8	1·3 ,, ,,
1·6	0·5	1·5	0·9 ,, ,,

·Dilute acids or alkalies in suitable concentrations gave the same effect. But I could never obtain the anti-ferment reaction without applying trop- istic stimulation.

Experiments with roots mechanically hindered in growth by gypsum also tally with the before-mentioned experiments. To work with roots fixed with gypsum, the seedlings (*Faba major*) were pinned into long and narrow wooden troughs filled with soft gypsum. They stayed in the solid gypsum for twenty-four hours in a vertical position, in damp air. And then, in those cases where geotropic stimulation was to be applied, they were placed horizontally for one hour. After being geotropically stimu- lated, the roots were freed from gypsum, their tips were cut off and the tests arranged. For such experiments the roots of *Vicia Faba major* were used, not being so easily injured as the roots of *Lupinus*: twenty-five *Faba* roots served for a series. The result of such an experiment is given; the numbers have the same significance as above.

Roots growing in sawdust :		Roots in gypsum :	
unstimulated.	stimulated.	unstimulated.	stimulated.
2·7	2·7	2·7	2·7 cc. $\frac{n}{10}$ AgNO$_3$
2·2	2·4	2·3	2·4 „ „
1·7	2·1	1·9	2·1 „ „
1·2	1·8	1·4	1·8 „ „
0·7	1·5	0·9	1·5 „ „

The anti-ferment reaction is therefore caused only by geotropic stimulus, even in this case.

The following experiments show that a wound cannot by itself produce the anti-ferment reaction. The roots were wounded by the amputation of 1 mm. from the tip.

Normal.	Decapitated and placed in vertical position.	Decapitated and placed for thirty minutes in horizontal position.
2·0	2·0	2·0 cc. $\frac{n}{10}$ AgNO$_3$
1·6	1·6	1·8 „ „
1·2	1·2	1·6 „ „
0·7	0·7	1·3 „ „
0·0	0·0	0·7 „ „

Neither can extreme degrees of temperature or light produce the anti-ferment reaction without a tropistic stimulus; and the sum of my experiences is to prove that tropistic stimulation exclusively is able to cause the alterations of metabolism described by me in root-tips, stems, cotyledons, &c.

I must therefore still differ from the view expressed by Noll (10) in the Botanische Zeitung, that the quantitative alterations of metabolism

discovered by me are an expression of general disturbance of the normal state of organs under anomalous conditions. But we must admit that the connexion with the other processes of geotropic stimulus is, at present, entirely hidden from us. What can be made out is given in the following chapters. Speaking generally, it may be said that it is a question of changes demonstrable at a very early stage of the process, and having probably an indirect relation to the phenomena of perception. At present it is hardly to be decided whether important processes or collateral phenomena of the physiological act are to be seen in the anti-ferment reaction. At any rate, the results of my experiments show that the anti-ferment reaction is a very important method for investigating tropisms, and one capable of throwing valuable light on a variety of points.

V.

Hitherto the geotropic curvature of roots was principally used for the investigation of the anti-ferment reaction. And I shall accordingly in this paper deal especially with the phenomena of geotropism.

First of all, it is of interest to know how long a period of stimulation in a horizontal position is needed for the appearance of the anti-ferment effect. The roots were kept horizontally for accurately measured periods of time (by means of a suitable apparatus), when they were placed vertically and underwent the anti-ferment test. Titrations were made, and the result is given in the following table (cubic centimetres of $\frac{n}{10}$ AgNO$_3$ per 5 cc. filtrate), at intervals of five days.

Time of induction, minutes:	0′	3′	4′	5′	6′	
	3·0	3·0	3·0	3·0	3·0 cc.	$\frac{n}{10}$ AgNO$_3$
	2·5	2·6	2·5	—	2·7 ,,	,,
	2·1	2·1	2·1	2·1	2·4 ,,	,,
	1·3	1·3	1·2	1·7	2·2 ,,	,,
	0·9	0·9	0·7	—	2·0 ,,	,,

