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A STUDY OF THE EFFECTS OF  
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ON POTATO PLANTS (SOLANUM  
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by  
Mary Langevin Anderson

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Doctoral Committee:

Professor Felix G. Gustafson, Chairman  
Assistant Professor James F. Hogg  
Professor Kenneth L. Jones  
Professor A. Geoffrey Norman  
Assistant Professor Warren H. Wagner, Jr.

## Abstract

# A STUDY OF THE EFFECTS OF GAMMA IRRADIATION (COBALT-60) ON POTATO PLANTS (SOLANUM TUBEROSUM L.)

by Mary Langevin Anderson

The initial purpose of the present investigation was to study the effects of ionizing radiation on the riboflavin, thiamine, and ascorbic acid content of Irish potato plants. Interesting developments which arose during the course of the investigation resulted in studies of morphological responses, starch accumulation, changes of dry weight, and respiration.

Potato plants were irradiated with gamma radiation from Cobalt-60 in doses ranging from 0 to 100 kilorep. The first visible evidence of irradiation effects was the discoloration and mottling of leaves. Later, the leaves became leathery and thickened, and newly formed leaves showed various abnormalities as to form and texture. Aerial stems became thickened and remained green longer than those of the control.

Riboflavin, thiamine, and ascorbic acid content of leaves and stems increased progressively with increasing magnitude of dose, except that the riboflavin content of leaves did not vary greatly from the control.

Starch accumulated in the stems of exposed plants, an increase with elapse of time after irradiation being

found. At the same time, tuber initiation and development were progressively inhibited until at the higher dosage levels few or no tubers were formed.

Respiration studies did not give significant changes, possibly resulting from inadequacies in sampling procedure. However, there appeared to be a trend toward a higher rate of respiration at the higher dosage levels.

Weight determinations revealed that the fresh weight and the dry weight of leaves, stems, and roots of exposed plants were greater than those of the control. Tuber yield progressively decreased with increasing magnitude of irradiation dose. Total fresh weight and total dry weight also decreased with increasing doses until at the 100 kilorep level the values were approximately half those of the control. The fact that total dry weight was progressively less with increasing doses suggests that there was less photosynthesis as a result of irradiation.

Although the underground systems were shielded by soil and a clay pot, the rhizomes and tubers exhibited unusual susceptibility to irradiation. This susceptibility is undoubtedly due to the fact that these organs remain meristematic throughout their period of development and growth. Shielding the underground portions of the plant with two-inch thick lead bricks prevented irradiation damage to the extent that tubers were formed at all exposures and starch did not accumulate in the stems and leaves.

It is concluded that gamma irradiation is deleterious to potato plants, particularly to the underground systems, and to a lesser degree to the aerial portions. Little variation from the normal was observed at the 5- and 10-kilorep dosage levels but with doses of 15 kilorep and above the effects were marked, rhizome and tuber formation being specifically inhibited with resultant accumulation of starch in leaves and stems.

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## INTRODUCTION

The repercussion of the bombs which devastated Hiroshima and Nagasaki in August, 1945, startled the world into the realization that it was living in an atomic age and subject to the influence of ionizing radiation. Unknown to the nation at large, developments which led to the exploration of atomic energy were started in the United States in 1937 (Dean, 1955). In January of that year, Niels Bohr, eminent Danish scientist, brought information to this country regarding atomic experimentation conducted in Italy and Germany during the 1930's. President Franklin D. Roosevelt, upon learning of the potentialities of atomic energy from a group of American scientists, arranged to finance further research. This resulted in the design and manufacture of the atomic bombs which helped bring an end to the war with Japan.

After the war, the Congress established the Atomic Energy Commission in 1946 and charged it with great responsibilities not only in relation to the production and control of fissionable material and the development of atomic weapons, but also in the exploitation of atomic energy for power, and the furtherance of research in all

aspects of science and human welfare that might bear upon or be influenced by the availability of isotopes or exposure to radiation.

On May 1, 1948, the regents of the University of Michigan, on recommendation of their War Memorial Committee, voted to "create a War Memorial Center to explore the ways and means by which the potentialities of atomic energy may become a beneficent influence in the life of man, to be known as the Phoenix Project of the University of Michigan" (R. P., 1945-1948). The project drew its financial support primarily from students, alumni, and friends of the University (Gomberg, 1956). In October, 1951, the construction of a laboratory with special facilities designed to handle, adequately and safely, large amounts of radioactive materials was authorized. The building was first occupied in July, 1955. Laboratories were available for chemical and biological experimentation, and, in addition, a greenhouse connected to the main building by a corridor was available for use in the fall of 1955. A high flux gamma source consisting of about 2500 Curies of Cobalt-60 made possible studies on the effects of ionizing radiation. In September, 1957, the Ford Reactor was licensed by the AEC and first went critical a few days later.

This combination of facilities suggested the possibility of a study of the effects of ionizing radiation on plant material. Although

some work has been done on the study of plant responses to ionizing radiation, it is a comparatively new field. The greater portion of the work has dealt with morphological changes at the cellular, tissue, or organ level, with little work on the biochemical responses to radiation and not a great deal more with regard to physiological effects. Furthermore, Professor F. G. Gustafson had directed a series of physiological investigations in his laboratories for several years which were concerned with the effects of various factors on the vitamin content of plants. After a consideration of the several facilities which were available, it seemed feasible to combine them for use in a study of the effects of ionizing radiation on the vitamin content of certain plant materials.

The ordinary Irish potato, Solanum tuberosum L., was selected for the studies because it is easily obtainable, can be grown the year around under suitable conditions, is fairly easy to grow, and produces a plant large enough to furnish ample material for assay.

This investigation was set up initially to determine the effects of ionizing radiation on the vitamin content of the potato plant; however, as the study progressed some unexpected and interesting observations on starch deposition in the plant caused an extension of the study to include changes in starch content attributable to

irradiation. This research was instigated and influenced by the opportunities at hand and serves as an example of the fact which Reed (1942) so aptly stated: "A scientist is a product of the time in which he lives. Thus, one will see reflected in the lives of most of them ideas which were borne in by the tides of human affairs."

