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I hereby recommend that the thesis prepared under my supervision by Gladys F. Smith

entitled EFFECTS OF X-RAYS ON PLANTS

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

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EFFECTS OF X-RAYS ON PLANTS.

A dissertation submitted to the

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of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

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by

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Introduction.

Since the discovery of x-rays by Rontgen in 1895, an abundance of publications dealing with the effects of x-rays on plants have appeared. These were concerned essentially with general physiological processes; seedling germination; rate of growth in so far as stimulation or retardation were involved; respiration; plant movements; etc. Morphological treatments were rather sketchy. Several studies were made on x-ray induced modifications in roots (4, 6) and also in aerial and underground stems (1, 2), but few if any detailed histological investigations are available among these early works.

During the last five to ten years, several histological studies have appeared in literature having the general title "Effects of X-Rays on ________ ". Studies on the x-ray induced modifications in leaf structure were made by Noguchi (7) and by Long and Kersten (5). Stem modifications were described by Johnson (3). It is true that investigations of this sort, which merely indicate the effects of x-ray treatment on tissues, are of little significance in themselves, but if they can be later employed as basis for more complex physiological investigations they are of value. Such structural inquiries must of necessity precede studies of a physiological nature. Consequently, with this as the ultimate use of data derived from morphological investigations, such investigations must be as detailed and as extensive as possible if any understanding of the real effects of x-rays upon developing tissues is to be accomplished. It might be mentioned that, the information contained in studies on x-ray induced stem and leaf structural modifications to date do not contribute adequate information to warrant their use as basis for later studies. Much is still to be done in the way of preliminary work in order to construct a worthwhile concept of the effects of x-rays on stem and leaf tissues.

Three investigations on root structure and x-rays have been conducted. These are included as part of this thesis (9, 10, 11). The investigations were designed to derive as clearly as possible data on the development and maturation of each root tissue under the influence of x-ray irradiation. But here also much work, in this case chiefly of a cytological nature, still remains to be done.

Thus one of the greatest needs of x-radiological research at the present time, is more complete and exact histological investigations. Without this, further work which is directing itself in the way of employing x-rays as a tool in physiological studies, will be considerably dampered. Much significant work on the effects of x-rays on tissues in both animals and plants still remains to be done.

It was mentioned above that a future use of x-rays in biological investigations, as indicated by present tendencies, will be as a research tool. In such a way, this radiation will have extreme significance. It is possible that many physiological phenomena which cannot be understood or demonstrated in a normal situation can be discerned in an x-ray induced pathological state. Successful use of x-rays in this way has been demonstrated in numerous investigations. Skoog (8) was able to indicate that as condition characterized by the formation of auxin destroying substances in shoots following x-ray treatment could be correlated with dwarfism in numerous plant varieties such as in "lazy corn".

Tytell and Kersten (13) were able to derive information of a biochemical nature on the physical character of urease and catalase contained in cells.

Included as a part of this dissertation is a study on the relation between secondary xylem wall thickenings and the extent of elongation of tissues surrounding the xylem elements (11). This relationship could be experimentally demonstrated by the use of x-rays. The rate and total extent of elongation of roots which were used as the test material, could be controlled by the x-ray treatment. Also included here is an investigation in which it was found possible to clearly indicate the differential character of the calines, a new group of specific plant hormones involved in stem, leaf and root growth (12).

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The writer wishes to acknowledge the assistance of several individuals who have made possible in various ways, the research included in this thesis.

First, I should like to thank Dr. H. J. Kersten of the Physics Department, University of Cincinnati, who was collaborator throughout all of the investigations presented here. Without his aid in the physical aspects of the investigations and his interest in biophysical problems in general, this work would have been impossible. As collaborator also, appreciation is given to Dr. H. V. Miller of the Department of Mathematics, Engineering College, University of Cincinnati, for his careful and thorough preparation of the statistical portion of the investigation entitled "The Stimulative Effects of X-Rays on Plants."

I am grateful to Dr. J. Hobart Hoskins of the Botany Department, University of Cincinnati, for the valuable aid derived from his discussions and generous considerations of the various ideas initiated and developed throughout the course of the research.

I wish to extend appreciation to Dr. F. W. Went of the California Institute of Technology for making available the facilities of the Kerckhoff Biological Laboratories of the California Institute of Technology where the physiological tests during work on "Auxin and Calines in Seedlings from X-Rayed Seeds", were carried out and al-

so for his interest and suggestions throughout the course of the investigation. In this particular study, also, the interest of Dr. D. M. Bonner in portions concerned with leaf growth factors is gratefully acknowledged as are also the suggestions and criticisms of Dr. James Bonner during the investigation, "The Failure of Excised Roots of Tomato Seedlings from X-Rayed Seeds to Grow in Vitro".

Acknowledgement is given to the Physics Department of the University of Cincinnati for generously supplying research materials and to the Botany Department for working space and laboratory facilities. In addition, I wish to thank both Dr. Hoskins and Dr. Went for reading several of the manuscripts prepared for publication.

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ROOT MODIFICATIONS INDUCED IN *VICIA FABA* BY IRRADIATING DRY SEEDS WITH SOFT X-RAYS.

Reprinted from Plant Physiology,
16: 159-170. 1941.

Introduction.

In a recent paper concerned with plants grown from dry or unsoaked seeds which had been irradiated with soft x-rays, Long and Kersten (1), described the occurrence of structural changes in the leaf tissue. Similar results had previously been reported by Noguchi (2), who used germinated seeds and x-rays of a shorter wavelength. This paper is a continuation of the study of plants germinated from unsoaked seeds which had been irradiated with soft x-rays; it describes some modifications which occur in the structure and development of their roots.

Methods.

The radiation was produced by the same apparatus as that used by Long and Kersten (1). It has an approximate intensity wavelength curve as shown in the upper part of figure 1, and a position in the electromagnetic spectrum as indicated by the arrow in the lower part of figure 1.

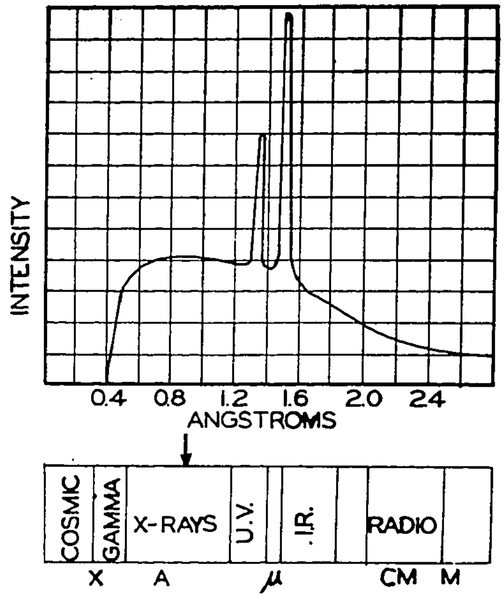

Fig. 1. Upper part: Approximate intensity wavelength curve for the radiation used. Lower part: General electromagnetic spectrum, with the position of the soft x-rays used, indicated by the arrow.

Unsoaked seeds of *Vicia faba* (Burpee's bush Lima beans) were irradiated by arranging them with their hilum sides 8 cm. from the focal spot of the x-ray tube. Preliminary tests were made to determine a dose of radiation which would produce pronounced root modifications. The treatment selected was one which, after an interval of growth, resulted in the phenomenon of "delayed killing" described by Maxwell and Kempton (3). This employed an exposure of 20 minutes while the tube was operated by 30 kv. and 10 ma. The irradiated Seeds were germinated in moist peat moss at 85°F. External developments were observed for 8 days, at the end of which time material was prepared for microscopical examination. The following observations, which were made with plants germinated from January 29th to February 6th, using seeds grown during the season of 1939, are in accord with identical experiments carried out during a three month period in the fall of 1939.

Fig. 2. A normal seedling of Vicia faba, and one grown from x-irradiated seed. Each seedling is 8 days old. The inset shows an enlarged view of a typical root grown from an irradiated seed.

Gross Observations.

During the first several days of growth there appear to be no external differences between control and irradiated seeds. The radicles appear at about the same time and continue development at a similar rate for several days. The group germinated from the irradiated seeds later shows a decreased root length, a poor development of the root tip, and the complete absence of lateral roots. By the eighth day, the condition of arrested development appears in the roots of the rayed group. At this time the average length of the primary root of the control plant, including the hypocotyl, is approximately 120 mm., and that of the rayed is approximately 44 mm. (fig. 2).

The inset in figure 2 shows an enlarged view of a typical root of the latter. The bulbous increase in diameter represents the beginning of the bypocotyl which occurs approximately 70 mm. from the tip.

An additional x-irradiation effect is seen when germinating control seedlings, and seedlings grown from x-irradiated seeds, (with combined growth of radicals and hypocotyls 12 to 15 mm.) are marked in mm. lengths. Observations 12 hours after marking show the results illustrated in figure 3.

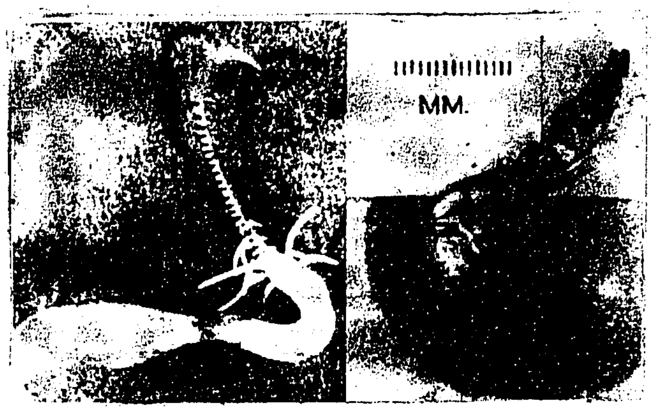

Fig. 3. Elongation in the primary root and the hypocotyl regions of a control plant, left, and in one germinated from an x-irradiated seed, right.

In the rayed group elongation does not occur within the first originally marked mm. of the primary root, as in the control. This suggests an inactivation of the root tip meristem soon after the radical appears. It is also seen that the elongation of the control hypocotyl is considerably greater than that of the germinating irradiated seed.

Microscopical Observations.

Material for a microscopical examination was collected from a great number of primary roots of germinating seeds which had been irradiated while unsoaked. This was done 8 days after irradiation when the arrested growth character became evident in the roots. Comparative studies were made with the primary roots of control plants of the same age. Paraffin sections were made and stained with iron-alum haematoxylin and gentian violet.

Examination of the root tip tissues shows many modifications in the case of plants germinated from the x-irradiated seeds as compared with the primary roots of control plants (fig. 4). In seedlings grown from the x-irradiated seeds, the root meristem begins approximately 0.63 mm. from the root tip, while in the primary root of the control, the meristem is approximately 0.225 mm. from the tip. In the root tip of a plant grown from an x-irradiated seed, vascular cells appear approximately 0.70 mm. from the tip, while in the primary roots of the control plants, the first vascular cells occur approximately 3.25 mm. from the root tip. In the rayed group, an obviously

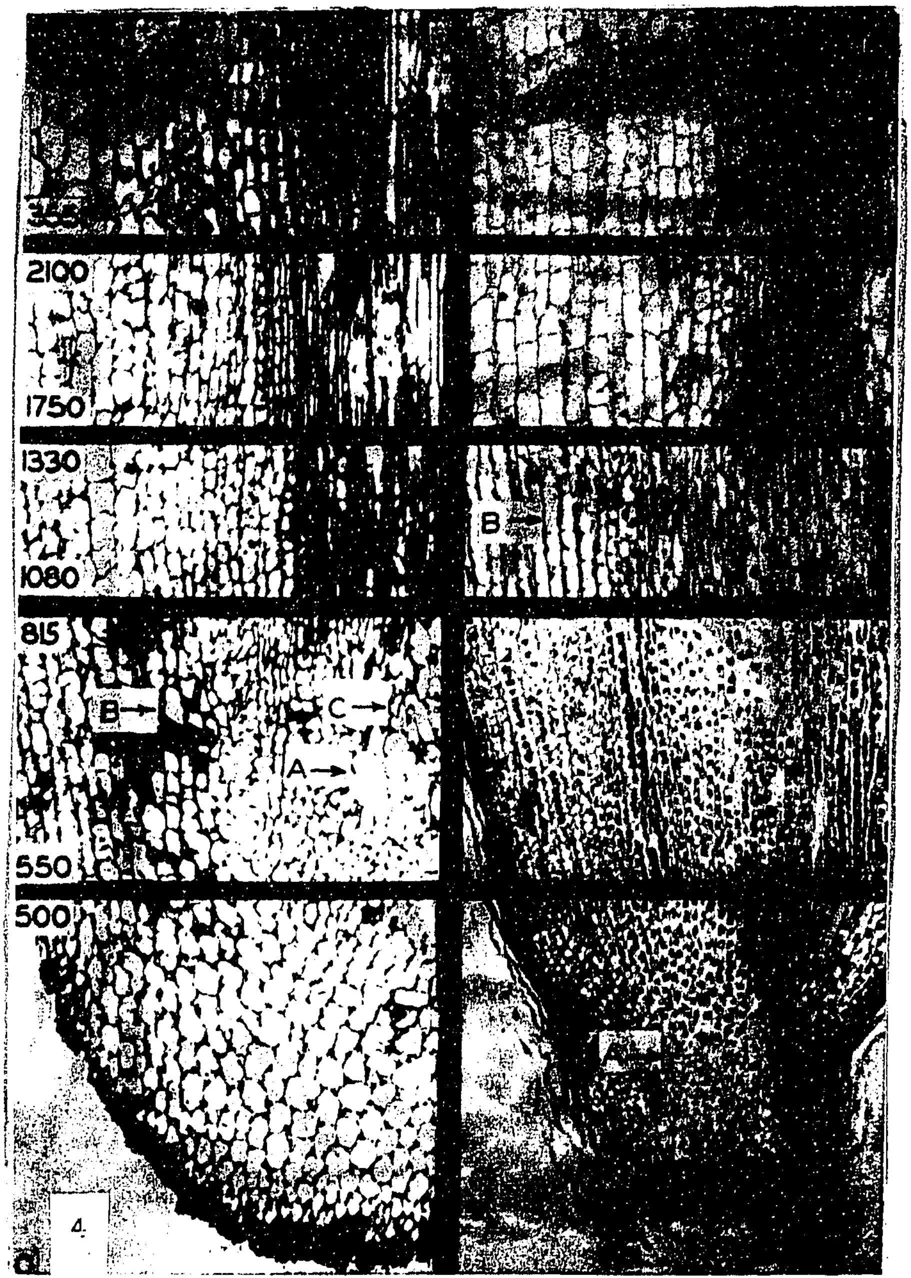

Fig. 4. Longitudinal sections of primary root tips of a normal seedling of <u>Vicia faba</u>, right, and one which had been germinated from an x-irradiated seed. Generative meristem, A: elongation, B: vascular cells, C. The numbers at the left show the distances from the tip in microns. x about 200.

peculiar situation is found in the first vascular cells. In many cases these cells possess a typically scalariform type of wall thickening; in others, the reticulate type may occur. This is a modification of the normal condition in Vicia faba, where the annular and spiral vascular elements extend for a considerable distance before the scalariform and the reticulate vessels of the metaxylem appear.

Elongation of the cells derived from the root tip meristem, as in the elongation region of normal roots, is but slightly indicated in the roots of seedlings grown from the x-irradiated seeds. In the latter, some suggestion of increased cell size has been observed approximately 0.675 mm. from the tip, while in the controls,

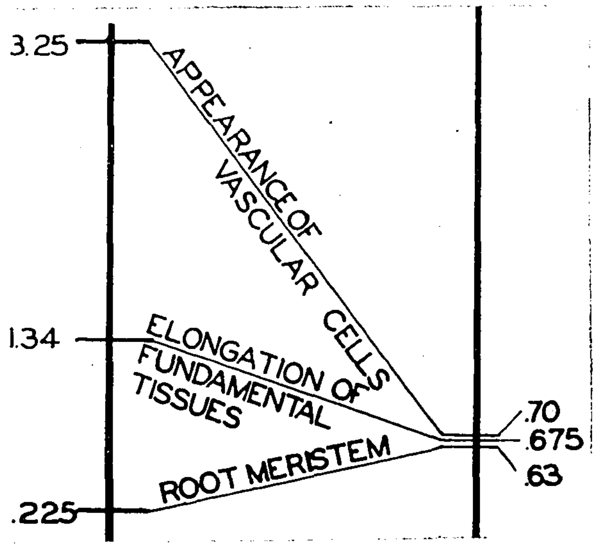

Fig. 5. Diagram of tissue developments in root tips of control seedlings and in those germinated from x-irradi-

ated seeds.

cell elongation is evident approximately 1.34 mm. from the tip. These observations are represented graphically in figure 5 and are listed in table I. Thus the condition found in the irradiated group is a foreshortening of the developmental regions in the root as suggested by the occurrence of the root tip meristem, cell elongation, and the appearance of vascular cells within a region of approximately 0.1 mm.; while in the primary roots of control plants, these developments are found in a region which extends between approximately 0.225 and 5 mm. from the root tip.

TABLE I.

Average cell lengths at various distances from the tips of Vicia faba seedlings grown from x-irradiated seeds as compared with controls.

Distance from Tip	Average cell length	
	Control	Irradiated
mm.		
0-1.............	8	40
1-2.............	12	45
2-3.............	30	45
3-4.............	37	63
4-5.............	45	64

Material for a microscopical study of the mature primary root tissue was taken approximately 2 mm. below the region of the rootstem transition. This position below the root-stem transition was accurately determined by making sections back from the hypocotyl. The region thus selected has a well developed pith region and is above the primitive radial root structure. Over 500 comparable sections of control seedlings and of seedlings which had been grown from x-irradiated seeds were examined.

In the transverse sections of control roots (fig. 6, top; fig. 9, left) a well-developed secondary vascular cylinder surrounds the tetrarch primary xylem. Lateral roots originate opposite the primary xylem arcs, which at this point, are often occupied by lacunae, bearing scattered peripheral protoxylem elements. The cylinder of xylem is surrounded by a zone of 10 to 12 cells which includes the cambium, phloem, pericycle and endodermis.

In the transverse sections of roots grown from x-irradiated seeds, (fig. 6, bottom; fig. 9, right) the primary xylem not only occurs in its normal tetrarch position, but also forms in a continuous band 2 to 3 cells in thickness between these points and is composed of cells having either the reticulate or scalariform type of wall thickening. No annular or spiral elements appear to be present as occur in the comparable sections of the primary roots of control seedlings.

