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(With One Plate)

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The most delicate available methods for the determination of plant growth-promoting substances involve the direct measurement of the effects of these substances on the growth of suitable plant test objects. The most commonly employed indicator is the decapitated *Avena* coleoptile. Two general techniques are available; one makes use of the elongation (straight growth) resulting from the application of the substance in question symmetrically with respect to the long axis of the coleoptile, the other utilizes the curvature produced by unilateral application of the growth-promoting substance. The latter method, which has been more widely used, has been described repeatedly (see *e.g.*, Boysen-Jensen, 1936; Went and Thimann, 1937; Avery, Burkholder, and Creighton, 1937) and need not be detailed here. As Went and Thimann (1937, p. 51) point out, "The convenience of curvature methods rests upon two facts: (1) the residual growth, after decapitation, is the same on both sides of the plant and thus is automatically eliminated from the measurement—no controls are necessary; and (2) only one measurement need be made; there is no zero reading." It should be noted, however, that the first condition, namely, the uniformity of the residual growth, is true only during the first 2 hours following decapitation (cf. fig. 20, Went and Thimann, 1937), and this limits the length of the test period.

Thus the curvature test measures not the maximum amount of curvature (growth) which can be induced by the applied substance, but rather the mean rate of curvature during a given period. During this period the rate is not constant and may even change in sign (cf. Schneider and Went, 1938). The factors which cause a reduction in the curvature rate and therefore in the amount of curvature at the end of the test period are: (1) gravity, which causes a geotropic curvature in the opposite direction; (2) the effect of "physiological regeneration" of the tip, and (3) the lateral transport of the applied growth-

promoting substance across the coleoptile, producing a growth acceleration on the far side of the plant. The influence of the last-named factor is very marked in the case of a number of substances which show relatively little or no activity by the curvature method but have considerable effect on straight growth (cf. table XII, pp. 137-139, Went and Thimann, 1937). This lateral transport is greater the higher the concentration of growth-promoting substance applied; the net result is a decrease in the sensitivity of the test.

Methods employing straight growth have been employed occasionally but have not come into routine usage, largely because of the inconvenience in measuring the growth. The present report describes an assay procedure utilizing straight growth of the coleoptile of *Avena sativa*,[1] in which the sensitivity of the response and the ease and accuracy of measurement are at least as great as in the commonly used curvature test. In addition, the method offers a number of other technical advantages.

The procedure will be outlined briefly first, and then each step will be discussed in greater detail.

SUMMARY OF METHOD

Seeds are planted on agar slants in small test tubes at a determined distance below the rim of the tube. The seedlings are germinated and grown under controlled conditions. When the coleoptiles have attained a given length they are decapitated level with the rim of the tube and the leaf is withdrawn completely. Blocks of agar, containing the growth-promoting substance to be tested, are placed terminally upon the entire cut surface of the stump. After some time a shadowgraph is made in the usual manner. The length of the coleoptile which extends above the rim of the tube represents the growth increment during the test period. It can be measured very easily with a dissecting microscope equipped with an ocular micrometer.

TEST TUBES AND RACKS

Soft glass or Pyrex tubes having an inner diameter of about 15 mm and a length of 7 cm have been found satisfactory. Trials with larger tubes indicate that within reasonable limits the size is immaterial. A simple rack for the tubes may be constructed by boring a row of holes in a wood block. The holes should be of such depth and

[1] *Avena sativa* var. Markton has been used exclusively. The seeds were obtained through the courtesy of Mr. T. Ray Stanton, of the U. S. Department of Agriculture.

diameter that the tubes slip in easily and stand upright; 2.5 cm between centers allows sufficient room to manipulate the tubes. The length of the block, of course, determines the number of tubes and will depend upon the size of the photographic paper, incubator, etc., which one uses. For ready identification of the racks and the shadowgraphs some suitable design (*e. g.*, a letter or number) may be punched or drilled in a strip of sheet metal which is fastened against the back of the rack (see pl. 1). Machine-made test tubes usually have sufficiently uniform rims; if the tubes are made by hand from glass tubing it is necessary to grind the rim at right angles to the long axis of the tube. A mark should be made on the tube at a given distance below the rim; if it is desired to change the depth of planting in different experiments a glass-marking pencil is convenient; otherwise, a scratch made with a file or carborundum wheel furnishes a permanent mark.

