CYTOLOGICAL STUDIES OF THE EFFECTS OF ADMINISTERING POTASSIUM CHLORIDE TO X-IRRADIATED SORGHUM SEEDLINGS

By

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PREFACE

In September of 1950, the author was assigned to work with Dr. R. M. Chatters as an Atomic Energy Commission Research Associate in the Botany and Plant Pathology Department of Oklahoma Agricultural and Mechanical College.

This work has been made possible through Atomic Energy Grant No. AT (11-1)-71, Project No. 2 which was obtained by Dr. R. M. Chatters under the auspices of the U. S. Atomic Energy Commission and the Research Foundation of the Oklahoma Agricultural and Mechanical College.

Experiments have been carried out based upon the assumption, initially accredited to Dr. R. M. Chatters, that ionizing radiations produce a potassium imbalance and that the administration of KCl following irradiation might aid in the restoration of this balance, thus preventing or retarding the appearance of radiation-induced morphological changes.

The author wishes to express his appreciation to Dr. R. M. Chatters, under whose supervision the experimental work has been carried out, for his constant source of encouragement and guidance, and for his helpful, advice and assistance, and constructive criticisms in compiling this data. Prof. O. E. Schultz and Dr. W. W. Hansen have given excellent advice in the critical reading of this thesis. To the above members of my committee and to Dr. Robert MacVicar and Dr. J. S. Brooks, appreciation is extended for their advice and assistance in the planning of my course of study.

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INTRODUCTION

Since the discovery of X-rays by Roentgen in 1896, biologists have devoted considerable effort towards the elucidation of the mechanism of action of ionizing radiations on living cells. It is well known that the absorption of ionizing radiations by tissues is associated with damage. In addition, exposures over periods of years may be cumulative to a varying degree. With the ever increasing number of scientific and industrial workers who are subjected to exposures of ionizing radiations, the need for an intensive study of the biological effects of exposures to radiations becomes of increasing importance. Likewise, it is desirable to determine by what means and to what extent the sequelae of the absorption of radiations can be altered by treatments instituted shortly after irradiation.

When living tissues are subjected to ionizing radiations, various biological effects may be manifested. Howard (34) states that the primary action of the radiation is causally linked by a chain of events. At the beginning of the chain, some information can be obtained through our present knowledge of the nature of the ions produced, their linear density, and their subsequent behavior. This behavior is modified according to the type of radiation and the absorbing medium. The biological changes observed will be determined by the complex array of normal life processes, which may be interrupted or terminated at any point between the time of primary ionization and this end result. At present, however, the intimate organization, on a molecular level, of even the simplest cell is largely unknown. Thus,

the first difficulty in the explanation of the observed biological action of radiations is the complex and obscure nature of the biological reactor.

Howard (34) feels that most information will be gained when those biological reactors are chosen about which we already have some knowledge, or which react in a quantitatively definite way to a given radiation stimulus. For this reason, much of the useful experimental work has been done on simple organisms, such as virus particles and yeasts, or on unit stable particles having definite quantitative effects, such as genes. With these materials, there is a better chance of working out the effect of the primary ionizations or excitations from the secondary effects which result from a change in some longer chain of events. However, Chatters (21) is of the opinion that total radiation effects are expressions of a multiplicity of complex simultaneously occurring chemical events which govern the total function of the organism.

Since the term ionization will be used very frequently, an explanation of the term will be advantageous. The atom is considered by Lea (41) to consist of a positively charged nucleus which is surrounded by a constellation of negative electrons. The whole is electrically neutral. When an ionizing radiation passes through matter, its principal means of energy dissipation results in the ejection of electrons from atoms through which it passes. An atom so ionized is left positively charged and is referred to as an ion. Possibly, some actions of radiations of biological significance are due to this separation of electrical charge, but in most cases it is more plausible to attribute it to chemical change resulting from the ionization. When an atom is ionized the molecule of which it is a part almost certainly undergoes chemical change. The chemical bonds which hold a molecule together

consist of electrons shared between the two atoms joined by the bond. Thus, it is to be expected that the removal of such a bonding electron from a molecule will lead to its dissociation or other chemical change. The removal of electrons other than bonding electrons may also be expected to lead to chemical change, since the energy involved in ionization exceeds the energy required to remove an atom from the molecule.

Excitation is listed by Lea (41) as a second method by which radiations dissipate energy in tissue. This means the raising of an electron in an atom or molecule to a state of higher activity, and it involves less energy for production than the complete ejection of an electron. Ultra-violet light and ionizing radiations are capable of producing excitation.

In the process of ionization as set forth by Lea (41), the electron which is ejected from an atom eventually becomes attached to another atom and makes it a negative ion. As far as the physical measurement of ionization is concerned, the positive and negative ions are equally significant, and one usually speaks of the production of ion-pairs. Since the energy involved in the attachment of an electron to an atom to form a negative ion is usually even less than the energy of excitation, it is probably safe to neglect negative-ion formation as a factor of biological importance. Thus, ionization refers to the production of a positive ion by the ejection of an electron. This ejected electron may have sufficient energy to ionize on its own account before it is brought to thermal energy and finally attached.

Practically all the energy dissipated in tissue by radiations ultimately becomes degraded to heat energy. A dose of 10^5 roentgens is sufficient to raise the temperature about $0.25^{\circ}C$. This small temperature rise for a large dose of radiation means that temperature change so produced is quite

inadequate to explain the biological effects of ionizing radiations. Lea (41) cites contrary evidence, however, by presenting Dessauer's point heat theory. This confines the energy dissipated in the tissue to a small proportion of the atoms, and in turn these atoms would experience a high rise of temperature which could produce biological effects.

X-radiation, like ultra-violet light, is an electromagnetic radiation emitted in quanta, but the difference in wave-length of the two types of radiations results in there being little similarity between the two. The absorption coefficient of X-rays does not depend on the chemical combination of the absorbing atoms, but it is dependent on their atomic number. The principal mechanisms by which high-energy electromagnetic radiations are absorbed in matter are presented by Lapp and Andrews (40) as the photoelectric effect, the Compton effect, and pair production. Due to the greater penetrating power of X-rays, it is usually inconvenient to measure the total energy absorbed on a surface. More accurate measurements are obtained by determining the amount of energy absorbed in a given volume. In a response to this fact the roentgen was set up as the unit of measurement of X- or gamma radiation and is defined by Lapp and Andrews (40) as,

> that quantity of X-radiation which will produce one electrostatic unit of ions in one cubic centimeter of air under standard conditions of temperature and pressure.[1]

The sequence of events of radiobiological action is summarized by Zirkle (83). The first of these is the absorption of radiant energy by molecules in the biological object or its medium. The second consists of a series of chemical changes, and the third event is the occurrence of

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[1]Lapp, R. E. and H. L. Andrews, Nuclear Radiation Physics, p. 90.

the biological effect. After the absorption of energy, our knowledge of the radiobiological action is limited until the biological effect appears. Having observed the varied and sometimes contradictory behavior of living organisms subjected to radiations, biologists have often been unwilling to accept the simple one-hit-one-effect interpretation which was first presented by Dessauer in 1922 and later reviewed by Howard (34). This theory is adequate to account for some of the genetic changes due to radiations, but it has to be supplemented when other aspects of experiments on radiations are considered. From the studies of Weiss (81), Barron (2), and others (3,4,5,14,15,61), it has been postulated that water decomposes upon irradiation, and there is formed four powerful oxidizing agents which are OH and O_2H radicals, H_2O_2, and atomic oxygen. They have concluded that the biological action of ionizing radiations is due mainly to the products of the irradiation of intracellular and extracellular water.

Twenty years have elapsed since Muller (49) discovered that rearrangements of parts of chromosomes can be induced by X-rays. In the intervening period, a wealth of information has been accumulated concerning the types of induced alterations which are produced in the various types of cells. Each type of response must be analyzed independently of the others if precise interpretations of the underlying mechanisms of ionizing radiations are to be formulated.

Radiobiological actions that are produced by ionizing particles are numerous. General expressions of the biological effects of radiations are summarized by Duggar (25), Lea (41), Hevesy (33), and others (2,10,11,27, 30,39,43,52,79). The inhibition of mitosis by radiations has been studied by Muller (48,49), and the effects of X-rays on chromosome structure has

been reviewed by Catcheside (18), Sax (60), and (6,12,17,29,59,72,73,74,76). The action of X-rays on nucleic acids has been investigated by Weis (61), Butler (14,15) and Sieburth (64). The oxidation of thiols by ionizing radiations has been demonstrated by Barron and Flood (5), and the depolymerization of thymonucleohistone and sodium thymonucleate has been accomplished by Sparrow and Rosenfeld (77). Studied on the effects produced by neutrons on biological materials have been made by Sparrow and Christensen (75), Chatters (19,20) and Zirkle et al (84), and the effects of alpha particles on plant cells have been reported by Zirkle (82) and Dobson (24).

Evidence of radiobiological changes produced by beta particles that are emitted from radioactive isotopes have been obtained and presented by Stanton and Sinclair (78), Mackie et al (45), and (32,36,38,53,58,70). Responses of the hematopoietic system to ionizing radiations other than from radioisotopes have been studied by Suter (80), Low-Beer and Aggeler (44) and other investigators (16,46,50).

While many of the biological effects of radiations described in the foregoing literature review are not deleterious, the changes which are undesirable biologically presuppose the need for research upon methods whereby radiation damage may be eliminated or at least attenuated to some degree. The results of a number of investigations on this subject have been published recently. In mice, for example, reduction in mortality by the administration of antibiotics following X-radiation has been demonstrated by Miller et al (47). They indicate that streptomycin provides the most effective protection. Limperos and Mosher (42) concluded from their studies that thiourea did and other reducing agents possibly would lower the mortality rate in irradiated mice because of the protection thus afforded to certain vital cellular

constituents. From the observations of Sokoloff et al (71) it appears that vitamin P compound, which contains four flavonoids naturally present in citrus fruit, gives considerable protection to rats against a total-body, near-lethal dose of X-radiation. Cysteine, some fuarans, glycerine in high concentrations, ethylene glycol and propylene glycol were reported by Hollaender (27) to afford protection to bacteria during the irradiation period. That glutathione protects a mechanism permitting orderly regeneration of the hematopoietic system of mice from ionizing radiations has been found by Cronkite et al (27). Patt and his co-workers (54) concluded that cysteine administered to rats prior to X-irradiation in the near-lethal range greatly diminished toxicity. When Rekers and Field (55) administered the flavonol glycoside, rutin, to dogs which had been given sub-lethal total-body X-irradiation, the mortality rate dropped 52 percent. Edelmann (26) reports that daily injections of desoxycorticosterone acetate enabled adrenalectomized rats to survive doses of 650 r-units of X-rays and concludes that the adrenal hormones play a part in resistance to the noxious effects of radiations. When the spleen of rats are shielded by lead from X-radiations, the mortality rate is decreased (35). Either forced exercise (37,65) or the administration of thyroid hormone (68,69) to irradiated mice increased the mortality rate. Allen et al (1) report that aureomycin and blood transfusions when administered together protect dogs from the after effects of lethal doses of X-rays. It was found by Chatters et al (22) that the administration of KCl solutions to irradiated sorghum seedlings produced an increase in growth in root length as compared with those seedlings which received X-radiation alone.

Although many types of radiobiological effects have been investigated at the cellular level, little attention has been given to the direct disturbing influence of ionizing radiations upon the distribution of electrolytes between cells and their surrounding medium. Sheppard (62) in his review on the role of potassium in cell metabolism has indicated that one of the factors controlling enzyme activity is the inorganic ionic environment within cells. Since the principal cation is potassium, it is not surprising that the rates of several enzyme reactions have been demonstrated to depend upon the concentration of this element. Boyer and his co-workers (8,9) have shown that the transfer of phosphate from 2-phosphoenolpyruvate to adenosinediphosphate required potassium, and Nachmansohn and John (51) found that the activity of choline acetylase is dependent upon the K concentration. Glycogen synthesis from glucose by rat liver slices was found by Buchanan et al (13) to be greater in a K-rich medium than in a medium containing Na as the principal cation. Deposition of glycogen in the liver has been shown by Fenn (28) to be accompanied by deposition of potassium. The effects of potassium depletion have been studied in Escherichia coli by Roberts, Roberts and Cowie (23,56,57). They conclude that potassium is present in the cell in an ionic and a bound state and that the rates of the incorporation of sulfur into proteins, and the incorporation of phosphorus into phospho-lipids and nucleic acids and growth phenomena are dependent on the content of the bound potassium in the cell.

If the processes described in the above paragraph are blocked by a potassium imbalance following treatment with ionizing radiation, it is evident that normal metabolic functions of the cell will diminish. Many investigators (2,7,63) have reported a physiological imbalance of potassium

in tissues exposed to ionizing radiations. A higher KCl tolerance, which indicates a potassium imbalance, in irradiated mice was reported by Smith (66,67).

Based upon the above observations, it has been assumed by Chatter et al (22) that the administration of KCl following irradiation might aid in the restoration of the potassium balance thus preventing or retarding the appearance of radiation-induced morphological changes. The present work is a report of investigations in support of this hypothesis.

METHODS AND MATERIALS

In order to determine cytologically the biological effects produced by X-radiations on plant material and to examine the results of post-administration of KCl to irradiated tissues, one of the stable varieties of sorghum, Sorghum vulgare var. Resistant Wheatland (G. C. 38288) was selected. After conducting experiments with corn, radish, barley, and sorghum, it was decided to use this variety of sorghum because of its small seed, appropriate primary root system, and its ready availability in quantity. The size of the seeds enabled several hundred of them to be included in a single X-ray exposure with reasonable certainty that all of the seeds received a uniform dose. Further, the small size of the seeds and seedlings made it possible to work with a large number of individuals and provided an opportunity for studying a multitude of different types of cells and tissues.

Pre-treatment was the same for all seeds until the time of X-ray exposure. In order to destroy surface-borne fungal spores existing on the seeds, a 5.0% solution of H_2SO_4 was added for a period of 15 minutes to a 50 c.c. beaker filled with seeds. The residual H_2SO_4 was removed by washing the seeds twice in distilled water at 15 minute intervals. Containers that were used for germination of the seeds were 6" X 8" X 1.5" Pyrex baking dishes (Fig. 1) which had been sterilized with 95% ethyl alcohol. The dishes were prepared for germination of the seeds by the addition of Shark skin general purpose filter paper which has been cut to cover the bottoms of the dishes and by moistening the paper with 20 ml. of Modified Crone's

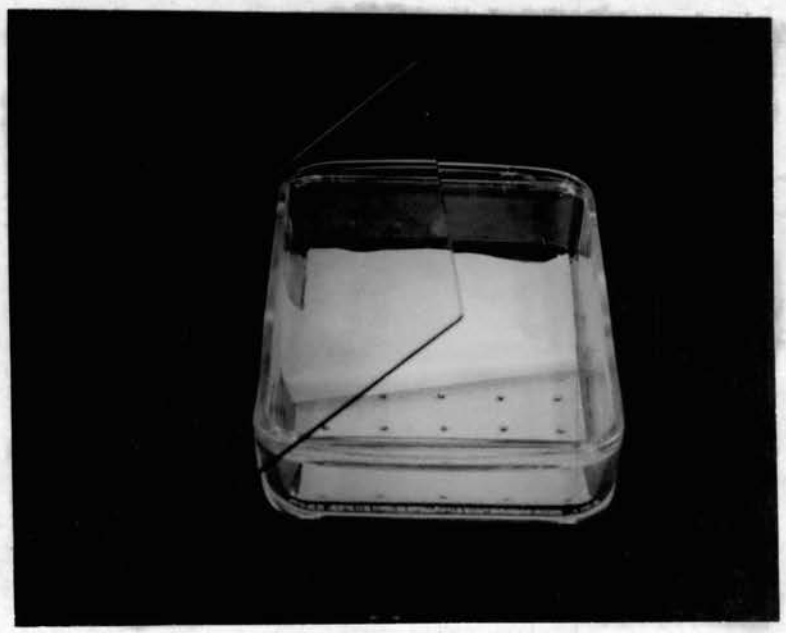

Fig. 1. Type of Containers Used for Germination and Growth of Seedlings.

Fig. 2. Exposure Tray.

Solution*. The sterilized seeds were spread over the moist surface and were covered with another filter paper. The dishes were closed with a glass plate and the seeds allowed to germinate for 16 hours in a 30°C incubator.