## HISTORICAL REVIEW

### Radiation

Shortly after the announcement of the discovery of X rays by Roentgen late in the year of 1895, papers began to appear describing the injurious effects of X-irradiation on plants. Becquerel's discovery of natural radioactivity in uranium salts in March, 1896, gave further impetus to investigations concerning the biological effects of ionizing radiations. For the next two decades, a great many types of biological effects were observed, most of which were mainly qualitative in character and the result of crudely measured doses of radiation. The apparent differences in effect which were noted at that time were due in part to the lack of satisfactory dosage measurements and in part to a lack of understanding of the inherent qualities of the radiations. Dose-measuring instruments became more reliable after 1920 and it was then possible to relate dose and biological response more accurately (Sparrow and Rubin, 1952).

Because of the extreme complexity of biological systems, a vast range of reactions and responses may be expected as a result of exposure to ionizing radiation. Only radiation which is absorbed

can be chemically active. Energy from radioactive sources initially causes excitation of the absorbing molecule; when the absorbed energy has reached a sufficiently high level, the molecule is ruptured with the ejection of an electron and the formation of free radicals and ions.

Water comprises the major part of physiologically active protoplasm and it is thought that a large part of the effects of ionizing radiation are caused by the activation of water. Irradiation of water is believed to result in the production of the highly oxidative hydroperoxyl radical, as well as in the formation of hydrogen peroxide, hydrogen ions, and hydroxyl ions (Gordon, 1957). These active radicals and oxidizing substances are thought to initiate changes at the molecular or submolecular level. The ensuing changes may alter the physical-chemical relationship directly; or, indirectly by the modification of the genic structure. Although ionizing radiation reacts with all types of atoms and molecules in a system, the various molecules concerned with vital processes are not present in equal quantities and they do not all have equal sensitivities. Radiation damage becomes critical only when a reduction in essential molecular species interferes with essential physiological processes (Sparrow and Rubin, 1952).

Early investigators were limited mainly to the use of X rays in their experiments. Radium was available but in very limited quantities and at prohibitively high prices. X rays were useful for seed and seedling irradiation and most of the early work was done on them or on small plants. The wide range of variation in effects reported at that time was due in part, at least, to lack of uniform dosage measurements because the various workers used several different units of measurement. After the adoption of the "roentgen" as an international unit in 1937, comparative studies were made possible. Irradiation of plants under growing conditions had to await the development of radioactive sources that could be placed in a field and plants grown near them. The first nuclear chain reaction in the United States occurred late in 1942; and, by 1949 a "gamma-field" was already in operation at Brookhaven National Laboratory.

#### Plant Responses to Irradiation

Several review articles on the responses of plants to ionizing radiation have appeared. The effects of X-irradiation were summarized by Johnson in an article which appeared in 1936 (a), and in a second article written by Breslavets in 1946. Tolbert and Pearson (1952), Gunckel and Sparrow (1954), Sparrow and Gunckel (1956),

and Sparrow, Gordon, Sax, Gunckel, and Konzak in a symposium which appeared in 1957 have given comprehensive reviews of later developments. Authorities agree that plant responses to ionizing radiation are variable depending upon physical environment, type of radiation, method of administration, dose rate and total dose, species and variety of plant used, radiosensitivity of the plant, age of the plant or plant parts used, and the physiological condition of the plant at the time of irradiation.

### Morphological Responses

The major portion of the work on plant responses to ionizing radiation has been at the gross descriptive level on the morphological changes which occur in the cell, the tissue, or the organ. Morphological abnormalities are usually produced in the embryo, seeds, and seedlings, and can occur in any organ or tissue of more fully developed plants. Although at the present time we have little specific information concerning the physiological and metabolic disturbances following irradiation, most of the effects observed on plant organs, with the exception of clearly demonstrated mutations or chromosome breakage or aberration, can probably be considered as secondary effects of induced biochemical or physiological disturbances (Gunckel, 1957).

### Growth habit

Growth habit and growth rate may be affected by irradiation. Growth is generally unaffected at low levels of irradiation but at medium or high levels it is partially or completely inhibited. When administered in excess of the threshold level, which is variable depending upon the plant, growth is inversely proportional to the dose rate or total dose. Stem dwarfing may occur due to the destruction or inhibition of the terminal meristem or partial failure of the internodes to elongate (Gunckel and Sparrow, 1954). Axillary buds may develop and cause a change in the general habit. Fasciation of stems or flowering stalks and localized or general stem swellings or thickening are common. Localized stem swellings may be accompanied by the formation of adventitious roots.

### Plant organ responses

Plant organ responses may show a wide range of variation from minute morphogenetic abnormalities to death.

Stems commonly show dwarfing, excessive branching, fasciation, dichotomy, altered phyllotaxis, local stem swellings, an increase in the amount of vascular tissues, occasional stem lesions, and development of adventitious roots (Gunckel, 1957; Gunckel and Sparrow, 1954; Sparrow and Gunckel, 1956).

Leaves exhibit various morphological responses to ionizing radiation. Sparrow and Gunckel (1956) stated, "In most plants, the leaves already present on the plant at the beginning of the exposure are changed relatively little. Rarely, these older leaves may have a restricted blade or show some growth of the blade between the veins; more commonly the leaf texture may be changed, becoming dry, stiff, coarse, or thickened, or leathery." They continue that young leaves formed during (chronic) irradiation show a wide range of responses such as dwarfing, asymmetrical development of the leaf blade, distorted venation, change in texture, and thickening of the blade. Puckering of blade, abnormal curvatures, curling of leaf margins, fusions, mosaic-like color changes or over-all color change, usually accompanied by increased pubescence, premature abscission, and even tumor formation as a result of local injury have also been reported.

Flowers and flowering show a variety of responses to irradiation. Treated plants may show delayed and/or reduced flowering; or, in some cases, premature flowering such as Johnson (1936b, 1948) observed in X-rayed Kalanchoe; or, often, increased flowering as observed by Gunckel (1957) in Impatiens. According to Sparrow and Gunckel (1956), "with chronic gamma irradiation, flowering is generally retarded at the higher doses and approaches that of the

controls with decreasing dosages. In some species, notably Tradescantia and Nicotiana, there is a critical dose range in which flowering is stimulated." Floral stalks may be increased in height and they may show fasciation and abnormal thickening (Gunckel, 1957). Flowers may show color changes and somatic mutations (Sparrow and Gunckel, 1956), a high degree of sterility, abscission, modifications in form and number of parts, especially of petals and stamens (Gunckel et al., 1953a), and vegetative growths may develop in the floral parts (Gunckel et al., 1953a, 1953b; Gunckel and Sparrow, 1954; Sparrow and Gunckel, 1956).

