Timing of the Auxin Response in Coleoptiles and Its Implications Regarding Auxin Action

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ABSTRACT The timing of the auxin response was followed in oat and corn coleoptile tissue by a sensitive optical method in which the elongation of about a dozen coleoptile segments was recorded automatically. The response possesses a latent period of about 10 min at 23°C, which is extended by low concentrations of KCN or by reducing the temperature, but is not extended by pretreatments with actinomycin D, puromycin, or cycloheximide at concentrations that partially inhibit the elongation response. Analysis of the data indicates that auxin probably does not act on the elongation of these tissues by promoting the synthesis of informational RNA or of enzymatic protein. Not excluded is the possibility that auxin acts at the translational level to induce synthesis of a structural protein, such as cell wall protein or membrane protein. While the data do not provide direct support for this hypothesis, the speed with which cycloheximide inhibits elongation suggests that continual protein synthesis may be important in the mechanism of cell wall expansion.

INTRODUCTION

A considerable amount of recent research effort has been directed toward showing that the developmental effects of hormones such as auxin can be ascribed to an activation of gene transcription and specific protein synthesis (5, 41, 42, 47, 62–64, and references there cited). The principal lines of evidence for this are (*a*) inhibition of hormonal responses by antibiotics that inhibit RNA and protein synthesis, (*b*) promotion by hormones of incorporation of labeled precursors into RNA and protein, and (*c*) promotion of synthesis of particular enzymes by hormones. It is widely recognized that these kinds of evidence are somewhat inconclusive as regards the actual means by which hormones influence development. The antibiotics will inhibit the developmental process if it depends in any way upon RNA or protein synthesis,

whether or not the hormone works by gene activation. It is to be expected that promoted growth will in general be accompanied by increased synthesis of RNA and protein even if the hormone does not act directly upon these processes. And although many inductive effects of hormones on enzyme synthesis are known in animals and several in plants (23, 33), it is a separate question whether such enzyme increases are the causal basis of particular physiological responses such as the cell elongation response to auxin.

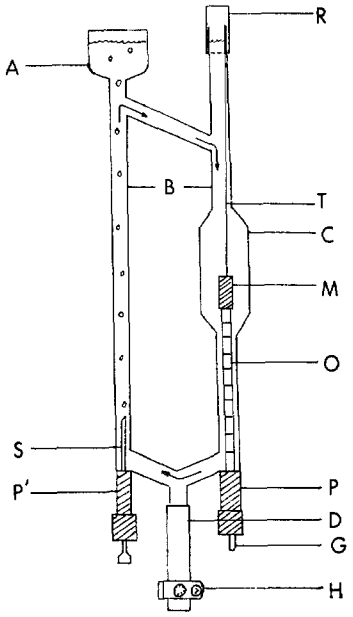

FIGURE 1. Growth measurement chamber, made from Pyrex glass. *A*, overflow reservoir, gas exit vent, and funnel for filling chamber. *B*, 7 mm O.D. glass tubing. *C*, spectrophotometer cuvette (10 mm square). *D*, drain outlet, Tygon tubing. *H*, screw clamp. *G*, glass plug. *M*, weight. *O*, row of coleoptile segments. *P*, *P'*, Teflon plugs. *S*, No. 25 syringe needle. *T*, thread.

The present paper reports an investigation of the timing of the elongation response of coleoptile tissues to auxin, conducted in order to explore the gene activation hypothesis of auxin action. A method was developed for recording automatically the elongation of about a dozen coleoptile segments with sufficient precision to display accurately the course of their elongation during the latent period of about 10 min that was known from previous work (52) to be involved in auxin action, and to permit determination of the effects of various treatments upon the time course of the auxin response.

MATERIALS AND METHODS

Plant Material Oats (*Avena sativa* var. Victory) were grown in an arrangement similar to that of Wiegand and Schrank (66). Immediately after planting, the dish containing the seeds was placed in a refrigerator at about 4°C overnight; the next morning, the dish was removed to a dark room kept at 26°C and placed under a 25 w ruby red bulb about 18 inches above the dish for the next 24 hr. The seedlings were

subsequently allowed to grow in complete darkness for 48 hr. Under dim red light segments 8 mm long were cut from coleoptiles 2.5–3.0 cm long, beginning 3 mm from the tip, using a double-bladed cutting device. The leaf was removed from each segment.

When corn coleoptiles were used, corn seeds (*Zea mays* var. Golden Bantam) were soaked for 2 hr in distilled water and grown in vermiculite in a dark room at 26°C. They were given red light as described above during the first 24 hr and then allowed

