THE ROLE OF AUXIN IN THE CORRELATIVE INHIBITION OF THE DEVELOPMENT OF LATERAL BUDS AND SHOOTS

by

J. H. G. FERMAN

(from the Botanical Laboratory of the State University, Utrecht).

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INTRODUCTION.

It is a long known phenomenon, that if in a plant the terminal bud of one of its shoots is removed, one or more of the axillary buds will develop. As long as men were interested in agriculture, horticulture and forestry, this facts must have been known, since it is the base of all pruning. In order to obtain tightly-stooled plants squeezing of the terminal bud is also often applied. In fir trees the phenomenon is particularly striking. When these for some reason have lost their terminal shoot, one, rarely more, of the lateral shoots quite close to it, growing in a horizontal direction first, will erect itself and grow in a vertical direction, thus physiologically replacing more or less the lost terminal shoot. An analogous phenomenon occurs after removing of the tip of the main root; one or more of the lateral roots below the tip change their direction of growing, bend downwards and physiologically replace the main root. This phenomenon, though known for a long time, remained unexplained until recently and then was only elucidated to a certain extent.

It was first explained by assuming that specific root- and shoot-forming substances existed, which were transported in opposite directions from apex to base and reversely. Another explanation was that the growing apex absorbed all the nutritive material available, so that the lateral buds became short of food and could not develop. On the other hand some other investigators thought, that the terminal shoots had an inhibiting influence on the lateral buds and shoots, on account of which it was called a phenomenon of correlative inhibition. Besides the terminal shoot, the leaves too appeared to exert such an inhibiting influence on the development of their axillary buds.

The problem was brought nearer to its solution, when it turned out, that here auxin, the phytohormone of cell elongation, is the correlation carrier. The auxin which is produced in large quantities by the terminal bud and by the young leaves

and is transported in basal direction would then inhibit the development of lateral buds and shoots in the intact plant. Soon, however, the difficulty arose how to explain this growth inhibiting action of auxin, as its action is generally growth-promoting. Several theories tried to solve this problem.

According to one of them the phenomenon would still be due to a direct action of auxin, but in this sense, that either the auxin coming from the terminal bud would prevent the lateral buds from producing auxin themselves and so from developing, or the high concentration of the auxin would inhibit this development. According to another theory, however, auxin would act here only indirectly. The auxin first promotes growth in the main stem and from this initial growth process a secondary growth-inhibiting influence acts upon the lateral buds and shoots. According to a third theory, besides auxin, at least two other specific substances, would be needed for the development of buds and shoots and these substances would be transported in acropetal direction. The function of auxin would only consist in attracting these substances to the production center of the auxin.

None of these theories gives an exhaustive explanation of the phenomena, as will be seen from the following discussion of literature. For this reason it seemed desirable to examine more closely and as quantitatively as possible the role of auxin in the correlative inhibition of lateral buds and shoots.

CHAPTER I.

Review and discussion of literature.

§ 1. *Early experiments: formative substances, nutritive exhaustion or inhibiting substance.*

SACHS (1874) in his essay „Ueber das Wachsthum der Haupt- und Nebenwurzeln II" points out, that after cutting off the tip of the terminal root of *Vicia Faba,* the lateral roots, growing out from the cut surface, will grow downwards much more perpendicularly than the lateral roots in an intact plant. He compares this to the behaviour of a lateral shoot a little beneath the terminal shoot, which, after the latter has been removed, will erect itself and grow perpendicularly upwards, in a way replacing the terminal shoot. SACHS (1880, 1882) tries to explain this by assuming that specific root-forming substances are flowing from the leaves to the roots in the intact plant, while

reversely shoot-forming substances are moving upwards to the terminal and lateral shoots.

As a matter of fact this was an organogenetic version of the theory of DUHAMEL DU MONCEAU (1758), who assumed two kinds of sapstreams, one flowing downwards from the leaves and serving for the formation of the roots, and the other moving upwards which promotes the growth of shoots and leaves.

DARWIN (1880) repeats the experiments of SACHS, but he does not find it necessary to cut off the tip of the main root for making one of the lateral roots replace the main root, as the same result could be obtained by pinching young radicles a little above their tips between the arms of a U-shaped piece of leaden wire. He considers this phenomenon as well as the analogous one, where the main shoot of plants and trees is removed, as a question of nutrition: the increased flow of sap into the lateral roots or shoots is the cause of their rapid development.

ERRERA (1904), however, concludes from his experiments that after the removal of the terminal shoot of *Picea excelsa,* the sapstream cannot be responsible for these phenomena. In intact fir trees the lateral shoots will develop quite nicely and do not make the impression of lacking anything. This makes him assume that the terminal shoot has a specific inhibiting effect on the lateral shoots, so to say of a catalytic nature.

GOEBEL (1902, 1903) originally tried to explain this correlation phenomenon by applying the theory of SACHS of the organ-forming substances as well as by the theory that the one part should monopolize the nutritive material to such an extent, that the other parts could not obtain sufficient to enable growth to go on. In GOEBEL's opinion the important cause is the direction in which the constructive material moves, the vegetative points acting as centers of attraction for the plastic material, their influence being weaker or stronger according to their position. Later (1908) GOEBEL thought that nothing but the concentration of the nutritive material could cause this correlation phenomenon.

MACCALLUM (1905) on the other hand, concluded from his regeneration experiments with leaves of *Bryophyllum calycinum,* that the means by which a terminal bud suppresses the development of the other meristematic cones of the plant do not lie in the withdrawal by the former of the nutritive materials or of the water, nor to a lack of a definite „formative substance". He believes in some influence independent of all these, which

an organ, acting perhaps along protoplasmic connections, is able to exert over other parts thus preventing their growth.

MOGK (1913) too, from his extensive experiments about the correlations of buds and shoots of various plants and trees, draws the conclusion that nutrition does not play an important part. The correlatively inhibited shoots rather seem to have lost the capacity of assimilating the available nutritive material.

According to LOEB (1915) the flow of material in the plant is responsible for phenomena of growth in leaves and stems of *Bryophyllum*. The apparent inhibition of growth in one place would be simply due to the fact that, under the conditions of the experiment, the substances required for growth flow to some other place and are retained there. Further the removal of inhibition creates conditions, which will force the substances to flow where we want growth to occur. In a later publication (1917) LOEB, however, tries to account for the correlative inhibition by assuming a geotropic hormone and shoot- and root-forming hormones. This would explain the fact that in certain fir trees a horizontal branch next to the apex may suddenly become negatively geotropic, when the apex is cut off. After the decapitation the hypothetical geotropic substance, which before was flowing to the apex, now can flow into the horizontal branches next to the apex and the one which by chance gets a little more of the substance than the others, will become vertical. From the fact that in *Bryophyllum* the apical bud prevents the lower ones from growing out LOEB (1917a) concludes, however, that there an inhibitory substance is send in the direction of the basal buds. The reason why the apical bud grows out first, would be that it is the first bud which is freed from this substance. In his later publications LOEB (1918, 1920) returns again to his first opinion, that the inhibitory influence of the growing stems or buds on the development of other shoots or buds is due to the automatic attraction of the material for growth by the stems or buds which grow out first.

APPLEMAN (1918, 1918a), studying the growth of potato sprouts, found that the buds on the apical end of the tuber grow out first and inhibit the growth of the more basal buds. If the tuber is cut into transverse slices the inhibitory influence of the apical buds is removed and there is a general growth of buds over the surface of the entire tuber. This proves that the slices still contain sufficient growth material to produce shoots and they were not prevented from doing so, because the terminal sprouts had automatically attracted the limited amoint of material for

growth, as LOEB (1918) had postulated.

Experimenting with isolated cuttings of *Citrus medica* REED and HALMA (1919) found that the dominant influence of the developing buds nearest the apex may be prevented from reaching lower buds by notching the phloem layers just above each bud. The experiments of APPLEMAN and REED and HALMA support the earlier view of LOEB (1917a), that the shoots developing nearest the apex form a substance which is capable of inhibiting the growth of other buds.

CHILD and BELLAMY (1919, 1920) succeeded in blocking the inhibiting action of the growing tip of *Phaseolus* upon other buds, or of a leaf of *Bryophyllum* upon buds of other leaves, by a zone of low temperature. 2 cm or more in length of the stem or petiole was surrounded by a coil of tubing through which a current of water flows at a temperature of 2,5 to 4° C. In their opinion this block does not prevent the flow of water and nutritive substances and the attempt to interpret this inhibition solely in nutritive terms, is therefore highly improbable. They conclude from their experiments that the inhibiting action in its passage from point to point depends upon the metabolically active protoplasm. Why, in their opinion, this should exclude the transport of an inhibiting substance, is not clear, as the movement of inhibiting substances might itself depend on the activity of living cells. By killing part of the stem of *Phaseolus* by means of steam, HARVEY (1920) succeeded in bringing about the same effect as CHILD and BELLAMY, namely the growing out of the axillary buds just beneath the dead zone, although the part above it remained alive.

By cutting potato tubers lengthwise into pieces and soaking them 2 hours in 4 per cent thiourea (NH_2CSNH_2) DENNY (1926) succeeded in disturbing the apical dominance of sprout formation. In many cases it was found that the apical buds of tubers treated with thiourea did not grow, but that growth first started in buds towards the basal end. The growth of the apical buds then was inhibited by the growth of the basal buds that had started first; for the apical buds started growth at once when cut off from these tubers and planted separately.

In the investigations treated in this section it was tried to find an explanation for the phenomenon of correlative inhibition of lateral buds and shoots by assuming an organ forming substance, a nutritive exhaustion, an inhibiting substance or the transmittance of some physiological process. The later experiments are strongly in favour of an inhibiting substance; curiously

enough, however, most of the experiments do not solve the question, whether a nutritive exhaustion or an inhibiting substance brings about the correlation. Experiments, which in the opinion of some investigators clearly prove one theory, are interpreted by other scientists in just the opposite way. Most experiments can be explained in favour of the one theory as well as of the other and we get the impression that it only depends on the investigator's original point of view which interpretation he will give of his experiments. It is only in the light of the investigations on the role of auxin in these correlation phenomena, that the early experiments become comprehensible.

§ 2. *Later experiments: the leaf as a source of inhibiting influence.*

A profound study of the correlation between leaf and axillary bud was made by DOSTÁL (1909, 1926) in *Scrophularia nodosa.* For his experiments he used isolated stem cuttings consisting of one pair of opposite leaves and a piece of internode above and below the node; the bases of these „pairs of leaves" were put in water. After cutting away one of the two leaves the bud in its axil began to develop while the one in the axil of the intact leaf did not. The same result could be obtained by covering one leaf with black paper. Simultaneously with the development of the axillary bud of the amputated leaf there was a strong root-formation at the base of the stem at the half of the intact leaf; on the side where the leaf had been removed no roots appeared. If both axillary buds are brought to development one of them will generally grow out much faster than the other. As DOSTÁL has pointed out, a similar correlation is found between them, as between terminal and lateral shoots. The one which grows fastest inhibits the growth of the other. Once the axillary buds having grown out, the inhibiting influence of the leaf is of little importance.

By longitudinally splitting at the base the internode of a „pair of leaves" of which one leaf had been removed and putting the basal half of stem, connected with the intact leaf, in a 0,2 % Knop's nutrient solution or in 1 n glucose, the other half with the removed leaf being put in water, the inhibiting influence of the intact leaf on its axillary bud was reduced by the Knop-solution, but was increased by the glucose. Full-grown leaves have the strongest inhibitory influence; this influence decreases with increasing age. In cuttings with two pair of leaves, a leaf is also capable of inhibiting an axillary bud above or beneath it.

According to DOSTÁL the assimilation products of the leaf regulate the growth of the axillary bud; therefore the leaf must be able to function normally and must not lack nutritive substances, light and water. He leaves undecided whether the inhibiting influence may be due to a specific inhibiting substance formed in the leaves during the assimilation.

We owe an important series of investigations on the correlative inhibition of the growth of axillary buds and shoots to SNOW (1925, 1929, 1929a, 1931, 1931a, 1932). SNOW (1925) split *Phaseolus* seedlings longitudinally from the roots up to about 2 cm of the epicotyl; the halves were then immediately bound tightly together again. One of the halves was completely isolated from the upper part by a transversal cut passing from the top of the split out to the side of the epicotyl. Further as a control part of the plants was decapitated. Then the bud in the axil of the cotyledon of the isolated halves grew out much more slowly than that of isolated halves of decapitated controls. Yet it was not likely that in the non-decapitated plants the growing apex could withdraw any nutritive substances from the isolated halves. Since the inhibition did act across a watery gap, SNOW concluded that it was very likely that it was conducted by the diffusion of a soluble substance. By ringing the epicotyl down to the wood, the inhibition was not yet interrupted, but only weakened if the axillary bud was left connected only by the pith with the main apex.

SNOW (1929) also made the following interesting experiment. Young seedlings of *Vicia Faba* were decapitated in the epicotyl, so that the two axillaries grew out. Of these the shorter one was decapitated above its second leaf and a bud was allowed to remain only in the axil of one of the two leaves of this shoot. The remaining bud did not grow out, but when the other shoot was also decapitated this bud grew out strongly. If the base of the shorter shoot was killed by scorching, the axillary bud above this zone was still inhibited by the apex of the longer shoot. If the longer shoot was decapitated too, no inhibition occurred. SNOW concluded from this experiment that an inhibiting substance, coming from the apex of the longer shoot, was drawn up through the dead zone with the transpiration stream. In later experiments SNOW (1929a) found that in seedlings of *Pisum sativum* the inhibiting effect exerted by the shoots upon their axillary buds comes from three or four of the developing leaves. Likewise as from the experiments of DOSTÁL (1909, 1926) it appears that the leaves are the source of the inhibition. In *Pisum*

sativum the leaf begins to inhibit at a length of 2 to 2,5 mm and it continues to inhibit strongly from the length of 3 to 15 mm; at about 20 mm the inhibition effect begins to fall off rapidly and at the final length of about 45 mm the leaf no longer inhibits at all or only very slightly. The strength of inhibition increases with the length of the interiacent stem, for in isolated decapitated pea-seedlings single developing leaves inhibit axillary buds that are from 70 to 100 mm below them more strongly than similar leaves in similar seedlings inhibit buds that are only from 5 to 15 mm below them (SNOW, 1931). This should make clear, why axillary buds of pea-seedlings grow out to a certain length, before they are stopped by inhibition. The axillary buds are at first not inhibited because they are too close to the developing leaves in or near the apical bud. They therefore grow until the growth of the main shoot separates them far enough from the developing leaves that inhibition is strong enough to stop them. SNOW does not say, how he explains this increasing inhibition with increasing length of interiacent stem.

By decapitating young pea-seedlings in the epicotyl SNOW (1931a) could obtain plants with two shoots springing from the axils of the cotyledons. If in such „two-shoot plants" one of the shoots has its leaves removed, until only those, 1 mm long or less, remain, it is rapidly arrested in growth and finally killed, whereas similar simple shoots similarly defoliated grow on rapidly and indefinitely. SNOW concluded that in these „two-shoot plants" the inhibiting influence coming from the two shoots counteract each other. If one of the shoots is defoliated or otherwise weakened, the influence coming from the other shoot travels up into it and arrests and kills it. Why this influence should travel acropetally in the defoliated shoot, while it normally travels basipetally in the intact shoots, SNOW does not make clear.

In a following publication SNOW (1932) recalls the fundamental investigations of JOST (1893) who showed that, as a rule, cambial growth in stems only takes place under the influence of growing leaves and further, that their influence travels only downwards. SNOW found that in *Vicia Faba* strips of mature stem survived and grew in thickness if they were attached by the top, so that they received the downward-moving cambial stimulus; but if attached by the base, they died. The killing of the defoliated shoot in „two-shoot plants" SNOW now explains in this way, that the young leaves transmit downwards, among other growth-regulators (either growth promoting or inhibiting), the cambial

stimulus, which overruns the inhibition by other factors in the parts which it reaches. It does not penetrate into lateral shoots or buds, however, whereas the inhibiting influence does penetrate into them. The growing leaves protect their shoot bij means of the cambial stimulus of being inhibited by another shoot. It seems a little surprising, however, that the growing leaves should transmit downwards growth promoting as well as -inhibiting influences, of which the latter could also travel upwards.

§ 3. *Recent experiments: the role of auxin in the inhibition.*

a. *The „direct" theory of* THIMANN *and* SKOOG *and the „indirect" theory of* LAIBACH.

The investigations of DOSTÁL and SNOW discussed in the preceding section made THIMANN and SKOOG (1933, 1934) assume that the correlative inhibition might be caused by the growth substance which was first demonstrated to occur in *Avena*-coleoptiles by PAÁL (1919), later quantitatively determined by WENT (1926, 1928) and first isolated in a pure form by KÖGL and HAAGEN SMIT (1931) [1]). Using the diffusion-method and the *Avena*-test as described by WENT (1928) with the procedure, size of agar blocks etc. as given by DOLK and THIMANN (1932) they determined the production of growth-substance of the terminal and lateral buds of *Vicia Faba*. The amount of growth substance, produced by the terminal bud was considerable (about 30 plant units [2]) per hour); in the undeveloped lateral buds (3,5 mm in length) there was little or no production, but during active growth they produced it in considerable amounts (about 20 plant units per hour). The growth-substance was produced by the leaves in smaller quantities than by the developing lateral buds, the amount decreasing with the age of the leaf. The production of growth substance thus closely parallels the bud-inhibiting effect found by DOSTÁL and by SNOW.

Further they applied to the top of decapitated seedlings of *Vicia Faba* agar blocks with growth substance, which were renewed every 6 hours. The growth substance was obtained from the ether extract of the culture medium of *Rhizopus suinus* under the conditions described by DOLK and THIMANN (1932).

[1]) For a survey of the nature of this growth substance or auxin and its role in plant growth I may refer to the excellent reviews of BOYSEN JENSEN, AVERY and BURKHOLDER (1936) and of WENT and THIMANN (1937).

[2]) The „plant unit" of DOLK and THIMANN (1932) is that amount of growth substance which when applied unilaterally to decapitated *Avena*-coleoptiles in a 10,7 mm^3 block of agar causes 1° curvature under the standard conditions.

The continued application of agar blocks with 1670 plant units growth substance per cm^3 suppressed the development of lateral buds to about the same extent as does the tip in intact plants. At the same time elongation of the stem was obtained by applying agar blocks with 800 plants units to decapitated and defoliated pea-plants and by submersing isolated preparations in a growth substance solution of 100 plants units per cm^3. These experiments leave no doubt that an auxin-like substance(s) is the inhibitor of bud-development in *Vicia Faba*.

The fact that more growth substance had to be applied than diffused from the tip (1670 against about 180 plants units per 6 hours), they ascribed to inactivation of the growth substance by wound substances and loss in non-transporting tissues. To explain the fact that a growth promoting substance might act as an inhibitor THIMANN and SKOOG supposed that the growth substance produced by the terminal bud reaching the lateral buds, prevented them in their own production of growth substance. As soon as the terminal bud is removed, this supply of excess growth substance ceases and the buds now commence to synthesize growth substance on their own account and therefore develop.

The same should occur in the physiological regeneration of a tip in a decapitated *Avena*-coleoptile. In the opinion of THIMANN and BONNER (1933) the presence of growth substance in sufficiently high concentrations prevents the production of growth substance in the lower zones of the coleoptile, and only when the supply is cut off by removal of the tip, production of growth substance occurs in the next lower zones.

In the case of the *Avena*-coleoptile, however, THIMANN and BONNER (1933) could show that there was a linear relationship between growth substance added and growth produced. But THIMANN and SKOOG do not give any explanation as to why, if growth substance reaches the lateral buds, these laterals are unable to use it for their development and are depending on growth substance of their own synthesis. Nor does their theory explain the inhibition of one developing bud by another, observed by them too. For from their own experiments they conclude that the transport of growth substance in *Vicia*-stems is apparently a polar phenomenon of the same type as that found in *Avena*-coleoptiles by WENT (1928) and VAN DER WEIJ (1932). It is therefore not probable that growth substance from the stronger developing bud travels up the stem of the shorter one.

LAIBACH (1933) also showed that pollinia of orchids applied

to the top of the decapitated epicotyl of *Vicia Faba* inhibit the development of the cotyledonary buds. Living pollinia secrete considerable amounts of growth substance, as was shown by LAIBACH before (1932). LAIBACH observed a considerable growth in thickness and length of the decapitated stem to which the growth substance was applied and he therefore supposes that the inhibition is not due to a direct action of the growth substance on the lateral buds, but that it first takes part in some growth process in the main stem and that this growth then secondarily inhibits the buds.

Against the experiments of LAIBACH this serious objection may be raised, that the growth substance is supplied "in extraordinarily high concentrations" as he himself says. For this reason too the swellings and thickenings, which are the results of this application, cannot be called normal and are by no means a reliable indication for what happens in the intact plant.

To test the possibility that the inhibition was not due to the growth substance itself but to a special inhibitor present in their impure preparations SKOOG and THIMANN (1934) repeated their earlier experiments with crystalline preparations isolated from urine and other sources by KÖGL, ERXLEBEN and HAAGEN SMIT (1934). An aqueous solution of these preparations was introduced every 8 hours into small paraffine cups which were moulded unto the cut surface of the stem of *Vicia Faba*. Of the three isolated growth hormones, auxin-a, auxin-b and hetero-auxin, auxin-a had lost most of its growth promoting activity and produced no inhibition, but auxin-b and hetero-auxin showed to be at least as active in causing inhibition as the impure preparation of *Rhizopus suinus*, when used in the same concentrations in growth promoting units (1000, 3000 and 5000 plants units per cm^3).

The correlative inhibition of leaves on the development of their axillary buds, was investigated closer by UHROVÁ (1934) in the light of what had meanwhile become known about the role of growth hormones in these phenomena. If he cut off both leaves of an isolated "leaf-pair" of *Bryophyllum* and then replaced one of them on its petiole, interplacing a thin layer of agar, this leaf would exert the same inhibiting influence on its axillary bud as if it had been left intact. The same results may be obtained by placing the leaf on agar and then applying the agar block to the cut petiole. When this agar block was placed unilaterally on decapitated *Avena*-coleoptiles, it gave a distinct growth curvature. The substances, diffusing from the

developing buds of *Syringa vulgaris,* the tips of *Avena-* and *Zea-*coleoptiles and flowerheads of *Bellis perennis,* have the same inhibiting influence on the axillary buds of *Bryophyllum.* Though UHROVÁ gives these substances the name of inhibiting substances, he yet draws the obvious conclusion that these substances are identical with growth substances.

BOYSEN JENSEN (1935) mentions in his book "Die Wuchsstoff-theorie", that he, ascribing the dormancy of resting plant-organs to a lack of growth substance, filled the hollow internodes of *Forsythia* with growth substance solutions or made this fluid flow slowly through twigs of *Salix, Syringa* and *Aesculus.* But it was not possible to observe any "forcing" effect in any of these experiments made during winter.

By applying to the decapitated stem some lanolin paste with growth substance extracted from orchid pollinia or urine as done by LAIBACH (1933), MÜLLER (1935) succeeded in inhibiting the development of the lateral buds in a great number of plants which as yet not had been tested in this respect. She obtained a marked, though not complete, inhibition in *Linum, Pisum, Antirrhinum, Godetia, Phaseolus, Zinnia, Sinapis, Helianthemum* and *Tradescantia;* no inhibition took place in *Impatiens, Polygonum* and *Tropaeolum.* In the decapitated shoots there appeared a strong growth connected with a great number of cell divisions under the influence of the growth substance supplied. Like LAIBACH, MÜLLER too thinks that the renewed growth phenomena are responsible for the inhibition of the axillary buds. The fact that she could not obtain a complete inhibition in her experiments MÜLLER explains by assuming that when the renewed growth has finished, the inhibition stops too. And indeed, with *Vicia Faba* she could obtain an almost complete inhibition by repeated decapitation and by continuously applying growth substance, which again and again caused new cell divisions. In the intact plant, however, these cell divisions will not occur to the same extent and it seems to me that they had better be considered as an attendant phenomenon in this abnormally high supply of growth substance.

b. *The theory of the two opposite streams of growth substance of* CZAJA.

From some experiments with an application of lanolin paste by the LAIBACH (1933a) method with growth substance prepared from urine, CZAJA (1935, 1935a) draws the conclusion, that a stream of growth substance flowing in a given direction in a

parallelotropic organ will morphologically polarize the cells of this organ. The swellings and growth inhibitions, which he observes in the case of supply of growth substance at right angles to the longitudinal direction of a stem or root, are due, in his opinion, to the direction of this supply, by which the cells can only enlarge themselves in a radiar direction. This leads him to assume that the growth inhibiting action of growth substance on roots is due to a mutual weakening of two opposing streams of growth substance, of which each in itself is again growth promoting. If one stream is stronger than the other, the cell in its polar behaviour will be mastered by the stream of the highest concentration. The correlative inhibition of the axillary buds by their leaves too, he explains in this way, namely that the stream of growth substance coming down from the leaves is opposed to the probably very weak one coming from the buds and so inhibits it altogether.

The existence of two opposite streams of the same substance in one and the same organ, even in the same cell, however, has not been proved by CZAJA. It is not clear, how to imagine such a transport.

SKOOG (1935) promoted the outgrowth of lateral buds by a treatment of the plant with X-rays. He showed that, when the apical end of a growing young *Pisum*-plant was exposed to X-rays, the production of auxin by the terminal bud was inhibited. Parallel with this the growth of the main stem was reduced and the buds in the stipules began to grow rapidly.

By treating the cut surface of decapitated *Nicotiana*-plants with lanolin preparations containing a high concentration of indole-acetic, indole-propionic, phenyl-acrylic, phenyl-propionic, indole-butyric, phenyl-acetic or naphtalene-acetic acid HITCHCOCK (1935) obtained a marked inhibition of the growth of the upper two or three buds. As these substances as far as known play no role in normal growth phenomena and as HITCHCOCK used abnormally high concentrations (from 3 in 10^3 till 1 in 10), these data are of little importance in explaining the phenomenon of bud inhibition. They only fit in a systematic research on the relation between structure and activity of growth promoting substances. The same holds for the communication by THIMANN (1935) that the supply of indene-3-acetic and coumaryl-1-acetic acid in lanolin paste in a concentration of 1 in 10^3 and 1 in 10^2 causes a strong inhibition of bud development in decapitated pea seedlings. Like HITCHCOCK he found that the effect of the substances used was weakened when the point of application was far from the bud, their trans-

port apparently being difficult.

c. *The "indirect" theory of* SNOW.

LE FANU (1936) then informs us of sone interesting new facts. Bij placing single-node stem cuttings of *Pisum sativum* in solutions of pure synthetic hetero-auxin of 2 in 10^6 and 4 in 10^6 she could obtain a strong inhibition of axillary buds. By placing intact shoots with their bases in hetero-auxin solutions of 2,5 in 10^5 and 5 in 10^5 the growth of the stem was inhibited too. The same result was obtained by inserting lanolin paste containing 5 in 10^5 hetero-auxin in a longitudinal split of the 4th internode of the stem, the growth of the 5th internode above it then was retarded. If the leaves of the plants were removed the same result was obtained with 5 in 10^6 hetero-auxin paste; but in plants with intact leaves hetero-auxin 5 in 10^6 hardly had any effect. As SNOW in 1931 LE FANU explains this last experiment in this way that the leaves protect a stem against inhibition. Of what nature this protection is, she leaves undecided, however. Nor can she explain the outgrowth of the buds in the axils of the leaves 2 and 3 after applying hetero-auxin paste 5 in 10^5 into the split in internode 4. She showed, that hetero-auxin of about the same concentration yet can exert a growth promoting influence by application of hetero-auxin paste 1 in 10^5 to the cut surface of decapitated and debudded pea seedlings in the dark. She then obtained an acceleration of the growth of the youngest internodes. LE FANU concluded from these experiments that the nature of auxin action, whether it is acceleration or inhibition is determined by the position of the auxin source relative to the organ to be affected. Travelling morphologically downwards auxin accelerates growth, coming from a morphologically basal part it inhibits.