The germination period produced seedlings whose radicles were approximately 1 mm. in length. Those seedlings which were to be exposed to X-radiation were transferred to a 2" X 2" piece of moist filter paper which was supported by a piece of photographic film backing. This support containing the seedlings was placed upon a piece of plastic refrigerator dish and covered with the top half of an 80 mm. petri dish (Fig. 2). The control seedlings remained in the germinating dishes while the others were being exposed.

Seedlings were exposed to a single dose of approximately 2800 r-units of X-radiation** delivered at 150 Kv, 4ma, at a distance of 20 cm. from the tube target. The dose rate was approximately 3.1 r-units per second. The arrangement of the physical set-up (Fig. 3) was of such a nature that the dosage had to be administered through the lower surface of the seedling container.

After irradiation, the seedlings were divided into control and treated groups. Irradiated seedlings which were used for controls were placed in lots of forty in Pyrex baking dishes prepared as previously described and moistened with 15 c.c. of Modified Crone's Solution. The treated groups

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*Modified Crone's Solution

$Ca(NO_3)_2$ ---------1.00 g.	$FePO_4$ ----------0.25 g.
$Ca_3(PO_4)_2$ --------0.25 g.	$CaSO_4 \cdot 2H_2O$ -----0.25 g.
$MgSO_4 \cdot 3H_2O$ -------0.25 g.	H_2O (dist.) -----1000 ml.

**Exposures were made by a Seifert Company (Hamburg) industrial type X-Ray unit provided by the Oklahoma A. and M. College Power and Propulsion Laboratory.

Fig. 3. Physical Set-up of X-Ray Unit

were prepared in the same manner but were moistened with 15 c.c. of a 0.25 percent KCl solution in Modified Crone's. These two lots of seedlings were designated as Modified Crone's X-ray and Modified Crone's plus 0.25 percent KCl X-ray. In order to have non-irradiated controls, seedlings in lots of 40 were taken from the germinating dishes and placed into two groups of Pyrex dishes containing 15 c.c. of Modified Crone's Solution and 15 c.c. of a 0.25 percent KCl solution in Modified Crone's, respectively. All seedlings were then placed in a 30°C incubator. Thus, four groups of seedlings, Modified Crone's X-ray control, Modified Crone's plus 0.25 percent KCl X-ray, Modified Crone's non-irradiated Control and Modified Crone's plus 0.25 percent KCl non-irradiated control, were prepared for study.

Beginning one hour after the X-ray exposure and continuing for 48 hours, root tips from the four groups of seedlings were removed at hourly intervals

for killing and fixing. For the latter process, a solution of Nawaschin's killing and fixing fluid was used. The tissues were processed by the butyl alcohol-paraffin technique, sectioned longitudinally at 8 microns, stained in a one percent aqueous solution of Safranin O, counterstained with a saturated fast-green in clove oil solution, and mounted in Clarite "X"*. Other tips from the four groups of seedlings were removed 72 hours following X-ray exposure.

All microscopic observations were made through a Bausch and Lomb binoucular research microscope (AD 5804) with the source of light being an American Optical Company coil filament research lamp (Model 370). In order to obtain maximum definition of tissues, green and frosted-glass filters were employed. Where photographs were made to furnish supporting evidence of tissue structure, a Leitz Micam camera and Kodalith Ortho-Type 2 film were employed.

An hourly "mitotic index"** was tabulated by counting the number of divisions in median-longitudinal sections which were selected from each of the four groups of seedlings. The types of tissues in which divisions occurred and the evidences of abnormal divisions were observed at this time.

In order to determine the effects of X-radiation on the process of growth at the cellular level, cell size characteristics, as seen in plane view, of the four groups of seedlings were obtained at three-hour intervals by securing an average cell area for epidermal, cortical, xylem, and

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*Clarite "X" is a mounting medium resin prepared by the Neville Co., Pittsburgh, Pa. The resin has a refractive index of 1.567 and a melting point ranging from 145-150°C.

**The term "mitotic index" has been employed to signify the number of mitotic divisions in a median-longitudinal section of a root tip.

meristematic cells. Measurements of the above first three types of cells were made at points beginning at 480 microns from the root tip, exclusive of the cap. These average areas of cells of each type tissue were calculated from measurements obtained from the first ten cells beyond this point. Meristematic cell areas were determined by measuring ten cells located immediately adjacent to the mid-point of the meristem where it adjoined the root cap.

OBSERVATIONS AND RESULTS

Growth was used as a basis to formulate a criterion for measuring radiation damage and for the effects of administering 0.25 percent KCl solution to irradiated seedlings. The growth processes of mitosis, elongation and maturation were observed in the control materials. These served as a standard and changes in these processes were attributed to X-radiation. Likewise, any differences existing between these processes in the irradiated seedlings and the irradiated KCl-treated seedlings were attributed to the administration of KCl.

Observations of the four groups of seedlings revealed that the expressions of ionizing radiations on the process of growth were numerous and variable. Some of the tissues experienced an extreme change, whereas others were altered to a limited extent. It was impossible to formulate a standard series of morphological changes that would apply to every tissue type at each hourly interval that observations were made. Thus, an attempt has been made to examine the various types of alterations produced in the processes of growth by X-irradiation and by post-administration of KCl solution to irradiated seedlings.

Cytological Observations on Irradiated Modified Crone's Seedlings

"Mitotic Block"*. The mitotic index (Table 1) illustrates that the number of divisions in the control materials was not consistent during the forty-eight hour period and that the administration of 0.25 percent KCl did not influence the number of divisions in the controls. Since such a great irregularity was found in the control materials, the number of divisions could not be used to determine radiation effects. However, the location of these divisions in the irradiated material was of significance and will be discussed later.

It has been reported by many investigators (18,31,33,41) that sub-lethal doses of X-radiation modify the process of mitosis in meristematic tissues. These modifications may be expressed as a proliferation of cell division, malignancies, or a stoppage of cell division. The length of time that the latter process is blocked depends upon the dose, dosage rate, atmospheric conditions during administration of the dose, and the resistance of the tissues to ionizing radiations. When sorghum seedlings were exposed to 2800 r-units of X-radiation, there was an immediate mitotic block (Table 1). Divisions reappeared approximately ten hours after irradiation and were restricted to those cells which initiated the vascular elements. It was thirteen hours after irradiation that divisions began in the meristematic, cortical and epidermal cells.

Mitotic Block with Elongation and Maturation Continuing. In some of the seedlings it was obvious that the process of elongation and maturation

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*The term mitotic block has been used to describe the prevention of mitotic divisions by X-radiation.

TABLE I

MITOTIC INDEX OF SEEDLINGS

Hours After Treatment	Number of Mitotic Divisions			
	Modified Crone's Control	0.25% KCl Control	Modified Crone's X-Ray	0.25% KCl X-Ray
1	14	8	0	0
2	14	26	0	0
3	0	0	0	0
4	4	0	0	0
5	37	10	0	0
6	33	20	0	0
7	18	44	0	0
8	12	19	0	1
9	22	18	2	0
10	18	13	0	7
11	16	4	2	0
12	24	19	2	2
13	16	4	11	0
14	10	10	7	8
15	0	20	11	0
16	26	20	12	7
17	18	22	9	12
18	28	16	10	24
19	30	41	17	9
20	38	29	6	11
21	23	17	7	15
22	25	32	15	10
23	28	7	16	5
24	30	14	20	10
25	13	31	19	15
26	8	34	7	5
27	16	26	13	11
28	21	25	9	20
29	12	40	5	11
30	3	3	3	3
31	1	3	0	15
32	27	20	11	12
33	16	7	0	10
34	15	18	0	8
35	14	9	2	0
36	3	0	2	11
37	15	37	12	2
38	27	19	5	12
39	25	7	5	6
40	22	10	4	5
41	27	13	6	8
42	15	16	16	8
43	15	19	11	12
44	17	20	0	9
45	19	11	15	10
46	18	15	15	8
47	14	9	18	6
48	16	23	10	7

had continued while the mitotic block was present. When recovery from the block occurred, cells which normally would have divided in the meristem several hours previously were far removed from the root tip. Thus, cells that were located 480 microns from the meristem (Plate 1, Figs. D,F; Figs. 6,7) were in the process of division. Even though these cells had reached a certain degree of maturity, they had never lost the potential of cell division. In the control materials (Figs. 4,5) there was no evidence of cell division at this level, whereas elongation and maturation were preceeding normally.

Permanent Mitotic Block with Elongation and Maturation Continuing. It was evident in some of the seedlings (Figs. 9-11) that the mitotic block continued for the entire period of observation. The processes of elongation and maturation of the meristem cells continued and produced seedlings that were devoid of meristematic tissue. In its place were mature xylem elements and cortical cells. This presented evidence that even though the mitotic process was blocked by X-radiation, the processes of elongation and maturation continued. Control material (Fig. 8) showed normal mitoses, elongation and maturation.

Mitotic Block and Stoppage of Elongation. There were other seedlings in which the process of mitosis was blocked for the entire growth period. In the meristemic area, maturation continued and produced mature cells. In its normal region, elongation was blocked, behind it, however, elongation and maturation took place resulting in the production of roots that were globose in part (Figs. 12,13). The hourly preparations of the seedlings showed that this condition started in the irradiated material approximately 37 hours after exposure. The control materials (Fig. 8) demonstrated a normal growth pattern.

Change of Cell Size. Another expression that ionizing radiations produced on the process of growth was a change in the size of the cells constituting the various tissue types. This expression was evidenced by the existence of an increase in cell size in the majority of irradiated seedlings. This should not imply, however, that all cell size differences were of this nature as there were areas of extremely small cells. This latter condition will be discussed later under the heading "Changes in the Plane of Division". An increase in cell size was more frequent and of greater magnitude in the epidermal cells and less so in the cells of the meristemic area. A comparison of figures four and five with figures six and seven illustrates cell size differences of the epidermal cells in the irradiated and non-irradiated seedlings. Further evidence that a difference in cell size existed between the irradiated and control seedlings was verified by calculations of individual cell areas for epidermal, cortical, xylem and meristematic cells (Figs. 38-45).

Four hours after exposure (Fig. 39), the average area of cells constituting the epidermis at 480 microns from the root tip in the Modified Crone's irradiated seedlings was 278 sq. microns, whereas, in the control seedlings (Fig. 38), the area was 195 sq. microns. This was evidence that after a short period of time following irradiation, the epidermal cells were in a process of increasing their size. This process continued in the Modified Crone's irradiated epidermal cells until an average cell area of 1669 sq. microns was reached 21 hours after exposure. The epidermal cells of the controls maintained a degree of uniformity in cell size throughout their period of growth. After 21 hours, the average area of non-irradiated epidermal cells at 480 microns from the root tip was 168 sq. microns. Thus, at the above time interval, the irradiated epidermal cells, as seen in plane view, were approximately ten times larger than the non-irradiated cells.

Epidermal cell sizes after this period of time were very inconsistent in the Modified Crone's irradiated cells. In some cases the cells began to slough-off, making accurate measurements impossible. It is of the utmost importance to note that this increase of area described above was due to growth in a lateral direction and not parallel with the long axis of the root. This has been verified by the experiments of Chatters et al (22) in which it was found that X-radiation decreased the length of the roots.

The same response of cell-size increase was evident in the cortical cells but was not expressed to such a great extent as that demonstrated in the epidermis. The average area of cells constituting the cortex at 480 microns from the root tip in the Modified Crone's irradiated seedlings (Fig. 41) was 326 sq. microns four hours after exposure, whereas, in the control seedlings (Fig. 40) the area was 170 sq. microns. Here again it was evident that the process of cell size increase started shortly after X-ray exposure. A cell area of 1,721 sq. microns was obtained as an average in the irradiated cortical cells 30 hours following irradiation. At the same time interval, the average area of non-irradiated cortical cells was 439 sq. microns.

The change in cell size of the irradiated xylem tissue was less pronounced. The average area of cells constituting the xylem (Fig. 43) at 480 microns from the root tip was 3582 sq. microns in the irradiated tissues 33 hours after exposure, whereas in the non-irradiated tissues (Fig. 42) it was 3429 sq. microns.

Measurements did not give evidence of a cell size difference existing between the cells comprising the meristems of the irradiated and non-irradiated seedlings (Figs. 44,45). However, these measurements gave a false impression of the true nature of this tissue following irradiation. As previously stated,

these were made by measuring ten cells located immediately adjacent to the lower mid-point of the meristem where it adjoins the root cap. Then an average cell size was determined from these ten measurements. Also it was stated previously, that in some of the irradiated seedlings the meristematic cells proceeded to elongate without going through a division. Thus, it would appear that a difference would have been found between the irradiated and non-irradiated meristematic cells. It soon became evident that the point of measurement was the final location of meristematic change. If the measurements had been made to the right or left of this point a difference would have been evident.

Chromosomal Changes. The literature review dealing with radiation effects reveals that chromosomal aberrations are expressions of radiation damage. In order to determine the prevalence of chromosome abnormalities, they were tabulated for the entire forty-eight hour period following irradiation. The chromosome complement of Sorghum vulgare consists of ten pairs of extremely small chromosomes. Due to their size, it was impossible to analyze the divisions for all types of chromosomal abnormalities. Bridge formation and fragmentation (Plate 1, Fig. B) at anaphase stages were the only aberrations that could be determined with any degree of certainty. Of all the seedlings examined during the forty-eight hours after exposure, only six cells contained abnormal chromosome formations of the above types. This should not imply that these were the only aberrations present in the irradiated material, but it does suggest that the dosage administered had an extremely small visible effect on the chromosomes.

Absence of Cell Wall Formation and Changes in the Plane of Division. Most evident of the effects of X-radiation on growth were the changes

produced in the planes of division during mitosis (Fig. 6; Plate 1, Figs. A,D). In the control material this event occurred at right angles to the long axis of the root (Figs. 4,5), whereas in the irradiated seedlings some of the planes were changed from a few degrees in some cases to 180° in others. Where these changes occurred, areas composed of small irregular cells were observed. There was no evidence to indicate that these irregular divisions were of a proliferating nature.

There were cells (Plate 1, Figs. A,C,D) in the irradiated seedlings in which nuclear divisions continued, but cell wall formation failed to occur. Such a condition produced multi-nucleated cells whose total cross-sectional areas were not changed to any great extent. This condition was infrequent, however, and the number of nuclei in these cells varied from two to four. These cells should not be confused with the control cells (Plate 1, Fig. E) which had two nucleoli.

Effects of the Administration of KCl Solution to Irradiated Seedlings

It has been assumed (22) that the administration of KCl following irradiation might aid in the restoration of the potassium balance thus preventing or retarding the appearance of radiation-induced morphological changes. Having set up growth as a criterion for measuring radiation damage, this criterion was used to determine the effects of administering KCl after irradiation. Any changes in the processes affecting the total expression of growth in the treated irradiated seedlings were attributed to KCl.

Mitotic Block. The administration of 0.25% KCl did not release the mitotic block (Table 1) any sooner than it was released in the Modified Crone's irradiated seedlings. The mitotic index shows that both groups of irradiated seedlings recovered from the mitotic block at approximately nine

hours following exposure. The significant fact was the difference in the location within the root of mitotic recovery in the two groups of seedlings. It has previously been stated that in the Modified Crone's irradiated seedlings the greatest morphological changes occurred in the epidermal cells and decreased as one moved inward to the xylem elements. This had a bearing on the mitotic recovery of the two groups of irradiated seedlings. Nine hours following irradiation, mitotic figures appeared in the primordial vascular elements of the irradiated Modified Crone's seedlings. It was not until thirteen hours after exposure that mitoses began to appear in the meristematic epidermal and cortical cells of these seedlings. In the irradiated KCl-treated seedlings, the first evidence of mitotic recovery was also nine hours after exposure, but these divisions were not only in the vascular primordia but also in the meristematic, cortical and epidermal cells.

Elongation and Maturation of the KCl-Irradiated Seedlings. The differences existing in the processes of elongation and maturation between the irradiated KCl-treated and the non-treated irradiated seedlings can best be demonstrated by a series of photomicrographs (Figs. 14-37). In the seedlings that were examined at hourly intervals prior to 12 hours following irradiation, there were no gross visible changes in the processes of growth. Cytological preparations of irradiated KCl-treated (Fig. 17) and irradiated non-treated seedlings (Fig. 16) 12 hours following exposure revealed that a slight difference in the pattern of growth existed between the two types. At this time the Modified Crone's irradiated seedlings were characterized by slightly larger epidermal cells which lacked to a limited degree a uniformity of shape and size. The above characteristics were not evident in the irradiated KCl-treated seedlings at this time. The growth pattern of the latter seedlings

was relatively uniform and approached that of the non-irradiated material (Figs. 14,15).