The shortest period giving a distinct anti-ferment reaction by placing the roots horizontally at 17° C. can therefore be considered as six minutes. The limit is found at five minutes, nor was the limit changed by raising the temperature to 30°. But it is possible that the anti-ferment is not formed as an immediate result of stimulation, but rather by after-effect. Therefore I resolved to place the roots, after being geotropically stimulated, in the vertical position for one-half, one, two and more hours, and then to test for the anti-ferment reaction. But I could not detect even in this way any anti-ferment reaction after less than six minutes of geotropic introduction. On the whole it seems that, under any circumstances, at least five minutes'

stimulation is necessary for anti-ferment formation. This is the shortest period in which geotropic stimulation has hitherto been shown to occur. Formerly I showed (11) that geotropic after-effect on the klinostat can only be detected in roots of *Lupinus* or other sensitive objects after more than fifteen minutes of geotropic stimulation. This 'presentation-time' for geotropic curvature is therefore much longer than the 'presentation-time' for the anti-ferment reaction. If the roots, having been horizontal during six minutes, are allowed to grow vertically, it is possible to find out how long the anti-ferment reaction continues. Under these circumstances the reaction is found slightly diminished after one and a half hours, very much diminished after two and a half hours, and it finally disappears after four hours at 17° C. Thus those parts of the geotropic process which are not externally perceptible come to nothing, precisely as is the case with the visible part of the phenomena. For a root left horizontal for fifteen minutes and then placed vertically shows no visible curvature. For stimulation of six minutes' duration the klinostat fails us as a method of observation, and we have only the anti-ferment reaction to rely on.

When the roots are placed horizontally for longer than six minutes the anti-ferment reaction is observed in about the same intensity as after an induction of six minutes, during at least three hours after the close of the stimulation. The maximum of the reaction is reached very quickly : thus between stimulation-periods of six and fifty minutes no quantitative difference can be observed in anti-ferment reaction ; it may be added that at a temperature of 15–20° curvature is usually beginning after fifty minutes. The anti-ferment reaction therefore differs from after-effect test, which occurs much more strongly after thirty to forty-five minutes than after fifteen minutes of geotropic induction. The duration of the anti-ferment reaction after the cessation of stimulation was also determined. When the stimulus lasted ten minutes the anti-ferment test could no longer be observed eight hours after stimulation. After inductions of twenty minutes the anti-ferment reaction seems to come to its end in eight hours. But when the roots were stimulated for thirty minutes the anti-ferment reaction did not disappear earlier than twenty hours after the close of the induction. With forty and fifty minutes' stimulation twenty-four and thirty hours are needed for the anti-ferment reaction to run its course. The effect is therefore more persistent the longer the geotropic reaction continues. As is known, the after-effect on the klinostat increases in a similar manner. The curvature due to after-effect disappears under the straightening influence of auto-tropism more easily in proportion as the stimulation is of short duration. In the same way the curvature appears later after a short stimulus than after a longer one. Thus the curvature of roots stimulated for twenty minutes is observed after twelve to twenty-four hours, of roots stimulated for thirty minutes after eight hours, of roots stimulated for forty minutes after

three hours, but of roots stimulated for fifty minutes after two hours. This phenomenon may be explained by assuming a superposition of two processes. In the anti-ferment reaction, however, there seems to be no opposing process, so that the curve shows no striking maximum.

The results of continued observation during the process of geotropic curvature are in harmony with the early occurrence of the maximal anti-ferment reaction and its prolonged persistence. The intensity of the anti-ferment reaction remains the same during the first beginning of the downward curvature, and it does not decrease before five hours have elapsed (at 16–17°), when the curvature is already finished. Since the root stays in the horizontal position about one hour, the anti-ferment reaction could not be expected to disappear in less than twenty-four hours, a supposition actually confirmed. Perhaps the result, that direct titration during the progress of the geotropic curvature shows after three hours no augmentation of the normal amount of homogentisinic acid, may seem to be a surprising one. But it is possible, on account of the very small quantity of substances to be estimated, that the method soon becomes useless. Perhaps also after the oxidation of the homogentisinic acid has been checked a regulative diminution in the production of homogentisinic acid takes place, so that even in the presence of the hindering antioxidase the amount of homogentisinic acid decreases a little.