By placing parts of the stem from both the shoots of "two-shoot" pea plants excentrically on *Avena*-coleoptiles LE FANU obtained after three hours strong curvatures with the stem parts of the stronger shoots, but no or scarcely any curvature with the same parts of the inhibited shoots. This absence of auxin in the inhibited shoots suggests that no auxin is transported into them in upward direction and therefore the inhibition cannot be due to a direct action of auxin. The conclusion is reached by LE FANU that the inhibition is probably a secondary process which originates from some primary process promoted by auxin in the inhibiting shoot. In her opinion not the abnormal stem swellings and cell divisions as found by LAIBACH (1933), but the cambial divisions shown by SNOW (1935)

as caused by auxin, might be considered as this primary reaction. However, in this case one meets the same difficulty as in the theory of CZAJA (1935, 1935a): one has to assume that in a two-shoot plant from each of the two shoots an inhibiting action will start which will travel upwards into the other shoot. In each of the two shoots therefore two of these effects will be transported in opposite directions, while the one from the fastest growing shoot will win.

The experiments of LE FANU are confirmed and extended by SNOW (1936). By putting a ring of concentrated hetero-auxin paste (5 in 10^2!) round the stem of pea seedlings, deprived of their leaves, close below one of the growing internodes, the elongation of the internode was at first accelerated and then strongly and increasingly retarded. When pea shoots, deprived of their youngest leaves except one at the top of the 4th internode, were placed with their bases in a hetero-auxin solution of 5 in 10^5 the 4th internodes were strongly retarded, but only if they were not more than about 5 mm long at the start. If, however, they were about 8 mm long at the start, the stage which normally comes just before they start their rapid elongation, they are never retarded at all. SNOW concluded that the retardation of very young internodes by hetero-auxin drawn up with the transpiration stream is probably brought about in a way different from that by hetero-auxin paste applied externally below; the retardation by hetero-auxin in the transpiration stream probably being more a direct action of hetero-auxin, the retardation by hetero-auxin paste an indirect phenomenon.

The researches of LE FANU (1936) and SNOW (1936) are of importance since we have to assume that a rather high concentration of auxin is present at the base of an inhibited bud or shoot in an intact plant too, coming from the inhibiting shoot. However, after the fundamental researches of VAN DER WEIJ (1932, 1934) the general opinion is that the transport of auxin in *Avena*-coleoptiles is strictly polar from tip to base. Later on this basipetal transport was found in several other organs such as stems of *Elaeagnus* (VAN DER WEIJ, 1933), hypocotyls of *Raphanus* (VAN OVERBEEK, 1933), stems of *Vicia Faba* (THIMANN and SKOOG, 1934), hypocotyls of *Lupinus* (DIJKMAN, 1934), stems of *Coleus* (MAI, 1934, GOUWENTAK and HELLINGA, 1935), leaf-veins of *Nicotiana* (AVERY, 1935). However, the polarity of this transport was denied by other investigators. For it appears from the researches of ZIMMERMAN and WILCOXON (1935) and of HITCHCOCK and ZIMMERMAN (1935) that hetero-auxin and other growth promoting sub-

stances can be transported in acropetal direction in stems of tobacco, tomato and marigold plants. LAIBACH and FISCHNICH (1936, 1936a) proved the same for leaves of *Coleus* and for tomato plants, SNOW (1936) for *Avena*-coleoptiles and hypocotyls of *Helianthus* and JOST and REISZ (1936) for *Avena*-coleoptiles too. An objection to the last mentioned researches is, however, that the growth promoting substances were supplied in unphysiologically high concentrations. One has to expect that these substances supplied at the base are taken along with the transpiration stream, but anyhow this is no proof against the conception, that in the intact plant the auxin is transported mainly in basal direction.

d. *The "diversion" theory of* WENT.

For the understanding of the correlative inhibition of lateral buds and shoots the investigations of SCHWANITZ (1936) in rhizomes of *Lathyrus* and *Agropyrum* are important. If the rhizome is cut into pieces directly after removal of the plant, each of the pieces will form the same number of shoots, but the longer one waits to divide, the stronger the regeneration will be restricted to the apical parts. SCHWANITZ sets up the hypothesis, that the growing-out of buds is brought about by a substance, transported polarly to the apex, while it was equally distributed over the rhizome before. He supposes that a growth substance is implied.

DOSTÁL (1936) did some work on the petiole of the cotyledon of soaked seeds of *Pisum sativum*. He found that the young epicotyl inhibits the elongation of the petiole of the cotyledon only slightly, while absolutely inhibiting the growth of the axillary buds of the cotyledons. The cotyledons too inhibit the growth of their axillary buds. The radicle however, inhibits the elongation of the petiole of the cotyledons very strongly, but promotes the growth of their axillary buds. If, for instance, the base of the radicle is cut on one side after the decapitation of the epicotyl, the axillary bud on the intact side of the root will grow out stronger than on the side above the cut, the petiole of the cotyledon on the intact side staying behind in growth to the one above the cut.

To explain this behaviour, a closer, quantitative examination is wanted of the role played by auxin in these phenomena. In connection to the later theory of WENT (1936), however, DOSTÁL's communication on the role of the radicle in the development of the axillary buds is of importance.

The removal of the epicotyl and of one of the cotyledons in pea seedlings results in a strong development of the bud in the axil of the amputated cotyledon. PLCH (1936) succeeded in inhibiting this outgrowth by applying to the cut surface some lanolin paste with 2 in 10^3 hetero-auxin and other growth promoting substances. The inhibition actually decreases with lower concentrations and when the application is made at a greater distance.

From some later experiments of DOSTÁL (1936a) with isolated pieces of tubers of *Scrophularia nodosa* it appears too that buds in the vicinity of intact roots develop faster as other buds in the vicinity of which the roots were removed.

In his "Allgemeine Betrachtungen über das Auxinproblem" WENT (1936) devotes a section to bud inhibition and describes an experiment with decapitated dark-grown pea seedlings placed with their roots in a 2 per cent saccharose solution. By the removal of the roots the growth of the developing axillary buds is strongly inhibited and the same happens, but to a less degree, when the cotyledons are removed. In non-decapitated seedlings the removing of the cotyledons causes a complete inhibition of the leaf growth, while the removal of the roots inhibits the growth to a less degree. From this WENT concluded, that for the elongation of the stem and for the growth of the axillary buds in decapitated plants at least two factors are wanted, besides auxin. The first factor is chiefly necessary for cell elongation and is produced in the roots, whilst the other generally stimulates or causes the leaf- and organ growth. The auxin directs the transport of these specific growth factors to the production center of auxin. So, under normal conditions, these substances are all transported to the terminal bud, but if the auxin production ceases by decapitation or by some other reason, the stream of specific growth factors in that direction is stopped. The greater auxin production of the lateral buds then draws this stream to themselves; so they can develop.

This "diversion theory" of WENT, as SNOW (1937) has called it, in fact includes two older theories, viz. that of SACHS (1880, 1882) about the existence of specific organ forming substances and that of GOEBEL (1903) and LOEB (1915) on the automatical attraction of the material for growth by the stems or buds which grow out first. To these theories the new hypothesis has been added that it is the auxin which brings about this attraction. Apart from the hypothetical character of this theory, it does not give either an explanation of

the fact, why in the intact plant no or very little auxin is produced by the lateral buds, whilst this auxin production is immediately increased as soon as the terminal bud is eliminated. If the auxin is really the initial phase of a series of processes, it should be made clear first, why in one case no auxin is produced and in another it actually is.

From the above-mentioned experiments of SCHWANITZ, DOSTÁL and WENT it appears, however, that a factor necessary for bud- and shoot development is transported acropetally upwards from the roots or cotyledons.

e. *The retardation of leaf development and the branching habit as related phenomena.*

In the young basal rosette of *Solidago sempervirens* one leaf is rapidly elongating at a given time and then retards the development of the younger leaves. By removing this leaf GOODWIN (1937) could obtain the elongation of the next succeeding leaves, but he could reproduce the retarding effect by applying hetero-auxin in a lanolin paste at a concentration of 2 in 10^4 to the petiolar stub of such an amputated leaf. The limitation in size of the leaves was brought about by an inhibition of cell enlargement. Diffusions from the cut bases of rapidly growing leaves surpassed in quantities of auxin those from leaves at any other stage. The retardation is, therefore, probably due, to an excess production of auxin by the inhibiting leaf.

In the young basal rosettes of *Solidago rugosa* no retardation of succeeding leaves occurs. Maximum diffusions of auxin from cut leaf bases were approximatively one-half as large as those from leaves of *Solidago sempervirens*. Hence GOODWIN suggests that in these plants the production of auxin is too small to cause periodic retardation.

No doubt this interesting phenomenon, as GOODWIN notes too, is of the same nature as the correlative bud inhibition. Whether, however, it is a direct action of auxin as THIMANN and SKOOG (1934) assume, seems to be questionable to him. For in that case polarity would not yet have become established in small buds and in very young leaves as the auxin must be transported into them from the older leaves below.

DELISLE (1937) showed that in two species of *Aster, Aster novae-angliae* and *Aster multiflorus*, of which the former has relatively few branches and the latter is more branched and bushier in habit, production of lateral buds and branches can be inhibited by applying hetero-auxin in lanolin paste 2 in 10^3

to the cut ends. The rate of lateral bud development in decapitated plants in which the five young leaves had been removed was consistently greater than that in decapitated controls with intact leaves. Diffusions indicated that the tip of *Aster multiflorus* produces only approximatively 74 per cent as much auxin as does that of *Aster novae-angliae,* and that of the hybrid only 84 per cent as much. Diffusions taken at varying distances from the tip showed a concentration gradient which is greatest at the tip, decreasing rapidly at the region of elongation below the tip and falling off gradually below this point. DELISLE concluded from his experiments that the problem of branching habit in these two species of *Aster* is largely correlated with the diferential production of auxin by the terminal bud and the growing young leaves. *Aster multiflorus* producing less auxin, has abundant lateral buds and branches, while *Aster novae-angliae,* producing considerable more auxin, has correspondingly little branching.

In this connection we must also refer to some previous investigations of VAN OVERBEEK (1935) on the dwarf type in corn and of ZIMMERMANN (1936) about the distribution of auxin in trees in space and in time. VAN OVERBEEK (1935) investigated the amount of auxin given off by coleoptiles of the normal corn and of the *nana*-form. He found, that the amount of auxin, given off by *nana,* was less than that given off by normal plants, this being probably a consequence of a higher destruction of auxin in *nana.* This smaller amount of available auxin will also result in a smaller growth in *nana* than in the normal plant. But if the dwarf growth in plants must be generally ascribed to a smaller amount of available auxin, it becomes clear — in connection with the above-mentioned investigations especially those of DESLISLE (1936) — that these dwarf types on the whole are also more strongly branched and bushier in habit than normal plants.

ZIMMERMANN (1936) determined in the same way the auxin content of buds and shoots of different trees by means of diffusion in agar slices and tests on *Avena*-coleoptiles. He found, that dormant buds contain no auxin, but that this amount rapidly increases when the buds begin to sprout, to decrease again slowly afterwards. Buds from the upper parts of the tree give off more auxin than buds in the same developing stage more at the base. He also finds a very high auxin content in the terminal bud of *Aesculus hippocastanum, Acer pseudoplatanus, Fraxinus excelsior* and of various conifers, whilst the auxin content of the lateral buds is lower and rapidly decreasing

towards the base. Parallel to this, the growth rate of the terminal shoot of these trees is much greater than that of the lateral shoots. ZIMMERMANN concludes, that the high auxin production of the terminal bud clearly inhibits that of the lateral buds. On the other hand in the case of *Tilia* the growth of lateral shoots is much stronger than that of the terminal shoot, but there the auxin content of the lateral buds is higher than that of the terminal bud. From these highly important investigations follows that the external architecture of trees can be ascribed to the difference in auxin production of terminal and lateral buds. The question which rising now is: what is the cause of this difference in auxin production?

f. *The controverse between the "direct", the "indirect" and the "diversion" theory.*

SNOW and SNOW (1937) showed that one of the effects of applying hetero-auxin — in lanolin paste at a concentration of 5 in 10^4 — to a part of the growing apex of *Lupinus albus* is an abnormal enlargement of the leaf primordium and axillary bud, which subsequently arises from that part. It appears, therefore, that the direct effect of hetero-auxin in bud growth is a promoting one.

NAGAO (1937) finds an inhibition of the elongation of *Helianthus*-hypocotyls by application of hetero-auxin lanolin pastes at a concentration of 4 in 10^3 till 5 in 10^4 to the cut surface of cotyledons whose upper halves have been removed. LE FANU (1936) however, did only find an inhibition of the growth of young internodes of non-decapitated pea seedling by application of hetero-auxin paste 5 in 10^5 to a part of the stem morphologically below and a growth acceleration by application of hetero-auxin paste 1 in 10^5 to the upper ends of decapitated dark-grown seedlings. NAGAO considers the inhibition found by LE FANU and by himself as due to an excess amount of auxin in the effected zone, and notes that the decapitation may play an important role in LE FANU'S experiments as it will reduce the amount of natural auxin present. However, the fact that LE FANU found a stronger inhibition by the removal of the leaves cannot be explained by an excess amount of auxin. As neither LE FANU nor NAGAO did determine the auxin content in the zones affected, this question must be left undecided.

In their review on phytohormones WENT and THIMANN (1937) point out that the influence of factors other than auxin may explain why some buds have a greater tendency to develop

than others. So they try to explain the increase with distance of the inhibition exerted by young leaves of *Pisum* in SNOW's experiments (1931), by assuming that the tendency to grow out is greatest in the basal buds. They conclude, that the mechanism of bud inhibition can probably not be understood until the fundamental mechanism of auxin action on the cell, and the role of other factors in bud growth are better reveiled.

One will agree with WENT and THIMANN that undoubtedly other factors as water, light and food, effect the development of lateral buds and shoots too. Since, however, THIMANN and SKOOG (1934) had proved quite plainly that auxin brings about the correlative inhibition of lateral buds and shoots, we must first try to elucidate which role auxin plays in this phenomenon.

Previously DOSTÁL (1926) already found that the leaves of *Scrophularia nodosa* inhibit the development of their axillary buds. Also in this case an action of auxin seems evident. Recent experiments of DOSTÁL (1937) showed that this is the case indeed. He cut off both leaves of a leaf-pair of an isolated section at the base of the stem, and to one of the cut surfaces he applied hetero-auxin paste 5 in 10^3, to the other one plain lanolin. The growth of the axillary buds was inhibited at the side where hetero-auxin was applied. Curiously enough, the development of higher axillary buds in similarly treated stems with more leaf-pairs was promoted by the application of hetero-auxin paste of the same concentration. According to DOSTÁL the different age of the implied sections causes this different behaviour.

Later THIMANN (1937) communicates some new experiments with *Pisum* seedlings. By applying lanolin paste with hetero-auxin at concentrations of 4 in 10^6 till 4 in 10^3 to decapitated dark-grown seedlings a marked inhibition of the development of lateral buds was obtained. This, however, was not accompanied, as LAIBACH (1933) had postulated, by any compensating increase of growth elsewhere in the plant and involved a real decrease in total dry weight.

According to the theory of WENT (1936) the auxin would act by attracting to itself no nutrients but specific factors for bud growth. THIMANN believes, one of these factors is to be a special substance, called by WENT "caulocaline", coming from the roots and decidedly different from auxin. If auxin is supplied to the bud itself, a kind of attraction will bring the bud factors to the bud and, instead of inhibiting, auxin will accelerate growth. Direct application of hetero-auxin in lanolin at concentrations of 4 in 10^6 till 4 in 10^3 to young lateral buds, 1 mm in length,

of decapitated seedlings, however, did *inhibit* their growth as compared with controls treated with plain lanolin. The higher concentrations, however, produced swollen buds and there was a marked increase in the dry weight of the buds per unit of length. These results thus contradict the above mentioned ones of SNOW and SNOW (1937) and do not endorse WENT's theory.

THIMANN then points to the parallel behaviour of buds and roots, which are both inhibited by auxin. However, as had been proved previously by FABER (1936), FIEDLER (1936), AMLONG (1936), THIMANN (1936) and GEIGER-HUBER and BURLET (1936), very low concentrations of auxin accelerate root growth, the response of roots to different concentrations of auxin thus showing an optimum curve. The parallel behaviour of roots and buds to auxin inhibition and the fact that very dilute auxin solutions increase root elongation make THIMANN suggest that roots, buds and stems all behave in a comparable way; their growth being inhibited by relatively high and promoted by relatively low auxin concentrations. Buds, therefore, in their response to auxin also should show an optimum curve and this curve should fit in between those of stems and roots; the normal auxin concentration in the stem of the growing plant being such as to stimulate the stem and to inhibit the buds.

This theory of THIMANN (1937) has the advantage of putting the various observed effects of auxin in roots, buds and stems on a uniform basis. He did not prove, however, that the amount of auxin in the inhibited buds is large indeed; on the contrary from the experiments of THIMANN and SKOOG (1934) follows, that the amount of auxin present in the undeveloped lateral buds in *Vicia Faba* is very low. He cannot explain either the inhibition of lateral shoots, a phenomenon closely related to that of the inhibition of lateral buds. In that case, according to THIMANN, the inhibition depends rather on competition for some other factor such as water or a second factor (caulocaline) which comes from the roots. Bud inhibition and shoot inhibition are not likely to be explained in two different ways, however.

In a recent publication SNOW (1937) also raises some serious objections against the "direct" theory. In one of his experiments from some "two-shoot" pea seedlings one of the shoots was decapitated just below the second leaf from the base. The decapitated shoot was placed inversely with its upper cut end in a little water in a glass tube. If the second shoot was left intact, the axillary buds at the first leaf nodes of the shoots in the tubes did not grow at all, but in decapitated controls they

grew vigorously. SNOW concluded from this, that in these plants the inhibition travelled from the intact growing shoots up through the cotyledonary nodes to the buds of the decapitated shoots, even against the transpiration stream.

In a following experiment young seedlings of *Vicia Faba* were split longitudinally with a median split through the main root, the cotyledonary node and upwards through the epicotyl nearly to the first node. Then the main shoot was decapitated higher up, above the third node, and when the two buds in the axils of the cotyledons had grown out and formed shoots one of these shoots was decapitated above its first leaf. The bud in the axil of this leaf then was inhibited by the other cotyledonary shoot across the zigzag path of tissue connecting the shoots. The inhibition thus travelled down the growing shoot, up and down through the halves of the split epicotyl and up again through the decapitated shoot. Yet there was no sign of cambial growth on the cut surface of the halves of the epicotyl.

From these experiments SNOW concludes that the inhibiting influence can travel where auxin cannot travel, and therefore the "direct" theory cannot explain this correlative inhibition of lateral buds and shoots. Since further no auxin from the growing shoots enters the epicotyl halves the inhibited bud cannot possibly have been deprived of any substances coming up from the half root system and cotyledon below it through any polarizing action of auxin, as WENT's theory would suggest. For this reason SNOW sticks to the "indirect" theory: the auxin travelling down a stem promotes its growth, and the growth of the stem then in some way inhibits the lateral buds secondarily. As SNOW indicates, the primary process promoted by the auxin in the stem need not always be actual growth, for in *Tamus communis*, a monocotyledon, he finds that if the tip of a shoot that stopped growing is cut off, several of the upper axillary buds soon grow out strongly, though in intact shoots the axillary buds do not grow out. Yet in these shoots there is, of course, no cambial growth and scarcely, if any, growth in thickness. In discussing the nature of this secondary inhibiting influence, SNOW assumes that this influence must be a soluble substance or substances of some kind. Accordingly he tried in various ways to extract some substance or substances from inhibited shoots to which the inhibition might be due, but up to this moment without success.

In a recent publication WENT (1938) gives evidence for the existence of the specific factors involved in bud- and leaf-growth

postulated by him before (1936). 4 cm tops of etiolated pea shoots were grafted on root systems (with attached cotyledons) of the same plants or of peas of different varieties. After the junction of the tissues, growth was resumed at approximately the initial rate. Since stem elongation, leaf growth, stipule growth and petiole growth were differentially affected by the pea varieties used as rootstock, WENT concludes that each of these processes is influenced by a different factor or set of factors. Special terms are suggested for these specific factors — viz. caulocaline for a factor coming from the roots and necessary for stem elongation, and phyllocaline for a factor indispensable for leaf-growth and in etiolated peas coming from the cotyledon.

In connection with the results of my own experiments and the theory set up in consequence of it, the literature treated in this chapter will be briefly discussed again in chapter X, § 3 (p. 274). In a previous publication (FERMAN, 1938) mention was already made of some of these experiments and of this new theory on the correlative inhibition of lateral buds and shoots.

CHAPTER II.

Materials and methods.

§ 1. *Plant materials.*

As experimental plant I used chiefly a pure line of *Lupinus albus*. The seed was obtained from the firm of HULLEMAN at Utrecht. The seeds were placed each separately in earthen pots filled with leaf-mould. From October till April the plants stood inside the greenhouse; for the remaining months they were dug in under glass outside. From January till April the plants were illuminated with a PHILIPS' neon tube from 16h—24h and from 6h—9h.

For some experiments seedlings of *Pisum sativum*, variety "*Kaapse groene*", were used, which had been grown in mould in the green house. Young twigs taken from a shrub of *Ligustrum vulgare* in the botanical garden at Utrecht served for some experiments with cuttings.

§ 2. *The application of auxin to the plants.*

For the application of auxin to the plant aqueous solutions and lanolin pastes of pure, synthetic hetero-auxin (indole-3-acetic acid) were used. This preparation had been obtained from Dr. FRAENKEL and Dr. LANDAU in Berlin-Oberschöneweide, Germany. The solutions were made by weighing 10 mg of

hetero-auxin and dissolving it into 100 cm^3 of tap water. From this standard solution 1 in 10^4 the dilutions wanted were then prepared. The lanolin pastes were made by mixing an equal volume of lanolin and hetero-auxin solution. The solutions and the pastes were kept in the refrigerator and renewed every 7 to 10 days.

The application to the plant of the aqueous hetero-auxin solutions and of the tap water to the controls was made via the decapitated main- or lateral stem. For this purpose small glass tubes, of about the same diameter as the stem, were fastened onto the cut stem by means of a little rubber tube, 10 mm long. — Only in experiment 1 these glass tubes were fixed watertightly to the outer side of the stem by means of paraffin —. In the experiments, made in 1936, these tubes had a length of 15 mm and a content of about 0,15 cm^3; in the later experiments they had a length of 20 mm and a content of about 0,2 cm^3. Once or twice a day, in the first case generally at 10h, in the second case at 9h and at 17h (in experiment 1 at 10h and at 22h), these glass tubes were filled with fluid by means of a pipette with a finely drawn-out point. Great care was taken that no air-bubbles could prevent the fluid from entering the stems. The fluids were absorbed regularly by the plants.

The investigations of KÖGL, HAAGEN SMIT and ERXLEBEN (1934) and of HEIJN (1935) have made it very plausible, that the auxin found in higher plants is auxin-a and certainly not hetero-auxin. An application of auxin-a is therefore preferable to an application of hetero-auxin. As auxin-a, however, could not be obtained in sufficient quantities and as, besides, it has the disadvantage of soon becoming inactive (KÖGL, HAAGEN SMIT and ERXLEBEN, 1933) we, like so many other investigators (see Chapter I, § 3, p. 187), ressorted to hetero-auxin, the effect of which on the growth of plants apparently is, on the whole, the same as that of auxin-a. There are some differences, however, — in the *Avena*-test hetero-auxin has ¼ of the molecular activity of auxin-a (KÖGL and KOSTERMANS, 1935), hetero-auxin is probably transported somewhat less readily (THIMANN, 1935; VAN OVERBEEK, 1936, 1936a), auxin-a is inactivated considerably by light, hetero-auxin very little or not at all (VAN OVERBEEK, 1936, 1936a; KONINGSBERGER and VERKAAIK, 1938) —, but on the other hand SKOOG and THIMANN (1934) found no differences in activity between auxin-b and hetero-auxin in bud inhibition in *Vicia Faba*. This makes us expect that the artificial application of hetero-auxin may give us also some information about the role

of auxin-a in the correlative inhibition of lateral buds and shoots in the intact plant.

§ 3. *The extraction of auxin from the plants.*

The auxin is extracted from the plants with ether after the method devised by VAN RAALTE (1937). This method is as follows.

First those plant parts of which the auxin content had to be determined were ground very finely in a mortar together with about an equal volume of chemically purified quartz sand and some drops of 0,5 n sulphuric acid. As soon as the plant parts had been crushed more or less, so much ether, freed from peroxide, was added that the pulp was entirely submerged in it and the grinding was continued. — The ether was freed from peroxide by distilling shortly before 400 cm^3 ether over 10 g $FeSO_4$, 1 g CaO and 40 cm^3 H_2O —. After the extraction the ether was decanted and the extraction repeated twice with new ether. The extract was washed twice with water, acidified with sulphuric acid to the conversion point of congo red. Finally the ether was evaporised over a hot water bath, till the volume was about 5 cm^3. The rest was put in a small tube with 0,2 cm^3 of a diluted buffer solution after MCILVAINE. — The buffer contained 0,04 mol citric acid and 0,02 mol Na_2HPO_4; its pH was $\pm$ 5,4 —. This tube was kept in a beaker with warm water and the evaporating ether was blown away by means of an air current. The residu solved in the 0,2 cm^3 of the buffer solution. The water insoluble substances were removed as far as possible by washing the preparation with petrolether. Two 3% agar slices of 8 $\times$ 6 $\times$ 0,9 mm were added and remained in the refrigerator in this solution overnight; the next day their auxin content was determined.

In order to prevent the auxin of becoming inactive by illumination (C. KONINGSBERGER, 1936) as much as possible, all the manupulations were carried out in weak orange light (filter O.G. 2).

For the determination of the auxin content of plant parts the extraction method is preferable to the diffusion method in which the plant parts are placed for a certain time on agar slices. In the diffusion method only that amount of auxin can be determined, which is given off by the plant part to the agar slice. The rate with which this is done and the percentage of the total amount of auxin present which is given off will strongly vary in different plant parts. Only by extracting the auxin, it will be possible to get an impression of the total amount of auxin,

present in the plant parts concerned. The following fact may serve as an example: older investigators (WENT, 1928; SÖDING, 1929) who worked with the diffusion method, could not prove any auxin to be present in the basal part of the *Avena*-coleoptile and from this fact they drew their conclusions. Later on, THIMANN (1934) being the first to apply several extraction methods, clearly showed that auxin is present in all sections of the coleoptile, though in the basal part less than directly under the tip. Besides, the extraction method has the advantage, that the auxin content of a certain part of a whole series of plants can be determined simultaneously, whilst in the diffusion method only the auxin delivered by small plant parts can be estimated.

§ 3. *The determination of the auxin content.*

The auxin content was determined by means of the *Avena*-test under standard conditions, as described by WENT (1928) and improved later on by VAN DER WEIJ (1931). For a description of this test method I may also refer to BOYSEN JENSEN, AVERY and BURKHOLDER (1936) and WENT and THIMANN (1937).

For the test a pure line of *Victory oats* (*Segre hafer*) was used, obtained from the Sveriges Utsädesförenings Institution at Svalöv, Sweden. The plants were grown in water culture in racks for 12 plants in a dark room in a relative moisture of 95%, a temperature of 22,5° C. and an orange illumination (filter O.G. 2). In the tables and figures the auxin content of the plants is always expressed by the curvatures (in degrees) of the *Avena*-coleoptiles. These data are the averages of 16—24 coleoptiles, the mean error being calculated from the formula $m = \pm \sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$. They are calculated on 10 plants and in stem parts on 10 mm of the stem.

As the reactivity of the test plants varies from day to day and even from hour to hour (KÖGL, 1933; KÖGL, HAAGEN SMIT and VAN HULSEN, 1936), the figures thus obtained on different days, are incomparable in an absolute sense. We tried to "gauge" the curvatures, found on different days, by always comparing them with the curvature brought about by a hetero-auxin solution of a known concentration. But this too yielded no uniform results. It is not improbable, that in spite of a 3 times repeated decapitation, this variability is due to the presence of small amounts of auxin, which are still present or constantly formed again by the supply of auxin-precursor (or -precursors) from the seed to the coleoptile (SKOOG, 1937). This

conversion of precursor into auxin seems to be very sensitive to slight alterations in the external conditions which even in rooms with constant moisture and temperature cannot be altogether avoided.

