Growth-Accelerating Substances in Cotton Fibers

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Elution of unprocessed cotton fibers with appropriate solvents produced crude extracts that accelerated growth of immature second internodes of young bean plants. The cotton fibers in chromatographic filter paper were a convenient source of these growth-accelerating substances. The amount of growth-accelerating substances obtained decreased with successive elutions of the fibers. The most effective solvents were 2-propanol and ethanol, when combined with ammonium hydroxide and water. Five growth-accelerating factors were detected in the eluates and three were partially purified by thin-layer chromatography. These growth factors were chromatographically and physiologically unlike indole-3-acetic acid and physiologically unlike six other endogenous compounds known to possess growth-regulating properties. They were chromatographically unlike gibberellic acid A_3, but resembled it on the basis of the stem elongation which they induced.

Hormones of higher plants that have physiological properties similar to those of gibberellic acid were detected in corn pollen and immature seeds of bean (*7*, *8*). The presence of gibberellic acid-like substances in bean seeds was later confirmed by Phinney *et al.* (*10*). More recently these cell-elongating hormones have been obtained from many kinds of higher plants and from various parts of plants, including all parts of both tall and dwarf peas (*2*). However, none of these cell-elongating substances have been obtained from higher plants in sufficient quantities for thorough chemical study and identification or in quantities suitable for studying their physiological effects and usefulness in controlling the growth and behavior of crop plants. Attention was directed to cotton fibers as a possible source of cell-elongating substances because of the rapid and relatively extensive lengthwise growth that occurs in these epidermal hairs.

Many compounds have been detected in mature cotton fibers—for example, the fibers contain on their outer surface a wax made up of numerous alcohols, fatty acids, and other carbon compounds (*4*). Nitrogenous substances have been isolated from the primary walls of the mature fibers (*11*). Also, a relatively large amount of protoplasmic residue, containing many compounds, often occurs in the lumen of mature cotton fibers (*3*). Atkinson and Allen (*1*) obtained a factor (or possibly factors) from cotton wax that was highly effective in stimulating the germination of self-inhibited wheat stem rust uredospores. This factor was also obtained from filter paper (composed of cotton fibers) and from nonabsorbent cotton.

Thus, evidently, a mature, dry cotton fiber contains a variety of residual substances, some of which were originally a part of the living protoplast. The purpose of this research was to learn whether potentially active cell-elongating substances can be obtained from mature cotton fibers.

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Methods

Strips of Whatman No. 1 chromatographic filter paper 30 cm. long and 4 cm. wide (weighing about 1 gram) were used as one source of mature cotton fibers. The paper used in these experiments had been stored in a laboratory for periods that varied from a few days to 4 years.

To extract the papers, the strips were enclosed in chromatographic chambers and then eluted for about 16 hours with a solvent system composed of 2-propanol, ammonium hydroxide, and water (8:1:1, v./v.). Ethanol, ammonium hydroxide, and water (8:1:1, v./v.) were also used to elute the fibers. Approximately 1 ml. of eluate was collected in a shell vial from each strip, evaporated at 50° C. to about 0.2 ml., and spotted across a thin-layer plate coated with activated aluminum oxide G. The plate was developed with the 2-propanol solvent system used for elution. Areas that fluoresced under ultraviolet light were assayed for growth-accelerating activity with the second internode method described below. The chromatographed growth factors were obtained by extracting the powder from the fluorescent areas with the 2-propanol solvent system. The powder was extracted three times and during the third extraction the temperature of the mixture was maintained at 50° C. for 30 minutes prior to stirring, centrifuging, and decanting the extract. Extract obtained from the powder was spotted on a thin-layer plate coated with activated silica gel H for further separation and the plate was developed with the same 2-propanol solvent system. The plates were examined under ultraviolet light. Fluorescent areas were removed and extracted as described, and the extracts were assayed for growth-accelerating activity.

Raw cotton fibers were also used as a source of growth-accelerating substances. These were harvested daily by hand from field-grown cotton plants (Coker-100 variety) as the bolls opened to prevent possible contamination of the fibers with pesticides. The seeds were also removed by hand and the fibers were stored under dry conditions for 4 years. A weighed sample

of the fibers was packed into a glass tube. Fifty milliliters of the 2-propanol solvent system was passed dropwise through the column. The eluate was concentrated at 50° C. and chromatographed on an aluminum oxide plate with the same solvent system. The unpurified extract and also partially purified factors obtained in this way were tested for growth-accelerating properties.

