Other Plant Hormones[1]

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CONTENTS

The interrelationships between different parts of plants involve, in addition to the auxins, a number of other hormones. Some of these have been studied in moderate detail, while of some the existence has only been inferred. Because the work on any one hormone has been somewhat

[1] The author is much indebted to Dr. F. W. Went for careful criticism of this chapter.

isolated from that on others, each group will be treated in a separate section, with its own bibliography. Interrelations with auxin, where these are indicated, will also be taken up in each section.

I. Wound Hormones[2]

A. Historical

When plants are injured, there typically results a stimulation of the growth of intact cells near the wound to produce scar tissue or "wound callus (*cf.* 2)." This phenomenon involves the resumption of cell division by cells apparently fully mature. More than fifty years ago Wiesner suggested that special substances may be produced by wounded cells which are responsible for this effect. A series of investigations by Haberlandt and co-workers went far to confirm this view. These experiments arose out of Haberlandt's first unsuccessful attempts to grow plant tissue cultures. In small pieces of potato tuber, renewed cell division leading to formation of a periderm took place only if (*a*) a fragment of phloem tissue was present and (*b*) crushed cells, or an extract of them, were applied (16). Control of cell division was therefore ascribed to two hormones, one from the phloem, called "leptohormone" and one from wounded cells—the "wound hormone" proper. The former was shown to be diffusible through agar and it may possibly be identical with auxin, though it has not been further studied. In the kohlrabi root, cell divisions could be prevented by washing the injured surface, and could be induced by covering the surface with crushed tissue of other plants (17,19). Finally, by careful dissection, uninjured cells were exposed in the leaves of succulents and shown to respond by cell division to the application of tissue juices from other plants. Reiche (26) obtained similar results by injecting petioles and stems with extracts of wounded tissue. Hence the substances involved are not species specific.

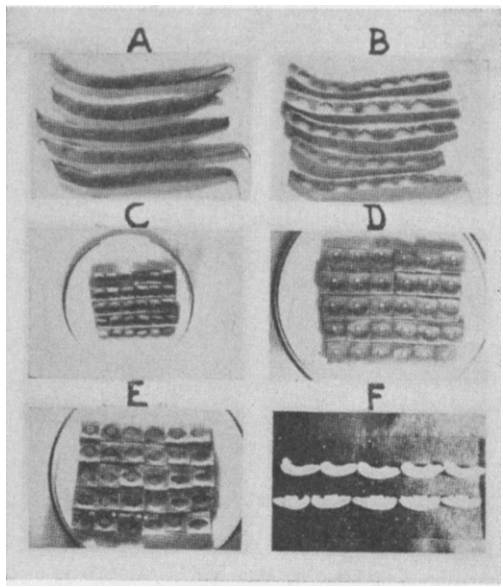
Search was made for suitable material for more extensive experiments, leading to the use by Wilhelm (40) of the parenchymatous lining of the hollow stem of the windsor bean, *Vicia faba*, and by Wehnelt (37) of the lining of the immature pod of the kidney bean, *Phaseolus vulgaris*. This latter test has been adopted in later work.

B. Assay Method

The only method extensively used is that of Wehnelt (37), modified by Bonner and English (4). When the unripe beans are removed from the pod the parenchymatous tissue beneath is the responsive material. A drop of the juice of crushed tissue (bean juice is very effective) applied to this layer causes a small intumescence a millimeter or two high to arise

[2] In this chapter, each section has its own list of references.

(Fig. 1). This consists of parenchyma cells elongating perpendicular to the axis of the pod and undergoing vigorous cell division. The height of



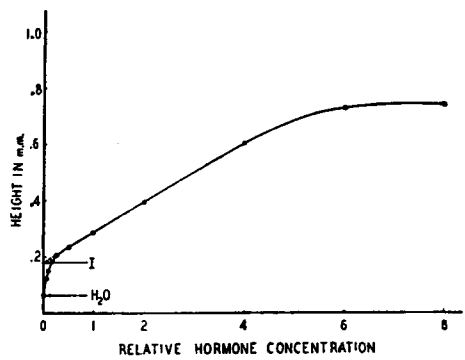


FIG. 1.—Upper: Stages in the bean test. A, fresh pods; B, pods slit and seeds removed; C, individual seed chambers in petri dish; D, drops of test solution in place; E, characteristic reaction to traumatic acid after 48 hours; F, cross section through seed chamber after 48 hours, top row, a control, lower row, reaction on the linear part of the curve.

Lower: Relation between concentration and height of the intumescence. Limit of nonspecific effect is shown at I. (From Bonner and English, 4.)

the intumescence after 48 hours, measured by a low-power microscope on a cross section, is proportional to the concentration of wound hormone. There is, however, a small reaction, producing an intumescence of about

one- fifth the maximum height, which is nonspecific in nature and may be caused by water, strong solutions of salts or sugars, toxic substances, etc. This nonspecific effect was encountered by Wehnelt (37), Wilhelm (40), and Jost (18), who obtained reactions from such nondescript material as 2% levulose and 0.01% citric acid. Such results have led to much confusion in the past. The intersection point I in Fig. 1 represents the highest nonspecific effect obtainable under the standard conditions, and its exact value varies from day to day. Above this the intumescence is due to wound hormone alone. The maximum height obtainable varies a good deal with the variety of bean; Bonner and English found "Kentucky Wonder, brown seed" the best. The test is given (beyond the nonspecific point) by the juices of many plants, by molasses and brewers' yeast, but not by urine, peptone, or meat extracts. The juice of the bean pod itself was, however, found to be the most active source, with brewers' yeast a close second. There seems little support for the suggestion of Silberschmidt and Kramer (30) that activity of plant extracts on the bean increases with increasingly close taxonomic relationship to the bean.

C. Purification and Chemical Nature

Numerous pure amino acids, auxins, vitamins, and other biochemicals were found inactive (4). Indoleacetic acid was found by Jost (18) to be active at 1000 mg./l., but this was doubtless a nonspecific effect, due to toxicity. It was also active, at 100 mg./l., on Wilhelm's test material (see above). In experiments by Orsos (23) on the kohlrabi root, tyrosine was found to be active. It was inactive in the bean tests of Bonner and English. Such differences suggest that different plants may have different limiting factors for the wound reaction, and that there may therefore be many substances interacting to produce the complete reaction.

By extracting bean pod juice first with acetone and then with ethyl acetate, at pH 2, extracting nonacid material with chloroform at pH 10, and forming barium salts, English, Bonner, and Haagen Smit (11) obtained a crystalline dibasic acid, $C_{10}H_{18}(COOH)_2$, which was active in the bean test. The name proposed is "traumatin" or "traumatic acid," and the structure is apparently that of Δ^1-decene-1,10-dicarboxylic acid:

$$HOOCCH{=}CH(CH_2)_8COOH$$

This was confirmed by synthesis (11). The yield was 18 mg. from 100 lb. fresh bean pods. The activity was increased about 50% by addition of $\frac{1}{2}$% sucrose (itself inactive) and was increased by a factor of two or more by adding some of the discarded acetone- and ethyl acetate-insoluble fractions (themselves of low activity only). This indicates that one or more cofactors, varying in amount in the test beans, participate in the

reaction (see above). The most marked "cofactor" of this sort is glutamic acid, which at 0.25% (almost inactive alone) enhances the activity of the traumatic acid some ten times (10,36). As little as 0.1 γ traumatic acid, in the presence of a solution of cofactors, gives a detectable response in the bean test. Furthermore the acid gives intense cell division in Haberlandt's test (15) on potato tubers (see introductory paragraph), and this too is enhanced by the cofactor solution.

Apparently traumatic acid is only one of many closely related substances having wound hormone activity. The saturated decane-1,10-dicarboxylic acid is about half as active as traumatic acid (12). The substances shown in Table I are all active to varying degrees according to English *et al.* (10,12).

TABLE I

DICARBOXYLIC ACIDS OTHER THAN TRAUMATIC ACID ACTIVE AS WOUND HORMONES

Slight Activity	
Hexane-1,6- (suberic)	Heptane-1,7- (azelaic)
Activity About Half That of Traumatic	
Octane-1,8- (sebacic)	Decane-1,10
Active in Presence of Cofactor Solution	
Δ^1-Octene-1,8-	Δ^2-Tridecene-1,13-
Δ^1-Nonene-1,9-	Δ^{1-7}-Octadiene-1,8-
Δ^2-Nonene-1,9-	5-Nonanone-1,9-
Δ^2-Decene-1,10- (isomer of traumatic)	5-Nonanol-1,9-
Δ^5-Undecene-1,11-	6-Undecanol-1,11-
Δ^1-Tridecene-1,13-	6-Undecanone-1,11-

Maleic acid showed very slight but definite activity, succinic acid none. This fact and the activity of the pairs—octane- and octenedioic acids and decane- and decenedioic acids—indicate that unsaturation, while not essential, increases the activity. Alcohol and ketone groupings in the chain do not remove the activity. No monocarboxylic acid of a large number tested was active. Activity appears, therefore, to be confined to dicarboxylic acids with a moderate number of carbon atoms in the chain.

In a study of the substance which carries the stimulus when the sensitive plant (*Mimosa pudica*) is touched, shaken, or damaged, Soltys and Umrath (35) found that their partially purified preparations were also active in Wehnelt's bean test. Study of other sensitive plants showed that activity on *Mimosa* could be separated from that on the bean test by chemical means, but activity on another plant, *Aeschynomene indica*, appeared to be brought about by the same substance as for the bean test. The substance of Soltys and Umrath (36) was prepared from leaf extract by precipitation with lead and mercuric acetates and extraction with alcohol. The final product appeared to be a dibasic hydroxy acid of

molecular weight about 420 with probably four acetylatable hydroxy groups. An apparent nitrogen content of about 2% may be due to impurities, since English and Bonner also found nitrogen consistently in their semipure preparations. Acetylation did not greatly reduce the activity. It is not possible to conclude definitely whether the substance is one of those found active by English in the above test; the two hydroxy acids mentioned there are both of too small molecular weight. Apparently final purification was not achieved.

