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PRESENT STATE AND PROSPECTS OF RESEARCH INTO NATURAL AND SYNTHETIC
PLANT GROWTH-REGULATING SUBSTANCES

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Summary. The present state is surveyed of our knowledge of the chemical nature of phytohormones, the determination of their amounts, rates of turn over, and localization in plant tissues, their biosynthetic pathways and modes of action.

This knowledge is a prerequisite for the design of refined programs for the production and application of chemosynthetic plant growth regulators (PGR's), that may act as supplements to insufficient endogenous levels or interfere with the biosynthesis, translocation, or metabolic conversion of phytohormones.

The beginning of a new decade offers a ready opportunity to look back and forward in order to evaluate the present position of PGR-research and its prospects. When we take a retrospective view on the seventies, at first sight it appears that less progress has been made than could be expected from the exciting fifties and sixties. This holds both for the practical application of PGR's, the sale of which amounts to less than one per cent of the plant protection market (1), and for the developments in the scientific understanding of the fate and mode of action of chemo- and biosynthetic PGR's in the plant. However, the following discussion of some of the achievements made in the past decade will indicate at least some hopeful perspectives for the development in the eighties.

IDENTIFICATION AND DETERMINATION OF PLANT HORMONES

To the five known groups of phytohormones: auxins, cytokinins, gibberellins, abscisins, and ethylene, no important new group of endogenous PGR's
has been added. The flowering hormone, if existing as a particular substance
at all, has not yet disclosed its chemical, possibly macromolecular, nature.
Yet, small advances have been made. The wound hormone, the first substance
known to exert a hormonal influence in plant tissues, was recently demonstrated to be a reduced form of the long-known traumatic acid (2). Also, from

40 kg rape seed pollen, collected by industrious bees, 4 mg of a steroid compound was isolated, which strongly promotes cell division and elongation in bean seedlings (3). Will this 'brassinolide' turn out to provide a link between phytohormones and the steroid hormones known from animal and human physiology? The different effects of steroidal oestrogens in plant growth and development open the possibility that this group will be recognized as true phytohormones (4).

Are there any new groups of phytohormones expected to be discovered at all? Some phenomena that cannot be satisfactorily explained from the actions of the presently known hormones point to this possibility. One of these is tuberization in potato for which Kumar and Wareing assume an unknown substance inducing lateral cell division in the stolon tip (5). Another was hit upon at our laboratory, where Yarga found that when a fertilized tomato ovary is severed from the mother plant all mitotic activity is promply interrupted. Upon culture in vitro no more cell divisions occur in the pericarp, placental or ovule tissues. However, when grafted back on the mother plant within a fortnight, the ovary regains its mitotic activity that cannot be restored in vitro by the addition of any cytokinin or other (group of) hormones in any possible way. The presence of roots seems not to be essential for this resumption, possibly the leaves are the site of production of a hitherto unknown substance essential for cell division.

The qualitative and quantitative determination of plant hormones has been greatly improved by the application of chromatographic and other analytical methods, allowing for the absolute identification of nanogram and picogram quantities (a.g. 6.7). However, the analysis requires scrupulous purification procedures that involve considerable risks. Scott at al. found no difference in the cytokinin contents of two crown gall cultures, one forming a callus only, the other producing sprouts. The recovery of their clean-up for GC-MS determination was only .6 - 2.7% of the activity determined with a bio assay (8). Apparently an internal standard is indispensable. The same issue of Planta in which this case was published, contained another serious warning, concerning the probable contamination with a commercial preparation of a sample of gibberellins for GC-MS determination (9).

Moreover, we have to be aware that the endogenous level of a hormone is not always a reliable indicator for its physiological importance. For example, although the main gibberellin in rice plants is GA_{19} , it is probably only the pool from which the active substance for stem growth, GA_{1} , is formed in only limited amounts (10). Also the accumulation of GA_{20} , in pea plants, coincides with a low growth rate. Under growth-promoting conditions the

level of GA_{20} decreases because of its conversion into GA_{29} which is the active gibberellin in pea stem elongation (11,12). These examples demonstrate that the amount of hormone need not correspond with its physiological significance and, moreover, need not be related to its effect, the relationship between amount and response may even be inverse.