On account of this it may be preferred in future to make all determinations of the auxin content with *Avena*-coleoptiles, of which the seeds after the method of SKOOG (1937) have been removed 18 hours ahead. These coleoptiles after decapitation are practically free from auxin and since no new precursor can be supplied, all the curvatures found at a certain amount of auxin will be equal. It also appears from the investigations of KONINGSBERGER and VERKAAIK (1938), that the variability in deseeded test plants is practically nothing.

CHAPTER III.

Inhibition of lateral buds by application of hetero-auxin solutions.

§ 1. *Experiments with seedlings of Lupinus albus.*

First of all we examined, whether it was possible to inhibit markedly the development of the lateral buds of decapitated seedlings of *Lupinus albus* by the application of hetero-auxin solutions via the decapitated stem.

Experiment 1 [1]).

80 4 weeks-old seedlings of *Lupinus albus* with an average of 8 expanded leaves were decapitated 20 mm above the first leaf from below, the second leaf being inserted about 2 or 3 mm

TABLE I. Development of the buds in the axils of leaf 1 and leaf 2 of seedlings of *Lupinus albus* decapitated above leaf 2 (experiment 1, 14/9/36 —28/9/36).

At application twice a day via the main stem of	Length in mm (average of 16 plants)					
	after 8 days			after 14 days		
	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together
hetero-auxin 5 in 10^{5}	3	4	7	21	29	50
hetero-auxin 1 in 10^{6}	8	10	18	32	49	81
hetero-auxin 5 in 10^{7}	13	15	28	55	72	127
hetero-auxin 1 in 10^{7}	13	20	33	48	70	118
tap water	11	11	22	48	53	101

[1]) This experiment has been partly published already in a previous paper (FERMAN, 1938).

above the first leaf. The plants were divided into 5 series of 16 plants each. In 4 series an aqueous solution of hetero-auxin in concentrations of respectively 5 in 10^6, 1 in 10^6, 5 in 10^7 and 1 in 10^7, and in one series tap water was applied twice a day to the plants via the decapitated stem. After 8 and after 14 days the lengths of the developing buds in the first and in the second leaf axil were measured. As appears from table I and figure 1, the development of the axillary buds was promoted weakly by application of hetero-auxin 1 in 10^7 and 5 in 10^7, but it was inhibited weakly (for about 20 per cent) by application of hetero-auxin 1 in 10^6, and the inhibition was very strong, though not complete, by application of hetero-auxin 5 in 10^6 (respectively 70 per cent after 8, and 50 per cent after 14 days), all series compared with the blank tap water series.

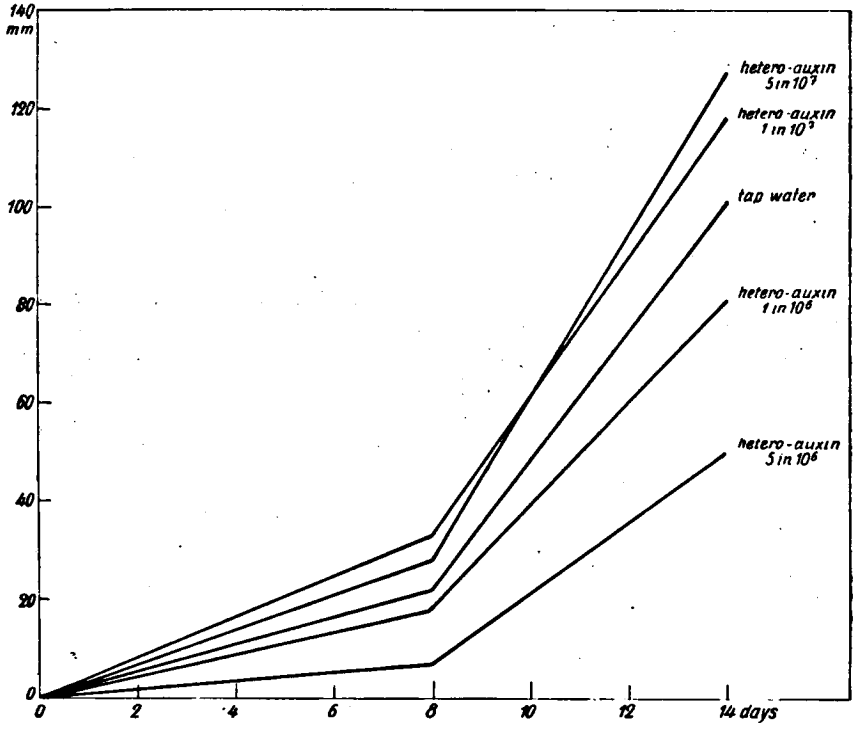


Figure 1. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus*, decapitated above the second leaf at application twice a day of hetero-auxin 5 in 10^6, 1 in 10^6, 5 in 10^7, 1 in 10^7 and tap water via the main stem (experiment 1, 14/9/36—28/9/36).

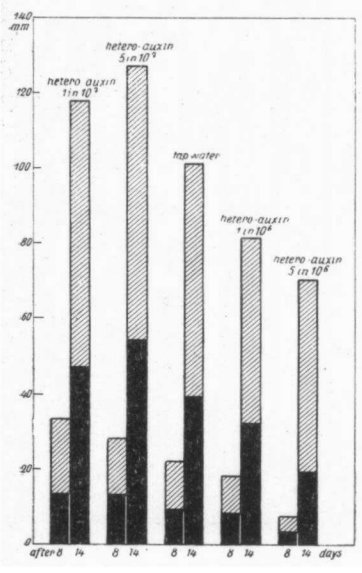


Figure 2. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application twice a day of hetero-auxin 5 in 10^6, 1 in 10^6, 5 in 10^7, 1 in 10^7 and tap water via the main stem; ■ slower bud, ▥ faster bud (experiment 1, 14/9/36—28/9/36).

It is obvious that the bud in the axil of the second leaf generally develops faster, than that in the axil of the first leaf, though the mean distance between the insertion of both leaves is only very small. Besides, this phenomenon occurs in the series where the growth of the axillary buds is inhibited as well as in the series where it is promoted. Arranging the buds per plant according to the rate of their development, the increase of the slower developing bud in the first 8 days as well as in the following 6 days proves to be smaller than that of the faster developing bud (see table II and figure 2).

Already DOSTÁL (1926) and later more particularly SNOW (1931a) have drawn attention to this phenomenon. Both investigators believe that a correlation between the two lateral shoots exists similar to that between terminal and lateral shoots in the intact plant. The experiments of chapter V (p. 226)

TABLE II. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 1, 14/9/36—28/9/36).

At application twice a day via the main stem of	Increase in length in mm (average of 16 plants)					
	first 8 days			next 6 days		
	slower bud	faster bud	ratio slower : : faster bud	slower bud	faster bud	ratio slower : : faster bud
hetero-auxin 5 in 10^6	3	4	7 : 10	16	27	6 : 10
hetero-auxin 1 in 10^6	8	10	8 : 10	23	40	6 : 10
hetero-auxin 5 in 10^7	13	15	9 : 10	41	58	7 : 10
hetero-auxin 1 in 10^7	13	20	7 : 10	34	51	7 : 10
tap water	9	13	7 : 10	30	49	6 : 10

will clearly show that the same phenomenon — inhibition of the growth of one lateral shoot by the other — occurs in *Lupinus albus*.

In some plants the axillary buds of the cotyledons also developed. As appears from table III, however, this development was so irregular, that no conclusion can be drawn. Only it is striking, that in the series, where the development of the axillary buds of the first leaf-pair was most strongly inhibited (the series with hetero-auxin 5 in 10^6) also the axillary buds of the cotyledons did not show the least development.

TABLE III. Development of the axillary buds of the cotyledons of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 1, 14/9/36—28/9/36).

At application twice a day via the main stem of	After 8 days			After 14 days		
	length in mm		number of plants with developed cotyledonary buds	length in mm		number of plants with developed cotyledonary buds
	average of 16 plants	total of 16 plants		average of 16 plants	total of 16 plants	
hetero-auxin 5 in 10^6	0	0	0	0	0	0
hetero-auxin 1 in 10^6	2	25	3	7	104	6
hetero-auxin 5 in 10^7	3	44	8	7	109	8
hetero-auxin 1 in 10^7	2	28	5	4	58	6
tap water	0	0	0	4	68	7

Simultaneously with the length of the axillary buds that of the epicotyl from the cotyledons to the first leaf was also measured. In none of the series, however, any increase in length of the epicotyl was observed, neither any swelling or thickening of the stem did occur.

Experiment 2.

30 seedlings of *Lupinus albus* with an average of 5 expanded leaves were decapitated 15 mm above the first leaf from below. The plants were divided into 3 series of 10 plants each. In two series an aqueous solution of hetero-auxin in concentrations of respectively 1 in 10^7 and 1 in 10^6, and in one series tap water was applied to the plants via the decapitated stem once a day. After 15 days the lengths of the developing buds in the axils of the first and the second leaf were measured. As appears from table IV and figure 3, the development of the axillary buds was not inhibited by application of hetero-auxin 1 in 10^7, but very

strongly by hetero-auxin 1 in 10^6, both compared with the blank tap water series.

TABLE IV. Development of the buds in the axils of leaf 1 and leaf 2 of seedlings of *Lupinus albus* decapitated above leaf 2 (experiment 2, 4/2/37—3/3/37).

At application twice a day via the main stem of	Increase in length in mm (average of 10 plants)					
	first 15 days with application			next 12 days without application		
	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together
hetero-auxin 1 in 10^6	5	4	9	30	31	61
hetero-auxin 1 in 10^7	15	11	26	38	32	70
tap water	12	14	26	42	57	99

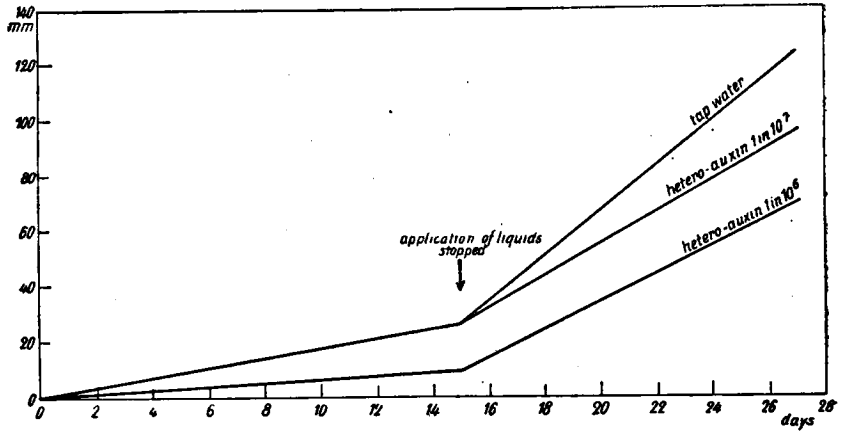


Figure 3. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application once a day of hetero-auxin 1 in 10^6, 1 in 10^7 and tap water via the main stem (experiment 2, 4/2/37—3/3/37).

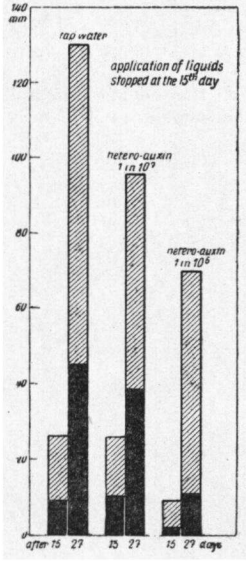
These results are similar to those of experiment 1. It is true that the development of the axillary buds in that experiment in all the series, is relatively much stronger than in this one, but this probably is due to different conditions. The plants of experiment 1, grown outside under glass during August and September, showed an abundant growth, those of experiment 2, on the other

hand, grew in the greenhouse during January and February, with an extra neon-radiation at night, but still under much more unfavourable conditions; they showed a less vigorous growth.

The application of the fluids 15 days after the beginning of the experiment having ended, the lengths of the axillary shoots were measured once more 12 days later. As appears from table IV and figure 3, the increase in length during these 12 days in the two series with the application of hetero-auxin was slighter than in the blank tap water series. So we find still an after-effect of

TABLE V. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 2, 4/2/37—3/3/37).

At application twice a day via the main stem of	Increase in length in mm (average of 10 plants)					
	first 15 days with application			next 12 days without application		
	slower bud	faster bud	ratio slower : faster bud	slower bud	faster bud	ratio slower : faster bud
hetero-auxin 1 in 10^8	2	7	3 : 10	9	52	2 : 10
hetero-auxin 1 in 10^7	10	16	6 : 10	29	41	7 : 10
tap water	9	17	5 : 10	36	63	6 : 10



the hetero-auxin supply during the preceding 15 days. The same is found in the series with hetero-auxin 1 in 10^7, in which the development of the axillary buds during the first 15 days equaled that of the series with water supply.

A faster development of the buds in the axil of leaf 2 than those of leaf 1, is only found in the series with tap water supply; in the two series with a hetero-auxin application the development of the buds in the axil of leaf 1 is similar to that of leaf 2. If, however, as was done in the preceding experiment, we arrange the buds of each

Figure 4. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application, once a day, of hetero-auxin 1 in 10^6, 1 in 10^7 and tap water via the main stem; ■ slower bud, ▥ faster bud (experiment 2, 4/2/37—3/3/37).

plant according to the rate of their development, we find here too that the increase in length of the slowly developing bud, during the first 15 days as well as in the following 12 days, is slighter than that of the faster developing bud (table V and figure 4).

Experiment 3.

In the two previous experiments the plants were decapitated just above the first leaf pair and we succeeded in inhibiting the growth of the axillary buds by applying hetero-auxin solutions. In the following experiment the plants were decapitated just above the second leaf pair and we tried to find out how the development of the axillary buds would be when applying hetero-auxin.

20 seedlings of *Lupinus albus* with an average of 6 expanded leaves were decapitated 10 mm above leaf 3. The plants were divided into 2 series of 10 plants each. In one series an aqueous solution of hetero-auxin in a concentration of 1 in 10^6 and in one series tap water was applied to the plants via the decapitated main stem once a day. After 15, 21 and 31 days the lengths of the developing buds in the axils of the cotyledons and of the first four leaves were measured.

As appears from table VI, it were chiefly the axillary buds of the lower pair of leaves which expanded in both series. Only in a few plants (their number is indicated in brackets) the

TABLE VI. Development of the buds in the axils of the cotyledons and of the first four leaves of seedlings of *Lupinus albus* decapitated above the fourth leaf (experiment 3, 15/2/37—18/3/37).

At application twice a day via the main stem of	Length in mm (total of 10 plants)														
	after 15 days					after 21 days					after 31 days				
	cotyledonary buds together	bud 1	bud 2	bud 1 and bud 2 together	bud 3 and bud 4 together	cotyledonary buds together	bud 1	bud 2	bud 1 and bud 2 together	bud 3 and bud 4 together	cotyledonary buds together	bud 1	bud 2	bud 1 and bud 2 together	bud 3 and bud 4 together
hetero-auxin 1 in 10^6	[1]) 14(1)	149	139	288	[1]) 27(2)	[1]) 67(2)	426	488	914	[1]) 91(3)	[1]) 170(2)	671	706	1377	[1]) 156(3)
tap water	11(1)	181	238	419	9(1)	29(3)	417	680	1097	30(1)	47(3)	699	934	1633	39(1)

[1]) between brackets the number of plants with developing cotyledonary buds or axillary buds of leaf 3 and 4.

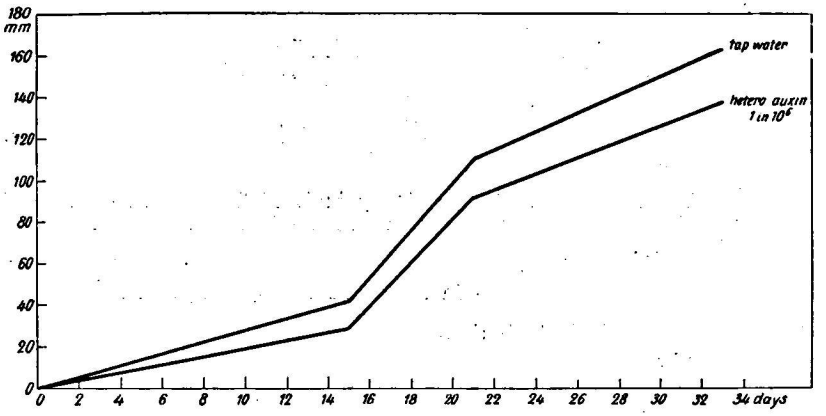


Figure 5. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the fourth leaf at application once a day of hetero-auxin 1 in 10^6 and tap water via the main stem (experiment 3, 15/2/37—18/3/37).

axillary buds of the cotyledons and of the second leaf pair developed (only in one case the axillary bud of leaf 4). As to the development of the axillary buds of the first pair of leaves, we find a smaller increase of length when applying hetero-auxin 1 in 10^6, than when tap water is applied (see also figure 5). During the first 15 days the inhibition was about 30 per cent, during the following 16 days 10 per cent. This difference is smaller than that in the two preceding experiments, probably as the place of application was farther removed.

In the series with the application of hetero-auxin 1 in 10^6 the development of the buds in the axil of leaf 1 as an average is the same as that of the buds of leaf 2; in the series with tap water supply the development

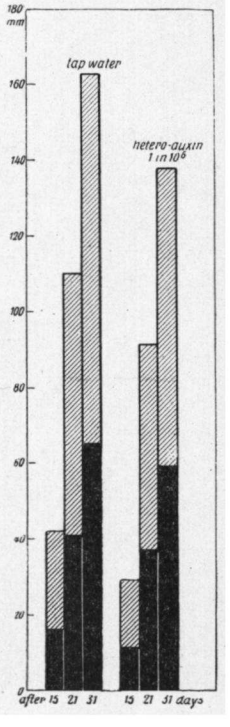


Figure 6. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the fourth leaf at application, once a day, of hetero-auxin 1 in 10^6 and tap water via the main stem; ■ slower bud, ▥ faster bud (experiment 3, 15/2/37—18/3/37).

of the axillary buds of leaf 2, however, is as an average much stronger than that of the buds of leaf 1. Here too an arrangement of the buds as to the rate of their development shows, that the buds which develop faster in the beginning, remain ahead as compared to their slower partners (see table VII and figure 6).

TABLE VII. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the fourth leaf (experiment 3, 15/2/37—18/3/37).

At application twice a day via the main stem of	Increase in length in mm (average of 10 plants)								
	first 15 days			next 6 days			next 10 days		
	slower bud	faster bud	ratio slower: : faster bud	slower bud	faster bud	ratio slower: : faster bud	slower bud	faster bud	ratio slower: : faster bud
hetero-auxin 1 in 10^6	12	17	7 : 10	25	37	7 : 10	22	25	9 : 10
tap water	16	26	6 : 10	25	43	6 : 10	24	29	8 : 10

Experiment 4.

The inhibition of the development of the axillary buds by the application of hetero-auxin solutions via the decapitated main stem, was weaker in the experiments 2 and 3, with an application once a day, than in experiment 1 with a twice-a-day application.

TABLE VIII. Development of the buds in the axils of leaf 1 and leaf 2 of seedlings of *Lupinus albus* decapitated above leaf 2 (experiment 4, 23/2/37—22/3/37).

At application twice a day via the main stem of	Length in mm (average of 10 plants)														
	after 13 days			after 16 days			after 20 days			after 23 days			after 27 days		
	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together
hetero-auxin 1 in 10^5	3	4	7	8	8	16	16	15	31	31	31	62	53	51	104
hetero-auxin 1 in 10^6	6	10	16	11	21	32	24	43	67	28	53	81	33	62	95
hetero-auxin 1 in 10^7	8	9	17	19	20	39	33	37	70	43	45	88	48	50	98
tap water	11	8	19	23	23	46	41	47	88	50	58	108	64	66	130

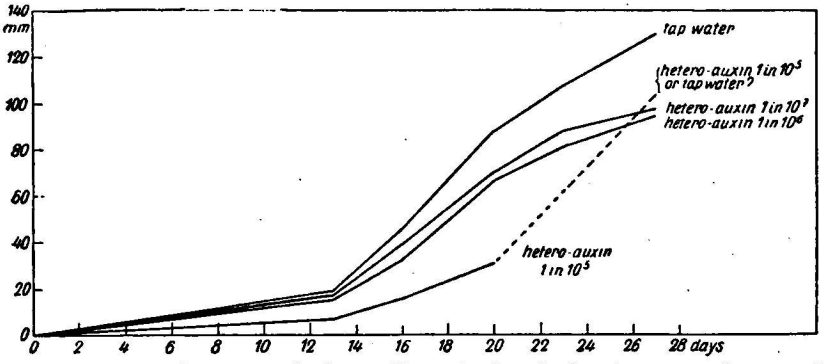


Figure 7. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application twice a day of hetero-auxin 1 in 10^5, 1 in 10^6, 1 in 10^7 and tap water via the main stem (experiment 4, 23/2/37—22/3/37).

For this reason another experiment was made with an application twice a day of hetero-auxin solutions of various concentrations.

40 seedlings of *Lupinus albus* with an average of 6 expanded leaves were decapitated 15 mm above the first leaf from below. The plants were divided into 4 series of 10 plants each. In 3 series an aqueous solution of hetero-auxin in a concentration of respectively 1 in 10^5, 1 in 10^6 and 1 in 10^7, and in one series tap water was applied to the plants via the decapitated stem twice a day. After 13, 16, 20, 23 and 27 days the lengths of the developing buds in the axils of the first and of the second leaf were measured. As appears from table VIII and figure 7 the development of the axillary buds was

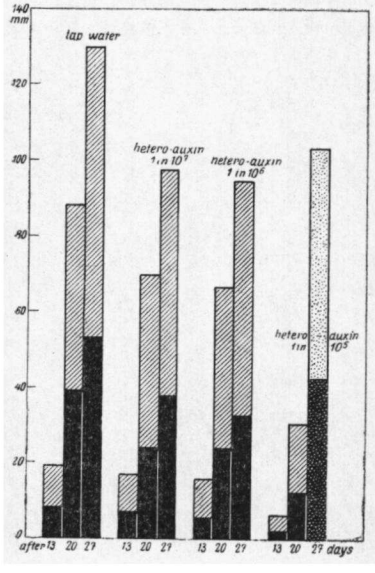


Figure 8. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application twice a day of hetero-auxin 1 in 10^5, 1 in 10^6, 1 in 10^7 and tap water via the main stem; ▬ slower bud, ▥ faster bud (experiment 4, 23/2/37—22/3/37).

inhibited weakly by hetero-auxin 1 in 10^7 and 1 in 10^6, but very strongly by hetero-auxin 1 in 10^5, at least during the first 20 days. During the last 7 days the axillary buds of the series with a hetero-auxin 1 in 10^5-application showed a remarkably rapid development. The more remarkable since the two other series with hetero-auxin supply particularly during these last 7 days still showed a decidedly smaller increase in length of the axillary buds than the series with tap water. We are wondering, whether some error has been made here, for instance an application of tap water instead of hetero-auxin 1 in 10^5. In the test protocols, however, no indication can be found for such a mistake so that these results must be given here unchanged.

On an average the development of bud 2 is not much faster than that of bud 1, except in the series with hetero-auxin 1 in 10^6, where bud 2 shows a decidedly stronger development. When arranging the buds according to the rate of their development, we find, however, again a constantly stronger increase in length of the buds, which developed more rapidly at the beginning (see table IX and figure 8).

TABLE IX. Development of the buds in the axils of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 4, 23/2/37—22/3/37).

At application twice a day via the main stem of	Increase in length in mm (average of 10 plants)														
	first 13 days			next 3 days			next 4 days			next 3 days			next 4 days		
	slower bud	faster bud	ratio slower : faster bud	slower bud	faster bud	ratio slower : faster bud	slower bud	faster bud	ratio slower : faster bud	slower bud	faster bud	ratio slower : faster bud	slower bud	faster bud	ratio slower : faster bud
hetero-auxin 1 in 10^5	3	4	7:10	3	6	6:10	7	8	9:10	14	17	8:10	16	26	6:10
hetero-auxin 1 in 10^6	6	10	6:10	5	11	5:10	13	22	6:10	4	10	4:10	5	9	6:10
hetero-auxin 1 in 10^7	7	10	7:10	7	15	5:10	10	21	5:10	8	10	8:10	6	4	15:10
tap water	8	11	7:10	12	15	8:10	19	23	8:10	7	13	8:10	7	15	5:10

§ 2. *Experiment with cuttings of Ligustrum vulgare.*

Experiment 5.

In the middle of February pieces of 2 years-old twigs of a shrub of *Ligustrum vulgare* were cut off at a length of 45 mm.

They were cut in such a way, that each piece had about the same thickness, and contained at 15 mm from the top two opposite, still dormant buds, 1 to 2 mm long. 40 of these cuttings were divided over 4 series of 10 each and planted in an earthen box with damp mould, which was put in the greenhouse. Once a day an aqueous hetero-auxin solution of a concentration of respectively 1 in 10^6 and 1 in 10^7 was applied to two series via the apical cut surface and to one series tap water was applied, whilst to the fourth series no fluid was supplied at all. The fluids were absorbed more slowly than in the preceding experiments with seedlings of *Lupinus albus*. After 8, 12 and 18 days the lengths of the developing buds were measured. As appears from table X and figure 9 the development of the buds was slightly promoted by the application of hetero-auxin 1 in 10^7 and inhibited to a slight degree by the application of hetero-auxin 1 in 10^6, in comparison with the development when tap water was applied. The development of the buds in the series without any application of fluid, was even much smaller, however, so that we must conclude that the mere application of water already promotes the developing of the buds.

TABLE X. Development of the lateral buds of single-node cuttings of *Ligustrum vulgare* (experiment 5, 12/2/37—2/3/37).

At application twice a day via the main stem of	Length in mm (average of 10 cuttings)											
	after 8 days				after 12 days				after 18 days			
	slower bud	faster bud	both buds together	ratio slower : : faster bud	slower bud	faster bud	both buds together	ratio slower : : faster bud	slower bud	faster bud	both buds together	ratio slower : : faster bud
								[1]				[1]
hetero-auxin 1 in 10^6	5	9	14	5:10	7	15	22	3:10	11	30	41	3:10
hetero-auxin 1 in 10^7	8	11	19	7:10	12	18	30	6:10	16	32	48	3:10
tap water	5	10	15	5:10	8	18	26	4:10	13	33	46	3:10
without supply of liquid	5	10	15	5:10	6	13	19	3:10	8	18	26	4:10

[1]) calculated on the increase in length during the last period of 4, respectively 6 days.

In none of the series anything could be observed of root formation in the cuttings during the experiment.

As the buds were inserted at exactly the same height of the

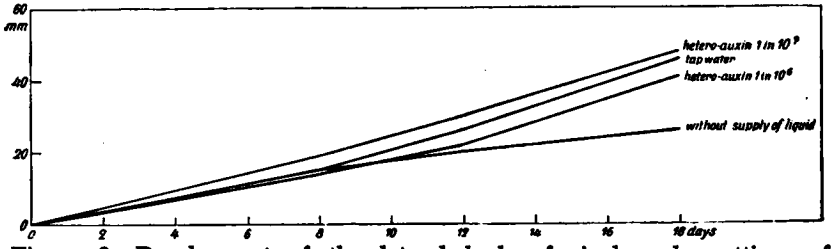


Figure 9. Development of the lateral buds of single-node cuttings of *Ligustrum vulgare* at application once a day of hetero-auxin 1 in 10^6, 1 in 10^7 and tap water via the apical cut surface, and without supply of liquid (experiment 5, 12/2/37—2/3/37).

twigs the place of their insertion cannot be responsible for the difference in rate of development. When arranging the buds according the rate of developing, however, we find the same as we did in the experiments with seedlings of *Lupinus albus:* the buds, developing faster in the beginning, keep on increasing their advance more and more. This phenomenon is even more striking in the *Ligustrum*-cuttings than in *Lupinus*-seedlings, for the ratio slower: faster bud is here even more unfavourable for the slower bud (see table X).

