

In the light of these considerations and of the sterical analysis of HERMANS and MAAN, who after a correspondence on the subject agree with these explanations, the observed behaviour of *all* diols towards boracic acid and acetone may consequently now be explained very satisfactorily.

The fundamental hypotheses, 1<sup>o</sup>. of the constant rhythmical intramolecular movements and 2<sup>o</sup>. of the mutual repulsion of the hydroxyl groups, may be maintained unaltered.

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**Botany. — Analysis and Integration of various auxin effects. I.** Bij F. W. WENT. (California Institute of Technology.)

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Three years ago the writer tried to analyze the action of auxin on different developmental processes in terms of a number of elementary reactions (WENT 1936). It was assumed that in root formation, growth, cambial activity, etc., the auxin not only reacted with specific other growth factors, named calines (WENT 1938), but that the auxin also conditioned the distribution of these calines. By consistent application of these two principles most auxin phenomena lined up nicely in a general picture. Nevertheless this theory of a multiple action of auxin had to cope with many difficulties. In the first place very few experiments were available to test the theory. And in the second place there were some rival theories, each accounting for some of the known facts. One of those, most clearly expressed by FITTING (1936), and adopted by BOYSEN-JENSEN (1938), AVERY (1938) and HITCHCOCK and ZIMMERMAN (1938) was that auxin was a "stimulant" for the cell, which practically precluded further analysis. Another theory of THIMANN (1935) was that auxin took part in one master reaction, which then led to either growth, or root formation, or bud inhibition, etc. THIMANN's main point was, that any substance which was found to cause growth by cell elongation also induced root formation or inhibited lateral growth. During the last three years many facts bearing on these theories have been collected and the theory of multiple auxin action has proven to be very fruitful as a working hypothesis. The author's recent papers, all dealing with different aspects of the growth problem in general, could not be published together. This review is intended to integrate these separate papers, to serve as their general discussion. For the experimental evidence on which this discussion is based, the reader is referred to the individual papers. The following subjects will be treated. On the one hand considerable data were collected on the existence of calines (WENT 1938, 1938a, BOUILLENNE 1938, BONNER, HAAGEN-SMIT and WENT 1939). On the other hand it was possible to differentiate each of the developmental processes studied (growth by cell elongation, 1939c, the pea test 1939b, bud inhibition 1939a and root formation 1939) in a chain of at least two successive reactions. And thirdly it was found, that not all substances active in one reaction could take part in other reactions. Lastly extremely interesting facts were published (D. BONNER 1938) substantiating and enlarging the experimental evidence, that in the growth reaction a stoichiometrical relationship exists between the applied growth substance and the resulting

growth. One by one these points will be taken up, and finally it will be shown that they fit into the theory of multiple auxin action.

### 1. *The existence of calines.*

When in peas, grown for a week in a darkroom, the stem is cut off above the cotyledons, and placed in different nutrient solutions, growth in length, development of leaves or of stipules is completely suspended (WENT 1938, 1938a). So far it has not been possible to substitute the factors necessary for growth in length, coming from root system and cotyledons by substances added to the nutrient solutions, so that it is not likely that these substances are identical with the simpler nutrients. Another reason for assuming that the lacking growth factors are not simple nutrients lies in the fact, that cutting off cotyledons or roots differentially affected growth, swellings upon auxin application and root formation. Still better arguments for the existence of a number of specifically different growth factors for leaf growth, stipule growth and growth in length were furnished by experiments, in which the tops of one variety of peas were grafted on a series of other varieties (WENT 1938a). Leaf growth was shown to be independent of stipule growth or growth in length, and also the latter two were not correlated. The best argument, however, lies in the demonstration that different plant organs require different sets of factors for growth. Roots can be grown indefinitely in a medium containing inorganic salts, sugar, vitamin B<sub>1</sub> and nicotinic acid (ADDICOTT and BONNER 1938) or vitamin B<sub>6</sub> (ROBBINS and SCHMIDT 1939). In such a medium excised buds will grow but little (BONNER and AXTMAN 1937). Leaves require a completely different set of factors (BONNER, HAAGEN-SMIT and WENT 1939, BONNER and HAAGEN-SMIT 1939).