Further investigations on the significance of the anti-ferment reaction were made in reference to the geotropic induction at different angles to the vertical. When the roots are stimulated for thirty minutes no anti-ferment reaction can be observed at deviations of from 1° to 6° from the vertical. The reaction becomes certain at an angle of 7° from the normal position. This therefore is the smallest stimulus able to cause the anti-ferment reaction. At a deviation of 10° and a stimulation of thirty minutes a nearly maximal anti-ferment reaction can be produced, and the progress of the reaction is now the same for all angles from 10° to 170°. Neither the horizontal nor the obliquely upward position have a stronger effect. The effect decreases at 176°, and at 179° the anti-ferment reaction is very little. The inverse vertical position never causes any anti-ferment reaction ; nor is any after-effect to be obtained in roots *fixed* in the inverse vertical position. I have shown elsewhere (12) that the maximal after-curvature is not to be obtained by stimulation in the horizontal position, but at positions of about 135° obliquely upwards, i. e. 45° above the horizontal. Therefore the inquiry whether the anti-ferment reaction does not after all show a maximum effect at some angle of deviation was an interesting one. The method employed was the weakening of the effect by the shortening of the period of induction. If the induction is diminished to six minutes, at a deviation of 10°, no distinct anti-ferment reaction can be observed, but a very distinct one at 170°. These positions had not differed from each

other when the roots were stimulated for thirty minutes. In this way it can also be established that the anti-ferment reaction is much weaker at 45° than at 135°, i. e. at the same distance from the horizontal position in the upper quadrant. But between 60° and 120° no difference could be detected, nor any distinct difference between 90° and 135°. The stimulus can be still more diminished if the roots after six minutes' stimulation stand vertically as long as possible. They may be even left vertical for three to four hours, since, as above shown, the anti-ferment reaction is not destroyed in such a period. Experiments carried out in this manner demonstrate that the anti-ferment reaction at 60° is distinctly weaker than at either 90° or 120°, and some experiments established a stronger anti-ferment reaction at 135–150° than in the horizontal position. The result of the anti-ferment test, therefore, can be taken as supporting my own view and that of Darwin (13), D. Pertz (14), and Nemec (15), that the positions obliquely upwards give a stronger geotropic stimulus to roots than the horizontal position. The detailed experimental proof of the results here only briefly communicated is reserved for an extensive paper to be published in German. Here I am restricting the discussion to the chief points of importance.

VI.

The behaviour of roots on the klinostat was found to be of great interest in reference to the anti-ferment test. I may state at once that the anti-ferment reaction is to be observed in roots rotating on the klinostat without undergoing any curvature. This is the only case where the anti-ferment reaction occurs (according with the general view) without geotropic or analogous induction. But since in other cases we find the anti-ferment reaction strictly connected with tropistic induction we may be right, supposing that geotropic stimulus occurs even in plants rotating on the klinostat; since no curvature takes place, it was hitherto impossible to demonstrate this effect directly. But the anti-ferment test is able to show it easily and certainly, while the phenomena of grass haulms (Elfving (16); Noll (17)) and pegs or heels of *Cucurbita* seedlings (Francis Darwin (18), Noll) have established similar conclusions in isolated cases. The facts and views recently published by Francis Darwin and Miss Dorothea Pertz (19), which are thoroughly confirmed by the chemical method, are of the highest interest in reference to the results mentioned above.

Several lots of seventy-five seedlings each (*Lupinus albus*) were placed in a box made of zinc-plate filled with sawdust, and fastened to the axis of the klinostat (Pfeffer–Albrecht model). The lot B was kept in rotation for fourteen minutes, the period of revolution being twenty-seven minutes, it made therefore half a revolution; lot C rotated for fourteen minutes with a period of revolution of fourteen minutes, i. e. C made a whole revolution.

Lot D rotated for the same time, but with a period of seven minutes, and made therefore two revolutions. Lot A consisted of control roots placed vertically. The temperature was 20° C. Lots B, C, D were, of course, treated one after the other on the same klinostat. The reducing power of the preparations made from these roots decreased at the following rate (measured at intervals of five days):—

A	B	C	D	
2·0	2·0	2·0	2·0 cc.	$\frac{n}{10}$ AgNO$_3$
1·6	1·7	1·7	1·8 „	„
1·2	1·4	1·4	1·5 „	„
0·8	1·2	1·2	1·3 „	„

In another similar experiment the klinostat made one turn in twenty-five minutes, and the lots of seedlings were allowed to rotate for the following times:—B, for fifteen minutes; C, thirty minutes; D, sixty minutes; A was again a control lot (temp. 20·3 C.).

