

PLANT PHYSIOLOGY

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HORMONES AND THE ANALYSIS OF GROWTH¹

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(WITH ELEVEN FIGURES)

It is now about twenty years since the carefully controlled experiments of PÁAL established finally that growth in plants is under the control of special growth substances or *hormones*. In the intervening period our knowledge has advanced considerably, though mainly only in respect to this one rather well-defined group of hormones, the *auxins*. These substances, as you know, have been isolated from both plant and animal sources, including both seed plants and fungi, and their chemical nature has been thoroughly elucidated. Furthermore, large numbers of synthetic substances have been prepared which have similar actions. Their chemistry will be discussed further below; it is enough for the moment to note that they have rather clearly defined similarities.

The physiology of these growth substances, however, shows much more diversity. The action studied by BOYSEN-JENSEN, PÁAL, SÖDING, WENT, and the other workers up until the last few years was an action upon cell enlargement, causing growth in the simplest sense of the word. The hormone was envisaged as the mechanism by which the influence of the tip of the plant was exerted on the growth of the part below it. However, it was not long before evidence was forthcoming that other quite different influences of one part of the plant upon another were brought about through the action of the same hormones.

The first instance of this was the *inhibition* of one bud by another. It had been previously indicated that this well-defined inhibiting action was perhaps due to a special inhibiting substance, and we were able to show not only that it is, indeed, but also that the substance is identical with auxin. Thus a substance which promotes growth in some tissues also characteristically inhibits it in others.

A more marked instance of the same thing is seen in roots. Here all but

¹ Fifth STEPHEN HALES address, read at the Indianapolis meeting, December 28, 1937.

the most extremely low concentrations of auxins inhibit elongation, so that root tissue, like bud tissue, is readily inhibited by auxin. However, it has recently been found both in our own and other laboratories that *excessively* low concentrations, of the order of 1 gram in 10 million liters, actually accelerate growth of roots. These are concentrations of almost unbelievable lowness. GEIGER has calculated that to dilute a gram of indole-acetic acid so that it is completely inactive on *Zea mays* roots would take an amount of water such that if it had to be brought by rail, it would require 400,000 trains, each of 50 ten-ton wagons. Such trains would comfortably extend around the world, or a quarter of the way to the moon.

This phenomenon of growth acceleration and growth inhibition is probably paralleled in all other plant material. Generally the inhibiting concentrations are much higher than in roots, but the difference is apparently quantitative rather than qualitative. The relation is something like that shown in figure 1.

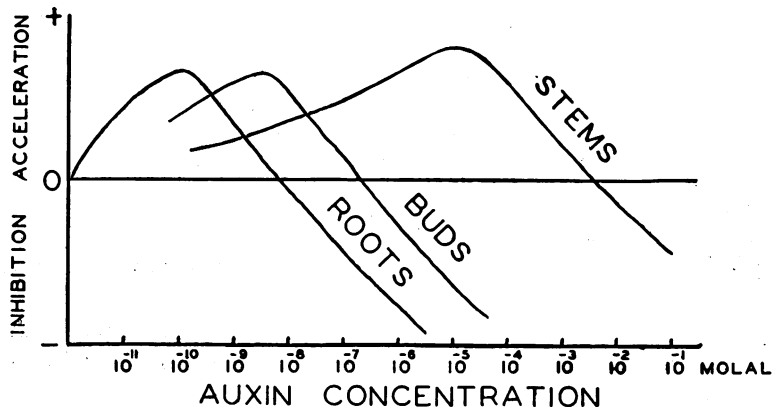


FIG. 1. Scheme for approximate ranges of growth acceleration for different plant organs.