Therefore, we must conclude, that if auxin affects different developmental processes, in each case it does so in combination with a different set of factors. The specificity is by definition due to the specificity of the calines. If now we get even in the same organ a different response to auxin under different conditions (e.g. swellings and root formation in stems (WENT 1938)), this must be due to variations in the caline content.

### 2. *Different approaches to the analysis of the action of auxin.*

WENT and THIMANN (1937) have pointed out two general lines of approach, the first being a physiological analysis, the second a chemical analysis of the action of auxin. An example of the chemical analysis, which uses the specific structure of the growth promoting substances as a starting point was stated by KOEPFLI, THIMANN and WENT (1938). DAVID BONNER (1938) has started an investigation taking into account the physical properties of these substances in relation with their activity.

The physiological analysis may be divided again in two groups. The

first is concerned with the ultimate changes due to auxin which can be measured in physical or chemical properties of the cell, such as plasticity and elasticity of the cell wall (see discussion in WENT and THIMANN 1937), osmotic pressure, cell sap constituents, etc. From these ultimately measurable effects the analysis is pushed forward towards earlier phases in the chain of reactions, starting with the first reaction of auxin and ending with growth or any other auxin induced phenomenon.

The following discussion is concerned with the second type of physiological analysis, which tries to identify the first reaction in which auxin is involved, and thus starts at the other end of the reaction chain. In 4 different cases a successful start in this direction has been made.

### 3. *The effect of auxin in the pea test.*

The effect of auxin in the pea test has been resolved in a chain of two successive reactions. As published before, the pea test consists of the inward curvature of longitudinally split stems of etiolated pea seedlings due to increased growth of the outer tissues when placed in auxin solutions. The details of the differential response of inside and outside are complex (WENT 1939b), but it is sufficient to know that the response is due to a definite growth reaction. It was now found that the actual growth reaction can be brought about by a much lower indole acetic acid concentration than was apparent in the earlier experiments. This is due to the fact that the indole acetic acid has to take part in two definite reactions, of which the first one requires a much higher concentration than the second. Since it was possible to make the first — the preparatory reaction — take place with certain substances (hemi-auxins) which do not influence the second or growth reaction proper, this second reaction could be studied independent of the preparatory reaction. It was also found that with substances which are slow in inducing the growth reaction a clear separation in time of the two reactions as carried out by the same substance was possible. With phenyl acetic acid in the first two hours only the preparatory reaction is induced, and from the third hour on the growth reaction occurs too. A further difference between the two reactions, which together lead towards the pea test curvatures was found in their pH sensitivity; the preparatory reaction being independent of pH, the growth reaction occurring in acid medium.

A few important conclusions can be drawn from these facts as far as the mechanism of the growth in the pea test is concerned. In the first place it shows that the reactions leading towards growth are complex, and that no single master reaction is responsible for it. But more important is the fact, that the action of the auxins in this process is dual. Therefore a substance must possess at least two sets of properties to show activity in the pea test. The first set must enable it to take part in the preparatory

reaction, the second set is required for the growth reaction proper. And as a general requirement such a substance must possess those properties which will allow it to enter the plant and the cells unaltered. The third conclusion is that there are conditions under which the growth reaction will not show up since the preparatory reaction was limiting. This is actually the case with very low indole acetic acid concentrations (e.g., 0.1 mg. per l. water and lower). This is interesting since the pea test requires higher concentrations of indole acetic acid than the Avena test. But this is mainly due to the fact that under the conditions of the Avena test the preparatory reaction does not limit the growth reaction. And the growth reaction proper in the pea test responds to the same low auxin concentrations as the Avena test.

