A STUDY OF THE EMBRYOLOGY OF *HORDEUM VULGARE* L. AND THE EMBRYONIC ABNORMALITIES INDUCED BY X-RAYS

By

Ali Mohammed Eunus

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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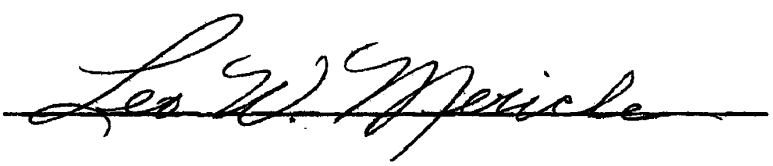
Approved 

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The aim of this study was to investigate the general embryological development of Hordeum vulgare L. and the X-radiated embryonal growth and development compared with the controls. Three sets of plants were raised in a temperature-light-moisture control-room. Flowering and pollination times were recorded and ranging from three to ten days after fertilization flowering spikes were treated with X-rays. Harder rays were applied to plants of the first and third sets of experiments while softer X-rays were used in the second set. During each set of experiments a group of control plants was maintained for general embryological study and for comparison with the radiated embryos.

Normal embryological study included a general development of microsporogenesis, the male gametophyte, megasporogenesis, the female gametophyte, the endosperm and embryogenesis.

Anthers are four-lobed with walls composed of an epidermal layer, two to three middle layers and a tapetum. The tapetum is glandular and uninucleate at the beginning but during the formation of microspores it becomes binucleate. Microspore mother cells by successive divisions usually give rise to microspore-tetrads of the isobilateral type. After the enlargement of microspores the nucleus divides and three-celled pollen grains are formed before pollination.

The gynoecium is composed of two carpels and bears a single ovule laterally near the base of the unilocular ovary. Ovules are crassinucellate, bitegmic and campylotropous and contain a

single hypodermal archesporial cell which functions directly as the megaspore mother cell. The megaspore mother cell by reduction divisions produces a linear tetrad of megaspores the lowermost of which functions and ultimately gives rise to an eight-nucleate "Polygonum type" embryo sac.

The primary endosperm nucleus always divides earlier than the fertilized egg and endosperm development follows the "Nuclear type".

In the study of embryogenesis a three-celled structure (consisting of ci, m, and ca) is found to be formed from the zygote by the first two divisions. Further divisions in tiers m and ca produce a proembryo consisting of tiers l, ph, h, d, f and ci of which the tier l engenders the cotyledon, tier ph the the stem-tip, while h gives rise to the hypocotyl, root, root-tip and the root cap, tiers d and f the coleorhiza and the upper part of suspensor, and ci the lower part of the suspensor of the mature embryo. Embryo development, therefore, follows the "Asterad type".

Examinations of the coleoptile through its gradual stages of development show that it is a single structure and originates from both the scutellum and stem-tip tiers.

The effects of X-rays (softer) as an external agent on the developing embryo were manifested by a number of abnormalities such as enlargement and vacuolation of cells, disturbances in cell walls, disintegration of some cells leading to the formation of cavities, disturbances in the distribution of meristematic tissues within the embryo, a number of proliferations in

different parts of the embryo, and retarded growth of the embryo during the early post-radiation period. In addition to these, deeply stained globular cytoplasmic bodies were observed in radiated embryonic cells. Almost similar kinds of abnormalities were produced by softer X-rays starting with 400r and including 800r. The frequency of occurrence and the degree of proliferations in embryos irradiated with 800r, however, were relatively higher than those produced by 400r and 500r. Treatments with 300r-units of irradiation induced only the appearance of deeply stained globular bodies within cytoplasm while no effects were visible with dosages below 300r.

Comparative measurements of cell-lengths of the treated and control embryos at undifferentiated as well as at differentiated stages showed an increase in cell-lengths within radiated embryos.

Harder X-rays were found to be less effective in inducing embryonic abnormalities than softer rays when equal calculated dosages of the two were applied to the flowering spikes of Hordeum vulgare L.

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A STUDY OF THE EMBRYOLOGY OF *HORDEUM VULGARE* L. AND THE EMBRYONIC ABNORMALITIES INDUCED BY X-RAYS

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Smilacina stellata Desf. New Phytol. 49: 149-155. 1950.

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INTRODUCTION

The science of plant embryology can be traced back to Aristotelian time (384 - 322 B.C.) but embryological study in the present form actually began with the publication of J. Hanstein's famous memoirs on the development of Monocotyledonous and Dicotyledonous embryos in 1870 (from Johansen 1950). Hanstein described the sequence of segmentation and development of embryos, grown under natural conditions, starting with the fertilized eggs and concluding with mature stages of the embryos. Until recently embryology consisted mainly of these descriptions of embryonic development plus some embryological studies comparing different groups of plants. In 1924 a new field of embryological study, experimental embryology, came into existence. Generally in this kind of study very tiny embryos are excised from ovaries and are then grown as artificial cultures. Their further growth and development are followed in order to determine the nutritional requirements of the embryos during their early stages of growth. Thus far only one study has dealt with the effects of external agents on growth and development of embryos growing intact on plants (Eunus and Mericle, 1954). Investigations concerning such effects opens up a new and interesting branch of embryological study. With this view in mind X-radiation was chosen as an external agent to investigate its effect on the embryonal growth and development of Hordeum vulgare L. (barley). X-rays were selected primarily because their dosage and quality can be regulated and because they are readily available for experimentation. Hordeum was selecd for the present study since it is considered one of the most suitable plant materials for testing the biological effects of irradiation and also, since a number of

investigations dealing with other aspects of barley have already been published by other workers. Moreover, a study of general embryological development (in broad sense) will aid in filling up gaps in the literature dealing with the embryology of Hordeum.

This investigation, therefore, involves a general developmental study of microsporogenesis, the male gametophyte, megasporogenesis, the female gametophyte, the endosperm and embryogenesis of Hordeum vulgare L. as well as a study of morphological abnormalities induced by X-radiation on developing embryos growing intact on the plants. It also includes a study of some of the qualitative effects of X-rays on Hordeum embryos.

HISTORICAL SURVEY

Survey of Radiation Physics

For a better understanding of the nature of X-rays and their actions on biological materials and to facilitate comparison between these actions and the effects observed in the present investigations, the process of X-ray production and the present day knowledge about the interaction of X-rays with matter is discussed briefly.

Discovery of X-Rays

X-rays were discovered by Röntgen in 1895 (from Weyl and Warren, 1951). While he was operating a gas-discharge tube in a darkened room he observed that a thin layer of barium platinocyanide on a cardboard, placed against the anode, was emitting light. By covering the gas-discharge tube with black paper and by placing objects of various thicknesses in between the gas-discharge tube and the barium platinocyanide screen, he demonstrated that radiant energy was being propagated from the discharge tube to the screen. These invisible rays Röntgen named X-rays.

Production of X-Rays

Today X-rays are produced in an evacuated hot-cathode X-ray tube (Weyl and Warren, 1951; Glasser, Quimby and Taylor, 1950). Electrons are supplied by a coil of incandescent tungsten wire heated by the passage of an electric current through it. The electrons emitted from the thermoionic cathode move toward the anode with an uniformly accelerated motion and collide with the latter. Following collision, X-rays of various wave-lengths are produced depending on how much of the energy of the impinging elec-

trons are transformed into in quanta of radiation (photons). Thus, X-rays produced in an evacuated hot-cathode X-ray tube are heterogenous. Since these heterogenous rays are unsuitable for therapeutic treatments, relatively homogenous rays are obtained from therapeutic machines by passing the original X-ray beam through a glass and oil layer, and then through some added filters. By filtration X-rays become relatively homogenous as well as harder (rays of shorter wave-lengths) since X-rays of longer wave-lengths are mostly absorbed by the filters.

Interaction of X-rays with Matter

Ionization. The principal means of energy dissipation by X-radiation is ionization. An X-ray on its passage through matter transmits some of its energy to an electron of an atom and causes the electron to leave the atom with a high velocity. An atom so ionized is left positively charged and referred to as an ion. The ejected electron in turn, after some time, becomes attached to a second atom making it a negatively charged ion. Thus during ionization a pair of oppositely charged ions are formed, which are assumed to cause chemical change in the molecules of which the ionized atoms are parts (Duggar, 1936; Lea, 1947; and Weyl and Warren, 1951).

Excitation. A second method by which radiation dissipates energy in tissue is by means of excitation. This means the raising of an electron in an atom or molecule to a state of higher energy and is a less drastic process than the complete ejection of electrons. In bringing about chemical changes in organic molecules,

excitation is much less effective than ionization (Duggar, 1936; Lea, 1947).

Changes in Primary Photons. A photon transfers its energy to matter through the intermediary of electrons which are set in motion and then ionize the material. There are two main methods by which the first step may be brought about. When soft X-ray radiation (long wave-lengths) is used, transference of energy from photon to matter takes place mainly through photoelectric absorption. In the case of hard X-rays a scattering of the rays occurs, in addition to the photoelectric effect, and most of the energy is spent by ionizing matter through Compton scattering. In photoelectric absorption all the energy of the photon is transformed at once and the electron leaves the atom of the absorbing substance as a photoelectron with a kinetic energy less than that of the photon by the amount necessary just to separate the electron from the atom. To bring about this drastic transformation of high-energy photons, the photon must collide with an electron which is capable of withstanding the full shock before running away. The photoelectron travels with a high speed in the direction of the incident photon and ionizes a large number of atoms before it finally becomes attached to another atom. The energy of the photon which is utilized in barely removing the electron from the atom reappears as another photon of greater wave-length as soon as a new electron takes the place of the one previously dislodged from the atom. This photon may again react with an electron of an atom, ionizing the latter (Duggar, 1936; Lea, 1947; Weyl and Warren, 1951).

In the case of Compton scattering the transference of

energy from the photon to the electron takes place according to the laws of elastic impact and depends on the angle which the path of the emergent electron makes with the path of the impinging photon. In contrast to the photoelectron, the Compton recoil electron is projected in any direction other than that of the photon of the primary beam, and the energy imparted to it is always less than that of the photoelectron; it has a slower speed and ionizes a smaller number of atoms on its passage. The energies of the Compton recoil electrons vary among themselves. The greater the angle of deviation of the Compton recoil electron from the direction of the primary photon the less is the energy it bears. The energy of the photon which is not transmitted to the electron by the Compton-effect process appears as a photon of lower energy and is emitted in a direction which is compatible with the laws of elastic impact (Duggar, 1936; Lea, 1947).

The interaction of irradiation and matter lead to very important results. Monochromatic radiation becomes polychromatic traversing even a small thickness of matter on account of the presence of lower energy in the transmitted beam of photons. The proportion of lower-energy photons in the transmitted beam increases with the thickness of the material, i.e., the radiation gradually becomes softer, and energy of the photons is transmitted rapidly to electrons within the next short distance (Duggar, 1936; Lea, 1947).

Secondary Photons and their Behavior. Secondary photons produced by reaction of the primary X-ray beam to matter are emitted in all directions and act as a new source of irradiation. The most important difference is

in the distribution of the rays around the new source. That part of secondary radiation which is due to the photoelectric effect, being extremely soft, is utilized *in situ* almost entirely. Secondary photons, liberated by the Compton effect, project mostly in the forward direction. They are capable of undergoing exactly the same transformation as with the photoelectric effect and Compton effect, *i.e.*, they can produce beta particles which further contribute to ionization of the medium. The transformation of photons into high speed electrons and into other secondary photons goes on indefinitely until practically all the energy of the original photons have been transferred to electrons through repeated steps of the above described processes (Duggar, 1936; Lea, 1947).

Biological Effects of Radiation

Biological effects of irradiation are usually considered to be the results of chemical reactions brought about by the ions liberated through the action of irradiation of matter (Duggar, 1936; Lea, 1947). The sequence of events are assumed to be: (a) ionization, (b) chemical changes, and (c) biological changes. Moreover, there may be further chemical changes as a consequence of the first biological changes.

Since the site of biological effeets of radiation is the cell it may be worthwhile to first describe some of the general changes in the cell brought about by radiation before discussing some of the views dealing with the explanation of radiation effects. The cell is composed of the cell wall, cytoplasm and nucleus. The nucleus is

usually considered to be the part of the cell most sensitive to radiation. It is possible, however, that the cytoplasm is also equally sensitive, if not more so, but cytoplasmic changes are not as easily detectable as nuclear changes.

As a result of irradiation, noticeable nuclear changes may occur; these are mainly exemplified by chromosomal changes. These chromosome changes may be in the form of breakage and structural rearrangements resulting from the joining, in various fashions, of the several broken ends present in a nucleus in which two or more chromatid-breaks have occurred. Breakage is thought to be caused by ionizing particles passing through or in immediate vicinity of the broken point in the chromosome. Irradiation also alters the surface properties of chromosomes so that they tend to stick together. This results in chromosomes at metaphase adhering where they happen to come in contact and interferes with the complete separation of sister chromatids at anaphase giving bridges. In severe cases the chromosomes may remain clumped at metaphase so that no further divisions ensue, or the bridges at anaphase may fail to break so that separate nuclei can not be formed (Lea, 1947; Catcheside, 1948). Changes of this type are assumed to be due to a general change in the surface properties covering the whole surface of the chromosome, while chromosome breakage and rearrangements by the union of broken ends are attributed to a localized action of radiation on the chromosome thread. The former is usually considered to be a "physiological effect" of radiation while the latter is assumed to be a "direct-hit effect".

The viscosity of cytoplasm is affected by X-rays.

The first effect of radiation is a liquifaction in the main mass of the cytoplasm as indicated by a decrease in cytoplasmic viscosity (Heilbrunn and Mazia, 1936). Then there takes place coagulation of cytoplasm associated with an increased swelling of cytoplasmic colloids which lead to an increase in viscosity. Liquifaction of plasmagel is caused by irradiation with an apparent release of calcium ions which diffuse to the interior of the cell and cause internal liquifaction and an ultimate coagulation of the main mass of the protoplasm (Heilbrunn and Mazia, 1936). Coagulation is often accompanied by the appearance of vacuoles in the cell. Heilbrunn and Mazia (1936) considered that this vacuolization of the cytoplasm is due to a specific chemical reaction peculiar to the living system.

Radiation usually increases the permeability of the cell membrane. According to Heilbrunn (1928), since the plasma membrane of a cell is a calcium gel, a loss of calcium ions following irradiation would weaken the gel and be responsible for an increase in the permeability of the cell membrane.

Theories Regarding Biological Effects of Radiation

It has already been mentioned that the biological effects of X-rays are due in some way to the chemical changes induced by irradiation. We are immediately confronted with the problem of explaining why marked biological effects are produced by doses of radiation which produce only a small degree of chemical change. Marked biological effects in different materials have been produced by X-ray doses ranging from 50r* to about 5×10^5r.

*An "r" unit (Röntgen) designates a measure of the ionizing effect of irradiation and is roughly the quantity of X-rays which will produce 2 billion ion pairs in a cubic centimeter of dry air at standard pressure and temperature.

The number of ionizations produced per cubic micron of tissue by a dose of 5×10^5r is about 10^5 and judging from the results of chemical experiments the number of molecules reacting will be of this order. The number of atoms in one cubic micron of tissue is, however, about 10^{11}. Thus even the very large dose of 5×10^5r can produce chemical changes in only about one-millionth of all the atoms (Lea, 1947; Zirkle, 1952) and the dose of 50r seems to be quite negligible from this point of view. There are, however, several ways by which small overall percentages of chemical changes have been visualized to be effective.

It is considered quite possible that products from the decomposition of proteins and other cell constituents by irradiation may be injurious to the cell even though present in a very low concentration and may be responsible for some biological effects (Lea, 1947).

In 1926 W. Casperi proposed an hypothesis which is known as the necrohormone hypothesis. According to this hypothesis some hormone-like substances in the cell are changed by the direct effects of X-rays and these changed hormonic substances diffuse to other cells causing visible, appreciable, biological effects.

It has been found that in sufficiently dilute solution a large percentage of chemical changes in the solute can be accomplished by a moderate dosage of irradiation. Dale (1940, 1942) argues that enzymes present in the cell in low concentrations may be destroyed by irradiation and produce appreciable, biological effects, since they are initially connected with so many biological functions.

