THE HORMONES

Physiology, Chemistry and Applications

Edited by

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Preface to Volume III

Seven years have passed since the appearance of Volume I of The Hormones. At that time it was suggested that the subject might have completed its first unfolding—virtually all the major hormones, especially in the mammals and man, having been discovered, bioassayed, and at least partially identified chemically. The subsequent period, it was felt, would be one first of consolidation, then of increasing emphasis upon physiological problems and the inner mechanism of the action of hormones. This prognostication, it now appears, was true only in part. It was true for the hormones of the thyroid, anterior pituitary, and gonads, which have always occupied so central a position in research and application. To a lesser extent it was true in other areas. But as regards the hormones of the invertebrates and insects, and in the realms of the posterior pituitary and the nervous systems, the appearance of new facts and ideas has opened new horizons. The concept of nerves as secreting and transmitting hormones, as well as the long-awaited isolation of the pure posterior pituitary hormones themselves, are both notable. Even in the plants, the discovery that auxin plays a role in flowering brings what was thought to be a growth hormone into relationship with the reproduction process. Not less to be regarded as new departures are the discoveries concerning the pituitary-adrenal axis and the widespread medical applications, just touched on in Volume II, which have followed.

The expected emphasis upon mechanism of action, on the contrary, has not yet developed to the degree expected, and the major discoveries in this direction are still probably for the future, although there have been interesting and suggestive developments. In the case of the animal hormones particularly, intensive investigations of their metabolic fate have been considered necessary preludes to studies of their specific roles in biochemical reactions; in several instances it has been indicated that a metabolite of the secretory product rather than the secreted substance itself may be responsible for the presumed specific effects. Furthermore, with the broadening of our knowledge of hormone interaction, the need for distinction between primary and secondary effects of a given hormone has become more evident. For example, the adrenocorticomimetic effects of estrogens are cited in the text; the effects are presumably exerted through stimulating the secretion of ACTH from the anterior pituitary. Another complication

which has emerged with clarity is that mammals differ more widely in their physiology than was formerly thought. For this reason, any attempt to study mechanisms of action as problems in general physiology must recognize species differences in response to hormones, and the past septennium has unearthed additional remarkable hormonal responses occurring in one species and completely absent in another.

For all these reasons the present volume is not merely a supplement to Volumes I and II; that is, it is not merely a chronicle of recent experiments, extending in detail what has already been laid down in outline. Instead, some parts of the volume supplant their predecessors, and certainly the majority of the chapters at least modify or recast the picture which had been presented. While part of its information is certainly supplementary an important part must be regarded as revision or perhaps as reassessment.

The partition of subject matter between the authors and the planned content of the individual chapters has been somewhat revised from that of the previous volumes to allow of more unification and changed emphasis. Some of the authors are those who already reviewed their subjects in Volumes I and II, but the regrouping of the subject matter, as well as death, retirement, or preoccupation with other affairs, has necessitated a number of changes. In any event the chapters reflect individual viewpoints as much as ever. It is hoped that the elements both of variety and of uniformity will combine to make the book a useful tool in the difficult task of integrating modern biology.

G. PINCUS

June, 1955 K. V. THIMANN

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CHAPTER I

Plant Growth Hormones

BY KENNETH V. THIMANN AND A. CARL LEOPOLD

CONTENTS

I. Introduction

The progress of any field of science is highly irregular; some parts may make a major break-through, whereas other parts—apparently no more complex—seem blocked by some difficulty of procedure or of concepts, so that ideas and experiments proceed in spirals without advancing. The study of plant hormones has shown the expected irregularity of advance during the six years since the appearance of Volume I of *The Hormones*. Some aspects, like that of the inhibition of the growth of buds, have not undergone any really fundamental change, whereas the role of auxin in flowering and the mechanism of auxin action in growth have developed with great rapidity. Recent evidence for an *in vitro* action of auxin, and

greatly increased clarity as to the naturally occurring substances which alter auxin action, strengthen the hope that a reasonable picture of the biochemical control of plant growth may become available rather soon.

Numerous books and reviews have appeared recently. A reissue of Chapters II and III of Volume I (290) with bibliography bringing the subject up through 1950, appeared early in 1952. The 1949 Wisconsin Centenary volume, *Plant Growth Substances*, edited by F. Skoog and with 39 individual contributions, appeared in 1951. In a monograph on "Growth and Differentiation in Plants," edited by W. E. Loomis, about half of the 18 contributed chapters deal in one way or another with hormone action. The Brookhaven Symposium, "Abnormal and Pathological Plant Growth" (44), though concentrating on abnormalities, is necessarily much concerned with the action not only of auxins but also of other growth factors, known and postulated. Finally, three full-length treatments of the whole field of plant hormones have appeared, one by H. Söding, *Die Wuchsstofflehre* in 1952, one by L. J. Audus, *Plant Growth Substances* in 1953, and one by A. C. Leopold, *Auxins and Plant Growth* in 1955. The several shorter reviews, published in the Annual Reviews of Plant Physiology and elsewhere, are referred to here and there in the following sections.

The matter of definitions was taken up in Volume I. The definitions there suggested have been accepted by Audus (10), and, with an interesting discussion of alternatives, by Gordon (98). There is therefore no reason to discuss the matter further in this chapter, though the proposal to name the compounds that cause root elongation "root auxins" should be noted (116). The term "shoot auxins" would be used for compounds stimulating elongation and multiplication of shoot cells and inhibiting the same processes in root cells. Problems of antiauxin terminology have been discussed by Burström (53).

II. Assay Methods

The speed and the direction in which an area of study proceeds are determined largely by the methods available for research in that field, and in the past few years a new set of tools has become available in the study of plant hormones. These new tools center around the chromatographic separation of auxins and related compounds.

Paper chromatography was first employed in the study of plant growth hormones by Jerchel and Müller (136) and Pacheco (224), who demonstrated the presence of indoleacetic acid in several plants, and by Bennet-Clark and co-workers (17, 18), who showed that growth-promoting and growth-inhibiting substances are present in several parts of the chromatogram. The compounds can be detected on the paper by color reactions (see below), by their fluorescence characteristics (261), and by bio-assay tests (13, 17, 18, 76, 77, 114, 281, 286).

Other techniques which have been recently developed for the separation of growth substances include column chromatography (180), countercurrent distribution (128), and electrophoresis of the paper chromatograph (76,77).

Another technique which has greatly facilitated research in the study of auxins is the development of color assays, especially for indole-acetic acid and 2,4-D. The Salkowski color reaction for indoleacetic acid and related compounds, which had been used as a qualitative test in many earlier researches, was first used quantitatively by Tang and Bonner (285) and improved by Gordon and Weber (100). This simple technique is used now almost to the exclusion of other tests in the study of the destruction of auxin. However, its sensitivity to interfering substances necessitates reservations in some instances (*u*)[1]. A number of other color reactions have been used in paper chromatography (17, 18, 281, 337). A color test for 2,4-D has been described (86) and subsequently improved (172).

The methods of bio-assay for auxins have undergone some changes, most of them minor. Improved methods for obtaining auxins by diffusion have been worked out, utilizing cyanide as a poison to prevent enzymatic destruction (276) or providing ascorbic acid as an alternative substrate (330). A fair number of workers have turned to the use of roots as an assay material. It has been known since the earliest days of the study of auxins that roots were the most sensitive material to auxins, and because of their sensitivity they have provided some very delicate assay methods (205, 334). It is a little unfortunate that most root assays utilize the inhibitory functions of auxins, while the action on shoots is typically growth promotion. In screening synthetic compounds, distinction between auxins (*i.e.*, "shoot auxins") and growth inhibitors is not easily made. By the proper selection of material, however, or by the use of isolated sections, root tests have been carried out using the growth promotion which results from very low auxin concentrations (205, 164, and see Section IV). Some variability in the sensitivity of the root promotion has been noted (205, 227) owing to the age of the roots or to some quality of the seed. For this reason it is not always possible to obtain consistent root growth promotions, though where they are obtainable they present valuable assay material. In a number of instances, synthetic compounds have been found to promote root growth over a wide range of concentrations and/or to remove the inhibition caused by auxins. These substances have been considered as "root auxins" or antiauxins (see 53). The removal of the auxin inhibition of root growth constitutes the best bioassay for antiauxin activity (see Section IV).

[1] The abbreviation (*u*) indicates unpublished data from the laboratories of the authors or supplied by correspondence.

The increasing array of chemical and biological techniques for assay has introduced some confusion, as might be expected. This will doubtless be removed as the new methods facilitate progress.

III. Natural Auxins

For some years one of the major problems of plant physiology has been the chemical identity of the native growth hormone, or auxin. With the passage of time, this problem has become broader, and certainly not simpler. Twenty years ago, when auxins *a* and *b* (di-*sec*-butylcyclopentene derivatives) and indoleacetic acid were isolated, the problem was merely to know which of these functioned in plants. Simple experiments on crude diffusates pointed to one of the two first-named, and indoleacetic acid (IAA) was relegated to the role of "heteroauxin," a substance formed by fungi but not by higher plants, and entering the growth process in higher plants only by some biochemical "backdoor." Ten years ago, indoleacetic acid began to be isolated from a few higher plants, and indirect evidence for its presence was obtained in many more. Indoleacetaldehyde was identified in several seedlings and in pineapple leaves. The isolation of auxins *a* and *b*, on the other hand, could not be confirmed, in spite of numerous trials. It seemed reasonable to conclude in Volume I (290), therefore, that "indoleacetic acid is widely distributed in higher plants, perhaps more widely than auxin *a* and *b*, and it is evidently a true plant hormone." In the last few years, however, the trend of opinion has gone further.

In the first place, the continued failure to reisolate auxins *a* and *b* has made their general occurrence very improbable.

In the second place, the study of analogues has not lent support to previous conceptions of these compounds. The cyclopentenyl (*i.e.*, unsubstituted) analogue of auxin *b* lactone has been synthesized in two laboratories (45, 145). The stability of this compound in the lactone form forced Jones and associates to the conclusion that the open-chain, free-acid structure could not exist as previously formulated. This casts fundamental doubt on the structures assigned. Furthermore, comparison of the structures with those of other compounds possessing auxin activity indicates that auxins *a* and *b* would not be expected to be appreciably active (291) for the following reasons: (*1*) No compound in which the ring structure is five-membered has so far shown appreciable activity; (*2*) side chains longer than three carbon atoms are associated with only low activity; (*3*) substitution of large alkyl groups on the ring lowers activity; and (*4*) the presence of hydroxyl groups on the side chain always drastically lowers activity. Yet auxins *a* and *b* have all four of these features.

In the third place, the original basis for identifying the auxin of such classical objects as the oat coleoptile with auxin *a* has been set aside. The

molecular weight of over 300, obtained by the diffusion method, has been found to fall to about 206 after a single ether extraction (332). (The calculated value for auxin *a* is 336, and for indoleacetic acid, 175.) The auxin of tomato shoots gave, as the mean of 24 careful determinations, a molecular weight of 202 (147), and a more recent study of the oat coleoptile auxin (236) gave values below 200 for both the acid and neutral fractions. The coleoptile auxin is destroyed by hot acid, as is indoleacetic acid (275). Because of the small discrepancy in the molecular weight, however, as well as other differences in behavior, Söding and Raadts concluded that the oat coleoptile contains, in addition to indoleacetic acid, a second auxin, chemically related, but of higher molecular weight. Terpstra (286) also found another auxin besides IAA in the coleoptile.

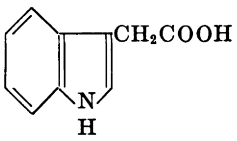
Although some of this evidence is circumstantial and might be set aside by new isolations, its cumulative weight appears now sufficient to justify the omission of auxins *a* and *b* from the present consideration of native plant hormones.