The inhibition of roots involves a very interesting *after-effect* which we have recently been studying, and which is a good example of one type of the analysis of growth. In this case an observed, rather ill-defined acceleration of growth can be traced back to the initial, much slighter acceleration of growth which caused it. If roots are treated with auxin so that they are inhibited, and the auxin afterwards removed, their subsequent growth is accelerated. They catch up with the untreated controls and even surpass them. Figure 2 shows this effect with *Avena* roots. *Triticum* behaves similarly. The higher the concentration of auxin used, the longer it takes for this acceleration to appear, but on the whole the greater the acceleration is when it does come. Thus the roots treated with lowest auxin concentrations are the first to pass the controls, and those treated with highest concen-

trations are the last, but even these plants treated with highest concentrations, whose roots scarcely grow at all during the treatment, afterwards have roots considerably longer than the controls.

At the same time, the very high auxin concentrations also increase the *number* of roots (at least in seedlings of certain plants). In *Avena* the treated seedlings may develop 20 or more roots as against 5 to 7 in controls of the same age (fig. 3). The result of both these responses is that the treated plants, although at first inhibited, subsequently have a far better root system. The roots are both longer and more numerous. As a result the development of the shoot is accelerated, since water supply is exceedingly

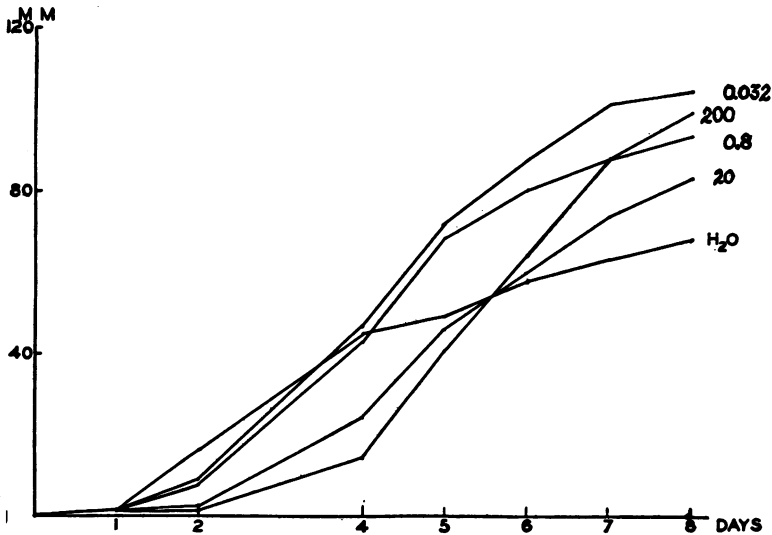


FIG. 2. Length of *Avena* roots, treated with indole-acetic acid at the concentrations marked (in mg. per liter) from the first to the second day of growth and then transferred to water.

important in the growth of young plants. This acceleration of the shoot can be very marked. Figure 4 shows such treated *Avena* plants, after 5 weeks in the cool greenhouse, with controls. Only the plants treated with highest auxin concentration (100 mg. per liter) are shown. The others behaved intermediately. Not only is there a general acceleration, but the leaves are broader and hence the photosynthetic area is increased. This leads to huskier plants. The formation of broader leaves has been obtained in other plant material also.

Thus we have here a growth acceleration resulting weeks later than the initial treatment, involving a number of changes such as increase in height, growth rate and leaf area, which can be analyzed back, with a high degree of probability, to the initial effect on the root system of the seedling. This

