Photo: Frank Salisbury

Kenneth V Neumann

PLANT GROWTH SUBSTANCES; PAST, PRESENT AND FUTURE[1,2,3]

By Kenneth V. Thimann

Biological Laboratories, Harvard University, Cambridge 38, Massachusetts

It is a third of a century, more or less, since the concept of auxins was placed upon a sound experimental footing by a small number of workers located in Western European laboratories. My own interest in the subject was aroused in 1930 due largely to the fact that Herman Dolk and I arrived at the California Institute of Technology almost at the same time. Went's thesis, which gave the unequivocal proof of the reality of the postulated "growth substance," and showed how to extract and measure it, had been finished nearly three years earlier and published in 1928; Cholodny's famous theory of tropisms had appeared in 1927. Söding's careful measurements of the growth rate of coleoptiles before and after decapitation and "reheading," and Stark's first (but unsuccessful) experiments on applying gelatine blocks to one side of decapitated plants, both of which had supplied the foundations for the above advances, had appeared in the mid-20's. Although all these studies (as well as my own at first) centered about growth promotion *sensu stricto,* some pointers toward a broader field of action of growth substances had appeared too. These included especially the papers by Snow (1) on correlative bud inhibition and Dostál (2) on the growth-regulating action of the leaf. It was these two pioneer studies that set me to thinking about a possible bud-inhibiting action of "growth substance" in the plant and led to the work with Skoog which demonstrated the role of auxin in apical dominance, thus implicating auxin as an integrative factor in plant growth. Later Went joined us from Java and we were able to follow up the careful study he had made there with Bouillenne, which had shown that the rooting of cuttings was under control of a hormonal factor from leaves and buds; this soon led us to yet another integrative action of auxin—root initiation. Very soon the work of Yasuda and of Gustafson on parthenocarpy of fruits helped to make it clear that there is no aspect of plant growth and development in which auxin does

not play an important role. Later work with gibberellin and kinetin has only served to establish this more firmly. On the other hand, we now have three types of naturally occurring growth regulators and a great many synthetic substances, some of which seem none too closely related to the hormones. How are we to assess the present status of all these materials and their role in regulating plant growth and development? What fundamental changes has the third of a century of intensive work brought about?

To evaluate the whole field in a few pages is impossible; Audus' book (3) has 553 pages and Pilet's (4) has 774. Even *Phytohormones* (5), which reviewed the literature through 1937, had 294. Numerous other books have appeared in various languages and a Fifth International Conference on the subject is about to be held. Book chapters, symposia, and reviews abound, e.g., in the *Annual Review* of *Plant Physiology* (6 to 15) and articles by Audus, Bentley, Cleland (51), Gordon, and Larsen in Volume 14 of the *Handbuch der Pflanzen-Physiologie*. Here only a few current and recent trends in the "pure" aspects of growth substance research will be presented and evaluated.

THE DOMAINS OF THE THREE NATURALLY-OCCURRING GROUPS

Unlike the animal hormones, each of which has its "target" organ or tissue, the most obvious property of the plant growth substances is not only that their functions are multiple but that they overlap. For any given process their actions may be similar or opposed, or synergistic, or entirely different. For instance, kinetin reacts with auxin to produce callus growth, it opposes auxin in lateral bud development, it resembles auxin in inhibiting root elongation, does strongly what auxin does only weakly in promoting protein synthesis, and acts in the same way as auxin to cause cell division; in this last case, however, auxin action may be dependent on endogenous kinins[4] already present, so that this action may really fall into the first category. Finally, it differs completely from auxin in not being readily transported.

Similarly, gibberellin acts like auxin in promoting elongation of etiolated stems and formation of parthenocarpic fruit (though it generally delays fruit-set), reacts with auxin in producing elongation of isolated green stems, acts far more powerfully than auxin on elongation of intact stems, does what auxin cannot do in causing flowering of long-day plants on short-day photoperiods and the elongation of monocotyledonous leaves and leaf

[4] The problem of terminology of kinetin and its relatives is acute. The name "kinin" has been pre-empted by animal physiologists for a group of polypeptides controlling the contraction of smooth muscle (16, 17). In any event its implied emphasis on movement is not quite what is needed. The writer has suggested the term *cytomin*, as indicating Cytos (Κυτοσ) = cell, and Tome (Τομη) = a cut or division (as A-tom = indivisible). Another possible candidate is *cytokinin*. General adoption of some such term is urgent.

sheaths.[5] Yet it acts in the opposite direction to auxin on root formation by cuttings and leaves (18, 19) and apparently also on the tensile properties of pea stems (20). Auxin favors formation of pistillate flowers, gibberellin of staminate. Generally, all gibberellins act in the same way as one another, and the same is qualitatively true for auxins, with certain exceptions.

The multiple actions of auxin have often been discussed. Here it needs only to be mentioned that the growth-inhibiting actions are probably at least as important as the growth promoting ones. The inhibition of lateral bud development is of major importance in integrating the plant body, and parallel phenomena to it are found in ferns (21) and mosses (22). Thus auxins should not necessarily be considered only as growth-promoting substances.

What underlies this miscellany of effects? Evidently two principles are to be distinguished. The first is that in any specific process which we modify with an externally applied growth substance, internal (endogenous) substances are normally interacting. Brian & Hemming's (23) evidence that auxin is necessary for gibberellin to promote the growth of stem sections, Kuse's similar evidence for *Ipomoea* petioles (24), Saebø's (25), Michniewicz' (26), and Kefford's (27) evidence for auxin-gibberellin interaction in leaves and coleoptiles, and Skoog & Miller's (28) evidence that only in the presence of auxin does kinetin cause mitosis and growth of callus tissue, are pointers to this broader generalization. Would auxin cause cambium cells to divide if they did not contain an endogenous kinin? Would it inhibit the development of lateral buds if they were not naturally deficient in such a kinin? In these buds, auxin-kinin balance appears to determine growth or inhibition (29). The fact that some fruits are induced to grow parthenocarpically by gibberellin, and others by auxin, strongly suggests that for this phenomenon both factors are needed and that the endogenous level of either one can be limiting. In stone fruits, unresponsive to auxin application, the limiting factor is evidently gibberellin, while in peppers, strawberries and squashes, in view of the parthenocarpic successes of 1936–38, it is evidently auxin; in tomatoes it may be either one, or, from the striking synergism reported by Wittwer & Tolbert (30), it may be both. Such interrelationship of factors may explain the peculiarity that gibberellin reverses the growth inhibition caused by chlorocholine and other inhibitors when used on intact plants, but not in tissue cultures (31), or the fact that gibberellin is nontoxic even at 1000 ppm to seedless grapes, but toxic at 25 ppm to seeded varieties (32). Brian *et al.* put it in the reverse way (33): "The failure of one hormone to induce a response in some given experimental system may be due to another hormone being limiting."