Indole compounds, on the other hand, have become much more important. In addition to the earlier isolations of indoleacetic acid (I), this compound has been identified by the Salkowski color reaction in oat coleoptiles (332) and by chromatographic methods in a wide variety of species. Its ethyl ester has recently been identified in apple seeds (288) and actually isolated from immature maize kernels (241). In the latter case, however, because the starting material was an ethanol extract, made by standing the corn in cold 95% alcohol, it is possible that esterification might have occurred during the extraction; Willstätter found ethyl chlorophyllide to be readily formed when leaves were similarly extracted with cold ethanol.

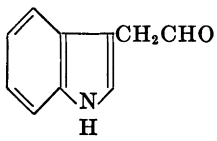
The wide occurrence of indoleacetaldehyde (II) appears to be well supported. First identified by Larsen (155) as a neutral material of very low activity, it was later found to be converted to a highly active acid auxin on treatment with soil or with aldehyde oxidase preparations (157). It has been found in etiolated seedlings of *Pisum* (155, 156, 157), in potato (120), in corn germ (337), and especially in pineapple leaves (99). A brei from these leaves converted tryptophane to neutral and acid auxins and also gave increased yields of auxin from tryptamine and from indolepyruvic acid. The preparation of Larsen formed an addition product with bisulfite, and that of Gordon and Nieva with dimedon as well. The bisulfite compound is, however, not specific, since indole forms a similar derivative (126). The acid auxin formed on oxidation of the aldehyde had a molecular weight close to that of indoleacetic acid (155). Both indole-3-acetaldehyde and naphthalene-1-acetaldehyde are converted to the corresponding acids by the juice of coleoptiles (158).

Next, the occurrence of indoleacetonitrile (III) has been established. This compound was isolated from cabbage in a yield of about 2 mg. per kilogram, crystallized, thoroughly characterized, and compared with a synthetic sample (124). Besides the cabbage and Brussels sprouts used in these experiments, broccoli contains a neutral auxin which is probably the nitrile (180), and chromatographic methods developed by Bennet-Clark and co-workers (17, 18) indicate its presence in potatoes, apple seeds, and rhizomes; it has been detected as well in young shoots of peach trees (*u*). A point of interest is that the nitrile is not destroyed on heating with 1 *N* acid at 100°C.—a character which has often been considered as *prima facie* evidence for the presence of auxin *a* (124).

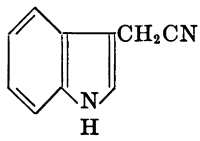
Whether the nitrile is really a true auxin is not certain. Although it is more active on *Avena* coleoptiles than is indoleacetic acid, it is converted to indoleacetic acid in good yield during the test, and the conversion has been proved by both biological and chemical means (294, 295, 281). It is almost totally inactive on pea stems (another standard test object) (21), and on corn coleoptiles and lupine hypocotyls it has only a low activity which is not proportional to concentration (294, 295). This behavior indicates that its activity is limited in these plants by the extent of its conversion to indoleacetic acid. Since the substance is quite stable in solution, the conversion must be enzymatic. Its activity in the agar-block test with *Avena* is lower than that of indoleacetic acid—a fact which may explain the apparent increase in yield of auxin (assayed by this test) which was long ago reported to occur on heating various plant materials with alkali.



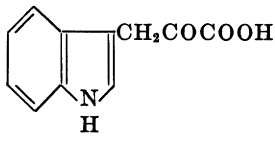
I



II



III



IV

Lastly, the presence of indolepyruvic acid (IV) has been shown in corn (var. Country Gentleman) by chromatographic methods (280, 281). Its chromatographic behavior, however, is such as to suggest strongly that it is identical with active substances observed by other workers in various plant

materials, including *Avena* coleoptiles themselves (286), wheat roots (177), and broad bean seedlings (17). Söding and Raadts' conclusion (275) that *Avena* contains, besides indoleacetic acid, another auxin of molecular weight about 200 could also be considered to support the presence of indolepyruvic acid (molecular weight, 203). Kramer and Went's value of 202 (above) agrees remarkably well. The value of about 360 sometimes found in fresh extracts has been ascribed to the possible occurrence of a dimer (enol ester) of indolepyruvic acid (281). The activity of indolepyruvic acid, though much lower than that of indoleacetic, appears to be exerted in all tests so far studied. The extent to which it is due to conversion to I is not known and would be difficult to assess because conversion takes place spontaneously in solution.

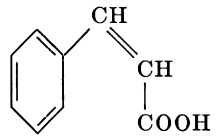
The corn variety Country Gentleman is probably exceptionally rich in IV, since another variety, Silver Bantam, yielded no evidence for it (315). In a third variety, Yamaki and Nakamura (337) found indoleacetaldehyde, II. The material was identified chromatographically and shown to be active (presumably by conversion to IAA) in the *Avena* test. However, in view of the ease with which pyruvic acid is decarboxylated, it seems not improbable that its indole derivative, IV, may provide a source for the aldehyde, II, which seems to occur widely in plants. Recently also, IV has been identified chromatographically in leaves of soybeans and tobacco (315a), particularly when these were grown under short-day conditions. The amounts of both I and IV seemed to be greatly reduced by long light periods.

IV. Synthetic Auxins

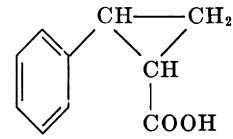
In recent years, the work on synthetic compounds having auxin activity has been less haphazard and more specifically directed towards the testing of theoretical ideas. It will be recalled (ref. 290, p. 19) that the earlier work demonstrated that growth-promoting activity involves two functions: *primary activity*, or the ability to cause cell enlargement when present in the cell, and *secondary activity*, or the ability to enter, to be stable in the cell, and to be transported from one cell to another. The requirements for primary activity were stated to be: (*1*) a ring system as nucleus, (*2*) a double bond in the ring, (*3*) a side chain containing a carboxyl (or an ester or amide readily convertible to a carboxyl) or certain other weakly acidic groups, (*4*) a distance of at least one carbon atom between this group and the ring, and (*5*) a particular spatial relationship between the acid group and the ring. Point (*4*) must now be abandoned, and much of the newer work bears on the last point. Some of it has already been reviewed in detail (291, 310).

A. The Relation between Structure and Activity

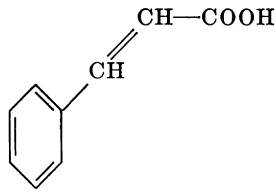
The effect of geometrical isomerism in the side chain is very clear. The activity of *cis*-cinnamic acid (V) and its derivatives, and the inactivity of the *trans*- isomer (VII), have been paralleled by the cases of tetralidene-1-acetic acid and naphthalene-1-acrylic acid (311), in both of which the *cis*-isomer is the only one with activity (119, 308). A further confirmation of this point is given by the 2-phenylcyclopropane-1-carboxylic acids, of which one form, shown indirectly to be the *cis*- isomer (VI) is active, the other (VIII) inactive (312).



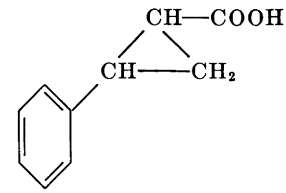
V



VI



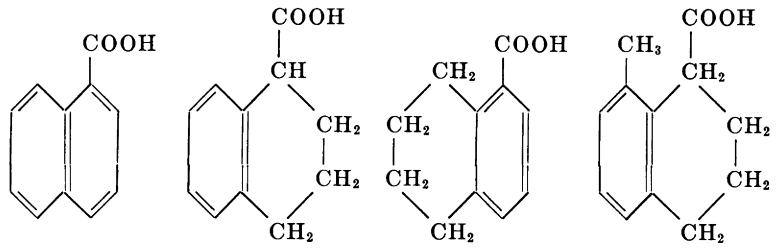
VII



VIII

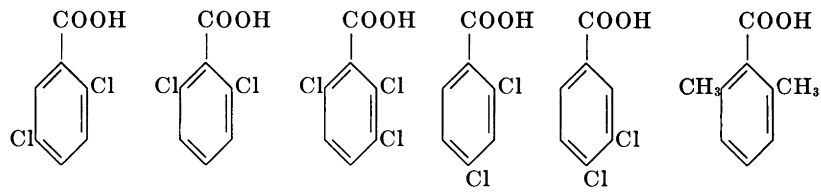
The association of activity with the *cis*- configuration was at first ascribed by Veldstra (307) to the necessity for the side chain to be as nearly as possible at right angles to the plane of the ring. More recently, this concept has been broadened to mean that the carboxyl is so situated that "on adsorption of the active molecule to a boundary (the non-polar part playing the most important role), this functional group will be situated as peripherally as possible" (310). Such an interpretation was applied to the nitrophenoxyacetic acids, in which only the *meta*-nitro form is active (308), and to the hydronaphthoic acids (313). α-Naphthoic acid, IX, which is the parent compound of these, has slight activity (which might be considered due to a spatial resemblance to phenylacetic acid) but its 1,2,3,4-tetrahydro derivative, X, is much more active. On the other hand, XI, which compares only to 2,3-dimethylbenzoic acid, is inactive. Also β-naphthoic acid and its reduced derivatives are inactive. All these effects were ascribed to the "puckering"of the reduced ring, which allows the carboxyl to leave the plane of the aromatic ring. However, introduction of a methyl group at position 8, which should much more strongly swing

the COOH out of the plane of the ring (XII), did not increase the activity at all. This rather critical test opposes the theory. So does the rather high activity of acenapthene-1-carboxylic acid, whose COOH would be expected to lie in the plane of the ring (137a). Other aspects of the theory have been discussed previously (291).



IX X XI XII

The most striking development in connection with all such theories of molecular orientation is the demonstration that certain substituted benzoic acids are active. The first reports of this, with 2-bromo-3-nitrobenzoic acid (338, 339), were with relatively unspecific biological tests, and could have been due to interaction with auxin in the green auxin-rich plants used. However, it is now clear that 2,5,- and 2,6,-dichloro-, and especially 2,3,6-trichlorobenzoic acids (XIII, XIV, and XV) have true auxin activity on etiolated, auxin-poor, test plants, (20, 210, 293). The corresponding aldehyde has weak activity (20), which is probably due to conversion to the acid, while several other substituted derivatives have weak activity (211). The 2,4- and 3,4-dichlorobenzoic acids (XVI and XVII) are inactive. Activity in this series can be quite high, that of 2,3,6-trichlorobenzoic acid being about twice that of indoleacetic acid in the pea test (293). The 2-chloro-, 2-bromo- and 2-nitro-benzoic acids also have distinct activity on *Avena*.

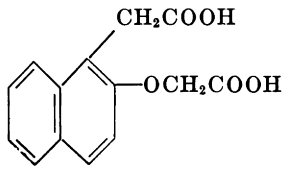


	XIII	XIV	XV	XVI	XVII	XVIII
Pisum:	—	—	200	0	0	0.2
Avena:	1.0	0.1	—	0	—	0.1[2]

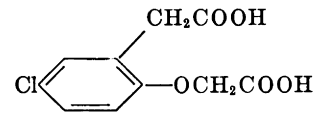
[2] See page 12.

Some of the data on pea curvature (293) and *Avena* straight growth (211) are given herewith, activities being expressed as per cent of that of indoleacetic acid. The relative activity of XVIII is much higher in another test.