D. Physiology and Interrelations with Auxin

It must be admitted that the physiology of wound growth is far from clear. For one thing, a considerable part is played by auxin. In woody plants, wound callus is produced at least in part by the vascular cambium, though Sharples and Gunnery (29) and Sass (28) have indicated that parenchyma of medullary rays is the main tissue whose division produces callus. At any rate cambium typically responds to wounding by cell division and formation of new wood (8,9,14). Now this reaction can also be produced by auxin, as was first shown by Snow (31) for bean seedlings and by Söding (32,33) for trees (see 38, p. 218, and Section VIII of the preceding chapter). The effect of pure indoleactic acid on poplar and willow was very striking, the new wood produced within 30 days being up to 1 mm. wide (32). In white pine the new wood so formed is of the "rot-holz" type (39). Nevertheless this effect is limited to a region about 3 cm. below the point of application of the auxin. Also in the experiments of Rogenhofer (27) on the formation of callus at the base of poplar twigs, the effect of auxin was limited to a distance of about 3 cm. below the point of application. In the work of Wershing and Bailey (39) on "rot-holz" the effect of auxin was not transmitted very far down in young plants. From a variety of experiments, however, we know that auxin is not limited to such short distances in its transport. Indeed, the activation of cambium in the spring, by the developing buds, travels all the way down the trunk, taking many weeks to do so (7). Presumably this stimulus is (at least in part) auxin, and indeed it was shown by Avery *et al.* (1) that auxin produced by the developing buds does in fact move down the shoot (apple trees were used) approximately parallel to the spread of the cambial activity. It appears that within a very limited period in the spring even externally applied auxin can produce cambial stimulation over long distances, up to 23 cm., as shown by Gouwentak and Maas (15) with ash trees (*Fraxinus ornus*). If auxin can indeed travel long distances, at least in the spring, and activate cambium, why then is the wound reaction of cambium limited to a few centimeters, and why is under most conditions the effect of applied auxin similarly limited?

Some light is thrown on this question by the work of Brown (5). By cutting incomplete rings with a bridge of bark remaining, in the balsam poplar (*Populus balsamifera*), Brown showed that the wound wood was formed only weakly below most of the ring, but was very strongly formed in a streamer below the bridge, such as would be produced by a substance being transported polarly in the bark (Fig. 2A). From this and other

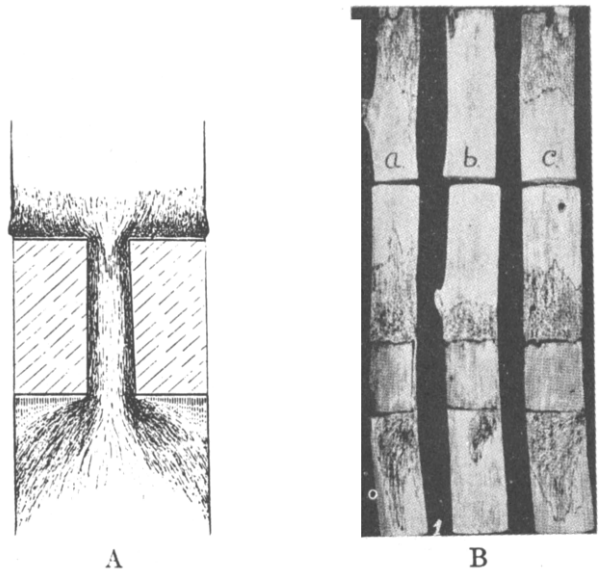


FIG. 2.—A. Cambial activity in relation to a longitudinally bridged ring, as shown by xylem formation under the bark in *Populus balsamifera*. The dotted lines indicate feeble cambial activity without differentiation of vessels or fibers. (From Brown, 5.)

B. Cambial activity in three units from the three-year-old portion of one leader shoot of *Populus balsamifera*. The upper (*a*) and lower (*c*) units were treated at the distal end (top) with indoleacetic acid, 1 mg./g. lanolin. The middle (*b*) unit treated with lanolin only. The longitudinal bridge (*cf.* Fig. 2A) in the lower sections is at the extreme right of each unit. (From Brown and Cormack, 6.)

experiments he concluded that two factors are involved in the wound reaction: the cambial hormone which moves basipetally downward in the phloem or cambium (33) (and is presumably auxin), and a wound substance whose effect is only local. Brown and Cormack (6) showed that, if the auxin is applied some 22 cm. above the wound, the wound reaction is much greater than without auxin but is still localized (see Fig. 2B).

The cambium cultures of Gautheret (13), which continued to grow indefinitely on culture media as largely undifferentiated callus, were con-

siderably stimulated by traces of auxin added to the medium; presumably the act of cutting from the tree produced wound substances, enough at least to start the growth (see Section VIII, A of the previous chapter). In this connection it is worth noting that a crude bean extract, rich in traumatic acid, greatly promoted the growth of fragments of bean parenchyma in culture medium (3). The fragments did not grow indefinitely, however, so that no true tissue culture resulted. All in all, it seems probable that the whole wound reaction involves in some way interaction of auxin with traumatic acid or other wound hormones. Where auxin appears to have no effect, as in the bean pod, we may suspect it is already present in optimal concentration.

There is a parallel for this in the case of root formation on cuttings: here auxin applied at the base frequently has a greater effect if one side of the cutting is wounded. This has been found by numerous horticultural workers, and especially by Rappaport (25) and La Rue (20). While it may be that the wounding improves the uptake of auxin, it seems unlikely that the effect can be due to this alone. Söding's finding (34) that cambium scrapings from one plant (*Acer*) stimulate cambial activity in another (*Helianthus*) is also suggestive in this connection.

Another important unknown is the biochemistry of the formation of traumatic acid. As early as 1929 Petri (24) suggested that the wound hormone must be an oxidation product of a compound normally present in living cells. The structure of traumatic acid would support this, and Nye and Spoehr (22) have pointed out that oxidation of C_{18} organic acids, particularly linolenic acid, could yield hexenal (which they isolated from *Ailanthus* leaves) and traumatic acid (see also 21). Certainly C_{18} acids occur in plants, but so little is known of the fatty acid metabolism of plant tissues that further discussion is valueless.

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II. Flower-Forming Hormones

A. Introduction

Unlike the hormones discussed above, flower-forming hormones or "florigens" have not been conclusively proved to exist. Extracts or preparations from plants, having flower-forming activity and capable of transport in the plant, have never been obtained in spite of many efforts. The evidence that a flower-forming hormone exists is thus indirect, although very strong, and it may be that flowering is controlled in some way by a balance between several substances.

Although Sachs in 1880 had put forward the concept of special flower-forming substances which would cause the growing plant to change over from the production of leaves to that of flowers, the early workers in

general considered that flowering was dependent on the condition of the whole plant. In 1907 Klebs developed evidence that flowering is induced by a low ratio of carbohydrates to soluble nitrogen, a view supported later by the work of Kraus and Kraybill (34) on the tomato. Never thoroughly established, however, this conception was weakened by numerous subsequent workers, and was rendered untenable when Knodel (32) showed that in the same species, with a given carbohydrate: nitrogen ratio, flowering may or may not occur, while the plants may flower with very different values of this ratio.

B. Photoperiodism

The whole subject was put on a practical experimental basis by the discovery of Garner and Allard (22) that flowering is controlled by the length of day. Some plants flower only when the day is shorter than a critical length (commonly ten hours or less), others only when it exceeds a critical length (commonly twelve or fourteen hours), while others again are essentially "day-neutral." It is not necessary that the prescribed length of day be maintained up to the time of flowering; frequently only a short treatment is necessary. For example, plants of dill (*Anethum graveolens*) when grown in a day length ("photoperiod") of nine hours remained in a vegetative condition for eleven months, but, after exposure to four long days (eighteen to nineteen hours) and then return to the short days, they flowered in a few weeks. Instead of the four photoperiods, continuous illumination lasting 84 hours would also cause flowering within a month (28). On the other hand, cocklebur (*Xanthium pennsylvanicum*), after growing vegetatively for many months on long days, could be induced to flower by treatment with a single short photoperiod. The former is termed a "long-day" plant, the latter a "short-day" plant; in general the subsequent production of flowering by exposure to a particular series of photoperiods is called "photoperiodic induction."

Many other examples, and detailed discussion of the large volume of work on photoperiodism, may be found in the reviews of Garner (21), Tincker (56), Loehwing (39), Adler (1), Hamner (24,26), Burkholder (7) and two recent books (58,63).

There are sundry secondary effects. The temperature prevailing during growth is of importance in some cases, the range of critical day length being a function of temperature; thus, to quote only one of many examples, *Baeria chrysostoma*, which requires long days for flowering, will not flower in long days or even continuous light if the temperature is above 25°C. (54). Soybean, on the other hand, although a short-day plant, will not flower on short days if the temperature is too low (50). In one case, that of dill, a long-day plant, wounding of the stem or of roots

greatly increases the tendency to flower (48). Nutrition sometimes exerts a modifying influence; in barley (a long-day plant), nitrogen deficiency may induce flowering in spite of the photoperiodic conditions, that is, on a nine-hour day (3). Intensity of light may affect the actual length of the effective photoperiod; in the case of *Xanthium* 30 minutes will suffice if the intensity is high enough (26). These secondary points need not concern us here. What is important, however, is that within a species, different varieties may have quantitatively different requirements. In extreme cases the requirements may be almost opposite; thus, in tobacco (*Nicotiana tabacum*), the variety Samson will flower on long photoperiods while Maryland mammoth is a short-day plant.

C. Experimental Basis of the Hormone Concept

It was shown in 1925 by Garner and Allard (23) that, when only a part of the plant was exposed to photoperiodic induction, the stimulus to flowering need not be limited to that part. When *Cosmos sulphureus*, a short-day plant, had the upper part completely darkened and the lower part exposed to short day, the lower part flowered, but on returning the whole plant to long days the upper part subsequently flowered. Garner and Allard did not make any deductions as to the significance of this translocation, and the development of this line of approach into the hormonal concept was only initiated ten years later, first being foreshadowed by the experiments of Knott (33), and later established, in 1936, simultaneously by five investigators, Cajlachjan (8,9,10a) and Moshkov (46) in Russia, Kuyper and Wiersum (36) in Holland, and Melchers (41,42) in Germany.

Cajlachjan's experiments with chrysanthemum, a "short-day" plant, were designed to study the importance of leaves in receiving the stimulus to flowering. After some preliminary work on millet, which indicated that the response to change in day length depended on the amount of leaf surface exposed, he set up a large group of chrysanthemums of equal age and size. The growing points and all the upper leaves and all lateral shoots except those in the upper part of the plants were removed, leaving, therefore, only leaves near the base and shoots near the apex. They were then divided into four groups as follows: group *1* received long day throughout; group *2* were also kept in long day, but the leaves were covered daily after ten hours; group *3* had the shoots covered daily after ten hours but the leaves were uncovered; and group *4* received short day (ten hours) throughout.

Thus we have: (*1*) leaves and shoots in long day; (*2*) shoots in long day, leaves in short; (*3*) leaves in long day, shoots in short; and (*4*) leaves and shoots in short day.

In another similar series the shoots left on were those near the base, the leaves were those near the apex, and the four groups the same as above. In both series, only the shoots of groups *2* and *4* flowered.