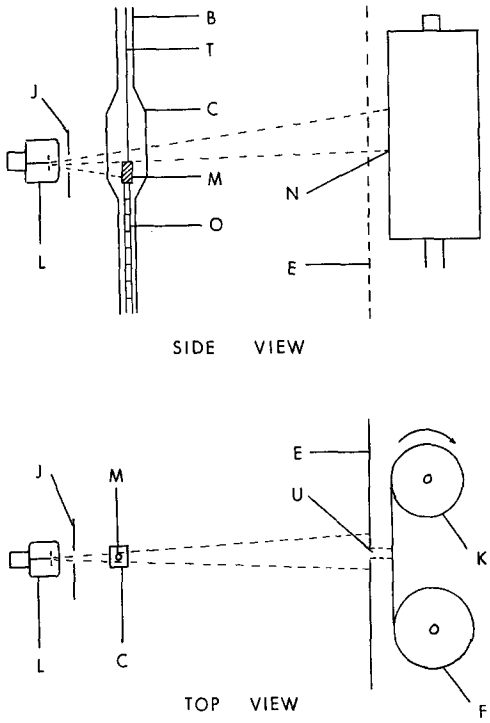

FIGURE 2. Schematic of shadowgraphic growth-recording apparatus (not to scale). *B*, *C*, *M*, *O*, *T*, parts of the growth measurement chamber as indicated for Fig. 1. *E*, baffle, with slit (*U*). *F*, roll of photographic paper. *J*, diaphragm. *K*, kymograph drum. *L*, zirconium arc lamp. *N*, upper edge of shadow of *M*.

to grow in complete darkness to a height of about 3 cm. Coleoptile segments were cut in the same manner as described above for oats.

Method of Measurement A piece of cotton thread about 40 cm in length was passed through a small hole in the Teflon plug *P* shown in Fig. 1. A knot was tied in the end of the thread to prevent it from slipping through the hole. The hollow coleoptile segments were then strung on the thread one at a time until a column of (usually) 13 segments was obtained. A weight consisting of a piece of dark red Pyrex glass capillary tubing weighing 92 mg, about 2 mm in diameter and 10 mm long, was then threaded so that it rested on the uppermost coleoptile segment. The Teflon plug (*P*) with thread (*T*) bearing the coleoptile segments (*O*) was inserted into the growth measurement chamber as shown in Fig. 1. The glass weight (*M*) fell within a spectrophotometer cuvette (*C*). The upper end of the thread was dropped over the upper edge of the glass tube above the cuvette and was secured by slipping a tight-

fitting rubber sleeve (*R*) over the glass tube and thread. The hole in Teflon plug *P* was stoppered with a small glass rod (*G*). Opening *D* was fitted with a small piece of Tygon tubing and a pinch clamp (*H*); this tube served as a drain. A syringe needle (*S*) fitted tightly into Teflon plug *P'* and served to gas the solution within the chamber with prepurified grade oxygen. The gassing caused the solution to circulate within the chamber as indicated by the arrows in Fig. 1. The total volume of the chamber was 14 ml. The chamber could be drained (via opening *D*) and refilled (via funnel *A*) with a different growth medium in a few seconds.

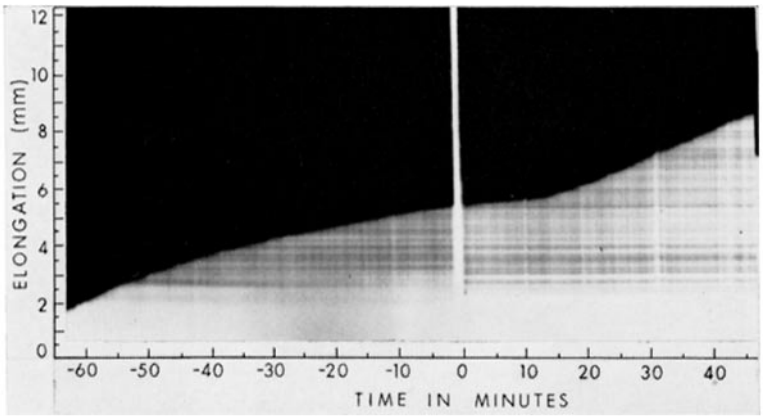

FIGURE 3. Record produced by the shadowgraphic apparatus (reproduced 0.25 times original size). Medium was changed from water to 3 μg/ml. IAA at the time corresponding to the vertical white line. The downward displacement of the shadow at the end of the record, made for calibration of optical magnification, represents 1.5 mm of actual vertical displacement. The rapid elongation that was occurring at the beginning of the record is the result of tactile stimulation of the oat coleoptile segments as explained in Methods.

The elongation of the entire column of coleoptile segments was recorded shadowgraphically as diagrammed in Fig. 2. A 2 w zirconium arc lamp (*L*) (Sylvania Type GZ2U Model C2) was positioned within the body tube of a horizontal microscope in place of the objective lens. At a distance of about 1 m from the stand supporting the growth chamber, a baffle (*E*) with a vertical slit (*U*) 2 mm wide was placed. Behind the baffle was located a spool (*F*) bearing a roll of Kodak polycontrast photographic paper (203 mm wide) and a multispeed kymograph drum (*K*), positioned as illustrated in the lower part of Fig. 2. The strip of photographic paper was pulled slowly by the kymograph drum past the slit. The speed of travel was usually 3.08 mm/min.

Light from the zirconium arc, which constitutes a nearly point source (0.127 mm in diameter), was limited by the diaphragm (*J*) so that a narrow cone of light passed through the cuvette (*C*) and fell upon the weight (*M*) but not upon the coleoptile segments. A sharp shadow (*N*) of the weight, within the circle of light, was thereby cast upon the slit as shown in Fig. 2. The light exposed that portion of the photographic paper (behind the slit) that lay above the edge of the shadow. As the segments

grew, this boundary moved upward and traced a record of the elongation which, after development of the photographic paper, appeared as in the example shown in Fig. 3. The vertical white line shown in Fig. 3 is an event marker and was produced by covering the slit during a solution change. All other growth curves presented in this paper are tracings of original records of the same kind.

