# AUXIN-INDUCED GROWTH INHIBITION A NATURAL CONSEQUENCE OF TWO-POINT ATTACHMENT*

BY ROBERT J. FOSTER, D. HAROLD MCRAE, AND JAMES BONNER

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

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*Introduction.*—It is characteristic of a great number of biologically active substances that the responses which they elicit are twofold, low concentrations of the material promoting a particular activity, and higher concentrations inhibiting it. This is the case with the auxin-induced growth responses of plants. An active auxin such as indole acetic acid (IAA) brings about and is essential to growth in length of stems, hypocotyls and other plant organs including the Avena coleoptile. Excised sections of this object grow in length when they are floated in solutions containing low concentrations of IAA and this growth is a simple function of auxin concentration as

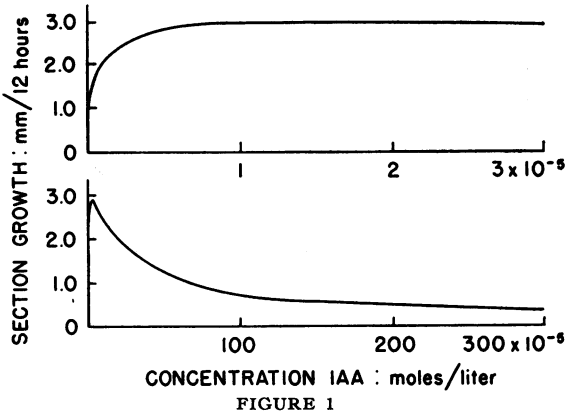


FIGURE 1

Avena coleoptile section growth as a function of IAA concentration: *a* (above), over a low concentrations range 0–3 × $10^{-5}M$; *b*, over a greater concentration range 0–300 × $10^{-5}M$.

is shown in figure 1a. With increasing auxin concentration, however, growth rises to a maximum only to decline as auxin concentration is still further increased (Fig. 1b).

It has been shown in earlier papers[1] that a molecule in order to be active as an auxin must satisfy a number of structural requirements among which is the requirement for two suitably positioned reactive groups. These two groups, a carboxyl group located in a side chain attached to an unsaturated cyclic nucleus[2] and a substitutable group of critical reactivity in the nucleus ortho to the side chain[3] appear to be the functional groups through which

the auxin molecule makes attachment to some receptor entity within the plant. The combined auxin, bound through its two reactive positions to two functional sites of the receptor entity, appears to be the form which is active in eliciting growth responses. Although the exact chemical nature and biochemical function of the auxin-receptor complex is not known, the existence and character of the complex can be deduced from kinetic considerations[4] and from the study of the relationships of chemical structure to physiological activity of synthetic auxin analogs.[5]

It is the purpose of this paper to show that the inhibition of growth induced by supra-optimal concentrations of auxin is a natural and indeed an inescapable consequence of the two-point attachment by which auxin is bound within the plant to form the functional entity.

*Materials and Methods.*—All of the experimental work reported here has been based on measurements of the rate of elongation of excised sections of Avena coleoptiles floated in solutions containing an active auxin.[6] Avena seedlings were grown in vermiculite contained in stainless steel trays and maintained in a dark room under low intensity red light at a temperature of 25–26°C. and a relative humidity of 90%. When the seedlings had attained an age of 82–84 hrs. they were selected for uniformity. Those plants with a coleoptile length between 2.75 and 3.25 cm. were decapitated by removal of a 2–3 mm. apical tip and the first leaves removed. Five mm. apical sections were then cut from each coleoptile with a double-bladed cutting tool. The sections were pooled and randomly distributed in lots of 20 to individual Petri dishes containing 20 ml. of the test solution. A basal medium containing 0.0025 *M* potassium maleate buffer (pH 4.5) and 3 per cent sucrose was employed. To this the varied auxins, adjusted to pH 4.5, were added as desired.

Sections were allowed to grow in the dark at 25–26° for a period of 12 hrs. and their length then measured under a microscope. This growth measurement yields the initial growth velocity since the growth of Avena coleoptile sections under the present conditions is linear with time for a period of 18 hrs. or more.

