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On the Nature of the Physiological Responses of *Avena* Stem Segments to Gibberellic Acid Treatment

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ABSTRACT

Gibberellic acid was found to cause elongation in *Avena sativa* (oat) stem segments whether it was applied continuously or as a short pulse. The shorter the pulse time became, the higher was the gibberellic acid concentration needed to cause elongation; the segmental growth apparently depends upon the amount of gibberellic acid taken up by the segments. *Avena* segments showed a decreased growth response to gibberellic acid if the treatments were initiated at increasingly later times after excision from the plant. This decreased responsiveness to gibberellic acid was inhibited by low temperature (0–4 C), but accelerated by anaerobiosis. On the other hand, growth stimulation by a gibberellic acid pulse at the start of incubation was not altered by cold treatment but was nullified by a nitrogen atmosphere. Both the readiness of the segments for growth stimulation by gibberellic acid and its action in promoting growth clearly involve temperature-dependent, aerobic metabolism.

Segments were able to use glucose, fructose, sucrose, and glycerol but not malate, citrate, pyruvate, glycine, or glutamine as substrate for growth. Since final elongation without exogenous substrate was highly correlated with initial content of total carbohydrate and reducing sugar, carbohydrates seem to be the major endogenous growth substrates.

The *Avena* segments are composed of three distinct morphological units: node, internode, and encircling leaf sheath. Although the node and leaf sheath do not grow, they must be present for maximal growth of the internode. Quantitative assessment of the roles of each part suggests that a substance other than gibberellin or sugar is necessary for maximal internodal growth and that this substance may be channeled from the leaf sheath to the internode through the anastomosing vascular tissue of the node.

During the past few years, our laboratory has been investigating the effects of GA_3 on the physiology of internodal elongation in excised oat stem segments. The growth response of the internode is extremely sensitive and specific to the exogenous application of gibberellin, whereas auxin, kinetin (6, 12), and ethylene (Kaufman, unpublished data) inhibit GA_3-induced growth. An application of gibberellin alone can stimulate growth to about two times the initial segment length (8). The growth response is further amplified to 3 to 10 times the initial length when 0.1 M glucose is added to the growth medium (1), but glucose alone affects the elongation very little or not at all. In addition to growth stimulation, gibberellin can increase invertase activity in these stem segments; this appears, however, not to be the direct cause of GA_3-induced growth (10).

Thus far, most studies have been carried out with a continuous supply of gibberellin in the medium. If gibberellin is needed simply to trigger a series of metabolic events leading to the elongation of the internode, application in short pulses should allow maximal growth. Indeed, with gibberellin pulses for the relatively short time of 1 hr, it has been shown that growth can be maximally induced when the pulse is given immediately after the preparation of the segments, while the final growth attained decreases with the time of incubation at which gibberellin pulses are given (11). However, the nature of this decrease in growth potential during incubation has not been fully explored.

The *Avena* stem segment consists of three morphologically distinct units: node, internode, and encircling leaf sheath (10). The internode contains the basally located intercalary meristem, and gibberellin has been shown to act on the cells within this meristem and on the elongating cells which are its immediate derivatives (9). The leaf sheath does not grow in response to gibberellin; it is customarily left intact because of experimental difficulty in excising it from each segment and because of experimental convenience in that it provides a structural support for the elongating internode portion (10). The removal of the node plus sheath from the segment decreases elongation to only about two times the initial length in a sugar plus gibberellin medium, as compared to four times for the intact segments (1, 2). The node and sheath seem to play important roles in internodal growth, but the exact nature of each of their roles still remains unexplained.

The present investigation concerns further efforts to clarify the mode of action of GA_3 in the regulation of segment growth. In particular, it attempts to answer the following three questions: (*a*) what is the nature of the decrease in growth potential which occurs with time after excision of the segments; (*b*) what is the primary growth substrate(s) in the segments; and (*c*) what roles do each of the three morphologically distinct parts of the segment play in gibberellin-induced growth?

MATERIALS AND METHODS

About 150 oat (*Avena sativa* cv. "Victory") plants were grown in a flat (17 × 11 × 3 inches) in the greenhouse for 40 to 45 days as in previous studies (10). Shoots of uniform height containing the internodes immediately below the peduncular node (p-1 internodes) with a length of 1 to 2 cm were carefully selected. One-cm segments including p-1 internodes and basally localized nodes were prepared from the shoots with a razor blade cutting device and placed upright in perforated Plexiglas frames on filter paper in 6-cm Petri dishes. Each dish

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contained 3 to 11 segments with their node ends in contact with 2 ml of treatment solution.