The authors developed an assay technique that involved growth of immature second internodes of intact bean plants. Seeds of the Pinto variety were germinated in a greenhouse. Plants were selected for size and uniformity when the tip of the center leaflet of the first trifoliolate leaf was slightly separated from the two lateral leaflets. At this stage the second internodes were from 1 to 2 mm. long.

Pointed tweezers were used to remove the bract that subtends the dorsal side of the center leaflet in the terminal bud and this left a smooth, well-defined scar. One side of the second internode was thus exposed. The distance from the lower edge of the scar to the base of the bracts on the third node was measured with an ocular micrometer and recorded as the initial length of the internode prior to treatment (Figure 1).

After eluting the fibers in chromatographic paper, and extracting the unprocessed cotton fibers (or extracting the powder from a thin-layer plate containing a chromatographed compound), the eluate or extract was evaporated to dryness (50° C.). One milliliter of ethyl ether containing 250 μg. of fractionated lanolin was added to the residue.

This nonphytotoxic fraction of lanolin, with a melting point more suitable for this assay than that of lanolin itself, was obtained by carefully warming on a steam bath 10 grams of anhydrous lanolin dissolved in acetone (6 parts of acetone by weight to 1 part of lanolin). After the mixture had reached about 50° C., it was stirred carefully until the lanolin was dissolved. The mixture was allowed to cool at room temperature overnight in a beaker covered with foil. The liquid portion of the mixture was recovered by decantation and warmed on a steam bath to evaporate the acetone. The resulting fraction is slightly more fluid than lanolin itself at room temperature. Under the proper temperature, this fraction can be applied to a plant surface as a semisolid, then readily liquefied to obtain excellent contact with the plant surface by exposing the treated area to a lamp for a few seconds. The lanolin fraction induced a slight increase in the rate of elongation of internodes used in the second internode test when 250 μg. was applied per plant. Growth stimulation owing to factors obtained from cotton fibers was, therefore, always calculated on the basis of controls treated with the lanolin fraction.

After the ether containing the fractionated lanolin was added to the residue, the solids were dissolved by stirring the mixture. The mixture was transferred with a coagulation capillary to the surface of a microscope slide. The tube was used as a micropipet and the mixture was delivered dropwise to a small area on the slide directly under a gently flowing stream of air. The ether was quickly evaporated in this way, leaving the compound to be tested thoroughly mixed with the fractionated lanolin.

The fractionated lanolin mixture was removed from the slide quantitatively with a razor blade in a way that left the paste piled on the beveled edge at one end of the blade. With the aid of a dissecting microscope, a narrow glass rod was used to remove the lanolin mixture from the blade and to place it quantitatively on the second internode of a test plant (Figure 2).

Plants treated in this way were placed in light intensity from Slimline fluorescent tubes of 700 foot-candles for 9 hours daily followed by darkness. The plants were grown at 22° to 24° C. during both the light and dark periods.

Four days after the plants were treated, the length of the second internode was measured with an ocular micrometer. Elongation of the internode during this period was calculated. In many experiments, subtraction of the initial length was unnecessary, since the plants were uniform, and relatively large responses were involved.

The sensitivity of the test plants varied from experiment to experiment, because the plants were grown in a greenhouse where the prevailing light intensity and other environmental conditions fluctuated. Therefore, a standard treatment consisting of 0.001 μg. of gibberellic acid A_3 was applied to indicate the sensitivity of the plants used in each experiment.