The zone of 10 or more cells in thickness (which has

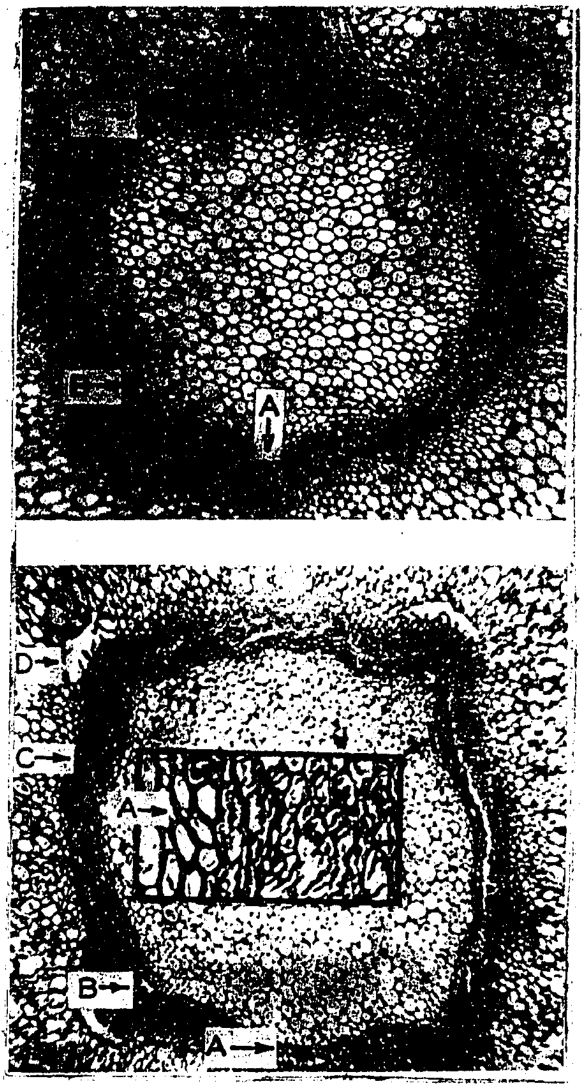

Fig. 6. Mature root development in a control plant of Vicia faba, top; and one germinated from an x-irradiated seed, bottom. Primary xylem, A; secondary xylem, B; phloem, C; distorted pericycle, D. x about 150.

previously been described in the control primary root, surrounding the xylem cylinder) is also present, as indicated in transverse section (fig. 6, bottom), in the roots of the plants grown from the irradiated seeds. It is impossible however, to identify the tissue types of this zone because it is undifferentiated and distorted. Its position and small cell size, however, is suggestive of, and corresponds to, a similar region in the primary roots of the control plants which is occupied by the cambium, phloem, pericycle, and endodermis. In the irradiated group, the inner part of this zone is consistently separated from the adjacent continuous primary xylem between the primary xylem arcs, because of a regular distortion of cells in this layer. It lies in contact, however, with a region of larger xylem cells external to the primary xylem arcs which may be secondary xylem. In the primary roots of the control plants, these inner cells (the cambiums) are in close contact with the secondary xylem. The distortion of plant cells which are able to divide (such as those already mentioned of the root tip meristem, and the cambium) is also apparent in a region external to the primary xylem arcs (fig. 6, bottom). This distorted area represents the place where the pericycle had been active in initiating lateral root formation but has now obviously degenerated.

One generalization which may be made up to this point, concerning the nature of root modifications which occur in plants of Vicia faba grown from x-irradiated seeds, is the partial or complete distortion of tissues whose cellular units are capable of dividing. Evidence of this is seen in the root tip meristem, the cambium, and the pericycle.

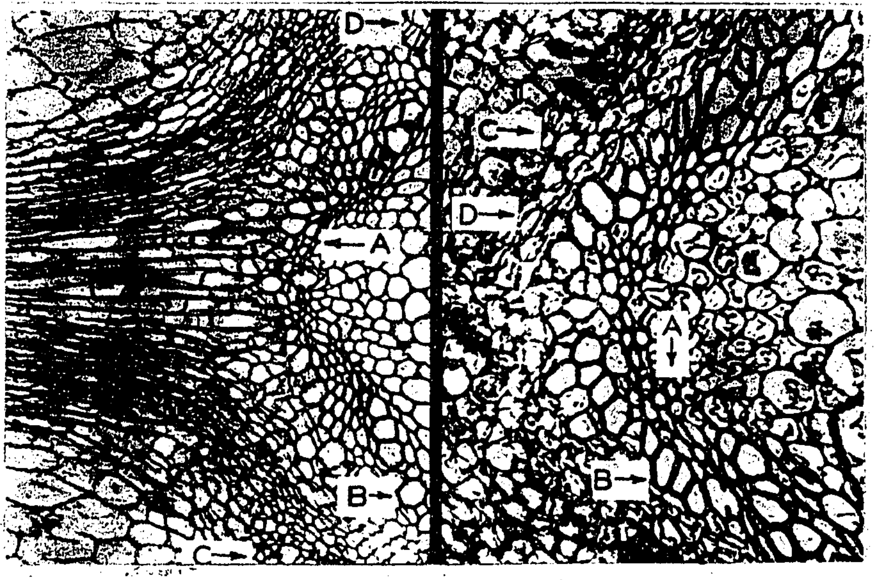

Fig. 7. Comparative developments at the points of lateral root initiation in a normal root of Vicia faba, left; and in one germinated from x-irradiated seed, right. Primary xylem, A; secondary xylem, B; phloem, C; distorted xylem, D. x about 900.

Measurement of the distribution of tissues in many transverse sections of the primary roots of control and of irradiated plants, demonstrates a greater development in the extent of the cortex and of the pith regions in the irradiated group than in the control. This is not caused by an increase in the number of cells as indicated by cell counts, but to an increase in cell size (table II).

TABLE II.

A comparison of root tissues in the transverse sections of control and in plants grown from x-irradiated seeds of <u>Vicia faba</u>.

Region	Control	Irradiated.
	μ	
Extent of cortex..........	900	1098
Extent of pith............	1449	2097
Average size of cortex cells..................	83	99
Average size of pith cells.	68	90

The hypocotyl of plants germinated from x-irradiated seeds possesses vascular tissue consisting solely of xylem cells showing the spiral type of wall thickening. These primary elements appear in groups of 2 to 4 cells lying internal to the phloem (fig. 8, right). They are similar in size and position to corresponding primary elements in the control section (fig. 8, left), but not so numerous.

The amount of phloem produced in the rayed hypocotyl is considerably less than that of the control and is poorly differentiated. There is but little noticeable variation in the cells of the cortical region of the rayed hypocotyl with the exception of a slight decrease in cell size. Numerous modifications, however, occur in the

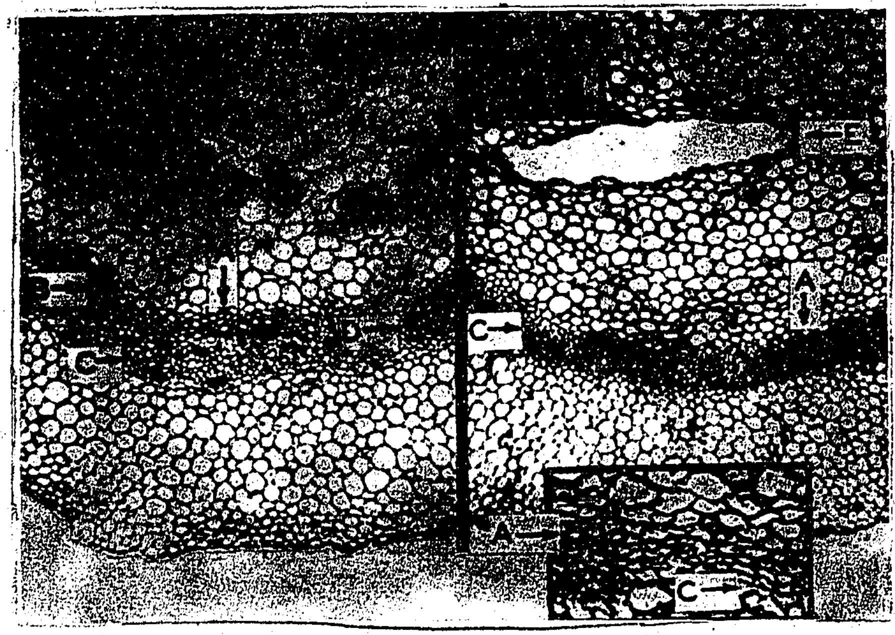

Fig. 8 Transverse sections of the hypocotyl of control seedlings of Vicia faba, left; and of plants grown from x-irradiated seeds, right. Primary xylem, A; secondary xylem, B; phloem, C; cambium, D; schizogenous cavity in the pith area, E. x about 150.

pith región. There is a marked decrease in both extent of pith and the size of the individual pith cells (table III).

TABLE III.

Comparison of pith size of control hypocotyl and that of a seedling germinated from an x-irradiated seed of Vicia faba.

	Control	Irradiated
	μ	μ
Extent of pith region......	3240	1520
Size of pith cells.........	105	51

A most interesting effect of the irradiation observed here is the constant occurence of a large intercellular cavity in the pith region of the hypocotyl (fig. 10, bottom). This is present when the delayed killing effect is evident in the roots. The cavity first appears in the hypocotyl near the region of the root-stem transition. Figure 9 shows its manner of origin in the pith. The cavity appears to be the result of a schizogenous splitting of the walls of successively formed cells. This abnormal situation may persist for various distances in the hypocotyl. In some cases it involves the entire pith area, as shown in figure 10.

Fig. 9. The origin of the cavity which occurs in the pith area of the hypocotyl of seedlings grown from x-irradiated seeds. x about 1500.

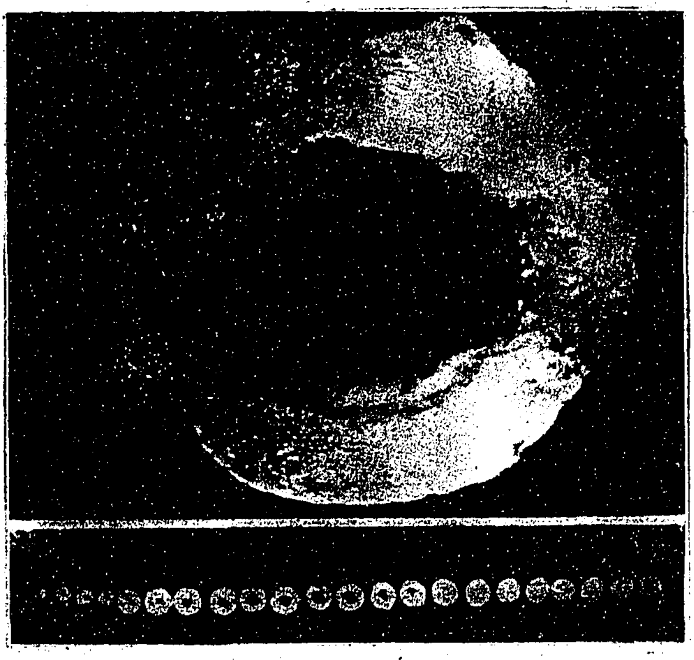

Fig. 10. A large cavity in the hypocotyl of a Vicia faba seedling grown from x-irradiated seed.

Summary.

External and microscopical observations of the primary roots of seedlings grown from seeds of Vicia faba (Burpee's bush Lima beans), which had been x-irradiated while unsoaked, are given.

The following external modifications are shown: (a) a general failure of lateral root formation; (b) an apparent poor development of the root tip as compared with controls; (c) the occurrence of "delayed killing" in the roots which becomes evident after a germination period of 8 days.

One thousand slides of the primary roots of germinating x-irradiated seeds and a like number of the pri-

mary roots of control plants, were stained and examined using standard methods of microtechnique. A microscopical examination of these slides has given rise to the following anatomical observations:

1. In the germinating irradiating seeds, the root meristem, the beginning of root elongation, and the first occurrence of vascular cells, appear within a span of approximately 0.6 to 0.7 mm. from the root tip; in the control plant, these developments appear over a region of approximately 5 to 6 mm.

2. In the upper limits of the primary root, the primary xylem tissues in the rayed sections appear as a continuous band surrounded by a region of poorly differentiated cells; if compared to tissues at a similar position in the primary root of the control plant, these are comparable to phloem, pericyclar, cambial and endodermal areas.

3. Cambial activity is indicated only at points opposite the primary xylem area; here a small band of large xylem cells are found which may be secondary xylem.

4. The degeneration of cells which normally retain their ability to divide is readily observed in the root tip meristem, the probable cambial region, and in the region where lateral roots are normally initiated in the pericycle.

5. Secondary vascular cells are not observed in the hypocotyl.

6. In the hypocotyl, small groups of primary spiral xylem cells appear at intervals internal to a region of cells which may be poorly differentiated phloem.

7. A schizogenous cavity is consistently and characteristically present in the pith region of the hypocotyl of the seedlings grown from the x-irradiated seeds when root growth permanently ceases.

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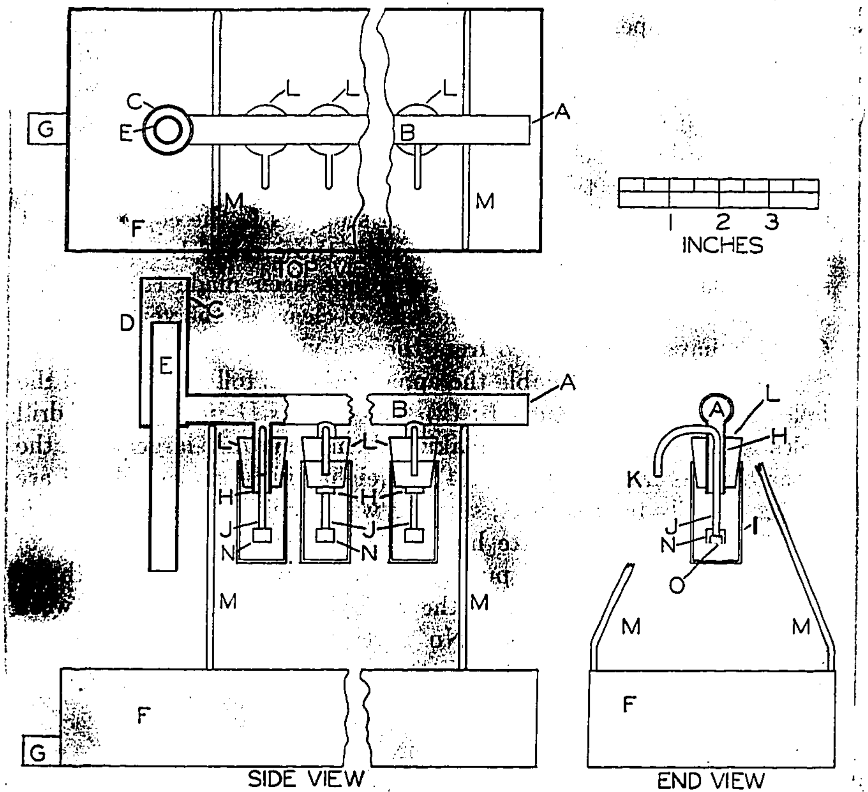
AN APPARATUS FOR WASHING TISSUE.

Throughout the course of these histological investigations on the effects of x-rays on plants, several devices were constructed to facilitate a more convenient handling of test materials used for study. One such development was an apparatus for washing tissues. It is described on the following pages.

Reprinted from Stain Technology,
Vol. 16, No. 4, October, 1941.

The apparatus shown below, which can be constructed in the ordinary departmental shop, has been found useful for washing large numbers of individual specimens preparatory to making histological sections and is particularly applicable in handling successive portions of relatively large structures which are to be studies in series.



In operation, water from the laboratory faucet enters a horizontal brass tube B, at A, by means of a hose not shown in the figure. The faucet is adjusted so that water fills the tube B, and the vertical tube C where a considerable portion overflows at D thru tube E into the pan F and out to the drain thru tube G and a hose not shown in the figure. This arrangement provides a constant head of water regardless of small variations in the tap water pressure. The specimens to be washed are placed in glass vials connected to the apparatus by the rubber stoppers L which normally remain attached to the tubes H. The way in which the water flows while washing the specimens can best be seen by examining the drawing at the lower right of Fig. 1, labeled "End View". Part of the water in tube A is diverted thru H into the glass vial I, then up thru the screen O, which is soldered to an enlarged opening, N, which in turn is soldered to the eighth inch copper tube J. The water leaves this tube at K, falls into the tray F and finally leaves at G with the water from the other vials and that from E. The rods M attach the tube B to the pan F.

In the figure only three vials are shown altho as many as may be desired may be attached to the tube B. In the model used by the authors there were twenty vials. The entire apparatus is made of brass with the exception of the rubber stoppers L, the copper tube J and the glass vials I. All joints except those made by the rubber stoppers, are soldered. The drawing has been made to the scale shown at the top right and for the sake of clearness the vertical tubes C and E have been omitted from the end view.

The best way to assemble the apparatus is as follows: (1) drill the holes for tubes H, in tube B; (2) solder tubes

H in place; (3) drill holes in tubes H for tubes J making them somewhat larger than the actual diameter of the latter; (4) the eighth inch copper tubes J are soft and easily bent so that, starting with straight tubes, one end is inserted in the appropriate hole in H, and with a combination of pushing and bending it is pushed inside H and finally bent with the fingers to the form shown in the end view; (5) the joint between J and H is then soldered. No particular order of assembly or instructions are needed for putting together the remainder of the apparatus.

ROOTT MODIFICATIONS INDUCED IN <u>ZEA MAYS</u> SEEDLINGS BY IRRADIATING DRY SEEDS WITH SOFT X-RAYS.

(WITH NINETEEN FIGURES)

In press--Plant Physiology.

Introduction.

This investigation is in part similar to a previous one which described the root modifications in *Vicia faba* seedlings grown from x-rayed seeds (1). Its purpose is to repeat and supplement this data, using *Zea mays*, so that it might be possible to state more generally the effects of x-rays on plant roots and also to test the opinion that one effect of x-raying seeds, which appears during germination; is on factors involved in cavity formation.

In *Zea mays*, such cavities of the lysigenous type occur normally in the roots. If the radiation were active in this respect, it might be expected that the process would show variations in rate and extent as compared with controls.

Methods.

The radiation referred to as soft x-rays was supplied by an apparatus described in previous papers (2, 3, 4). Unsoaked seeds of *Zea mays* were oriented with their embryo sides toward the source of radiation, 8 cm. from the focal spot of the x-ray tube of the gas type, having a copper target, so that the most intense part of its radiation consisted of the Ka line (1.54 A^o). The dose of radiation selected was 30 peak kv., 10 ma. with an exposure time of 100 minutes which according to preliminary tests produced growth cessation during germination. Any exposure time over 30 minutes under these conditions also results in this characteristic growth cessation although to a less marked degree. Variations in growth with increasing times of exposure are given in Fig. 1.

Following the irradiation, the seeds were germinated in moist peat moss at 85°F. The approximate germination time before growth stops in such seedlings is 8 days. External observations were recorded for this period and histological studies made of 3, 5 and 8 day old roots. Fifty roots of each were examined microscopically using standard methods of microtechnique. Due to the irregularity in arrangement and extent of tissues in the seedlings grown from x-rayed seeds, it was found to be quite impossible to secure sections with evenly arranged cells.

Observations.

Fig. 2 indicates the general external appearance of 8 day old seedlings grown from x-rayed seeds as compared with a control of the same age.

External modifications in the primary root may be briefly listed as: decreased root length, an average of 3 cm. in the rayed plants as compared with an average of 18 cm. in controls; absence of lateral roots; absence of adventitious roots in the region of the first internode, as compared with controls.

