INTRACELLULAR MECHANISM OF GROWTH INHIBITION BY RADIANT ENERGY[1, 2]

JAMES A. LOCKHART

DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Visible radiant energy retards stem growth of most higher plants. Growth inhibition is due principally to a decrease in cell elongation with little effect on cell numbers. This conclusion is supported by cell counts (22) and by the finding that growth inhibition occurs in the morphological region of elongation (11; Lockhart, unpubl.). An investigation has been made of the influence of radiation on various physical properties of elongating stem cells, to learn what phase of the cellular growth process is affected by irradiation.

Elongation of stem cells takes place through the uptake of water and concurrent stretching of the cell wall. It has been established that the factor which causes cell elongation and controls rate is an increased plasticity of the cell wall. Yielding of the cell wall under stress of turgor pressure results in an increased diffusion pressure deficit (DPD) and resultant uptake of water (1, 15, 21). The reduction in growth rate of a plant stem as a result of irradiation could be due to effects on any of three factors which influence DPD.

Radiation could act by 1) reducing osmotic concentration within the growing cells, 2) by reducing the plasticity of the cell walls, or 3) by reducing the rate of water movement through the tissue.

Du Buy (2) investigated the effect of blue radiation on the plasticity of *Avena coleoptile* sections. Dark-grown, auxin-treated coleoptile sections showed an increase in plasticity (after 180 min) while irradiated sections showed no corresponding increase. Gessner (5) found that visible radiation, as well as other treatments which inhibit growth, reduced the plasticity of Helianthus hypocotyls.

Priestley (17) examined the effect of brief daily irradiations on plasmolysis of epidermal cells of the pea epicotyl. He reported a difference in the apparent plasmolysis of dark-grown and light-treated tissues which he interpreted as a difference in the degree to which the protoplast adheres to the cell wall. It is noteworthy that he did not find this effect when the plants were irradiated for 3 hours immediately prior to analysis. De Haan and Gorter (6) reported no difference in osmotic values of greenhouse-grown tall and dwarf peas. Galston and Hand (4) found the uptake of indole-3-acetic acid by stem sections was decreased by prior irradiation of the plants.

In the present paper, effects of visible radiation on all three factors controlling cell enlargement have been investigated.

MATERIALS AND METHODS

Except when otherwise specified, the experiments reported here were performed with stem sections cut from 7- or 8-day-old etiolated Alaska pea seedlings (*Pisum sativum*). The seedlings were grown in complete darkness at 25 ± 0.5° C with only brief exposures to low intensity green radiation. The details of culture have been described previously (12). For experiments with stem sections, 1.5 or 2 cm sections were cut 1 to 2 mm from the tip of plants having the third internode at least 35 mm long. Any plant with a node visible at the apex was rejected. Sections were cut with a double-bladed cutter and immediately placed in distilled water or other appropriate solution.

The radiation source for all experiments described here was a single pink 48 inch fluorescent tube wrapped in two layers of 0.005 inch red cellulose acetate. Seedlings were placed approximately 30 cm from the tube giving an intensity at the growing region of about 150 ergs $\cdot$ cm^{-2} $\cdot$ sec^{-1}. For reasons discussed below, the standard irradiation period was 3 hours. Immediately after irradiation the sections were cut for use. All experiments were carried out under weak green radiation.

For determining the time-course of growth, vigorous, uniform plants were selected and transferred intact to 2 dram polyethylene vials containing enough distilled water to immerse about a third of the root system. The plants were supported by cotton wrapped around the base of the stem and inserted into the vials. Plant growth was completely normal in these tubes over the duration of the experiments.

The time course of growth was determined with an instrument developed to make shadowgraphs of the upper portion of the stems at 20 minute intervals. The shadowgraphs were made on 100 ft (30.7 m) rolls of Kodak 35 mm microfile (M 417) film. The film was advanced automatically between exposures. The light source for exposing the film was a 100 watt projection bulb in a 35 mm slide projector from which the projection lens had been removed. Light was filtered through a 12 mm Corning "Sextant green" glass filter. Plants were exposed to 3 seconds radiation (ca. 5 ergs $\cdot$ cm^{-2} $\cdot$ sec^{-1}) each 20 minutes. Exposure to this green radiation had no detectable effect on growth. Red radiation was from the same red source described above wired through the shadowgraph instrument so that it would be turned off when the film shutter was open (15 sec every 20 min).

[1] Received June 5, 1959.