A	B	C	D	
2·0	2·0	2·0	2·0 cc.	$\frac{n}{10}$ AgNO$_3$
1·5	1·7	1·7	1·8 „	„
1·1	1·5	1·5	1·6 „	„

A third experiment with the same time of revolution (25′) was designed to investigate the effects of more prolonged rotation. Lot A consisted of control roots; B was rotated for three hours, C for six hours, D for twenty-four hours. Care was taken that the roots of the different lots were of the same age at the end of the experiment.

A	B	C	D
2·0	2·0	2·0	2·0
1·6	1·8	1·8	1·7
1·2	1·5	1·6	1·5
0·8	1·2	1·4	1·2

Retardation in the oxidation of homogentisinic acid was therefore to be observed in every case. I may here briefly mention that the accumulation of homogentisinic acid can be demonstrated by direct titration with silver nitrate in roots rotated on the klinostat. We must conclude from these experiences that roots in rotation on the klinostat perceive the geotropical stimulus in the same time as roots standing vertically, and even that this stimulus lasts during the whole time of rotation. Now that it has been shown that the anti-ferment reaction occurs in roots placed horizontally after five to six minutes, the view I formerly held must be given up, viz. that the effect of the klinostat may be caused by the plant remaining in each position for too short a time to allow of perception. Why curvature does

not occur on the klinostat is a question for further investigations. I should suppose that probably extremely small curvatures do take place, which are easily neutralized by the ortho-autotropism of roots, i. e. the tendency to grow in a straight line ; moreover, there must exist interferences between the curvatures.

VII.

My researches on various other applications of the anti-ferment test to geotropical questions, e. g. to the problem of the geotropism of secondary roots, will be reserved for another paper. Here I shall only enter upon the use of the method in a single problem—the question of the geotropic sensitiveness of the root-tip, which has received so much attention since the famous experiments by Charles and Francis Darwin. This problem has gained a new interest from the views established by Nemec (21), Haberlandt (22), Francis Darwin (23), the so-called 'statolith hypothesis.'

As is known, Nemec supposes that the displacement of starch-grains in the young cells of the root-cap supplies the mechanism of perception, and that only the root-tip (more exactly the part containing the young root-cap cells) can perceive the geotropical stimulus. To this view, which has been supported recently by experiments published by Francis Darwin, which harmonize with other physiological experiences, I have especially objected as follows : that in my experiments with glass tubes much more than the root-cap must be bent; at least 1·5 mm. of the root-tip must be bent laterally before the perception-zone is entirely separated from the zone of growth and curvature. I further succeeded in demonstrating, in one of my later papers, that the accumulation of homogentisinic acid in decapitated roots could only be hindered by cutting off 1·5 mm. of the root-tip.

There remains to complete these experiments by applying the better and stricter anti-ferment test.

One hundred roots of *Lupinus albus*, from which 0·5 mm. of the tip had been removed, were placed in a vertical position in sawdust ; another lot of one hundred roots treated in the same manner were placed horizontally for thirty minutes. Then all the tips were ground and prepared for the test. The reducing power decreased in the following ratio :—

| Unstimulated roots | 2·0 | 1·6 | 1·2 | 0·7 cc. $\frac{n}{10}$ AgNO$_3$ |
| Stimulated roots . | 2·0 | 1·7 | 1·4 | 1·2 ,, ,, |

The same experiment, cutting off 1·0 mm. of root-tip :—

| Unstimulated roots | 2·0 | 1·6 | 1·2 | 0·7 | 0·0 cc. $\frac{n}{10}$ AgNO$_3$ |
| Stimulated roots. . | 2·0 | 1·8 | 1·6 | 1·3 | 0·7 ,, ,, |

The same, cutting off 1·5 mm. :—

Unstimulated . . .	2·0	1·6	1·2	—	0·4 cc.	$\frac{n}{10}$ AgNO$_3$
Stimulated . . .	2·0	1·7	1·4	—	0·6 ,,	,,

The same, amputating 2 mm. :—

Unstimulated . .	2·0	1·6	1·1	—	0·4 cc.	$\frac{n}{10}$ AgNO$_3$
Stimulated . . .	2·0	1·6	1·2	—	0·5 ,,	,,

It was shown by a preliminary experiment that decapitation is not able to produce the anti-ferment reaction to any degree without geotropic stimulation.

