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GROWTH REGULATORS AND PLANT PROPAGATION

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In our modern botanical literature the substances which influence the growth of plants are frequently called "growth regulators." This is a collective term which includes vitamins and hormones, as well as certain inorganic and organic chemicals that are important. The growth regulators may be classified as follows:

1. Hormones, viz., substances which, being produced in any one part of the organism, are transferred to another part and there influence a specific physiological process. Hormones are not considered to have a direct nutritive effect upon the organism (Went and Thimann 1937; Boysen Jensen 1936).*

2. Vitamins, viz., substances which act as accessory food factors and have a direct nutritive effect upon the organism.

3. Other growth regulators, viz., the various nutritive elements, such as iron and phosphorus—and certain drugs, notably colchicine—which affect plant growth in diverse ways.

An immense literature pertaining to these substances has appeared in our professional journals, and no individual could give, in one short paper, any adequate review of the work that has been done. You will appreciate the truth of this statement when I tell you that there are available for study over three hundred scientific publications dealing exclusively with the effect of the drug colchicine upon growth in plants.

The present discussion will deal largely with hormones, and only to a lesser extent with vitamins and other growth regulators.

Someone has said that "less than 1/2000 of an ounce of thyroxine is all that stands between Einstein and imbecility." Thyroxine is a hormone which regulates metabolism (assimilation and energy relationships) in the animal body. Imbecility is a condition which is of little importance to plants, except in so far as plant propagators may be involved, but hormones are of the utmost importance—they influence the growth of plants.

The earliest hints as to the possible presence of hormones in plants were obtained from studies of growth curvatures (tropisms) in plants. It is well known to every housewife that plants will curve or bend toward a window; and to prevent this, a careful housewife is likely to turn her plants daily. It is almost equally well known that the stems of a potted plant, placed in a horizontal position, will curve or bend upward. Darwin (1881) was perhaps one of the first individuals to study these phenomena critically; and his book "The Power of Movement in Plants" is a botanical classic. One of the most interesting experiments reported in this book is one dealing with the effect of unilateral, or one-sided illumination, upon grass seedlings. In order to understand the experiments of Darwin and those of other more recent investigators, it is necessary to know something of the structure of a young grass plant. Surrounding the leaves of a young grass seedling is an interesting, tubular, sac- or sheath-like structure known as the coleoptile; apparently the coleoptile helps protect the young leaves from injury as the shoot of the seedling grows upward through the soil. After emergence from

* Complete citations of these papers and others mentioned later may be found in the bibliographies of the publications given at the end of this paper.

the soil the leaves of the seedling rupture the tip of the coleoptile and become exposed to the light.

Darwin found that the coleoptiles of grass seedlings normally would bend toward a source of light, but that when he shaded the tips of the coleoptiles by the use of light foil caps, bending did not occur. When he shaded the lower portions of the coleoptiles but left the tips exposed, the coleoptiles would bend in the usual manner. Darwin concluded from this "that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend." Although Darwin apparently thought the influence to be a substance, he did not go so far as to call it a "hormone", for the word "hormone" had not been used in plant or animal physiology until just after the beginning of the twentieth century (Starling 1906, 1914; Fitting 1910). Darwin's conclusions were confirmed by Rothert in 1892.