[2] This work was supported in part by the Herman Frasch Foundation.

After the film was developed the height of the stems was measured by projecting the shadowgraphs through a microfile reader. Heights were read against mm-square graph paper, and the data converted to natural scale.

For determinations of plasticity the apical 5 mm of each 15 or 20 mm section was inserted in a hole drilled in an aluminum or stainless steel rod, which served as a weight. The basipetal end was inserted 5 mm into a holder at an angle 45° from the vertical. Thus, a section of growing tissue either 5 or 10 mm long as close as 7 mm to the stem apex was subjected to the lateral force. Since the stem sections are seldom perfectly straight, the natural curvature of the sections was measured before the load was applied, and the curvatures were measured every 2 minutes for 15 minutes. (The first reading was made 1 min after the weight was applied.) The weights were then removed and the final, plastic curvature was measured 1 to 2 minutes later. Generally, the plasticity of seven sections was measured simultaneously on a sawtooth-shaped Plexiglas holder. Plasticity determinations were made in an atmosphere with a relative humidity in excess of 90 %.

The method used for the determination of osmotic pressure has been described previously (14). Essentially, it consists of incubating stem sections in various concentrations of mannitol, then determining the deformability of the sections by bending. When a relatively light load is imposed (e.g., 150 mg), sections bend only when plasmolysed. Furthermore. the magnitude of bending is found to be a linear function of the degree of plasmolysis. This makes it possible to interpolate the experimental points to the osmotic pressure at incipient plasmolysis.

The osmotic pressure of the expressed sap of dark-grown and irradiated tissues also was measured. Usually 20 mm stem sections were harvested and immediately frozen on solid CO_2. The tissue was then placed in 2 ml hypodermic syringes and the sap expressed into stoppered tubes. The sap was frozen and thawed immediately prior to osmotic pressure determination. Osmotic pressures were determined by the general method of Hill (10) as modified by Roepke (18). In essence this consists of measuring the temperature increase due to heat of condensation when a drop of the cell sap is placed in a water-saturated atmosphere. Two thermisters are suspended in a flask, a drop of cell sap is suspended on one, and a drop of water on the other. The thermisters are two arms of a wheatstone bridge. One of the other resistors is varied to measure the resistance (and the temperature) of the thermister surrounded by the drop of cell sap. The instrument is calibrated with sucrose solutions of known concentration.

The statistical limits in the various experiments are given as standard deviations of the means.

Results

Time-Course of Stem Growth Inhibition by Radiation: It is of both theoretical and practical interest to establish the time-course of the response of etiolated seedlings following the onset of irradiation. The time-course of radiation inhibition is illustrated in figure 1. In this experiment the growth of eight Alaska pea seedlings (7 days old) was measured. Irradiation was begun at the time marked "Radiation". The growth rate slightly decreased almost immediately, but the main growth inhibition occurred approximately 100 to 120 minutes after the onset of irradiation.

The results of this experiment indicate that the changes responsible for stem growth inhibition must occur within 120 minutes after the beginning of irradiation. Thus, in the experiments described in this paper, irradiation treatments have been standardized at 3 hours. Stems are harvested immediately after irradiation and the appropriate experiments performed. The use of this irradiation time should measure early changes causing growth inhibition and should minimize secondary effects which may be the result of either of growth inhibition or radiation.

Effect of Irradiation on Osmotic Pressure: One possible mechanism of growth inhibition is through a reduction in the osmotic pressure of the tissue. To test this possibility a method for determination of osmotic pressure has been developed (14), and used to compare dark-grown and irradiated