Thus the photoperiodic stimulus is (a) received by the leaves and (b) transmitted along the petioles, the main stem, and the side shoots to the buds. Cajlachjan (8) states:[3]

"As in the processes of growth the regulatory function is performed by the hormone of growth, so in the processes of development this role is performed by a specific hormone of flowering. The flowering of the plants and subsequent seed formation is due to the sufficient amounts of this hormone, which is formed in the leaves and translocated into the growing points."

Moshkov had been working on frost resistance. He found (45) that white acacia can be prevented from freezing in the winter by subjecting it to short days in the latter part of the preceding summer. Defoliated branches, however, could not be protected in this way. Hence he came to consider that frost resistance, like flowering, is conferred by a photoperiodic stimulus received by the leaves. His experiments on chrysanthemum (46) were similar to those of Cajlachjan, but more elaborate. They confirmed the latter in showing that exposure of the buds alone to short day did not induce flowering. Exposure of the leaves, but not the buds, to short day, induced flowering consistently. Of the leaves, the two youngest were slightly effective, while the next four, *i.e.*, those young but fully developed, were the most effective in receiving and transmitting the stimulus. This point was confirmed by Borthwick and Parker (5) for soybeans, in which the most effective leaf was found to be that which had most recently attained its full size. The same workers (4) confirmed also that application of the photoperiod to the growing point alone does not initiate flowering; only the leaves can receive the stimulus.

Among other interesting results, Moshkov showed that exposure of alternate leaves along the plant to short day did not induce flowering, so that there is an inhibiting effect exerted by those leaves which are in the long day. This also has been confirmed by Borthwick and Parker (4) using soybeans with one branch in short day and one in long; the latter flowered only when it was defoliated.

The experiments of Kuyper and Wiersum (36) were also with soybean (*Glycine max*, var. Vilmorin), another short-day plant. Two series of plants were grown, one in short days (9.5 hours) and one in long (thirteen to seventeen hours). Those in the long day produced no flowers

[3] The work of Cajlachjan, Moshkov, and others is most unfortunately largely published in Russian. For careful and critical translations the author is much indebted to Miss K. Zarudnaya. Cajlachjan's conclusions, but not the main experiments, are set out in English (10a).

throughout the experimental period. Apical parts of plants grown in short day, and already bearing flower buds, were grafted to bases of long-day plants, and the plants then maintained in long day. After about seven weeks all the basal parts produced one to several flower buds. Thus the flower-forming substance or stimulus was transported from the plant grown in short day, across the graft, to produce flowering in the part which had never received short day. The experiments were later confirmed and extended (35), but were not successful with another variety; they believe that this is because with this variety the short-day graft continued to grow and blossom so freely in the long day that it used up all the flower-forming substance in itself.

This latter phenomenon was noted by Cajlachjan (11) in very similar experiments with *Perilla nankinensis*. The "hormone-donating" shoot, which had been given short day, was again grafted on to a "hormone-acceptor" stock which had had only long day. When the donator had only leaves and the acceptor only shoots, the acceptor flowered freely. But when the donator had shoots, or the acceptor had leaves, transference of the flowering stimulus was weak or absent. Very similar results, but with the stock treated with short days and the scion in continuous light, were obtained by Moshkov (47a) (see also 27).

Melchers' experiments (41,42) were carried out with black henbane (*Hyoscyamus niger*), of which he used a biennial race, *i.e.*, one which flowered only in the second year. By grafting into the crown of one-year old plants, close to the growing point, a shoot of the two-year old, the growing point of the one-year old plant was caused to flower. The material appears to graft very readily, and numerous variations of the experiment are possible (see below).

D. Transport of the "Hormone"

In the experiments of Cajlachjan the flower-forming stimulus traveled with apparently equal facility either up or down the stem; exposure of basal leaves caused flowering of apical shoots and *vice versa*. This is in strong distinction to the movement of auxin, which (see Chapter 2, Section IV) under normal conditions travels in a strictly polar (basipetal) direction. There are some indications that the flowering stimulus travels more readily down the stem than up. Thus, in Moshkov's earlier work (45) on the frost resistance of acacia, the stimulus was found to travel downward from exposed branches into the trunk, but not upward. Similarly, Kuyper and co-workers obtained upward movement of flowering stimulus (*i.e.*, stock on short day, scion on long) in only one plant out of 23, while the reverse movement took place in nearly all cases. It must be remembered, however, that transport of the substance

is being deduced from observations of its effect. In view of the opposing influence of the leaves on long day, shown in Moshkov's experiments above, and also in those on cocklebur by Hamner and Bonner (27), and confirmed by numerous others, transport upward could well occur without resulting in flowering. Borthwick and Parker (5) found in soya that transport occurs equally in both directions, and the experiments discussed below all agree in this respect: transport is not polar (see also 44,52).

It appears that transport may occur in any tissue except the wood. Cajlachjan (11) showed with *Perilla*, a short-day plant, that, if the leaves which were given short day were separated from the buds by a section of stem in which a one-sided cut was made, the buds still received the stimulus and subsequently flowered; indeed, the side of the shoot directly above the cut flowered just as soon as the opposite side. This is taken to show transverse as well as longitudinal movement, but this deduction depends on the number of nodes between the stimulated leaves and the receiving buds. However, he also showed that chrysanthemum leaves, of which the main vein was cut through, thus remaining attached to the stem only by parenchyma tissue at the base, could donate the flowering stimulus to buds on the main stem. Although Lubimenko and Buslova (40) were unable to obtain this result with *Perilla ocymoides*, Cajlachjan later (12) repeated it successfully on *Perilla nankinensis*. There seems no doubt, therefore, that the "hormone" can travel in parenchyma.

In the experiment with *Perilla* mentioned above, if instead of a one-sided cut the shoot was completely girdled, cutting all phloem, the stimulus was not transmitted. In later experiments (12) the "hormone" was shown to move from one side to the other of a *Perilla* stem slit longitudinally all the way down to the base. In this case transport was from the apical leaves down to the base through the bark, then transversely through cortical parenchyma and up again through the bark to the growing points of the lateral shoots.

All the evidence therefore supports the view that the "hormone" travels in any direction in the plant, but only in living tissue. Since living tissue is involved, it is not surprising to find that local application of low temperature to the stem between the donating leaves and the receiving buds greatly delays transmission of the stimulus (6,13). Application of ether or chloroform to an internode also completely inhibited transport (13).

E. Later Work on Hormonal Nature of the Stimulus

In the work discussed above, flowering has been envisaged as an "all-or-none" phenomenon: either the plant forms flower buds or it does not. A valuable step forward, therefore, was made when Hamner (25)

introduced the measurement of the *number* of flower buds formed. With this procedure he was able to show that, for a fixed cycle of nine hours light and fifteen hours dark, the effect, *i.e.*, the number of flower buds, is linearly proportional to the number of such cycles (see Fig. 3). Such quantitative results very strongly support the hormonal nature of the stimulus. By the same procedure it was also shown that both the light and the dark periods[4] are needed for completion of the flower-forming process in the short-day plants soybean and cocklebur. This is not true for long-day plants, some of which, such as dill, will flower in continuous light.

The attempts made so far to extract an active hormone preparation have been suggestive but not convincing. Hamner and Bonner (27)

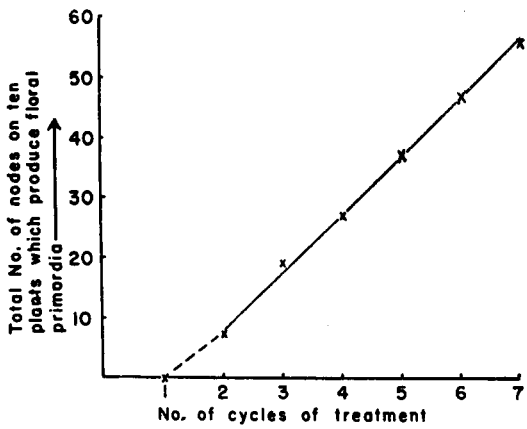

FIG. 3.—Effect of number of cycles, each consisting of a nine-hour photoperiod and a thirteen-hour dark period, on the number of floral primordia produced by Biloxi soybean. (From Hamner, 25.)

made grafting experiments with whole plants of *Xanthium* in which one plant with leaves was given short days, the other, defoliated, given long days. The graft was of the veneer type, *i.e.*, both plants on their own roots. After the graft had taken, the acceptors, *i.e.*, the plants on long day, flowered. When the experiment was repeated, but with lens paper inserted in the graft, the long-day plants also flowered. Unfortunately this latter experiment, which is crucial, could not be satisfactorily repeated, and Withrow and Withrow (59) have subsequently pointed out that, where transmission of the stimulus is observed, growth of tissue

[4] To parallel the term "photoperiod" Went has suggested "nyctoperiod" (Gr. *Nus, Nukti* = night) for the dark period. The more exact meaning of darkness would be given by "skotoperiod" (Gr. *Skotos* = darkness) but, since this might lead to phonetic confusion, nyctoperiod may be preferable.

through the lens paper has occurred. It is probable, therefore, that the successful result was due to a small amount of cellular connection. A more striking claim was made in 1937 by Moshkov (47), who grew chrysanthemum under continuous illumination, removed the first four leaf blades, and attached to their petioles glass tubes filled with water. Into these were inserted leaves from plants growing in short day and therefore containing the "hormone." Moshkov states:

> "No coalescence took place, nor could have done so, if only because the leaves were changed every day. Even so, some of the chrysanthemum plants subjected to such treatment formed flower buds, whereas the control did not form any."

To the author's knowledge, no confirmation or extension of this most important experiment has been reported, nor is any unpublished work on this point mentioned in Cholodny's book (16). It only remains to be added that neither Hamner and Bonner (27), Sivori and Went (54), nor any other workers have obtained a flower-forming effect with any combination of known growth substances or vitamins applied to leaves or roots, except in the pineapple (see Section J, page 96). However, the number of flowers may sometimes be increased by a variety of chemicals, in plants which are already flowering.

About the only safe conclusion from these experiments is that the flower-forming material *may* pass outside the tissue, but it is not proved. It seems certain, however, that quite small amounts of material are involved, and that small amounts of living tissue suffice to transmit it. In some respects the data are suggestive of the behavior of viruses.