This indicates that the determination of the turn over rate of a hormone may be far more relevant than that of its absolute amount. Turn over rates can be determined by addition of the substance involved containing a radioactive label and following the fate of the label, but in such studies the problem of compartmentalization may easily interfere. For example, radioactive GA_{2Q} , added to pea stem tissue, is quite stable, indicating a low rate of turn over. However, endogenous GA20 is readily converted into an inactive catabolite. If this different fate of exogenous and endogenous molecules of the same substance is not due to an isotope effect, then it points to a different localization in the tissue, probably within the cells. The endogenous GA₂₀ being located in a compartment in which it is enzymatically converted and which is inaccessible for the exogenously applied molecules (12). Similarly, whereas radioactive IAA, applied to hypocotyl segments of light-grown sunflower seedlings, is basipetally translocated at a rate of 7 mm h⁻¹ (13), the endogenous IAA in the tissue was found at our laboratory to be hardly transported at all (14). It is apparently pooled in a compartment such as the vacuole from which it is hardly released. Therefore, exogenously applied PGR's may arrive at other sites than where the corresponding endogenous hormones reside and, accordingly, their fate and function may be very different.

These examples underline the necessity to perform exact determinations, not only of the amounts of the phytohormones, but of their turn over rates and localization as well. This will allow, on the one hand, a better understanding of the mode of action of applied PGR's and, on the other hand, a verification of hypotheses based on the earlier, less specific and accurate bio assays and to further unravel the interactions of phytohormones in plant growth and development. As a final example of this topic, the physical determination of endogenous IAA in the phototropically stimulated sunflower hypocotyl showed that no lateral gradient in the IAA-concentration occurs. Therefore, the venerable Cholodny-Went theory of tropic curvature, from the age of the bio assay, does not apply. Instead, the abscisin, xanthoxin, was found to accumulate at the illuminated side and may, by its growth-inhibiting action, account for the curvature (14,15). Similarly, abscisic acid is replacing 1AA in the geotropic curvature of roots (16).

BIOSYNTHESIS AND MODE OF ACTION OF PLANT HORMONES

The biosynthetic pathways of the phytohormones have been further analyzed, also with the aid of cell-free systems and radioactive presursors. Particularly the progress in the gibberellins is notable, while the elucidation of the biosynthesis of ethylene in higher plants is a real breakthrough (17). The further evaluation of the immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) and, particularly, of the inhibitor of its formation, aminoethoxyvinylglycine (AVG), may lead to applications of considerable practical importance (18,19). However, one should keep an open mind for the possibility of alternative biosynthetic pathways, especially for auxins and ethylene, while the biosyntheses of the cytokinins and of xanthoxin are not yet established with certainty.

On the contrary, really disappointing is the progress, during the last decade, in the understanding of the mode of action of phytohormones. The acid-growth theory of auxin-induced cell elongation is still a matter of controverse (cf. 20 and 21). But the central question is: Do phytohormones act in a similar way as the steroid hormones in vertebrates? After the promising results of Matthijsse in 1969 (22), cytoplasmic hormone-protein complexes have only rarely been identified with certainty (23). The specific activity of such a complex in cell-free, RNA-synthesizing systems, has not vet unequivocally been established. Membrane-bound receptors are mainly of a carrier-type character (24). The interaction of plant hormones with membranes is as little understood as their interaction with, e.g., phytochrome. The poor results to detect and investigate hormone-protein and hormonemembrane complexes, and the lack of specificity of many hormone actions, as demonstrated by their manifold activities and their mutual interactions, led Trewavas (25) to suggest the abolition of the model presented by the vertebrate steroid hormones. He is inclined to consider plant hormones just as 'some of a number of signals or substances which can modify development' and finds it 'simpler to suppose that one critical metabolic event may be induced by many substances without the necessary intervention of receptor proteins'.

An interesting example of how a phytohormone can act just as one 'of a number of signals or substances which can modify development', has been described at our laboratory by Van Loon (26). He studies the role of ethylene in tobacco plants reacting hypersensitively to infection with tobacco mosaic virus (TMV). In these N gene-carrying tobacco varieties, TMV cannot spread through the plant to develop mosaic symptoms. Instead, the virus is confined to the surroundings of the necrotic spots developing at the sites of infection. The appearance of lesions is preceded by a peak of ethylene evolution and accompanied by large changes in protein constitution, in

peroxidase isoenzyme patterns, and in the induction of systemic resistance in non-infected leaves against further infections. Van Loon found that the local application of ethylene, by pricking healthy leaves with needles moistened with ethephon, gave rise to all these symptoms: occurrence of local necroses, a qualitatively and quantitatively similar re-direction of protein metabolism, and even development of systemic resistance against virus infections. Therefore, among the various agents able to evoke the syndrome of the hypersensitivity reaction, ethylene apparently is one of the key substances. However, we have no idea as to how this loose double bond acts in fundamentally re-adjusting the genetically controlled protein-synthesizing apparatus of the plant cells.