THE AGAR SLANT

The same purified agar which is used for the test blocks is suitable. The concentration of agar should not be less than 0.8 percent. Greater concentrations, up to 2 percent, give equally good results; 0.9 to 1 percent agar has been routinely used. The agar may be made up in nutrient solution if desired but tap or distilled water have given uniformly satisfactory results. The growth rate of the coleoptile will be found to depend upon the composition of the agar. The simple device used in bacteriological laboratories is very convenient for filling the tubes with the melted agar. This consists of a funnel connected by a short length of rubber tubing to a glass tip and provided with a pinch clamp. The tubes are placed in the rack, filled up to the mark and the whole rack is tilted backward through about 60° so that the agar solidifies in a slant. The angle of the slope (and of the planted seed) should be such that the coleoptile grows erect without being required to curve. One hundred tubes can be charged with agar in about 8 minutes.

PLANTING AND GERMINATING SEEDS

The husked seed is pressed gently against the surface of the agar slant with the groove side down and the embryo at the level of the mark on the tube. The seeds may be soaked in water before planting or planted without previous soaking. Soaking does not affect the growth rate or the sensitivity of the plants. Furthermore, husked seeds planted dry on the agar absorb water nearly as rapidly as

if they are immersed in water, so that there is no advantage in preliminary soaking. Planting of dry seeds obviates a second handling of the seedlings. Under the conditions routinely employed in this laboratory (growth continuously from the time of planting in red light (Wratten Safelight, series 0) at 25° C. and about 90 percent relative humidity) the coleoptiles attain a length of 25 mm at about 65 hours after planting. The growth rate at this time, and for the next 24 hours, is approximately 0.9 mm per hour. Plants grown on agar slants in small tubes as described have shown less individual variability than those handled in any other way, as on filter paper, or sand, in the usual glass *Avena* holders, or on porous stone wicks. One hundred seeds can be planted in the tubes in about 10 minutes.

LENGTH OF COLEOPTILE

In connection with the size of the coleoptile used for the test, three factors have been studied. These are the total length of coleoptile, the length of the tip decapitated, and the length of the stump used.

The growth rate of the basal portion of the coleoptile decreases as the total length of the coleoptile increases, and if 20 to 25 mm of the coleoptile tip are removed, the stump makes practically no growth when a plain agar block is applied. If coleoptiles are used under these conditions no controls are necessary. However, the sensitivity (used here as the amount of growth in excess of the control which is produced by application of a given amount of growth substance) of the basal portion also decreases rather rapidly as the total length of the coleoptile increases. The 20-mm stumps of 40-mm coleoptiles have practically no residual growth under the conditions of the test, but do have a rather high sensitivity. It is possible, therefore, to use coleoptiles of this length without controls. More commonly, however, 13-mm stumps of 24- to 27-mm coleoptiles have been used because the plants are ready for the test nearly a day earlier. A control set must, of course, be included.

It is not intended to suggest that it is necessary to employ plants of just this length or even that the described conditions are optimal, but merely to indicate the technique which has given satisfactory results. As a matter of fact, since the test is essentially comparative, a few millimeters variation in the length of the test plants is of no consequence provided the plants are randomized throughout the different sets.

DECAPITATION

It is essential that the cut surface of the coleoptile be exactly at the level of the test tube rim and that the cut be clean and horizontal (at right angles to the long axis of the coleoptile). Otherwise the plants may bend and measurement will be difficult. Decapitation can be performed quite rapidly by making a small cut partially through one (or two opposite) sides of the coleoptile with a thin safety razor blade held flat against the rim of the tube and bending the coleoptile toward the cut with the fingers or forceps until it breaks. The leaf is pulled out completely. It is often possible to break off the coleoptile and withdraw the leaf in a single motion. One hundred coleoptiles can be decapitated in 30 minutes or less.