*Kinetic Treatment of Avena Section Growth.*—The elongation of excised *Avena coleoptile* sections in response to exogenous auxin may be formally treated by the enzyme kinetics of Michaelis and Menten.[7] For this treatment, the initial growth velocity of the sections ($v$) is determined as a function of substrate (IAA) concentration [$S$]. Classical enzyme kinetics are based upon the hypothesis that substrate combines with enzyme ($E$) to form an enzyme-substrate complex ($ES$) which then decomposes to form the product of the reaction (in this case growth) and to reform $E$. In this formulation which is outlined in equation (1), $K_s$ and $k$ are constants which express, respectively, the affinity constant for the formation of $ES$ from $E$

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k} \text{growth} + E \qquad (1)$$

and $S$ and the constant characterizing the rate of decomposition of $ES$ to end product.

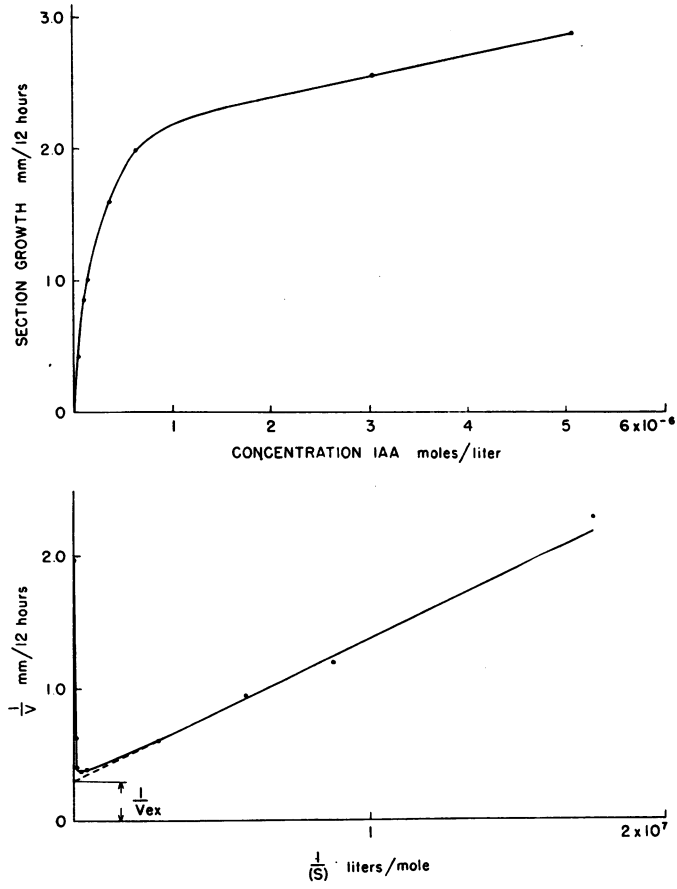


FIGURE 2

Avena coleoptile section growth as a function of IAA concentration: *a* (above), growth rate plotted as a function of IAA concentration; *b*, reciprocal of growth rate plotted as function of reciprocal of concentration.

Equation (1) may be expressed in terms of reaction velocity ($v$) which is dependent, as shown in equation (2) upon $[S]$ and the constants $K_s$ and

$$v = \frac{V_{max}\,[S]}{K_s + [S]} \qquad (2)$$

$V_{max.}$ The latter is determined as the reaction velocity of the system in the presence of non-limiting substrate concentration and in which all $E$ is therefore combined as $ES$. The relationship between $v$ and $[S]$ for any system which follows the formulation of equation (2) must be expected to be a hyperbolic one in which $V_{max.}$ represents the value of $v$ which is asymptotically approached as $[S]$ is increased and in which $K_s$ is the value of $[S]$ at which one half of the maximum reaction velocity is attained. Figure 2a shows how the data relating growth rate of Avena coleoptile sections to IAA concentration fall smoothly on such an hyperbola.

A critical test of the applicability of equation (2) to a particular system consists in relating graphically the reciprocal of reaction velocity $(1/v)$ to the reciprocal of substrate concentration $(1/[S])$. Any system following Michaelis-Menten kinetics is expected to yield a straight line for which the intercept on the ordinate is $1/V_{max.}$ and the slope $K_s/V_{max.}$. Figure 2b shows that the data for Avena section growth as a function of IAA concentration fit this formulation with precision over the lower range of substrate concentration. The systematic deviation of the data for the higher concentrations, which is an expression of a depression in growth rate at the higher auxin levels, may be understood in the light of an extension of the Michaelis-Menten treatment as will be shown hereafter.