§ 3. *Discussion of the results.*

When we examine the results of the preceding experiments, it appears very clearly, that it is possible to inhibit the growth of axillary buds of decapitated seedlings of *Lupinus albus* by an application, via the cut surface of the stem, of aqueous hetero-auxin solutions of a sufficient concentration. The experiment with cuttings of *Ligustrum vulgare* is less convincing, but points in the same direction. It appears at the same time that hetero-auxin concentrations lower than 1 in 10^6 have no inhibiting but rather a promoting effect on the development of the axillary buds. On the whole, we find a strong inhibition only when hetero-auxin solutions of a concentration 5 in 10^6 and 1 in 10^5 are applied. These concentrations are of about the same order of magnitude as the auxin concentration, which may be expected to be present in intact plants. It may be possible that still higher concentrations might cause a stronger inhibition; it did not seem important to us to find that out, since little value can be attached to the results obtained with so unphysiologically high concentrations.

Our experiments endorse the results of THIMANN and SKOOG

(1933, 1934) who clearly showed, that the terminal bud has an inhibiting influence on the development of the lower lateral buds and that after decapitation auxin, applied via the decapitated main stem, has a similar inhibiting effect. However, the character of the action of auxin in this correlative inhibition still remains in the dark. Whether a direct action of auxin as a consequence of its high concentration is in the play, has to be discriminated by determination of the auxin content (see chapter VII and VIII, p. 238 and 242).

As the exact measuring of lengths in experiment 1 did not give any indication for growth in the main stem and as swellings could not be observed anywhere either — as a matter of fact, we do never find the latter in intact plants —, the idea of LAIBACH (1933) that growing processes in the stem themselves have an inhibiting influence on the axillary buds, seems rather improbable.

Besides, several facts must to be accounted for which hardly fit in the existing theories. For instance: notwithstanding the slight difference in place (on the average only 2 tot 3 mm), bud 2 nearly always developed faster than bud 1. It is true, that in some series no difference was found between the average lengths of the two buds, but in none of the series bud 1 developed faster than bud 2. Further it is remarkable that in experiment 3, where the plants were decapitated a little above the second pair of leaves, the development of the axillary buds of the second pair of leaves was so slight, as well at application of hetero-auxin as at application of tap water. These facts require a further explanation.

The fact, that of two developing axillary buds of a plant, one generally develops faster than the other must be considered too as a correlative inhibition of one lateral shoot by the other; it will be examined more closely in chapter V (p. 226).

CHAPTER IV.

Inhibition of lateral buds by application of lanolin hetero-auxin pastes.

In addition to the application of aqueous hetero-auxin solutions we also tried to inhibit the development of the lateral buds by applying lanolin hetero-auxin pastes. The advantage of these pastes is, that they can be applied everywhere on the plant and thus also in the immediate surroundings of that part of the plant of which we try to affect the growth. In the application

of aqueous hetero-auxin solutions some tissue of the stem always was included too. A disadvantage of the paste method is, however, that we can never be sure how much hetero-auxin is absorbed by the plant from the paste and neither, whether in the application of hetero-auxin pastes of various concentrations, the quantities absorbed are proportional to these concentrations.

§ 1. *Experiments with seedlings of Lupinus albus.*

Experiment 6.
(This experiment runs parallel with experiment 2).

40 seedlings of *Lupinus albus* with an average of 5 expanded leaves were divided into 4 series of 10 plants each. In two series the plants were decapitated 15 mm above leaf 1 and in two other series as closely as possible above leaf 2. The distance between leaf 1 and leaf 2 was 3 mm on an average. In each set of 2 series the cut surface of the stem of one of the series was supplied with an amount of lanolin hetero-auxin paste 1 in 10^6, whilst in the other two series this was done with lanolin paste without hetero-auxin. The pastes were renewed daily. After 15 days the lengths of the developing buds of the first leaf-pair were measured. As appears from table XI and figure 10, in the series with the application 15 mm above leaf 1 as well as in the series with the application just above leaf 2, the development of the axillary buds was inhibited by the application of lanolin hetero-auxin paste 1 in 10^6 for about 50 per cent, as compared

TABLE XI. Development of the buds in the axils of leaf 1 and leaf 2 of seedlings of *Lupinus albus* decapitated above leaf 2 (experiment 6, 4/2/37—3/3/37).

At application onto the the cut surface of the main stem of	Place of application	Increase in length in mm (average of 10 plants)					
		first 15 days with application			next 12 days without application		
		bud 1	bud 2	bud 1 and bud 2 together	bud 1	bud 2	bud 1 and bud 2 together
lanolin hetero-auxin paste 1 in 10^6	15 mm above leaf 1	3	6	9	25	43	68
plain lanolin paste		6	12	18	26	36	62
lanolin hetero-auxin paste 1 in 10^6	just above leaf 2	4	2	6	45	20	65
plain lanolin paste		10	3	13	46	19	65

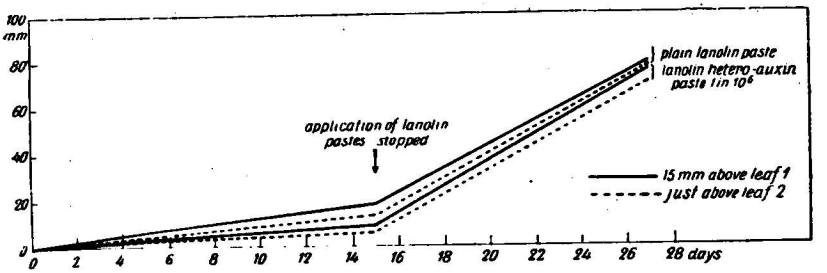


Figure 10. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application to the cut surface of the main stem of lanolin hetero-auxin paste 1 in 10^6 and plain lanolin paste, 15 mm above leaf 1 and just above leaf 2 (experiment 6, 4/2/37—3/3/37).

to their development in the corresponding series with an application of blank lanolin paste. If we compare both series with the paste just above leaf 2, with the corresponding series with the paste 15 mm above leaf 1, we see that in the former series the development of the lateral buds is about 30 per cent smaller than in the corresponding of the latter. Since also in the application of blank lanolin paste just above leaf 2, the development was smaller than with the blank paste 15 mm above leaf 1, we can only conclude, that the decapitation and the application of paste just above the axillary buds has a detrimental influence on their development. This also appears from the fact, that in the series where the paste was applied just above leaf 2 the development of the bud in the axil of this leaf was slighter than that of the bud in the axil of leaf 1. This phenomenon was not found in any of the other experiments.

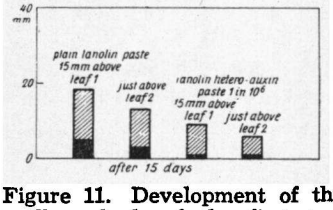


Figure 11. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application onto the cut surface of the main stem of lanolin hetero-auxin paste 1 in 10^6 and plain lanolin paste, 15 mm above leaf 1 and just above leaf 2; ■ slower bud, ▥ faster bud (experiment 6, 4/2/37—3/3/37).

After the 15th day the application of paste was stopped. 12 days afterwards the lengths of the axillary buds were measured once more and it appeared (see table XI) that the increase in length was about the same in all the series. So here we do not find an after-effect of the hetero-auxin application, as we did in experiment 2

TABLE XII. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 6, 4/2/37—3/3/37).

At application onto the the cut surface of the main stem of	Place of application	Increase in length in mm (average of 10 plants)					
		first 15 days with application			next 12 days without application		
		slower bud	faster bud	ratio slower: : faster bud	slower bud	faster bud	ratio slower: : faster bud
lanolin hetero-auxin paste 1 in 10^6	15 mm above leaf 1	1	8	1 : 10	9	59	2 : 10
plain lanolin paste		5	13	4 : 10	25	37	7 : 10
lanolin hetero-auxin paste 1 in 10^6	just above leaf 2	1	5	2 : 10	17	48	4 : 10
plain lanolin paste		3	10	3 : 10	18	47	4 : 10

with the application of hetero-auxin solutions.

If arranging the buds according the rate of their development, we also get the same relations here between more slowly and faster developing buds as we did in the experiments of the preceding chapter (see table XII and figure 11).

Experiment 7.

20 seedlings of *Lupinus albus* with an average of 6 expanded leaves were decapitated 10 mm above leaf 1. The plants were divided into two series of 10 plants each. A ring of lanolin paste was put around the stem at the insertion of the first leaf-pair, in one series containing hetero-auxin at a concentration of 1 in 10^6, in the other one only tap water. The pastes were renewed every 3 days. After 13, 19, 26, 30 and 34 days the lengths of the developing buds were measured. As appears from table XIII and figure 12, the development of the axillary buds in the series with the application of hetero-auxin paste 1 in 10^6 was smaller than in the series with plain lanolin paste.

If we examine the increase in length in the successive periods (see table XIV), it appears, that this increase during the first 19 days in the series with hetero-auxin 1 in 10^6 was about 35 per cent smaller than in the control series; during the next 7 days the difference in increase is only about 20 per cent, whilst during the last 8 days the axillary shoots of the series with hetero-auxin increased in length more than 20 per cent faster than the control series. During these last 8 days the hetero-auxin

TABLE XIII. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 7, 17/2/37—23/3/37).

At application as a ring around the stem at the insertion of the first leaf-pair of	Length in mm (average of 10 plants)				
	days after the decapitation				
	13	19	26	30	34
lanolin hetero-auxin paste 1 in 10^6	14	57	124	149	172
plain lanolin paste	22	87	171	191	210

has no longer an inhibiting effect. That in the whole the bud inhibition in this experiment was weaker than in the preceding one, may be caused by the fact that in this experiment the pastes were renewed every 3 days, whilst in the preceding experiment this was done every day. In both series of this experiment the development of bud 2 was on an average stronger than that of bud 1. By arranging the buds according to the rate of their development, we obtained results which equaled those of the preceding experiments too. It seems superfluous therefore to mention them again for this experiment in a separate table and figure.

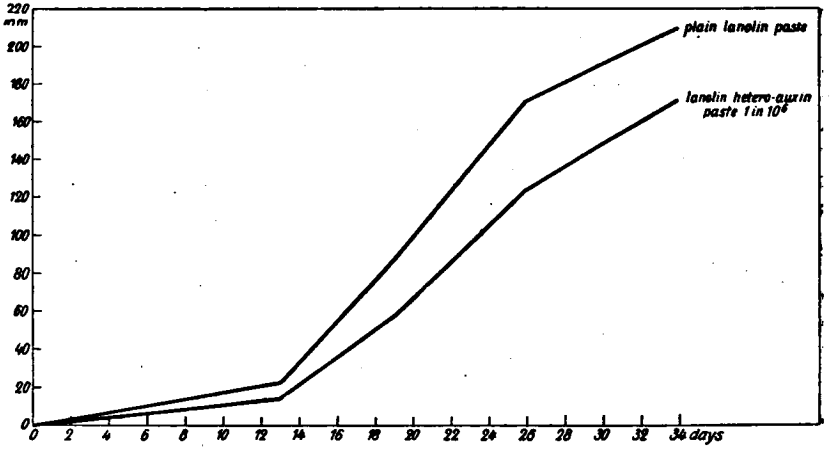


Figure 12. Development of the axillary buds of the first two leaves of seedlings of *Lupinus albus* decapitated above the second leaf at application as a ring around the insertion of the first leaf-pair of lanolin hetero-auxin paste and plain lanolin paste (experiment 7, 17/2/37—23/3/37).

TABLE XIV. Development of the axillary buds of the first two (leaves of seedlings of *Lupinus albus* decapitated above the second leaf (experiment 7, 17/2/37—23/3/37).

At application as a ring around the stem at the insertion of the first leaf-pair of	Increase in length (average of 10 plants)									
	first 13 days		next 6 days		next 7 days		next 4 days		next 4 days	
	in mm	ratio	in mm	ratio	in mm	ratio	in mm	ratio	in mm	ratio
lanolin hetero-auxin paste 1 in 10^6	14	6	43	7	67	8	25	12	23	12
plain lanolin paste	22	10	65	10	84	10	20	10	19	10

§ 2. *Experiments with cuttings of Ligustrum vulgare.*

Also some experiments with an application of lanolin hetero-auxin paste were made with cuttings of *Ligustrum vulgare.*

Experiment 8.

In the middle of February the stems of 2 years-old twigs from a shrub of *Ligustrum vulgare* were cut into 20 pieces all as equal as possible. These pieces were 45 mm long and contained one dormant bud, about 5 mm long, at 15 mm distance from the lower side; the opposed bud was removed. Then these single-node cuttings were split into two equal halves from the base to a little over the node. Into this split some lanolin paste was applied on the level of the dormant bud. In one series of 10 cuttings this paste contained hetero-auxin in a concentration 1 in 10^6, in the other series of 10 cuttings only tap water. The cuttings were planted in an earthen pot with leaf mould which was placed in the greenhouse. Every 2 days the pastes were renewed. After 7 and after 14 days the lengths of the developing buds were measured. The average length of the series with the application of lanolin hetero-auxin paste 1 in 10^6 was respectively 13 and 28 mm, for the series with the application of plain lanolin paste it was 14 and 28 mm. We see, from this that between the two series not the least difference in the development of the lateral bud could be observed.

Experiment 9.

On account of the negative results of the preceding experiment a new experiment was made with hetero-auxin paste of a higher concentration and in a somewhat different way of application. For this purpose 30 single-node cuttings of *Ligustrum vulgare* were used, each with a length of 30 mm and with an equal

thickness of stem. The upper part of these cuttings was cut off obliquely in such a fashion that from the two opposing buds at the apex of the cutting one was removed. The remaining bud had a length of 1 tot 2 mm. The pieces were divided into 3 series of 10 and in all the series lanolin paste was applied to the oblique cut surface at the apex, in two series with hetero-auxin in a concentration of respectively 1 in 10^5 and 1 in 10^6 and in one series without hetero-auxin. The cuttings were planted in an earthen pot with mould and placed in the greenhouse. The pastes were renewed very 2 days. After 6, 9, 13 and 15 days the length of the remaining bud was measured. As appears from table XV, there is some difference between the development with the hetero-auxin pastes and the plain lanolin paste. The difference, however, is so slight, that we need not attach much value to it.

TABLE XV. Development of the lateral bud of single-node cuttings of *Ligustrum vulgare* (experiment 9, 9/3/37—24/3/37).

At application onto the apical cut surface of	Length in mm (average of 10 cuttings)			
	after 6 days	after 9 days	after 13 days	after 15 days
lanolin hetero-auxin paste 1 in 10^5	$2^1/_2$	4	$8^1/_2$	10
lanolin hetero-auxin paste 1 in 10^6	2	4	$8^1/_2$	10
plain lanolin paste	3	$5^1/_2$	$9^1/_2$	$11^1/_2$

§ 3. *Discussion of the results.*

It clearly appears from the results with lanolin hetero-auxin paste 1 in 10^6 to decapitated seedlings of *Lupinus albus* that also by this method of auxin application the growth of lateral buds can be inhibited. The inhibition in the application of a hetero-auxin paste 1 in 10^6 in the immediate surroundings of the axillary buds, was as strong as in the application 12 mm higher up on the stem. According to the theory of LAIBACH (1933) the auxin would first bring about growth processes and cell divisions in the stem and by these secondarily an inhibiting influence would be exerted on the growth of the axillary buds. Between the place of auxin application or production and the axillary buds in question, some tissue must necessarily be present, capable of this growth. In our experiment with the application of hetero-auxin paste quite close to the axillary buds no growing interiacent tissue is present, but still we find an inhibition not slighter than in the hetero-auxin application

12 mm higher on the stem. From this it appears that LAIBACH's theory hardly can be right.

In the cuttings of *Ligustrum vulgare* the application of lanolin hetero-auxin paste 1 in 10^6 as near as possible to the still dormant lateral buds did not cause any inhibition of their development, whilst in experiment 5, taken at about the same time, an application of aqueous hetero-auxin solutions indeed had some effect. The reason probably is, that the hetero-auxin is absorbed from the lanolin paste by these woody cuttings only to a slight degree, whilst in the applications of the aqueous solutions the hetero-auxin together with the fluid is much more easily absorbed by the plant. Also on account of these results, we are of opinion, that wherever it is possible, the application of auxin in the form of aqueous solutions, is to be preferred to its application as lanolin pastes.

CHAPTER V.

Inhibition of lateral shoots by application of hetero-auxin solutions.

The experiments, treated in both preceding chapters, showed the possibility of inhibiting the growth of lateral buds in decapitated seedlings of *Lupinus albus* by applying aqueous hetero-auxin solutions or lanolin hetero-auxin pastes to the cut surface of the stem. This made it highly probable, in view of the analogic behaviour of hetero-auxin and auxin-a (see chapter II, § 2, p. 203), that in the correlative inhibition of lateral buds by the terminal shoot auxin acts as a correlation carrier. However, the exact role of auxin in this correlation, still remains in the dark. At the same time our attention was drawn to a phenomenon which also could be considered as correlative inhibition, namely the fact, that in the case of two developing axillary buds inserted on the same level of the decapitated stem, one of the buds generally develops more rapidly than the other. It seemed important to find out, first whether actually inhibition of the growth of one lateral shoot by the other occurs and secondly, whether auxin is the correlation carrier here too.

§ 1. *Experiments with "two-shoot plants" of Lupinus albus.*

Experiment 10 [1]).

From a lot of seedlings of *Lupinus albus,* decapitated just

[1]) This experiment has already been published in a previous paper (FERMAN, 1938).

above the first pair of leaves and of which the two axillary buds of leaf 1 and leaf 2 had developed, so called "two-shoot plants", 30 plants were selected, of which the two lateral shoots were distinctly unequal in length. The plants were divided into 3 series of mutually comparable plants. Of all the plants the longer lateral shoot was cut off at 10 mm above its base. To two of the series an aqueous hetero-auxin solution of a concentration of resp. 1 in 10^5 and 1 in 10^6 was applied twice a day via the decapitated longer lateral shoot and to one series tap water. Every 3 or 4 days the length of the remaining shorter shoot was measured from its base to the apical bud. From the results summarized in table XVI and figure 13, it appears, that during the test period of 18 days, the shorter lateral shoot showed rather a good growth when tap water was applied via the decapitated lateral shoot; when applying hetero-auxin 1 in 10^6 its growth was slightly less (about 10 per cent), whilst in

TABLE XVI. Growth of the shorter lateral shoot of „two-shoot plants" of *Lupinus albus* (experiment 10, 4/3/37—22/3/37).

At application twice a day via the decapitated longer lateral shoot of	Length of the longer lateral shoot before its decapitation, in mm (average of 10 plants)	Length in mm (average of 10 plants) — days after decapitation of the longer lateral shoot						Increase in length in mm after 18 days (average of 10 plants)
		0	4	7	11	14	18	
hetero-auxin 1 in 10^5	13	4	5	5	7	8	9	5
hetero-auxin 1 in 10^6	17	5	7	8	13	16	22	17
tap water	13	4	6	8	12	16	23	19

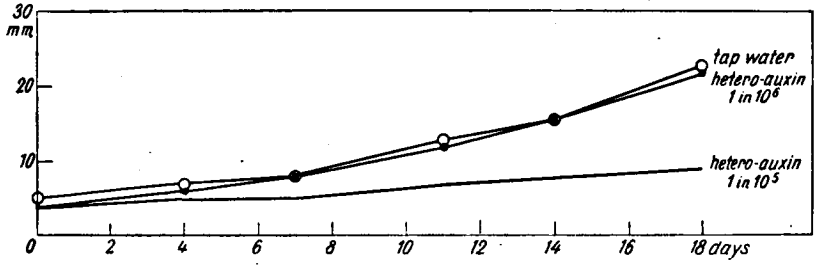


Figure 13. Growth of the shorter lateral shoot of „two-shoot plants" of *Lupinus albus* at application twice a day of hetero-auxin 1 in 10^5, 1 in 10^6 and tap water via the decapitated longer lateral shoot (experiment 10, 4/3/37—22/3/37).

the application of hetero-auxin 1 in 10^5 the growth of the shorter lateral shoot was strongly inhibited (for about 75 per cent), as compared with the growth when tap water was applied.

Some corresponding experiments with two-shoot plants of *Lupinus albus* (the experiments 27—30) will be treated in chapter IX, § 2, p. 258). The results of these experiments, however, all point in the same direction. Thus in experiment 27 with a test-duration of 6 days we find an inhibition of about 70 per cent of the growth of the shorter lateral shoot, when applying hetero-auxin 1 in 10^5 via the decapitated longer lateral shoot, compared with the growth of the shorter lateral shoot of plants of which the longer one had been decapitated, without any fluid being applied. In experiment 28 the increase in length of the shorter lateral shoot after 14 days, when applying hetero-auxin 1 in 10^5, is about 50 per cent of that of plants to which tap water was applied. At the same time in a set of intact two-shoot plants the length increase of the shorter lateral shoot was about 65 per cent of that of the longer lateral shoot.

In experiment 29 the length increase of the shorter lateral shoot at an application of hetero-auxin 1 in 10^5 is inhibited in one series after 7 days for about 30 per cent, in another series after 14 days for about 75 per cent, compared with the growth of the shorter lateral shoot of corresponding series with an application of tap water via the decapitated longer lateral shoot. Finally we find in experiment 30 that, when applying hetero-auxin 1 in 10^5 via the decapitated *shorter* lateral shoot the growth of the *longer* lateral shoot in one series is inhibited after 7 days for about 10 per cent, in another series after 15 days for about 40 per cent, compared with the growth of the longer lateral shoot of corresponding series with an application of tap water.

§ 2. *Discussion of the results.*

The experiments mentioned above showed the possibility of inhibiting the growth of the shorter, or of the longer, lateral shoot in two-shoot plants of *Lupinus albus* by applying an aqueous hetero-auxin solution 1 in 10^5 via the decapitated second (resp. longer, or shorter) lateral shoot, compared with the growth of these lateral shoots when applying tap water or no liquid at all. This makes it very probable that in the intact two-shoot plant too, the growth of the shorter lateral shoot is inhibited by the longer one and that also in this case of correlative inhibition auxin is the correlation carrier. The correlative

inhibition of axillary buds by the terminal shoot and the correlative inhibition of the one lateral shoot by the other, should therefore be considered as phenomena of an analogous character. This is of importance in our later efforts to find an explanation for these phenomena.

CHAPTER VI.

Inhibition of young shoots and lateral buds by application of hetero-auxin solutions from below.

From the experiments of LE FANU (1936) and SNOW (1936) it appeared that the growth of the young shoots and the axillary buds of decapitated shoots of *Pisum sativum* can be inhibited by hetero-auxin when applied to them from below. This phenomenon may be linked to that of the correlative inhibition of lateral buds and shoots, so that it seemed important to make the same experiments with seedlings of *Lupinus albus* too.

§ 1. *Inhibition of young shoots of Lupinus albus.*

Experiment 11.

40 two weeks-old seedlings of *Lupinus albus* with as an average 1 expanded leaf were cut off 40 mm below the cotyledons. They were divided over 4 series of 12 shoots. Each shoot was put separately in a glass tube 10 cm long and with a diameter of 15 mm. The tubes in 3 series were filled with 14 cm^3 of an aqueous hetero-auxin solution of a concentration of resp. 1 in 10^5, 4 in 10^6 and 1 in 10^6 and in one series with tap water. The

TABLE XVII. Growth of shoots of seedlings of *Lupinus albus* cut off 40 mm below the cotyledons (experiment 11, 7/10/36—16/10/36).

Placed with their basal ends in glass tubes with	Length of internode 1 in mm (average of 12 shoots)				Total length in mm (average of 12 shoots)				Number of expanded leaves (average of 12 shoots)			
	days after starting the experiment											
	0	3	6	9	0	3	6	9	0	3	6	9
hetero-auxin 1 in 10^5	—	6	7	9	43	60	65	84	1	1	2	2
hetero-auxin 4 in 10^6	—	8	16	19	45	68	93	103	1	2	2	3
hetero-auxin 1 in 10^6	—	8	14	15	44	69	88	97	1	2	2	3
tap water	—	9	13	16	45	76	91	102	1	2	2	3

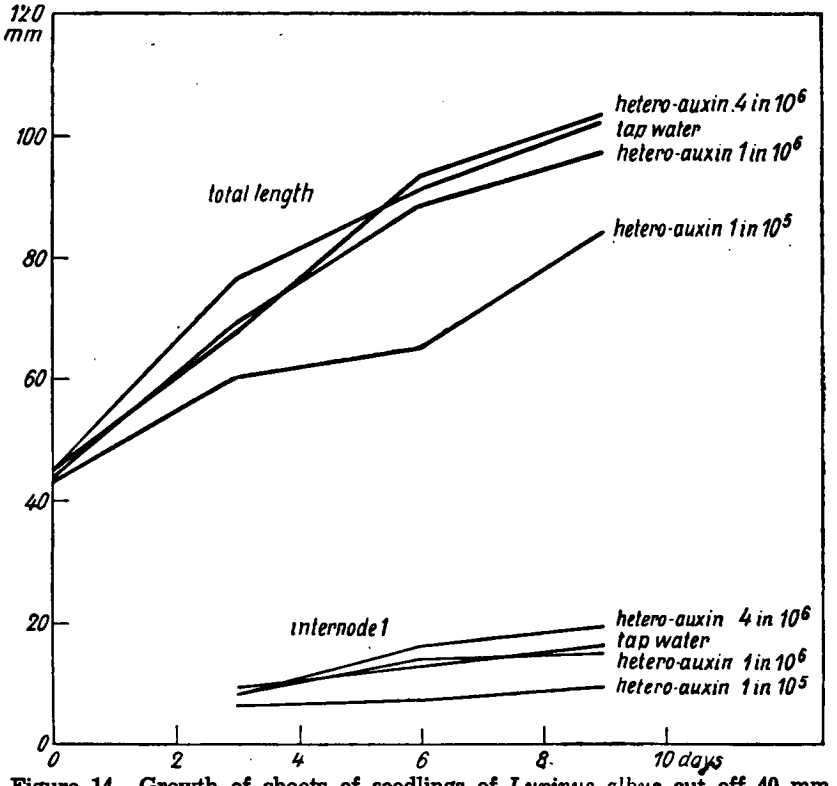


Figure 14. Growth of shoots of seedlings of *Lupinus albus* cut off 40 mm below the cotyledons and placed with their basal ends in glass tubes with hetero-auxin 1 in 10^5, 4 in 10^6, 1 in 10^6 and tap water (experiment 11, 7/10/36—16/10/36).

shoots rested with their cotyledons on the edge of the glass tubes and their hypocotyl was immersed in the liquid for about 2 cm. Every 2 or 3 days the hetero-auxin solutions were renewed. At the beginning of the experiment and after 3, 6 and 9 days the shoots were measured, that is a) the length of internode 1 (from the cotyledons to leaf 1), b) the greatest measurable length from the cotyledons to the end of a leaf and c) the number of expanded leaves. It appears from table XVII and figure 14 that the hetero-auxin solutions 4 in 10^6 and 1 in 10^6 had no influence on the development of the shoots, as compared with their growth in tap water. The hetero-auxin solution 1 in 10^5, on the other hand, produced a strong inhibition.

The development of these shoots by hetero-auxin 1 in 10^5 is influenced very unfavourably, as appeared also from the fact, that the petioles of these shoots showed a distinct epinastic curvature 3 days after the beginning of the experiment. Further the leaflets were somewhat folded inward alongside their midrib. After 9 days all the cotyledons in this series showed a brownish-yellow discoloration and in half of the cases they had already dropped off.

Experiment 12.