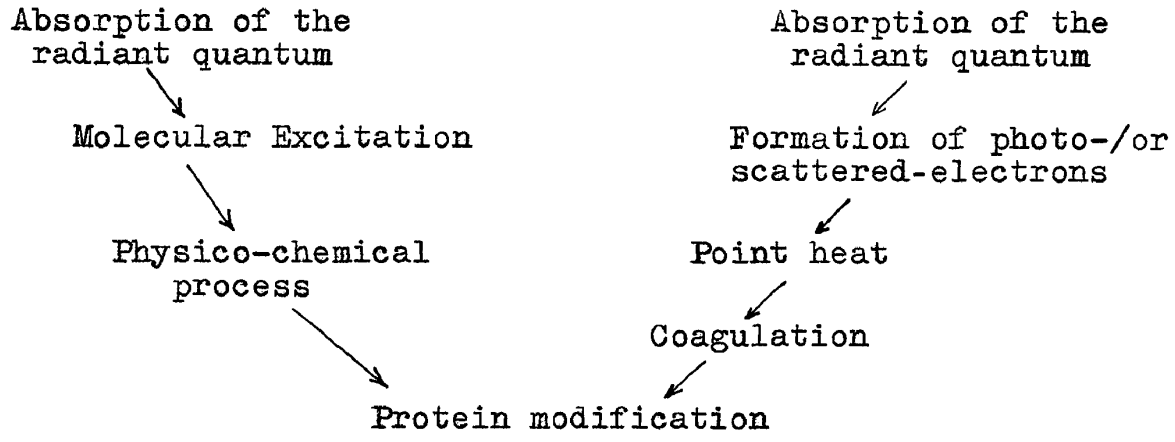
In 1932 F. Dessauer proposed a theory known as the "Point Heat Theory" to account for the action of X-rays

on biological objects. His explanation is based on the premise that electrons which are generated by the absorption of X-rays in matter are the bearers of transformed energy. These electronic energies are degraded on colliding with atoms of protein molecules. In this process the excited atoms collide with nonexcited atoms and transform the energy of excitation into energy of translation, i.e., into the basic process of heat. The theory assumes that these processes occur at isolated points in the molecule and that the action of radiation is to be regarded as a nonspecific process, dependent on temperature. The theory also presumes a wave-length dependency of irradiation effect, hard X-rays being assumed to produce greater ionizations.

Another hypothesis concerning biological action of radiation is the "Photochemical Theory" proposed by Holthusen in 1933. The energy required for radiation effects originates, according to this theory, from the state of excitation of the molecules and atoms following absorption of quanta of radiation. The energized molecules and atoms become capable of reactions of which they would otherwise be incapable. According to this theory radiation effect is dependent only upon the exciting, dissociating action of the radiation, but independent of wavelength and temperature.

Ellinger (1935) presented a schematic representation of radiation effects. His idea is a combination of the "Point Heat Theory" and the "Photochemical Theory".

It may be shown as follows:



The theory which is best known, especially concerning nuclear changes, is the "Target Theory" or "Quantum Hit Theory". According to this theory exceptionally radiosensitive volumes are assumed to be present in the cell (Holweck and Lacassagne, 1931) and only absorption phenomena (hits) which occur within these radiosensitive volumes are effective. Structure of these radiosensitive bodies being vital for living cells, any change in their structure is recorded as biological change. Genes and chromosomes in the nucleus are assumed to be such radiosensitive structures since any change in their structure causes appreciable change in the organism. Lea (1947) feels that the "Target Theory" can satisfactorily explain the genic changes following irradiation.

Another mechanism suggested by L. H. Grey and elaborated by Zirkle (1952) takes account of the fact that ionizations produced in water lead to the production of active radicals inside or immediately outside complex organic molecules including genes and chromosomes. These active radicals are capable of producing chemical changes, including, presumably, the disruption of bonds (within a

a definite distance from the place where ionization occurs) and cause visible changes in the organism.

Previous Embryological Work on Barley

Most older literature concerning studies of the embryology of Hordeum is fragmentary. As early as 1858 Hofmeister gave a brief description and diagrams of a few stages of embryonal development of Hordeum (from Schnarf, 1931). Later, Norner (1881) studied the early stages of embryos of Hordeum, Avena, Triticum and Secale by dissecting out whole embryos and mounting them in glycerine for observation; the oldest stages illustrated were those just beginning to show gross differentiation. He also attempted to classify the arrangements of the cells according to the manner in which they divided. Celakovsky (1897), in attempting to explain the true nature and origin of the coleoptile and the scutellum, gave a short description and diagrams of five stages in the development of the upper part of the embryo of Hordeum and considered the coleoptile a part of the scutellum.

Krauss (1933) in his study on the developmental history of the fruits of Hordeum, Triticum, Bromus and Poa described the changes which occurred during the transformation of the fertilized ovule into the mature seed. He stated that the ovules were anatropous with two integuments and that the embryo sac was eight-nucleate. He also studied the gradual transformations of nucellus, integuments and ovarian wall.

Harlan and Pope (1925) and Pope (1946) studied pollination in barley and noted that the presence of one pollen tube in the stigma seemed to inhibit the growth of

another tube in the same region. They always found only one pair of male nuclei in an embryo sac. Pope (1946) observed that temperature influenced the growth of pollen tubes. Between temperatures of 30°--35°C pollen tubes entered the embryo sacs twenty minutes after pollination, while below 5°C one hundred and forty minutes were necessary.

Erdtman (1944), in a study on the pollen of cereals, found that pollen grains of barley were comparatively small (about 43.5 by 45 microns) and contained one germ pore. Jones and Newell (1948) found the diameter of pollen grains of Hordeum pusillum to be about 39 microns and those of H. jubatum to be about 43 microns.

The most elaborate work on Hordeum was published by Merry in 1941. He made a detailed study of the development of the embryo of Hordeum sativum. He described the origin and development of scutellum, coleoptile, first and second leaves of the plumule, root, coleorhiza and suspensor. He particularly noted the time of initiation of the respective organs and also the time of their vascularization.

In the course of his study Merry (1941) followed the sequence of the first two divisions of the fertilized egg. He considered further divisions to be irregular and was unable to locate the individual tiers of the pro-embryo which gave rise to the different parts of the mature embryo. This is in contrast to the description given by Souèges (1924) for the embryonal development of Poa annua. Soueges indicated that in Poa there was a definite arrangement of cells in the proembryo and the cells of each tier had a definite destination in the formation of the mature embryo.

Radiation Work on Animal Embryos

It has already been mentioned that no attempt has yet been made to study the effects of X-rays on the development of plant embryos. A good deal of work, however, has been reported in the literature dealing with X-radiation effects on the embryonic development of animals. A review of this work should be very useful in interpretating the results of the present study. With this view in mind a brief survey of the literature covering studies on the effects of X-radiation on animal embryos is given.

All the earlier work up to 1935 dealing with the effects of X-radiation on animal embryos were reviewed by Job, et al. (1935). The effects of X-radiation reported in these early works included abnormal outgrowths in different parts of the embryos, disturbances and the disorganization of some systems (e.g. nervous, circulatory etc.), retarded growth, delayed development as well as the death of some embryos as a result of X-ray exposures to pregnant organisms.

Earlier workers did not take into consideration the stage of embryonic development at the time of treatment. Job, et al. (1935), first attempted to determine whether a critical period existed in the development of rat embryos. With doses below 100r they were able to produce eye abnormalities with considerable high incidence following irradiation on the tenth day of gestation (period of gestation for rat is 21 days), and with lower incidence on the ninth day and jaw defects from irradiation on the eleventh day of gestation when equal doses were used in both cases. Doses between 95r and 200r killed all embryos if applied on the eighteenth day after conception. They further claimed that sex ratio was changed in favour of

males if irradiation was given during the critical stages of sex organ development.

Similar to Job, et al, Wilson and Karr (1951), Wilson, Brent and Jordan (1952, 1953) and Wilson, Jordan and Brent (1953) radiated pregnant rats on the tenth, ninth, and eighth days of gestation. On the respective days of gestation pregnant rats were opened surgically and shielded with lead plates in such a manner as to permit direct irradiation on two to five implantation sites without exposure of the mother or the remaining implantation sites. Dosages used were 12.5r, 25r, 50r, 100r and 200r. 200r proved lethal for all three days of gestation but embryos treated on the eighth day were resistant to any lethal effects of dosages less than 200r, whereas those radiated on the ninth or tenth days showed variable responses. The growth-retarding effects of irradiation, on the other hand, were more pronounced after treatments on the eighth day than at older ages. Further, in sharp contrast to the results of the ninth and tenth day exposures, no developmental malformations were observed from treatments on the eighth day. Of treatments on the ninth and tenth days the former produced more abnormalities. Eyes, brains, aortic arches, heart, spinal cord, situs inversus and the urinary tract were affected from the exposure of X-rays on the ninth day of gestation while from similar exposures on the tenth day of gestation only the eyes, urinary tract and the brain were affected. Moreover, the percentage of effects on the respective organs was more in the former group. The authors explained these differential effects by assuming that the X-ray reaction varied depending on the critical stage of embryonic development.

In contrast to the low doses employed by Job, et al. (1935), and Wilson and his co-workers (1951, 1952, 1953), Warkany and Schraffenberger (1947) applied doses ranging from 190r to 1,120r to pregnant rats. Irradiation was given in single acute exposures at stages ranging from the tenth to sixteenth day of gestation. A great variety of malformations resulted but there was little correlation between the time of application of the dose and the resulting defect. This was probably a result of the very high dosage-levels which disturbed primordia in stages other than the ones at which they showed maximum sensitivity.

Kaven (1938a, 1938b) irradiated pregnant albino mice. Each female received a single acute exposure to dosages ranging from 170r to 230r at stages between the seventh and seventeenth days of gestation. Treatment at the seventh and eighth days of gestation caused brain hernias in 2.4 per cent and 16.1 per cent of the new borns respectively. In the general survey Kaven found irradiation at the latter stages produced tail abnormalities (critical period 12th to 13th days), male sterility (critical period 14th to 17th days), cataract (18th to 19th days), and skin changes (possibly 13th to 14th days).

Russel (1950) made an elaborate study of the effects of X-radiation on the development of mice embryos. She treated pregnant mice with dosages of 100r, 200r, and 300r units of X-rays on the ½, 1½, 2½ ..... 13½ days of pregnancy, and with 300r and 400r on the 14½, 15½ .....18½ days. The latter showed no appreciable effect of irradiation but in the former group, 300r of X-rays produced varying degrees of lethal effects depending on the age of the embryo at the time of treatment. On the other hand, radiation with 100r and 200r caused a decrease in the

size and weight of litters at birth as well as various kinds of structural abnormalities. These abnormalities occurred on the eye, head, tail, anus, urogenital system, and the feet. In addition to these, bloating, situs inversus of the stomach and kidneys, and outgrowths on the shoulder were observed.

In 1951 Schneller subjected 33-hour and 66-hour chick embryos to X-rays. She observed that exposures to dosages less than 500r produced no significant effects on survival of the embryos. The percent of survivals progressively decreased following exposures of 500r--900r. Exposure to 1000r produced death to embryos within 24--36 hours following irradiation. Irradiated embryos showed abnormalities of the eye, head and legs. Multinucleate, giant cells developed in the brain, the heart-tissues, and the head mesenchyme when irradiated with 1000r. Many of the normal-sized cells had pycnotic nuclei. Schneller (1951) failed to observe comparable lethal effects and histological abnormalities when she exposed the yolk even to 1,500r, keeping the embryo shielded, but exposure of 5,000r to the yolk caused death to many embryos. She, therefore, thought that there was some indirect effect of X-rays.

Review of Radiatiation Work on Green Plants

Although no study has yet been undertaken to investigate the effects of irradiation on the development of plant embryos, there have been a number of investigations of the effects of irradiation on green plants. Most of these investigations have dealt with changed behaviors of embryos and abnormalities of plants obtained from irradiated seeds. The knowledge and understanding of biological

effects of radiation obtained in these observations will help to compare and interpret the results obtained in the present study, so a brief review of the previous work concerning the effects of X-radiation on green plants has been included.

Radiation on Seeds

An exhaustive account of the work up to 1936, dealing with the effects of X-irradiation on seeds, has been given by Johnson (1936) in her article "X-ray effects on green plants". Many conflicting results about irradiation effects are cited in this article. While the majority of writers considered that low doses of X-rays stimulated growth and higher doses produced retardation, a number of others were of the opinion that no such stimulation was obtained by low dosages. Objection was raised to the validity of the reports about stimulative effects of X-radiation, since most of the workers belonging to the former group based their conclusions upon the study of only a few seeds and they did not give sufficient consideration to variations observed within each group. Stimulative effects with low dosages of X-rays were reported by Maldiney and Thouvenin (1898, Convolvulus and Lepidium), by Koernicke (1904, Vicia and Brassica), Schwarz (1914, Vicia faba), Miege and Coupe (1914, Raphanus and Lepidium), Pfeiffer and Simmermacher (1915, Vicia faba), Jüngling (1920, Vicia faba), Altmann and Gleichgewicht (1923, Phaseolus vulgaris), Jacobson (1923, Potato), Komuro (1924, Oryza sativa), Arntzen and Krebs (1925, Pea), Iven (1925, Vicia faba), Rivera (1926, Ricinus communis), Goodspeed and Oslon (1928, Nicotiana), Goodspeed (1929, Nicotiana), and by Johnson

(1931, 1936, Tomato, Sunberry, Sunflower and two species of Vetch). On the contrary, Ancel (1924, 1926), Komuro (1923), Sprague and Lenz (1929), Capizzaro (1926), Horlacher and Killough (1931) and Schwarz, Czepa and Schwindler (1924) obtained nonstimulative and retarding effects of radiation, depending on the dosages of X-rays used. Retarding effects with higher dosages of X-rays were also reported by Koernicke (1915), Pfeiffer and Simmermacher (1915), Jüngling (1920), Halberstaedter and Simons (1922), Geller (1924), Iven (1925) and by Shull and Mitchell (1933).

In 1943 Kersten, Miller and Smith observed an apparent X-ray induced stimulation in the growth of the primary root of Zea mays. Stimulative effects of X-radiation on the germination of barley seeds were reported by De Fazi (1930) and Mezzadroli and Vareton (1930). Leopold (1949) reported that irradiation resulted in an increased tillering presumably by opposing or destroying auxin. Tascher (1929) tested the possibility of X-ray stimulation rather thoroughly with barley and other crop plants and concluded that there was no evidence of improved yields resulting from light dosages of X-rays.

Tascher (1929) also studied X-radiation tolerance of dormant seeds of twenty-four crop plants including barley. He found the dose which killed approximately 50 per cent of the seeds ranged from 4,000r for rye to 85,000r for alsike clover, with a corresponding lethal dose for barley at 18,000r. Gustafsson (1937a) made a similar kind of study with a larger number of crop seeds. He concluded that Leguminous seeds generally were more sensitive to X-radiation, fatty or oily seeds more resistant, while cereal seeds occupied an intermediate position.

Resistance to X-radiation decreases in barley with longer storage period (Freisleben and Lein, 1943; and Gustafsson, 1936, 1937a, 1937b). Irradiated seeds stored at low temperatures showed more survival than those stored at high temperatures.

Increase in moisture content decreases the tolerance of the seeds to irradiation (Froier and Gustafsson, 1941; Gustafsson, 1936, 1937b, 1940; Hayden and Smith, 1949; Stadler, 1928, 1930; Tascher, 1929; and Wertz, 1940). Tascher (1929) showed that only about one-half the dosage of X-rays was required to kill seeds after six hours of soaking, at which time the moisture content was 34 per cent as compared with 10 per cent in unsoaked seeds. He also noted that sensitivity to X-ray injury and the respiration rate increased markedly during the first 40 hours of aeration after the soaking period.

The temperature of seeds during radiation (Tascher, 1929 and Stadler, 1931) as well as before or after radiation (Camus, 1928; Gustafsson, 1947; Smith, 1943, 1946; Smith and Caldecott, 1948; and Tascher, 1929) also affects the degree of injury. Dormant seeds treated in solid CO_2 tolerated approximately 1-2/3 times the dose tolerated at room temperature. A heat treatment preceding irradiation has been shown to give seeds a measure of protection from subsequent irradiation (Camus, 1928; Gustafsson, 1947; Smith, 1943, 1946; and Smith and Caldecott, 1948). Heat applied after irradiation may add to, or subtract from, the radiation sensitiveness depending upon the combinations of treatments applied (Camus, 1928; Smith, 1943, 1946; and Smith and Caldecott, 1948). Injury to dormant seeds increased if the seeds were kept at 45°C instead of at 22°C during the intervals between intermittent X-ray treatments.

Irradiating dormant seeds, or germinating seeds in a

vacuum or in nitrogen, showed a distinct reduction in injury as compared with the injury received from similar dosages of X-rays applied while seeds were in air or oxygen (Hayes and Garber, 1927; and Smith, 1946).

Froier and Gustafsson (1944), observed that seeds with "hulls" were more resistant to X-radiation than "hull-less" seeds.

A number of mutants were obtained from irradiated barley seeds (Stadler, 1930, 1931; Smith, 1950b; Moh and Smith, 1951; and Stubbe and Bandlow, 1947). Of all mutants produced by irradiation, mutation in chlorophyll and in spike characters were most common.

Various types of chromosomal aberrations were obtained from X-irradiated seeds (Caldecott and Smith, 1948, 1952;; Gelin, 1941; Gustafsson, 1936, 1937a; Smith, 1943, 1950b; and Smith and Caldecott, 1948). Rod and ring fragments and translocated chromosomes as well as the absence of chromosomes were observed in root tip cells obtained from the germination of irradiated dormant seeds. Frequencies of these aberrations per r-unit increased with increased moisture content (De Fazi, 1930; and Gustafsson, 1936, 1937a, 1937c). Aberrations persisted in the sporogenous cells of flowers where bridges and rings were formed during meiosis (Caldecott and Smith, 1952).