At 18 hours following irradiation the Modified Crone's irradiated seedlings (Fig. 20) showed to a greater extent broken, jagged, enlarged epidermal cells. Such a condition gave this tissue an irregular appearance. Also the cortical cells of these seedlings were larger than those of the non-irradiated controls (Figs. 18,19). The irradiated KCl-treated seedlings (Fig. 21) began to show signs of irregular epidermal and cortical cells at this time. These changes in the pattern of growth were minor as compared to those of the irradiated non-treated seedlings.

The Modified Crone's irradiated seedlings (Fig. 24) 25 hours after exposure were characterized by extremely large epidermal cells which had begun to slough-off. The processes of elongation and maturation had begun to displace the meristem. In the irradiated KCl-treated seedlings (Fig. 25) at this time, the only evidence of a change in the growth pattern was an increased size of the epidermal cells. Even though they were larger than the epidermal cells of the control seedlings (Figs. 22,23), they had not become irregular and broken as those of the Modified Crone's irradiated seedlings.

It was quite evident that the processes of elongation and maturation had displaced the meristematic region of the Modified Crone's irradiated seedlings (Fig. 28) 32 hours after exposure. Not only were the cortical cells larger than those in the controls (Figs. 26,27), but they had taken on characteristics of mature cells. The epidermal cells completely lacked uniformity and were extremely large and irregular in their growth pattern. Thirty-two hours following exposure, the irradiated KCl-treated seedlings

(Fig. 29) began to acquire the characteristics of irradiated material. At this time there existed in these seedlings large, irregular epidermal cells and a pre-maturation of the cortical tissue in the meristematic region. However, these seedlings were comparable to the Modified Crone's irradiated seedlings 12 hours after exposure.

At 40 hours following exposure, the Modified Crone's irradiated seedlings (Fig. 32) had very little meristematic tissue remaining. There was no true epidermal tissue, and elongation and maturation of the cortical and xylem cells had continued to displace the meristematic tissue. All cell types had become irregular in shape, size and maturity. In the irradiated KCl-treated seedlings (Fig. 33) at this time, the meristem was still functional even though the processes of elongation and maturation had displaced it to some extent. The epidermal cells lacked uniformity of growth, but no more so than the modified Crone's irradiated seedlings at 25 hours following exposure.

Observations of the Modified Crone's irradiated seedlings (Fig. 36) 48 hours following exposure, gave evidence that these seedlings were practically devoid of meristematic tissue and that elongation and maturation had continued to the lower limits of the root tip. Not only had these processes continued in the meristematic region, but there was an increase in cell size of the cortical and epidermal cells at this level. That is, these cells went beyond a pre-maturation point and attained large sizes. The irradiated KCl-treated seedlings (Fig. 37) at this hour of observation also showed pre-elongation and pre-maturation, but these processes did not displace the meristem to the extent of that in the Modified Crone's irradiated material. The irradiated KCl-treated seedlings maintained an active meristematic region.

Cell Size. A quantitative expression for radiation injury was obtained by determining the cell size for any cell constituting the various tissue types at comparable locations. Individual measurements of ten epidermal, cortical and xylem cells at a distance of 480 microns from the root tip showed a marked difference in cell size between the irradiated Modified Crone's and the irradiated KCl-treated seedlings.

It was previously stated that after a short period of time following irradiation, the epidermal cells of the Modified Crone's irradiated seedlings (Fig. 39) began to increase in size. Further, this process continued until the average area of cells constituting this tissue at 480 microns from the tip was 1669 sq. microns 21 hours after exposure. This increase in cell size of the epidermis was present in the irradiated KCl-treated seedlings (Fig. 39) but not to the extent that it was expressed in the irradiated Modified Crone's material. The area of cells constituting the epidermis of the irradiated KCl-treated seedlings 21 hours after exposure was 292 sq. microns. This area is much more comparable to the area of 168 sq. microns of the control seedlings (Fig. 38) than 1669 sq. microns of the Modified Crone's irradiated material. These differences in epidermal cell sizes are best illustrated in the photomicrographs of Figures 4-7.

The same response existed in the cortical cells but not to the extent that it was expressed in the epidermis. The average area of cells constituting the cortex at 480 microns from the root tip in the irradiated Modified Crone's seedlings (Fig. 41) was 326 sq. microns four hours following exposure, whereas, in the irradiated KCl-treated seedlings (Fig. 41) the area was 217 sq. microns. This great a difference was maintained through the period of observation. Even though there were some inconsistences in the irradiated KCl-treated seedlings at 12 and 15 hours after exposure,

they maintained a smaller average cortical cell area than the irradiated Modified Crone's seedlings after this time interval. At 30 hours following irradiation, the average area of cortical cells in the irradiated Modified Crone's seedlings was 1,721 sq. microns, compared to 157 sq. microns in the irradiated KCl-treated material. This was an extremely large difference, and it should be noted that the cortical cells of the irradiated KCl-treated material at this time interval were smaller than the non-irradiated seedlings.

The difference in sizes of the xylem cells of the irradiated Modified Crone's and irradiated KCl-treated seedlings (Fig. 43) was less pronounced. It was not until 27 hours after exposure that the irradiated KCl-treated seedlings showed a consistent tendency to have xylem cells which were smaller in area than those of the irradiated Modified Crone's material. At this time interval, measurements revealed cells whose average areas were 2289 sq. microns and 3247 sq. microns, respectively. A less pronounced difference between the two types of irradiated seedlings continued to exist and at 36 hours following exposure the average area of cells constituting the xylem of the irradiated KCl-treated seedlings was 2418 sq. microns and 2618 sq. microns for the irradiated Modified Crone's material.

Measurements of the two types of irradiated seedlings (Fig. 45) did not provide evidence that a difference existed between their meristems. It has previously been stated that these measurements gave a false impression of the true nature of this tissue type following irradiation and that a difference actually existed which measuring did not verify.

DISCUSSION AND CONCLUSIONS

The growth processes of mitosis, elongation and maturation were observed in the control material and served as a basis for a criterion of radiation damage. In turn, this criterion served a two-fold purpose. First, it was a standard that was used in determining the effects of administering KCl to irradiated tissues. Second, it suggested phenomena that might be efficacious in irradiation therapeutics.

Experiments were carried out in such a manner that any differences existing between the growth processes in the irradiated seedlings and the irradiated KCl-treated seedlings were attributed to the administration of KCl.

Microscopic examination of the irradiated material at hourly intervals following exposure revealed that the expressions of ionizing radiations on the processes of growth were numerous and variable. All irradiated seedlings varied to a certain degree in their expression of radiation damage. This was to be expected when it was kept in mind that each seedling was an individual and that the possibility was small that the same biological system was disturbed to the same extent in every seedling. The various types of responses to X-radiation are discussed under the following headings.

Cytological Effects of X-Radiations

Mitotic Block. Since the seedlings were exposed to X-radiation at a very early age, the process of enlargement of the embryonic cells was probably at a maximum, whereas cell division was possibly just starting.

If the seedlings had been in a more active state of cell division, it is feasible that the mitotic block would have been for a longer period of time. This assumption is made through the knowledge that prophase stages of mitosis are the most susceptible to radiation damage and that the process of cell division is stopped when cells at this mitotic stage are subjected to ionizing radiations.

When sorghum seedlings were exposed to 2800 r-units of X-radiation, there was an immediate mitotic block. Divisions reappeared approximately ten hours after irradiation and were restricted to those cells which initiated the vascular elements. It was thirteen hours following exposure that divisions began in the meristematic, cortical and epidermal cells. This suggests that the major portion of the dose administered was absorbed by the meristematic epidermal and cortical cells due to their position instead of a greater susceptibility to ionizing radiations.

The number of cell divisions in the control materials was not consistent during the forty-eight hour period of observation, and the administration of 0.25 percent KCl did not influence the number of divisions in the controls. Since such a great irregularity was found in the control materials, the number of divisions was not used to determine radiation effects.

Mitotic Block With Elongation and Maturation Continuing. In some of the seedlings the processes of elongation and maturation continued in those cells which were experiencing a mitotic block. This presupposes that mitoses are more sensitive to ionizing radiations than the processes of elongation and maturation. Further, it suggests that the growth processes of mitosis, elongation and maturation are possibly independent of each other and are not necessarily a continuous chain of events which require a definite order

of occurrence. In other words, once a cell has been formed by cell division it does not have to undergo a division before it elongates and matures even though it retains the potential to divide upon maturity.

When recovery from the block occurred and cell divisions reappeared, those cells which normally would have divided in the meristem several hours previously were far removed from the root tip. In these cells it appeared that ionizing radiations had stopped the process of division for a period of time, but the processes of elongation and maturation had continued. Further, it was evident that even though these cells had reached a certain degree of maturity, they had never lost the potential of cell division. Such a response could be applicable to therapeutic measures in cases which required a limited amount of mitotic blockage and the desirability of returning the tissue to a pre-irradiated condition.

Permanent Mitotic Block with Elongation and Maturation Continuing. Another example of radiation damage was a permanent mitotic block in the meristem for the entire period of observation. The processes of elongation and maturation of the meristem cells continued and produced seedlings that were devoid of meristematic tissue. Maturation continued until mature xylem elements and cortical cells were present in the lower limits of the root tips. This presented evidence that even though the mitotic process was blocked by X-radiation, the processes of elongation and maturation continued and did not require the precursor of cell division.

Mitotic Block and Stoppage of Elongation. A related expression of the above mentioned radiation damages was evident in seedlings in which the process of mitosis was blocked for the entire period, and the process of elongation was stopped in the region in which it normally occurred.

Maturation continued and produced mature cells in this region and in the meristem. Beyond these locations, however, elongation and maturation continued. Such a condition produced roots that were globose immediately behind the normal position of the meristem. Hourly preparations of the seedlings showed that this condition started in the irradiated material approximately 37 hours after exposure.

Since a blockage of mitoses and elongation did not prevent the process of maturation from continuing, additional evidence is furnished to suggest that the processes are possibly independent of each other.

It is most significant that the mature cells which were present in the region occupied normally by the meristematic area were active metabolically and were not examples of dead cells. Therapeutically, this would be of value. If irradiated cells were to undergo a pre-maturation which permitted metabolic functions to continue but prevented a continuation of cell division, the concept that the therapeutic value of X-radiations lies in their ability to kill cells could possibly be altered. Doses could be administered which would produce a state of pre-maturation with less danger of permanent damage to tissues.

Change of Cell Size. A quantitative expression for radiation injury was obtained by determining the cell size for any cell constituting the various tissue types at comparable locations. Individual measurements of the epidermal, cortical and xylem cells at a distance of 460 microns from the root tip showed a marked difference in the size of the cells in the irradiated Modified Crone's and the non-irradiated seedlings.

It is of the upmost importance to note that this increase in cell size found in the epidermal, cortical and xylem cells was in a lateral direction and not parallel with the long axis of the root. This phenomenon could be

related to the processes of elongation and maturation. It was previously stated that in some of the irradiated seedlings these processes displaced the meristematic region. This in itself would account for an increase in cell size in the irradiated seedlings at 480 microns from the root tip. However, there was not only a pre-elongation and pre-maturation of the cells at this level but also a general increase in cell size. Thus, at 480 microns from the root tip in the irradiated seedlings, it appeared that a pre-maturation of the cells occurred and that these cells were slightly larger than those cells of the control material.

This increase in cell size should not alarm the radiation therapist who is interested in preventing the rapid enlargement of organs due to a proliferation of cell divisions in certain types of adnormal growth. A mature cell slightly larger than normal would be more advantageous and would occury less space than the final products of the same cell if it were of a proliferating nature.

In the control seedlings four hours after exposure, the average area of cells constituting the epidermis at 480 microns from the root tip was 195 sq. microns, whereas, in the Modified Crone's irradiated seedlings the area was 278 microns. This indicated that after a short period of time following irradiation, the epidermal cells were in a process of increasing their sizes. This process continued in the Modified Crone's irradiated epidermal cells until a cell area of 1669 sq. microns was reached 21 hours after exposure. The epidermal cells of the controls maintained a degree of uniformity in cell size throughout their period of growth. After 21 hours, the average area of non-irradiated epidermal cells at 480 microns from the root tip was 168 sq. microns. Thus, at the above time interval, the

irradiated epidermal cells were approximately ten times larger than the non-irradiated cells.

The same response of cell size increase was evident in the cortical cells but was not expressed to such a great extent as that demonstrated in the epidermis. Four hours after exposure any cortical cell constituting the irradiated Modified Crone's seedlings 480 microns from the root tip had an average area of 325 sq. microns, whereas, in the controls the measurement was 170 sq. microns. Thirty hours after exposure the measurements were 1,721 sq. microns and 439 sq. microns, respectively.

The change in cell size of the irradiated xylem tissue was less pronounced. The average area of cells constituting the xylem at 480 microns from the root tip was 3582 sq. microns in the irradiated tissues 33 hours after exposure, whereas in the non-irradiated tissues it was 3429 sq. microns.

Chromosomal Changes. The fact that the seedlings were exposed to ionizing radiations at a very early age might account for the small number of chromosomal aberrations that were observed. Since the seedlings were exposed to X-radiations shortly after germination, the process of cell size enlargement of the embryonic cells was probably at a maximum, whereas cell division was possibly just starting. If the seedlings had been in a more active state of cell division, the possibility of more chromosomal aberrations would have been greater. This assumption is made through the knowledge that chromosomes in an active state of mitotic division are more susceptible to radiation damage.

Due to the small sizes of the ten pairs of chromosomes of Sorghum vulgare, it was impractical to analyze the divisions for all types of chromosomal abnormalities that are produced by ionizing radiations. Bridge

Bridge formations and fragmentations at anaphase stages were the only aberrations that could be determined with any degree of certainty. Of all the seedlings examined during the 48 hours after exposure, only six cells contained abnormal chromosome formations of the above types. This should not imply that these were the only aberrations present in the irradiated material, but it does suggest that the dose administered had an extremely small visible effect on the chromosomes.

Absence of Cell Wall Formation and Changes in the Plane of Division. In the control material, the planes of division during mitosis were at right angles to the long axis of the root, whereas, in the irradiated seedlings some of the planes were changed from a few degrees in some cases to 180° in others. Where these changes occurred, areas composed of small irregular cells were observed. There were no indications that these irregular divisions were of a proliferating nature. In those cells which revealed a change in the plane of division, there were no visible evidences of abnormal chromosome behavior. Since the figures of each process of mitosis experienced the change, it appeared that this type of damage was induced prior to cell division. The middle lamella also experienced the change as its formation consistently divided the chromosomes into equal complements. There was no evidence that divisions occurred in one plane and cell wall formation in another. A change in the plane of division suggests that the radiation effect occurred prior to cell division. Thus, it was an expression of a radiation damage that occurred in an inactive cell but was not manifested until the onset of cell division.

An infrequent condition that occurred in the irradiated seedlings was the continuation of nuclear division without cell wall formation. This produced multinucleated cells whose total areas were not changed to any

great extent. Cells were observed in which the number of nuclei varied from two to four. By observing cells in which one of the two nuclei present was in the process of division, it appeared that the latter process was normal. This fact suggests that the processes of nuclear division and cell wall formation are governed by different mechanisms.

Effects of the Administration of KCl to Irradiated Seedlings

Although many types of radiobiological effects have been investigated at the cellular level, little attention has been given to the direct disturbing influence of ionizing radiations upon the distribution of electrolytes between cells and their surrounding medium. A review of the role of potassium in cell metabolism has indicated that one of the factors controlling enzyme activity is the inorganic ionic environment within cells. Since the principal cation is potassium, it is not surprising that the rates of several enzyme reactions have been demonstrated to depend on the concentration of this element. If these processes are blocked by a potassium imbalance following treatment with ionizing radiation, it is evident that normal metabolic functions of the cell will diminish.

It has been assumed (22) that the administration of KCl following irradiation might aid in the restoration of the potassium balance thus preventing or retarding the appearance of radiation-induced morphological changes. Having set up growth as a criterion for measuring radiation damage, this criterion was used to determine the effects of administering KCl after irradiation. Any changes in the processes affecting the total expression of growth in the treated irradiated seedlings were attributed to KCl.