A corollary to this principle is that two or more hormone-secreting sources must normally be present. They need not necessarily function at

[5] A more complete list of the differences between auxins and gibberellins is given in Galston and Purves' review (9).

the same time, and indeed in fruit development the point has been stressed elsewhere (34) that as far as auxin is concerned there is typically a succession of transient auxin sources. These produce "waves" of auxin which play a major role in determining the stages of fruit development. Van Overbeek recently proposed that successive "waves" of dependence on auxin and gibberellin play a normal part in numerous growth phenomena (35).

The alternative to this concept of the normal, universal, interaction of growth substances is that the same process may be quite differently controlled in different plants, which is improbable and unattractive. More reasonable is the view that it is always physiologically the same, only the limiting factor being different.

The second principle, disappointing but inescapable, is that in these observed responses of organs or tissues we are far removed from the actual molecular processes catalyzed by each class of growth substance. For the occurrence of overlap, independence, opposition or synergism, depending on the system studied, shows clearly that we are not observing the primary process, but a derivative one, modifiable at many points after the initial reaction. The arguments advanced 25 years ago for a single "master reaction" catalyzed by auxin hold equally well for the other factors. Substances which have one auxin activity, *e.g.*, promoting coleoptile growth, have all the others, such as promoting cambial division, root formation on cuttings, and parthenocarpy, while they inhibit root elongation and axillary bud development. Differences are quantitative, varying from 0.01 per cent to 1200 per cent of that of IAA (indole-3-acetic acid), or, where they are truly qualitative, are due to differences in secondary properties such as transportability or sensitivity to oxidation. Each molecule is therefore considered to have the essential minimum of structure for auxin activity. Corresponding arguments hold for gibberellins and, so far as they have been studied, for kinins.

The Relation Between Structure and Activity

This second principle leads directly to the question: what is the minimal structure essential for each of these activities?

In the case of auxin, the relation between structure and activity has been the subject of continuing researches over the years (for reviews see 36, 37, 38). The result is still inconclusive. Every few years the discovery of a new active compound modifies what were up to then believed to be the requirements. The need for a sidechain disappeared with the advent of the benzoic acid auxins. The need for an unsaturated ring as nucleus was modified by the activity of the thiocarbamates to need for a planar structure, (but perforce unsaturated, otherwise it would not be planar). The need for a carboxyl group, however, has survived the challenge of indole acetonitrile, which is active only on hydrolysis (39), while the need for a fixed distance between the carboxyl and the planar structure has been strongly

vindicated by the proof of Wain and co-workers (40, 41) that sidechains of 3 or more carbon atoms on the aromatic auxins are subject to rapid and efficient β-oxidation. The most recent proposal (42) is that the critical property is a distance of about 5.5 Å between the carbon of the carboxyl and a fractional positive charge in the nucleus (Fig. 1). This explains the relative activities of most of the phenoxy, phenylacetic and benzoic acids, as well as a number of peculiarities such as the high activity of 2,3,6-trichlorobenzoic acid and the inactivity of 3,5-dichlorophenoxyacetic acid. The great differences in the activity of closely related compounds are seen to be largely due to the effects of substitution on the location and the magnitude of the fractional positive charge. There must be
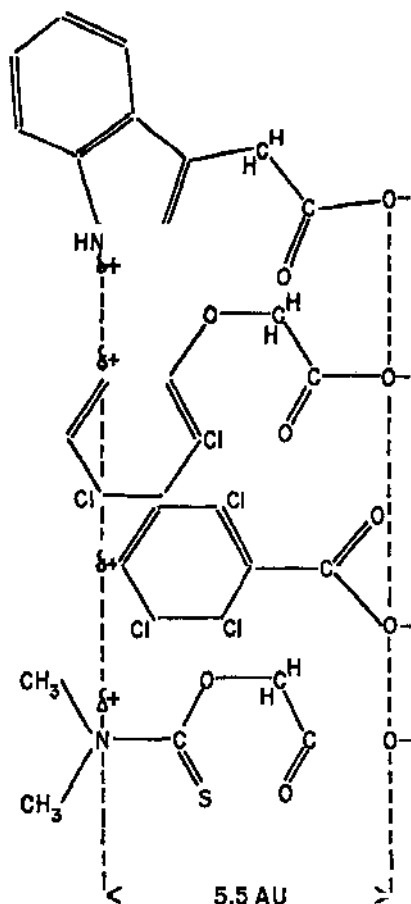

FIG. 1. IAA and the most active of the auxins in three other series. Four examples of the charge-distance relationship postulated by Porter & Thimann (42). For simplicity of reproduction the diagrams are presented as planar. AU = Angstrom units.

several secondary influences; solubility in lipids is one, and the angle of orientation of the side-chain, particularly when it contains oxygen, is another. For naphthalene derivatives the application is not so clear. But a general pattern can be discerned, and it is supported by work with synthetic compounds.

Much less can be said about the other groups of substances. For kinetin-like activity, only adenine derivatives have so far been explored, and indeed a number of the naturally-occurring compounds now being actively isolated in Australia, New Zealand, U.S.A., and France show signs of being adenine derivatives. Among the synthetic 6-amino purines the N-benzyl is generally the most effective substituent (8), and the effects of

TABLE I

Approximate Relative Activity of Gibberellins as Per Cent of That of GA[7]

	Dwarf pea stem[a]	Dwarf maize leafsheath[a]		Lettuce hypocotyl[a]	Cucumber hypocotyl[a]	Lettuce seed germination in dark[a]	Formation of staminate flowers on gynoecious cucumbers[b]	Parthenocarpic growth of tomatoes[c]
		Dwarf-1	-3	-5					
GA[1]	100	100	33	20	2	2	3	8	50
GA[2]	33	10	5	10	2	2	0	40	11
GA[3]	330	100	50	100	50	2	10	27	33
GA[4]	16	100	50	33	16	100	100	80	100
GA[5]	33	10	100	100	2	0.2	3	2	330
GA[6]	33	10	5	20	0.5	0.2	0	3	25
GA[7]	100	100	100	100	100	100	100	100	100
GA[8]	3	0.3	0.5	1	0	0.02	0	<2	3
GA[9]	0	0.3	100	33	16	100	0	40	20

[a] Data adjusted from (47).
[b] Inverse of the concentration needed to form two staminate flowers (48).
[c] Inverse of the concentration needed to produce 9 mm growth in 9 days (48).

chlorine and other substitutions on its benzene ring suggest that, as with the auxins, the distribution of charges is the major determining factor (43).