Experiments involving a nitrogen atmosphere were carried out using air-tight glass baby food jars. A 6-mm hole was made in the center of each lid, and a piece of metal tubing was soldered in the hole; this allowed the jar to be connected to a vacuum pump (Cenco-Hyvac 2) and a prepurified nitrogen tank (Union Carbide Corp., Linde Division) with a 3-way pressure gauge. Each jar contained 3 to 11 segments and 2 ml of test solution as cited above. Nitrogen was introduced into the jars after evacuation and filling with nitrogen twice. When segments were incubated in nitrogen, they were always transferred to fresh medium for growth.

Segments were routinely allowed to grow at 28 C in the dark to avoid the effects of photosynthesis (1), and all manipulations were carried out under a dim green light (475–550 nm) with a maximal transmission at 510 nm and an energy of 200 ergs cm^{-2} sec^{-1}. This light was constructed by covering a fluorescent lamp with one sheet each of green and amber Plexiglas G (Cadillac Plastic Co., Detroit). GA_3 was administered as a short pulse, either by allowing the segments to lie on their sides in the hormone solution or by placing them in a large test tube containing the hormone solution and shaking for a specified time period. After exposure to GA_3, the segments were quickly rinsed with 500 to 600 ml of distilled water. The lengths of the segments were measured with a mm ruler. Experiments were repeated at least twice. Results were analyzed statistically by methods described in Simpson et al. (16).

Carbohydrates were extracted from lyophilized segments by grinding with a mortar and pestle in cold 3% (v/v) perchloric acid for 10 min. The perchlorate was precipitated by the addition of sufficient 1 M K_2CO_3 to raise the pH to 2 to 3. Total carbohydrate was determined using the anthrone method of Roe (15) and reducing sugar by the method of Nelson (13).

Gibberellic acid was generously supplied by Mr. Douglas Broadbent of Imperial Chemical Industries, Ltd., England and "Victory" oat seed was purchased from the Swedish Seed Association, Svälof, Sweden.

RESULTS

Pulse Treatments with GA_3. In order to examine effective concentrations of GA_3 for subsequent experiments, segments were treated either continuously or given 5- or 20-min pulses with various concentrations of GA_3 immediately after preparation. Figure 1 summarizes the results of a typical series of experiments. The results of several similar sets of experiments indicated that there were certain batch-to-batch variations and some heterogeneity in the segments, as exemplified by the maximal growth attained and by the size of the standard errors. Nevertheless, it is clear from the results that (*a*) the general shapes of the three curves resemble one another, although concentration ranges which induce growth are shifted towards higher concentrations with decreasingly shorter GA_3 pulses, and (*b*) the GA_3 concentrations needed for half-maximal stimulations are about 0.2, 10, and 30 μM for continuous GA_3, 20-min pulse, and 5-min pulse treatments, respectively. These results strongly suggest that the elongation response depends upon the amount of GA_3 taken up by the segment. Since a 5-min pulse of 300 μM GA_3 is saturating, subsequent GA_3 pulse experiments were carried out with 300 μM GA_3.

Avena stem segments show a decreased responsiveness to GA_3 if the pulse treatments are given at increasingly longer times after excision of the segments (11). Figure 2A shows the growth curves for segments which were transferred to continuous 300 μM GA_3 plus 0.1 M glucose from 0.1 M glucose