Much of Veldstra's earlier reasoning was based on the idea that the auxin functions in a surface membrane, where the "planarity" of the molecule would be of importance. However, this view has been largely abandoned (308). Another of his suggestions was that activity requires a certain balance between lipophilic and hydrophilic parts of the molecule (309); thus, increased activity of phenoxyacetic acid on chlorine-substitution was ascribed to increased lipophily, but the decrease in activity when more than three chlorine atoms were introduced was held to indicate a degree of lipophily above the "optimum." Since the optimum remains undefined, and is probably different for each molecular type, this view is very flexible. Recently it has been again invoked to explain the fact that compounds with two COOH groups are always inactive or nearly so. Thus 2-carboxymethoxy-naphthalene-1-acetic acid, XIX, contains the side chains of two highly active compounds, yet has very low activity (137b). Similarly the compound XX, which has one COOH added to the highly active "MCPA," is quite inactive (137b). Numerous aryloxy-succinic acids are also inactive (137d). It is true that the extra COOH would give more hydrophily, but one would expect that 2,4,5-trichlorination would redress the balance somewhat; it does not. The effect of a second acid group therefore probably has another explanation.



XIX



XX

Optical isomerism, like geometrical isomerism, has a great influence on activity. The first case of this, with the (+) and (−) forms of α-(3-indole)-propionic acid (146), was ascribed to an effect of the optical activity on transport of the auxin in the plant, since the two enantiomorphs were equally active when the coleoptile sections were immersed in the test solution. However, the case of α-(dichlorophenoxy)-propionic acid is different; here the (+) form is twice as active as the racemic, which means the (−) form is virtually inactive, and this holds in the pea test, carried out in solution, where transport is not a critical factor (291). Similar considerations hold for the enantiomorphs of *X* and of the corresponding 1,4-dihydro compound (312, 203, 204), and of α-allyl-phenylacetic acid (312).

Clear differences exist in the optical and biological activities of several other compounds of the α-aryloxy propionic type (2, 273, 5, 321).

Recently an extensive study of this whole subject, undertaken by Fredga and collaborators in Sweden, has been reported in detail (2, 3, 197; also 84, 85). In this work, some 19 pairs of phenoxy and naphthoxy compounds have been resolved into their enantiomorphs and tested, principally against growth of flax roots. In most cases the optical activity has been sterically connected to that of glyceraldehyde. The main conclusion is that the D-forms of all the compounds are more active than the L-. The ratio of

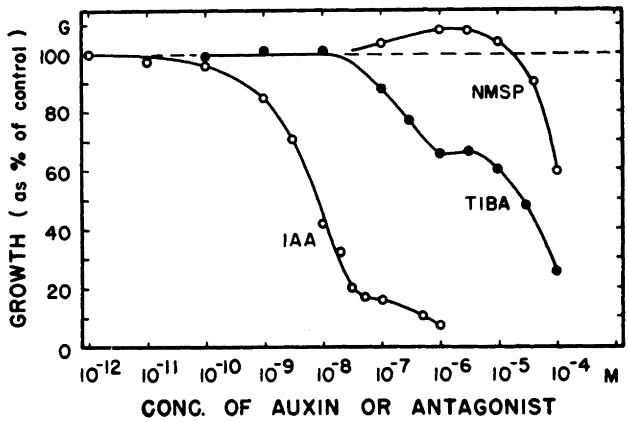


FIG. 1. Effects of three compounds on root elongation of flax seedlings. Concentrations molar and on logarithmic scale. IAA, indoleacetic acid, inhibition only (auxin action). N.M.S.P., α(1-napthylmethyl-sulfide)-propionic acid, promotion at low concentrations, inhibition at high (auxin antagonist). TIBA; 2,3,5-triiodobenzoic acid, shape of curve ascribed to synergism with auxin. From Åberg, 1953 (2).

biological activity, D:L, varies from 2 to over 1000; even when the ratio is high, however, the activity of the L-form seems not to be due to optical impurity, but to persist through very careful repeated fractionation (197). The highest activities found were for D-α-(2-methyl,4-chloro-phenoxy)-propionic acid and D-α-(2-naphthoxy)-propionic acid (XXI, p. 15). In a number of instances the L-forms acted as auxin antagonists, *i.e.*, they restored the growth of roots which were inhibited by 2,4-D or other auxins (2, 3). These antagonists included especially the 2-naphthoxy, 2-methyl-4-chlorophenoxy, and the trichlorophenoxy compounds. In contrast, the L-forms of phenoxy and 2,4-dichlorophenoxy compounds were inactive both as auxins and as antagonists. The antagonists generally gave root growth promotion at subinhibiting concentrations (*cf.* Fig. 1). Introduction of an isopropyl or a *n*-butyl group at the asymmetric carbon atom

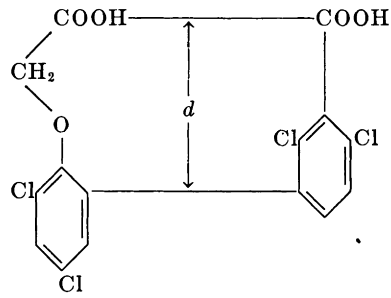
greatly reduces auxin activity, but in many cases leaves the substances with a marked auxin-antagonistic action. All these facts point strongly toward the existence of multiple points of attachment between the auxin and its substrate (3; see below).

Substitution in the ring has, of course, a large effect on activity, and the above work adds many instances of this. Other compounds have been made and tested by many workers (see 310, 137a–f). With certain exceptions (see below), their activity confirms or extends the principles already discussed (290, 291), but one new theory has been suggested. Because 2,6-dichloro- and 2,4,6-trichloro-phenoxyacetic acids are much less active than the other di- and tri-substituted acids, Muir and Hansch (209–212) have proposed that combination takes place with the substrate at the position *ortho* to the side chain. Thus compounds with both *ortho* positions substituted would be unable to combine. The postulated auxin-substrate complexes involve formation of a ring with (*1*) the carboxyl group, (*2*) cysteine or a similar sulfur-containing residue in the presumed substrate, and (*3*) the *ortho* carbon of the ring; the suggested rings contain up to 11 atoms, which should make their formation somewhat difficult.

The evidence for this proposal has been examined critically, and it has been pointed out that actually 2,6-dichlorophenoxyacetic acid is far from inactive, having about 4% of the activity of indoleacetic acid in the pea test (293). Also 2,6-dimethylphenoxyacetic acid is active (322). Even 2,6-dimethylbenzoic acid, XVIII, has real, though weak, activity. Furthermore, when tested on slit coleoptiles by the curvature method, its relative activity is some 40 times greater than that shown for straight growth (*u*). Thus, both phenoxy and benzoic acid derivatives can be active when both groups *ortho* to the side chain are occupied. The activity of 2,6-dichlorobenzoic acid (XIV above) was ascribed to liberation of one of the Cl atoms in the reaction (115), but this explanation could hardly apply to methyl groups. Besides, it was shown that 2,4-dichlorobenzoic acid, which is inactive, liberates almost as much chlorine as the 2,6 acid. Equally difficult for this theory is the fact that 3,5-dichlorophenoxyacetic acid, which has *both ortho* positions free, and therefore should have full activity, is in fact totally inactive (293; *cf.* also 322). The low activity of the 2,6-disubstituted phenoxy acids may alternatively be due to steric blocking of the free rotation about the ring-oxygen bond. While 2,4,6-trichloro- and 2,4,6-tribromophenoxyacetic acids admittedly have extremely low activity, yet the 2,4-dichloro-6-fluoro and 2,4-dibromo-6-fluoro acids are reported highly active (321). These facts clearly point away from any absolute requirement for the unsubstituted *ortho* position as far as chemical reactivity is concerned.

The increase of activity by introduction of methyl groups into the ring

is important in this connection, for it has been pointed out (293) that halogen atoms, which generally confer auxin activity, are known to organic chemists as the least effective substituents in "deactivating" the benzene ring, whereas methyl groups are the least effective in "activating" it. Thus, these two types come nearest to merely occupying a position on the ring. If the ring has to combine with some substrate it was suggested that it may do so at the 2, 4, or 6 positions, and hence occupation of any one of these would promote activity at the others. This, of course, would explain the activity of 2,4- or 2,6-di-substituted chlorophenoxy acids and the inactivity of the 3,5 acid mentioned above. It also would explain the high activity of the 2,4,5-trichloro- and the virtual inactivity of the 2,4,6-trichlorophenoxyacetic acids. In general, the only groups other than halogens which confer activity are CH_3 groups. Although Muir and Hansch (212) draw the opposite conclusion, their data show this effect very clearly; the activity of phenoxyacetic acid in their test is increased some 7 times by *ortho* methylation and 2 times by *meta* or *para* methylation, 17 times by 2,4-dimethyl substitution, and 7 times by 2,5-dimethyl substitution. *Para* methyl substitution also imparts auxin activity against roots (4). The great activity as a weed killer of 2-methyl-4-chlorophenoxyacetic acid, "MCPA," is a familiar example of the same principle. The difference between phenoxy acids and benzoic acids falls into line with this view also. In the benzoic acids 2,6 di-substitution *favors* activity, which means the 3- or 4-position is probably the one most favorable for combination with the substrate (293); in the phenoxy series 2,6 di-substitution, although it does not prevent activity, does reduce it, *i.e.*, the 2-position is favored for combination with the substrate. The distance *d* between the carboxyl and the presumed point of attachment would be essentially the same in each case:



The oxygen atom in the side chain is of course not simply an inert "spacer." Its place can be taken by sulfur in some phenylthioacetic acids (291). In other compounds both —S— and —SO—, but not —SO_2—, could take its place without serious loss in activity (137, 138). However,

—NH—, which has properties very different from —O— or —S—, will apparently not substitute for it, since the chlorinated derivatives of γ-phenylaminocrotonic acid are quite inactive (137f), while the corresponding derivatives of γ-phenoxycrotonic acid are active (137e). In tests on roots, substitution of —O— by —S— or —NH— lowers, but does not abolish, the activity (2, 3). Many thio-acids cause callus formation (138).

The influence of side-chain length, per se, is, however, somewhat puzzling. Activity usually alternates with increasing numbers of CH_2 groups in the side chain; indoleacetic and indolebutyric acids have long been known to have much higher activity than indole-carboxylic, -propionic, or -valeric acids, etc. In the chlorinated phenoxy series the same is true, the acetic, butyric, and even caproic acids being active, the propionic and valeric inactive. Fawcett *et al.* (81) have related the inactivity of the unsubstituted phenoxy acids of uneven numbers of carbon atoms in the side chain to the ease with which such acids are metabolized down to phenol. The acids with even numbers of carbons (acetic, *n*-butyric, *n*-caproic, etc.) were not metabolized to phenol to any appreciable extent, and presumably were oxidized only to the acetic level, there exerting their auxin action. 1-substituted propionic acids, like XXI, in which such oxidation is blocked, are generally highly active. However, in the unchlorinated phenoxy series the order is reversed, the acetic and butyric acids being inactive, the propionic and valeric active (321). At the other end of the molecule, long chains introduced in the *para* or 4-position oppose auxin activity, and with increasing chain length the antiauxin activity on roots increases steadily (4). Branched chains and alkoxy groups act similarly.

It is evident that the combination of the auxin with its substrate has real "chemical" specificity and cannot be wholly explained in simple physicochemical terms of absorption on to membranes, etc. It is also evident that the explanations so far advanced for the nature of the combination are in general too simple, and that a full explanation must involve both electrical and spatial considerations.