In this recording method the elongation of the coleoptile segments was multiplied by the optical lever arm *LN/LM*. This magnification was calibrated at the end of each experiment, by moving the lamp up or down a known distance using a vertical

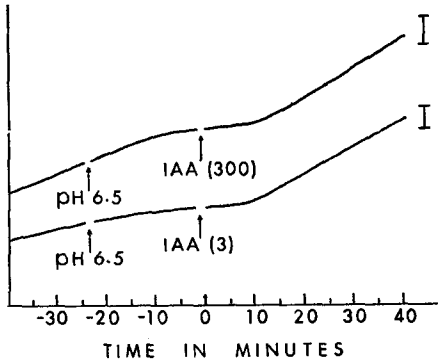

FIGURE 4. Effect of widely different IAA concentrations on timing of elongation response of oat coleoptile segments. At the first arrow water in chamber was replaced with Na citrate buffer, 10^{-3} M, pH 6.5; at the second arrow this was replaced with the same medium containing either 3 or 300 μg/ml IAA as indicated. The vertical bar by each curve in this and subsequent figures represents 1.0 mm of elongation, for that particular record, for the entire row of coleoptile segments.

micrometer screw in the lamp mounting, thus causing a displacement of the shadow boundary which appeared on the photographic record (cf. Fig. 3). In most experiments the optical magnification was between 15 and 20. This calibration allowed the absolute elongation to be calculated from the shadowgraph record. With each growth curve a vertical bar is given which indicates 1 mm of elongation (by the entire row of coleoptiles) for that growth record.

Chemicals Actinomycin D was generously supplied by Merck, Sharp and Dohme, West Point, Pa. Puromycin and cycloheximide were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Tissue was treated with antibiotics in neutral, unbuffered solution.

Preconditioning of Segments Oat coleoptile segments were normally floated on distilled water (and/or in some pretreatment medium) for at least 30 min after having been strung on the thread, prior to insertion into the growth measurement chamber and commencement of recording. This preconditioning was necessary because immediately after being strung on the thread the segments showed a burst of rapid elongation which lasted about half an hour. This is illustrated in the left-hand part of Figs. 3 and 7 (C and D), which are records that were begun shortly after the segments had

been strung on the thread. During this period of rapid elongation a promotion by auxin could not be obtained. Investigation indicated that this initial growth burst was not due to disappearance of endogenous auxin nor to manipulations accompanying insertion into the growth chamber but was the result of mechanical stimulation of the coleoptile segments by the thread when they were moved along it. Analogous effects of tactile stimuli on coleoptile elongation have been described previously (6, 13). We found no such tactile effect on the elongation of corn coleoptile segments,

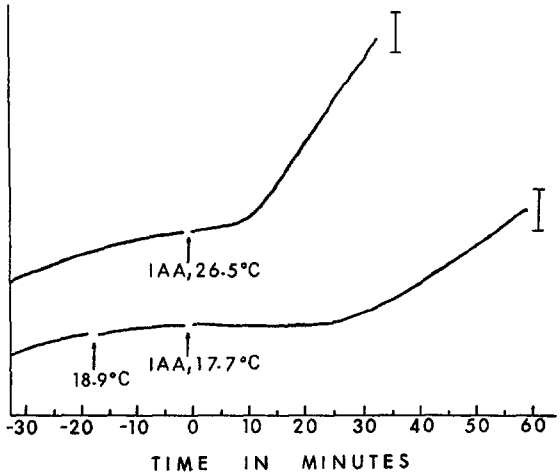

FIGURE 5. Timing of the auxin response at 17.7°C as compared with 26.5°C. Temperatures were maintained by continuously circulating through the growth measurement chamber water or IAA solution (3 μg/ml) held in large reservoirs in a constant temperature bath. Temperatures monitored using a copper-constantan thermocouple placed adjacent to the row of coleoptile segments. In upper curve, medium changed from water at 26.5°C to IAA at 26.5°C at the arrow. In lower curve, medium changed from water at 26.5°C to water at 18.9°C (first arrow) and then to IAA at 17.7°C (second arrow). In this experiment each curve was obtained using 10 coleoptile segments.

which may be because their much larger diameter results in little contact between the thread and the surface of the coleoptile's central cavity.

RESULTS

Timing of the Auxin Response Fig. 3 illustrates the response of oat coleoptile segments to 3 μg/ml IAA. After the addition of IAA, the segments continue to grow at a low rate for about 10 min, at which time a rather sudden increase in elongation rate occurs. Within about 3 additional min a rate of elongation about six times the previous rate is attained, the rate thereupon becoming steady. This type of result has been obtained many times in the course of the present work.

The auxin response of corn coleoptiles, illustrated in Fig. 7 A, was similar in possessing an absolute latent period (period of no increase in rate) of about

10 min. At the end of this period the elongation rate rose rapidly, but did not attain a steady maximum value until about 25 min after the beginning of exposure to IAA. This difference could be due to the much greater tissue thickness of corn coleoptiles with a resultant greater time dependence of penetration of IAA into the interior.