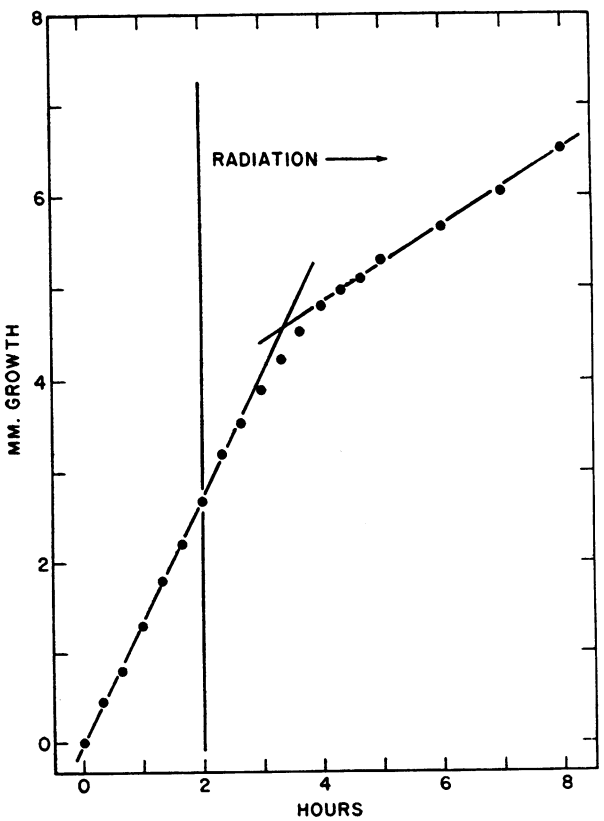


Fig. 1. Time-course of growth inhibition of Alaska pea seedlings. Red radiation (ca. 100 ergs · cm^{-2} · sec^{-1}) began at 2 hrs as indicated.

TABLE I

OSMOTIC CONCENTRATION OF STEM CELLS OF DARK-GROWN AND IRRADIATED PLANTS

By incipient plasmolysis	
Dark	0.35 M
Irradiated	0.36
By expressed sap	
Dark	0.39 M
Irradiated	0.39

tissues (see Materials and Methods). The osmotic pressure of dark-grown and irradiated stems is equal, as determined by this method, within the limits of experimental error (table I).

Osmotic pressure of the expressed sap of irradiated and non-irradiated stem tissue was also found to be the same (table I).

TIME-COURSE OF EQUILIBRATION IN DARK-GROWN AND IRRADIATED TISSUE: In spite of the fact that the osmotic concentration of dark-grown and irradiated plants was found to be the same, a difference in bending as a result of irradiation was observed in the plasmolysis method. Stems of irradiated plants were less bendable than those of dark-grown plants after plasmolysis in equal mannitol concentrations for standard time periods. Such a difference could, presumably, be due either to greater mechanical strength (of the cell walls) of irradiated stems or to a decrease in permeability of irradiated tissues. A decrease in permeability (which would be of interest) would cause a delay in establishing equilibrium in irradiated tissues and would affect the validity of results with this technique. Equilibration time has been directly determined (fig 2). Twelve sections were used for each point; the conditions of this experiment were the same as those for osmotic concentration determinations. It is clear that the difference observed in the magnitude of bending of dark-grown and irradiated plants is not due to a difference in permeability of the two tissues, since the half-times for attainment of equilibrium in mannitol solution are substantially the same.

PERMEABILITY OF PROTOPLASTS TO WATER: Growth inhibition might also be conceived as a radiation-induced reduction in the permeability of the cell to water. Two separate permeability factors must be distinguished. First, the true permeability of the cell membrane (either the plasmalemma or tonoplast) and, second, the rate of water movement across the tissue, presumably through the cell walls (discussed above). When water must be moved into and out of the whole tissue, membrane permeability probably is seldom limiting.

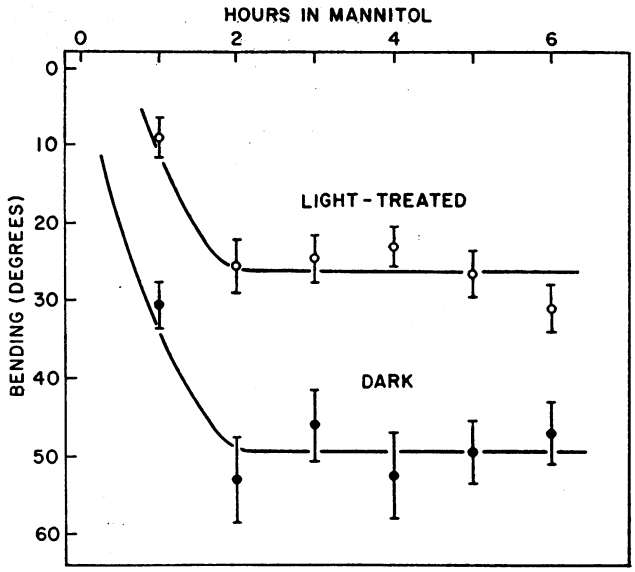
Nevertheless, the effect of irradiation on water permeability of protoplasts was measured. A pea stem section, when split longitudinally and placed in a hypotonic solution, will curve outward to an equilibrium position. Plasmolyzed stem sections show no curvature while maintained in a plasmolyzing solution, but when transferred to distilled water they rapidly attain a marked outward curvature (fig 3). The absence of an epidermis in the region absorbing water and the short (maximum path ca. 0.3 mm) water path makes it highly unlikely that diffusion through the tissue would be limiting. In fact, the water probably must enter only the cells nearest the cut surface to cause curvature. This conclusion is confirmed by the observed rate of curvature (deplasmolysis). The half-time for curvature using this technique was found to be 20 to 25 seconds. The value was very reproducible. This figure compares favorably with known rates of water permeability of other types of cells (15). While this method cannot be claimed to be quantitative, comparisons between similar tissues measure relative water permeability.