24 seedlings of *Lupinus albus* with on an average 2 expanded leaves were cut off 30 mm below the cotyls. The shoots were divided over 2 series of 12 plants each and placed separately in glass tubes in the same way as in the previous experiment. These tubes were filled in one series with an aequeous hetero-auxin solution 1 in 10^6 and in the other with tap water. These liquids were renewed every 3 days. The hypocotyl of these shoots was immersed in the liquid for about 1 cm. At the beginning of the experiment and after 3, 6, 8, 12 and 17 days the lengths of the shoots were measured, that is the length of internode 1 (from the cotyledons to leaf 1) and the greatest measurable length from the cotyledons to the end of a leaf. From table XVIII and figure 15 it appears, that by placing the shoots in

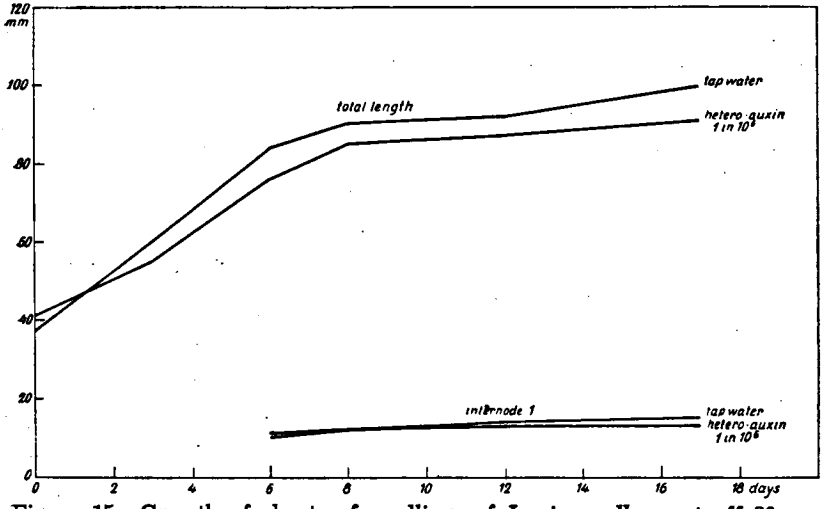


Figure 15. Growth of shoots of seedlings of *Lupinus albus* cut off 30 mm below the cotyledons and placed with their basal ends in glass tubes with hetero-auxin 1 in 10^6 and tap water (experiment 12, 8/1/37—25/1/37).

TABLE XVIII. Growth of shoots of seedlings of *Lupinus albus* cut off 30 mm below the cotyledons (experiment 12, 8/1/37—25/1/37).

Placed with their basal ends in glass tubes with	Length in mm (average of 12 shoots)										
	internode 1				total length						
	days after starting the experiment										Increase in length after 17 days
	6	8	12	17	0	3	6	9	12	17	
hetero-auxin 1 in 10^6	10	12	13	13	41	55	76	85	87	91	50
tap water	11	12	14	15	37	60	84	90	92	100	63

the 1 in 10^6 hetero-auxin solution the growth is slightly inhibited, in comparison with their growth in tap water. This inhibition is not strong and actually only occurs during the first 3 days of the experiment, the increase of length of the shoots in the hetero-auxin 1 in 10^6 then being about 40 per cent less than of the shoots in tap water. Afterwards the development of the shoots in both series runs about parallel. After the 8th day the growth is only very slight. The two curves in figure 15 than begin to look like BLACKMAN-curves and so we must accept, that between the 6th and the 8th day one or more factors begin to act as limiting factors for the growth.

Experiment 13.

In this experiment which runs almost parallel with the preceding one, 36 seedlings of *Lupinus albus* with on an average 2 expanded leaves were cut off 30 mm below the cotyledons. The shoots were divided over 2 series, one of 24 and one of 12 plants, and placed again separately as in the previous experiment in glass tubes. In the series of 24 these tubes were filled with a hetero-auxin solution 1 in 10^7 and in the other series with tap water. Every 3 days the liquids were renewed. In the beginning of the experiment and after 3, 5, 11 and 16 days the shoots were measured, that is the length of internode 1 and the greatest measurable length beginning at the cotyledons. From table XIX and figure 16 it appears, that the growth of the shoots in the hetero-auxin solution 1 in 10^7 was about the same as the growth in tap water.

In order to make the results of this experiment and those of the preceding one more visually comparable, the joined results of these two experiments were plotted in figure 16. This was done by shifting the curves of the hetero-auxin series in both

TABLE XIX. Growth of shoots of seedlings of *Lupinus albus* cut off 30 mm below the cotyledons (experiment 13, 9/1/37—25/1/37).

Placed with their basal ends in glass tubes with	Length in mm (average of 24 and of 12 shoots)										
	internode 1					total length					
	days after starting the experiment										Increase in length after 16 days
	0	3	6	11	16	0	3	6	11	16	
hetero-auxin 1 in 10^7	—	—	12	13	14	39	58	81	85	89	50
tap water	—	—	12	13	15	43	65	93	94	96	53

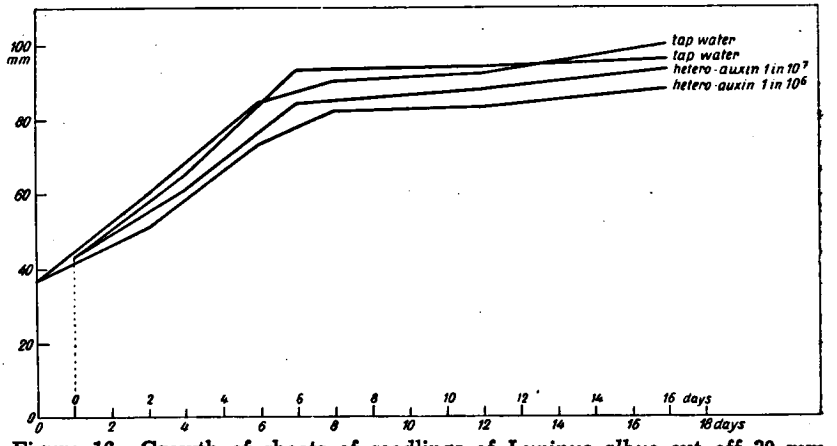


Figure 16. Growth of shoots of seedlings of *Lupinus albus* cut off 30 mm below the cotyledons, and placed with their basal ends in glass tubes with hetero-auxin 1 in 10^7, 1 in 10^6 and tap water (experiment 12 and 13, 8/1/37—25/1/37).

experiments to such an extent that their initial point coincides with that of the series in tap water. As appears from the figure, there is a good conformity between the two series in tap water, while we find a slight inhibition for the series in hetero-auxin 1 in 10^7 and a somewhat stronger inhibition for the series in hetero-auxin 1 in 10^6.

§ 2. *Inhibition of young shoots of Pisum sativum.*

Experiment 14.

An analogous experiment was made with shoots of *Pisum sativum*, variety "*Kaapse groene*". 24 two weeks-old seedlings with 2 to 3 expanded leaves were used; 16 of the seedlings

were cut off at 40 mm below leaf 3, and these shoots, like the shoots of *Lupinus albus* in the preceding experiment, were placed separately in glass tubes. In one series of 8 shoots the tubes were filled with a hetero-auxin solution 4 in 10^6 and in another series of 8 with tap water. The shoots rested with their 3rd leaf on the edge of the glass tubes and thus their bases were immersed in the liquid for about 2 cm. Every 2 or 3 days the liquids were renewed. The remaining 8 plants were left intact in the same place where they had also been grown, namely in mould in the greenhouse; only their first two scales

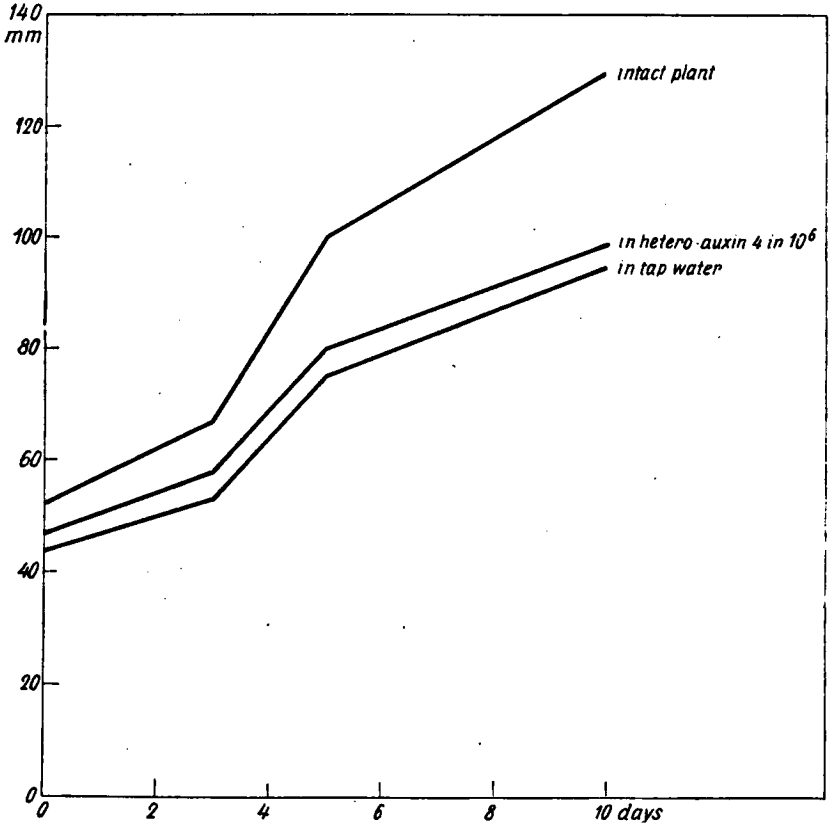


Figure 17. Growth of shoots of seedlings of *Pisum sativum* cut off 40 mm below leaf 3 and placed with their basal ends in glass tubes with hetero-auxin 4 in 10^6 and tap water and of shoots of intact plants (experiment 14, 21/9/36—7/10/36).

TABLE XX. Growth of shoots of seedlings of *Pisum sativum* (experiment 14, 21/9/36—7/10/36).

Cut off 40 mm below leaf 3 and placed with their basal ends in glass tubes with	Length in mm (average of 8 shoots)																							
	after 0 days				after 3 days					after 5 days						after 10 days								
	internode			total length	internode				total length	internode					total length	internode							total length
	4	5	6		4	5	6	7		4	5	6	7	8		4	5	6	7	8	9	10	
hetero-auxin 4 in 10^6	24	19	4	47	24	25	8	1	58	25	26	18	9	2	80	25	27	19	15	11	2	0	99
tap water	23	17	3	43	23	23	6	1	53	24	24	18	8	1	75	24	25	20	15	10	1	0	95
of intact plants	21	23	8	52	22	27	16	2	67	22	28	24	20	6	100	23	28	25	23	21	9	1	130

beginning of the experiment and after 3, 5 and 10 days the lengths of the internodes above leaf 3; internode 4 (from leaf 3 to leaf 4), 5 (from leaf 4 to leaf 5), etc. were measured. As appears from table XX and figure 17 the growth of the shoots, when placed in hetero-auxin 4 in 10^6 was the same as when placed in tap water; the growth of the intact plants, however, was much stronger.

§ 3. *Inhibition of lateral buds of young shoots of Lupinus albus.*

It seemed important to investigate whether by placing young, decapitated shoots of *Lupinus albus* in hetero-auxin solutions of various concentrations, the growth of the developing buds would be inhibited too. In the following experiment we also tried to find out how much the auxin content of these shoots increased by placing them in these hetero-auxin solutions.

Experiment 15.

From 90 four weeks-old seedlings of *Lupinus albus* with on an average 3 expanded leaves, internode 1 having an average length of 76 mm (from cotyledons to leaf 1), 60 were cut off 30 mm below the cotyledons and decapitated just above leaf 2. The cut-off shoots, 10 together, were put into small glass trays. These trays were covered with paraffined card-board lids, in which 10 holes had been punched, through which the hypocotyls of the shoots could be put. The glass trays were filled with 250 cm^3 liquid, two with an aqueous hetero-auxin solution of concentration 1 in 10^5, two with hetero-auxin 1 in 10^6 and two with tap water. The shoots rested with their cotyledons on the card board lids and their hypocotyl was immersed in the liquid for about 1 cm. Every 3 days the liquids were renewed. From the 30 remaining plants 20 were decapitated just above leaf 2, but further left intact. Of the 10 remaining plants the

auxin content was determined of 10 mm of the stem at the node of the first pair of leaves (the method has been described in chapter II, § 3 and 4, p. 203 and 205).

7, 11 and 14 days after the beginning of the experiment, of respectively 7, 7 and 6 shoots of each of the 4 series the auxin content was determined of 10 mm of the stem at the node of the first pair of leaves. The results of these determinations of the auxin content, have been summarized in table XXI and figure 18. Before determining the auxin content the lengths of the axillary buds of each set of shoots were measured; the results of these measurings too have been summarized in table XXI. (leaves 1 and 2) with their axillary buds were removed. At the

TABLE XXI. Auxin content of 10 mm of the stem at the node of the first leaf-pair of shoots of *Lupinus albus* decapitated just above the second leaf, and development of the axillary buds of these shoots (experiment 15, 15/4/37—29/4/37).

Placed with their basal ends in glass tubes with	Auxin content				Length of the axillary buds			
	days after starting the experiment							
	0	7	11	14	0	7	11	14
hetero-auxin 1 in 10^5	—	17,1°±1,9°	22,0°±1,6°	15,0°±1,2°	0	0	2	7
hetero-auxin 1 in 10^6	—	16,7°±1,1°	24,0°±1,6°	15,0°±1,8°	0	0	2	5
tap water	—	4,7°±0,9°	6,3°±1,1°	6,0°±0,8°	0	0	2	6
of intact, merely decapitated plants	12,5°±1,0*	12,9°±1.3°	— **	14,7°±1,0°	0	0	10	15

*) the auxin content of 10 mm of the stem above and below the first leaf-pair was resp. 11,6° ± 1,0° and 17,7° ± 1,2°.

**) extract lost.

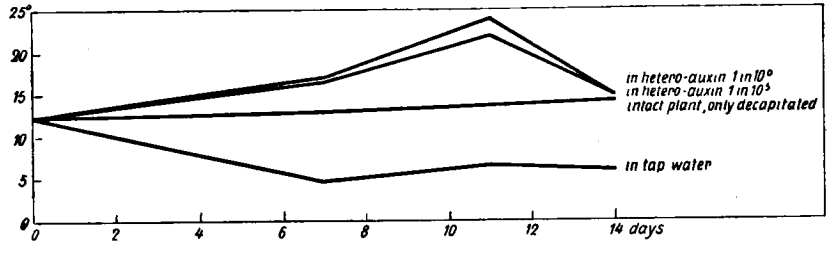


Figure 18. Auxin content of 10 mm of the stem at the node of the first leaf-pair of shoots of *Lupinus albus* decapitated just above the second leaf and placed with their basal ends in glass trays with hetero-auxin 1 in 10^5, 1 in 10^6 and tap water, and of intact, merely decapitated plants (experiments 15, 15/4/37—29/4/437).

It is obvious from these results that the auxin content of the stem at the level of the first pair of leaves of the shoots, placed in hetero-auxin 1 in 10^5, and 1 in 10^6, is higher than of the shoots placed in tap water. This shows that the hetero-auxin has been absorbed by the shoots and transported upwards. Between the shoots put in hetero-auxin 1 in 10^5 and in 1 in 10^6 there is no difference, however, as regards the auxin content at the node of the first leaf-pair. In the merely decapitated controls the auxin content in the same place is somewhat lower than in the shoots in hetero-auxin solutions, but it is considerably higher than in the shoots in tap water. The development of the axillary buds of these shoots, however, is strikingly uniform: between the series in hetero-auxin and in tap water there is but little difference. These series, however, distinctly remain behind the series of decapitated plants, still standing in the soil. The shoots in hetero-auxin 1 in 10^5 showed a toxic effect exerted on them by this solution. One day after the beginning of the experiment the petioles were already epinastically curved and the leaflets were turned inward alongside their midrib. 6 days later the leaves became yellowish green and wilted; 11 days after the experiment had begun the leaves and cotyledons from part of the shoots dropped off.

§ 4. *Discussion of the results.*

The experiments discussed in this chapter show the possibility of inhibiting cut off shoots of *Lupinus albus* by placing them in an aqueous hetero-auxin solution. This inhibition is not strong, however, as compared with the growth in tap water and also proved to be rather dependent on the age of the shoots, which corresponds with the results of SNOW (1936). In shoots of plants with one expanded leaf a hetero-auxin solution 4 in 10^6 and 1 in 10^6 caused no inhibition, but a hetero-auxin solution 1 in 10^5 did. In another experiment with shoots with two expanded leaves a hetero-auxin solution 1 in 10^7 already caused a very slight inhibition of growth, this inhibition being somewhat stronger in hetero-auxin 1 in 10^6.

In the only experiment with *Pisum sativum* no inhibition of the growth could be obtained by placing the shoots of plants with 4 to 5 expanded leaves in hetero-auxin solution 4 in 10^6. Also the development of the axillary buds of decapitated shoots of *Lupinus albus* was not influenced by placing them in hetero-auxin solutions 1 in 10^6 and 1 in 10^5, as compared with their growth in tap water. It is, however, striking that the growth of the shoots

and the axillary buds in hetero-auxin and in tap water is *always* less than that of shoots of control plants left in the soil. The growth-curves of the cut-off shoots after some time all show a type of BLACKMAN-curves. This means that after some time one or more factors act as limiting factor.

From the determination of the auxin content of the decapitated shoots in hetero-auxin 1 in 10^5, 1 in 10^6, in tap water and of plants still rooted in the soil, it appeared that the auxin content of the shoots in tap water was distinctly less than that of plants in the soil, whilst that of the shoots in the hetero-auxin solutions was still higher. From this we may conclude: 1) the auxin content decreases after the cutting off of the shoots and placing them in tap water, 2) when placing the shoots in hetero-auxin solution this hetero-auxin is absorbed by the shoots and transported (probably by the transpiration stream) in acropetal direction. At the same time, however, it was proved, that hetero-auxin 1 in 10^5 applied in this way had a toxic effect, noticeable in the epinastic movement of the petioles, the folding of the leaflets, the yellowish-green discoloration, and the dropping of cotyledons and leaflets.

CHAPTER VII.

The auxin content of the intact plant.

It was beyond the scope of my actual subject to investigate systematically the auxin content of the growing plant. To enable myself, however, to compare decapitated plants in which the development of lateral buds and shoots had been artificially inhibited by hetero-auxin with intact plants, the auxin content of the latter was determined too.

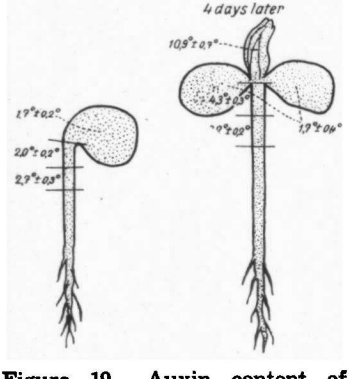


Figure 19. Auxin content of young seedlings of *Lupinus albus* (experiment 16, 25/1/37-29/1/37)

§ 1. *The auxin content of young seedlings of Lupinus albus.*

Experiment 16.

Of a set of 40 one week-old seedlings of *Lupinus albus*, of which the cotyledons had not yet split, the auxin content of 20 plants was determined, i.e., of the cotyledons and of two successive 15 mm long sections of the hypocotyl exactly below the cotyledons, the total

length of the hypocotyl being about 65 mm. The crushing of the cotyledons when extracting the auxin, was rather difficult. The possibly imperfect extraction may be responsible for the low auxin content found in the cotyledons. 4 days later in the remaining 20 seedlings the cotyledons had split and the plumule had grown out to a length of 35 mm, the hypocotyl having an average length of 107 mm. The auxin content of these plants was determined too, i.e., of the plumule, the cotyledons and two successive pieces of 20 mm of the hypocotyl directly below the cotyledons. The results of these determinations have been summarized in figure 19.

The amount of auxin that could be extracted from the cotyledons appears to be equally low at both stages. After the splitting of the cotyledons and the growing out of the plumule, however, we find a distinct increase of the auxin content of the hypocotyl. The plumule too proves to contain a fairly considerable amount of auxin.

§ 2. *The auxin content of older seedlings of Lupinus albus.*

Besides very young seedlings of *Lupinus albus*, the auxin content was also determined of a number of seedlings, which were already well developed.

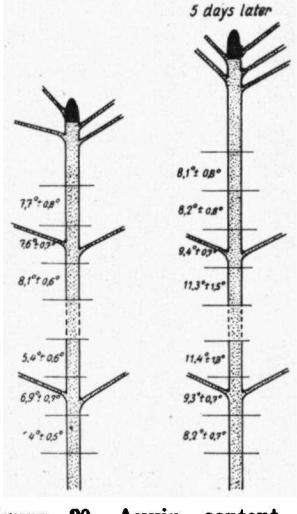


Figure 20. Auxin content of the stem of older seedlings of *Lupinus albus* (experiment 17, 16/7/37—21/7/37).

Experiment 17.

Of 10 plants of a set of 20 5 weeks-old seedlings of *Lupinus albus* with on an average 8 expanded leaves the auxin content was determined in stem sections of 10 mm, a) above the node of the 2nd leaf-pair, b) at this node, c) below this node, d) above the node of the 1st leaf-pair, e) at this node and f) below this node. Internode 2 in these plants (from leaf 1 to leaf 3) had an average length of 39 mm and internode 3 (from leaf 3 to leaf 5) of 28 mm.

5 days later the auxin content was determined of the remaining 10 plants of the same stem sections (and besides also of a piece of 10 mm just above a)). In these plants internode 2 had an average

length of 37 mm and internode 3 of 45 mm; the average number of expanded leaves was 9.

The results of these determinations have been summarized in figure 20.

From this figure we can see that the auxin content of the stem is rather high all over its length; there is little difference in the auxin content of the stem at different points. In the first set of 10 plants the auxin content was a little lower at the base than near the top, in the second set of 10, 5 days later, the auxin content was higher in the middle than above or below it. These differences, however, in our opinion are too slight, to evaluate them. This experiment clearly proves, that there is no essential difference between the auxin content of the higher and of the lower part of the stem.

Experiment 18.

For this experiment 20 8 weeks-old seedlings of *Lupinus albus* were taken with as an average 10 expanded leaves and 4 developed internodes. The plants were not in optimum condition, the leaves lower on the stem were already dropping off. The auxin content of 10 of these plants was determined in stem sections of 20 mm, a) and b) two successive pieces just below the terminal bud, c) a piece half-way between b) and d), d) a piece just above leaf 2 and e) a piece just below it. From the cotyledons to the terminal bud these plants had an average length of 172 mm.

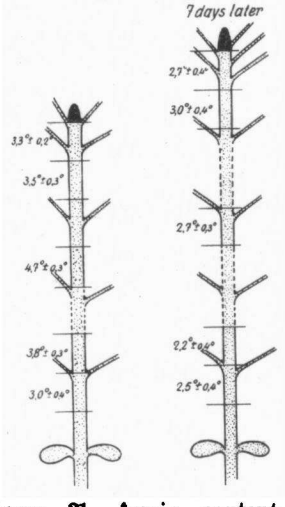


Figure 21. Auxin content of older seedlings of *Lupinus albus* (experiment 18, 26/8/37—2/9/37).

7 days later the auxin content was determined of the same stem sections of the 10 remaining plants. These plants then on an average had 13 expanded leaves and their mean length from cotyledons to terminal bud was 208 mm.

The results of these determinations have been summarized in figure 21.

Probably in consequence of the less favourable conditions of the plants, the auxin content of the stem is lower than that in the preceding experiment. In the second series it is even lower than in the

first one, 7 days earlier. Also in these far developed plants, however, we find an equal auxin content all over the stem, also in the basal part, where the leaves had already dropped off for the greater part. In this feature this experiment confirms the preceding one.

The buds in the axils of the first leaf-pair, where they had an average length of 1 mm excepted, the axillary buds had not developed at any point of the stem, neither in this experiment, nor in the preceding one.

Experiment 19.

In this experiment besides the auxin content of the stem, that of the leaf-pairs was determined too. 10 7 weeks-old seedlings of *Lupinus albus* were used for it. The plants were all in good condition, had as an average 9 expanded leaves and from cotyledons to terminal bud measured 122 mm. The axillary buds had not developed, except those of the lowest leaf-pair, 2 mm long on an average. The auxin content was determined in: the terminal bud including the 5th leaf-pair, the 4th, the 3rd, the 2nd and the 1st leaf-pair, as well as of pieces of the stem 20 mm long, that is of, a) and b) two successives pieces just below the terminal bud, c) a piece half-way b) and d), d) a piece just above leaf 2, and e) a piece just below it. The results have been summarized in figure 22.

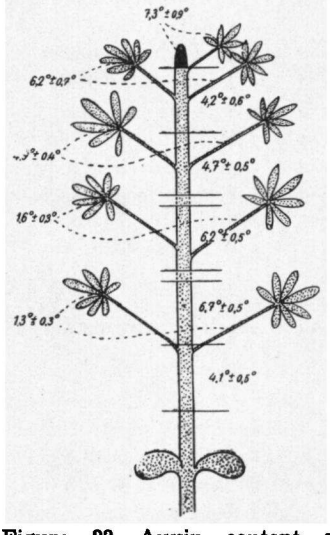


Figure 22. Auxin content of the stem and of the leaves of older seedlings of *Lupinus albus* (experiment 19, 15/9/37).

Here too the auxin content of the stem proves to be about the same all along the stem; perhaps the auxin content in the middle of the stem actually was a little higher than above or below it. On the other hand the auxin content of the leaf-pairs at the top of the stem proves to be the highest; it decreases gradually in basal direction. So the auxin content decreases with increasing age.

Also in the experiments 22 and 23 in chapter VIII (p. 246) the auxin content of several parts of the stem of intact lupine-seedlings was determined. These both expe-

riments made with seedlings with on an average 6 expanded leaves, point in the same direction. Here too, we find that at different points of the stem the auxin content was equal. In experiment 20 (p. 243), where the auxin content of the first and the second leaf-pair was determined, it proved to be the highest in the youngest leaf-pair too.

§ 3. *Discussion of the results.*

From these experiments it appears first, that the auxin content of the stem of seedlings of *Lupinus albus* is about equal all along the length of the stem. If the development of the axillary buds should be connected with the auxin content of the stem, this equal distribution of the auxin, would explain why in the intact plants of *Lupinus albus* the axillary buds do not develop either at the top nor at the bottom of the stem.

Only in the second series of experiment 18 we found an extremely weak development of the axillary buds of the first leaf-pair in far developed plants with an exceptionally low auxin content of the stem.

We also found, that in young lupine-seedlings the auxin content of the hypocotyl directly after the splitting of the cotyledons and the first development of the plumule clearly shows an increase. In far developed plants a decrease of the auxin content of the leaves is found with advancing age. These last two facts make it highly probable, in our opinion, that also in *Lupinus albus* the terminal buds and the young developing leaves are production centers of auxin. DIJKMAN (1934) and JAHNEL (1937) believe, however, that in *Lupinus albus* such production centers of auxin are lacking. We will discuss this further in § 3 of the next chapter (p. 253).

CHAPTER VIII.

The auxin content of plants with artificially inhibited lateral buds.

In the experiments of chapter III we succeeded in inhibiting the development of the axillary buds of decapitated seedlings of *Lupinus albus* by application of aqueous hetero-auxin solutions. We found at the same time that the degree of inhibition depends on the concentration of the hetero-auxin applied. It seemed worth while to repeat these experiments and to determine the auxin content of the stem of these plants simultaneously, as this might give us some information on the real nature of this phenomenon.

§ 1. *Experiments with seedlings of Lupinus albus.*

Experiment 20.

Of a set of 40 seedlings of *Lupinus albus* with on an average 4. expanded leaves the auxin content was determined of 8 plants, a) in the terminal bud including the not yet expanded leaves, b) the 2nd leaf-pair, c) the 1st leaf-pair and d) in a 10 mm section of the stem at the node of the 1st leaf-pair. The remaining 32 plants were decapitated 10 mm above leaf 1. After 5, 10, 13 and 14 days of every 8 plants the auxin content was determined in the 1st leaf-pair and in a 10 mm section of the stem at the node of the 1st leaf-pair. At the same time the lengths of the developing buds in the axils of the 1st leaf-pair were measured. When determining the auxin content of the stem sections these axillary buds were included; only in the last two determinations the buds were large enough to be tested separately on their auxin content. The results of these determinations and of the measurings have been summarized in table XXII and figure 23.

TABLE XXII. Auxin content and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 10 mm above leaf 1. (experiment 20, 27/4/37—11/5/37).