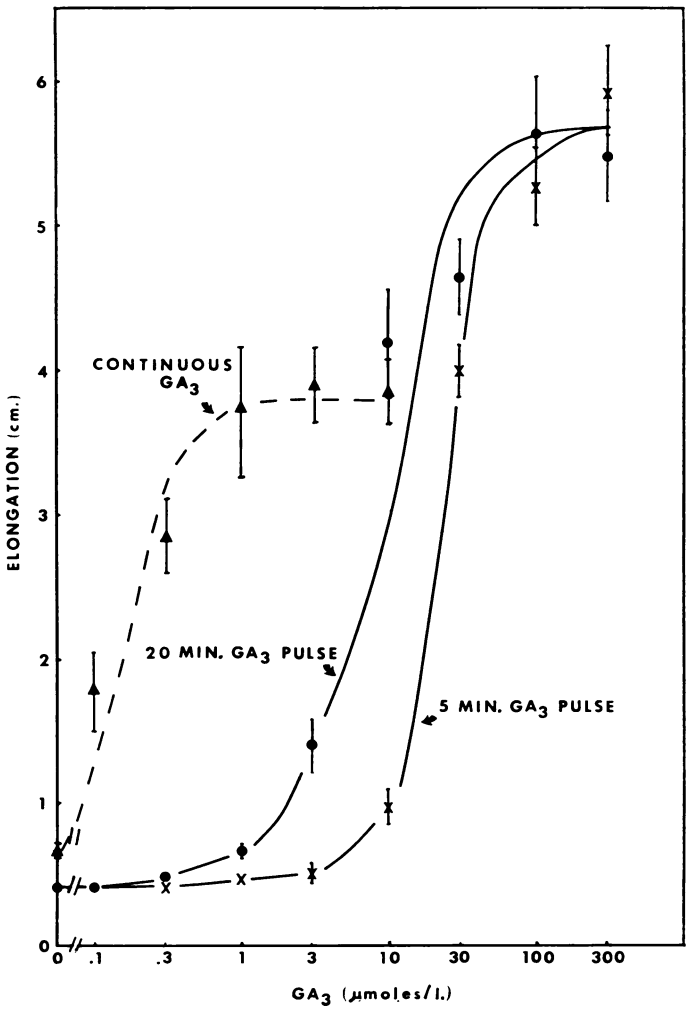

FIG. 1. Dose-response curves for continuous and pulse application of GA_3. The means of 3 to 11 replicates are plotted with standard errors. The same batch of segments was used for the 5- and 20-min pulses, but a different batch was used for continuous GA_3 application. In all cases, 0.1 M glucose was supplied.

alone after 0, 12, and 24 hr of dark incubation. Figure 2C shows the growth curves of segments which were pulsed with 300 μM GA_3 for 10 min after the same lengths of incubation time in 0.1 M glucose. The two sets of growth curves are remarkably similar: (*a*) in both cases there is about a 6-hr lag period before the rate of growth becomes appreciable; (*b*) growth kinetics are practically identical; and (*c*) regardless of the time of exposure to GA_3, segments ceased to elongate after about 60 hr. In Figure 2, B and D, the final elongations attained as shown in Figure 2, A and C, respectively, are plotted as a function of the time after excision at which segments were exposed to the hormone. In both cases, it is apparent that the final level of elongation decreases linearly with the time of dark incubation, and the slopes (approximately −0.11 cm/hr) of the two linear decay curves are statistically indistinguishable using the *t* test for the difference between two regression coefficients (16). The dashed line in Figure 2D is the result obtained from a separate experiment in the absence of glucose: here again, the decay is linear, but with a slope of approximately −0.022 cm/hr. These results clearly indicate that GA_3 pulse treatments should be made immediately after the excision of the segments in order to ensure maximal growth response. Furthermore, since both the continuous and the pulse appli-

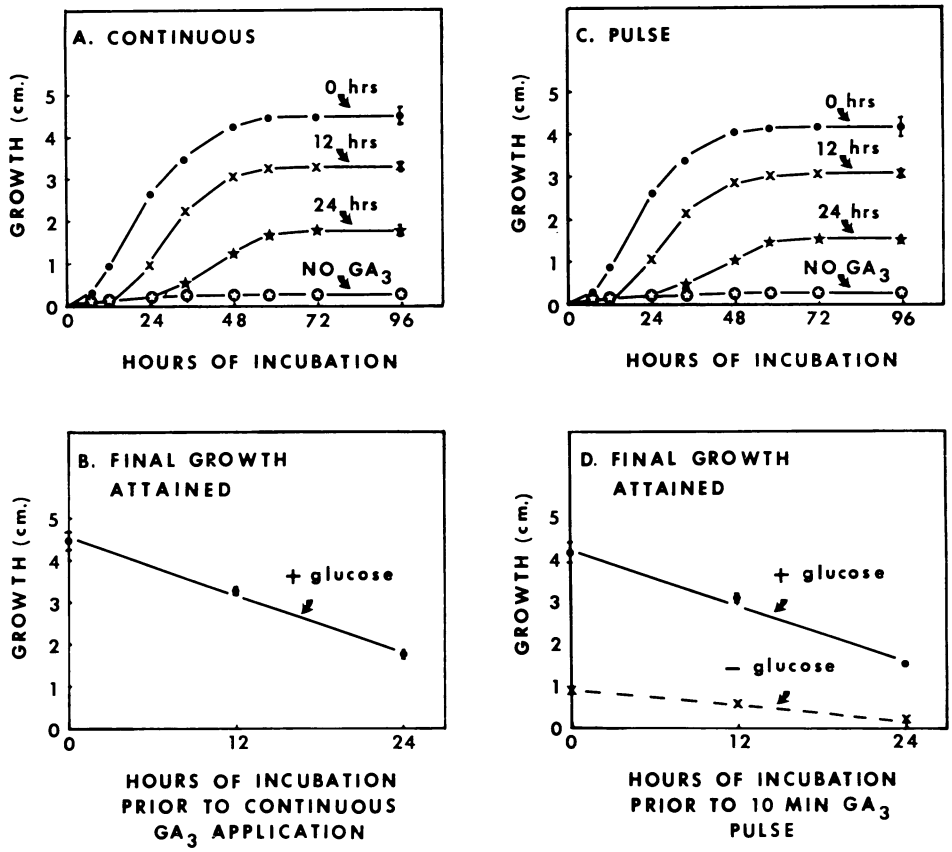

FIG. 2. Effects of delayed GA_3 application on time course patterns of growth (A and C) and on the final growth attained (B and D). Left figures (A and B) show the results of continuous application of 300 μM GA_3 starting at 0, 12, and 24 hr after the beginning of dark incubation at 28 C; right figures (C and D) show the results of 10-min pulse application of 300 μM GA_3 after the same dark incubation as in the left figures. Except for those grown without glucose (dotted line in D), segments were maintained on 0.1 M glucose both before and after exposure to GA_3. Means of 6 to 10 replicates are plotted with standard errors presented for the final growth attained.

cations of hormone resulted in the same amount of decrease in responsiveness, the loss of response cannot be explained by a decrease in the capacity of the segments to take up the hormone.