A little information is developing as to the structural requirements for the activity of gibberellins. Loss of the lactone ring destroys all, or almost all, the activity, but otherwise the relative activities of the different compounds differ widely in different bioassays. Nine compounds are so far known and doubtless more will appear in due course; indeed, gibberellin-like substances have recently been isolated which appear to be of new types (44, 45). Of the nine, GA[7] shows the highest activity in nearly all tests. On light-sensitive lettuce seeds the combination of GA[4] and GA[7], indeed, is 100 times as active as GA[3] (46). From the published cases where quantitative comparison has been made, the approximate relative activities shown in Table I have been calculated. GA[7] was chosen as a reference

compound here because of its rather uniformly high activity. The main chemical differences are the ring double bonds in 3, 5, and 7, and the location and numbers of hydroxyl groups present in all but 9. The table shows no clear correlation of activity with the double bond, since GA_4 is one of the most active, but the hydroxyl groups show some relationship. If we group the compounds according merely to the numbers of OH groups, we get the data in Table II. Optimal activity is associated with one OH group; in 4 and 7 it is in ring A, in 5 it is in rings C and D. Except in GA_3, additional OH groups clearly lower activity. There is a curious partial parallel with the auxins, in which any OH groups greatly lower activity (36). This has been ascribed to the power of OH groups to donate electrons to ben-

TABLE II

GIBBERELLINS ARRANGED ACCORDING TO NUMBERS OF HYDROXYL GROUPS

No. of OH groups	Gibberellins Nos.	Relative Activities (means of values in Table 1)	Average relative activity
0	9	34	34
1	4, 5, 7	66, 64, 100	77
2	1, 2, 3, 6	35, 13, 78, 11	34
3	8	<2	<2

zene rings. To make the same deduction for the saturated rings of the gibberellins is dubious but suggestive.

The data of Michniewicz & Lang (49) do not lend themselves readily to the calculation of relative activities, but in general they agree well with those in Table I. In four of the five plants whose flowering was studied, GA_7 was the most active (or one of the most active group), namely in *Myosotis, Silene, Crepis,* and *Bryophyllum,* while in the fifth, *Centaurium,* the responses are not quite clear, but GA_7 appears second in activity to GA_3. Certainly 7, 4 and 3 are the most active, 2, 6 and 8 the least.

An interesting complication follows logically here. Suppose that further synthetic and isolation work gives us eventually a clear understanding of the essential structures for activity of all three groups of substances; from this we can deduce something of the nature of the surfaces with which they react, as recent work with penicillin has done for the bacteria. The problem then will be: how can these substances *interact*? Does the interaction take place at one and the same reacting surface or is it mediated through a series of steps? If the interactions are real they cannot be separated very far in time or place. On the other hand the problem is certainly much more complicated if a substrate capable of reacting simultaneously with two or even three growth substances must be envisaged. These considerations lead directly to the next major group of problems.

MECHANISM OF ACTION

A third of a century has given us no firm basis for visualizing the mode of action of auxin. From the spatial and structural requirements above we can derive some idea of the surface with which the auxin combines, both in shape and in charge distribution. But we know neither what it is nor where it is located. Auxin does not appear to attach to any particle (50), though there is some evidence for attachment of 2,4-D to a soluble protein (9, 50). Auxin cannot act in the absence of oxygen, or in presence of inhibitors of oxidation, phosphorylation or SH-enzymes (51). It does not cause cell enlargement in the absence of any turgor. No known *in vitro* enzyme system is catalyzed by it. These limited and mainly negative statements are similar to those which must be made about most animal hormones, for as Szent-György (52) recently said,

The biochemist will proudly show the row of vials containing these mysterious hormones mostly in the form of nice crystalline powders (and) will be able to give us the structural formula of most of these substances. The really intriguing problem, however, is not what these substances *are* but what they *do,* how they act on the molecular level, how they produce their actions. There is no answer to this question.

Naturally most work has been directed towards cell enlargement, the most typical of the auxin functions. In this process the amounts of cell wall, and of all its constituents, increase proportionately to the amount of growth, and show little sign of "thinning out." Besides, no one constituent appears to change more than another. For a while it appeared that auxin might cause elongation by promoting the methylation of the carboxyl groups of pectin, especially in the hot water-soluble fraction. However, while this does occur in the coleoptiles of oats (53) and maize, it does not occur in mesocotyls of maize; for Cleland's measurements of growth and the transfer of methyl groups from C^{14}-methionine to the hot water-soluble fraction of pectin
ing ratios between treated and control sections (54):

| | Ratio IAA/Control | |
	Growth Rate	C^{14} from Methionine in Pectin
Avena coleoptile	2.5	1.9
Zea coleoptile	2.1	1.6
Zea mesocotyl	2.0	1.0

Furthermore, the basis for the above proposal was that the carboxyl groups limit growth by being linked together in pairs by calcium; methyl ester formation would promote growth by occupying the carboxyl, so that the calcium salt could not form. In support of this was the observation that

chelating agents cause a slight increase in elongation, and hence auxin was thought to act like a chelating agent. Aside from the fact that the chelating power of most auxins is vanishingly small, it developed that chelating agents exert their most marked effects in the presence of IAA (55). Furthermore no such action occurs with NAA or 2,4-D. Thus the chelating agents act by saving IAA from destruction or binding and not by influencing the primary process of growth or auxin action. Besides, neither auxin alone nor auxin plus the chelating agent EDTA liberate appreciable calcium from the coleoptile tissue (55, 56). It is evident then, that there is no real experimental basis for the methylation and decalcification concept, and whatever the way in which auxin modifies the plastic extensibility of the cell wall, it must be even more intimately associated with metabolism, and probably with oxidations, than that concept suggests.