Morphological and Histological Effects

Johnson (1926, 1931) noted almost universal production of leaf abnormalities on plants grown from the X-irradiated seeds. Unfolding leaves presented a pebbly appearance soon after treatment. As the leaves grew older, light green areas intermingled with normal areas gave a mosaic or

variegated aspect. Simple leaves were often notched at the apex, deeply forked, occasionally split into two independent leaves. Compound leaves were often twisted and the leaflets fused.

Poorly developed leaves with very irregular margins were observed by Rivera (1926) in Vicia faba. Similar irregularities on leaves were observed by Sprague and Lenz (1929) in Solanum tuberosum. In addition to leaf anomalies in shape, Horlacher and Killough (1931) noted irregularities in leaf color and sectorial chimeras in Gossypium.

Ancel (1926) found increased axillary bud development when terminal buds were X-radiated. A similar increased development of the axillary buds of underground stems of Freesia corms was obtained by Morgan (1932).

Excessive branching of stems of irradiated seedlings and stem fasciation were described by Johnson (1926, 1931) for species of Helianthus and Lycopersicum. Stems showed fasciation three weeks after irradiation; they were generally cylindrical at the base, but the apex was diffusely branched. The stems after becoming flattened usually showed splitting somewhere along their lengths, the resulting branches occasionally remaining unfasciated. The percentage of fasciations increased with an increase of dosage given to young seedlings. Tomato plants irradiated with a medium dose of X-rays assumed a bushy appearance due to increased branching.

Altmann and Gleichgewicht (1923), from a microscopic study of the irradiated stems of Phaseolus vulgaris, found a greater development of mechanical tissue than was present in the controls. The xylem was increased at the expense of the pith. Similar results were reported by Johnson (1936) in the hypocotyl of mature Helianthus plants. Miege and

Coupe (1914), likewise, found more developed vascular tissue in X-rayed plants of Raphanus and Lepidium. Goodspeed (1929) observed that cell number, form and arrangement were the same in X-rayed and controlled plants, but an increase in cell volumes in treated materials accounted for the abnormal leaf thickness in Nicotiona.

The effects of X-irradiation on the anatomy of leaves and stems of barley as well as a number of other species were studied by Walcott (1937). He found that irradiated leaves were thicker, shorter and less symmetrical than untreated leaves. Irradiation resulted in a proliferation of colorless parenchyma and spongy tissue as well as an increase in cell size. Some cell walls seemed to lack "reinforcement" and vascular tissue was delayed in development.

Smith and Kersten (Vicia faba, 1941, and Zea mays, 1942) found a general failure of root formation as well as a poor development of the root tip in plants raised from irradiated seeds. Vascular tissue was formed within a distance of approximately 0.6 to 0.7 mm from the irradiated root-tips while in the controls vascularization normally occurred basipetally 5 to 6 mm from the tips. The degeneration of cells which normally retained their ability to divide was observed in the root-tip meristem. They also observed lysigenous and schizogenous cavities in the pith of treated roots of Zea mays and schizogenous cavities only in Vicia faba.

Quantler, Schertiger and Stewart (1952) found retarding growth in the root of Phaseolus aureolus. They observed that neither individual cell elongation nor mitosis were appreciably hindered. Hence they considered the radiation effect on growth must affect some mechanism which operates on the cell after early differentiation.

Christensen (1954) was able to induce adventitious root formation in Xanthium, Nicotiana, Lycopersicum, and Phaseolus by localized irradiation on small lengths of stems of intact seedlings. The author considered that a phloem block occurred at the radiation site which impaired downward transport beyond that point causing an accumulation of organic materials at the irradiated site as well as the induction of roots from the swollen regions.

Comparative Studies of Different Kinds of Radiations

Stadler (1928) demonstrated that radium produced an increase in the frequency of mutation in seedlings raised from irradiated seeds. No difference was observed in types of genetic changes produced by radium and X-rays on barley.

Exposure to atomic-bomb-induced radiation resulted in effects comparable to those produced by X-radiation (Smith, 1950b;and Moh and Smith, 1951). Comparison of radiation effects from the two sources included observations on germination, mottling on the first leaves, chimeras, seedling mutation frequencies,and chromosomal interchanges in X_1 plants. The bomb-induced effects were about equivalent to those produced by 16,000r of X-rays.

Comparative studies on the effects of X-rays and neutrons on barley as well as on wheat seeds have been carried out by Gustafsson and Mac Key (1948), Caldecott, Frolik and Morris (1952), Mac Key (1951, 1952 and 1954), Ehrenberg, Gustafsson and Nybom (1952). Gustafsson and MacKey (1948) reported a higher frequency of chimeras, greater sterility in the N_1 generation, and a higher seedling mutation frequency in the N_2 than those produced by X-radiation in the respective generations. Caldecott,

Frolik and Morris (1952) observed seedlings from X-rayed seeds had a wide range in height distribution on the 14th day whereas those from thermal neutron irradiation had a very narrow range.

In producing chromosome aberrations neutron radiation is more than ten times as effective as X-rays (Caldecott, Frolik and Morris, 1952; Mac Key, 1951, 1952, 1954; Ehrenberg, Gustafsson and Nybom, 1952), about 50 to 100 times more effective in producing sterility in the first generation and the mutation rate in the second generation (Ehrenberg, Gustafsson and Nybom, 1952; and Mac Key, 1951, 1952, 1954). For similar chromosomal aberration frequencies, however, there was less seedling injury in seeds subjected to neutrons than in seeds treated with X-rays. These differential effects were considered by Caldecott, Frolik and Morris (1952) to be due to the fact that X-rays had proportionately more effect on the extrachromosomal elements of the cell (as compared to neutron bombardment), while according to Mac Key (1951, 1952), X-rays produced "physiological effects" in addition to chromosomal aberrations.

Mac Key (1951, 1952, 1954) reported that seeds treated with X-rays and neutrons showed retarded and weakened sprouting. The retarding effects of neutrons on sprouting was uniform and severe while the X-rayed seeds gave rise to seedlings with quite varied degrees of effects with each treatment. The decreased sprouting ability followed a sigmoid relation to doses in the X_1 generation but an exponential one in the N_1. Mac Key thought that the biological events causing the retarded growth were generally induced by "direct hits" of neutrons, while a cummulative effect should be characteristic of X-rays.

Ehrenberg, Gustafsson and Nybom (1952) observed that both X-rays and neutrons were more effective on presoaked seeds than on dry seeds. Seeds were treated with 5,000r to 25,000r of X-rays and also with 100 to 1,000r of neutrons. The number of surviving plants obtained from neutron treated seeds were more than those from X-rayed seeds, but plants grown from X-rayed seeds showed about 70% fertility while fertility in neutron treated plants approached zero per cent. Mutation rate was observed to be about 40 times higher in neutron radiated plants.

MATERIALS AND METHODS

Raising of Plants

Materials used in this investigation were obtained from plants raised from seeds of Hordeum vulgare var. "Mars", furnished by the Farm Crops Department of Michigan State College through the courtesy of Dr. K. Frey. The variety, "Mars", is an inbred line of long-day plants

Three sets of experiments were carried out, two during the spring and fall of 1953 and the third the winter of 1954. Plants were grown in wooden-flats about two feet by two feet by six inches for the first two sets of experiments while those for the third set were grown in earthen pots of ten inches diameter. Soil used was a mixture of one part vermiculite, one part sand and three parts loam. Flats and pots were placed on four movable platforms inside a temperature-control room, in which night temperature was 55°F to 60°F and day temperature 68°F to 72°F. A constant intensity of light was applied for a period of fifteen hours per day by means of eight 40 watt incandescent light bulbs and twenty 40 watt cool-white, standard fluorescent lights, with a time switch for regulating the "day-length". Platforms were frequently rotated at definite time intervals to provide, as near as possible, a uniform exposure of light to all plants. Humidity of the air was maintained within a range of 75 per cent to 85 per cent by use of a spray device. Plants were regularly watered with 200 cc of water per plant on every fourth day during the first two weeks after germination and on every third day thereafter.

Bamboo supports were provided for each plant when seedlings were five to six days old and were continued for

the rest of the growth period. Nutrients were added to soils three times during each set of the experiments. Stock solutions were prepared by dissolving each one of the following salts in a liter of distilled water: 14.6 gms potassium phosphate (KH_2PO4), 68.9 gms calcium nitrate ($Ca(NO3)_2\ H_2O$), 714 gms ammonium sulphate ($(NH_4)_2SO4$), 69.9 gms magnesium sulphate ($MgSO4.\ 7H_2O$) and 1.6 gms ferrous sulphate (FeSO4). Each plant during the course of the development received, on the average, 2 cc of ferrous sulphate solution and 9 cc of each of the remaining four nutrients.

Plants flowered about nine weeks after germination. The time of pollination was recorded and three to four days later plants were grouped and labelled and then transferred to the Veterinary Department for radiation treatment. Immediately following irradiation plants were returned to the control-room and allowed to grow to full maturity.

Radiation

A therapeutic X-ray machine (G. E. Max. 250 III) was used for the radiation. This was calibrated with a standardized Victorian r-meter. The X-ray beam was produced by the following factors: 200 killo-volt of power, 15 milliampere (ma) of current, inherent filtration of 3 mm aluminum, and a 0.25 mm copper and 1 mm aluminum added filter. Further characteristics were: 0.75 mm copper half-value-layer (hvl) 50 cm focal spot distance (fsd), and 400 sq cm beam size.

Plants of the first set were divided into eight groups, seven of which were given exposures of 50r, 100r, 150r, 200r, 250r, 300r, 350r units of X-rays while the eighth was kept as a control. In each case radiation treatment was given by a single exposure.

Plants of the second set were divided into nine groups eight of which were treated separately with 100r, 200r, 300r, 400r, 500r, 600r, 700r, and 800r of X-rays with the ninth group being kept as control. As with the previous case single exposure was given to flowering spikes of each group, but the X-ray machine was somewhat altered for the proper accomodation of plant heads within the area of X-ray beam. The cone (29 cms long) which is usually a part of the machine for therapeutic treatment, was removed, thus reducing the focal spot distance. With a reduction of focal spot distance softer rays were increased in the X-ray beam, since the X-rays of longer wave lengths were given less chance for absorption in air. X-rays used in the second set of experiments, therefore, were softer. At the time of treatment a lead sheet 2 cm thick was used to cover each group of plants being irradiated, so that exposure of neighboring groups to X-rays could be avoided.

Since the second set of experiments showed some interesting effects it was decided to group plants of the third set in various stages of embryonic development and treat them with 500r units of irradiation to determine if possible any "critical stages" to radiation during their development. Accordingly four groups of plants were selected for treatment on four dates, approximately two, five, eight, and ten days following fertilization time. Further considered, was the least dose required to produce visible radiation effects. To aid in the determination of this threshold dose, two sets of four groups each, were selected to be radiated with 150r, 200r, 250r, and 300r on two dates, about five and eight days after fertilization.

Since the potted plants were found to be quite suitable

for X-ray exposure with the usual fittings of the X-ray machine for therapeutic treatment, it was decided to treat plants in exactly the same manner as in the first set of experiments, assuming that the harder X-rays would produce almost similar effects to those of softer rays. It was also felt that even if somewhat different effects were obtained with harder rays, it would be worthwhile to report since conflicting statements concerning effects of hard and soft X-rays are found in the literature. Plants were radiated according to the above mentioned scheme with an additional group of plants treated with 800r and another group kept as a control.

Fixation

In the first and second sets of experiments a few ovaries in each group were killed and fixed immediately after irradiation in order to know the probable embryonal stage of development at the time of treatment. In the first set one additional fixation was made at harvest-time, while in the second irradiated set ovaries were collected and fixed on three dates: four, eight and fifteen days after treatment. In the case of the third set three to four ovaries from each plant belonging to each group were killed and fixed just prior to irradiation treatment to record the exact state of development at the time of exposure. Further killing and fixation of ovaries from each plant were made six and twelve days after treatment.

The fixative used was formalin-acetic-alchohol in a proportion of one part formalin, one part acetic acid, six parts 95 per cent ethyl alchohol and six parts distilled water.

At the time of fixation glumes were removed from the

ovaries. Older ovaries were treated with 1 : 1 solution of hydrofluoric acid and 50 per cent ethyl alchohol for five to seven days to soften the fruit-seed-coat and to facilitate infiltration. Standard methods of dehydration with ethyl-butyl alchohol mixtures and paraffin embeding were followed. Paraffin blocks were cut at thicknesses of seven to sixteen microns depending on the materials used and their stages of development.

Most of the slides were stained with Chlorozol Black E and counter-stained with Fast Green. Some of the slides were stained with Safranin-Crystal violet-Fast Green. Chlorozol Black E was found to be more suitable for photomicrography. A few slides were hydrolyzed with one-normal hydrochloric acid for five minutes in a 58°C oven and then stained by the Feulgen technique in order to verify whether or not any Feulgen positive bodies developed within the cytoplasm of treated embryonic cells.

Germination

Control seeds and those radiated with 400r, 500r, 600r 700r and 800r in the second set of experiments were germinated in paper towels in order to note any variation in germinability caused by radiation. Due to the lack of an adequate number of seeds only fourteen seeds belonging to each group were germinated. Five days after the complete germination of control seeds the number of germinated seeds were recorded.

Microscopical Examination

The slides were examined with 10X low and 45X high power, as well as with 90X oil-immersion objectives, and a 12.5X ocular. A ribbon-filament lamp with a B-green filter

placed between the lamp and microscope was used for illumination.

For a comparative study of cell-length of control and treated (500r) embryos two sets of measurements were taken from undifferentiated (young) and differentiated (mature) embryos. The first set involved random measurements of five hundred cells taken from fifteen slides each of treated and control undifferentiated embryos, while in the second set two hundred-fifty cells from each part of the differentiated ones (coleorhiza, first leaf, coleoptile and cotyledon) were similarly measured.

Drawings were made with the aid of a camera lucida for general embryological development while photomicrographs were taken for representative embryonic abnormalities.

OBSERVATIONS

Embryology of Hordeum vulgare

Anther, Microsporogenesis and Male Gametophyte

There are three stamens in each flower. Anthers of the stamens are bilobed with two locules in each lobe, each locule containing two long rows of sporogenous cells. The wall of the anther is composed of an epidermis, 2--3 middle layers and a tapetum (fig. 1). Of the middle layers two degenerate very early leaving one layer in between the epidermis and the tapetum. The persisting layer at a later stage becomes the endothecium. The tapetum is glandular. It is uninucleate prior to the division of the microspore mother cells, later however, during the formation of microspores it becomes binucleate (fig. 3).

Microspore mother cells divide by successive divisions. Two diad cells are formed as a result of heterotypic division of the microspore mother cells (fig. 2). Spindles of the dividing nuclei of diads are not always oriented in the same manner which results in the formation of different kinds of tetrads. Isobilateral tetrads are most common but T-shaped tetrads are also seen as well as a few tetrahedral tetrads (figs. 2-3).

After enlargement of the microspore its nucleus divides and a vegetative nucleus and a generative nucleus are formed. The generative nucleus divides again giving rise to two lenticular sperms, each surrounded by a hyaline cytoplasmic sheath. At this three-celled stage pollen grains are shed from the anther (fig. 4). Similar reports have been made for species of Zea, Avena, Anthoxanthum, Secale, Triticum, Andropogon and Festuca (from Schnarf, 1931).

Reduction division of microspore mother cells are, thus, successive, and tetrads are isobilateral, T-shaped or tetrahedral. Pollen grains are shed at the three-celled stage. The tapetum is glandular and binucleate.

Ovule, Megasporogenesis and Female Gametophyte

The gynoecium is composed of two carpels with a single cavity inside the ovary. Close to the base of the ovary only one ovule develops laterally showing that the placentation is parietal (fig. 5). Ovules are crassinucellate and campylotropous. They have two integuments of which only the inner takes part in the formation of the micropyle (fig. 6) unlike the case in Pennisetum typhoideum (Narayanswami, 1953). Randolph reported (1936) however, that in Zea mays the micropyle is formed by the inner integument. Krauss (1933) thought that the same is true for the entire family Gramineae.

A single archesporial cell directly functions as the megaspore mother cell. The cells of the nucellar epidermis, however, may divide anticlinally and the megaspore mother cell may appear 1--2 layers below the epidermis (fig. 7). This condition has been reported fairly common in the Gramineae (Schnarf, 1931), although Guignard (1901) in Cornucopiae nocturnum, Weatherwax (1919) in Zea mays and Rangaswami (1935) in Pennisetum typhoideum observed that archesporial cells divided and gave rise to a wall cell and a megaspore mother cell. Narayanswami (1953), however, demonstrated that Rangaswami was incorrect in P. typhoideum.

A linear tetrad of megaspores is formed as a result of the meiotic division of a megaspore mother cell (fig. 8). The lowermost megaspore enlarges and becomes the embryo sac primordium. Its nucleus by three successive divisions

NORMAL DEVELOPMENT

Fig. 1 T.S. of a young anther lobe showing two middle layers, uninucleate tapetum and a group of microspore mother cells. (x 1054).

Fig. 2 Separation wall being formed after heterotypic division of meiosis. (x 1054).

Fig. 3 Two tetrads of microspores and a binucleate tapetal layer. (x 1954).