After Darwin's book was published, a period of over 25 years elapsed before additional critical experiments with growth curvatures were performed. Then Fitting (1907), and later Boysen Jensen (1910, 1911), became interested in the problem, and Boysen Jensen concluded from his carefully conducted experiments on the coleoptiles of grass seedlings that a growth promoting substance is synthesized in the tip of a coleoptile, and that when the coleoptile is lighted from one side only, the growth substance migrates down the darkened side, and, by stimulating the cells of the darkened side to elongate, causes the coleoptile to bend toward the light. These conclusions were confirmed and amplified by Paál (1918), Söding (1925), Stark (1917), and others. Went (1928) performed some ingenious experiments in an attempt to isolate the substance responsible for the bending of seedlings. One of his experiments is especially worthy of attention. Went cut the tips from grass coleoptiles and placed them with their cut surfaces in contact with small agar blocks, so that the juices from each coleoptile tip could diffuse into the agar. Later, when these agar blocks, soaked by the juices from the coleoptile tips, were attached to one side of a grass seedling, the seedling was observed to bend in a direction away from the agar block, thus indicating the presence of growth substances in the agar. Later work by Dolk (1929), Van Overbeek (1933), Boysen Jensen (1933) and Dijkman (1934) demonstrated that a growth substance is formed in the tips of grass seedlings, that the growth substance is transported from the tip, and that the path of its conduction may be displaced by the stimulus of light or gravity. Thus, if a plant is lighted only from one side, the growth substance or "hormone" (Fitting 1910) is conducted down the darkened side, causing the plant to bend toward the light; if the plant is turned on its side, the hormone is conducted largely in the lower half, causing the plant tip to bend upward. Cholodny (1926) was able to demonstrate that the hormone retards, rather than accelerates, the growth of roots, a fact which helps explain the downward curvature of the roots of horizontally placed plants. An excellent review of the literature pertaining to the subject may be found in the English translation (by Avery and Burkholder) of *Die Wuchsstofftheorie* by Boysen Jensen, and a somewhat shorter account is to be found in Went and Thimann's *Phytohormones*.

The function of the growth substance or substances involved in tropic responses apparently is restricted to cellular enlargement—that is, cellular division is not accelerated. However, this statement does not apply to the hormone's influence upon the cells of the cambium, as was shown by Söding, an European investigator. Söding (1926) was able to demonstrate that mitotic activity in the cambium is accelerated by the presence of growth hormones, and it is now thought that the resumption of cambial activity each spring in the twigs of woody plants is associated with the production of growth hormones in the expanding buds.

Kögl (1932) proposed that those growth substances which bring about cellular enlargement be known as auxins. Three auxins have been isolated and analyzed chemically by a large number of workers, (Nielson 1930; Kögl and Kostermann 1934; Thimann 1935; Kögl 1935; and others). All of the auxins are monobasic acids with one double bond, viz., auxentriolic acid —auxin-*a*; auxenolonic acid—auxin-*b*; 3-indole acetic acid—heteroauxin. These auxins have been isolated from a great variety of substances, including yeast, molds, corn oil, and human urine. Their properties are now well known to plant scientists, and the significance of one of them (heteroauxin or 3-indoleacetic acid) in the field of plant propagation is very great.

It is to be expected that the effects of the three recently discovered auxins upon the various tissues of the plant would receive thorough study by plant scientists, and it is a fact that several hundred scientific papers dealing with these unique substances have been published. However, from the first it has been clear that heteroauxin is the most versatile of the three, and we shall confine ourselves to a consideration of this interesting substance. In an amazingly short period of time it was found that heteroauxin (3-indoleacetic acid) "can promote cell enlargement in the shoots of higher plants, inhibit the development of lateral buds, stimulate cell division in the cambium, promote callus formation, produce galls, initiate the formation of roots but at the same time inhibit their growth in length, etc." (Avery 1938).

No definite information is available concerning the possible function of heteroauxin in flowering, but it appears from the researches of Cajlachjan (1938) and Melchers (1939) that floral initiation and development may be regulated by other hormones (florigen and vernalin) of unknown chemical composition.

The amount of auxin necessary to bring about a reaction is exceedingly small. For instance, heteroauxin may be mixed with lanolin in concentrations of from 0.4 to 2% and applied as a salve to the stems of marigolds, tomatoes, sweet peas, cabbages, and a host of other common plants with the result that roots appear on the areas treated with the salve (Zimmerman and Wilcoxon 1935).

The auxin also may be applied dissolved in water, and in this medium it has been shown to stimulate root formation in tomato plants in concentrations ranging from 1 p. p. m. to 100 p. p. m. (Zimmerman and Wilcoxon 1935). The remarkable efficiency of heteroauxin as a medium for inducing the initiation of lateral roots has been revealed by the researches of LaRue (1939), who was able to produce roots from the petals of roses. It has been found recently that seeds treated with hormones give increased yields (Stier and DeBuy 1938).