A histological study of the primary root tip material shows a relatively normal arrangement of cells

(Fig. 3) at the meristem in the seedlings examined on the 3d and 5th days of germination. A root tip section of an 8 day old seedling, however, indicates considerable distortion and irregularity of the cells with extensive lignification about the meristem (Fig. 4).

Fig. 6 indicates the inactivity of the pericycle in lateral root formation. Lateral root initiation is indicated but the dividing cells break down after a few divisions. At other regions of the mature root the pericycle is realtively normal in arrangement and cellular composition. These are sections of the 5 day old seedlings grown from x-rayed seeds within the region of 10 to 15 mm. from the root tip. Comparable sections of control seedlings of the same age are also given in Fig. 5, Fig. 8 shows the first internode accounting for the failure of adventitious root formation in the Zea mays seedling grown from x-rayed seeds. This formation in a control seedling is found in Fig. 7.

An interesting series of observations on the root modifications in Zea mays seedlings due to the x-irradiation of seeds involves the formation of lysigenous cavities in the pith region of the primary roots. Normal development as found in the control roots are listed in Fig. 9-11. This includes enlargement of specific pith cells which form cavities by the reabsorption of the cellular contents and also enlargement of certain other cells which divide forming adjoining divisions, which continue to increase in size and later form a single large cavity as the cell walls between them and their protoplasmic contents are reabsorbed. The end of the process is a more or less definite distribution of 7 or 8 cavities in the pith, each surrounded by regularly arranged corticle cells (Fig. 12).

In the roots of the 3 and 5 day seedlings grown from the x-rayed seeds, it is found that this cavity formation occurs only as a result of cell enlargement followed by the reabsorption of the protoplasm. No indications of cavity formation incorporating dividing cells are observed. Stages of cavity formation in the rayed plants are given in Fig. 13 and 14. The approximate distances from the root tip at which each change may be observed are also given. A diagram comparing the approximate portion of the root in which these developments occur with the condition in control roots of the same age are given in Fig. 19. The modification is very great and may be associated with decreased length.

It is observed further in the 5 day old material that after the lysigenous cavity formation is complete to the extent observed in control roots, cell wall splitting occurs in the central portion of the pith (Fig. 15 and 16)

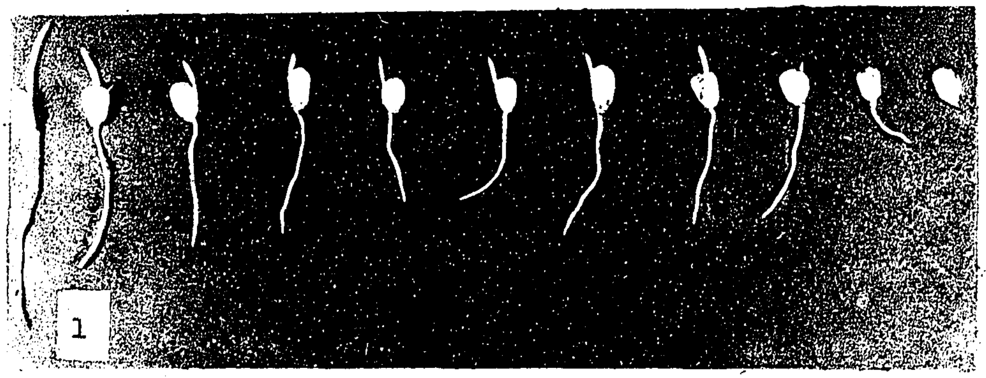


Fig. 1. Decreasing root length with increasing x-ray treatment. Fig. 2. Comparative root, length of a control seedling and of a seedling showing "delayed killing".

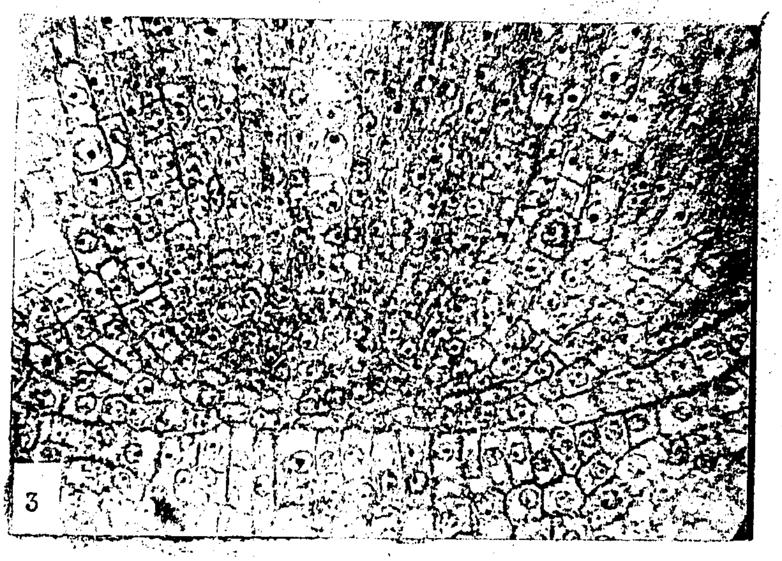
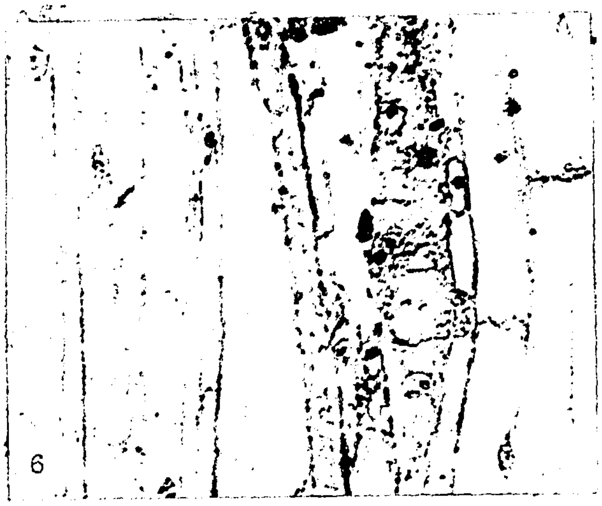


Fig. 3. Normal root-tip meristem structure in 3 to 5 days old seedlings from x-rayed seeds. (x 225).

Fig. 4. Root tip meristem in 8 day old seedlings from x-rayed seeds. (x 225)



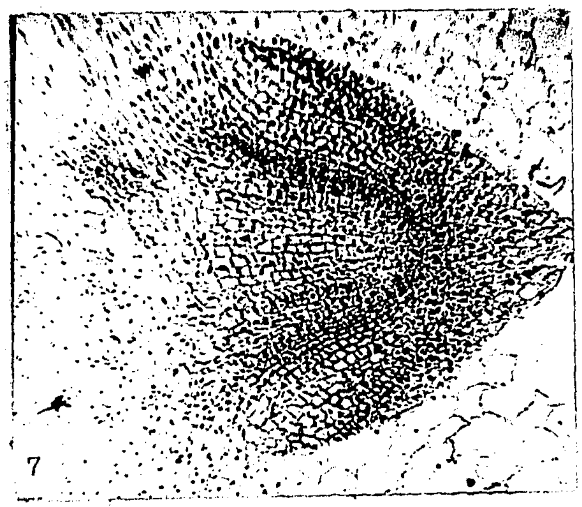


Fig. 5. Normal lateral root initiation. (x225)

Fig. 6. Pericycle disentigration in lateral root formation in seedlings from x-rayed seeds. (x 225)

Fig. 7. Initiation of adventituous roots in normal seedlings. (x 225)

Fig. 8. Failure of adventituous root formation in seedlings from x-rayed seeds. (x 225)

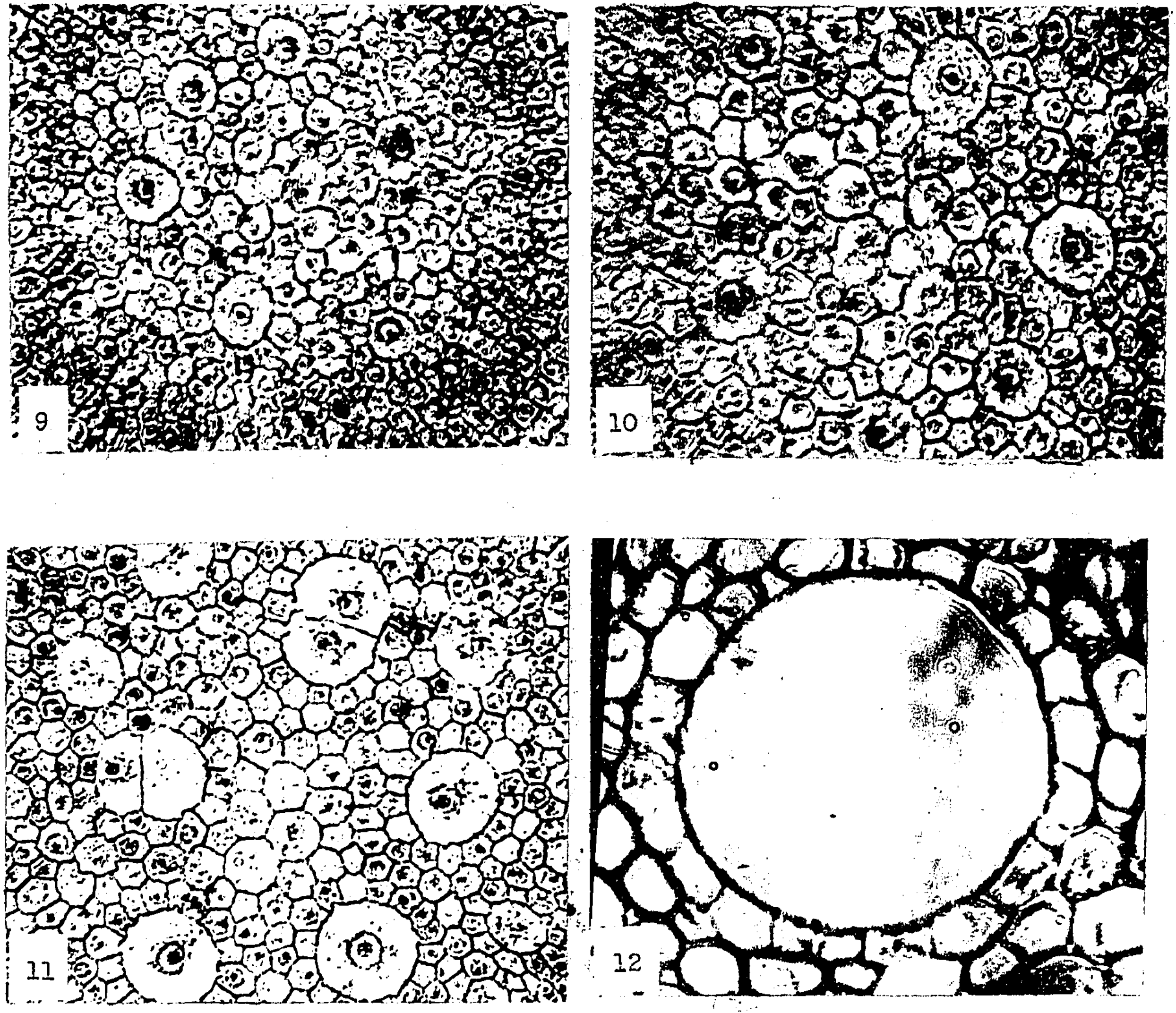
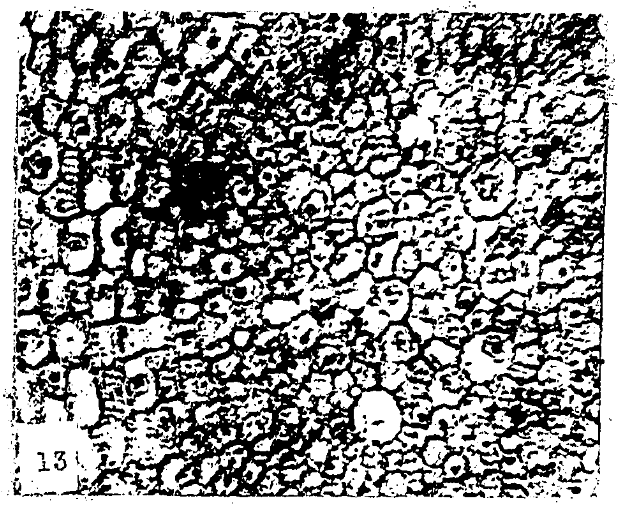


Fig. 9.-12. Steps in the formation of lysigenous cavities in normal roots of corn.

Fig. 9. Enlargement of specific pith cells; (x 125). Fig. 10. Reabsorption of cell contents; (x 125); Fig. 11. Division of adjoining pith cells, (x 125). Fig. 12. Complete lysigenous cavity, (x 500).

Fig. 13-18. Cavity formation in roots of seedlings from x-rayed seeds; Fig. 13. Pith cell enlargement. (x 125). Fig. 14. Reabsorption of cell contents; (x 125). Fig. 15. Schizogenous cavity formation in central pith cells. (x 125). Fig. 16. Enlarged pith cells showing schezogenous splitting of cell walls; (x 375). Fig. 17. Schizogenous cavity formation between previously formed lysigenous cavities; (x 375) Fig. 18. Extent of excessive cavity formation into the root tips of seedlings from x-rayed seeds. (x 225).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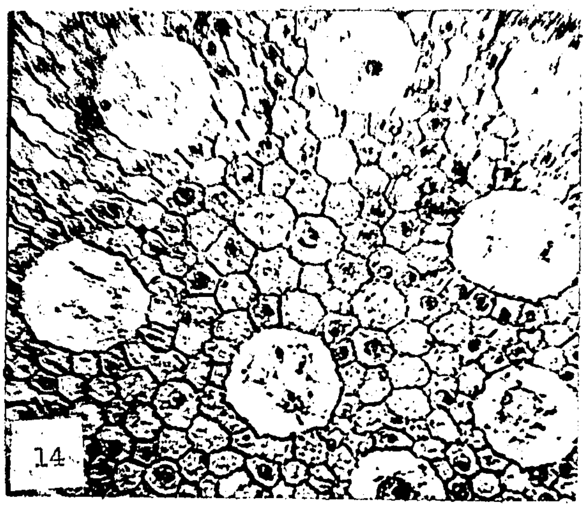
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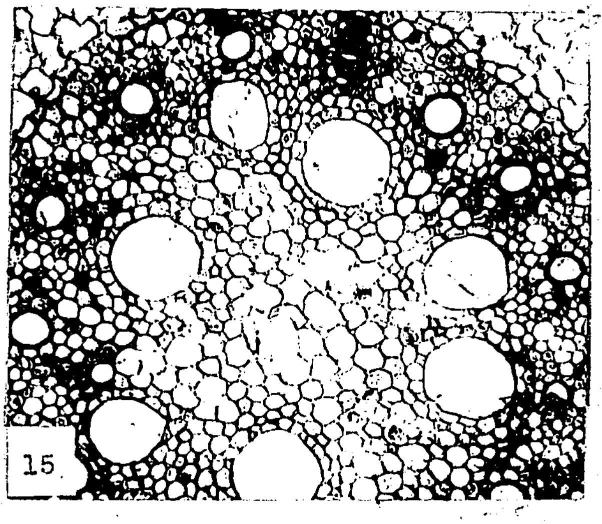
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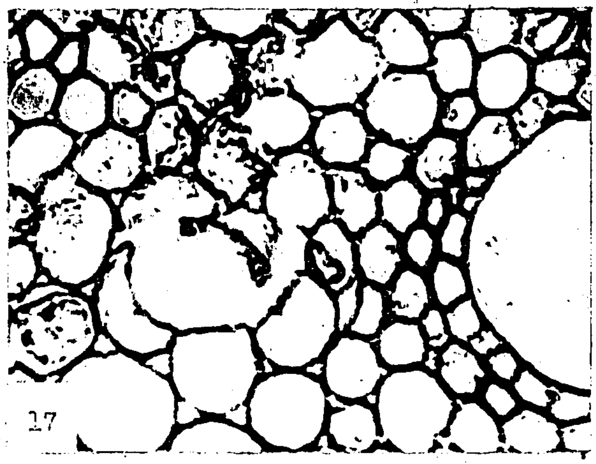
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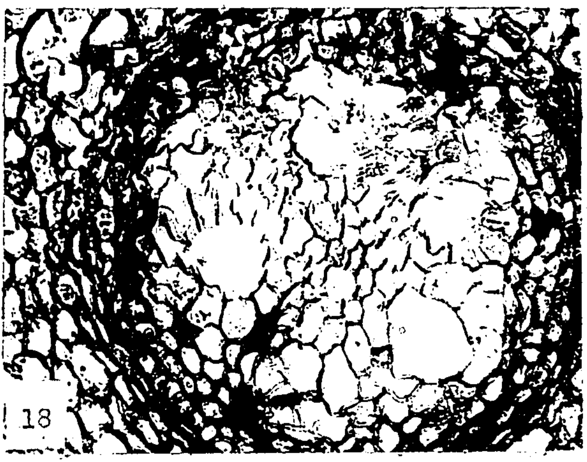
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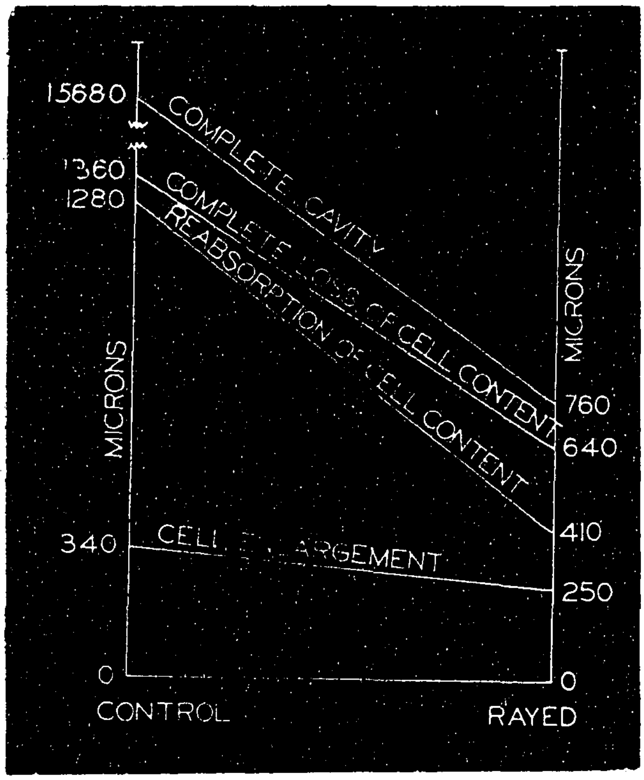

Fig. 19. Diagram indicating approximate distances from the root tip of various steps involved in cavity formation in roots of control seedlings and in roots of seedlings from x-rayed seeds.

resulting in a schizogenous cavity, and also in areas between the originally formed lysigenous cavities (Fig. 17) resulting in schizolysigenous formations. In the 8 day old seedlings, these two processes are found to involve the entire pith portion of the root in its mature region and following this to extend downward to include the pith region of the root tip (Fig. 18).

These observations are difficult to interpret. Variations in lysigenous cavity formation as compared with that in plants grown from non-irradiated seeds, and the additional schizogenous and schizo-lysigenous cavities seem to be two separate effects of the x-irradiation.