Root growth may show inhibition or stimulation depending upon the dose administered. The root is fairly well shielded from irradiation by the soil but it has been observed that on plants exposed to fairly high dose rates during growth the root systems frequently suffer severe damage (Sparrow and Gunckel, 1956). As mentioned previously, adventitious roots may be formed at the site of localized stem swellings. Such roots may also be developed above irradiated stem sectors and are presumably due to phloem blockage (Gunckel and Sparrow, 1954; Sparrow and Gunckel, 1956). Gunckel concluded his recent review by saying that most of the morphological irregularities described are probably secondary effects due to physiological imbalances.

## Cytological and Genetical Responses

The publications of Müller (1927) and Stadler (1928) showed that X rays could speed up dramatically the frequency of mutations and induce hereditary changes in animals and plants. By the ready production of radiation-induced mutations, our knowledge concerning the genetics of higher plants has been greatly increased. McClintock (1938) has confirmed genetic linkage by means of induced chromosome breakage and by employing special chromosome translocations and rings induced by irradiation. Anderson (1956) has been able to obtain data for gene mapping, and genetic studies have been extended to include loci the individual effects of which are not readily distinguished (Konzak, 1957).

Little work was done in this country to employ radiation as a tool in plant breeding until the late forties because many geneticists believed that radiation caused only destructive genetic changes. More recently, the greater availability of various types of radiation and the greatly increased knowledge of hereditary materials, together with the successful efforts of European workers in producing useful plant mutations, have stimulated interest in the use of ionizing radiation as a tool in agriculture and plant-breeding programs (Konzak, 1957). In Sweden, Nilsson-Ehle, Gustafsson, and their associates

have produced superior strains of cereals with higher yields, stiffer straw, and earlier ripening period. In North Carolina, experimentation on the peanut, Arachis hypogea L., has produced some mutants which are resistant to leaf spot disease, and there are other mutants which indicate higher yields. Investigators in Minnesota are able to report changes in disease resistance characters in cereals. By 1955, well over one hundred workers throughout the United States and Canada were investigating the effects of ionizing radiation on more than sixty crop plants (Singleton et al., 1956). Even though relatively few radiation-induced varieties have been released for commercial production, radiation as a tool in plant breeding has made a modest beginning. In Sweden, Primex oil mustard was released in 1950, and Svalof Regina oil rape in 1953; in this country a new early bean variety, Sanilac, with bush habit was released by the Michigan Experimental Station in 1956. New superior strains of barley, peas, and tobacco are undergoing final tests before release. Useful mutants have been induced in horticultural crops such as sweet cherries, black currants, and apples; flower color has been changed in carnations, cyclamen, and African violet, Saintpaulia (Konzak, 1957). Ehrenberg et al. (1956) concluded that "the induction of mutations by means of ionizing radiations have given and can give hereditary changes of high production capacity in agricultural plants. If the

mutants, although high-productive, are not suited for direct marketing, they form a source of variation valuable for the normal continued breeding."

## Physiological Responses

### Growth

Most of the early work on plant irradiation was concerned with the effects of acute doses of X rays on green plants. In a review appearing in 1936 (a), Johnson reported that the majority of the published papers dealing with the subject since the discovery of X rays in 1895 were concerned with the effect of X-irradiation on physiological processes, including rate of seed germination, growth, respiration, and movements. A review of the evidence presented indicated that medium and heavy doses of X rays are injurious to growth. A controversy of thirty-five years' duration concerning the so-called stimulative action of light doses remained to be solved at that time. Stems were shown to be less sensitive to X rays than roots; lateral roots much more susceptible than main ones; dry seeds particularly resistant and seedlings much more sensitive. Respiration of treated seedlings paralleled growth. Nyctinastic movements were reported to be inhibited by X rays of sufficient intensity.

Johnson (1936a) also reviewed the results of a number of experiments performed on potatoes during this period. She reported that Jacobson had demonstrated that irradiation caused an increase of 84 per cent in weight of the crop of one variety of potatoes over the control, and that another variety showed an increase of as much as 200 per cent. Tubers were larger than normal and there was an increase in the total number of tubers. Johnson (1936a), in an extensive series of experiments over a five-year period, found that irradiating unsprouted tubers of the Colorado wild potato, Solanum Jamesii, with a dose of 1500 r-units caused a slightly greater production than controls. The same dose on sprouted tubers caused an increase of over 40 per cent in the average number of tubers per hill, with an even greater increase in the average weight per hill. Sprague and Lenz (1920), in a preliminary experiment, found that strong doses may reduce the number of tubers formed, but that the tubers may attain a greater size so that yields are not lowered. In no case did they find that the crop was increased.

In 1952, Tolbert and Pearson reviewed the work of Sparrow and Christensen at the Brookhaven National Laboratory. For three years prior to the report, various plants had been grown in the "chronic gamma-field." In this field, the intensity of radiation was varied by differing the distance of the plants from the source, the

intensity being inversely proportional to the square of the distance from the source. The authors reported that, "although plants vary in radiation sensitivity, all plants are affected by sufficiently high exposure. It is important to note that no stimulation has been observed at any radiation intensity." White potatoes were used to study the effects of acute doses of X rays and fast neutrons and of chronic gamma irradiation from Cobalt-60 upon germination and growth of plants. Tubers irradiated at various dosages before planting gave decreased yields. Equivalent seasonal doses applied as low-level continuous irradiation had no adverse effects on growth. Chronic gamma irradiation in which the cumulative dose varied from 28 r to 8529 r for the season totals showed no consistent relationship between dosage and yield. There was no visible difference in size or apparent vigor of plants. It was noted that fast-neutron, acute irradiation of plants is at least four times more detrimental than acute X-irradiation. Chronic irradiation was found to be much less damaging physiologically than the same amount of radiation delivered in a short exposure. All of the possible explanations stem from the fact that ionization density does not reach a very high level in chronic exposure. Plants are thus given a much better chance to recover from damage than if given a single heavy dose.