F. Specificity of the Material

Numerous experiments show that the "hormone" is not species specific, and, what is more important, that the flowering "hormones" of long-day and short-day plants are the same. Moshkov (47) used Samson tobacco, grown in continuous light, as hormone donator, and Maryland mammoth as acceptor. Grafts of Samson on the latter caused it to flower in continuous light, provided only that the grafted scion was fairly large (25–30 cm. long). Short scions (4–5 cm. long) were inadequate, perhaps because they did not contain fully developed leaves (see above). Cajlachjan (11) similarly used sunflower (*Helianthus annuus*) as donator and artichoke (*Helianthus tuberosus*) as acceptor in grafting experiments and obtained good flowering in the latter. Heinze *et al.* (30) made numerous grafts of soybean varieties on one another, and obtained good transmission of the flower-inducing stimulus, particularly when the acceptor plant was defoliated. Where single leaves were the donator, it was necessary for them to stay on for four days to cause flowering in the acceptor.

More remarkable is the nonspecificity in Melchers' experiments (43), in which shoots, or even single leaves, of the short-day Maryland mammoth tobacco were grafted close to the growing point of one-year-old plants of *Hyoscyamus niger*. Both plants are in the same family (*Solanaceae*), but separate genera. The *Hyoscyamus* was thus induced to flower, but the curious result was obtained that it flowered equally whether the tobacco had been grown on long days or short. This evidently means that even in long days the tobacco produces the "hormone," but is prevented from flowering either because there is not enough of it, because some other factor is needed as well, or because an antagonistic substance is also present (see below). In later experiments a leaf of *Hyoscyamus* grown in long day, grafted on to the Maryland mammoth tobacco induced the latter to flower in long day. Whatever the explanation of these phenomena, it is quite clear that the "hormone" is nonspecific.

G. The Light-Sensitive System

At least in the case of the long-day plant, it is evident that the "hormone" must be produced by light. Considerable interest therefore attaches to the photosensitive system involved, particularly since it must be mainly present in mature leaves. Moshkov from the first considered chlorophyll and the ordinary photosynthetic system to be responsible, and he explained the difference in effectiveness between young and mature leaves as due to differences in the amount or activity of chlorophyll. But only recently has this view had any direct support.

The first evidence that photosynthesis is involved came from the experiments of Parker and Borthwick (50a), who showed that carbon dioxide must be supplied to the plant in order for photoinduction to lead to flowering. That this is due to the need for carbon dioxide in the actual photoinduction process, and not for the general life of the plant, was made clear by Harder and Witsch (29) using an individual leaf of *Kalanchoe* as hormone donator, and showing that carbon dioxide must be specifically supplied to that leaf, while it is on short day.

As early as 1933 Rasumov showed that red light behaves like white for the photoperiodic effect, while blue and green act like darkness. Withrow and Benedict (60) and Katunskij (31) confirmed this in general, though with some differences in regard to the effect of blue on certain long-day plants. Funke (20), however, finds the effects of red and blue light are different for different plants. More careful spectral studies using illumination of equal intensities (59) have shown that both in long- and short-day plants the longer wavelengths of the visible spectrum between 5770 and 7000 A. are the most effective (see Fig. 4). This

FIG. 4.—Influence of color of light on flowering. The light was given at 100 ergs/cm.^{2} (with blue also at 400 ergs/cm.^{2} in center pot) as supplement to natural day to make a total of 24 hours illumination.

Above: *Scabiosa atropurpurea*, Scabious, after 81 days; below: *Spinacia oleracea*, Spinach, after 37 days. (From Withrow and Withrow, 61.)

obviously suggests the spectrum of chlorophyll, and indeed Katunskij (31) specifically noted a secondary maximum in the blue and concluded that the effect of different wavelengths "well correlates with spectra of chlorophyll absorption."

Recently Parker *et al.* (51) have made a more thorough study with a specifically designed spectrograph to test this. Instead of giving the whole illumination by selected spectral bands, with all the accompanying

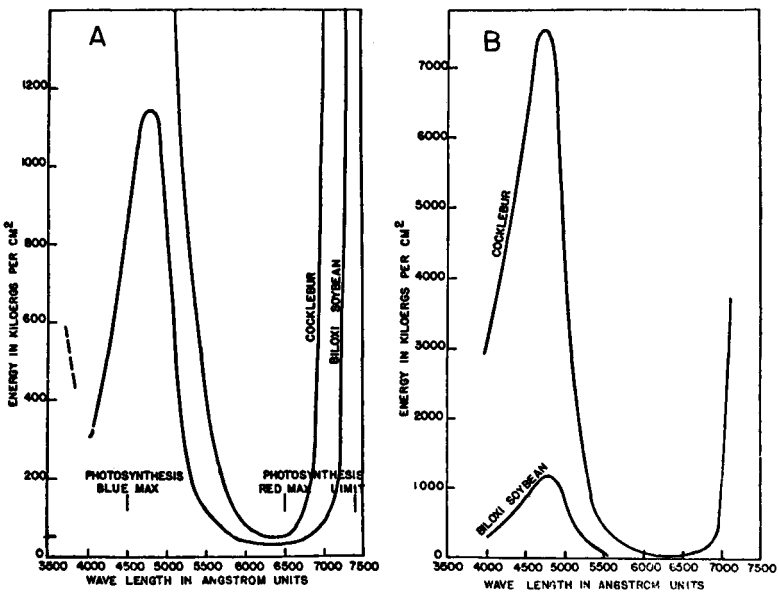

FIG. 5.—Composite action spectrum for suppression of floral initiation in soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*), plotted on two different ordinate scales. The soybean curve represents energy required at middle of fourteen-hour dark period to prevent floral initiation: the cocklebur curve represents energy similarly required at middle of twelve-hour dark period. (From Parker, Hendricks, Borthwick, and Scully, 51.)

complications due to different amounts of etiolation and photosynthesis, they used the spectral bands to interrupt the dark period. With Biloxi soybean and with cocklebur a brief interruption of the minimum dark period, providing this interruption occurs near the middle of the period, prevents flowering (see 25). The minimum energy needed to prevent initiation of flower buds is plotted against wavelength in Fig. 5. The position of the cut-off at the red end, and the sharp drop between 4900 and 5400 A., are particularly suggestive, but the agreement with the chlorophyll spectrum at the blue end is not so good. . The tentative con-

clusion is "the action spectrum is due to a porphyrin-like material which is *probably* chlorophyll."

H. Theoretical

Hamner (26) has put forward the following theory to explain in general terms the phenomena discussed above for short-day plants: (*1*) A substance or condition A is produced by light; its rate of production varies with temperature and with light intensity, and it decomposes slowly in darkness or in weak light. In both short- and long-day plants A increases up to a maximum with increasing time of illumination. (*2*) A substance or condition B is produced in darkness, also increasing up to a maximum with increasing dark time. Brief exposure to light destroys B at once (one minute's lighting during the dark period prevents flowering of *Xanthium*, 27). (*3*) When B reaches threshold concentration it interacts with A to produce the flowering hormone or flowering condition C. The stability of C varies in different plants, as shown by differing degrees of transfer in grafting experiments, etc. The minimum dark period for flowering of short-day plants is thus the time needed to reach threshold concentration of B.

The situation in long-day plants is less clear. Since some long-day plants can flower in continuous light, B would either have to be light stable in these plants or else conceivably not needed at all, *i.e.*, as soon as A reaches the threshold concentration C is formed.

An entirely different theory for long-day plants is that of Melchers and Lang (44a), according to which the failure to flower in short days is due to the breakdown of some essential carbohydrate. In *Hyoscyamus niger*, feeding of sugar allows flowering in short days; glucose, fructose, mannose, sucrose, and maltose were equally effective. Flowering was also induced in short days by placing the plants in pure nitrogen during the dark period; this, according to Melchers and Claes (44b) reduces the carbon dioxide production of the leaves. The normal Pasteur effect, however, would lead one to expect an accelerated carbohydrate breakdown in nitrogen. It is conceivable, therefore, that the striking results of these workers may have another explanation.

J. Role of Auxin

The relation between auxin and flower formation is somewhat obscure. In a general way auxin exerts an influence which opposes flowering. For instance, conditions leading to vigorous growth, and presumably therefore to active auxin formation, tend to delay flowering. An example is high nitrogen fertilization, which generally promotes vegetative growth and may delay flowering; see also Borodin's result (3) with low nitrogen given above. In tobacco, high nitrogen promotes high auxin formation

in the stem tip (2); however, this is not the case in tomato (57), which shows no correlation at all between growth rate, auxin production, and added nutrients. In oats, general nutrient deficiency (including nitrogen deficiency) hastened flowering (13a), but in millet it caused a slight delay.

The most striking instance of the antiflowering action of auxin is given by the experiments of Dostál and Hosek (19) on *Circaea*. In this plant isolated nodes from the apex will form flowers, those from the center will form leafy shoots, and those from the base storage organs. The flowering of the most apical nodes is, however, as was long ago observed by Dostál, dependent on presence of the leaf. If now the cut surface is treated with indoleacetic acid in lanolin ("auxin-paste") flowering is completely inhibited, and the bud forms instead either vegetative runners or tubers. The experiments were carried out under presumably long-day conditions (Brno, Czechoslovakia, in July). Here evidently the auxin has acted strongly against flowering. Another experiment with auxin is unfortunately by no means so clear-cut. Obsil (48a) reports that application of indoleacetic acid in lanolin to young shoots of *Lycopus* very strongly inhibited flowering, as compared to controls. The shoots were halved longitudinally, each pair of opposite buds thus furnishing one treated and one control bud, in the same stage of maturity. But since the criterion adopted was the actual opening of flowers, it is most probable that the effect was the normal inhibition of buds by auxin, which would be expected to occur, and which is discussed in Section VII of the preceding chapter. An isolated fact which may prove significant is the observation of Zimmerman and Hitchcock (62) that triiodobenzoic acid applied to tomato plants causes axillary buds to develop into flowers. There is reason to believe (20a) that this substance is an antagonist of auxin (in high concentrations),[5] since in soybeans it inhibits elongation and promotes lateral bud development, while it decreases auxin curvatures in the *Avena* test. Treatment of soybeans with 200 mg./l. triiodobenzoic acid increased the average number of flowerbuds from 3.2 to 36.2. However, it did not cause flowering on long days. Galston (20a) concludes that there is normally antagonism between auxin and the flowering hormone.

A very interesting and suggestive experiment of Sokolovsky (cited in 16) should be mentioned in this connection. It will be remembered that in Moshkov's 1936 experiments, the plants in which alternate leaves along the plant were given short day did not flower. Sokolovsky found that if these plants were decapitated they did flower. A similar phenomenon was observed by Reece, Furr, and Cooper in the mango (53), in which removal of the terminal bud during the flowering causes the axillary

[5] In lower concentrations triiodobenzoic acid actually promotes the effect of auxin (54a).

buds, which would have remained vegetative or dormant, to differentiate into flowers. Since the terminal bud is the major source of auxin in the plant, it might be suggested that removal of this source is enough just to turn the balance between auxin and flowering "hormone."