Neither wounded nor stimulated	2·0	1·6	—	0·6	0·2 cc.	$\frac{n}{10}$ AgNO$_3$
Decapitated, not stimulated . .	2·0	1·6	—	0·6	0·3 ,,	,,
Decapitated and stimulated . .	2·0	1·7	—	0·9	0·6 ,,	,,

The parts cut off were immediately ground and added to the other parts of the root-tips used for the test.

I may mention that the anti-ferment reaction can be investigated in isolated root-tips of 5 mm. length placed horizontally.

These experiments show that the anti-ferment reaction is to be obtained in spite of removing the tissues of root-tip up to the motor zone, and that at least 1·5 mm. of the root-tip must be removed to hinder the anti-ferment reaction. This is in complete agreement with my previous experiments, and contradicts the statolith hypothesis in a manner which demands an explanation. Nemec (24) recently expressed the view that possibly cells containing statolith starch could still exist in a length of nearly 1·0 mm. of the root-tips. But according to my experience, in roots of *Lupinus* decapitated to 1·0 mm. there are never any statolith cells, nor can there be any regeneration of such cells in the period of stimulation, viz. half an hour.

Therefore other possibilities must be examined. Thus it is not impossible that the starch-cells of the root-cap are to be considered as being the chief organs for perception of the geotropic stimulus, but that the displacement of bodies contained in other cells may be available for the statolith function, though in a less effective manner. There is even the possibility that such displacements produce anti-ferment reaction, but no complete or normal curvature. That there exist processes connected with the anti-ferment reaction, but never producing any visible curvature, can be shown by the phenomena of roots laterally illuminated, as mentioned in the sequel. Unfortunately, in the case of geotropism this is difficult to decide, because the stimulus of wounding has itself an influence, and the failure of curvature may just as well be due to the effect of wounding as to the non-perception of the geotropic stimulus.

Finally, I cannot exclude the possibility that the anti-ferment reaction in decapitated roots is stronger in the part of the root-tip remaining after amputation than is normally the case in this upper part of the root-tip. Here also the decision is a difficult one.

On the whole the application of the anti-ferment reaction to the question of the localization of the sensory zone demonstrates that all stimulation effects are not absent when all cells containing statolith starch are as carefully as possible removed. Therefore it may still be doubted whether geotropic perception is caused only by means of such cells. But the principle of the statolith hypothesis is not yet refuted by the anti-ferment experiments, and according to the present state of my experience and deliberations I cannot consider the appearance of anti-ferment reaction in decapitated roots as refuting directly the hypothesis of Nemec. I say so because it is, generally speaking—and not merely in this case—dangerous to draw conclusions as to normal processes from the result of operations. The statolith hypothesis, however, must explain many other difficulties before it can rank as a permanent acquisition to our knowledge. At any rate, we gain from it a valuable impulse to new experimental studies. What position may now be ascribed to the anti-ferment reaction in the chain of processes constituting geotropic action in roots? As has been shown, the anti-ferment reaction takes place long before the curvature, and occurs in the root-tip before any alterations can be discovered in the motor zone. Further, the anti-ferment reaction is not influenced by the shock of decapitation in the same degree as those phenomena of irritability which are seriously interfered with when heliotropic and geotropic curvatures are for a time checked by injuries to grass seedlings and roots.

Let us imagine these processes schematically expressed as following each other in a chain of consecutive changes [1]. We might, for example, have :

1. Statolith effect.
2. Anti-ferment reaction.
3. The processes which are hindered by shock.
4. Stimulus transmitted to the motor zone.
5. Curvature.

According to this scheme no curvature can exist without anti-ferment reaction (or some unknown process in causal connexion with the anti-ferment reaction). But statolith action can take place without anti-ferment reaction, which is shown by roots placed inversely undergoing movement of the statoliths, but showing no anti-ferment reaction. It is at present unexplained why the displacement of statoliths produces no geotropic stimulation in roots inversely placed, in contrast with roots in any other positions. In contemplating these remarkable phenomena we are reminded of the macula lutea of the retina.

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[1] These actions might in reality go on in part simultaneously, and not one after the other.

VIII.

I had already shortly mentioned in a previous paper (25) that the anti-ferment reaction occurs in roots laterally illuminated, which nevertheless show no trace of heliotropic curvature, even on the klinostat. As a source of light incandescent gas was used. It was found that roots of *Lupinus albus* do not respond to illumination by curvatures under any conditions. But they nevertheless show a distinct anti-ferment reaction when laterally illuminated.