Elongation and Maturation of the KCl-treated Irradiated Seedlings. Cytological observations at hourly intervals over a 48 hour period following exposure revealed a difference in the growth patterns of the irradiated non-treated and irradiated KCl-treated seedlings. The most obvious difference was the extent to which elongation and maturation occurred in the meristematic regions of the two types of irradiated seedlings. In the irradiated non-treated seedlings these processes were occurring in practically all parts of the meristematic area 48 hours after exposure, whereas, in the irradiated KCl-treated seedlings these processes were limited in this area, and an active meristem was maintained throughout the period of observation.

This suggested that the administration of KCl helped to maintain a more active meristematic region in the irradiated-treated seedlings. If this assumption be correct, the administration of KCl might serve as a tool in radiation health physics. The tolerance level (40) for total or limited body exposure is 0.1 rem. in any 24 hour period. No person should knowingly expose himself or cause others to be exposed to a quantity that exceeds this figure. If it be assumed that an individual so exposed to ionizing radiations developed a potassium imbalance, such cases might be benefited by the administration of KCl thus aiding in the restoration of the potassium balance.

It is proposed that following irradiation the response of nausea, vomiting, diarrhea, and a reduction in the number of lymphocytes and neutrophils might partially be associated with a potassium imbalance. If this be true, an administration of KCl might be beneficial in attenuating these radiation effects.

Mitotic Block. That the administration of KCl was slightly beneficial in releasing the mitotic block earlier in those cells which had demonstrated the greatest radiation damage was verified by a tabulation of the reappearance of cell divisions and determining their locations. This suggests that potassium is possibly a part of the governing mechanism of cell division. If this proposal be correct, the mitotic block produced by ionizing radiations might possibly be attenuated by restoring the potassium balance through the administration of KCl.

It had been found in the Modified Crone's irradiated seedlings that the greatest morphological changes occurred in the epidermal cells and decreased as one moved inward to the xylem elements. This had a bearing on the pattern of mitotic recovery of the two groups of irradiated seedlings. After nine hours following exposure in the irradiated KCl-treated seedlings, cell divisions were found in the primordial xylem, cortical and epidermal cells. It was not until thirteen hours after exposure that mitoses began to appear in all tissue types of the irradiated Modified Crone's seedlings.

Cell Size. Numerical values of the cell sizes existing in the irradiated KCl-treated epidermal, cortical and xylem cells gave evidence that KCl was beneficial by preventing a pronounced difference in the size of the cells constituting these tissues at 480 microns from the root tip. Thus, the administration of KCl to irradiated tissues appears to be beneficial in helping maintain uniformity in the meristematic region. This in turn would produce cells more uniform in size at 480 microns from the root tip.

If cell size can be used as a criterion for the determination and location of the processes of elongation and maturation, the difference existing between the cell sizes of the irradiated non-treated and the

irradiated KCl-treated seedlings is in agreement with the microscopic observations.

SUMMARY

Experiments have been carried out under the assumption that ionizing radiations produce a potassium imbalance and that the administration of KCl following irradiation might aid in the restoration of this balance, thus preventing or retarding the appearance of radiation-induced morphological changes.

Cytological Effects of X-Radiations

The processes of mitosis, elongation and maturation in roots of sorghum seedlings that were exposed to 2800 r-units of X-radiation were affected either by (1) a temporary mitotic blockage with elongation and maturation continuing at a normal rate in those cells which were experiencing the blockage, or (2) a permanent mitotic blockage with elongation and maturation continuing at a normal rate in those cells experiencing the permanent stoppage of cell divisions, or (3) a permanent mitotic blockage and a stoppage of the process of elongation with maturation continuing at its normal rate in the cells in which these processes were blocked. This indicates that mitoses are more sensitive to ionizing radiations than the processes of elongation and maturation. Further it suggests that the growth processes of mitosis, elongation and maturation are possibly independent of each other and are not necessarily a continuous chain of events which require a definite order of occurrence.

A quantitative expression for radiation injury was obtained by determining the cell size for any cell constituting the various tissue types at

comparable locations. If cell size can be used as a criterion for the determination and location of the processes of elongation and maturation the differences existing between the cell sizes of the irradiated and non-irradiated seedlings are in agreement with the microscopic observations.

A limited number of chromosomal bridge formations and fragmentations were observed in the irradiated non-treated seedlings. This should not imply that these were the only aberrations present in the irradiated material, but it does suggest that the dosage administered had an extremely small visible effect on the chromosomes.

In the irradiated non-treated material, some of the cells demonstrated a lack of cell wall formation which produced multinucleate conditions, and in other cells a change in the plane of division occurred which produced areas composed of small irregular cells.

Effects of the Administration of KCl to Irradiated Seedlings

The most obvious difference which the administration of KCl produced in the irradiated seedlings was the extent to which the processes of elongation and maturation were reduced in the meristematic area of the materials. In the irradiated non-treated seedlings, these processes were occurring in practically all parts of the meristematic area 48 hours after exposure, whereas, in the irradiated KCl-treated seedlings these processes were limited in this area, and an active meristem was maintained throughout the period of observation. This suggests that the administration of KCl helped to maintain a more active meristematic region in the treated-irradiated seedlings.

Numerical values of the cell sizes existing in the irradiated KCl-treated seedlings presented evidence that the administration of KCl was

beneficial by preventing a pronounced difference in the size of the cells at 480 microns from the root tip. If the criterion of cell size can be used for the determination and location of the processes of elongation and maturation, the difference existing between the cell sizes of the irradiated non-treated and the irradiated KCl-treated seedlings is in agreement with the microscopic observations.

The administration of KCl was slightly beneficial in releasing the mitotic block earlier in those cells which had demonstrated the greatest radiation damage. This suggests that potassium is possibly a governing mechanism of cell division, and its administration might possibly attenuate the stoppage of cell division produced by ionizing radiations.

It is postulated that the administration of KCl to irradiated tissues is beneficial by releasing the mitotic block and by maintaining a more active meristematic region which in turn would produce fewer morphological changes. It is evident that KCl is not a "cure-all" for alleviating all types of radiation damage. However, its application with therapeutic agents now in use might possibly be more effective in preventing radiation damage.

Plate 1

Explanation of Figures

A. Irradiated non-treated material demonstrating changes in the plane of division and multinucleate condition.

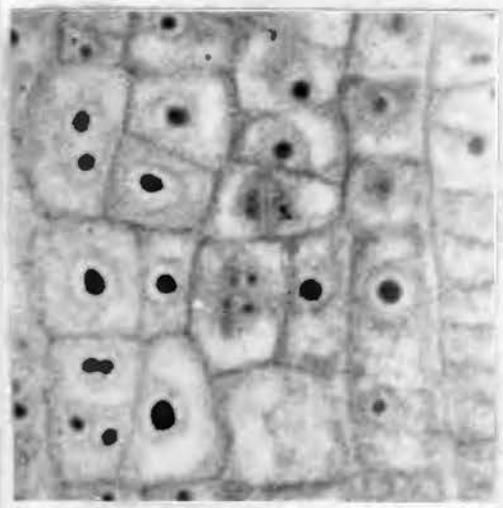
B. Irradiated non-treated material illustrating the chromosomal aberrations of bridge formation and fragmentation.

C. Irradiated non-treated material demonstrating multinucleate condition with one of the nuclei in the process of division.

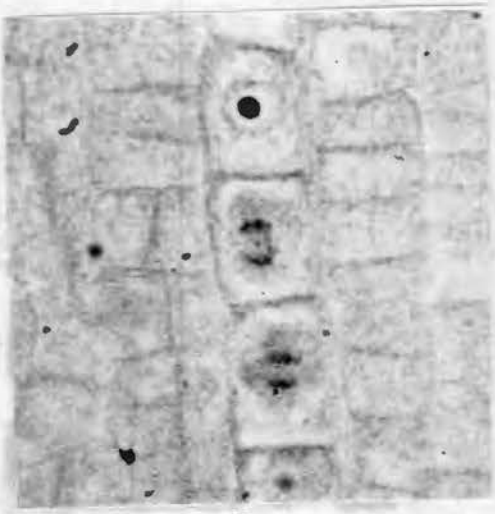
D. Irradiated non-treated material illustrating multinucleate condition and change in the plane of division.

E. Non-irradiated material showing cells with two nucleoli.

F. Irradiated non-treated material illustrating divisions in relatively mature tissue at 480 microns from the root tip.



A



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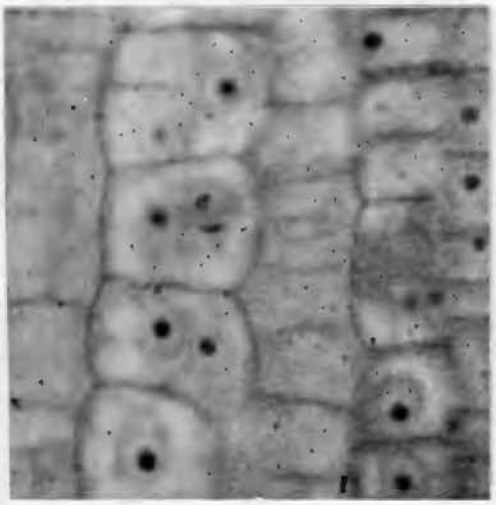

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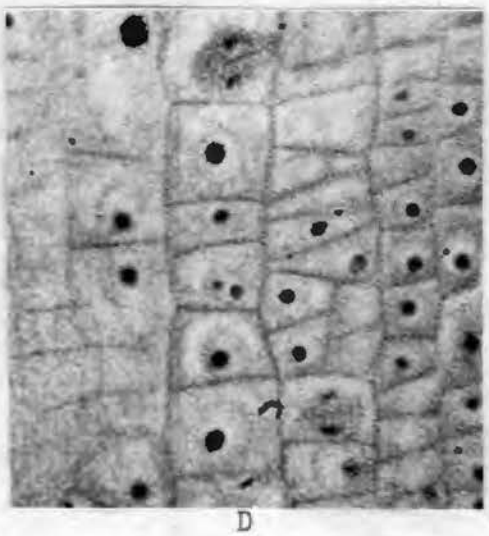

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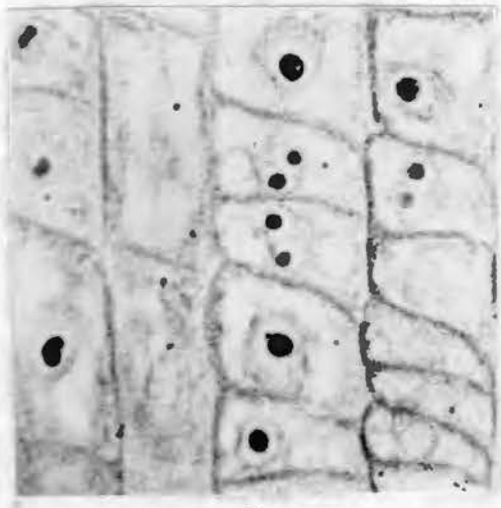

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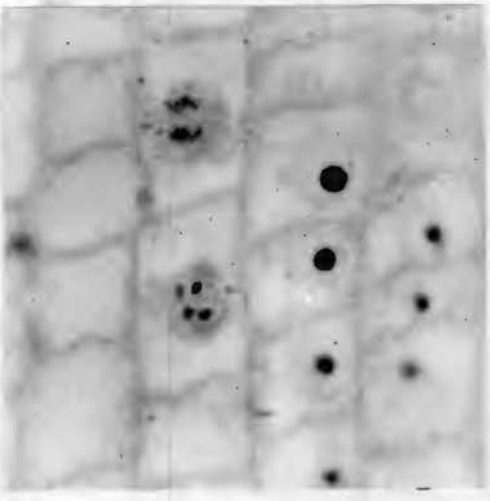

F

Plate 1

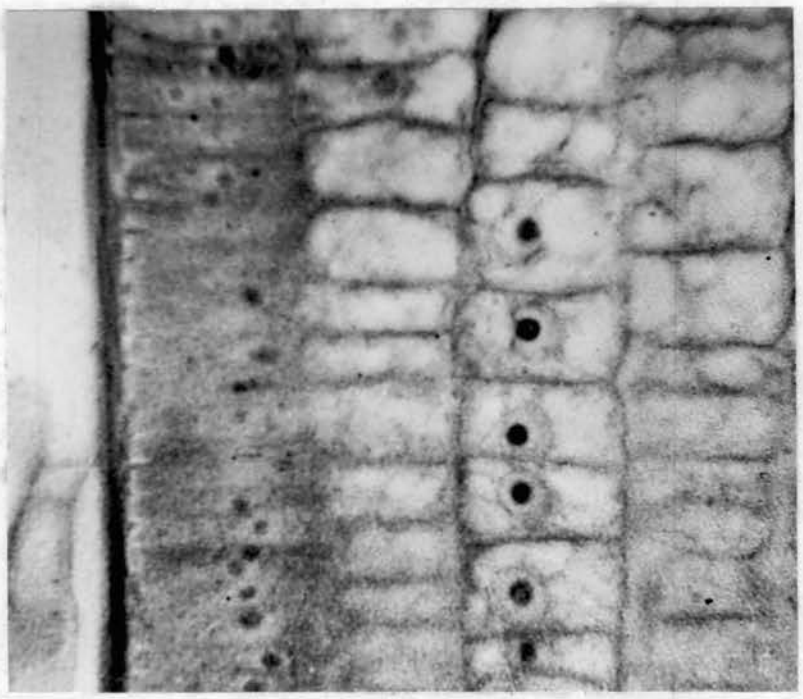

Fig. 4 21 Hours Modified Crone's Control Epidermal and Cortical Cells at 480 Microns from Root tip

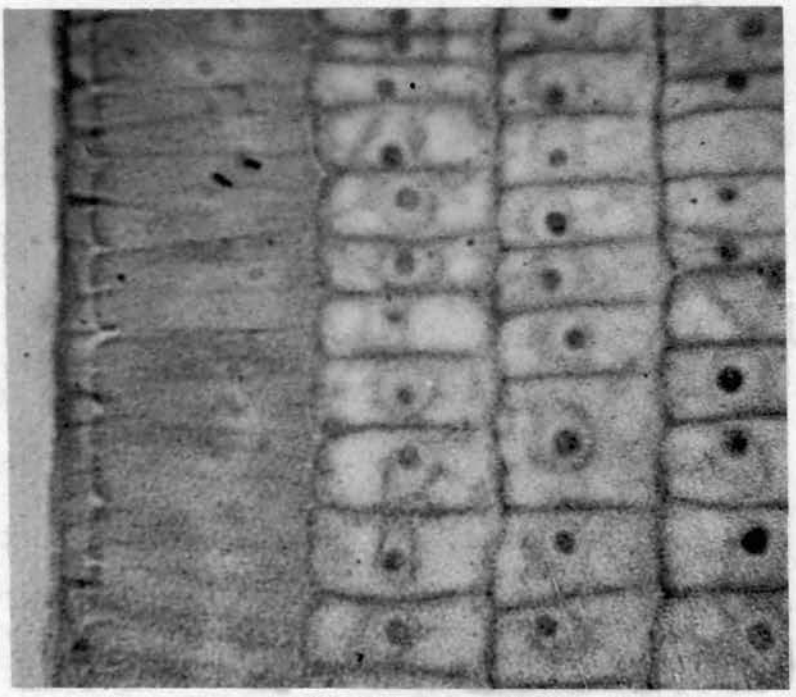

Fig. 5 21 Hours 0.25% KCl in Modified Crone's Control Epidermal and Cortical Cells at 480 Microns from Root tip

