IRRADIATION IN SUCCESSIVE GENERATIONS: COMPARATIVE RESPONSES OF DEVELOPING AND DORMANT EMBRYOS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

William F. Campbell

1964

This is to certify that the

thesis entitled

IRRADIATION IN SUCCESSIVE GENERATIONS:
COMPARATIVE RESPONSES OF DEVELOPING
AND DORMANT EMBRYOS

presented by

William F. Campbell

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Radiation Botany

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Date 28 April 1964

ABSTRACT

IRRADIATION IN SUCCESSIVE GENERATIONS:
COMPARATIVE RESPONSES OF DEVELOPING
AND DORMANT EMBRYOS

by William F. Campbell

The aims of this investigation were: one, to study the effects on the germ line of Hannchen barley following the application of recurrent irradiation to each of one, two, and three successive generations at specific stages of embryogeny and at dormancy; two, to compare the responses of seedlings and mature plants arising from these embryos irradiated either during their embryonic development or at dormancy; and three, to determine if Hannchen barley (a diploid, two-rowed variety) possesses the potentialities for the induction of radiation resistance or susceptibility.

Hannchen barley was chosen as the experimental material since it produces many seeds per head with embryos in relatively the same stage of development provided that the four basal and the four terminal grains are discarded. In addition, a great deal of information concerning its normal development and radiosensitivity responses are already known. X-radiation was selected because the intensity could be easily regulated and it was the only readily available source of irradiation at hand for treating the developing embryos. Dormant embryos were exposed to one of a number of different dosages (0 r; 4,000 r; 7,500 r; 12,500 r; 15,000 r; 30,000 r),

while four specific stages (EP = early proembryo; LP = late proembryo; ED = early differentiation; LD = late differentiation) of developing embryos were each given one exposure of 400 r each generation. Effects on germination, seedling vigor, survival to maturity, fertility, and induced abnormalities were observed.

Germination was very good following irradiation either of dormant embryos at the various dose levels or at the four specific stages of embryogeny, except for the early proembryo stages. With irradiation at the 15,000 r and 30,000 r dose levels (given dormant embryos) and at the 400 r exposure level applied to the early and late differentiation stages of developing embryos, germinability tended to decrease with each successively irradiated generation. This reduction in germination appeared to result from the induction of both dominant and recessive lethals. Seeds of the X-2 generations of both developing and dormant embryos germinated 94 to 100 percent.

Growth inhibition of seedlings arising from irradiated dry seeds tended to increase as the level of radiation increased. Also the number of roots emerging from irradiated dormant embryos, with few exceptions, remained similar to those of the controls indicating that the centers of origin, or pre-existing cells, of the roots are already present in the mature, dormant embryo at the time of irradiation. On the other hand, seedling responses, based upon coleoptile height, number of roots, and dry weights of

roots and shoots, following irradiation of developing embryos generally exhibited differential stage specific responses, the early proembryo and late differentiation stages showing greater inhibition. Following the second generation of radiation of both the developing and dormant embryos the shoot growth generally decreased. This was followed by an increase after the third irradiated generation. Delayed and reduced cell divisions, rather than an effect on cell elongation, have been discussed as the probable primary cause of growth reduction. Special emphasis was given to the fact that decreased growth, followed by an increase with a subsequent generation of radiation, may have resulted from a build-up of genetic burdens (presumably chromosomal interchanges) followed by an elimination (involving haplontic and/or diplontic selection) of the aberrant cells. With few exceptions, the induced variability was greater following the irradiation of developing embryos than that of the dormant embryos for all criteria used to measure seedling vigor. Interestingly, irradiation of 30,000 r given dormant ambryos produced seedling responses similar to those of 400 r applied to the early proembryo and late differentiation stages of developing embryos.

Following the first generation of radiation of both developing and dormant embryos, the frequency of seedling abnormalities was higher in the developing embryo groups than in dormant ones. Moreover, the irradiation during

embryonic development induced a wider spectrum of seedling mutants. In addition, the leaf and coleoptile abnormalities produced were correlated with the specific stage irradiated in developing embryos and were entirely absent in plants arising from irradiated dormant embryos. Seedling abnormalities were extremely rare in the X-2 generation following each succeeding generation of radiation and may have resulted from sampling error. The "coleoptile-only" mutant appearing in seedlings of the X-2 generations following the irradiation of dormant embryos suggests that it may be a recessive segregant.

The experimental results of this study strongly indicate that when measuring radiation sensitivity careful consideration should be given the criterion used as an index of effect. In addition, the ontogenetic stage of development irradiated should also receive special attention. Overall, increased seedling vigor (as measured by shoot height) and survival to maturity, following the third successive generation of radiation given both developing and dormant embryos, suggests that the Hannchen germ line is capable of adapting to irradiation as an additional environmental stress. This appears to be accomplished by an alternating cycle, initially a build-up of the genetic burdens in one generation followed by their elimination in a subsequent irradiated generation. Furthermore, from the data obtained in this study, it does not appear feasible to use recurrent irradiation, during successive generations, to increase and retain the frequency

of mutations in this diploid variety of barley mainly because of its high level of discrimination (through haplontic and/or diplontic selection) against these induced mutations.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1964

ACKNOWLEDGEMENT

The author wishes to express his sincere gratitude to Dr. L. W. Mericle for his deep interest, encouragement, and guidance throughout the course of this investigation.

Thanks and appreciation are also extended to other members of the committee: Drs. W. B. Drew, G. B. Wilson, and A. Kivilaan, all of whom gave willingly of their time, to Dr. F. C. Elliott, who served as a substitute, for his constructive criticism and reading of the manuscript, and to Dr. R. P. Mericle for many constructive suggestions and stimulating conversations.

Others whose aid was greatly appreciated were Dr. U. Mostosky of the College of Veterinary Medicine, Michigan State University and Mr. L. A. Schairer of the Biology Department, Brookhaven National Laboratory, Upton, New York for the irradiation, Miss Heidi Schlosser for the drawing of Figures 1 and 4, and Mr. Gaylon Peyton for reading parts of the manuscript.

A very special thanks is extended to the author's wife, Phyllis, for help in typing and editing the manuscript and for the constant inspiration and encouragement during the entire period of college training.

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INTRODUCTION

In recent years a search has been on for some means of protection (radio-resistance: acquired or induced) against ionizing radiation. The net result has been the discovery that radiosensitivity of organisms can indeed be influenced. For example, scientists have reported inducing resistance in mice (Luchnik and Kulinkova, 1956) and rats (Maisin et al., 1960) to doses of irradiation which would otherwise have been lethal, by prolonged weak irradiation during the fetal stages of development. Other investigators (Bloom, 1950; Pierce, 1948; Cronkite et al., 1950; Nuzhdin et al., 1960) have observed acquired radio-resistance in crypt epithelial cells of the small intestine in animals following repeated low-dosage x-irradiations.

Recently Spalding and Strang and their co-workers (1961, 1962) have attempted to determine what happens to future generations of mice when the irradiation is continued over several succeeding generations of progenies. They observed heritable genetic damage and a shortening of the life span on the unirradiated F-6 offspring. Others, working with mice (Stadler and Gowen, 1961, 1962, 1963) and rats (Brown and Krise, 1961) exposed to continuous gamma irradiation for a 22-23 hr./day throughout their lifetimes and that of their descendants for each succeeding generation,

found that an exposure level of 2 r/day allowed sufficient reproduction to continue the germ line through 11 generations of mice and 5 generations of rats. Several other studies dealing with acquired and/or induced radio-resistance in animals are noted in a review by Dacquisto (1959).

By collecting data on germination, survival (at the end of 6 weeks) and seedling vigor (as measured by shoot height and dry weight), Abrams and Frey (1958) studied the effects of acute irradiation applied to dry seeds of oats once in each of several successive generations. They observed a reduction in germination and in the number of surviving seedlings but found an increase in vigor of those surviving. Mewissen et al. (1959, 1960) collected Andropogon filifolius seeds from an uraniferous soil, with radioactivity of the superficial layers ranging from 10 to 50 times background, and from a non-uraniferous, but otherwise similarly mineralized, soil. Following single acute X-ray exposures, seeds from the long-continued low-level irradiated population exhibited higher vitality. There appeared to be an enhancement of the biological potentialities rather than a degeneration.

Caldecott (1961) and Caldecott and North (1961) have recorded an increase in chlorophyll mutations induced in hexaploid oats each generation through 6 successive generations of re-irradiation. They also observed greater variability for plant height, panicle type, and maturity date following the second irradiated generation. Yamaguchi (1962), using dry

seeds of barley and rice, observed an increase in germination and fertility of the X-2 generation over the X-1 following recurrent radiation. This method also increased the frequency and broadened the mutation spectra.

Information concerning the effects of ionizing radiation applied to developing plant and animal embryos during more than one generation assumes particular importance for two reasons. First, in most biological organisms the developing embryo and young are much more sensitive than the mature individuals of the same species (i.e., actively dividing cells which are undergoing syntheses of new materials are the most sensitive). For example, Mericle (1960) and Mericle and Mericle (1961) recently reported that immature barley embryos developing in situ on the mother plant are some 40-50 times more radiosensitive as regards their ability to survive to sporophytic maturity than dormant embryos used in the dry seed experiments of other investigators. Russel, L. B. et al. (1960) have also noted comparable results when the embryonic young of mice were irradiated. Secondly, there is possible genetic danger to all future organisms from chronic exposure of the present population to low doses of radiation. This has recently been supported by Sparrow and Schairer (1962), who have shown that in the event of high levels of fallout radiation many of our native and cultivated plants would suffer serious damage at about the same radiation levels which would produce serious effects on mammals. Further

evidence by Sparrow (1963) and Mericle et al. (1962) shows that average dose rates of 3-5 r/day of chronic gamma radiation from Co-60 causes complete lethality of Pinus rigida, while approximately 12 r/day produces visible abnormalities in oak trees. It is of interest to note also that chronic dosages of 12 r/day result in sterility and loss of the germ line in mouse populations (Stadler and Gowen, 1962, 1963). Thus, it appears that some plants, at certain stages during their life cycle, are equally as sensitive as animals. This point is even more strongly emphasized when comparing the recent Tradescantia data of Mericle, L. W. et al. (1963) with those of mice (Miller, 1963).

Due to their ease of handling, dormant embryos in dry seeds have been the choice of investigators for most mutation induction in plants, instead of irradiating the more delicate pollen or unwieldy developing embryos in situ on the mother plant. One disadvantage in irradiating dry seeds is the small size of the mutated sector recovered. It has been shown that irradiation applied early in the development of barley embryos will yield comparable mutation frequencies among survivors, larger isomutant-carrying sectors (possession of the same induced mutation) and consequently a much larger number of isomutant X-2 plants (Mericle and Mericle, 1962). Many investigations have been reported on the radio-sensitivity responses of developing embryos of barley after the first generation (Mericle and Mericle, 1957, 1959, 1961, 1962, 1963) while recurrent irradiation in successive generations has been

carried out only on the dormant embryos of barley, oats, and rice (Abrams and Frey, 1958; Caldecott, 1961; Caldecott and North, 1961; Yamaguchi, 1962). Thus far there have been no attempts to study the effects of ionizing radiation applied recurrently to successive generations of developing plant embryos. Investigations dealing with such effects opens up a new and interesting field of study. With the above views in mind Hannchen barley was chosen as the experimental material, since previously it has been shown to be especially suitable for radiation biology research (Mericle and Mericle, 1957, 1961), and since so much cytologic and genetic work has already been done with barley (see review by Smith, 1951; Caldecott, 1956; Ehrenberg, 1955; Gustafsson, 1947; Konzak, 1957). X-rays were selected as the form of irradiation since the dosage and intensity could be regulated experimentally and because they were the most readily available source of irradiation for developing embryos. Employing a wider range of criteria than those used by previous investigators, the study reported herein was designed to study the effects on the germ line of Hannchen barley of recurrent irradiation applied to each of several successive generations at specific stages of embryonic development. Dormant embryos of dry seeds were also exposed to different dose levels once in each of several successive generations for comparison with the developing embryos of this study as well as to compare with the results of Abrams and Frey (1958), Caldecott (1961),

Caldecott and North (1961), and Yamaguchi (1962), all of whom have used dry seed. Finally to determine if Hannchen barley (a diploid variety) possesses the potentialities for induced radiation resistance or susceptibility.

BACKGROUND

Moisture Content and Radiosensitivity of Dormant Embryos

It has long been known that the radiosensitivity of dormant seeds was in some way dependent on their water content during and after irradiation. This dependency has been demonstrated by Gelin (1941), who found that about 50 percent of barley seeds soaked in water for 23 hours prior to irradiation showed fragmentation and bridging of chromosomes. He observed that with 15 percent water content the frequency of chromosomal aberrations decreased to 28 percent and with 10 percent moisture, to 13 percent. In the case of barley seeds, the differential moisture response apparently related mainly to processes associated with germination.

Wertz (1940) considered in some detail the relationship between moisture content of seeds and X-ray injury. He took measurements on growth of roots, coleoptiles, and leaves. In general, injury was directly correlated with moisture content at the time of treatment. Roots were most reduced in length and coleoptiles the least.

The sensitivity of dry barley seeds to X irradiation has been shown to increase by five- to seven-fold upon soaking them in water prior to treatment. Their sensitivity to neutrons does not increase as greatly, but it is still a

two- to three-fold increase (Ehrenberg, 1955). Changes in survival and fertility (MacKey, 1951), mutation rate (Ehrenberg et al., 1953; Stadler, 1928), and chromosomal aberrations in root-tip cells (Ehrenberg et al., 1953) have been shown to increase in sensitivity to X-rays.

Kempton and Maxwell (1941) noted that survival and mean height were greater in maize seedlings grown from seed containing 2 percent water than from those containing 8 percent at the time of X-irradiation. By contrast, Gustafsson and Simak (1958) reported that pine seeds of low moisture content (equilibrated at 0 percent relative air humidity) were more sensitive to ionizing radiation than seeds of high moisture content (equilibrated at 40-100 percent relative air humidity).

Osborne et al. (1963 a,b), in an extensive investigation, exposed the dormant embryos of 10 species of plants from 7 families to gamma ray doses from 9 to 400 kr after equilibrating at relative humidities from 10 to 85 percent. They reported that while in most cases radiosensitivity changed drastically with alterations in relative humidity, the relative humidity exhibited very little influences on seed radiosensitivity of Hordeum and Festuca.

It has been noted (Caldecott, 1955) that dormant barley seed X-irradiated immediately after storage at 0 percent relative humidity apparently were more radiosensitive than seed stored over 32 or 75 percent relative humidity, while seed stored at 98 percent relative humidity were again more

sensitive to irradiation. Gustafsson and Simak (1958) also have data supporting Caldecott's results.

Caldecott (1956) stored dormant barley seed over solutions with different vapor pressures until the seed had reached weight equilibrium. The water contents of the embryos ranged from 4 to 16 percent. Following X-irradiation and germination, seedling height measurements were made at the end of 7 days. His data showed that the sensitivity of the seed decreased as the water content of the embryo increased from 4 to 8 percent. He observed that a plateau was reached at about 8 percent after which added increments of water up to about 16 percent gave little or no further increase of radiosensitivity. In general, between 7 and 20 percent moisture, barley showed an inverse relation between moisture and sensitivity to X-rays (Caldecott, 1955; Ehrenberg, 1955). This inverse relation was also found when the water content of embryos alone within the grains was determined (Caldecott, 1955).

Stimulation Versus Non-stimulation

The question of whether ionizing radiation can or cannot stimulate plant growth has been much discussed. Certainly the acceleration of seed germination and initial stages of development of agricultural crops, as well as raising their yields, would be a boon to farmers and commercial seedsmen as seed can now be irradiated at relatively low cost. Saric

(1958a, 1961) has recently reported an increase in the number of ears produced per plant on inbreds and hybrids of corn with dose levels ranging from 2,500 to 25,000 r of X-irradiation. However, all of these ears were not fertile. On the average, only one ear per plant set seed, and for almost all dose levels the ears that were produced were of lighter weight than the controls. Hence, the stimulative effect of irradiation for the production of extra ears in corn certainly does not appear profitable at this time.

Some authors give data which demonstrate unequivocally that low doses of X-rays increase germinability and dry matter production (Shull and Mitchell, 1933; Breslavets, 1946, 1960). Other investigators have reported either no effects or slight increases on rates of respiration (Mikaelsen and Halvorsen, 1953), photosynthesis (Vasil'ev and Rybalka, 1958), transpiration (Bowen and Cawse, 1960) and mineral uptake (Vasil'ev and Rybalka, 1959) following low doses of irradiation, while high doses tended to depress these rates. Many conflicting reports of stimulation on the physiological functions of seeds and plants following treatment with ionizing radiations have been reviewed by Johnson (1936), Breslavets (1946), Sax (1955), and Kuzin (1956), (see also Sparrow, Binnington and Pond, 1958). The contradictory findings cited in these reviews suggest that in the experimental materials used, certain radiosensitive, inhibitory factors are present in some cases but absent in others.

Reports are also found in the literature which reveal that X-irradiation of dry seeds (Caldecott, 1958; Curtis et al., 1958) as well as growing plants (Sparrow and Evans, 1961; Gunckel and Sparrow, 1953; and many others) result in growth inhibition. In dry seeds this growth inhibition has been shown to be closely correlated with chromosomal aberrations (Bozzini et al., 1962; Caldecott, 1955, 1961). Growth inhibition of the growing plants has been attributed in large part to an inactivation of the growth hormone (auxin) producing mechanism at the shoot apex. From work reported in 1954 and 1956 Gordon showed that ionizing radiation has a depressing effect on the enzyme system responsible for the transformation of tryptophane into the auxin, indoleacetic acid. This could, in part, explain the data obtained earlier by Skoog (1935), who found that dormant Syringa buds, i. e., those with decreased sprouting ability, could be forced to sprout with moderate doses of gamma rays. Leopold (1949) and Leopold and Thimann (1949) reported that small doses of irradiation resulted in an increased number of tillers and floral primordia, presumably by "opposing or destroying auxin production." Micke (1961) found it possible to stimulate the growth of dry sweet clover seeds by irradiation with thermal neutrons but not by X-rays. He also obtained plants that became dichotomous instead of maintaining their monopodial structure. In the discussion that followed his paper it was assumed that the bifurcated stems produced were a result of

physiological disturbances in auxin levels, but no supporting data were given.

Gunckel and Sparrow (1953) have presented data and an excellent review on the characteristic stem responses to irradiation, such as dwarfing, fasciation, and tumor-like growths. Also it has been noted that ionizing radiation can enhance the growth of various plant organs such as roots (Kuzin, 1956; Breslavets, 1960), seeds (Breslavets, 1946, 1960), bulbs, tubers, and rhizomes (Gunckel and Sparrow, 1953; Sax, 1955; Spencer, 1955), sometimes leading to earlier flowering (Kuzin, 1956; Sax, 1955) and maturation (Breslavets, 1960).

A number of investigators have demonstrated a stimulation of growth and development and have raised the yield of some agricultural crops. Single exposures of X-irradiation (750-1,000 r) on rye seed increased the subsequent generation yield by 21 percent (Kuzin, 1956), while chronic gamma irradiation of less than one-third these dose levels gave a 40 percent increased yield of buckwheat. Also prolonged gamma irradiation at a dose of 341 r for 27 days accelerated cell division in root meristems of rye by 6 percent as compared with cell division in unirradiated control cells (Breslavets, 1960). Breslavets et al. (1960) irradiated radish and carrot seedlings with 1,000 r and noted improved growth and increased yields of 11-33 percent in field trials over a three-year period. Seed of these crops given 2,000 r

to 4,000 r showed an increase germinating capacity, and the roots of the irradiated seedlings were 20 to 40 percent longer than those of the non-irradiated controls. Radish and carrot plants, grown from irradiated seed, began to ripen 5 to 6 days earlier than the controls. The production of chlorophyll was stimulated in corn plants when dry seeds were exposed to low dose levels (1,000 r to 2,000 r) of X- and gamma-irradiation. Exposures to higher levels had no such stimulating effect, and when dosages were raised above 20,000 r there was a decrease in chlorophyll content (Sidorenko, 1962).

While it is probably worthwhile to view the reports of increased yields or growth stimulations following X-irradiation of seeds with some skepticism because of inadequate controls or insufficiently controlled experiments, there are a few critical works supported by statistical analyses.

Feher (1950), in Hungary, claimed to have obtained increased yields in tobacco and cotton of as much as one-third by using radioactive fertilizers. His data, however, are in doubt since Alexander (1950), from carefully planned experiments encompassing some 20 Land-Grant Experimental Stations in the United States, showed no statistically significant effect (neither beneficial nor harmful) of radioactive material in fertilizers.

Dittrich et al. (1949) reported an increase in the growth of barley leaves, as compared with the controls,

following irradiation of the seed with electrons, but found no stimulation with X-rays, a fact substantiated later by Micke (1961). The electron-treated seed grew faster following exposure to moderate doses, but the X-rayed seed germinated more slowly than the controls in all cases. Saric (1958a, 1961) also failed to obtain increased growth or yield in inbred and hybrid corn following X-ray exposure.

Kersten et al. (1943) with a carefully controlled experiment were able to show a statistically significant increase in growth of X-rayed roots of corn seedlings. Sax (1955), in a critical study of X-radiation effects on dry seeds of various crop plants, noted some stimulation of growth, but found no significant effects on yields. In the same paper, Sax also reported that Gladiolus bulbs subjected to 4,000 r of X-rays flowered significantly earlier and suggested that higher dosages might be even more effective.

Irradiation of bulbs and corms of 16 varieties from 12 genera of cultivated monocotyledonous plants with 5,200 r of X-rays resulted in significantly earlier flowering for seven varieties the first season after treatment (Spencer, 1955). However, no significant differences in average dates of flower initiation for the second year were noted.

While investigating the biological effect of X-rays on the first stages of seedling development of various plants, Lallemand (1929) could not establish a definite inhibition or retardation of sprouting. He did find, however, that

decreases in lengths of stems, roots, and lateral roots were directly proportional to increases in dosage received by the seed.

Growth inhibition, resulting from the effects of ionizing radiation, has been well established. The extent of this inhibition, however, is dependent upon the radiation conditions, the species of plants being irradiated, the kinds of radiation, and the radiation levels involved (Beard et al., 1958; Spencer and Cabanillas, 1956; Gunckel and Sparrow, 1961; Sparrow, 1961; and Sparrow et al., 1958). For example, in perusing the literature one finds data that show that most plant species subjected to chronic gamma irradiation are killed or injured at high intensities with generally no visible effects on growth at lower exposures. However, there are exceptions. Sparrow and Christensen (1953) reported evidence of stimulating effects on the growth of Antirrhinum plants when subjected to moderate doses in the gamma-field at Brookhaven National Laboratory. Also with chronic gamma irradiation, flowering was generally retarded at the higher dose ranges but approached that of the controls as dosages were decreased (Gunckel and Sparrow, 1953). In some species there was a critical dose range, varying with the plant. In Tradescantia, for instance, at an exposure rate of between 12 and 37 r per day, the number of flower buds per inflorescence was markedly increased, but the flowers were largely sterile or highly abnormal (Gunckel et al., 1953). On the

other hand, tobacco plants growing in a radiation area of 2.5 to 670 r per day all formed flower buds at about the same time, yet those which received between 100 r to 350 r per day actually bloomed much sooner (Sparrow and Singleton, 1953).

The controversy as to whether mild doses of X-rays do or do not stimulate the growth of seeds still exists and will probably continue as long as there are radio-biologists active in this field. There is no doubt, however, that high doses of ionizing radiation are injurious to plants (Caldecott and Smith, 1948; Collins and Maxwell, 1936; Froier and Gustafsson, 1944; and Stadler, 1930). Injury may be expressed in a variety of ways, such as chimeras, reduced rate of growth, sterility, abortion, tumors, non-germination, floral abnormalities, or killing (Stadler, 1931; Gunckel and Sparrow, 1961; and Sparrow et al., 1958).

Irradiation During Embryo Development

As early as 1930 Stadler suggested that irradiation of immature embryos might be advantageous when working with certain plants, since at some stage during embryo development there should be a single cell responsible for forming all of the seeds produced by the plant. It was not until the Mericles began their work in the early fifties, however, that any effort was directed toward elucidating the radiosensitivity responses of plant embryos irradiated during the

various phases of their embryological development in situ on the mother plant. They (Mericle and Mericle, 1962) have now presented evidence confirming Stadler's suggestion.

Bergonie and Tribondeau (1906) formulated the principle that actively proliferating tissues are the most sensitive to radiation and that the radiosensitivity of a tissue varies inversely with its degree of differentiation. In accord with this concept, embryonic germinal centers and plant meristematic tissues should be the most readily damaged by ionizing radiations. This can be confirmed by comparing the data of Mericle and Mericle (1961, 1962) with that of Gaul (1961). Although the mutation rate was similar in both studies Gaul found it necessary to use 40 times more irradiation on dry seeds as did the Mericles on developing embryos. Furthermore, it is interesting to note that following the 30,000 r dose level Gaul obtained 10 to 46 percent survival to maturity but 48 percent sterility, whereas Mericle and Mericle (1961, 1962) reported 35 to 80 percent survival with less than half the sterility. Gustafsson and Simak (1958), from germination studies of spruce seed, indicated that ripe seed was 2 to 3 times more tolerant to the same irradiation dose as unripe seed. Also, in animals, the embryo is more sensitive than the fetus, and the fetus is more sensitive to irradiation than either the young or adult animal (Wilson and Karr, 1950; and Russell and Russell, 1956).

Mericle et al. (1955) found a general agreement with the

law of Bergonie and Triobondeau when the sensitivity of various stages of barley embryos to irradiation was based upon histological effects and germination ability. Later they (Mericle and Mericle, 1957) reported some interesting exceptions to the "inverse law." The zygote and two-celled stages appeared somewhat less sensitive to radiation when observed histologically, yet were very sensitive when evaluated on the basis of germination, seedling lethality, and total non-survival to X-1 maturity. After further, extensive investigations it now appears that these inverse relationships are borne out from the zygote through the mid and late proembryos and early differentiation stages (Mericle and Mericle, 1961). They have suggested that "hidden" or physiological effects sustained by these developing embryos may account for their patterns of sensitivity and are expressed in their most serious form at germination, during seedling development, and non-survival to maturity. Irradiation of embryos with doses in the range of 50-800 r applied at mid-proembryo through differentiation stages produced more effects when measured histologically than were apparent from their data on germination and seedling lethality. They concluded that embryos could function with a large number of induced abnormalities, or that they were capable of limited repair before they reached maturity.

The sensitivity of the organs developing within the embryo also demonstrated an inverse relationship between

degree of differentiation and the effect produced by radiation (Mericle and Mericle, 1957, 1961). The scutellum, shoot, and root all exhibited similar sensitivities if the embryo was irradiated prior to differentiation. If, however, the embryo was irradiated after differentiation began, the scutellum and shoot were much less sensitive than the root. This differential sensitivity has been supported and related to effects on the DNA content of these various organs (Chang, 1961; and Chang and Mericle, 1962). Since the root is last to develop it is ontogenetically younger, and this, the Mericle's assumed, apparently explained its more sensitive nature.

Eunus (1954, 1955), a student of Mericle's, investigated the general embryological development of Hordeum vulgare L. and some of the embryonic abnormalities that were induced by X-rays. The plants were given single exposures ranging from 50 to 800 r, 3 to 10 days following fertilization. Histological samples were taken 4, 8, and 15 days after treatment. In general, similar kinds of abnormalities (cells enlarged and vacuolated and small cavities formed by degeneration of cells) were produced starting with 400 r and including 800 r. The frequency of occurrence and the degree of proliferation in embryos irradiated with 800 r, however, were relatively higher than those produced by 400 r and 500 r. There were no visible effects with dosages below 300 r.

Saric (1957, 1958b, 1961), of Yugoslavia, working with

cereals, approached the problem of radiosensitivity from a different point of view than other investigators. His method was to harvest the seed as young as possible but still get germination and survival. Thereafter he harvested seed every third or fourth day until he had what he termed 10 stages of developing embryos with moisture contents ranging from 80 percent to 20 percent. Since previous workers (Gelin, 1941; Caldecott, 1956; and others) had demonstrated that moisture content of the dormant embryos (at the time of irradiation) was a factor in their response, Saric eliminated this variable by drying seeds of the various developmental stages to the same moisture content. In order to insure further uniformity of the barley seed lot Saric followed the same technique used earlier by Chang (1957), that of discarding the four grains at the top and bottom of each spike, having the remaining grains for all practical purposes in the same stage of embryonic development. X-ray dosages of 15,000 r; 20,000 r; and 25,000 r were applied as a single dose to all seed lots. From his results he concluded that ontogenetically younger seeds are more radiosensitive than older ones, a fact also substantiated by Gustafsson and Simak (1958). From Saric's data and from his photomicrographs it is estimated that the earliest embryo stage he irradiated corresponded to a late stage 5 (a differentiating embryo, late in development) of the stages mentioned by Mericle and Mericle (1957).

Rabideau (1954) investigated chlorophyll abnormalities

in bean plants which were irradiated during embryo development, and Reinholz (1954) studied the changes in cotyledon number following irradiation of developing Arabidopsis thaliana embryos. Neither work, however, correlated the changes produced with the exact stages of embryogeny irradiated as did the work of Mericle and Mericle (1957, 1961, 1962). In later studies carried out on Arabidopsis, Reinholz (1959) applied a single irradiation dose of 5,000 r to the inflorescences. This dose caused death or developmental anomalies of the embryos enclosed in the pods. She observed that organ formation could be influenced throughout the entire developmental period between fertilization and differentiation of tissues, and also that sensitivity to irradiation was different at different developmental stages.

In an attempt to obtain a more fundamental understanding of some of the biochemical changes that take place following irradiation, Chang (1961) and Chang and Mericle (1962, 1963) analyzed the nucleic acid changes which occurred in irradiated barley embryos. Following a single acute dose of 450 r X-rays applied to barley embryos at specific stages during their development, the amount of DNA and RNA present was analyzed at subsequent stages. The rate of DNA reduction during the first 24 hours following irradiation and its residual depression at embryonic maturity were related to the embryonic stage irradiated. The youngest embryos showed a more immediate reduction after irradiation, while the oldest

embryos underwent the least recovery. Although RNA content was also markedly affected, the direction and magnitude of effects appeared to be independent of embryonic stage. The amount of DNA depression in the scutellum, shoot and root regions also reflected the sensitivity of these organs to radiation. Irradiation of the embryo before differentiation caused little difference in the DNA content of these organs when measured in the mature embryo. Irradiation after differentiation, however, produced a greater depression of DNA in the root than in the shoot or scutellum. This agrees with earlier sensitivity studies of Mericle and Mericle (1957, 1961) based on histological examination. Again the depression of RNA due to irradiation appeared to be unrelated to the degree of differentiation. DNA content of the embryo was reduced by the radiation more than was RNA during the early stages of development, while in the mature embryo RNA was more affected. Analyses of individual base content and molar base ratios in DNA and RNA following irradiation showed uracil and thymine to be the most sensitive.

Other biochemical changes induced by irradiation of developing barley embryos have been noted by Mericle and Mericle (1959) who found that a single acute dose of 400 r X-rays applied during embryonic development resulted in a decrease in the total amino acid content at embryonic maturity. The ratios of various amino acids were also changed. Glutamic acid and sometimes tyrosine attained levels above the controls,

while proline, threonine, valine, and cystine/cysteine were sometimes depressed to the point of non-detection. No stage or radiation specificity was observed for any of the amino acids investigated.

Nybom et al. (1956) have attempted to determine if barley had any special mutation sensitive period (i.e., a time at which mutants could be induced and recovered in the greatest number) during its vegetative and floral development. Using chronic gamma irradiation, they divided the irradiation exposure into what they referred to as part-periods: (a) germination to heading; (b) flowering to maturity; (c) heading to maturity; and (d) germination to maturity. At the highest dose that permitted survival of the plants, complete sterility occurred. Irradiation applied during the early embryo formation also resulted in complete sterility. With the lowest exposures used the genetic effects of chronic irradiation were truly additive, both with regard to chromosomal re-arrangements leading to sterility in the second generation as well as to recessive mutations appearing in the third generation.

Later Hermelin (1959) used Co-60 to apply acute exposures to barley in different phases of ontogeny. These were: (a) premeiotically; (b) during meiosis; and (c) after meiosis, to the beginning of endosperm formation. An increased sensitivity was displayed at meiosis with doses of less than 1,000 r. In a more comprehensive experiment, Kozhushko (1961)

gave chronic gamma irradiation to two varieties of wheat and two varieties of barley during 4 phases of their development: (a) from sprouting until the beginning of the formation of reproductive tissue; (b) from the formation of the reproductive primordia to flowering; (c) the week following the beginning of flowering; and (d) from the beginning of flowering to seed maturation. He reported that irradiation of plants in phase "a" led to suppression of vertical growth and a decrease in the length and number of spikes. Irradiation in all phases was accompanied by a decrease in the fertility of the plants as compared with controls, the highest sterility occurring in phases "a" and "b." All plants in the first half of the vegetative period (i.e., from sprouting to the beginning of flowering) showed increased sensitivity to gamma-irradiation.

In plants, Sax et al. (1955) showed that fractionation of X-ray doses decreased production of "two-hit" chromosome and chromatid aberrations, if there was sufficient time interval between treatments to permit restitution of breaks induced by the first exposure before any new breaks were induced by the second exposure. More recently Sax (1961) showed that if the time interval between treatments were relatively long, the first dose (given during the resting stage) increased the sensitivity of the chromosomes to the second dose (given at prophase).

Nybom (1956) fractionated into 12-hour periods the gamma irradiation given to flax, barley, and beans. He found no

significant change in the tolerance of the plants, nor was there any apparent difference between irradiations carried out during the day as contrasted with those given at night.

Induced Radiation Resistance Versus Radiation Susceptibility

In any investigation dealing with the sensitivity of a biological organism to the action of ionizing radiation, the questions of individual radiosensitivity, capacity for repair following radiation damage, and the possibility of adaptation to radiation are of paramount importance. In an attempt to answer these questions, Mewissen et al. (1959, 1960) collected seeds of a perennial grass Andropogon filifolius growing in an uraniferous soil (in which radioactivity of the superficial layers ranged from 10 to 50 times background) and from non-uraniferous, but otherwise similarly mineralized, soils. Following a single acute radiation exposure seed originating from the uraniferous soil exhibited a higher percentage of germination and had a greater average length of stems and roots. Kiselev et al. (1961) reported that micro-organisms isolated from radio-active waters were 3 to 10 times as radio-resistant as similar micro-organisms inhabitating non-radioactive water basins. After 2,000 to 4,000 years of background irradiation to the ancestral germ line. The primordia of Sequoia gigantea still produced seeds which were 70 percent viable (Tahmisian and Brar, 1960). Since both the male and female primordia are present in each tree

the germ plasm of both of the pre-zygotic gametes have been subjected to cumulative irradiation. Calculations of cumulative dose indicated that the germ line of the primordia had received 115 roentgens per thousand years, whereas the roots had received 150 roentgens.

McCormick and Platt (1962) studied spring and summer annuals exposed to gamma-irradiation with a total cumulative dose of 8,000 r to 130,000 r over a three year period and found both immediate and long-range effects upon individual species and community attributes. Certain post-irradiation changes in the spring flora were interpreted as "stimulation effects" at radiation doses of 8,000 to 30,000 r. Following irradiation of the parental generation at these levels, the first filial generation of Arenaria brevifolia was observed to increase in density, distribution, and growth at the expense of a competitive species, Diamorpha cymosa. These "stimulatory" effects, apparent only in the first generation, made it possible for this species to continue as the dominant species in the succeeding two generations observed. Other changes in the spring floral were brought about through lethal and inhibitory effects of radiation upon plant growth. It was concluded further that changes in the summer flora resulted from a selective elimination of radiation-sensitive species with a concomitant positive selection pressure toward radiation-resistant species. Under all environmental conditions studied, species which become dominant following the

irradiation period consistently exhibited greater physiological tolerances to radiation than did the normally dominant species which they replaced.

Lüning and Jonsson (1958) X-irradiated two stocks of Muller-5 fruit flies, Drosophila melanogaster (one of which had received 5 r/hr. for 6 years, the other with no known radiation history) and considered the possibility of mutational adaptation due to an incorporation into the population of mutational isoalleles with lower mutability than the alleles originally present. Their results indicated no adaptation due to mutational isoalleles.