After all, the primary action of auxin can hardly be sought in the cell wall, for while cell elongation is certainly the most characteristic result of auxin action, it is by no means the only one, or even the first to appear; acceleration of protoplasmic streaming occurs at least as rapidly. Several other responses, especially cambial division, inhibition of lateral bud development, and root formation, do not appear to rest on changes in the properties of the cell wall. As noted above, inhibition is probably just as typical and important. Thus the effects on the wall, like other effects, are almost certainly secondary, and the primary or "master reaction" eludes us. Even the peculiar effect of auxin treatment in decreasing the heat-coagulability of the tissue protein (57) is probably a result, rather than a cause, of the growth, for it is a relatively long-term effect. It was because of the need for a primary reaction that an effect mediated via the nucleus was sought, but in the alga *Acetabularia,* which can be readily enucleated, the typical response to auxin can occur (albeit less strongly) after enucleation (58).

A phenomenon perhaps insufficiently brought into consideration is the powerful interaction between auxin and metallic ions in growth phenomena. Calcium, as we long ago found, inhibits growth drastically (59). It markedly inhibits lignification in tissue cultures (60), and lignification in turn is strongly and perhaps directly promoted by auxin (61, 62, and earlier work). Potassium, on the other hand, promotes growth in many tissues and organs. There is good reason to believe that the effect of auxin on respiration entails potassium ions. Manganese promotes IAA oxidation strongly in the enzyme system from higher plants and appears essential for its action (14, 63). The enzyme from *Omphalia* appears to operate without manganese, though its action is modified and the oxidation carried further when Mn is added (64). The same is true for the purified enzyme from *Lupinus* (64a). Surprisingly, Mn in relatively high concentrations actually promotes the growth induced by IAA in coleoptile sections (65). So does iron, and $FeSO_4$ has been used to increase the sensitivity of the *Avena* curvature

test (66). Since iron acts like chelating agents, its primary action may be to inhibit the destruction of IAA, although it also seems to promote translocation of both IAA and NAA (67). Cobalt is particularly active on a number of tissues (68) and since it acts equally well with IAA and NAA its action cannot be due to protecting IAA from oxidative destruction. The fact that cobalt is not normally required by higher plants makes this action difficult to explain in terms of a normal process, though this need not impair its value as a possible guide to the site of auxin action. Perhaps cobalt, like EDTA, interacts with another metal or metal-combining site (55). These metal effects might well be more actively exploited.

TROPISMS

The upward geotropic curvature of stems is obviously of crucial importance to the seedling, doomed to germinate at all angles in the soil. Similarly, the positive phototropic curvatures of stems and petioles are critical for green plants growing in conditions of partial shade. Yet, somehow, this great ecological importance of the tropisms does not engender much study, and the field attracts little attention from most plant physiologists. Actually it has a special experimental clarity because of the fact that nowhere is the precise control of growth by growth substances so evident as in the tropisms.

That old-established ideas on tropisms should be continually subject to re-examination is of course only healthy, but it is perhaps surprising that the apparently well-founded concept that auxin undergoes redistribution under the influence of light and gravity should have been so easily abandoned by so many workers. The onslaught on the facts so early established by the Utrecht school began with the finding that IAA is inactivated by light in the presence of riboflavin, which led to the statement in a 1959 review (69) that "light-induced changes in auxin metabolism, and not a direct influence of light on auxin transport, are the primary causes of phototropic movements." In this case "metabolism" refers primarily to destruction, but in fact no evidence that IAA is actually destroyed by light in tissues had been given, or even seriously looked for. Next came the series of claims (70) that when C^{14}-IAA was applied to various organs, no asymmetric distribution of the C^{14} occurred under the influence of light, or indeed under the influence of gravity either. Since in *Avena* coleoptiles phototropic curvature (at low light dosages) results only when the extreme tip is illuminated, this result could be interpreted as meaning that light acts not on the auxin itself but only on an auxin precursor, or the precursor → auxin converting system, which would be expected to be limited to the tip. But the result with gravity seemed to prove too much, since we know from Dolk's work that auxin applied to sub-apical sections of coleoptiles could suffer clear-cut redistribution by gravity. Fortunately, re-examination of the phenomenon has now shown unequivocally that C^{14}-auxin does undergo redistribution under the influence of gravity (71a). Such ex-

periments are subject to three sources of error, any one of which can ob-
scure the effect: (a) impurities in the C^{14}-IAA, which carry radioactivity
but are not subject to the effect of gravity on IAA transport, (b) over-
loading of the IAA transport system, which occurs in *Avena* coleoptile
sections when 800 μg per liter (or more) are transported (72), and (c)
fixation of C^{14} in the tissue, which occurs rapidly, though to varying ex-
tents, in all tissues examined. Where this third source of error is large it
can be avoided by examining agar blocks into which the IAA-C^{14} has been
transported, rather than the tissue itself.

Preliminary experiments with light, under conditions causing both first
and second types of positive phototropic curvature, show that here too a
true redistribution of auxin occurs.[6] We must conclude that the mechanism
which transports auxin polarly through the plant is readily modified by
external conditions. In the case of gravity the inhibition of bud growth
on the lower side of horizontally-growing shoots and the frequent forma-
tion of "rotholz" or compression wood there, thus receive a logical explana-
tion. In the case of light, scattering and reflection are probably the reasons
why such clear-cut effects have not been reported. It is worth noting that
the auxin transport system is also very sensitive to chemical inhibitors and
auxin analogues (73 to 76). Many substances which have been reported to
inhibit geotropic curvature without much influencing growth (76) or photo-
tropism (77) evidently act by modifying the transport system, or specifi-
cally that part of it which is sensitive to gravity.