APPLICATION OF PLANT GROWTH REGULATORS

I stress this point of our ignorance of the mode of action of this and other phytohormones because it is not only a matter of academic interest. If the application of PGR's in practice and the development of new chemosynthetic PGR's are to be more than haphazard enterprises, then we have to know, not only how the natural PGR's, the hormones, are synthesized, translocated, and metabolized, but first of all how they act in the metabolism of the plant cells. Only then can we try to design refined programs for the production and application of chemosynthetic PGR's.

For the time being, there are two ways along which exogenous PGR's can interfere with the endogenous, hormonal pattern. On the one hand, they can interfere with the biosynthesis, translocation, or metabolic conversion of plant hormones. On the other hand, they can replace or supplement phytohormones when the level of the latter is suboptimal.

Examples of the latter are the application of gibberellins to promote germination of barley and lettuce seeds, to enlarge petioles and berries in bunches of table grapes and, especially, to induce flowering in coniferous trees. An interesting finding here has been that flowering in the economically important Pinaceae cannot be obtained by gibberellic acid, GA_3 , but rather with the gibberellins GA_{4+7} that lack one of the hydroxyl groups of GA_3 . Under conditions promoting cone production, e.g., water stress or nitrate fertilization, just these less polar gibberrellins appear in the needles, indicating that the exogenous treatment replenishes a very specific endogenous shortage (27).

Similarly, the ceiling in soybean productivity can possibly be broken at last by the local application of benzyladenine (BA). This treatment not only prevents abortion of the remaining flower buds after the first fruit set, but also leads to increased numbers of seed per pod and weight per seed. This indicates the replenishing of an endogenous cytokinin shortage.

which is further corroborated by the observation that the cultivars with the lowest zeatin content in their ovaries show the strongest response to the treatment (28).

Shortage of ethylene can be overcome by treatment with ethephon, or by stimulation of its biosynthesis with auxin, ACC or, possibly, daminozide. The applications are manifold: for the induction of flowering in bromeliacean plants, the promotion of ripening and abscission of fruits, growth retardation in flax.

Nearly as versatile are the growth retardants that inhibit gibberellin biosynthesis, such as chlormequat and ancymidol. They are not only used for stem shortening in cereals and ornamentals, but also to improve flower formation of fruit trees. In all these applications the main effect seems to be a shift in the endogenous gibberellin:ethylene ratio, and sometimes ethylene promotors and gibberellin inhibitors are applied together. An example is Terpal, a growth regulator in winter barley and cotton, in which ethephon is combined with the onium compound, DPC (dimethylpiperidinium chloride) (29). Hopefully, the eighties will also see the coming into practice of a suitable retardant for our lawns.

This survey of PGR-applications is of necessity very incomplete, it only presents some main lines of development, leaving out of scope, s.g., the many subtle treatments applied to regulate flowering, fruiting, and harvesting in fruit culture (18) or the important field of defoliants and desiccants which at least partly overlaps with the area of herbicides. It also omits the new and extremely interesting field of the plant anabolics, that possibly act directly in the plant metabolism rather than through the hormonal pattern. Glyphosine is an example of such a compound, already in use in sugar cane (30). Plant anabolics may enhance the production of such primary and secondary plant products as carbohydrates, proteins, lipids, pigments, and aromatic and medicinal substances. But this is largely music for the future. At present, the number of important applications of PGR's is still rather limited, one would have hoped for many more possibilities to regulate vegetative and reproductive growth and development, dormancy and senescence, to improve resistance against unfavourable conditions, to facilitate harvesting, and to enhance the keeping quality of harvested commodities.

Hopeful data for the future are the increased efforts in the phytopharmaceutical industry to develop new PGR's and also the growing readiness to cooperate as, for example, in the large-scale testing of abscission chemicals in Citrus at the Florida Department of Citrus at Lake Alfred. For the time being, research for the development of new PGR's will maintain much of its haphazard characteristics, although a more sophisticated approach occurs in the research connected with, e.g. AVG, biostable abscisins, and onium compounds. May the

coming decade bring us not only a few casual hits but above all a deeper insight in how both bio- and chemosynthetic PGR's perform their jobs in helping mankind to produce more and better food, fodder, flower, fibre and fuel crops.

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