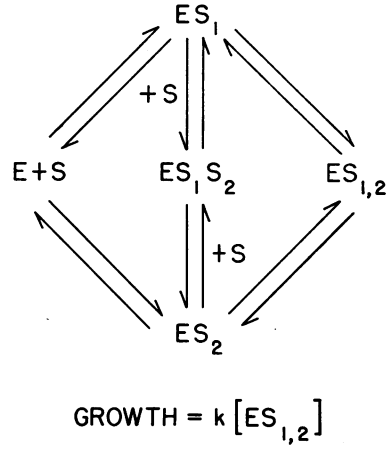


FIGURE 3

Equilibria relating the formation of active receptor-substrate complex ($ES_{1,2}$) to the formation of inactive receptor-substrate complex ($ES_1S_2$).

*Kinetic Treatment of Two-Point Attachment.*—The treatment thus far has dealt implicitly with systems in which attachment of substrate to enzyme is consummated through a single point. Let us now consider the manner in which two-point attachment may be expected to alter this picture.[8] A molecule of auxin ($S$) might be expected to become first associated with one of the two receptive sites on the enzymatic entity and then, by becoming associated with the second receptive site, to consummate the two-point attachment essential to enzymatic (growth) activity. This sequence of reactions will predominate at low auxin concentrations. At higher auxin concentrations, however, there is an appreciable probability that two auxin molecules may simultaneously approach the receptor entity and simultaneously react with it, each combining through a single reactive group. Such interaction will yield an inactive auxin-receptor complex in which

each auxin molecule prevents the other from consummating a two-point attachment.

The several equilibria which relate the monomolecular and bimolecular substrate-receptor complexes are illustrated in figure 3. These equilibria involve the following quantities:

$E$ = Receptive entity of the plant.
$S$ = Auxin.
$ES_1$ = Auxin-receptor entity complex combined through point 1.
$ES_{1,2}$ = Auxin-receptor entity complex combined through points 1 and 2. This complex, the enzymatically active one, may be presumed to be formed from either $ES_1$ or $ES_2$.
$ES_1S_2$ = Bimolecular auxin-receptor entity complex in which two auxin molecules are involved, one attached through point 1, the other through point 2. This complex is presumed to be enzymatically inactive.

In the classical enzyme kinetics only the equilibria which result in the formation of the active complex, $ES_{1,2}$ are considered. If we include in the consideration the formation of the complex $ES_1S_2$, the classical expression relating reaction velocity to substrate concentration may be shown to be altered to the form given in equation (3).

$$v = \frac{V_{ex}\,[S]}{K_s' + [S] + [S]^2/C} \qquad (3)$$

This expression differs from that of classical kinetics in that it includes a term $[S]^2/C$ which is a measure of the probability that a second molecule of substrate will become attached to the receptor entity before the first molecule has consummated its two-point attachment. The reaction velocity $v$, which is proportional to $ES_{1,2}$, is expressed in terms of $[S]$ and the experimentally determinable constants $V_{ex}$, $K_s'$ and $C$. Equation (3) predicts a maximum reaction velocity (growth rate) at a substrate concentration of $\sqrt{K_s' \cdot C}$. This maximal rate is decreased below the rate which would be achieved in a classical system by the amount to which the inactive complex $ES_1S_2$ is formed in the system at the substrate concentration $\sqrt{K_s' \cdot C}$.

Equation (3) will now be applied to data derived from experimental determination of the relation of auxin concentration to rate of Avena coleoptile section growth. We shall first evaluate the constants $V_{ex}$ and $K_s'$ which correspond to the constants $V_{max.}$ and $K_s$ of classical enzyme kinetics. These two constants may be determined in the low range of auxin concentration in which the formation of the bimolecular complex $ES_1S_2$ is negligible. This is most readily done on the reciprocal plot of figure 2b. $V_{ex}$ is the extrapolated intercept on the ordinate while $K_s'$ is the slope

divided by the intercept. The remaining constant $C$, which is a measure of the probability that two molecules will react with the two receptor sites of the reactive entity before either has had a chance to consummate its two-point attachment, must be evaluated in the region of high concentration in which the formation of this complex predominates. This is done as