Summary.

External and histological modifications which occur in the primary roots of Zea mays seedlings grown from x-rayed seeds are described. Microscopical observations of the primary roots of 3, 5, and 8 day old seedlings grown from dry seeds, irradiated at 30 kv., 10 ma., for 100 minutes are listed with comparable observations of seedlings grown from non-irradiated seeds.

External modifications due to the radiation are similar to those found in Vicia faba. A decreased root length, a failure of lateral root formation and growth cessation after a short period of germination; in addition, adventitious roots, do not form in the internodal regions of the seedlings.

Histological observations indicate the following modifications:

1. The primary root tip meristem of the 8 day old seedlings is extensively distorted and lignified. The effect however is not apparent when the plants are from 3 to 5 days old. At this time the meristem cells appear to be relatively normal in arrangement.

2. A breakdown of the pericycle in lateral root initiation is found; also a distortion of cells of adventitious root primordia in the first internode is apparent. These correspond to the failure of secondary roots to appear externally.

3. Lysigenous cavities occur normally in the pith region of the primary root of *Zea mays*. In the roots of the seedlings grown from the x-rayed seeds, there are slight variations in the formation of these normally present cavities; this striking modification is found in their very early and rapid appearance.

4. In addition to these lesigenous cavities in the primary roots, a schizogenous cavity forms in the central region of the pith as a result of cell wall splitting when the seedlings of the x-rayed seeds are approximately from 5 to 8 days old. In the 8 day old seedlings, schizogenous cavities are observed, due to continuous cell wall splitting between the previously formed lysigenous cavities and

evidences of this cell wall splitting are found in the pith region of the root tip.

5. It is not known whether factors involved in lysigenous and schizogenous cavity formations are comparable. They seem to be separate effects of the radiation.

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THE RELATION BETWEEN XYLEM THICKENINGS IN PRIMARY ROOTS OF VICIA FABA SEEDLINGS AND ELONGATION, AS SHOWN BY SOFT X-RAY IRRADIATION.

Bulletin of the Torrey Botanical Club.
69 (3); 221-234. March 1942.

Previous studies on the root structure of Vicia faba seedlings grown from x-rayed seeds reported modifications in the secondary wall thickenings of the xylem elements (6). One observation indicated an alteration in the normal sequence of secondary wall thickenings including the annular, spiral, scalariform and pitted types. In the roots of these treated seedlings, very prominent pitted vessels occurred slightly above the apical meristem with only an occasional spiral or scalariform element in some specimens.

Morphology texts such as those of Jeffrey (2) and Haberlandt (1) discuss the relationship between these vessel types and the extent of elongation in the stem or root. A more complete study of this point is given in an early work of Stover (7) on the vascular anatomy of Calamovilfa longifolia. In it he maintains that the vessel types are the result of elongation. His observations indicate that annular and spiral xylem elements, which are rarely present in the rhizome of Calamovilfa because of the failure of internodal elongation, are present in the rapidly elongating aerial stems, as are also the later formed reticulate and pitted elements.

It is further implied by Stover, that the first thickening of a desmogen cell is of the pitted type, being laid down in the arrangement of a pitted cytoplasmic cell lining, the latter being a result of a cellular vacuolation which occurs early in the development of young cells of desmogen strands. After the formation of the original pitted thickening, division and elongation of surrounding parenchymatous cells causes it to be torn apart and to form annular, spiral, scalariform, or pitted arrangements, depending upon the extent of stretching. Consequently a pitted vessel of the metaxylem remains as such if the surrounding cells are no longer dividing or enlarging.

If the extent of elongation, therefore, determines the type of wall thickening of the xylem elements, variations in both of these factors in the roots of seedlings germinated from x-rayed seeds ought to show some relationship. On this basis, together with the fact that, within limits, the extent of elongation of the primary roots of plants grown from x-ray treated seeds decreases as the dose of irradiation increases, it seemed likely that a study of a series of *Vicia faba* primary roots with successively less elongation correlated with increasing x-ray irradiation might contribute information on the relationship existing between the types of vessel secondary wall thickening and total elongation.

Methods.

The soft x-ray apparatus used here and the general character of its radiation have been described previously

(3, 4). Dry Vicia faba seeds were placed with their hila toward the source of radiation in small glass dishes 8 cm. from the focal spot of the x-ray tube. The tube was operated at 30 peak kv. and 10 ma. Exposure times included 0, 15, 30, 60, 90 and 120 minutes.

Fig. 1. Decreasing root lengths of 5 day-old Vicia faba seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 0, 15, 30, 60, 90 and 120 minutes.

After irradiation, the seeds were soaked for 5 hours and later germinated in moist peat moss at 25°C. Material for histological studies was collected after five days, when the seedlings germinated from the seeds exposed for 120 minutes displayed the characteristic cessation of growth associated with the "delayed killing" effect in seedlings from x-ray irradiated seeds, as described by Maxwell (5).

Primary roots of 50 seedlings of each experimental lot were prepared for microscopical study with standard methods of microtechnique.

In studying the histological developments in seedlings grown from x-ray treated seeds, it is necessary to prepare and observe a great quantity of sections because of the resulting irregularity in arrangement and formation of tissues. Consequently the longitudinal sections used as illustrations in this paper are not medial throughout their entire extent, but they approach this position as nearly as possible, and the developments indicated represent data from several hundred slides.

Observations.

Figure 1 indicates the extent of primary root elongation in seedlings grown from seeds which had been exposed to soft x-rays for periods of 0, 15, 30, 60, 90, and 120 minutes. The characteristic decrease in root length with increasing doses of x-ray irradiation is shown. The average primary root lengths, including the hypocotyl, of seedlings of each lot, are listed in Table I.

TABLE I.

	Time of exposure to x-rays in minutes.					
	0	15	30	60	90	120
Average length of primary root in centimeters.......	8.0	5.8	5.0	4.0	3.6	3.0

The failure of lateral root formation related to x-ray treatments of seeds is also observed. A discussion of this has already been given (6).

Medial longitudinal sections of the root tips of these seedlings, showing types of secondary wall thickenings of the xylem elements, are illustrated in figures 2-7. Figure 2 shows the occurence of the vessel elements and the order of their formation in normal primary roots of _Vicia faba_ seedlings. The relative range of prominence of each vessel type, as it appears in the roots of the seedling groups studied, is diagrammed in figure 8.

In examining longitudinal root sections of the seedlings germinated from x-ray irradiated seeds, the most evident modification of the normal arrangement is the appearance of large pitted vessels in the most distal part; vessels of other types are found in the earlier formed regions above (figs. 3-7). If pitted vessels are associated with the least elongation of the structure in which they are forming, as is suggested by Stover, and if types formed before these indicate more extensive elongation, one is led to assume that the appearance of elements related to least elongation during later development indicates a decline in growth so far as elongation is involved.

This implication becomes plausible when one considers the phenomenon of "delayed killing" or growth cessation (5) which occurs in seedlings from seeds given a medium dose of x-rays. After an apparently normal onset of germination, inasmuch as the radicles appear beyond the cotyledons at approximately the same time in both control and treated seedlings, there is a progressive decline in rate of elongation resulting ultimately in death. The total extent of elongation and the length of their germination period before subsequent decline are dependent upon the magnitude of the x-ray treatment. They are found to decrease with increasing treatments. It seems justifiable to proceed further and to associate histological data with these external observations.

It would be expected that indications of decline in extent of cellular elongation in the roots would appear first in the most recently formed vessels. This is observed in all seedlings from the x-ray treated seeds; in them, portions of the root tip have become completely permanent in aspect by the increasing presence of xylem elements with pitted secondary wall thickenings. The effect is found to become more pronounced with higher x-ray treatments. It is very prominent in the root tip sections of seedlings grown from seeds irradiated for 60, 90, and 120 minutes (fig. 5, 6, 7). It seems quite possible, therefore, that there is a relationship existent between the decreasing total elongation of the apical portions of

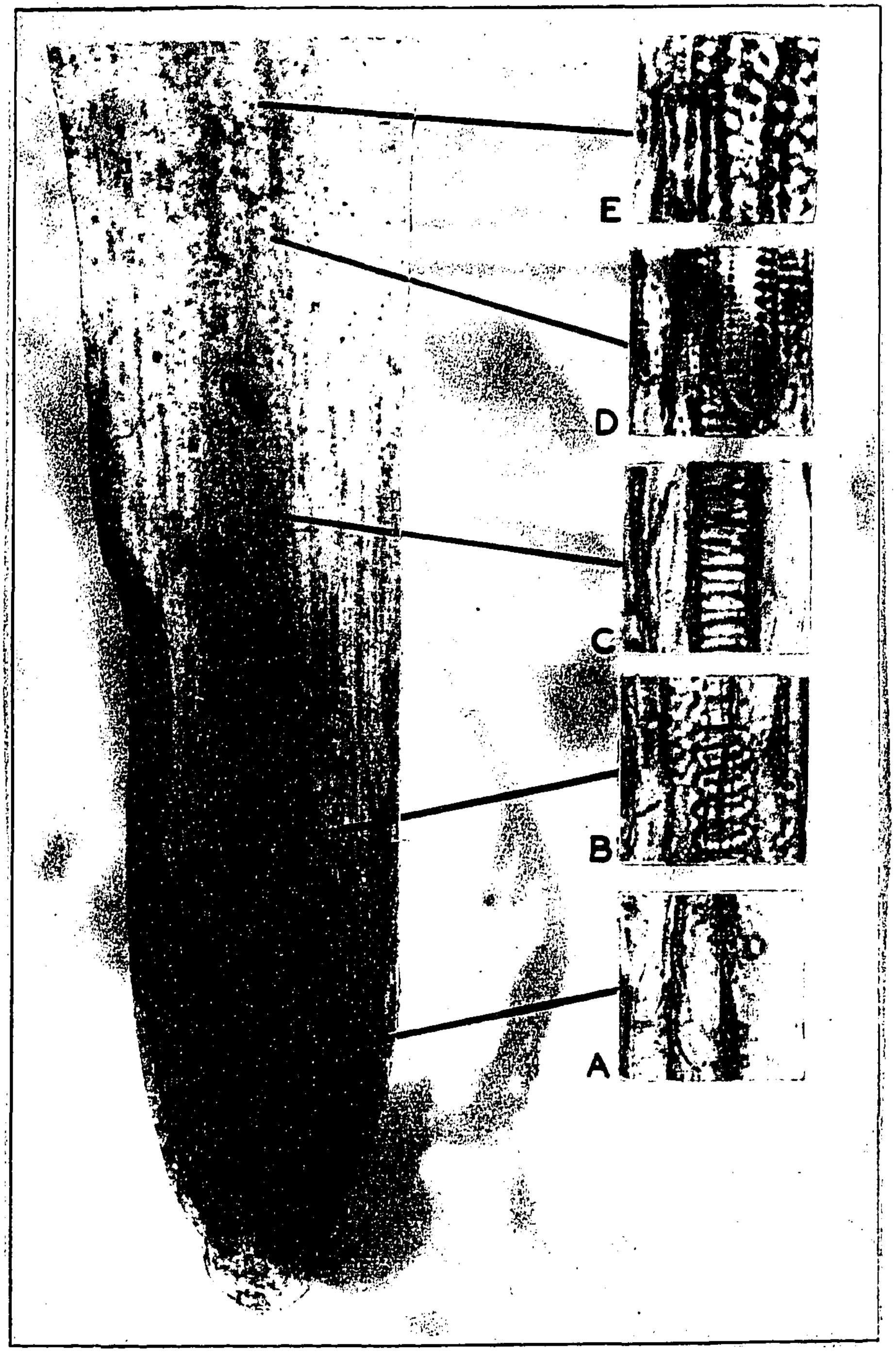

Fig. 2. Medial longitudinal root tip section of a normal Vicia faba seedling showing the occurence of vessel types. x 40 and 675.

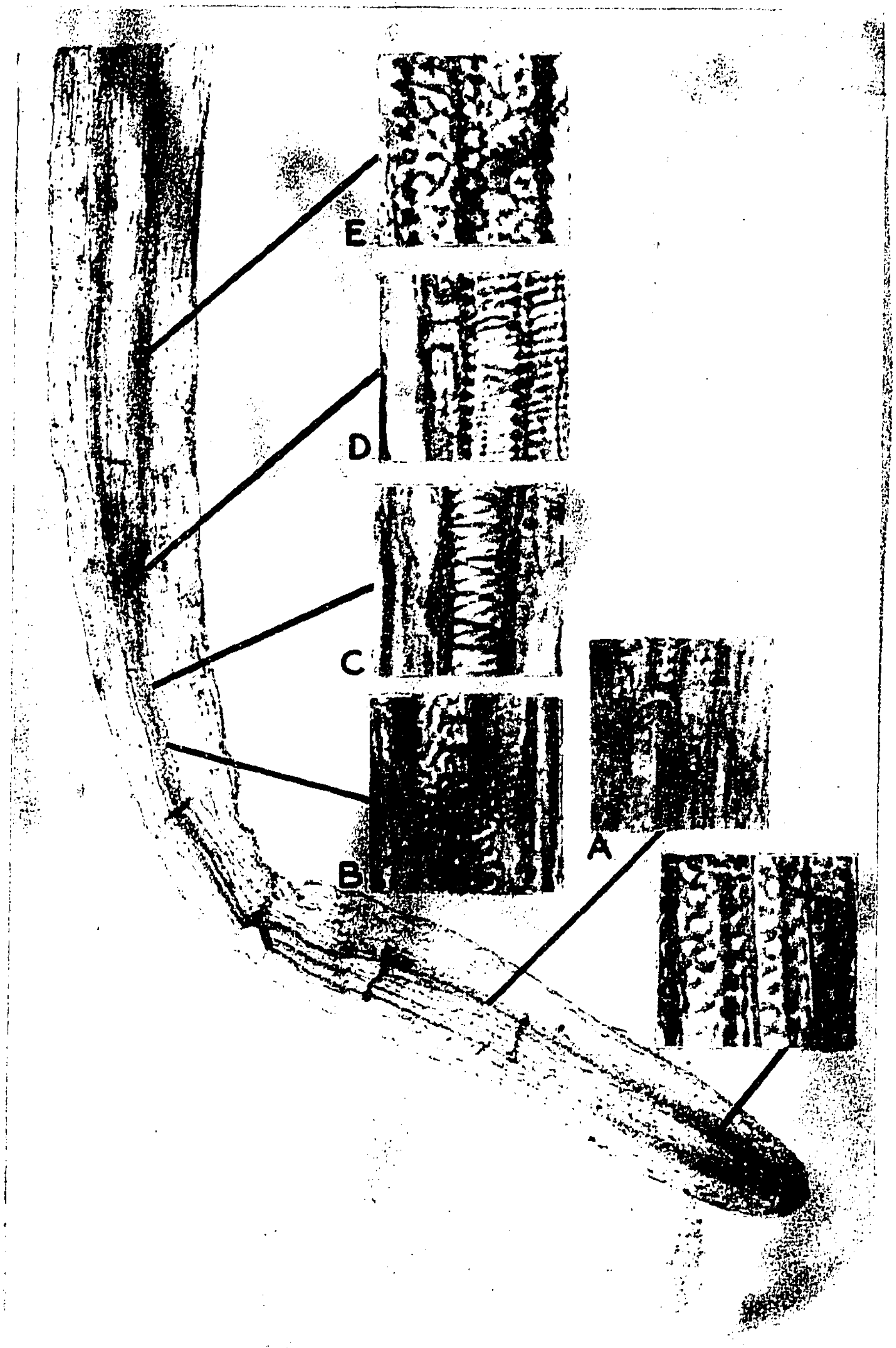

Fig. 3. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 15 minutes. x 20 and 675.

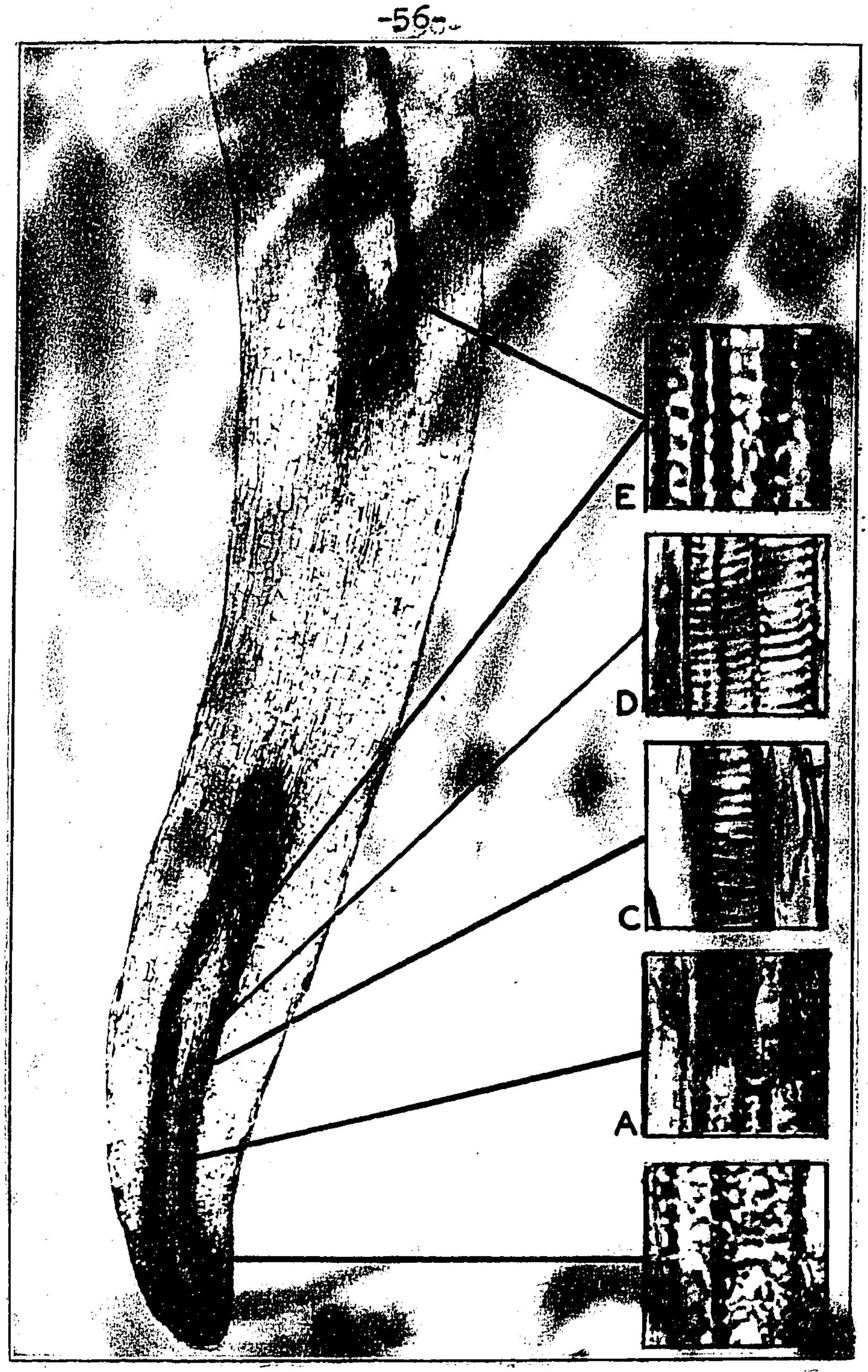

Fig. 4. Medial longitudinal root tip section of a Vicia faba seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 30 minutes. x 20 and 675.