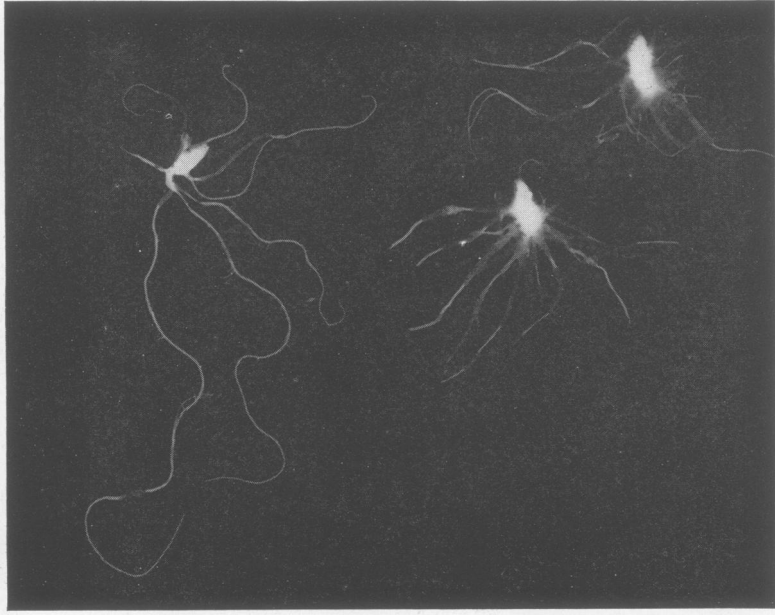


FIG. 3. Right, two *Avena* seedlings treated with indole-acetic acid. Left, control in water.



FIG. 4. Right, *Avena* plants grown from seedlings treated during first and second day of growth and transferred to water. Left, controls treated only with water. Five weeks old.

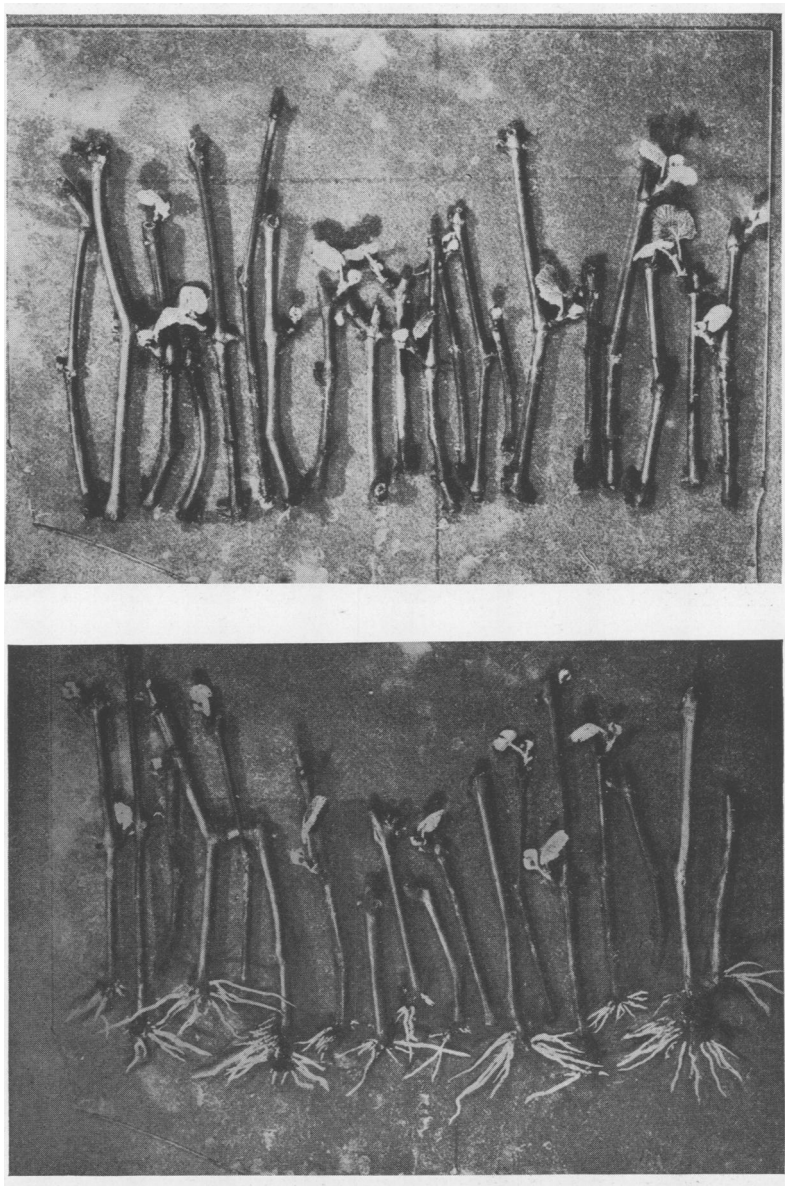


FIG. 5. Concord grape cuttings (one-year wood). Below, treated with indole-acetic acid, 200 mg. per liter for 24 hours at base. Above, controls treated with water. Photographed after 14 days in unheated sand.

shows how complex the analysis of growth phenomena can be, particularly when they are long-term effects.