Gunckel and Sparrow (1954) noted a variation in the growth rate response in plants exposed to chronic gamma irradiation similar to that reported after acute exposures; but most species showed no detectable stimulation after chronic irradiation; in a few cases, however, notably Antirrhinum majus, a definite increase in height occurred in a certain dose range. Nicotiana plants which grew at 15 r per day were much taller and bloomed well in advance of either the control or of any of the plants grown at higher dosage rates. The growth pattern of Tradescantia paludosa was also changed drastically at certain doses. Irradiation of dormant bulbs, tubers, and corms induced growth inhibition. These investigators found that in plants irradiated at later stages of development growth was nearly normal at low dosages and progressively decreased with higher doses until completely inhibited.

The controversy concerning growth stimulation caused by low dosages of irradiation still continues at the present time. Sax (1955) reported that there is some critical evidence of growth stimulation in a few cases. Ionizing radiation appears to be effective in stimulating cell growth, increasing the rate of growth of seeds and bulbs, and producing earlier flowering in some cases. Sax suggested that "these positive results would seem to justify further work on the effect of ionizing radiation with economic and ornamental plants."

Sparrow and Gunckel (1956) did not consider the two examples of apparent stimulation which they observed in Antirrhinum and Nicotiana as typical responses for plants in general. The 1956 report of the National Research Council of the National Academy of Sciences on The Biological Effects of Atomic Radiation stated, "There is no evidence that plant growth is stimulated or crop yields increased by exposure to low levels of radiation, despite earlier well-publicized claims to this effect."

### Respiration

Investigators do not agree about the influence of irradiation upon respiration. Lyon (1936) reported that the results obtained on yeast, on Helianthus annuus seeds, and on Vicia faba seedlings were in part contradictory. The results indicated either no influence with nondestructive doses or a depression of the respiratory intensity, especially with doses which are so injurious as to prevent continued growth of the plants involved. Mikaelson and Halvorsen (1953) found that doses of X-irradiation from 2500 r to 15000 r had negligible effect on germination of barley seeds and that respiration appeared unaffected at the 2500 r level. Doses from 5000 r to 15000 r did not affect the initial respiratory increase, but after four or five days the respiration of the irradiated seeds was only about half that of

the controls. Sussman (1953) reported increases of  $Q_{O_2}$  up to 60 per cent as a result of gamma irradiation of potato tubers over a wide range of doses. Gustafson et al. (1957) demonstrated that, following gamma irradiation, oxygen consumption in potato tubers decreased during the first week following an initial rise, then increased again. Tubers that had received doses of 50, 100, and 200 kilorep respired at a higher rate than the controls. Potato slices from tubers receiving doses of 5, 15, and 25 kilorep showed a high rate of respiration for several weeks, after which there was a drop in oxygen consumption, and from the seventh week on they respired at approximately the same rate as the controls. Mikaelson et al. (1956) reported that the respiration rate of neutron-irradiated barley seeds, measured as oxygen uptake per unit dry weight, was unimpaired during the first two days after germination, but that a pronounced reduction in rate of respiration appeared from the third to the seventh days. This reduction increased with increasing dose. Bjørnseth et al. (1957) found that neutron irradiation of barley seeds caused a reduction in the respiration rate which was correlated with reduced growth of the seedlings. If the respiration rate was determined as oxygen uptake per any given number of seeds, the irradiated seeds showed a considerably reduced respiration as compared to control seeds. If, however, the rate was based on

the amount of "protein-N" in the embryo, there was no difference between irradiated and untreated seeds. The investigators concluded that the respiration per unit active protoplasm was unchanged and the respiration process itself was apparently not inhibited by the radiation. Boell (1952) stated, "Respiration is an over-all expression of reactions by which energy is liberated in cells and tissues. Since the respiration process involves a multiplicity of enzyme-catalyzed steps and events, the type of result which one obtains on irradiating intact living systems will depend upon which steps are affected and to what extent damage has been done."

#### Metabolic changes

Medium and high doses of ionizing irradiation cause a variety of physiological and metabolic disturbances in both plants and animals (Nickson, 1952). Little specific information regarding plant metabolism is available. Auxin levels are known to be reduced following irradiation (Skoog, 1935; Gordon, 1957), and changes in enzyme activity occur (Blinks, 1952). Cooke (1953) has shown that radiation affects the ascorbic acid level in plants and has also demonstrated some correlation between the ascorbic acid content of the plant and its radiosensitivity. Growth inhibition after irradiation has been attributed to the inability of cells to utilize available

materials (Quastler and Baer, 1950; Riley, 1953). Pelc and Howard (1953) have reported inhibition of DNA synthesis in the irradiated roots of Vicia faba. Apparently, a great deal of work remains to be done in this area.

### Statement of Objectives

In the preceding discussion an attempt has been made to set forth the pertinent aspects of ionizing radiation and to review briefly the salient points in the existing literature in regard to its effects on plants. Extensive studies have been conducted on the morphological responses and a considerable amount of work has been done on the cytological and genetic effects. Although some investigations have been made on the physiological and biochemical responses, much yet remains to be done in these fields. The initial purpose of the present investigation was to determine the effects of ionizing radiation on the riboflavin, thiamine, and ascorbic acid content of treated plants since these compounds are all essential in normal plant metabolism. Interesting developments which arose during the course of the investigation have resulted in studies of morphological responses, starch accumulation, respiration, and dry-weight determinations.

## MATERIALS AND METHODS

### Plant Material

Plants of the Saco variety of the Irish potato, Solanum tuberosum L., were the exclusive plant material used in this study. This variety was selected for use because it has a relatively short dormant period. A continuing supply of tubers for propagation was obtained from the United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Branch, through the courtesy and cooperation of Dr. Robert V. Akeley and Dr. A. E. Schark. Most of the tubers were shipped from the Preque Isle, Maine, Station but one lot was received from the Beltsville, Maryland, Station. They were stored at 4° to 5° C. to prevent sprouting until the time of planting.