In order to determine what physiological processes are involved in the linear loss of responsiveness to GA_3, segments were first incubated in the cold (0–4 C) for 24 hr with or without an anaerobic atmosphere, then pulsed with 300 μM GA_3 for 10 min, and lastly allowed to grow in air until growth ceased. Table I shows the results of such experiments, carried out in the presence of 0.1 M glucose throughout the treatments. As expected, an incubation at 28 C for 24 hr (treatment 3) resulted in a considerable decrease in the growth response with respect to GA_3 treatment given at zero time (treatment 2). With incubation at 0–4 C for 24 hr (treatment 4), however, internodal elongation was virtually identical to that obtained by pulsing the segments with hormone immediately after excision (treatment 2). While a nitrogen atmosphere at 28 C (treatment 5) greatly accelerated the rate of decline in the responsiveness of the segments to GA_3, cold treatment administered to segments concomitantly with an anaerobic atmosphere (treatment 6) overcame the effects of anaerobiosis. In a parallel series of experiments with a continuous supply of 10 μM GA_3, the results were practically identical to those shown in Table I. These findings indicate that the loss of responsiveness to GA_3 involves processes dependent upon temperature and accelerated by anaerobiosis. It may be of interest to note that segments which had been left on glucose in the cold (0–4 C) for as long as 5 days retained their responsiveness to GA_3.

Table I. *Effect of Preincubation in the Cold or in a Nitrogen Atmosphere or both on GA_3-induced Growth of Avena sativa Stem Segments*

GA_3 (300 μM) was administered as a 10-min pulse in air at 25 C. Segments were allowed to grow at 28 C in air after GA_3 treatment and maintained on 0.1 M glucose both before and after pulsing. Net growth is given as the mean of six replicates.

Treatment	Net Growth (± SE)
	cm
1. Control (no GA_3)	0.35 ± 0.03 (a)*
2. GA_3 pulse at 0 hr	4.67 ± 0.24 (b)
3. 28 C in air for 24 hr → GA_3 pulse	2.18 ± 0.21 (c)
4. 0–4 C in air for 24 hr → GA_3 pulse	4.60 ± 0.32 (b)
5. 28 C in N_2 for 24 hr → GA_3 pulse	0.05 ± 0.05 (d)
6. 0–4 in N_2 for 24 hr → GA_3 pulse	4.86 ± 0.36 (b)

* Means followed by the same lower case letter in parentheses are not significantly different at the 5% level.

To clarify the effects of cold or anaerobiosis or both further, segments were pulsed for 10 min with 300 μM GA_3 immediately after excision. They were then placed in 0.1 M glucose and immediately treated with cold or nitrogen atmosphere or both for 24 hr before being transferred to the air at 28 C for growth. The results (Table II) are similar to those in Table I. Cold overcame most of the deleterious nitrogen effect in these experiments; the small inhibitory effect caused by nitrogen in the cold may be ascribed to technical difficulties, such as failure to

achieve immediate lowering of the temperature of the segments after exposure to GA_3. These results further show that GA_3 action on growth involves a temperature-dependent, aerobic type of metabolism.

Endogenous Substrate(s) for Growth. The question of the endogenous substrate(s) used by the segments during growth has been only indirectly approached by examining the effects of supplying exogenous substances; sucrose, fructose, and glucose were shown to serve equally well as substrates for growth when GA_3 was supplied (1). In search of other substances which may support GA_3-induced growth, we have investigated the effects of various concentrations of citrate, malate, pyruvate, glycine, glutamine, and glycerol on the growth of GA_3-treated segments; of these substances, only glycerol promoted growth (2.5-fold at 0.1 M) over the GA_3 control growth. It appears, therefore, that among the compounds tested, carbohydrates constitute a major source of exogenous substrate for growth in these segments.