Fig. 4 shows that in buffered media there is no difference in the timing of the response of oat coleoptile segments to IAA at concentrations of 3 and 300

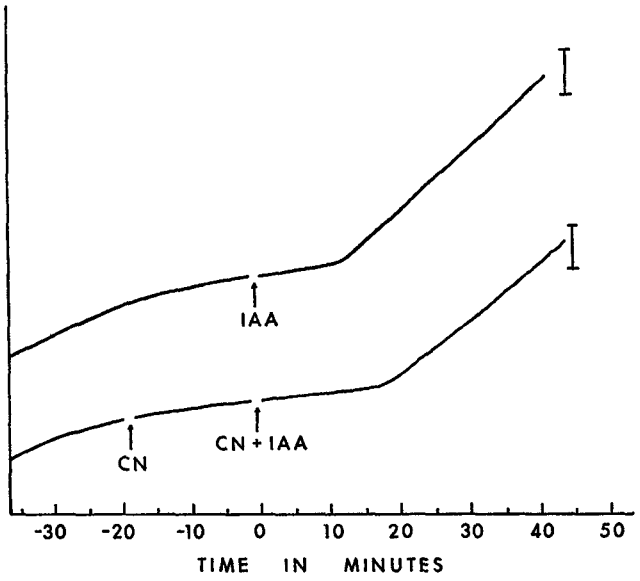

FIGURE 6. Effect of 5×10^{-5} M KCN on timing of auxin response. Upper curve shows control. Medium changed from water to 3 μg/ml IAA at arrow. Lower curve, medium changed from water to cyanide at first arrow and then, after 20 min, to cyanide plus 3 μg/ml IAA (second arrow).

μg/ml. Similar results were obtained at intermediate concentrations of IAA. This finding agrees with earlier observations using single coleoptile segments (52).

In unbuffered media an elongation response to 300 μg/ml IAA was found to occur within 1 min. This rapid effect proved to be caused by the low pH of this medium. This effect of pH on elongation has been investigated and will be considered in a later paper.

Figs. 5 and 6 show the effect of cyanide and low temperature on the length of the latent period. The absolute latent period in response to auxin at 17.7°C was almost three times as long (23 min vs. 8 min) as the absolute latent period at 26.5°C. A 20 min pretreatment with 5×10^{-5} M KCN nearly doubled the latent period while reducing the magnitude of the response to auxin by 25%.

Effect of Actinomycin D Fig. 7 (A-D) shows the effect of a 1 hr pretreatment with 20 μg/ml actinomycin D on the response to IAA in corn and oat coleoptile segments. Upon transfer to IAA, the maximum rates of elongation attained by pretreated segments were 55 and 32% of the control

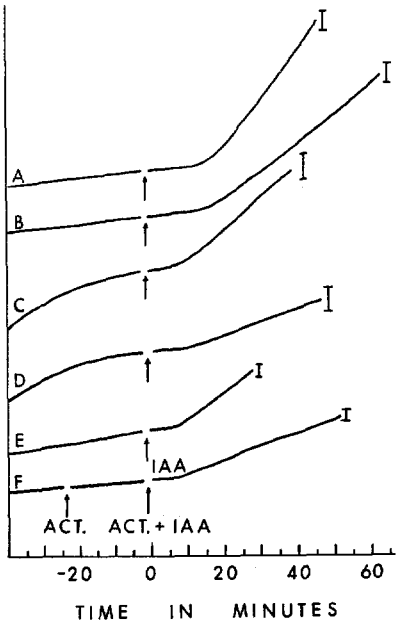

FIGURE 7. Effect of actinomycin D on response of coleoptile segments to IAA. A and B, response of corn coleoptile segments to 3 μg/ml IAA (added at arrow) after pretreatment for 1 hr in either water (curve A) or 20 μg/ml actinomycin D (curve B); segments were pretreated outside the growth measurement chamber, then introduced into the chamber, which contained water only, just before the record was begun. C and D, response of oat coleoptile segments to 3 μg/ml IAA with or without pretreatment similar to that in A and B, respectively; the part of the record prior to the arrow shows response to the tactile stimulation of placing segments onto thread, as explained in Methods. E and F, comparison of oat coleoptile segments transferred from water to IAA (curve E), and from water to 20 μg/ml actinomycin D and then, after about 20 min, to the same medium containing 10 μg/ml IAA (curve F).

rates, respectively. There was no difference in the length of the latent period between control and actinomycin-pretreated segments, with either corn or oats. Similar results were obtained if segments were pretreated with actinomycin in the growth chamber and then transferred to actinomycin plus IAA, as illustrated in Fig. 7 (E and F).

Table I summarizes data from several experiments with actinomycin. A 20 min pretreatment with 20 μg/ml was almost as effective as a 60 min pretreatment with the same concentration. Inhibition of elongation by actinomycin D

was in no case accompanied by a significant change in the length of the latent period.