Fig. 4 A three-celled pollen grain. (x 1054).

Fig. 5 L. S. of an ovary showing lateral origin of the ovule. (x 105).

Fig. 6 L. S. of a campylotropous ovule showing micropyle formed by the inner integument only. (x 105).

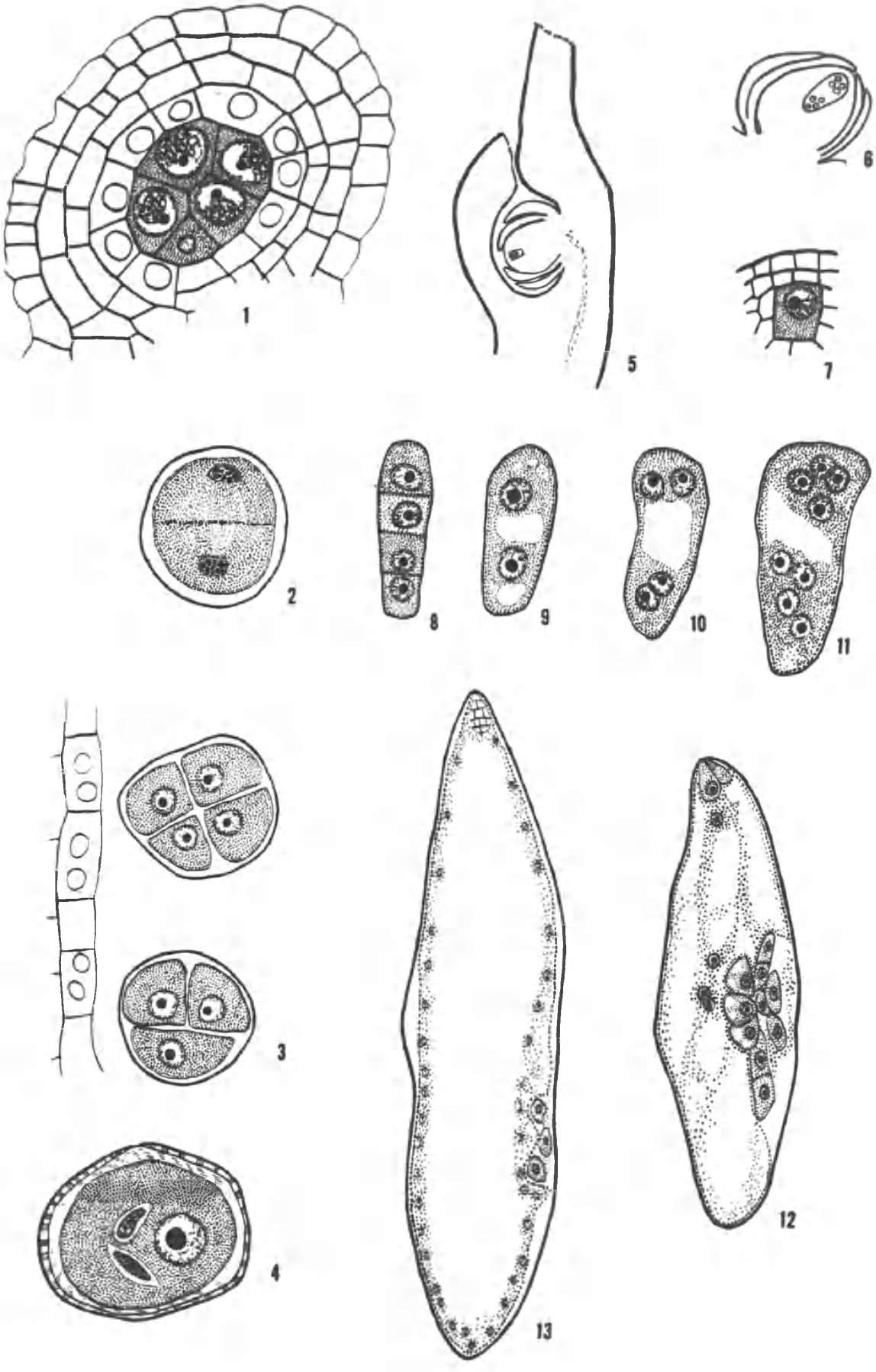
Fig. 7 Megaspore mother cell below two layers of nucellar cells. (x 703).

Fig. 8 A linear tetrad of megaspores. (x 703).

Figs. 9-11 Bi-, four- and eight-nucleate embryo sacs. (x 703).

Fig. 12 L. S. of a post-fertilized embryo sac showing the zygote, remnant of a synergid, four endosperm nuclei and laterally placed antipodal cells. (x 494).

Fig. 13 An elongated post-fertilized embryo sac with a small embryo and peripherally disturbed endosperm nuclei. (x 105).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gives rise to eight nuclei (figs. 9-11). The nuclei are then organized into a "Polygonum Type" of embryo sac (Maheshwari, 1948). The "Polygonum Type" of embryo sac has been mentioned for members of the Gramineae (Schnarf, 1931) with the exception of Cornucopiae nocturnum (Guignard, 1901), Melica nutans and M. altissima (Fischer, 1880) where the development of the embryo sac follows the "Scilla Type".

The embryo sac consists of an egg apparatus of three cells, three anti-podal cells and a secondary nucleus (the fusion product of the two polar nuclei). The antipodal cells are arranged laterally at one side of the embryo sac (fig. 12). A similar condition has been reported by Narayanswami (1954) in some Indian millets. During early divisions of the endosperm nuclei the antipodal cells appear densely protoplasmic but later on "fade out" and disappear.

It may therefore be concluded that the ovules are crassinucellate, bitegmic, and campylotropous with parietal placentation. The embryo sac is monosporic, eight-nucleate and of the "Polygonum Type".

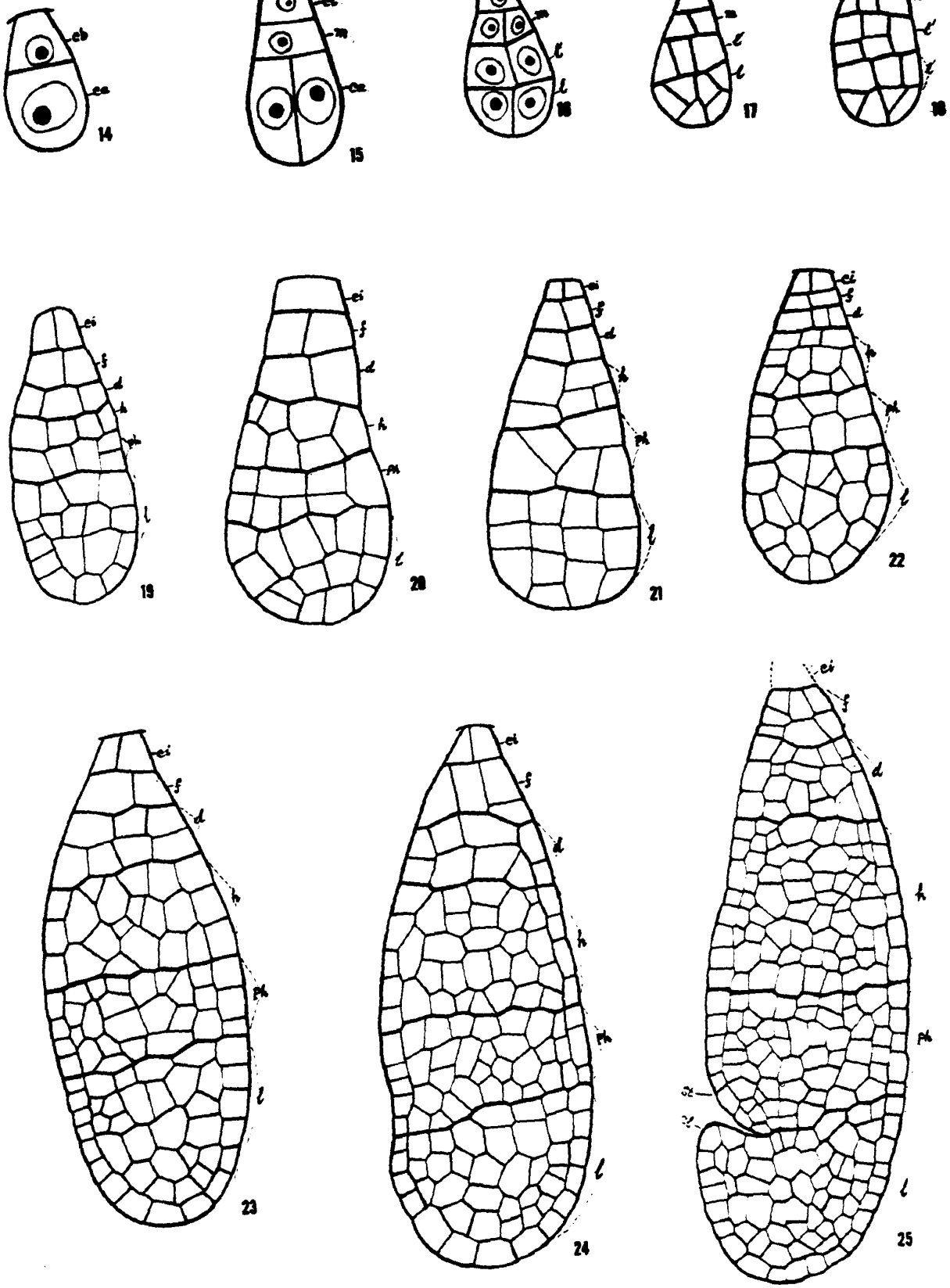
Endosperm

Following fertilization the primary endosperm nucleus always divides earlier than the fertilized egg (fig. 12). Most of the endosperm nuclei, resulting from the first few divisions accumulate around the antipodal cells and only one or two nuclei lie near the egg. When the embryo sac becomes elongated the endosperm nuclei take up a uniform distribution along the peripheral region of the embryo sac membrane (fig. 13). Wall formation begins at this

NORMAL EMBRYOGENESIS

Figures 14 - 15 Stages in embryo development (for explanation see text).

Figs. 14 - 16 (x 850)
Figs. 17 - 24 (x 398)
Fig. 25 (x 277)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region and proceeds centripetally and the entire inner space becomes occupied by the growing endosperm. The endosperm is, thus, nuclear in Hordeum vulgare.

Embryogenesis

The first division of the zygote is transverse and results in the formation of a large terminal cell ca and a smaller basal cell cb (fig. 14). This is followed by a transverse division in the basal cell cb into tiers ci and m and a vertical division in the terminal cell ca (fig. 15). The two cells of the tier ca then divide transversely producing tiers 1 and 1'. Meanwhile the two cells of the tier m divide by a transverse wall giving rise to tiers d and f (figs. 16-18). By this time the basal cell ci divides by a vertical wall and forms the lower part of the suspensor. The upper part of the suspensor develops from the lower part of the tier f (fig. 19).

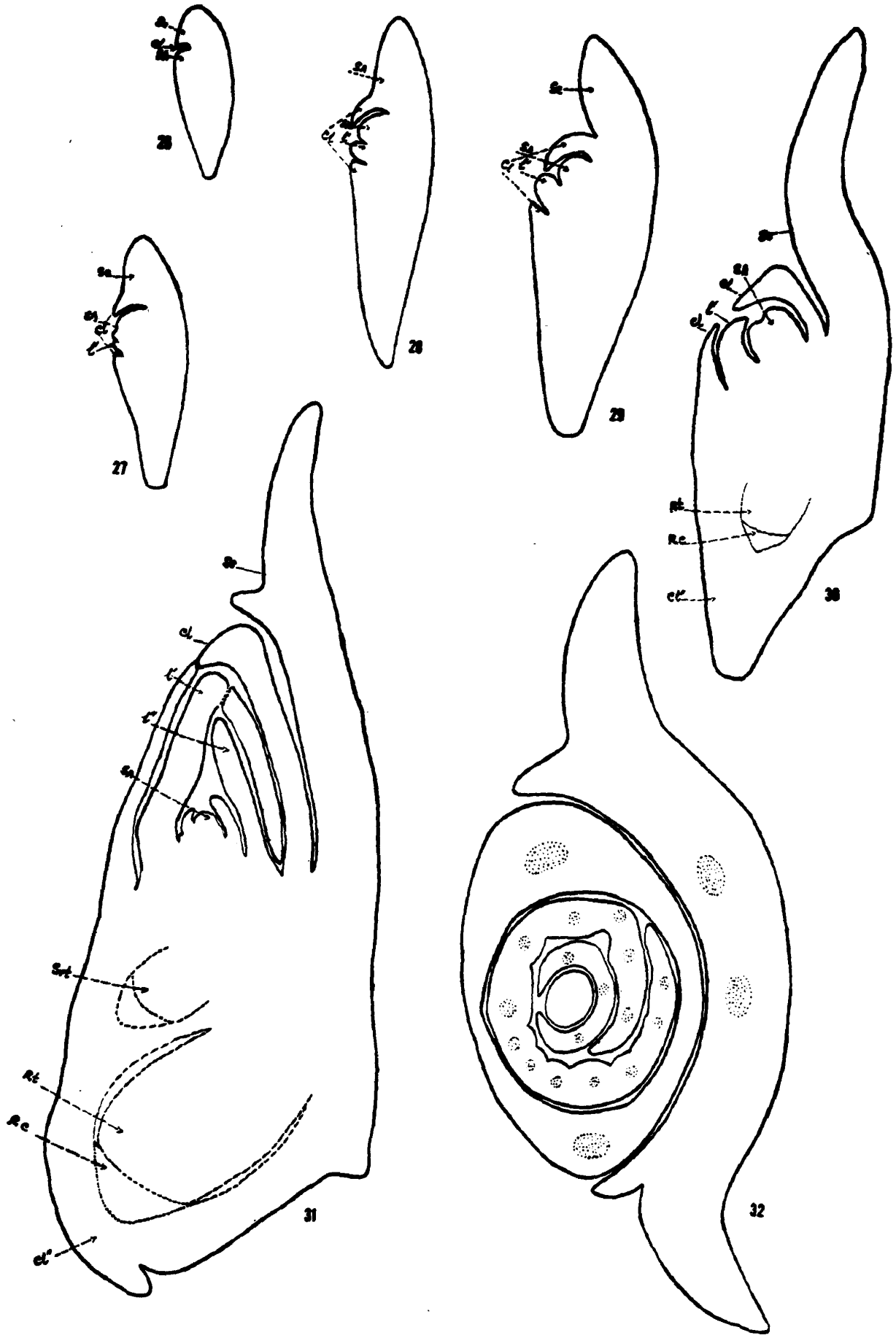
In the tier l the first anticlinal walls appear at the stage of embryonic development as shown in figures 17-19. These are followed by periclinal walls, thus delimiting the wall layer of the scutellum (figs. 17-24). The inner cells by further divisions add more cells to the body of the scutellum. Similar divisions take place in the tiers ph, h, and f and the wall layer is differentiated. The lower portion of the tier h broadens and contributes to the formation of the root tip and root cap while tiers d and f give rise to the coleorhiza and upper part of the suspensor in a similar way. By this time cells of the tier ci become disorganized and eventually disappear (fig. 25).

At the moment the embryo begins to exhibit laterality the depression which marks the separation of the cotyledon and the hypocotyledonary axis appears. This depression is

STAGES IN THE NORMAL DEVELOPMENT OF THE COLEOPTILE

Figs. 26--31 L.S. of a series of embryos showing gradual stages of the coleoptile development. (Sc - scutellum, Cl - coleoptile, SA - stem apex, 1' - first leaf, 1" - second leaf, SRT - seminal root, Rt - root, Rc - root cap, Cl" - coleorhiza)
(Figs. 26--29 x 87; Figs. 30--31 x 63).

Fig. 32 T.S. of a mature embryo showing relative distribution of cotyledon, coleoptile, first and second leaves, and shoot. (x 63).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observed to be at the common limit of the tiers l and ph (figs. 24-25). Active divisions in cells around a group of comparatively inactive cells situated at one side of the tier ph bordering the tier l causes this depression. Somewhat later, however, this inactive region shows high activity and gives rise to the stem tip.

When the laterality of the embryo is established, the coleoptile begins to develop as a ring-like outgrowth around the lateral shoot apex. From the beginning, the coleoptile shows a differential growth (on different sides). As a result the coleoptile appears as a lip-like structure projected from the base of the scutellum when seen in longitudinal section (fig. 25). Figures 26-32 show gradual stages of coleoptile development. In early stages the coleoptile appears to grow much faster on the side adjacent to the scutellum but in later stages the rate of growth seems to shift to the opposite side of the scutellum. In the mature embryo the coleoptile encircles the shoot apex almost completely (figs. 30-32), leaving only a small slit or pore opposite the stem tip. Thus, the coleoptile appears to originate from both the tiers l and ph.

