Gradient of Growth, Spontaneous Changes in Growth Rate and Response to Auxin of Excised Hypocotyl Segments of *Phaseolus aureus*1

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**ABSTRACT**

Spontaneous growth was studied in excised mung bean (*Phaseolus aureus* Roxb.) hypocotyl segments. Measurements were made with a growth-recording apparatus using displacement transducers on single 5- to 6-millimeter samples excised from the growth zone immediately below the hook.

Even for a given zone and under controlled experimental conditions, there are differences in the spontaneous growth of individual explants. Nevertheless, in every case, two phases of endogenous acceleration are found at 15 to 20 minutes, and 120 to 150 minutes after excision. Accelerations were separated by steady growth phases. Knowledge of the spontaneous growth curve appears important for the choice of the time of application of experimental stimuli. Auxin was added at various times after excision (0 to 6 hours). The classical biphasic response to auxin was obtained when the hormone was added during a steady phase of growth. However, the response was difficult to interpret when the hormone was added during an acceleration phase.

Spontaneous and indoleacetic acid-induced growth were studied along the hypocotyl. Spontaneous growth rate and growth potential revealed by indoleacetic acid changed markedly along the growth gradient. The nature of spontaneous changes according to experimental time and state of differentiation of the cells is discussed.

The physical and chemical factors which control the elongation of plant cells have been analyzed in great detail (4, 5, 16, 20). Numerous studies of cell growth have been made, but there are conflicting results between studies concerned with short term effects (7, 9, and references therein), and those looking only at long term over-all growth. It is only since 1974 that the precise characteristics of endogenous growth have begun to be understood (6, 10, 17).

Growth of an excised segment is not stable with time. This instability has been attributed to a readjustment to a new, lower and stable rate of growth and has led various workers (8, 23, 34) to preincubate tissue for 30 to 90 min after excision before any experimental treatment. These phenomena are, however, more complex. Cline *et al.* (6) have shown that phases of spontaneous acceleration can occur during the growth of coleoptile segments. The nature of the spontaneous changes in the growth rate has been analyzed by Evans (10, 12, 13). More recently, MacDowell and Siros (19) stressed the need to take these spontaneous changes into account when interpreting responses to exogenous stimuli. It is, accordingly, necessary to adapt the experimental conditions to the particular biological material.

We have used mung bean (*Phaseolus aureus* Roxb.) hypocotyls, for which numerous data concerning changes in wall polysaccharides (3, 14, 29), in osmotic pressure and vacuolation (27), in glucanase activities (15 and references therein), and in polysaccharide metabolism and biochemistry (30, 36) during growth have been previously accumulated. Precise studies of responses to auxin have been performed using the closely related soybean hypocotyl (33-35). It is necessary to carry our experiments to define both the time course of endogenous growth (short and long term experiments) and differences in growth characteristics between cells at various stages of elongation.

Recent progress in the precise measurement of plant growth is largely attributable to the development of growth-measuring apparatus using optimal amplification (microscopic [23, 28], shadowgraphic [11], photometric device [18], laser method [21]) or displacement transducers (1; see 24 and references therein).

The potential for cell growth can now be studied as a function both of time and of degree of differentiation. In actively growing organs, important differences in the rate of growth are often observed, according to the particular region studied. The results presented here are of two types: in our experimental plan we wanted to define exactly the characteristics of endogenous growth so as to be able to choose the best determined experimental conditions. We then proceeded to a study of the development of the potentialities for endogenous and auxin-induced growth. The existence of a well defined gradient of growth may permit a clearer understanding of growth regulation.

**MATERIALS AND METHODS**

Seedlings of mung bean (*P. aureus* Roxb.) were grown on moist Vermiculite at 26 C in the dark for 3 days. The hypocotyls were 40 ± 10 mm in length. Segments were excised from different levels of the straight part of the plumular hook. In experiments to measure long term over-all growth, 20 segments of 20-mm length were marked at 2-mm intervals with India ink and incubated with 20 ml of medium in Petri dishes for 24 hr in the dark with constant agitation. The incubation medium was 2 mM Na/K phosphate buffer (pH 6.5) plus or minus auxin (IAA, 10 μM). For long term growth experiments, 1% sucrose was added to the medium. The growth of individual 5- to 6-mm segments in incubation chambers with a continuous flow of test medium was measured using Philips displacement transducers. Growth curves were registered for four segments simultaneously with a 2-min interval between each measured point and the next. A detailed description of the apparatus has been previously given (26). The rate of growth was calculated directly from the chart recordings in μm/2 min with an accuracy of ± 0.2 μm.