AGAR TEST BLOCKS

In the development of the method, weighed amounts of dehydrated agar were mixed with aqueous solutions of known concentrations of indole-3-acetic acid, or of auxin-a.[2] Similar results have been obtained with both of these growth-promoting substances. The test blocks were prepared with an apparatus similar to that described by DuBuy (1938).

Thimann and Schneider (1938) have reported that the concentration of agar in the test blocks is of considerable importance in the *Avena* curvature test. In general they found that a given concentration of indole-3-acetic acid produced larger curvatures the lower the agar concentration. Similar, although less marked, differences have been found in the present study of straight growth. The use of 1.5 percent agar has been adopted as a general procedure.

SIZE OF TEST BLOCKS

Went (1928) concluded that with 0.9 mm^3 blocks the curvatures are proportional to the absolute amount of growth substance in the blocks. Van der Weij (1931) and Thimann and Bonner (1932) concluded that the curvatures are proportional to the concentration of growth substance in the block. The data of Thimann and Bonner indicate that the rate at which the growth substance passes from the block to the plant is proportional to its concentration in the block at any moment. The change in concentration of growth substance in the block during any given period will be less the greater the volume of

[2] A solution of pure crystalline auxin-a was very generously supplied by Prof. F. Kögl, of the University of Utrecht.

the block. Therefore the larger the block the longer the time during which the induced growth will be proportional to the original concentration of applied growth substance. Much larger blocks can be applied terminally than can be applied unilaterally.

With blocks as large as 26 mm^3 it has been found that the straight growth rate of the decapitated coleoptiles remains constant for at least 6 hours. As blocks of this size can be manipulated very conveniently they have been adopted.

The test blocks are applied so as to cover the entire cut surface of the coleoptile stump. A small drop of water or gelatin solution may be previously applied to the cut surface in order to insure good contact with the block. About 20 minutes are required for the application of 100 blocks.

Schneider and Went (1938) have shown that the length of time between decapitation and application of the blocks is of considerable importance in the curvature test. This has been confirmed by Thimann and Schneider (1938). In the straight growth method, on the other hand, the response to applied growth substance has not been found to be significantly influenced by the interval between decapitation and application of the blocks, at least within the limits of 5 to 120 minutes.

LENGTH OF TEST PERIOD

The greatest sensitivity is obtained with the longest test period during which the growth in excess of the control is proportional to the concentration of applied growth-promoting substance. That is, under such conditions the absolute useful amount of growth is the greatest, and consequently the measurement can be made with greatest accuracy. Actually, it has been found that a test period of 3 to 4 hours is quite adequate. In the present study a 4-hour period has been generally used. In practice it is not essential to use a test period of any exactly predetermined length. Hence, if it is inconvenient to terminate the test at precisely 4 hours, there is no objection to making the test period several minutes shorter or longer. In any comparable series, of course, the test periods for all the sets should be the same.

ENVIRONMENTAL CONDITIONS OF TEST

Thimann and Schneider (1938) have reported that the growth response of coleoptile sections to indole-3-acetic acid depends upon the conditions of illumination of the seedlings during the previous development; maximal response was found when the plants received red light during the first several hours of germination and were kept

in darkness thereafter. It has not been determined whether this is true also of the coleoptile stumps attached to the seeds as employed in the present technique. As has been mentioned previously, constant illumination with red light has been used, since this permits absolute reproducibility in successive lots of plants and is much more convenient when successive lots are grown concurrently in a single dark room. It has been found, however, that the sensitivity is not appreciably different whether the plants are kept in darkness or given red light during the test period itself.

With an adequate water supply to the roots of the plants, considerable differences in atmospheric humidity do not influence the sensitivity. No significant difference in growth rate was found between plants at 100 percent and at 75 percent relative humidity even though the test blocks shrink very considerably at the lower humidity.