A quite different mechanism for phototropic curvature in opposite-leaved
plants has been made probable by Shibaoka & Yamaki (78). Here the pro-
duction of auxin increases with increasing light intensity on the leaf (to an

---

[6] Phototropism occurs only in response to blue and near ultraviolet light, not in
the red, although red may influence the total growth rate. In this connection the
remarks of Mohr in the volume preceding this one (72a) are worth noting. Mohr
says, referring *inter alia* to a paper of ours (72b), "There has been general agree-
ment that any radiation influencing the rate of elongation of an organ can induce a
phototropic curvature when applied unilaterally." He then concludes that this is in-
correct because the growth rate can be influenced by light which is phototropically
inactive (i.e., > 600 mμ). As a matter of fact there never was any such "agreement",
and the statement is certainly not made in three of the four papers he cites. In so
far as it is implied by the concept that phototropism is due only to a difference in the
light-growth reactions on the two sides of a growing organ, this ancient view (due to
Blaauw) was specifically referred to by us as "naive" and "essentially improbable."
The fact is, of course, that phototropism does not simply involve, as Mohr supposes,
"a reduction of the rate of cell elongation"; it involves (in coleoptiles at least) a
decrease on one side and an increase on the other. This is specifically the result of
lateral auxin migration towards the shaded side, whose growth is accelerated. Such
migration is caused only by blue (and UV) light. Thus there is no mystery "as to
why no bending occurs with unilateral visible radiation at longer wavelengths"; these
wavelengths, though they may affect total growth rate, simply do not cause lateral
auxin movement.

optimum at 2000 lux) and hence the stem receives more auxin from the leaf which is more nearly perpendicular to the light. Thus the plant curves until the two leaves receive the light at equal angles of incidence.

Growth Inhibitors

Prominent among explanations of the control of growth have been the roles assigned to growth inhibitors. Many experiments have been believed to establish their function in bud dormancy. Unfortunately the mere presence of a substance potentially able to inhibit growth does not establish any such function for it *in vivo*. Two reasons in particular must make us sceptical of such explanations. Firstly, the growth inhibition is usually not tested on the object assumed to be inhibited, but on sections of oat or wheat coleoptiles, which doubtless have a very different susceptibility.[7] Secondly, even on the test objects employed, little attempt is usually made to relate the extent of inhibition to the amount or concentration of the inhibitor. It is impossible to tell, therefore, whether the level of inhibitor in the organ from which it was extracted was high enough to make it effective. The fact that the supposed inhibitors are often not characterized chemically, or even purified, is a less important objection in principle than the other two. In a few instances, notably naringenin from peach buds (80), decanoyl-acetaldehyde from *Phaseolus* stems (81), and quercetin and kaempferol glucosides from pea stems (82, 83), they have indeed been identified, but this does not establish their *in vivo* function. In the case of naringenin, the fact that its inhibition of the germination of lettuce seeds is partly reversed by light, and more completely by gibberellin, does give the phenomenon an appearance of naturalness (84). But the general lack of quantitation is a serious barrier. This is brought out strikingly in some recent papers in which the condition that is supposed to increase the inhibitor in the buds operates only on one chromatographic fraction, with R_f 0.55, while an apparently more potent fraction with another R_f remains unchanged. In the absence of any evidence as to the relation between concentration and effect of these substances, we can only suppose that this relation is likely to be far from linear, and hence the unchanged fraction may well exceed the other in amount by a power of ten or more. The continued use of one-dimensional chromatograms on relatively crude extracts reminds one of Brefeld's remark about the use of impure cultures, from which, he said, "one obtains nothing but nonsense and *Penicillium glaucum*." Indeed Nitsch & Nitsch (85) early noted that on such chromatograms an auxin and an inhibitor could so overlie one another that the effect of both is masked.

Reservations as to the adequacy of the test method are even more justified in work with growth promoters. What are we to think when coleop-

---

[7] An exception must be made for the inhibitor produced by *Helianthus* leaves which, though primarily assayed on coleoptiles, was also shown to inhibit growth of *Helianthus* hypocotyls (79).

tile sections, whose growth is known to be promoted by sugars, organic acids and potassium ions, are floated on water and then used to test aqueous extracts of plant tissue which are well known to contain these three constituents (86)? Obviously the findings justify no deductions about water-soluble auxins.

There is, however, one hard-core group of inhibitors whose effects cannot be argued away. These are the substances which, at low concentrations, promote growth specifically in the presence of IAA and not of other auxins, and inhibit only at higher concentrations. Such characteristic behavior indicates interference with the system destroying IAA. The behavior of guaiacol on rice coleoptile sections (87) is a clear example; it can promote growth as much as 50 per cent in suboptimal IAA but has little effect in NAA. At $3 \times 10^{-3}M$ it inhibits at all IAA and NAA levels. Chlorogenic acid, which has been isolated from rice coleoptiles (88), belongs in the same category, while caffeic and sinapic acids, both common in plants, have similar but weaker action (89). The recent claim that caffeic acid is a natural auxin of major importance (90) rests on this effect (91). As opposed to these diphenols, several monophenols like 2,4-dichlorophenol and p-coumaric acid, which occurs in pineapple (92), promote the oxidation of IAA (14) and correspondingly inhibit the growth which is due to endogenous or applied IAA. A development from this is the isolation from the bud of etiolated pea seedlings of a p-coumaryl-triglucoside of the monophenolic flavanoid kaempferol, while the similar seedlings grown in full light yielded, in addition, the corresponding derivative of the diphenolic flavonoid, quercetin (83). This latter is believed to be the natural IAA oxidation-inhibitor previously shown to occur in green tissue. Like the diphenolic acids, it promotes IAA-induced growth at low concentrations and inhibits at high. The parallel with the phenols is not clear, however, since the kaempferol derivative was first reported to inhibit IAA oxidation, and free kaempferol to be even 20 times more active as an IAA oxidase inhibitor (82). The difference is probably a matter of the concentration used, since Furuya *et al.* (83) do find kaempferol and its derivative to promote the oxidation at low concentrations.

In addition to the phenols, the chelating agent EDTA and the metal ion Fe^{++} act in the same way, as noted above. If IAA oxidation is mediated by a cyclic free-radical mechanism (64, 93), then no doubt other compounds with "chain-breaking" activity will be found to synergize with IAA. Perhaps indole, which shows marked synergism (94), is one of these.

FUTURE OUTLOOK

First we must note that there are a number of serious gaps in our knowledge, which will have to be filled before really fundamental advances can take place. While it is always hard to say that one field of research is more important than another, yet it seems as though these gaps interrelate so strongly with more than one area that they would justify special attention.