Souèges reports (from Johansen, 1950) that the upper and lower lips of the coleoptile of Poa annua originate from the bases of the scutellum and stem tiers, respectively, as if the two lips are separate, distinct structures. On the other hand, Merry (1941), while conceiving the coleoptile as an unitary structure, considers that the coleoptile originates as a crescent-shaped ridge from the base of the scutellar region above the stem meristem. He writes, "(the

coleoptile) first appeared as a crescent-shaped ridge above the stem meristem. This ridge was then extended downward until it encircled the growing point and then grew out from the rest of the embryo as a sheathing tube. The sides of the tube gradually approach each other so as to leave a narrow vertical slit or pore, through which the shoot emerges during germination." In contrast to the statement of Merry (1941), the coleoptile in the present investigation is found to originate from both the scutellum and stem tiers and unlike Soueges' description is a continuous ring-like outgrowth around the stem tip.

A developmental study of the scutellum, first and second leaves of the shoot, root, root cap, coleorhiza and seminal roots shows that their growth and development exactly coincide with the description of Merry (1941) on Hordeum sativum.

On the whole, therefore, the embryonal development in Hordeum vulgare follows the "Asterad Type" (Maheshwari, 1950). The coleoptile, which originates from the tiers l and ph is a continuous covering around the plumule with a narrow slit opposite the projecting shoot apex.

Embryonal Abnormalities Induced by X-rays

Harder X-rays were used in the first and third sets of experiments while softer rays were used in the second set. Since the first set of experiments were performed mainly for exploratory purposes and produced no visible effects, descriptions will include only observations made in the second and third sets of experiments. Furthermore, since effects produced by softer and harder rays were not the same, the two will be described separately.

Effects of Softer X-rays

Single exposures of each of 100r, 200r, 300r, 400r, 500r, 600r, 700r and 800r were given to flowering spikes. Visible morphological variations were found in embryos treated with irradiation as low as 400r, while 300r of X-rays produced only cytoplasmic effects and no visible changes were induced by dosages lower than 300r. Cytoplasmic effects were manifested by the appearance of numerous deeply stained globular bodies within the cytoplasm. Similar effects were observed in embryos treated with higher dosages. These globules proved to be Feulgen negative so presumably do not contain D N A.

The kinds of abnormalities observed were almost identical in embryos radiated with X-rays starting with 400r and including 800r. A general description of all abnormalities obtained from irradiation, therefore, will be given under 500r followed by brief remarks about the effects induced by 800r.

Effects with 500r. In a comparative study of all slides it was apparent that the effects of X-rays were not uniform on all embryos of the same spike. Embryos exhibited a wide range of variable irradiation effects. Some embryos were highly affected and showed profuse proliferations in later stages, some behaved almost like normal embryos, while others were intermediate types between the two extremes (figs. 33-39 and 43-52).

X-ray effects also varied depending on the stage of development of the embryos at the time of irradiation. Younger embryos appeared more sensitive to X-rays than older ones. Embryos radiated after differentiation showed no abnormalities four days after treatment, while visible abnormalities were evident at the corresponding time in those which were treated before differentiation. Moreover, all cells of the same embryo generally were not equally affected (figs. 34-36 and 50-51). In some cases cells of a treated embryo were enlarged and vacuolated more or less uniformly (X-ray effects, in this case, were presumed equally distributed). In the majority of cases, however, differential effects were found in different parts of an embryo. As a result of this differential effect, "patches" of densely staining cells appeared scattered within the embryo (figs 34 and 49). These "scattered patches" of tissues were surrounded by vacuolated cells; they ordinarily appeared to contain even denser cytoplasm than the corresponding regions in the control embryos. Close examination at higher magnification, however, showed no significant difference between the two. These "patches" of cells were apparently regions of high metabolic activity and behaved like "apical meristems" giving rise to a number of proliferations. Once scattered "meristematic" tissues

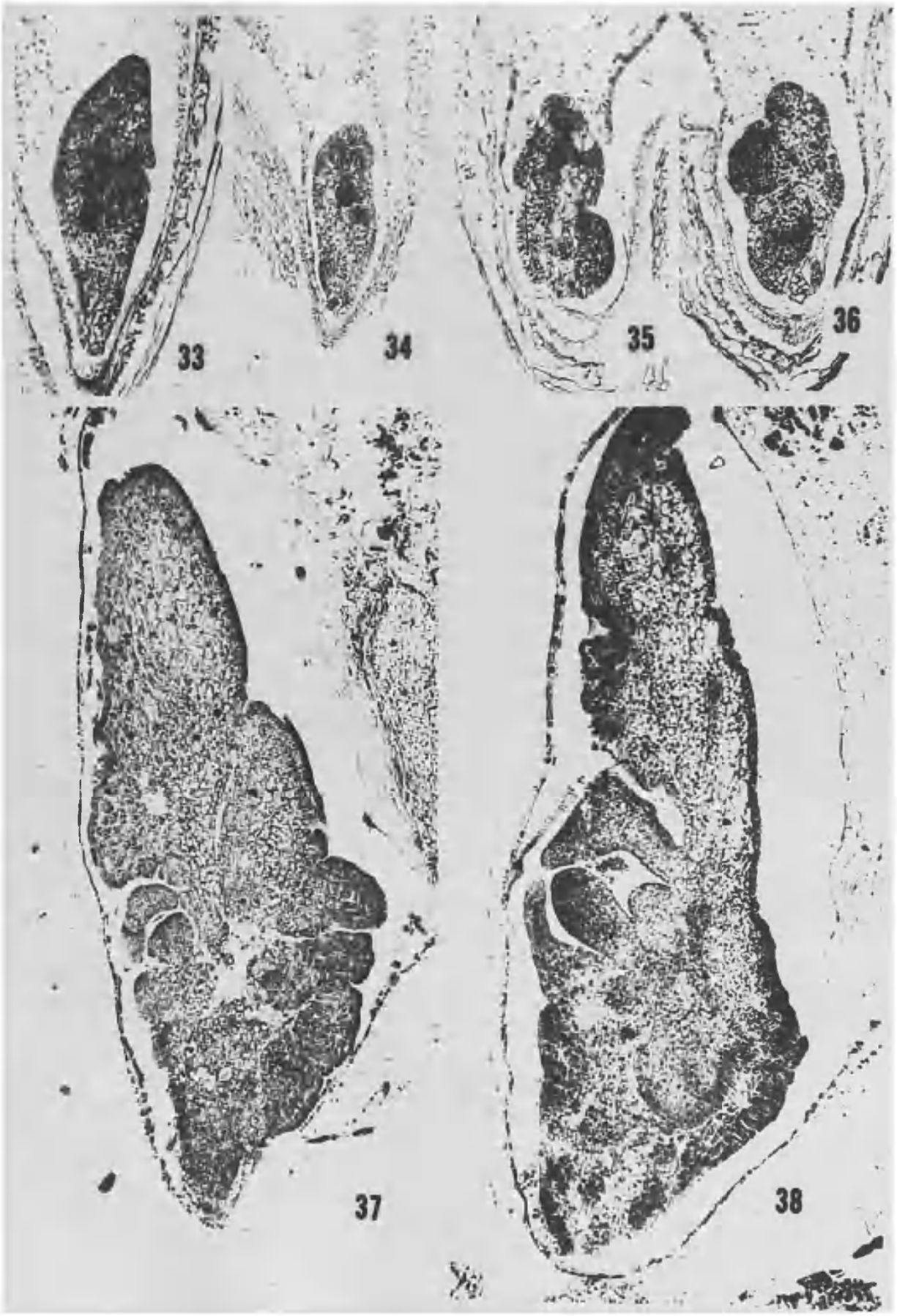
EFFECTS OF SOFTER X-RAYS (500r)

Fig. 33 L. S. of almost uniformly affected embryo observed four days after irradiation. (x 75).

Fig. 34 L. S. of unequally affected embryo collected four days after treatment. Enlarged, vacuolated cells, disturbances in the distribution of meristematic tissues, and "scattered patches" of tissues with denser cytoplasm are visible. (x 75).

Figs. 35 --36 L. S. of embryos (collected eight days after irradiation) showing enlarged, vacuolated and disorganized cells, and a number of proliferations. (x 75).

Figs. 37 --38 L.S. of older embryos (fifteen days after irradiation) showing lysigenous cavities, proliferations and enlarged, vacuolated cells. (x 75).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were established, growth occurred in various directions and produced outgrowths at different places. There was no definite place, sequence, or regularity for the development of such outgrowths. Their occurrence and direction of growth probably depended directly on the place of origin of the "scattered meristems" and indirectly on the passage of X-rays and their absorption. That portion of the embryo where absorption of X-rays was relatively high but unequal showed many such "scattered meristems" which in turn produced proliferations (figs. 34--38 and 49--53). The frequency of such proliferations, however, were noted more at the region near the base of the cotyledon and directly opposite the plumule or nearby. Some of the proliferations at this region resembled a second plumule of the embryo (fig. 37).

The first visible X-ray effects were enlargements and vacuolation of cells. In some cases enlargement and vacuolation led to a disintegration of cells and lysigenous cavities were formed. The formation of cavities in this way appeared to occur at any place in an embryo either in the cotyledon, coleoptile, hypocotyl or the root regions. It seemed that formation of such cavities depended on the amount of X-rays absorbed in specific places. Disintegration of cells which led to the formation of cavities possibly occurred as a result of greater X-ray absorption in the specific region while absorption of lesser amounts presumably led only to cell enlargement and vacuolation. Occurrence of such cavities were noted to be relatively more frequent in regions of the hypocotyl and root (figs. 37 and 53) and less frequent in regions of root cap, coleorhiza and suspensor. It should not be stated, however, that regions of coleorhiza and suspensor were less

NORMAL AND IRRADIATED EMBRYOS

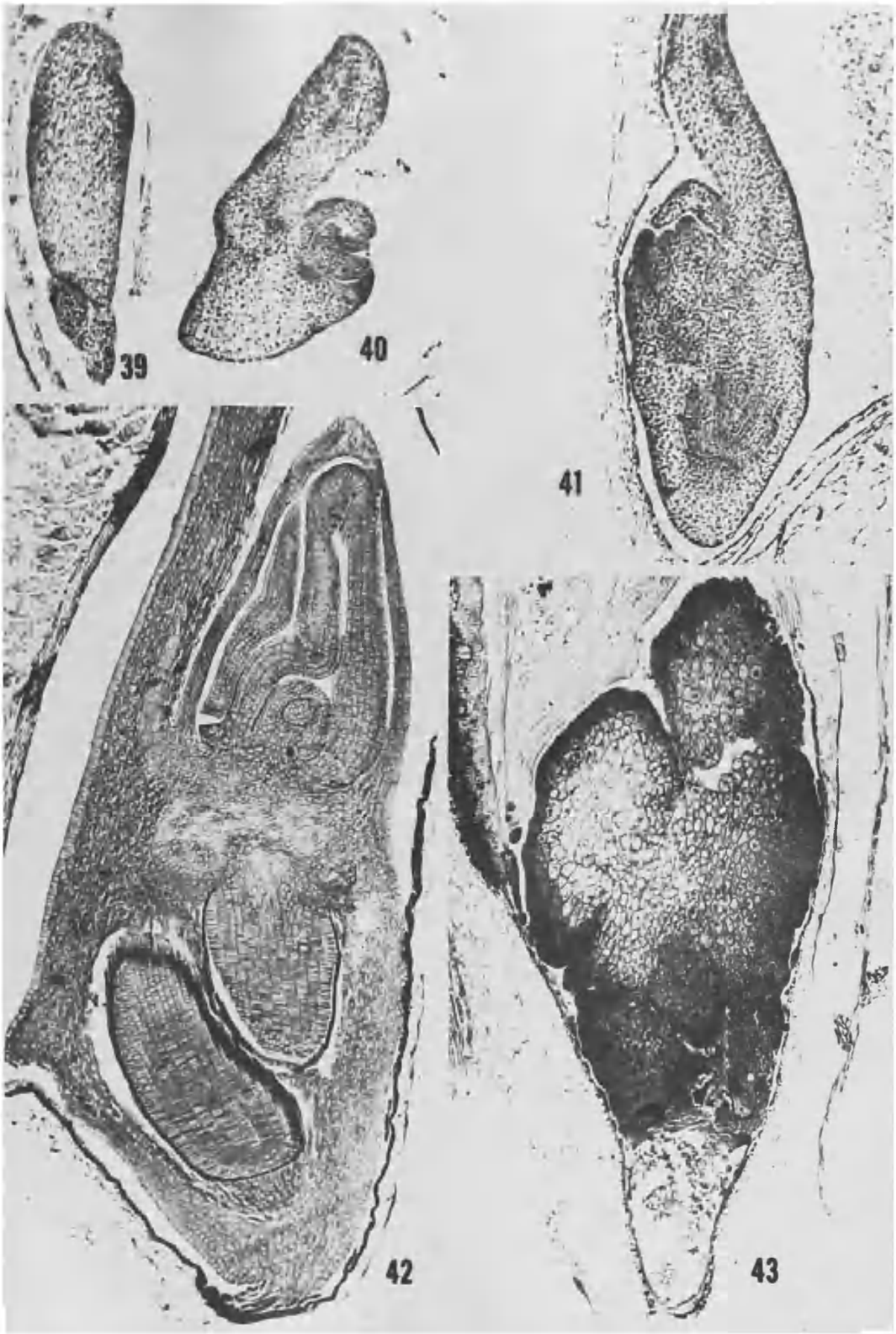
Fig. 39 Embryo X-rayed with 500r of softer rays and collected eight days after treatment. The basal end of the embryo is severely affected. (x 75).

Fig. 40 Embryo radiated with 800r of harder X-rays and collected six days after treatment. Cells are enlarged and vacuolated. (x 75).

Fig. 41 Differentiated control embryo. Approximately the same age as those of figs. 35 - 36 and 50-51. (x 75).

Fig. 42 L. S. of a mature control embryo with seminal roots developed. It is the same age as those of figs. 37-38 and 52-53. (x 75).

Fig. 43 Embryo treated with 500r of softer X-rays and collected fifteen days after radiation. The basal part is highly affected and other parts show proliferations. (x 75).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susceptible to X-rays. Figs. 39 and 43 show that the basal region of the embryo may also be severely affected.

While individual cell-sizes increased, general growth as well as development of embryos appeared to be retarded. The treated embryos were smaller and less differentiated than the control of the same age. The control embryos were not only larger but also had differentiated into cotyledon, coleoptile, plumule, root, root cap and coleorhiza (figs. 35, 36 and 41). Treated embryos, however, seemed to regain the rate of growth and appeared almost of the same size as the control when samples were collected fifteen days after treatment, although at that time they lacked complete differentiation (figs. 37, 38, 43, 52 and 53).

An origin of many "scattered meristems" capable of profuse proliferations appeared to destroy the polarity of embryos when they were examined four to eight days after irradiation. Examination of older embryos, however, showed that in spite of so much irregular growth and proliferation an inherent tendency persisted for them to undergo differentiation ultimately into plumule and root.

Some of the highly proliferating embryos in later stages appeared to be composed of strikingly regular cells like those of control. This was apparently due to the fact that these cells were formed entirely from the growth of "scattered meristems" while the intervening affected cells disintegrated and the empty spaces, thus created, were occupied by proliferating tissues.

The embryos which were older at the time of treatment or which were affected more or less uniformly by X-rays, showed growth and development somewhat different from what has been described in the foregoing paragraphs. Four to

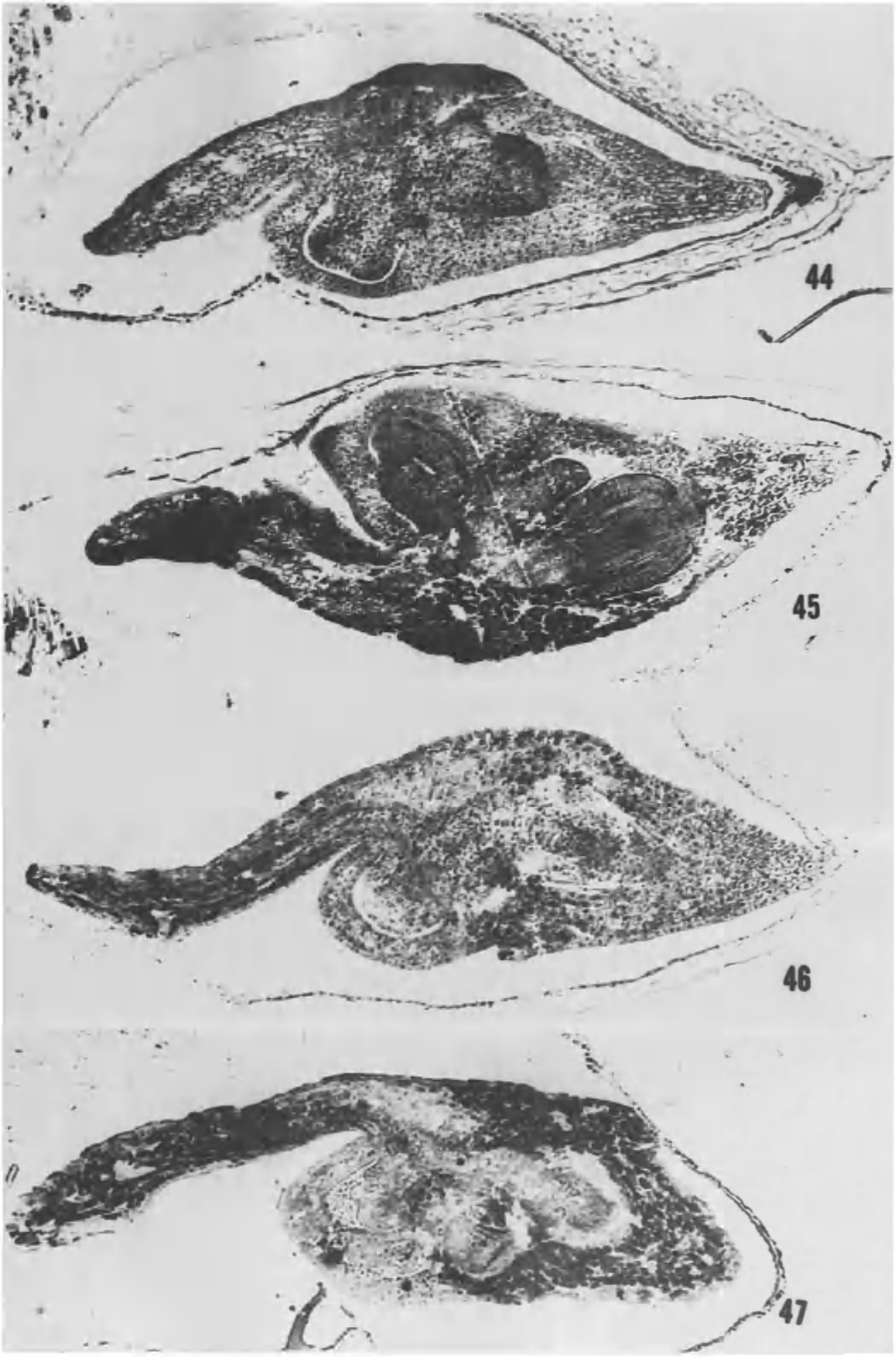
EFFECTS OF SOFTER X-RAYS

Fig. 44 L. S. of embryo radiated with 500r at an older stage and collected eight days after treatment. Cells are vacuolated and enlarged; small cavities have been formed by the degeneration of cells but the distribution of meristematic tissues is undisturbed. (x 75).