In addition to 3-indoleacetic acid (heteroauxin) Zimmerman and Wilcoxon (1936) tested several other related organic compounds for their ability to cause rooting responses in various plants. Included in the list of chemicals used effectively were naphthaleneacetic acid, indolebutyric acid, indolepropionic acid, phenylacetic acid, fluoreneacetic acid, and anthraceneacetic acid. I believe that the list has now been extended to include over forty chemicals. Indolebutyric acid is now sold commercially to gardeners (auxilin), and seems to be somewhat more effective in rooting certain plants than the hormone heteroauxin (hormodin). It has been used successfully in propagating arborvitae, barberries, camellias, carnations, clematis, dogwood, elms, geraniums, grapes, holly, juniper, honeysuckles, magnolias, peaches, spirea, viburnums, and many others, including such difficult plants as the spruce and yew.

In 1936 Gustafson, of the University of Michigan, discovered that heteroauxin has properties previously unsuspected. It is well known that pollination is usually necessary for the production of fruits as well as seeds. It had been assumed by some that the young embryo in the seed secretes a hormone which diffuses into the wall of the ovary and stimulates the latter to develop into a fruit. However, in certain interspecific and intergeneric crosses of flowering plants, fruits frequently develop after pollination even though no seeds, and consequently no embryos, are found. These facts led Gustafson to suspect that the pollen grain or pollen tube provides a substance, which, upon diffusion into the walls of the young ovary, causes it to enlarge greatly and develop into a fruit. Having an idea that the substances involved in fruit growth might be the same as those which cause plants to bend and roots to be initiated, Gustafson applied heteroauxin, as well as several related chemicals, to the stigma and to the styles of flowers of the tomato, snapdragon, petunia, various squashes, cucumbers, watermelons, egg plant, and pepper. Naturally, he had to remove the stamens of the flowers involved in the experiment, for these structures would certainly cause confusion by contributing pollen to the stigma. With stamens safely removed and the experimental flowers carefully covered with small sacks, the effects of the heteroauxin were observed. Generally speaking, remarkable success was achieved, especially with tomatoes, and it is now an easy matter to produce seedless tomatoes in our own greenhouses. Gustafson was somewhat less successful with watermelons, but one of his students succeeded in producing a seedless, if somewhat disfigured, watermelon about two years ago.

Of course, much experimentation was stimulated by Gustafson's work. Although the results of these researches may not be of tremendous, immediate importance to agriculture, there are possibilities of development. For instance, the home gardener, desirous of obtaining increased production of ornamental fruits such as holly, might try spraying his bushes at the time of flowering, with an aqueous solution of auxin. Gardner and Marth (1939) have written a paper describing the technique and results (90% successful) obtained with our native holly, *Ilex opaca*.

In connection with our discussion of auxin-treated fruits it is worth while to mention that auxins, in certain concentrations, have a restraining effect upon bud expansion, as shown by Guthrie (1939). Thus it may soon be possible, by spraying fruit trees, to prevent their buds from expanding during warm spells in late winter. This should be of especial significance in Oklahoma, where fruit crops are so frequently destroyed by late frosts. A still further use of auxin in horticulture has been suggested by Gardner (1939), who demonstrated that certain concentrations, when applied as sprays to ripening fruit, prevent fruit drop and thus allow a better maturation of the crop.