In a large glass trough were placed 200 roots of *Lupinus* ; 100 of them were in direct contact with the glass wall, so that they could be illuminated laterally ; 100 other roots were completely surrounded by earth and kept in darkness. At a distance of one meter was placed the gas lamp, and the trough was laterally illuminated during two hours. The temperature near the roots in the earth was at first 20° C., at the end of the experiment 21° C. The roots were then taken out and prepared. The reducing power was decreased in the following manner :—

Illuminated	2·0	1·8	1·5	1·2	0·9 cc. $\frac{n}{10}$ AgNO$_3$
Darkened .	2·0	1·5	1·1	0·8	0·4 ,, ,,

The same results were obtained with *Sinapis alba* (cultivated in water), *Phaseolus, Faba, Zea.* Of these only the roots of *Sinapis* showed distinct heliotropic curvature. In all other species of roots I could not obtain distinct curvatures even by means of the klinostat.

The anti-ferment reaction was, however, very distinct and certain in all cases, and this behaviour proves that the investigated roots were all sensitive to lateral illumination, even if they were mostly not able to show curvature.

The effect of coloured light was investigated by means of ruby glass and of gelatine paper of several colours. Behind the ruby glass seedlings of *Avena*, which are very sensitive to the heliotropic stimulus, showed no curvature. Behind red, yellow, or blue gelatine paper, heliotropic reaction was observed. The anti-ferment test (with *Lupinus* roots) gave results strictly analogous to the direct observation of curvature in *Avena*:—

Red gelatine paper .	2·0	1·8	—	1·1 cc. $\frac{n}{10}$ AgNO$_3$	
Control	2·0	1·6	—	0·6 ,,	,,
Yellow gelatine paper	2·0	—	1·2	0·9 ,,	,,
Control	2·0	—	0·8	0·4 ,,	,,
Ruby glass	2·0	—	0·9	0·4 ,,	,,
Control	2·0	—	0·8	0·4 ,,	,,
Blue gelatine paper .	2·0	—	1·4	— ,,	,,
Control	2·0	—	0·6	— ,,	,,

Roots of *Lupinus* or of other seedlings equally illuminated on all sides never gave any anti-ferment reaction. This is proved by the following experiment with roots of *Lupinus*. Two lots of 100 roots were placed vertically in damp air in glass troughs; their cotyledons were wrapped in wet cotton wool. One of the troughs was covered with an opaque box, the other was illuminated from both sides by equidistant incandescent lights during two hours. The reducing power in this experiment decreased in the following rate :—

Darkened .   2·0   1·6   1·1   0·7 cc. $\frac{n}{10}$ AgNO$_3$

Illuminated . 2·0   1·7   1·2   0·8 ,,   ,,

There was, therefore, no distinct difference ; in other words, only lateral illumination gives an effect. In the above experiments the heating effect of the lamps has not been considered. To test this possibility, large glass dishes 20 cm. wide were placed before the troughs containing the roots, and through the dishes a stream of cold water was allowed to flow. During the two hours that the experiment lasted the temperature was taken at three places, and it was clearly ascertained that the temperature on the illuminated side was not higher than on the dark side. The roots were then prepared and tested in the usual way. The decrease in reducing power was :—

Control .   2·0   1·6   1·1   0·7 cc. $\frac{n}{10}$ AgNO$_3$

Illuminated   2·0   1·8   1·5   1·2 ,,   ,,

In spite of excluding any local effects of higher temperature the anti-ferment reaction was found to be distinct.

Another experiment, analogous to this, but made with roots growing in damp air, gave the following results :—

Control .   2·0   1·6   1·2   0·8 cc. $\frac{n}{10}$ AgNO$_3$

Illuminated   2·0   1·8   1·5   1·2 ,,   ,,

I consider it, therefore, an undoubted fact that lateral illumination alone (under the conditions generally necessary for heliotropic curvature) is able to produce anti-ferment reaction in all seedling roots. We have certainly a right to consider this a rudimentary tropistic reaction, and these experiments are the only ones up to the present time that can demonstrate the general occurrence of heliotropism in roots. We may hope that the anti-ferment reaction will prove to be an available method for demonstrating sensibility to tropistic stimuli where no curvature or only uncertain reactions are observable. I have here in mind chemotropism, osmotropism, thermotropism in roots and in other plant organs. Investigations of these questions will be carried out in my laboratory.

H

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