Inhibition of oat coleoptile elongation by actinomycin was not reversible within a 50 min period, after transfer from actinomycin plus IAA to IAA alone. This explains why actinomycin pretreatment of the segments only prior to

TABLE I

EFFECT OF ANTIBIOTICS ON LATENT PERIOD AND MAGNITUDE OF AUXIN RESPONSE IN COLEOPTILE SEGMENTS

Experiment No. and antibiotic	Pretreatment	Latent period		Auxin-promoted elongation rate		Auxin-promoted elongation rate of treated segments, as per cent of control
		Control	Treated	Control	Treated	
	min	*min**		*mm/hr × segment*		
Actinomycin D (20 μg/ml)						
1	60	15.9	17.8	1.30	0.72	55
2	60	11.3	11.7	0.62	0.20	32
3	20	8.8	10.0	0.89	0.53	60
4	20	11.7	11.0	0.70	0.28	40
5	20	11.4	11.0	0.88	0.43	49
Cycloheximide (5 μg/ml)						
6	10	6.8	5.9	0.68	0.29	43
7	15	14.6	7.8	0.55	0.24	44
8	15	12.3	7.2	0.41	0.21	51
9	20	10.4	3.3	0.62	0.21	34
10	20	13.3	5.5	0.35	0.20	57
Puromycin (8×10^{-4} M)						
11	180	12.4	12.6	1.00	0.36	36

* Estimated as the time at which elongation rate had become one-half of its steady final value.

Coleoptile segments (oats, except experiment 1 in which corn was used) were pretreated for the indicated times in antibiotics, while controls were pretreated with water; they were then treated with 10 μg/ml IAA containing, in the case of the antibiotic-pretreated segments, the same concentration of antibiotic as in the pretreatment; except in experiments 1, 2, and 11 in which segments were treated as described for Fig. 7 (curves A-D). Elongation was followed for at least 40 min after addition of IAA.

their introduction into the growth chamber was just as inhibitory as transfer from actinomycin pretreatment to actinomycin plus IAA (Fig. 7).

In several experiments actinomycin was added after segments had responded to IAA. Actinomycin caused no inhibition of elongation within at least 60 min after its addition to IAA-promoted segments.

These findings agree in general with some observations on single coleoptile segments given by Masuda and Wada (43), although they found a much slower effect of actinomycin pretreatment. The difference may possibly be due

to the fact that our 20 min pretreatments were given with the coleoptiles totally submerged in the medium, rather than floating on it. Unpublished experiments using our oat coleoptile material show that inhibition of RNA synthesis by actinomycin D becomes fully established within 30 min after its addition.

Cycloheximide and Puromycin Table I summarizes the results of five experiments in which oat coleoptile segments were pretreated with 5 μg/ml cyclo-

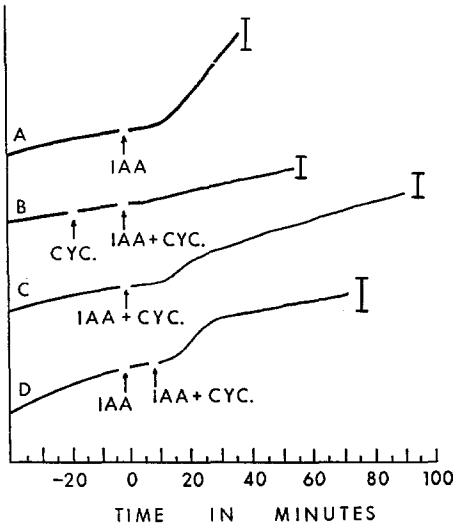

FIGURE 8. Effect of cycloheximide on auxin-induced elongation of oat coleoptile segments. Cycloheximide was added before (curve B), at the same time (curve C), and after (curve D) the IAA. Curve A shows the control (IAA only). Chamber contained water prior to the additions indicated. Cycloheximide concentration was 5 μg/ml in B and 10 μg/ml in C and D; IAA concentration was 10 μg/ml in B and 3 μg/ml in A, C, and D.

heximide and then transferred to cycloheximide plus IAA. The inhibitory action of cycloheximide on elongation was accompanied by no lengthening of the latent period of auxin action; to the contrary, the latent periods found for the cycloheximide-treated segments were in all cases less than those of the controls.

In the experiment shown in Fig. 8, cycloheximide was added before, at the same time as, or after the addition of IAA. Inhibition began within 15 min after addition of cycloheximide and became complete within 20 min. Similar experiments demonstrated that cycloheximide, unlike actinomycin, is capable of inhibiting within 15 min even when given after the establishment of auxin-promoted elongation. Also in contrast to actinomycin, inhibition by cycloheximide was completely reversible within 30 min after removal of the segments from the antibiotic.

Inhibition by puromycin had no effect on the latent period, after a pretreatment that reduced by 64% the elongation response of oat coleoptile segments to auxin. These data are given in Table I.

DISCUSSION

Since the latent period for auxin action on elongation is independent of the externally supplied concentration of IAA over a wide range, whereas the rate of IAA absorption is strongly concentration-dependent (7, 26, 49), the latent period appears not to be mainly time required for uptake of IAA by the cells. However, this does not exclude the possibility that a concentration-independent transport of auxin within the cells might contribute to the timing of the response, nor is the conclusion valid if the auxin response were to depend upon the concentration ratio between different cell or tissue compartments rather than simply upon concentration at a site of auxin action.

Since cyanide and reduced temperature extend the latent period, metabolic processes seem to be involved. The latent period (minus any time required for auxin uptake) may be regarded as the time that is required for that process or property which is directly affected by auxin, to give rise to an effect on the cell wall and thus on the rate of cell enlargment via whatever metabolic and transport processes couple the latter to the former (the primary action of auxin).

The possibility has been suggested (45, 65) that IAA may be physiologically active only after biochemical conversion to a different compound; in this event the time required for such conversion would also contribute to the latent period.