### Culture Methods

The tubers were removed from cold storage two or three days before planting. They were allowed to stand at room temperature for about twenty-four hours after which they were cut into pieces weighing approximately sixty grams ( $60 \pm 2$  g.) and having

two eyes or buds (Thompson, 1949). The tubers were cut through the apical end in order to divide the cluster of eyes, and, in so far as possible, compact pieces were cut because they were less apt to dry out than were slender ones. The pieces were allowed to cure at room temperature for two or three days until the cut surface had suberized and a wound periderm formed beneath, which protected the pieces from rotting prior to sprouting. They were then planted in six-inch clay pots in sandy loam composted soil which had been prepared at the Botanical Gardens. This soil had a pH of 7.0 and was composed of 50 per cent decayed sod, 25 per cent sand, 12.5 per cent well-rotted manure, and 12.5 per cent German peat. The plants were grown in the greenhouse of the Phoenix Memorial Laboratory. No attempt was made to maintain constant conditions of temperature, humidity, or lighting. However, all of the plants in any given experiment were grown under the same conditions and subject to the same environmental factors in the greenhouse.

#### Irradiation Techniques

When the plants had attained a height of twelve to fifteen inches, they were carefully selected for uniformity and removed to the radiation cave where they were placed in a circle of appropriate radius around the Cobalt-60 source. The prescribed doses

of gamma irradiation were then administered by exposure to the source for various periods of time. Dosages of 0, 5, 10, 15, 25, 50, and 100 kilorep were administered. Dosimetry, the actual radiation, and technical problems concerned with it were handled by the staff of the Phoenix Memorial Laboratory. The plants were returned to the greenhouse after irradiation, where they remained until they were transferred to the Natural Science Building for assay and analysis.

### Sampling Techniques

Riboflavin and thiamine determinations were made upon approximately 25 g. samples of plant material. Composite samples were used representing aliquot portions of three plants in any given category, as a rule. For leaf assays, three leaves from the top, midsection, and lower portion of each plant were collected (fig. 1). If the combined weight of these nine leaves was over 25 g., the leaves were split down the midrib and aliquot portions taken until a sample of the desired weight was secured. If, on the other hand, these leaves weighed under 25 g. additional leaves from the same plants were harvested. In some cases every other leaf was used in order to secure the necessary weight of sample. For stem

Fig. 1. Habit sketch of the potato plant, Solanum  
tuberosum L.

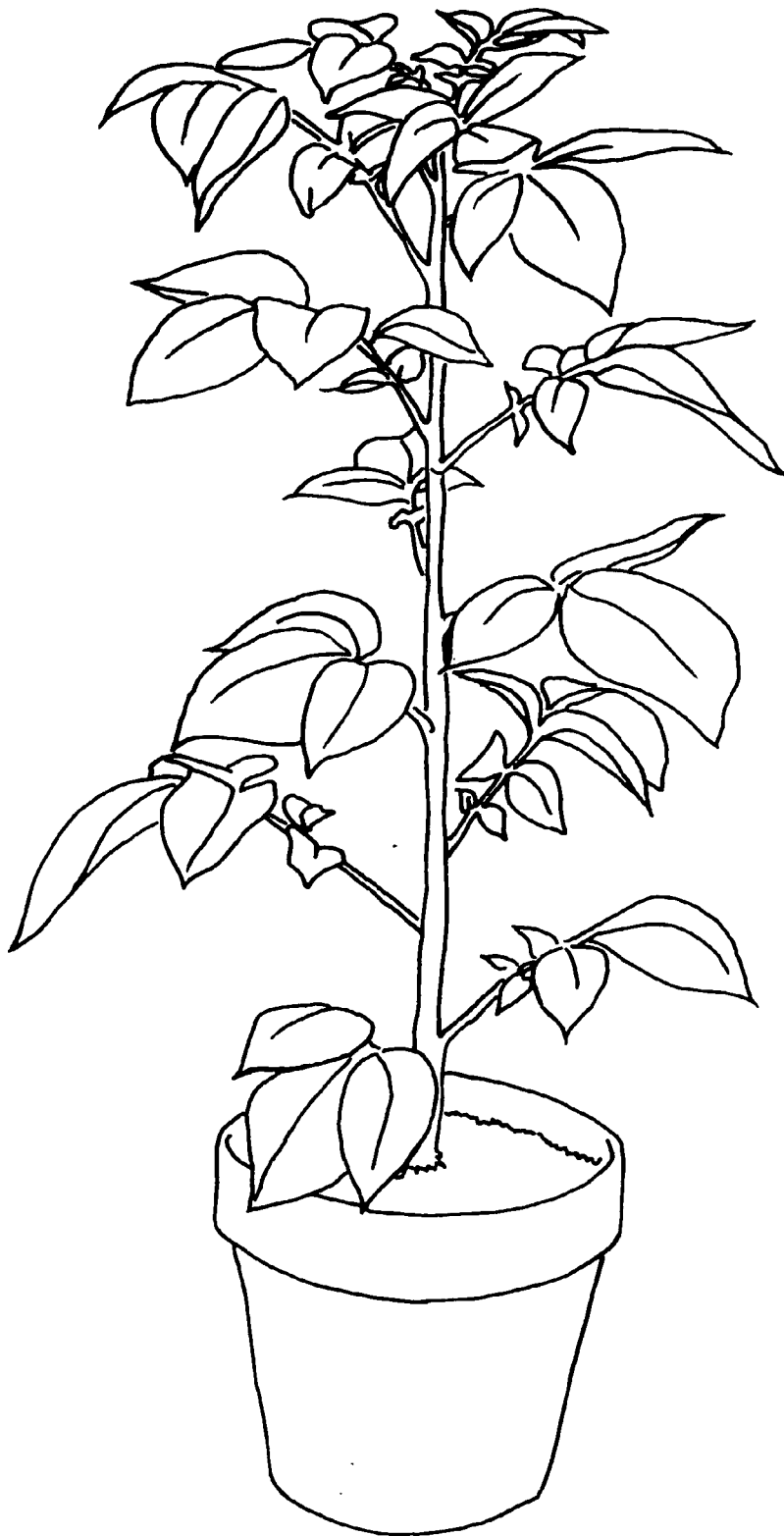


**FIGURE I.**

assays, stems were split longitudinally and aliquot portions taken up to the required amount.

Ascorbic acid determinations required samples weighing approximately 5 to 10 g. Leaves and stems were harvested in a manner similar to that used for the material for riboflavin and thiamine assays, and since smaller samples were required aliquot portions were taken.