Defoliation acts in a similar way. In *Hyoscyamus niger*, Lang and Melchers (38) obtained flowering on both short and long day when the plants were completely defoliated; one leaf regrafted and maintained in short day was enough to prevent flowering (37). Leaves are of course a source of auxin though not so powerful as the terminal bud.

When seeds are treated with auxin and growth acceleration results (15,55) there is often a slight delay in flowering.

A striking exception to this generally somewhat antagonistic effect of auxin to flowering is furnished by the pineapple. Here a brief treatment with any one of several auxins (indoleacetic, naphthaleneacetic, and 2,4-dichlorophenoxyacetic acids, in particular) induces flowering promptly and almost quantitatively (17,18,49). Some varieties respond only in certain seasons (18), others at all times and with a treatment of only 0.25 mg. per plant (49). No other plant of all those used in the various types of auxin or of flowering experiment responds in this way, so that for the present this behavior must be regarded as quite exceptional.

Cholodny, in his book (16), attempts to support the thesis that the flower-forming stimulus is exerted by a group of substances, one of which is auxin. They are supposed to be effective only in certain specific proportions. However, the possibility that auxin plays at least some part in promoting flowering had been considered by Cajlachjan and Zdanova (14), who made some experiments designed to show that leaves produce the most auxin under conditions in which they do not produce much flower-forming "hormone." They diffused auxin from leaves into agar blocks, and applied these to the outside of coleoptiles—a somewhat insensitive method—and the results, so far as leaves are concerned, were inconclusive. They did show clearly, however, with stem tips that auxin production increases with the duration of illumination, and that this is so for short-day (hemp, chrysanthemum), long-day (lupine, mustard) and day-neutral (sunflower) plants (14). Production of flower-forming hormone, of course, is not a simple function of illumination, and at least in short-day plants must decrease with increasing illumination. The fact that the mature leaves have greatest flower-forming effect, as mentioned above, also shows that auxin is not the active agent, since mature leaves produce much less auxin than very young ones.

We may conclude that auxin, if it plays any part at all in flower formation, is in most plants an antagonist to the process. Whether flowering results from a balance between the flowering "hormone" and auxin or other antagonistic substances is not proven as yet, but the

phenomena of flowering do strongly suggest that at least two factors are working in opposite directions, and that the difference between short- and long-day plants is due to differences in the relative rates of synthesis or destruction of these factors.

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III. Leaf Growth Substances

As was mentioned in Chapter II, expansion of the leaf blade does not seem to be under the control of auxin, while growth of the veins probably is. Growth of the blade is very sensitive to light, leaves of seedlings grown in complete darkness being always very small and unexpanded. When equal energy exposures are given, the green region of the spectrum

is much less effective than the rest (15, and literature cited therein). The process is not, however, a simple function of photosynthesis, for Gregory (5) found in cucurbits that its temperature coefficient differs from that of photosynthesis, and deduced that a special photochemical reaction produces a substance which causes leaf expansion. In plants growing on controlled photoperiods, the size of the leaves is often a function of the length of the photoperiod (7), though the night temperature is also a controlling factor. Vyvyan (12) showed that leaf growth was dependent on the presence of cotyledons, and Went (13,14) confirmed and extended this, showing clearly that in the dark-grown pea seedling some factor or factors, stored in the cotyledons, controls expansion of the leaf blades. Part of his results are summarized in Table II.

TABLE II

LEAF AREA OF ETIOLATED PEA SEEDLINGS TEN DAYS AFTER OPERATIONS INDICATED

Condition of Plant	Total Area of First and Second Leaves, Mm.[2]
Before treatment	24
Roots and cotyledons removed	24
Cotyledons removed	24
Roots removed	41
Intact	42

It is evident that the cotyledons, but not the roots, promote leaf growth. Bonner, Haagen Smit, and Went (3) therefore examined the effectiveness of the diffusate from pea cotyledons in promoting leaf blade growth. They used discs cut from the bases of young tobacco or radish leaves grown in the light. The discs grew about 40% more in pea diffusate plus 1% sucrose than in the sucrose alone. The reaction is independent of pH between 4 and 7. Certain amino acids, particularly proline and asparagine, and some purines, particularly adenine, were active (2), but the greatest increase of growth obtained was only about 20%. Auxin, thiamin, and other vitamins were inactive. Embryonic pea leaves showed a much greater effect when cultured in the pea diffusate (3). As shown in Fig. 6, they reached a larger size on this medium in darkness than they would have done on the plant. In experiments of the greenhouse type, adenine was found to increase the leaf area of *Cosmos* plants grown in sand culture (2). It is of interest that adenine promotes the rooting of leaf cuttings (10) and that purines are known to be among the important nitrogenous constituents of leaves (11). Whether these substances really act as leaf growth hormones in the plant is, however, not proven. In cultures of isolated stem tips of rye (*Secale*

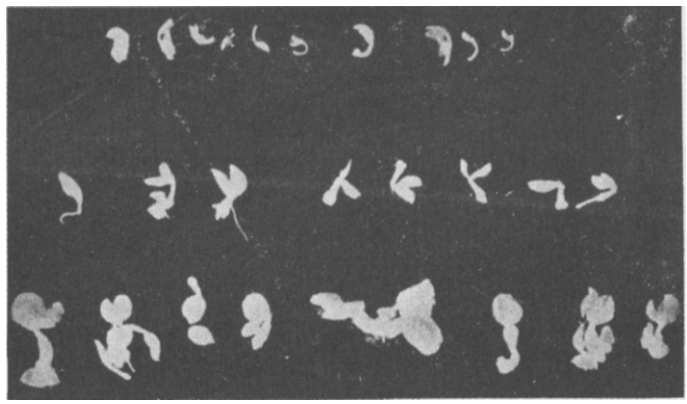

FIG. 6.—Growth of leaves excised from etiolated pea seedlings in culture solution after one month. Top row: in water alone; middle row: in inorganic salt medium plus 1% sucrose. Bottom row: in the same plus 1% standard pea diffusate solution. (From Bonner, Haagen Smit, and Went, 3.)

FIG. 7.—Left: Tomato shoot with simplified leaves and enclosed growing point (+). Right: Double leaf of tomato with fused petioles. Both from buds treated with auxin. (From Laibach and Mai, 6.)

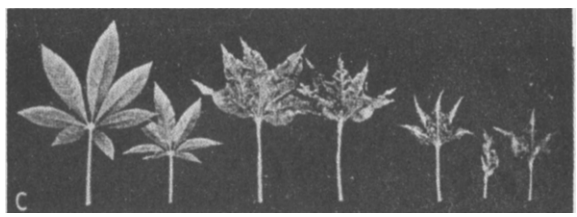

FIG. 8.—Leaves of *Cleome*. Left: Two leaves from control plants. Right: Five leaves from plants exposed to vapors of ethyl esters of 2,4-dimethylxyleneoxyacetic and α(2,4-dimethylxylenoxy)-propionic acids. (From Zimmerman *et al.*, 16.)

cereale) on a sucrose–salts medium, De Ropp (4) found no promotion of growth of the leaf by pea diffusate or any other plant extract, nor by any vitamins; hence the situation in monocotyledons may be quite different. Thus the whole problem remains in a suggestive, rather than a convincing, state.

Although auxins do not appear to promote growth of the leaf blade in formed leaves, they do so in the rapidly developing leaf primordia. This was first observed by Laibach and Mai (6), who showed that, when buds were treated with auxin, the subsequently developed leaves showed various abnormalities, including fusion of petioles and the growth of leaf tissue all round the growing point to enclose it like that of a monocotyledon (Fig. 7). That auxin applied to buds actually increases the size of leaf primordia was shown by Snow and Snow (8) and Ball (1). Recently a number of experiments with the vapor of esterified auxins has been carried out by Zimmerman and co-workers, from one of whose papers (16) Fig. 8 is taken (see Chapter 2, pp. 17–21, 51). It shows clearly that leaf blade (mesophyll) tissue has extended laterally under the influence of the auxin. Similar abnormalities were obtained by Ball (1) in *Tropaeolum*, the widening of the foliar primordia being particularly clear-cut and often leading to coalescence of two leaves at the base. An extensive histological examination of this phenomenon will be found in the paper of Ball. It is not easy to interpret such observations; embryonic leaves when damaged can regenerate their parts (9), so that some of these effects may be due to recovery after injury rather than to growth promotion proper. In any event, such responses seem to be limited to very young primordia.

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IV. Vitamins, Steroids, and Carotenoids as Plant Hormones

Since vitamins are produced in plants, and since they take part in reactions of fundamental and quite general importance, it is hardly surprising that they should, to some extent, act as hormones in the plants in which they are produced. The following is a very brief survey of the main aspects of their hormonal activity. The early work has been reviewed by Bonner (3) and a full review published by Schopfer in 1943 (49), of which Chapters 6 and 7 are particularly pertinent.

A. Vitamins of the B Group

1. *Thiamin*

The early work of Robbins in 1922 (39,42) and Kotte (28) showed that isolated excised root tips will grow for a time in a medium containing only inorganic salts and sugar, but better when yeast extract or peptone is added. By studying carefully the optimal concentrations of all constituents of the medium, White (1934) eventually was able to make continuous subcultures of tomato roots and thus to achieve "potentially unlimited growth."[6] The factor in yeast extract mainly responsible for the growth was shown simultaneously in 1937 by Bonner (2) for pea roots, and Robbins and Bartley (41) and White (56) for tomato roots, to be thiamin. Isolated roots can be grown indefinitely in the salts-sugar medium with added thiamin, although their growth is not as rapid as with yeast extract (see below).

The discovery that thiamin is a growth factor for higher plants was actually made, before the work on root cultures, by Kögl and Haagen Smit in 1936 (27), who used isolated embryos of peas, freed from the cotyledons, cultivated in the dark on a nutrient gelatin medium. They found that *biotin* greatly improved the growth of the shoot, but also that pure thiamin ("aneurin") at 0.01 mg./l. increased both the length and the branching of the roots. A selection of their results is given in Table III.

The response of pea embryos to thiamin as well as other factors was further studied by Bonner and Addicott (9). Many tissue cultures, growing in light, appear not to require thiamin (Gautheret, 22,23). Roots, like many microorganisms, can utilize a mixture of the thiazole and pyrimidine moieties instead of the intact thiamin molecule. Bonner

[6] For a complete discussion of plant tissue cultures see the reviews of White (55,58,60) and Gautheret (22,23).