In order to better examine the significance of the observations regarding the latent period, we first consider the kind of timing that is to be expected for changes in output of a generalized reaction sequence

$$q_o \xrightarrow{k_o} q_1 \xrightarrow{k_1} q_2 \xrightarrow{k_2} \cdots \xrightarrow{k_{n-1}} q_n \xrightarrow{k_n} \text{output} \qquad (1)$$

in response to a step-up change at the start of the sequence.

In this model it is assumed that the reaction steps are irreversible and first order, the differential equation for concentration of each intermediate $[q_i]$ being

$$\frac{d[q_i]}{dt} = k_{(i-1)}[q_{(i-1)}] - k_i[q_i]. \qquad (2)$$

$[q_o]$ is assumed to be constant; the step-up change is initiated by an increase in $[q_o]$ or k_o (or both). The n steps include whatever kinetically significant transport processes are involved in the operation of the reaction chain. A particular q_i may be either a substrate *or* a catalyst (enzyme, coenzyme, template, etc.) for the succeeding step, but

in the latter case k_i in equation (2) is the rate coefficient for inactivation of q_i whereas the rate at the $(i + 1)$th step [which produces $q_{(i+1)}$] is to be written $K_i[q_i]$ where K_i is the specific catalytic activity (turnover number) of q_i at the prevailing concentrations of substrates and other cofactors of the $(i + 1)$th step.

As an illustrative case, all the k_i's (and K_i's) are now assumed to be equal, so that n represents the number of "kinetically comparable" steps. This assumption is justifiable because steps whose k_i's are much larger than the rest will not contribute significantly to the timing of the response; the assumption of k_i's all equal greatly simplifies equation (3). The assumption of irreversibility is likewise made in the interest of mathematical simplicity, and is justifiable because reversible steps can exert a significant controlling effect on the timing only if they lie relatively far from

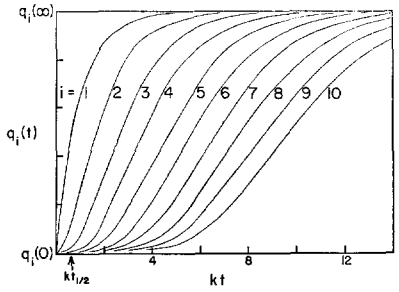

FIGURE 9. Time courses of concentration change predicted by equation (3) for intermediates in a linear pathway as represented in equation (1), following a step-up change at the beginning of the pathway, under simplifying assumptions explained in the text. $q_i(0)$ is initial concentration, $q_i(\infty)$ is final steady-state concentration, and the number i given by the curves indicates the number of kinetically comparable steps preceding the compound in question.

equilibrium (cf. references 27, 28). The assumption of first-order dependence of the reactions upon their substrates (equation [2]), also adopted for mathematical convenience, is not critical to the use that we shall make of the results. We are interested mainly in the general form of the response as a function of the number of steps, not in the detailed shape of the individual curves for concentration change.

The set of equations (2) for the q_i's can then be integrated to give the time course for increase in q_n following the step-up change:

$$[q_n](t) = [q_o]\left(1 - e^{-kt}\left[1 + kt + \frac{k^2t^2}{2!} + \frac{k^3t^3}{3!} + \cdots + \frac{k^{n-1}t^{n-1}}{(n-1)!}\right]\right) \quad (3)$$

These time courses for different numbers of steps n are drawn in Fig. 9.

The curves in Fig. 9 show that an appreciable absolute latent period (time interval between the step-up and the beginning of increase in output) is to be expected if four or more kinetically comparable steps are involved in the chain of response.

Another source of absolute latency in the response will be the time required to assemble from their monomers complete chains of polymers such as RNA or protein, if the formation of these is involved in the response. These different sources of latency should have additive effects.

In Fig. 9 one can also visualize the relationship between the time that is required to attain a steady-state response [i.e., to attain q_n (∞)] and the metabolic "half-life" $t_{\frac{1}{2}}$ of the components q_i as would be measured in turnover or decay experiments. This half-life, which is $(\ln 2)/k_i$, is equal (if all k_i's are equal) to the time for the first intermediate q_1 to attain half of its steady-state concentration change in the step-up response, and is indicated as $t_{\frac{1}{2}}$ in Fig. 9. It is clear that the time required to attain a steady state is at least five times $t_{\frac{1}{2}}$ even for q_1, and is still several times longer if the sequence involves several steps. More generally, it can be concluded that to attain a steady-state response requires at least five times the half-life of the component q_i that has the *longest* half-life (cf. reference 19).

The Gene Activation Hypothesis If auxin causes cell enlargment by inducing the synthesis of certain species of RNA which code(s) for protein that promotes the cell enlargement process, it follows that within the latent period is subsumed the time required for the induced synthesis of this informational RNA and its homologous protein(s), as well as for other obligatory steps such as the transport of mRNA (messenger RNA) from DNA template to ribosomes, transport of the growth-specific protein(s) or of metabolites derived from the action thereof to the cell wall, and the interaction of these with cell wall polymers in some manner.

On the principle that the epigenetic system should have a relaxation time orders of magnitude greater than that of the metabolic system (19), we expect that, under the gene activation hypothesis of auxin action, the latent period will be determined mainly by the timing of induced RNA and protein synthesis.