#### 4. The evidence for a dual effect of auxin in *Avena* coleoptile growth.

As mentioned above in the growth reaction measured by the Avena test a preparatory reaction does not seem to be involved, and the induced growth seems a simple reaction. Still a number of observations (e.g. WENT 1935) led already to the conclusion that auxin not only caused growth, but high concentrations also increased the maximal growth response, which was taken to mean that auxin increased the available food factor supply. Recently (WENT 1939c) the latter point could be definitely established by using hemi-auxins. When  $\gamma$  phenyl butyric acid is applied to the tip of the coleoptile, especially near the tip, it increases the response to subsequently applied indole acetic acid. This could be shown when the latter substance was unilaterally applied, since the curvatures were 2—3 times as large when  $\gamma$  phenyl butyric acid had been put on the tip for 2—3 hours before decapitation. But also when the coleoptiles thus treated were cut into short sections, and the growth of these cylinders was measured when immersed in a sugar and auxin solution, the increased response was clearly evident. When phenyl acetic acid was used, the effect of pre-treatment was also clearly pronounced, but with indole acetic acid the effects were less clear, and only indirectly or in special cases could the increase in responsiveness be seen. Thus with substances without or with slow growth activity it could be established that in *Avena* coleoptiles the growth reaction may be preceded by a facilitation reaction, which increases the response of the cells to a subsequent auxin application. This reaction is in many ways distinct from the growth reaction proper, just as in the pea test, and in the coleoptile occurs even with the relatively low auxin concentrations which are naturally present in the growing plant. This fact made it more difficult to establish the double function of auxin in ordinary cell elongation. In this case it also could be made very probable, that this facilitation or preparatory reaction actually is a transport of "food factor" towards the regions where auxin is applied, this "food factor" being distinct from sugar.

#### 5. The explanation of bud inhibition on the basis of the dual effect of auxin on growth.

In the preceding paragraph the experiments mentioned only involved the growth of the *Avena* coleoptiles. A few experiments were performed on the elongation of the main stems of etiolated peas which led to the same conclusion (WENT 1939c). Therefore it is reasonable to assume that for cell elongation everywhere auxin takes part in two distinct and separate reactions. Also for the pea test this was shown (section 3).

Now we know that in lateral bud growth auxin also acts in two ways. In the first place, as long as it is produced by the apical bud, it inhibits the lateral buds. But once the laterals are growing, auxin is required for their elongation. This double action has been explained in different ways, e.g. by assuming that the different reactions were due to different concentrations of the auxin (THIMANN 1937). This view could be disproven (VAN OVERBEEK 1938, FERMAN 1938) since under experimental conditions bud inhibition occurred at the same auxin concentrations where bud growth could take place. The experiments in the preceding sections, however, suggest that by differentiating between the two auxin reactions a ready explanation of bud inhibition by auxin can be found. If auxin causes the upward movement of other growth factors, essential for stem elongation (see section 1), towards the region of auxin production, as was shown in the previous section, and if the supply of these other growth factors is limited (see WENT 1938), then we can easily conceive a condition in which all these factors move towards the growing apical bud, leaving the lateral bud without further supply of them. The direct experiment to test this explanation was very positive (WENT 1939a). When an etiolated pea stem is decapitated about 20 mm above a lateral bud, this bud will start to grow out immediately. But if a low auxin concentration (1 part indole acetic acid in 20,000 parts lanolin) is applied to the cut surface of the main stem, the lateral bud will be inhibited. Simultaneously with this inhibition it was experimentally determined that an accumulation of bud growth factors had occurred in the main stem just below the applied auxin, and above the lateral bud. This shows that auxin, by directing the bud growth factor flow towards its place of application, decreases the growth of lateral buds, or deflects these bud growth factors from them. Therefore, by its facilitation reaction, auxin increases growth of the regions just below its place of production, and inhibits growth of all regions further away from it, which leads either to the growth inhibition described for the lower regions of the *Avena* coleoptile (WENT 1939c) or to lateral bud inhibition. This explanation does not exclude further contributing factors to bud inhibition, but it gives a satisfactory explanation of the dual effect of auxin on bud growth based on direct experiments. The inhibition is mainly due to the preparatory reaction of the auxin, and it is interesting to note that some hemi-auxins like  $\gamma$  phenyl butyric acid actually cause bud inhibition.

(see also VAN OVERBEEK 1938), although they are unable to take part in the growth reaction proper.