Several workers have dealt with the problem of induced radio-resistance of an organism as a result of its being previously irradiated. These workers have applied either: (a) a single preliminary sublethal dose of irradiation to the organism; or (b) a series of fractionated doses, each followed by a subsequent lethal dose. An exposure of 10 r X-rays to the mouse fetuses at 15.5 days post-fertilization increased considerably their survival to LD-50 in 30 days when exposed as adults (Rugh and Wolff, 1957). Higher "pre-conditioning" exposures (25 r to 300 r) to the fetuses at any stage were detrimental when the mice were exposed to X-rays in later life (Rugh and Wolff, 1957). Luchnik and Kulinkova (1956) employed sublethal dose levels in the range of 15 to 200 r, followed 10 days later by an exposure of 700 r X-rays. Control mice with no previous "pre-conditioning"

dose showed 92 percent mortality, while the pre-conditioned mice exhibited a mortality range of only 37 percent to 83 percent. Another group of Russian scientists (Nuzhdin et al., 1960) used preliminary irradiation exposures of 15 to 400 r followed by a second exposure to 600 r 7, 14, 28, or 42 days later. "Pre-conditioning" dose levels of 200 r and below increased survival by 25 percent, while above this exposure the protection was insignificant.

Cronkite et al. (1950) used a "pre-conditioning dose" of 144 r whole-body radiation each week for a total of three weeks. Thirty days after the last exposure, 703 r was given to previously treated mice and controls. The control mice showed a 41 percent mortality at the end of 28 days, while the experimental group exhibited only 26 percent mortality. According to Bloom (1950) 10 or more exposures of 60 r per day induced limited resistance in mice to a subsequent acute exposure of 200 r X-rays as indicated by negligible debris and persistent mitotic activity in the crypt epithelial cells of the duodenum. This resistance was not evident, however, when the subsequent exposure was raised to 600 to 1,200 r. In rats, fractionated irradiation decreased the number of primordial ovarian follicles. By increasing the interval between irradiations, however, the damaging effect of irradiation decreased (Kitaeva, 1960). In contrast, fractionated doses aided in the preservation of the primordial ovarian follicles, and the fertility of mice subjected to a

fractionated dose was greater than when a single dose was given, particularly 6 months after irradiation (Kitaeva, 1960).

Recurrent Irradiation

Many papers have been published dealing with the influence of ionizing radiation on cytological, genetic, morphological, and physiological processes in plants and animals. These studies, however, with few exceptions, have been carried through only one generation of treatment. In many cases, data have been collected from only short-term experiments in which the investigators did not allow the plants or animals to grow to maturity, hence they were not able to judge how irradiation affected the entire life cycle of the organism. Information concerning the effects of recurrent irradiation through successive generations is not completely lacking, however, as evidenced by a few papers which have appeared in the literature in recent years.

Cells that are cytologically deficient in their chromosome complement may often be incapable of competing with more normal cells during development. Thus, the net result is that many detrimental effects such as reciprocal interchanges and deletions disappear within one generation. However, it is conceivable that certain chromosomal disturbances (near breaks or deletions of one or more alleles in polyploids) could persist but remain hidden. If this be the case, it is

entirely possible that with repeated irradiation treatment through successive generations these chromosomal disturbances could be cumulative. In an attempt to test this hypothesis, Abrams and Frey (1958) studied the effects of recurrent X-radiation in one, two, or three successive seed generations on germination and seedling vigor in oats. Under both greenhouse and field conditions they noted that germination percentage and the number of surviving seedlings decreased but found an increase in vigor of those surviving. On the basis of these analyses they postulated that genetic burdens of the following types had accumulated within the germ line: (a) weakened intrachromosomal linkages making the chromosomes more subject to actual breakage resulting in a greater number of aberrations after repeated radiations; and (b) an accumulation of chromosomal disturbances at homeologous locations in the different genomes. Their second postulation, however, would only apply to polyploids.

Caldecott (1961), Caldecott and North (1961) and Yamaguchi (1962) have indicated that further research should be conducted in developing techniques for an induction of sizeable mutation effects on various characters and for a selection of desirable mutations from a number of induced mutant lines. Such techniques, according to these workers, should greatly aid the geneticists and plant breeders in screening for agronomically useful mutants. They have suggested administering irradiation to a sequence of

generations, i.e., "recurrent" irradiation to successive generations as one method by which the mutation frequency may be increased. Yamaguchi (1962) using this method with dormant seeds of barley and rice reported that fertility of the populations was not reduced, rather, a striking improvement in seed fertility appeared for the X-2 population as compared with that for the X-1. He also found an increase in the mutation frequency and a broadening of the mutation spectra.

Caldecott and North (1961) chose to select for re-irradiation only seed from those spikes or panicles which did not exhibit gross chromosomal anomalies. They found, in the course of their investigations, that a polyploid oat apparently had been "diploidized," and that, in spite of the rigorous selection methods employed, the incidence of chlorophyll mutations had increased with each generation through a total of six successive generations. They also gave supporting data on the incidence of chlorophyll mutations in wheat following irradiation for two successive seed generations.

Employing dry seed of barley Gaul (1961) has investigated the size of mutated sectors. By following the mutation frequency in X-1 spikes he found that the average size of mutated sectors increases with increasing dosages. The increased dose level also increased the amount of sterility. According to Mericle and Mericle (1962) irradiation of barley

proembryos developing within the ovaries of young spikes will permit recovery of mutant-carrying sectors 2 to 20 times larger and with a frequency and fertility equal to or greater than those usually found following dry seed irradiation. As a consequence, the size of their isomutant (possession of the same induced mutation) test populations increased 5- to 25-fold. The size and frequency of isomutant populations which could conceivably be obtained by applying recurrent irradiation techniques to proembryos makes for interesting speculation.

Stadler and Gowen (1961, 1962, 1963) exposed several strains of mice to eight different dosages of Co-60 irradiation over periods averaging 22 hours a day throughout their lifetimes, and to their descendants in each succeeding generation. Dosages of 1-2 r per day allowed sufficient reproduction to continue the germ lines through 11 generations, while dosages of 12 r day resulted in sterility and loss of the germ line. They have found that a long term continuous irradiation of the germ plasm allowed phenotypically vigorous mice to be produced within the highly inbred strains. No mutations were observed during the course of their investigation.

Brown et al. (1963) reported that the exposure of six successive litters of female albino rats with continuous gamma-radiation (2, 5, and 10 r/day) affected neither fertility nor reproductive capacity. However, radiation

at the 20 r/day level did decrease fertility and the total number of litters that a female could produce. They also noted that offspring from the third litter borne by mothers continuously in the radiation chamber were fertile in those groups which received doses of 2 and 5 r of gamma-radiation, while the corresponding animals receiving daily doses of 10 and 20 r from the time of conception were sterile when inbred or when bred to known fertile unirradiated males. Their data substaniated the observations of Stadler and Gowen (1961, 1962, 1963), in that continuous irradiation up to 2 r/day did not impair reproduction sufficiently to prevent continuation of the germ line.

In a critical experiment dealing with recurrent radiations Spalding et al. (1961) concluded that 200 rads of X-radiation delivered to male mice for five consecutive generations produced sufficient genetic damage to affect significantly the breeding characteristics of the offspring. In other studies, Spalding and Strang (1961) have found that the offspring of ten generations of irradiated male mice were approximately 24 percent less resistant to protracted gamma-ray stress than were the controls. On the basis of all of their studies they have postulated that heritable genetic burdens (i.e., near chromosomal breaks or other sublethal mutations) accumulate in the germ line.

For comparison purposes it is interesting to note that average dose rates of 3 to 5 r/day of chronic gamma-irradiation

from Co-60 resulted in lethality of entire trees of Pinus rigida (Sparrow, 1963), while approximately 12 r/day produced visible abnormalities in oak trees (Mericle et al., 1962). Embryo abortion, decreased survival, and a reduction in height of oak seedlings were observed following 6 to 12 r/day chronic gamma-radiation (Mergen and Stairs, 1962). Thus, it appears that some plants are no less sensitive to radiation than are animals. This point is even more strongly emphasized when comparing the recent data on Tradescantia (Mericle, L. W. et al., 1963) exposed to 0.25 mr/hour with that on mice (Miller, 1963) exposed to 0.20 mr/hour.

MATERIALS AND METHODS

Experimental Procedures

In order to investigate the effects of recurrent ionizing radiation (once in each of several successive generations) upon viability and fertility, a long inbred, highly uniform two-rowed strain of barley, Hordeum distichum L., emend. Lam. cv. Hannchen (C. I. 531) was used as the experimental material. In this two-rowed variety the lateral florets of the flowering spike remain sterile. As a result the "flat" head produced is particularly well adapted for X-irradiation of the developing embryos, since it eliminated the possibility of partial shielding by other grains; this not being true for four- or six-rowed varieties. This particular variety produces many seeds per head with embryos in relatively the same stage of development provided the four basal and the four terminal grains are discarded (Chang, 1957, 1963b). In addition, this variety has been extensively used in irradiation experiments at Michigan State University for the past 10 years, and a great deal of information is already at hand (Mericle and Mericle, 1957, 1959, 1961, 1962, 1963).

The study consisted of two parts, namely: (a) embryos irradiated with 400 r X-rays at one of four different specific stages of embryonic development, repeated at each of three

successive generations (Figure 1) and (b) dormant embryos of dry seeds X-irradiated with one of five different dosages, repeated at each of three successive generations, for comparison with developing embryos as well as to compare with earlier work on dry seeds.

Handling of dry seeds for dormant embryo irradiation

Lots of 100 dry seeds, equilibrated to 5 to 10 percent moisture content and selected for uniformity in size were used for each treatment. Seeds were stored at room temperature and prior to irradiation, moisture content of the grains was stabilized over anhydrous $CaCl_2$ for 1 to 2 weeks (Caldecott, 1956). For irradiation, all grains were oriented embryo-side down and mounted on blotters by means of double-gummed masking tape (Figure 2) in order for the embryos to receive the best uniformity of absorbed does (Watson et al., 1954). The mounted grains were sealed in petri dishes then enclosed within plastic bags (Figure 3) and shipped to Brookhaven National Laboratory for radiation treatment. Control seed lots were handled in an identical manner except for receiving irradiation. In addition, after the first generation, 50 seeds of a uniform-sized control lot, as well as 50 seeds from the previous generation which had not yet been irradiated, were always included with the present generation treatment as a double set of controls and also to provide more data for comparison.

Figure 1. Diagram illustrating application of X-irradiation to Hannchen barley embryos either during their embryonic development or during dormancy in each of three successive generations

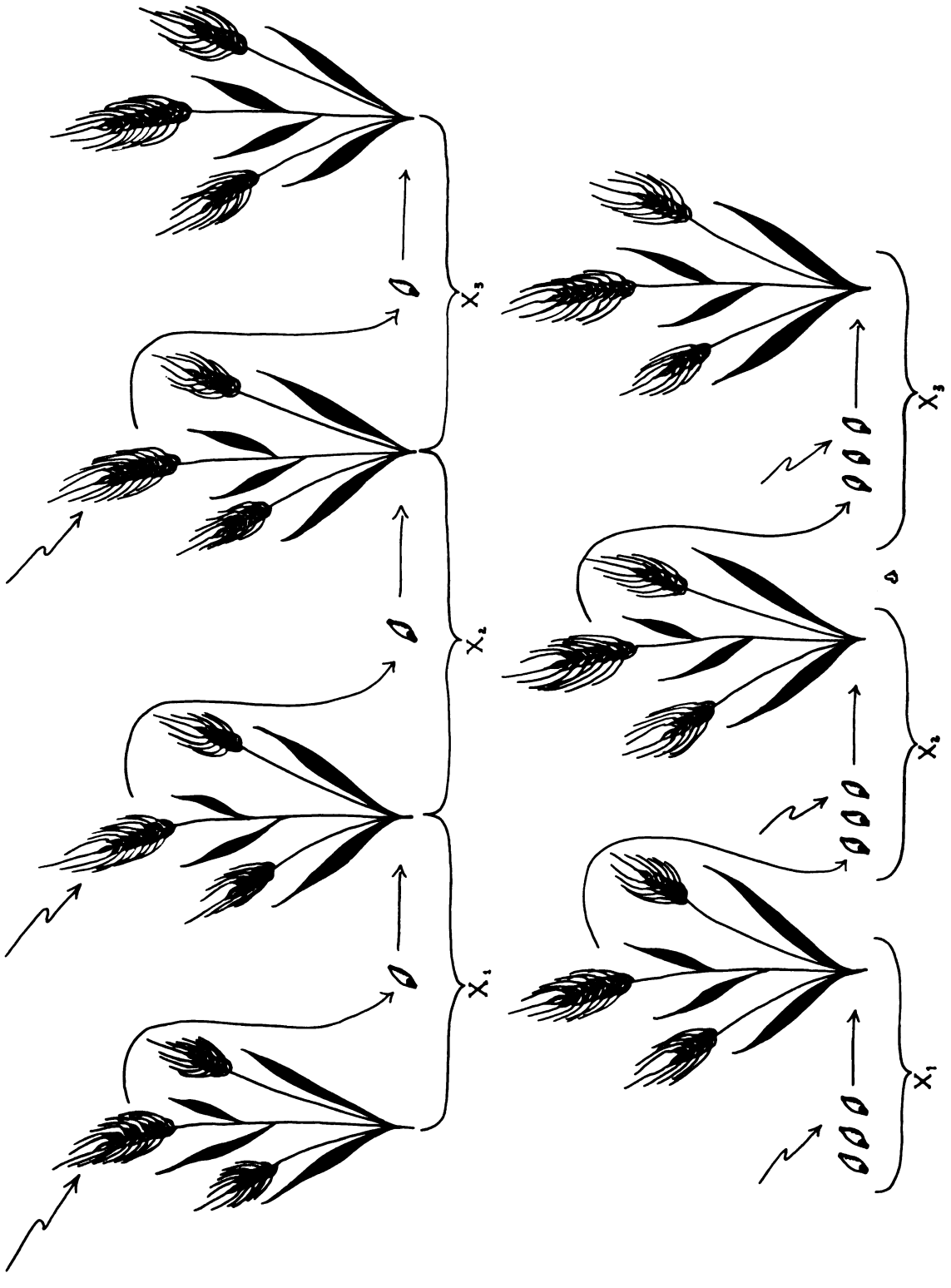

Figure 1

Figure 2. Photograph illustrating the position of Hannchen barley seed during X-irradiation. (All grains were oriented embryo-side down).

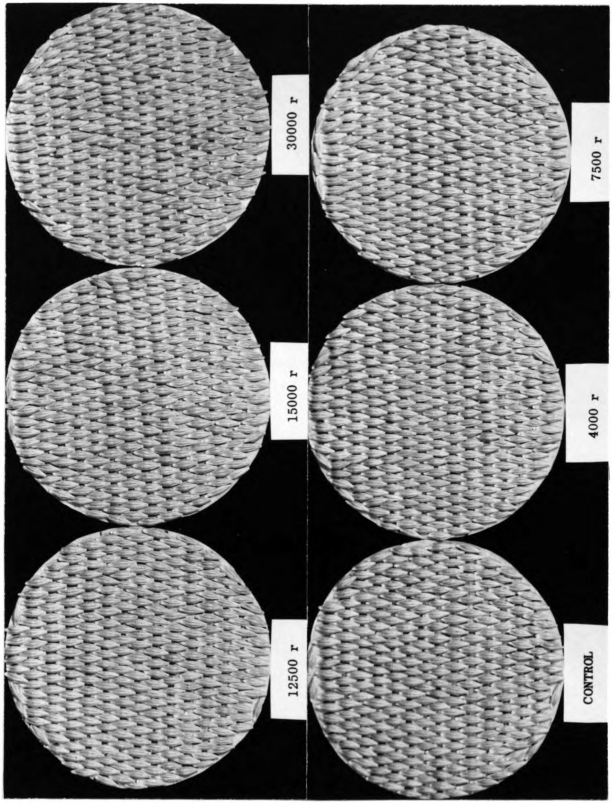

Figure 2

Figure 3. Photograph illustrating method used to maintain constant moisture of Hannchen barley seed during shipment to Brookhaven National Laboratory for X-irradiation.

Figure 3

For dry seed irradiations, a G. E. Maxitron 250 X-ray machine with a beryllium window tube was used, physical factors being 250 KVP, 30 ma, 1 mm Al filtration and a target distance of 30 cm. Exposure doses of 4,000 r; 7,500 r; 12,500 r; 15,000 r; and 30,000 r were delivered to the various seed lots at dose rates of 1,000 r per minute as measured by a Victoreen Integron.

Upon return from Brookhaven National Laboratory, the grains were germinated in replicates by rolling 10 seeds per replicate in wet paper toweling. Prior to rolling, the grains were positioned equidistant along a straight line 8 to 10 cm from the top of the toweling, with their long axes parallel and vertical. Rolls were then placed upright in glass jars with the lower ends immersed in approximately 1 inch of distilled water after which they were placed under bell jars during seed germination. At the end of 5 days, data were collected on germination lethality, semi-lethality, coleoptile height, shoot height, root length, number of roots, and any observed abnormalities.

The fifth day was selected as the best time to take the above data since earlier, preliminary work showed that seedling growth per day slowed down after 5 days. Also Mericle, R. P. et al (1963a, b) have reported that shoots and roots usually reach the edges of the toweling by the eighth day, and that the food supply stored within the endosperm tends to become a limiting factor by that time.

There is some controversy concerning the value of coleoptile measurements, since Caldecott (1955) has mentioned that, given sufficient time, the coleoptiles of treated seeds will elongate about equally well even though they may be subjected to doses of X-rays of upwards to 500,000 r. Thus, measurements of coleoptiles of developing and dormant embryos were not initiated until after preliminary data on developing embryos indicated that there might be different responses between the embryonic stages following their irradiation.

Shoot heights were measured as one index of radiation damage, since Caldecott (1955, 1961) and Bozzini et al. (1962) showed that a good correlation existed between the degree of seedling injury, as measured by seedling height and the frequency of chromosomal aberrations in the shoot tips. The longest roots were measured as a matter of convenience since preliminary work showed that the total root length, average root length, and longest root length gave identical curves when plotted as percent of control. Measurements, in centimeters to the nearest millimeter, were made at the junction of the shoot and root.

Dry weights of shoots and roots were also taken as a measure of seedling vigor (Abrams and Frey, 1958). The shoots and roots were separated at the base of the seed.

Following the observations made at the end of 5 days, 30 seedlings per each dose level were transplanted to 6 inch

pots (5 seedlings per pot) in the greenhouse and allowed to grow to maturity. Pots of plants of each generation were placed on the greenhouse bench in a completely randomized arrangement.

Main heads were harvested when the moisture content of the grains reached approximately 10 to 20 percent. Also at this time, survival to maturity, fertility, and sterility data were taken. After harvest the kernels were removed from the spikes and placed in lots arranging them according to dose and generation. For subsequent radiation exposures, seeds of uniform size were selected from each lot. Selection of seeds for uniformity of size was carried out because there appears to be some controversy in the literature over the irradiation sensitivity of large seeds versus small ones. Some investigators (Froier and Gustafsson, 1944; Gustafsson, 1947; Mikaelson and Halvorsen, 1953) have presented evidence which shows that larger barley and wheat seeds have a greater tolerance to irradiation than smaller seeds. A similar relationship has since been demonstrated in pine seeds (Simak et al., 1961). In contrast, Gonzales and Frey (1959) have shown that larger seeds of oats are affected more than smaller ones when reduced germination and seedling vigor are used as criteria of radiation damage. When this study was more than half completed, Caldecott (1961) and Caldecott and North (1961) reported a method of selecting seed for re-irradiation which they considered especially desirable. Their method was

always to select only those heads which contained no sterile florets, since Gaul (1958) had shown that mutations and chromosomal aberrations are independently induced events, therefore, seeds from the fertile heads should carry as many mutations as seed from the semi-sterile heads.

Handling of plants for developing embryo irradiation

The G. E. Maximar 250 III therapeutic X-ray machine located in the large animal clinic of the Veterinary Medicine Building at Michigan State University was used for irradiation of developing barley embryos. Physical factors were as follows: power 200 KVP, current 15 ma, inherent filtration of 3 mm Al and HI localizer FND, 33 cm focal spot distance, and a beam size of 400 cm^2. Irradiation in each of three successive generations was given as a single acute exposure dose of 400 r delivered at a rate of 200 r per minute, as measured with a Victoreen Integron.

For irradiation, the flowering spikes (heads) were taped flatwise to cardboard orienting them in such a way that all developing embryos would be exposed as nearly as possible to the same amount of irradiation.

Thirteen stages of embryonic development in Hannchen barley have been designated by Mericle and Mericle (1957) in their irradiation studies, and two additional stages have been recognized by Chang (1957, 1963b). Of these, four representative stages (Figure 4) corresponding to stages a, g, and 2, of

Figure 4. Developmental stages of Hannchen barley embryogeny. EP, early proembryo or zygote; LP, late proembryo; ED, early differentiation; and LD, late differentiation, stages. All drawings at same magnification, ca. 50X.

Mericle and Mericle (1957) and 6c of Change (1957, 1963b) were chosen for recurrent irradiation in this study.

Initially five seeds were planted per 6 inch pot. Flowering occurred between eight and nine weeks after germination. During this time plants were observed very closely to note the time pollen was shed. Since fertilization is completed within 24 to 36 hours after pollination, the first irradiation of each generation was always carried out in the latter part of this period in order to catch the zygote stage. From the work of Chang (1957) it was possible, by a combination of measuring the size of the caryopsis and counting the number of days after pollination, to determine with a good degree of accuracy the other embryonic stages observed in this study. Final determination, however, was always made histologically. Whenever plants were thought to be in the developmental stage wanted, they were transported, along with a control, to the Veterinary Medicine Building for radiation treatment. Immediately after irradiation, samples of the developing ovaries (one from the middle of each spike irradiated) were taken for histological confirmation of developmental stage at the time of irradiation. The ovaries were killed and fixed in formalin-acetic acid alcohol (FAA) and dehydrated in an ethyl-tertiary butyl alcohol series. Following their embedding in Tissuemat, they were sectioned in a dorsi-ventral plane at 12 u and stained with safranin-fast green (Johansen, 1940). The

remaining ovaries were allowed to grow to maturity and were used for study in the subsequent generation.

On several occasions the time for radiation treatment of certain stages occurred on a weekend. This was not a serious problem in the first generation since a later stage could be irradiated a few days later. When this situation occurred in the second and third successive generations, however, these plants were lost for this sequence, and new material had to be started.

Harvesting of the main heads was carried out after the plants had fully matured and when the grains had reached a moisture content of approximately 10 to 20 percent. After setting around the lab a few weeks the seed had attained a moisture content of 5 to 10 percent (Caldecott, 1956). Seeds of this portion of the experiment were then germinated in the manner described earlier for the dormant embryos of dry seeds. Also similar data were taken as in the dry seed portion of the investigation. Twenty seedlings per embryonic stage were transplanted into 6 inch pots (5 seedlings per pot) and placed on the greenhouse bench in a completely randomized arrangement.

All barley plants, from both developing and dormant embryo portions of this study, were grown to maturity in the greenhouse where average temperatures were 75° F during the day and 60° F at night. It is particularly important to maintain the lower night temperature to avoid sterility

problems that are often encountered with higher average night temperatures. In order to hasten flowering supplemental, artifical lighting was used to extend the day length to 20 hours. Plants were grown in 6 inch pots in a soil mixture of 2 parts silt loam, 1 part sand, and 1 part manure. Supplemental nutrient solution was added every 10 days, beginning two weeks after transplanting until plants had flowered. Each pot of 5 plants received 300 ml of nutrient solution each time. The nutrient solution was made up in the following manner after Mericle (1950):

0.19 millimoles $(NH_4)_2SO_4$ per liter
0.34 millimoles KH_2PO_4 per liter
0.94 millimoles $MgSO_4 \cdot 7H_2O$ per liter
1.25 millimoles $Ca(NO_3)_2 \cdot 4H_2O$ per liter

Statistical Procedure

Data were analyzed statistically by standard analysis of variance methods and the significance of differences determined by Student's "t" test.

Bases for Criteria Used in Analyses

To facilitate a better understanding for the selection of the criteria used in this investigation a brief explanation follows. Germination of seeds is perhaps one of the easiest of criteria to observe in radiation biology studies. It is a criterion which gives a reliable measure of the immediate radiation damage done to dormant embryos of dry seeds, germinability decreasing with increasing dose level (Lamarque et al.,

1958; Saric, 1958a, b; and others). Furthermore, non-germination has been shown to increase with and parallel the increase in mitotic disturbances in pine embryos (Simak et al., 1961).

In any comparison of radiation sensitivity between developing and dormant embryos, however, the following should be kept in mind: (a) the developing embryo may have one cell, a few, or many cells which may or may not continue to divide and later to differentiate; whereas (b) the embryo within the dry seed is a fully formed and differentiated multi-cellular organism in a state of extreme desiccation and mitotic arrest. Thus, as Mericle and Mericle (1961) have pointed out, those embryos irradiated during embryonic development have the added stress of continuing cell division and eventually undergoing differentiation. Therefore, observations on certain criteria (e.g., germination) would tend to bias the comparison in favor of the dormant embryos.

Following the initial germination, seedling growth measurements in barley (i.e., measurement of the coleoptile, shoot, and root) have previously been employed as a routine procedure to determine radiation damage (Caldecott, 1955, 1961; Moutschen, 1958; Saric, 1958a, b; and others). It is known that the coleoptiles grow chiefly by cell elongation with very little cell division (Moutschen et al., 1956; Moutschen, 1958). The shoots emerge by the combined processes of cell elongation and cell division (Mikaelsen and Halvorsen,

1953; Sicard and Schwartz, 1959), while the roots grow primarily by cell division with some cell elongation (Wertz, 1940). Measurements of these structures should give some indication of relative radiation damage to these two processes in developing and dormant embryos. Dry weights of seedlings are not only a good measure of seedling vigor but also in conjunction with growth measurements they are a good indication of sensitivity between the processes of cell elongation and cell division (Evans and Sparrow, 1961). Finally, observations on germination and growth usually do not harm the seedlings, and thus, the same embryos or seedlings may be observed again at later stages in their life cycle. Such is not the case when the seedlings are sacrificed to obtain dry weight or cytological data.

From experience it has been found that this variety of barley normally has one primary root and 5 or 6 seminal roots. In dry seed most of these roots or root primordia are apparently already formed, whereas this would not be the case with developing embryos (Figure 4). Thus, observations on the number of roots that emerge should shed some light on the sensitivity of the centers for root origin.

An alternative to germination and seedling growth measurements would be to observe some criterion much later in the life cycle of the plant and thus eliminate entirely this stage of treatment variable (Mericle and Mericle, 1961). With this in mind, survival of the seedlings to maturity,

total number of florets formed per main spike and their fertility have been recorded each generation in these experiments as an adjunct to and for comparison with the germination and seedling growth data.

RESULTS

Germination

The frequency of germination was recorded at the end of five days since earlier, preliminary work showed that seedling growth per day slowed down after five days. Also Mericle, R. P. et al. (1963a, b) have reported that shoots and roots usually reach the edges of the toweling by the eighth day, and that the food supply stored within the endosperm tends to become a limiting factor by that time. Absolute values are listed in Table 1 and are plotted as percent of control at each embryonic stage (Figure 5A), dose level (Figure 5B), and generation time (Figure 5C). Many seeds which were classified as having germinated under the conditions of this study (i.e., within rolls of paper toweling) would probably not have emerged had these seeds been planted directly in the soil.

Following the first, second, and third generations of radiation the percentage of germination among embryonic stages of developing embryos ranged from 77 to 98 percent, 83 to 92 percent, 74 to 97 percent, respectively (Table 1). Further examination of these data shows that the ranges in germinability for these embryonic stages spanning the three generations were: early proembryo 74 to 83 percent, late

Table 1. Effect of X-irradiation on the percentage of germination with seeds treated either as developing or dormant embryos. (Actual values)

	X-1	X-2	X-1/2	X-2	X-1/2/3	X-2
			Developing Embryos			
Control	98	100	99	100	99	100
400r EP[a]	77**	100	83**	100	74**	98
400r LP	98	100	92*	100	97	100
400r ED	95	100	92*	100	90*	94
400r LD	94	100	92*	100	85**	100
			Dormant Embryos			
Control[b]	100	100	100	100	96	100
Control[c]	100	100	100	100	99	100
4,000 r	100	100	96	100	97	100
7,500 r	98	98	94	98	95	100
12,500 r	100	98	100	100	95	100
15,000 r	95	100	93	98	90	98
30,000 r	95	98	99	96	90	100

[a]EP, early proembryo "zygote" stage; LP, late proembryo; ED, early differentiation; and LD, late differentiation, stages.
[b]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[c]Control for 4,000 r, and 12,500 r dose levels.
* $P < 5\%$
** $P < 1\%$

Figure 5A. Influence of X-irradiation on germination, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

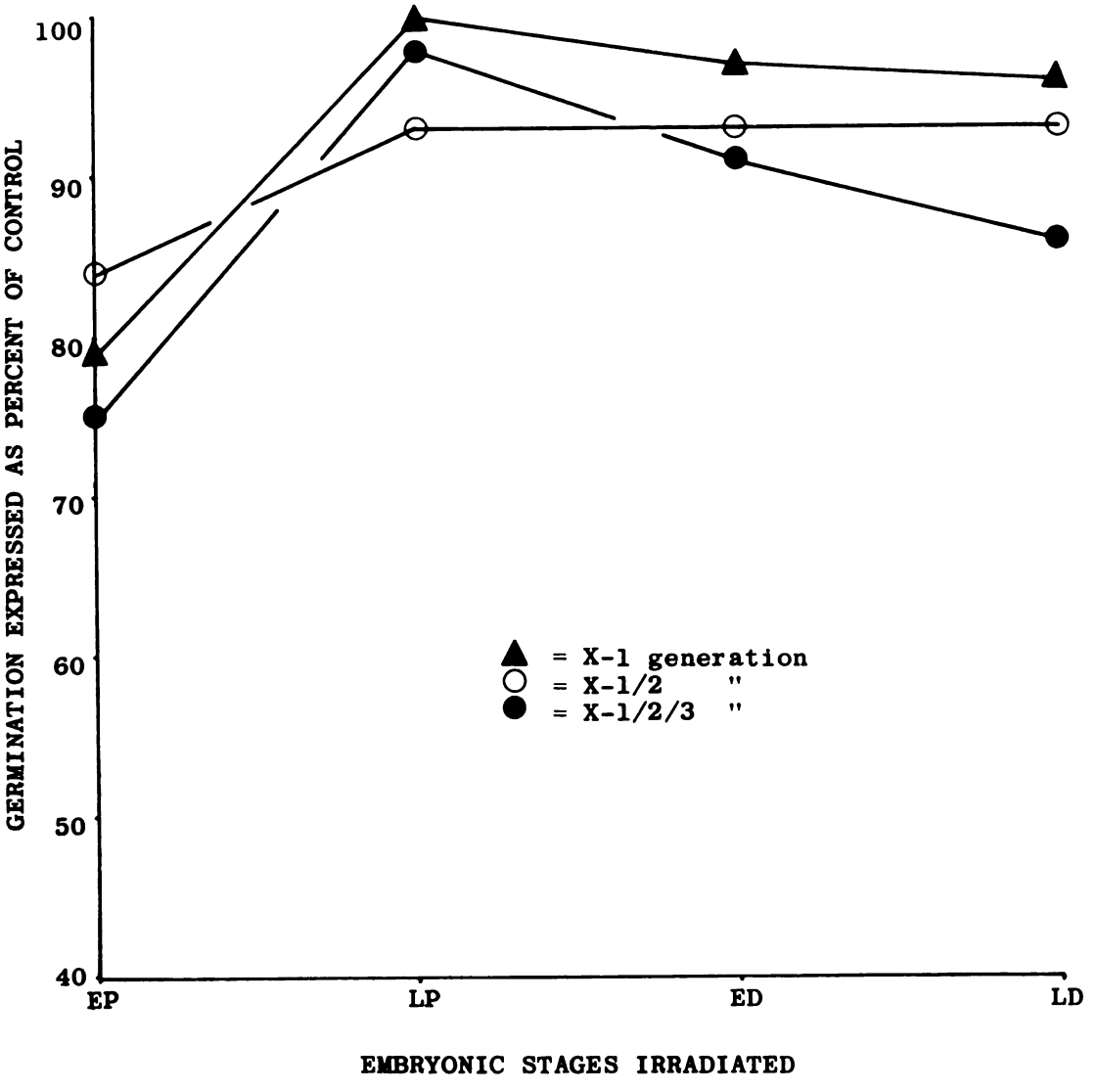
100
90
80
70
60
50
40
GERMINATION EXPRESSED AS PERCENT OF CONTROL
= X-1 generation
= X-1/2 "
= X-1/2/3 "
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 5A

Figure 5B. Influence of X-irradiation on germination, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

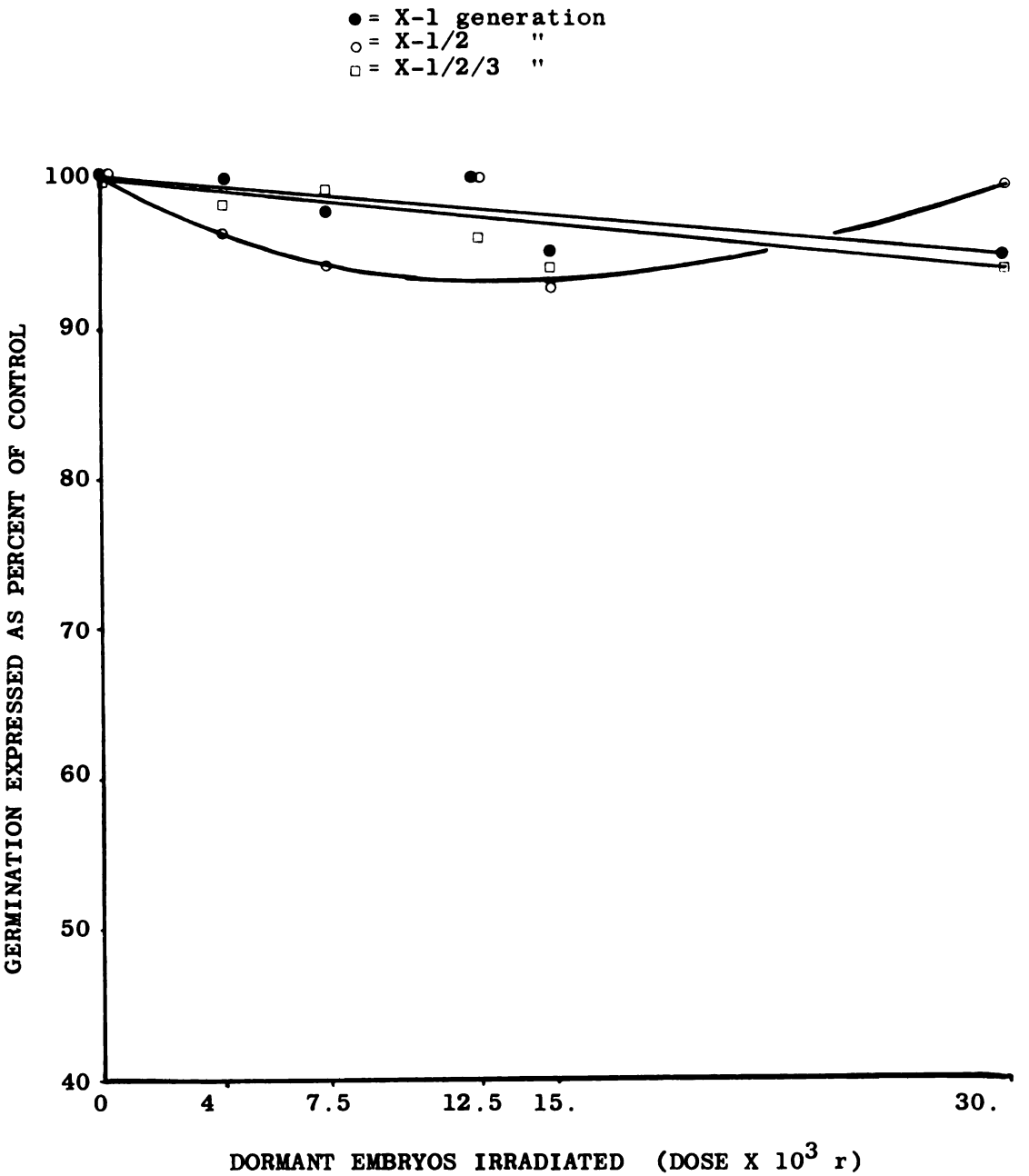
● = X-1 generation
○ = X-1/2 "
□ = X-1/2/3 "
100
90
80
70
60
50
40
GERMINATION EXPRESSED AS PERCENT OF CONTROL
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10³ r)