MEASUREMENT OF GROWTH

For measurement of the shadowgraphs a dissecting microscope equipped with a 14 × ocular and a 2 × objective has been used. The ocular is provided with a 1-cm scale subdivided into 100 divisions. One mm on the scale (10 divisions) corresponds to 0.5 mm on the shadowgraph so that the length can be read directly to 0.05 mm. The uncertainty in measuring is of the order of one scale division. Since the growth of the control plants in four hours is about 0.7 mm, this corresponds to an error of about 7 percent; in the test plants, which make more growth, the error of measurement is correspondingly reduced. Furthermore, the error tends to be minimized when the average of a number of plants is taken.

RESULTS

The usefulness of the *Avena* coleoptile as a test object rests upon the fact that the induced growth is proportional, within certain limits, to the concentration of applied growth-promoting substance. That such a proportionality does exist was demonstrated by Thimann and Bonner (1933), and has been confirmed repeatedly in the present study. Figure 1 represents the relationship between growth and growth substance concentration which has been found with the procedure here employed. It will be seen that the curve is a typical Blackman curve with a very short transition region, very similar to that obtained originally by Went (1928) for the curvature test. The work of Thimann and Schneider (1938) indicates that, in the curvature test

at least, the form of the curve may vary greatly according to the technique used. No evidence of a similar situation in the straight growth response has been obtained as yet.

DISCUSSION

In addition to the theoretical preferability of utilizing straight growth rather than curvature, the present method offers several technical advantages. The usual glass holders, which are time-consuming to make, difficult to clean and easily broken, are eliminated. The solid

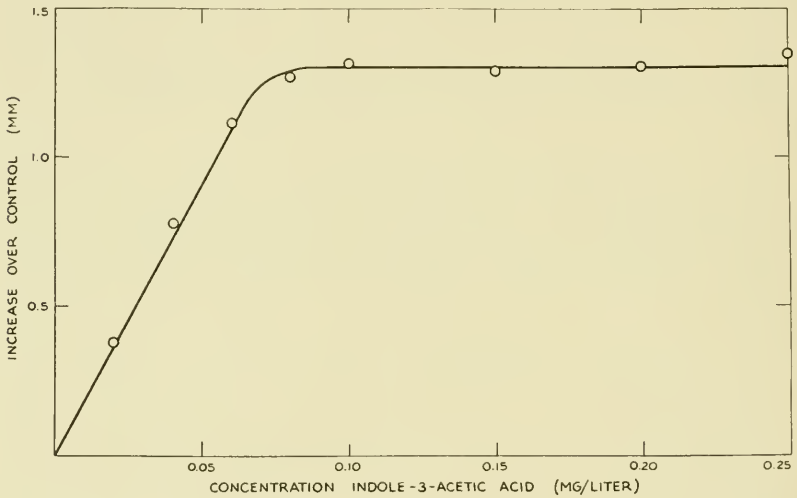

FIG. 1.—Relation between concentration of applied growth substance and straight growth.

root medium provides firm anchorage for the seedlings which facilitates the operations of decapitation, removal of the leaf, and application of the block. The use of test tubes greatly expedites the removal and rearrangement of the plants in the racks in preparing uniform sets. The large test blocks are more easily applied and make better contact when applied terminally. No handling of the seedlings is necessary between the time of initial planting and of testing.

These practical advantages, as well as some others, apply also to the use of agar in test tubes for the growth of seedlings to be used in the curvature test. The seeds may be planted very close to the top of the tubes so that practically none of the coleoptile is obscured in photographing. The tubes allow the plant to be revolved about its long axis

so that the plane of curvature can be placed parallel to that of the photographic paper. Deseeding may be very easily accomplished with a small section lifter or with forceps.

Some disadvantages of the method should be mentioned also. The size of the plants used is a relatively critical factor in comparative studies. It has been found that with the uniform conditions employed the time at which the seedlings will be ready for use can be predicted, at the time of planting, to within 2 or 3 hours. It is essential that this be considered in planning the various operations. The individual variability of the seeds used is such that only about 75 percent of the plants are ready at one time. In a limited series, therefore, there will be considerable waste. In an extended series of tests, involving a few hundred plants, if the larger plants are used first the smaller ones will attain a suitable size by the time they are needed so that more than 90 percent of the planted seeds can be utilized.