The first major area of ignorance—the mode of action—has been dis-

cussed above. Perhaps more surprising, since it seems so much more concrete a problem, is that of auxin biogenesis. That at this stage we should still not know for certain whether IAA normally comes from tryptophan, tryptamine, indoleacetonitrile, or some other precursor, or is more directly synthesized, seems remarkable. Some evidence points in each direction, but none is conclusive. The very limited occurrence in the plant kingdom of the enzyme converting indoleacetonitrile to IAA (39) seems to eliminate the nitrile as a precursor. The nitrile itself is readily derived by breakdown of the glucoside, glucobrassicin (95), and if this is its normal origin then it doubtless lies remote from the direct path of formation of IAA. The route from tryptophan via indolepyruvic acid is attractively simple, and certainly occurs in fungi and bacteria, but its status in higher plants is still uncertain. The route from tryptamine, supported by the growth-promoting effectiveness of tryptamine in some instances, and the common occurrence of amine oxidases, is equally uncertain. To the author the most attractive route is a direct synthetic one, rather than one depending on breakdown of the amino acid or amine. But firm evidence, obtained under natural conditions with physiological concentrations of substance, is much needed.

Another curious gap is that of the hormonal control of root growth. From early days the action of auxin in inhibiting root elongation has been clear, and much of the work on auxin antagonists is based on their ability to restore growth in roots inhibited by auxin. The less negative role of auxin in controlling vasculation and promoting the formation of lateral root primordia (96) has also been worked out, especially by Torrey (97). The control of growth and cell division in roots by nutrients, vitamins, etc., has likewise been extensively studied. But whether there are truly hormonal relations between the root tip and the remainder of the root, or between growing and mature segments, needs clarification, and the relative participation of auxins or other, unknown, factors needs to be worked out. Somewhat similar problems exist for the relations between lateral roots and nodules in legumes (see Nutman, 98).

In these and in all other cases, the question needs to be clearly asked— what is the limiting factor? When many hormones participate, this question is especially likely to prove critical.

If we may assume that the researches now going on in several laboratories will culminate in the isolation of kinetin-like substances from several plant sources, then there are three known groups of naturally occurring growth promoting substances. The members of each group are chemically related among themselves, but only distantly related (if at all) to those of the other groups. Two of the groups qualify as genuine hormones; kinetin and those of its congeners so far studied do not, for present evidence indicates that they are not at all readily translocated. They are local growth substances, though none the less potent for their spatial restriction. In addition, it has long been recognized that thiamine and other B-vitamins qualify as hormones, since they are synthesized in leaves and exported to the roots, whose

growth is dependent on them (99). They are the only growth-promoting hormones for roots whose existence is established as yet. The naturally occurring inhibitors, while not exactly growth substances in the ordinary sense, may well control growth in specific instances, as auxin does in roots, though whether they are truly hormones remains to be seen.

Then there are the hormones whose existence has as yet only been postulated. Evidence that something which initiates flower formation migrates from leaves to apices makes the existence of a flower-forming hormone at least likely (though too easily accepted by some without the still-needed proof). Roots certainly promote the growth of shoots, though the hormonal basis for this remains unsure. Abscisin, the postulated abscission hormone, rests on still less real evidence (100), and the control of senescence, although ascribed to a transmitted "signal" (101), has not yet been given the shape of a hormone. But in all reasonableness we do have to face the fact that a third of a century is a short time in the history of science, and that other hormonal factors will almost surely be turned up before long. Some time soon we shall be visualizing any one of the organs of a plant as a veritable Times Square of intersecting streams of traffic, with specific hormones crossing and recrossing on predictable paths, some entering a cell together, there to activate specific biochemical processes, others accumulating or decaying, and every external influence playing its part in changing their fate. And while we may thus see the machinery so much more completely, the problem of visualizing the wholeness of the plant—the balanced and integrated organism—will be as elusive as ever.

LITERATURE CITED

1. Snow, R., *Ann. Botany*, **39**, 841–59 (1925)
2. Dostál, R., *Acta Soc. Sci. Natl. Moravicae*, **3**, 83–209 (1926)
3. Audus, L. J., *Plant Growth Substances* (Interscience Publishers Inc., New York, 553 pp., 1959)
4. Pilet, P. *Les Phytohormones de Croissance* (Masson et Cie., Paris, 774 pp., 1961)
5. Went, F. W., and Thimann, K. V., *Phytohormones*, (The Macmillan Co., New York, 294 pp., 1937)
6. Fawcett, C. H., *Ann. Rev. Plant Physiol.*, **12**, 345–68 (1961)
7. Stuart, N. W., and Cathey, H. M., *Ann. Rev. Plant Physiol.*, **12**, 369–94 (1961)
8. Miller, C. O., *Ann. Rev. Plant Physiol.*, **12**, 395–408 (1961)
9. Galston, A. W., and Purves, W. K., *Ann. Rev. Plant. Physiol.*, **11**, 239–76 (1960)
10. Phinney, B. O., and West, C. A., *Ann. Rev. Plant Physiol.*, **11**, 411–36 (1960)
11. Steward, F. C., and Shantz, E. M., *Ann. Rev. Plant Physiol.*, **10**, 379–404 (1959)
12. Gruen, H. E., *Ann. Rev. Plant Physiol.*, **10**, 405–40 (1959)
13. Bentley, J. A., *Ann. Rev. Plant Physiol.*, **8**, 47–80 (1957)
14. Ray, P., *Ann. Rev. Plant Physiol.*, **9**, 81–118 (1958)
15. Leopold, A. C., *Ann. Rev. Plant Physiol.*, **9**, 281–310 (1958)
16. Collier, H. O. J., *Sci. Am.*, **207**, 111–18 (1962)
17. Lewis, G. P., *Physiol. Rev.*, **40**, 647–76 (1960); *Nature*, **192**, 596–99 (1961)
18. Brian, P. W., Hemming, H. G., and Radley, M., *Physiol. Plantarum*, **8**, 899–912 (1955)
19. Schraudolf, H., and Reinert, J., *Nature*, **184**, 465–46 (1959)
20. Yoda, S., and Ashida, J., *Nature*, **182**, 879–80 (1958); (a) Hasman, M., Inanc, N., and Verter, C., *Physiol. Plantarum*, **14**, 290–302 (1961)
21. Albaum, H. G., *Am. J. Botany*, **25**, 124–33 (1938)
22. Von Maltzahn, K. E., *Nature*, **183**, 60–61 (1959)
23. Brian, P. W., and Hemming, H. G., *Ann. Botany (London)*, **22**, 1–17 (1958); *Plant Growth Regulation,*