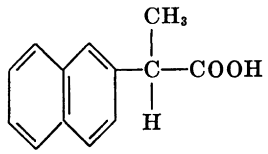
B. Phenomena of Antagonism and Synergism

As long ago as 1942 it was shown (271) that γ-phenylbutyric acid, itself virtually inactive, could oppose the action of indoleacetic acid in the *Avena* curvature test. Since that time, a number of compounds having structures similar to that of the auxins have been found to promote or reduce the action of auxin. Promotion is termed synergism, and inhibition is termed antagonism. Weak auxins, in concentrations too low to show growth promotion by themselves, greatly increase curvature in the pea test (329). So do many compounds which have all but one of the structural requirements for auxin activity (329). At first it was thought that these compounds had to

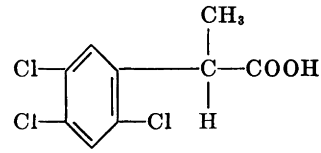
be applied before the auxin, to "prepare" the plant, but it seems now that synergism is as good or better when both compounds are applied together (293). 2,3,5-Triiodobenzoic acid (TIBA) is particularly potent, causing very large synergism in the pea test, smaller effects in other tests, and clear antagonism in still others (89, 297, 21). Its role in promoting flower formation in some plants was discussed in Volume I (see also Section VI,C); its production of tumors may be due to synergism (241a).

It is essential to establish that the antagonism is really competitive with auxin, for in some instances "antagonism" has been claimed, which was only nonspecific inhibition, such as is produced by enzyme inhibitors. The inhibition by 2,4-dichloroanisole has been ascribed to this latter type (12, but see 192). In the cases of 2,6-dichlorophenoxyacetic acid, which is virtually inactive on *Avena* (191), and *trans*-cinnamic acid, which is inactive in the pea test (223), the interaction between indoleacetic acid and the antagonist seems, however, to be well supported.

Antagonism is particularly clear in roots, for auxins produce only very slight growth promotion therein (sometimes none at all) and only at a narrow range of low concentrations; all higher concentrations inhibit growth. Hence an auxin antagonist is readily detected by its ability to restore growth in roots inhibited by auxin (48, 49). Such effects are exerted not only by compounds of the same general structure as auxins, but very strongly by the optical isomers of auxins discussed above. In the 2-naphthyl and phenyl derivatives the L(−) forms are active as antagonists (or antiauxins) whereas in the phenoxy series the L(−) forms are simply without any activity, positive or negative (3). In the straight growth of *Avena* sections, similar behavior appears, the (+) isomer of α-(2,4,5-trichlorophenoxy)-propionic acid, XXII, being strongly antagonized by its (−) isomer, to give only one example (321).



XXI



XXII

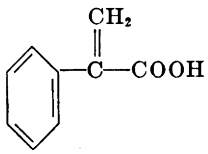
Phenoxyacetic acid is inactive, but the introduction of a large alkyl group in the *para* position makes for strong auxin antagonism (4). A methyl group in the same position makes the molecule an auxin, suggesting that the *para* position plays an important part in the growth reaction.

These phenomena naturally indicate that the auxin and its antagonist compete for the same locus. Skoog and co-workers in 1942 suggested that the auxin, acting as a kind of coenzyme, had to combine with both

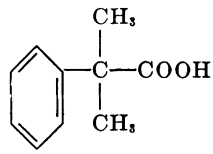
enzyme and substrate and pointed out that excess of auxin would mean that different molecules of auxin could combine separately with the enzyme and with the substrate. Hence these two materials would not be brought together, and inhibition would result at high auxin concentrations. Thimann (291) envisaged a combination similar to that between succinic dehydrogenase and a protective agent like maleic acid and pointed out that growth promotion or inhibition would then depend on the relative affinities (for the enzyme) of the auxin and its natural antagonist in the plant. Both views require combination at not less than two points on the auxin molecule (see Section X,A), but Wain (321) concluded from the importance of optical activity that three points of attachment must be involved, namely, the ring, the carboxyl, and the H atom of the side chain. Supporting this is the fact that some compounds in which the side chain does not contain a free H atom, like α,α-dimethyl toluic acid, XXIV, and α-(2-naphthoxy)-isobutyric acid, XXV (321), are inactive. Others, in which a methylene group is substituted for 2 H atoms, however, as in XXIII, have definite, though low, activity, and this makes the suggested need for a free H atom less attractive.

On the other hand, Åberg (2, 3) has laid stress on the varying effects, in different aromatic series, of the change in configuration, *i.e.*, the fact that some enantiomorphs antagonize and some do not, etc. For these reasons, he believes that there are probably numerous points of attachment and contact between the auxin and its receptor molecules. Burström (53) in a valuable discussion, concludes that in roots antagonism must be exerted not only at the site of growth action, but also at the sites of uptake or transport; the very numerous data cannot be explained on a simpler basis.

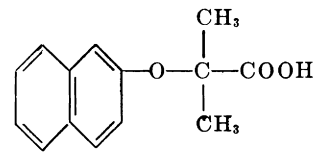
All in all, it seems that the study of the relation between structure and activity has come to a point where some definite lead from another direction is called for. Evidence of such a type, as to the systems with which the auxin combines, will be discussed in Section X.



XXIII



XXIV



XXV

V. The Transport of Auxin

Two types of auxin transport have long been recognized—an apex-to-base polar transport, which is linked to metabolism, and an "upward" transport, occurring mainly in the transpiration stream. The former moves indoleacetic acid at a rate of about 1 cm. per hour, and synthetic

auxins more slowly; the latter may involve rates enormously higher and essentially independent of the nature of the auxin. Any solute which penetrates to the xylem will, of course, be carried upward in the transpiration stream.

In addition to these two types, there is now evidence that nonpolar transport may occur in other ways. Much of this evidence, but not all, has been obtained with 2,4-D, whose transport by the polar system is very slight. From the observation that darkened plants were unable to transport 2,4-D (244), it was suggested that light may serve to permit this transport through the production of photosynthetic products (251). To verify this concept, the leaves of darkened plants to which 2,4-D had been applied were treated with sugar; auxin transport took place at once, even without light. A wide variety of sugars can invoke auxin transport in this way, and it is indicated that auxins are transported by a translocation system which normally carries sugars (179, 324). Indeed, the application of auxins can even bring about an increase in carbohydrate translocation (314). Borate, which accelerates the rate of sugar transport, also influences the rate of movement of 2,4-D (202).

It has been calculated that the rate of carbohydrate translocation is in the vicinity of 80 cm. per hour (314, and many earlier researches) and the nonpolar transport of auxin has been estimated to be in the same range (74). The values were arrived at by applying 2,4-D at different time intervals to each of two opposite bean leaves. Application to one leaf was made at the leaf base and application to the other leaf was made at a point 4 cm. distal to the base. If the two applications were made simultaneously, the plant would curve first away from the leaf with the basal application. If the distal application were made sufficiently earlier than the basal one, the auxin from the two applications would be transported to the stem simultaneously and no curvature would result. Such a lack of curvature was obtained when a 5-minute interval was permitted to elapse between the two applications. It is deduced that 5 minutes was the time required for 4 cm. of transport; hence the rate of transport is approximately 48 cm. per hour. Repetitions of this experiment gave results varying from 10 to 100 cm. per hour.

Several pieces of evidence show that while the movement of physiological amounts of auxin in stems is principally basipetal in its polarity, as in the *Avena* coleoptile, acropetal transport can occur. *Phaseolus* hypocotyls transport a fraction of their natural auxin acropetally, though the movement of applied auxin is polar (132). Transport through the apical "hook" may be very weak. There is also evidence of acropetal movement in the developing female flower of corn (43). Furthermore, there appear to be certain internal conditions under which a strict polarity may be modi-

fied. With the advent of flower buds, the polar transport system in *Coleus* stems is markedly weakened (163). Consequently, while vegetative stem tips show strict basipetal polarity, flowering stems do not. In fact, the phototropic stimulus has been shown to move upward in flowering stems, whereas it can not move upward in vegetative stems. Again, the strict polarity of auxin movement in *Coleus* stems exists only in young tissue. Stem sections taken at increasing distances from the tip show weaker and weaker polar transport characteristics. This weakening gradient is illustrated in Fig. 2, which shows that stem sections taken from vegetative apices

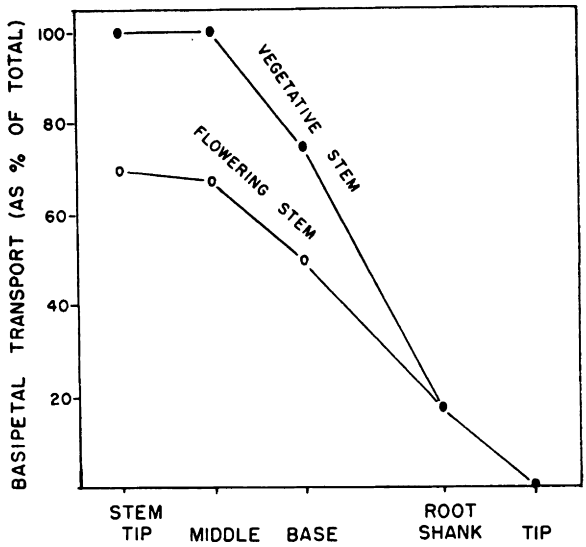


FIG. 2. The polarity gradient in stem and root sections of *Coleus* plants. From Leopold and Guernsey, 1953 (163).

show only basipetal auxin transport, whereas root tip sections show only acropetal transport. Stem and root sections taken from intermediate positions show a declining gradient from one extreme to another.

There is also some evidence that environmental factors may modify the polar transport of auxin. High humidities retard the polar translocation of the morphogenic effects of applied auxin in cuttings, and therefore presumably retard transport of the auxin itself (7). Transpiration also exerts an influence on lateral transport of auxins in roots (227).

There is one synthetic compound which can bring about the loss of strict polarity of auxin transport in a manner very suggestive of the substance formed in *Coleus* flowers. Niedergang and Skoog (215) have shown that the application of 2,3,5 triiodobenzoic acid (TIBA) to tobacco stem

sections results in a loss of polarity such that callus tissues, which would be normally formed only at the base of the stem, are formed at random over the entire stem section. The same compound has been shown to prevent the transport of the phototropic stimulus down the plant, and to prevent the inhibition of bud growth by auxins applied higher on the stem (151). The interference of flowering with polarity in *Coleus*, discussed above, may be due to some kind of natural inhibitor, similar to TIBA, since a diffusible substance or substances from the flower buds can bring about the loss of strict polarity (163).

From studies on the diffusible auxin from the roots of lentils, Pilet (227, 228) suggested that there are actual currents of auxin transport. He conceives of a cyclic transport in the roots, with auxin first moving away from the tip, then being transported laterally some 6 to 16 mm. from the root tip, and returning to the tip by a basipetal movement. He also presents some evidence that such a cyclic flow of auxin is altered by the age of the root, by the presence of leaves on the stem, and of course by various tropistic stimuli.

The picture of the total transport of auxin thus appears to be one of transport in several directions occurring naturally and simultaneously, and by several independent systems. Whether it is correct to conceive of these as cyclic currents of auxins as visualized by Pilet is far from clear. Auxin carried upwards in stems in the transpiration stream is continually diffusing into the living tissue and being retransported downwards therein (269). This constitutes a kind of cycle, though only for auxin applied externally. Most indigenous auxin moves in a polar manner down the aerial parts, and transport toward the plant apex occurs naturally only in limited places and circumstances.