SUMMARY

An assay method for growth-promoting substances, which utilizes straight growth of the *Avena* coleoptile, is described. The method appears to possess a number of theoretical and practical advantages over the widely used curvature test.

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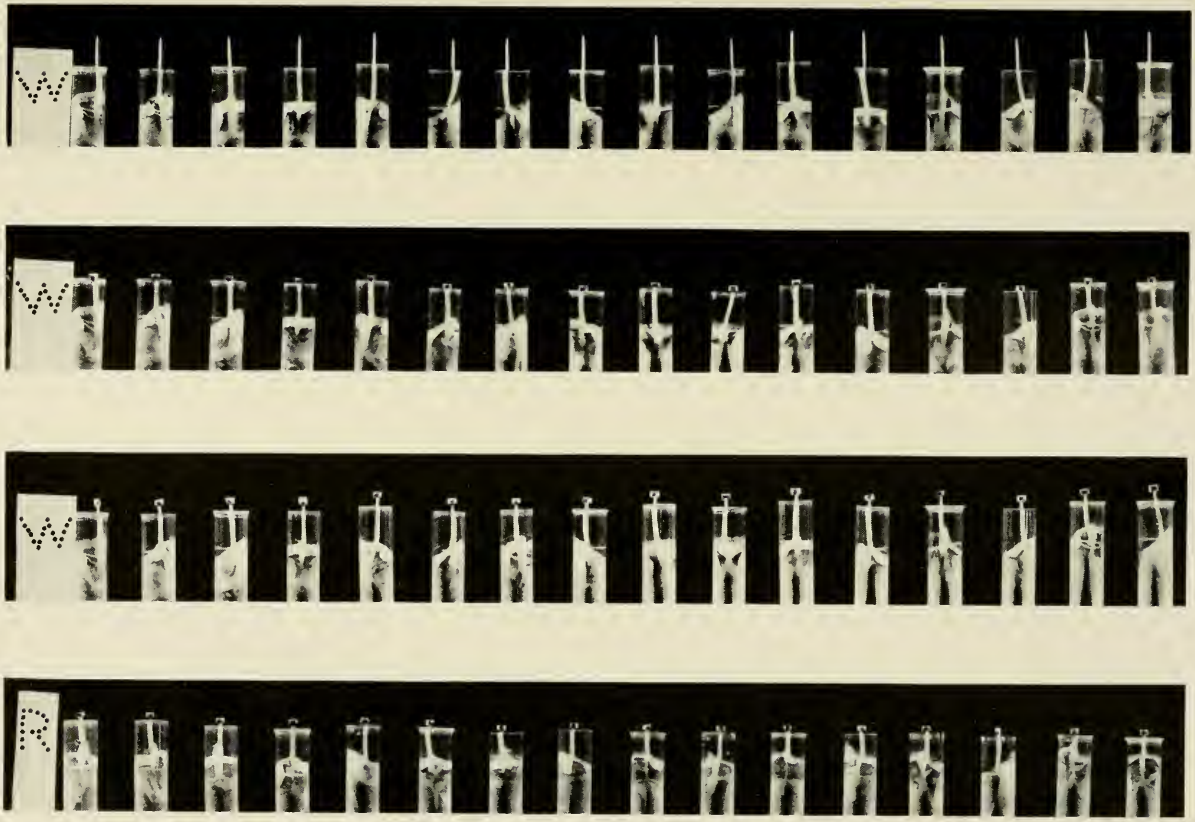

SHADOWGRAPHS SHOWING FROM TOP TO BOTTOM: INTACT PLANTS PRIOR TO DECAPITATION, DECAPITATED PLANTS IMMEDIATELY AFTER APPLICATION OF AGAR BLOCKS, PLANTS 4 HOURS AFTER APPLICATION OF BLOCKS CONTAINING GROWTH SUBSTANCE, PLANTS 4 HOURS AFTER APPLICATION OF PLAIN AGAR BLOCKS.