Fig. 45 L. S. of a differentiated embryo with many cavities and degenerating cells. It was similarly treated as in fig. 44 but collected fifteen days after radiation. (x 75).

Fig. 46 L. S. of embryo radiated with 800r at an older stage and collected eight days after radiation. Vacuolated, enlarged cells and scattered cavities have been formed by the degeneration of cells but the distribution of meristematic tissues is undisturbed. (x 75).

Fig. 47 Differentiated embryo with a degenerating appearance as a whole. It was similarly treated as in fig. 46 but collected fifteen days after irradiation. (x 75).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eight days after irradiation, embryos were observed to be composed of somewhat enlarged and vacuolated cells but with an almost normal arrangement of meristematic regions in them (figs. 33 and 48). There were practically no proliferations in these embryos. Time of embryonal differentiation was similar to that of the control. A further distinguishing feature was the appearance of individual cells. Cells were mostly filled with dense cytoplasm with numerous deeply stained globular bodies. Some cells were seen with two nuclei. Cell walls seemed to lack middle lamellae showing a tendency of formation of schizogenous cavities. Occasionally a few small cavities or gaps were found scattered within the embryos (figs. 44-47). They were probably formed as a result of greater absorption of X-rays in localized cells followed by a disintegration of these cells.

Not only were X-ray effects dependent on the degree of absorption of X-rays and the age of embryos as a whole, but they also appeared to vary with the age of the individual parts. Thus the treatment of X-rays prior to differentiation of the embryo seemed to produce more effects on the coleoptile than on the first leaf while radiation at the time of differentiation of the embryo produced almost similar effects on the two. The primary root usually showed somewhat irregular and retarded growth while seminal roots grew like those in the control embryos. This differential effect probably was due to the fact that irradiation is likely to produce more effects in embryo parts which are at their primordial stage of development or at least close to that stage.

Retarding effects of irradiation on growth was almost uniform on all parts of the embryo. The first leaf of the

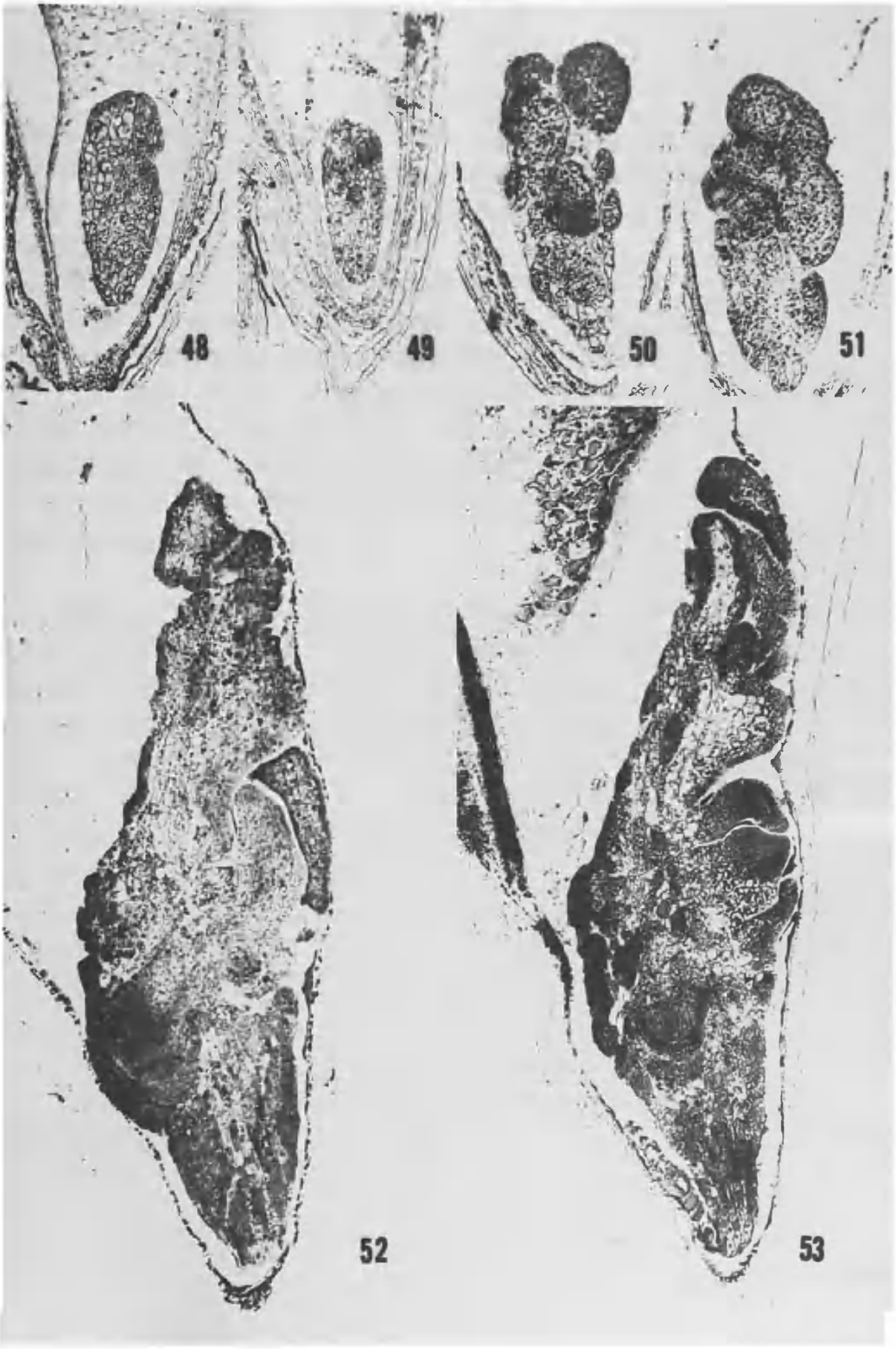
EFFECTS OF SOFTER X-RAYS (800r)

Fig. 48 A uniformly affected embryo collected four days after irradiation. Cells are enlarged and vacuolated. (x 75).

Fig. 49 Unequally affected embryo (observed four days following treatment) showing enlargement and vacuolation of cells and origin of "scattered patches" of denser cytoplasmic tissues. (x 75).

Figs. 50-51 Embryos collected eight days after irradiation. Vacuolation, enlargement and degeneration of cells and proliferations are visible. (x 75).

Figs. 52-53 L. S. of older embryos (fifteen days after treatment) showing proliferations, lysigenous cavities and also enlarged, vacuolated cells. (x 75).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plumule, however, appeared to be relatively thicker, and showed greater growth than the other surrounding parts and at times seemed to project through the plumule sheath (figs. 38 and 52-53).

It may thus be concluded that in addition to globular cytoplasmic bodies in embryonal cells other abnormalities such as enlargement and vacuolation of cells, disturbances in dell walls, disintegration of some cells leading to the formation of cavities, and disturbances in the distribution of meristematic tissues within the embryo during early post-radiation period were induced by irradiation dosages of 500r.

Effects Induced by 800r. It has been mentioned that similar kinds of abnormalities were produced by X-rays of dosages from 400r to 800r. The frequency of occurrence and the degree of proliferation in embryos radiated with 800r, however, were relatively higher than those produced by 400r and 500r (figs. 48---52). Furthermore, 800r of X-rays seemed to produce more physico-chemical after-effects in cells which resulted in "unhealthy" cells of unusual appearance even in differentiated mature embryos (fig. 47). It also became apparent that the latent period required in producing visible variations in radiated embryos appeared less in the case of the 800r dosage.

Comparison of Cell-lengths of Control and Treated Embryos (500r). Since smallness of cell-breadths as well as the contraction of cytoplasm from cell walls made the measurements of cell-breadths very difficult, only cell-lengths were measured in order to obtain an indication of comparative growth of treated and controlled embryonic cells.

Measurements were taken with the aid of an ocular micrometer using a high power (45 X) objective and a 12.5X eye-piece. In the course of the measurements one small division of the ocular micrometer (one small division was equal to 2.75 microns) was considered as a unit of length (to avoid long decimal figures) since only relative lengths of cells were essential for a comparative study. Cell-lengths were measured separately prior to gross differentiation of the embryo as well as at the mature stage of development. In the latter case cell-lengths of different parts of embryos were measured in order to obtain a closer comparison. Data obtained from measurements are shown in Table I and II. Table I shows that following irradiation there had been a considerable increase in the length of treated cells. In the case of undifferentiated embryos the average length of treated cells was 13.31 units while that of the control was less than half that amount (6.52 units). As embryos continued to grow the relative difference in cell-lengths between control and radiated embryonic cells diminished, although it remained statistically highly significant. Average cell-lengths measured 11.8 units in the coleorhiza, 10.86 in the cotyledon, 8.18 in the coleoptile and 6.50 in the first leaf of treated differentiated embryos, while average cell-lengths were 8.64, 5.70, 4.94 and 6.98 units in the corresponding regions of the control. Thus while mean lengths of treated cells in cotyledon, coleoptile, and first leaf were one and one-third times larger than control cells in the corresponding parts, those in the coleorhiza were about two times larger than the control. Less reduction in cell-lengths in the coleorhiza during further

growth and development can probably be interpreted by assuming that relatively fewer cell divisions occurred in this region.

In addition to the determination of the relative mean-cell-lengths and the respective standard deviations, the relative distribution of cells of different lengths were compared graphically by plotting cell-lengths along the abscissa and the number of cells along the ordinate (figs. A, B, C, D, and E).

Table I. Comparison between relative cell-lengths of control and treated embryos (with 500r) at the time of differentiation.

Kind	No. of cells measured	Mean-lengths	Standard Deviation
Control	500	6.52± 0.078	1.75
Treated	500	**13.31± 0.138	3.08

Table II. Comparison between relative cell-lengths of control and treated embryos (with 500r) at the mature stage.

Kind	Part of embryo	No. of cells measured	Mean-lengths	Standard Deviation
Control	Cotyledon	250	8.64± 0.155	2.47
"	Coleoptile	"	5.70± 0.11	1.73
"	First leaf	"	4.94± 0.069	1.07
"	Colerhiza	"	6.98± 0.073	1.16
Treated	Cotyledon	250	**10.84± 0.095	1.5
"	Coleoptile	"	** 8.18± 0.108	1.7
"	First leaf	"	6.50± 0.089	1.4
"	Colerhiza	"	**11.68± 0.156	2.48

** Significant at one per cent level.

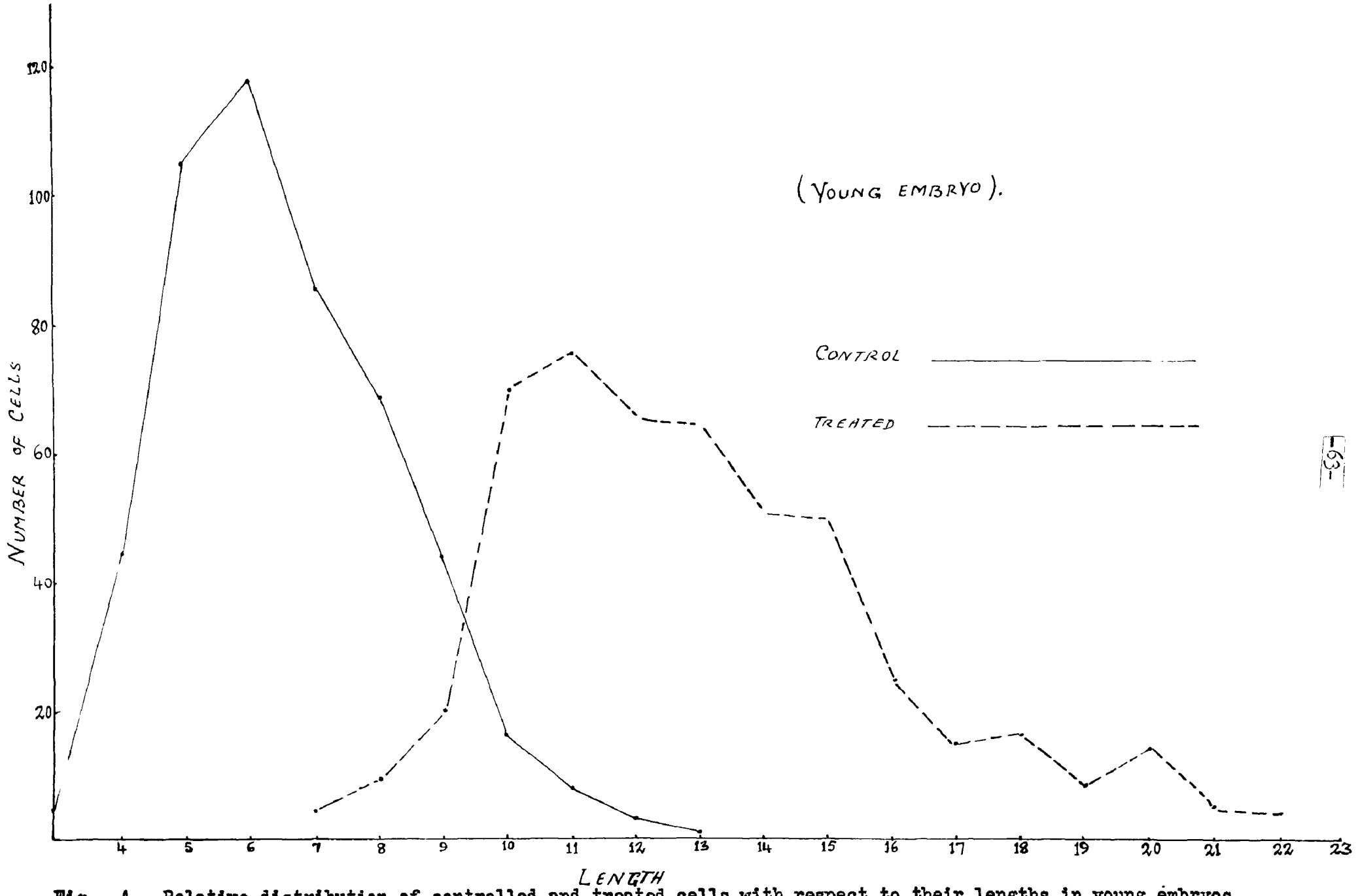

Fig. A Relative distribution of controlled and treated cells with respect to their lengths in young embryos.