Induction and derepression of the synthesis of various enzymes in *E. coli* and other bacteria involve latent periods of 2–10 min. In certain cases this latency is known to be determined mainly by the time required to complete the transcription of the relevant cistrons (2, 3, 29, 36, 67); it is lengthened by conditions that slow down RNA synthesis. Assembly of a messenger RNA molecule probably requires at least as long a time in higher organisms.

In animal systems the time required for assembly of complete protein chains has been estimated as 1.5–4 min in particular instances (14, 39). Goodwin (19) concluded that a typical time for assembly of a complete protein molecule by cells of higher organisms would be 5 min. It is probable that in a plant cell, as in bacteria, the time required for mRNA assembly is even longer than that for assembly of the homologous polypeptide.

These values for the latency of completion of transcription and translation thus fall in the same range as the absolute latent period of about 10 min that is seen in the elongation response to IAA. If the latter were in fact determined by the former, we would expect that actinomycin and cycloheximide, which slow down the rate of transcription (53, and personal communication from E. Reich, 1968) and translation (37, 38), respectively, would extend the latent period of the growth response. However, these agents, at concentrations giving partial inhibition of the final growth response, did not extend the latent period significantly. This implies either that IAA does not act by gene activation or that the transcription and translation responsible for any growth-specific protein that is induced by IAA are completed in a small fraction of 10 min. This at least weakens the gene activation hypothesis because it conflicts with available indications of the time required for transcription and translation in higher organisms.

We then want to know how the gene activation hypothesis would account for the fact that at the conclusion of the latent period of about 10 min the elongation rate of oat coleoptiles rises within 2–3 min to a maximum and does not increase further. Two alternative explanations can be considered: During the latent period, (*a*) steady-state levels of the growth-specific mRNA and protein(s) are attained (*steady-state hypothesis*) or (*b*) the level of growth-specific mRNA and/or protein rises, as a result of its auxin-induced synthesis, to the point that some step in the chain of processes connecting transcription with cell enlargement becomes rate-limiting (*saturation hypothesis*).

Steady-State Hypothesis If a steady state in respect to mRNA or protein is attainable because its rate of degradation or inactivation increases with its concentration while its rate of formation does not, as expressed in equation (2), then the time required to attain the steady state will be determined only by its metabolic half-life (i.e. by k_i in equation 2) and not by its rate of synthesis (56, 57). Thus we would expect that the latent period would not be extended by antibiotic treatments giving partial inhibiton of RNA or protein synthesis and of the growth response; this conforms with the observations presented.

Attainment of such a steady state requires, as previously mentioned, at least five times the half-life of the component having the longest half-life. Therefore, by the steady-state hypothesis the mRNA and specific protein that are induced by IAA must both have half-lives of not greater than about 3 min.

While the half-life of certain kinds of mRNA in bacteria has been estimated as about 2 min (e.g., reference 36), such a short half-life is in serious conflict with available evidence about the stability of mRNA in higher organisms, which indicates half-lives of hours to days (8, 12, 16, 20, 22, 25, 30, 32, 44, 53, 58, 59). In the present case, treatment with actinomycin D *after* IAA did not cause inhibition of elongation within the following hour, although the

inhibitory effect of actinomycin D on RNA synthesis becomes fully established within 0.5 hr. Similar results with other auxin-sensitive growing tissues have been interpreted as indicating that the growth-specific mRNA has a lifetime of hours (10, 47).

Likewise the requirement for a half-life of the order of 3 min for the growth-specific protein supposedly induced by IAA is incompatible with the extensive evidence regarding protein turnover in higher organisms, which indicates half-lives of hours to days (1, 18, 23, 24, 54, 55).

Saturation Hypothesis If the IAA-induced mRNA and protein were stable enough to build up, by the end of the latent period, to the point at which some step beyond transcription and translation became saturated and rate-limiting, then a steady growth rate would ensue, but not a true steady state (except at steps beyond the "saturated" step), because IAA-induced mRNA and protein would continue to accumulate. If synthesis of RNA or protein were partially inhibited, the growth-saturating levels would still be attainable, but merely in a longer period of time, provided the inhibitions were not too severe; thus the latent period would be extended. It follows a fortiori that the latent period should be extended if RNA or protein synthesis were inhibited strongly enough so that the final growth response would be reduced; i.e., when the rate-saturating level of mRNA or protein cannot be attained. This is clearly inconsistent with the observed effects of antibiotics on the latent period.

One might suggest that the length of the latent period is actually determined mainly by the dynamics of steps that follow transcription and translation, the saturation of the later steps by these earlier ones taking place in such a small fraction of the normal latent period that the extension of the latent period by inhibitors is not detectable. But this seems unsatisfactory since it demands a complete response of the epigenetic system in a time that is short compared even with what appears to be required to complete the assembly of single RNA and protein molecules.

The observations conflict, therefore, with the gene activation hypothesis of auxin action, and we conclude that this hypothesis is probably not a correct explanation of the promotion of cell elongation in coleoptile tissue by auxin. As a number of authors have pointed out, the inhibition of a hormonal response by inhibitors of RNA and protein synthesis may equally well be ascribed to a requirement for the availability of particular gene products either in order for the hormone to act, or in the mechanism of the hormone-sensitive physiological process. In order to maintain, against the kinetic evidence, the gene activation hypothesis for the growth response of coleoptiles to auxin, it would be essential first to demonstrate a substantial effect of IAA on RNA synthesis in this material *within the latent period* of 10 min, and to show that this promoted synthesis involves RNA that has a half-life of only a few minutes.