In order to correlate the endogenous carbohydrates and GA_3-induced growth, a more direct approach was employed. Segments were excised from the plants at different times of the day and were either (*a*) frozen immediately in liquid nitrogen, followed later by extraction and analysis for carbohydrate or (*b*) grown on continuously supplied 10 μM GA_3 in the dark without an exogenous supply of glucose. The results (Fig. 3) clearly show that there are parallel diurnal changes in (*a*) GA_3-induced growth, (*b*) the amount of anthrone-positive carbohydrate, and (*c*) the amount of reducing sugar present in the segments at the time of removal from the plant. All three parameters decreased from 8:30 PM on through the night, reaching a minimum at 8 AM (when the light intensity in the greenhouse began to increase appreciably), and rose to a maximum at mid-afternoon, followed by a decline toward evening. Anthrone-positive material presumably represents all available carbohydrate soluble in 3% perchloric acid. Fructose and glucose are by far the most plentiful reducing sugars in *Avena* segments, each constituting aprpoximately one-half of the total reducing sugar pool (see also ref. 1). Our data indicate that the maximal amount of growth obtained by the segments is dependent, at least in part, upon the endogenous level of carbohydrates, which presumably reflects the relative photosynthetic activity of the plant. These considerations strongly suggest that carbohydrates are the major endogenous growth substrates in this system.

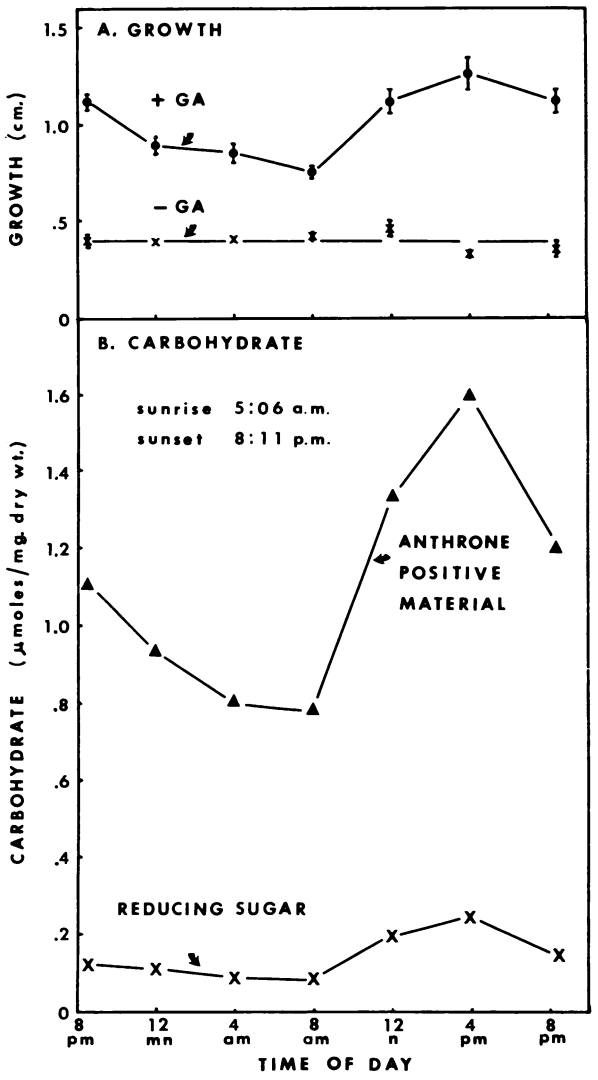

FIG. 3. Growth (A) and carbohydrate content (B) of segments excised from greenhouse-grown plants at various times of the day. GA_3 (10 μM) was supplied continuously. Segments were collected on 10 and 11 July, 1971. Those used for carbohydrate analysis were frozen in liquid nitrogen immediately after excision and lyophilized. Content of anthrone-positive material and reducing sugar are expressed using glucose as a standard. Growth is plotted as the mean of 4 to 5 replicates with standard errors.

Table II. *Effect of Cold Temperature and a Nitrogen Atmosphere after a Pulse of GA_3 on the Growth of Avena sativa Stem Segments*

A 10-min pulse of 300 μM GA_3 was administered in air at 25 C followed by incubation of segments on 0.1 M glucose in the cold or in a nitrogen atmosphere or both for 24 hr. The segments were then transferred to 0.1 M glucose and allowed to grow at 28 C in air. Net growth is given as the mean of five to six replicates.

Treatment	Net Growth (±SE)	
	Experiment 1	Experiment 2
	cm	
1. Control without GA_3	0.47 ± 0.04 (a)*	0.47 ± 0.02 (a)
2. Control with GA_3 pulse	4.92 ± 0.26 (b)	4.82 ± 0.17 (b)
3. GA_3 pulse → 0–4 C in air for 24 hr	4.07 ± 0.62 (bd)	4.32 ± 0.21 (bd)
4. GA_3 pulse → 28 C in N_2 for 24 hr	0.00 ± 0.00 (c)	0.00 ± 0.00 (c)
5. GA_3 pulse → 0–4 C in N_2 for 24 hr	3.22 ± 0.49 (d)	3.80 ± 0.34 (d)

* Within each experiment, means followed by the same lower case letter in parentheses are not significantly different at the 5% level.