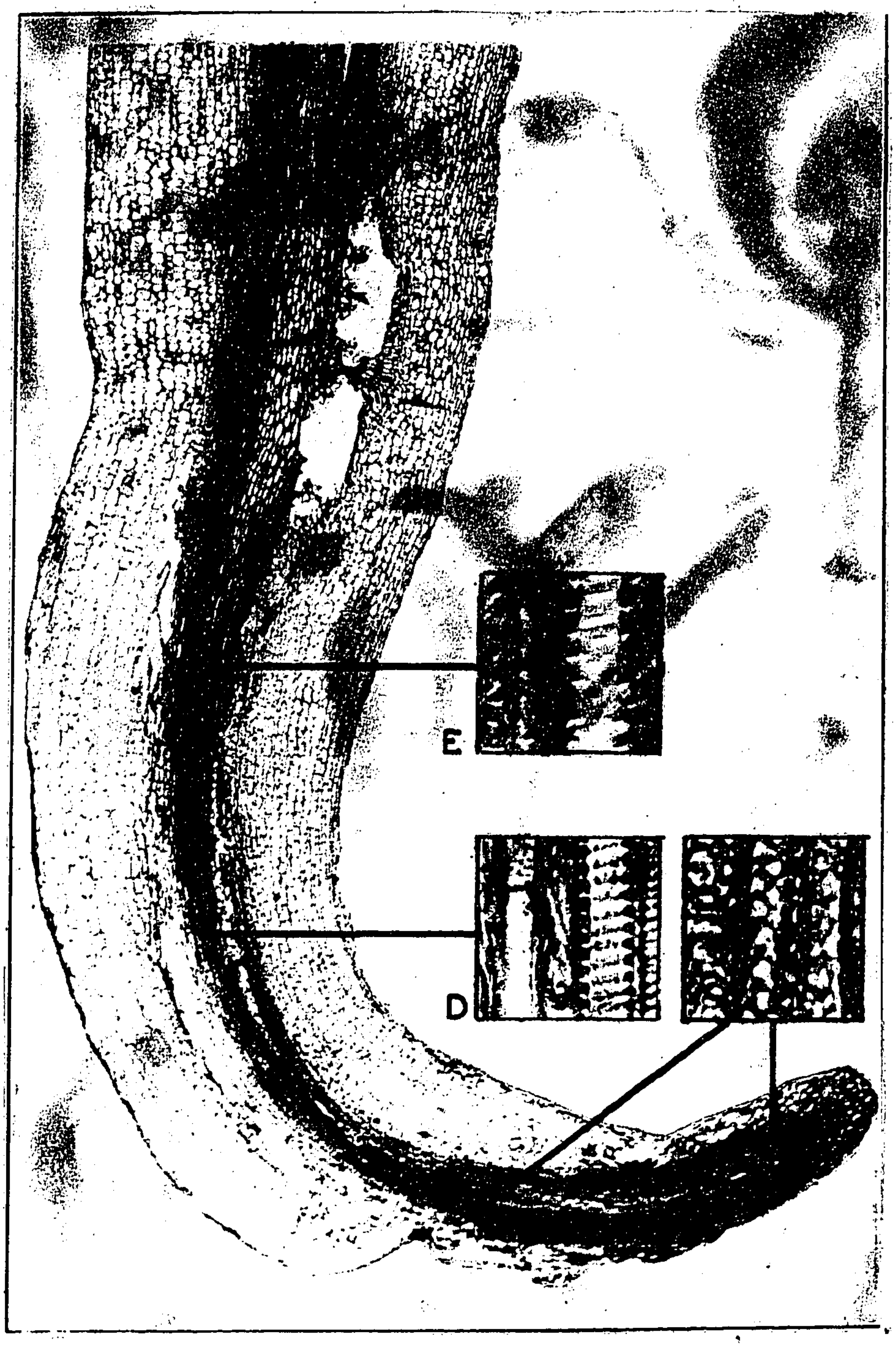

Fig. 5. Medial longitudinal root section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 60 minutes. x 20 and 675.

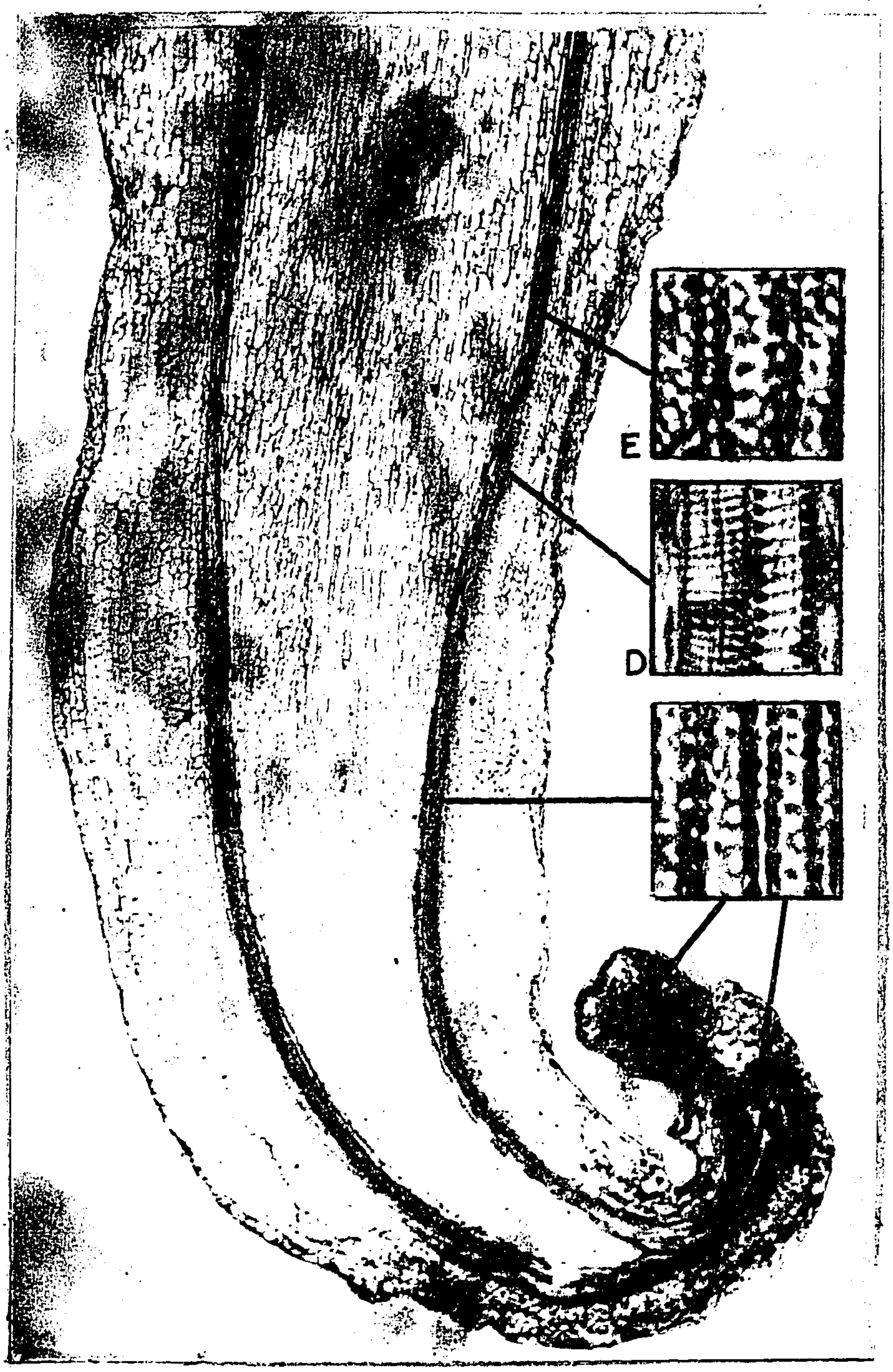

Fig. 6. Medial longitudinal root tip section of a Vicia faba seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 90 minutes. x 20 and 675.

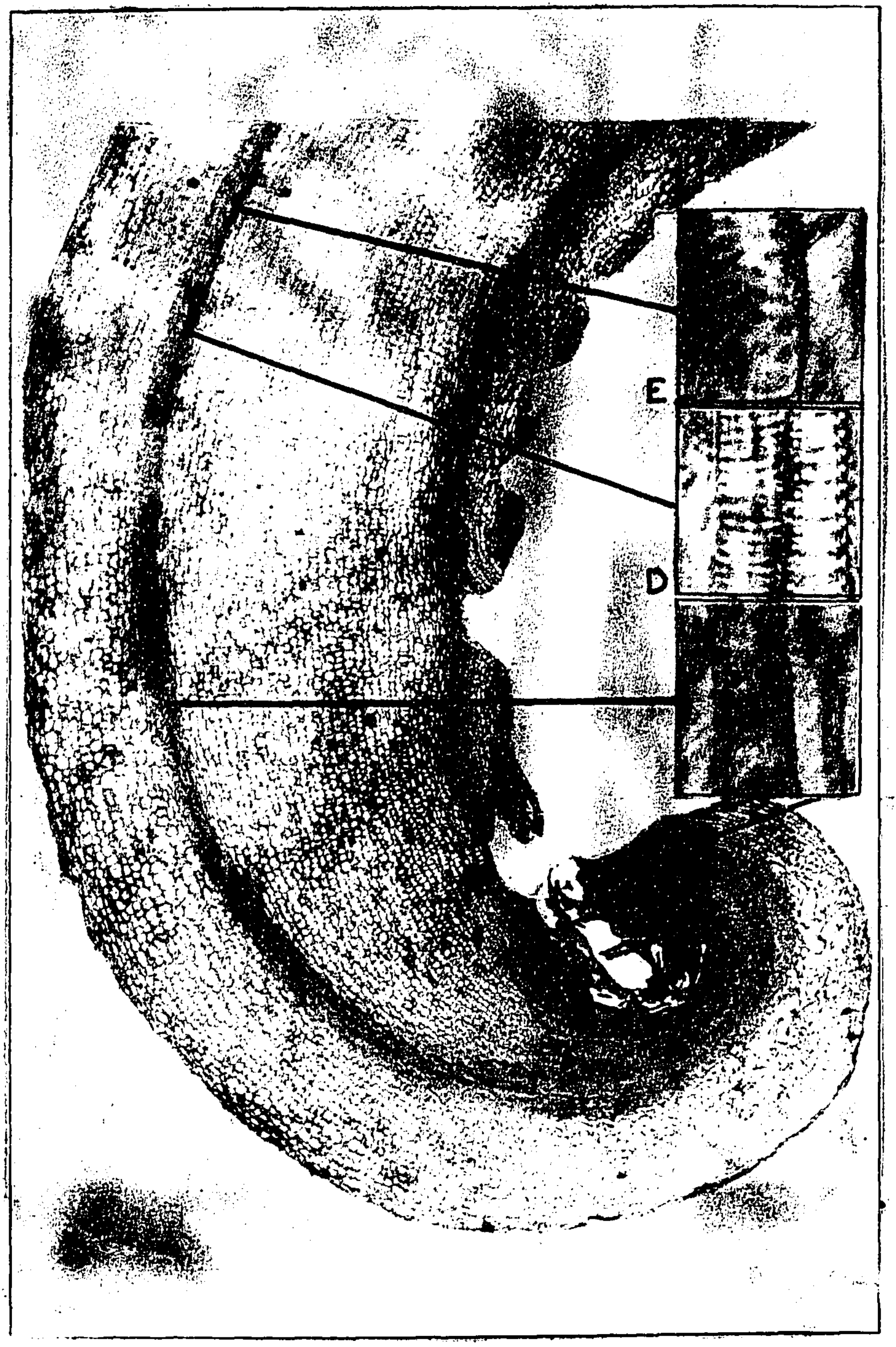

Fig. 7. Medial longitudinal root tip sectionof a <u>Vicia faba</u> seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 120 minutes. x 20 and 675.

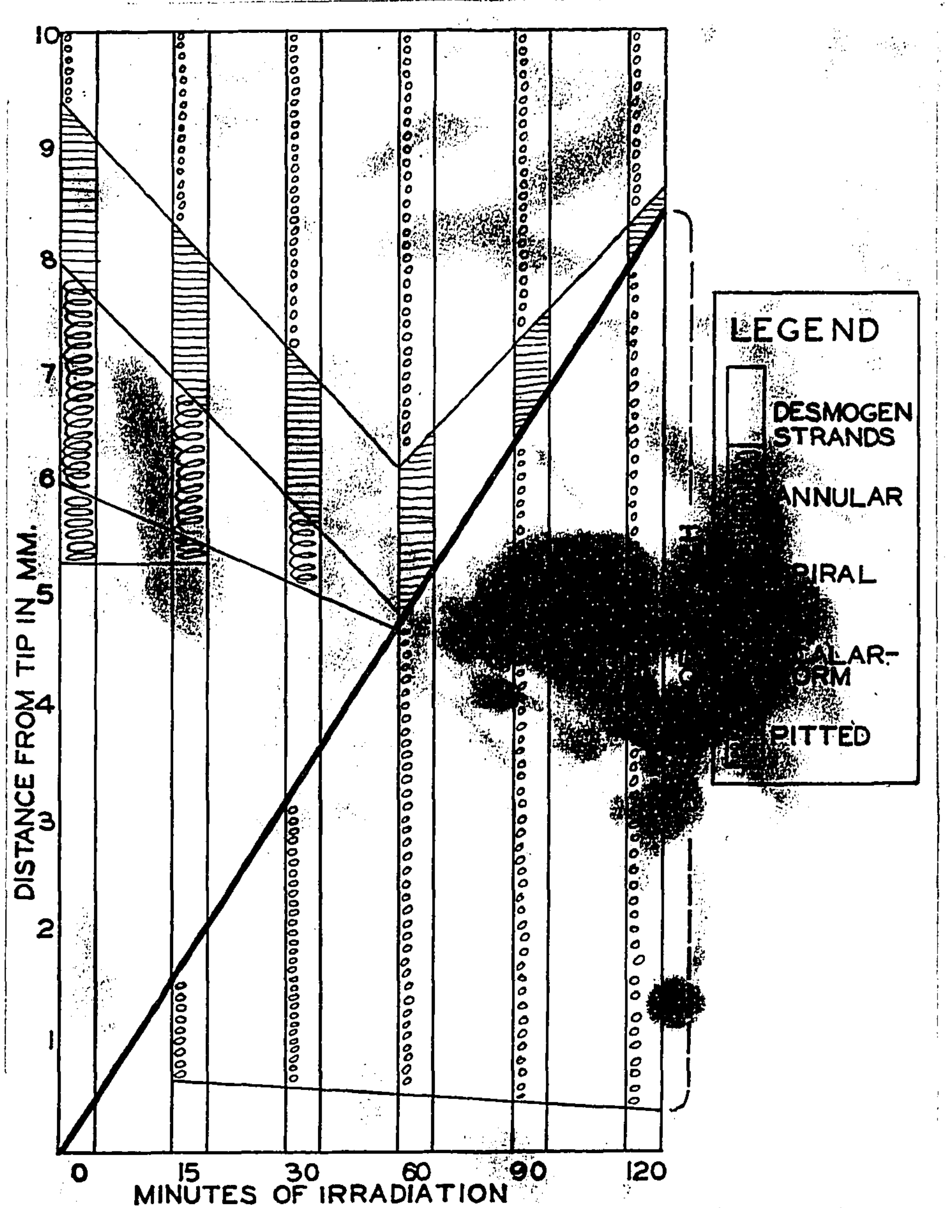

Fig. 8. Diagram showing the order and relative range of vessel types in the root tips of control Vicia faba seedlings and of seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 15, 30, 60, 90 and 120 minutes.

the root and the lignification of desmogen cells going on at that time.

In these roots, it is observed that the inner walls of the young desmogen cells, show an immediate pitted lining of cytoplasm (figs. 3A. 4A), which with subsequent secondary wall thickening, forms the initial pitted vessel character. The fact that this pitted wall thickening is maintained can be related to the arrested elongation of surrounding cells.

The occurrence of pitted vessels in the tips of roots of seedlings grown from x-ray treated seeds, may therefore be interpreted as indicating a period of declining growth so far as it includes elongation, and as portraying the time of initiation of this decline by the extent of these pitted elements upward from the root apice. This suggests a turning point in normal elongation during the early part of germination to a decline resulting finally in a growth cessation. In the seedlings from seeds irradiated for 120 minutes, growth cessation as recognized by the failure of a further increase in length was apparent when the material was taken for histological examination, so that in them it is possible to inspect the extent of elongation during early germination and growth decline before "delayed killing" based on the types of secondary wall thickenings of the xylem elements. Other seedlings throughout the dose range of x-rays selected represent conditions further removed from "delayed killing" as the treatment was decreased. All, however, show similar indications of a decrease in extent of cellular elongation by the presence of pitted wall thickenings in the most recently formed xylem elements. This information is diagrammed in figure 8.

The manner of elongation in the roots of the treated seedlings indicated in normal elongation and in decline of elongation is also shown in the diagram. Normal elongation is recognized by the formation of the annular, spiral, scalariform and pitted vessels, as they may be, in the upper portion of the elongation region. Growth decline, climaxing with cessation of growth, based on the failure of further increase in length, is observed by the presence of pitted vessels in the lower part of the region of root elongation. The extent of normal growth is found to decrease, and the extent of growth decline is found to increase, with increasing x-ray treatments.

The observations are briefly summarized in the following paragraphs:

1. Normal *Vicia faba* seedlings (fig. 2). Vessel formation starts with vacuolation of desmogen cells, causing the cytoplasm to form a pitted lining on the inner wall (fig. 2, A). The first vessel secondary wall thickening is annular, which is followed basipetally by spiral, scal-

ariform, and pitted formations. These are considered to be related to the extent of elongation taking place in the region of the root in which they are being formed; annular and spiral in the region of rapid elongation, and scalariform and pitted in regions of slight elongation.