Response curves were made for quantitatively estimating the growth-accelerating activity of factors obtained from fibers in terms of the effectiveness of

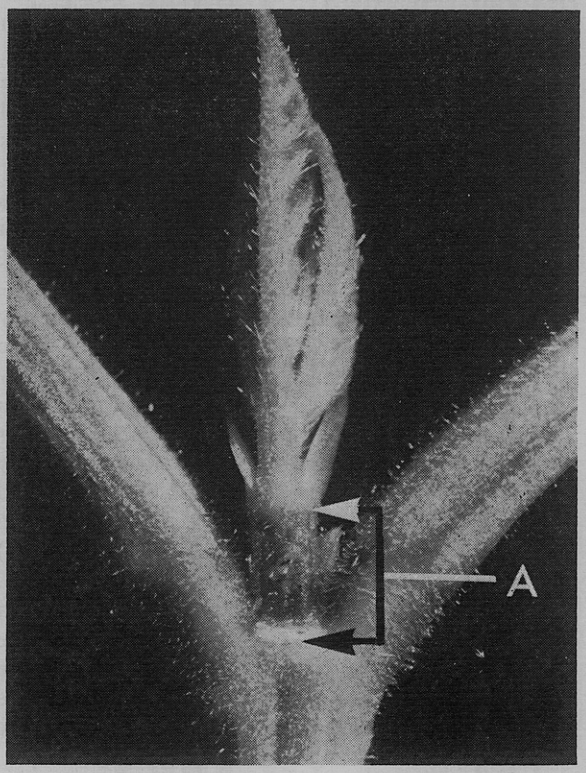

Figure 1. Terminal bud of young bean plant

Shows scar left after removal of bract and distance measured to determine length of second internode, *A*

gibberellic acid A_3. Response of the second internodes was essentially a straight line when a dosage range of 0.001 through 0.0001 μg. of the acid was used. The results were plotted on semilog paper as the average per cent increase in length over that of control internodes treated with an equal amount of the fractionated lanolin carrier.

Results

Growth-Accelerating Factors from Cotton Fibers. Elution of cotton fibers with the 2-propanol–ammonium hydroxide and the ethanol–ammonium hydroxide solvents resulted in crude extracts that accelerated growth when applied to immature bean internodes. When used without the ammonium hydroxide, 2-propanol and ethanol failed to elute the accelerators, as did methanol when used alone and with the ammonium hydroxide. Ammonium hydroxide in water, and several other solvent systems tested, also failed to elute the growth-accelerating substances.

Sufficient accelerating activity was obtained from 1 gram of fibers to increase the growth of one plant significantly. The accelerators were readily obtained from ground or unground filter paper and from unprocessed cotton. On the basis of plant response, growth substances eluted from unprocessed cotton and those obtained from filter paper were alike. Substances from both sources behaved the same chromatographically, and fluoresced the same when exposed to ultraviolet light. The amount of growth-accelerating substances obtained from fibers decreased with successive elutions of the sample (Figure 3).

Thin-layer fractionation of the crude eluates on aluminum oxide resulted in growth-accelerating factors that fluoresced under ultraviolet light with approximate R_f values of 0.3, 0.6, 0.88, and 0.98. Areas at R_f 0.88 and 0.98 possessed the greatest amount of accelerating activity but overlapped. When combined extracts from areas with R_f values of 0.88 and 0.98 were chromatographed on silica, three distinct growth factors were obtained: Factor I, R_f 0.6; Factor II, R_f 0.4; Factor III, R_f 0.9.

All three growth factors accelerated elongation of the bean internodes significantly. There was no significant difference in the magnitude of acceleration induced by Factors I and II. Factor III induced marked acceleration, resulting in a growth rate about 10 times that of the controls, and the effect of Factor III was significantly greater than the acceleration induced by Factors I and II (Table I).

The second internodes responded quantitatively to gibberellic acid applied over a dosage range of 0.001 through 0.0001 μg. under the conditions described. Expressed in terms of gibberellic acid activity, 0.25, 0.28, and 0.31 μg. of Factors I, II, and III, respectively, were obtained from 1 kg. of chromatographic paper.