#### 6. *The dual effect of auxin on root formation.*

In the three preceding sections it was shown that for different types of growth by cell elongation which depend on auxin the latter takes part in two successive reactions, which can be differentiated in time, pH sensitivity and required auxin concentration. It even has been found that certain substances, for which the common name hemi-auxins is used, induce the first reaction only without affecting the growth reaction proper. Now it is known that many of the auxins not only cause cell elongation, but also are involved in inducing root formation. Since the latter phenomenon is a typical "organization", involving differentiation of tissues and initiation of organ primordia, it was questionable whether the same rules would hold as for the effect of auxin on cell elongation. An examination was made as to whether auxin takes part also in two reactions leading towards root formation. It was found (WENT 1939) in fact that a preparatory reaction, requiring auxin or a hemi-auxin, does precede the actual root forming reaction. The latter requires an auxin, but not all substances inducing the growth reaction proper are active. A notable exception was found in phenyl acetic acid, which can cause cell elongation by itself, but does not induce root formation unless its action is supported by indole acetic acid or a similar substance. It is not thought that the inability of phenyl acetic acid to induce the actual root forming reaction is merely a quantitative problem, for although it is quantitatively less effective in the growth reaction (according to D. BONNER 1938, it has one sixth the activity of indole acetic acid), it very definitely takes part in the preparatory root forming reaction at only slightly higher concentrations than indole acetic acid. And in the actual root forming reaction it shows no trace of activity, not even in the highest concentrations which are close to the toxic limit.

In the case of root formation some experiments gave indications that the preparatory reaction can be identified with the effect on the redistribution of rhizocaline (see WENT 1938, COOPER 1938), which was shown to occur under the influence of auxin. When a stem has grown under conditions favourable for auxin production, apparently this auxin is sufficient for causing the actual root forming reaction, so that any substance inducing the preparatory reaction will seem active as a root forming substance. This may seem to be in contradiction to the idea of specificity, but the distinction between preparatory and actual root forming reaction restored most of the specificity of the latter reaction.

#### 7. *Effect of auxin on carbohydrate translocation.*

A number of papers recently appeared, in which the effect of applied auxin on dry weight, sugars and other constituents of the plant was

described. Since this may have a direct bearing on the effect of auxin on translocation, this effect should be considered. In the papers (MITCHELL and MARTIN, 1937; HAMNER and MITCHELL 1938, CZAJA 1938, STUART 1938, ALEXANDER 1938, MITCHELL and STUART 1939) a distinction was made between the effect of applied auxin on the rate of carbohydrate synthesis and the increase in size and dry weight of the treated portion of the plant. The former effect, on carbohydrate synthesis, does not have to be considered in connection with our present problem. The latter effect, however, has a direct bearing on our considerations. Whether the auxin effect is direct on carbohydrate and nitrogen translocation, or only indirectly connected with it, is difficult to decide.

We can envisage this auxin effect in different ways. In the first place the primary process may be an accumulation of carbohydrates and nitrogenous substances, which secondarily will lead towards growth. In this case accumulation should precede the growth, and would be comparable with the effects of auxin on translocation of growth factors, mentioned in sections 4, 5 and 6, which definitely precede the growth. From the published data it is difficult to form an opinion as to whether an accumulation of carbohydrates and nitrogenous substances occurs prior to the increased growth. STUART (1938) has followed from day to day the dry weight, carbohydrates and total nitrogen in bean cuttings treated with indole acetic acid. Since no fresh weight data are given, the increase in volume (i.e. growth) due to the treatment can not be correlated with the data concerning cell contents, but it seems that they parallel each other, with probably growth in the lead.

As a second alternative concerning the auxin effect on translocation of organic materials inside a plant we can start with its wellknown effect in decreasing the turgor, which is caused by yielding of the cell wall against the internal pressure. It is also known that all young cells have a continuous supply of osmotic material, which, however, lags behind the decrease in turgor in the case of rapid growth. As a mental picture for this continuous supply of osmotic material we can use MÜNCH's hypothesis of a pressure flow. In this case we would expect that expansion of a tissue, accompanied by a decrease in turgor, would lead to an inflow of osmotic material, especially sugars. In this way a translocation of organic materials can be caused by auxin, although it is a secondary phenomenon, and not a direct effect of auxin on translocation itself. The data as published by STUART and others fit this explanation, so that there is no direct evidence as yet that auxin directly affects the translocation of carbohydrates and nitrogenous food materials. If this proof could be brought, it would be the strongest support for the author's contention that auxin affects the movement of materials inside the plant.