TABLE III

GROWTH OF ISOLATED PEA EMBRYOS IN THE DARK ON SUCROSE—INORGANIC SALTS—GELATIN MEDIUM[a,b]

Addition, mg./l.		Shoot, wt.	Root, wt.
Thiamin	Biotin		
0	0	92	47
0.0008	0	96	57
0.008	0	107	61
0.04	0	104	61
0.4	0	119	62
0	0.01	127	48
0.004	0.004	112	55
0.2	0.02	137	62

[a] Fresh weight in milligrams after eight weeks.
[b] From Kögl and Haagen Smit (27).

(4) showed also that certain changes may be made in the molecular structure without impairing the availability of these compounds for growth. A hydroxyl group in the thiazole, and the 6-amino group in the pyrimidine seem to be essential. The requirements have been compared to those for numerous microorganisms in the review of Knight (26).

Evidence that thiamin promotes growth in plants does not necessarily establish it as a hormone, of course. The hormone function of thiamin in the plant derives from our knowledge of its production and distribution. The distribution of thiamin in the plant has been studied by the use of the fungus *Phycomyces blakesleeanus*, whose growth in a standard medium was shown by Schopfer (48) to be strictly proportional to the thiamin present. The method was worked out by Schopfer and Jung (50) and applied to plant tissue by Rytz (46), Burkholder and McVeigh (17), and Bonner (6). With this method it has been shown that the growing apex has the highest concentration of thiamin, and that there is a gradient of concentration from the youngest to the oldest leaves. Roots have a relatively low concentration; the thiamin is transported there from the leaves (15). The data of Burkholder and McVeigh (1940) for two varieties of corn (*Zea mays*) are summarized in Fig. 9A, and those of Bonner (1942) on tomato in Fig. 9B. It is of interest that in these different plants the absolute concentrations are very similar; 20 γ/g. dry weight in the tomato apex is about 0.07 millimoles/kg. dry weight, while 60×10^{-7} moles/kg. fresh weight in the corresponding tips

of the corn is about 0.06 millimoles/kg. dry weight. The relative concentration in the roots is, however, lower in corn than in tomato, averaging in fourteen hybrids only a quarter of that in leaves of medium age, while in tomatoes the value is about two thirds.

These concentration data do not give any indication of direction of movement. Bonner's experiments on girdling (6) show clearly, however, that the thiamin travels out of the mature leaves to the young leaves and

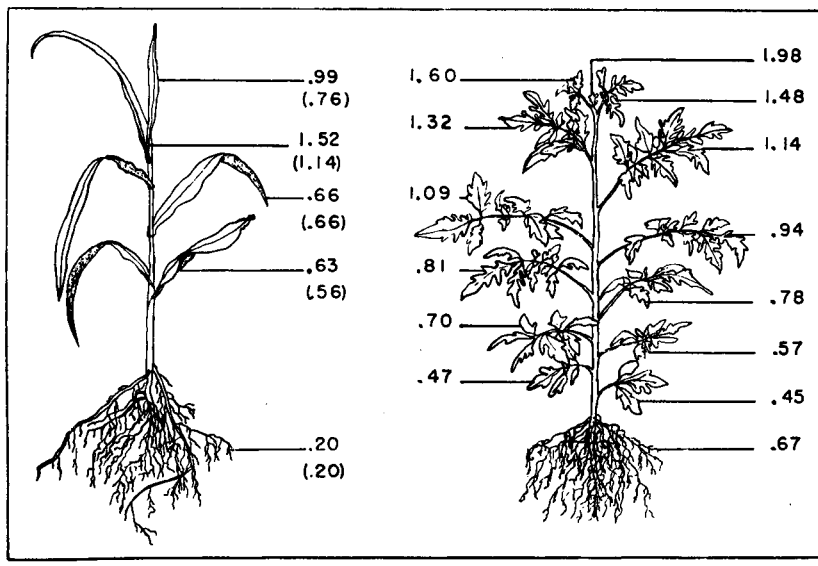

FIG. 9.—Distribution of thiamin in leaves, buds, and roots, expressed as γ/g. fresh weight. A: corn, data of Burkholder and McVeigh (17). The figures in brackets are determinations on another variety. B: tomato, data of Bonner (6). Bonner's data are given on a dry weight basis and have been corrected to fresh weight assuming 90% water content.

growing point, and to the roots. When the petiole of a mature leaf was girdled, thiamin accumulated above the girdle; when the main stem was girdled just below the apex and the youngest leaves, it accumulated below the girdle. When the main stem was girdled near the base (above the second node), however, thiamin accumulated above the girdle. These data not only show the direction of movement, *i.e.*, from mature leaves to the growing apex and to the roots, but indicate that at least most of the transport of thiamin takes place in the cortex. What the function of thiamin is in the growing leaves and terminal bud is not clear, but certainly in the roots it is essential for growth, as discussed above.

The actual function in roots is the same as in animal tissues, namely in decarboxylation of pyruvic acid. Horowitz and Heegaard (25) have shown that the carboxylase of pea roots uses thiamin pyrophosphate as coenzyme. The thiamin seems to be very closely linked to protein; during the action on pyruvate the enzyme loses much of its activity through the splitting off of pyrophosphate, but the thiamin remains protein-bound. Thiamin is therefore a hormone produced in the leaves and transported to the roots to induce growth there, *i.e.*, a true growth hormone.

2. *Pyridoxine*

In investigating the question as to why growth of isolated tomato roots was better when brown sugar was used instead of pure sucrose (the usual inorganic salts and thiamin being present), Robbins and Schmidt (43,44,45) studied the influence of various possible impurities in the brown sugar. The ash was only very slightly beneficial, while amino acids and nicotinic acid were without effect, but pyridoxine (vitamin B_6) had a large and immediate effect. The average weight of roots in 50 ml. of culture solution was raised from 3.4 mg. with 5 γ thiamin to 16.1 mg. with 5 γ thiamin plus 1 γ pyridoxine. Robbins and Schmidt consider, therefore, that on thiamin alone the roots synthesize enough pyridoxine for slow, but not for maximum, growth. Curiously enough, White (57) could not at first confirm this effect of pyridoxine either with his or with the Robbins and Schmidt strain of tomato. Nevertheless, Bonner and Devirian (12) did confirm it with another strain, and Bonner (8) again found pyridoxine essential for growth with three clones of tomato root and also (5) for roots of sunflower (*Helianthus annuus*). Subsequently White in 1943 (59) did find an acceleration of growth when pyridoxine was used as supplement to thiamin in the tomato root clones of all three groups of workers.

If it is accepted that pyridoxine is essential for root growth, at least in some plants, then data on the distribution and movement of this substance in the plant are needed to establish its hormonal nature. It is evidently not synthesized in the roots themselves. Bonner and Dorland (13), using a *Neurospora* mutant for bioassay of pyridoxine, find the highest concentration in the young (but not the youngest) leaves and a steady decrease throughout the older leaves. There is also a gradient in the stem from apex to base, although the roots appear to contain more (14 γ/g.) than the basal part of the stem (4–9 γ/g.). Girdling experiments show, again, accumulation above a node near the base and below a node near the apex, also above a girdle in the petioles of mature leaves.

It is evident, therefore, that pyridoxine is mainly produced in young but mature leaves (*cf.* the flowering "hormone" discussed above) and transported both to the growing apex and to the roots. Since it promotes growth at least in the roots, pyridoxine must be classed as a growth hormone.

3. *Other Compounds*

The situation for the other vitamins of the B group is not so clear.

Nicotinic acid was originally shown to be essential for pea roots and for tomato (12); but neither White nor Robbins and co-workers could at first confirm the effect. Later, however, Robbins (40) and Bonner (8) showed that different strains or clones of tomato roots vary greatly in their need for nicotinic acid. White (59) finds a small beneficial effect of nicotinic acid when glycine, thiamin, and pyridoxine are all present. By analogy with other such cases, particularly among microorganisms, it is probable that all roots require nicotinic acid for growth, but that many strains can synthesize sufficient for their needs. As yet no data are available on the distribution and transport of nicotinic acid, but since some roots at least do not produce it in optimum quantities, it is likely that they will be stimulated by any which reaches them from the shoot; this would make nicotinic acid a sort of growth hormone, at least in certain strains of tomato and pea.

Pantothenic acid shows a gradient of concentration from apex to base in the tomato plant according to Bonner and Dorland (14), but the concentration in the roots (29 γ/g. dry weight) is about equal to that in the apex and youngest leaf (35.7 and 23.3 γ/g., respectively), so it is possible that it is synthesized in the roots. In any event, it is not certain that there is a real requirement for pantothenic acid in roots or any other part, though a growth-promoting effect in the pea embryo has been reported (10). Its accumulation at girdles indicates transport similar to that of thiamin and pyridoxine. Riboflavin, on the other hand, though showing a gradient of concentration from apex to base, did not accumulate much above girdles on the stem or on petioles, and Bonner (6) has found evidence that it is synthesized in root tips of tomato and four other plants.

Biotin promotes growth in isolated pea embryos, especially of the shoot (Kögl and Haagen Smit, 27) (see Table III, above), and is evidently supplied to the growing seedling from the cotyledons, in which most of the biotin is stored. Furthermore, biotin promotes the formation of roots in response to auxin, when ample auxin is supplied at the same time (see 54, Chapter XI). It has no effect on the growth of isolated oat coleoptiles. In addition to the limited experiments with pure

biotin, Dagys (19) has made a number of determinations of the distribution of "bios II." The bios activity was determined on yeast growth. It may be identical with biotin, or with biotin plus thiamin. The bios II content of buds increases sharply in the spring when the buds begin to develop, and remains high during the summer in mature and growing leaves. In the growing seedling it decreases in the cotyledons and increases in the embryo. Thus, although its activities are not entirely clear, biotin may well prove to be a plant hormone.

In the above discussion, attention has been centered on substances which behave as hormones in the strict sense of the word, not merely as "growth factors." Thus ascorbic acid definitely promotes growth in isolated pea embryos (11) and in whole tobacco plants (20) and to a smaller extent in wheat (24); riboflavin promotes growth of eggplants (20) etc., but its role as a hormone is not clear. The following two sections will summarize briefly a large quantity of experimental work whose significance for the hormonal control of growth and development is much more debatable.