	Auxin content				
	days after the decapitation				
	0	5	10	13	14
Terminal bud and not-expanded leaves	15,5°±1,5°	—	—	—	—
second leaf-pair	17,1°±1,9°	—	—	—	—
first leaf-pair	3,2°±0,9°	8,0°±1,3°	4,1°±1,3°	—	9,1°±0,8°
axillary buds of the first leaf-pair	—	—	—	4,0°±0,5°	4,0°±0,6°
10 mm of the stem at the node of the first leaf-pair	19,0°±1,6°	6,5°±1,0°	5,1°±0,9°	4,9°±0,8°	5,8°±0,6°
	Length in mm (average of 8 plants)				
axillary buds of the first leaf-pair	0	0	8	13	22

From these results it appears that the auxin content of the stem at the node of the first leaf-pair clearly shows a decrease after the decapitation. At the same time the axillary buds of the first leaf-pair begin to develop. As is proved by the determination of the auxin content, these buds in their turn begin to produce

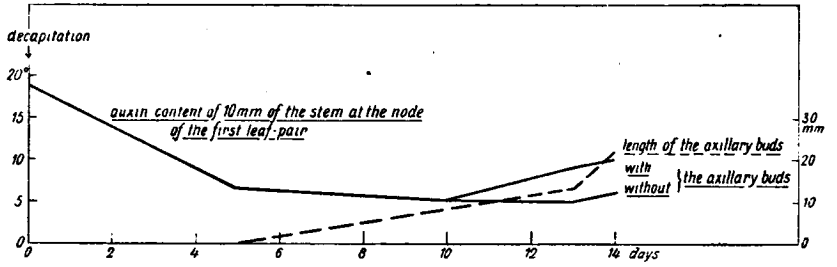


Figure 23. Auxin content of 10 mm of the stem at the node of the first leaf-pair and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 10 mm above leaf 1 (experiment 20, 27/4/37—11/5/37).

rather much auxin. It seems allowed to assume that this makes the auxin content in the stem stop to decrease and even slightly increase.

Experiment 21.

86 seedlings of *Lupinus albus* with on an average 6 expanded leaves were used for this experiment. 80 of these were decapitated 20 mm above leaf 1 and to 30 of them a hetero-auxin solution 1 in 10^5 was applied once a day via the cut surface, to 30 others tap water, whilst no liquid was applied to the remaining 20. 6 plants were left intact. 4 days later of 10 plants in each series and of the 6 intact plants the auxin content was determined in a 10 mm section of the stem at the node of the first leaf-pair, including the developing axillary buds. The length of these axillary buds were measured simultaneously. On the 9th and the 16th day after the beginning of the experiment another lot of 10 plants from the 3, resp. 2 series, the auxin content was determined in a 10 mm section of the stem at the node of the first leaf-pair. The auxin content of the developing buds was determined separately after measuring their lengths. The results of these determinations and of the measurings have been summarized in table XXIII and figure 24.

The results of the determinations of the auxin content teach us first of all, that the auxin content of the merely decapitated plants and of the decapitated plants to which tap water had been applied is strongly reduced as compared to that of the intact plants. It was taken for granted and indicated in figure 24 by dotted lines, that the auxin content of the intact plants at the beginning of the experiment is the same as 4 days later. When

TABLE XXIII. Auxin content of 10 mm of the stem at the node of the first leaf-pair and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 20 mm above leaf 1 (experiment 21, 1/6/37—17/6/37).

At application once a day via the main stem of		Auxin content			Length of the axillary buds in mm (average of 10 plants)		
		days after the decapitation			days after the decapitation		
		4	9	16	4	9	16
hetero-auxin 1 in 10^5	axillary buds 10 mm of the stem	12,5°±0,5°	0,0°±0.0° 5,1°±0,4°	1,5°±0,6° 0,8°±0,2°	0	7	22
tap water	axillary buds 10 mm of the stem	4,6°±0,6°	2,0°±1,0° 0,5°±0,2°	3,1°±0,6° 4,2°±0,5°	3	17	46
without application of liquids	axillary buds 10 mm of the stem	4,9°±0,5°	0,0°±0,0° 0,6°±0,2°	—	3	12	—
intact plant	10 mm of the stem	19,5°±1,0°	—	—	0	—	—

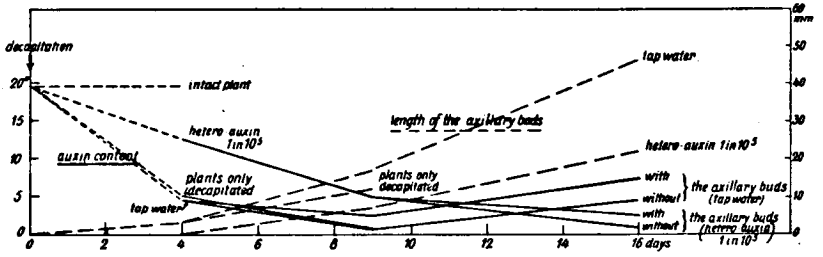


Figure 24. Auxin content of 10 mm of the stem at the node of the first leaf-pair and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 20 mm above leaf 1, at application once a day of hetero-auxin 1 in 10^5 and tap water via the main stem, and without any application of liquids (experiment 21, 1/6/37—17/6/37).

applying hetero-auxin 1 in 10^5 the reduction in the auxin content is only very small, probably as a consequence of this auxin supply. This only holds good for the results of the first 9 days, however, for at the end of the experiment, 7 days later, the auxin content of the plants with hetero-auxin 1 in 10^5 is but extremely small, the plants with an application of tap water then showing some increase again. It seems rather strange that the auxin content of the stem at the node of the first leaf-pair in the series with hetero-auxin 1 in 10^5 shows such a strong decrease at the

end of the experiment. Can this possibly be due to a transport of the hetero-auxin to the basal parts of the stem or to inactivation of the hetero-auxin? If, however, this low auxin content of the stem in the application of hetero-auxin 1 in 10^5 at the end of the experiment is taken as a matter of fact, the simultaneous increase of the auxin content in the series with tap water can easily be ascribed to the higher auxin production by the axillary buds in this series developing faster than in the series with hetero-auxin 1 in 10^5. These facts correspond with the results of the analogous experiments of chapter III. It also appears from the determinations of the auxin content of the buds separately, that the buds which develop faster, contain more auxin than the slower inhibited buds.

Experiment 22 [1]).

As in the former experiment a remarkable low auxin content was found in the stem when applying hetero-auxin 1 in 10^5, it was decided to repeat this experiment with an application of hetero-auxin and tap water to more developed plants, so that the application could take place above the second leaf-pair, the auxin content thus being easily determinable at different points in the stem.

For this experiment 70 seedlings of *Lupinus albus* with on an average 6 expanded leaves were used. The auxin content in 10 of these plants was determined of 10 mm sections of the stem a) at the node of the second leaf-pair, b) below this node, c) above the node of the first leaf-pair, and d) at this node. After 3 days the remaining 60 plants were decapitated above the second leaf-pair (15 mm above leaf 3), while the cotyledons and the first leaf-pair were taken away. To 20 of these plants twice a day an aqueous hetero-auxin solution 1 in 10^5 was applied via the cut surface, to 20 others tap water. The remaining 20 plants did not get anything at all. 8 and 9 days, respectively 14 and 16 days after the decapitation, of 10 plants of each series the auxin content was determined exactly in the same way as we did with the intact plants mentioned above. At the same moment the length of both developing buds in the axils of the first and second leaf-pair was measured. The auxin content of these buds was determined together with the stem parts at the nodes of the leaf-pairs. Only in two cases the buds were large enough to determine their auxin content separately. The results

[1]) This experiment was already published in a previous paper (FERMAN, 1938).

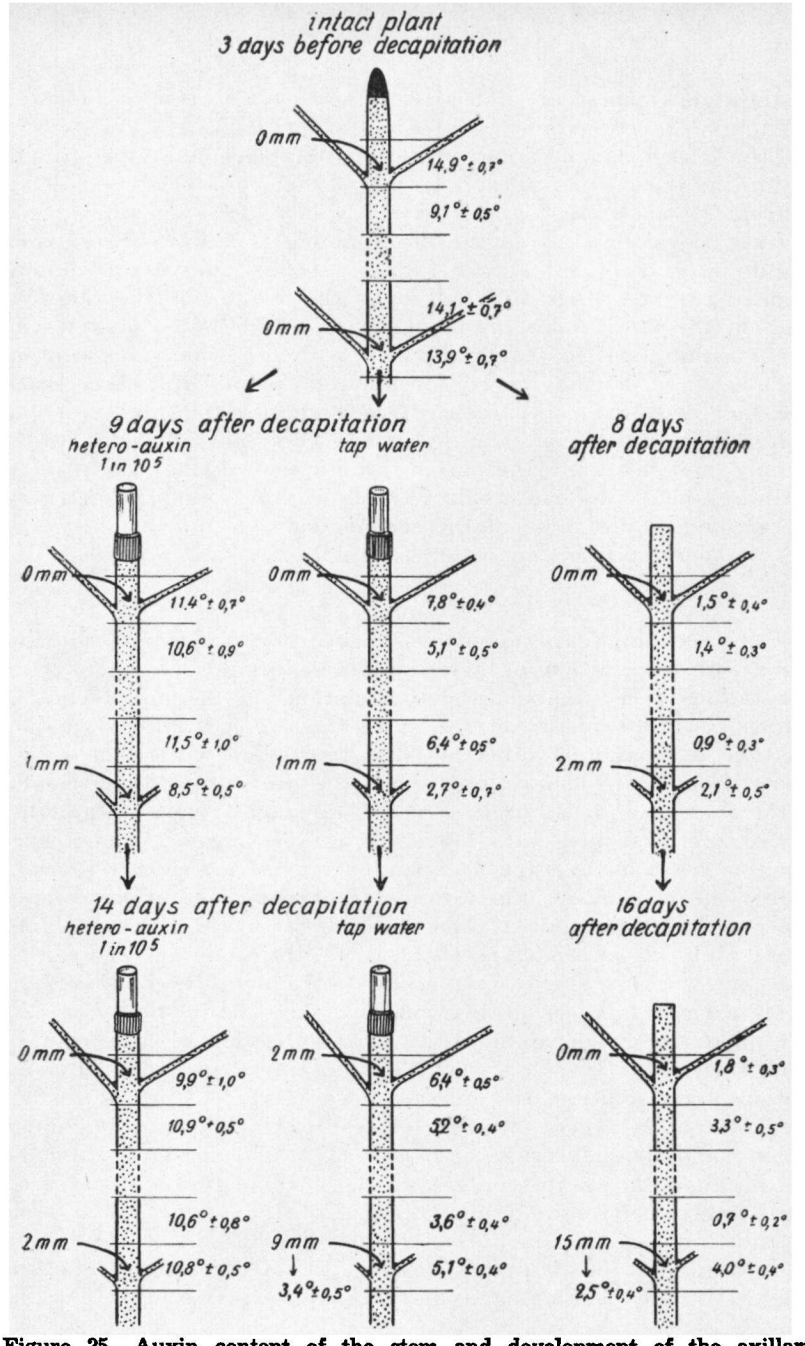


Figure 25. Auxin content of the stem and development of the axillary buds of the first two leaf-pairs of seedlings of *Lupinus albus* decapitated 15 mm above leaf 3, at application twice a day of hetero-auxin 1 in 10^5 and tap water and without application of liquid via the main stem (experiment 22, 9/6/37—28/6/37).

of these determinations and measurements have been summarized in figure 25.

From these data it appears in the first place, that the growth of the axillary buds at application of hetero-auxin 1 in 10^6 is extremely small. By supply of tap water, however, there is a marked development and the development is still stronger when no liquid is supplied at all. Parallel to this the auxin content appears to be rather high all over the length of the stem as well in the intact plant as in plants to which hetero-auxin 1 in 10^5 was supplied. In plants supplied with tap water this content is lower and in the merely decapitated plants still less. From the fact that the auxin content is higher with supply of tap water than without any liquid can be concluded that the water supply has favoured the auxin production of the plant. It is striking that only the axillary buds of the first leaf-pair are developing, while those of the second leaf-pair do not show any development, unless tap water is supplied.

Experiment 23.

The preceding experiment was once more repeated, but this time with application of hetero-auxin solutions of various concentrations. The number of determinations of the auxin content which could be made on the same day being limited, we had to take a smaller number of stem parts, of which the auxin content had to be determined, when the number of experimental series was extended. For this reason the plants were decapitated above the first leaf-pair. 130 seedlings of *Lupinus albus* were used with on an average 6 expanded leaves, internodes 1 and 2 were well developed, the terminal bud being found just above leaf 4. The auxin content was determined in 10 of these plants, of 10 mm sections of the stem a) at the node of the second leaf-pair, b) just below this node, c) above the node of the first leaf-pair and d) at this node. 8 days later the remaining 120 plants were decapitated 15 mm above leaf 1 and divided into 4 series of 30 plants each. To 3 of these series an aqueous hetero-auxin solution was applied twice a day via the cut surface of the stem in a concentration of resp. 1 in 10^5, 5 in 10^6 and 1 in 10^6, and to one series tap water. 2, 9 and 16 days after the decapitation the auxin content in every 10 plants of each series was determined of a 10 mm section of the stem at the node of the first leaf-pair, including the developing axillary buds and of a stem part of 10 mm just below it. At the same time the lengths of the developing axillary buds were measured. At the

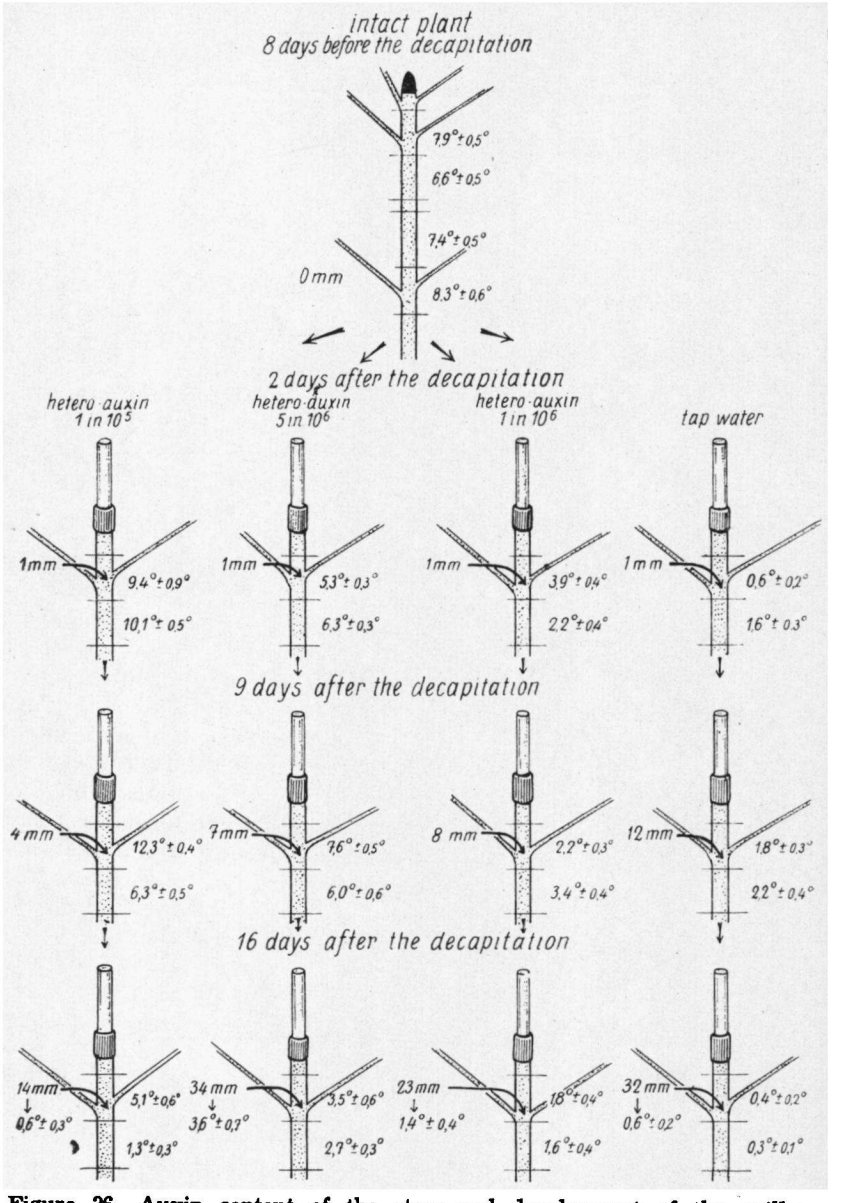


Figure 26. Auxin content of the stem and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 15 mm above leaf 1, at application twice a day of hetero-auxin 1 in 10^5, 5 in 10^6 and 1 in 10^6, and tap water via the main stem (experiment 23, 12/7/37—5/8/37).

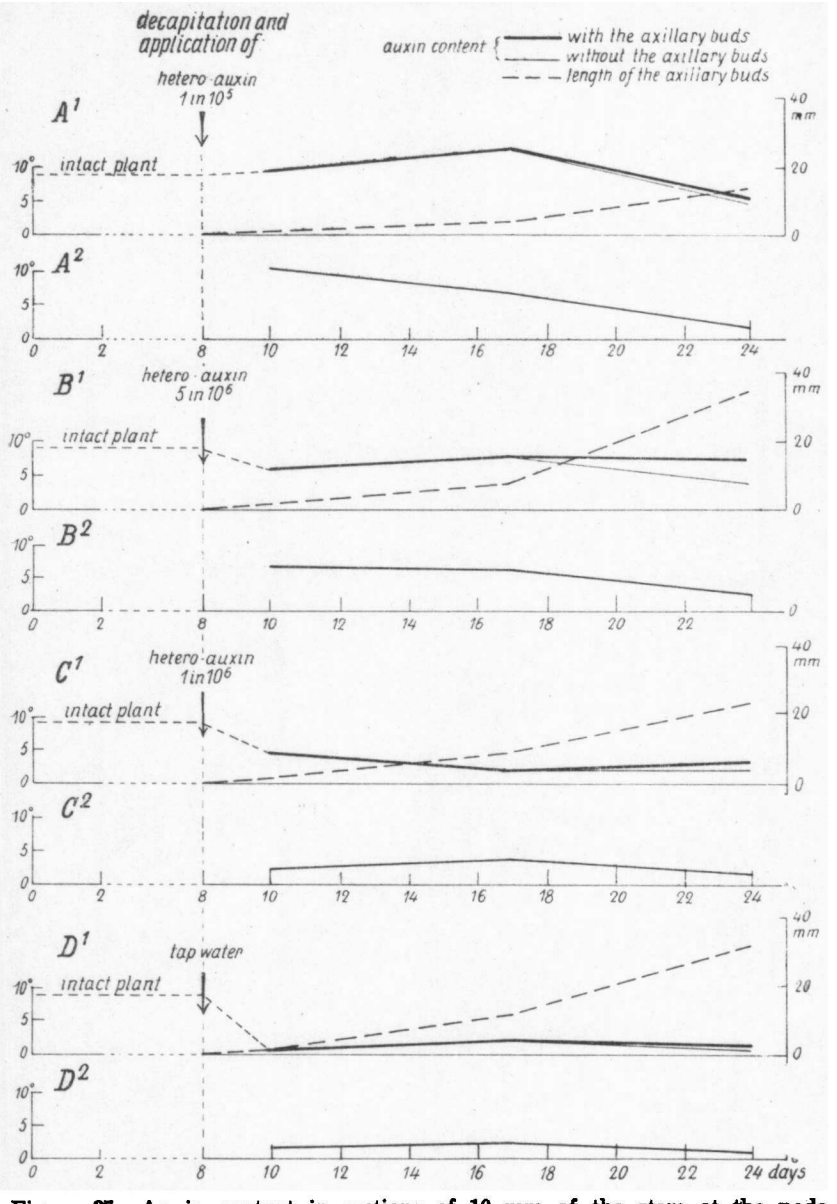


Figure 27. Auxin content in sections of 10 mm of the stem at the node of the first leaf-pair (1) and of 10 mm of the stem below this node (2), and development of the axillary buds of the first leaf-pair of seedlings of *Lupinus albus* decapitated 15 mm above leaf 1, at application twice a day of hetero-auxin 1 in 10^5 (A), 5 in 10^6 (B), and tap water (C) via the main stem (experiment 23, 12/7/37—5/8/37).

time of the determination of the auxin content, 16 days after the decapitation, the axillary buds were large enough to have their auxin content examined separately. The results of these determinations and measurings are summarized in the figures 26 and 27.

First of all it appears from the determinations of the auxin content in the intact plants that here too the auxin content all over the stem is the same. When comparing the auxin content of the decapitated plants, to which hetero-auxin or tap water has been applied artificially, we see a strong decrease of the auxin content when tap water is applied. — In figure 27 it was taken for granted and indicated by dotted lines, that the auxin content of the intact plant at the time of the decapitation was the same as 8 days before. — When comparing the auxin content of the plants of the different series, we find, that 2, 9 as well as 16 days after the decapitation, the auxin content of the series with hetero-auxin 1 in 10^5 is the highest. It is distinctly less in the series with hetero-auxin 5 in 10^6, still lower in the series with 1 in 10^6 hetero-auxin and reaches a very low value in the series with tap water. The auxin content of the stem at the node of the first leaf-pair is sometimes higher, in other cases lower than that of the stem part 10 mm lower. The differences, however, are small and on the whole we can say that the auxin content at these two places is about the same for each series. The values of the auxin content, found 16 days after the decapitation, in all series are lower than those found on the 2nd and 9th day. This may have been caused by a low reactivity of the *Avena*-coleoptiles on the day, when the extracts were tested.

As appears clearly from figure 27, the development of the axillary buds is smallest in the series with the highest auxin content of the stem and highest in the series with tap water, that is: in the series where the auxin content was lowest. In figure 27 this all is clear since the crossing of the lines, indicating the auxin content of the stem at the node of the first leaf-pair and those, indicating the length of the axillary buds shifts to the left in the successive series: a lower auxin content of the stem coincides with a faster growth of the axillary buds.

§ 2. *Discussion of the results.*

The experiments discussed in this chapter clearly prove, that the application of aqueous hetero-auxin solutions to decapitated seedlings of *Lupinus albus* brings about a higher auxin content

of the stem, than when tap water is applied or no liquids at all. From experiment 23 it also appears that this auxin content, within certain limits, corresponds with the concentration of the applied hetero-auxin solutions. At the same time we see, just as in the experiments treated in chapter III (p. 218), that the development of the axillary buds is inhibited by the application of hetero-auxin. The only conclusion from these two observations — in addition to those on the auxin content of the intact plant, discussed in this chapter and the previous one — is, a) that the growth of the axillary buds is inhibited if the auxin content of the stem is high, and b) that when this auxin content decreases, this inhibition decreases too. These results thus seem to endorse the supposition of THIMANN and SKOOG (1934) that this correlative inhibition is caused by a direct action of the auxin, and especially also the idea of THIMANN (1937) that this inhibition is proportional to the concentration of the auxin.

As has been demonstrated, however, in chapter I, § 3a and 3f (p. 187 and p. 198) already, some serious objections may be moved against this "direct" theory. Further it appears from our experiments that the phenomenon is not mastered only by the direct effect of a too high auxin content of the stem. If the supposition of THIMANN and SKOOG would be right, a high auxin concentration should be present in the inhibited buds. Our experiments, however, show — as do also the experiments of THIMANN and SKOOG (1934) themselves — that inhibited buds contain, or give off, less auxin than buds, which are not inhibited. Besides, our experiments with decapitated plants, to which tap water was applied, or no liquid at all, teach that parallel with, and probably as a consequence of the development of the axillary buds, the auxin content of the neighbouring stem-parts increases. This increase, however, does not cause again inhibition of the growth of the axillary buds, as one should expect when agreeing with the theory of THIMANN and SKOOG. The results of experiment 22 do not fit entirely in the "direct" theory either.

In the first place the axillary buds of the first leaf-pair develop in all series inversely proportionate to the auxin content of the adjoining stem parts. The axillary buds of the second leaf-pair, however, do not develop — excepted to a slight degree in the tap water series — although the auxin content for each series is about the same all over the stem. It is also striking, that the tap water series was the only one in which some development of the axillary buds of the second leaf-pair was observed. In

this series, however, the auxin content of the stem was distinctly higher than in the series without application of any liquid. Another accompanying feature is that the auxin content of the stem is increased by the application of tap water, as compared with plants without any supply of liquid. The application of tap water to these decapitated plants therefore must have promoted their auxin production.

Summarizing the results obtained so far, we can say that there is some correlation between the auxin content of the stem and the inhibition of the lateral buds, but a direct action of the auxin on the growth of these buds seems rather improbable. The experiments of the next chapter will show with certainty, that as far as the growth of lateral shoots is concerned a direct action does *not* occur.

§ 3. *On the existence of auxin-producing centers in Lupinus albus.*

This question was earlier investigated by DIJKMAN (1934) and JAHNEL (1937), who both concluded that an auxin producing center is absent in *Lupinus*. Our own investigations, however, point in a different direction, and a closer examination of the experiments by DIJKMAN and JAHNEL tought us, that their conclusions are rather premature and can easily be attacked.

DIJKMAN as well as JAHNEL determined the auxin content of the plants by means of the diffusion method. As already has been explained in chapter II, § 3 (p. 203), this method is less reliable than the extraction method. In the diffusion method one is always dependent on the readiness with which the plant part gives off its auxin and this need not necessarily be the same for the various parts, of which the auxin content has to be determined. It is proved to be possible to indicate the presence of auxin by the way of extraction in places, where this was said not to occur on account of results obtained by diffusion.

DIJKMAN (1934) working with dark-grown seedlings of *Lupinus albus*, decapited a number of 6 days-old seedlings just below the cotyledons and found the growth of the hypocotyl during the first 2 tot 3 days about equal to that of intact plants.

After that period growth stopped entirely whereas in the intact plant the hypocotyl then still increases very rapidly in length. When decapitating 2 days-old seedlings just above the cotyledons the growth of the hypocotyl, for the following 7 days, is equal to that of intact plants. It is obvious, that in these

etiolated seedlings the cotyledons contain a stock of auxin materials. But though DIJKMAN could show the presence of auxin in the epicotyl, in the etiolated leaves and in various sections of the hypocotyl, he does not succeed in getting some auxin by diffusion from the cotyledons. From this he concludes, that auxin-producing centers are absent in *Lupinus*, and that the cells of the growing parts apparently are capable of producing their own auxin.

JAHNEL (1937) removed the terminal bud, one cotyledon or both cotyledons of young seedlings of *Lupinus albus* growing in the open air, and as a consequence of this he found a slight decrease in growth of the hypocotyl for the next two days. This decrease was strongest when the two cotyledons and the terminal bud had been removed. When applying auxin by means of orchid-pollinia no retarding of growth occurred, excepted when the terminal bud had been taken away. JAHNEL thinks, that this retardation is not due to lack of auxin, but to the checking of the supply by the cotyledons of nutritive matter. The removal of any auxin-producing center, according to him, already should show its effect directly after the decapitation and to a much higher degree. His observations, however, do not last longer than 48 hours after the decapitation, whilst, in our opinion, the auxin present in the hypocotyl is sufficient to enable the only slightly retarded growth to go on during this time. DIJKMAN (1934) did not find a distinct influence of the removal of cotyledons and terminal bud either until on the second or third day afterwards. JAHNEL also determined the auxin content of different parts of the plant. His figures are too irregular to enable to conclusive comparisons. From the cotyledons he could obtain but very little auxin. Young leaves gave of more auxin than older ones and on the whole he found an equal distribution of auxin in the hypocotyl. In the stem the auxin content was higher in the higher sections than in the lower ones. From the terminal bud he could get relatively little auxin. This does not justify his conclusion, however, that actually no auxin producing center is present in *Lupinus*.