In an effort to arrive at a physiological explanation of the polar transport of auxin, a great deal of work has been carried out on bioelectric potentials in the *Avena* coleoptile. The existence of bioelectric gradients was recognized as early as 1907 by Bose, and the suggestion that such a gradient may be involved in the polar transport of auxins was made by Went in 1928, the electrical field being considered to move the auxin anion by electrophoresis. This concept is somewhat clouded by the more recent finding (259) that an electrical field imposed on a coleoptile results in an apparent movement of the auxin toward the negative charge, whereas one would expect an auxin anion to move toward the positive charge. However, the base of the coleoptile is positive to the apex, which means that the natural transport of auxin does take place toward the positive pole. Schrank has brought out strong evidence that stimuli which cause tropistic curvatures (light, gravity, and mechanical contact) bring about changes in the bioelectric gradient, which may be able to account for the lateral transport of

auxins directly, causing the tropistic response. These bioelectric changes precede the redistribution of auxin (258), and in the case of stimulation by gravity, they persist for about as long a time interval as does the geotropic growth response (323). It is important to note that bioelectric changes alone will not cause curvature, a supply of auxin being essential (260). Limitations of the theory at present appear to be: (*1*) the bioelectric gradient in the coleoptile shows a peak in negative potential 5 to 8 mm. below the coleoptile tip, and no reflection of this has been found in auxin transport; and (*2*) imposing artificial electrical fields upon a coleoptile results in auxin movement opposite to that which would be predicted on the basis of simple electrophoretic movement (259).

VI. The Role of Auxin in Physiological Inhibitions

A. Apical Dominance and the Inhibition of Buds[3]

It has been recognized for many years that auxin produced at the stem apex inhibits the growth of lateral buds—a phenomenon termed apical dominance. This is the classical case of correlative inhibition due to auxin. The concept that auxin brings about apical dominance by inhibiting bud development has been challenged in several ways in recent years.

In the first place, a number of instances have been described where growth-promoting rather than growth-inhibiting influences seem to control growth of lateral buds. In woody plants whose branches are growing with unusual vigor, lateral buds which normally would be inhibited may grow out (58, 62a). The diffusible auxin content of these branches is higher than that of controls (62a). In *Cicer arietinum*, certain laterals develop in the middle of the stem, where the diffusible auxin content should be near its peak (62, 62a). In these cases, bud development clearly parallels general growth vigor. Another type is that of seedlings of *Impatiens* and others, where the buds in the cotyledonary axils are usually inhibited by the cotyledons, or probably by auxin coming therefrom; the relationship is sometimes reversed and the bud becomes promoted by the cotyledon in whose axil it grows. In *Bidens pilosus* the cotyledon promotes its axillary bud at first, and then inhibits it (57). This may be explained by the observation that in daylight, where the cotyledons yield more auxin than in artificial light, the inhibition comes on sooner and is more complete. The inhibition is therefore probably still due to auxin, while the promotion is due to nutritive factors (59). Champagnat (60) has also shown that the inhibiting action of the cotyledons can be suppressed by the young leaves in the terminal bud, but not by the apical meristem. In general, it is the

[3] This section is based in part upon a review presented to the Section on Physiology at the International Botanical Congress (294a).

young leaves which inhibit lateral bud development, but their action appears here to be exerted on the auxin *production* from the cotyledons.

In the second place, an inhibition of a different type has come to light. In *Ginkgo biloba* the growing point does not inhibit the lateral buds from developing, but merely from elongating (105, 106). The resulting "short shoots" produce auxin for a very brief time only and exert no appreciable inhibiting influence on other buds. If the terminal bud is removed early in the season, from one to three laterals may develop into long shoots with many leaves and with greatly prolonged auxin production. Substitution of a little naphthaleneacetic acid for the terminal bud maintains the laterals in the "short-shoot" form. The transition from short to long shoots is thus clearly controlled by auxin. Yet the short and long shoots differ from one another in their auxin production; the long shoot forms auxin throughout its elongation. A characteristic of these shoots is that the auxin is not formed mainly in the young leaves, or even in the apex, but all through the stem. The leaves may contribute a precursor, but the stem, whether still elongating or having just ceased to do so, is the "auxin-forming center." This type of auxin production is probably general in woody plants.

Comparison between the auxin content of organs and their ability to inhibit buds has been made in tissue cultures of chicory (*Cichorium intybus*). Camus (55) showed that developing buds, whether arising spontaneously or grafted in from another culture, inhibited the growth of other buds in the tissue, and that the inhibition is exerted polarly in the direction shoot–root. He then determined the auxin content by extraction with ether for 6 hours. The basal end yielded more auxin than the apical end for the first few days, but by the ninth day, when the buds were beginning to develop and hence the auxin content beginning to increase, the difference became very small. The buds at the basal end, nevertheless, remained only one-half to one-third as long as those at the apical end. The auxin level was thus qualitatively correlated with bud development; nevertheless, because the rate of bud growth was not inversely proportional to the extracted auxin found, Camus concluded that auxin "plays no role" in the inhibition. Such a conclusion is too drastic, for three reasons: (*1*) although the auxin differences were admittedly small, the bud inhibition was also only partial; (*2*) there is no assurance that a 6-hour ether extraction measures the auxin available to the bud-forming tissue; and (*3*) in nearly all auxin actions, the presence of other substances in the tissue can greatly alter the effectiveness of the auxin action (Section VI,C below).

In experiments on growth, a close correlation with auxin content as determined by extraction has always been hard to establish. In addition to the older work, von Abrams recently (6) has found in dwarf and normal

peas no parallelism between the growth type and either the production or inactivation of auxin. Yet one knows that auxin does control growth rapidly and precisely. The apparent deduction is, then, that the inhibition is due to auxin, as has been thoroughly established with many different plants, but that it can be enhanced or lessened by other factors.

These factors may be numerous. The influence of general growth vigor was mentioned above. Probably more important—certainly more concrete—are nutritional factors. High phosphate nutrition will partly overcome the inhibition by auxin. Added adenine specifically promotes the formation of buds in tobacco callus (272, 276), and other purines in the presence of adenine have similar effects; deoxyribose is also effective. Probably, therefore, bud differentiation depends on the synthesis of nucleic acids. In line with this conclusion, Silberger and Skoog (267) observed that concentrations of IAA too low to cause appreciable increase in growth cause a 40% to 50% increase in both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) within 7 days. There are other substances effective in promoting the growth and development of buds. Among synthetic substances, maleic hydrazide breaks apical dominance sharply (213, 14), as does triiodobenzoic acid (151). Eosin, which destroys auxin in light, causes rapid development of lateral buds in *Perilla* (183, 26). Some of these synthetic materials may well be similar in their action to natural antagonists of auxin or auxin-destroying systems. The many naturally occurring materials which lower auxin effectiveness are taken up in Section VI,C below.

Another fact which has led several workers to doubt the role of auxin is that inhibition is occasionally exerted in an "upward" or acropetal direction. To the older observations on this score may be added three new ones. Removal of lateral buds in *Coleus* stimulates elongation of the terminal bud and growth of its young leaves (133). Removal of adult leaves of lilac accelerates the development of lateral buds higher up on the stem (61). Removal of lateral buds of *Cercidiphyllum* enables the apical shoot, which would otherwise have regularly abscised, to remain on and to continue to grow (303). But in recent years the strictness of polarity of auxin transport has been shown to be subject to modification. The data in Section V show that the polarity may be modified by both internal and external factors. It follows that when an inhibition is exerted on organs situated morphologically "upward," this is not necessarily evidence that the inhibition is not mediated by auxin. Furthermore, acropetal inhibitions are in the majority of cases less marked than basipetal ones, so that only a small acropetal auxin transport would be needed.

It is still possible that auxin exerts inhibition indirectly, through being converted to an inhibitor in the basal tissue of the inhibited bud or other

receptor organ. Several pieces of evidence point, though not conclusively, in this direction. Dormant potatoes and dormant ash buds, on extraction, yield a growth inhibitor, while later in the season when dormancy is over the inhibitor seems to disappear (121; see Section VI,C). Extracts from dormant maple buds inhibit carrot tissue cultures, and again the inhibition disappears with onset of the growing season (278). Chromatograms from extracts of *Vicia Faba* showed a strong inhibitor of coleoptile growth to be present in lateral inhibited buds and much less in the growing apex (139). Also in apple buds and leaves, chromatograms show that the amount of inhibitor (as measured by growth inhibition of *Avena* coleoptiles) roughly parallels the amount of auxin. Both materials are present in largest amount in the tip and are almost absent from mature leaves (114). If these data are taken at their face value, it could be deduced that many tissues form an inhibitor *from* auxin or under the influence of auxin. Further, it is possible that this substance inhibits a process leading to auxin *formation.*

B. Inhibition of Root Growth

The role of auxins in root inhibition has been discussed in Section IV, and little need be added here. The relation between inhibition by externally applied auxin and the mutual inhibitions exerted within the root has never been thoroughly explored. In the first place, a clear inhibition exerted by the main meristem and axis on the development of lateral roots has long been known. Some material has now been extracted from pea root tips which is apparently responsible for the inhibition by the tip of root growth and lateral root formation (129). The material is clearly not an auxin. In the second place, root decapitation almost always accelerates lateral root growth. Street and Roberts (283) have now shown that in tomato roots this is to some extent mutual, *i.e.*, that laterals inhibit the growth of the main axis. Red light inhibits growth of laterals (in pea roots), and, perhaps as a result, this too stimulates elongation of the main axis (304). Very low intensities of white light have a similar effect on tomato roots (282). In a comparable way, the nodules of legumes inhibit one another's development (220); the main root tip also inhibits the development of nodules. It is the meristem of the nodule, and not the bacterial tissue, which is effective. The inhibitors of nodule formation seem also to be secreted by the root into the external medium. In none of these cases does the inhibitor really behave like an auxin.

Inhibitions of root growth by different substances have very different morphological and anatomical bases. Torrey (305) finds that indoleacetic acid and enzyme poisons like iodoacetate or dinitrophenol all inhibit elongation, but the auxin accelerates at least one physiological process, and indeed

one which it also accelerates in shoots, namely, the differentiation of xylem, whereas the enzyme poisons, by contrast, appear to inhibit much more generally. The action of auxin in promoting xylem differentiation is particularly marked in tissue cultures (55) and in wounded stems (132). Iodoacetate and other inhibitors are certainly not true auxin antagonists.

The geotropic responses of roots can be altered under conditions where elongation itself is not much affected. This results from treatment with some synthetic substances, such as the α- and β-naphthylphthalamic acids (199), 2,4,6-trichlorophenoxyacetic acid (46), or indoleisobutyric acid (255). In consequence, the phenomenon has been used (255) in the analysis of the geotropic reaction. It seems possible that these substances specifically affect auxin transport, just as TIBA (see above) does in stems. In any hormonal system, effects exerted on the movement or circulation of the hormone must be distinguished from those exerted on its production or action, though the distinction is not easy to make.

C. Agents Which Accentuate Auxin Action

For many years it has been known that various compounds which were not themselves auxins could increase the effectiveness of an auxin application. Sugars are of course necessary for the growth of many isolated plant parts and in their presence the effect of auxin may be greatly enhanced. Sugar phosphates cannot be substituted (299), though some organic acids are moderately effective.

Unsaturated lactones are a class of compounds with this synergistic character. Detailed studies of the effects of coumarin and protoanemonin have shown that these lactones can synergistically increase growth in the presence of auxins by some 20% to 60% (298). Some lactones, particularly coumarin and scopoletin, are known to be of common occurrence in plants, and the interesting possibility exists that this property may permit them to alter growth in the natural condition. At higher concentrations lactones act as growth inhibitors, and at least in one case their naturally occurring concentrations may be high enough to cause such an inhibition (97). The inhibition of the growth of stems and coleoptiles is reversed by BAL (2,3 dimercapto-1-propanol), suggesting that the effect of these compounds is through an attraction for sulfhydryl groups (298); such a characteristic of lactones was originally suggested by Cavallito and Haskell (56). Another effect of some unsaturated lactones relates to the enzymatic destruction of auxin. Scopoletin, for example, appears to spare auxin destruction (8). Another lactone, umbelliferone, stimulates the enzymatic oxidation of auxins (9); this, however, would result in a growth inhibition.