#### 8. *Summary of the different reactions in which auxin takes part.*

For each of a number of different processes the dual effect of auxin in

bringing them about has been demonstrated. The question can be raised whether any of these reactions are identical, e.g., whether the preparatory reactions for growth and root formation are the same. This cannot be analysed by any direct methods, since the reactions themselves are insufficiently known. But table 1, which summarizes the preparatory, growth and root forming reactions for 10 different substances, as far as they have been determined individually, suggests certain answers. Whenever a substance listed has not been tried in a given test a blank space is left. For the others either a positive or a negative effect is marked, without comparing the effectiveness in a more quantitative way. Only when a complete analysis of all reactions is carried out, would a quantitative comparison between the reactions gain significance. Most of the figures of table 1 are based on extensive experiments, involving about 50.000 individual reactions.

We can recognize definite groups of substances, according to their activity in the various reactions. The first group, comprising various indole compounds, such as the indole acetic, propionic and butyric acids, and naphthalene and anthracene acetic acids, are able to take part in all of the 7 reactions studied. In the same group belong auxin *a* and *b*, although for most of the reactions their effectiveness is only indirectly concluded from a consideration of the activities of intact plants, and from the data obtained with some crystals of auxin *a* and *b* which were received many years ago from Professor F. KÖGL.

To the second group belongs phenyl acetic acid, which is effective in all preparatory reactions, and in the growth reactions proper, although its maximal activity does not equal that of indole acetic acid (see section 9). But the important difference with group I is that it cannot induce the root forming reaction. The number of experiments which were carried out to test its activity on root formation (on approximately 1000 pea stems in 8 separate experiments) seems to preclude the possibility of overlooking its effect, even if it had been small. And in each of these experiments the indole acetic acid was active. It is possible that cis-cinnamic acid belongs to this group, but since no conclusive experiments on its root forming activity are available, this cannot be concluded with certainty.

The third and fourth groups are alike inasmuch as neither of them are able to take part in the growth or root forming reactions proper, but both can induce the preparatory reaction of one or more processes. Thus they both fall under the group name hemi-auxins, substances which can perform only the first of the two reactions which are carried out by the true auxins. The difference between groups III and IV only lies in the number or type of preparatory reactions in which they can take part. To group III belong probably most substances which can give the preparatory reaction in the pea test. Group IV is wholly inactive in the pea test, but is very effective in preparing stems for root formation. Most of the substances listed by TRAUB (1938) will probably turn out to belong to group IV. Ethylene can be listed under group III, according to the work of MICHENNER (1935, 1938).

TABLE 1.  
Activities, marked + for activity and — when substance is unable to induce the particular reaction, and a blank when no tests are available, of 10 different substances in seven different reactions, connected with growth or root formation.

Class	Preparatory reaction in			Growth reaction proper in			Root forming reaction proper on pea stems
	Avena elongation	Pea test	Bud initiation	Root formation on peas	Avena test	Pea test	
Indole acetic acid	I	+	+	+	+	+	+++-
Indole butyric acid	I	+	+	+	+	-	---
Naphthalene acetic acid	I	+	+	+	+	-	---
Phenyl acetic acid	II?	+	+	+	+	-	---
Cis-cinnamic acid	III	+	+	+	+	-	---
? Phenyl butyric acid	III	+	+	+	+	-	---
Cyclohexane acetic acid	IV	+	+	+	+	-	---
Sodium naphthol sulfonate	IV	+	+	+	+	-	---
Potassium anthraquinone sulfonate	V	+	+	+	+	-	---
Benzoic acid							

In a last group (V) are listed all substances which are completely inactive in any of the preparatory or growth or root forming reactions. Most substances belong here, such as benzoic, indole carboxylic and the aliphatic acids, but since their characterisation is wholly negative, they will not be considered further.