In our experiment 16 (p. 239) the presence of auxin in the cotyledons of *Lupinus albus* was proved by means of the extraction method, although the quantity of auxin was not large. Further this experiment showed that directly after the splitting of the cotyledons and the developing of the plumule, the auxin content of the hypocotyl increased. This already

suggests that in *Lupinus* the plumule, which proved to contain a considerable quantity of auxin itself, is a production center of auxin. Besides, in experiment 19 (p. 241) the auxin content of the successive leaf-pairs of well-developed plants decreases with advancing age. Particularly the terminal bud and the young leaves are rich in auxin and therefore must be considered as production centers. The final proof, however, that in *Lupinus albus* production centers of auxin actually occur is given by the experiments mentioned in this chapter. In the experiments 20, 21, 22 and 23 (p. 243) after the removal of the terminal bud or of the shoot above the first or second leaf-pair, the auxin content of the stem part just below regularly and clearly shows a decrease. This decrease does not change into an increase again until lateral buds are developing. The only possible explanation of these facts is that in *Lupinus albus* too the terminal bud and the young leaves are production centers of auxin, and that the removal of these production centers makes the auxin content of the stem decrease. It does not increase, until the developing lateral buds start to act as new production centers.

Since the extraction method actually showed the presence of auxin in the cotyledons of *Lupinus albus*, the continued growth of the hypocotyl after decapitation just above the cotyledons as observed by DIJKMAN — whilst after decapitation under the cotyledons the growth after two days stopped — can easily be explained by accepting that, during these early stages, auxin is delivered by the cotyledons of *Lupinus albus*. By VAN OVERBEEK (1932) a similar auxin production by the cotyledons was found in seedlings of *Lepidium* and *Raphanus*.

The presence of production centers in *Lupinus* does, of course, not exclude that other parts of the plant too may be capable of forming auxin. So, for instance the tip of the *Avena*-coleoptile normally is the auxin production center but after decapitation lower zones of the coleoptile prove to be able to produce auxin too. Exactly the apparent lack of a similar physiological regeneration of the production center in *Lupinus albus* makes that, in our opinion, the own production of auxin by other parts cannot play an important part.

CHAPTER IX.

The auxin content of plants with inhibited lateral shoots.

From the experiments discussed in the preceding chapter it

appeared already that the auxin content of slowly developing (inhibited) lateral buds was lower than that of fast developing ones. It was difficult, however, to get comparable results in these experiments. If the buds were still very small they could not be extracted separately, and also in better developed buds it was not possible as yet to determine the auxin content of successive sections, so that only the auxin content of buds of unequal length could be compared. If, however, we determine the auxin content of inhibited lateral shoots, one is enabled to determine the auxin content of successive sections and besides, in intact two-shoot plants, we can compare the auxin content of the inhibiting and of the inhibited shoot. The determination of the auxin content of intact two-shoot plants and of two-shoot plants with an artificially inhibited lateral shoot, looked promising for the explanation of the correlative inhibition of lateral buds and shoots.

§ 1. *The auxin content of intact "two-shoot plants" of Lupinus albus.*

Experiment 24 [1]).

In 9 plants of *Lupinus albus,* decapitated above the first leaf-pair, both axillary shoots had developed, but widely differed in length. Of these 9 two-shoot plants, which were of about the same habit, the auxin content was determined in a) 10 mm of the base of the longer lateral shoot, b) 10 mm of the stem above a), c) 10 mm of the base of the shorter lateral shoot, d) 10 mm of the stem above c) inclusive the apical bud, e) 10 mm of the main stem between both lateral shoots and f) 10 mm of the main stem below e); the average of the stem from the base to the apical bud of the longer lateral shoot being 23 mm, of the shorter one 11 mm. The results of these determinations have been summarized in figure 28 and show that the auxin content of the shorter "inhibited" shoot is lower per length unit than that of the longer "inhibiting" shoot. The auxin content of the main stem was of the same order as that of the longer lateral shoot.

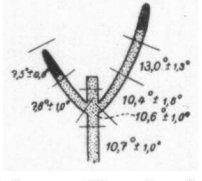


Figure 28. Auxin content of a set of two-shoot plants of *Lupinus albus* (experiment 24, 20/4/37).

Experiment 25.

In this experiment, like in the preceding one, of 10 two-shoot

[1]) This experiment too was published already in a previous paper (FERMAN, 1938).

plants of *Lupinus albus*, with lateral shoots of unequal length, but of about the same habit, the auxin content was determined, namely of 3 successive stem parts of 10 mm of the base of the longer lateral shoot, one stem part of 10 mm of the base of the shorter lateral shoot and one part of 10 mm of the main stem between both lateral shoots. In these plants the longer lateral shoot had an average length of 52 mm, the shorter one of 15 mm. The results of these determinations have been summarized in figure 29.

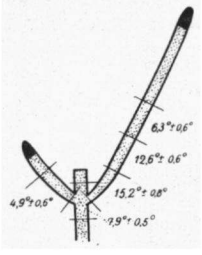


Figure 29. Auxin content of a set of two-shoot plants of *Lupinus albus* (experiment 25, 3/5/37).

Here too, the auxin content of the longer lateral shoot is much higher than that of the shorter one; the auxin content of the main stem between both lateral shoots being about between these two.

Experiment 26.

For comparison with the two preceding experiments, the auxin content of a number of two-shoot plants with lateral shoots of about the same length was determined too. Two sets of 10 plants were used, each set consisting of plants of about the same habit. Of the first set, the length of the one, as well as of the other lateral shoot was on an average 21 mm; the number of expanded leaves was 5 on an average. For the second set of plants these figures were resp. 44 mm and 6. The auxin content of these plants was determined in stem parts of 10 mm, this being in the first set of two, in the second set of three successive sections from the base of both lateral shoots, and further of a part of the main stem between both lateral shoots and of a part just below it. The results have been summarized in figure 30.

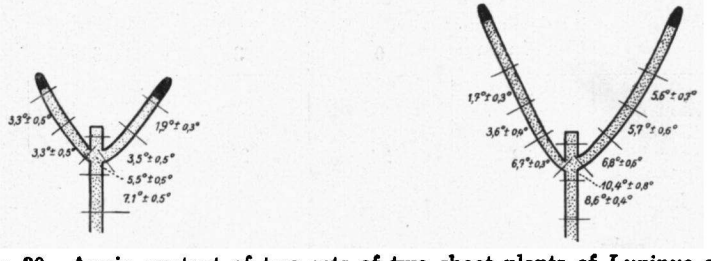


Figure 30. Auxin content of two sets of two-shoot plants of *Lupinus albus* with shoots of equal length (experiment 26, 14/7/37).

Though some deviating values occur, the general impression, obtained from these determinations, for both sets is that the auxin content of both lateral shoots of the same length is about equal. In both sets the auxin content of the main stem is higher than that of the lateral shoots.

§ 2. *The auxin content of "two-shoot plants" of Lupinus albus with artificially inhibited lateral shoot.*

In the experiments discussed in chapter V (p. 226) the growth of one of the lateral shoots of two-shoot plants of *Lupinus albus* was artificially inhibited by application of hetero-auxin solutions via the decapitated other lateral shoot. These experiments were repeated and at the same time the auxin content of the plants was determined.

Experiment 27 [1]).

For this experiment 12 two-shoot plants of *Lupinus albus* of an almost uniform habit were used. In 4 of these plants the auxin content of two successive stem parts of both lateral shoots of respectively 5 and 10 mm and of a part of the main stem between both lateral shoots, 10 mm long, was determined. The average length of the stem from the base to the apical bud of the longer lateral shoot was 24 mm, that of the shorter one 12 mm. The remaining plants were divided into two series of 4. In both series the longer lateral shoot was cut off at 10 mm above its insertion and in one of these series once a day a hetero-auxin solution 1 in 10^5 was supplied to cut surface of this shoot. On the first

TABLE XXIV. Growth of the shorter lateral shoot of two-shoot plants of *Lupinus albus* (experiment 27, 4/5/37—10/5/37).

	Length of the longer lateral shoot before its decapitation in mm	Length in mm (average of 4 plants)			Increase in length in mm after 6 days (average of 4 plants)
		days after decapitation of the longer lateral shoot			
		0	3	6	
At application once a day via the decapitated longer lateral shoot of hetero-auxin 1 in 10^5	39	20	21	22	2
Longer shoot only decapitated	30	17	20	23	6

[1]) This experiment too was published earlier (FERMAN, 1938).

day and after 3 and 6 days the length of the remaining shoot was measured. From table XXIV it appears, that here too the growth of this shoot is inhibited strongly by the application of hetero-auxin 1 in 10^5 via the decapitated longer shoot, as compared with the blank controls.

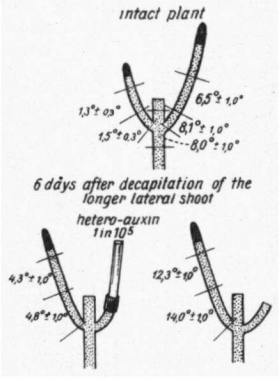


Figure 31. Auxin content of the shorter lateral shoot of two-shoot plants of *Lupinus albus*, a) intact plant, b) application once a day of hetero-auxin 1 in 10^5, c) longer shoot merely decapitated (experiment 27, 4/5/37—10/5/37).

Subsequently of two successive parts of 10 mm of the shorter shoot of both series the auxin content was determined. The results of these and the preceding determinations are shown in figure 31.

From the determinations of the auxin content of the intact plant it appears again, as in the experiments of the preceding section, that the auxin content of the longer lateral shoot is higher than that of the shorter one and that the auxin content of the main stem is of the same order as that of the longer lateral shoot. It is remarkable, that in the series where the growth of the shorter lateral shoot is inhibited by hetero-auxin 1 in 10^5 via the decapitated longer shoot, its auxin content is again distinctly less than in the series where the longer lateral shoot was merely decapitated and the shorter lateral shoot was not inhibited in its growth.

Experiment 28.

The previous experiment was repeated once more with 40 two-shoot plants of *Lupinus albus* of about the same habit which unfortunately were in a less vigourous state. The auxin content in 10 of these plants was determined, a) of the terminal bud of both lateral shoots, b) of the stem parts of the shorter lateral shoot, c) of the upper and lower half of the stem part of the longer lateral shoot and d) of a 10 mm section of the main stem between both shoots. The average length of the stem of the shorter lateral shoot was 7 mm, of the longer one 16 mm. In the same time the longer lateral shoot of 20 of the 30 remaining plants was decapitated at 10 mm above its insertion. To 10 plants an aqueous hetero-auxin solution 1 in 10^5 was applied twice a day to 10 others tap water. The remaining 10 plants were left intact. On the first day and after 6 and 14 days the length of

TABLE XXV. Growth of the shorter lateral shoot of two-shoot plants of *Lupinus albus* (experiment 28, 16/6/37—30/6/37).

At application twice a day via the decapitated longer lateral shoot of	Length in mm (average of 10 plants)			Increase in length in mm after 14 days (average of 10 plants)
	days after decapitation of the longer lateral shoot			
	0	6	14	
hetero-auxin 1 in 10^5	13	13	16	3
tap water	13	14	19	6
intact plant *)	6 (17)	7 (20)	8 (23)	2 (6)

*) between brackets the length of the longer lateral shoot.

the lateral shoots was measured. The results of these measurings have been summarized in table XXV. Probably as a consequence of the unfavourable condition of the plants, the growth of the lateral shoots in all the series was very slow. Yet it is striking, how well the increase in length of the shorter lateral shoots in the intact two-shoot plants agrees with that of the shoots with the application of hetero-auxin 1 in 10^5 via the decapitated longer lateral shoot. Also the increase in length of the longer lateral shoot in the intact two-shoot plants agrees well with that of the shorter lateral shoot with the application of tap water.

On the 14th day the auxin content of all plants of the three

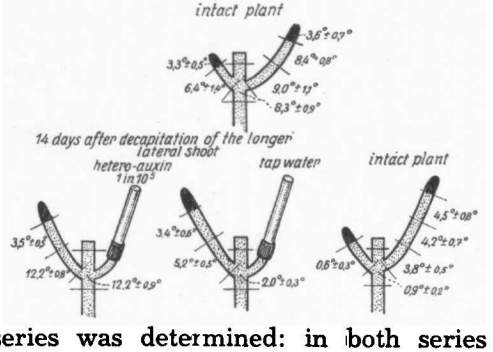


Figure 32. Auxin content of the shorter lateral shoot of two-shoot plants of *Lupinus albus*, a) intact plant, b) at application twice a day via the decapitated longer lateral shoot of hetero-auxin 1 in 10^5, c) idem, at application of tap water, d) intact plant (experiment 28, 16/6/37—30/6/37).

series was determined: in both series with decapitated longer lateral shoots: that of the upper and of the lower half of the stem of the shorter lateral shoot and of 10 mm of the main stem between both shoots, and in the series of intact two-shoot plants: that of the stem of the shorter lateral shoot, of three successive parts of equal length of the longer lateral shoot and of 10 mm of the main stem between both lateral shoots. The results of

these and the preceding determinations of the auxin content have been summarized in figure 32.

The intact plants at the beginning of the experiment show an equally high auxin content in the apical bud of the shorter and of the longer lateral shoot, but the auxin content of the stem of the longer lateral shoot was higher again than that of the shorter one. The same is found in the series of intact two-shoot plants 14 days later. The series with hetero-auxin 1 in 10^5 compared to that with tap water shows that the main stem between both shoots and the lower half of the shorter shoot in the former have a rather high auxin content. A much lower auxin content is found in the upper half of the shorter lateral shoot. The auxin content found in the shorter lateral shoot and in the main stem of the series with tap water is about equal to that of the longer lateral shoot in the intact plants.

Experiment 29.

This experiment too is a replication of experiment 27. This time we used 50 two-shoot plants of *Lupinus albus*, all of them with lateral shoots of unequal length. The auxin content was determined in 10 of these plants of about the same habit: a) of two successive parts of 10 mm of the bases of the longer and b) of the shorter shoot and c) of a piece of 10 mm of the main stem between both lateral shoots. The shorter shoot of these plants was on an average 18 mm long, the longer one 31 mm; the average number of expanded leaves of these shoots being resp. 4 and 5. 7 days afterwards the remaining 40 plants were divided into 4 series of 10 plants each, each series consisting as much as possible of plants of the same habit. Of all these plants the longer lateral shoot was decapitated 10 mm above its insertion and an aqueous hetero-auxin solution 1 in 10^5 was applied

TABLE XXVI. Growth of the shorter lateral shoot of two-shoot plants of *Lupinus albus* (experiment 29, 19/7/37—2/8/37).

At application twice a day via the decapitated longer lateral shoot of	Length in mm (average of 10 plants)			Increase in length in mm after 7 or 14 days (average of 10 plants)
	days after decapitation of the longer lateral shoot			
	0	7	14	
hetero-auxin 1 in 10^5	35	40	—	5
tap water	24	31	—	7
hetero-auxin 1 in 10^5	12	14	15	3
tap water	9	15	21	12

to these plants twice a day via the cut surface in two series; in the two other series tap water was applied. On the first day, and after 7 and 14 days the lengths of the lateral shoots were measured. The results of these measurings have been summarized in table XXVI.

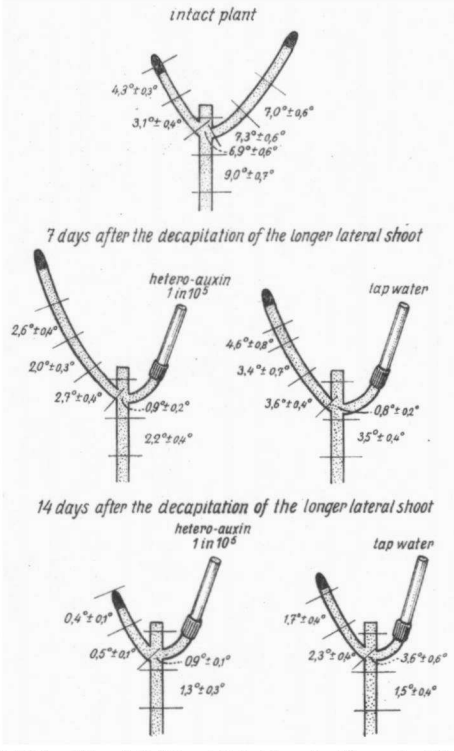


Figure 33. Auxin content of the shorter lateral shoots of two-shoot plants of *Lupinus albus* at application twice a day via the decapitated longer lateral shoot of hetero-auxin 1 in 10^5 and of tap water (experiment 29, 12/7/37—2/8/37).

In none of these series the rate of growth of the lateral shoots was high, but here too in the series with hetero-auxin 1 in 10^5 the growth of the shorter shoot was distinctly less than in the series with tap water.

7 days after the decapitation of the longer shoot the auxin content was determined in the two series mentioned at the top of the table, i.e. a) in the series with hetero-auxin 1 in 10^5 of 3 successive parts of 10 mm of the shorter shoot upward from its insertion and b) in the series with tap water also of 3 successive parts, 2 of 10 mm and 1 of 5 mm, and besides c) in both series of a part of 10 mm of the main stem between both shoots and d) of a part of 10 mm just below. Again 7 days later the auxin content was also determined of the plants of the two other series i.e. a) of two successive parts of 10 mm of the stem of the shorter lateral shoot upward from its base and b) of a part of 10 mm of the main stem and of a part of 10 mm directly below it. The results of these determinations have been summarized in figure 33.

The longer shoots in the intact plants also here show a higher auxin content than the shorter ones. The auxin content of the main stem corresponds to that of the longer lateral shoot. The differences in the later determinations are only small, but still a lower auxin content is found in the shorter shoots inhibited by hetero-auxin 1 in 10^5 via the decapitated longer shoot than in those of the series with tap water. Curious enough, a very low auxin content was found in the main stem in the series with hetero-auxin 1 in 10^5, whereas the experiments of the preceding chapter showed that the auxin content is increased all over the length of the stem by application of hetero-auxin 1 in 10^5 via the decapitated stem.

It seems likely that here the auxin has been inactivated or moved to lower sections of the stem.

Experiment 30.

Finally an experiment was made, with a number of two-shoot plants of *Lupinus albus* with lateral shoots of unequal length in which the shorter shoot, and not the longer one, was decapitated. We now tried to inhibit the growth of the longer shoot by application of hetero-auxin 1 in 10^5 via the shorter one. 50 plants were divided over 5 series of 10 plants, each series with plants of about the same habit. In 4 of these series the shorter lateral shoot was decapitated 10 mm above its base and twice a day an aqueous hetero-auxin solution 1 in 10^5 was applied via the cut surface to two of the series and tap water to the two other series. In the 10 remaining plants the auxin content was determined one day later: a) of the terminal buds of both shoots, b) of the stem part of the shorter shoot, c) of the upper and

TABLE XXVII. Growth of the longer lateral shoot of two-shoot plants of *Lupinus albus* (experiment 30, 11/8/37—26/8/37).

At application twice a day via the decapitated shorter lateral shoot of	Length of the shorter lateral shoot before its decapitation in mm (average of 10 plants)	Length in mm (average of 10 plants)			Increase in length in mm after 7 or 15 days (average of 10 plants)
		days after decapitation of the shorter lateral shoot			
		0	7	15	
hetero-auxin 1 in 10^5	24	44	66	—	22
tap water	24	38	63	—	25
hetero-auxin 1 in 10^5	13	20	33	46	26
tap water	19	25	41	69	44

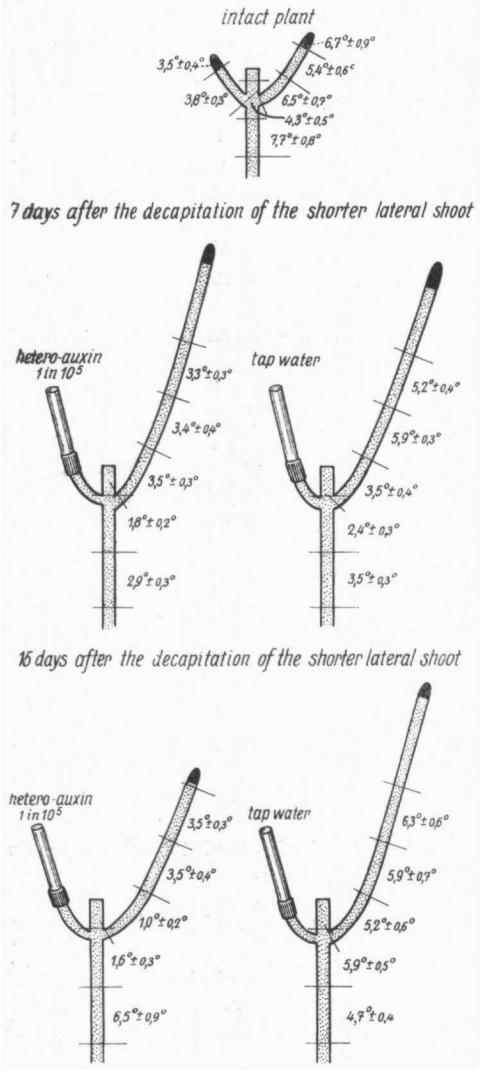


Figure 34. Auxin content of the longer lateral shoot of two-shoot plants of *Lupinus albus* at application twice a day via the decapitated shorter shoot of hetero-auxin 1 in 10^5 and tap water (experiment 30, 19/7/37—2/8/37).

lower half of the stem part of the longer lateral shoot, d) of 10 mm of the main stem between both shoots and of 10 mm directly below it. This series consisted of smaller plants than the other series; the shorter shoot had an average length of 11 mm, the longer one of 18 mm; the average number of expanded leaves was resp. 3 and 4. Just before the decapiation of the shorter shoot and 7 and 15 days afterwards the length of the lateral shoots was measured in the 4 other series. The results of these measurings have been summarized in table XXVII.

From this, it appears that it is also possible to inhibit the growth of the longer lateral shoot by an application of hetero-auxin 1 in 10^5 via the decapitated shorter lateral shoot. During the first 7 days the difference is not yet very distinct, but after 15 days we find that the increase in length of the longer lateral shoot in the series with hetero-auxin 1 in 10^5 is decidedly less than in the series with tap water. The small difference af-

ter the first 7 days — which is in contrast with the preceding experiments — is quite understandable, since here the growth of the strongly growing longer lateral shoots had to be inhibited, whereas in the preceding experiments the growth of the shorter lateral shoots was influenced, which had already been inhibited by the longer lateral shoot before.

On the 7th day after the decapitation the auxin content was determined of the plants of the 2 series, mentioned at the top of the table, on the 15th day of the two last-mentioned series, always of 3 successive stem parts of 15 mm of the longer lateral shoot from its base upward and of two successive parts of 15 mm of the main stem from the insertion of the highest lateral shoot to the base. The results of these and the preceding determinations have been summarized in figure 34.

The auxin content of the intact two-shoot plants again perfectly agrees with the results of the previous experiments; only the higher auxin content in the terminal bud of the longer lateral shoot, as compared to that of the shorter one differs from the results of experiment 28. 7 days after the decapitation of the shorter shoot there is little difference in the auxin content between the series with hetero-auxin 1 in 10^5 and with tap water. The auxin content of the longer shoot in the former series is slightly lower than that of the second series; this again runs parallel with the growth of the longer shoot, which in the first series was but little less than that in the second series. In both series, in which the auxin content was determined 14 days after the decapitation, the difference between the lower auxin content of the longer shoot in the series with hetero-auxin 1 in 10^5 and the higher auxin content in the series with tap water is much more pronounced than 7 days before. As we have seen already, just during these last 7 days a distinct difference in growth of the longer lateral shoot was found between the two series.

§ 3. *Discussion of the results.*

The results of the experiments discussed in this chapter clearly show in the first place that in intact two-shoot plants of *Lupinus albus* with lateral shoots of unequal length, the auxin content per length unit of the longer shoot is higher than that of the shorter one. The large number of determinations made on this subject, all yielded the same results and place the phenomenon beyond any doubt. The auxin content of the adjoining part of the main stem in these plants generally was of the same order

of magnitude as that of the longer shoot, sometimes a little lower, but still always higher than that of the shorter shoot.

We also observed that in two-shoot plants of *Lupinus albus* with lateral shoots of equal length, the auxin content of both shoots was about the same and that of the main stem higher than of each of the shoots separately.

The determinations of the auxin content of two-shoot plants, of which the growth of one of the shoots had been inhibited by application of hetero-auxin 1 in 10^5 via the decapitated other shoot, yielded less uniform results. On the whole, however, the auxin content of the inhibited shoot was found to be lower than that of the uninhibited shoot from the series in which — via the other decapitated, lateral shoot — tap water had been applied or no liquid at all. These results were not only obtained with plants where the growth of the shorter shoot was inhibited, but also in plants where we succeeded in inhibiting the growth of the longer lateral shoot by application of hetero-auxin 1 in 10^5 via the decapitated shorter shoot. It is remarkable that we did not find an increase of the auxin content of the main stem just below the insertion of both lateral shoots after the application of hetero-auxin, as was the case in the experiments discussed in the preceding chapter. Only in experiment 28 this was actually the case, but there, as an exception, the auxin content of the base of the shorter shoot was higher in the series with hetero-auxin 1 in 10^5 than in the tap water series.

These experiments clearly show that it is highly improbable that either in the intact two-shoot plant, nor in two-shoot plants with artificial inhibition of one of the shoots, auxin is transported from the inhibiting shoot into the inhibited one. Only for experiment 28 some restriction must be made. The theory of THIMANN and SKOOG (1934) and also that of CZAJA (1935, 1935a), explaining the phenomena of correlative inhibition by a direct action of the auxin, postulates that the inhibition of lateral shoots is caused by the transport of auxin from the inhibiting shoot into the inhibited one. Since our results contradict this postulate, this theory must be discarded.

We rather get the impression that there is a distinct relation between the auxin content and the growth. In case of a low auxin content we also find a slow growth, and reversely. When the difference in auxin content is small, we also find a small difference in the increase in length; a striking example of this was given in experiment 30.

All our experiments clearly show that in these phenomena

of correlative inhibition auxin is the correlation carrier. Since a direct action of auxin in these inhibition phenomena is excluded, the question rises how to explain this inhibition — in fact: a decrease of the auxin content in the inhibited parts. In the next chapter a final discussion is devoted to this question.

CHAPTER X.

General discussion of the results.

§ 1. *Discussion of the experiments.*

A complete survey of the results of our successive experiments is given in the Summary (p. 278). In broad outline these results are the following. Application of hetero-auxin solutions to decapitated seedlings of *Lupinus albus* could inhibit the development of the axillary buds, though not so completely as the terminal bud if left intact. As application of tap water causes no inhibition, it is highly probable indeed that in this correlative inhibition of lateral buds auxin is the correlation carrier. In this respect our experiments corroborate the earlier experiments of THIMANN and SKOOG (1933, 1934) with *Vicia Faba*. Also an application of lanolin hetero-auxin pastes to decapitated seedlings of *Lupinus albus* inhibits the development of the axillary buds. In cuttings of *Ligustrum vulgare* the application of aqueous hetero-auxin solutions only caused a slight inhibition of the lateral buds and when lanolin hetero-auxin pastes were applied, there was none at all. Probably these woody cuttings had much difficulty in absorbing the hetero-auxin from the lanolin pastes.

When measuring the lengths of the developing lateral buds of decapitated lupine seedlings nearly always the axillary bud of the second leaf proved to develop faster than that of the first leaf from below, even though the average distance between the insertion of the two leaves was only 2 to 3 mm. Besides it appeared, when arranging the buds according to the rate of their development, that the start which the faster developing shoot had of the more slowly developing one, gradually increased. So here too we apparently had again another case of correlative inhibition as suggested already by DOSTÁL (1926) and SNOW (1929). Our later experiments proved indeed this to be the case. Here too auxin proved to be the correlation carrier. If one of the two lateral shoots was decapitated and a hetero-auxin solution 1 in 10^5 was applied to the stump the growth of the other shoot was inhibited, whilst an application of tap water or no liquid at all

failed to do so. We may therefore conclude that in the correlative inhibition of lateral buds as well as in shoots, auxin is the correlation carrier. Interesting as these results in themselves may appear, they do not yet elucidate the actual character of the activity of auxin in this inhibition. As has been said already in chapter I (p. 180) different theories try to explain this phenomenon.

The "indirect theory" of LAIBACH (1933) and of SNOW (1932, 1937), according to which the auxin primarily participates in a growth process in the main stem and from this a secondary effect inhibits the lateral buds and shoots, seems rather improbable to us. In this case some tissue capable to these growth processes necessarily has to be present between the place of the auxin production or -application, and the inhibited organ. In our experiments with application of lanolin hetero-auxin pastes to decapitated lupine seedlings, however, we found a strong inhibition of the lateral buds as well when the hetero-auxin was applied in the immediate surroundings of the buds, as when applied at 12 mm higher up on the tsem. Neither did we find in the latter case any indications of growth in length or of stem swellings.