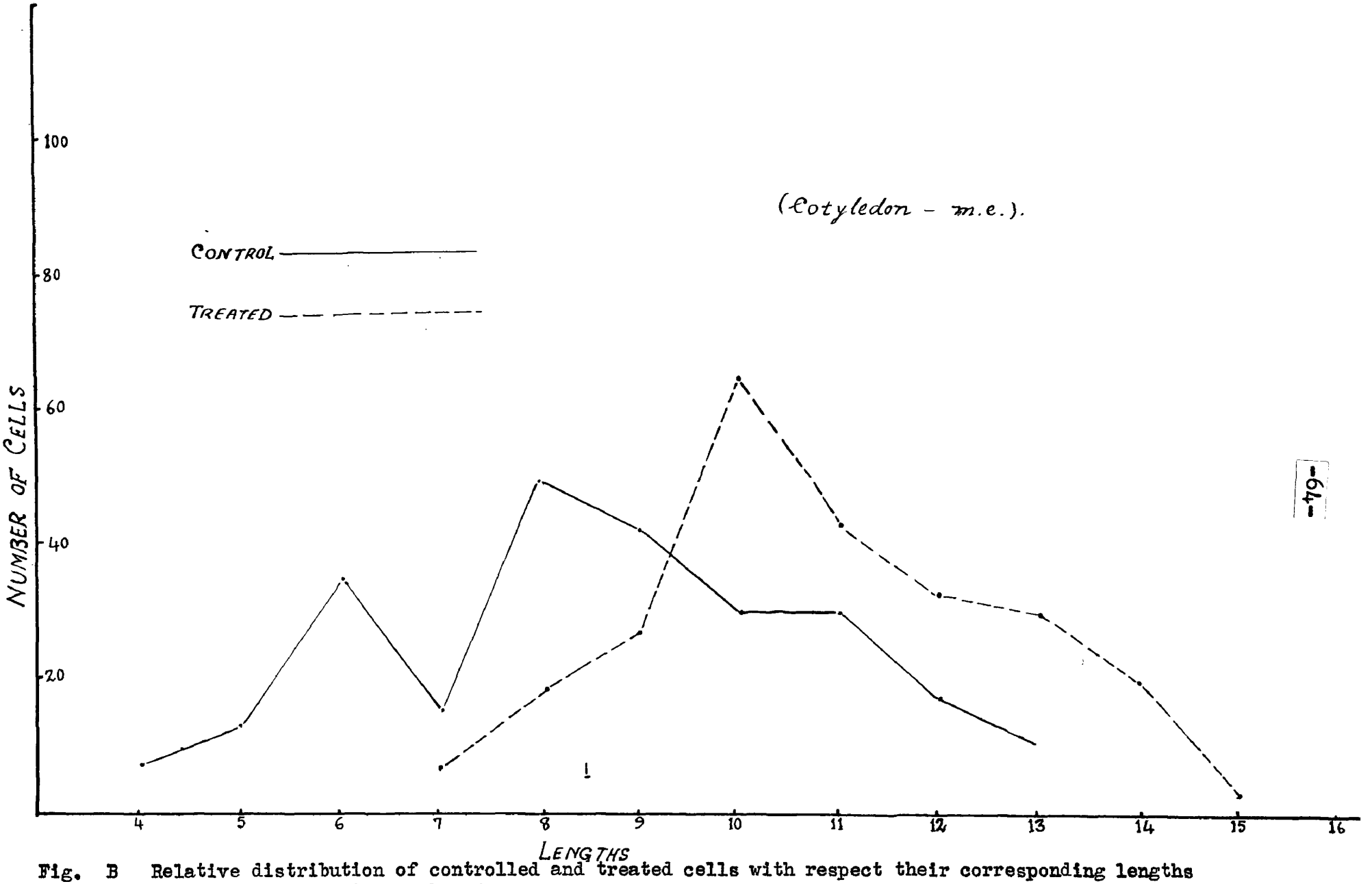

Fig. B Relative distribution of controlled and treated cells with respect their corresponding lengths in cotyledon of mature embryos.

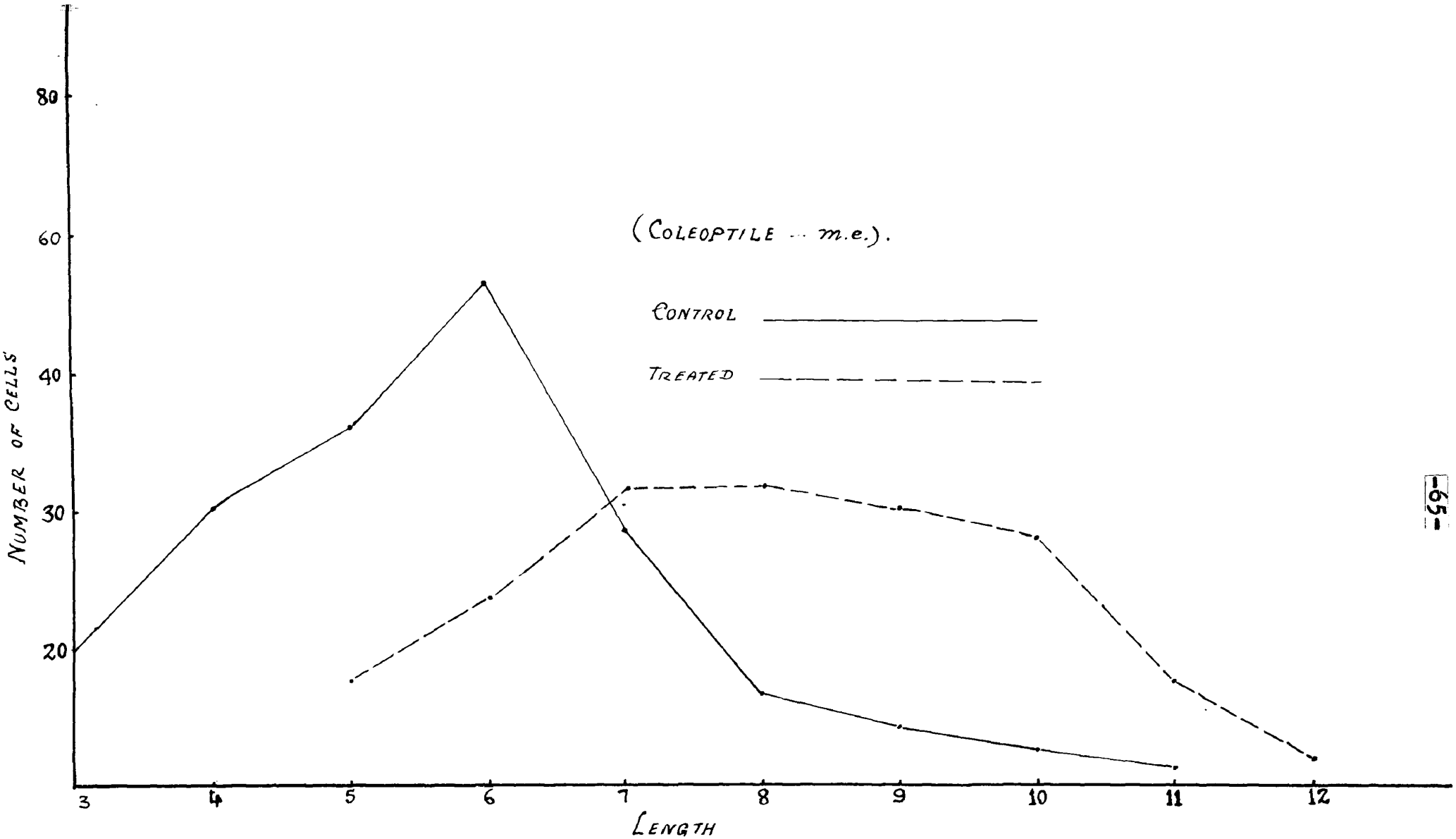

Fig. C Relative distribution of controlled and treated cells with respect to their corresponding lengths in coleoptile of mature embryos.

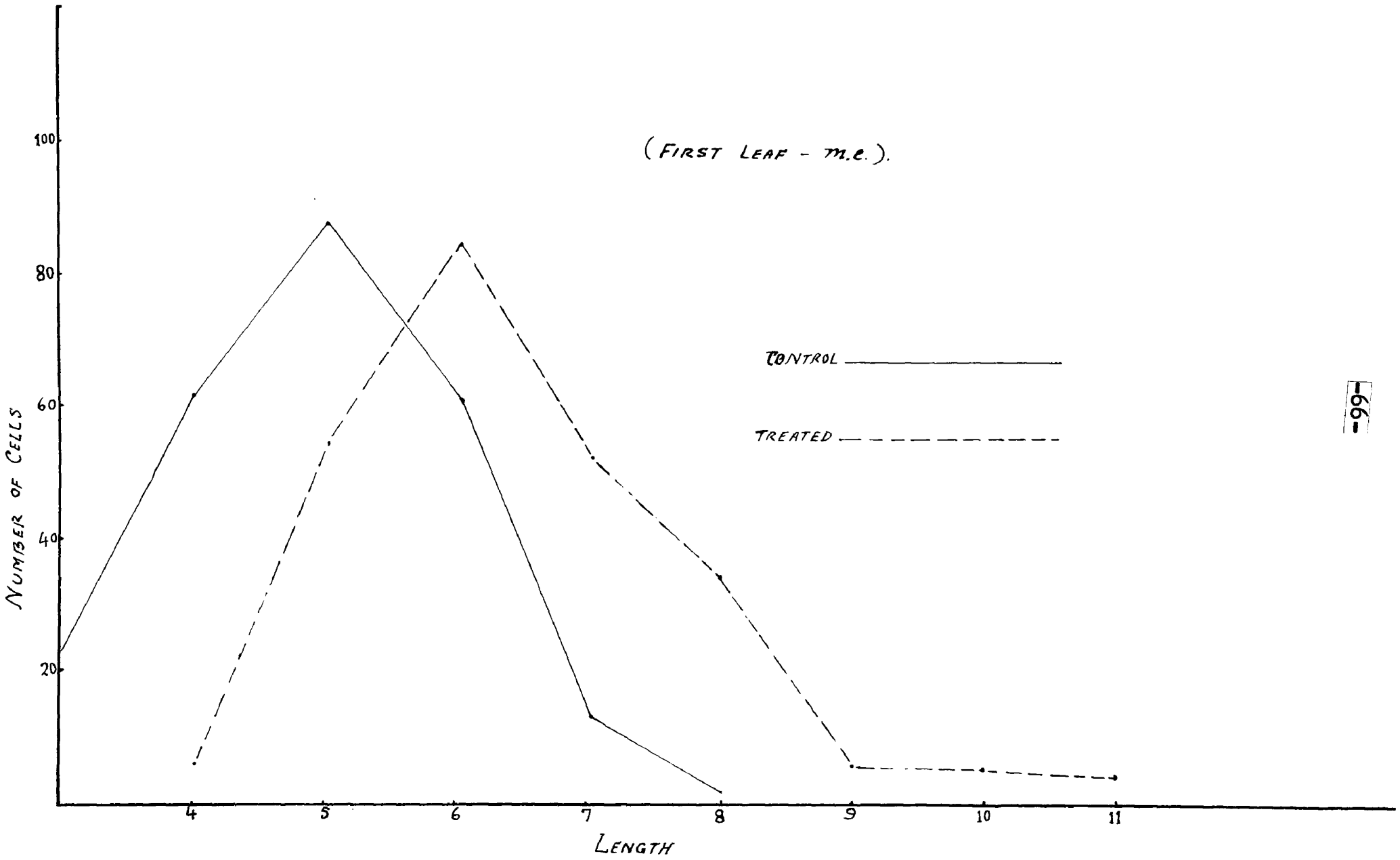

Fig. D. Relative distribution of controlled and treated cells with respect to their corresponding lengths in first leaf of mature embryos.

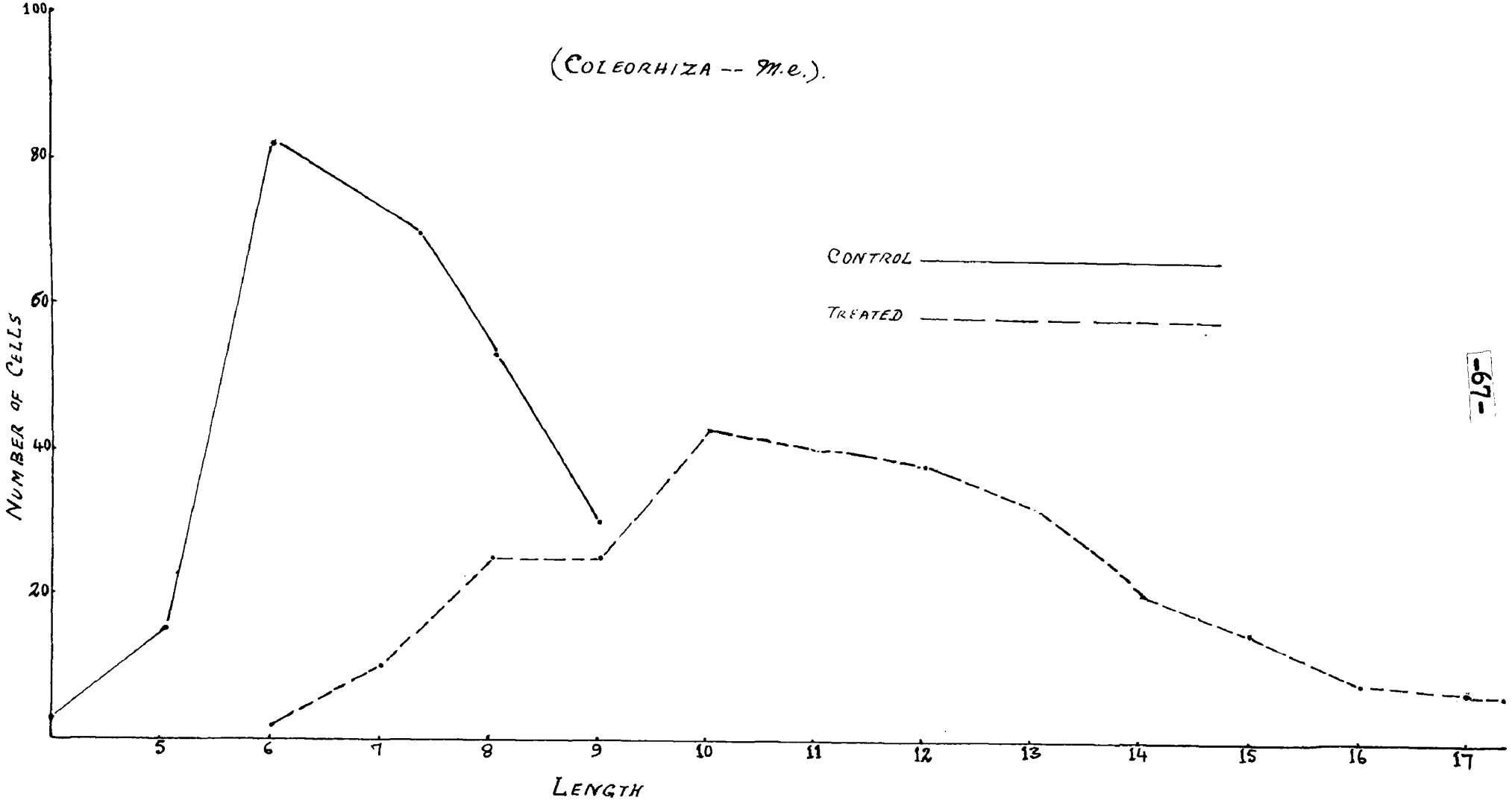

Fig. E Relative distribution of controlled and treated cells with respect to their corresponding lengths in coleorhiza of mature embryos.

Germination Tests. Germination tests were carried out in order to observe how the pattern of germination of X-rayed seeds was affected. For want of a sufficient number of seeds only fourteen seeds belonging to each group were used in the test. Although no dependable conclusions can be drawn from these tests, they may, however, give some indications.

Five days after the complete germination of control seeds final countings of all germinated and non-germinated seeds were made. Table III shows the data obtained from the test. While all fourteen control seeds germinated, only two seeds treated with 400r and one in each of the 500r and 600r groups showed normal germination. In addition to this, some treated seeds showed partial growths. Seminal roots during germination emerged from six more seeds irradiated with 400r four of which exhibited growth of plumule while none showed growth of the main root (radicle). Almost similar conditions were met with seeds treated with 500r and 600r. In the case of seeds treated with 700r and 800r neither radicle nor plumule emerged while seminal roots appeared from four of the fourteen seeds during germination. It may thus be inferred that X-rays were relatively more effective in inhibiting the growth of the main root (radicle) during germination, while seminal roots were less affected in this respect.

Table III. Germination Tests

Kind	No. of seeds used	No. of normally germinated seeds	Germination with seminal roots and shoot but no main root	Germination with seminal roots only	No. of non - germinated seeds
Control	14	14	-----	-----	-----
400r	14	2	4	2	6
500r	14	1	-----	5	8
600r	14	1	2	4	7
700r	14	-----	-----	4	10
800r	14	-----	-----	4	10

Effects of Harder X-rays

Single exposures of 500r of harder X-rays were given on four dates, approximately two, five, eight and ten days after fertilization, in order to study radiation effects on the critical stages of embryo development. Elaborate fixation and preparation of slides were made for the purpose but with an unexpected outcome. All four exposures of 500r produced neither visible morphological nor cytoplasmic variability. Similarly, preparations for the purpose of determining threshold dosages did not show any irradiation effects. A dosage of 800r of harder X-rays, however, induced some effects. Vacuolation and enlargement of cells (fig. 40) as well as occasional disorganization of a few scattered cells were observed when irradiated embryos were examined six days following treatment, but even this dose did not disturb the distribution of meristematic tissues within the embryos and induced no abnormal outgrowths which were characteristic of those irradiated with softer X-rays. Treated embryos collected twelve days after irradiation showed neither enlarged vacuolated cells nor any disorganized cells. This was probably due to the fact that these vacuolated cells recovered from the shock of X-ray treatment, became more densely cytoplasmic and the empty space (s) created by a degenerated cell, or cells, was filled up by the growth of the adjoining cells.