Roles of Node and Sheath in Internodal Elongation. Figure 4A is a schematic diagram of a longitudinal section of an *Avena* stem segment as used in our experiments. The anatomical parts are labeled in this figure[2] according to presently accepted nomenclature (4). The basal stippled region, which contains a plexus of vascular elements (4), is the area most strongly stained red by phloroglucinol, although certain parts of the joint and sheath are also stained positively. This is due to the

[2] The term "node" has been used somewhat loosely in our earlier publications (1, 11) to include both the "node" and the "joint" in Figure 4A.

presence of highly lignified tracheidal elements in vascular bundles at these loci. The joint or leaf sheath base includes geotropic receptor cells in its periphery (P. B. Kaufman, unpublished results). The intercalary meristem resides in the basal part of the internode (cross-hatched region), slightly above the node. While almost all of the internodal cells can grow in these segments, there is a gradient of growth along the internode with its basal region (basal 3–4 mm) showing greatest cell elongation in the presence (9) or absence (1) of exogenous GA_3.

Ng and Audus (14) found that gibberellin promotion of *Avena* seedling first internode growth was enhanced by the presence of the node located between the internode and the coleoptile. Similarly, in our system, while the node and the leaf sheath do not grow appreciably as compared to the internode when exposed to gibberellin, they must be present in order for maximum internodal elongation to occur (1, 2). The ways in which the node or the sheath contribute separately to internodal elongation have not been clearly explained. In order to determine whether the node and sheath are required for maximal elongation throughout the period of GA_3-induced growth, GA_3 was supplied either continuously (experiment 1) or as a 5-min pulse (experiment 2). For both experimental series, GA_3 was administered immediately after excision of the segments from the plants, and the nodes and joints were removed from the segments with a razor blade at various times during the course of dark incubation, thus severing their vascular connection with the internode. The internodes (plus unconnected leaf sheaths) were then returned to the original medium for the total growth period of 96 hr. Figure 5 shows the results where two different batches of segments were used. The shapes of the two curves are practically identical. Excision immediately after GA_3 treatment causes essentially the same amount of growth as GA_3 treatment of the internode itself (Table III). These results indicate that the node and shealth must be connected to the internode throughout the time course of growth for maximal elongation to occur. Their removal at any time, even when GA_3 is supplied continuously, results in reduced elongation.

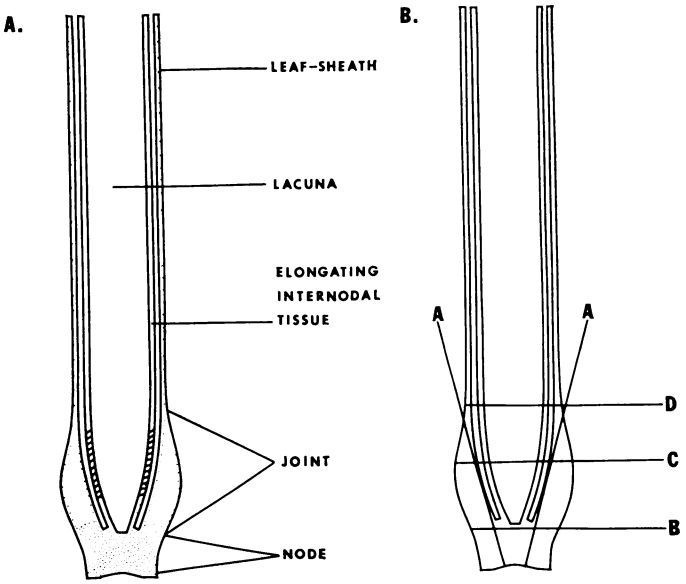

FIG. 4. A: Schematic diagram of a longitudinal section of an *Avena* stem segment. The stippled region indicates the area with large amounts of vascular tissue and the cross-hatching indicates the approximate position of the intercalary meristem. B: Diagram indicating the surgical procedures used for removal of the node and sheath as presented in Table III.

In order to attempt to distinguish the roles of node, joint, and leaf sheath, portions of the node or the joint or both were removed from the segment with or without the leaf sheath (Fig. 4B and Table III). When the leaf sheaths were removed from the segments (last two columns of Table III), they were peeled from the internode and broken off just above the joint; the node, joint, and internode remained intact. Treated and control segments were grown on a continuous supply of 10 μM GA_3 and 0.1 M glucose in the dark. The results are shown in Table III where both the net growth and percentage of maximal growth are indicated. In addition to the expected growth of control segments in the presence of GA_3, the data indicate that (*a*) the removal of the leaf sheath alone reduced internodal elongation to 57% of the maximal growth (line 2, last column), (*b*) partial removal of both node and sheath resulted in 41 to 44% of the maximal value (line 3), (*c*) the removal of the entire node further reduced the growth to 31 to 38% (line 4), and (*d*) the removal of the entire node plus one-half (line 5) or the total (line 6) joint provided essentially the same results as occurred with removal of the entire node. It should be pointed out that the internode alone (line 6) is capable of a fairly marked growth response to GA_3 (approximately five times greater than the glucose control). These results clearly indicate that the sheath and the node play significant roles in the attainment of maximal elongation by the internode. Since the node is the site of