Figure 5B

Figure 5C. Influence of X-irradiation on germination, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

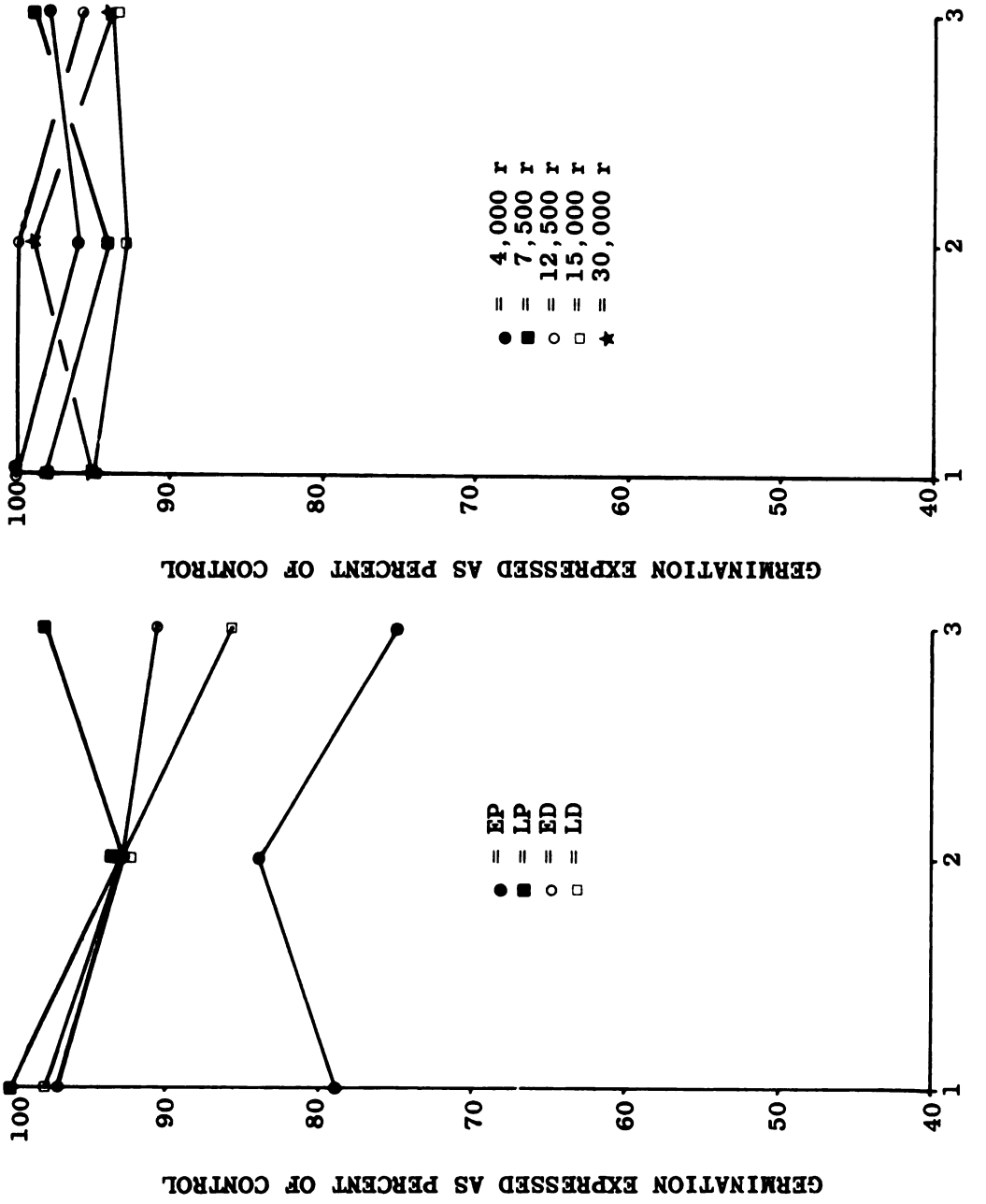
GERMINATION EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
GENERATIONS IRRADIATED
4,000 r
7,500 r
12,500 r
15,000 r
30,000 r
GERMINATION EXPRESSED AS PERCENT OF CONTROL
DEVELOPING EMBRYOS
EP
LP
ED
LD

Figure 5C

proembryo 92 to 98 percent, early differentiation 90 to 95 percent, and late differentiation 85 to 94 percent. Thus, it can be seen that the early proembryo stage is consistently more radiosensitive, as measured by germination frequency, than the older embryonic stages. This fact is more easily seen when these values are expressed as percent of control and plotted against embryonic stage (Figure 5A) and generation time (Figure 5C). In addition, the radiation sensitivity of the late differentiation stage is slightly greater than that of the late proembryo and early differentiation stages following the first and third generations of radiation. These germination data agree, in general, with the differential stage specific responses observed by Mericle and Mericle (1957, 1961, 1962, 1963), Chang (1961), and Chang and Mericle (1962, 1963). The percent germination of embryos irradiated as early proembryos in each treated generation is considerably higher in this study than that reported for this stage by Mericle and Mericle (1957, 1961), whereas the corresponding values for the late proembryo stage agree essentially with their results. These data on germination of developing embryos are much higher than those obtained earlier by Eunus (1954, 1955). Furthermore, when these germination data (expressed as percent of control) are plotted against generation time (Figure 5C) it may be seen that the early proembryo stage exhibits a slight increase in germination following the second generation of radiation followed by a decrease after the third

treated generation. In contrast, the late proembryo stage shows a decrease in germinability following the second treated generation followed by an increase after the third generation of radiation. Germinability of the early and late differentiation stages decreased following each successively treated generation. Moreover, it may be observed that following the third generation of radiation of the embryonic stages the germinability was lower than it was after the first treated generation. This observation is in agreement with the results of Abrams and Frey (1958).

Germinability at the different dose levels applied to dormant embryos following the first, second, and third generation of radiation ranged from 95 to 100 percent, 93 to 100 percent, and 90 to 97 percent, respectively (Table 1). In addition, the ranges in the percent germination at the different exposure levels were: 4,000 r, 96 to 100 percent; 7,500 r, 94 to 98 percent; 12,500 r, 95 to 100 percent; 15,000 r, 90 to 95 percent; and 30,000 r, 90 to 99 percent. Thus, it may be seen that germination was very good at all exposure levels following each generation of radiation. The percent germination of dormant embryos irradiated at the 12,500 r and 30,000 r dosages, however, was higher than that of the other treatments following the second successive generation of radiation. Otherwise, germinability decreased slightly after the first generation (Table 1, Figure 5C). This decrease in germinability of the dormant embryos agrees

with the data of Abrams and Frey (1958). However, a detailed comparison shows that the germination frequencies in these data are much higher than those obtained by Abrams and Frey (1958).

An interesting comparison may be made between the results of irradiating the early proembryo or one-celled stage and applying the different dose levels to dormant embryos. Dormant embryos sustained a total dosage of 10 to 75 times that of the early proembryo stage, yet the maximum depression in germinability was only 10 percent, whereas the one-celled stage showed a maximum depression of 25 percent over that of the controls. Generally, with the exception of the late differentiation stage following the third generation of radiation, the other embryonic stages of developing embryos exhibited germinability within the range of that from the different exposures on dormant embryos. These data agree with the germination results of Gustafsson and Simak (1958) and Saric (1958b). The germination percentages of the X-2 generations of the developing and dormant embryos treated in one, two, or three successive generations are listed in Table 1. These seeds germinated 94 to 100 percent following each radiation cycle. Thus, a maximum difference of only 6 percent is observed, with the maximum difference occurring in the third treated generation. Abrams and Frey (1958) noted a maximum difference of 4 percent when germinability of the X-2 generation was compared with controls. Their maximum difference

likewise occurred following the last generation of radiation. They (Abrams and Frey, 1958), however, noted a gradual decrease with each treated generation. Germinability in the X-2 generations of the irradiated groups of the developing embryos in this study are in agreement with those reported by Mericle and Mericle (1963). They (Mericle and Mericle, 1963) found a reduction in germination of almost 5 percent at the youngest proembryo stage as opposed to 2 percent in this study, and no reduction from the controls at the late proembryos, which agrees well with these results.

Seedling Responses

Coleoptile height

As mentioned earlier, in control plants the coleoptiles normally emerge by cell elongation with very little cell division taking place. Following irradiation, however, even this small amount of cell division may be delayed (Mikaelsen and Halvorsen, 1953) or completely inhibited (Cherry, 1962; Moutschen et al., 1956; and others). Furthermore, cell division is inhibited at much lower doses than cell elongation (Moutschen, 1958; Cherry, 1962; and others). In this study the mean coleoptile heights of all groups irradiated as developing embryos were significantly lower (1 percent level) than the controls in each generation (Tables 2 and 11). Following the first generation of radiation the induced variability of the coleoptiles increased about two- to

Table 2. Effect of X-irradiation on coleoptile heights. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations—heights measured in cm at the end of the fifth day).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	122	5.1	0.04	0.42	100	143	5.0	0.04	0.48	100	79	4.9	0.07	0.62	100
400r EP[a]	67	3.9**	0.18	1.45	76	48	4.1**	0.20	1.36	82	49	4.2**	0.18	1.28	86
400r LP	100	4.2**	0.15	1.60	82	59	4.5**	0.11	0.86	90	54	4.6**	0.07	0.50	94
400r ED	120	4.1**	0.14	1.52	80	91	4.3**	0.12	1.18	86	50	4.5**	0.12	0.87	92
400r LD	99	3.8**	0.15	1.54	75	93	4.1**	0.13	1.24	82	47	4.2**	0.27	1.82	86
Dormant Embryos															
Control	90	5.7	0.06	0.56	100	90	5.6	0.06	0.52	100	90	5.6	0.06	0.56	100
4,000 r	88	5.2**	0.11	1.07	91	88	5.4[b]	0.10	0.92	96	85	5.4[b]	0.05	0.41	96
7,500 r	88	5.1**	0.08	0.78	90	81	5.3**	0.07	0.62	95	90	5.5[b]	0.06	0.53	98
12,500 r	88	4.9**	0.07	0.66	86	89	5.2**	0.09	0.84	93	82	5.2**	0.07	0.66	93
15,000 r	88	4.9**	0.10	0.95	86	86	5.2**	0.07	0.62	93	83	5.1**	0.08	0.77	91
30,000 r	87	4.3**	0.09	0.83	75	84	4.1**	0.09	0.80	73	89	4.4**	0.04	1.07	79

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from the controls
* $P < 5\%$
** $P < 1\%$

three-fold over that of the controls (Table 2). Except for the late differentiation stage following the third generation of radiation, variability decreased at all embryonic stages with each treated generation (Table 2). Irradiation applied at the early proembryo and late differentiation stages caused the greatest maximum and minimum depressions each treated generation, while treatment at the late proembryo and early differentiation stages showed the least (Table 2, Figure 6A). This pattern is maintained following each generation of radiation and is easily seen by comparing the ranges of growth depression of the embryonic stages: early proembryo; 14 to 24 percent; late proembryo, 6 to 18 percent; early differentiation, 8 to 20 percent; and late differentiation, 14 to 25 percent. Growth retardation among the embryonic stages, however, ranged from 18 to 25 percent, 10 to 18 percent, and 6 to 14 percent following the first, second, and third generations of radiation, respectively. These data show that the minimum and maximum percentages of depression decreased with each treated generation. This is an observation that is more clearly seen when the growth retardation values (expressed as percent of control) are plotted against generation time (Figure 6C). It is of further interest to note that the differences in these ranges are fairly constant each irradiated generation. Coleoptile height curves of these irradiated stages mirror the residual DNA depression curves of Chang (1961) and Chang

Figure 6A. Influence of X-irradiation on coleoptile height, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

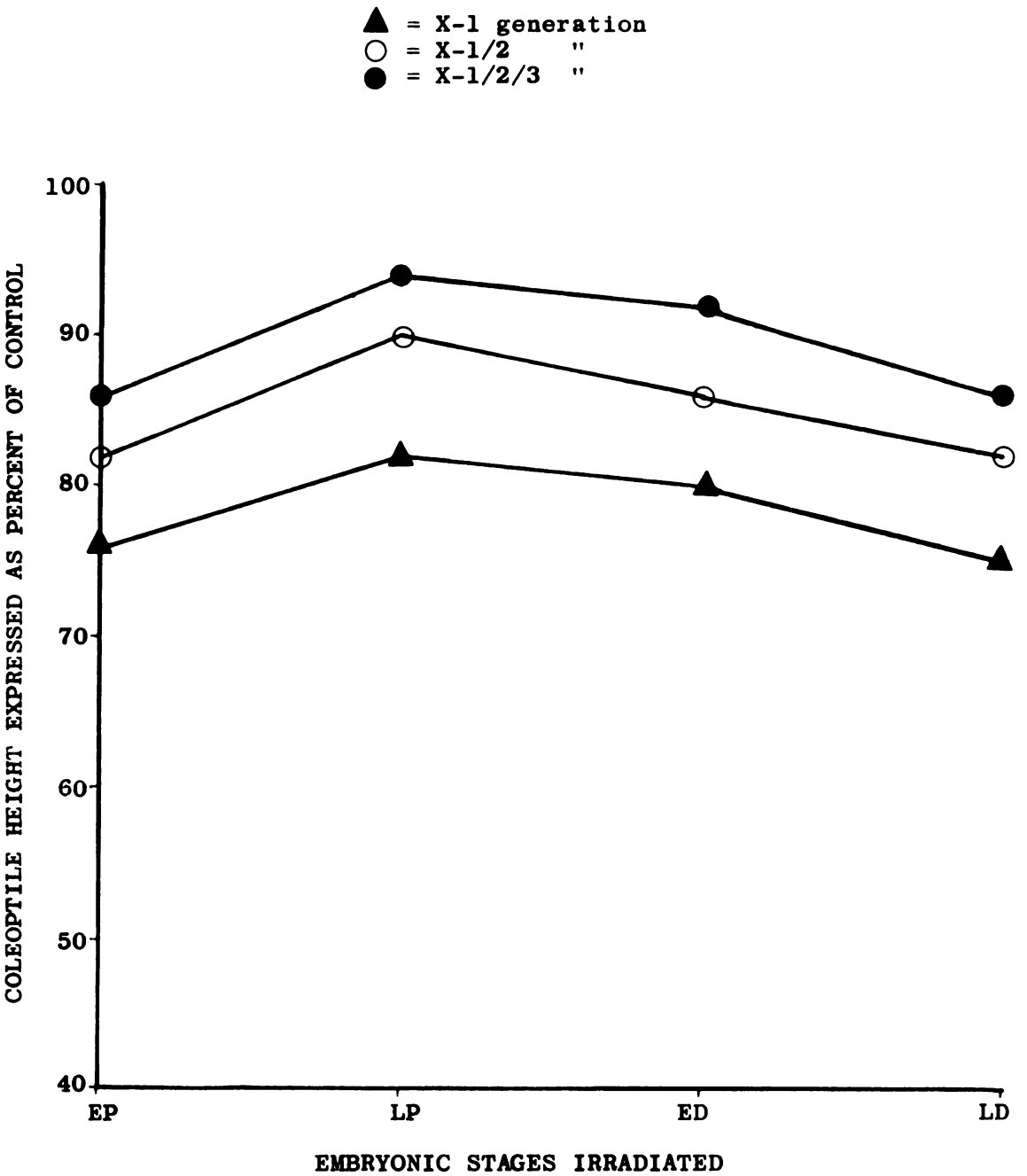
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
100
90
80
70
60
50
40
COLEOPTILE HEIGHT EXPRESSED AS PERCENT OF CONTROL
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 6A

Figure 6B. Influence of X-irradiation on coleoptile height, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

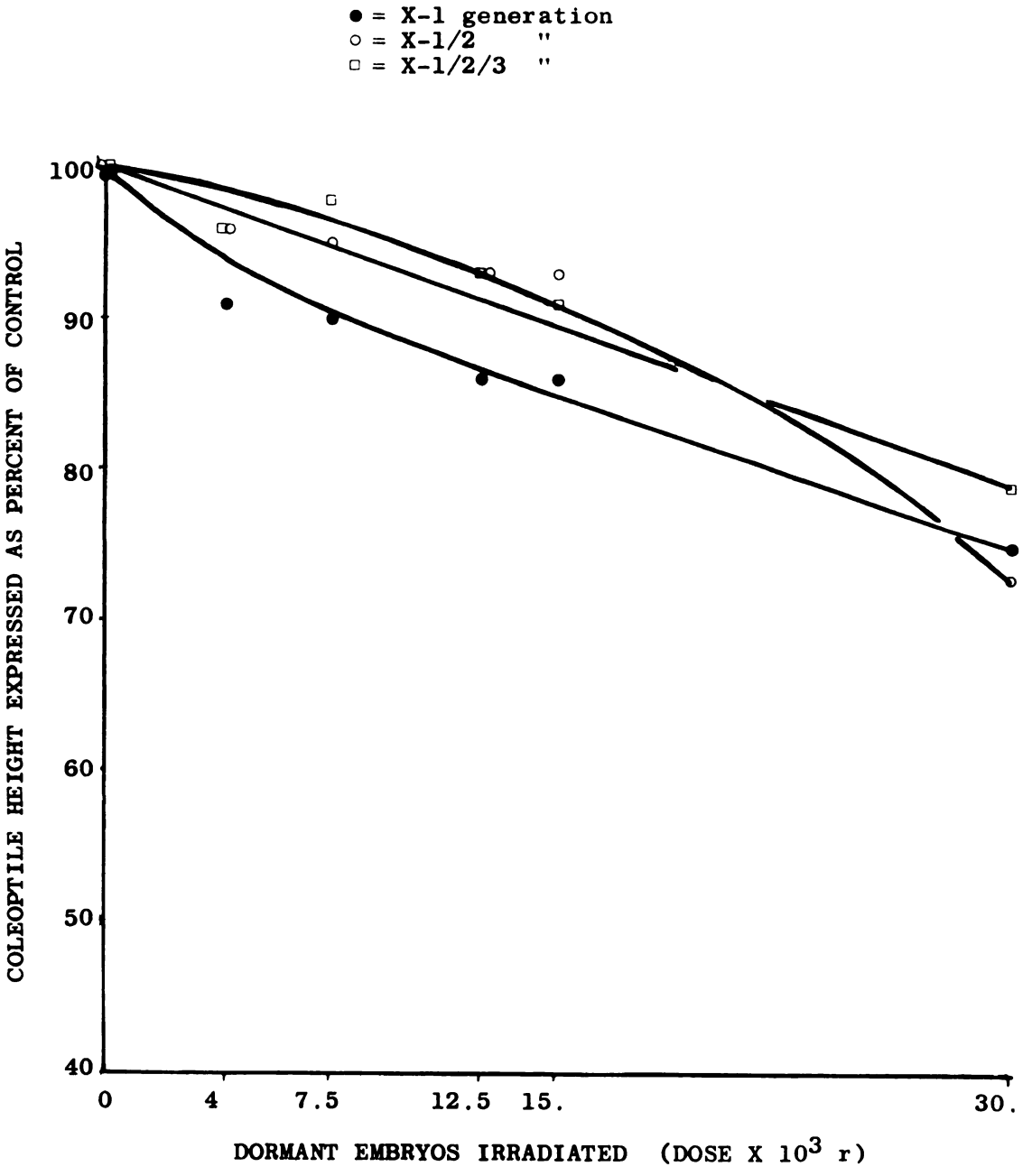
● = X-1 generation
○ = X-1/2 "
□ = X-1/2/3 "
COLEOPTILE HEIGHT EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10³ r)

Figure 6B

Figure 6C. Influence of X-irradiation on coleoptile height, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

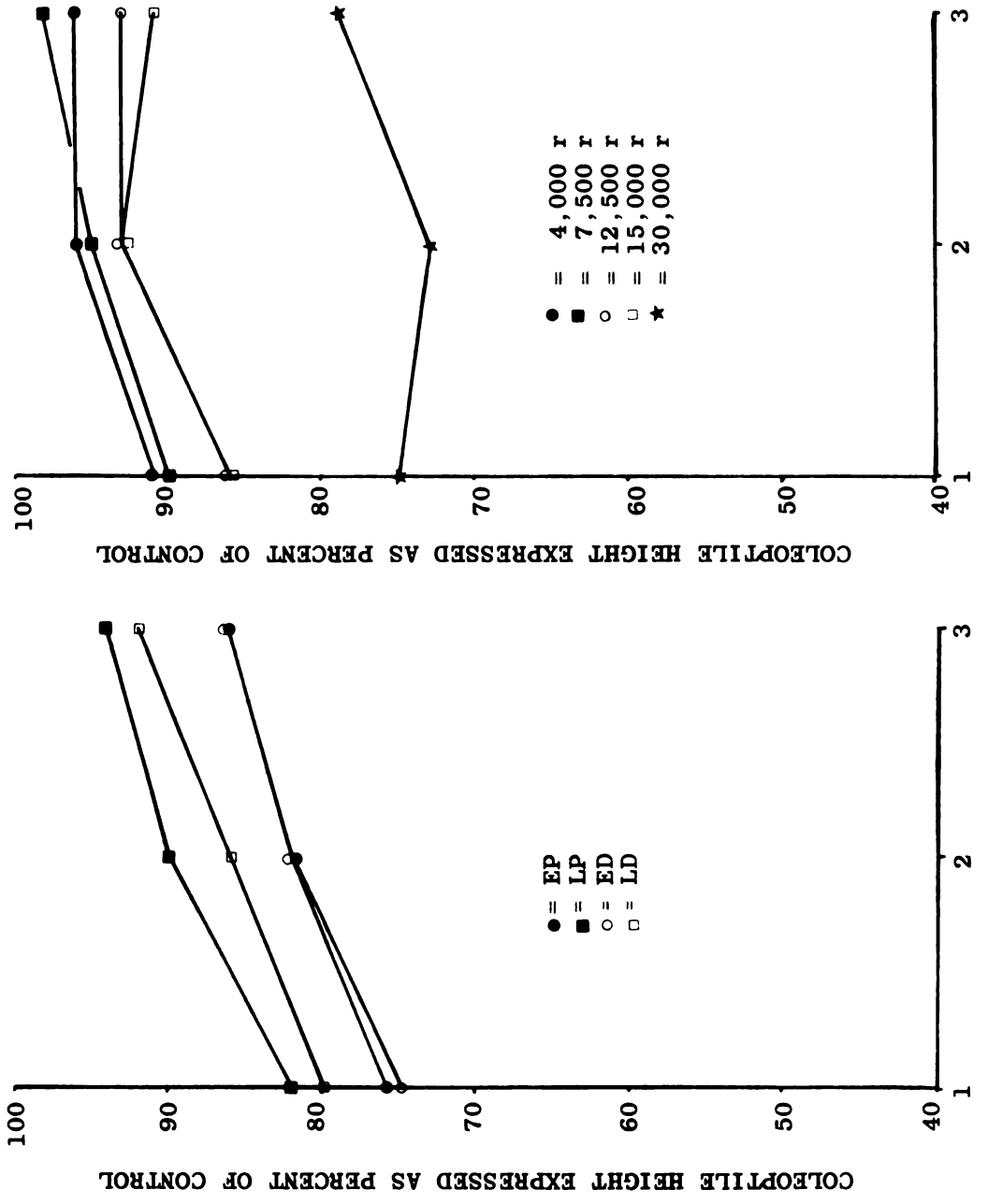
COLEOPTILE HEIGHT EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r
COLEOPTILE HEIGHT EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DEVELOPING EMBRYOS
● = EP
■ = LP
○ = ED
□ = LD
GENERATIONS IRRADIATED

Figure 6C

and Mericle (1962, 1963). The greater radiosensitivity of the early proembryo stage (measured by coleoptile inhibition) as compared with the late proembryo and early differentiation stages supports earlier data of Mericle and Mericle (1957, 1961, 1962, 1963). Although the late differentiation stage has not been previously investigated by the Mericles as extensively as the other embryonic stages, the sensitivity of this stage (based upon coleoptile inhibition) also parallels, to some extent, the P-32 incorporation data of Chang (1961, 1963a).

The mean coleoptile heights of seedlings from dormant embryos receiving different dose levels were either not significantly different from the controls or were significantly lower (1 percent level) each irradiated generation (Tables 2 and 11). Except for the 4,000 r and 7,500 r dose levels following the third generation of radiation the induced variability in coleoptile heights is slightly greater than that of the controls. In general, the average coleoptile heights of the 4,000 r and 7,500 r exposures were very close, while those of the 12,500 r and 15,000 r dose levels were, or very nearly, equal in each generation. The average coleoptile height for the 30,000 r dose level, however, was considerably lower than those of other exposures (Table 2, Figure 6C). For the first, second, and third generations of radiation, growth inhibition of coleoptiles ranged from 9 to 25 percent; 4 to 27 percent; and 2 to 21 percent,

respectively (Table 2). Growth retardation ranges at the different dose levels were: 4,000 r, 4 to 9 percent; 7,500 r, 2 to 10 percent; 12,500 r, 7 to 14 percent; 15,000 r, 7 to 14 percent; and 30,000 r, 21 to 27 percent. From this it may be seen that the 4,000 r and 7,500 r exposures exhibited the least growth depression on coleoptiles, while the 30,000 r dose level showed the greatest. When the mean coleoptile heights (expressed as percent of control) are plotted against exposure level an overall decrease is noted (Figure 6B). These results agree with those of Moutschen et al. (1956) and Moutschen (1958). When these data (expressed as percent of control) are plotted against generation time, there is, with few exceptions a slight increase in mean coleoptile height following each generation (Figure 6C). The average coleoptile height for the 30,000 r dose level exhibited a decrease after the second generation of radiation followed by an increase in the third treated generation.

The induced variability in coleoptile heights was greater following radiation applied to developing embryos than that given dormant ones (Table 2). Variability resulting from treatment of developing embryos, however, generally decreased with each irradiated generation, while that resulting from exposure of dormant embryos fluctuated considerably. There was a slight increase in the average coleoptile heights among generations at irradiated stages of developing embryos, while those of the different dose

levels given dormant embryos remained fairly uniform. It is of interest to note that the coleoptiles from embryos irradiated in early proembryo and late differentiation stages showed minimum and maximum depressions comparable to those of the dormant embryos treated with 30,000 r. Further, the minimum and maximum depressions of the late proembryo and early differentiation stages fall within the ranges of the 12,500 r and 15,000 r exposure levels. In using the coleoptile height as a criterion to measure the radiation sensitivity these data indicate that some radio-resistance has been induced by recurrent irradiation in successive generations. In this respect these data agree with earlier recurrent work on barley (Yamaguchi, 1962) and repeated irradiation exposures (consisting of daily exposure to sublethal doses for a finite number of days, followed by a lethal dose) given to one generation of animals (Bloom, 1950; Pierce, 1948; and others).

Shoot height

Irradiation at all embryonic stages of developing embryos had an inhibitory effect on seedling shoot height (Table 3). Except for the early proembryo stage following the second generation of radiation, the mean shoot heights at all irradiated stages were significantly lower (1 percent level) than the controls, each treated generation (Tables 3 and 11). With the exception of the late differentiation stage following the first generation, greater variability was induced by irradiation at the early proembryo and late differentiation stages

Table 3. Effect of X-irradiation on shoot heights. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations—heights measured in cm at the end of the fifth day).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x}$	± SE	SD	%C	N	$\bar{x}$	± SE	SD	%C	N	$\bar{x}$	± SE	SD	%C
Developing Embryos															
Control	122	11.2	0.20	2.21	100	143	10.1	0.23	2.82	100	79	11.7	0.33	2.91	100
400r EP[a]	78	6.5**	0.41	3.63	58	46	9.3[b]	0.56	3.83	92	48	8.2**	0.58	4.05	70
400r LP	104	8.4**	0.35	3.56	75	57	6.8**	0.31	2.38	67	54	9.8**	0.40	2.92	84
400r ED	118	8.4**	0.34	3.75	75	87	7.1**	0.35	3.28	70	49	9.4**	0.44	3.07	80
400r LD	91	8.1**	0.37	3.54	72	90	7.4**	0.38	3.57	73	46	9.6**	0.48	3.25	82
Dormany Embryos															
Control[c]	288	9.3	0.14	2.39	100	151	9.5	0.29	3.63	100	205	9.4	0.17	2.47	100
Control[d]	189	8.5	0.15	2.06	100	188	10.0	0.14	1.90	100	109	8.2	0.20	2.14	100
4,000 r	188	7.8**	0.16	2.22	92	172	8.8**	0.23	3.03	88	111	7.6[b]	0.22	2.39	93
7,500 r	278	7.6**	0.20	3.30	82	137	7.3**	0.28	3.32	77	205	8.8**	0.23	3.36	94
12,500 r	188	6.8**	0.25	2.58	80	175	8.3**	0.25	3.34	83	102	7.4**	0.21	2.14	90
15,000 r	262	7.5**	0.21	3.36	81	140	6.3**	0.23	2.73	66	184	8.3**	0.25	3.35	88
30,000 r	266	6.8**	0.17	2.84	73	140	4.2**	0.18	2.08	44	189	7.1**	0.21	2.91	76

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from the controls.
[c]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[d]Control for 4,000 r, and 12,500 r dose levels.
* $P < 5\%$
** $P < 1\%$

than in the other groups irradiated. In addition increasing variability was observed with each succeeding generation of radiation at the early proembryo stage, whereas the variability induced at other embryonic stages fluctuated. By using growth inhibition of the shoots as a measure of radiosensitivity, it can be seen that irradiation at the early proembryo and late differentiation stages also showed the greatest reduction in shoot height from the controls following the first generation of radiation (Table 3, Figure 7A). In the second treated generation, except for the early proembryo stage, the mean shoot height exhibited an inverse correlation with the stage of differentiation. Following the third generation of radiation, however, the average shoot heights again showed a tendency to follow the same pattern as in the first generation. Thus, when these data (expressed as percent of control) are plotted against embryonic stage (Figure 7A) it is easily seen that they parallel in part, the residual DNA depression curves observed by Chang (1961) and Chang and Mericle (1963). Following the first, second, and third generations of radiation, maximum growth inhibition among the embryonic stages ranged from 25 to 42 percent, 8 to 33 percent, and 16 to 30 percent, respectively (Table 3). It is interesting to note that the maximum depression decreased each treated generation. A more detailed examination, however, shows that the ranges in growth inhibition of the irradiated stages were: early proembryo, 8 to 24 percent;

Figure 7A. Influence of X-irradiation on shoot height, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

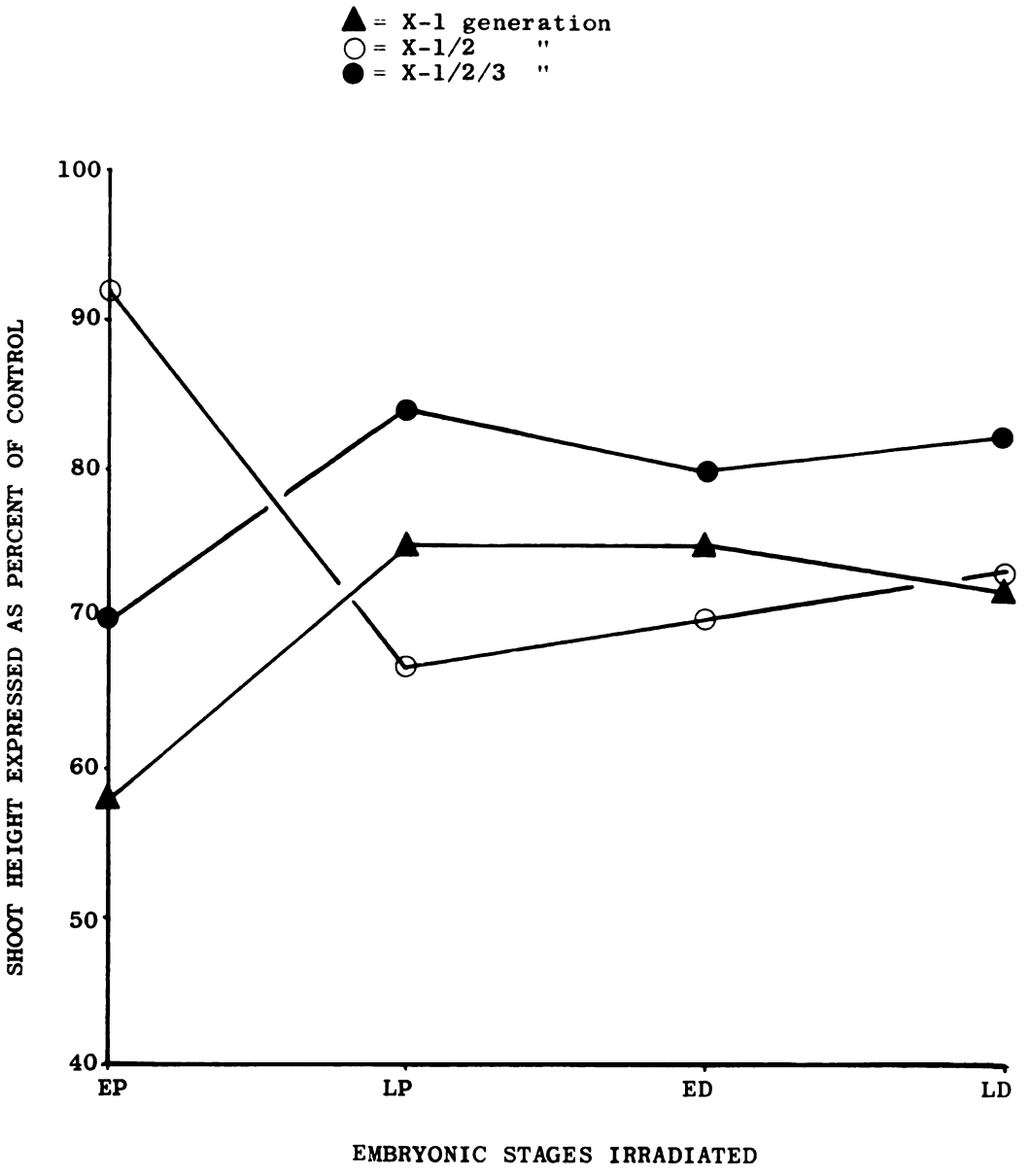
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
100
90
80
70
60
50
40
SHOOT HEIGHT EXPRESSED AS PERCENT OF CONTROL
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 7A

Figure 7B. Influence of X-irradiation on shoot height, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