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RESULTS

Spontaneous Variation of Growth in Zone of Elongation. Comparable segments taken from the zone of elongation, just below the hook, show a considerable variation of rate and total amount of growth (Figs. 1 and 2). In spite of these individual variations, all segments show alternate phases of rapid and slow growth. An initial acceleration of growth occurs 15 to 20 min after excision (arrow 1). The rate then decreases and stabilizes for 30 to 60 min between 0.4 and 1 μm/2 min. A second acceleration (arrow 2) occurs 100 to 150 min after excision. The rate then rises to 2 to 3 μm/2 min, then decreases and stabilizes at a low level (0.7 ± 0.4 μm/2 min). The duration of this stable phase is variable, and a third acceleration (arrow 3) can occur 4 to 7 hr after excision, but is often of low amplitude, and does not occur in all segments.

Effect of IAA Removal. When segments are kept in IAA during the time of excision, the growth rate is very high and fluctuates considerably (from 3 to 8 μm/2 min). When IAA is removed after 1 hr (Fig. 3a) or 2 hr (Fig. 3b), a considerable acceleration of growth occurs after 10 to 20 min (arrow 1). This oscillation could be only retarded effect of auxin action. The rate then progressively falls, stabilizes between 0.4 and 1 μm/2 min, then a second acceleration (arrow 2) occurs every time about 2 hr after IAA removal.

Fig. 1. Simultaneous recording of the elongation of four different segments S1, S2, S3, and S4 (length = 6 mm) excised from immediately below the hooks of similar seedlings. Datum points are at 2-min intervals. Note the acceleration of growth about 2 hr in each case. Length in μm; time in hr.

Fig. 2. Rate of elongation calculated in μm/2 min from curves S1, S2, S3, and S4 of Figure 1. Spontaneous variations of rate are clearly demonstrated (arrows 1, 2, and 3). First and second phases of endogenous acceleration appear reproducibly at 15 to 20 min and 90 to 150 min, respectively, after excision. Third phase is not observed. When IAA is not added during the steady-state of growth (i.e., before 3 hr; Fig. 4, a, and b), the response is less characteristic and seems to interfere with the spontaneous accelerations of growth.

Fig. 3. Effect of IAA removal on spontaneous growth. (■): time of exposure to IAA which was added at the time of excision and removed after 1 hr (a) or 2 hr (b). Note the two spontaneous accelerations of growth (arrows 1 and 2) 15 min and 130 min after IAA removal, in both cases. Rate of elongation in μm/2 min; time in hr.

Fig. 4. Interactions between endogenous growth and exogenous stimulation by IAA. (●): time of exposure to IAA, which was added after different periods of spontaneous growth of the segment, and left until the end of the experiment. In each case, the usual endogenous accelerations are seen, before the addition of IAA (a and b, arrow 1; c and d, arrows 1 and 2). Biphasic response to auxin was observed (c and d) with a lag time of 12 min. Response was complex when the hormone was added at the first 2 hr (a and b). A third endogenous acceleration (arrow 3') was present in several cases (a, b, and c) during incubation with IAA. Rate of elongation in μm/2 min; time in hr.

Variation in Response to IAA According to Time of Application. IAA was added to similar segments at different stages of spontaneous growth. When IAA was added after the 3rd hr after excision (after the second spontaneous acceleration), the different parameters of the biphasic response defined by Vanderhoof (34) could be recognized (Fig. 4, c and d). The lag phase of the first transitory phase is from 12 to 14 min. The rate of growth decreases from 1 ± 0.5 μm/2 min to 4 to 5 μm/2 min. It is multiplied by 4 to 10 according to the sample. The maximum rate is attained after 30 min. The second phase, weaker in general, reaches its maximum rate between 100 and 120 min after hormone application. Depending upon the relative intensity of the two phases, the two peaks can be well separated (Fig. 4c) or partially overlap (Fig. 4d). When IAA is not added during a steady-state of growth (i.e., before 3 hr; Fig. 4, a, and b), the response is less characteristic and seems to interfere with the spontaneous accelerations of growth. A spontaneous acceleration often exists after the response to auxin (arrow 3', Fig. 4, a, b, and c) and has characteristics analogous to the endogenous acceleration (arrow 3, Fig. 2), observed in the absence of auxin. Only the stimulations effected after the 3rd hr in the stable phase give reproducible responses, and even then, there may be an interference by unpredictable spontaneous acceler-
SPONTANEOUS CHANGES IN GROWTH RATE