A synergistic compound which may be of even more general occurrence in plants is chelidonic acid. This compound has slightly less synergistic

activity than coumarin, but its occurrence in large amounts in nearly two-thirds of the many plants which have been tested suggests that it may be important in influencing growth (170).

Synergism depends strongly on the growth process involved. Thus, indole was found to increase the effectiveness of a given auxin concentration when incorporated with root-inducing auxin treatments (237, 238). However, in the growth of pea stem and coleoptile sections its action was only inhibitory (*u*). Triiodobenzoic acid shows powerful synergism in the split pea stem test (297, 293) but only moderate synergism in the straight growth of *Avena* (5) and small synergism over a limited concentration range, or none at all, in the agar-block *Avena* test (297, 316). A number of compounds inactive or weakly active as auxins show synergism in the split pea stem test (see Section IV). These include indoleacetonitrile (221), which, as mentioned in Section III, is almost inactive in *this* test though active in other systems.

A variety of other compounds of physiological interest have been shown to promote growth in one or another test system in the presence of auxin; among these are several vitamins (256, 123), thiourea (225), giberellin (138), and some antibiotics (*u*). Mention must also be made of coconut milk and similar endosperm preparations; these greatly promote growth of young embryos in culture (239) and are used widely in tissue culture media. Their active constituents have not yet been identified.

Since synergistic effects are obtained with such a variety of compounds, it seems rather difficult to attempt to interpret them all as having effects on growth through the same specific system. It is entirely clear, however, that a wide variety of naturally occurring substances may be able to influence auxin effects. It follows that simple measurements of the quantity of auxins in a plant tissue do not necessarily describe its potential ability to respond to auxin.

D. Auxin Inhibitors

A great variety of compounds can inhibit the action of auxin on growth. In many cases, the identity of the inhibiting compounds has not been established, and in some cases it is not clear whether the inhibition is exerted on those growth reactions which are controlled by auxin or on some other part of metabolism. The widespread occurrence and diverse nature of these compounds is evident from the review of Evenari (80), and their importance in processes of growth and especially of dormancy is gradually becoming evident.

In ecological terms, growth inhibitors may play a limited role in the control of the distribution of plants. For example, toxic materials can be extracted from leaves of a variety of desert plants, and as the leaves ac-

cumulate under the parent plant and their toxic consituents are leached into the soil they may prevent the establishment of competing plants in the immediate area (19). One of these compounds is 3-acetyl-6-methoxybenzaldehyde (101). Several other toxic substances which have been separated from plants may be of similar ecological significance, including juglone (73), absinthin (27), and *trans*-cinnamic acid (33).

Investigations of dormancy have revealed that in several instances the dormancy of buds and tubers can be correlated with the presence of inhibitors (120–122). These inhibitors may be assayed by their capacity to

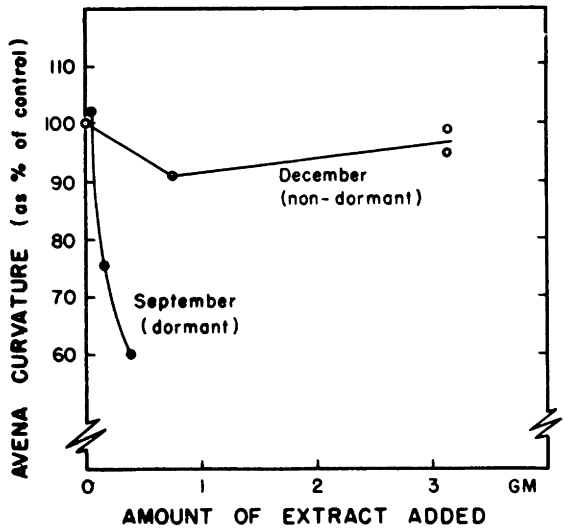


FIG. 3. Effect of extract of *Fraxinus* buds (made at two different seasons) on the curvature caused by a constant amount of IAA. From Hemberg, 1949 (121).

inhibit the auxin stimulation of growth in the *Avena* test. As dormancy is broken, the inhibitor content of the tissue falls drastically, as shown for ash buds in Figure 3. The similar inhibitors in dormant apple seeds also disappear as dormancy is broken, in this case by cold treatment (187, 188). However, because no absolute correlation was found between the breaking of dormancy and the disappearance of inhibitor, Luckwill could not conclude that the inhibitors were directly responsible for the dormant condition. Treatment of potato tubers with chemical agents which break dormancy results in a similar disappearance of inhibitor (122). Hemberg has pointed out that the effectiveness of the inhibitors may be lowered naturally by the increase in glutathione content which occurs in potatoes with the termination of dormancy. Although he did not specifically propose that these dormancy-inducing inhibitors may be sulfhydryl reagents, the fact

that glutathione can protect against them suggests that they may in fact be sulfhydryl inhibitors. The possible importance of sulfhydryl compounds in dormancy is somewhat heightened by the suggestion that the mechanism of the auxin stimulation of growth may be through a direct reaction with a sulfhydryl group (see below).

An inhibitor of quite a different type is formed in *Datura* embryos (240). This material, which appears to be a nucleic acid, inhibits growth of the embryos both *in vivo* and in tissue culture. It does not appear to be the cause of the abortion of the ovules in incompatible crosses, as was at first hoped. However, its apparent multiplication or reproduction within the embryo sac makes this material of great interest.

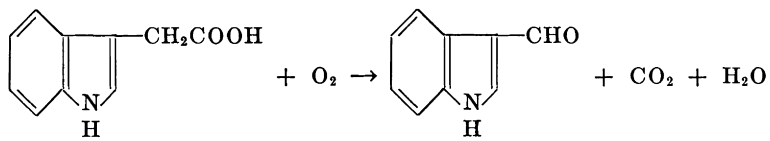
The germination of seeds is another physiological phase which may be under the control of auxin inhibitors. The role of unsaturated lactones as germination inhibitors was discussed in Volume I, and their effects on growth in Section VI, C above. Their interaction with auxins in this function has been demonstrated by Mayer and Evenari (198), who found that the inhibition of germination could be reversed, at least in part, by compounds which protect sulfhydryl groups, such as thiourea and cysteine. Further evidence of the sulfhydryl-combining nature of germination inhibitors has been advanced by Elliott and Leopold (79), who assayed for the material by measuring inhibition of the enzyme amylase, which is very sensitive to sulfhydryl reagents. They correlated the extent of this inhibition by natural inhibitors with their effectiveness in inhibiting germination. The inhibition of germination by lactones can be reversed by light, as was first shown by Nutile (219), and indeed the germination of several types of seeds is known to require light. The effective wavelengths have been carefully determined on lettuce seeds by Borthwick *et al.* (36), who find that whereas red (*ca.* 6800 A.) erases the inhibition, allowing the seeds to germinate, far-red (*ca.* 7200 A.) reinstates it. The inhibition can be reversed and re-established an indefinite number of times by exposure to the appropriate wavelengths. Apparently, therefore, the effect of light may involve the conversion of a pigment reversibly from one form to another and back. Whether the pigment itself acts as a germination inhibitor, or whether it is only the first member of a chain of reactants, is not yet established.

VII. The Formation and Destruction of Auxin

It was shown in Section III that indoleacetic acid can be produced in plant tissue from indoleacetaldehyde, indoleacetonitrile, and indolepyruvic acid. It can probably be formed from tryptamine also. Strong, though essentially circumstantial, evidence points to tryptophane as the overall parent substance (158, 98, 295). Enzymes converting tryptophane to

indoleacetic acid directly have not been much studied, but spinach leaf sections, infiltrated with tryptophane, or a preparation from the cytoplasm of the leaves, will form some auxin (333). Tryptophane applied to tissue cultures, under sterile conditions, causes formation of auxin (149a). Hot alkali treatment of various proteins will certainly produce auxin, though in small yield (257, 337). The evidence summarized in Volume I indicates that proteolysis of plant tissues yields auxin, and this doubtless derives from tryptophane, which is present in most proteins. The existence of an "auxin-protein" as an auxin precursor, for which evidence was earlier adduced, has not been confirmed, although a complex of this sort may well be involved in the functioning of auxin (see Section X). Since tryptophane may be synthesized by a condensation of indole with serine, as in *Neurospora*, the indole deriving from anthranilic acid, we can sketch at least a skeleton of the paths of biogenesis of auxin.

The disappearance of auxin from the plant is less well understood. This process is probably of critical importance in controlling growth rates, but it remains elusive. The destruction of auxin by light has been the most amenable to study. This reaction is catalyzed by various dyes, including eosin (268, 25), riboflavin (91), chlorophyll (38), and almost any fluorescent compound (82). Photodestruction with riboflavin involves disappearance of the acid group, with concomitant shift of pH (38, 40) and uptake of a mole of oxygen (91, 93). The initial product has been tentatively identified as indolealdehyde (77):



The identification rests on the R_f and color reactions in chromatographic and electrophoretic separations. It is probable that the destructive reactions do not terminate here, however. Brauner (39) has noted that the indole ring is ruptured soon after the acid group is removed. The later stages of the reaction, involving the ring, undoubtedly account for the divergent conclusions of earlier workers as to whether the ring was destroyed or not (90, 285).

Auxin is also destroyed in an enzymatic reaction, first demonstrated in *Helianthus* leaf brei in 1934 (289). The enzyme, later called "indoleacetic acid oxidase" (285), is apparently a peroxidase. Like tryptophane peroxidase (143, 144) it appears both to produce and to use peroxide. Pure peroxidase, plus H_2O_2, also destroys indoleacetic acid (96, 229). Indoleacetic acid can, of course, be chemically destroyed by H_2O_2, but for this more peroxide is required than exists in physiologically natural conditions. Catalase inhibits the enzyme reaction, and blue light reverses the

inhibition (93). The enzyme is more or less specific for indoleacetic acid, though reports on this differ (318). It contains a heavy metal, probably Fe^{+++}; it is inhibited by Mn^{++} ions and the inhibition is apparently reversed by light. The reaction itself is promoted by light, but it is not excluded that this may perhaps merely be light-catalyzed oxidation of the type mentioned above, since a flavin is present. The product is certainly not indolealdehyde, but apparently 3-methyl, 3-hydroxy-oxindole (279). Probably two reactions occur in sequence.

The enzyme is produced in numerous higher plant tissues and also by a fungus parasitic on leaves, where it is responsible for the resulting leaf-fall (263). It is well understood that leaf abscission occurs when the auxin supply from the blade ceases (see Section VII,C of 290; also ref. 264).

Finally it should be noted that several compounds can protect auxin from enzymatic oxidation. Cyanide acts, of course, by inhibiting the enzyme (285), and this makes possible the improved technique of "diffusing" auxin from cut surfaces described in Section II above (277). Scopoletin, a coumarin derivative, appears also to inhibit the enzyme (8), whereas ascorbic acid, which has been shown to increase auxin effectiveness in some circumstances, probably protects auxin by acting as a reducing agent (330, 39, 40). In artificial systems, peroxidase substrates such as protocatechuic acid protect IAA, perhaps by acting as alternative substrates for the peroxidase enzyme (229).

It remains genuinely uncertain whether peroxidative auxin destruction actually occurs in intact cells, especially at the rapid rates characteristic of the usual experiments. Indeed, such destruction may be mainly a phenomenon of wounds and cut surfaces, and *in vivo* auxin destruction may proceed much more slowly (229). Further work is needed in this area.