Table III. *Roles of the Node, Joint, and Leaf Sheath in Internodal Elongation in Avena sativa*

Segments were grown on 0.1 M glucose and continuous 10 μM GA_3 was supplied except to the controls. Net growth is given as the mean of four to six replicates with standard errors.

Treatment to Node and Joint	Leaf Sheath Present		Leaf Sheath Absent	
	Net growth	Maximal growth*	Net growth	Maximal growth
	cm	%	*cm*	%
Control without GA_3 (node and joint intact)	0.33 ± 0.03	6.9	0.34 ± 0.05	7.1
Control with GA_3 (node and joint intact)	4.82 ± 0.23	100.0	2.75 ± 0.17	57.1
Cut at A** (internode intact with surrounding joint tissue)	2.10 ± 0.24	43.6	1.96 ± 0.09	40.7
Cut at B (entire node removed)	1.82 ± 0.17	37.8	1.48 ± 0.09	30.7
Cut at C (entire node plus ½ of the joint removed)	1.60 ± 0.31	33.2	1.62 ± 0.15	33.6
Cut at D (entire node and joint removed)	1.52 ± 0.04	31.5	1.50 ± 0.11	31.1

* The maximal net growth of 4.82 cm in the presence of GA_3 is taken as 100%.

** Excision was made from four sides to remove as much joint tissue as possible without damaging the basal part of the internode. See Fig. 4B for a schematic diagram of the surgical procedures used here.

anastomosing vascular bundles from the leaf sheath and the internodes above and below, it is highly probable that a substance or substances, other than gibberellin or sugar, required for internodal growth, must be channeled from the leaf sheath (including joint) into the internode through the nodal vascular system.

DISCUSSION

The ability of *Avena* segments to respond to a short pulse of GA_3 (Fig. 1) is in decided contrast to the barley aleurone system where gibberellin must be present in constant supply for continued synthesis of α-amylase (3). Once GA_3 is taken up into a segment, it appears to be able to trigger or enhance the metabolic event(s) leading to growth. Clearly, a given amount of gibberellin must be present in the segment for maximal growth; this amounts to at least 300 pmoles/segment (2). The observation that higher concentrations of GA_3 are needed for increasingly shorter pulses (Fig. 1) may suggest that GA_3 uptake by the tissue does not involve an active process. It should be mentioned that the dose-response relationship for continuous supply of GA_3 to upright segments (Fig. 1) is identical to that obtained with segments placed horizontally and also to that in the absence of exogenous sugar (also see ref. 1). An earlier report from this laboratory which indicated that some 100-fold less GA_3 was needed to cause growth (10) is now considered to be a computational error in GA_3 concentrations.

The loss of responsiveness to GA_3 with time (Fig. 2) may be taken as physiological senescence of the segment, which is clearly sensitive to temperature and anaerobiosis (Table I). In order to test whether the senescence process is somehow connected with the leaching of endogenous substances from the tissue, a glucose medium which had been used to incubate a set of segments for 24 hr in air at 28 C was used to support the growth of a new set of segments pulsed with GA_3. The new segments grew to the same length as the control set of segments grown on fresh glucose medium. Further tests were made in the absence of glucose; again, the results indicated that a leaching process is probably not involved in the loss of responsiveness to GA_3. Experiments with anaerobiosis, however, gave a different picture; segments placed under a nitrogen atmosphere before or after a GA_3 pulse never regained their full growth potential unless anaerobiosis was given at 0 to 4 C. Furthermore, the medium which had been used for anaerobic treatment could not support the full growth of new segments; segments grown on this medium attained only approximately 30% of the control growth. The addition of 0.1 M ethanol to fresh medium did not mimic this effect. Under an anaerobic atmosphere at 28 C, therefore, segments undergo an irreversible aging process by secreting substances which are inhibitory to the growth of new segments. This irreversible process is apparently sensitive to temperature (Tables I and II) and is probably different from the physiological aging process under aerobic conditions.