Respiration studies were made on leaf discs. Three 12-mm. discs were cut from the top, midsection, and bottom leaf of each of three plants as quickly as was consistent with good technique (fig. 2). They were then stored in a moist chamber until samples from the various dosage levels could be harvested, after which they were set up for the determination of respiration.

### Analytical Methods

#### Determination of Riboflavin and Thiamine

The procedure employed for the riboflavin and thiamine assay was an adaptation of the method outlined by Conner and Straub (1941) as finally revised in this laboratory in July, 1954, for use in connection with plant materials. By this procedure, it was possible to make a combined determination of thiamine and riboflavin upon a

Fig. 2. Sketch of a potato leaf from which 12-mm. discs had been removed for respiration studies.

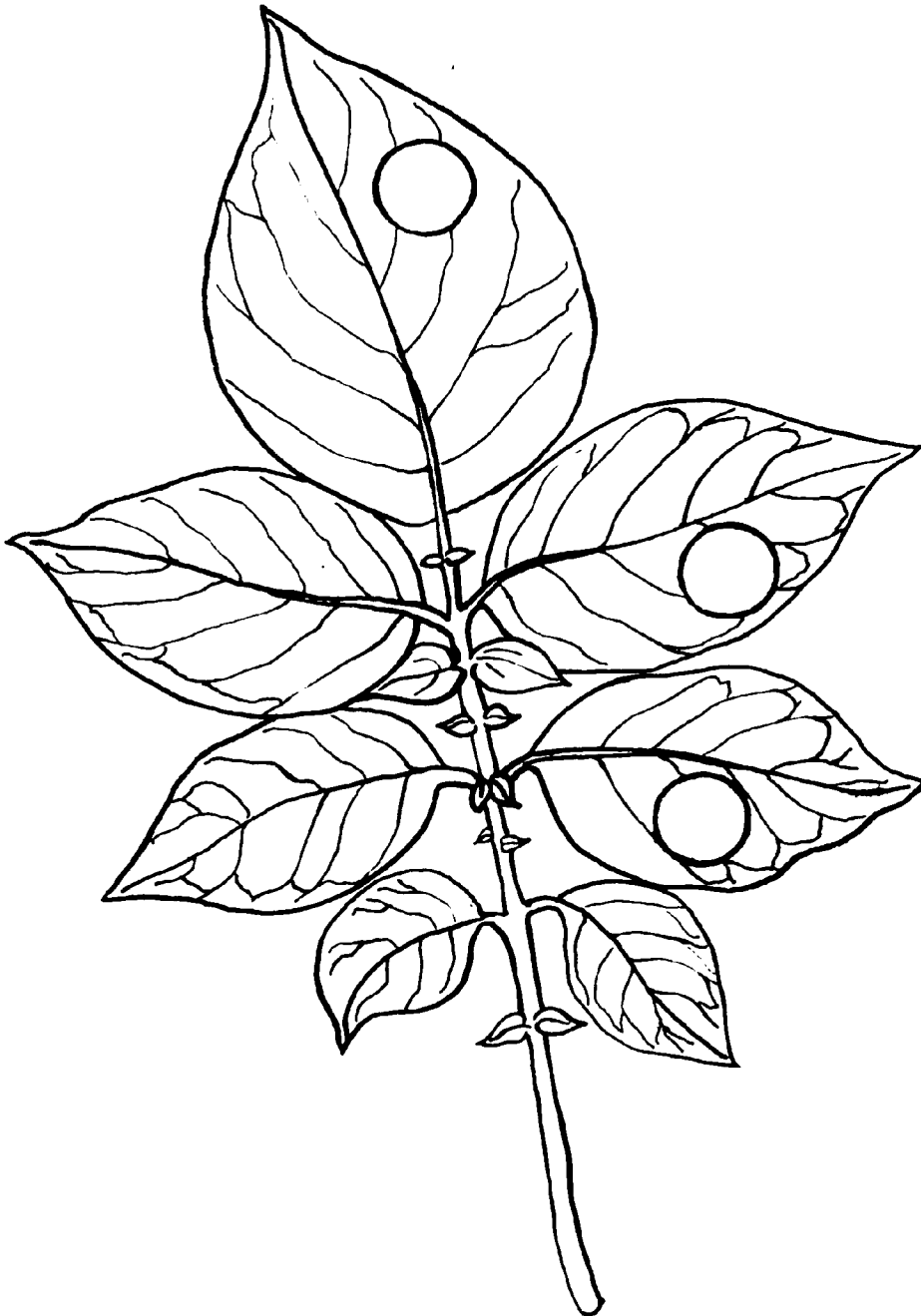


FIGURE 2.

single sample of a given material. After extracting the vitamins from the sample in an acid medium, they were then separated by the use of selective adsorbing agents.

Thiamine occurs in natural foods and biological materials, either free or in a combined form, as a protein complex, as a phosphorus-protein complex, or as the pyrophosphoric acid ester cocarboxylase (Association of Vitamin Chemists, 1947). The determination of thiamine depends upon its oxidation to thiochrome and the fluorometric measurement of the latter compound.

In living cells, riboflavin generally occurs combined either with phosphoric acid, or with phosphoric and adenylic acids, both of which may be combined further with specific proteins to form oxidative enzymes. In most analytical procedures, it is necessary to treat natural products with acids or enzymes to insure the liberation of riboflavin from its protein combination and to make it more readily extractable.

Riboflavin is very sensitive to both visible and ultraviolet light (Williams and Cheldelin, 1942; DeMerre and Brown, 1944) and all operations for its assay must be performed in the absence of strong light. Riboflavin concentrates give off a yellow-green fluorescence upon activation by light of certain wave lengths. In acid

solution, lumichrome (6,7-dimethylalloxazine) is formed which is characterized by a blue fluorescence.

Thiamine and riboflavin determination in plant material required two days. On the first day, samples were prepared as previously discussed, and macerated in a Waring Blendor with a quantity of distilled water four times the weight of the plant material (4 g. water/1 g. plant material). After the material was finely subdivided, a quantity of 0.1N sulfuric acid equivalent to the total volume of the mixture (5 ml. sulfuric acid/1 g. plant material) was added and mixed for a short time to insure thorough blending. The pH (1 to 2) of this extraction mixture was optimal for the extraction of the vitamins and at the same time sufficiently acid to prevent their destruction. The resulting brei was strained through several thicknesses of cheesecloth. Fifty milliliters of the strained mixture was transferred to a 125-ml. Erlenmeyer flask which was then stoppered with a cotton plug and properly tagged.