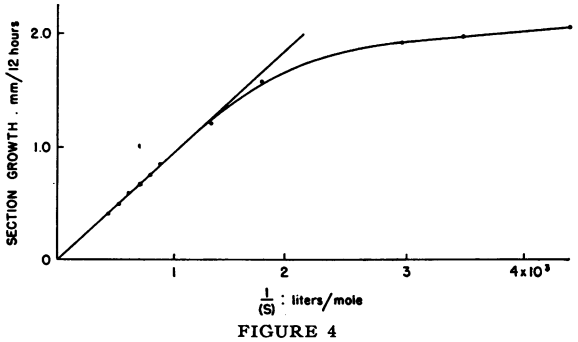


FIGURE 4

At high auxin concentration, growth of Avena coleoptile sections is inversely proportional to the substrate concentration. The limiting slope of the lower portion of this curve = $V_{ex} \cdot C$.

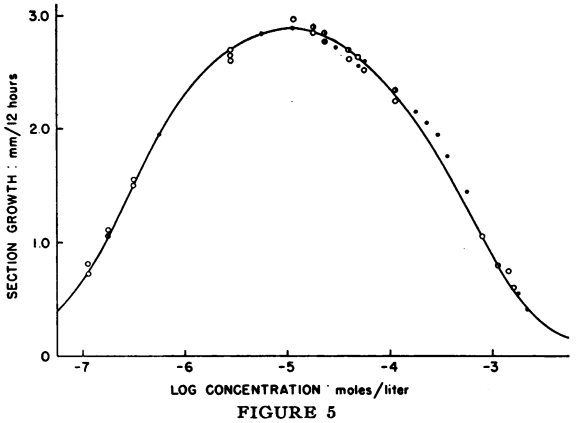


FIGURE 5

Avena section growth as a function of IAA concentration. The solid line is that for equation (3); the points represent data from five separate experiments.

shown in figure 4 which presents growth velocity as a function of the reciprocal of the substrate concentration. It can be seen that as the auxin concentration is increased, the growth velocity becomes inversely proportional to it and from the limiting slope which is $V_{ex}C$, the value of $C$ may be determined.

It appears then that at the extremes of the auxin concentration range auxin affects growth in the manner predicted by equation (3). This equation describes accurately the effects of auxin on the growth response of Avena coleoptile sections over the entire range of auxin concentrations to which this organ is sensitive, a range of one hundred thousand fold. That this is so is shown in figure 5. Since the range of concentration is so large,

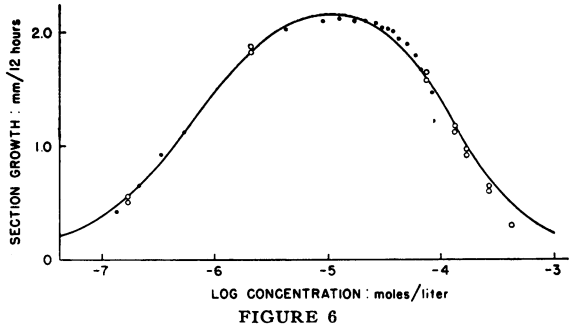


FIGURE 6

Avena section growth as a function of 2,4-D concentration. The solid line is that for equation (3); the points represent data from two separate experiments.

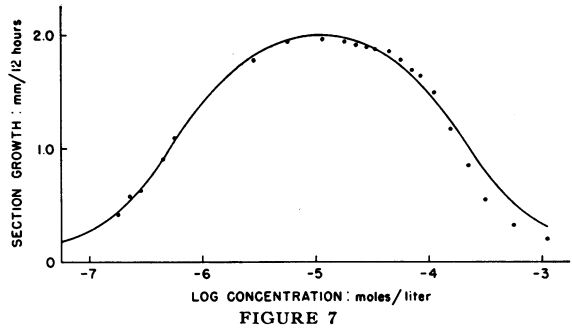


FIGURE 7

Avena section growth as a function of NAA concentration. The solid line is that for equation (3); the points represent experimental data.

the data are presented in the form of initial growth velocity as a function of the log of auxin concentration. The curve of figure 5 is that for equation (3) using the values for the constants determined as outlined above. The individual points of figure 5 represent data from 5 separate and complete experiments. These fit the curve within experimental error over the entire 100,000 fold range of concentrations. That the maximum growth rate occurs at a substrate concentration of $\sqrt{K_s' \cdot C}$ is also verified by the data.