Our results when placing cut shoots of *Lupinus albus* and of *Pisum sativum* seedlings in hetero-auxin solutions are somewhat irregular. On the whole we found, as did LE FANU (1936), that the higher auxin concentrations inhibit the growth in length of these shoots, as compared with their growth in tap water. So auxin has a specifically inhibiting influence, when applied from the base. To this kind of experiments there is the objection, however, that in tap water too, the growth will soon stop, probably in consequence of a deficient supply with nutrients, since the growth of shoots of intact plants is always much stronger. The auxin content of the shoots, placed in hetero-auxin, was higher than of those, placed in tap water; so we must assume that the auxin was transported acropetally with the transpiration stream. This normally, however, does not occur as appeared from a distinctly toxic effect of the hetero-auxin 1 in 10^5 on these shoots.

The question rises whether in all these correlation phenomena auxin, as assumed by THIMANN and SKOOG (1934) acts directly and whether this direct activity must be ascribed to too high a concentration of auxin (THIMANN, 1937). In order to answer this question, we determined the auxin content of a great number of intact plants and of plants with lateral buds and

shoots artificially inhibited by application of hetero-auxin. We found indeed in both cases a rather high auxin content all along the entire length of the stem. After mere decapitation and when tap water was applied, we found a lower auxin content and, parallel with it, also a stronger development of the lateral buds. Though this matches with the theory of THIMANN and SKOOG, the fact that in inhibited buds a lower auxin content was found than in the uninhibited ones, does not fit in it. This conflict still becomes more urgent, when comparing the auxin content of intact two-shoot plants and of two-shoot plants of *Lupinus albus* of which one of the lateral shoots had been artificially inhibited. In these two-shoot plants we always found a distinctly lower auxin content in the inhibited than in the inhibiting shoot. The auxin content of the main stem appeared to match most with that of the inhibiting shoot. The theory of THIMANN and SKOOG must therefore also be discarded. Inhibition is not a consequence of too high an auxin concentration, but rather of a too low one. These facts urged us to explain this phenomenon of correlative inhibition in a different direction. Already in a previous publication (FERMAN, 1938) mention was made of this.

§ 2. *A new theory on the correlative inhibition of lateral buds and shoots.*

In all our experiments we found that the auxin content in the correlatively inhibited parts, in the axillary buds as well as in the lateral shoots, is lower than in the uninhibited parts. It is plausible that a slighter growth is the result of this. We actually found a distinct parallel between the auxin content and the rate of growth of the concerned parts. It is remarkable in this phenomenon, however, that in this correlative inhibition auxin is also the correlation carrier, as our experiments and also those of others have clearly proved. This means that the auxin, produced in the inhibiting organ, causes a lack of auxin in the inhibited one. As our experiments have proved, there cannot be any question of a direct effect of the auxin caused by too high a concentration in the inhibited organ.

The only safe starting point, ascertained by our experiments, is that in the inhibited organ the auxin content is lower and a slighter growth the result of it. When moving the question on which factor the production of auxin depends, we find as yet but few data on this subject in the literature. In this connection we must mention, however, the important investigation of SKOOG

(1937) with coleoptiles of *Avena* deprived from their seed. He found, that the formation of auxin in the tip of the *Avena* coleoptile is dependent on some other substance (or substances) which are transported from the seed to the tip in acropetal direction. This substance, the nature of which still being unknown, he calls auxin precursor. SCHWANITZ (1935), DOSTÁL (1936, 1936a) and WENT (1936) too found that in seedlings of peas, rhizomes of *Lathyrus* and *Agropyrum* and tubers of *Scrophularia nodosa* a substance or substances, necessary for the growth of the shoots, is transported in acropetal direction. These data are as yet rather vague, but if we assume that in all these cases auxin is the limiting factor for the developing of the organs concerned, we might conclude from it that yet another factor is necessary for the formation of this auxin, which is transported in acropetal direction to the place where the auxin is formed.

The correlative inhibition of lateral buds and shoots can be explained then in a simple and plausible way by means of the following two hypotheses. First the assumption that in all these cases a substance, or substances, are involved which are needed in the production of auxin and which we, according to SKOOG, call the auxin-precursor. Secondly this precursor must be transported acropetally and particularly to the spots where the auxin production is intensive, viz. where the precursor is quickly converted into auxin.

During the first developmental stages of the young seedling the precursor will be transported from the cotyledons acropetally to the terminal bud and converted there into auxin. Subsequently this center of auxin production will attract the precursor at an increasing rate. Consequently the terminal bud and the young unfolding leaves, allready preformed in that bud, are supplied with precursor. The dormant, hardly developed axillary buds, however, remain deprived from precursor and therefore cannot produce auxin. Since without auxin no growth, they cannot grow out.

If the terminal bud is taken away, the "attraction" of the precursor stops. The latter merely will accumulate at the apical cut surface and also the young axillary buds will get a part of it. They will convert it into auxin and consequently develop.

If one of the two developing axillary buds of the same leaf-pair for some reason — e.g. by its slightly higher insertion on the stem — gets a little more of the precursor than the other, it will also produce more auxin. The more intensive production

of auxin by one of the lateral buds subsequently will attract more of the precursor than the slower auxin production by the other bud. The "inhibition" exerted by one lateral shoot on the other or by the terminal shoot on the axillary buds or lateral shoots below thus is explained by the distribution of the precursor: the "inhibiting" shoot attracts the available precursor and deprives more or less the "inhibited" lateral bud or shoot from it.

The remaining difficulty is how to visualize this "attraction" of the precursor by the auxin. We might think of a chemical balance, the precursor moving to the places, where it is converted most rapidly and where, consequently, its concentration is low. Another more plausible notion of this mechanism we owe to CURTIS.

In his survey on "The translocation of solutes in plants" CURTIS (1935) mentions the probability that any treatment that starts the activity of a group of cells in a shoot meristem may also initiate the streaming activity of the conducting cells leading to this particular region, thus establishing an active conductive system connecting the meristem tissue with a supply of necessary solutes. The supply of these solutes to the particular tissue enables it to continue its activity and thus also to continue its connection with the source of supply.

It does not seem unlikely that in our case the auxin is the activator which effects the attraction of the auxin-precursor together with that of other soluble substances.

Recent investigations by THIMANN and SWEENEY (1937) showed an increase of the rate of protoplasmic streaming in the epidermal cells of the *Avena*-coleoptile brought about by hetero-auxin at concentrations between 5 in 10^7 and 2 in 10^9. It seems therefore in no way improbable that the auxin would have an activating effect on the plasmic streaming of the conducting cells.

According to this, our conception is that the auxin produced by the terminal bud and the growing leaves is given off to the stem and will bring the cells where it arrives to a higher activity and this, to a certain extent, proportionately to the concentration of the auxin supplied. The results will be o.a. a greater activity of the cells conducting the nutritive substances, among which also the auxin precursor, to the growing parts. The inhibited parts in the beginning will produce less auxin; the cells, conducting the nutritive substances, therefore, will be brought to a less high activity, and consequently a smaller amount of precursor will be supplied to these parts. Once behind

in auxin content the inhibited parts thus automatically must remain behind.

Though this theory gives a rather plausible and simple explanation of the phenomenon of correlative inhibition, still the question remains how to explain in our experiments the inhibition by artificial hetero-auxin supply. We can hardly assume that here too, this hetero-auxin leads to a greater activity of the conducting cells, for then the precursor, like in the decapitated controls, would have to accumulate at the apical cut surface, and this would have to result in a strong development of the most apical axillary buds. This is not the case, however. For that reason we believe the artificial inhibition must be explained differently by assuming that in this case the supply with auxin all over the cut surface of the stem, blocks up already in the basal parts of the stem the tracks of the upward transport of the precursor or at least seriously hampers the transport. Also the growth inhibition, found when the cut shoots are placed in hetero-auxin solutions and when hetero-auxin pastes are applied to the morphologically lower side (Le Fanu, 1936; Snow, 1936), can be explained in a similar way. In both cases the application of hetero-auxin — in concentrations as a rule probably higher than those found in the intact plant — would block up the upward transport of the precursor. The result then must be a reduced auxin production and consequently a reduced growth of the apical parts.

This theory, unlike the older ones, does not lead to controversies, and further it has the advantage to match with the two old and generally accepted hypotheses: a) the growth of the aerial parts is — within certain limits — proportional to the amount of available auxin, and b) the transport of auxin is strictly basipetal.

Various experimental results, which formerly could not be readily explained, fit well in this theory and can now be understood. For instance the stronger development of the axillary bud 2 (as compared to bud 1) in decapitated plants can be ascribed to the fact that in the acropetal transport of the percursor after decapitation, the latter is accumulated somewhat more in the neighbourhood of the higher bud than in that of the lower one. Even though this difference should be only very slight at first, the somewhat stronger auxin production of the higher bud would attract more of the precursor. Consequently the bud being ahead in the first beginning can keep its start and even increase this more and more.

The inhibition of the growth of cut shoots when placed in hetero-auxin solutions, can be explained by our theory by assuming that the high hetero-auxin concentrations in the base of these shoots blocks up the transport of the precursor still present there. These shoots therefore are more deficiently supplied with precursor then the control shoots in tap water. The difference between these two, however, can only be small since there is but little precursor and we actually find only a sligth inhibition. The growth of the shoots in tap water is also soon stopped, which proves that other factors, probably nutritive substances too, may soon act as limiting factors.

In experiment 26 with seedlings of *Lupinus albus* decapitated just above te second leaf-pair we found that the apical supply with tap water promoted the auxin content of the stem already, as compared with plants without water supply. Besides, the axillary buds of the second leaf-pair also developed, though weakly, in these plants which was not the case in any of the other series. In our opinion this may be explained in this way that the water supply to the stem promoted the upward transport of the precursor and its conversion into auxin. This ready upward transport of the precursor enabled the higher axillary buds to develop too.

The fact that in all the other series only the axillary buds of the first leaf-pair developed, proves once more that for the developing of the axillary buds a factor is needed, which comes from the basal parts. Even if the axillary buds of both leaf-pairs would receive an equal amount of this factor, the auxin precursor, in the beginning, the axillary buds of the second leaf-pair will already soon become deprived from precursor since by the longer distance the effect of their attraction is smaller.

In intact two-shoot plants with lateral shoots of unequal length the auxin content of the stem proved to be of about the same value as that of the longer shoot. In our opinion this may be caused by an auxin delivery by the longer shoot. This must, according to our theory, also bring about — at the place of insertion of the two shoots — a higher activity in the cells of the tracks of transport of the nutritive substances and precursor in the direction of the longer lateral shoot than in the direction of the shorter one. Consequently also this supply with nutrients and with auxin precursor must be stronger in the direction of the longer shoot. In two-shoot plants, where the growth of one lateral shoot was inhibited by applying hetero-auxin via the decapitated other shoot, this must be ascribed again to a blocking

up of the precursor supply of the intact lateral shoot by the hetero-auxin in the main stem.

We leave undecided where exactly the auxin precursor is produced and along which tracks of transport it is transported upward. Further investigations will be necessary to shed more light on this question and also on the nature of the auxin precursor (or precursors).

§ 3. *Discussion of literature in the light of this theory.*

When comparing this new theory, discussed in the preceding section, with the older theories on the correlative inhibition of lateral buds and shoots, one will find back in it several intems of the older ones. One of the essential elements, the acropetal transport of a substance, necessary for the formation of shoots, is found already in SACHS' theory (1880, 1882), with this modification however, that we now assume, that this substance must be first converted in the vegetation points into another substance, which is transported in basipetal direction and which initiates the growth of shoots.

The opinion, upheld especially by GOEBEL (1903) and LOEB (1915), that one has to deal here with the fact of one organ depriving another of nutritive substances, we find back in our theory in the attraction, exerted by the parts which are activated by the auxin, on the transported nutritional substances and auxin precursor to those parts.

Their experiments and also those of APPLEMAN (1918, 1918a), REED and HALMA (1919), CHILD and BELLAMY (1919, 1920), HARVEY (1920) and DENNY (1926), all refered to in chapter I, § 1 (p. 180), can be easily explained by assuming an acropetal transport of an auxin precursor, which is interrupted by the various experimental treatments and results into a development of the axillary buds just below this interruption.

Our new theory also enables us to explain the experiments of DOSTÁL and SNOW (chapter I, § 2, p. 184). In the experiments of DOSTÁL (1909, 1926) with leaf-pairs of *Scrophularia nodosa* we must assume that at first the stream of nutritional substances and precursor was directed towards the leaves and that only after the amputation of a leaf, its axillary bud was supplied with these substances. The inhibiting influence of the leaves, the axillary buds once developing, being only slight, as found by DOSTÁL (1926), can be well understood since the developing buds will produce more auxin and will exert a stronger attraction than the leaves.

The interesting experiments of SNOW (1925—1937) are all interpreted by him by means of an inhibiting influence, which the terminal bud, the young leaves or some growth process in the main stem exert on the concerned parts. All his experiments, however, can be explained in a much simpler way than by assuming that the inhibiting influence moves towards the inhibited parts, viz.: these parts remain deprived of a growth-promoting influence, in the first place of auxin precursor. So we should not see the phenomenon of correlative inhibition as an inhibition of the growth, but rather as an absence of growth-promotion. The attracting effect, exerted by the parts activated by means of the auxin, on the substances wanted for their growth, causes other parts to be deprived of these substances.

The experiments of SNOW, in which his "inhibiting" influence was weakened by a ringing of the epicotyl as far as the pith (1925) or by decapitation or by removing the leaves of the inhibiting shoot (1929), in our opinion must be explained as follows. By the manipulation the auxin flow coming from the inhibiting shoot is blocked up or weakened and consequently also the attraction by this shoot. The result is that the previously inhibited parts in their turn can attract the nutritional substances and precursor.

The fact, noticed by SNOW (1931), that the axillary buds in *Pisum sativum* first grow out to a certain length, before being inhibited by the terminal bud, according to us, must be explained as follows. The anatomical structure of the vegetation point of *Pisum sativum* is such that in the terminal bud the young axillary buds, at almost the same level with the terminal bud, also receive some precursor; consequently they can produce some auxin, develop to a slight degree and exert some attraction on new precursor and nutritional substances. The terminal bud, however, will constantly receive more precursor and thus be able to exert a stronger attraction. By the growth of the terminal shoot the axillary buds will get farther removed from the terminal bud and no longer share any precursor simultaneously with it. Therefore they will no longer be able to compete with the strong attraction by the terminal bud. The consequence will be that, after some time, the growth of the axillary buds stops.

According to SNOW (1931) and LE FANU (1936) the leaves of a shoot will protect it against the inhibiting effect of another shoot. According to us, however, the inhibition, occurring after the removal of the leaves of one of the shoots, is the consequence of the smaller auxin production and thus of a weaker attraction

by this shoot.

Our theory makes clear why cut shoots with removed leaves, when placed in a hetero-auxin solution, are inhibited more strongly than shoots with intact leaves. The shoots with leaves will be better enabled to attract the precursor, which was blocked up by the hetero-auxin (see preceding section), than shoots without leaves.

The recent experiments by SNOW (1937), to which we refered on p. 200, too can be readily explained by means of our theory. The experiment, in which the inhibiting influence of the one lateral shoot would travel into the axillary bud of the other shoot even up against the transpiration stream, can be explained by assuming that the intact lateral shoot, when not yet decapitated, attracts all nutritive substances available. After its decapitation the axillary bud of the other shoot may succesfully exert some attraction. In a following experiment the inhibiting influence of one shoot would act on the axillary bud of the other decapitated shoot, travelling down the growing shoot, up and down through the halves of the split epicotyl and up through the decapitated shoot. In this case we assume that the attraction of the non-decapitated shoot, via the two halves of the epicotyl also acts in the cotyledon of the half with the decapitated lateral shoot. If not knowing the experimental results, we would not have expected this. Our explanation, however, still is simpler than that of SNOW, who has to assume that his inhibiting influence acts in basipetal, in acropetal, in basipetal and again in acropetal direction, whilst for us the only difficulty is, that we must assume that in one half of the split epicotyl the attraction is continued in basipetal direction.

WENT's "diversion" theory (1936) has one important point in common with our theory viz. the attraction, which the parts activated by auxin are believed to exert on the transport of other substances. WENT (1936), however, describes these other substances as two specific factors, necessary for the growth of stem and axillary buds, and for the growth of leaves. These factors should be clearly different from auxin. As we have mentioned already, he fails to account for the fact that in the intact plant no or only very little auxin is produced by the dormant lateral buds, whilst their auxin production immediately increases after decapitation of the terminal bud.

In two recent articles, which reached us just before the printing of this manuscript, WENT (1938, 1938a) mentions a number of interesting experiments which should prove the existence of

these specific substances. By removal of roots or cotyledons of etiolated pea seedlings the growth of stems or leaves drops off rather rapidly, indicating a rapid depletion of a factor necessary for their growth. WENT (1938a) concludes that the roots form, and the cotyledons store to some extent a factor, required for growth of the stem and proposes to call this substance *caulocaline*. Besides, WENT finds indications for the existence of a substance, necessary for the growth of the leaf, which he calls *phyllocaline*. Together with the *rhizocaline,* necessary for the formation of roots, they form a new group of phytohormones, the "calines". It is remarkable, that the activity of the caulocaline shows itself in promoting the growth in length, a quality which formerly used to be ascribed to auxin. Therefore we believe that the substance which WENT calls caulocaline is identical with the auxin precursor of SKOOG (1937) and that this substance does not promote the growth in length directly, but only after its conversion into auxin in the vegetation point. Only by assuming that an auxin precursor is involved, it becomes possible to explain the correlative inhibition of lateral buds and shoots. The explanation given by WENT by means of his caulocaline is insufficient as we have already demonstrated. We are, however, much pleased that the clever and remarkable investigations by WENT (1938, 1938a) have given new indications for the existence of such an auxin precursor.

In a recent publication ALBAUM (1938) mentions some experiments with prothallia of *Pteris longifolia,* which are also important for the phenomenon investigated by us. He finds that adventitious outgrowths only appear from cut pieces of these prothallia when such pieces lack an actively growing meristematic region of when a junction of dead cells lies between an actively growing region and more basal regions. Auxin is supposed to be transported from the apex through the cells of the prothallium to the base. The auxin producing center of the young sporophyte is the primary leaf. The latter produces auxin which not only inhibits adventitious outgrowths from the prothallium, but also the oudgrowth of other embryos. This function of the primary leaf may be taken over by hetero-auxin applied in lanoline.

ALBAUM concludes from his experiments that the more active apical regions draw up materials from less active basal regions and use them in growth. According to him some relationship appears between the supply of synthetic material and the production of growth hormone. The inhibition of outgrowth of

adventitious prothallia or of more primary leaves he explains, according to THIMANN (1937), as an inhibition by too high a concentration of auxin. A clear indication for this, however, he does not find. It seems more probable to us, that here too we have another case where by the activity of the auxin in the apical region the nutritional substances and auxin precursor are attracted from the more basal region.

Finally a few remarks on the investigations of VAN OVERBEEK (1935), ZIMMERMANN (1936), GOODWIN (1937) and DELISLE (1937). They all find in herbs and trees with a high auxin production of the terminal shoot a weak development of the lateral shoots and in the case of a lower auxin production of the terminal shoot, a strong development of the lateral shoots. This difference in auxin production of terminal and lateral buds therefore appears, by means of their correlative inhibition, to account for the difference in structure of herbs, shrubs and trees. According to us this difference in auxin production must be ascribed by a difference in their supply with auxin precursor. The question on which this depends in its turn, must be answered by the anatomical structure of the young parts in their earliest stages of development. The important question is which parts at the start will receive more of the precursor. These parts will be the first to convert it into auxin and by their higher auxin production they will be able in future to attract nutritional substances and auxin precursor at an increasing rate. So finally the anatomical structure of the tracks of transport in the young vegetation points determines the distribution of the precursor, predestinates the result of the next competition and therefore must be responsible for the external architecture of plants.

SUMMARY.

Experimental results.

1. The development of the axillary buds of seedlings of *Lupinus albus* decapitated above the first or second leaf-pair was inhibited by applying — via the cut surface of the stem — aqueous hetero-auxin solutions of a concentration of 5 in 10^6 and 1 in 10^5; hetero-auxin concentrations lower than 1 in 10^6 mostly had no inhibiting but rather a promoting effect on the development of the axillary buds, as compared with their growth when tap water was applied.

2. No increase of length of the epicotyl, neither any swelling

or thickening of the stem was observed as a consequence of this hetero-auxin supply.

3. In the series where the growth of the axillary buds of the first leaf-pair was inhibited, as well as in the series where it was promoted, the bud in the axil of the second leaf generally developed faster than that in the axil of the first leaf, though the mean distance between the insertion of both leaves is only 2 to 3 mm.

4. In single-node cuttings of *Ligustrum vulgare* placed in the greenhouse the development of the still dormant lateral buds was weakly promoted by an application of hetero-auxin 1 in 10^7 and inhibited to a slight degree by the application of hetero-auxin 1 in 10^6 via the apical cut surface, in comparison with their development when tap water was applied; without any supply of liquid the development of the buds was still much smaller.

5. An application of lanolin hetero-auxin paste 1 in 10^6 to seedlings of *Lupinus albus* decapitated above the first leaf-pair caused an inhibition of the growth of the axillary buds. This inhibition was as strong in an application in the immediate surroundings of the axillary buds as when applied 12 mm higher up to the stem.

6. An application of lanolin hetero-auxin 1 in 10^6 as near as possible to the still dormant lateral buds of single-node cuttings of *Ligustrum vulgare* placed in the greenhouse did not cause any inhibition of their development.

7. In seedlings of *Lupinus albus,* decapitated just above the first leaf-pair, with axillary shoots of different length (so called "two-shoot plants") the growth of the shorter or of the longer lateral shoot was inhibited by applying an aqueous hetero-auxin solution 1 in 10^5 to the cut surface of the decapitated second (resp. longer or shorter) lateral shoot, compared with the growth of these lateral shoots when applying tap water or no liquid at all.

8. The growth of cut off shoots of *Lupinus albus* with one expanded leaf placed in a hetero-auxin 1 in 10^5 solution was inhibited, as compared with the growth in tap water; a hetero-auxin solution 4 in 10^6 and 1 in 10^6 caused no inhibition. In shoots of plants with two expanded leaves a hetero-auxin solution 1 in 10^7 already caused a very slight inhibition of growth, this inhibition being somewhat stronger with hetero-auxin 1 in 10^6.

9. In *Pisum sativum* no inhibition of the growth could be

obtained by placing the shoots of plants with 4 to 5 expanded leaves (the scales included) in hetero-auxin 4 in 10^6, as compared with the growth in tap water. The growth of the shoots of intact plants, however, was much stronger.

10. The development of the axillary buds of decapitated shoots of *Lupinus albus* was not influenced by placing the shoots in hetero-auxin solutions 1 in 10^6 and 1 in 10^5, as compared with the growth in tap water. In decapitated control plants left in the soil, however, a much stronger development of the axillary buds was observed. The auxin content at the level of the first leaf-pair of the shoots in tap water was distinctly less than that of the plants in the soil, whilst that of the shoots in the hetero-auxin solutions was still higher. At the same time a toxic effect of the hetero-auxin 1 in 10^5 solution was observed.

11. The auxin content of the stem of seedlings of *Lupinus albus* is about equal all along the length of the stem.

12. In young seedlings of *Lupinus albus* the auxin content of the hypocotyl increases directly after the splitting of the cotyledons and the first development of the plumule; the plumule then contains a fairly considerable amount of auxin too.

13. In far developed plants of *Lupinus albus* the auxin content of the leaves decreases with increasing age.

14. The application of aqueous hetero-auxin solutions to decapitated seedlings of *Lupinus albus* via the cut surface of the stem brings about a higher auxin content of the stem, than when tap water is applied or no liquid at all. The auxin content corresponds, within certain limits, with the concentration of the applied hetero-auxin solutions.

15. The application of tap water to decapitated seedlings of *Lupinus albus* increases the auxin content of the stem, as compared with plants without any supply of liquid.

16. The development of the axillary buds of the first leaf-pair of decapitated seedlings of *Lupinus albus* is, within certain limits, inversely proportionate to the auxin content of the main stem.

17. In seedlings of *Lupinus albus* decapitated above the second leaf-pair, in all series the axillary buds of the second leaf-pair almost do not develop, whereas those of the first leaf-pair do, though in each series the auxin content at both levels of the stem is about the same.

18. "Inhibited" axillary buds of seedlings of *Lupinus albus*

contain less auxin than buds which are not inhibited.

19. In decapitated seedlings of *Lupinus albus* to which tap water has been applied or no liquid at all, parallel with the development of the axillary buds the auxin content of the neighbouring stem parts increases.

20. In intact "two-shoot plants" of *Lupinus albus* with lateral shoots of unequal length the auxin content of the longer shoot per length unit is higher than that of the shorter shoot. The auxin content of the adjoining part of the main stem generally is of the same order of magnitude as that of the longer shoot, and always higher than that of the shorter shoot.

21. In intact "two-shoot plants" of *Lupinus albus* with lateral shoots of equal length, the auxin content of both shoots is about the same and that of the main stem higher than that of each shoot separately.

22. In "two-shoot plants" of *Lupinus albus* of which the growth of one of the shoots has been inhibited artificially by an application of hetero-auxin 1 in 10^5 via the decapitated other shoot, on the whole, the auxin content of the inhibited shoot is lower than that of the uninhibited shoot of two-shoot plants in which — via the other decapitated, lateral shoot — tap water has been applied or no liquid at all.

Conclusions.

1. In the correlative inhibition of the development of lateral buds and shoots auxin is the correlation carrier.

2. In *Lupinus albus* the terminal bud and the growing leaves are production centers of auxin.

3. As the auxin content of the inhibited organs is always lower than that of the inhibiting ones, the theory of THIMANN and SKOOG (1934), and also that of CZAJA (1935, 1935a), postulating that the inhibition of lateral buds and shoots is caused by a direct action of auxin transported from the inhibiting shoot into the inhibited organ, must be discarded.

4. As no indication has been found for the necessity of some primary growth process — or stem thickenings and swellings — in the main stem, from which, according to the "indirect" theory of LAIBACH (1933) and SNOW (1932, 1937), a secondary influence would inhibit the growth of the lateral buds and shoots, their theory is highly improbable.

5. Besides, against the above-mentioned theories a serious objection is that one has to assume that in the inhibition of

one lateral shoot by another, either auxin or an inhibiting influence or substance from either shoot has to travel upwards into the other one. It seems very improbable that two identical factors would be transported in opposite directions in one and the same organ or even in the same cells.

6. The "diversion" theory of WENT (1936) does not give an adequate explanation of the correlative inhibition of lateral buds and shoots, as it fails to explain why no — or scarcely any — auxin is produced by the lateral buds in the intact plant, and why this auxin production immediately increases as soon as the terminal bud has been eliminated.

7. In a new theory on the correlative inhibition of lateral buds and shoots, the production of auxin is assumed to depend upon the supply of a precursor, or precursors, transported acropetally and chiefly attracted to those spots where auxin is most intensively produced. Consequently, those parts which received a little more of the precursor than the other parts in the beginning, by their originally higher production of auxin, will continue to receive more of the precursor. Other organs, such as young, hardly developed axillary buds, remain deprived from the precursor and therefore dormant, since they cannot produce auxin and consequently cannot grow out. In the same way the "inhibition" of lateral shoots is caused by too deficient a supply with the precursor.

8. In experiments with artificial (hetero-)auxin supply to decapitated plants via the apical cut surface it is assumed that this (hetero-)auxin supply prevents or seriously hampers already in the basal parts of the stem the upward movement of the precursor in its tracks of transport. In the same way the growth inhibitions caused by a hetero-auxin supply from a place, morphologically below the parts concerned, are explained as a blocking up of the upward transport of the precursor.

9. WENT's assumption of the existence of a new phytohormone, called caulocaline, coming from the roots and necessary for the elongation of the stem or lateral buds (WENT, 1938, 193a) seems superfluous. The phenomena described by him simply — and preferably — can be explained in terms of the new precursor theory.

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