A "complete blank" consisting of all the reagents used throughout the method, but devoid of plant material, was run through the entire procedure. In the case of the riboflavin, the value for the blank was subtracted from fluorescence readings of the sample.

The flasks containing the brei were autoclaved for 15 minutes at 15 pounds pressure to ensure complete extraction of the vitamins.

After cooling to room temperature, 10 ml. of a freshly prepared suspension of the commercial enzymatic reagent Clarase (5 per cent in a 1 per cent sodium acetate-acetic acid buffer) was added to each flask. This increased the pH to the range of 4 to 5 and afforded optimal conditions for the enzymatic hydrolysis of any thiamine pyrophosphate present in the extract. Digestion with Clarase accomplishes two things. It liberates bound riboflavin and thiamine, and, because of its diastatic activity, it is effective in preparing extracts of starchy materials. After the addition of a few drops of toluene to cover the surface of the liquid in each flask, the brei was incubated at 37°C. for about 18 to 20 hours.

On the second day, about 45 ml. of the plant extract was removed with a pipette from below the toluene layer in the flasks and placed in labeled centrifuge tubes. These tubes were then centrifuged at 2500. to 2600 revolutions per minute for 45 minutes and a clear supernatant liquid was obtained. Meanwhile, the adsorption apparatus was set up (fig. 3). This consisted of two glass adsorption columns approximately 2.5 cm. in diameter which were connected by a two-hole rubber stopper so that the filtrate from the upper column fell directly onto the lower column. The stopper was provided with a rubber tube and screw clamp to regulate the flow of liquid through the upper column. The combined columns were set up on a

Fig. 3. Sketch of apparatus assembly for riboflavin and thiamine determinations.

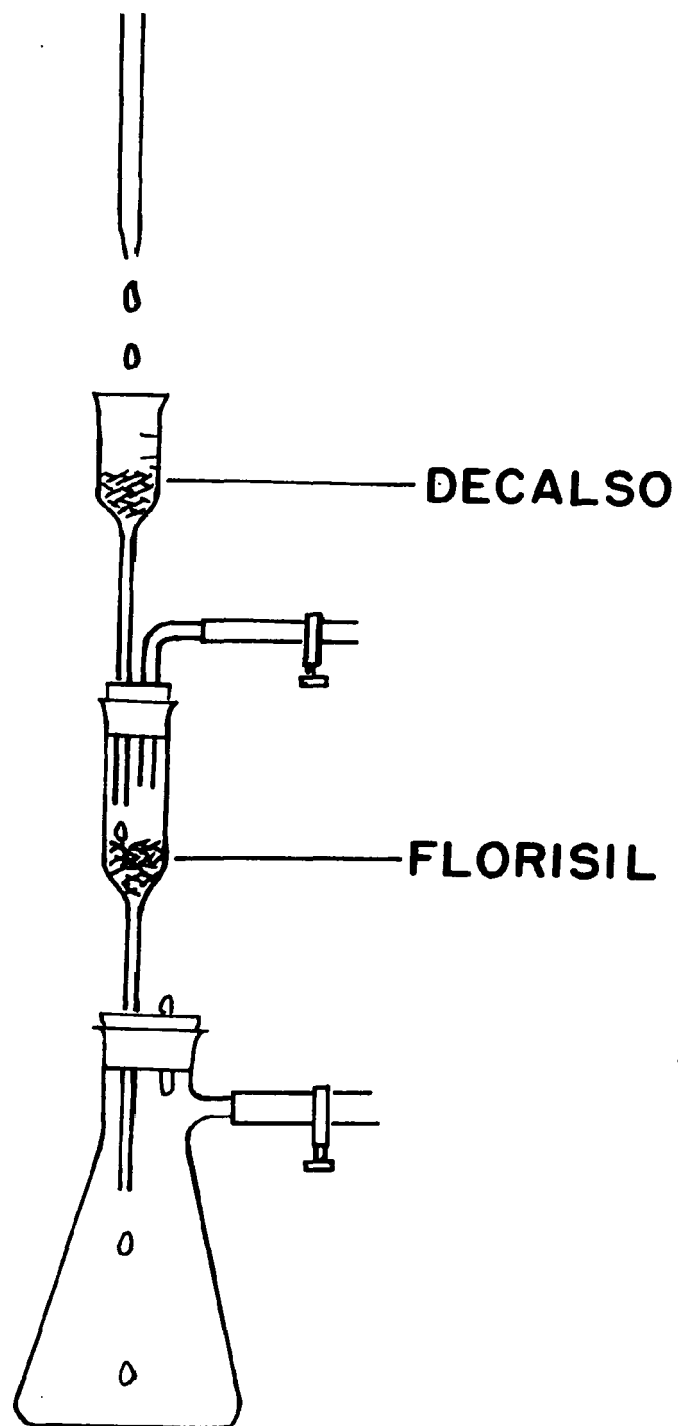


**FIGURE 3.**

suction flask connected to a suction outlet. Discs of fine glass wool were cut and a disc fitted into the bottom of each column or tube. Properly activated Decalso was added to the upper column to a depth of approximately 1.5 cm. and a similar amount of activated Florisil was placed in the lower column.

Twenty-five milliliters of the supernatant from the centrifuged samples was allowed to drop slowly from a pipette and to pass through the two columns at the rate of about 1 ml. per minute, the flow being regulated by adjusting the screw clamps between the columns as well as that on the suction flask. Thiamine was adsorbed on the Decalso in the upper column and riboflavin was adsorbed on the Florisil in the lower column. Both columns were thoroughly washed down with about 35 to 40 ml. of boiling distilled water, drained by suction, and the washings discarded. After draining, the upper column containing the adsorbed thiamine was set aside until after the completion of the riboflavin assay. In order to protect the riboflavin from light, the room was darkened before removing the tubes from the centrifuge and kept dark until after completion of the assay, and the containers and receivers for the vitamin were covered with black paper.