Translational Control Auxin could act at the translational level to

promote synthesis of growth-specific protein(s), as has been proposed regarding certain animal hormones (17, 33, 60). If we suppose that the induced proteins are effective upon elongation by virtue of catalytic (enzymatic) activity, the alternatives of steady-state and saturation hypotheses again have to be considered. As discussed previously, the saturation hypothesis must be rejected, from the fact that puromycin and cycloheximide did not extend the latent period, and the steady-state hypothesis seems to be virtually ruled out because it requires that the half-life of growth-active protein(s) be of the order of 3 min.

However, the observation that cycloheximide, when added after IAA, causes a strong inhibition of elongation in 15 min might be interpreted as indicating a short half-life for a protein essential to the cell enlargment process, as has been concluded in an analogous instance involving an animal hormone effect (17). But there is an alternative explanation that does *not* conflict with plausible conceptions about protein stability, namely, that cycloheximide inhibits rapidly because elongation depends upon *continual synthesis* of new protein (rather than upon catalytic activity of some unstable protein previously synthesized). This type of hypothesis has been suggested to explain the rapid effect of cycloheximide in certain other systems (31, 40, 46). It may then be proposed that auxin promotes the rate of formation of this protein.

By this hypothesis the steady rate of elongation, reached after auxin application, requires not the attainment of a new steady-state level of growth-active protein, but merely the attainment of a steady rate of formation of such protein. Since stability of the protein is irrelevant to the timing of the response, this obviates the contingency that defeats the steady-state hypothesis in its previously discussed form. That translational level promotion of protein synthesis by auxin could take place within 10 min is made plausible by such phenomena as the induction of protein synthesis by fertilization in sea urchin eggs (15) and the induction of nitrate reductase formation by nitrate in *Aspergillus* (11), both of which have latent periods of about 10 min.

The protein synthesis envisaged under the continual formation hypothesis cannot represent simple accumulation of protoplasmic protein, as might naturally be assumed in a bacterial or animal growth system, because such accumulation cannot cause expansion of the cell wall which is critical to the plant cell enlargment phenomenon being measured, and in addition it is known that total protein does not increase during auxin-induced elongation of coleoptile segments (4). The growth-active protein must be assumed to be a minor component of total protein. Moreover, it is inherent in the continual formation hypothesis, and essential to its superiority over the steady-state hypothesis, that the growth-active protein function only at the time of its formation; i.e., not possess catalytic activity. This implies a structural protein, and excludes a wide range of suggested mechanisms of auxin action that envisage induction of enzymes that degrade or otherwise modify the chemical structure and mechanical properties of the cell wall.

One possibly structural protein that comes immediately to mind under the continual formation hypothesis is cell wall protein (35). Promotion by auxin of incorporation of precursors into hydroxyproline-rich cytoplasmic and cell wall protein has been reported (9, 34). This and other results have suggested that this protein may be involved in the process of cell wall expansion; however, the available evidence indicates that the immediate synthesis of this protein is not required for cell enlargment in coleoptiles (9).

Another class of structural protein that may be considered is membrane protein. Formation and secretion of cell wall polysaccharides may be involved in the mechanism of cell wall expansion (51). Polysaccharide synthesis and transport are believed to occur via the Golgi system (48). Formation of Golgi vesicles or analogous transport structures might well require continued synthesis of structural components of membranes and thus might account for a dependence of cell enlargment upon continued protein synthesis.

However, lest we uncritically accept as a fact the hypothesis that continual protein synthesis is required in the mechanism of cell enlargement, it would be well to bear in mind the possiblility that the cell possesses secondary regulatory mechanisms that brake the process of cell enlargment if and when the cell is unable to synthesize protein. Since such a feedback loop would probably be advantageous it might well have been selected for; if it existed, experimental results with inhibitors of protein synthesis could lead us erroneously to think that protein synthesis participates directly in the mechanism of cell wall expansion.

If elongation does depend upon continual synthesis of structural protein this does *not* necessarily mean that auxin promotes by activating protein synthesis. The latter hypothesis should be seriously considered only if it can be shown that auxins promote synthesis of certain proteins maximally within 10 min; published evidence is a long way from meeting this requirement (e.g., references 62, 63). The observation that cycloheximide shortened the latent period for the auxin response of coleoptiles suggests the contrary possibility that auxin action actually competes with protein synthesis; for example, for energy or for metabolites.

From the comparative point of view it is striking that a wide variety of hormonal effects on enzyme or protein synthesis that are known in animal and plant systems exhibit latent periods of about 1–2 hr, with even longer periods required to attain a steady rate of increase or a steady state (23, 33, and references there cited). The elongation response of coleoptiles to auxin, attaining completion as it does in little over 10 min, would thus be quite exceptional if it were in fact a hormonal activation of protein synthesis (whether at the transcriptional or translational level). This is even less probable for the promotion by auxin of protoplasmic streaming in coleoptile cells, which can occur in less than a minute (61). While there is no proof that the protoplasmic streaming effect leads to the effect on elongation, the idea that influence on a

transport process may be the way in which auxin affects elongation is suggested by the fact that various nonspecific stimuli can cause an elongation effect similar to that of auxin; for example, the tactile effect noted above under Methods, and the effect of cyanide (50) or CO_2 (21).

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