Endogenous Gradient of Growth and Auxin Response. After excision, the gradient of growth is conserved (Fig. 9). Successive segments were removed from along the zone of growth, starting at the hook, and their spontaneous growth was followed over 6 hr (Figs. 5 and 6). Strong endogenous accelerations near the hook diminish toward the bottom of the hypocotyl. The basal rate stabilizes after 4 hr to 1 μm/2 min under the hook. It becomes progressively lower toward the hypocotyl base and is almost zero after the 12th mm.

Auxin was added to the same specimens after 8 hr growth (Figs. 7 and 8). In the short term, auxin stimulates all of the regions with an amplitude which is approximately inversely proportional to the distance from the hook. The maximum rate in the first phase of growth ranges from 5 μm/2 min for the first segment to 0.5 μm/2 min at 15 mm from the hook. A second phase exists in all cases. Its maximum rate decreases along the endogenous growth gradient. It should be noted that although the absolute stimulation observed in the segment situated 12 to 18 mm from the hook is low, it is very significant when compared to the negligible basal rate in this region.

Studies on the short term growth were compared with long term over-all growth. Segments 20 mm in length were removed from the zone of growth, marked at 2-mm intervals, and then measured after 24 hr. The growth profile along the hypocotyl is completely changed by treatment with IAA (Fig. 9). The growth of the regions proximal to the hook, which have the highest endogenous growth rate, was inhibited by 10 μM IAA, while the basal regions (from the 5th mm down) were stimulated by the same concentration. To resolve this apparent contradiction, experiments, segments removed from different levels were placed in the measuring apparatus and the spontaneous or the auxin-induced growth was followed over a long duration (Fig. 10). Under these conditions, the region proximal (A, Fig. 10) to the

[Diagram of growth curves and measurements]

FIG. 5. Simultaneous recording of the growth of three segments A, B, and C (length = 6 mm) removed successively from along the zone of growth of the same hypocotyl. Length in μm; time in hr.

FIG. 6. Rate of elongation calculated in μm/2 min from curves A, B, and C of Figure 5. A spontaneous acceleration (arrow) is strong for A, still visible in B, but poorly resolved in C. Rate stabilizes after 4 hr at 1 μm/2 min for A, 0.4 μm/2 min for B, and below 0.2 μm/2 min for C.

FIG. 7. Response to auxin of the same three segments A, B, and C removed from the zone of growth of the hypocotyl (Figs. 5 and 6). Auxin was added after 8 hr growth in buffer.

FIG. 8. Growth rate calculated from curves of Figure 7. Lag time is similar for all three segments (12 min). Amplitude of first and second phases decreases from the top to the bottom of the hypocotyl.

FIG. 9. Long term over-all spontaneous growth (●) auxin-induced growth (○). A segment containing the whole of the growing zone was marked at 2-mm intervals with India ink and incubated at 27 °C in the buffer with 1% sucrose ± 10 μM IAA. The intervals were then measured after 24 hr. Each point is the mean of 20 replicates. Ordinate indicates percentage of length increase (ΔL%); abscissa indicates distance below the hook in mm.

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hook had a very high endogenous growth rate which continued throughout the experiment. Auxin stimulated growth for several hr. Then the growth rate fell to below that in the absence of auxin. After 12 hr, the growth curves (± IAA) had converged: there was no significant difference in the total growth between auxin-treated and control samples. In contrast, in the region situated just below (B, Fig. 10), spontaneous growth rate was slow. IAA action was more long lasting, which is manifested as a large increase in total growth after 12 hr.

**DISCUSSION**

**Endogenous Accelerations of Growth.** The use of a precise measuring apparatus allows a detailed analysis of the capacity for elongation of plant cells to be made. Interpretation of responses to endogenous stimuli requires a prior knowledge of endogenous growth characteristics. The essential problem is to know when the growth of a specimen is stable so as to be able to choose the most favorable time for experimentation. The usual 30- to 90-min preincubation now seems insufficient. In *Avena* coleoptiles, spontaneous accelerations of growth can arise at the moment of an experimental stimulus and be a potential source of error (6, 12, 13, 19 and references therein).