VIII. Hormones and Reproduction

The physiological mechanism which controls reproduction in plants is a subject of great interest but not very complete understanding. The auxins, as *growth* hormones, strongly influence those phases of reproduction which involve growth, but their influence on the transition from vegetation to reproduction is less clear. It has been suggested in several different quarters that the growth hormones antagonize reproductive activity—a generalization which has been made improbable by the multitude of instances where auxin has been found to promote reproductive activity, especially floral initiation (see p. 31).

A. The Humoral Stimulus Controlling Flowering

After the discovery that flowering is in many plants controlled by the length of day and night (photoperiodism), it was soon learned that the flowering stimulus originates primarily in leaves. And since the re-

sponse must occur in the buds, it was deduced that flowering may be in fact controlled by a hormone (see discussion in Vol. 1, Chapter III). Attempts to extract such a hormone have never been successful, or the reported successes have not been repeatable. A substance, apparently a calcium soap, has been extracted from plants in the flowering state which is absent from the vegetative state (247). This material has been reported to have antagonistic effects on auxin and may have a slight effect in promoting the flowering of plants to which it is applied. However, it is not a "flowering hormone" in the usual sense. Nevertheless, in spite of the absence of an identifiable hormone, the flowering stimulus does move through plants, and some factors concerning its translocation have been identified.

Whatever the nature of the stimulus, there is good evidence for believing that it is the same in each of the photoperiodic classes of plants. By ingenious experiments in which plants of long-day type, short-day type, and intermediate type have been grafted together in various paired combinations, it has been shown that if one member of such a pair is induced to flower, the other can become induced by the translocation of the flowering stimulus across the graft (see ref. 154). Other evidence pointing in the same direction is found in the observations of Holdsworth and Nutman (127) that the parasitic *Orobanche* becomes reproductive only when the host plant upon which it is growing has been induced to flower. A somewhat weaker, though clear, response was obtained with the parasite *Cuscuta*, which was promoted in flowering by the flowering of its host plants (75).

In brief, then, it can be concluded that a flowering stimulus exists which is probably common to most plants and which can move about in the plant. It is quite clear too that the flowering stimulus is not identical with auxin, for it is nonpolar in movement and can not be substituted for by auxin except in the special case of pineapple.

B. Auxins and Flowering

The demonstration that auxins could induce flowering in the pineapple is the most dramatic influence of auxin on reproduction yet recorded (67). The application of small amounts of nearly any substance with auxin activity can induce flowering, whereas the application of relatively large amounts can completely prevent flowering (67, 222). An interesting complication has been observed in that indoleacetic acid is almost without effect in this regard, which has led to the suggestion that the application of synthetic auxins induces flowering by lowering the effective auxin level in the plant (31). A more probable explanation is that pineapple leaves are rich in enzymes which bring about the destruction of indoleacetic acid (99),

resulting in the lack of effectiveness of that auxin. It seems quite likely that the ability of auxin to induce flowering in the pineapple may be through removing some secondary limitation to flowering when the primary flowering stimulus is already present. The arguments for such a point of view have been summarized elsewhere (160).

The effects of auxin in *modifying* flowering of plants generally follow an optimum curve, similar to that which describes the effects of auxin on growth and other physiological functions. Low concentrations of auxin can promote flowering in many plants (171, 66, 163, 182), whereas higher concentrations inhibit flowering. The promotive effects on reproduction begin at concentrations of auxin which are too low to bring about detectable vegetative responses (118). The promotion effect is generally not large, and auxin treatment cannot substitute for the requirement for a particular photoperiod or temperature cycle, although it can slightly modify the threshold photoperiodic treatment required for flowering (182). The inhibiting effect, however, can be virtually complete, and a number of instances of the auxin inhibition of flowering have been described in the last few years (33a, 171, 78, 262). The inhibiting effect on flowering is most pronounced in short-day species, but these species too can show promotion of flowering by auxin under special environmental conditions (163). This may be still another instance in which cofactors are involved in determining the net effect of a given auxin concentration in plants.

The modification of flowering by auxin is brought out also by the action of auxin antagonists. These compounds cannot in general *produce* flowering, although a small effect has been reported just at the threshold of photoinduction (29). The effect of the antiauxin was erased if auxin was added simultaneously. However, the antiauxins used included 2,4-dichloroanisole and 2,3,5-triiodobenzoic acid; the former of these is not necessarily a true auxin antagonist (12), while the latter sometimes exerts synergistic effects with auxins; this may account for the fact that other experiments attempting to use such compounds to increase flowering have not always been successful (117). Nevertheless, promotions of flowering have been recorded in various plants for triiodobenzoic acid (89, 171, 317).

Auxin has also been invoked to explain the need of a dark period for flowering (181). There are several instances in the older literature indicating that the auxin diffusing from leaves and stems decreases in the dark. Growth experiments on the effects of red and far-red light led to the proposal that the function of the dark period in photoperiodism is to permit the lowering of the active auxin content to a level at which the flowering stimulus can be formed. On this basis, light would counteract the effect of the dark period by causing the re-formation of auxin from some bound form or auxin complex. Although such a theory is certainly interesting, it is dif-

ficult to adapt it to the findings that: (*1*) auxins sometimes promote flowering, (2) auxins may increase in leaves during the night (328), and (*3*) some plants, especially of the rosette type, actually show greatly accelerated growth during flowering.

The fact that auxin cannot imitate the control of flowering exerted by photoperiodism, nor yet that exerted by vernalization (see below), strongly suggests that auxin plays some secondary role in flower initiation. The variable effects of auxin applications—sometimes promoting and sometimes inhibiting—bear out this relegation of auxins to a secondary role. They are able to modify flowering or sometimes even prevent it, but they do not seem to constitute the primary control of flower initiation.

Besides modifying flowering in a quantitative way, auxins can also exert an influence, though apparently only a small one, on the sexuality of flowers. The application of lanolin pastes of auxin to gherkin plants has been found to increase the proportion of female to male flowers (153). Similar results have been obtained with acorn squash (218), though only at one node. A much more far-reaching control of sexuality is exerted by cool nights or long photoperiods.

Quite another effect of auxins on flowering is the inhibition of development of flower buds (102), which is simply another case of bud inhibition. Some attempts have been made to utilize this property horticulturally by applying auxins to retard blooming of fruit trees.

C. Auxins and Vernalization

In those cases where temperature rather than light controls flower initiation, it is interesting to find that auxins also play a role. In the study of flower initiation by cold, or vernalization, extensive researches, principally by Gregory and Purvis, have been directed towards understanding why some plants require a cold period in order to flower. These workers have established that the influence of low temperatures is perceived mainly by the embryo of the grains (104). The cold reaction can be reversed by several environmental manipulations, including exposure to nitrogen gas or to high temperatures (103, 235). In order to vernalize excised embryos on agar, sugar or other organic nutrient must be supplied (232). In addition, the endosperm has a pronounced effect in facilitating the response of seeds to cold, and this effect cannot be replaced entirely by sugars (233). The endosperm is known to be a rich source of auxin to the seedling, and it seems possible at least that when it is present during vernalization, it might serve to supply auxin to the tissue. However, auxin alone will not cause vernalization, as has been shown in many earlier experiments on seed treatment (see Vol. I, Chapter III, Section VIII,C). After a period of eclipse, this subject was reopened in 1952 by the finding that the treatment of seeds

with auxin before chilling could considerably increase the vernalization effect (234, 160). Some species of plants whose flowering is not promoted by vernalization became susceptible to vernalization after auxin treatment (163, 164). These include peas, teosinte, soybeans, and corn. Experiments with excised embryos of winter rye indicated that an improved vernalization effect could be brought about by pretreatment of the seed with auxin whether the endosperm was present or not. Analysis of the factors influencing the auxin response (166) has led to the suggestion that auxin may be specifically required for some metabolic reactions which go on at low temperature. After the auxin low-temperature step, there appears to be a requirement for CO_2, as indicated by the finding that very rapid devernalization can be brought about by exposing the seeds to CO_2-free air.

D. Hormones in Fruit-Set and Fruit Development

After flowers have been formed on a plant, an entirely different requirement for auxin sets in, namely, for setting of the fruit. This subject has been reviewed recently (218) and may be treated relatively briefly here.

At fertilization, auxin is carried to the ovule by the pollen. However, the supply of auxin from the pollen is considerably less than the amounts found in the ovary of tobacco flowers after pollination (207, 208). It appears, therefore, that the pollination causes a large increase in the enzymatic production of auxin by the ovary itself. This spurt of auxin production shortly after fertilization is probably the key factor in the commencement of fruit growth. If this is correct, then the phenomenon of fruit-set is in fact the release of an enzymatic system for the production of auxin.

Some biochemical studies of the responses of flower ovaries to pollination and to auxins indicate that several enzymatic changes are brought about by these agents. Large increases in the activity of catalase and in salt and water uptake have been observed upon pollination of orchid embryos (130). In several other types of ovaries either auxin or pollen brings about marked increases in the activity of phosphorylase and some dehydrogenases, and the apparent mobilization of considerable amounts of carbohydrates (287, 195). Starch is also synthesized (193, 130). These changes in enzymes and in carbohydrates are 1 or 2 days slower in appearance following pollination than they are following auxin treatment. The slower response is probably due to the time required for the release of the auxin-forming apparatus in the ovary, a process which requires from 48 to 60 hours (207).

For either auxins or pollen to be effective in bringing about fruit-set, there appears to be a strong requirement for the presence of mature leaves on the plant (169). By culturing excised flowers, the nature of this dependence has been studied, and a wide variety of materials has been found

to substitute for leaves. It is probable that the function of mature leaves is simply to supply substrates necessary for any of several metabolic pathways. Ovary growth is promoted especially by malate, which is perhaps decarboxylated in the process (162).

The sources of auxin to the enlarging fruit have been studied by Nitsch (217) and Luckwill (187–189). It appears that, at least in some fruits, the auxin source changes with the stage of development. Upon fertilization, the initial auxin supply is apparently derived from the ovary itself, but as the endosperm enlarges, the auxin source shifts to that structure. By the time the endosperm has developed to the stage where it is clearly segmented, the embryo has developed sufficiently to take over the auxin-producing function. The embryo then serves as the auxin source for the final stages of fruit growth, and when this supply ceases the fruit is abscised from the tree (189). In the strawberry, the "seeds" or achenes are on the outside of the fruit and therefore readily accessible for experimentation (216–218). If they are removed, the "fruit," or receptacle, at once stops growth; application of auxin reinstates it, causing the formation of a sort of parthenocarpic fruit. The application of auxin cannot take the place of pollination in the strawberry but can take the place only of the young achenes on the enlarging fruit. Fig. 4 presents the stages in the apple.

Auxins have particularly strong effects upon the young developing embryos in fruits. The promotive and inhibitory effects of indole acetic acid upon the various parts of *Datura* embryos have been compared, and the

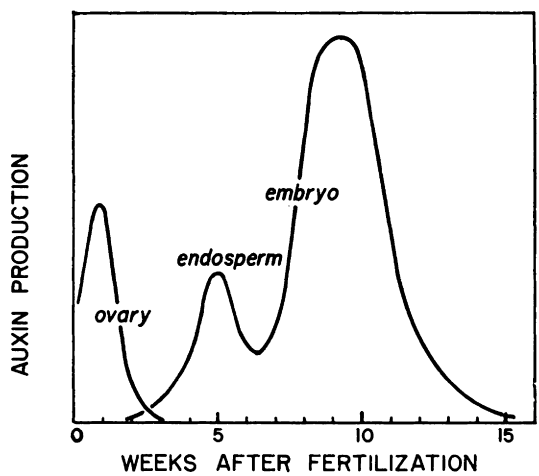


FIG. 4. The production of auxin during the development of the apple fruit. The curves for endosperm and embryo are averages of several experiments. The relative values for the ovary in relation to the other two are highly uncertain. Plotted from data of Luckwill, 1953 (189).

development of roots and cotyledons found to be particularly sensitive to high auxin levels (245). If the auxin supply is sufficiently high, the embryos may completely abort, and as a consequence fruit growth may be terminated (284). This abortion of young fruits by auxin is one of the bases for the agricultural use of auxins in thinning young apple fruits.