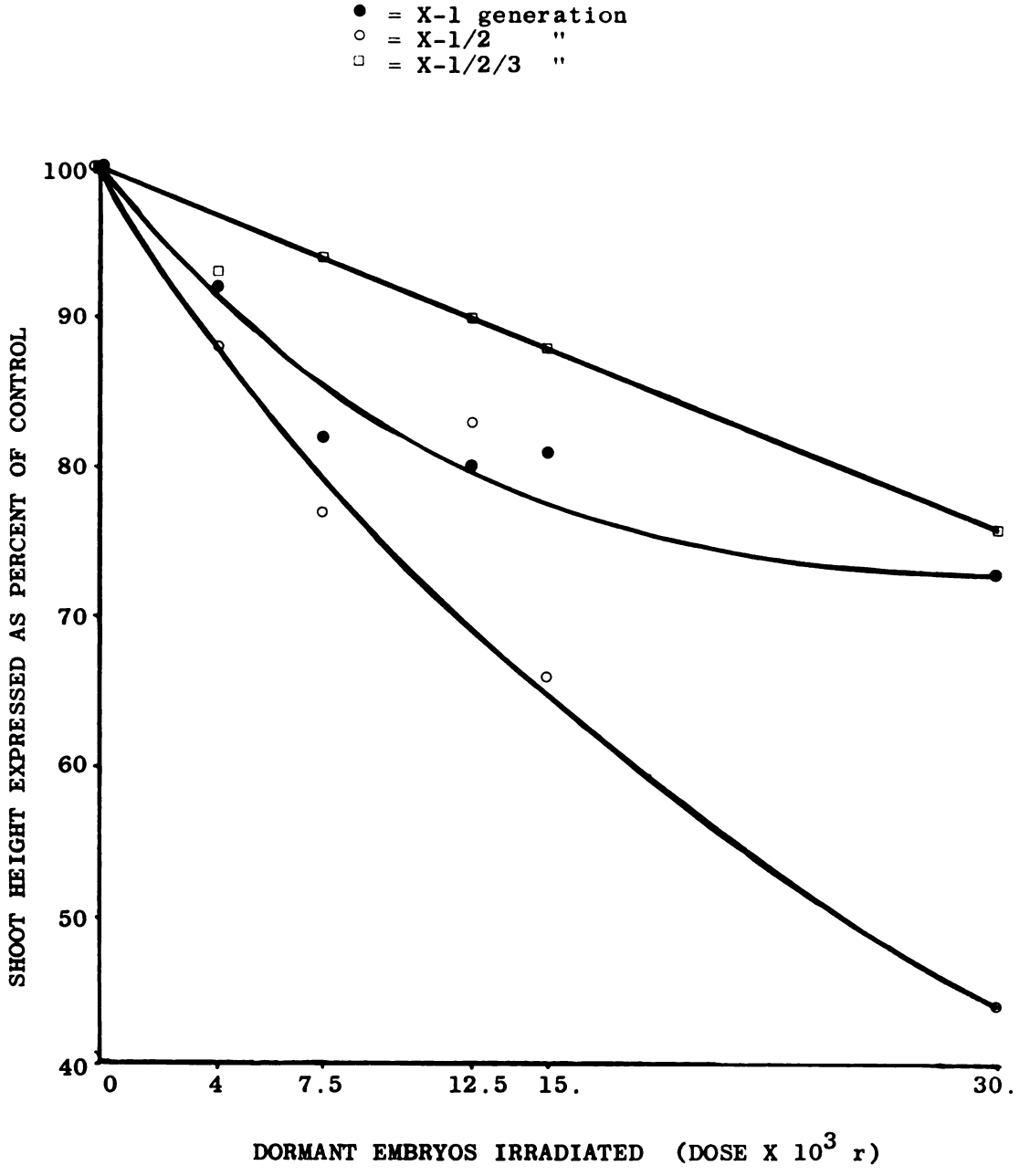
● = X-1 generation
○ = X-1/2 "
□ = X-1/2/3 "
100
90
80
70
60
50
40
SHOOT HEIGHT EXPRESSED AS PERCENT OF CONTROL
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10^3 r)

Figure 7B

Figure 7C. Influence of X-irradiation on shoot height, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

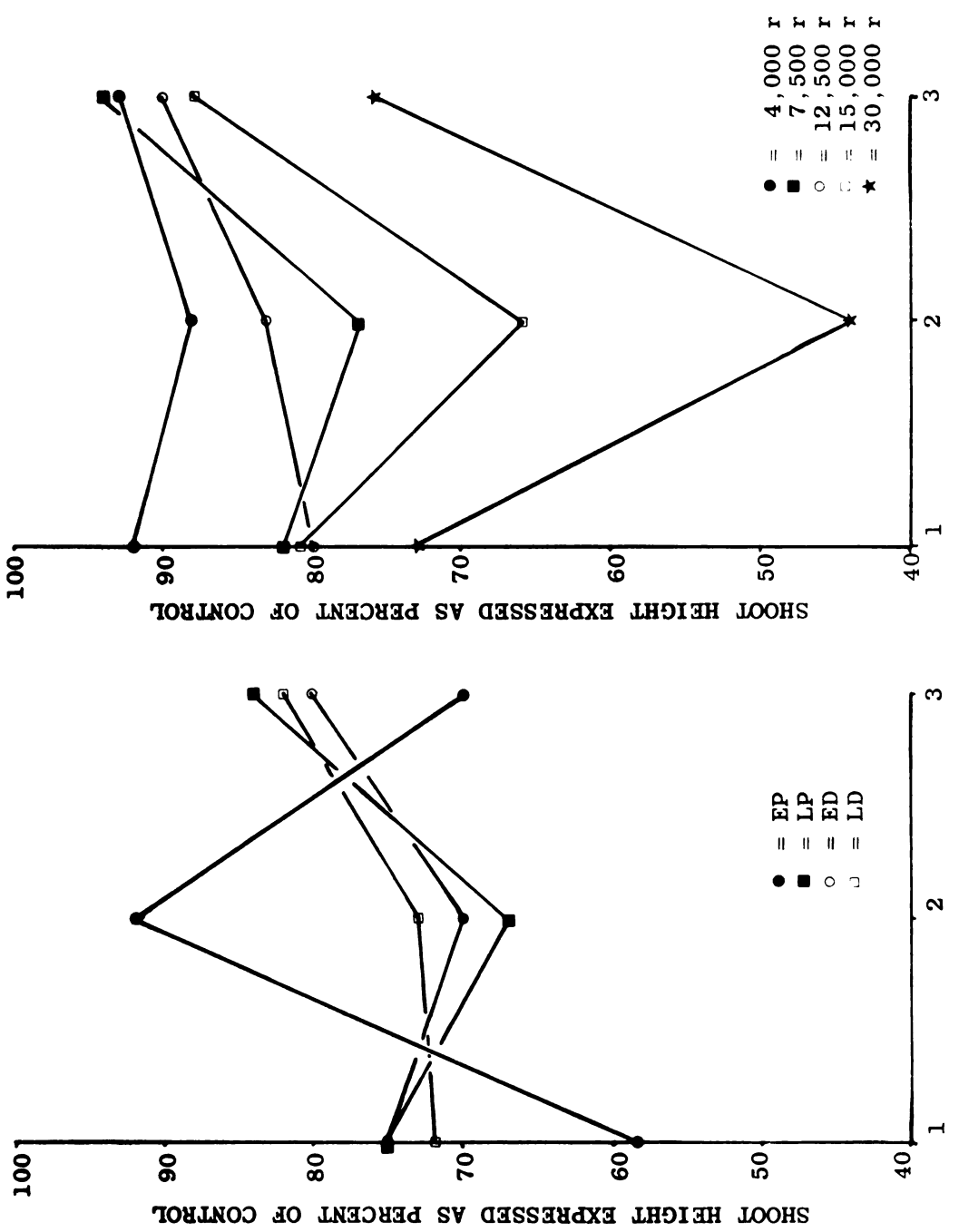
SHOOT HEIGHT EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
EP
LP
ED
LD
DEVELOPING EMBRYOS
4,000 r
7,500 r
12,500 r
15,000 r
30,000 r
DORMANT EMBRYOS
GENERATIONS IRRADIATED

Figure 7C

late proembryo, 16 to 33 percent; early differentiation, 20 to 30 percent; and late differentiation, 18 to 28 percent. When the average shoot heights (expressed as percent of control) are plotted against generation time (Figure 7C) the early proembryo stage exhibited a large increase following the second generation of radiation but decreased again after the third treated generation. In contrast 400 r of X-rays at the late proembryo and early differentiation stages caused a decrease after second generation followed by significant increase after the third successively treated generation. The late differentiation stage exhibited a slight increase each generation of radiation.

In general, the irradiation of dormant embryos at the various exposures had an inhibitory effect of seedling growth in terms of shoot height levels (Table 3). In no instances were the shoot heights greater than the controls. In this respect, these data disagree with those of Saric (1958b), Breslavets (1946, 1960), and Kuzin (1956) all of whom found an increase over controls following irradiation. From Tables 3 and 11 it may be seen that the mean shoot heights, except for the 4,000 r and 7,500 r dose levels following the third generation of irradiation, were significantly lower (1 percent level) than the controls. Induced variability at the different dose levels was, with one exception, greater than that observed for the controls (Table 3). It is of interest to note that the variability following irradiation at the 30,000 r dose

level was in a few cases less than that at the lower exposure levels, always less than that at the 15,000 r dosage, and in one instance less than that of the control. This is probably a real thing and not due to sampling error considering the number of plants measured. Also this same phenomenon has recently been observed by Mericle et al. (1964) in this same variety of barley using a dose of 50,000 r X-rays.

When growth inhibition (expressed as percent of control) is plotted against exposure level (Figure 7B) there is a tendency for the effects to be directly correlated with the amount of irradiation applied to the dry seeds. However, when these same values are plotted against generation time (Figure 7C), except for the 12,500 r dose level, there is a decrease in the mean shoot height after the second generation of radiation followed by an increase in the third treated generation. Viewed in this respect, the growth inhibition of the shoot becomes more severe with an increase in exposure level. A closer examination of the growth depression at the different dose levels shows that the ranges over three generations were: 4,000 r, 7 to 12 percent; 7,500 r, 6 to 23 percent; 12,500 r, 10 to 20 percent; 15,000 r, 12 to 34 percent; and 30,000 r, 24 to 56 percent. Except for the 12,500 r dose level the difference in the ranges increased with increased exposure level. Following the first, second, and third generations of radiation the ranges in percent of growth inhibition among the different exposure levels were: 8 to

27 percent; 12 to 56 percent; and 6 to 24 percent, respectively (Table 3).

With few exceptions, the differences in shoot heights of the control plants and those obtained from seedlings irradiated either as dry seed or as developing embryos were significant (1 percent level). The shoot heights were quite variable, however, the amount of variability induced by the irradiation each generation was greater in the developing embryos than in the dry seed material. It is interesting to note that, in general, 400 r of X-rays at the early proembryo stage of developing embryos showed growth inhibition comparable to that at the 30,000 r dose level given dormant embryos. Irradiation at the other embryonic stages exhibited growth depressions similar to the 15,000 r exposure level. Following the second successive generation of radiation of both developing and dormant embryos the shoot heights, except for the early proembryo stage, showed a large decrease followed by an equally large increase after the third irradiated generation. This appears to be good evidence for a build up of genetic defects (presumably chromosomal interchanges) such as was reported in plants by Abrams and Frey (1958) and in animals by Spalding and Strang (1962) following recurrent irradiation in successive generations. The fact that there is a recovery following the subsequent irradiated generations indicates that selection against dominant and recessive mutant cells is occurring (Ehrenberg et al., 1953;

Gaul, 1958). Whether this alternating cycle would continue with future subsequent irradiated generations is open to speculation and remains to be determined. In this respect it does not appear that the shoots are any more sensitive to irradiation following the third generation than they were after the first.

Root length

With the exception of the early proembryo and late differentiation stages following the second generation of radiation, the root lengths of all groups irradiated as developing embryos were significantly reduced (1 percent level) from the controls (Tables 4 and 11). From Table 4 it can be seen that the induced variability following irradiation at the early proembryo and late differentiation stage was greater than that observed at the late proembryo and early differentiation stages. Moreover, this occurred after each irradiated generation. In addition, irradiation applied at the early proembryo stage produced the greatest degree of variability each treated generation, while that at the late proembryo stage was the least. This differential stage specific response of the induced variability of the embryonic stages agrees with the stage specific responses noted by Mericle and Mericle (1957, 1961, 1962, 1963) and the residual DNA depression data of Chang (1961) and Chang and Mericle (1963). Using the mean root length as criterion expressed as percent of control and plotted against embryonic stages

Table 4. Effect of X-irradiation on root length. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations—heights measured in cm at the end of the fifth day).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	122	10.5	0.31	3.42	100	143	9.9	0.26	3.08	100	79	11.5	0.39	3.44	100
400r EP[a]	86	8.9**	0.55	5.12	85	53	8.9[b]	0.68	4.96	90	54	8.7**	0.72	5.27	76
400r LP	116	8.1**	0.34	3.66	77	60	8.6**	0.44	3.39	87	58	9.6**	0.53	4.07	83
400r ED	122	7.0**	0.36	4.02	67	94	8.0**	0.41	3.96	81	55	9.4**	0.63	4.70	82
400r LD	101	7.0**	0.43	4.32	67	94	8.9*	0.42	4.06	90	51	8.7**	0.68	4.86	76
Dormant Embryos															
Control[c]	288	9.3	0.19	3.26	100	152	10.1	0.24	2.92	100	206	10.2	0.19	2.68	100
Control[d]	189	9.2	0.18	2.47	100	189	10.4	0.17	2.30	100	110	11.1	0.26	2.75	100
4,000 r	188	7.6**	0.22	2.97	83	181	8.5**	0.25	3.36	82	112	10.3**	0.31	3.30	93
7,500 r	278	7.1**	0.20	3.33	76	137	8.4**	0.25	2.86	83	208	8.0**	0.25	3.57	78
12,500 r	188	7.4**	0.21	2.90	80	177	7.9**	0.26	3.43	76	106	9.1**	0.30	3.09	88
15,000 r	263	7.5**	0.22	3.55	81	142	8.7**	0.26	3.16	86	188	8.6**	0.26	3.62	82
30,000 r	267	6.3**	0.17	2.73	68	148	6.5**	0.22	2.73	64	197	6.7**	0.21	2.95	66

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from the controls
[c]Controls for 7,500 r, 15,000 r, and 30,000 r dose levels.
[d]Controls for 4,000 r and 12,500 r dose levels.
* $P < 5\%$
** $P < 1\%$

(Figure 8A), the early proembryo stage, in contrast to its increased variability showed greater radiation resistance following the first and second generations of radiation than the other stages. Following the third generation, however, the root responses for embryos irradiated at different stages of development exhibited a curve similar to those of residual DNA depression (Chang, 1961; Chang and Mericle, 1963). When these values are plotted against generation time (Figure 8C) they show an increase after the second generation of radiation followed by a decrease in the third generation. Root growth inhibition ranges following irradiation over three treated generations were: early proembryo, 10 to 24 percent; late proembryo, 13 to 23 percent; early differentiation, 18 to 33 percent; and late differentiation, 10 to 33 percent (Table 4). From these data it can be seen that irradiation at the early and late differentiation stages caused a greater depression from the controls than the early and late proembryo stages. Following the first, second, and third successive generations of radiation growth inhibition of the roots of the different embryonic stages ranged from 15 to 33 percent; 10 to 33 percent; and 17 to 24 percent, respectively.

Following the irradiation of dormant embryos there was a tendency for root length to decrease with an increase in dose level (Table 4, Figure 8B). Except for the 4,000 r and 12,500 r dose levels following the third successive generation of radiation, there does not appear to be any great effect

Figure 8A. Influence of X-irradiation on root length, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

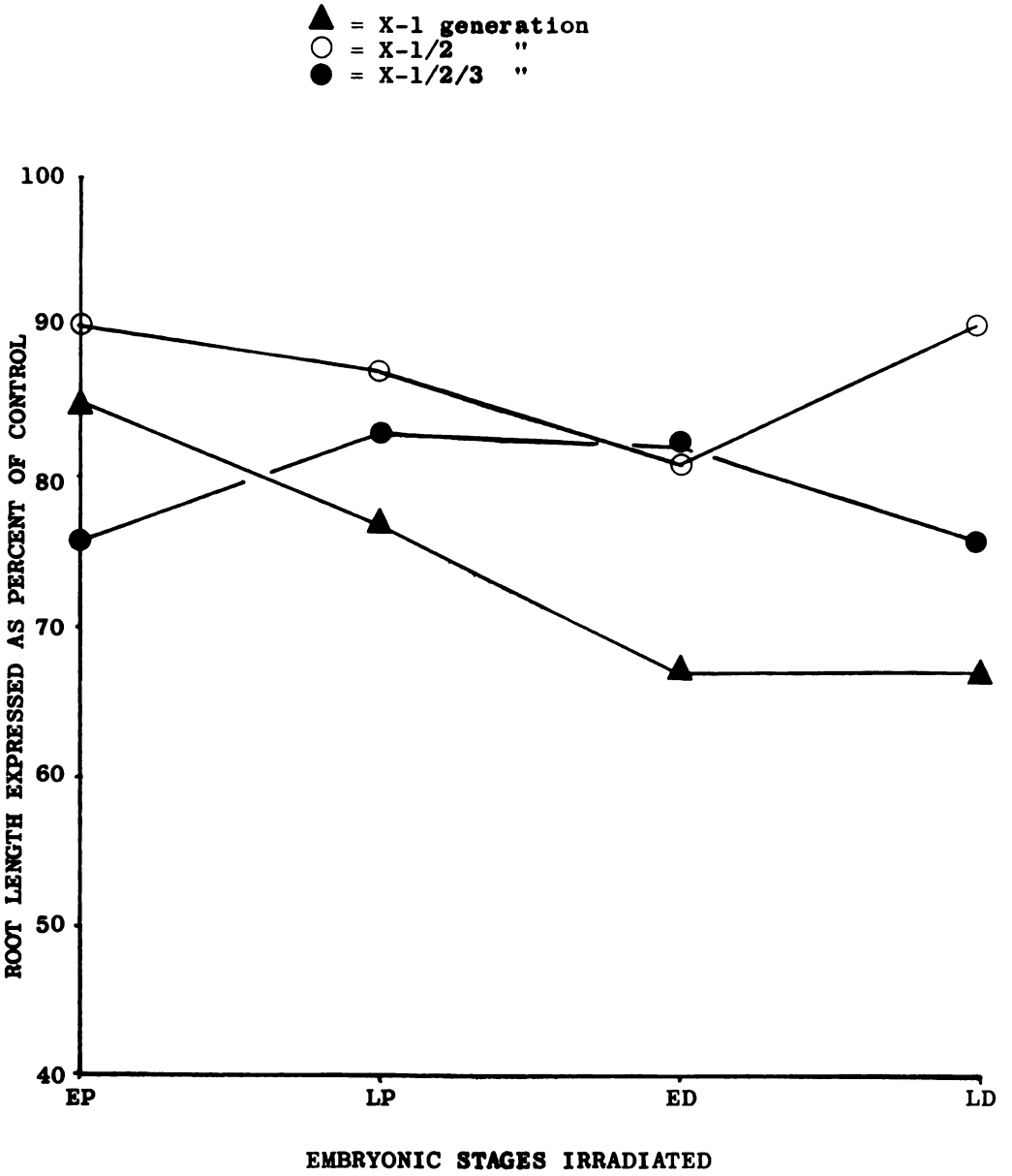
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
100
90
80
70
60
50
40
ROOT LENGTH EXPRESSED AS PERCENT OF CONTROL
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 8A

Figure 8B. Influence of X-irradiation on root length, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

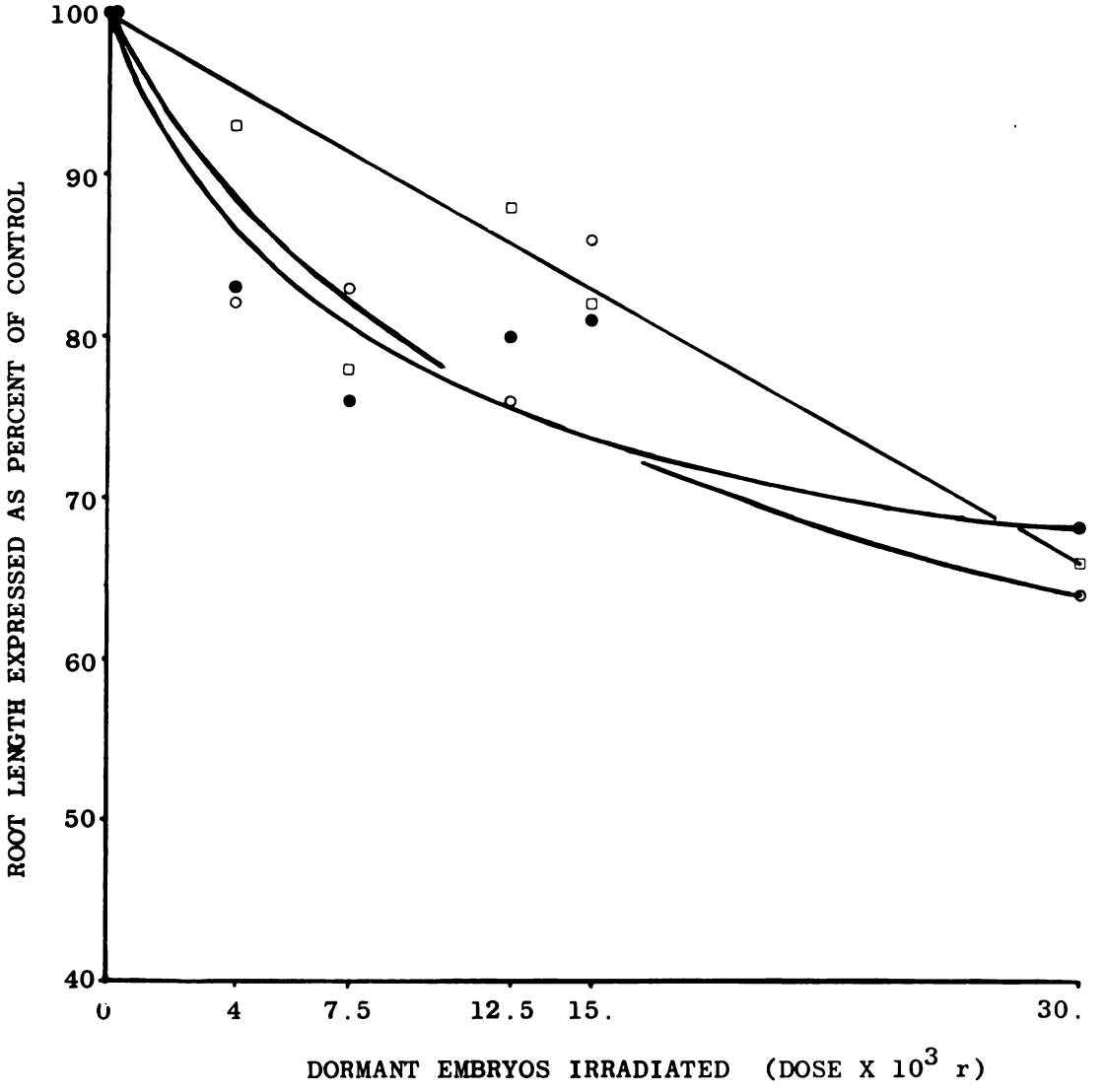
100
90
80
70
60
50
40
ROOT LENGTH EXPRESSED AS PERCENT OF CONTROL
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10^3 r)

Figure 8B

Figure 8C. Influence of X-irradiation on root length, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

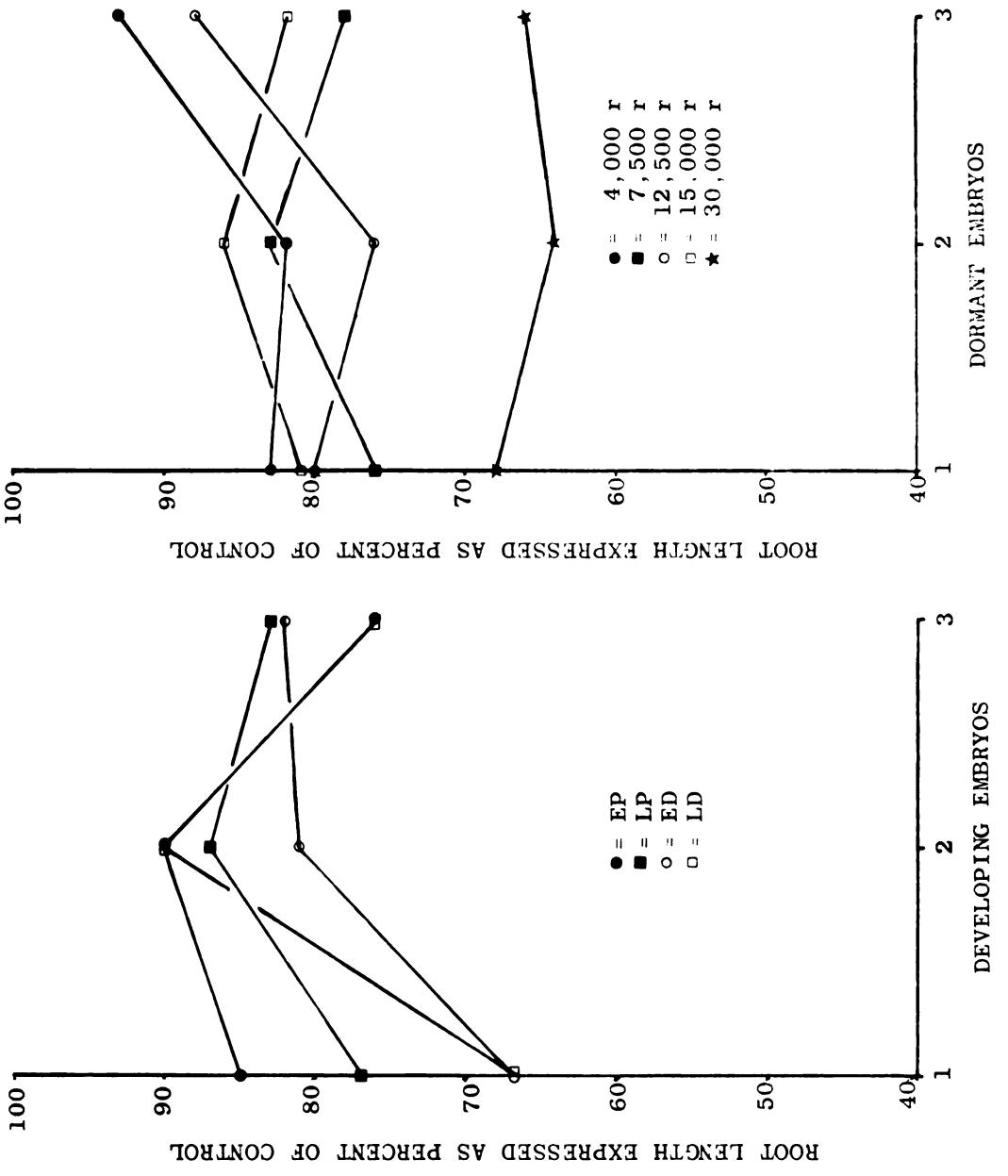
ROOT LENGTH EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r
ROOT LENGTH EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DEVELOPING EMBRYOS
● = EP
■ = LP
○ = ED
□ = LD
GENERATIONS IRRADIATED

Figure 8C

when these data are plotted against generation time (Figure 8C). From Tables 4 and 11 it can be seen that at all exposure levels the root growth from irradiated dormant embryos was significantly less (1 percent level) each generation when compared with the controls. Interestingly, the variability observed following the 30,000 r dose level was less than that at the lower dose levels and in two instances even less than that observed for the controls. This observation is in agreement with some recent data of Mericle et al. (1964). Growth depression of roots from dormant embryos exposed to different dose levels over three generations ranged as follows: 4,000 r, 7 to 18 percent; 7,500 r, 17 to 24 percent; 12,500 r, 12 to 24 percent; 15,000 r, 14 to 18 percent; and 30,000 r, 32 to 36 percent (Table 4). From these data it may be seen that the maximum depression at all exposure levels below and including 15,000 r are within 6 percent of one another. In contrast, the maximum depression of the 30,000 r dose level is 12 to 18 percent greater than that of the lower exposure levels. Corresponding values at different exposures within a generation following the first, second, and third successive generations of radiation ranged from 17 to 32 percent; 14 to 36 percent; and 7 to 34 percent, respectively. It is interesting to note that the difference between these ranges increased each generation.

From Table 4 it may be seen that greater variability was induced in the population by irradiating developing

embryos than by the different dose levels applied to dormant embryos. These data indicate that the dormant embryos are much more stable to irradiation each generation than are developing embryos. The maximum depression in root growth of the early and late differentiation stages of developing embryos is comparable to that of the 30,000 r dose level given dormant embryos, while the early and late proembryo stages fall within the range exhibited by the other exposure levels. Due to the fluctuations of the root growth following irradiation of both developing and dormant embryos no definite conclusion can be drawn concerning whether or not resistance or susceptibility has been induced by recurrent irradiation.

Number of roots

At all embryonic stages irradiated the number of roots which had emerged by the end of the fifth day was significantly lower (1 percent level) than controls (Table 5 and 11). In addition, the variability induced each irradiated generation was two- to three-fold greater than that observed for the controls. Following the first, second, and third generations of radiation the reduction in the number of roots among the embryonic stages ranged as follows: 11 to 24 percent, 10 to 19 percent, and 8 to 23 percent, respectively. From these data it can be seen that the maximum and minimum reduction in the number of roots were rather comparable each generation. A closer inspection of these data shows that irradiation applied to the early proembryo and late

Table 5. Effect of X-irradiation on the number of roots. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	122	6.6	0.05	0.54	100	143	6.7	0.05	0.56	100	79	6.5	0.08	0.75	100
400r EP[a]	86	5.2**	0.19	1.75	79	53	5.4**	0.24	1.78	81	54	5.4**	0.24	1.75	83
400r LP	116	5.9**	0.15	1.63	89	60	6.0**	0.14	1.12	90	58	6.0**	0.19	1.45	92
400r ED	122	5.9**	0.17	1.91	89	94	5.5**	0.15	1.49	82	55	5.7**	0.23	1.67	85
400r LD	101	5.0**	0.14	1.41	76	94	5.5**	0.11	1.05	82	51	5.0**	0.19	1.35	77
Dormant Embryos															
Control[c]	288	6.7	0.05	0.85	100	152	6.8	0.06	0.72	100	206	6.6	0.04	0.61	100
Control[d]	189	6.7	0.05	0.63	100	189	6.7	0.06	0.79	100	110	6.7	0.06	0.63	100
4,000 r	188	6.7[b]	0.07	0.93	100	181	6.5[b]	0.11	1.44	97	112	6.8[b]	0.10	1.03	100
7,500 r	278	6.5*	0.08	1.26	97	137	6.8[b]	0.07	0.82	100	208	6.5[b]	0.10	1.46	98
12,500 r	188	6.6[b]	0.05	0.70	98	177	6.6[b]	0.10	1.28	98	106	7.0**	0.09	0.94	103
15,000 r	263	6.3**	0.08	1.39	94	142	6.7[b]	0.07	0.82	99	189	6.6[b]	0.10	1.44	100
30,000 r	267	6.6[b]	0.02	1.04	98	148	6.4**	0.12	1.48	95	197	6.3**	0.09	1.25	95

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from control.
[c]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[d]Control for 4,000 r and 12,500 r dose levels.
* $P < 5\%$
** $P < 1\%$

differentiation stages produced the greatest reduction in root number as compared with the controls. These values for the irradiated stages were: early proembryo, 17 to 21 percent; late proembryo, 8 to 11 percent; early differentiation, 11 to 18 percent; and late differentiation, 18 to 24 percent. Thus, it is easily seen that when the number of roots (expressed as percent of control) is plotted against embryonic stage (Figure 9A) the early proembryo and late differentiation stages show greater radiation sensitivity. Furthermore, these data (based upon root number) exhibit curves similar to those observed earlier for coleoptiles and to the residual DNA depression curves obtained by Chang (1961) and Chang and Mericle (1963). When these data are plotted against generation time (Figure 9C) there is a slight increase in the mean number of roots.

From Table 5 it may be observed that, with few exceptions, irradiation applied at the different dose levels to dormant embryos reduced the number of roots. However, this reduction is significant in only a few instances (Tables 5 and 11). In one case (12,5000 r) there was a significant increase in the number of roots over that of the controls. While the number of roots remained relatively constant each generation the induced variability fluctuated considerably. At all dose levels, however, the induced variability is greater than that of the controls. Following the first and third generations of radiation the induced variability of the 30,000 r does level is less than that at the 7,500 r and 15,000 r

Figure 9A. Influence of X-irradiation on the number of roots, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

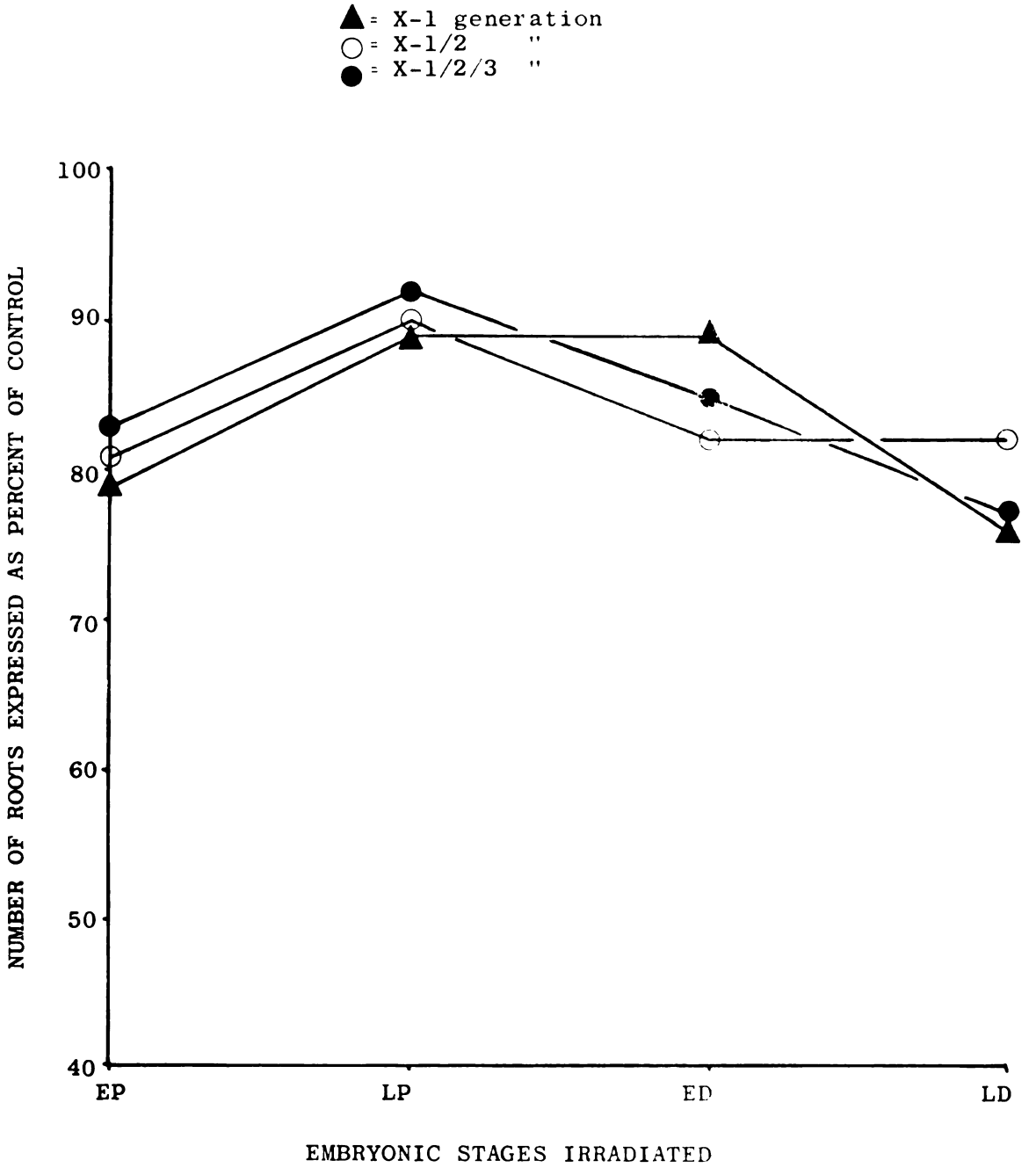
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
100
90
80
70
60
50
40
NUMBER OF ROOTS EXPRESSED AS PERCENT OF CONTROL
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 9A

Figure 9B. Influence of X-irradiation on the number of roots, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