Now, as you know, another action of the auxins, and it is common, in varying degree, to all the different substances which are auxins, is the induction of root formation. Like other auxin effects this is common to the majority of plants. Figure 5 shows the effect of twenty-four hours' treatment with indoleacetic acid on the rooting of Concord grape cuttings. Many similar photographs have been published recently for a variety of plants. In the normal plant, root-formation is achieved by the migration, to the base, of auxin produced in the growing buds and in the leaves, the transport which obtains normally in the plant being polar. This polarity of the transport of auxin brings about the formation of roots predominantly at the *bases* of cuttings, just as it is also responsible for the influencing of growth by buds or other auxin-forming organs below rather than above them. Of course, if we apply auxin to the plant externally, this polarity of its transport may be obscured by the upward and outward movement to which auxin (like salts or any other externally applied material) is subject. Nevertheless in the intact plant it is evident that there is a rather strict polarity of auxin transport, and the fact that under the right experimental conditions it can be demonstrated and even measured is important for the analysis of growth problems.

The first stages of root development involve rapid cell division and the formation of a root initial. Since this is brought about by auxin, it is perhaps not surprising to find that cell division in other tissues is also activated by auxin. This is mainly true only for the cambium. In tissues other than the cambium, however, cell division can be stimulated, though usually only by unphysiologically high auxin concentrations such as are produced by artificial application, or by the invasion of pathogenic micro-organisms. There does not, therefore, seem to be so clear a distinction between the factors causing division and those causing enlargement as was formerly thought. The influence of auxin in causing the development of parthenocarpic fruits, recently demonstrated by GUSTAFSON, must be considered in the same connection. It is of interest also that the wound hormone, recently studied by BONNER and ENGLISH on the pericarp of the bean, may cause either enlargement alone or enlargement accompanied by cell division according to the variety of bean used as test object. Evidently here also the factors causing the two processes are somewhat interlocked.

We are faced, therefore, with the fact that this one group of hormones, the auxins, bring about a variety of processes of growth and inhibition, and the effect that they exert evidently depends on the kind of cell they enter and on its physiological state. In the earlier days of this work, some four to five years ago, when only the action on cell enlargement was recognized,

it was customary to consider that the auxin causes growth by combining with the cell wall in some way. However, we were able to show that there is no stoichiometrical relation whatever between the auxin that enters the cell and any of the constituents of the cell walls. Further, this variety of physiological functions obviously indicates that the auxin action is exerted upon some very fundamental process in the cell, some kind of "master reaction." The action has been aptly compared to the unlocking of a door; once unlocked, the doorway no longer controls who or what shall pass in or out; these depend upon the conditions on either side of the door. However, the problem of the unlocking of the door becomes more interesting and important than ever because so fundamental. Similar problems of physiology are associated with the action of all hormones and vitamins, and in no case is there a really clear understanding of their actual mode of action.

There have been two main lines of approach to this unlocking problem; one consists in the analysis of the *key*, *i.e.*, the determination of just what properties the hormones must have in order to act—the other is the analysis of the *lock*, *i.e.*, an attempt to determine more nearly the nature of the master reaction and the other processes brought about by auxin. I will discuss each of these briefly.