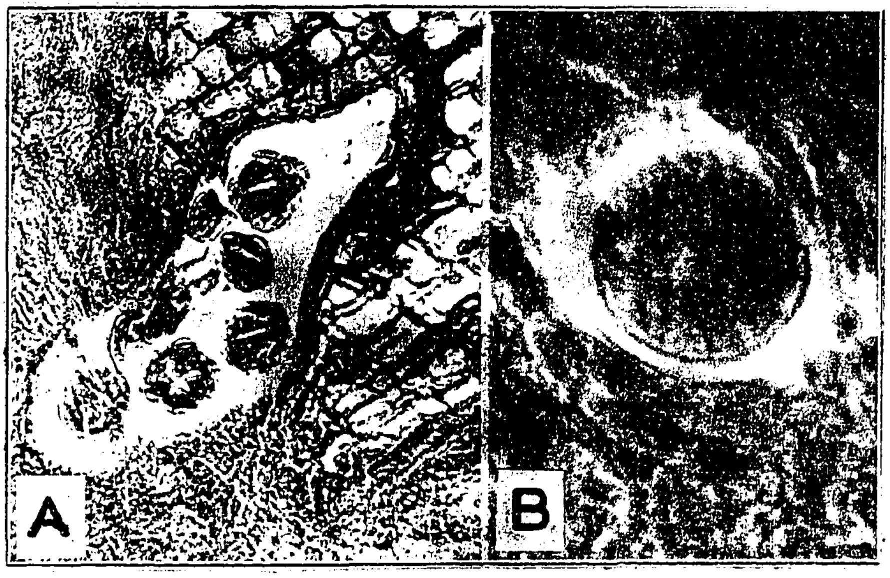

Fig. 9. Modifications about the root tip meristem in seedlings grown from seeds x-ray irradiated at 30 kv., 10 ma., for 120 minutes. A. Extensive lignification with cavities containing giant nucleus-like structures. x 450. B. Enlargement of a structure shown in A. x about 1000.

2. Seedlings grown from seeds irradiated for 15 minutes (fig. 3). In the roots of these seedlings above the pitted vessels of the tip, a region of desmogen strands is present but is less extensive than that observed in control roots. The first vessel type is represented by a few an-

nular elements. Spiral and scalariform thickenings are far more abundant, and are followed by a prominent region of larger pitted vessels.

3. Seedlings grown from seeds irradiated for 30 minutes (fig. 4). Above the pitted vessel present in the lowermost portion of the region of elongation, desmogen cells with vacuolated cytoplasm are evident, but these are rapidly followed by spiral vessels with scalariform and pitted elements appearing in regions above. No annular vessels are observed.

4. Seedlings grown from seeds irradiated for 60 minutes (fig. 5). In roots of these seedlings few desmogen cells are found above the pitted vessels in the tip. Thickenings of the scalariform type appear very rapidly, and a great prominence of pitted vessels are found above. Annular and spiral elements are observed only occasionally in these roots.

5. Seedlings grown from seeds irradiated for 90 minutes (fig. 6). The region of pitted vessels is very pronounced and extends upward in these roots for approximately 6.5 mm. This is followed by a few desmogen strands which soon show wall thickenings of the scalariform type. These, however, are considerably less in number than in preceding lots. The pitted vessels appearing next are found throughout the remaining part of the region of root elonga-

tion.

6. Seedlings grown from seeds irradiated for 120 minutes (fig. 7). The vessel type in roots of these seedlings is almost completely pitted; a few scalariform elements are found approximately 8.5 mm. from the tip (fig. 8). Further inspection of these roots indicates a very extensive lignification of the tip, in which cavities are observed containing large structures which seem to represent giant nuclei. These structures are shown in figure 9, but no explanations are attempted here. The very pronounced curling of the root tip appears characteristically in seedlings irradiated with this dose of x-rays.

Summary.

1. Roots of Vicia faba seedlings grown from unsoaked seeds irradiated with soft x-rays, show a decreasing total elongation as increasing doses of x-rays are employed.

2. A histological study of such roots and of control roots affords adequate data to demonstrate experimentally that the type of wall thickening found in vessels is determined by the extent of elongation in surrounding tissues.

3. After the onset of "delayed killing" in seedlings grown from x-ray irradiated seeds, evidenced by a decline in growth as it involves elongation, only pitted vessels

are formed. This is aparently related to a markedly decreased extent of elongation of cells surrounding the vessels.

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FAILURE OF ROOT TIPS OF TOMATO SEEDLINGS GERMINATED FROM X-RAYED SEEDS TO GROW IN VITRO.

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Vol. 17, No. 2. April 1942.

Histological studies of roots of Vicia faba and of Zea mays seedlings grown from seeds given a medium dose of soft x-rays, indicate considerable distortion and irregularity about the apical meristem and surrounding tissues after the first week of germination (7, 8). These are not apparent during the early part of germination when the radicles first appear; the meristem is relatively normal in arrangement as compared with that in nontreated control seedlings (8).

In view of this effect, it was considered of interest to determine the ability of root tips of seedlings germinated from x-rayed seeds to continue growth in vitro; the results of which might indicate whether the abnormalities found at the apical meristem are localized effects of the x-ray radiation, or effects influenced more directly by functional irregularities occurring elsewhere in the root or in the seedling as a whole.

Unsoaked beef steak tomato seeds were irradiated 8 cm. from the focal spot of a copper target gas x-ray tube operated at 30 peak kv., and 10 ma. for 30 minutes. It was observed by separate tests that lateral roots do not appear during later germination of tomato seedlings from seeds given this x-ray treatment. The culture methods employed followed procedures listed by Bonner (4), Bonner and Addicott (5), Addicott (1), Addicott and Bonner (2), Addicott and Devirian (3), and Robbins and Bartley (6).

When root lengths of approximately 1 cm. were attained in aseptic seedlings from both irradiated and non-irradiated seeds, 5- to 10-mm. root tips (50 seedlings of each group) were cut and transferred to a previously sterilized nutrient medium containing: 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 81 mg. KNO_3; 36mg. $MgSO_4 \cdot 7H_2O$; 65 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. Fe-tartrate; 20 gm. sucrose, per liter of Pyrex redistilled water. Nicotinic acid (0.5 mg.), vitamin B_1 (0.1 mg.) and vitamin B_6 (0.1 mg.) were added per liter of nutrient.

The root tips of both seedling groups appeared to be alike in growth rate and size at the time of isolation. In view of the uniformity of the data, one repetition of the original results seemed adequate to confirm the observations made.

In figure 1A is shown the extent of growth of the excised root tips of control seedlings after four weekly transfers; B indicates that which occurred in the tissues excised from seedlings from the irradiated seeds. In the latter, the cultured root tips generally display only a very slight increase in length with some increase in thickness and, in addition, become inactive and browned at the apices soon after isolation.

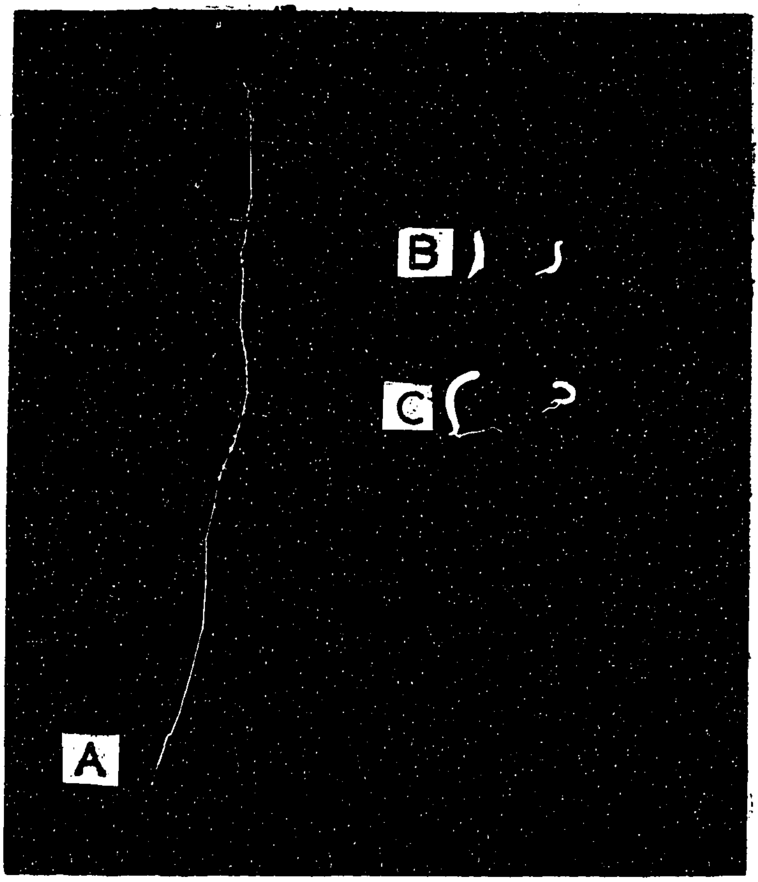

Fig. 1. Shadow photographs of excised root tips of tomato. A, normal, at the end of four transfers; B, root tips of seedlings from x-rayed seeds, at the end of four transfers; and C, at the end of six transfers.

When transfers of tissues from the treated seeds were continued for five to six weeks, an activation of a few cells above the root tip frequently occurred, resulting in secondary root formation at these points (C). This occurrence might be attributed to a continued functioning of a few cellular elements having meristematic activity, which escaped effects induced by the x-ray radiation treatment of the seeds.

The inability of excised root tips of tomato seedlings grown from seeds given a medium dose of soft x-rays, to grow in vitro is probably associated directly with factors active in continuous meristem activity in the root tip, present in the seed at the time of irradiation. The fact that the tissues did not grow in a nutrient medium adequate to support the continuous growth of normal root tips of tomato, seems to remove the alternative supposition that the effect is dependent upon modifications present in other parts of the germinating seedlings.

This work was repeated at the California Institute of Technology. Appreciation is expressed to Dr. F. W. Went and to Dr. James Bonner for comments and use of their laboratories.

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STIMULATIVE EFFECTS OF X-RAYS ON PLANTS, ROOTS OF _ZEA MAYS_ FROM X-RAYED SEEDS.*

(WITH ELEVEN FIGURES.)

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Introduction.

The majority of previous investigations dealing with the effects of x-rays on plants have been concerned with the question of growth stimulation of the aerial portion of the plant resulting from treatments with weak doses of x-rays (4, 5, 6, 8, 9, 13, 14). The methods generally employed, involved an x-ray treatment of unsoaked or soaked seeds, or of growing points. Observations recorded during subsequent growth, particularly at maturity, were examined for evidences of stimulation. Reports of the stimulative effects of x-rays on the growth of the aerial portions of the plants are numerous, but in only a few cases, is the evidence conclusive. Inadequate sampling and limited test periods have been given as reasons for regarding the evidence as inconclusive.

The view that a consistent growth stimulation effect might be obtained by using a specific x-ray dose, was emphasized by Shull and Mitchell (21). They employed metallic screens, high voltage, low amperage and short exposures. The use of metallic screens eliminated the soft x-rays.

In addition to the x-ray treatment however, it is also necessary to consider the condition of the test material. Chemical alterations, generally destruction and re-synthesis are commonly affected by x-ray irradiation and in biological materials, these may well be the fundamental factors concerned. Such x-ray induced changes would undoubtedly alter plant growth in view of its chemical nature due to the presence and activities of auxins, calines, growth inhibitors and other growth factors (18). The experiments reported in this investigation, however, were not designed to determine this chemical aspect of growth stimulation, but it is worthy of consideration in future experiments of this sort.

It is the purpose of the work described here to investigate the question of stimulation of primary root growth in seedlings of seeds given a small dose of soft x-rays. Previous work by Rivera (16, 17) reported stimulative effects of treating roots with small doses of x-rays. This however, was not a study on root growth stimulation directly, but rather on the resulting growth acceleration of aerial portions of plants following the irradiation of the roots.

In experiments described in this paper, corn was found to be most suitable for the method of germination employed. Stimulation as it will be used throughout refers to an increase in root length, or a greater wet or dry weight of roots of seedlings from treated seeds as compared with that occuring in the roots of normal seedlings. Observations were limited to the first five days of germination and the data presented refer solely to that period.

Methods.

The x-ray radiation was provided by a gas type x-ray tube (12) having a copper target, operated at 10 ma. and at various voltages mentioned later. The seeds which were irradiated were arranged with embryo toward the source of radiation at a distance of eight cm. from the focal spot of the tube. The window of the tube which was made of aluminum foil and Cellophane, transmitted the K. and K^{8} characteristic radiation (1.54 and 1.38 A°.) as well as that of both longer and shorter wavelength radiation of the continuous spectrum of the copper target. The shortest wavelength emitted in any experiment was approximately 0.5 A°. In every case, the time of exposure was 10 sec.

After considerable preliminary work for the purpose of determining the requirements necessary to obtain sufficient data it was decided to construct an apparatus which would permit 2000 seeds to be germinated simultaneously in water culture under controlled conditions. The apparatus, Fig. 1, consisted of a large wooden cabinet in the bottom of which was a tank containing tap water kept at 29°C. by thermostatic control. A uniform temperature of the water was maintained by the use of a stirring motor equipped with paddles to circulate the water in the tank. A constant flow of fresh water through the tank was made possible by small entrance and exit tubes.

The frame which supported the seeds during germination consisted of a rack suspended from above by four cables.

The position of the rack below or above the water level was automatically controlled by means of a crank operated by an electric motor. Across the rack were placed forty metal rods, each equipped with fifty fine nickel wire hooks. The purpose of each hook was to support a corn seed in its normal position by passing the hook through the endosperm portion of the seed at a point removed from the embryo.

The corn used in the experiments was Stowell's Evergreen Sweet Corn, 1940 crop. Badly formed and inferior kernels were discarded. The selected seeds were prepared for germination, the first part of which involved a twenty-four soaking period, as follows: fifty corn seeds were placed in each of forty copper wire baskets, designed as seed soaking containers. Twenty of these baskets of seeds were arbitrarily chosen as controls and the seeds in each of the remaining twenty baskets were given identical x-ray treatments.

It was found by trial that fifty roots, that is the content of one rod, could be examined for length and wet weight in a ten minute interval. Consequently, in order to have all plants of the same age when observations on root growth were made, the following procedure was adopted: fifty seeds were irradiated, replaced in a particular basket and the basket suspended from the rod to which the seeds were to be attached after soaking. Ten minutes later a basket containing non-irradiated seeds was suspended from the next rod. After another ten minute interval a basket of irradiated seeds was suspended from the third rod. This procedure of starting the soaking alternately of control and irradiated seeds every ten minutes was continued for each of the forty rods.

After a 24-hour soaking period the seeds were attached to the hooks of the rod which had suspended the basket. When the seeds were arranged on the wire hooks, a mechanism was started which automatically controlled the movement of the entire rack to positions below or above the water level. Preliminary experimentation indicated that good germination was possible if the germinating seeds were immersed in water for five minute periods followed by two hour intervals during which time they were suspended above the water.

At the end of the germinating period of four days, the rods were removed from the apparatus at ten minute intervals in the same order as they were originally arranged, so that the age of the seedlings from the time that the seeds were first put to soak until they were harvested, was exactly five days. As each rod with its lot of seedlings was taken from the rack, the roots of the plants were severed as close to the seed as possible. The length of each root was measured and the total wet weight of the roots from the seedlings on this rod was determined. The roots were then placed on paper and dried in the open air in the laboratory for seven days, after which time the total dry weight of the roots were determined. A seedling which did not produce root growth of at least two mm. in length was considered as not germinating.

Results.

The results obtained show such a degree of variability that it is difficult to make a reliable interpretation of them without the use of statistical analysis. Tests of significance (7) were applied in comparing the experimental values obtained from the irradiated seeds with those obtained from the non-irradiated controls to determine whether the differences in these values would be ascribed, without much doubt, to a difference in the treatments received by the samples or whether they could be due to random sampling.

A comparison of the results obtained has been made with regard to the following variates:

The number of seeds germinating on each rod, x_1.

The total root length per rod, x_2.

The average root length per seed germinating on each rod, x_3.

The length of the longest root on each rod, x_4.

The total length of the 25 longest roots on each rod, x_5.

The total wet weight of roots per rod, x_6.

The average wet weight of root per seed germinating on each rod, x_7.

The total dry weight of roots per rod, x_8.

The average dry weight of root per seed germinating on each rod, x_9.

Each of the nine variates was tested in two ways. First, the control seeds and irradiated seeds were regarded as separate and distinct samples chosen at random from the same population group. The difference between the mean value of a variate for the controls and for the irradiated seeds was tested to see if this difference was significant. Second, each rod of irradiated seeds was paired with an adjacent rod of control seeds. The differences of the variates for each pair was tested to see if its mean value differed significantly from zero. The first test lead to a probability value p_1, which measured the probability of choosing from the same population group two random samples having mean values which differed by at least as much as that observed. The second test lead to a probability value p_2 which measured the probability of choosing from a population group having a zero mean value a random sample having a mean value which differed from zero by as much or more than that observed. The usual convention of selecting a probability level of 5 percent or less as being significant has been adopted.

The percentage stimulation based upon the mean value of each variate for the control and for the irradiated seeds is given in Table I. The parentheses enclose those values for which one or both tests for significant difference indicated a real difference in the treatments of the controls and irradiated seeds. Except in one case, the only significant differences were obtained from samples irradiated at 17.5 and 20 peak kv. This one exception was in Ex. 5 for which the voltage was zero. Since there was no difference in the treatments of those seeds marked rayed and those marked control, this difference must be attributed to chance.

Using a 15 mm. class interval for the frequency tables of the root lengths of seedlings, the modal class for the seedlings from the control and irradiated seeds are given in Table II. The percentage increases in the midpoint of this interval for the seedlings from the irradiated seeds over that for the control seedlings are given in this table. The averages of the percentage increases at 0 kv. and 20 kv. are 3.8 and 13.1 respectively. The tests of significance do not indicate that the averages differ significantly from zero or from each other. The average of the increases at 17.5 and 20 peak kv. is 13.5 percent.

This value does differ significantly from zero but not from the results obtained at zero kv.

The percentages of stimulation at the various voltages are represented graphically in Fig. 2-11.

TABLE I

			Percent stimulation of variate (based on mean value of variate)								
			x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9
Experiment number	Peak kilovolts	r units per minute	No. of seeds germinating	Total root length per rod	Ave. root length per seed germinating on each rod	Length of longest root on each rod	Total length of 25 longest roots on each rod	Total wet wt. of roots per rod	Ave. wet wt. of root per seed germinating on each rod	Total dry wt. of roots per rod	Ave. dry wt of root per seed germinating per rod
1	20	1000	(6.2)	(10.5)	(4.2)	3.0	(5.4)	(10.1)	3.7	(10.2)	4.0
2	15	450	- 1.1	2.6	3.9	2.8	2.3	2.5	3.2	2.6	3.3
3	0	0	-17.0	1.4	3.4	4.2	2.1	1.2	3.3	2.2	4.3
4	17.5	750	- 0.1	2.6	2.2	1.2	2.7	(8.6)	(8.3)	4.3	4.3
5	0	0	- 2.2	-1.3	0.1	-0.5	-1.8	3.2	(5.2)	0.6	2.6
6	22.5	1300	0.1	-0.8	-0.3	-1.1	-1.0	-3.1	-2.7	- 0.6	-1.0
7	20	1000	(7.2)	(10.2)	2.2	1.4	(3.6)	(8.8)	1.2	(9.2)	1.4
8	25	1700	0.4	-1.6	-2.2	0.7	-1.0	-5.6	-5.7	- 0.8	-1.6
9	20	1000	- 3.0	0.4	(3.3)	-1.5	1.0	0.6	3.7	0.3	(3.3)

Numbers in parentheses are those values for which one or both the tests for significance of differences indicated a real difference in the treatments of the controls and the irradiated seeds.

TABLE II.

Most probable length of root (15 mm. interval).