B. Steroids

Accelerative effects of steroid preparations on plant growth have been claimed by numerous workers in the past fifteen years. At first, the presence of auxin in many of the crude steroid preparations engendered doubts, but more recently clear-cut effects have been obtained. Pure estrone was shown to promote growth in the pea embryo by Kögl and Haagen Smit (27) and in other isolated embryos (10). Various investigators, especially Scharrer and Schropp (47), have found acceleration of flowering or growth promotion on treating whole plants, or even fields of crops, with animal sex hormones. However, many negative results have also been reported (see the reviews of Thimann, 52, Bonner, 3, and Bomskov, 1). Some of these may be due to lack of control of other conditions; for instance, Chouard (18) found that dihydrofolliculin (estradiol) accelerates growth and flowering of asters, but only when on an eight-hour day; when given 15 to 22 hours of illumination no effect of the sterol was observed. With *Fuchsia*, Burkhardt (16) found that high dosages of estrone only gave growth promotion when the "microelements" were added to the nutrient solution. Lower estrone concentrations promoted growth and flowering under all conditions of mineral nutrition. A clear acceleration of growth and increase in dry weight were obtained in three varieties of a grass, *Poa alpina*, by Zollikofer (61). Interestingly enough, Zollikofer subsequently found (63) that diethylstilbestrol is also active

in promoting vegetative growth, and for a given concentration appears somewhat more active then estrone. This certainly suggests something in common between the effects on plant and animal tissue.

If there is really a requirement of steroids for plant growth, then it is evident that plants vary a great deal in their ability to synthesize enough for their needs. Although steroids do occur in plants, evidence that they are produced and transported as true hormones is wholly lacking. Presence of steroids of the estrogen type was first shown in plant material by Loewe and Spohr (35), and by Dohrn *et al.* (21) as early as 1926. There is some evidence for the occurrence of male hormones also (see Bomskov, *loc. cit.*).

At first it was thought possible that the steroid sex hormones might control sex in plants, but the effects observed can, with one exception, be ascribed to an influence on growth generally (see Zollikofer, 62). The exception, however, is provided by the interesting work of Löve and Löve (33,34) on various types of normal and intersexual flowers of *Melandrium dioecium*. Crystalline estrone, estradiol, and estradiol benzoate, applied in lanolin paste to the axils of leaves in which flower buds would later develop, definitely shifted the subsequent flowers toward the female side, suppressing the development of anthers and promoting that of the gynecium. Testosterone and its propionate had the opposite effect, promoting maleness. These results apparently establish that animal sex hormones *can* control the sex expression of plants. It remains to be seen, of course, whether such control is exerted by these substances under physiological conditions and in the concentrations normally present.

C. Carotenoids

Apart from their role in absorbing the light responsible for phototropic curvature, (see Section V of the previous chapter), the claimed hormonal effects of the carotenoids are few. Lazar (32) found that carotene promotes root formation in *Impatiens* seedlings. Such an effect has not been reported in other plants, and remains unconfirmed. More remarkable are the experiments of Moewus (36) and of Kuhn, Moewus, and co-workers at Heidelberg (29,30,31). According to this work, the unicellular green alga *Chlamydomonas eugametos* is controlled in many of its activities by the carotenoids crocetin and safranal and their derivatives, which are excreted from the cells into the surrounding solution. Crocin, or crocetin gentiobioside, whose excretion is promoted by red light, causes motility of the gametes. Crocetin dimethyl ester causes copulation of these gametes, and the sex affected depends on the previous irradiation of the solution. There are eight sexes, from the strongest female through intermediate forms to the strongest male, and the copu-

lation of each requires a specific period of irradiation with blue light. This was traced to a conversion by light of the *cis* into the *trans* isomer. Thus 95% *cis* and 5% *trans* activates the strongest females, 85% *cis* activates the next group, 75% the next, and so on; finally 5% activates the strongest males. Further, safranal causes maleness and a glucoside of safranal, picrocrocin, causes femaleness. The published results have certain inherent improbabilities, which are discussed by Philip and Haldane (38), Thimann (53), and Murneek (37); and, though Smith (51) did find a small effect of light in promoting copulation of gametes of three Californian strains, no other part of the work has been confirmed elsewhere. The interpretation is made more complex, too, by the later finding (28a) that the activity of picrocrocin is probably due to an impurity of 10^5 times higher activity. This substance, obtained from a *Crocus* species, appears to be a methyl ether of quercetin and thus quite unrelated to the above carotenoids. An excellent summary of this work has been given by Lang (31a).

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V. Additional Postulated Hormones

We have seen that, in the case of flower formation, the observations point strongly to the existence of a flower-forming hormone or "florigen,"

but that proof of the existence of such a hormone has not been forthcoming. In two other cases there is evidence for the functioning of a special substance or hormone, but proof of its existence has not been obtained. These have been brought out by the work of Went, who has referred to the postulated substances as "calines."

A. Rhizocaline

When in 1925 van der Lek (11) carried out his early experiments on root formation in cuttings, he postulated that the developing bud forms a hormone which moves downward in the cutting through the phloem and accumulates at the base, producing roots there. Went later (20) found that the diffusate of leaves promoted root formation, and Bouillenne and Went (2) showed that the active substance is transported polarly from apex to base; it appeared to be stored in buds and cotyledons, and formed by leaves in light. To this hormone they gave the name "rhizocaline." When it was subsequently found that the root-forming hormone was identical with auxin (see preceding chapter, Section VI), the conception of rhizocaline as a specific root-forming factor was retained by Went (21,22), and the idea put forward that auxin causes root formation primarily by inducing the accumulation of rhizocaline in the basal zone of the cutting. On the basis of experiments with hypocotyls of *Impatiens* seedlings, which form large numbers of roots without auxin and show very little increase when treated with any concentration of indoleacetic acid, Bouillenne and Bouillenne (1) insisted that auxin is not "the root-forming factor." In an extensive study of plant tissue cultures, Gautheret also concluded (8) that although root formation is due to hormones produced in buds, these hormones are not identical with auxin. The experiments of Howard (10) on root formation in kale at first led him to the conclusion that auxin converts leaf initials into root initials, but he later showed that new root initials were formed very close to the auxillary bud. Whether a shoot initial once formed can ever be *converted* into a root is thus not clear.

It should, of course, be remembered that sucrose and thiamin are required for the roots to grow out, and in some plants also nicotinic acid and pyridoxine. Thus auxin is certainly not the only factor controlling the formation of visible roots. Indeed, in the kidney bean (*Phaseolus vulgaris*) Thimann and Poutasse (19) showed that a supply of available nitrogen, particularly potassium nitrate, asparagine, or adenine, promotes root formation much more strongly than does auxin, which presumably is present in nearly optimum concentration. These materials exerted their effect partly by promoting the maintenance of the cutting, an effect which was also exerted by the leaves (see below). In *Impatiens*,

too, the amino acids glycine and alanine had an effect on the general maintenance of the hypocotyl cuttings (1). These substances, however, are essentially external factors. There are clearly internal factors other than auxin involved in root formation. Many plants do not root from cuttings even with optimum auxin treatment. The peculiar fact that cuttings from young plants may form roots freely while cuttings consisting of tissues of the same age, but from older plants, do not do so was first noticed by Gardner (7). This was extended to various trees, especially pines and spruces, by Thimann and Delisle (18). They showed that this difference in rooting ability persists even in presence of optimum auxin treatment. There is also a difference between the responses of different types of cuttings made from the same plant. Recently van Overbeek and Gregory (15) studied the parallel case of rooting and nonrooting varieties of the same plant. Leafy scions of red ("rootable") *Hibiscus* were grafted to woody stocks of the white nonrooting variety and the resulting cuttings, after auxin treatment, formed roots readily. This experiment strongly indicates that an internal transportable factor, coming from the leaves (*cf.* 4,19), cooperates with auxin in root formation. Indeed many workers have found a strong effect of leaves in promoting rooting of a variety of cuttings (see Section VI of the preceding chapter). On closer analysis (14) this factor supplied by the leaves of *Hibiscus* proved to consist of carbohydrate and nitrogen nutrients, and to be wholly replaceable by known substances, particularly sugar, ammonium sulfate, or arginine, in physiologically reasonable concentrations. The concept of a "hormonal" factor, therefore, receives no support from this work.

Evidence for the mobilization of rhizocaline by auxin treatment was brought by Cooper (3), who treated lemon cuttings at the base with 170 or 500 mg./l. indoleacetic acid and after 15 hours cut off $\frac{3}{4}$ in. of the base. On now re-treating with auxin, very few roots were formed—in fact no more than when the bases were cut off without a re-treatment. Controls from which the bases were not cut off rooted freely. The portion removed is thus thought to have contained the rhizocaline. However, Hellinga (9), Pearse (16), and Dorfmüller (6) repeated Cooper's experiments with various other plants and found no such effect. Indeed, Cooper himself obtained this result only with certain auxin concentrations and times of treatment. In Hellinga's experiments with *Coleus*, it was necessary to apply sugar to the cuttings. Went (24) points out that in Pearse's willow cuttings most of the roots are formed from pre-existing primordia and not developed *de novo*, and shows that, in pea seedlings treated basally with 500 mg./l. indoleacetic acid, cutting off the base and retreating does not produce as many roots as in controls treated first

with water. To some extent the same treatment may be applied unwittingly when cuttings are treated basally with too high an auxin concentration. For instance, Thimann and Delisle (18) showed that with blue spruce the treated base, which presumably would contain the mobilized rhizocaline, dies completely but roots are then formed above the dead portion.

Somewhat more indirect though very suggestive evidence is given by Went's experiments (23) on root formation at the base and apex of auxin-treated seedlings. When auxin is applied to dark-grown pea seedlings at the apex, the location of the resulting roots depends on the auxin concentration used. At low concentration the polarity of transport is normal, the auxin goes to the base, and all roots are formed at the base. At high concentrations the transport system is overloaded or paralyzed (30) and some of the roots occur at the region treated, *i.e.*, at the apex. When this happens, however, the number of roots at the base does not remain maximal but actually decreases. In other words "the roots at the apex are formed at the expense of those at the base" (22). Went concludes, therefore, that the total number of roots is limited by a factor other than auxin.

Phenylacetic acid is quite inactive for root formation in cuttings of etiolated pea seedlings, but such cuttings, if first treated with phenylacetic acid, afterward give an increased rooting response to auxin (24). This curious behavior is explained by Went in terms of the mobilization of rhizocaline by the phenylacetic acid, which in this respect is considered to act like a true auxin. He thus envisages root formation as a dual process: (*1*) the accumulation of rhizocaline at the base, which may be brought about by substances inactive or only weakly active as auxins, and (*2*) the activation of the rhizocaline, resulting in the formation of roots; this requires true auxins. The only reasonable conclusion from all these experiments is that there probably is more than one internal "root-forming" factor, but the evidence that auxin "mobilizes" such material is as yet far from convincing.