Let us consider first the key. The formulae of figure 6 show that the

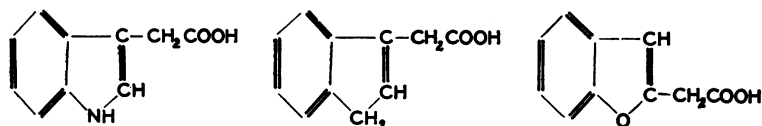
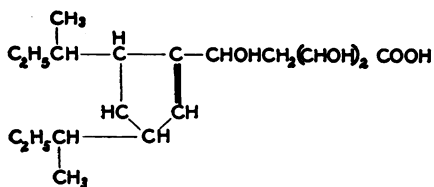


FIG. 6. Above, auxentriolic acid (auxin a) according to KÖGL, HAAGENS MIT, and ERXLEBEN. Below, left to right, indole-3-acetic acid, indene-3-acetic acid, benzofurane-2-acetic acid.

substances auxin a and indole-acetic acid, the two most highly active auxins, both have an unsaturated 5-membered ring with an acid group in the side-chain. One has a nitrogen atom, the other not. The first subject for study was, therefore, the activity of comparable compounds without the nitrogen atom. Both these substances (formulae to the right) were found to be active, though much less so than indole-acetic acid. A very interesting difference lay, however, in their behavior in the *Avena* test, in which the auxin

is applied in agar to one side of the coleoptile. In this test, indole-acetic acid produces curvature distributed down the coleoptile for 15 mm. or so, but indene-acetic acid, the compound with carbon instead of nitrogen in the ring, produces only localized curvatures (fig. 7). To produce curvature there

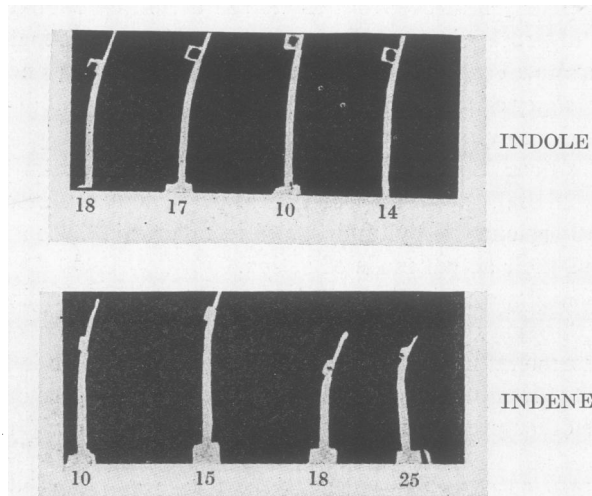


FIG. 7. Decapitated *Avena* seedlings supplied with auxin in agar on one side. Above, indole-3-acetic acid, curvature distributed. Below, indene-3-acetic acid, curvature localized.

must be a marked differential growth and hence the substance must be restricted to the side to which it is applied. If it does not move far on that side it can cause only local curvature—if it spreads out equally on to the other side it can cause no curvature at all. The physiological concentrations of indene-acetic acid evidently do not move as rapidly through the tissues as indole-acetic acid, *i.e.*, their transport is poorer, and this is supported by the fact that indene-acetic acid causes excellent rooting of cuttings if applied at the base, *i.e.*, at the point where its action will occur, but little or no rooting if applied at the tip, from whence it will have to move to the base to bring about its effect.

The second difficulty, that of spreading out, is shown by the oxygen derivative, cumaryl-acetic acid, or benzofurane-acetic acid, which causes straight growth but no curvature. A later analysis of the 3-derivative, benzofurane-3-acetic acid has shown it to be essentially similar though a little more active. Hence here we are dealing with properties which are not essential for *growth* activity, but which control the way in which the growth comes to be manifested; in this case they influence the ability of the substances to be linearly transported.