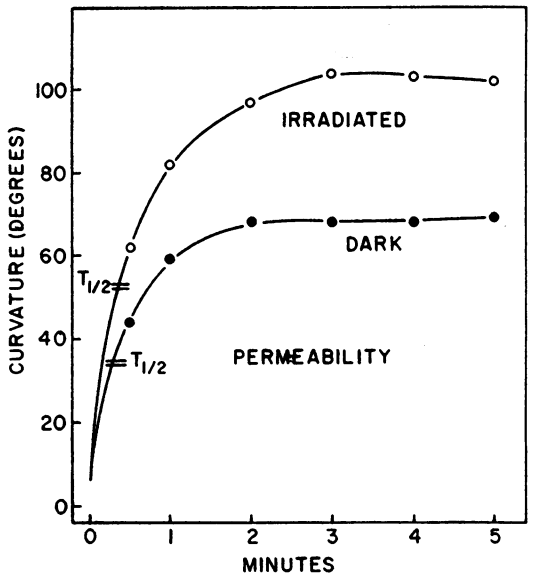
Twenty millimeter sections from irradiated and non-irradiated plants were plasmolysed in 1.0 M mannitol for 2.5 hours. They were then split longitudinally for a distance of 10 mm from the apical end and returned to the mannitol solution for 15 minutes. They were moved individually to redistilled water and the angle between the two arms was measured periodically for 5 minutes. Irradiated and non-irradiated sections were measured alternately. The results of one such experiment are illustrated in figure 3. The average half-time for equilibration was about 22 seconds in each case using four sections per treatment. Thus, no difference in permeability of cells of the two tissues can be detected.

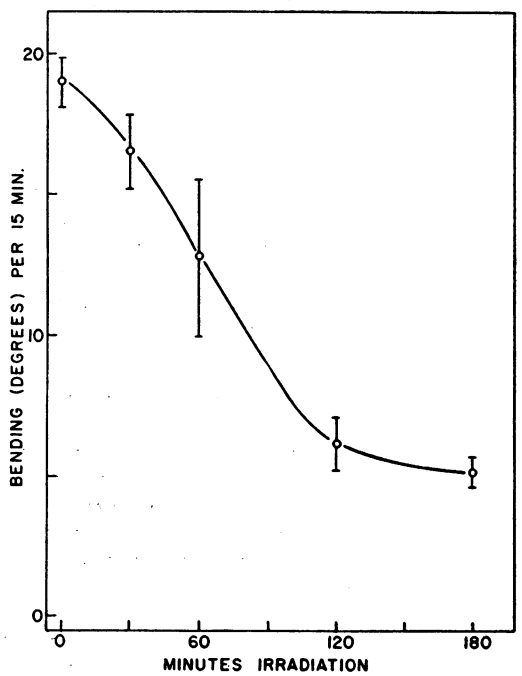
Irradiated tissue always showed a greater final angle of opening than dark-grown tissue on transfer to distilled water. When sections were transferred to M/20 phosphate buffer (pH 6.2), however instead of distilled water the equilibrium angles were equal, although the half-times to equilibrium were the same as in distilled water. The reason for the different responses in water and buffer is not understood.

VAPOR PRESSURE OF WATER IN STEM TISSUES: Availability of water to the cells might also influence cell enlargement. It may be calculated that the irradiance necessary to give maximum stem inhibition (1.0 erg cm^{-2} sec^{-1}) could, at most, raise the temperature of the plant by about 1×10^{-4}° C.

There is, then, no direct effect of radiation on water tension. Thus, any effect of radiation on water availability would have to be on water movement up the stem, or transversely from the xylem to the enlarging cells. (Plants without roots are also inhibited by light; therefore the roots could not be responsible for reduced water movement.) A reduction of water supply would restrict the amount of water moving into the elongating cells regardless of the plasticity of these cells. The rate at which water is lost from the tissue is a measure of the vapor pressure or DPD of the water in the stem. If water deficiency is, in fact, the cause of growth inhibition, the vapor pressure of water in the stem must be lower in irradiated plants. If the growth inhibition is due only to a decrease in cell wall plasticity, however, there should be no significant change in the potential rate of water loss.