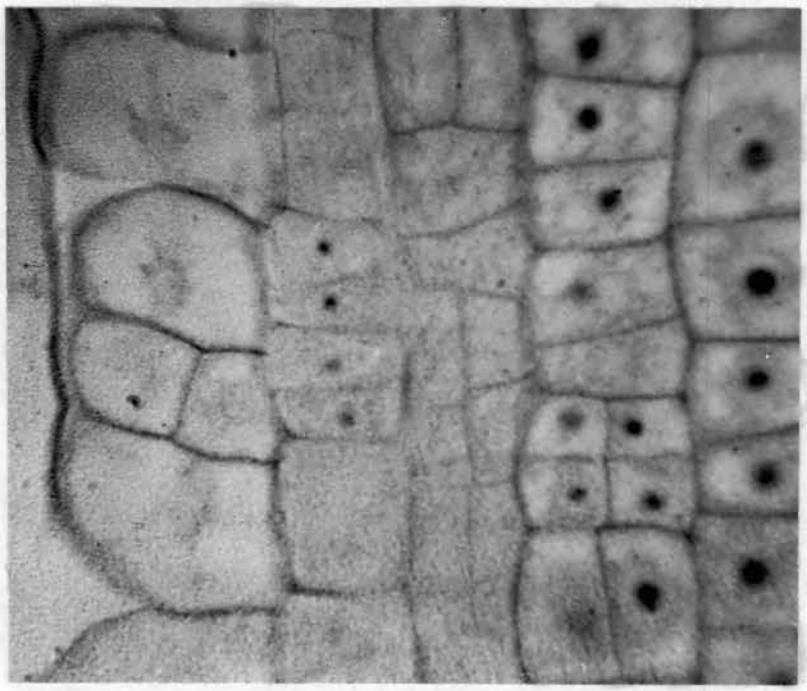

Fig. 6 21 Hours Modified Crone's X-Ray Epidermal and Cortical Cells at 480 Microns from Root tip

Fig. 7 21 Hours 0.25% KCl in Modified Crone's X-Ray Epidermal and Cortical Cells at 480 Microns from Root tip

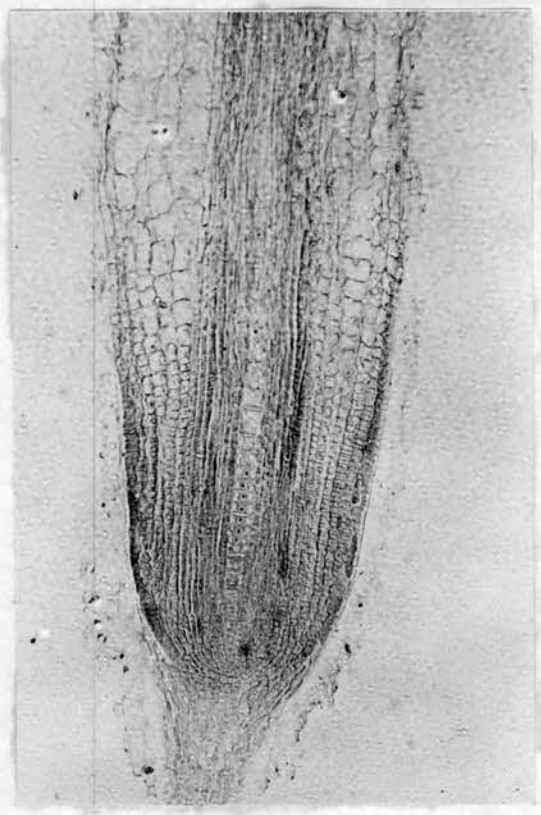

Fig. 8

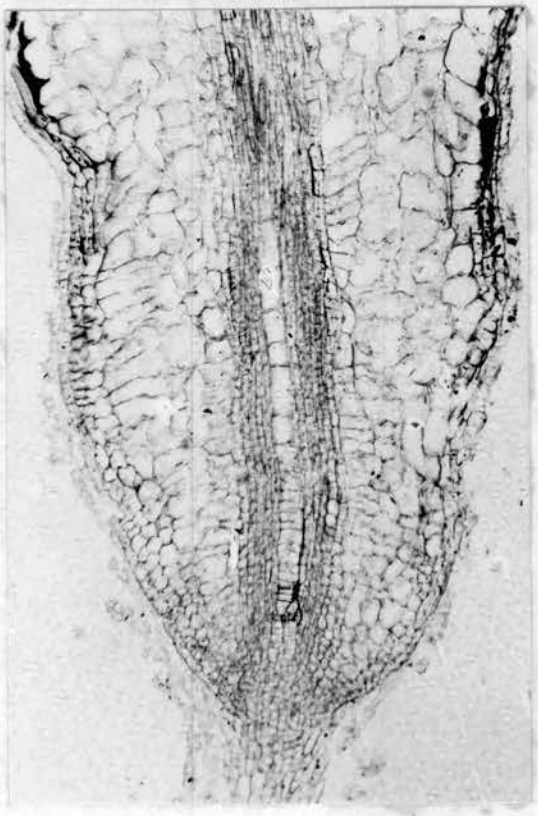

Fig. 9

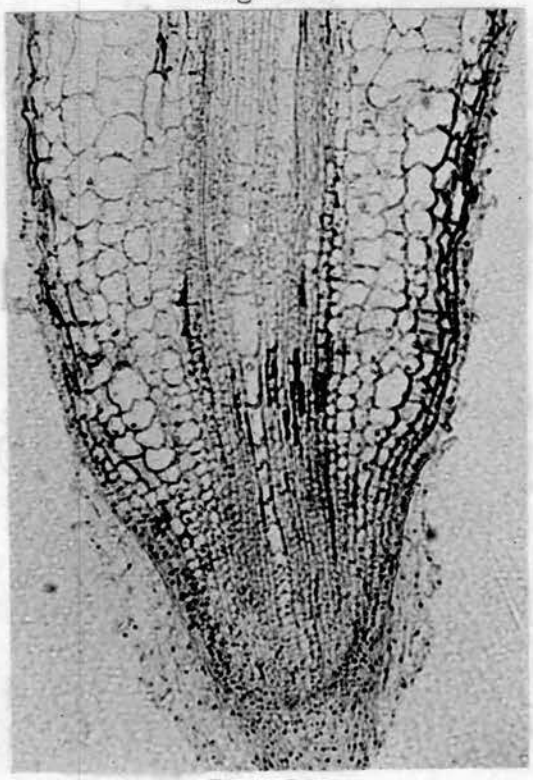

Fig. 10

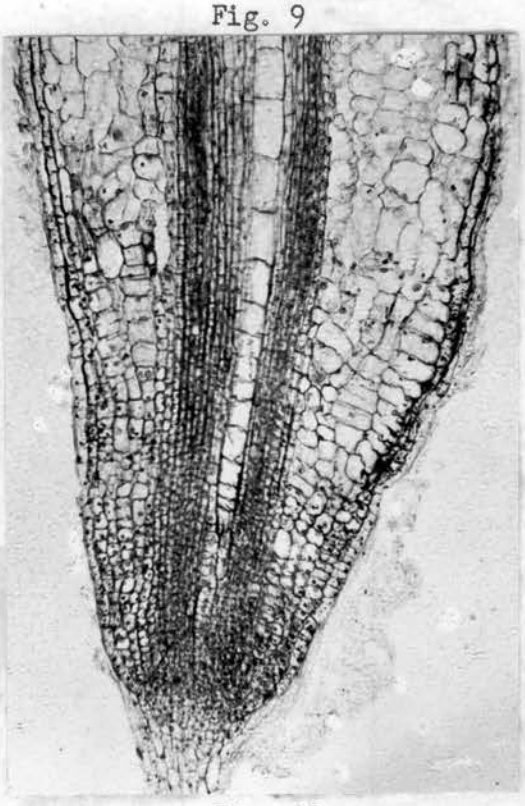

Fig. 11

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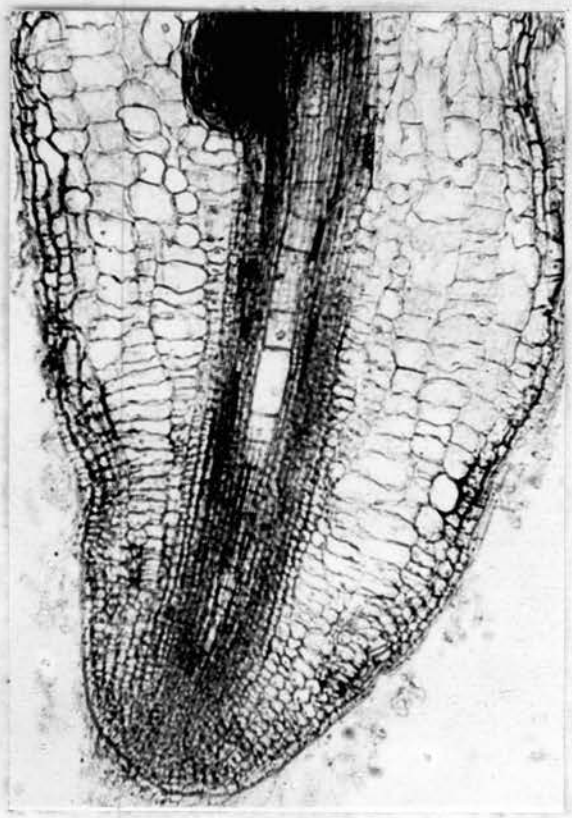


Fig. 12

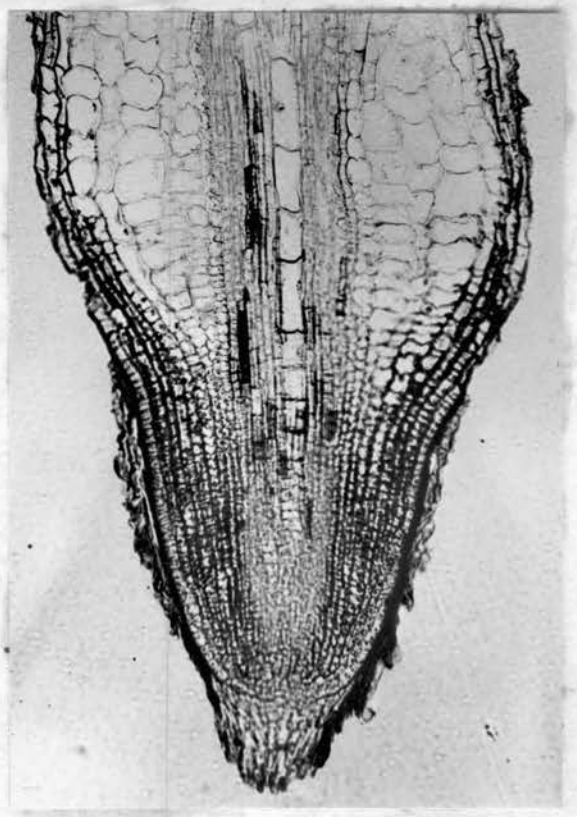


Fig. 13

Explanation of Figures 8-13

Fig. 8 Non-irradiated control seedling after 72 hours of growth.

Fig. 9, 10, 11 Irradiated non-treated seedling 72 hours following exposure demonstrating various degrees of mitotic blockage with the processes elongation and maturation continuing in the meristematic region.

Fig. 12, 13 Irradiated non-treated seedlings 72 hours following exposure illustrating various degrees of mitotic blockage and the stoppage of elongation with the process of maturation continuing in the meristematic area.