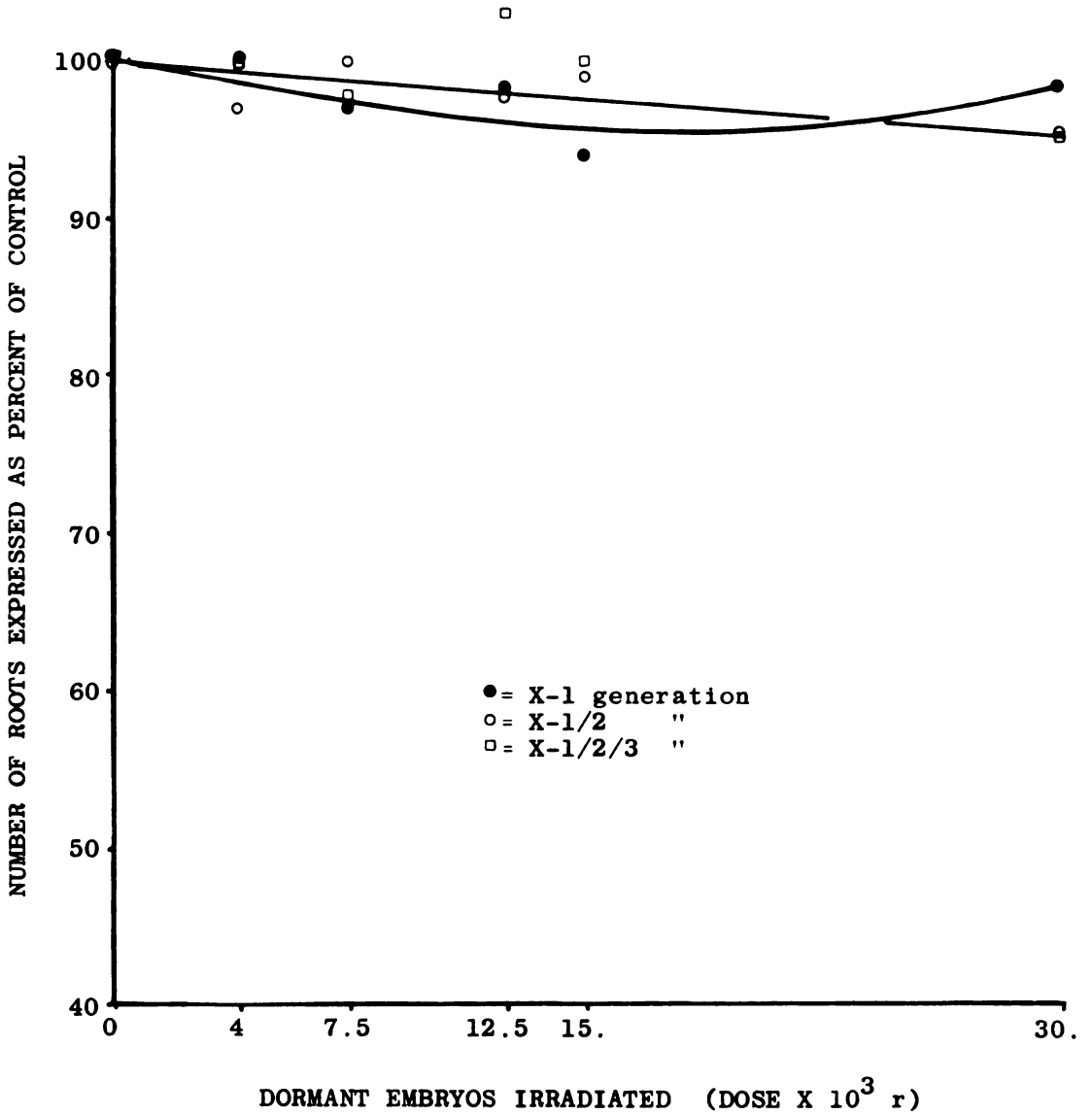
100
90
80
70
60
50
40
NUMBER OF ROOTS EXPRESSED AS PERCENT OF CONTROL
●= X-1 generation
○= X-1/2 "
□= X-1/2/3 "
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10^3 r)

Figure 9B

Figure 9C. Influence of X-irradiation on the number of roots, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

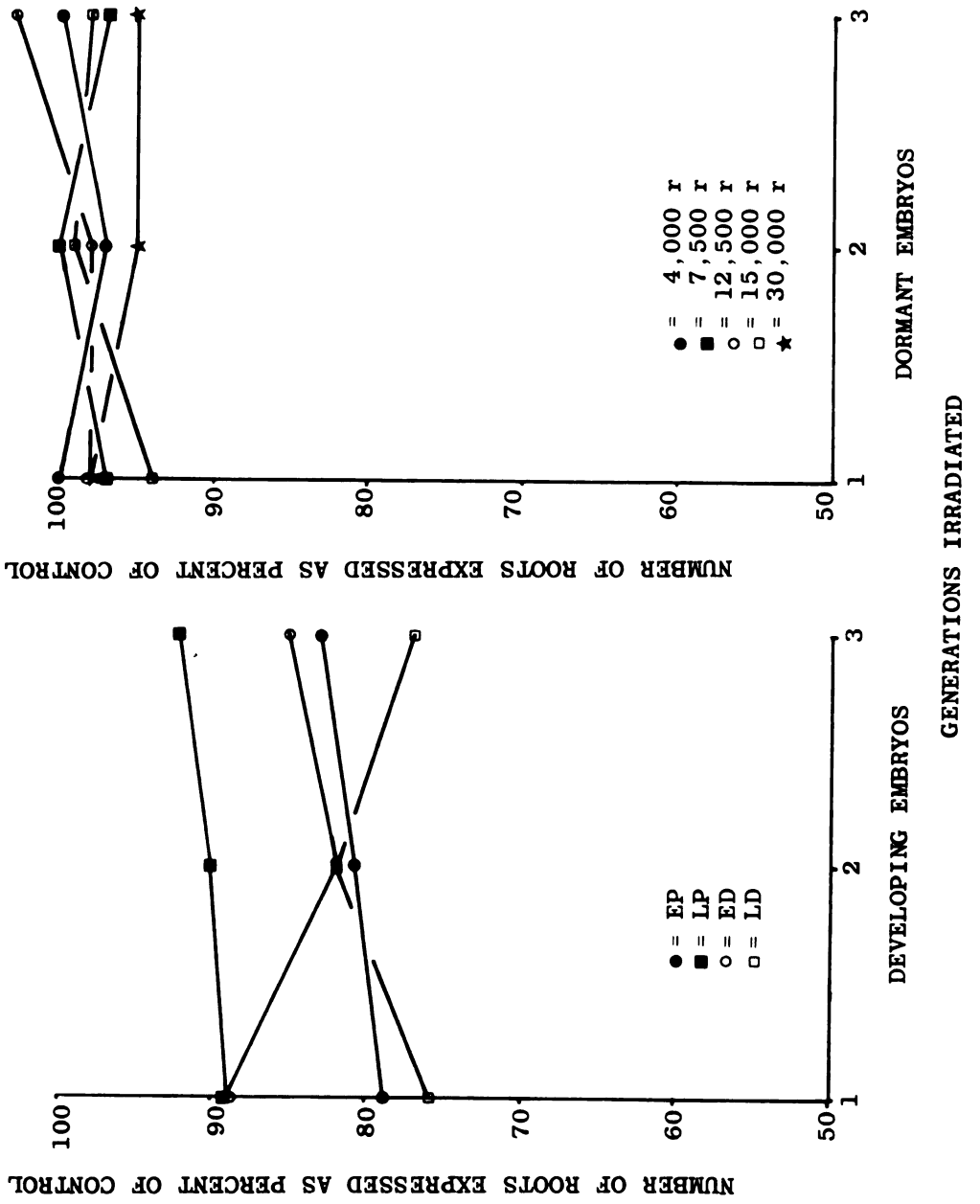
NUMBER OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
1
2
3
DORMANT EMBRYOS
4,000 r
7,500 r
12,500 r
15,000 r
30,000 r
NUMBER OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
1
2
3
DEVELOPING EMBRYOS
EP
LP
ED
LD
GENERATIONS IRRADIATED

Figure 9C

exposure levels.

Based upon the controls of this and other studies (Mericle, R. P. et al., 1963) it has been found that this particular variety of barley normally has one main root (radical) and 5 or 6 seminal roots which emerge upon germination. From Table 5 and Figure 9C it may be seen that X-rays are relatively more effective in inhibiting the number of roots emerging in seedlings that have been treated as developing embryos than as dormant embryos. The reduced number of roots observed in these data are in agreement with the results of Eunus (1954, 1955) and Saric (1958b). The number of roots appearing after irradiation of dormant embryos, with few exceptions, was not significantly reduced from the controls, while all groups of embryos irradiated during development were significantly lower than the controls (Tables 5 and 11). However, after a cumulative dose of 60,000 r and 90,000 r over two and three generations, respectively, there was a significant reduction in the number of roots over that of the controls. In general, greater variability appeared following irradiation of different embryonic stages of developing embryos than at the different exposure levels applied to dormant embryos each treated generation. Using the number of roots as a criterion to study the effects of recurrent irradiation on the germ line indicates that there is, in general, a slight increase in resistance. Moreover, this is evident following irradiation of either developing or dormant embryos.

Dry weights of shoots

From Table 6 it may be seen that irradiation applied to embryonic stages of developing embryos has had an inhibitory effect on the growth and development of the seedling shoots. The shoot dry weights were, with two exceptions, significantly lower (1 percent level) than the controls. Except for the first generation the induced variability of the shoot dry weights was greater following irradiation at the early proembryo and late differentiation stages each treated generation (Table 6). In all cases it was greater than that observed for the controls. Moreover, with one exception, the induced variability was greater at the early proembryo stage than at the other embryonic stages. Following one, two, or three generations of radiation growth inhibition ranges were: 7 to 39 percent, 12 to 40 percent, and 14 to 30 percent, respectively (Table 6). A closer examination of the responses at each generation shows that the growth retardation of seedlings from embryos irradiated at the early proembryo stage ranged from 30 to 40 percent; the late proembryo, 12 to 31 percent; the early differentiation, 7 to 24 percent; and the late differentiation, 26 to 37 percent. When these data (expressed as percent of control) are plotted against embryonic stage (Figure 10A) it is clearly seen that the early proembryo and late differentiation stages exhibited greater radiation sensitivity than the other irradiated stages. Moreover, this pattern occurred with each generation irradiated.

Table 6. Influence of X-irradiation on dry weights of shoots. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations—dry weights expressed in mg).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	18	10.5	0.24	1.01	100	15	9.0	0.47	1.81	100	18	10.3	0.28	1.17	100
400r EP[a]	9	6.4**	1.26	3.78	61	14	5.4**	0.95	3.56	60	17	7.2**	0.95	3.90	70
400r LP	10	7.2**	0.77	2.42	69	17	7.9[b]	0.61	2.52	88	19	8.7**	0.31	1.36	84
400r ED	10	9.8[b]	1.31	4.13	93	20	6.7**	0.51	2.27	74	14	8.9**	0.34	1.26	86
400r LD	15	6.6**	0.45	1.74	63	21	6.0**	0.62	2.86	67	20	7.6**	0.63	2.80	74
Dormant Embryos															
Control[c]	48	8.8	0.26	1.81	100	14	8.8	0.60	2.24	100	23	8.5	0.30	1.44	100
Control[d]	35	8.6	0.32	1.94	100	24	9.2	0.29	1.41	100	10	8.5	0.44	1.39	100
4,000 r	40	5.9**	0.44	2.80	69	21	9.2[b]	0.87	4.01	100	10	8.4[b]	0.32	1.01	99
7,500 r	45	6.8**	0.35	2.39	77	15	8.3[b]	0.43	1.65	94	22	9.2[b]	0.42	1.95	108
12,500 r	37	6.1**	0.39	2.40	71	19	7.5*	0.62	2.69	82	10	7.9[b]	0.41	1.29	93
15,000 r	46	6.8**	0.38	2.59	77	14	7.9[b]	0.37	1.39	90	21	8.1[b]	0.51	2.33	95
30,000 r	47	6.3**	0.28	1.91	72	15	5.0**	0.56	2.18	57	22	6.0**	0.56	2.63	71

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from control.
[c]Controls for 7,500 r, 15,000 r, and 30,000 r dose levels.
[d]Controls for 4,000 r and 12,500 r dose levels
* $P < 5\%$
** $P < 1\%$

Figure 10A. Influence of X-irradiation on dry weights of shoots, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

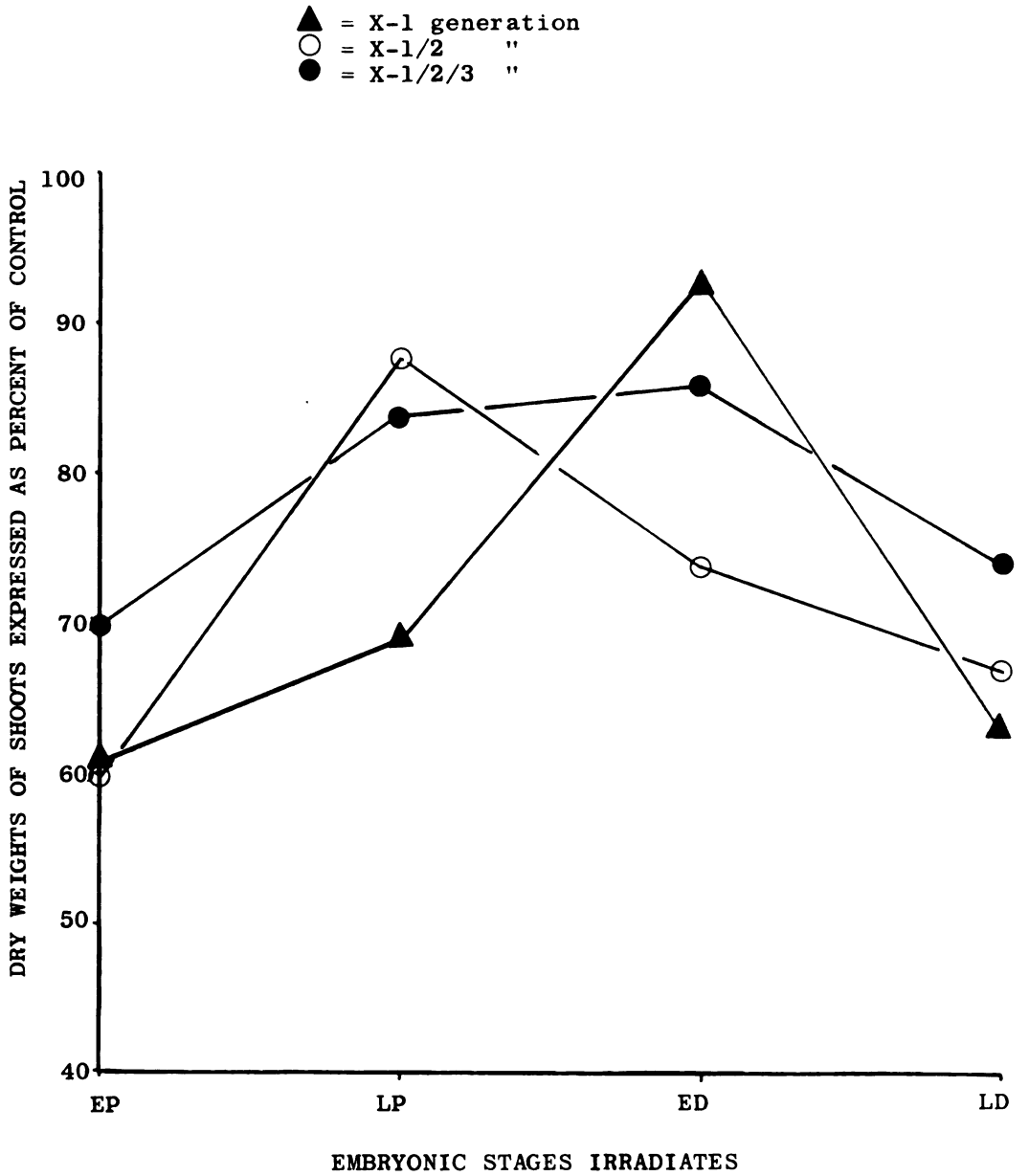
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
DRY WEIGHTS OF SHOOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATES

Figure 10A

Figure 10B. Influence of X-irradiation on dry weights of shoots, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

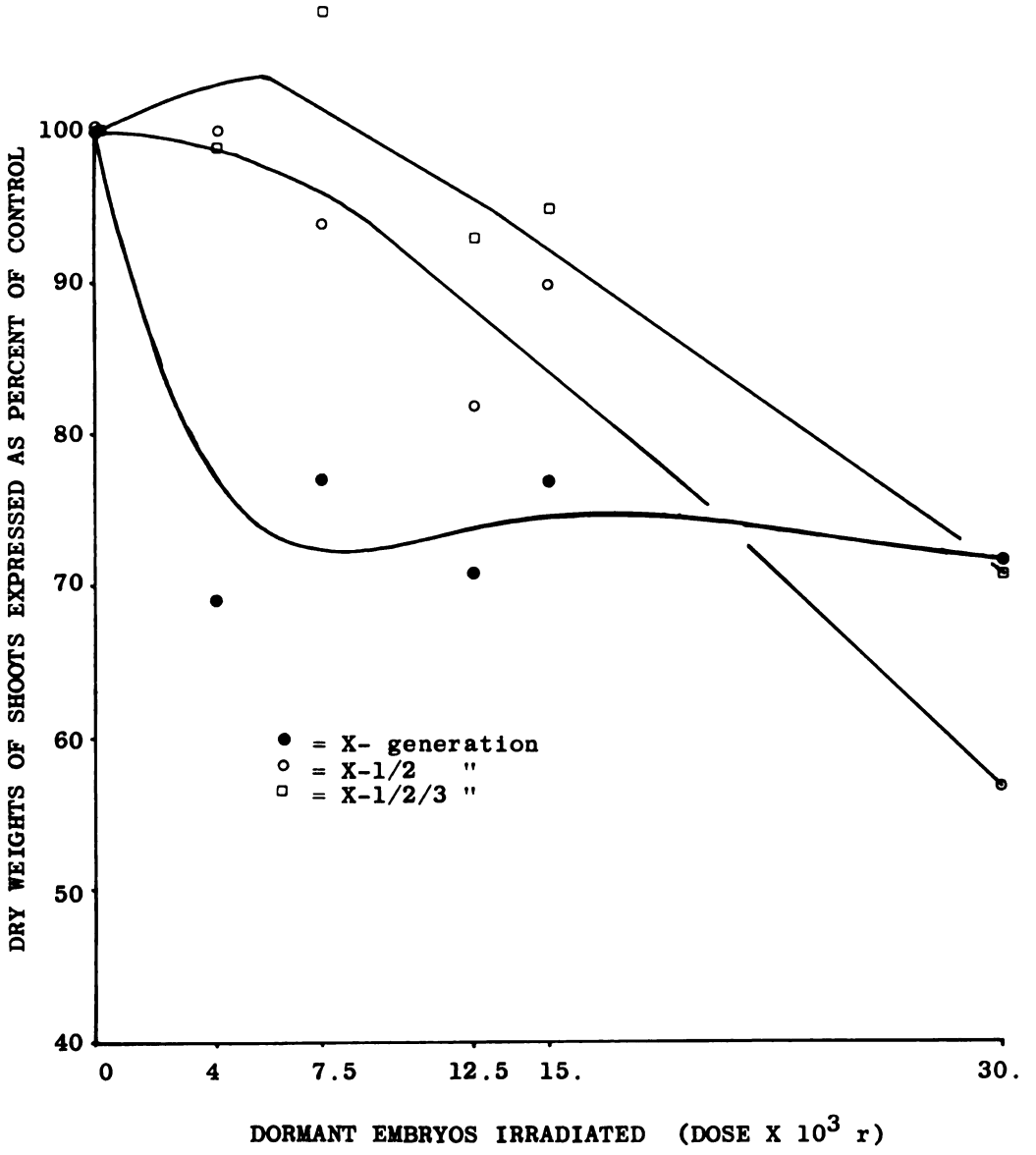
DRY WEIGHTS OF SHOOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
● = X- generation
○ = X-1/2 "
□ = X-1/2/3 "
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10³ r)

Figure 10B

Figure 10C. Influence of X-irradiation on dry weights of shoots, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

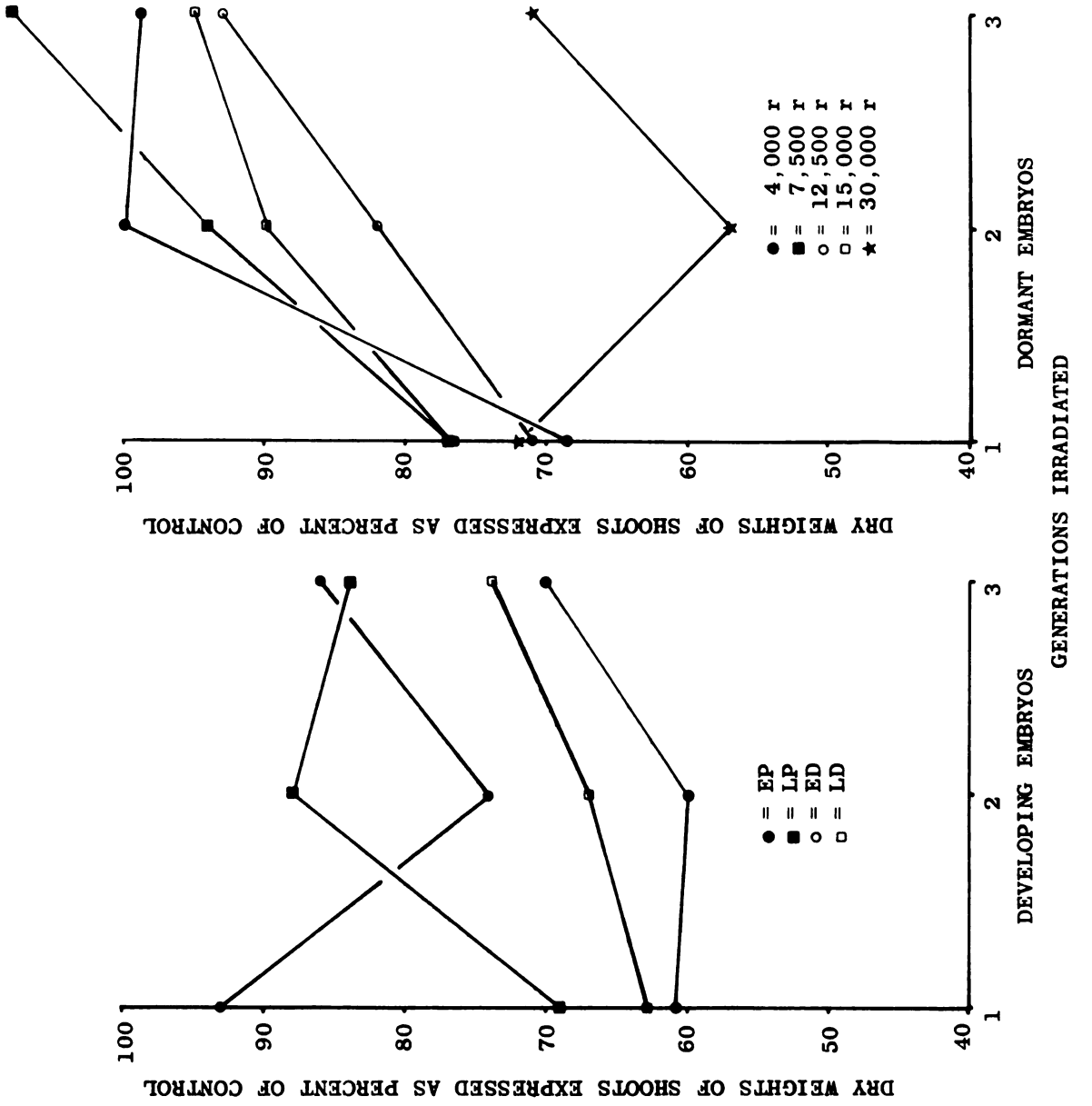
DRY WEIGHTS OF SHOOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r
DRY WEIGHTS OF SHOOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DEVELOPING EMBRYOS
● = EP
■ = LP
○ = ED
□ = LD
GENERATIONS IRRADIATED

Figure 10C

These data agree with those on coleoptile heights and number of roots observed earlier in this study as well as with the residual DNA depression data obtained by Chang (1961) and Chang and Mericle (1963). When considered from the standpoint of generation time (Figure 10C) it is seen that, in general, there is a slight increase in dry weights of shoots after three successive generations of radiation.

Following the first generation of radiation the mean dry weights of shoots at the different dose levels given dormant embryos were all significantly lower (1 percent level) than the controls (Tables 6 and 11). This level of significance carries through the second and third treated generations only at the 30,000 r dose level. Calculation of the standard deviations showed that, with few exceptions, the induced variability at all exposure levels was greater than that of the controls. A closer examination shows that at the 30,000 r dose level the variability increased each treated generation. Analyses of the dry weights of shoots at the different exposure levels gave curves of the type shown in Figures 10B and 10C. The 7,500 r dose level exhibited stimulatory effects following the third generation of radiation (Table 6, Figures 10B and 10C). This observation agrees with the results of Breslavets (1946, 1960) and Kuzin (1956). In general, there was a considerable increase in shoot dry weights after three successively treated generations. Growth inhibition ranges among generations were:

4,000 r, 0 to 31 percent; 7,500 r, 8 to 23 percent; 12,500 r, 7 to 29 percent; 15,000 r, 10 to 23 percent; and 30,000 r, 28 to 43 percent (Table 6). Following the first, second, and third generations of radiation corresponding values among the different dose levels ranged from 23 to 31 percent, 0 to 43 percent, and 8 to 29 percent, respectively.

It may be observed in Table 6 that following irradiation of both developing and dormant embryos shoot dry weights were, in general, reduced from that of the controls. Furthermore, with two exceptions, this reduction was significantly lower (1 percent level) at all embryonic stages of developing embryos following each treated generation (Tables 6 and 11). At the different dose levels given dormant embryos, however, this level of significance is apparent only following the first generation of radiation and at the 30,000 r dose level after the second and third irradiated generations. Likewise, with few exceptions, the irradiated stages exhibited greater variability than the different exposure levels. Upon comparing the maximum and minimum growth depression, the 30,000 r dose level on dormant embryos and the 400 r dosage given the early proembryo and late differentiation stages of developing embryos show about equal sensitivity. The late proembryo and early differentiation stages of developing embryos exhibited maximum and minimum growth retardation within the range of the other exposure levels given dormant embryos. Using shoot dry weight as the criterion to measure radiation

sensitivity these data, in general, show that some resistance to irradiation is being induced.

Dry weights of roots

Growth of the root systems as indicated by their dry weights was greatly inhibited by the X-radiation of developing embryos. In the first generation the root dry weights were significantly reduced (5 percent level) from that of the controls only following irradiation applied to the late differentiation stage (Tables 7 and 11). With one exception, the root dry weights of all irradiated stages in the second and third generations were significantly lower (1 percent level) than the controls. Generally, root retardation was most accentuated when irradiation was applied to the early proembryo and late differentiation stages following the first and third generations, while root growth was inhibited for all stages irradiated in the second generation (Table 7, Figure 11A). The overall root dry weights among irradiated embryonic groups following one, two, or three generations ranged from 8 to 25 percent, 49 to 54 percent, and 10 to 34 percent, respectively (Table 7). A detailed inspection of the irradiated groups over three generations shows that the growth depression ranges were: early proembryo, 18 to 53 percent; late proembryo, 10 to 49 percent; early differentiation, 8 to 54 percent; and late differentiation, 25 to 50 percent. From these data it can be seen that the maximum depressions of the root as indicated by their dry weights

Table 7. Influence of X-irradiation on dry weights of roots. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations—dry weight expressed in mg).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	18	5.1[b]	0.27	1.13	100	15	7.0	0.41	1.57	100	18	6.1	0.45	1.89	100
400r EP[a]	9	4.2[b]	0.67	2.01	82	14	3.3**	0.59	2.19	47	17	4.0**	0.49	2.01	66
400r LP	10	4.6[b]	0.49	1.55	90	17	3.6**	0.30	1.24	51	19	4.1**	0.29	1.24	67
400r ED	10	4.7[b]	0.44	1.38	92	20	3.2**	0.29	1.30	46	14	5.5[b]	0.24	0.91	90
400r LD	15	3.8*	0.57	2.22	75	21	3.5**	0.30	1.38	50	20	4.0**	0.35	1.54	66
Dormant Embryos															
Control[c]	48	5.6	0.22	1.53	100	14	6.4	0.25	0.92	100	23	6.2	0.54	2.58	100
Control[d]	35	5.9	0.27	1.62	100	24	5.9	0.23	1.13	100	10	7.7	0.58	1.82	100
4,000 r	40	3.5**	0.27	1.70	59	21	5.4[b]	0.29	1.31	92	10	6.1*	0.37	1.16	79
7,500 r	45	4.4**	0.21	1.41	79	15	5.3**	0.26	1.00	83	22	5.2[b]	0.32	1.48	84
12,500 r	37	3.6**	0.22	1.37	61	19	4.0**	0.33	1.46	68	10	6.1*	0.44	1.41	79
15,000 r	46	4.4**	0.26	1.75	79	14	5.5*	0.36	1.35	86	21	4.9[b]	0.38	1.73	79
30,000 r	47	4.2**	0.21	1.41	75	15	4.1**	0.42	1.65	64	22	3.3**	0.34	1.62	53

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation stages.
[b]Not significant from control
[c]Controls for 7,500 r, 15,000 r, and 30,000 r dose levels.
[d]Controls for 4,000 r and 12,500 r dose levels.
* $P < 5\%$
** $P < 1\%$

Figure 11A. Influence of X-irradiation on dry weights of roots, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

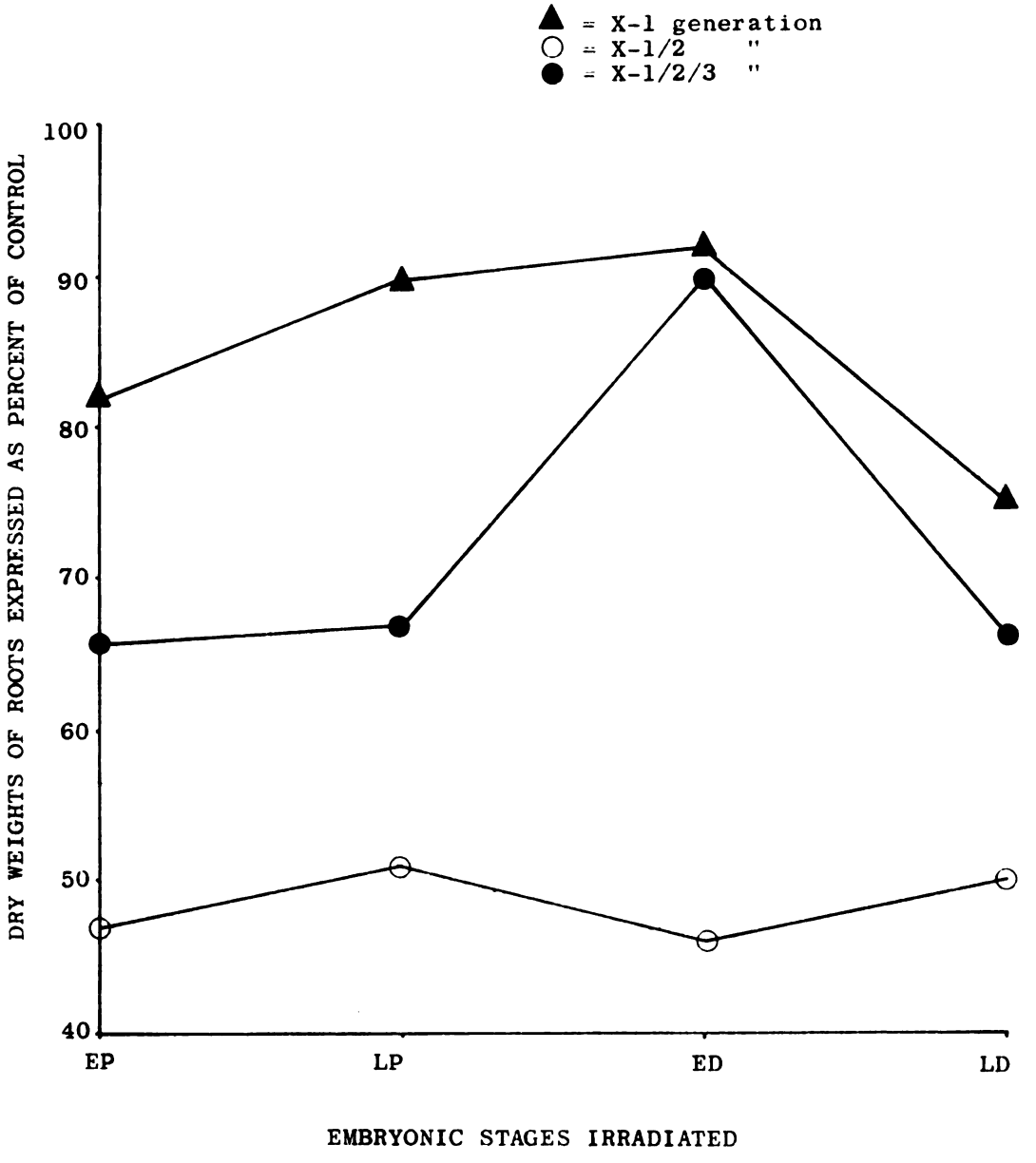
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
DRY WEIGHTS OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 11A

Figure 11B. Influence of X-irradiation on dry weights of roots, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

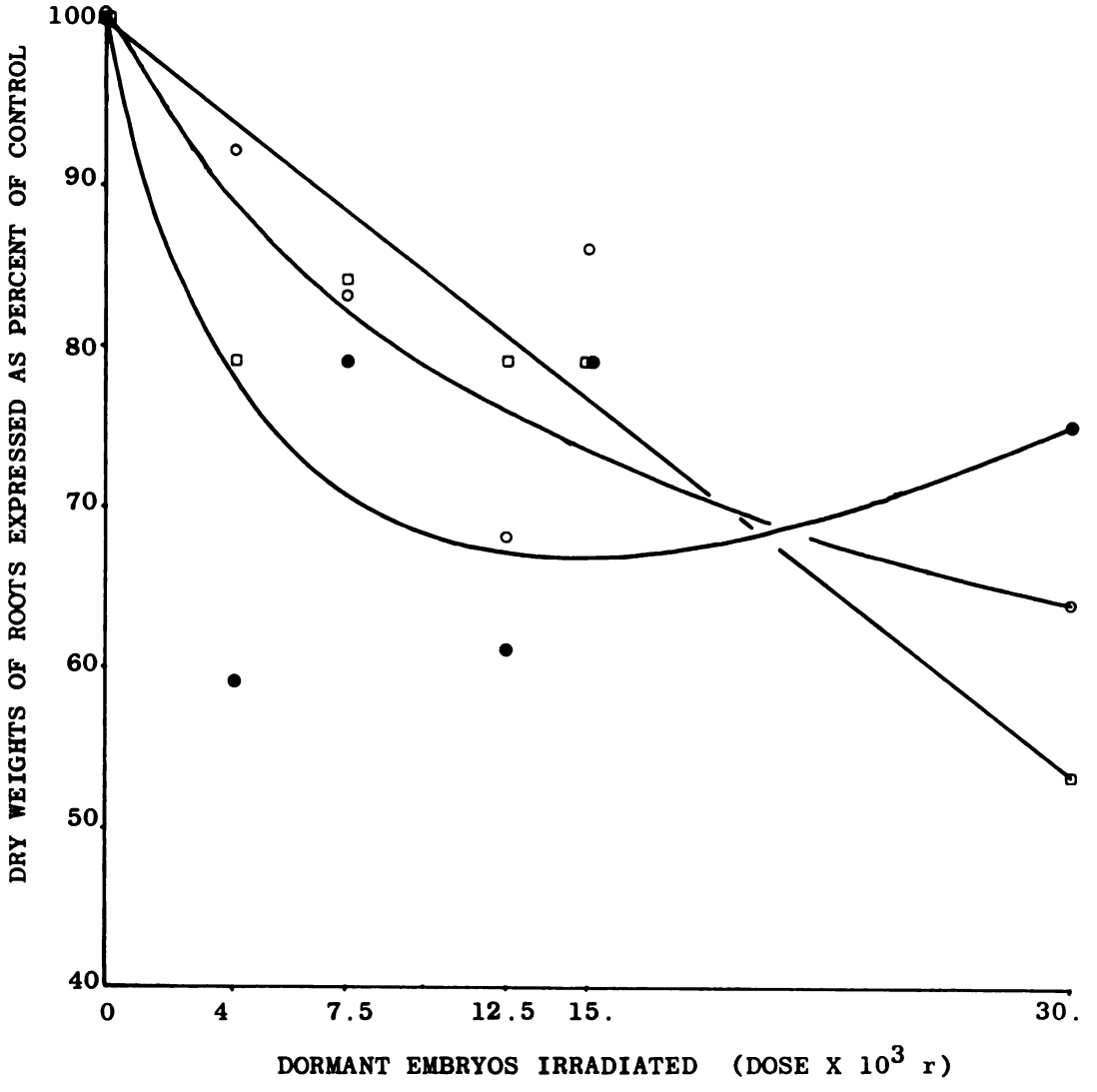
DRY WEIGHTS OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
0
4
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10^3 r)

Figure 11B

Figure 11C. Influence of X-irradiation on dry weights of roots, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

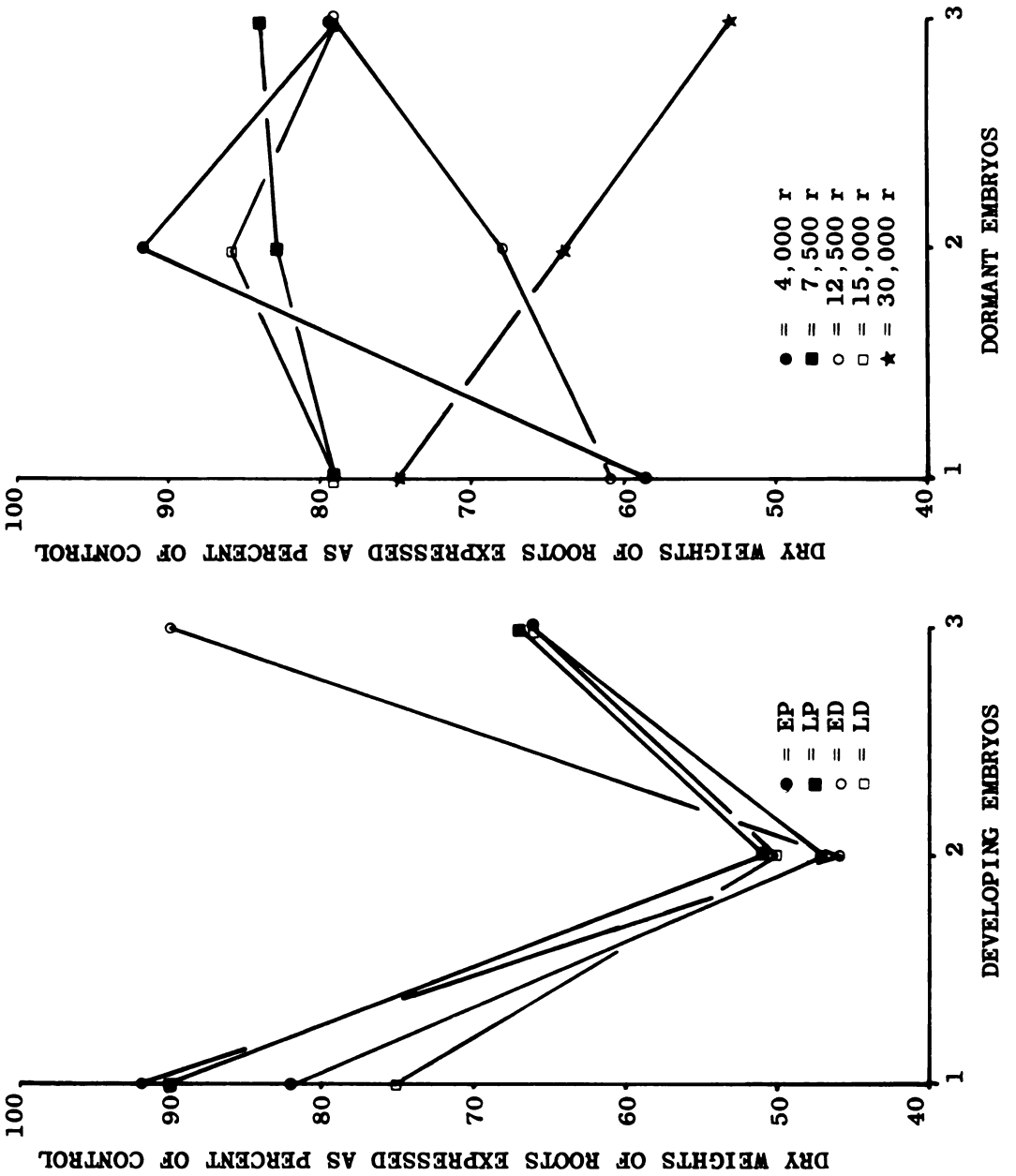
DRY WEIGHTS OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DEVELOPING EMBRYOS
GENERATIONS IRRADIATED
● = EP
■ = LP
○ = ED
□ = LD
DRY WEIGHTS OF ROOTS EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r

Figure 11C

were comparable at all irradiated stages. These high values, however, result from the large depression observed following the second generation of radiation and are more easily seen when root growth inhibition (expressed as percent of control) is plotted against generation time (Figure 11C). Although the roots were greatly depressed they showed recovery following the subsequent generation of radiation. In general, root growth inhibition as well as the amount of induced variability was greater following irradiation at the early proembryo and late differentiation stages than at the other embryonic groups. Except for the second generation the root dry weight curves of these irradiated stages mirror the residual DNA depression data of Chang (1961) and Chang and Mericle (1963).