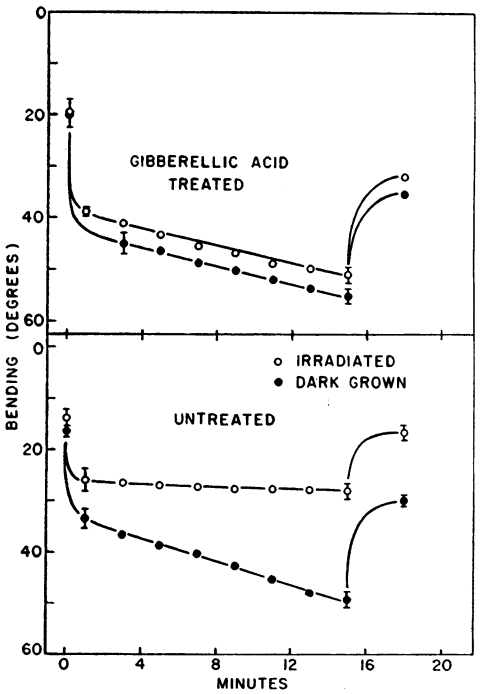


FIG. 2 (*upper left*). Rate of plasmolysis (in 0.5 M mannitol) of 20 mm apical stem sections from dark-grown and irradiated plants. Average of 12 sections per point.

FIG. 3 (*upper right*). Water permeability of Alaska pea stem cells measured as the rate of opening of plasmolysed, split stem sections. Average of four sections per treatment.

FIG. 4 (*lower left*). Change in stem plasticity with time of irradiation. Seedlings were irradiated for the times indicated, then sections were cut and plasticity immediately determined. Average of six sections per point.

FIG. 5 (*lower right*). Time-course of bending as a measure of plasticity of stem sections. The first point in each treatment is the natural curvature of the stem before the weight was added and the final point is the residual bending after the weight was removed. Average of six sections per point. The effect of gibberellin treatment on dark-grown and irradiated plants is shown.

The rate of water loss from dark-grown and irradiated stem tissue was determined by measuring the loss of weight of cut stems with time, and also by measuring the change of relative humidity of dry air passed over the stems.

Stems were cut from seedlings and the bases immediately sealed with wax (50 % beeswax–50 % paraffin oil) melting at ca. 40° C. Stems were placed in a chamber and dry air (from a compressed air cylinder) swept over the tissue, then past a set of humidity sensing elements (American Instrument Company, Silver Spring, Md.). After appropriate amplification resistance of the sensing elements was recorded on a Brown recorder as a change in current. The area under the time curve is a direct measure of the amount of water lost by the stems. Several experiments failed to show any changes in rate of water loss as a result of previous irradiation (table II).

TABLE II

WATER LOSS FROM ALASKA PEA STEMS

	IRRADIATED	DARK CONTROLS
By water measurement (in mg H_2O/plant)*	9.8	9.6
By weight loss (in mg H_2O/plant/hr)	10.4	11.1
	8.9	8.8
	12.6	10.6

The entire stems of 7-day-old Alaska seedlings were cut, the bases sealed with wax, and the measurements started immediately. Irradiation consisted of 3 hrs exposure to red radiation (ca 75 ergs $\cdot$ cm^{-2} $\cdot$ sec^{-1}) immediately prior to harvest and measurement.

* Total water lost during first 30 min (the period of rapid water loss) in completely dry air. Calculated from the area under plot of current. Flow rate over ten plants, 1.5 L per min.

Alternatively, groups of stems were cut, the bases sealed, weighed and allowed to stand in a dark room (60 % R.H.). Stems were reweighed each hour for 3 hours. They were then oven dried and the dry weight determined. Typical results are presented in table II. In no case was the rate of water loss affected by prior irradiation.

If growth inhibition actually was caused by water deficiency in the elongating region it might be expected that inhibition would be prevented, or at least reduced, in a completely water-saturated atmosphere. A large sheet of blotting paper with its base immersed in water was placed around the plants in a glass chamber. The plants were irradiated (ca. 75 ergs cm^{-2} sec^{-1}) continuously. Radiation inhibition was compared with that of plants irradiated in a normal (85 % R.H.) atmosphere. In no case was decreased inhibition of growth observed in a water-enriched atmosphere. This result, then, verifies the previous conclusion that growth inhibition is not caused by changes in water availability in the tissue.