The synthetic growth substances such as 2,4-dichlorphenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA) are, like the naturally occurring IAA, active in promoting the growth of Avena coleoptile sections at low concentrations, active in inhibiting growth at higher concentrations. That the kinetic relations of 2,4-D and of NAA in their effects on Avena section growth are also in accord with the two-point attachment concept is evident from the data of figures 6 and 7. This is true even though the constants $V_{ex}$, $K_s'$ and $C$ for these substances differ appreciably among themselves and differ also from those determined for IAA, a fact summarized in table 1.

*Discussion.*—The concept that auxins are bound within the plant to an appropriate receptor entity through which auxin-induced growth responses are mediated was originally based not only on the known binding of auxins to plant proteins[9] but also and more specifically on the fact that the kinetics of auxin action are in close accord with the expectations of classical

TABLE 1

SUMMARY OF THE PARAMETERS WHICH RELATE GROWTH OF AVENA COLEOPTILE SECTIONS TO AUXIN CONCENTRATION

| Auxin | $V_{ex}$, mm./12 hrs. | $Ks'$,[a] moles/liter | C,[b] moles/liter | $\sqrt{Ks' \times C}$,[c] moles/liter |
|---|---|---|---|---|
| IAA | 3.1 | $3.4 \times 10^{-7}$ | $4.0 \times 10^{-4}$ | $1.2 \times 10^{-5}$ |
| 2,4-D | 2.3 | $5.5 \times 10^{-7}$ | $1.4 \times 10^{-4}$ | $0.9 \times 10^{-5}$ |
| NAA | 2.1 | $6.4 \times 10^{-7}$ | $1.7 \times 10^{-4}$ | $1.1 \times 10^{-5}$ |

[a] Concentration of auxin at which growth rate is 0.5 $V_{ex}$ and $ES_{1,2} = E_{free}$.

[b] Concentration of auxin at which growth rate is 0.5 maximal due to formation of the complex which contains two auxin molecules. $ES_{1,2} = ES_1S_2$.

[c] Concentration of auxin at which growth rate is maximal.

enzyme kinetics.[4] The extension of this concept to include the specification that the binding must be consummated through a two-point attachment of the auxin molecule to the receptor entity was first suggested by the observation of Muir, Hansch and Gallup[3] that a free ortho group of critical reactivity as well as a free carboxyl group are essential to auxin activity. The hypothesis that both of these groups are in fact essential to auxin activity and by implication essential to auxin binding has been strengthened by the finding that auxin-like molecules in which either of the two essential groupings is blocked or abolished are not only inactive as auxins but also are in fact competitive inhibitors of auxin action.[10] The matters considered in this paper constitute a critical experimental test of the two-point attachment concept as applied to auxin action. If two-point attachment of auxin to its receptor is essential for the formation of the complex which induces growth it follows that inhibition of growth by the formation of bimolecular complexes may be expected at higher auxin concentrations. The fact that auxin-induced growth inhibition not only does occur but also

that it follows in elegant detail the kinetics of bimocular complex formation provides strong support of the whole two-point attachment concept.

Of what practical use may it be to express and to think about auxin-induced growth inhibition in terms of the kinetics of two-point attachment? One evident application may be in the study of the herbicidal action of synthetic growth substances such as 2,4-D. It is a peculiarity of these materials that although they are exceedingly deleterious to particular plants at higher concentrations, they act at lower concentrations in a manner indistinguishable from that of IAA itself. We might therefore inquire in how far the herbicidal action of 2,4-D and related materials may be owing to the formation of bimolecular complexes of the kind here described and we might attempt to answer this question by determination of the extent to which herbicidal activity is a function of the second power rather than of the first power of the applied growth substance concentration.

The twofold action of auxin on plant growth appears then to have its basis in a requirement for two-point attachment of the auxin to its receptor, a requirement which makes possible the formation of an inactive bimolecular complex at higher auxin concentrations. It will be of evident interest to discover whether the twofold action of other biologically active substances may in some instances similarly reside in a requirement for multiple attachment.

*Summary.*—The relationship between Avena coleoptile section growth rate and auxin concentration over a wide range has been demonstrated to be predictable on the basis of a requirement for two-point attachment of the auxin molecule to some receptive entity in the plant. In particular, the inhibition of growth at higher auxin concentrations, which may be the cause of herbicidal activity of certain chemicals, has been shown to be a natural and predictable consequence of the two-point attachment of the auxin molecule to the receptor entity within the plant.

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