Analyses of the dry weights of roots of the different dose levels given dormant embryos following recurrent irradiation in one, two, or three successive generations gave curves of the type shown in Figures 11B and 11C. With two exceptions, root dry weights following the first and second generations of radiation were significantly lower (1 percent level) than the controls (Tables 7 and 11). This level of significance, however, was observed only at the 30,000 r exposure following the third irradiated generation. In general, the root dry weights were irregular following the first and second generations of radiation, while the responses after the third treated generation were related to the increase in exposure level (Figure 11B). This irregularity is again

noted by the variability induced by irradiation (Table 7). Following the first, second, and third successive generations of radiation a comparison with the control seedlings shows that the root dry weights exhibit a depression of 21 to 41 percent; 8 to 36 percent; and 16 to 47 percent; respectively. Interestingly, the difference in ranges increased slightly with each treated generation. Growth inhibition ranges at the different exposure levels among generations were: 4,000 r, 8 to 41 percent; 7,500 r, 16 to 21 percent; 12,500 r, 21 to 39 percent; 15,000 r, 14 to 21 percent; and 30,000 r, 25 to 47 percent. From these data it may be seen that the maximum depressions of the 4,000 r and 12,500 r dose levels were comparable, while those of the 7,500 r and 15,000 r exposure levels were equal. The 30,000 r dose level showed both a greater maximum and minimum depression than the other dosages. When the mean dry weights of roots (expressed as percent of control) of dormant embryos irradiated at different dose levels were plotted against generation time (Figure 11C) the 30,000 r dose level exhibited a decrease each treated generation. All other exposure levels showed an increase following the second generation of radiation. However, only the 7,500 r and 12,500 r dose levels showed this increase after the third irradiated generations. Dry weights of roots at the 4,000 r and 15,000 r exposure levels exhibited a decrease following the third generation of radiation.

The growth of the root systems of both developing and

dormant embryos was greatly inhibited by irradiation, as exemplified by their dry weights (Table 7). In all cases the dry weights were reduced when compared with the controls. Following the first generation of radiation dormant embryos showed a greater significance from the controls than did developing embryos (Tables 7 and 11). After the second and third treated generation, however, this situation was reversed. With few exceptions, the variability induced by the irradiation of developing embryos did not differ greatly from the dormant ones. It is interesting to note that the maximum root dry weight reduction observed following irradiation at the 30,000 r dose level is, in part, comparable to that observed after treatment at the early proembryo and late differentiation stages following the first and third generations. Due to limited sampling of root dry weights it is doubtful if any definite conclusions can be drawn, however, these data may offer a suggestion concerning the response to recurrent irradiation in successive generations.

Comparison of coleoptiles and shoots

Irradiation produced an inhibitory effect on the mean coleoptile and shoot heights of developing embryos treated at specific stages (Tables 2 and 3). With one exception, the growth of both coleoptiles and shoots was significantly reduced from the controls (Tables 2, 3, and 11). Following one, two, or three successive generations of radiation the maximum reduction among the embryonic stages was greater in

the shoots than in the coleoptiles, being 25 percent, 18 percent, and 14 percent for the coleoptiles and 42 percent, 33 percent, and 30 percent for the shoots. It is of interest to note that the maximum depression decreased for both the coleoptiles and shoots each treated generation. A comparison of the irradiated stages shows that, with few exceptions, the growth reduction in both coleoptiles and shoots is greater in the early proembryo and late differentiation stages than in the late proembryo and early differentiation stages (Tables 2 and 3). As mentioned earlier these data on differential radiosensitivity support those on residual DNA depression (Chang, 1961; Chang and Mericle, 1963) as well as earlier results of Mericle and Mericle (1957, 1961, 1962, 1963). Overall the coleoptiles showed rather uniform but slightly increased growth each treated generation following the irradiation of developing embryos. In contrast, shoot heights for the different embryonic stages were quite irregular. This irregularity agrees with the earlier findings of Caldecott (1961) and Caldecott and North (1961).

In Tables 2 and 3 data are shown to illustrate the influence of different dose levels on coleoptile and shoot heights of dormant embryos. At all dose levels growth of the coleoptiles and shoots were reduced as compared with the controls. With few exceptions, this reduction was significant (1 percent level) at all dosages. In general, when the amount of growth inhibition of both coleoptiles and

shoots (expressed as percent of control) was plotted against dose level the effect was related to the amount of irradiation applied to the dormant embryos (Figures 6B and 7B). Again as with developing embryos, the coleoptile heights of the dormant embryos were rather uniform each generation, while the shoot heights were highly irregular. The irregularity of the shoots as compared with the coleoptiles may be observed further by the amount of induced variability (Tables 2 and 3). In all cases this variability was 2 to 4 times greater than that of the coleoptiles. This is probably a result of the large difference in mitotic activity of these two tissues (Sicard and Schwartz, 1959). The non-uniformity of the shoots has been previously observed by Caldecott et al. (1952), Caldecott (1961), and Caldecott and North (1961). Following one, two, or three generations of radiation the maximum growth inhibition among the different dose levels was 25 percent, 27 percent, and 21 percent for the coleoptiles and 27 percent, 56 percent, and 24 percent for the shoots. With one exception, these maximum depressions are comparable one to the other.

Comparison of roots and shoots

The effects of radiation on seedling development may be expressed in terms of the root-shoot ratio. Root-shoot dry weight ratios for five day old irradiated and unirradiated seedlings were determined and are listed in Table 12. In Tables 3 and 4 it may be seen that X-irradiation applied to

developing embryos induced more variability in the root lengths than in the shoot heights. When the dry weights of shoots and roots were compared, however, this pattern was reversed (Tables 6 and 7). Of further interest is the fact that, with few exceptions, irradiation given during the early proembryo and late differentiation stages produces a greater variability than during the late proembryo and early differentiation stages in so far as both dry weights and growth measurements of roots and shoots are concerned (Tables 3, 4, 6, and 7). Variability for both was, in general, greater following treatment at the early proembryo stage. Following irradiation of the early and late proembryo stages there occurred a greater growth depression of the shoots than of the roots (Tables 3 and 4). When treatment was applied at the early and late differentiation stages this situation was reversed. The maximum depression between the roots and shoots of these irradiated stages, however, differed by only 3 to 5 percent. Compared with the controls the shoot heights of the irradiated stages are reduced to a greater degree than the root lengths. When the dry weights of the roots and shoots are compared, the roots, with one exception, show a greater depression from the controls (Tables 6 and 7).

Root-shoot dry weight ratios were rather constant for seedlings from dormant embryos exposed to different levels of radiation in the first generation (Table 12). Following the second and third generations, however, irradiation applied

to dormant embryos caused considerable fluctuation in the root-shoot ratios of the seedlings. In some instances a slight increase in the ratios were observed. An increase in root-shoot ratio has been observed and attributed to more dry matter being built up in the roots than in the shoots, and thus, resulting in the development of remarkably longer roots (Ehrenberg and von Wettstein, 1955). With very few exceptions, greater variability was induced in the root length than in the shoot height by irradiating dormant embryos each treated generation (Tables 3 and 4). These data are supported by recent results obtained by Mericle, R. P. et al. (1964). From Tables 3 and 4 it can also be seen that at the 30,000 r dose level less variability was induced in shoot heights and root lengths than at some of the lower dosages, in all cases less than that induced at the 15,000 r exposure level, and in two instances less than that in the controls. Mericle, R. P. et al. (1964) have also observed similar results following 50,000 r of X-rays applied to dormant embryos of this same strain of barley. In the dry weights of seedlings from irradiated dormant embryos this scheme for the variability did not follow that of the growth measurements (Tables 6 and 7). In one case the induced variability at the 30,000 r dose level, however, was lower than that of the controls. In general, it can be seen from Figures 7B and 8B that the seedlings from dormant embryos exhibited growth responses directly related to the amount of irradiation applied. A

comparison of the growth inhibition (based upon both dry weights and height and length measurements) on the roots and shoots shows that, with one exception, roots are depressed to a greater degree than are the shoots (Tables 3, 4, 6, and 7). A closer examination shows that the maximum depression generally occurred at the 30,000 r exposure level each treated generation. With few exceptions, the other dose levels exhibited comparable maximum retardation values. Root and shoot dry weights of seedlings from embryos irradiated either as dry seed or during development did not behave identically (Figures 10C and 11C). However, there was closer agreement between roots and shoots of seedlings from irradiated dormant embryos than developing ones. This difference may or may not be due to sampling error or to the stage of life cycle in which the irradiation was applied.

Comparison of coleoptiles and roots

Irradiation applied at the embryonic stages of developing embryos produced seedlings in which the coleoptile heights and root lengths were reduced from those of the controls (Tables 2 and 4). Except for the early proembryo and late differentiation stage following the second irradiated generation this reduction was significant (1 percent level). In addition, it may also be observed that greater variability was induced in the roots than in the coleoptiles at each generation of radiation. In nearly all instances increased variability was about three- to four-fold. Following one, two, or three

generations of radiation the maximum depression from the controls was 25 percent, 18 percent, and 14 percent on the coleoptiles and 33 percent, 19 percent, and 24 percent on the roots. Thus, from these data it can be seen that the roots were reduced from the controls to a greater extent than the coleoptiles. Following irradiation at different embryonic stages the growth of coleoptiles showed differential stage specific responses, the early proembryo and late differentiation stages being more radiation sensitive than the late proembryo and early differentiation stages. This differential stage specificity was apparent each generation on coleoptiles (Figure 6A). In roots, however, this differential response was only observed following the third generation of radiation (Figure 8A). Following the first and second generations of radiation the root responses of the different embryonic stages appeared to show no specificity.

Different dose levels given dormant embryos generally produced seedlings in which the coleoptile heights and root lengths were directly correlated with the amount of irradiation given to the dry seed (Figures 6B and 8B). In no instances were stimulatory effects observed. In this respect, these data disagree with those of Breslavets (1946, 1960) and Kuzin (1956), who found longer and heavier roots from seeds X-irradiated with 2,000 r to 4,000 r as compared with controls. On the contrary, in this study, all dosages reduced the coleoptile heights and root lengths over that of the controls (Tables 2

and 4). With few exceptions, this reduction was significant (1 percent level). Following the first, second, and third generation of radiation the roots were depressed to a greater degree than were the coleoptiles, maximum depression being 25 percent, 27 percent, and 21 percent on coleoptiles and 32 percent, 36 percent, and 34 percent on roots. While the maximum depression of roots was greater than that of coleoptiles each generation, the roots entertained a difference of only 4 percent in maximum depression as compared with 6 percent on the coleoptiles. Of further interest is the fact that in both coleoptiles and roots maximum depression occurred at the 30,000 r dose level (Tables 2 and 4). Interestingly, the difference in the maximum deviations between the 30,000 r dose level and the lower exposure levels was 13 percent for coleoptiles and 12 percent for roots. Irradiation applied to either developing or dormant embryos gave rise to seedlings in which the root lengths exhibited a greater depression from the controls than the coleoptile heights (Tables 2 and 4). In addition, with few exceptions, both coleoptiles and roots were significantly reduced (1 percent level) from the controls following each successive generation of radiation.

Mature Plant Responses

Survival to maturity

The killing effect of non-survival at maturity is often used as a criterion in radiation studies. Following the

first generation of radiation of developing embryos the non-survival of plants at maturity exhibited an inverse correlation with degree of differentiation (Figure 12A). This is in agreement with the first generation data of Mericle and Mericle (1961). Following the second generation, however, the early differentiation stage showed a decrease in survival, while the late differentiation stage exhibited a slight decrease after the third irradiated generation. Thus, it may be seen that this inverse correlation did not occur with each treated generation. Following the first, second, and third successive generations of radiation non-survival ranged from 2 to 38 percent, 7 to 23 percent, and 10 to 20 percent, respectively (Table 8). A closer examination shows that lethality among generations were: early proembryo, 17 to 38 percent; late proembryo, 7 to 12 percent; early differentiation, 5 to 17 percent; and late differentiation, 2 to 13 percent. Thus, these data show that the early proembryo stage had a greater maximum and minimum death rate than the other embryonic stages over the three generations of radiation. The maximum non-survival of the other embryonic stages differed by only 4 to 5 percent, while their minimum differed by 2 to 5 percent. In general, when the survival to maturity (expressed as percent of control) is plotted against generation time (Figure 12C) all irradiated stages show a decrease in survival.

Dormant embryos irradiated at different dose levels

Table 8. Influence of X-irradiation on survival to maturity. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

	X-1			X-1/2			X-1/2/3		
	# Plted	# Survived	%	# Plted	# Survived	%	# Plted	# Survived	%
				Developing Embryos					
Control	80	80[b]	100	40	40[b]	100	25	25	100[b]
400r EP[a]	60	37[b]	62	40	33[b]	83	20	8	80[b]
400r LP	60	53	88	40	37	93	20	18	90[b]
400r ED	60	57	95	40	33	83	20	9	90[b]
400r LD	60	59	98	40	37	93	20	13	87[b]
				Dormant Embryos					
Control	110	110	100	90	90	100	45	45	100
4,000 r	90	89	99	60	58	97	30	30	100
7,500 r	90	81	90	60	56	93	30	29	97
12,500 r	90	82	91	60	57	95	30	29	97
15,000 r	90	80	89	60	57	95	30	27	90
30,000 r	90	79	88	60	42	70	30	26	87

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation.

[b]Sets were discarded due to heavy damage by powdery mildew and aphids. Results based on limited number of plants.

Figure 12A. Influence of X-irradiation on survival to maturity, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

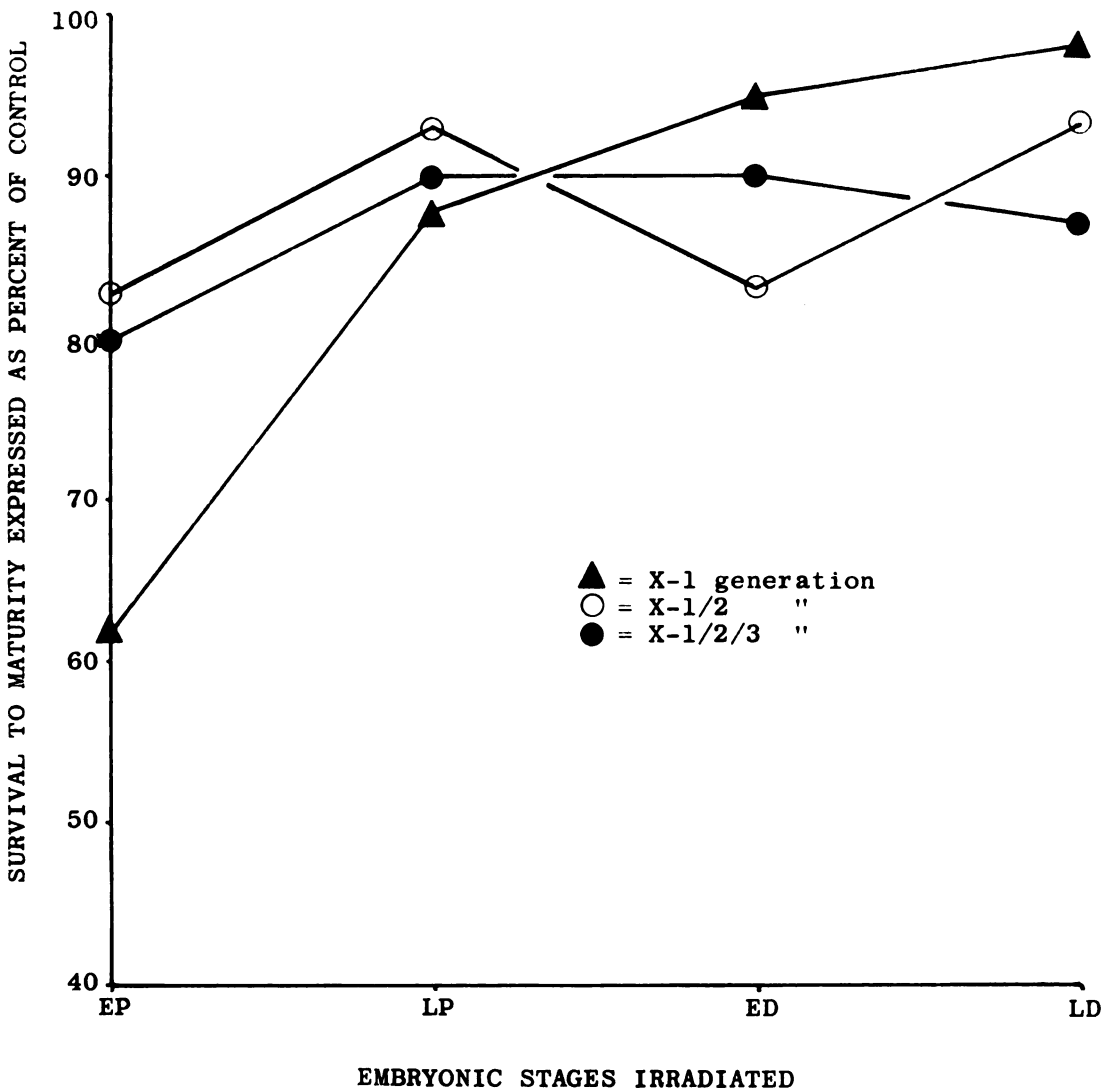
100
90
80
70
60
50
40
SURVIVAL TO MATURITY EXPRESSED AS PERCENT OF CONTROL
▲ = X-1 generation
○ = X-1/2 "
● = X-1/2/3 "
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 12A

Figure 12B. Influence of X-irradiation on survival to maturity, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

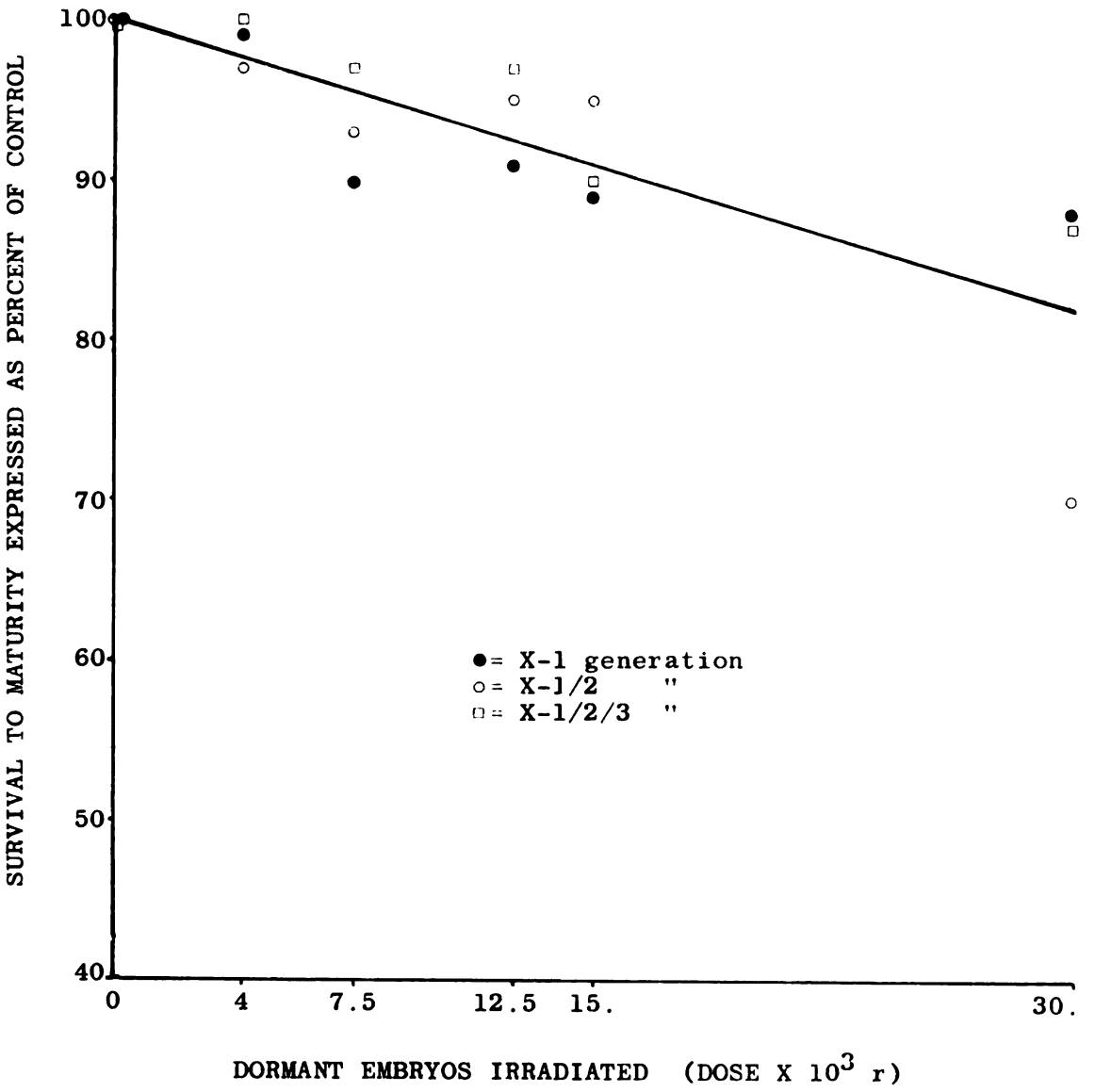
SURVIVAL TO MATURITY EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
0
4
7.5
12.5
15.
30.
●= X-1 generation
○= X-1/2 "
□= X-1/2/3 "
DORMANT EMBRYOS IRRADIATED (DOSE X 10^3 r)

Figure 12B

Figure 12C. Influence of X-irradiation on survival to maturity, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

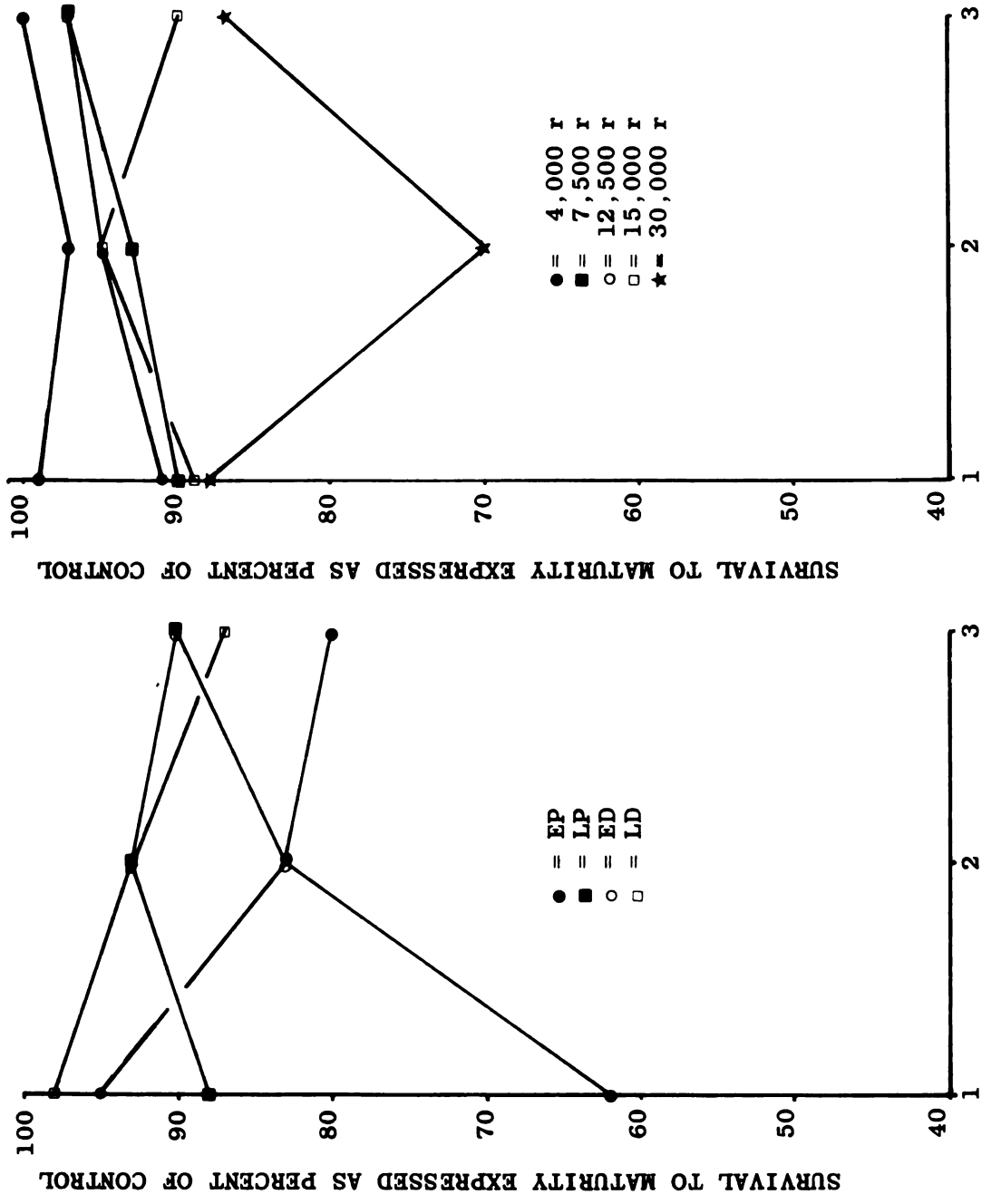
SURVIVAL TO MATURITY EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DORMANT EMBRYOS
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r
SURVIVAL TO MATURITY EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
DEVELOPING EMBRYOS
● = EP
■ = LP
○ = ED
□ = LD
GENERATIONS IRRADIATED

Figure 12C

produced plants which showed non-survival ranges at maturity of 1 to 12 percent, 3 to 30 percent, and 3 to 13 percent following one, two, or three generations, respectively (Table 8). Thus, while non-survival was in good agreement after the first and third generations of radiation, the non-survival in the second generation increased 17 to 18 percent over that of the first and third generations, the increase being due to the 30,000 r dose level (Table 8, Figure 12C). Examination of the individual dose levels among generations shows that the non-survival ranges were: 4,000 r, 0 to 3 percent; 7,500 r, 3 to 10 percent; 12,500 r, 3 to 9 percent, 15,000 r, 5 to 11 percent; and 30,000 r, 12 to 30 percent. From these data it can be seen that when survival to maturity (expressed as percent of control) is plotted against dose level the effect of irradiation on lethality is related to the total dosage applied to the seeds (Figure 12B). In this respect these data disagree with those of Caldecott (1953), Bhaskaran and Swaminathan (1962), and MacKey (1951), all of whom found a sigmoid type of curve. Survival, although higher in this study than that reported by Ehrenberg (1955), is slightly less than that observed by Notani and Gaur (1962) and Sarvella et al. (1962). Further comparison at the 30,000 r dose level is lower than the maximum non-survival observed at the lower exposure levels. In addition, maximum lethality at the 30,000 r dose level is 19 to 27 percent lower than that at the lower dose levels. When survival to maturity

(expressed as percent of control) is plotted against generation time (Figure 12C) the 7,500 r and 12,500 r dose levels show an increase in survival with each succeeding irradiated generation. At the 15,000 r exposure level survival increased following the second generation of radiation followed by a decrease after the third treated generation. In contrast, the 4,000 r and 30,000 r exposure levels exhibited a decrease after the second generation followed by an increase after the third irradiated generation.

Interestingly, the lethality of plants from dormant embryos given 30,000 r dose level falls within the range exhibited by developing embryos given 400 r of X-rays at the early proembryo stage (Table 8). Maximum non-survival at the early proembryo stage, however, was 8 percent greater than the maximum at the 30,000 r dose level. In addition, it is noted that the maximum lethality at the late proembryo and late differentiation stages is 2 to 4 percent greater than that at the 7,500 r, 12,500 r, and 15,000 r exposure levels applied to dormant embryos. Viewed from an overall standpoint lethality of plants derived from seed irradiated during embryonic development was higher each generation than that among plants produced from dormant embryos exposed to different dose levels. In addition, survival to maturity of plants from the irradiated stages decreased with an increasing number of generations, while survival of plants from irradiated dry seed did not show any major effect

(Figure 12C). Thus, in using survival to maturity as a criterion to measure the radiation sensitivity it appears that irradiation applied to developing embryos is more effective than when dry, dormant seeds are irradiated.

Fertility

Fertility of the plants from seeds irradiated during their embryonic development was, with one exception significantly less (1 percent level) than that of the controls following each generation (Table 9). In addition, the variability induced by irradiating the embryonic stages ranged from one and one-half to four times that observed in the controls. Among the embryonic stages, however, the variability fluctuated considerably. With one exception, the standard deviations were greater following irradiation at the early proembryo stage. Following one, two, or three successive generations of radiation the maximum and minimum spike fertilities among these embryonic stages were: 66 to 76 percent; 68 to 84 percent; and 75 to 89 percent, respectively. From these data plotted against generation time (Figure 13C) it can be observed that fertility is, with few exceptions, increasing with each treated generation. These data support those of Yamaguchi (1962). When these data (expressed as percent of control) are plotted against embryonic stage (Figure 13A) there is a tendency, except for the early differentiation stage following the first and third generations of radiation, for the spike fertility to

Table 9. Influence of X-irradiation on the number of fertile florets per spike. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	80	19.1	0.26	2.37	100	40	19.6	0.39	2.47	100	25	21.8	0.43	2.18	100
400r EP[a]	35	12.7**	0.83	4.87	66	32	13.3**	1.35	7.67	68	8	16.4**	3.11	8.81	75
400r LP	50	14.3**	0.78	5.54	75	34	13.8**	1.16	6.76	70	18	18.6**	1.52	6.25	85
400r ED	55	13.2**	0.72	5.21	69	30	16.4**	1.21	6.66	84	9	17.7**	1.86	5.58	81
400r LD	58	14.6**	0.59	4.48	76	36	15.4**	0.61	3.65	79	13	19.3[b]	1.69	6.11	89
Dormant Embryos															
Control	110	17.0	0.07	2.24	100	90	17.5	0.27	2.57	100	45	16.3	0.50	3.13	100
4,000 r	89	16.3[b]	0.37	3.47	96	58	16.0**	0.40	3.02	91	30	15.3[b]	0.62	3.42	94
7,500 r	81	14.5**	0.48	4.36	85	55	16.9[b]	0.50	3.67	97	29	15.3[b]	0.63	3.42	94
12,500 r	78	13.8**	0.57	5.01	81	56	14.4**	0.64	4.78	82	28	13.6**	0.58	3.08	83
15,000 r	79	14.5**	0.45	4.03	85	57	15.2**	0.50	3.75	87	27	13.4**	1.08	5.61	82
30,000 r	75	13.1**	0.51	4.39	77	36	9.5**	0.93	5.59	54	24	9.8**	0.93	4.56	60

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation, stages.
[b]Not significant from the controls
* $P < 5\%$
** $P < 1\%$

Figure 13A. Influence of X-irradiation on spike fertility, expressed as percent of control. (Irradiated at specific stages of embryogeny in each of three successive generations).