EFFECT OF IRRADIATION ON ELASTICITY AND PLASTICITY: When stem tissue is placed under a tension, either internal (turgor pressure) or external (linear or transverse), two components of deformity may be distinguished (9). The reversible component is called elastic and the irreversible component plastic deformity. The irreversible or plastic stretch of cell walls is the component responsible for growth in length (together with the applied force), while the elastic component is apparently a coincidental property of the tissue (7). In classical plasticity determinations the tissue is subjected to an external force. The deformity is measured initially, as it continues with time under the imposed load, and after the load is removed. Plastic deformability may be measured either as the rate of deformation with time (after the preliminary elastic deformity) or, after the weight has been removed, as the amount of irreversible deformation. Elasticity is the immediate, reversible component of deformation.

For the present investigations the bending procedure developed by Heyn (8) has been adopted as the primary technique. Two principal objections have been raised to the validity of conclusions drawn as a result of using this technique. First, the amount of irreversible bending observed may be, at least in part, due to the movement of water from the cells under compression to the cells under tension. Second, the irreversible bending may arise, in part, through a deformation of cell walls perpendicular to the stem axis. Further techniques developed by Heyn (cf. 9) have been used to eliminate the first possibility. After bending has occurred the stem tissues are placed in 1.0 M mannitol until completely plasmolysed (3–4 hours), and the deformity of the tissue is again measured. Thus deformation of the tissue may be measured in the absence of all turgor pressure.

Table III shows the results of a typical bending experiment, in which elasticity and plasticity of the growing region of untreated (dark-grown) plants is compared with that of plants irradiated for 3 hours immediately prior to the experimental determinations.

TABLE III

EFFECT OF RED RADIATION ON PLASTICITY OF ALASKA PEA STEMS

TREATMENT	INITIAL CURVATURE (—WT)	FINAL CURVATURE (—WT)	PLASTICITY	ELASTICITY
Irradiated	11.5 ± 1.3°	20.0 ± 2.0°	8.5 ± 1.6°	12.5°
Dark	8.8 ± 0.8°	35.0 ± 2.2°	26.2 ± 2.0°	23°
	*After plasmolysis**			
Irradiated	11.5 ± 1.3°	10.7 ± 1.0°	0°	
Dark	8.8 ± 0.8°	19.2 ± 1.6°	10.4°	

Fifteen mm stem sections bent under a load of 1.5 g for 15 min.

Irradiation 3 hours. Plasmolysis in 1.0 M mannitol for 2.5 hours.

* Only the final curvatures were determined after plasmolysis.

Six plants were used for each treatment. A comparison of irreversible deformation of the stems and deformation after plasmolysis is included. The most conservative estimate of plasticity would, presumably, be the amount of curvature after plasmolysis. Regardless of the detailed interpretations it is obvious that plasticity of pea stem sections was greatly decreased as a result of 3 hours irradiation.

The change in stem plasticity with irradiation time is illustrated in figure 4. It is again evident that there was a substantial decrease in plasticity as a result of irradiation, measured here as the rate of bending. Furthermore, both the decrease in plasticity and inhibition of growth evidently occurred simultaneously (see fig 1). In fact, of course, it is impossible to conceive that cell wall stiffening, even as the cause of growth inhibition, could actually precede growth inhibition in time. Any change in plasticity would simultaneously appear as a change in the rate of elongation.

Determination of Plasticity with a Longitudinal Force: The second problem in interpretation of the bending technique is that irreversible bending may arise in part through a deformation of cell walls perpendicular to the stem axis (16). While this seemed unlikely, it could not be ruled out a priori. It was considered desirable, therefore to measure plasticity also by stretching stem sections parallel to the axis of the stem.

In order that stem tissues be stretched to a measurable extent it was necessary to use tissues at zero turgor pressure. Killing the tissues by rapid freezing on crushed solid CO_2 has proved to be satisfactory. The tissues were thawed in distilled water immediately prior to the test. For stretching stem sections, the stationary end was clamped between two aluminum blocks and the other end clamped in a needle holder, whose shank had been cut off and a hook attached. Both the stationary clamp and the needle holder had been drilled out to about half the diameter of the stem, and then tapped. (The success of this system was not high, but it was the best which could be readily contrived). The lower clamp weighed 1.5 g and the load which was attached to the hook, 6.7 g.