A primary target tissue for GA_3 action in growth metabolism appears to be the internode itself. This is based on the fact that the internode is the only growing tissue in these segments, and also because of the fact that GA_3 is capable of increasing the growth of isolated internodes about five times above that of the nonhormone control (Table III). However, in order for GA_3 to induce a maximal growth response in the internode, the presence of the node, joint, and leaf sheath is required. We consider that the leaf sheath plays an important role, particularly since (*a*) the removal of the leaf sheath results in about a 40% reduction in maximal growth, and (*b*) the removal of the entire node, where vascular bundles from the sheath join with those from the internode, reduces the growth by 65 to 70% (Table III). In somewhat similar fashion, other workers have noted the importance of cotyledons in gibberellin-promoted hypocotyl growth of cucumber (7) and soybean (5). In our system, two alternative interpretations can be proposed to explain the role of the leaf sheath: (*a*) gibberellin may act directly on the leaf sheath, causing the synthesis and transport of some factor(s) which is necessary for maximal internodal elongation, or (*b*) the site of action of gibberellin is in the internode whose high metabolic demands cause a mobilization of the factor(s) from the leaf sheath. The second alternative appears to be the more likely one in view of the observation that the node, joint, and leaf sheath must be present throughout the growth period (Fig. 5). The factor(s) cannot be gibberellin or carbohydrate, since nonhormone growth is equally low regardless of the presence or absence of the leaf sheath, and also, since the decrease in growth is caused, even in the presence of exogenous substrate, by the excision of the leaf sheath or the node. In search of this diffusible factor(s), isolated internodes were allowed to grow on a glucose medium containing GA_3 together with a number of leaf sheaths, split in half longitudinally. The resultant growth was never above the control value. The "sheath factor(s)," then, could be a water-insoluble substance such as a vitamin or even a nongibberellin hormone which must be transported to the internode continuously throughout the growth period (Fig. 5). It is unlikely that the factor(s) is auxin, since the application of several concentrations of IAA failed to promote internodal growth, even in the absence of node and

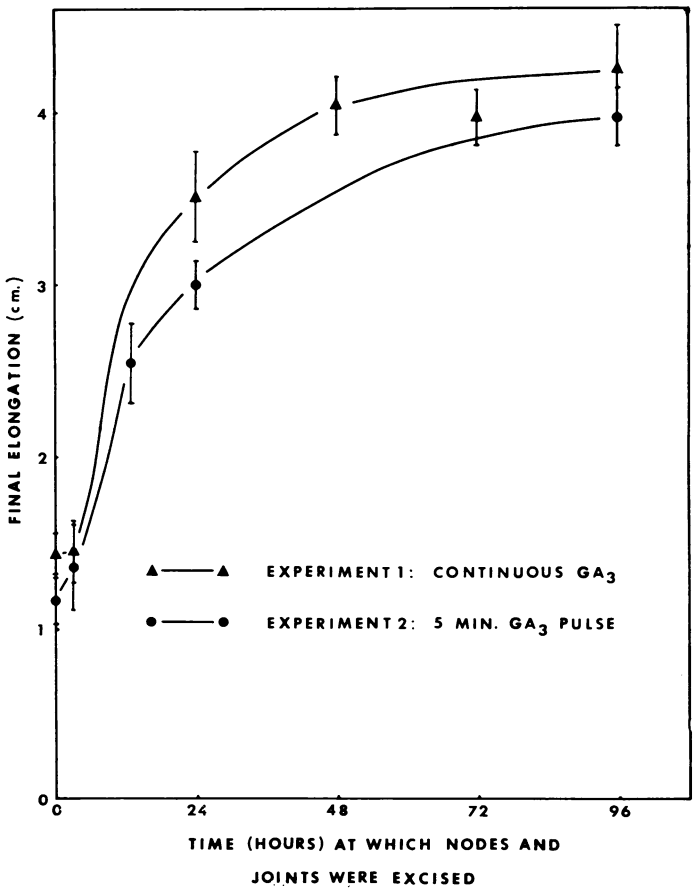

FIG. 5. Effect of excising the nodes and joints from segments at various times during the course of growth. Final elongation after 96 hr is plotted as the mean of 5 to 7 replicates with standard errors. Experiment 1 (continuous 10 μM GA_3) and experiment 2 (5 min 300 μM GA_3 pulse) involved different batches of segments. In both experiments, 0.1 M glucose was supplied.

sheath (see also 12). A decreased transport of the factor(s) may be associated with the physiological aging discussed earlier.

Adams (1, 2) has found that the dry weight of the node plus sheath decreases when segments are grown on gibberellin without exogenous substrate. When viewed in the context of the present results, this strongly suggests that growth substrate, presumably carbohydrate, is transported from the sheath to the internode through the node. Such mobilization may be caused directly by gibberellin, or it may be the result of increased demands for substrate by the growing internode. Exogenous carbohydrate, however, did not overcome the effect of the removal of node or sheath or both in our experiments, which indicates the additional transport of some other "sheath factor(s)," as discussed above. Furthermore, since growth occurs without an increase in internodal osmotic pressure (1), the "sheath factor(s)" is probably not an osmoticum.

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