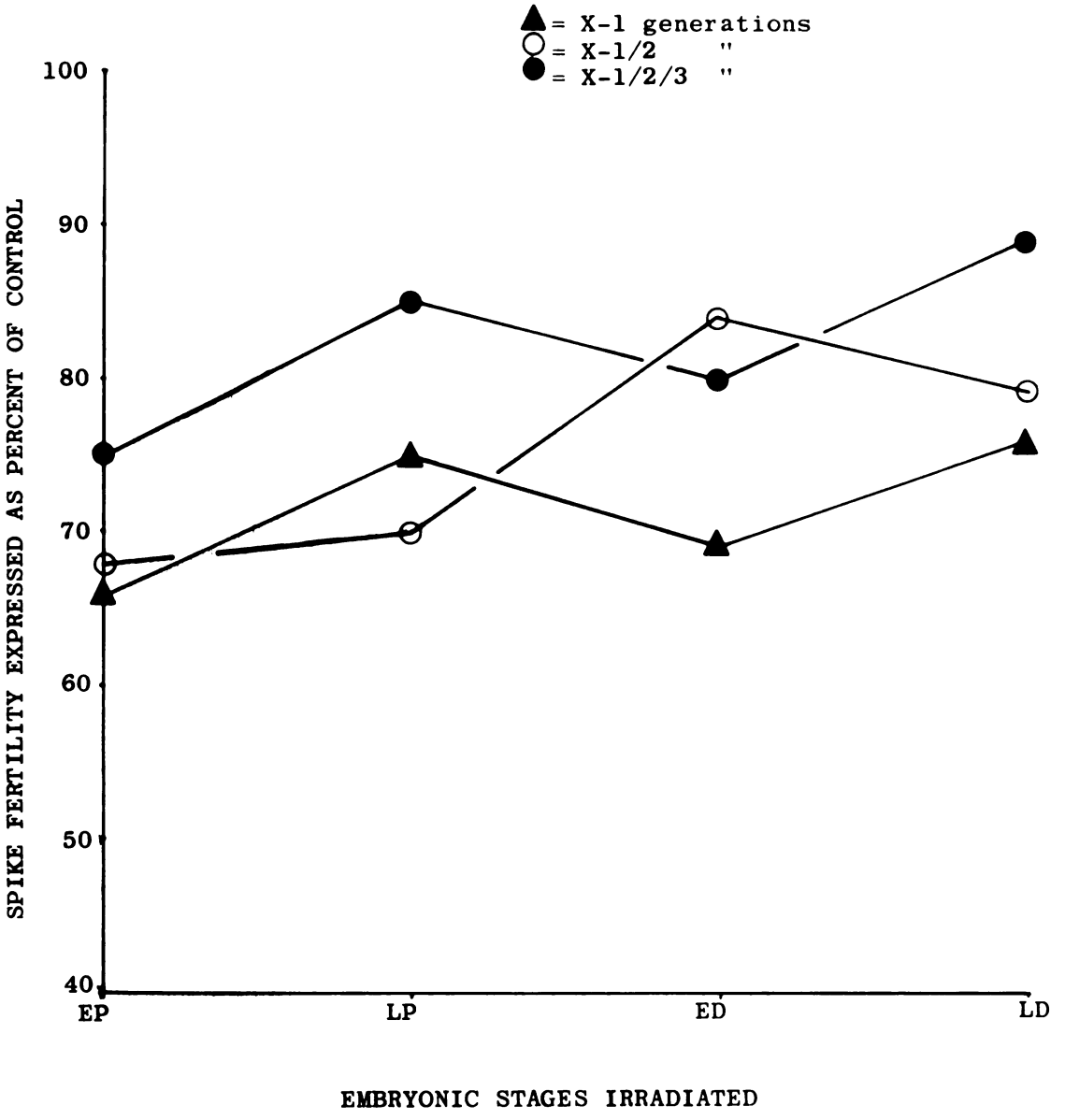
▲ = X-1 generations
○ = X-1/2 "
● = X-1/2/3 "
100
90
80
70
60
50
40
SPIKE FERTILITY EXPRESSED AS PERCENT OF CONTROL
EP
LP
ED
LD
EMBRYONIC STAGES IRRADIATED

Figure 13A

Figure 13B. Influence of X-irradiation on spike fertility, expressed as percent of control. (Irradiated as dry seed in each of three successive generations).

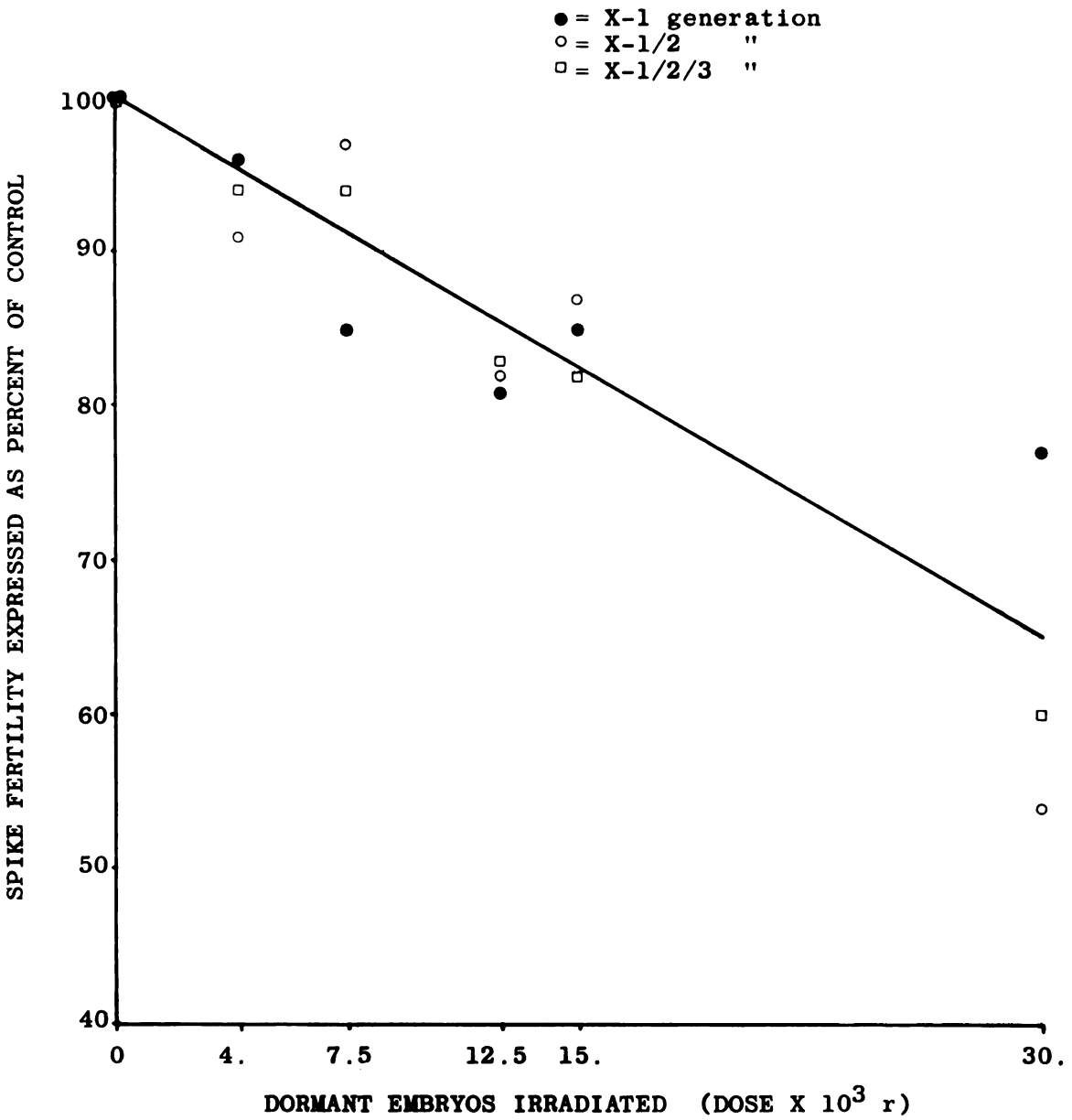
● = X-1 generation
○ = X-1/2 "
□ = X-1/2/3 "
SPIKE FERTILITY EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
0
4.
7.5
12.5
15.
30.
DORMANT EMBRYOS IRRADIATED (DOSE X 10³ r)

Figure 13B

Figure 13C. Influence of X-irradiation on spike fertility, expressed as percent of control. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

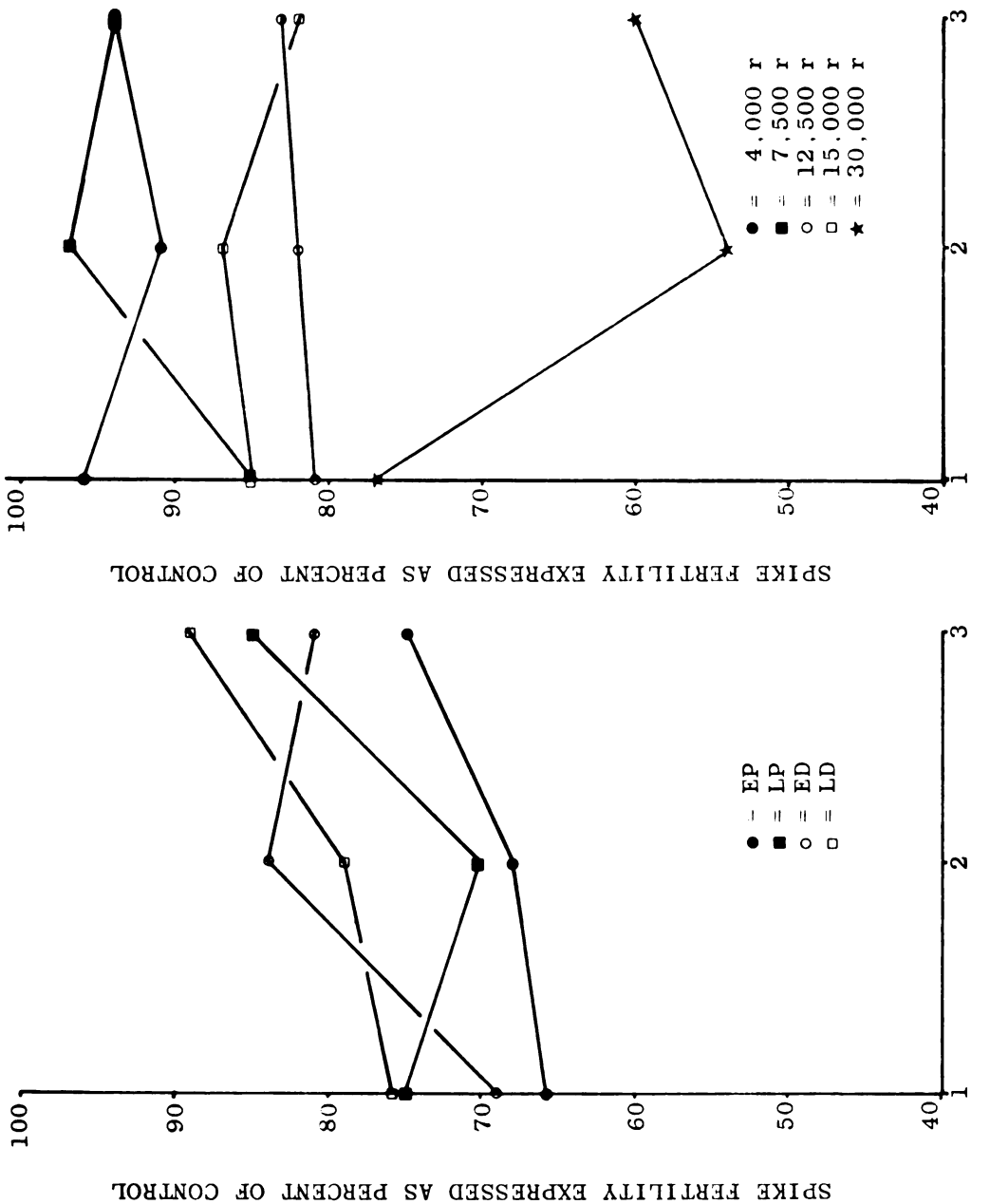
SPIKE FERTILITY EXPRESSED AS PERCENT OF CONTROL
100
90
80
70
60
50
40
1
2
3
● = EP
■ = LP
○ = ED
□ = LD
DEVELOPING EMBRYOS
GENERATIONS IRRADIATED
SPIKE FERTILITY EXPRESSED AS PERCENT OF CONTROL
● = 4,000 r
■ = 7,500 r
○ = 12,500 r
□ = 15,000 r
★ = 30,000 r
DORMANT EMBRYOS

Figure 13C

be inversely correlated with the degree of differentiation. After the second generation of radiation, however, it is the late differentiation stage that does not follow this inverse correlation. Fertility ranges were: early proembryo, 66 to 75 percent; late proembryo, 70 to 85 percent; early differentiation, 69 to 84 percent; and late differentiation, 76 to 89 percent (Table 9). The greatest reduction in spike fertility occurs after irradiation of the early proembryo stage with the least reduction after the late differentiation stage. In addition, the maximum and minimum spike fertilities are almost equal following irradiation of the late proembryo and early differentiation stages.

From Table 9 it can be seen that, with few exceptions, the fertility of plants derived from dry, dormant seed exposed to different levels of radiation was significantly reduced (1 percent level) from the controls. Following the first, second, and third generations of radiation the maximum and minimum fertilities among these different levels of radiation ranged from: 77 to 96 percent, 54 to 97 percent, and 60 to 94 percent, respectively. Thus, while the minimum reduction in fertility showed a difference of 1 to 3 percent, the maximum reduction was 17 to 23 percent over the three treated generations. When spike fertility (expressed as percent of control) is plotted against generation time (Figure 13C) the 12,500 r dose level shows an increase with each treated generation. The 7,500 r and 15,000 r exposure levels

exhibited an increase in fertility after the second irradiated generation but showed a decrease in the third treated generation. In contrast, the 4,000 r and 30,000 r dosages decreased following the second successive generation of radiation and increased after the third successively treated generation. This reduction as well as the subsequent increase was greater at the 30,000 r than at the 4,000 r dose level. Thus, it can be seen (Figure 13C) that there was considerable fluctuations in the spike fertility of the individual dose levels. This fact is again substantiated by the amount of variability induced by irradiation at the different dose levels, variability being greater than the controls in all cases and, in general, increasing as the level of radiation increased. When the fertility (expressed as percent of control) is plotted against dose level (Figure 13B), effect of irradiation is related to the total amount of radiation applied to the dry seed each generation. Spike fertility ranges in plants from seed exposed at the different dose levels were: 4,000 r, 91 to 96 percent; 7,500 r, 85 to 97 percent; 12,500 r, 81 to 83 percent; 15,000 r, 82 to 87 percent; and 30,000 r, 54 to 77 percent over the three treated generations (Table 9). From these data it can be seen that the highest degree of fertility following irradiation at the 30,000 r dose level is 4 to 14 percent lower than the lowest degree of fertility at the other exposure levels. These fertility data are comparable, in part, to

those of Ehrenberg (1955).

When irradiation of developing embryos is compared with irradiated dormant embryos (Table 9), the fertility of the plants was in all cases reduced from that of the controls. With few exceptions this reduction was significant (1 percent level). Following one, two, or three successive generations of radiation the spike fertility ranged from 66 to 76 percent; 68 to 84 percent; and 75 to 89 percent among the embryonic stages and from 77 to 96 percent; 54 to 97 percent; and 60 to 94 percent among the dormant embryos. Thus, while the overall fertility among embryonic stages increased after three successive generations of radiation that among the different dose levels generally decreased. On the other hand, the spike fertility among generations ranged from 66 to 75 percent, at the early proembryo; 70 to 85 percent, at the late proembryo; 69 to 84 percent, at the early differentiation; and 76 to 89 percent, at the late differentiation, stages and from 91 to 96 percent, at the 4,000 r; 85 to 97 percent, at the 7,500 r; 81 to 83 percent, at the 12,500 r; 82 to 87 percent, at the 15,000 r; and 54 to 77 percent, at the 30,000 r exposure levels. Therefore, the reduction in spike fertility was greatest following irradiation at the 30,000 r dose level (on dormant embryos) and at the early proembryo stage of developing embryos. It is of further interest to note that, with the exception of the 30,000 r dose level given dormant embryos, spike fertility was reduced to a

greater degree following irradiation of the embryonic stages. In addition, Table 9 shows that, with few exceptions, the amount of variation induced by irradiation was generally greater after the irradiation of developing embryos than following the irradiation of dry seed materials. Further comparison shows that the reduction in fertility of dormant embryos was, in general, directly related to the increase in dose level (Figure 13C). On the other hand, the reduction in fertility following irradiation of developing embryos appeared, in general, to be inversely correlated with the stage of embryonic development (Figure 13A). When fertilities (expressed as percent of control) were plotted against generation time (Figure 13C) it is seen that the fertilities of the populations were not reduced with a recurrence of irradiation. On the contrary, a slight increase was noted. Thus, it seemed that with repeated irradiation in successive generations of developing or dormant embryos the seed setting ability did not become more sensitive to the treatment. However, it should be pointed out that due to the limited number of plants available in the third generation these results may offer only a suggestion concerning the seed setting ability of plants whose germ line has been exposed to recurrent irradiation in successive generations.

Total number of florets per main spike per plant

Data from Table 10 indicates that the average number of

Table 10. Influence of X-irradiation on the average total number of florets per spike. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

	X-1					X-1/2					X-1/2/3				
	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C	N	$\bar{x} \pm$	SE	SD	%C
Developing Embryos															
Control	80	22.2	0.31	2.78	100	40	22.9	0.42	2.67	100	25	26.3	0.37	1.92	100
400r EP[a]	37	19.6**	0.61	3.73	88	33	18.2**	1.52	8.72	79	8	22.4**	2.11	5.98	85
400r LP	53	20.6**	0.30	2.18	93	37	21.1[b]	0.82	4.94	92	18	25.9[b]	0.62	2.64	98
400r ED	57	18.7**	0.40	3.06	84	33	22.6[b]	0.67	3.88	99	9	25.9[b]	1.06	3.17	98
400r LD	59	20.2**	0.43	3.30	91	37	21.7*	0.35	2.15	95	13	26.1[b]	0.82	2.96	99
Dormant Embryos															
Control	110	20.7	0.17	1.82	100	90	21.2	0.20	1.93	100	45	20.8	0.31	2.07	100
4,000 r	89	21.7**	0.22	2.08	105	58	21.3[b]	0.20	1.54	100	30	21.1[b]	0.34	1.87	101
7,500 r	81	20.7[b]	0.27	2.46	100	56	21.9[b]	0.32	2.40	103	29	21.0[b]	0.30	1.63	101
12,500 r	82	20.7[b]	0.44	4.01	100	57	21.3[b]	0.30	2.30	100	29	21.0[b]	0.42	2.27	101
15,000 r	80	20.4[b]	0.31	2.82	99	57	21.9*	0.29	1.99	103	27	20.8[b]	0.60	3.06	100
30,000 r	79	19.8*	0.41	3.68	96	42	18.4**	0.65	4.24	87	26	19.9[b]	0.67	3.42	96

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; LD, late differentiation, stages.
[b]Not significant from the controls
* P < 5%
** P < 1%

Table 11. Summary of significance for Tables 2, 3, 4, 5, 6, 7, 9, and 10

Generation	Criteria	Developing Embryos				Dormant Embryos				
								X10^3		
		EP[a]	LP	ED	LD	4.	7.5	12.5	15.	30.
X-1	Coleoptile	-**	-**	-**	-**	-**	-**	-**	-**	-**
	Shoot height	-**	-**	-**	-**	-**	-**	-**	-**	-**
	Root length	-**	-**	-**	-**	-**	-**	-**	-**	-**
	No. of roots	-**	-**	-**	-**	0	-*	-**	-**	0
	Dry wt. shts.	-**	-**	0	-**	-**	-**	-**	-**	-**
	Dry wt. rts.	0	0	0	-*	-**	-**	-**	-**	-**
	Total Florets	-**	-**	-**	-**	+**	0	0	0	-*
	Fert. Florets	-**	-**	-**	-**	0	-**	-**	-**	-**
X-1/2	Coleoptile	-**	-**	-**	-**	0	-**	-**	-**	-**
	Shoot height	0	-**	-**	-**	-**	-**	-**	-**	-**
	Root length	0	-**	-**	-*	-**	-**	-**	-**	-**
	No. of roots	-**	-**	-**	-**	0	0	0	0	-**
	Dry wt. shts.	-**	0	-**	-**	0	0	-*	0	-**
	Dry wt. rts.	-**	-**	-**	-**	0	-**	-**	-*	-**
	Total Florets	-**	0	0	-*	0	0	0	+*	-**
	Fert. Florets	-**	-**	-**	-**	-**	0	-**	-**	-**

Table 11. Continued

Generation	Criteria	Developing Embryos				Dormant Embryos				
								X10^3		
		EP[a]	LP	ED	LD	4	7.5	12.5	15.	30
X-1/2/3	Coleoptile	-**	-**	-**	-**	0	0	-**	-**	-**
	Shoot height	-**	-**	-**	-**	0	-*	-**	-**	-**
	Root length	-**	-**	-**	-**	-**	-*	-**	-**	-**
	No. of roots	-**	-**	-**	-**	0	0	+**	0	-**
	Dry wt. shts.	-**	-**	-**	-**	0	0	0	0	-**
	Dry wt. rts.	-**	-**	0	-**	-*	0	-*	0	-**
	Total Florets	-**	0	0	0	0	0	0	0	0
	Fert. Florets	-**	-*	-**	0	0	0	-**	-**	-**

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; and LD, late differentiation, stages.
- = Less than that of the control.
+ = Greater than that of the control.
0 = Not significant from the control.
* P < 5%
** P < 1%

Table 12. Influence of X-irradiation on root-shoot (dry weight) ratios at specific stages of embryogeny and on dry seeds in each of three successive generations.

Generation treatment	X-1	X-1/2	X-1/2/3
		Developing Embryos	
Control	0.49	0.78	0.59
400r EP[a]	0.65	0.60	0.55
400r LP	0.64	0.46	0.48
400r ED	0.48	0.48	0.62
400r LD	0.57	0.57	0.53
		Dormant Embryos	
Control[b]	0.64	0.73	0.73
Control[c]	0.68	0.64	0.90
4,000 r	0.60	0.59	0.72
7,500 r	0.65	0.64	0.57
12,500 r	0.60	0.54	0.76
15,000 r	0.64	0.69	0.61
30,000 r	0.66	0.82	0.56

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; and LD, late differentiation, stages.
[b]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[c]Control for 4,000 r and 12,500 r dose levels.

total florets per main spike of plants from seed which received 400 r of X-irradiation during their embryonic development was less than that of the controls each treated generation. The reduction in the mean number of total florets per spike was significant (1 percent level) at all irradiated stages following the first generation of radiation. After the second and third successively treated generations, however, only plants from seed treated during the early proembryo stage of embryonic development maintained this level of significance. Except for the late proembryo stage following the first generation of radiation and the late differentiation stage after the second treated generation, the induced variability was greater than that of the controls each irradiated generation. Treatment at the early proembryo stage, however, showed the greatest degree of induced variability of all the embryonic stages treated following each generation of radiation. Following one, two, or three successive generations of radiation the average number of total florets per spike among embryonic stages were: 84 to 93 percent, 79 to 99 percent, and 85 to 99 percent, respectively. A closer examination of the data shows that the total number of florets per spike among generations were: early proembryo, 79 to 88 percent; late proembryo, 92 to 98 percent; early differentiation, 84 to 99 percent; and late differentiation, 91 to 99 percent. These data show that the mean number of total florets per spike was reduced to the

greatest degree following irradiation at the early proembryo stage. The total number of florets per spike at the other irradiated stages is much the same.

After the radiation of dormant embryos at the different dose levels the average number of total florets per main spike per plant was, except for the 30,000 r dose level, equal to or slightly higher than those of the controls following each generation of radiation (Table 10). The ranges (expressed as percent of control) at the various exposure levels were: 4,000 r, 100 to 105 percent; 7,500 r, 100 to 103 percent; 12,500 r, 100 to 101 percent; 15,000 r, 99 to 103 percent; and 30,000 r, 87 to 96 percent among generations. Thus, irradiation has had a slight stimulatory effect on the total number of florets per spike. These data agree with those of Saric (1958a). In addition, the greatest reduction in total number of florets per spike occurred at the 30,000 r dose level following each generation of radiation. Table 10 also shows that following the first and second treated generations the total number of florets per spike was significantly reduced from the controls. Furthermore, with few exceptions, the degree of induced variability was greater than that of the controls and increased each generation as the level of radiation increased.

It is apparent (Table 10) that irradiation applied to dormant embryos does not have as great an effect on the total number of florets per spike per plant as when applied to

developing embryos. The radiation sensitivity of developing embryos, as measured by the average number of total florets per spike, appears to be inversely correlated with the degree of differentiation. In contrast, the radiosensitivity of dormant embryos is not affected by dose levels at or below 15,000 r. In fact there appears to be a slight stimulation effect. In this respect these data agree with those of Saric (1958a). There was, however, a decreased number of florets per spike at the 30,000 r exposure level ranging from 4 to 13 percent below that of the controls over the three treated generations. These data also show that irradiation of the early proembryo stage of developing embryos exhibits a greater reduction in the mean number of florets per spike than any dose level given dormant embryos. Irradiation of other embryonic stages also shows a greater reduction in the total number of florets per spike than that observed by any exposure levels to dormant seeds at or below 15,000 r. In addition, variability induced was greater each generation following irradiation at the early proembryo stage than at any exposure level applied to dormant embryos. Variability of the other embryonic stages was, with few exceptions, comparable to that of the 30,000 r dose level given dormant embryos. While there was a slight increase in the mean number of total florets per main spike per plant following irradiation of dry seed the fertility generally was directly correlated with the increase in the level of radiation (Figure 13B). Hence, as

was found by Saric (1958a) the stimulative effect of irradiation for the production of extra yield of grain does not appear profitable at this time. These data contradict those of Kuzin (1956), who found that a dose range of 750 r to 1,000 r of X-rays applied to seeds of rye increased the subsequent generation yield by 21 percent.

Abnormalities

Lethality at germination

Lethality at germination was a rarity in control plants and very seldom occurred at the lower dose levels applied to dormant embryos after the first generation of radiation (Table 13). In this respect these data are in agreement with those of other workers (Natarajan and Maric, 1961; Ehrenberg, 1955; Saric, 1958b; and others). Following one, two, or three generations of radiation, however, there was a slight tendency for maximum germination lethality to increase each treated generation, being 5 percent, 7 percent, and 10 percent among dose levels, respectively. This tendency to increase agrees with the results of Abrams and Frey (1958). Lethality ranges at the different dose levels among generations were: 4,000 r, 0 to 4 percent; 7,500 r, 2 to 6 percent; 12,500 r, 0 to 5 percent; 15,000 r, 5 to 10 percent; and 30,000 r, 1 to 10 percent. Thus the lethality among generations increased slightly as the level of radiation increased. The maximum lethalities at the three lowest dose levels are almost identical.

Table 13. Seedling abnormalities observed following irradiation of embryos during either their development or dormancy in each of three successive generations.

	# Seedlings	Lethal	% Semi-lethal	% Leaf abnorm.	% Cleft Coleoptile		
					Single	Double	Partial
				X-1			
				Developing Embryos			
Control	122	2.00	0.00	0.00	0.00	0.00	0.00
400r EP[a]	86	23.00	36.04	0.00	0.00	0.00	0.00
400r LP	116	1.00	16.38	2.38	7.70	0.61	1.21
400r ED	122	5.00	5.74	1.64	25.40	1.60	15.60
400r LD	101	4.00	10.89	0.00	3.00	1.00	3.01
				Dormant Embryos			
Control[b]	288	0.00	0.00	0.00	0.00	0.00	0.00
Control[c]	190	0.00	0.00	0.00	0.00	0.00	0.00
4,000 r	188	0.00	0.53	0.00	0.00	0.00	0.00
7,500 r	278	2.00	2.88	0.00	0.00	0.00	0.00
12,500 r	188	0.00	7.44	0.00	0.00	0.00	0.00
15,000 r	263	5.00	6.46	0.00	0.00	0.00	0.00
30,000 r	267	5.00	7.13	0.37	0.00	0.00	0.00

Table 13. Continued

	# Seedlings	Lethal	% Semi-lethal	% Leaf abnorm.	% Cleft Coleoptile Single	Double	Partial
				X-1/2 Developing Embryos			
Control	143	1.00	0.00	0.00	0.00	0.00	0.00
400r EP[a]	53	17.00	13.20	1.89	0.00	0.00	2.20
400r LP	60	8.00	13.32	0.00	4.20	0.00	2.11
400r ED	94	8.00	13.82	0.00	0.00	0.00	0.00
400r LD	94	8.00	7.45	0.00	0.00	0.00	0.00
				Dormant Embryos			
Control[b]	152	0.00	0.00	0.00	0.00	0.00	0.00
Control[c]	189	0.00	0.53	0.00	0.00	0.00	0.00
4,000 r	181	4.00	4.42	0.00	0.00	0.00	0.00
7,500 r	137	6.00	3.64	0.00	0.00	0.00	0.00
12,500 r	189	0.00	3.70	0.00	0.00	0.00	0.00
15,000 r	140	7.00	5.00	0.00	0.00	0.00	0.00
30,000 r	148	1.00	21.60	0.00	0.00	0.00	0.00

Table 13. Continued

	# Seedlings	Lethal	% Semi-lethal	% Leaf abnorm.	% Cleft Coleoptile		
					Single	Double	Partial
			X-1/2/3				
			Developing Embryos				
Control	79	1.00	0.00	0.00	0.00	0.00	0.00
400r EP[a]	54	26.00	14.81	0.00	0.00	0.00	0.00
400r LP	58	3.00	8.63	5.17	10.41	0.00	4.20
400r ED	55	10.00	14.51	1.82	4.90	0.00	2.52
400r LD	51	15.00	15.69	0.00	0.00	0.00	0.00
			Dormant Embryos				
Control[b]	206	4.00	0.00	0.00	0.00	0.00	0.00
Control[c]	110	1.00	0.00	0.00	0.00	0.00	0.00
4,000 r	112	3.00	0.00	0.00	0.00	0.00	0.00
7,500 r	208	5.00	0.96	0.00	0.00	0.00	0.00
12,500 r	106	5.00	2.83	0.00	0.00	0.00	0.00
15,000 r	188	10.00	11.19	0.00	0.00	0.00	0.00
30,000 r	197	10.00	12.69	0.00	0.00	0.00	0.00

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; and Ld, late differentiation, stages.
[b]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[c]Control for 4,000 r and 12,500 r dose levels.

Following the first, second, and third successive generations of radiation lethality at germination among irradiated stages of developing embryos ranged from 1 to 23 percent, 8 to 17 percent, and 3 to 26 percent, respectively. After the first and third successively treated generations the maximum and minimum lethalities were comparable. Among generations the lethality ranges were: early proembryo, 17 to 26 percent; late proembryo, 1 to 8 percent; early differentiation, 5 to 10 percent; and late differentiation 4 to 15 percent. Thus, the early proembryo and late differentiation stages showed greater lethality at germination than the late proembryo and early differentiation stages. Maximum lethality at the late proembryo and early differentiation stages showed very good agreement, differing by only 2 percent. The minimum lethality at the late differentiation stage exhibited good agreement with that of the early differentiation stage, being 1 percent less. It is of further interest to note that the minimum lethality following irradiation at the early proembryo stage was 2 percent greater than the maximum lethality observed at the late differentiation stage and 7 to 9 percent greater than the other irradiated stages. Further examination of the maximum lethalities of the early proembryo and late differentiation stages shows that lethality at the early proembryo stage was 11 percent greater than that observed at the late differentiation stage and 16 to 18 percent greater than the maximum lethalities at the other treated embryonic stages.

These data, in part, agree with those of Mericle and Mericle (1961).

Among generations, the maximum and minimum lethalities following irradiation at the late proembryo, early and late differentiation stages of developing embryos are comparable to those shown by the 15,000 r and 30,000 r dose levels given dormant embryos (Table 13). Further, germination lethality increased with each treated generation at the early and late differentiation stages, while only the 15,000 r exposure level given dormant embryos showed a slight increase with each generation of radiation.

Semi-lethality

Plants having only coleoptiles, shoot, and/or roots, albinos, and those failing to survive to maturity in the greenhouse have been classified as semi-lethals. Semi-lethality of seedlings ranged from 6 to 36 percent, 7 to 14 percent, and 9 to 16 percent following one, two, or three successive generations of radiation on embryos during their embryonic development. Among generations, however, semi-lethality ranges were: early proembryo, 13 to 36 percent; late proembryo, 9 to 16 percent; early differentiation, 6 to 15 percent; and late differentiation, 7 to 16 percent. Maximum seedling lethality was observed after irradiation of the early proembryo stage. Furthermore, it was 20 to 21 percent greater than that observed at any other irradiated stages spanning the three generations. On the other hand,

the maximum and minimum semi-lethalities following irradiation of the late proembryo, early and late differentiation stages were nearly equal. Following the first generation of radiation at the early proembryo stage semi-lethality decreased and remained relatively constant thereafter (Table 13). Also, following each successive generation of radiation semi-lethality decreased only at the late proembryo stage and increased at the early differentiation stage. At the late differentiation stage it decreased after the second irradiated generation but increased again following the third treated generation.

Semi-lethality ranges among generations following irradiation at the different dose levels given dormant embryos were: 4,000 r, 0 to 4 percent; 7,500 r, 1 to 4 percent; 12,500 r, 3 to 7 percent; 15,000 r, 5 to 11 percent; and 30,000 r, 7 to 12 percent. Thus, the maximum semi-lethality observed at the different dose levels increased as the level of radiation increased. Maximum and minimum semi-lethalities at the 4,000 r and 7,500 r exposure levels showed very good agreement, while those at the 12,500 r and 15,000 r dose levels were near to one another. At the 30,000 r dose level the minimum semi-lethality, with one exception, was as great or greater than the maximum semi-lethality observed at the other exposure levels. Maximum semi-lethality at the 30,000 r dose level, however, ranged from 11 to 18 percent greater than that of all other dose levels.

In comparing the semi-lethality of seedlings arising from embryos irradiated during their development or dormancy it can be seen that the maximum seedling death at the early proembryo stage is much greater than that observed from different dose levels applied to dormant embryos (Table 13). Maximum semi-lethality observed from irradiation of the late proembryo, early, and late differentiation stages 4 to 5 percent greater than that at the 15,000 r dose level and 8 to 11 percent greater than that at the three lowest exposure levels. Except for the 30,000 r dose level, the least semi-lethality observed at the early proembryo stage was greater than the maximum occurring following irradiation of dormant embryos.

Seedling abnormalities

Leaf. One type of leaf abnormality not noted previously has been described as a split first leaf (i.e., a split, either partially or entirely down the first leaf occurring on either side of the midvein). This abnormality occurred following irradiation at the late proembryo and early differentiation stages in the first and third generations and also occurred after irradiating the early proembryo stage in the second generation. The frequency of this leaf defect increased only slightly when irradiation was applied to the early differentiation stage but increased two-fold in seedlings irradiated at the late proembryo stage. Other leaf abnormalities observed following irradiation of the developing embryos

were the appearance of "dwarf" and "grassy" individuals and three plants with tubular or onion-like leaves. These latter leaf abnormalities were first noted and described by Mericle and Mericle (1957). The dwarf and grassy individuals and two of the plants with tubular seedling leaves appeared after irradiating the late proembryo stage in the first generation. The third plant with a tubular leaf appeared in plants arising from embryos irradiated at the late differentiation stage of development in the second generation. This leaf defect appears to be specific for plants that have arisen from embryos irradiated during their embryonic development since it was completely absent in plants from irradiated dry seed.

Coleoptile. The "coleoptile-only" (i.e., coleoptile present, but no shoot) abnormality appeared in seedlings arising from embryos irradiated either during their development or dormancy (Table 14). It occurred, however, with a much greater frequency after the embryos were irradiated during their embryonic development. Following each succeeding generation this mutation increased in frequency when the embryos were given 15,000 r and 30,000 r of X-rays during their dormancy following each succeeding generation of radiation. At the lower dose levels it occurred only following the second and third irradiated generations. In contrast to the increased frequency of the coleoptile-only mutation observed at the 15,000 r and 30,000 r exposure levels, the

Table 14. Seedlings having coleoptiles only (no shoots) and albino mutations. (Irradiated at specific stages of embryogeny and as dry seed in each of three successive generations).