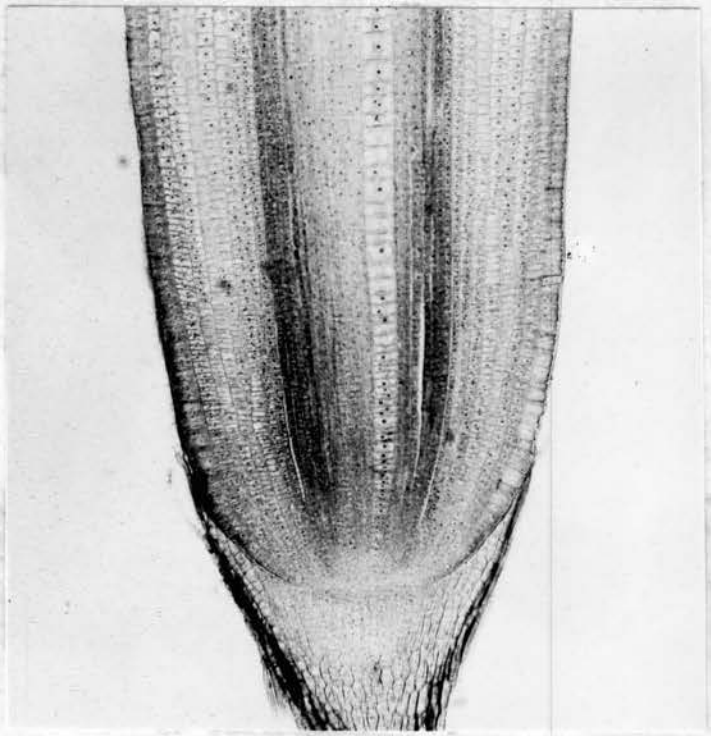


Fig. 14 12 Hours Modified Crone's Control

Fig. 15 12 Hours 0.25% KCl in Modified Crone's Control

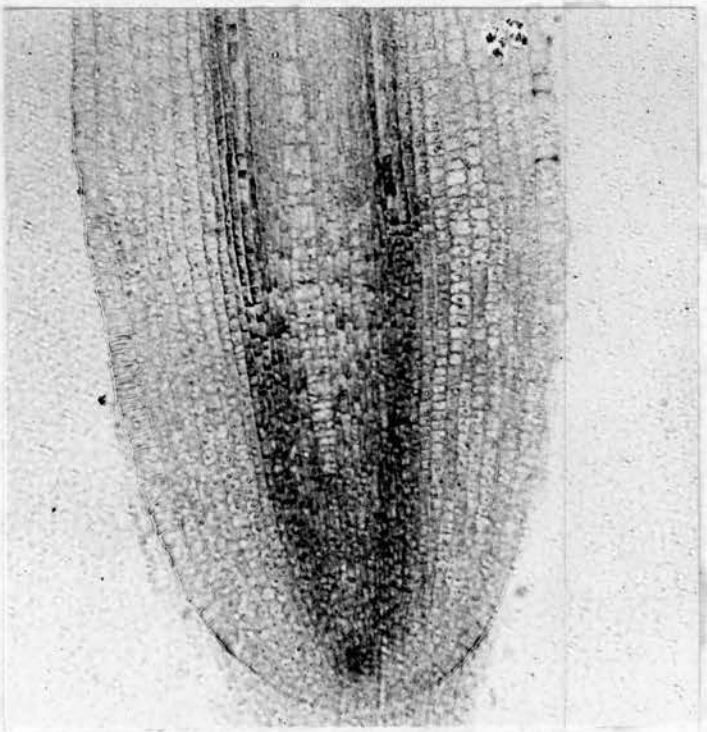


Fig. 16 12 Hours Modified Crone's X-Ray

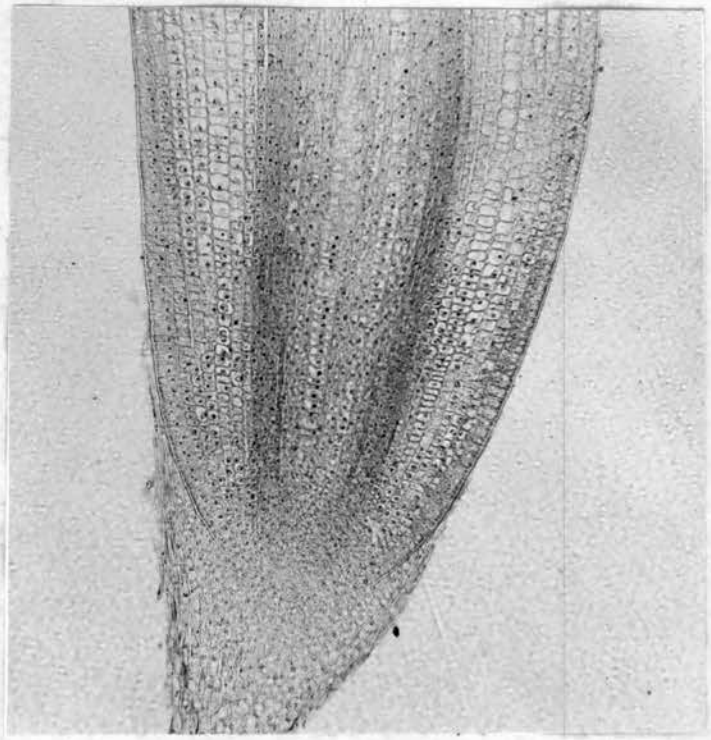


Fig. 17 12 Hours 0.25% KCl in Modified Crone's X-Ray

Fig. 18 18 Hours Modified Crone's Control

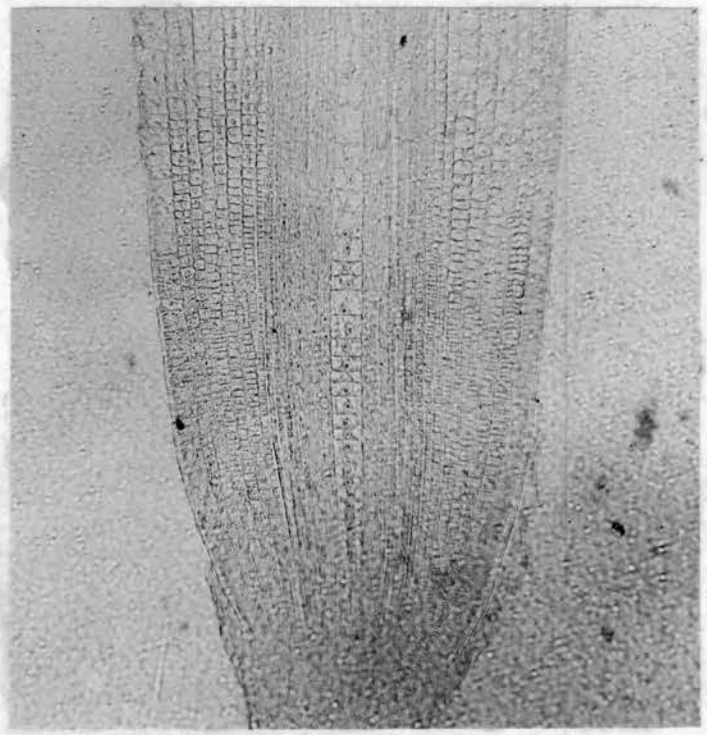
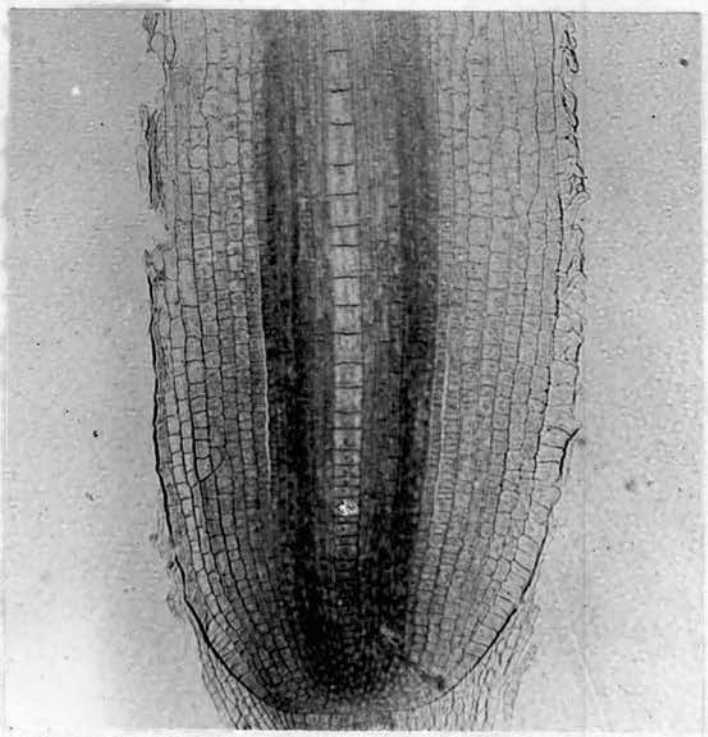


Fig. 19 18 Hours 0.25% KCl in Modified Crone's Control



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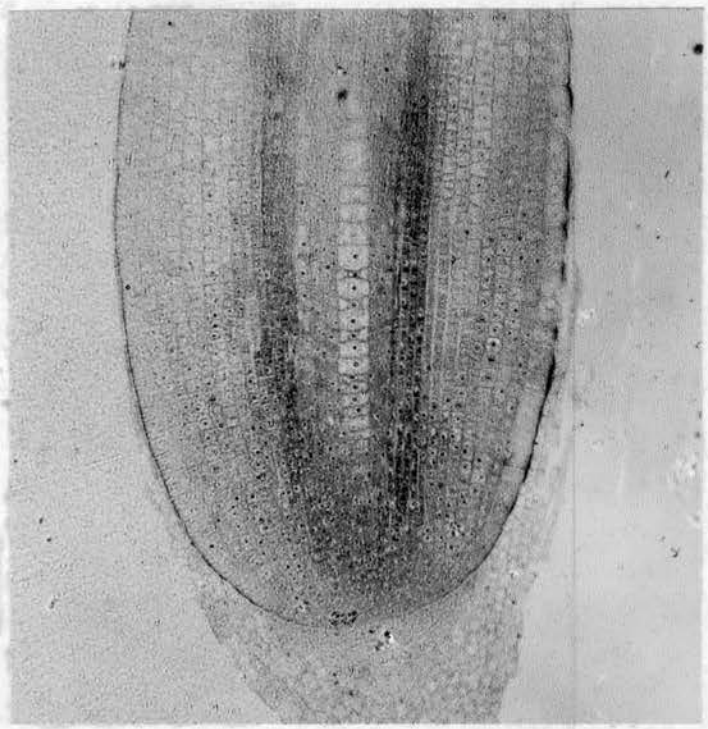

