STUDIES ON THE GROWTH HORMONE OF PLANTS. IV. ON THE MECHANISM OF THE ACTION

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Introduction.—It has been known for some time that among the higher plants cell elongation as well as tropistic response is governed by a well-defined chemical substance which is secreted by young leaves and by the apical portions of stems.[1,2] The mechanism by which this substance exerts its effect upon plant cells has, however, remained obscure and it is relatively recently that an attack upon this problem has been made.

In an earlier paper it was shown that the amounts of this "growth substance" which enter the *Avena* coleoptile are far too small to bear any simple stoichiometrical relation to the substances which are formed in the cell wall during the resultant growth.[3] The growth substance is therefore a true hormone in that it is active in minute amounts and in some indirect manner. The work of Heyn,[4] of Heyn and van Overbeek,[5] and of Söding[6] has shown that *Avena* coleoptiles which are artificially supplied with growth substance exhibit a marked increase in the plasticity of their cell walls. From this result it seemed possible that the growth substance itself might possess the property of being able, merely by its presence, to decrease the viscosity of the fluid in which the cellulose micelles of the cell wall are embedded. The cell wall could then be readily stretched by the force at hand, which would, under normal conditions, be the force due to the osmotic pressure of the cell contents. It was found in this laboratory, however, that if the osmotic force be replaced by the force due to a suitable weight, no increase in plasticity of the coleoptile results from the application of growth substance. It seemed, therefore, more probable that the hormone exerts its influence upon the cell wall through the mediation of the cell protoplasm. The present paper will describe briefly experiments which demonstrate that this is actually the case. A fuller account will appear elsewhere.

Materials and Methods.—*Avena* plants of the pure line, "Sieges Hafer," kindly supplied by Dr. Åkermann of Svalöf, were used throughout. The plants were grown under the usual rigidly controlled conditions of temperature and humidity.[9] All experiments were carried out under red light, to avoid photropic stimulation of the plants.

The conventional method of applying growth substance dissolved in agar blocks to the stumps of decapitated *Avena* coleoptiles is not well adapted to the study of matters concerned with metabolism. It was

found, however, that short sections (three mm. long) cut from the coleoptile and suspended in growth substance of suitable concentration elongated rapidly, even in the absence of their usual food source (i.e., the seed). The various substances whose effects upon the action of growth substance are to be investigated may be conveniently added to the growth substance solution in which the sections are suspended. In order to obtain sections which were initially relatively free of growth substance, the hormone-producing tip was removed from each plant two hours before the short sections were cut from the coleoptile.

Experimental.—Avena coleoptiles have not previously been grown, as in the present case, suspended in solutions of growth substance. It is therefore of interest to enumerate, as follows, some of the factors which were found to control elongation under these conditions:

(*a*) Sections immersed in water or solution of growth substance do not exhibit "regeneration" of the capacity to produce growth substance which is characteristic of decapitated plants grown in the usual manner with only their roots in water.

(*b*) Sections from near the apex of a previously decapitated coleoptile elongate more rapidly in growth substance of a given concentration than sections from near the base of the same plant. This indicates that cells which have already undergone considerable elongation are less reactive to the hormone than those which have elongated only slightly.

(*c*) The rate and extent of the elongation vary greatly with the growth substance concentration. There is an optimum concentration above which the hormone is toxic and may even bring about a loss of turgor.

The growth of coleoptile sections under the influence of growth substance was found to be completely inhibited by 10^{-3} normal KCN, and by 0.05 per cent phenyl-urethane. This inhibition suggested a relation between the action of growth substance and respiratory activity. Coleoptile sections were, therefore, immersed in a solution of growth substance under an atmosphere of nitrogen. The sections were not harmed by a prolonged lack of oxygen since they grew normally if placed in air. Nevertheless, neither elongation nor any action of growth substance preliminary to elongation took place in nitrogen. This result might be due either to a failure of the hormone to penetrate into the cells in the absence of oxidative processes, or to the participation of growth substance itself in an oxidative process. In the first case it would hardly be expected that the respiration of a section should be effected by the addition of the hormone, since Steward has shown that, in the case of potato tissue at least, the uptake of ions, while dependent upon aerobic metabolism, nevertheless does not increase the production of carbon dioxide.[7] In the second case it would be difficult to predict *a priori* whether or not an increase in respiratory activity would occur upon the addition of growth substance.

A preliminary study of the effect of growth substance upon the respiration of coleoptile sections was made, using the standard Warburg technique.[8] The results may be summarized as follows:

(*a*) Growth substance in low concentrations stimulates the respiration of coleoptile sections by as much as 27 per cent during the first two hours after its addition. In high concentrations growth substance greatly inhibits the respiration. There is apparently some discrepancy between the effects of hormone concentration on growth and on respiration, although the optimal concentrations are similar.

(*b*) The respiration of sections is inhibited by the same concentrations of KCN and of phenyl-urethane which were found to inhibit elongation under the influence of growth substance.

(*c*) The additional respiration observed upon the addition of growth substance exhibits the same sensitivity to KCN and to phenyl-urethane as does the normal respiration and is therefore probably identical with it.

(*d*) Growth substance which has been inactivated for growth by oxidation with hydrogen peroxide does not stimulate respiration.

Discussion.—From the foregoing results it seems possible that an increase in respiration is one member in the chain of processes by which growth substance brings about elongation. It is as yet unsafe to conclude that this is actually the case. In the first place, the hormone preparation used was, while of relatively high purity, still only approximately one per cent hormone. It is possible that an impurity in the preparation is responsible for the stimulation of respiration. In the second place, this stimulation may be a secondary phenomenon attending the presence of growth substance in the cell, and have in reality no bearing upon growth. These questions, as well as a possible relation of respiratory rate to cell wall plasticity, are now being investigated.

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[1] Went, F., *Rec. Trav. bot. neerl.*, **25**, 1 (1928).
[2] Kögl, F., Haagen-Smit, A., and Erxleben, H., *Zeit. Physiol. Chem.*, **214**, 241 (1933).
[3] Thimann, K. V., and Bonner, J., *Proc. Roy. Soc. London.* (In press.)
[4] Heyn, A., *Rec. Trav. bot. neerl.*, **28**, 113 (1931).
[5] Heyn, A., and van Overbeek, J., *Proc. kon. Akad. Wetensch. Amster.*, **34**, 1190 (1931).
[6] Söding, H., *Jb. Wiss. Bot.*, **74**, 127 (1931).
[7] Steward, F., *Protoplasma*, **18**, 208 (1933).
[8] Warburg, O., *Stoffwechsel der Tumoren*, Berlin, (1926).
[9] Dolk, H., and Thimann, K. V., these PROCEEDINGS, **18**, 30 (1932).