Intern. Conf., 4th, Yonkers, N.Y., 1959, 645–56 (Univ. Iowa Press, Ames, Ia., 1961)
24. Kuse, G., *Botan. Mag. (Tokyo)*, **71**, 151–59 (1958)
25. Saebø, S., *Physiol. Plantarum*, **13**, 839–45 (1960); **14**, 393–99 (1961)
26. Michniewicz, M., *Acta Soc. Botan. Polon.*, **30**, 133–48 (1961)
27. Kefford, N. P., *Plant Physiol.*, **37**, 380–86 (1962)
28. Skoog, F., and Miller, C. O., *Symp. Soc. Exptl. Biol.*, **11**, 188–231 (1957)
29. Thimann, K. V., and Wickson, M., *Physiol. Plantarum*, **11**, 62–74 (1958); Wickson, M., and Thimann, K. V., *Physiol. Plantarum*, **13**, 539–54 (1960)
30. Wittwer, S. H., and Tolbert, N. E., *Plant Physiol.*, **35**, 871–77 (1960)
31. Sachs, R. M., *Am. J. Botany*, **49**, 656–57 (1962), (Abstr.)
32. Weaver, R. J., *Nature*, **187**, 1135–36 (1960)
33. Brian, P. W., Grove, J. F., and MacMillan, J., *Fortschr. Chem. Org. Naturstoffe*, **18**, 350–433 (1960)
34. Thimann, K. V., *Fundamental Aspects of Normal and Malignant Growth*, Chap. 10, 748–822 (Elsevier Publ. Co., Inc., Amsterdam, 1960)
35. Van Overbeek, J., in *Proc. Plant Sci. Symp., Camden, N.J., 1961*, 37–56 (Campbell Soup Co., N.J., 1962)
36. Thimann, K. V., in *Plant Growth Substances*, 21–35 (Univ. of Wisconsin Press, Madison, Wisc., 1951)
37. Veldstra, H., *Ann. Rev. Plant Physiol.*, **4**, 151–98 (1953)
38. Thimann, K. V., and Leopold, A. C., *Hormones*, **3**, 1–56 (1955)
39. Thimann, K. V., and Mahadevan, S., *Nature*, **181**, 1466–67 (Abstr.); (a) *Intern. Biochem. Congr., 5th Moscow*, 1961, but see (b) Libbert, G., and Ballin, G., *Naturwissenschaften*, **46**, 532–34 (1959)
40. Wain, R. L., and Wightman, F., *Proc. Roy. Soc. (London) Ser. B*, **142**, 525–35 (1954); (a) Fawcett, C. H., Wain, R. L., and Wightman, F., *Plant Growth Regulation, Intern. Conf., 4th, Yonkers, N.Y., 1959* (Iowa State Univ. Press, Ames, Ia., 1959)
41. Fawcett, C. H., Wain, R. L., and Wightman, F., *Proc. Roy. Soc.*

(London) Ser. B, **152,** 231–54 (1960)

42. Porter, W. L., and Thimann, K. V., *Abstr. Intern. Botan. Congr., 9th, Montreal, 1959,* 305–36 (Univ. of Toronto Press, Toronto, 1959); see also (*a*) Thimann, K. V., *Plant Growth Regulation, Intern. Congr., 4th, Yonkers, N.Y., 1959,* 444–447 (1959); (*b*) Porter, W. L., *Molecular Structure and Auxin Activity: The Fourth Specificity Requirement for Auxin Action* (Doctoral thesis, Harvard Univ., Cambridge, Mass., 1962)

43. Shantz, E. M., and Steward, F. C., *Plant Physiol.* (In press 1963)

44. Nitsch, J. P., and Harada, H., *Proc. Intern. Symp. Environ. Control Plant Growth, Canberra, 1962* (In press)

45 Katoh, J. (Personal communication)

46. Ikuma, H., and Thimann, K. V., *Science* (In press 1962)

47. Brian, P. W., Hemming, H. G., and Lowe, D., *Nature,* **193,** 946–48 (1962)

48. Wittwer, S. H., and Bukovac, M. J., in *Proc. Plant Sci. Symp., Camden, N. J., 1961,* 65–83 (Campbell Soup Co., N.J., 1962)

49. Michniewicz, M., and Lang, A., *Planta,* **58,** 549–63 (1962)

50. Nakamura, T., Ishii, H., and Yamaki, T., *Plant Cell Physiol. (Tokyo),* **3,** 249–56 (1962)

51. Cleland, R., *Handbuch der Pflanzer Physiology,* 754–83 (Springer-Verlag, Berlin, 1961)

52. Szent-Györgyi, A., *Introduction to a Submolecular Biology,* 135 pp., (Academic Press, Inc., New York, 135 pp., 1960)

53. Ordin, L., Cleland, R., and Bonner J., *Plant Physiol.,* **32,** 216–20 (1957)

54. Cleland, R., *Nature,* **185,** 44 (1960)

55. Thimann, K. V., and Takahashi, N., *Plant Growth Regulation, Intern. Conf., 4th, Yonkers, N. Y., 1959,* 363–80 (Iowa State Univ. Press, Ames, Ia., 1961)

56. Cleland, R., *Plant Physiol.,* **35,** 581–84 (1960); cf. also Ng, E. K., and Carr, D. J., **12,** 275–87 (1959)

57. Galston, A. W., and Kaur, R. *Proc. Nat. Acad. Sci. U.S.,* **45,** 1587–90 (1959); *Abstr.* IXth *Intern. Botan. Congr., 9th., Montreal, 1959,* 129 (Univ. of Toronto Press, Toronto, 1959)

58. Thimann, K. V., and Beth, K., *Nature,* **183,** 946–8 (1959); also see reference 51.

59. Thimann, K. V., Slater, R. R., and Christiansen, G. S., *Arch. Biochem.,* **28,** 130–37 (1950); (*a*) Thimann, K. V., and Schneider, C. L., *Am. J. Botany,* **25,** 270–80 (1938); cf. also (*b*) Cooil, B. J., and Bonner, J., *Planta,* **48,** 696–723 (1957)