Fig. 21 18 Hours 0.25% KCl in Modified Crone's X-Ray

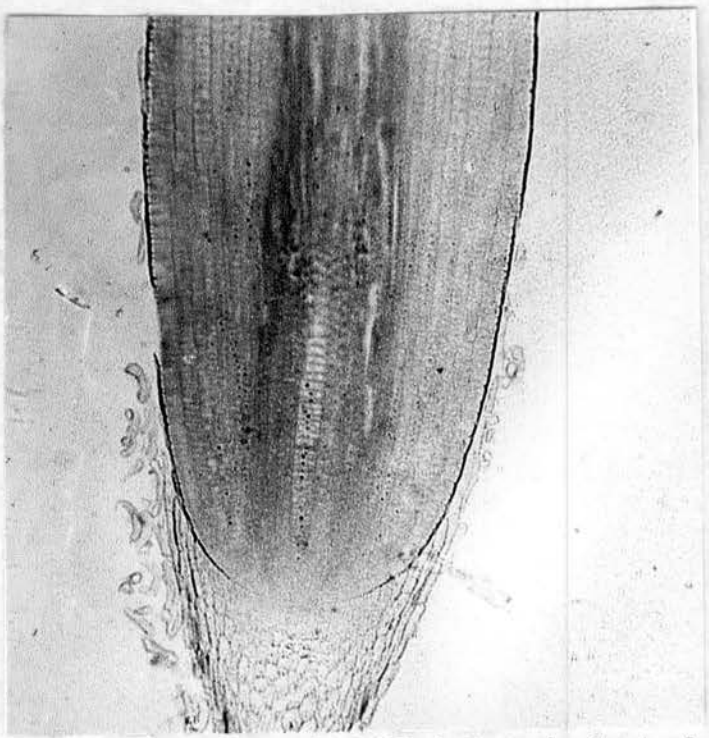

Fig. 22 25 Hours Modified Crone's Control

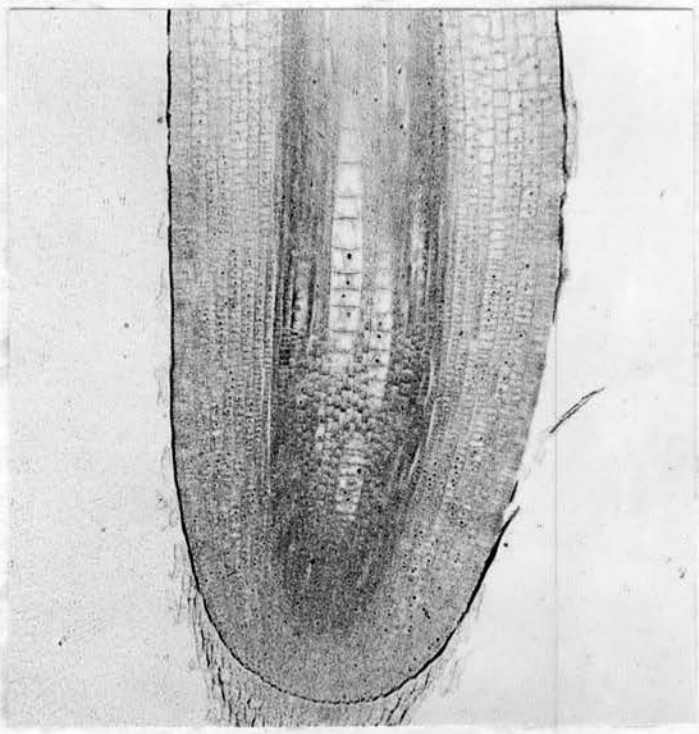

Fig. 23 25 Hours 0.25% KCl in Modified Crone's Control

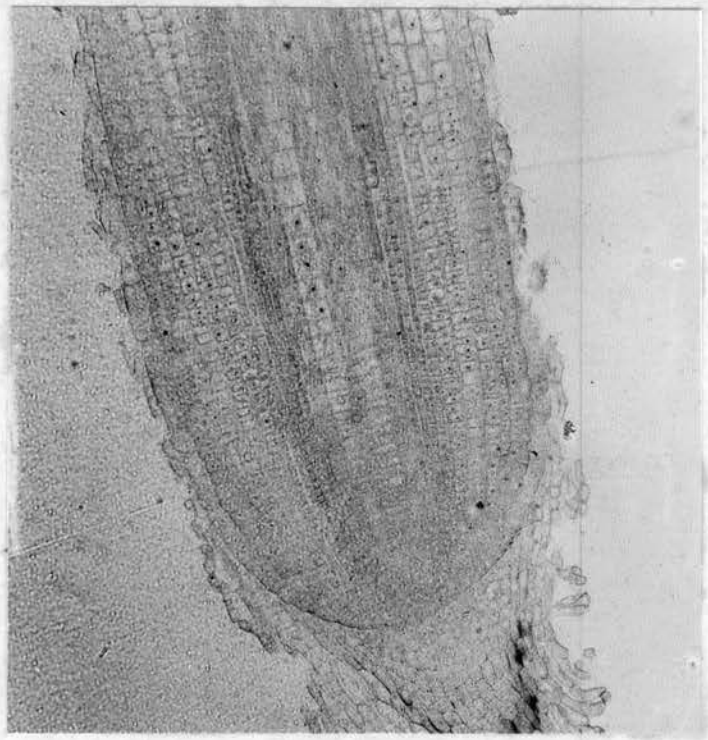

Fig. 24 25 Hours Modified Crone's X-Ray

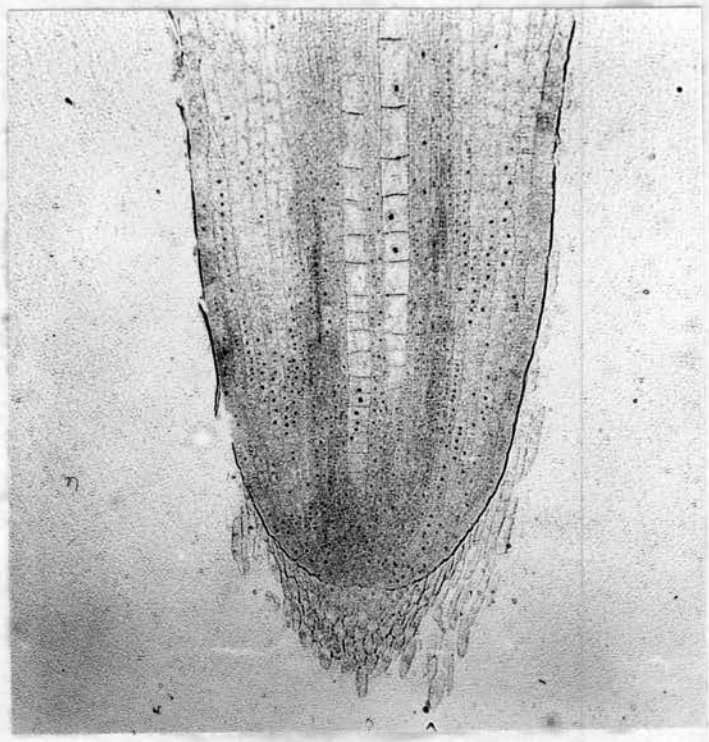

Fig. 25 25 Hours 0.25% KCl in Modified Crone's X-Ray

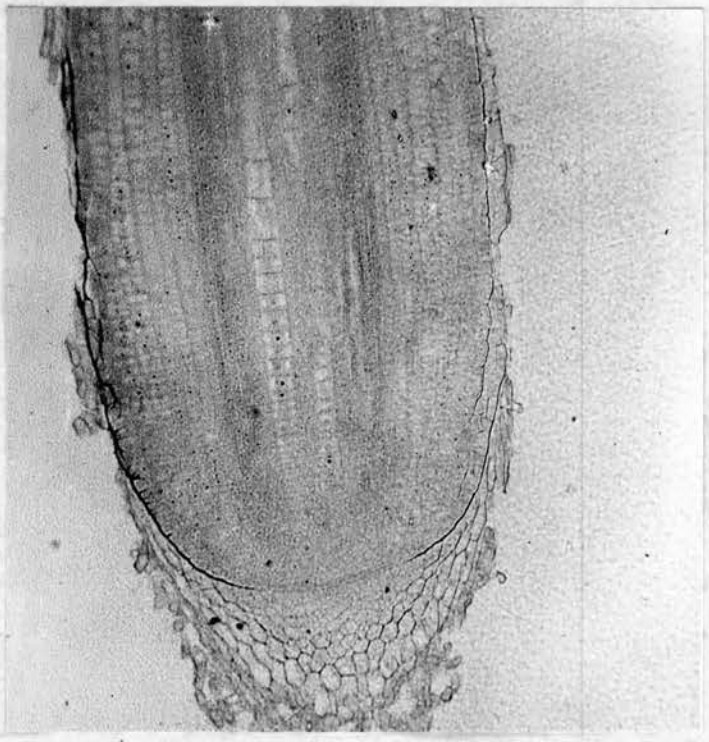

Fig. 26 32 Hours Modified Crone's Control

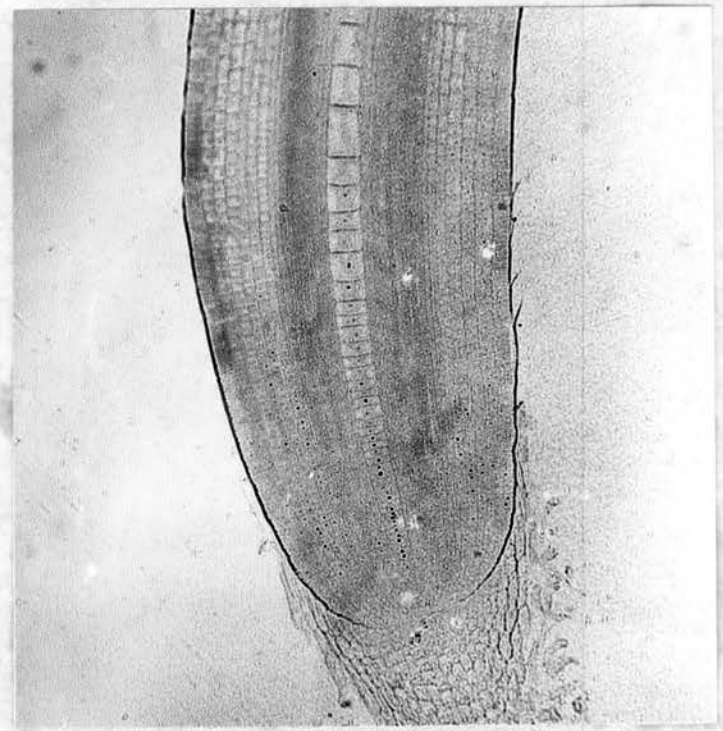

Fig. 27 32 Hours 0.25% KCl in Modified Crone's Control

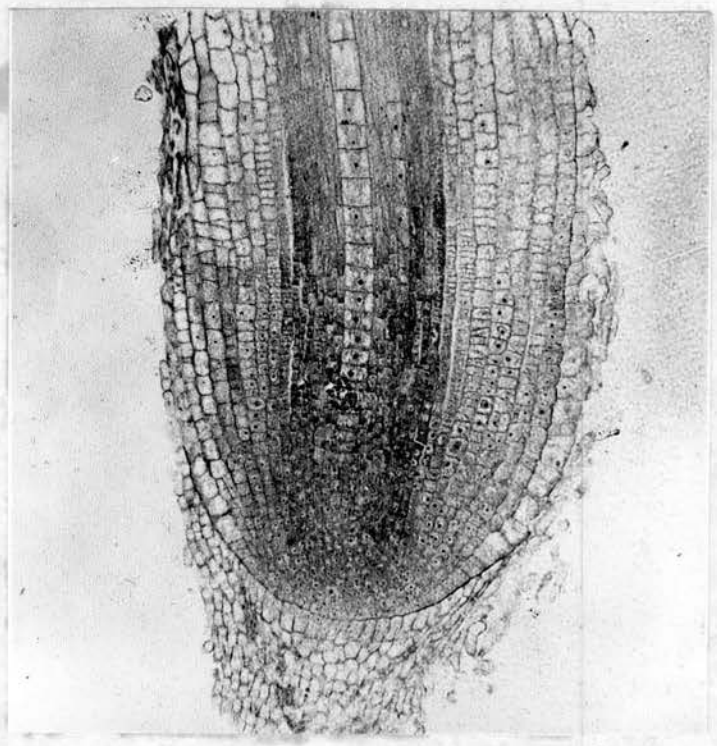

Fig. 28 32 Hours Modified Crone's X-Ray

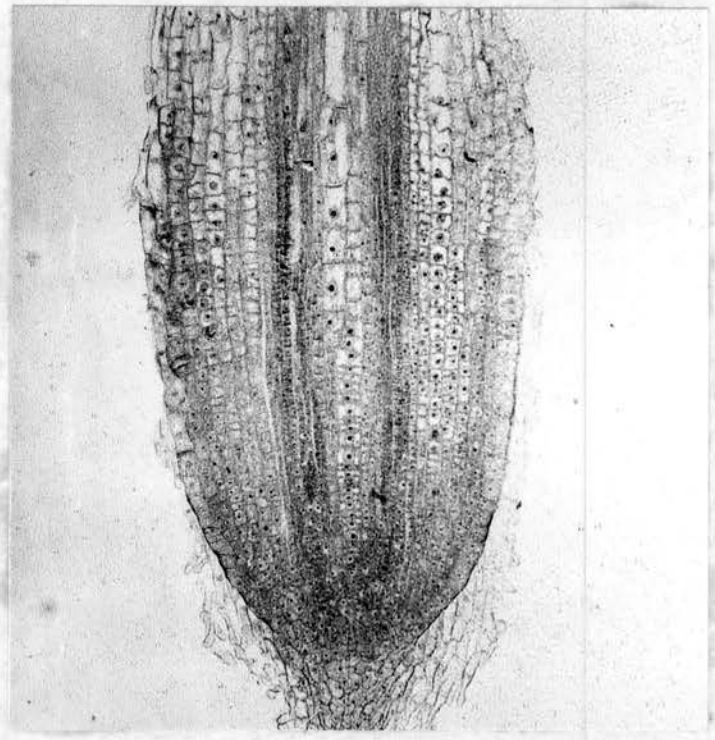

Fig. 29 32 Hours 0.25% KCl in Modified Crone's X-Ray

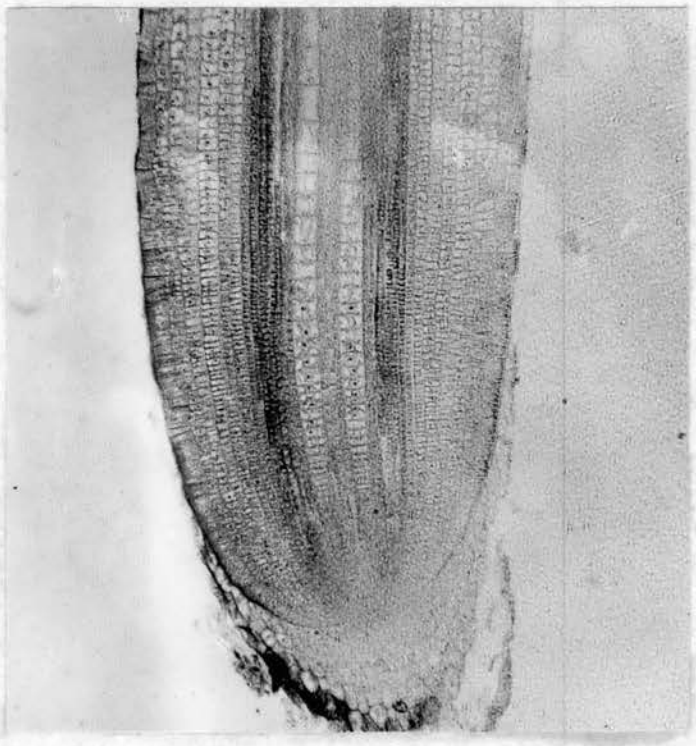

Fig. 30 40 Hours Modified Crone's Control

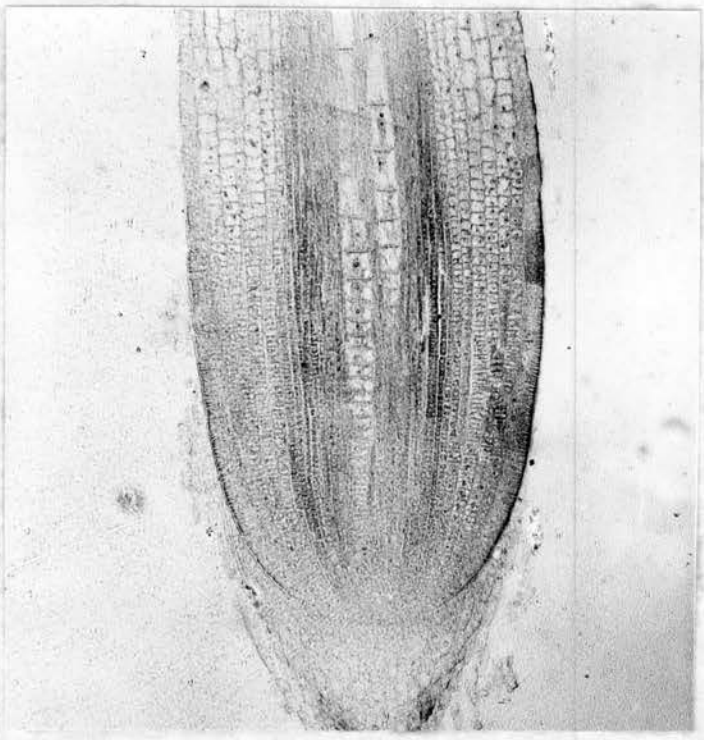

Fig. 31 40 Hours 0.25% KCl in Modified Crone's Control

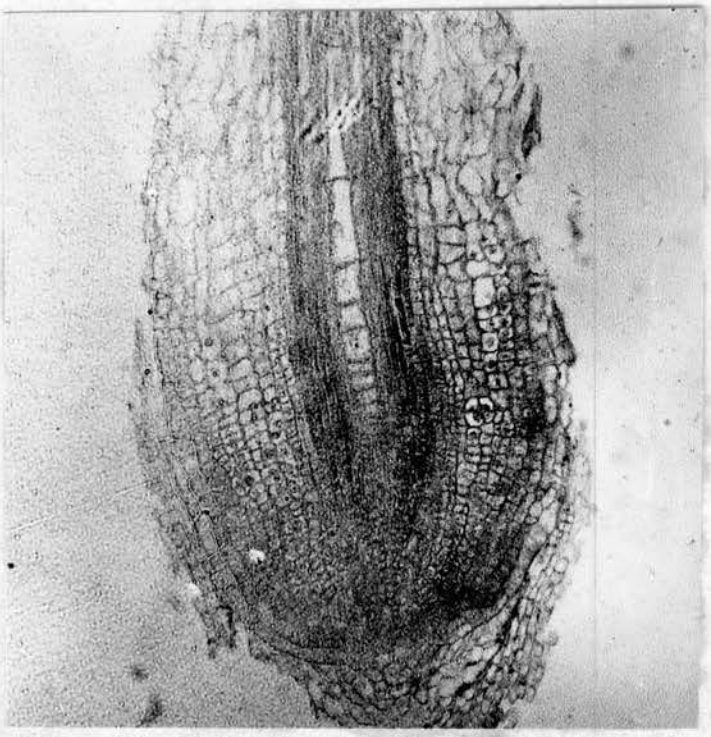

Fig. 32 40 Hours Modified Crone's X-Ray

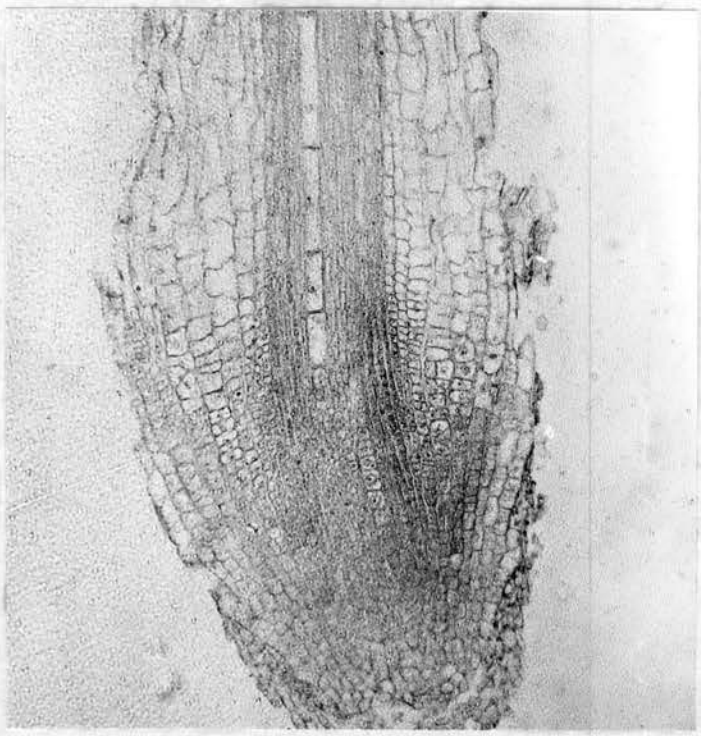

Fig. 33 40 Hours 0.25% KCl in Modified Crone's X-Ray

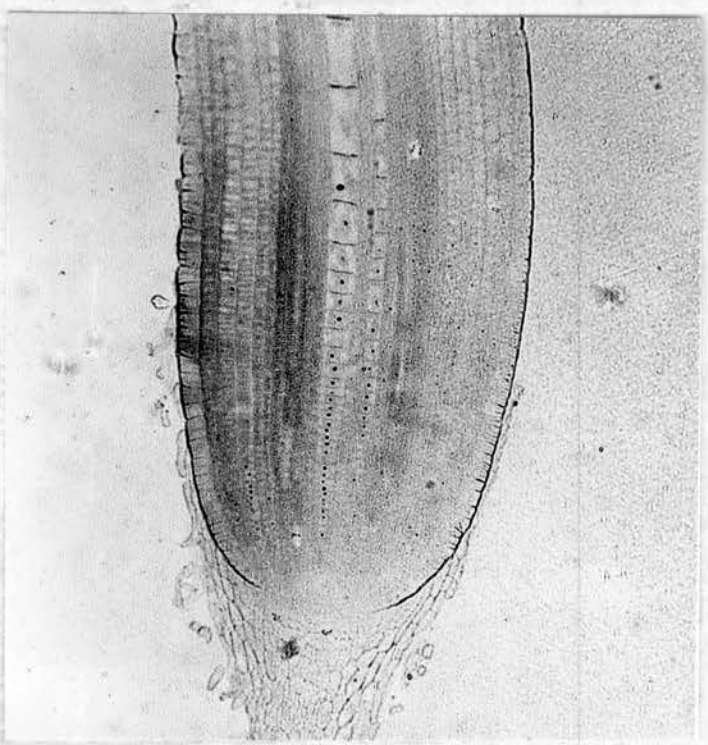

Fig. 34 48 Hours Modified Crone's Control

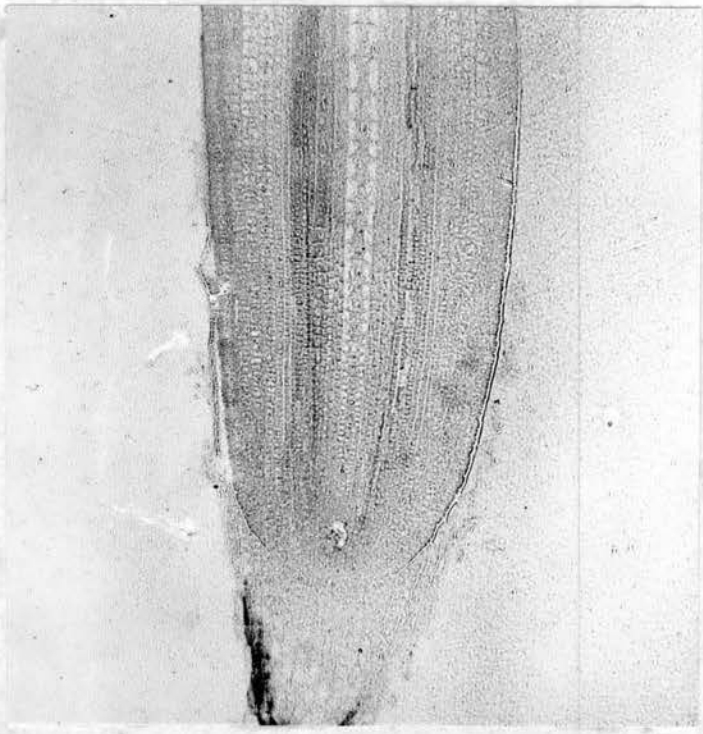

Fig. 35 48 Hours 0.25% KCl in Modified Crone's Control

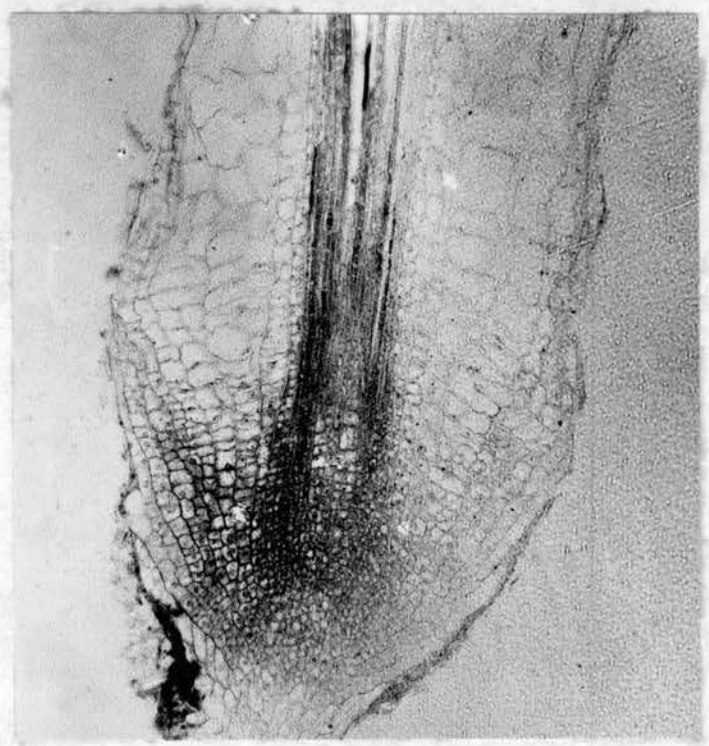