KOEPFLI, THIMANN and WENT (1938) have determined the minimal structural requirements of a molecule to have growth activity. What they actually determined were the structural requirements for the preparatory and the growth reaction combined, but since so far no substances have been found which had growth activity but lacked preparatory activity, their conclusions may be applied to growth activity by itself. Now it was found that in most molecules only a fraction, carrying a ring double bond and a carboxyl group in a side chain, was essential for the growth reaction. This was surprising, since auxin *a* and *b* molecules, occurring in the higher plants, seemed much more complex than the growth reaction would call for. Now it becomes evident, that this complexity is not connected with the growth reaction proper, but with other functions or properties of auxin *a* and *b*. Phenyl acetic acid, which has the required molecular structure for growth activity, cannot induce root formation. For the latter reaction a more active double bond than the one in the benzene nucleus seems necessary, such as in the indole nucleus. If on the other hand the structure of phenyl acetic acid is simplified, by removal of the double bonds, then the resulting cyclohexane acetic acid is only active in the preparatory reaction, but not in the growth reaction proper any more. Therefore it seems a reasonable assumption, that due to its more complex nature, the molecules of auxin *a* and *b* can take part in certain reactions which cannot be performed by indole acetic acid. As soon as plant physiologists have freer access to auxin *a* and *b* this assumption can be put to the test. There are only very few indications supporting the assumption. One is the different behavior inside the plant of auxin *a* and *b*, and of indole acetic acid, when subjected to light (VAN OVERBEEK 1936, KONINGSBERGER and VERKAAIK 1938). Another is the difference in activity when tested in the pea rooting test (WENT and THIMANN 1937). Some other differences need further confirmation. The structural and physical properties which the hemiauxins have in common will be considered in a later paper.

It seems of considerable interest, that in three different cases the preparatory reactions can be described as a redistribution of other growth or root forming factors under the influence of the auxins or hemiauxins (Avena growth, bud inhibition and root formation). Whereas for each one of these processes other explanations have been put forward, it seems that the explanation offered, based on experimental evidence, which can be applied to all known cases, has great advantages. It is true, that no physical explanation of the effect of auxin on the direction movement of other substances can be given, but we cannot give an entirely satisfactory explanation for the ordinary movement of other substances in the plant either.

There is another interesting point in this connection. The preparatory reaction is dependent upon the movement of auxin and correspondingly the correlation effects of auxin are exerted in this period. The auxin during its transport must be under different conditions than the auxin inside the cell (WENT and THIMANN 1937: free moving auxin as contrasted with auxin inside the cell, see also WENT 1938b). Now it is interesting to note, that the preparatory reaction is independent of pH, whereas the growth and root forming reaction proper, which presumably take place inside the cell with the auxin present there, are pH dependent. This suggests that the pH effects the combination of auxin with its carrier or substrate inside the cell. During its transport, before combination with a substrate, no pH effect would be expected.

If it had not been evident from each of the sections 3 to 6, comparison of the data of table I would have been sufficient to show, that auxin does not take part in one single master reaction which leads to all different responses, but that we must consider, that the auxin is involved in a number of different reactions, each of them with its own specificity. This conclusion, tentatively proposed as hypothesis some years ago (WENT 1936), seems now firmly established.

By considering the number of different groups in table 1 and further evidence concerning activity we may conclude that:

1. The growth and root forming reactions proper are different.
2. The preparatory reactions for growth and for root formation are different.
3. The preparatory reactions in Avena coleoptile growth and pea test are probably identical.
4. Also the growth reactions proper in Avena and pea can be considered as identical.
5. Bud inhibition shows only preparatory reaction, which seems identical with the preparatory reactions for Avena and pea growth.

Therefore we have at least 4 distinct reactions in which auxins can take part. The possibility that the differences in reactivity in the various reactions are due to differential entry or destruction in the cells can be ruled out, since in the pea stem indole acetic acid can take part in all 4 reactions. Until we obtain direct evidence for the identity of any of the four reactions: preparatory reactions both for growth and for root formation, the growth reaction proper and the root forming reaction proper, it will be necessary to consider them strictly separate, to avoid further confusion, especially concerning the question of specificity.