60. Lipetz, J., *Am. J. Botany,* **49,** 460–64 **(1962)**

61. Camus, G., *Rev. Cytol. Biol. Vegetales,* **11,** 1–199 (1949)

62. Wetmore, R. H., and Sorokin, S., *J. Arnold Arb.,* **36,** 305–17 (1955)

63. Abramovitch, R. A., and Ahmed, K. S., *Nature,* **192,** 259–60 (1961)

64. Ray, P. M., *Arch. Biochem. Biophys.,* **96,** 199–209 (1962)

64a. Stutz, R. E., *Plant Physiol.,* **32,** 31–9 **(1957)**

65. Bonner, J., *Am. J. Botany,* **36,** 323–32 (1949)

66. Shibaoka, H., and Yamaki, T., *Bot. Mag. (Tokyo),* **72,** 152–8 (1959)

67. Shibaoka, H., and Yamaki, T., *Bot. Mag. (Tokyo),* **72,** 203–14 (1959)

68. Miller, C. O., *Plant Physiol.,* **27,** 408–12 (1952); **29,** 79–82 (1954); Thimann, K. V., *Am. J. Botany,* **43,** 241–50 (1956)

69. Reinert, J., *Ann. Rev. Plant Physiol.,* **10,** 441–58 (1959)

70. Bünning, E., Reisener, H. J., Weygand, F., Simon, H., and Klebe, J. F., *Z. Naturforsch.,* 11b, 363–66 (1956); (*a*) Ching, T. M., and Fang, S. C., *Physiol. Plant.,* **11,** 722–27 (1958); (*b*) Reisener, H. J., and Simon, H., *Z. Botan.,* **48,** 66–70 (1960)

71. Gillespie, B., and Thimann, K. V., *Experientia,* **17,** 126–29 (1961)

71a. Gillespie, B., and Thimann, K. V., *Plant Physiol.,* **38,** (In press, 1963)

72. Goldsmith, M. H. M., and Thimann, K. V., *Plant Physiol.,* **37,** 492–505 **(1962)**

72a. Mohr, H., *Ann. Rev. Plant Physiol.,* **13,** 465–88 (1962)

72b. Thimann, K. V., and Curry, G. M., *Phototropism, in Light and Life,* 646–72, (McElroy, W. D., and Glass, B., Eds., Johns Hopkins Press, Baltimore, Md., 1961)

73. Kuse, G., *Mem. Coll. Sci., Univ. Kyoto, Ser. B,* **20,** 207–15 (1953)

74. Niedergang-Kamien, E., and Leopold, A. C., *Physiol. Plant.,* **10,** 29–38

(1957); (a) Niedergang-Kamien, E., and Skoog, F., **9**, 60–73 (1956)

75. Hay, J. R., *Plant Physiol.*, **31**, 118–20 (1956)

76. Mentzer, C., Molho, D., and Pacheco, H., *Bull. Soc. Chim. Biol.*, **32**, 572–82 (1950); see Larsen, P., in *Handb. Pflanzen-Physiol.*, **17**, 2, 153–99 (1962), for a discussion of these phenomena in roots

77. Vander Beek, L. C., *Plant Physiol.*, **34**, 61–65 (1959); (a) Schrank, A. R., *Plant Physiol.*, **35**, 735–41 (1960)

78. Shibaoka, H., and Yamaki, T., *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, **9**, 105–26 (1959)

79. Shibaoka, H., *Plant Cell Physiol. (Tokyo)*, **2**, 175–97 (1961)

80. Hendershott, C. H., and Walker, D. R., *Science*, **130**, 798–800 (1959)

81. Isogai, Y., *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, **10**, 73–85 (1960)

82. Mumford, F. E., Smith, D. H., and Castle, J. E., *Plant Physiol.*, **36**, 752–56 (1961)

83. Furuya, M., Galston, A. W., and Stowe, B. B., *Nature*, **193**, 456–57 (1962)

84. Phillips, I. D. J., *Nature*, **192**, 240–41 (1961)

85. Nitsch, J. P., and Nitsch, C., *Biol. Pflanzen*, **31**, 387–408 (1955)

86. Britton, G., and Housley, S., *Phyton (Buenos Aires)*, **16**, 1–19 (1961)

87. Wada, S., and Nagao, M., *Sci. Rept. Tohoku Univ., Fourth Ser.*, **26**, 181–88 (1960)

88. Wada, S., *Sci. Rept. Tohoku Univ., Fourth Ser.*, **27**, 237–49 (1961)

89. Tomaszewski, M. M., *Bull. Acad. Polon. Sci., Classe II*, **7**, 127–30 (1959); (a) *Abstr. Intern. Congr. Biochem., 5th Moscow*, **15**, 94 (1961)

90. Vendrig, J. C., and Buffel, K., *Nature*, **192**, 276–77 (1961)

91. Thimann, K. V., Tomaszewski, M. M., and Porter, W. L., *Nature*, **193**, 1203 (1962)

92. Gortner, W. A., Kent, M. J., and Sutherland, G. R., *Nature*, **181**, 630–31 (1958)

93. Yamazaki, I., *Proc. Intern. Symp. Enzyme Chem., Tokyo Kyoto, 1957*, 224–29 (Maruzen Co., Ltd., Tokyo, 1958)

94. van Raalte, M. H., *Koninkl. Ned. Akad. Wetenschap., Proc., Ser. C*, **54**, 21–29, 117–25 (1951)

95. Gmelin, R., and Virtanen, A. I., *Ann. Acad. Sci. Fennicae, Ser. A. II*, **107**, (1961)

96. Torrey, J. G., *Physiol. Plant.*, **9**, 370–88 (1956)

97. Torrey, J. G., in *Cell, Organism and Milieu*, 189–222 (The Ronald Press Co., New York, 1959)

98. Nutman, P. S., *Biol. Rev. Cambridge Phil. Soc.*, **31**, 109–51 (1956)

99. Bonner, J., and Bonner, H., *Vitamins Hormones*, **6**, 225–75 (1948)

100. Osborne, D. J., *Nature*, **176**, 1161–63 (1955); Leopold, A. C., and Rubinstein, B., *Science*, **134**, 1883 (1961)

101. Leopold, A. C., *Science*, **134**, 1727–32 (1961)