Experiment number	Peak kilovolts	r units per minute	Midpoint of interval		Frequency		Percent Stimulation.
			C	R	C	R	
1	20	1000	101	112	172	183	10.9
2	15	450	94	102	154	148	8.2
3	0	0	86	89	104	221	3.5
4	17.5	750	75	86	185	202	14.7
5	0	0	71	74	171	180	4.2
6	22.5	1300	81	76	212	218	-6.2
7	20	1000	86	105	173	193	22.1
8	25	1700	130	127	159	133	-2.3
9	20	1000	119.5	127	216	201	6.3

Discussion

No very great stimulation in primary root growth of corn seedlings from seeds given weak x-ray treatments as compared with that of control seedlings was obtained. However, statistical analysis of the data indicates beyond a reasonable doubt that the treatment of these seeds by x-rays employing a voltage in the neighborhood of from 17.5 to 20 peak kv. did produce a significant increase in the average length and in the wet and dry weights of the roots of the seedlings. Voltages of 22.5 and 25 peak kv. did not produce a significant change in any of the variates examined.

It is suggested by the data that the amount of radiation as well as the condition of the biological material to be treated which might induce growth stimulation may be very sharply defined. A slight change from the optimum condition for growth stimulation may fail to produce sufficient stimulation to detect or may even induce growth inhibition.

Summary.

1. An apparatus for seedling germination which fulfills requirements of adequate sampling necessary to obtain reliable conclusions in investigations of x-ray induced growth stimulation has been described.

2. Under the conditions of these experiments it is possible to obtain an apparent x-ray induced stimulation in the primary root growth of Zea mays seedlings. Statis-

tical analysis of the date obtained indicates beyond a reasonable doubt that stimulation of root growth was obtained by irradiating dry seeds at voltages in the neighborhood of 17.5 to 20 peak kv. The analysis shows that differences as much as those observed would occur in fewer than five cases in one hundred trials if the x-ray treatment made no essential difference in root growth.

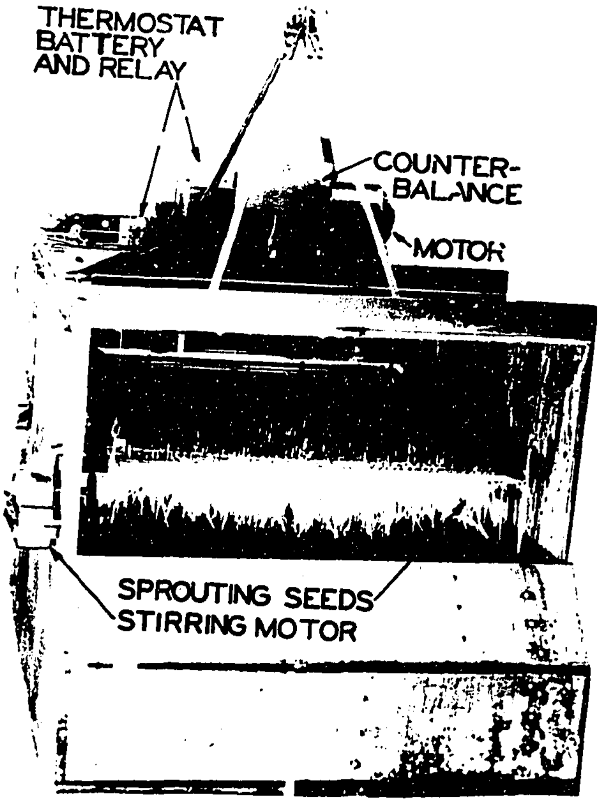

Fig. 1. Apparatus for seedling germination under controlled experimental conditions.

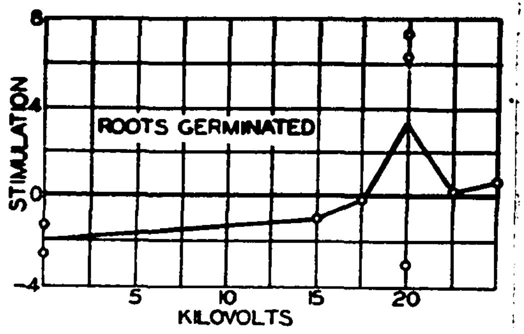

Fig. 2. Percent stimulation determined from the number of seeds on each rod (x_1) as a function of voltage.

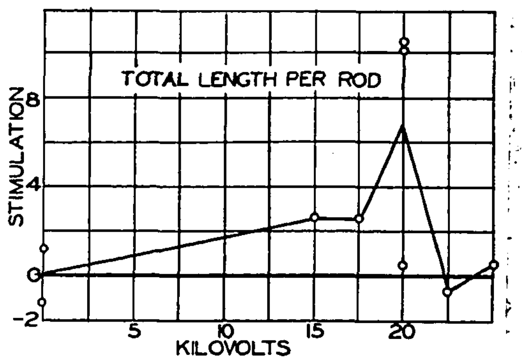

Fig. 3. Percent stimulation determined from the total root length per rod (x_2) as a function of voltage.

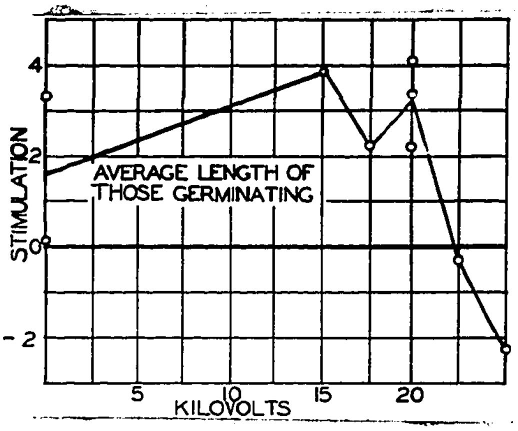

Fig. 4. Percent stimulation determined from the average root length per seed germinating on each rod (x_3) as a function of voltage.

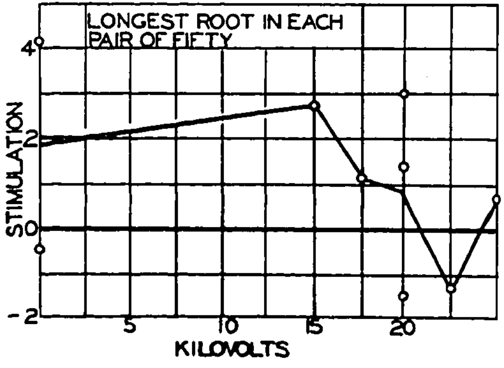

Fig. 5. Percent stimulation determined from the length of the longest root on each rod (x_4) as a function of voltage.

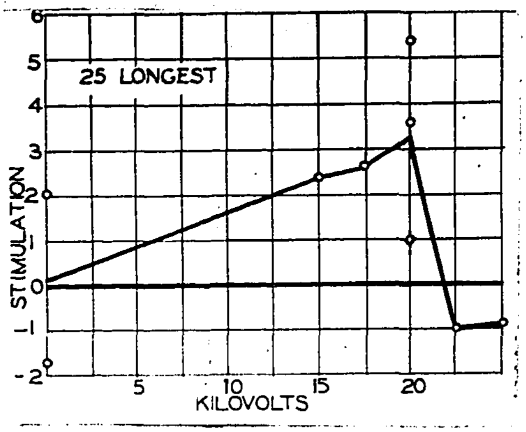

Fig. 6. Percent stimulation determined from the total length on the 25 longest roots on each rod (x_5) as a function of voltage.

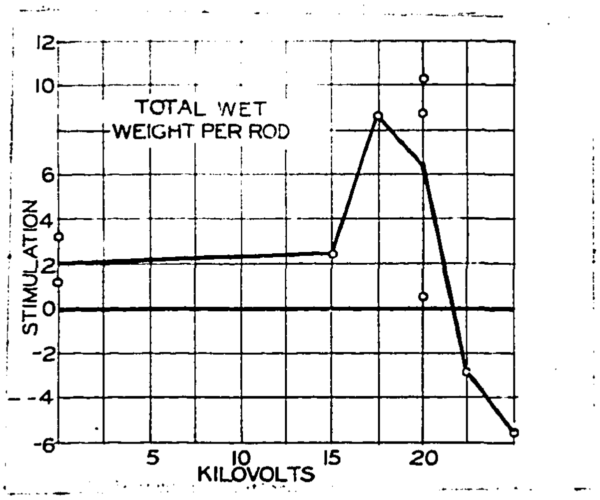

Fig. 7. Percent stimulation determined from the total wet weight of roots per rod (x_6) as a function of voltage.

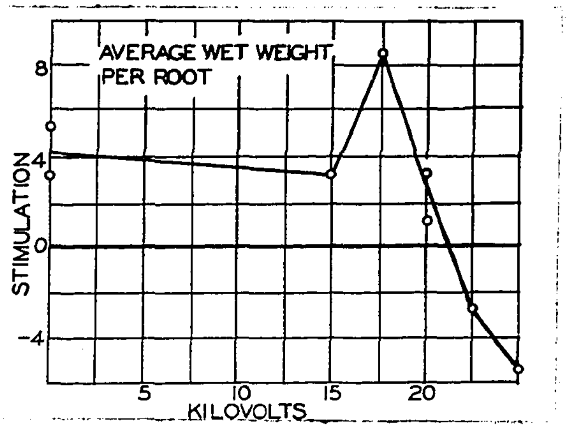

Fig. 8. Percent stimulation determined from the average wet weight of root per seedling germinating on each rod, (x_7) as a function of voltage.

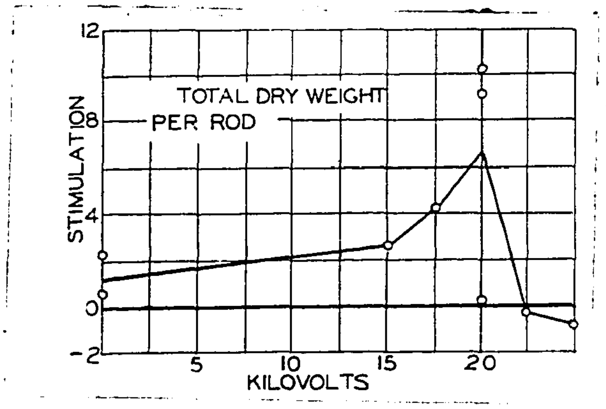

Fig. 9. Percent stimulation determined from the total dry weight of roots per rod (x_8) as a function of voltage.

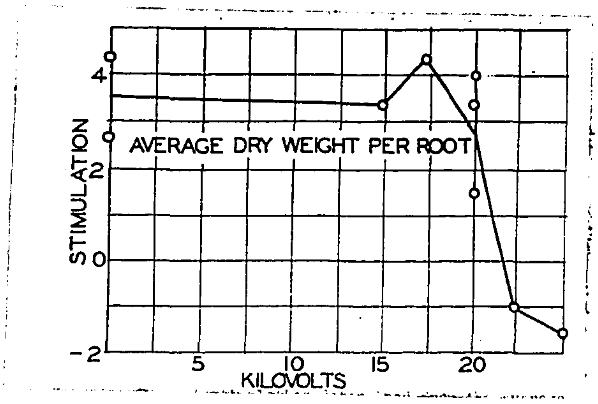

Fig. 10. Percent stimulation determined from the average dry weight of root per seed germinating on each rod (x_9) as a function of voltage.

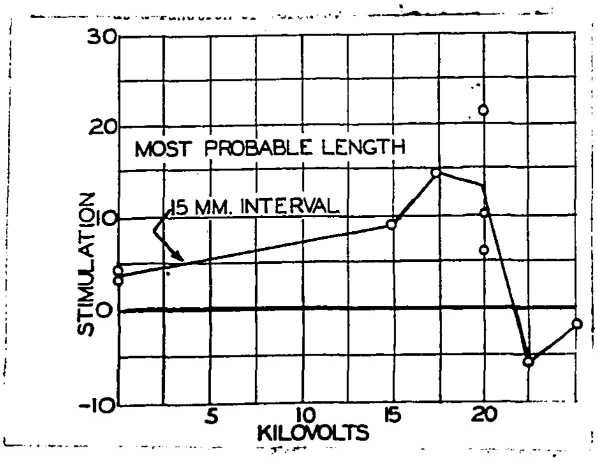

Fig. 11. Most probable length (15 min. intervals) as a function of voltage.

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AUXIN AND CALINES IN
SEEDLINGS FROM X-RAYED SEEDS.

Amer. Jour.. Bot. in press.

AUXIN AND CALINES IN SEEDLINGS FROM X-RAYED SEEDS.[1]

It has been previously established by Went (1938, 1939) that certain specific factors other than auxin, are involved in the growth of plants. These have been placed in a group of plant harmones termed "calines", having the essential character of being transported through living tissue only. Included are: rhizocaline, obtained from the cotyledons in the case of etio-lated pea seedlings, which with auxin is responsible for root formation; caulacaline, formed in the roots, which is necessary in conjunction with auxin for stem elongation; and phyllocaline, a requisite for leaf growth, is auxin independent and in the case of pea seedlings germinated in the dark, is derived solely from the cotyledons. The schemes of activity of these factors as correlated with auxin in both light and dark germinated pea seedlings are diagrammed in Figs. 1 and 2.

Originally the presence as well as the differential character of the calines were indicated by tests in which the supply of one or another of the calines was experimentally removed by the excision of various plant parts. For

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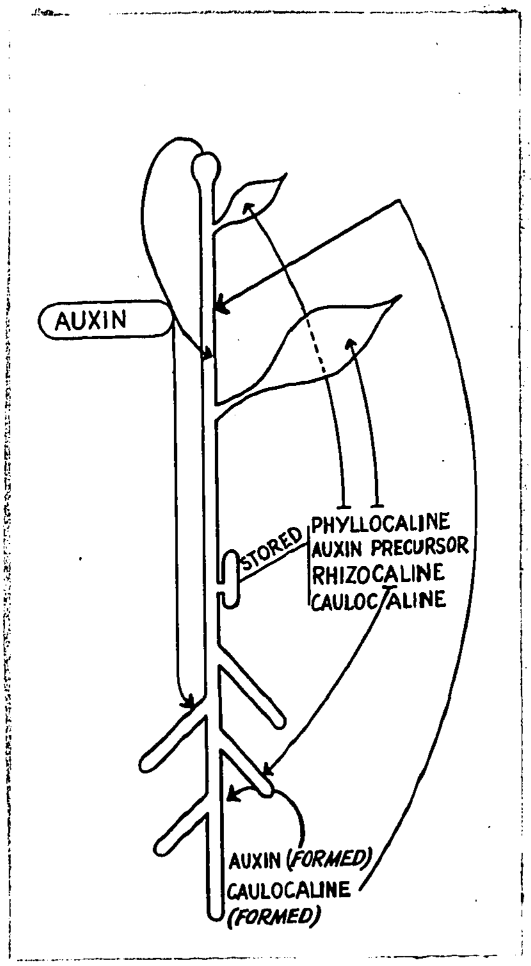

Fig. 1. Factors involved in dark-room germinated peas.

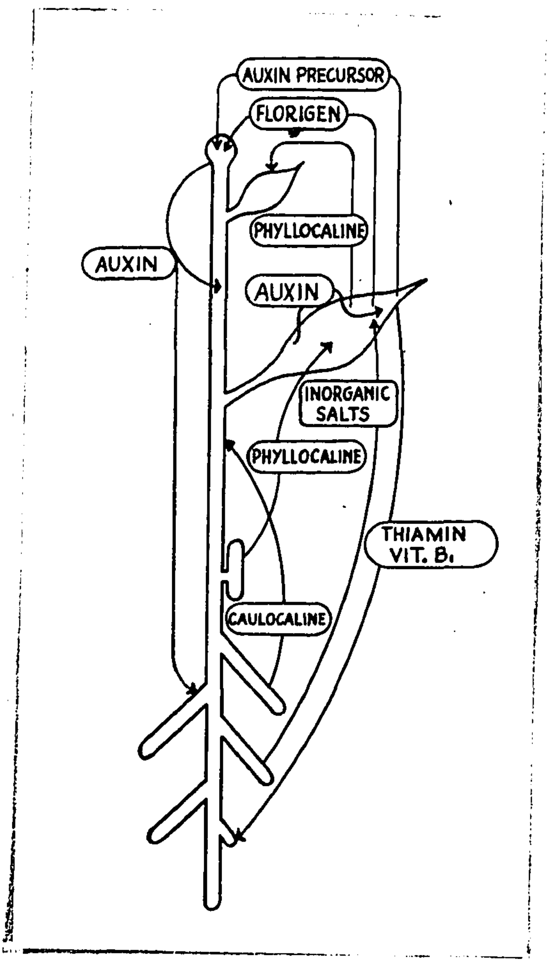

Fig. 2. Factors involved in light germinated peas.

example, a removal of the cotyledons from etiolated pea seedlings was found to hamper root and leaf development, while de-rooted pea seedlings exhibited modifications in the elongation of stems in which growth in length ultimately ceased, Went (1939).

Additional evidence for the existence of calines as well as for their habit of transport through living tissues only, was assembled by performing experiments in which various amounts of calines were added by grafting experiments with peas using bases of varieties having different contents of some caline, Went (1938). In all instances it was found essential that a graft union incorporating vascular tissue continuity be accomplished between stock and scion before any influence of the base type be exhibited, Hayward and Went (1939).

The investigation described in this paper supplies further data for the presence of calines and especially stresses their differential character as they are involved in the growth of both intact and grafted seedlings. The experimental method employs one variety of pea seedling, Alaska, in which it had been found possible to alter the content of one or another of the calines as present in the seed, by treating the seeds with x-rays prior to germination. Tests to determine auxin conditions in seedlings from x-rayed seeds which indicate results comparable to those obtained by Skoog

(1937) in his study on the effect of x-rays on auxin and plant growth, are also given.

Methods.

As to the x-ray treatment of seeds, it will suffice to mention that the radiation was of the soft x-ray type, supplied by a copper target gas x-ray tube, equipped with a window of aluminum and cellophane, which transmitted the K_A and K_B lines of copper as well as the longer and shorter wavelengths in the continuous spectrum, Kersten (1934). Because of the great amount of plant material required for the investigation, the seeds were oriented at random during treatment, at a distance of 8 cm. from the focal spot of the x-ray tube, which was operated at 30 peak kv. and 10 ma. Under these conditions the intensity of the x-ray beam was approximately 2000 r/min. Different exposures used will be indicated later.

All procedures were carried out in a physiological dark room, 24-26^{o}C and 85 percent humidity. The pea grafting technique employed was that described by Went (1938). One week-old etiolated pea seedlings were used. The stem portion between mid-first and mid-third internodes was removed by two oblique cuts made at the same angle by use of a small mitre box-like structure through which a razor blade could be passed over the stem. The facing cut surfaces of the remaining stem portions were brought into contact through a

TABLE I

Maximal growth rate, leaf and stipule surfaces, petiole and tendril lengths, of first, second and third leaf developed in Alaska pea seedlings of control seeds and of seeds given a small x-ray radiation treatment (30 kv, 10 ma, 8 cm, 15 min.) after 2 weeks germination. Values given are the mean of 40 plants

	Leaf			Petiole	Stipule			Tendril	Maximal growth per day
	Length	Width	Surface	Length	Length	Width	Surface	Length	mm.
Control									40.2
First leaf	6.1	3.9	23.8	6.3	4.3	3.1	13.3	2.9	
Second leaf	7.7	4.8	37.0	8.1	6.1	3.9	23.8	5.2	
Third leaf	7.3	5.0	36.5	9.9	5.9	3.9	23.0	9.7	
X-rayed									37.5
First leaf	4.4	2.8	12.3	4.7	3.5	2.7	9.5	1.5	
Second leaf	6.0	4.0	24.0	6.9	4.8	3.4	16.3	3.8	
Third leaf	6.6	4.7	31.0	10.1	5.5	3.8	20.9	8.3	

5 mm. piece of glass tubing having a bore which approximated the diameter of the seedling stem.