For further analysis we have therefore tried to study growth activity in

such a way that transport does not enter in. For this purpose immersed plant parts, such as the slit pea stems whose curvatures was shown by WENT to be so convenient a tool for auxin work, have been used. Figure 8 shows

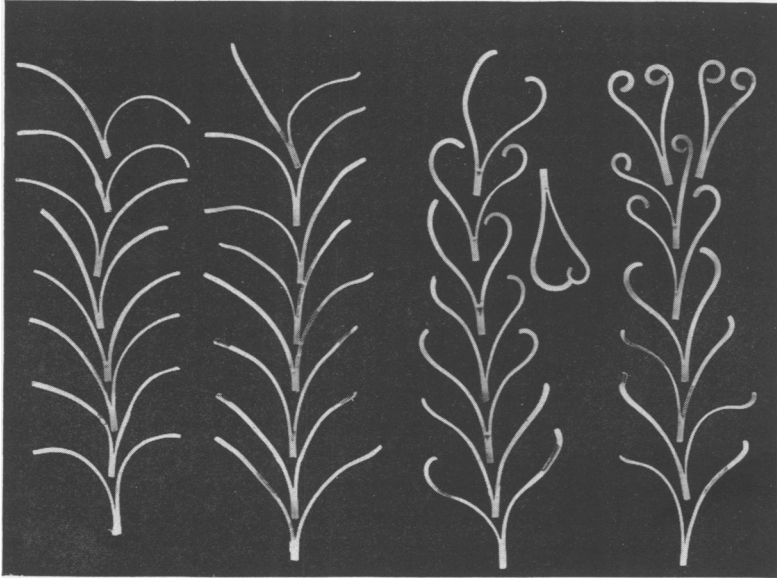


FIG. 8. Curvatures of slit internodes of etiolated pea stems. Left to right, water, 0.1, 1.0 and 4.0 mg. per liter indene-acetic acid.

single young internodes of etiolated *Pisum*, slit longitudinally and immersed in water or auxin solutions. The stem halves, which curve slightly outwards in water, curve inwards in auxin solution on account of differential response to auxin. The inward curvature varies roughly with the logarithm of the auxin concentration. Being immersed, the auxin is applied all round and transport difficulties need not concern us, except insofar as the rates of entry might be different at the different surfaces. One or two interesting points as to the relation between structure and activity have been brought out in this work. First as to the need for the double bond in the auxin molecule. KÖGL found that the saturation of the double bond in indole-acetic acid and auxin caused complete loss of activity (fig. 9). The same thing is shown by phenyl-acetic acid (active) and cyclohexyl-acetic acid (inactive). Cyclohexene-acetic acid, with one double bond, on the other hand, is active. If the double bond is in the sidechain and not in the ring, as in cyclohexylidene-acetic acid, the activity is again lost, hence not only must the double bond be present, but it must be in the ring. Up to now all open-chain compounds have been found inactive.

Secondly as to the structural arrangement (fig. 10). The acid group cannot be next to the ring, since indole-carboxylic and benzoic acids are inactive. Apparently it must be free to reach a certain position in space which is about one carbon atom distance from the ring. Introduction of one methyl group in the side chain does not affect this orientation, but the two methyl groups on the same place prevent it from doing so; to a lesser degree it is prevented from reaching this position by being farther out on the chain, for, in general, activity decreases with increasing length of the sidechain, (phenyl-acetic > β phenyl-propionic > γ phenyl-butyric). A special case is

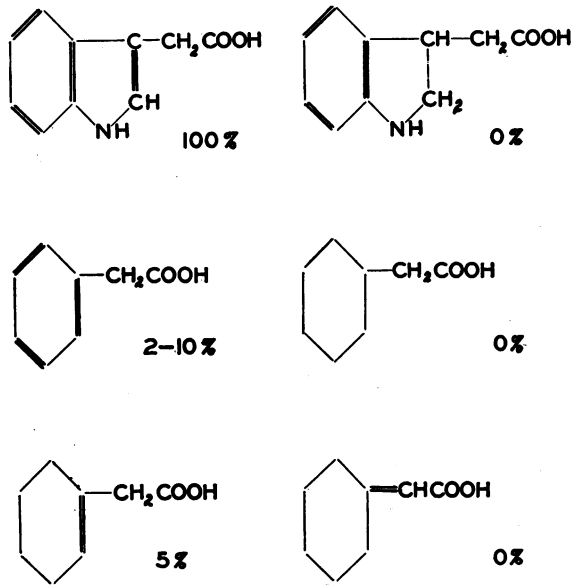


FIG. 9. Top, left, indole-acetic acid; right, dihydro-indole-acetic acid. Middle, left, phenyl-acetic acid; right, cyclohexane-acetic acid. Bottom, left, cyclohexene-acetic acid; right, cyclohexylidene-acetic acid.