For stretching, two marks were made with lanolin-carbon ink on the stem about 1.5 mm apart. Distance between the two marks was measured (with a horizontal microscope equipped with an ocular micrometer) before the load was added, 1 to 2 minutes after it had been added, and again after the weight was removed. Results of two experiments are presented in table IV. Five stems were used for each treatment.

Table IV

Plasticity of Killed Alaska Pea Stems Measured by a Longitudinal Force

Treatment	Plasticity	Elasticity
Irradiated	0.4 ± 0.8	2.2 ± 0.5
Dark	3.8 ± 0.7	4.4 ± 0.6
Irradiated	0.8 ± 0.3	4.2 ± 0.6
Dark	5.2 ± 0.6	3.2 ± 0.9

Stretching of frozen-killed stem sections in micrometer units (50 micrometer units per mm). The load was 6.7 g. Limits are standard deviations of the means, five sections per treatment. Two separate experiments are illustrated.

It is evident that plasticity, i.e., irreversible deformation, of dark-grown stem tissue was markedly greater than that of plants exposed to 3 hours of red radiation. This experiment, then, fully confirmed the results of bending experiments. Irradiation markedly reduced cell wall plasticity of the growing stem.

In this experiment the potential plasticity (at the time of killing) was determined, while the bending experiments measured a combination of potential plasticity and the plasticity which was generated during the 15 minute bending period (cf. 3).

Influence of Gibberellic Acid on Cell Wall Plasticity: It has been reported previously that application of gibberellic acid will completely prevent growth inhibition as a result of visible irradiation (12). It was, therefore, of critical interest to determine whether gibberellic acid also would prevent reduction in cell wall plasticity caused by irradiation.

Uniform seven day old seedlings were selected and marked. Half were treated with 1.0 μg gibberellic acid (in 4 μl ethanol) and the other half kept as controls. After 2 hours a portion of the treated and a portion of the untreated plants were irradiated for 2.5 hours. Immediately after irradiation stem sections were cut and plasticity was determined by the bending method described above.

The results of a typical experiment are illustrated in figure 5. It is clear that applied gibberellic acid completely prevented irradiation-induced decrease in cell wall plasticity. This result was entirely consistent with the observed prevention of growth inhibition by applied gibberellic acid. Gibberellic acid has no effect on the plasticity of the dark-grown plants, just as it has, generally, no effect on growth of Alaska pea seedlings in darkness.

Discussion

These investigations were carried out on plants which had been irradiated for 3 hours immediately prior to the beginning of the analyses. This technique should minimize secondary effects of irradiation. Changes observed in the tissue 12 to 24 hours after irradiation might well be the result of the reduced growth or of unrelated effects of irradiation. It is considered of critical importance to analyze the elongating tissue as it exists in the growing plant. Growth responses of excised pea stem sections are clearly different from those of comparable intact tissue (4); therefore, changes which occur as a result of treating excised sections may not reflect similar changes in the intact plant. In all experiments reported here intact plants were irradiated and the

physical properties of the cells were determined immediately after excision from the plant.

Experimental results presented here demonstrate that visible radiation retards stem growth by preventing normal plasticity increases in the cell wall. It has already been demonstrated that radiation-induced stem inhibition takes place through a reduction in the effective gibberellin level in the plant (12, 13). This means, then, that a reduction in endogenous gibberellin results in a decrease in cell wall plasticity. This is supported here by the demonstration that applied gibberellic acid prevents the radiation-induced plasticity decrease. Thus, it may be concluded that the mechanism of action of gibberellin is through an effect on cell wall plasticity.

Summary

The intracellular mechanism by which visible radiation inhibits stem growth has been investigated in *Pisum sativum*, variety Alaska.

It is shown that the inhibition of growth begins approximately 120 minutes after the onset of radiation. Therefore, the physical properties of stem tissue were studied approximately 180 minutes after the onset of irradiation (i.e., very soon after growth inhibition was evident), and compared with the properties of non-irradiated tissue.

No difference was found in the osmotic concentrations or in the water permeability of cells of irradiated and non-irradiated tissue. Plasmolysis proceeded at equal rates in dark-grown and irradiated tissue. Vapor pressure of water in dark-grown and irradiated tissue was found to be equal, indicating that water deficiency is probably not causally related to irradiation effects.