It thus appeared that harder X-rays were less effective than softer X-rays in producing abnormalities in barley embryos when 500r and 800r were used.

DISCUSSION

Anther and Pollen

The anther tapetum of Hordeum vulgare is glandular and binucleate, which was considered by Schnarf (1931) to be the usual condition for the family Gramineae although Narayanswami (1953) reported the presence of a uninucleate tapetum in Pennisetum typhoideum. The shedding of pollen grains at the three-celled stage was reported by Jones and Newell (1948) for Hordeum pusillum and H. jubatum and by Hirayoshi (from Smith, 1950a) for H. murinum and H. spontaneum. These reports coincide exactly with the present findings in H. vulgare. Uninucleate pollen grains, however, were observed by Rangaswami (1935) in Pennisetum typhoideum although reinvestigation of the species by Narayanswami(1953) showed the presence of three-celled pollen grains.

Embryogenesis

Soueges (1924) made a detailed study of embryonal development of Poa annua. He observed that the zygote divided by a transverse wall producing a terminal cell ca and a basal cell cb. Further divisions in these two cells produced a proembryo consisting of tiers p, o, m, n, and q of which the terminal tier q and the adjoining tier m were derived from ca with the rest from the basal cell cb. During subsequent development of the embryo Soueges noted that the cotyledon was derived from the terminal tier q; stem tip and hypocotyledonary axis from m; the tier n engendered root, root cap, and coleorhiza; while tiers

o and p gave rise to the suspensor of the mature embryo. Merry (1941), on the other hand, reported that while the first two divisions of the zygote could be followed easily, further divisions in the proembryo were very irregular. He, therefore, thought that the subsequent sequence of segmentation in the embryo was of no importance. The condition met in Hordeum vulgare is almost similar to that reported by Soueges for Poa annua and dissimilar to Merry's report on Hordeum sativum. In H. vulgare a three-celled structure (consisting of tiers ci, m and ca) is formed from the zygote by two divisions. Further divisions in tiers m and ca produce a proembryo consisting of tiers l, ph, h, d, f, and ci. Although it is true that divisions of cells within each tier are irregular, it was not a very difficult task to trace the individual tiers through later stages of growth and development up to the mature embryo in which the terminal cotyledon was derived from the tier l, stem-tip from ph, hypocotyl, root, root-tip and root cap from h, coleorhiza and the upper part of suspensor from d and f and the lower part of the suspensor from ci. Hordeum vulgare, therefore, follows a definite pattern of embryo development which is known as the "Asterad Type" (Maheshwari, 1950).

Enlargement, Vacuolation and Disintegration of Cells

Wolcott (1937), during his study of the influence of X-rays on stem and leaf anatomy in barley, observed that cell-size increased following irradiation. He also found that cell walls appeared to lack "reinforcement". A similar increase in cell-size following irradiation has been observed in both animals and

plants by a number of other investigators. Smith and Kersten (1941, 1942) noted that an enlargement of cells was accompanied by vacuolation and that in some cases these vacuolated cells disintegrated giving rise to lysigenous cavities and in other cases schizogenous cavities arose as a result of the disorganization of the middle lamellae of the cell walls. The results of the present experiments corroborate these findings. Following irradiation, cells became vacuolated and increased in size. Affected cells often disintegrated giving rise to cavities. Sometimes they appeared disconnected from one another apparently due to a disorganization of the middle lamellae of the cell walls.

Disintegration of cells leading to the formation of cavities was presumed to take place in those areas where absorption of X-rays was higher. Heilbrunn and Young (1930) and Heilbrunn and Daugherty (1933) reported that calcium ions are released from "the cell cortex" following irradiation and enter the cytoplasm causing liquefication and coagulation of the latter. Since the middle lamellae of young plant cells are primarily composed of calcium compounds, a loss of calcium ions following radiation may account for the disorganization of middle lamellae and hence the disorganization of cells which later account for the formation of cavities. Likewise vacuolation of cells may be interpreted as a result of the liquefaction and coagulation of cytoplasm caused by the action of calcium ions liberated by the interaction of X-radiation on cells as Heilbrunn and Mazia (1936) visualized. Disturbances in metabolism due to an inactivation

and/or destruction of enzymes or hormones after irradiation may also be involved in the formation of vacuoles. That X-radiation inactivates and destroys enzymes was reported by Dale (1940, 1942), Mitchell (1942) and Hevesy (1952), among others, while inhibitory and destructive effects of X-rays on hormones were studied by Skoog (1935) and Leopold (1949). Cell enlargement, on the other hand, may be attributable to the continued growth of cells while cell divisions were delayed following irradiation (Lea, 1947; Catcheside, 1948).

Growth-Retarding Effects of X-Rays

General retardation of growth was manifested by a proportionate smallness of affected embryos. Similar effects were observed in animal embryos as have been pointed out earlier in this work. In plants retarding growth effects from irradiation were observed by Rasch (1951) and Quantler, Schertiger and Stewart (1952) among others with the application of higher doses of X-rays, while a great many earlier workers (from Johnson, 1936) found stimulative influences at low doses.

Smith and Kersten (1941) reported that during earlier post-radiation periods the radiated roots of Vicia faba showed growth rates similar to those of the controls but in later stages of development their growth was retarded. In contrast to their findings this investigation shows that the growth of X-rayed barley embryos was retarded for a short time following irradiation but assumed a normal rate of growth during later stages of development.

Wilson and Karr (1951) and Wilson, Jordan and Brent (1953) tried to explain the growth-retarding effects of X-rays on rat embryos. They thought that this effect might be attributed either to a reduction in mitotic rate in radiated embryonic cells or to an outright destruction of a certain percentage of cells. They further felt that both the processes might be involved in producing the effect. A similar explanation may be plausible for the retarded growth of radiated barley embryos. A reduction in cell division as well as a destruction of some cells in radiated embryos were apparent during the early part of the post-radiation period.

Bergonie and Tribondeau (1906) made a generalization with regard to radiosensitivity which has often been referred to as the "Bergonie-Tribondeau Law". The law holds that the sensitivity of cells varies directly with their reproductive capacity and inversely with the degree of differentiation. Accordingly therefore, younger embryos are more sensitive to radiation than older embryos. V$_a$lidity of the law has been verified by a number of investigators with many animal embryos. Chick embryos, however, have been reported to be one exception to this law. Strangeways and Fell (1927) and Schneller (1951) observed that the sensitivity of chick embryo tissue increased as it grew older. The results of the present study, however, are in complete accordance with the law. The younger irradiated embryos were more affected than the older ones. Embryos irradiated at early stages in development showed disturbances in the distribution of meristematic tissue and profuse proliferations in addition to

vacuolation and enlargement of cells, disintegration of middle lamellae leading to the formation of schizogenous cavities and disintegration of some cells forming lysigenous cavities. Embryos treated at older stages, on the other hand, exhibited no disturbances in the distribution of meristematic tissues and no proliferations, but showed disintegration of a few scattered cells, and less vacuolation and enlargement of cells.

X-Ray Effects on Critical Stages of Development

X-radiation effects on critical stages of development of animal embryos have been studied by Job, et al. (1935), and Wilson and his co-workers (1951, 1952, 1953) in rats, and by Kaven (1938a, 1938b) and Russel (1950) in mice. They found that predetermined and undifferentiated primordia were more sensitive than organs already differentiated. Wilson, Jordan and Brent (1953) attempted to explain their findings in terms of the "Theory of Critical Moment" enunciated by Stockard (1921). Stockard in his study concerning the effects of temperature and oxygen on developing sea minnows noted that a single teratogenic agent was capable of producing a variety of developmental defects but the production of any particular defect was dependent upon the action of the agent at a specific time ("particular developmental moment"). Results of the present experiments are not decisive in this respect; they, however, indicate that the "Theory of Critical Moment" may also hold good. For example, it was observed that X-radiation prior to differentiation of barley embryos seemed to produce more effects on the coleoptile than on the first leaf, while radiation applied at the time of differentiation produced almost similar effects on the two.

Unequal Effects of X-Rays

Uniform effects of X-rays were not observed in barley embryos of the same spike. Some were highly affected, showing profuse proliferations, some behaved almost normally while others were intermediate types between the two extremes. While it was conceived that a uniform exposure of X-rays to all flowers of a spike was not possible, it, however, was not expected that flowers borne on the same side of a spike would show differential radiation effects. Nevertheless this differential effect did occur and may be due to the fact that all flowers of a spike often are not in "exactly" the same stage of embryonic development. Similar non-uniform effects of irradiation were reported by Horlacher and Killough (1931) and by Caldecott, Frolik and Morris (1952). Horlacher and Killough (1931) while working with cotton seedlings raised from X-rayed seeds noticed that the seedlings could be grouped into three classes; normal, intermediate and dwarf. Likewise, Caldecott, Frolik and Morris (1952) found that seedlings from X-rayed barley seeds had a wide range in distribution, whereas those from thermal neutron radiation had a very narrow range.

Differential Effects of Softer and Harder X-Rays

Highly differential effects of harder and softer X-rays were experienced in the present study. Embryos treated with as low as 400r of softer X-rays showed enlarged, vacuolated cells, formation of schizogenous and lysigenous cavities and numerous

proliferations. On the other hand exposure of 500r of harder X-rays to embryos failed to induce any visible variations. With 800r feeble irradiation-effects (enlargement and vacuolation of embryonic cells and disintegration of a few randomly scattered cells) were observed during the earlier post-radiation period, yet other embryos appeared similar to those of the control. These findings raise the question: do X-radiation effects on biological materials vary with the quality of X-rays used? This was a very lively problem for experimentation during the 1920's and 1930's (Duggar, 1936). A number of research papers were published with two sets of contradictory results. While one group maintained that softer and harder X-rays produced differential effects, the other showed that similar irradiation effects were obtained in cases where the two kinds of X-rays were of equal intensity. Packard (1936) reviewed all the literature pertaining to the subject and concluded that according to the opinions of the majority of investigators both soft and hard X-rays resulted in similar biological effects when equal "quantity" and "intensity" of the two were applied to the irradiated organism. He, himself, considered that similar results should be obtained if all physical phenomena of ionizing radiation are taken into consideration at the time of irradiation.

It has already been stated that the primary action of irradiation is to bring about in the matter ionizations which in turn produce visible biological effects, presumably through chemical reactions between the ions produced. Biological effects of

radiation therefore, depend on the number of ions produced per unit area by radiation in the irradiated object, i.e., they depend indirectly on the amount of energy absorbed from the X-ray beam. The amount of X-rays, however, absorbed from a beam (by a given area) varies with the quality of rays used. Harder X-rays with shorter wave-length, have greater penetrating capacity and a certain amount of the beam emerges without being absorbed if the irradiated object is thin. On the other hand, softer rays with longer wave-length, are absorbed within a short distance and therefore, produce more ionizations per unit area when equal calculated doses of soft and hard X-rays are used. It, therefore, becomes evident that though equal calculated doses of harder and softer X-rays were used in the present study, the "effective intensity" (the number of photons absorbed per unit area from a particular X-ray beam) of the two might not be the same, and hence, differential irradiation effects would probably be the natural outcome. In addition to this probable differential "effective intensity" a "quantitative difference" of X-rays may also be involved in spite of the fact that the dosages of X-rays used were equal in both cases. "True quantity" of X-rays is the amount of X-rays passed through a unit area of the field of exposure. In the present study the beam size was 400 sq cm in the case of harder radiation, while no beam size was used in the softer radiation and hence, the "true quantity" of X-rays in the two cases was not determinable.

Mechanisms Involved in Biological Effects of X-Rays

The last and most important phenomena to be discussed are the mechanisms involved in the biological actions of irradiation. A number of hypotheses dealing with these phenomena have been stated briefly in the introduction. The "direct hit" hypothesis is supported by a majority of the investigators as an explanation for X-ray effects on biological objects. The theory presumes that X-ray absorption which occurs directly in the radiosensitive volumes of the nucleus (genes and chromosomes) are responsible for the biological effects. Since no cytological and genetic examinations were carried out in this investigation, it was not possible to determine whether or not any such reactions were involved in producing the abnormalities in the irradiated embryos.

Sax (1942) in dealing with mechanism of X-ray effects stated that "although the major effect of X-rays appears to involve chromosomal alterations produced by 'direct hits' there is evidence of a general physiological effect". He considered the temporary cessation of cell division to be a result of the "physiological effect" of irradiation, while according to Scott (Sax, 1942) "physiological effects" involved changes in metabolic rate, alteration in cytoplasmic viscosity and permeability, and an effect on the enzyme systems resulting in the ultimate injury and death of irradiated cells. He, however, thought these effects to be of secondary importance. Recent data of MacKey (1951, 1952, 1954), Caldecott, Frolik and Morris (1952), and Ehrenberg,

Gustafsson and Mybom (1952) are quite illuminating in this respect. They made comparative studies of the effects of neutrons and X-rays on barley and wheat. They observed that for similar chromosomal aberration frequencies there were more seedling injuries and death in seeds treated with X-rays than in seeds subjected to neutrons. MacKey (1952) further noted that the sprouting of X-radiated seeds showed a sigmoid curve in the X_1 generation while neutron-bombarded seeds showed an exponential relationship in the N_1. MacKey concluded that X-rays produced "physiological effects" in addition to chromosomal aberrations and that their effects were cumulative, while neutrons induced biological effects by "direct hit" only. Likewise Caldecott, Frolik and Morris (1952) thought that the differential effects of neutrons and X-rays might be due to more extra-chromosomal effects of X-radiation.

"Physiological effects" of X-radiation may also be involved in the production of abnormalities in irradiated barley embryos. Retarded growth rates during the early post-radiation period, vacuolated, enlarged cells, the origin of "scattered patches of cells", and the profuse proliferations occurring in treated barley embryos may be attributable to this effect. Probable explanations for the enlargement and vacuolation of embryonal cells have been discussed in a foregoing paragraph. Disturbances of the metabolism of cells through inactivation and/or the destruction of enzymes or hormones by irradiation have been visualized as a cause of the vacuolation of cells. They may also be indirectly responsible for the origin of "scattered patches

of meristematic cells" and hence for proliferations. Due to an unequal absorption of X-rays apparently some cells became vacuolated and enlarged following irradiation, while some "scattered patches of cells" were left unaffected and undisturbed with presumably normal hormones and enzymes. The "patches of cells" with all the necessary constituents for further growth were in an advantageous position and hence produced the proliferations.

While an inactivation and destruction of hormones and enzymes may be directly or indirectly concerned in the vacuolation and enlargement of cells, and in proliferations, irradiation in the course of inactivating and destroying enzymes or hormones may be involved in any one or all of the following theories: "photochemical action", "point heat action" and "action of active radicals". It seems apparent, therefore, that no single hypothesis alone is completely adequate for a fully satisfactory explanation of the biological action of irradiation but that perhaps all the processes may be operating in the induction of the ultimate, visible, biological effects.

SUMMARY

This investigation involved a study of the general embryological development of Hordeum vulgare and the embryonic abnormalities induced by X-rays as an external agent. The study of general embryology included the anther, microsporogenesis, the male gametophyte, the ovule, megasporogenesis, the female gametophyte, the endosperm and embryogenesis. Conclusions made during the course of the observations are as follows:

1. The anther wall is composed of an epidermal layer, two to three middle layers, and a tapetum. The tapetum is glandular and binucleate.

2. The microspore mother cells, by successive divisions, usually give rise to microspore tetrads of the isobilateral type. Pollen grains are shed at the three-celled stage.

3. Ovules are crassinucellate, bitegmic, and campylotropous. The placentation is parietal.

4. A hypodermal archesporial cell functions directly as a megaspore mother cell which by reduction divisions gives rise to a linear tetrad of megaspores, the lowermost of which produces an eight-nucleate "Polygonum type" embryo sac.

5. The primary endosperm nucleus divides earlier than the fertilized egg. The endosperm is a "Nuclear type".

6. Hordeum vulgare follows the "Asterad type" of embryo development.

7. The coleoptile is a single structure and originates from both the scutellum and stem-tip tiers.

8. Irradiation dosages of 500r of X-rays with longer wave lengths induced a number of abnormalities such as enlargement and vacuolation of cells, disturbances in cell walls, disintegration of some cells leading to the formation of cavities, disturbances in the distribution of meristematic tissues within the embryo, a number of proliferations in different parts of the embryo, and retarded growth of the embryo during the early post-radiation period. In addition to these, deeply stained globular cytoplasmic bodies were observed in radiated embryonic cells. Almost similar kinds of abnormalities were produced by softer X-rays starting with 400r and including 800r. The frequency of occurrence and the degree of proliferation in embryos irradiated with 800r, however, were relatively higher than those produced by 400r and 500r. Treatments with 300r-units of irradiation induced only the appearance of deeply stained globular bodies within cytoplasm while no effects were visible with dosages below 300r.

9. Harder X-rays were found to be less effective in inducing embryonic abnormalities than softer rays when equal calculated dosages of the two were applied to the flowering spikes of Hordeum vulgare.

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