Stability of Growth Factors Obtained from Cotton Fibers. The growth-accelerating substances obtained from fibers were relatively stable. For example, unchromatographed eluate from 1 gram of the filter paper, eluted after the paper had been stored in a laboratory for 4 years, increased growth of the internodes an average of 273%. Eluates from fibers retained their growth-accelerating properties for more than 14 months when stored in the 2-propanol solvent system at 1° to 4° C. The eluates also retained their activity when the solvents were evaporated and when

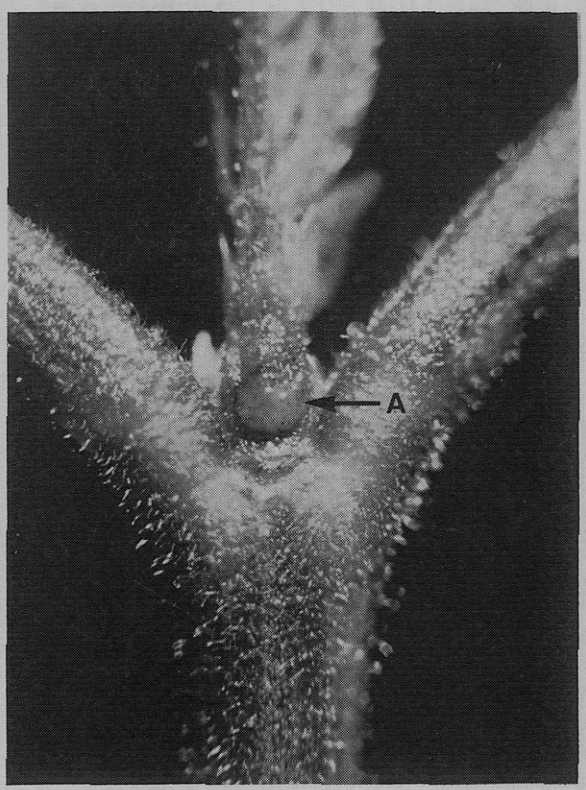

Figure 2. Mixture of 250 μg. of fractionated lanolin and fiber extract applied to second internode, *A*.

Young bean plant after bract at base of internode had been removed

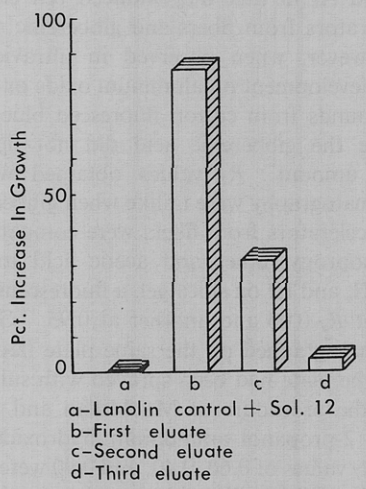

Figure 3. Growth-accelerating properties of extracts

Extracts obtained by successive elution of a fiber sample compared with that of fractionated-lanolin carrier used alone

Table I. Accelerated Growth of Bean Second Internodes Induced with Factors Isolated from Cotton Fibers in 4 Grams of Filter Paper

Treatment	R_f	Av. Initial Length, Mm.	Av. Final Length, Mm.	Gain[a]	Increase over Controls, %
Lanolin fraction		1.43	2.82	1.39-c	
Factor I	0.6	1.45	10.06	8.61-b	519
Factor II	0.4	1.49	11.52	10.03-b	622
Factor III	0.9	1.49	16.52	15.03-a	981

[a] Unlike letters following figures indicate statistically significant difference at 5% level.

the mixture was reconstituted by redissolving the solids in an amount of the solvent system equal to the original volume. Eluates reconstituted in this way were also stored for long periods without measurable loss of activity.

Comparison of Growth Accelerators from Cotton Fibers with Other Regulating Substances. Based on the second internode test, response to indole-3-acetic acid (IAA) was physiologically unlike the response obtained with any one of the growth factors from the fibers. The IAA inhibited growth when relatively large doses were used and smaller amounts failed to accelerate growth in this assay (Figure 4). The fiber eluates also inhibited growth when relatively large doses were used but greatly accelerated growth when smaller amounts were applied. The accelerators obtained from cotton and the IAA fluoresced when illuminated with ultraviolet light. R_f values obtained with thin-layer chromatography were unlike, however, when IAA was compared with accelerators from the fibers. Separation on aluminum oxide with the 2-propanol solvent system described above resulted in R_f values of 0.43 for the IAA and 0.88 and 0.95 for the two areas from which Factors I, II, and III were obtained with subsequent fractionation on silica.