The plasticity of turgid and of killed stem sections was markedly reduced as a result of prior irradiation. The time-course of change in plasticity closely paralleled the change in growth rate. It is concluded that visible radiation inhibits stem elongation through an interruption or blocking of normal cell wall extension.

Gibberellic acid treatment is known to prevent radiation-induced growth inhibition. It has been shown here to prevent radiation-induced plasticity decrease as well. It may be concluded from these results that the mechanism of action of gibberellin is through an effect on cell wall plasticity.

Acknowledgments

The author wishes to thank Dr. Gordon Alderton for performing the thermoelectric osmotic concentration determinations and Prof. James Bonner for his continued counsel. The expert technical assistance of Mrs. Jane Pilcher with the early experiments is also acknowledged.

Literature Cited

1. Bonner, J., Ordin, L. and Cleland, R. Auxin-induced water uptake. In: The Chemistry and Mode of Action of Plant Growth Substances, R. L. Wain and F. Wightman, eds. Pp. 260–270. Butterworths Scientific Pub., London 1956.
2. DuBuy, H. G. Über Wachstum und Phototropismus von *Avena sativa*. Rec. trav. bot. néerl. 30: 798–925. 1933.
3. Cleland, R. A separation of auxin-induced cell wall loosening into its plastic and elastic components. Physiol. Plantarum 11: 599–609. 1958.
4. Galston, A. W. and Hand, M. E. Studies on the physiology of light action. I. Auxin and the light inhibition of growth. Amer. Jour. Bot. 36: 85–94. 1949.
5. Gessner, F. Wachstum und Wanddehnbarkeit am Helianthus-Hypokotyl. Jahrb. wiss. Bot. 80: 143–168. 1934.
6. DeHaan, I. and Gorter, C. J. On the differences in longitudinal growth of some varieties of *Pisum sativum*. Rec. trav. bot. néerl 33: 434–446. 1936.
7. Heyn, A. N. J. Der Mechanismus der Zellstreckung. Rec. trav. bot. néerl. 28: 113–244. 1931.
8. Heyn, A. N. J. Récherches sur les relations de la plasticité des membranes cellulaires et la croissance des végétaux. Compt. rend. acad. sci. 194: 1848–1850. 1932.
9. Heyn, A. N. J. The physiology of cell elongation. Bot. Rev. 6: 515–574. 1940.
10. Hill, A. V. A thermal method of measuring the vapor pressure of aqueous solutions. Proc. Roy. Soc. (London) A. 127: 9–19. 1930.
11. Idle, D. B. Studies in extension growth. II. The light-growth responses of *Vicia faba* L. Jour. Exp. Bot. 8: 127–138. 1957.
12. Lockhart, J. A. Reversal of the light inhibition of pea stem growth by the gibberellins. Proc. Natl. Acad. Sci., U.S. 42: 841–848. 1956.
13. Lockhart, J. A. Studies on the mechanism of stem growth inhibition by visible radiation. Plant Physiol. 34: 457–460. 1959.
14. Lockhart, J. A. A new method for the determination of osmotic concentration. Amer. Jour. Bot. 46: 704–708. 1959.
15. Ordin, L. and Bonner, J. Permeability of *Avena coleoptile* sections to water measured by diffusion of deuterium hydroxide. Plant Physiol. 31: 53–57. 1956.
16. Pohl, R. Ein Beitrag zur Analyse des Streckungswachstums der Pflanzen. Planta. 36: 230–261. 1948.
17. Priestley, J. H. Light and growth. II. On the anatomy of etiolated plants. New Phytologist 25: 145–170. 1926.
18. Roepke, R. R. The thermoelectric method of measuring vapor pressure. Jour. Phys. Chem. 46: 359–366. 1942.
19. Solberg, R. A. and Higinbotham, N. The effects of indoleacetic acid, sucrose, and eugenol on differentiation in segments of etiolated pea epicotyls. Amer. Jour. Bot. 44: 769–776. 1957.
20. Tagawa, T. and Bonner, J. Mechanical properties of the *Avena coleoptile* as related to auxin and to ionic interactions. Plant Physiol. 32: 207–212. 1957.
21. Thimann, K. V. and Samuel, E. W. The permeability of potato tissue to water. Proc. Natl. Acad. Sci., U.S. 41: 1029–1033. 1955.
22. Thomson, B. F. The effect of light on cell division and cell elongation in seedlings of oats and peas. Amer. Jour. Bot. 41: 326–332. 1954.