In *P. aureus* hypocotyls, the same distinct phases of endogenous growth acceleration are found as have been described for coleoptile segments. The first phase consistently occurs 15 to 20 min after excision. The second phase is routinely found (in the present study) 120 ± 30 min after excision, but has a variable amplitude.

In *Avena* coleoptile, Cline et al. (6) point out that a reaction related to the experimental conditions (tactile effect) cannot be excluded. The growth rate of excised pea stem sections can be influenced by a mechanical stress (22) and the excision itself could have an effect. For Evans et al. (12) the "spontaneous growth response" of *Avena* and corn coleoptile segments could be induced by an auxin derepression caused by excision. In the mung bean hypocotyl segments, there is a certain similarity between the response induced by exogenous auxin removal and response induced by excision. These data support the hypothesis of Evans (12). Nevertheless, significant variations in the rate of growth (arrow 3; Fig. 4) occur even in the presence of IAA.

**Time of IAA Application.** IAA stimulates elongation with a precise lag phase which varies according to the experimental material. In the present study (for *P. aureus*) it is 12 to 14 min, very close to that found by Vanderhoef (34) in soybean. This response to IAA is biphasic (23, 25). Mean values obtained from several experiments correspond well with the parameters defined by Vanderhoef (lag time, maximum of the two phases, minimum). Nevertheless, for specimens taken individually, the relative importance of the two phases can vary by sizable amounts.

According to the time of application, the response can interfere with endogenous phenomena and the growth curves obtained are then irregular. This reiterates the possible difference between endogenous acceleration of growth and direct response to auxin. A precise knowledge of the growth curve of a specimen, of its endogenous fluctuations, and of the amplitude of individual variations is an indispensable experimental prerequisite. A preincubation of 1 hr 30 min is insufficient, as specimens are then susceptible to important endogenous accelerations in the minutes following stimulation. To avoid these interferences, samples are kept in the measuring apparatus from the time of excision, and a stimulation is only applied when the second phase of acceleration has finished and the growth rate decreased and stabilized—i.e. 3 to 4 hr after excision. Even then, a third phase of spontaneous acceleration may occur unforeseeably and affect the experimental reaction.

These data are in accord with other results which show that with time, coleoptile segments present variations of IAA permeability (19) or IAA sensitivity (13).

**Growth Gradient.** The existence of a gradient of growth rate in the hypocotyl introduces another variable. Experiments using long specimens (> 10 mm) give mean values, but in so doing, averages are obtained for neighboring levels having different growth characteristics. The short term and long term responses to IAA can appear contradictory. The magnitude and the duration of the response were different according to levels. In the biphasic response to auxin, the first phase is always transitory and the second phase can have a variable duration. For regions situated just below the hook, the spontaneous growth was important. The magnitude of the response to IAA was strong but the duration limited. The growth rate quickly decreased to the initial growth rate. The over-all growth in 24 hr was weakly stimulated. For regions situated below, the spontaneous growth was almost zero. IAA gives a response of weak magnitude but long duration. The growth over-all in 24 hr was strongly stimulated.

Decrease of the concentration of auxin present or of the tissue sensitivity to auxin could explain these results. Cessation of growth could occur in two steps: first, a cessation of endogenous stimulation during which cells retain the potentiality for growth, and second, a disappearance of this potentiality. The cells then no longer react to either endogenous or exogenous stimuli. This total cessation of growth could be associated with ultrastructural changes, especially of the cell wall which is directly implicated in cell enlargement (2, 4, 20, 31, 32).

It will be interesting to compare the physiological changes along the growth gradient with biochemical and fine structure changes of the cell wall. The material can yield valuable information concerning the conditions which control the initiation, maintenance, and termination of cell growth.

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**LITERATURE CITED**

3. BAILEY RW, H KAISER 1974 Extraction of hydroxyproline-containing proteins and pectic substances from cell walls of growing and non growing mung bean hypocotyl segments. Planta 119: 223-245
SPONTANEOUS CHANGES IN GROWTH RATE

31. Roland JC, B Vian, D Reis 1977 Further observations on cell wall morphogenesis and polysaccharide arrangement during plant growth. Protoplasma 91: 125-141