B. Caulocaline

The experiments of Went (22,23), which indicate the storage of a leaf-forming factor in pea cotyledons, were discussed above (p. 99). Very similar data were obtained which suggest the production in roots of a stem-forming factor. Seedlings were decapitated and the stem length of the resulting lateral branches was measured. The clearest experiment is shown in Table IV. It is evident that stem growth is dependent on the roots, but not on the cotyledons. The factor responsible for stem growth was termed "caulocaline."

TABLE IV

STEM LENGTH OF AXILLARY BUDS AFTER DECAPITATION AND PLACING BASES IN 2% SUCROSE[a]

Condition of Plants[b]	Stem Length of Buds, Mm.
Cotyledons and roots removed	1.0
Roots removed	1.9
Cotyledons removed	21.2
Intact	26.3

[a] From Went (22).

[b] Dark-grown plants, kept in dark throughout.

The provision of sucrose solution obviates the possibility of carbohydrate as a limiting factor and goes some way toward eliminating the role of water. The role of roots in promoting stem growth might, however, be due to improved water supply, as was suggested by de Ropp (5) in connection with his observation that stem tips of rye show greatly increased growth when they form roots. A demonstration of increased stem growth without the participation of roots is therefore desirable. This has been furnished by Went and Bonner (29), who cut off tomato stems at the base and kept them in darkness with various solutions applied to two of the leaves, the bases being in water. Such stems grow little and do not respond to auxin appreciably, though they do grow after roots have been formed. The application of coconut milk to one leaf, however, definitely increases stem growth (see Table V). The use of coconut milk was suggested by the finding of van Overbeek, Conklin, and Blakeslee (13) that this material promotes the growth of plant embryos in tissue culture. Pea diffusate and, to a lesser extent, yeast extract or potassium nitrate solution were also active.

TABLE V

ELONGATION OF TOMATO STEMS IN DARKNESS[a]

First leaf in	Second leaf in	Growth, mm.[b]	
		First day	Second day
Water	Water	0	Dead
Sucrose 10%	Water	2.7	0.3
Sucrose 10%	Coconut milk 100%	4.2	2.9
Sucrose 10%	Coconut milk 50%	4.5	3.1

[a] From Went and Bonner (1943).

[b] Mean of six plants.

Extracts of roots were, however, inactive. This experiment certainly indicates that some factor besides auxin or sugar, though not necessarily

a hormone, is necessary for stem growth. Another experiment of Went (26) goes far toward eliminating the factor of water supply as an explanation of the effect of roots on stem growth. In this a part of the root system was submerged in nutrient solution, the other part allowed to grow in moist air. Such plants showed greater stem growth than controls with the roots wholly immersed even though vigorously aerated. Went concludes that the oxygen requirement for caulocaline production is greater than that for uptake of salts and water.

In other experiments Went (27,28) has attempted to determine what are the limiting factors for growth of the entire plant. Neither in peas nor in tomatoes is the ether-extractable auxin content of the tip correlated with general growth rate. In tomatoes the water supply from the roots also does not limit growth. In peas, in which different stem growth rates were obtained by means of different grafting combinations, Went concludes (23,27) that growth rate depends primarily on a factor coming from the stock, *i.e.*, stem base and root system; this factor is designated as the caulocaline.

Strong evidence that roots are not *essential* for stem growth, however (though they appear to promote it), comes from two recent studies. Loo (12) succeeded in growing isolated stem tips of asparagus in a simple nutrient medium and making apparently unlimited transfers. These rootless stem tips grow indefinitely in light, though on the rare occasions when roots were formed the growth rate of the stem tips increased three- or four-fold, as was noted also by de Ropp (5) with rye stem tips. The other is that of Skoog (17) with tissue cultures of callus formed by a tobacco hybrid, described and first cultured by White. White showed (31) that these calluses, which grow as organless tissue when on the surface of solid media, readily produce stems when *immersed* in the culture solution, and Skoog's observations make clear that such stems are formed and elongate freely, quite independently of roots. Roots indeed are very rarely formed, though occasionally a well-developed stem with leaves will give rise to a root. Skoog concludes that no "caulocaline" is necessary for stem growth. Internal factors may, of course, play an important part in controlling growth and differentiation "but in contrast with calines these substances must be present in all cells" (17). It is, of course, not excluded that they may be produced more vigorously in roots than in stems.

Finally the proposed role of caulocaline in bud inhibition may be mentioned briefly. As shown in Chapter II, pp. 39–41, the application of auxin in place of the terminal bud causes the continued inhibition of development of the lateral buds. Went (25) brought forward a number of experiments to show that this action is due to the mobilization of

caulocaline by the auxin, *i.e.*, it is accumulated at the point where the auxin is applied, so that none is available for growth. But (as was described in Section VII, A of the preceding chapter) lateral buds may be inhibited when the auxin is applied directly on them, and not elsewhere on the stem, and isolated lateral buds growing in nutrient solution are strongly inhibited by auxin in the solution. It is possible, of course, that such inhibition *in vitro* may not be the same phenomenon as inhibition of buds on the intact stem, but evidence for this is lacking. Although the phenomena of inhibition are very puzzling, such facts make it difficult to invoke the mobilization of a bud growth factor to explain them.

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VI. Hormone-Like Substances in Fungi

Compared to the amount of work on higher plants, the physiology of the fungi has been surprisingly little investigated. Nevertheless, there

are a number of instances in which some process has been either postulated or proven to be controlled by a substance produced within the organism. Most of these are connected with the sexual reaction. The influence of externally applied substances, particularly vitamins, on sexual development or on the production of fruiting bodies will not be discussed here. This work has been reviewed, together with all effects of vitamins on fungi, in the book by Schopfer (18).

The first evidence of the sort here considered was brought for members of the *Zygomycetes*, in which hyphae of + and − strains fuse to form zygospores at their point of contact on a solid medium. As long ago as 1924, Burgeff (4) showed that in *Mucor mucedo*, before the two mycelia come into contact, there is inhibition of elongation, followed by characteristic swelling and branching, which he considered as the initial stages in the sexual reaction. By separating the + and − strains with a collodion membrane these effects were proved to be due to a diffusible substance (or substances), both strains being affected.

Burgeff's findings were confirmed by Kohler (7) and also, with another organism, *Phycomyces blakesleeanus*, by Ronsdorf (16), who obtained evidence that, as might be expected, two diffusible substances were concerned, one produced by each strain. The intensity of the sexual reaction was greatly increased by adding histamine to the medium. Thiamin was shown by Schopfer (19) to have a similar effect on *Phycomyces*, while in *Melanospora destruens* Hawker (6) has shown that both thiamin and the balance between carbohydrates supplied control the formation of zygospores. In a third organism, *Pilobolus crystallinus*, Krafczyk (8) again obtained similar results, showing clearly that, as Burgeff had indicated earlier, there are at least three distinct processes under hormonal control, namely, the branching and swelling ("telemorphosis"), the growth of special hyphae toward one another ("zygotropism"), and the delimitation of the gametangia.

Very similar phenomena occur in the aquatic forms, and here progress has been much greater. Couch in 1926 (5) observed some distance effects, corresponding to those of Burgeff, with *Dictyuchus monosporus*, but he could obtain no direct evidence for diffusible substances, the collodion membrane experiment being negative. However, Bishop (1) with *Sapromyces reinschii*, obtained much clearer evidence and was able to cause increased branching in the tips of the hyphae of the male plant by adding the water in which the female plant had grown. The extensive studies of Raper (1939–1942) with two species of *Achlya*, *A. bisexualis* and *A. ambisexualis*, include a similar experiment, as well as one with a cellophane membrane *à la* Burgeff. From observations of this type, as well as from the rigid sequence of events in the sexual reaction, Raper

(10) deduced that four substances are involved, as follows: Hormone A*, produced by the female plant, which starts the reaction by inducing the formation of antheridial branches near the tips of the male hyphae (*cf.* "telemorphosis," above); Hormone B, produced by the male plant after the above reaction, causing the formation of oögonial initials on the tips of the female hyphae; Hormone C, produced by the oögonial initials (and not by other hyphae of the female plant), which causes the antheridial hyphae to grow toward these initials (*cf.* "zygotropism," above), and also induces the delimitation at their tips of the male gametangia, or antheridia; and Hormone D, presumably produced by the antheridia, which causes delimitation of the oögonia from their stalks, and subsequent development of the oöspheres. Since this stage takes place usually after direct contact with the antheridia, the evidence that it is controlled by a diffusible substance or hormone is not fully convincing.

The existence of at least the first three substances was pretty well proved by exposure of plants at the appropriate different stages of development to diffusates from cultures of the opposite sex. The two *Achlya* species evidently use and produce the same hormones, though the production rates and sensitivities are different. However, chemical experiments so far are limited to Hormone A. Using a standardized measure of antheridial branch formation, Raper (11) obtained temperature, pH, and concentration curves, and discovered a marked, but irregular, diurnal periodicity in the response. Addition of 2.10^{-4} *M* malonic, glutaric, or pimelic acid greatly increased the production by the female plant. Concentration of Hormone A from large-scale cultures by Raper and Haagen Smit (12) through many stages led to a 70,000 times enrichment, but not to a pure preparation. It was concluded that the substance is a neutral ketone, and is active in a concentration of 1 in 10^{12}. Activity is destroyed completely by 2,4-dinitrophenylhydrazine, and partially by the reagent of Girard and Sandulesco. A number of barbiturates showed activity, but only at relatively high concentrations. Further chemical work will be awaited with great interest.

A reaction of another kind is that of the aggregation of individual amebae into a fruiting body, one of the stages in the life cycle of the *Acrasiales*. The spores of these organisms germinate into myxamebae which grow and multiply for a time, feeding on bacteria, and then suddenly flow together into a sort of mound, termed a pseudoplasmodium. In *Dictyostelium discoideum* the life cycle has been worked out in detail by Raper (13,14,15) and Bonner (2), who have considered the aggregation stimulus to be chemical in nature. This was virtually proved by the experiment of Runyon (17), who placed a cellophane membrane over an aggregating mass of myxamebae and found that additional myxamebae

*Hormone A was subsequently shown to be a complex of four substances (see Supplementary References).

above this would follow the aggregation of the pattern below. Bonner (3) has carried out many similar experiments, particularly with aggregation under water, and concludes that aggregation is due to the gradient of a substance, "acrasin," produced by all myxamebae, but unstable enough to be constantly breaking down, so that the gradient is maintained. No chemical work has yet been carried out. The phenomena of polarity and dominance observed in the aggregation are in many ways suggestive of those due to auxin in higher plants.

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Addendum

Papers which have appeared since this chapter on other plant hormones was written are listed below under the section headings which are used in the chapter. Readers should refer also to the supplementary bibliography of the preceding chapter.

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I. Wound Hormones

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D. Physiology and Interrelations with Auxin

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