Fig. 36 48 Hours Modified Crone's X-Ray

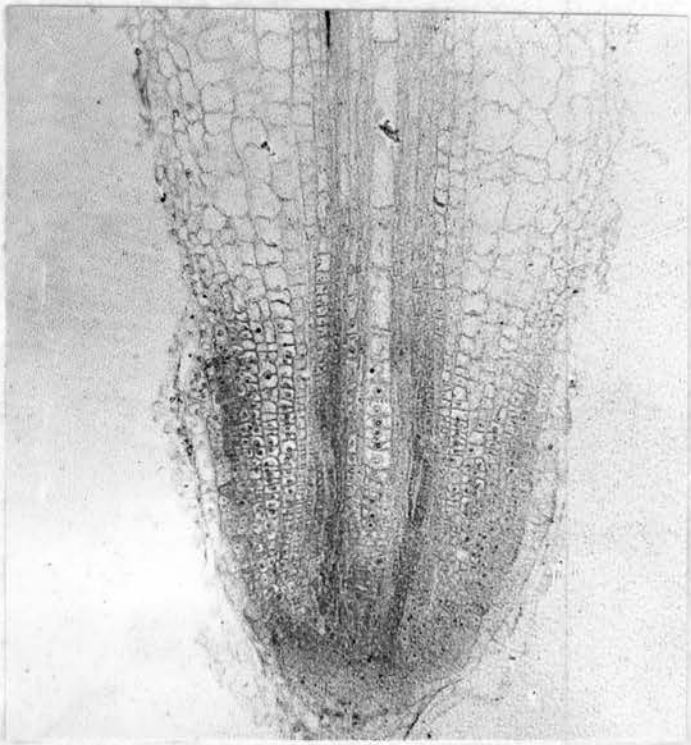

Fig. 37 48 Hours 0.25% KCl in Modified Crone's X-Ray

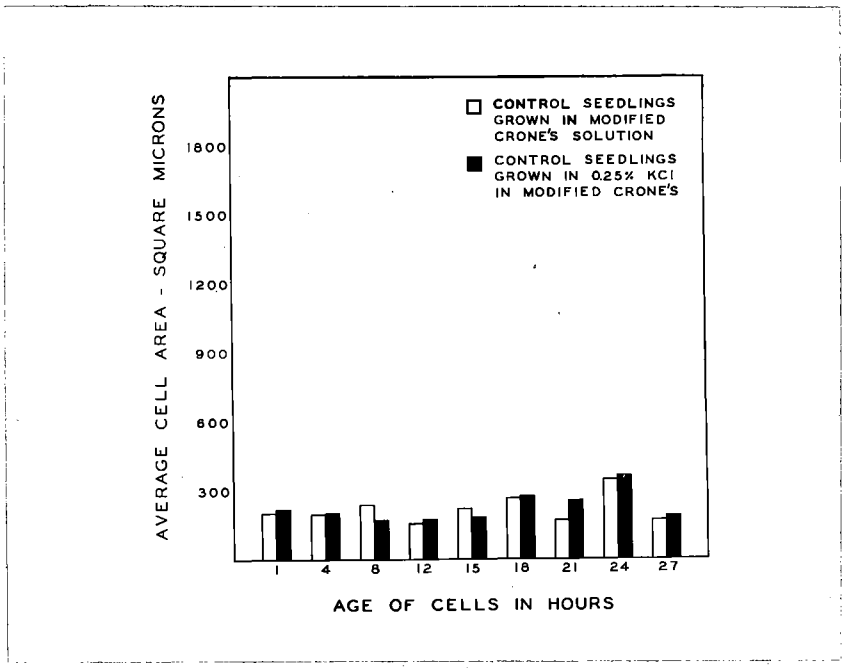

Fig. 38 Average Area for cells constituting the epidermis at 480 microns from the root tip of non-irradiated materials.

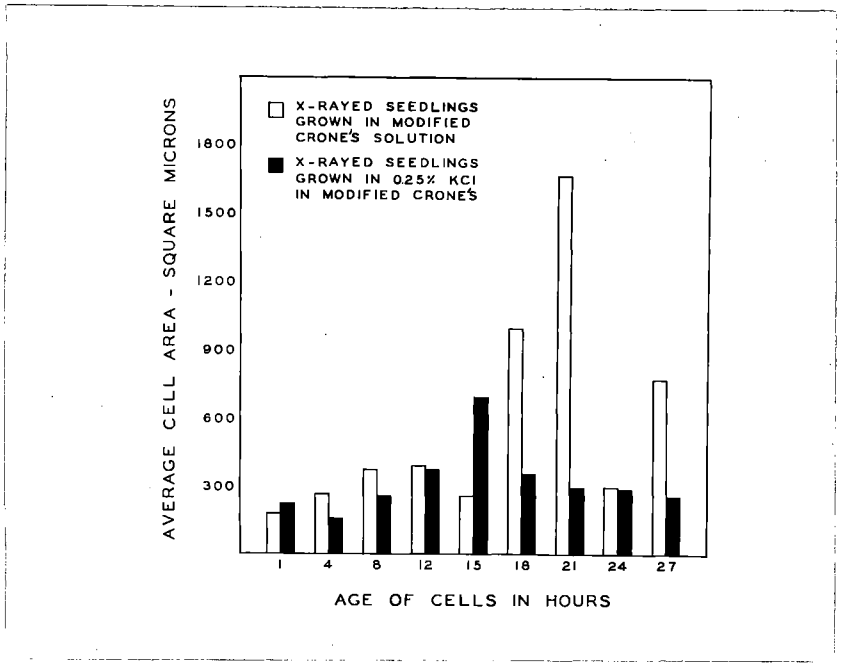

Fig. 39 Average Area for cells constituting the epidermis at 480 microns from the root tip of irradiated materials.

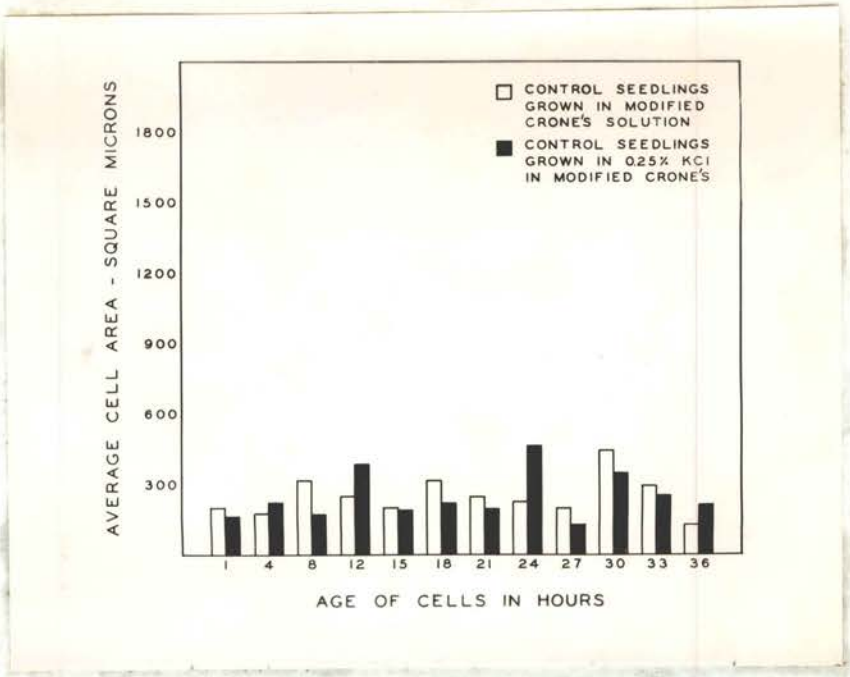

Fig. 40 Average Area for cells constituting the cortex at 480 microns from the root tip of non-irradiated materials.

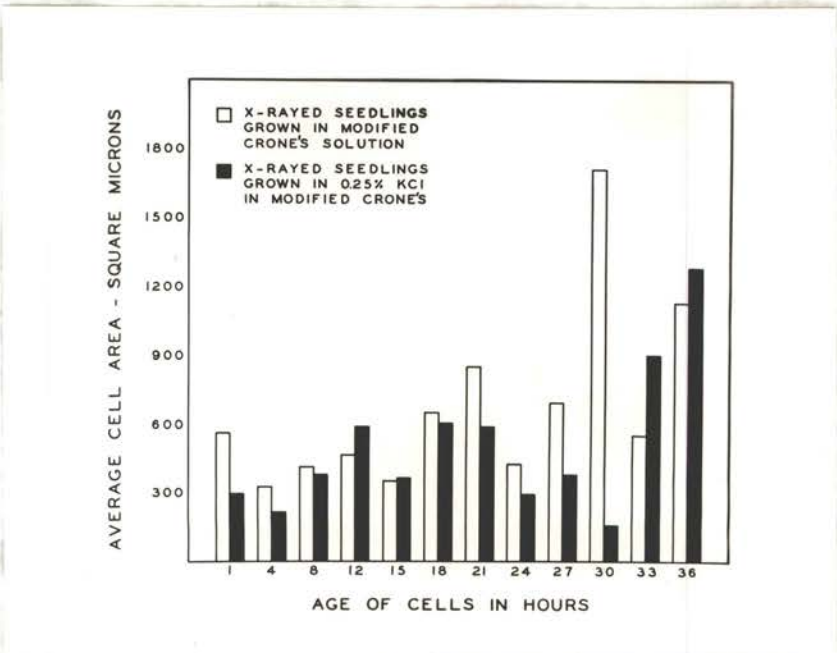

Fig. 41 Average Area for cells constituting the cortex at 480 microns from the root tip of irradiated materials.

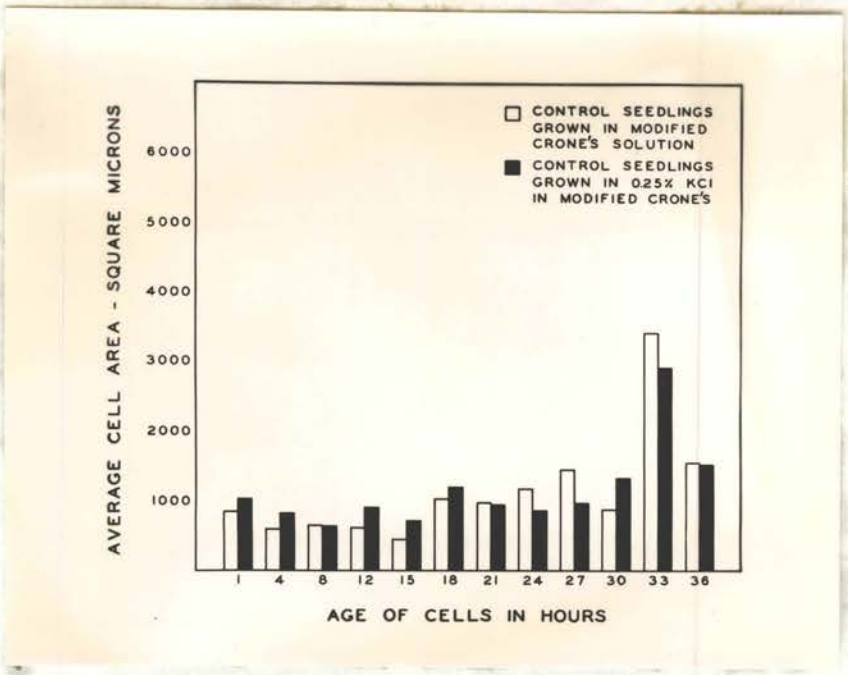

Fig. 42 Average Area for cells constituting the xylem at 480 microns from the root tip of non-irradiated materials.

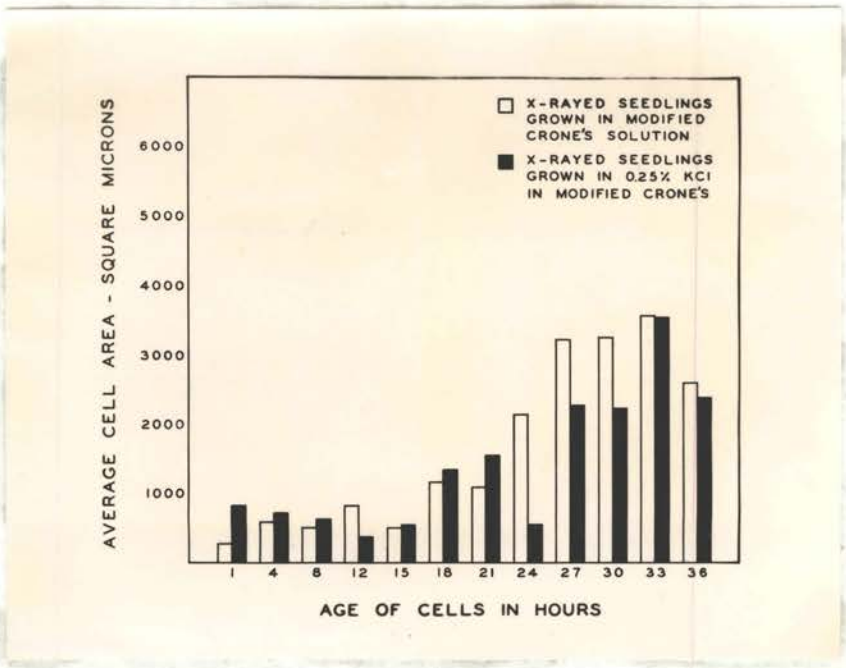

Fig. 43 Average Area for cells constituting the xylem at 480 microns from the root tip of irradiated materials.

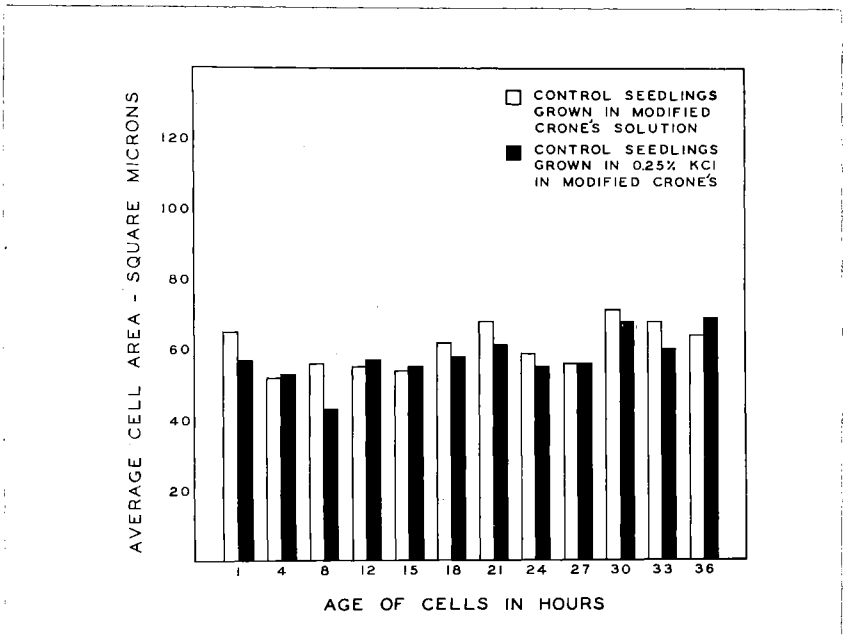

Fig. 44 Average Area for cells constituting the meristem of non-irradiated materials

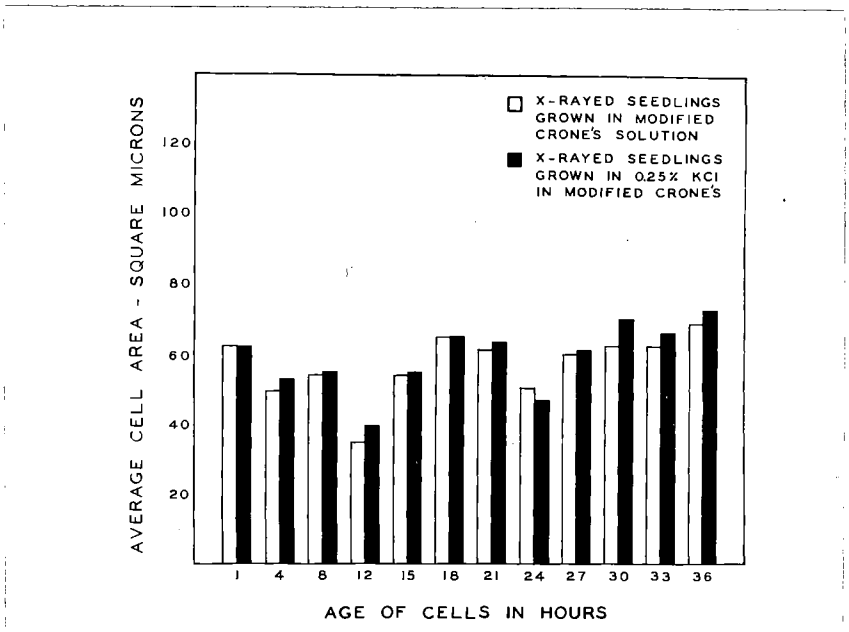

Fig. 45 Average area for cells constituting the meristem of irradiated materials

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