that in which the double bond in the sidechain prevents free rotation; *cis*-cinnamic, in which the approximate position can be reached, is active, while *trans*-cinnamic, in which the COOH is held out at arm's length, so to say, has no activity. The same holds for derivatives of *cis* and *trans*-cinnamic acids. It is evident that within this group of substances the requirements for activity are quite strict, and the action is therefore rather *specific*. When this is more clearly understood we shall be in a position to formulate possible reactions in which the auxin may take part and begin to find out which one occurs.

The other approach is by analysis of the lock. In this, an important piece of evidence is the fact that auxin acts in the tissues together with other sub-

stances. Of these, the best understood is sugar. In order for auxin to cause growth in the coleoptiles, sugar is essential. If the sugar supply is first exhausted from the plant, auxin can cause no growth. If auxin alone is supplied, sugar greatly increases the growth. Not only sugar, however, but other factors are also concerned. This is particularly true in root formation

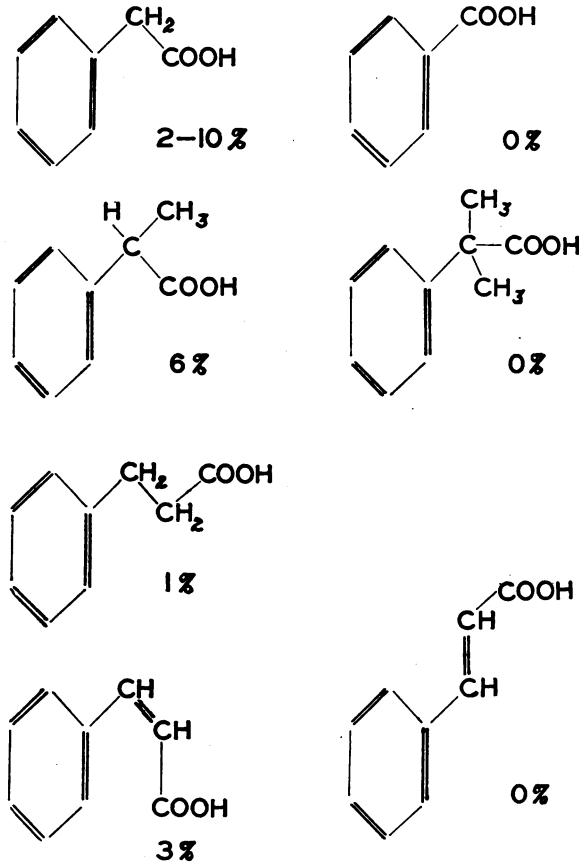


FIG. 10. Top, left, phenyl-acetic acid; right, benzoic acid. 2nd row left, α -methyl- α -toluic acid; right, $\alpha\alpha$ -dimethyl- α -toluic acid. 3rd row, β -phenyl-propionic acid. 4th row, left, *Cis*-cinnamic acid; right, *trans*-cinnamic acid.

on cuttings (fig. 11). Here, in *Pisum* cuttings, sugar alone makes about two roots per plant, probably using the auxin already there. Holding the sugar constant and optimal, the number of roots increases proportional to the *auxin* concentration, to a maximum of about 10 roots per plant. Then, holding the auxin constant and maximal, we can increase the next factor (biotin, yeast

growth factor) to reach a new maximum. When this is held at the maximum value, there is evidence for a fourth factor.