All three accelerators from fibers resembled gibberellic acid A_3 in that they induced cell elongation. The accelerators from fibers and gibberellic acid were unlike, however, when observed in ultraviolet light following development on aluminum oxide or silica gel. The compounds from cotton fluoresced blue to gray-blue, while the gibberellic acid did not fluoresce a detectable amount. R_f values obtained with thin-layer chromatography were unlike when gibberellic acid and the accelerators from fibers were compared.

Using isopropyl ether and acetic acid to develop Factors I, II, and III on silica gel, a fluorescent area was observed at R_f 0.00 and another at 0.95. Gibberellic acid chromatographed on the same plate fluoresced at 0.12 after the plate had been sprayed with sulfuric acid following the procedure of MacMillan and Suter (*6*). Using the 2-propanol–ammonium hydroxide system, the usual R_f values of 0.60, 0.40, and 0.90 were obtained on silica gel. Gibberellic acid, chromatographed on the same plate, gave an R_f of 0.48 which was evident after the sulfuric acid spray was used.

In comparing other endogenous regulating substances with those obtained from fibers, 3-indoleacetonitrile; indole-3-carboxaldehyde; indole-3-carboxylic acid; indole-3-acetic acid, methyl ester; indole-3-acetamide; and α-phenylacetamide failed to accelerate growth, indicating that these compounds were physiologically unlike the accelerators obtained from cotton.

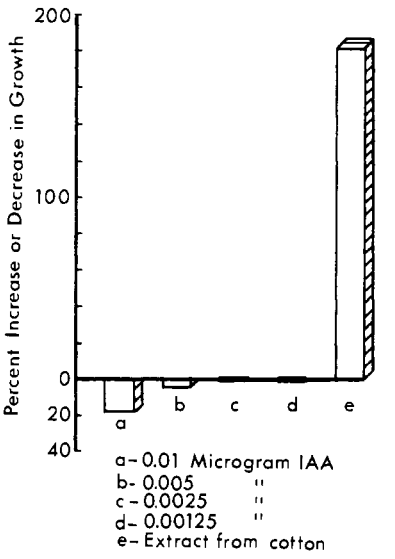

Figure 4. Growth-regulating property of IAA compared with that of unfractionated fiber extract

Discussion

During the development of a cotton fiber, elongation proceeds at a relatively rapid rate and within a few weeks the final length of the cell is sometimes about 2000 times its width (*4*). Although cell-elongating substances were detected in eluates from mature cotton fibers, it is not known that these substances had been a part of the living fibers, nor that they had played a part in the development of these fibers. However, the presence of cell-elongating substances in mature fibers suggests that a system of hormones may be associated with this process of fiber elongation. The system concept is also supported by the fact that all five growth factors induced growth responses that were qualitatively the same but varied in magnitude. Pegg (*9*) recently reported that a complex of growth-promoting substances whose action is physiologically similar to that of the gibberellins, was obtained from tomato seeds and seedlings.

These cell-elongating hormones may be analogous to, but chemically unlike, gibberellins capable of inducing elongation of rice and many other kinds of plants. As in the case of gibberellins, differences in the physiological activity of the various fiber-growth factors may be evident after sufficient amounts of these substances have been isolated to permit studies with a variety of test plants. Results with cotton fibers suggest that cells and hairlike organs which elongate rapidly, such as other kinds of fibers, pollen tubes, the beak of achenes of dandelions, and the stigmata of corn, may represent sources of specific hormones that control cell elongation.

The cell-elongating substances obtained from cotton fibers appear to be relatively stable, since they retained activity when stored under various conditions for at least 4 years. There is, of course, the possibility that hormones of a labile nature may be involved in the development of cotton fibers and would not be present in the mature fibers, and that the constitution of a hormone system, such as the one suggested here, may vary in the amount and kind of hormone involved during development of the fiber (*5*).

Results presented here support the hypothesis that plant growth involves a variety of different kinds of hormones. In fact, there is evidence that some plants may produce a group of related hormones, a hormone system, which may control a single growth process such as the elongation of an epidermal hair. In the cotton fibers studied, the compounds detected appear to be structurally unlike gibberellic acid and also unlike various endogenous indole compounds known to possess growth-regulating properties.

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