STEADY STATE GROWTH OF AVENA COLEOPTILE SECTIONS IN HIGH AUXIN CONCENTRATIONS[1]

N. P. KEFFORD & JAMES BONNER[2]

DIVISION OF PLANT INDUSTRY, C.S.I.R.O., CANBERRA, AUSTRALIA

The excised Avena coleoptile section constitutes a convenient system for studying the enlargement of plant cells in response to applied auxin. A portion of the convenience lies in the fact that it has proven possible to arrange conditions such that auxin-induced extension of the section takes place at a rate which is constant with time over periods of several hours. Under these steady state conditions, the powerful methods of chemical kinetics may be applied to the analysis of the interaction of auxin with tissue (4).

The conditions required for steady state extension of Avena coleoptile sections in solutions of suboptimal auxin concentration (less than ca. 10^{-5} M indole-3-acetic acid) have been achieved by Foster, McRae, and Bonner (6), Bennet-Clark and Kefford (2), Bonner and Foster (3), and others. Less general agreement has however been achieved concerning the nature of the response of sections to supraoptimal (ca. 10^{-5} M & higher) concentrations of auxin. Concentrations of auxin up to 10^{-3} M have been reported by Foster et al (6) and by Bonner and Foster (3) to elicit section growth rates which are constant with time but less than that elicited by the optimal concentration. Other investigators including Bennet-Clark (1), Bennet-Clark and Kefford (2), Hancock (7), Housley et al (8) and Marinos (9) find that high auxin concentrations elicit an initial rapid extension, followed by a progressive and continuing decline in extension rate. The present work has been done to try to find any differences in technique which might cause this difference in extension behavior and to discover how to establish reproducible steady state extension rates for coleoptile sections grown in supraoptimal auxin concentrations.

MATERIAL & METHODS

Avena sativa L. variety Siegeshafer from Svalöf was used throughout. The seedlings were grown in one of three different ways.

I. In one type of experiment an attempt was made to reproduce as exactly as possible the conditions used by Foster et al (6). Seeds were soaked for 1 hour in distilled water and then planted in moistened (with distilled water) vermiculite obtained from Pasadena and of North American (Montana) origin.

II. In the second type of experiment, dehusked seeds were soaked for 1 hour in distilled water and then planted on filter paper over distilled water.

III. In the third type of experiment, seeds were soaked for 1 hour in distilled water and then planted in moistened (with distilled water) vermiculite obtained in Canberra and of South African origin. This vermiculite had been either thoroughly washed with water or with a complete Hoagland's nutrient solution.

In all cases germination and growth of the seedlings were allowed to take place in a standard Avena room in red light at 25° C. After 70 hours, coleoptiles 2.5 to 3.0 cm in length were selected and one section 6 mm long was cut 3 mm from the tip of each.

The sections as cut were collected either into distilled water or into a basal nutrient solution consisting of 3 % sucrose and potassium maleate buffer, 2.5×10^{-3} M, pH 4.5. After a minimum period of 30 minutes in the collecting medium, the initial length of each section was measured using a microscope equipped with an ocular micrometer. The sections were then transferred in lots of 20 to the several growth media of the experiment. These media all contained 3 % sucrose, potassium maleate buffer 2.5×10^{-3} M, pH 4.5 and indole-3-acetic acid (IAA) in the concentrations indicated below. Stock solutions of IAA were made up by dissolving in hot water or dilute potassium hydroxide.

Incubation of the sections took place at 25° C in red light. Lengths of the sections were measured at intervals of two to three hours as described above.

Since the amount of North American vermiculite available was small, only one experiment could be done with plants grown according to method I. This was considered sufficient because, as will be shown below, the results were the same as those obtained many times under identical conditions in Pasadena by Bonner and Foster (3, 4). Experiments were done with plants grown according to methods II and III on five occasions for each method.

[1] Received November 14, 1960.