Perhaps no substances of biological interest have received greater publicity than the vitamins. However, their function in plant growth has not been emphasized, possibly because they are synthesized by plants and therefore are usually present in the various tissues in amounts adequate to insure normal growth. Bonner and Greene (1938, 1939) investigated the effects of vitamin B_1 (thiamin) on plant growth by watering seedlings with aqueous solutions. They were puzzled to find that whereas certain plants, notably cosmos, cocklebures, and mustard, were benefited greatly, others, such as tomatoes and peas, seemed unaffected. But when leaf analyses of the various plants were made, it was found that those species which responded markedly to vitamin treatment usually had relatively small amounts of

B_1 in their tissues at the beginning of the experiment, while those which exhibited little or no response had larger, adequate amounts. Since Bonner and Greene reported their results, vitamin B_1 has received considerable publicity, and many a home gardener has performed wholesale, if somewhat haphazard, experiments in his own back yard. The varying results reported for these experiments indicate that the vitamin does not always behave in the manner publicized by its admirers, although it is quite generally agreed that some plants benefit greatly by its use. Also, in some cases the negative results may be explained by the over-enthusiasm of many amateur gardeners who reason that "if a little is a good thing, a lot should be better."

In any event it should be emphasized that vitamins will not do anything for an organism except to maintain it in a normal, healthy condition. Unlike hormones, vitamins cannot cause the production of superstructures. Thus, ironically enough, a careful gardener who has always kept the soil in his garden well cultivated and fertilized, is not likely to profit much from adding vitamin B_1. There must be a deficiency in the soil if vitamin B_1 is to be of value.

Perhaps one of the most important uses of vitamin B_1 is in making cuttings. In many instances plants which are hard to propagate may be rooted by the use of heteroauxin, and then induced to grow vigorously by applying B_1. Except for vitamin B_1, and possibly vitamin B_6 (pyridoxine) (Robbins and Bartley 1939), the value of adding any vitamin in plant propagation has not been established.

The third group of growth regulators, including the nutritive elements and drugs that affect plant growth, is a large one. However, it is not necessary for us to discuss the nutritive elements here, and as for the drugs, certainly the most important one is colchicine. In 1937 a young plant scientist named Eigsti, employed as a research assistant at the Carnegie Institution, heard from a zoologist friend of some strange effects produced by colchicine upon the tissues of mice. Curious to determine what the effects of the drug upon plant tissues might be, he propagated an onion with its root tips hanging in a solution of colchicine. The root tips exposed to the colchicine swelled to about twice the usual size. A cytological investigation revealed that the number of chromosomes in the cells of the enlarged onion roots had been doubled, tripled, or even quadrupled. Further investigation showed that the increase in chromosomes occurs as a result of the destruction of the spindle fibers during the metaphase of dividing cells (Eigsti 1938). With spindle fibers destroyed, the chromosomes, though split in halves, do not separate, and the single restitution nucleus therefore is equipped with the two sets of chromosomes that had been destined for two daughter cells. Recognizing the potentialities of the drug, Eigsti and other investigators applied it to seeds of many different plants, and to the shoots of others. Lanolin, water, and mineral oil were used as solvents. The results of these experiments have been encouraging, for in many instances, larger, more vigorous and otherwise desirable specimens have been secured, including improved cotton, periwinkles, zinnias, cosmos, and tobacco. By the use of colchicine the Burpee Company has developed a new giant marigold that is a decided improvement over the older varieties. At the present time Eigsti, now with the University of Oklahoma, is carrying on an ingenious experiment involving the use of colchicine, in cooperation with gardeners in Oklahoma. He supplies to anyone interested a vial of colchicine salve with directions for applying it to garden plants. At the end of the experiment each cooperating individual makes a detailed report on a mimeographed outline sent out by the University. During the past growing season, many interesting results were obtained, and the work will be continued during 1941.

At the present time experiments with various growth regulators are proceeding at such a rapid pace that it is difficult even to imagine what the future may bring forth, and in this connection I feel as Franklin must have felt when he said, "It is impossible to imagine the height to which may be carried in a thousand years the power of man over matter." However, I feel safe in making three predictions, viz.: (1) Methods of propagating plants on a commercial basis, by the use of hormones and vitamins, will be revolutionized; (2) many new and desirable varieties will be produced through the use of colchicine and possibly some of the related drugs; and (3) as a result of the development of techniques for using growth regulators, the scope of home gardening will be widened and popularized.

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