IX. Auxins and Pathological Growth

The various types of tumors induced in plants by bacteria and viruses show considerable similarity to the swellings induced by auxins. The suggestion that the pathogens act by causing an overproduction of auxin was therefore a natural one and some of the evidence for this idea was discussed in Volume I of *The Hormones*. There are several reasons for suspecting, however, that whereas auxin plays a part in the growth of tumors, it is not the initial causative agent. For one thing, swellings induced by excess auxin are usually accompanied by the formation of roots, whereas tumors very seldom differentiate any root primordia. More important is the fact that bacterially induced tumors of crown gall can give rise on transplantation, or even by heat treatment, to secondary tumors which are bacteria-free. This has led to the concept of a special tumor-inducing principle, T.I.P. Since tumors can be transmitted only through grafts, the principle must be nondiffusible and able to move only through living tissue (252, 253). Clearly this concept has much in common with that of the Rous Sarcoma virus and other virus-induced transplantable tumors of animals. The properties of T.I.P are discussed by Braun (in 44).

Avirulent strains of crown-gall bacteria have been made virulent by being cultured in a synthetic medium containing deoxyribonucleic acid (DNA) purified from the cells of a virulent strain (140). Klein has also found that within a short time after inoculation with crown gall the DNA content of host tissue increases rapidly (141). Crown gall which has been mildly heat-treated so as to lose all or most of its tumor-inducing potency has a much smaller effect on the DNA level (142). The significance of these observations, however, is weakened by the finding (267; see Section VI) that auxin alone can cause a marked increase in DNA content of stem tissue.

However, the facts are consistent with an interpretation that the crown-gall organism, *Pseudomonas* (or *Agrobacterium*) *tumefaciens*, carries a virus-like or DNA-like agent, perhaps similar to the *Pneumococcus* transforming substance; that this agent, in presence of sufficient auxin to stimulate at least initial growth of the tissues, can multiply therein; and that in so doing it affects the auxin relations of the plant in such a way as to cause continued further growth (see Kehr and Smith, and Klein, in ref. 44).

Similar to the bacteria-free secondary crown galls are the spontaneous

tumors which arise in tissue cultures. These are distinguished from the original cultures by their ability to grow on the same medium but without added auxin (95, 206). They can be transplanted to healthy host tissue where they form smaller and slower growing tumors than crown gall; also they can be propagated indefinitely in tissue culture. Apparently these tumors represent mutations, perhaps in several steps, towards auxin-independence.

Whatever the initial tumor-inducing agent, the various tumor types will all characteristically grow in culture without added auxin. It follows, then, that their common character is the ability to produce auxin spontaneously (95) or else perhaps to destroy it less rapidly than normal tissue (24, 125). Ether extraction of such auxin-independent tissues does in fact show that their auxin content is higher than in normal tissue (148, 149). Attempts to find significant differences in enzymes which might be concerned with auxin metabolism were unsuccessful, however (125), perhaps because the tissues used had been lyophilized. In "model" auxin-destroying systems, using horseradish peroxidase, both gall and normal tissues were found to contain materials which protected indoleacetic acid from destruction (229). Paradoxically, though, it was the normal that contained the more protective agent, and this actually is the only real metabolic difference yet found between the two types. The possibility arises that perhaps these very "protective" agents, which appear to be phenolic in nature, may have growth-inhibiting properties, which account for the reduced growth of normal tissue (229).

In line with the concept that the *in vivo* growth of tumors is strictly a function of their effective auxin content is the observation that factors which destroy or reduce the auxin content of healthy tissues have generally been found to limit the growth of tumorous tissues. This is apparently not a prevention of the *inception* of tumorous activity, but rather an inhibition of the growth phase. The irradiation of tumors with X-rays greatly reduces their growth (319), and the effects of these radiations on auxin destruction are well known. Maleic hydrazide, which acts as an antiauxin in several ways (168, 8), has been found similarly to reduce tumor growth (320, 150). In addition a number of antivitamins, especially folic acid antagonists, inhibit tumor growth at quite low concentrations (254).

The action of auxin in tumorous growth appears not to be a simple one, for some types of galls are dependent for their growth upon yeast extract (252) or upon various amino acids (135). It is curious that the ammonium ion was also somewhat effective in the presence of an auxin. There is some evidence that the nitrogenous materials which facilitate the action of auxin in cabbage tumor growth may be synergistic with auxin (134).

The invasion of plants by pathogenic organisms appears to be strongly

affected, though in different directions, by auxins and related compounds. On the one hand, nonpathogenic strains of crown-gall bacteria can be rendered gall-forming by simultaneous application of an auxin (302). On the other hand, it has been reported that auxin treatments may actually reduce the severity of infection by potato virus (178), tobacco mosaic virus (152), and bean brown spot (71). The ability of a substance to control virus does not seem to be dependent upon its auxin activity as we know it in growth reactions, since good control of the bean virus has been reported with antiauxins such as 2,4,6-trichlorophenoxyacetic acid (72). The significance of these observations, as well as their potential value in agriculture, remains to be evaluated.

X. Mode of Action of Auxin

Our concept of the means by which auxin acts to cause growth developed considerably in the last few years. Some of the advances in this field have sprung from the studies of molecular structure in relation to auxin activity, and some from studies on the metabolism which takes place during growth.

A. The Combination of Auxin with Its Substrate

It was first suggested by Skoog *et al.* (271) that auxin may function by serving to link an enzyme to its substrate, thus making possible some biochemical reaction essential for growth. The molecule would essentially be one which can combine simultaneously with two different substances. This theory has received new support recently from kinetic studies, which have been interpreted as showing that auxin does react at the molecular level in two positions.

A study of the rate of growth of coleoptile sections in the first few hours, as distinguished from their total growth, led McRae and Bonner (191, 192) to the conclusion that the combination of auxin with its receptor in the coleoptile can be competitively inhibited by structurally related compounds (*cf.* Section IV,B). Evidence was adduced that these compounds inhibit the reaction by combining at either of two positions on the molecule. In agreement with the concept of Skoog and co-workers it was assumed that when auxin molecules are in excess they inhibit the growth reaction by interference with one another. Thus if two separate auxin molecules, instead of one, combine at the two points of attachment, then the enzyme and substrate are held apart instead of being linked together, and growth inhibition will result. Analysis of the growth performance of inhibitory levels of auxins (83) indicates that the inhibition obtained was precisely what would have been predicted from known enzyme kinetics, assuming two points of attachment. These workers were able to estimate numerically the affinity of several different auxins for the positions of attachment. It is interesting

to note that indoleacetic acid was found to have the greatest estimated affinity for the receptor complex and also the greatest auxin activity of all the compounds tested (the nitrile not being included).

These kinetic analyses have been interpreted in terms of the view (*cf.* ref. 271) that auxins must have at least two characteristics for activity: (*1*) the proper configuration for attachment to the receptor materials, and (*2*) the reactivity to carry through the growth reaction. Any compound which will satisfy only one of these requirements will be an antiauxin. Consequently there could be three types of antiauxins: those which can combine only at the first position of attachment, those which can combine only at the second position, and those which can satisfactorily combine at both positions but are of such low reactivity that they fail to bring about growth. McRae and Bonner point out that if the two points of attachment are located in the ring and in the acid side chain, respectively, one would predict the existence of antiauxins with improper ring structures, such as the excessively substituted 2,4,6-trichlorophenoxyacetic acid, or antiauxins with an insufficient side chain such as 2,4-dichloroanisole. Evidence for two such types of antiauxins was advanced, and the third type, that with low reactivity, may perhaps be fulfilled by phenylacetic acid (131) and γ-phenylbutyric acid (271).

Thus the kinetics of auxin stimulation of growth in shoots appear to be consistent with the assumption that auxin reacts at two different points of attachment. Whether two points of attachment are sufficient to explain all the observed phenomena is uncertain, and the role played by optical isomerism in the side chain (*cf.* Section IV) suggests strongly that the situation is more complex than this. The further fact that 2,4-dichloroanisole does not show true antiauxin activity in some tests is disturbing. Skatole would be predicted to be a strong antiauxin on the basis of its structure, but unpublished experiments have not found it to be so either. No doubt further work will clear up some of these discrepancies.

In roots, unfortunately, these relatively simple considerations do not seem to apply. Burström has pointed out (53) that compounds which remove the auxin inhibition of growth (*i.e.*, antiauxins) do not necessarily promote root elongation (see also references 2, 3). Further, these presumed antagonists may actually antagonize each other. He concludes that antagonism is not necessarily exerted at the growth locus (*cf.* Section IV). If true, this would make antagonism studies of little value for elucidating the mechanism of auxin action.

B. Metabolic Functions of Auxin

The fact that the stimulation of growth by auxin is commonly associated with a stimulation of respiration has been reviewed in Volume I of *The*

Hormones. The problem of the relationship between the respiratory effects and the growth effects has received considerable attention since that time.

One fact which has emerged with new clarity is that the metabolic pathways by which auxin stimulates growth are strictly aerobic. Singularly small differences in oxygen availability to pea and *Avena* sections in auxin solutions have been found to alter the growth responses (265, 296). Experimental alteration of oxygen tension, with potato discs, has provided a direct demonstration of the linear dependence of auxin function upon aerobiosis (112a). The increases of respiration caused by auxin are usually smaller than the effects on growth, though parallel to them; in *Avena* coleoptiles, indeed, they have often been reported to be zero and appear to depend on the conditions used. In phosphate buffer the optimum rise in respiration is only 14%, but the curve does parallel that for growth (336). In *Pisum* stem sections the increase approximates 20% (64), and in potato tuber slices it is of somewhat larger magnitude but is delayed for about 48 hours in its onset, just as is the effect on growth (113). In artichoke sections, however, the increase is 400% and is immediate (112); the effect on growth is equally rapid and even larger.

Another aspect which has been established firmly is the significant role of sulfhydryl materials in auxin action. That sulfhydryl inhibitors such as iodoacetate, arsenite, organic mercurials, and the unsaturated lactones profoundly inhibit auxin functions has been established for the respiratory response to auxin (112, 113), for the uptake of water in response to auxin (111, 112a), and for growth by elongation (69, 297a). It is noteworthy that each of the current theories of the molecular functioning of auxins suggests a direct role of sulfhydryl compounds. The two-point attachment theory as originally proposed by Muir *et al.* (212) suggested that a sulfhydryl may be the point of attachment for the ring or nucleus of the auxin; Leopold and Guernsey (165) observed a measurable utilization of sulfhydryl groups in the presence of auxin (see below); and Siegel and Galston (266) have reported that the attachment of auxin to a pea root protein material could be severed by the addition of Coenzyme A, a sulfhydryl compound.

Some evidence concerning a relationship between auxin action and phosphorylation has been brought forward (88, 32). It has been pointed out that compounds with ring structures common to certain auxins (phenoxy series) but without the acidic side chain may often be uncoupling agents between oxidative metabolism and phosphorylation reactions (88). Both types of materials can elicit respiratory increases, auxins with an associated growth stimulation, and the uncoupling agents such as dinitrophenol with a growth inhibition. It has been suggested, as also by Bonner earlier (28), that both types of compounds may stimulate respiration by making phos-