[2] Present address: Division of Biology, California Institute of Technology, Pasadena. The visit during which the present work was done, was supported by the Commonwealth Bank of Australia.

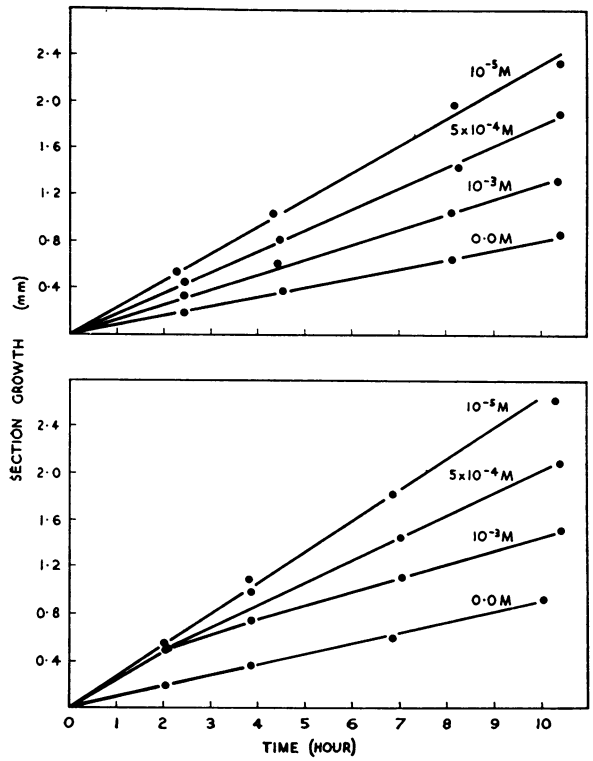

FIG. 1 (*upper*). Progress curves for elongation of coleoptile sections in varied concentrations of indole-3-acetic acid (IAA). Sections from seedlings grown in North American vermiculite moistened with distilled water.

FIG. 2 (*lower*). Progress curves for elongation of coleoptile sections in varied concentrations of IAA. Sections from seedlings grown on filter paper over distilled water.

RESULTS

The data of figure 1 concern an experiment with coleoptile sections produced by method I above and representing, as exactly as possible, a duplication of the material used by Foster et al (6). It is clear that the growth rates of such sections are constant for periods of at least 10 hours. This is true both for sections in media containing IAA in approximately optimal concentration (10^{-5} M) and for sections in media containing supraoptimal IAA concentrations, such as 10^{-3} M. Thus the results of Foster et al (6) and of Bonner and Foster (3) are confirmed.

The data of figure 2 concern an experiment conducted with coleoptile sections cut from seedlings germinated and grown on filter paper over distilled water (method II above). The progress curves of figure 2 show that while the rate of elongation of sections in near-optimal auxin concentration (10^{-5} M) approximates constancy with time from zero time on, the rates of elongation of sections in higher auxin concentrations assume steady and characteristic values only after an initial period of ca. two to four hours. During this initial period, the growth rate of sections in solution containing 10^{-3} M IAA approaches that of sections in near-optimal auxin concentrations (see also 5).

We have at present no explanation to offer for the differences in initial growth rate between these sections and those from seedlings grown according to method I. If one is concerned only with steady state growth rates, it is convenient to regard the initial 2 hour growth period as a pre-incubation during which the sections are immersed in the medium and allowed to equilibrate with it. The progress curves of figure 2 show that the growth rates, of coleoptile sections from seedlings germinated in distilled water, are constant with time after the initial 2 hour period and show further that the absolute growth rates elicited are essentially identical to those obtained with sections grown in the same auxin concentrations but cut from seedlings grown by method I.

The growth of sections obtained from plants grown by method III, namely in South African vermiculite equilibrated with distilled water or Hoagland's nutrient solution, in general resembles qualitatively that of sections from plants grown over distilled water. Their extension rates, however, were lower and times required to achieve steady state extension rates longer than for sections from seedlings grown by either of the other two methods.