After the graft preparation, measurements of the stem lengths were taken over a period of 18-20 days by which time maximal growth had been achieved in most cases and at this time, leaf, stipule, petiole and tendril measurements were generally recorded according to purposes of the particular experiment. Leaf part measurements were supplemented by results of bio-assays for leaf growth factors as outlined by D. B. Bonner, A. J. Haagen-Smith and F. W. Went (1939). Auxin conditions were determined by the standard *Avena* test as specified by Went and Thimann (1939).

Throughout the paper, terms such as x-rayed or treated seedlings, tops, or bases, will be used to refer to seedlings or parts of seedlings from x-rayed seeds.

Growth of intact seedlings with x-ray modified caline content. A number of experiments were run with Alaska peas from seeds given brief x-ray exposures. The treated seedlings as well as an equal lot of control seedlings were germinated individually in 20 cc. bottles. Daily growth rates were recorded for 18 to 20 days, by which time maximal growth had generally occurred. Measurements of leaf, tendril, petiole and stipule of the first, second and third leaf developed were taken. Table I lists data from a typical test in which the exposure time was 15 minutes. Leaf and stipule surfaces were calculated by determining the products of mean lengths and mean widths.

Data presented in the table indicate that the maximal growth rate value for both treated and normal intact seedlings, is relatively constant. Normal variations in Alaska peas generally fall within the range of 34-42 mm. per day, so that the slightly lesser value in the x-rayed set is probably of no experimental significance.

There is, however, a definite decrease in the size of foliar parts of all seedlings from the x-ray treated seeds. This is also illustrated in Fig. 3.

Grafting experiments with peas from control seeds and from x-rayed seeds: Later experiments were carried on with seedlings from seeds exposed to x-rays for 60 minutes while oriented at random. Due to this random orientation, a single effect of the treatment was not apparent. The types of seedlings from seeds treated in this way can be arranged into four arbitrary groups, Fig. 4. These are: (1) normal maximal growth rate, reduced leaf size, normal root development: (2) reduced maximal growth rate (about 1/3 normal), reduced leaf size, normal root development; (3) reduced maximal growth rate (about 1/10 normal), normal root development; (4) reduced maximal growth rate (about 1/10 normal), poorly developed roots.

Grafting experiments with seedlings from group 1, em-

Fig. 3. Seedling tops from control seeds (left) and from seeds given a 15 min. exposure to x-rays having an intensity of approximately 2000 r/min.

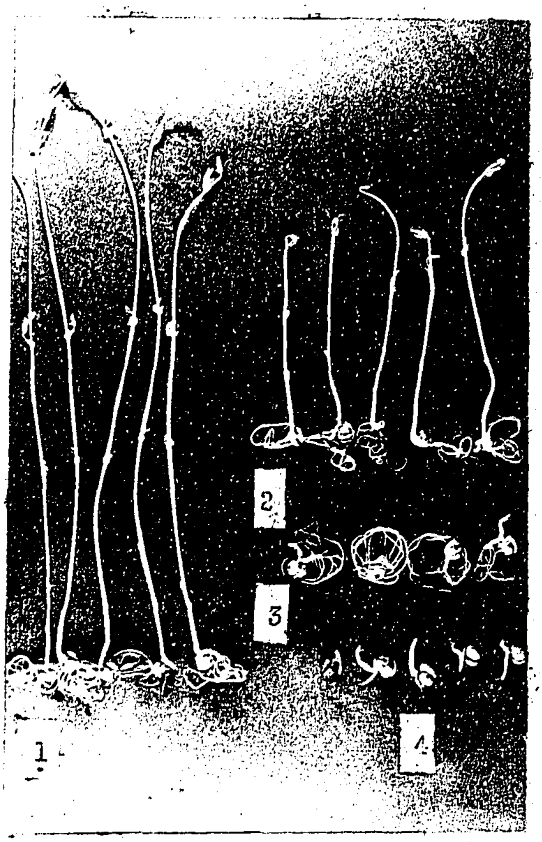

Fig. 4. Seedling groups from seeds oriented at random while being exposed for 60 minutes to x-rays having an intensity of approximately 2000 r/min.

ploying various combinations of tops and bases of control and treated seedlings, gave results shown in Table II. The following effects are indicated: (1) uniform maximal growth rates for intact and grafted control and treated seedlings; (2) similar size of foliar parts of control-intact and of control-self-grafted seedlings; (3) similar size of foliar parts of x-rayed intact and x-rayed self-grafted seedlings; (4) reduced size of all foliar parts of x-rayed intact and of x-rayed self-grafted seedlings as compared with values given for control seedlings; (5) decreased size of foliar parts of control tops grafted on x-rayed bases, as compared with normal leaf size; (6) increased size of foliar parts of x-rayed tops grafted on control bases, as compared with treated leaf size.

Apparently the effect of this particular x-ray seed treatment is on the phyllocaline supply which in dark-room germinated seedlings, is available solely from the cotyledons. To check this conclusion further, assays for leaf growth factors were run, using methods described by D. M. Bonner, A. J. Hagen-Smit, and F. W. Went (1939). Seed diffusate of Alaska peas was used as a source of leaf growth factors. Circular disks of leaf tissue, 19.5 mm. in area were cut from young primary leaves of radish. Twelve such disks were floated on 2 cc. of a culture medium in Syracuse watch glass dishes and grown for 30 hours at 25°C. Total

TABLE II

Maximal growth rate, leaf surface, stipule surface, petiole and tendril lengths of first, second and third leaves of intact and grafted seedlings of control seeds and of seedlings grown from x-rayed seeds referred to as group 1 in Fig. 4. Mean values given represent 15 to 30 plants.

	Leaf			Petiole	Stipule			Tendril	Maximal growth per day
	Length	Width	Surface	Length	Length	Width	Surface	Length	mm
Control intact									51.5
First leaf	6.2	3.9	24.2	6.5	4.3	3.2	13.8	3.2	
Second leaf	7.8	4.9	38.2	8.8	6.5	3.9	25.4	5.9	
Third leaf	8.7	5.8	50.5	10.5	6.3	4.2	26.5	9.3	
X-rayed intact									39.8
First leaf	4.1	3.0	12.3	4.3	3.6	2.7	9.7	1.5	
Second leaf	5.3	3.6	19.1	5.4	4.7	3.5	16.5	3.8	
Third leaf	6.2	4.2	26.0	6.7	5.3	3.7	19.6	7.1	
Control self-grafted									40.5
First leaf	6.0	3.9	23.4	6.8	4.6	3.4	15.6	3.1	
Second leaf	7.8	4.7	36.7	8.6	5.9	3.8	22.4	5.4	
Third leaf	9.0	5.8	52.2	8.5	6.6	4.6	30.4	10.4	
X-rayed self-grafted									40.8
First leaf	4.2	2.9	12.2	4.9	4.0	3.1	12.4	1.8	
Second leaf	5.9	3.7	21.8	5.9	4.8	3.5	16.8	3.5	
Third leaf	6.1	4.3	26.2	6.5	5.4	3.8	20.5	6.9	
Control on X-rayed									41.4
First leaf	6.5	4.0	26.0	5.8	4.9	2.9	14.2	3.7	
Second leaf	6.2	4.0	24.8	6.0	5.3	3.7	19.6	3.9	
Third leaf	6.3	4.3	27.1	6.0	5.5	3.5	19.3	6.7	
X-rayed on control									39.1
First leaf	5.4	3.5	18.9	5.3	3.3	2.6	8.6	1.7	
Second leaf	7.2	4.4	31.7	6.8	5.3	3.6	19.1	4.5	
Third leaf	7.7	5.1	39.3	9.0	6.5	4.2	27.3	8.8	

weight of all 12 sections was determined by direct weighing. The culture medium consisted of a basic medium of 1 percent sucrose plus various concentrations of a standard solution having a dry weight of 10 mg. per cc. Diffusates of normal and of x-rayed seeds were used in testing. Results of typical experiments are summarized in Fig. 5. The results are expressed in terms of leaf units (L.U.). One L.U. being defined as the activity of a solution containing 1 mg. dry weight of normal pea diffusate per cc. of 1 percent sucrose solution. Values given are the mean of 5 to 15 tests.

In the assays using diffusate of seeds x-rayed for 60 minutes before extraction, there is considerably less growth of the cultured leaf disks than in cultures on normal diffusate for all concentrations of the diffusate employed. Diffusate of seeds x-rayed for 150 minutes displays a still greater decrease in leaf growth activity. In the latter, there is little activity over that apparent in cultures on the basic medium of 1 percent sucrose with no added diffusate. From this it is evident that the effect of reduced leaf size of the treated seedlings is a result of the x-ray modified phyllocaline content of the cotyledons.

Results of grafting experiments with seedlings of

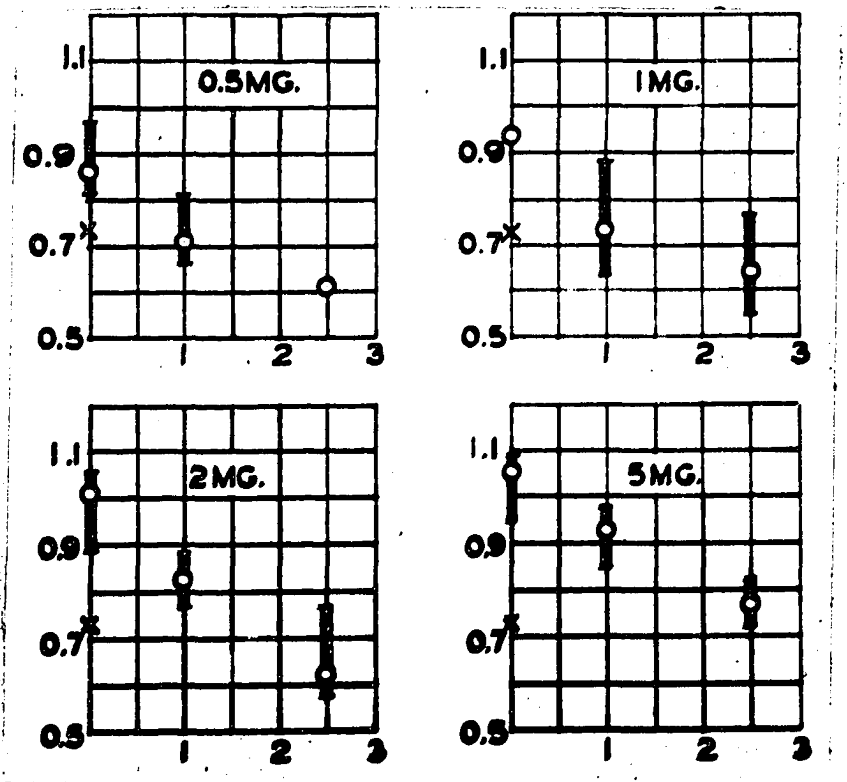

Fig. 5. Results of leaf growth factor assays employing diffusates from non-irradiated seeds and from seeds irradiated for 60 and 150 minutes. The vertical scale indicates leaf units of activity of the diffusate tested and the horizontal scale represents hours of exposure to x-ray irradiation of approximately 2000 r/min. The graphs represent values obtained throughout a dilution series of 0.5, 1.0, 2.0, and 5.0 mg. dry wt. of pea diffusate per cc. of the basic medium of 1 percent sugar. The mean value of tests using 1 percent sugar without added diffusate is indicated by X.

TABLE III

Mean length in mm. of leaf, stipule, petiole and tendril of Alaska pea seedlings of control seeds and of x-rayed seeds, the latter seedlings belonging to the group referred to as 2 in Fig. 4. Measurements were taken of the first and second leaf developed, after maximal growth had been achieved.

	Leaf	Stipule	Petiole	Tendril	Maximal growth per day mm.
Control intact					52.0
First leaves	6.4	5.7	6.5	3.6	
Second leaves	9.6	7.3	9.8	5.8	
X-rayed intact					14.3
First leaves	4.2	4.6	4.0	3.0	
Second leaves	3.9	4.6	3.7	3.5	
Control on control					43.0
First leaves	4.9	6.2	4.6	4.6	
Second leaves	6.4	6.6	5.0	8.6	
X-rayed on x-rayed					45.5
First leaves	4.1	4.3	2.8	2.5	
Second leaves	4.1	4.6	3.5	3.1	
Control on x-rayed					44.5
First leaves	6.6	5.0	5.6	3.3	
Second leaves	6.0	5.3	7.0	3.3	
X-rayed on control					43.7
First leaves	4.8	4.5	3.6	3.6	
Second leaves	6.7	5.4	8.4	6.6	

the second group of treated plants, Fig. 4, characterized by reduced leaf size as well as by reduced maximal growth rate, are listed in Table III. The following results are found: (1) similar leaf size effects in intact seedlings and in various grafting combinations as observed in the experiments with treated seedlings of groups 1; (2) reduced maximal growth rate of intact x-rayed seedlings; (3) normal growth rate of self-grafted x-rayed seedlings.

The only difference between the intact and the self-grafted x-rayed seedlings involved the removal of the mid-first to the mid-third internodal stem portion, as specified by Went's pea grafting technique. Consequently if the problem were one of stem elongation factors, auxin or caulocaline, it would be a matter of transportation or destruction rather than of content. The significance of this mid-first to mid-third internode stem elongation of the treated seedlings is further indicated in grafting experiments employing interstocks. Representative data are given in Table IV. The following results are recorded: (1) control base, interstock and top results in a normal maximal growth rate; (2) x-rayed base, interstock and top, results in a reduced maximal growth rate; (3) control base, x-rayed interstock, control top, results in a reduced maximal growth rate; (4) x-rayed base, control interstock, x-rayed top, results in a normal maximal growth rate.

Experiments designed to test phloem transport through the stem portions in question, of the treated seedlings by use of externally introduced indicators have been started, but conclusive results are not available to date.

Auxin conditions in seedlings from x-rayed seeds.- Since auxin is also involved in stem elongation in conjunction with caulocaline, it was investigated in various stem regions of group 2 treated seedlings, Fig. 4. Methods of determining auxin production, transportation, and destruction, described previously by Michener (1937) were used. Quantitative results were obtained by the *Avena* test method.

TABLE LV.

Maximal growth rates in grafted Alaska peas incorporating interstocks using various combinations of control seedlings and of seedlings from x-rayed seeds. Values given are the means of 15-25 plants.

Seedling type	Maximal growth rate mm./day.
Control intact..............	42.5
Control top, interstock, and base.....................	35.9
Control top and base, x-rayed interstock..........	17.4
X-rayed top, base and interstock..................	12.0
X-rayed top and base; control interstock..........	37.7

Auxin production determined by the auxin diffusion tests from 1 cm. stem sections taken at intervals throughout germination,. is found to be less during the first 4-6 days in the treated seedlings than in controls, but recovery is generally observed by the 8th day; Table V. Auxin transportation tests, indicated no effect of the seed treatment.

Auxin destruction was found to be exceedingly greater in the stem portions of the treated seedlings than in controls. Typical results are given in Table VI. The auxin destroyed is expressed in degrees of curvature in Avena, Table VI. The effect of auxin destruction in the treated seedlings is seen to increase over controls from the stem apex to the base and to be more pronounced in older seedlings.

The condition of increased auxin destruction about the stem apice, seemed to have had no impeding effect on stem elongation when employed in grafted combinations of group 2 treated seedlings. The pronounced effect of auxin destruction in the basal portions of the treated seedling stems, may, however, have significance in regulating the transport or production of caulocaline from the roots. No information is available to support this relationship, from experiments to data.

Summary.

A study has been made to determine the conditions of auxin and calines in dark room germinated pea seedlings from seeds given small to medium x-ray treatments. The effects

TABLE V

Effect of x-ray radiation on auxin production in stems of Alaska pea seedlings grown from control seeds and from seeds given medium x-ray treatment (30 kv, 10 ma, 8 cm, 60 min.) indicated by auxin diffusion tests made at intervals during germination. The treated seedlings were of the type referred to as group 2 in Fig. 4.

			°curvature indicated by Avena test			
			Stem part			
Plant	Age	Tip	middle	base	0 IAA/L	50 IAA/L
Control	4 days	4.0°	3.2°	1.0°	0°	
X-rayed	4 days	0.3°	-3.6°	1.0°		12.3°
Control	6 days	6.16°	2.1°	2.4°	0°	18.8°
X-rayed	6 days	1.1°	0.3°	0.0°		
Control	8 days	4.6°	3.8°	3.1°	0°	18.0°
X-rayed	8 days	4.0°	3.0°	5.3°		
Control	8 days	2.2°	0.7°	0.1°	0°	18.8°
X-rayed	8 days	2.3°	2.3°	0.0°		

TABLE VI

Auxin destruction in Alaska pea seedling stems of control seeds, and of seeds given a medium dose of x-rays (30 kv, 10 ma, 8 cm, 60 min.) referred to as group 2 in Fig. 4; determined at intervals during germination.

Plant	Age	Auxin destruction expressed as °curvature 1 cm. stem portions from--			°curvature produced by	
		Top	Middle	Base	0 IAA/L	50 IAA/L
Control	6 days	0.6°	1.8°	5.7°	0°	18.8°
X-rayed	6 days	1.6°	8.3°	7.0°		
Control	8 days	0.0°	0.0°	3.8°	0°	18.0°
X-rayed	8 days	4.5°	5.4°	6.0°		
Control	8 days	0.8°	2.5°	1.2°	0°	18.8°
X-rayed	8 days	5.2°	6.4°	4.1°		

of treatment on auxin production, destruction, and transport, were determined by the usual test methods and quantitative results were obtained by the *Avena* test method. Methods used to determine conditions of the calines included direct measurement of parts of intact seedlings, and of grafted seedlings incorporating various combinations of control and treated seedlings. In the case of phyllocaline, direct determination of the x-ray effect was made by testing diffusate from x-rayed seeds for leaf growth activity using the assay method of D. M. Bonner, A. J. Hagen-Smit and F. W. Went (1939).

Data indicated that auxin production and transport were relatively unaffected by the seed treatment, while auxin destruction was noticably increased in seedlings from the treated seeds, particularly in the basal stem portions. Phyllocaline was found to be definitely altered by the x-ray treatment by all test methods used. Seed diffusate from x-rayed peas had little to no leaf growth activity in the leaf test. No apparent effect was found in caulocaline production or activity, but its translocation seemed to be hindered. This effect is concerned with the mid-first to mid-third internode region of the stem since recovery in growth rate results in grafted seedlings prepared by removing this stem region. Various graft combinations employing interstocks of this stem region of seedlings from x-rayed seeds, also show retarded stem growth.

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