Thus the analysis of the lock must take into account the other parts of the lock besides the key. WENT has suggested that these other factors, or certain of them, are really the substances which are specific for the different processes, special substances in the sense of SACHS. A better understanding of the rôle of these other factors will depend on further experiments.

Lastly, as to the nature of the first reaction into which the auxin enters. We now have excellent evidence that it is an oxidation process. I will not

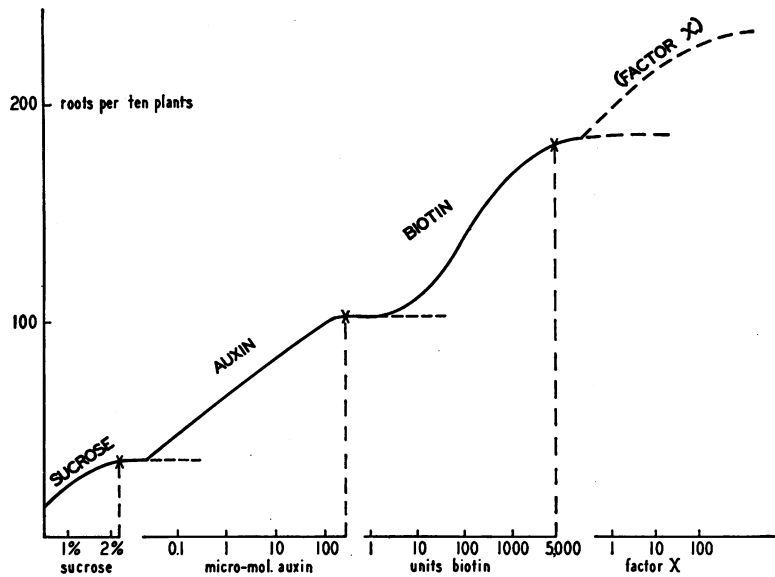


FIG. 11. Interaction of factors causing root formation in *Pisum* cuttings. Ordinate, roots per 10 plants; abscissa, concentration (log. scale) of the factor which is limiting root formation. (From WENT and THIMANN, *Phytohormones*. New York, Macmillan. 1937).

discuss this in detail now, but will only say that when auxin is applied to the cell there is an immediate acceleration of the rate of protoplasmic streaming. This acceleration is observed much sooner than any growth can be detected. It is like growth in that it needs sugar for its effect to be fully realized. It also needs oxygen; in absence of oxygen, streaming is retarded; and in auxin, the consumption of oxygen by this reaction is increased. Hence auxin probably has as its first effect an increase in the rate of one special oxidation process, a process whose substrate is sugar, and which directly controls the rate of streaming, and, probably through this, the rate of growth. The streaming of protoplasm in the cell is something so fundamental that it is easy to see how changes in its rate can bring about a variety of subsequent

changes. It is not yet known whether we can say that this reaction which controls streaming and growth is the primary door which auxin unlocks; if not, it is very near it.

In conclusion I would like to say two things about this analysis of growth problems through hormones. In the first place, it must be remembered that it is plant *physiology*, not *pharmacology*, *i.e.*, we do not, as some people imagine, simply apply all kinds of substances externally to plants, but we are engaged in the study of processes which go on in the normal plant.

Secondly, it is not purely academic. One immediate result of the work has been as you know, the elucidation of the action of auxins in causing root formation on cuttings, a finding of immediate practical utility. In this respect it would have been strongly approved by STEPHEN HALES, who, more than 200 years ago, pointed out that the only sound basis, both of agriculture and horticulture, must be plant physiology; (I quote from his vol. 1): "As the art of Physick has of late years been much improved by a greater knowledge of the animal œconomy, so doubtless a farther insight into the vegetable œconomy must needs proportionably improve our skill in Agriculture and Gardening, which gives me reason to hope that enquiries of this kind will be acceptable to many, who are intent upon improving those innocent, delightful and beneficial Arts: Since they cannot be insensible that the most rational ground for Success in this laudable pursuit must arise from a greater insight into the nature of Plants."

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