It has been shown by Foster et al (4), for their experiments, that the steady state growth rate of coleoptile sections becomes inversely proportional to auxin concentration at high auxin concentrations (10^{-5} to 10^{-3} M). This relationship is that predicted on the basis of the concept of two point interaction between auxin and auxin-binding entities of the coleoptile. That the same relationship is found for experiments of the type illustrated in figure 2 is indicated in figure 3.

DISCUSSION

It is now generally agreed (1, 4) that among the more important provisions needed to ensure steady

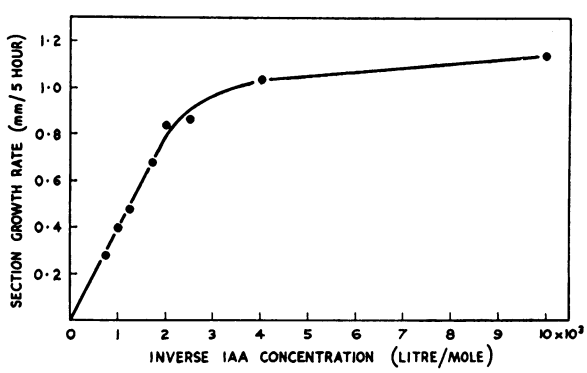

FIG. 3. Steady-state elongation rates of coleoptile sections in concentrations of IAA greater than 10^{-5} M as a function of reciprocal IAA concentration. Sections from seedlings grown on filter paper over distilled water.

state growth of Avena coleoptile sections in optimal or suboptimal auxin concentrations are (4):

I. An external supply of absorbable solutes, such as sucrose, so that the tissue may maintain its internal osmotic concentration.

II. Constancy of external pH, auxin concentration, and other factors.

III. Equilibration of sections in water or growth medium lacking auxin prior to addition of auxin to minimize osmotically induced transient volume changes.

Under these conditions steady state extension is established soon after the sections are immersed in auxin-containing solution. Equilibration of sections with solutions containing low concentrations of auxin is therefore rapid.

The considerations noted above may be expected to apply equally to the maintenance of constant growth rates of sections immersed in solutions of supraoptimal auxin concentration. In fact it has been shown that for sections from seedlings grown in North American vermiculite, steady state extension rates are established quickly in supraoptimal auxin concentrations. Sections from seedlings grown by the other two methods however, behave as if equilibration of the sections with auxin may take longer with high auxin concentrations than with low. The same conclusion may be drawn from the work of Cleland and Bennet-Clark (5).

The present experiments have been concerned with auxin concentrations up to 1×10^{-3} M. Still higher auxin concentrations, as for example 2.5×10^{-3} M or higher, have been found to cause section shrinkage and ultimate loss of turgor, which is observable after 4 to 8 hours of incubation and suggests irreversible damage to the section. Cleland and Bennet-Clark (5) have found that small amounts of alcohol in the test solution lead to the ultimate shrinkage of sections. Some instances of shrinkage reported in the literature may thus be explained by the use of alcohol to bring IAA into solution.

Summary

Progress curves for Avena coleoptile sections incubated in supraoptimal (10^{-5} M & higher) concentrations of the auxin indole-3-acetic acid have been found to be characterized in general by a long period of steady state growth rates preceded in some instances by an initial transient period of more rapid growth. In the present experiments the conditions of growth of the Avena seedlings determined whether coleoptile section steady state growth rates were achieved immediately or after a transition period. In order to establish steady state growth of sections in solutions of supraoptimal auxin concentration it may therefore be necessary to preincubate sections for approximately two hours in basal medium containing auxin in the desired final concentration. When this provision is observed, it is possible to obtain reproducible, steady state growth of Avena coleoptile sections in solutions of high auxin concentration. The steady state growth rates are inversely proportional to auxin concentration as expected on the basis of the two point interaction concept.

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