	X-1		X-2		X-1/2		X-2		X-1/2/3		X-2	
	% Col. only	% Alb.	% Col. only	% Alb.	% Col. only	% Alb.	% Col. only	% Alb.	% Col. only	% Alb.	% Col. only	% Alb.
						Developing Embryos						
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
400r EP[a]	9.30	0.00	0.00	0.00	2.78	0.00	0.00	0.00	1.85	0.00	0.00	0.00
400r LP	6.54	0.00	0.00	0.00	5.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00
400r ED	1.64	0.00	0.00	0.00	3.19	0.00	0.00	0.00	3.64	0.00	0.00	0.00
400r LD	6.28	0.00	0.00	0.00	3.19	0.00	2.00	0.00	3.92	0.00	0.00	0.00
						Dormant Embryos						
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,000 r	0.00	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7,500 r	0.00	0.00	2.00	0.00	0.72	0.73	0.00	0.00	0.48	0.00	0.00	2.00
12,500 r	0.00	0.00	2.00	0.00	1.59	2.28	0.00	6.00	0.94	0.94	0.00	4.00
15,000 r	0.76	0.00	0.00	2.00	0.76	0.00	0.00	0.00	1.14	4.30	0.00	0.00
30,000 r	0.37	0.00	0.00	0.00	0.75	0.00	2.00	0.00	2.99	4.10	0.00	0.00

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation, LD, late differentiation.

appearance of this mutation at the 4,000 r, 7,500 r, and 12,500 r dose levels decreases with an increased number of successively treated generations, being entirely absent at the 4,000 r dosage following the third generation of radiation.

When the embryos were irradiated at the early and late proembryo stages of their development seedlings having only coleoptiles decreased with each treated generation (Table 14). Following irradiation of the early differentiation stage this mutation showed a decrease in the second generation followed by an increase after the third irradiated generation. Irradiation applied to the late differentiation stage on the other hand, produced a decrease in this abnormality in seedlings in the second generation followed by an increase after the third treated generation.

A detailed comparison of the mutation data (Table 14) shows that the coleoptile-only mutation occurred very infrequently in the X-2 generations following irradiation of both developing and dormant embryos. Further examination shows that whenever the coleoptile-only mutation is induced, there is, with few exceptions a reduction in the root length and number of roots (Table 15) as compared with the root length and number of roots of seedlings not showing this mutation in plants arising from embryos irradiated either during their development or dormancy.

Another type of abnormality that was observed in seedlings

Table 15. Root length and number of roots of the coleoptile-only (coleoptile present but no shoot) mutant

	# coleopt. N only		$\bar{x}$ LR	$\bar{x}$ #rts.	N	# CO	$\bar{x}$ LR	$\bar{x}$ #rts.	N	# CO	$\bar{x}$ LR	$\bar{x}$ #rts.
						Developing Embryos						
Control	122	0	0.0	0.0	143	0	0.0	0.0	79	0	0.0	0.0
400r EP[a]	86	8	4.0	2.9	53	2	2.5	4.0	54	1	6.2	6.0
400r LP	116	11	5.7	5.7	60	3	3.5	4.3	58	0	0.0	0.0
400r ED	122	2	2.9	6.0	94	3	6.4	3.7	55	2	2.6	3.5
400r LD	101	12	2.5	4.0	94	3	3.0	4.0	51	2	1.6	2.5
						Dormant Embryos						
Control[b]	288	0	0.0	0.0	152	0	0.0	0.0	206	0	0.0	0.0
Control[b]	190	0	0.0	0.0	189	0	0.0	0.0	110	0	0.0	0.0
4,000 r	188	0	0.0	0.0	181	8	2.4	3.6	112	1	14.2	7.0
7,500 r	278	0	0.0	0.0	137	0	0.0	0.0	208	1	1.4	5.0
12,500 r	188	0	0.0	0.0	189	3	2.2	5.3	106	2	6.1	4.5
15,000 r	263	2	0.9	1.5	140	1	3.6	6.0	188	2	2.8	4.5
30,000 r	267	1	2.3	4.0	148	7	3.9	1.7	197	6	5.0	4.1

[a]EP, early proembryo; LP, late proembryo; ED, early differentiation; and LD, late differentiation, stages.
[b]Control for 7,500 r, 15,000 r, and 30,000 r dose levels.
[c]Control for 4,000 r, and 12,500 r dose levels.

from irradiated developing embryos but did not appear in plants arising from treated dormant embryos was the split or cleft coleoptile (Table 13). This mutation was noted as a single or a double split, extending the full length of the coleoptile (Mericle and Mericle, 1957), or a partial split, extending one-fourth to one-half way down, the coleoptile. The single cleft coleoptile appears to be extremely stage specific, occurring most frequently in seedlings arising from embryos irradiated at the late proembryo and early differentiation stages of their development. This mutation, however, did occur at times following the first generation of radiation at the late differentiation stage. The double and partial clefts do not show this strict stage specificity. The double cleft is entirely absent following the second and third treated generations. Furthermore, the single and partial clefts occur very infrequently after the second successive generation of radiation. Further, this mutation was not observed in the X-2 generation following the irradiation of either the developing or dormant embryos.

Albino mutations. Control plants were entirely devoid of albino mutations throughout this investigation. However, albino mutants did occur infrequently among the seedlings derived from irradiated developing or dormant embryos (Table 14). Following the second generation albino mutants occurred in seedlings of embryos irradiated with 400 r of X-rays at the late proembryo stage of their embryonic development and

7,500 r and 12,500 r during their dormancy. After the third successive irradiated generation albino mutants were recovered only after the three highest exposure levels, the frequency of albinism being almost 4 times greater at the 15,000 r and 30,000 r dose levels than at the 12,500 r dosage. Examination of the data in Table 14 shows that the frequency of albinism was low in the X-2 generations following the irradiation of dry seed material. Further, this mutation was completely absent in the X-2 generations following irradiation of developing embryos. The low incidence of albinism in the X-2 generations could have resulted from sampling error, however, since only 50 seedlings were available in some instances. This possibility is further supported by the fact that Mericle and Mericle (1961) have reported that the incidence of albinism is extremely low in this variety of barley.

Other abnormalities. Abnormalities restricted to seedlings arising from embryos irradiated during their dormancy were of the following types: (a) a white stripe about 4 cells wide appeared near the margin of the fifth leaf of one seedling from dry seed exposed to 30,000 r of X-rays in the first generation; and (b) the spike of one plant mutated from the normal two-rowed to the six-rowed barley following an exposure of 30,000 r to the dry seed in the third generation. The lateral florets, however, remained sterile. The germ plasm of this lot of seed had at this time a cumulative dose of

90,000 r. Stadler (1930) and Mericle and Mericle (1957) have earlier reported the occurrence of white stripes in barley.

DISCUSSION

Germination

Reduced germination of seeds following the irradiation of the developing embryos has been attributed to induced dominant lethality resulting from a physiological effect, either genic or non-genic, as a possible explanation for the non-germination of embryos which appeared histologically normal (Mericle and Mericle, 1957, 1961). Simak et al. (1961) have reported that reduced germination increased with and paralleled an increase in mitotic disturbances incurred after the irradiation of the embryos of pine. Others (Gustafsson and Simak, 1958; Saric, 1957, 1958b) have demonstrated that ontogenetically younger seed, which differ much physiologically from the ontogenetically older seed, sustained lower germinability following irradiation than did older seed. Working with seeds of barley collected at different developmental ages, Saric (1958b) found that at 15,000 r of X-rays the difference in germinability between control and treated seed was 15 percent for the youngest but only 1 percent for the oldest seed. At a higher exposure level (25,000 r) the respective differences were 42 percent and 6 percent. Gustafsson and Simak (1958) reported that 500 r of X-rays applied to the youngest pine seed stages reduced subsequent

germinability by 50 percent, while a 1,500 r dose level was required to achieve the same results with the oldest stage. Following one generation of radiation, Mericle and Mericle (1957), found that 400 r of X-rays reduced germinability by 44 percent following irradiation at the zygote stage but only 3 percent if the late proembryo stage was irradiated. In this investigation it has been shown that the difference in germinability between controls and embryos irradiated at specific stages of development was maximal (ranging from 17 to 25 percent) for the early proembryo or zygote stage and minimal (ranging from 2 to 8 percent) for the late proembryo stage (Table 1) over the three treated generations. Thus, it can be seen that the germinability data on embryonic stage of irradiations obtained in the present study show fairly good agreement with those of the above workers.

Except for those irradiated at the early proembryo stage, germinability of all irradiated embryos whether treated during embryo development or with different dose levels as dormant embryos was very good, even though the differentiation stages and 15,000 r exposure level showed some tendency for decreased germination with an increase in the number of successively treated generations (Figure 5C). This reduction in germination with increasing number of irradiated generations, as well as the reduction in germinability of the X-2 generations supports, in part, the results obtained by Abrams and Frey (1958) after irradiating

dry seed of oats. These workers concluded from the reduced germination percentages which they observed following each succeeding generation of radiation that there were some carryover effects due to the previous generation(s) of radiation.

This study also shows that radiation exposures up to and including even the highest dose level (30,000 r) given dry seed caused a maximum reduction of only 10 percent on germination proper, i.e., the emergence of root, coleoptile, and shoot. This, however, did not occur until after the third successive generation of radiation (Table 1). Following the first treated generation of dormant embryos germination was 95 to 100 percent at the different exposure levels. MacKey (1951) has observed normal germination for dry, dormant barley seeds (two-rowed, Bonus variety) even after the heaviest dose applied (25,000 r X-rays). Caldecott et al. (1957) have reported that barley seeds will almost always begin germination and develop to a certain extent even at their higher doses. Their highest dose level, however, was only 45,000 r of X-rays. A similar view has been expressed for maize seed (Schwartz and Bay, 1956; Sicard and Schwartz, 1959) and cereals (Moutschen et al., 1956; Moutschen, 1958; Haber et al., 1961) at dose levels up to 500,000 r of X-rays. Mericle and Mericle (1961) have reported that a dose of 1,000 r X-rays, however, will almost completely inhibit the emergence of root and shoot at germination if it is applied during the

early stages of embryogenesis. On the other hand, to achieve complete suppression of germination of embryos treated during their dormancy required an exposure of 1 to 2 million r (Collins and Maxwell, 1936; Micke and Wöhrmann, 1960). In this respect these data agree rather well with those mentioned above.

According to Mikaelsen and Halvorsen (1953), Schwartz and Bay (1956), and Haber et al. (1961) emergence of embryonic tissues (root, coleoptile, and shoot) in barley is initiated by cell elongation of pre-existing cells within the embryos prior to the first mitosis. Approximately 24 hours after germination the number of mitoses approaches a maximum (Sicard and Schwartz, 1959) in unirradiated controls. Following this initial emergence, growth of the coleoptile, however, continues to be primarily by cell elongation with cell division contributing only a small portion, while continued growth of the shoot and root depends on the combined processes of cell elongation and cell division (Mericle et al., 1964; Moutschen et al., 1956; Mikaelsen and Halvorsen, 1953; Sicard and Schwartz, 1959; and others). Following irradiation cell division may be delayed (Mikaelsen and Halvorsen, 1953) or inhibited entirely (Moutschen et al., 1956; Haber et al., 1961). Moreover, cell division is inhibited before cell elongation (Cherry, 1962; Haber et al., 1961; Moutschen, 1958; and others). Mikaelsen and Halvorsen (1953) found that pre-mitotic elongation proceeded normally at all radiation doses

up to and including 15,000 r of X-rays without any delay or inhibition. This is not surprising since Moutschen et al. (1956), Moutschen (1958), Schwartz and Bay (1956), and others, working with cereals, have found that, following complete mitotic inhibition by irradiations of 2×10^5 r gamma rays, cell elongation may continue up to a dose of 4×10^5 r, after which it decreases. Haber et al. (1961) have shown that "entire seedlings" can emerge as a sole result of cell elongation. In this case the young plants increased in dry matter, protein, and RNA. Moreover, it was demonstrated that these seedlings could perform photosynthesis by fixing carbon from CO_2 into sugar phosphates, sucrose, amino acids and organic acids. The plants were "limited in growth," however, since it was found that they could neither synthesize DNA nor undergo mitosis. Ehrenberg and von Wettstein (1955) have also noted that increase in dry matter is not checked simultaneously with growth.

Mikaelsen and Halvorsen (1953) have found that 15,000 r of X-rays delayed mitosis in root cells of barley approximately 24 hours. In spite of this delay, they observed that all embryonic cells in the dormant seed were able to complete at least the first mitotic division. It is during this first division, however, that nuclear disturbances may become manifest. These disturbances (genetic losses due to chromosomal damage), if serious enough, may cause elimination of valuable nuclear material, resulting in deficient daughter cells which

are unable to undergo further mitotic divisions. This explanation has been proposed by Caldecott et al. (1952), Evans and Sparrow (1961), and others as a possible factor involved in growth inhibition. Van't Hof and Sparrow (1964) have recently suggested mitotic arrest as another factor contributing to growth depression. The growth retarding effects of irradiation in rat embryos has also been attributed to a reduction in the mitotic rate of the embryonic cells or to an outright destruction of a certain percentage of the cells (Wilson and Karr, 1950). The possibility that both might be involved in producing the effect was also considered. Eunus (1954, 1955) considered it conceivable that similar events were taking place following irradiations of developing barley embryos.

Since further growth and survival depends upon a period of fairly rapid cell multiplication, it is reasonable to expect the growth rate to be closely related to the number of cells capable of mitotic division (Mikaelsen and Halvorsen, 1953). Accordingly, a marked growth inhibition in seedlings derived from irradiated dry seed would be expected, giving rise to a straight line relationship between the dose level applied and the amount of growth retardation, assuming the effects were due to single "hit" events (Lea, 1955; Sparrow, 1961). It should, however, be pointed out that the regeneration or substitution of killed or weakened cells of the shoot and root apices together with the selective effect on

chromosomal aberrations by a high number of successive mitoses could change the original picture considerably. Certainly, the ability to eliminate and replace mutant cells in the meristems is an important factor in determining the survival and recovery of the seedlings. Evans and Sparrow (1961) have suggested that in young plants cell division may indeed be a prerequisite for their survival. Although the irradiated dormant embryos in this study germinated well even when subjected to dose levels as high as 30,000 r of X-rays, they did not all survive to sporophytic maturity. Hence, the real criterion of resistance to irradiation cannot be based entirely upon the percentage of germination, but must also be the capability of producing a second generation to sporophytic maturity. It is a known fact that by irradiating seeds, young plants are obtained in which normal biochemical and physiological processes have been deranged (see review by Gunckel and Sparrow, 1961). This happens most commonly following larger doses of radiation and may be seen best by comparing the results obtained on percentage germination with those obtained on percentage survival.

Growth and Survival

The reactions of plants to ionizing radiation, in general, take several forms. In addition to the cytologic and genetic effects of irradiation, reports of growth stimulation as well as growth inhibition are quite numerous (see

reviews by Breslavets, 1946; Johnson, 1936; Kuzin, 1956; Sax, 1955; Sparrow et al., 1958). The only instance of radiation induced stimulation or increase over the controls in the present investigation occurred in the number of roots at the 12,500 r dose level (Table 5) and the dry weights of shoots at the 7,500 r exposure level (Table 6) following the third generation of radiation and in the average number of total florets per spike per plant at the lower radiation dose levels given dormant embryos (Table 10) following each treated generation. No stimulation was observed following irradiation at any developmental embryonic stage in any treated generation.

In this study the most frequently observed effect in young seedlings was a reduction in the amount of growth as compared with controls at the end of five days. However, the shoot heights and root lengths were admittedly rather variable within each treated generation. Caldecott et al. (1952) have also noticed a considerable range in seedling heights between X-ray exposures of 10,000 r and 20,000 r. At the 20,000 r exposure level they observed a range of 1 to 18 cm on 14 day old seedlings, while the controls had an average height of 17.7 cm. In a recurrent irradiation study, Caldecott (1961) and Caldecott and North (1961) have observed a further increase in the variability of seedling heights over that of the controls following the second generation of radiation and increasing yet more with each successively

treated generation.

In the present study roots arising from embryos irradiated during their embryonic development or dormancy showed a greater degree of variability than the shoots. Mericle et al. (1964) have recently observed similar results following 50,000 r X-irradiation of dry seeds of this same variety of barley. Moreover, this situation occurred in the present study following each successive generation of radiation. Roots from embryos treated during embryonic stages have been shown to be more radiosensitive than the shoots providing the irradiation is applied after differentiation has begun or is about to begin (Mericle and Mericle, 1957, 1961; Chang, 1961; Chang and Mericle, 1962, 1963). This greater radiation sensitivity of the root region has been attributed to the fact that during embryo differentiation when irradiation is applied the roots are ontogenetically younger than the shoots (Mericle and Mericle, 1957, 1961). Such an explanation appears to be satisfactory for the increased variability observed at the early and late differentiation stages and perhaps also for the different dose levels applied to dormant embryos. It does not, however, account for the increased variability induced as a result of irradiation in the early and late proembryo stages since in these instances the roots are not yet formed at the time of irradiation (Mericle and Mericle, 1957, 1961; Saric, 1958b).

While it is well known that ionizing radiation induces

more variability into the population than that normally exhibited by the controls, the extent to which the degree of variability increases with increasing dose level is apparently limited. This appears to be the situation following a 30,000 r exposure to the dry seed in this experiment since both shoot heights and root lengths showed less variability than that exhibited by the lower dose levels, in all cases less than that at the 15,000 r dosage and in a few instances even less than the controls. This is most probably not due to sampling error considering the number of shoots and roots measured and is further substantiated as a "real" effect by the results obtained by Mericle et al. (1964) following X-irradiation of dormant barley embryos with 50,000 r. An explanation for the reduced variability observed with high radiation doses may be due to the fact that in the normal distribution of the cell population those cells suffering the greatest chromosomal damage at this dose level are no longer able to compete with those remaining normal and so are eliminated from any further contribution.

In almost all instances greater variability was induced into the population by the irradiation of developing embryos than of dry seed material. The fact that less variability was induced following irradiation of dry seed may indicate that the dormant embryos were more stable among different exposure levels and successive generations than were the developing embryos. Such stability might be due to the

rather unique biological situation exemplified by the dormant embryos, i.e., the combination of extreme desiccation and division arrest. Still another possibility is that the cells of the dormant embryos contain a 4C amount of DNA (Stein and Quastler, 1963). The difference between developing and dormant embryos may be better understood by a simple analogous comparison of a wrench thrown into the gears of a machine. When the machine is running even a small wrench thrown into the gears may have enough force to create havoc. On the other hand, when the machine is stopped it requires a force far greater than that of which a small wrench is capable to cause much damage to the machine's gears. Thus, the greater variability observed following irradiation of developing embryos as compared with dry seed material may be nothing more than the inherently different response of a resting and an actively metabolizing system (Curtis et al., 1958). Another possibility is that during sporophytic development following irradiation of dormant embryos the aberrant cells have a greater amount of time to segregate out.

Fertility

The differences between irradiation of dry seed with the different exposure levels and irradiation of the different embryonic stages in this investigation can be indicated by other viability characteristics, for example, the average

number of total flowers per spike per plant and spike fertility. Gelin (1941) found a pronounced correlation between chromosomal disturbances observed in the first mitotic cycle after germination of irradiated seed and the reduced fertility observed in the X-1 generation. Since that time seed setting ability has often been used as an indication of genetic disturbances (Gustafsson, 1947). With acute irradiation (X-rays) reduced fertility has been found to range from 10 to 50 percent, being due primarily to chromosomal rearrangements (inversions and translocations) (Caldecott and North, 1961; Gaul, 1961; Nybom, 1956; and others). The reduced fertility of X-1 spikes, however, never decreases below 40 to 50 percent following X-irradiation, owing to intrasomatic (Ehrenberg et al., 1953) or haplontic and diplontic selection (Gaul, 1958) in the damaged plants. The reduced fertility exhibited by plants arising from irradiated dormant embryos in this study agree quite well with that reported by the other workers referred to above. The overall increase in average spike fertility of the plants from embryos treated during their embryonic development (Table 9, Figure 13C) agrees with the data reported by Yamaguchi (1962), who also found that spike fertility of barley increased slightly following three successive generations of radiation.

Mutations

The spontaneous mutation rate for albinism, as well as for other morphological and physiological variants, is extremely rare in the Hannchen variety of barley (Mericle and Mericle, 1957, 1961). Even after irradiation this variety is relatively resistant to the induction of albino mutations (Mericle and Mericle, 1957, 1961, 1962), having a frequency of only 5 percent after a total dose of 800 r gamma irradiation given to proembryos at a dose rate of 10.9 r per minute. The low incidence of albinism observed following irradiation of embryonic stages of development in this study lends support to the Mericles' results. Following X-irradiation of dormant embryos of Haisa II variety of barley with 30,000 r Gaul (1961) has obtained 12 percent chlorophyll abnormalities. However, Stadler (1930), Caldecott (1953, 1955), and others have reported that albino mutants comprise about 50 to 60 percent of all chlorophyll mutations. On this basis if the values (4 percent) obtained at the 15,000 r and 30,000 r dose levels in this investigation are doubled they are only a little lower than those obtained by Gaul (1961) but compare favorably with those of Mericle and Mericle (1962) in their studies with developing embryos.

Since the effects of radiation were measured in the X-1 generation as well as in the irradiated X-1/2 and X-1/2/3 generations and in regard to germination of the X-2 following each combination of irradiation either as developing or

dormant embryos, any mutation which appeared in the X-1 would have to be a dominant, or after this time could be either a dominant or a homozygous recessive one. The frequency of dominant mutants is extremely rare in irradiated seed material unless very large doses of irradiation are given (Abrams and Frey, 1958; Caldecott, 1961; Stadler, 1930; and others). Effects which might be interpreted as dominant mutations (such as non-germination and white striping of seedling leaves), however, have been recorded by Mericle and Mericle (1957) following irradiation at various embryonic stages during development. Stadler (1930) and Smith (1950) have also observed dominant mutations in the form of white and yellow stripes on tiller leaves of barley. Stadler (1930) attributed this to be the result of some direct cytoplasmic effects of the X-ray treatment. The appearance of a double cleft (split) coleoptile abnormality first observed and described by Mericle and Mericle (1957) as well as the single clefts reported by Mericle and Mericle (1961), both of which occurred in the present study may conceivably arise from dominant lethal mutations of certain cell initials.

The frequency of recessive mutations occurring simultaneously in the two genes at a given locus is the product of their independent mutation rates. The probability of this occurring in the X-1 generation is rather remote. This, however, does not negate the fact that mutations of this type

can occur, even if rarely, and to a certain extent perhaps accumulate with recurrent irradiation in successive generations. That recessive lethal mutations do accumulate is supported by the reduced germination data in X-2 populations following irradiation (Abrams and Frey, 1958; Mericle and Mericle, 1963) and certain instances in this investigation.

Recurrent Irradiation

The frequency of mutations in a population can be further increased by applying the method of recurrent irradiation in successive generations (Caldecott, 1961; Caldecott and North, 1961; Yamaguchi, 1962). Moreover, it has been shown that chlorophyll mutations in barley and rice (Yamaguchi, 1962) as well as the variability for plant height, panicle type, and maturity date in oats (Caldecott, 1961; Caldecott and North, 1961) increase with each succeeding generation of radiation after the second generation. All of their work, however, has been done with irradiated dry seed. On this basis the data in the present study derived from irradiation of dormant embryos tends, in part, to support their view.

The interpretation of the present data with regard to the events which are taking place during recurrent irradiation is not yet clear. However, two opposing hypotheses may be considered. First, if it is assumed that living organisms (e.g., plants) do possess the ability to adapt to

an environmental stress, such as irradiation, via intrasomatic (Ehrenberg et al., 1953) or haplontic and diplontic (Gaul, 1961) selection, then it is reasonable to expect that each successive generation, after radiation, would contain less induced damage than the previous one due to the survival of only the more vigorous and genetically stable individuals. Abrams and Frey (1958) indeed have observed increased vigor in surviving oat plants with an increasing number of generations of radiation. This is also exemplified by the present data on survival to maturity. The increase in mean height coupled with a reduction in induced variability of the coleoptiles following irradiation of developing embryos in this investigation lends further support to this theory. Also the fertility data of Yamaguchi (1962) for barley and rice and for irradiated developing embryos of barley in this study supports this view. In addition, the low incidence of albino mutants in seedlings arising from embryos irradiated both during embryonic development and dormancy in this study is also in agreement. Thus, using survival to maturity, fertility, and the frequency of induced albino mutants as criteria for evaluation of radiosensitivity it appears that adaptation is indeed possible.

For a second hypothesis we might assume that if the damage done by recurrent radiation in each successive generation is cumulative then it is equally conceivable to expect that irradiation causes not only heritable genetic damage to

the current population but also that this effect accumulates in the germ line, as has been recently shown in animals (Spalding and Strang, 1962; Stadler and Gowen, 1961, 1962, 1963). When observing germination percentages (Table 1, Figure 5A) and the "coleoptile-only" anomaly in these data following irradiation of dormant embryos (Table 14) it appears that the latter hypothesis is more likely to be true, since there is an overall tendency for lower germination percentages with each successive generation of radiation of both developing and dormant embryos. This is in agreement with the work of Abrams and Frey (1958), who found lower germination percentages with an increasing number of successive generations of radiation on dry seeds of oats. Caldecott (1961) and Caldecott and North (1961), despite rigorous selection for high fertility still found increasing incidence of mutations for chlorophyll deficiencies.

The possibility that both of these forces may be operating in an alternate or opposite manner (i.e., genetic burdens built up, are eliminated, perhaps to build up again, only to be eliminated in a yet subsequent generation, etc.) should also be considered. This explanation, in fact, appears to be substantiated by the shoot height data obtained following irradiation of both developing and dormant embryos. This point is further illustrated by combining all of the data for seedling abnormalities (Tables 13 and 14). Furthermore, in nearly all of the criteria used in this study to measure

radiation sensitivity, this alternating cycle is apparent at the 30,000 r exposure level given dormant embryos. While this latter possibility is suggested by certain criteria of this study, it is still open to speculation and remains to be confirmed by further investigation as to whether this alternating cycle would continue perhaps ad infinitum.

Irradiation of Developing Embryos

The "law" of Bergonie and Tribondeau (1906) states that the radiosensitivity of cells varies directly with their reproductive capacity and inversely with their degree of differentiation. Accordingly, then, younger embryos should be more sensitive to radiation than older ones. This has been verified especially with regard to lethality responses of animal embryos (Wilson and Karr, 1950; Russell and Russell, 1956; Russell, L. B. et al., 1960) and plant embryos (Eunus, 1954, 1955; Mericle et al., 1955; Mericle and Mericle, 1957, 1961, 1962, 1963). Other investigators (Saric, 1958b, 1961; Gustafsson and Simak, 1958) have also shown that ontogenetically-younger seeds with which they worked were the most radiation sensitive.

The early proembryo and late differentiation stages were more radiation sensitive following each generation of radiation than the late proembryo and early differentiation stages for nearly all criteria observed on the seedlings. Considering each embryonic stage separately and reflecting on the possible

cause or causes of this differential sensitivity it is necessary to consider many factors. First the early proembryo stage: this stage is a single cell, completely totipotent, and containing complex systems carrying on all of the physiological functions required of life. In addition, this zygote or one-celled stage contains a vast network of phospholipid membranes with one of their chief functions to keep certain substances and enzymes apart. Any factor or factors that interfere with or extensively modify this highly complex cellular organization or disrupts these barriers, either by forming holes or altering their selective permeability in some way, can be considered, in a biological sense at least, to cause some biochemical defects or even to kill the zygote cell. The release of degradative enzymes, for example, could produce damage to cellular constituents capable of digesting the entire cell (de Duve, 1963). If by chance, however, the defects are small the final outcome might depend on whether these defects are repaired, amplified, or bypassed as the complex cellular machine proceeds to operate in its faulty condition. There appears, in general, to be an "all-or-nothing" effect, i.e., the zygote cell being either able of withstanding the radiation and proceeding through normal organogenesis, or incurring damage so severe that the embryo aborts (Mericle and Mericle, 1957, 1961). Similar circumstances have been reported in mice (Russell and Russell, 1956; Russell, W. L. et al., 1960). In many cases, however, there are "hidden effects"

which do not show up during organogenesis but appear later in development as dominant lethals at the time of germination (Mericle and Mericle, 1957).

This greater sensitivity of the early proembryo stage may also be related to other factors such as nuclear volume, water content and amount of DNA. The nuclear volume has been shown to decrease approximately eight-fold from the zygote stage to the late proembryo and later stages of embryogeny in Hannchen barley (Mericle and Mericle, 1961). Moreover, Sparrow et al., (1963) have demonstrated a two-fold change in nuclear volume between dormant and actively growing plants. The increased nuclear volume of the zygote implies that it has a greater amount of nuclear material and/or water or perhaps both. It remains to be determined, however, whether it has a larger nuclear volume because of a proportionately greater water content. Mericle and Mericle (1961) have indicated that this stage may contain 90 percent, or more, of water. If this be the case, and with the known effects of ionizing radiation on water (Weiss, 1944), then much speculation can be made as to the reactions involved, since theoretically many active radicals could be formed. These active radicals could react with nearly all of the important components within the zygote cell. Every protein as well as the nucleic acids within the cell have many points to which active radicals could attach. Thus, they may selectively destroy certain groups in a side chain of the

protein which may be essential for enzymatic activity. Recent evidence (Conger and Randolph, 1959), however, indicates that more harmful effects are sustained because long-lived free radicals are produced which combine with biologically important molecules within dry seeds (2 to 4 percent moisture), since at this time there are few water molecules, with which to compete. In wet seeds (12 to 15 percent moisture), on the other hand, these free radicals react with water molecules and hence are not biologically very active. Whether developing embryos behave similarly to dormant embryos of dry seeds in this respect remains to be determined.

It has been suggested, although not yet verified, that the DNA content within the zygote or one-celled stage may be in a tetraploid or a higher ploidy condition (Mericle and Mericle, 1961). Brewbaker and Emery (1962) have presented some interesting probabilities advocating that sperm DNA in pollen of Tradescantia (and probably ovary DNA as well) is duplicated prior to anthesis. Synthesis and accumulation of DNA up to 8C values and possibly higher quantities without further cell division have been reported in stamen hair cells of Tradescantia (Davies and Wimber, 1963). Moreover, the radiation sensitivity of plants has been shown to increase as the size of the nucleus and DNA content increase (Sparrow et al., 1961; Sparrow and Evans, 1961; Sparrow and Miksche, 1961).

For most of the criteria used to measure sensitivity in

the present study the late proembryo stage appeared to be more radiation resistant each generation than any other embryonic stage treated. This observation is supported by earlier results obtained by Mericle and Mericle (1957, 1961, 1962, 1963). Chang and Mericle (1963) have suggested that this embryonic stage apparently sustains less initial injury and/or that it possesses a greater capacity for intra-cellular repair. However, for certain organ abnormalities (e.g., cleft coleoptile) this stage has been shown to be stage specific (Mericle and Mericle, 1957, 1961, and these data). If other abnormalities occurred which would be detrimental to the continued development of the embryo then abortion should result. The fact that abortion is low at this stage refutes this reasoning. On the other hand, if we assume, as was suggested by Mericle and Mericle (1957, 1961), that each cell of the developing embryo at this embryonic stage remains sufficiently totipotent to establish new centers of organogenesis, then this stage should stand a better chance of recovery than embryonic stages already differentiated.

In addition, the late proembryo stage has roughly 1000 cells (estimate of R. P. Mericle) as compared with one cell of the early proembryo. Any cells that were induced to mutate by irradiation, unless the mutations were lethal would now exist in a heterozygous condition as opposed to the homozygous non-mutated cells of the embryo. Thus, tissue incompatability could result within the embryo and/or between

the endosperm and the embryo (Mericle and Mericle, 1961; Mericle et al., 1961; Chang and Mericle, 1963). Although this incompatability theory still lacks complete verification, there are indications that biochemical reactions between the embryo and endosperm do occur (Meletti and D'Amato, 1961). Meletti and D'Amato (1961) irradiated mature embryos of wheat and transplanted them to non-irradiated endosperm. The resulting plants showed improved survival. In contrast, non-irradiated embryos placed in contact with irradiated endosperm produced seedlings with retarded root and shoot growth and reduced survival.

Mericle and Mericle (1959) have reported that the building blocks of proteins (amino acids) within developing embryos are effected by irradiation. They reported that proline, threonine, valine and cystine/cysteine observed in differentiated embryos were sometimes depressed to the point of non-detection by X-irradiation applied during early embryogeny. Furthermore, proline, valine, and cysteine have uracil as part of their RNA code "words" (Nirenberg, 1963). Since Chang (1961) and Chang and Mericle (1962, 1963) have shown uracil to be highly radiation sensitive, the reduction of these amino acids may result because of uracil deficiency and an alteration in the coding system.

Late differentiation stages treated in this experiment showed greater radiation sensitivity than either the late proembryo or early differentiation stage. These data are in

contrast to earlier results which indicate that those cells which are actively dividing are much more sensitive to acute irradiation than those which are not (Sparrow et al., 1961). In addition, cells in an active stage of metabolism are much more radiosensitive than those which are relatively quiescent. These results may be explained, however, by the data of Chang (1961, 1963a), who found that the relative amount of phosphorus-32 incorporation into DNA per cell, per unit time, decreased gradually as the growth rate of embryos slowed down and differentiation occurred. Chang (1961, 1963) explained his results on the basis that a cell which has completed its last division and is already differentiated does not synthesize additional DNA but retains only the DNA transmitted to it by its parent cell. Also Mericle and Mericle (1961) and Chang and Mericle (1963) have suggested that this late differentiation stage appears to be either incapable or less capable of intracellular repair.. If we make the assumption, therefore, that no further cell divisions or DNA syntheses takes place following irradiation of the late differentiation stage, then any damage that is induced in the genetic material cannot be eliminated (by diplontic selection) until the cells of the dormant embryo commence germination and resume DNA syntheses and cell divisions. Chang and Mericle (1963) have further stated that the late proembryo and early differentiation stages are apparently capable of intracellular repair. This "repair" is probably due to selective elimination

of the faulty cells since at these stages there are still other undamaged cells apparently capable of taking over (Mericle and Mericle, 1961; Chang and Mericle, 1963). This ability, however, would be less at the early differentiation stage than at the late proembryo stage because of the fewer cells present.

SUMMARY

1. When germination percentage and percent survival to maturity are used as indices of effects following irradiation of embryos during their embryonic development there is, in general, an inverse correlation between the stage of differentiation and the degree of effect.
2. Growth inhibition of seedlings, as measured by their dry weights, coleoptile heights, and number of roots, generally showed differential stage specific responses following the irradiation of embryonic stages of developing embryos, the early proembryo and late differentiation stages being affected to the greatest extent each irradiated generation.
3. In general, shoot height and root length of seedlings from embryos treated during their embryonic development and the spike fertility of the resulting plants showed great variability among the irradiated stages treated and do not appear to be correlated with the specific stages irradiated.
4. Differential exposure levels of irradiation used in this study when applied to dormant embryos had little or no effect on germinability nor on the average number of roots per seedling, while seedling growth (as

measured by height and dry weight), survival to maturity, and spike fertility generally exhibited responses directly related to the amount of irradiation applied to the dry seed.

5. Root length of seedling from embryos irradiated either during embryonic development or at dormancy showed greater variability than the shoot height. In general, the variability induced by irradiating dormant embryos with a dose of 30,000 r was less than that of the lower dosages and in a few instances less than that of the controls. Greater variability was induced following the irradiation of developing embryos than when dormant embryos were irradiated. Generally, the greatest variability was induced following irradiation at the early proembryo stage.
6. A wider spectrum of seedling abnormalities was produced by irradiating the developing embryos than when the dormant embryos of dry seeds were irradiated. Although irradiation of the first generation of developing embryos may produce a larger isomutant sector than can be obtained when the dormant embryos of dry seed are irradiated, it does not follow that the size of the isomutant sector can be increased further by recurrent irradiation in successive generations, since there is a tendency for the plant to selectively eliminate those induced mutations in later treated generations.

7. Lethality at germination, following irradiation of the early and late differentiation stages of developing embryos, and following the 15,000 r dose level given dormant embryos, suggests some carryover of genetic defects from each previous generation of irradiation.
8. Results of five of the nine criteria used to measure radiation sensitivity of developing and dormant embryos indicate an increase in resistance with each treated generation.
9. Repeatedly, the seedling and mature plant responses produced, following an X-ray dose of 30,000 r given dormant embryos, were comparable to a dose of 400 r X-rays applied at the early proembryo and late differentiation stages of developing embryos.

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