The tubes containing riboflavin were mounted on vacuum receivers and the riboflavin eluted with 35 to 40 ml. of a solution of

20 per cent pyridine in 2 per cent acetic acid, the flow through the column being adjusted to 1 ml. per minute. Suction was applied to the columns. The eluates were withdrawn from the vacuum receivers into 50-ml. volumetric flasks and made up to volume with pyridine-acetic acid solution. The flasks were shaken to insure mixing and covered with black paper to protect the vitamin from light. Duplicate 15-ml. aliquots of the riboflavin solutions were pipetted into 125-ml. brown Erlenmeyer flasks. Then, working with two flasks at a time, 1 ml. of freshly prepared 4 per cent potassium permanganate solution was added to each, with shaking. This oxidizes extraneous matter but not riboflavin. Three milliliters of 3 per cent hydrogen peroxide was added, the reaction mixture shaken vigorously, and then allowed to stand until all effervescence had ceased. Fifteen milliliters of the mixture was then transferred to a cuvette and the fluorescence readings made with a Lumetron Fluorescence Meter (Lumetron Mod. 402 EF). The Lumetron was adjusted by inserting a cuvette containing 15 ml. of a sodium fluorescein solution (1 mcg./ml.), setting the dial at 50 and adjusting the balance photocell until no deflection showed on the galvanometer. Then, with the dial set at 0, a cuvette containing pyridine-acetic acid was inserted in the Lumetron and the zero suppressor moved until no deflection showed on the galvanometer when contact was made.

The reading for the blank was subtracted from the individual readings to compensate for any fluorescence of the ingredients other than riboflavin. The readings were then translated into micrograms of riboflavin per gram of material by the use of a chart prepared previously for use in this laboratory. A standard curve representing values of riboflavin from 0.005 to 1.42 micrograms was prepared first. Then, a dilution factor of 3.84 for the unknowns was applied to extrapolations from this curve and the values set up in a table so that dial readings could be translated directly into micrograms of riboflavin per gram of fresh weight.

Thiamine was also assayed by fluorescence. The adsorption columns were placed on graduated bases or tubes and the vitamin eluted with 30 to 35 ml. of boiling acid potassium chloride solution (25 per cent KCl, 0.85 per cent HCl). Suction was applied to the columns, the adsorption sections removed, and the volume in the collection tubes made up to exactly 40 ml. with boiling acid potassium chloride solution. The eluates were withdrawn into 125-ml. Erlenmeyer flasks which were shaken to mix the contents. Five-milliliter samples of each were taken in triplicate and placed in 50-ml. volumetric flasks. One of the triplicates of each sample was used as a blank, the other two for the oxidation of the thiamine to the fluorescent thiochrome. Beginning at this point and working

as expeditiously as possible in order to obtain consistent results, the samples were run through a uniform procedure. The blanks were processed first. To each of two flasks was added 3 ml. of 15 per cent sodium hydroxide solution. The flasks were shaken for one minute by a stop watch. Fifteen milliliters of reagent isobutyl alcohol was then added from a burette and the mixtures shaken for one minute by the stop watch. The mixtures were transferred to centrifuge tubes and centrifuged at 2500 to 2600 revolutions per minute for 15 seconds; then the centrifuge was shut off and allowed to coast 45 seconds more and stopped by braking at the end of this time. The supernatant alcohol was pipetted off and placed in test tubes containing about 1.5 g. of anhydrous sodium sulfate in order to remove any residual water.

The other sets of replicates were treated in a like manner except that they were oxidized by the addition of 3 ml. of freshly prepared mixed reagent (4 ml. of 1 per cent potassium ferricyanide and 96 ml. of 15 per cent sodium hydroxide) instead of the nonoxidizing sodium hydroxide solution. Potassium ferricyanide in the presence of strong alkali oxidizes thiamine to thiochrome (Association of Vitamin Chemists, 1947). This compound is soluble in isobutyl alcohol and upon irradiation with ultraviolet light fluoresces blue. After oxidation of the samples, extraction of the thiochrome with

isobutyl alcohol, and drying with anhydrous sodium sulfate, all of the tubes were centrifuged for four or five minutes to eliminate any suspended particles of sodium sulfate.

Following this treatment, the isobutyl alcohol layer was decanted into a cuvette and the fluorescence measured in the Lumetron which had been standardized using quinine sulfate solution (0.3 mg. per liter) with the dial set at 35 and isobutyl alcohol with the dial set at 0. The reading of the blank from each sample was subtracted from the readings obtained from the oxidized material. Finally, the readings were translated into micrograms of thiamine per gram of plant material from the table of values which had been prepared in a manner similar to that used in preparing the chart for riboflavin. In this instance, the dilution factor for thiamine was 1.60.

#### Determination of Total Ascorbic Acid

The 2,4-dinitrophenylhydrazine colorimetric method of analysis was used in the present investigation. This procedure was originally proposed and perfected by Roe and his associates (1944, 1948) and had been adapted and employed in this laboratory in earlier experimentation. Ascorbic acid is alkali-sensitive and easily oxidized in aqueous solution. It is believed to exist in the enediol form

which may be reversibly oxidized by the removal of two hydrogen atoms from carbon atoms 2 and 3 with the formation of dehydroascorbic acid. This oxidation may be carried out by the enzyme ascorbic acid oxidase (Bonner, 1950), or it may be catalyzed by inorganic copper. In plant tissue, a variable proportion of the total ascorbic acid occurs in this reversible oxidized state (Bonner, 1950). Gustafson and Cooke (1952) found that ascorbic acid is rapidly converted to dehydroascorbic acid at low temperatures. Since it was necessary to store aliquot portions in the deep-freezer at about  $-20^{\circ}\text{C}$ . and thus induce an increase in the proportion of dehydroascorbic acid, only total ascorbic acid (reduced plus dehydro) has been determined and reported. Suitable precautions were practiced in the investigation to minimize error because of the lability of the compound by maintaining a low pH, avoiding contact with heavy metals, and by working as rapidly as was consistent with good technique.

A representative sample of the material, generally between 5 and 10 g., was comminuted in a Waring Blendor with a sufficient quantity of freshly prepared mixed acid (5 per cent metaphosphoric acid and 10 per cent acetic acid). The quantity of acid used was such that the final concentration was usually 2.5 g. of leaves per 100 ml. of brei, 5 g. of stems per 100 ml. of brei, or 10 g. of

tuber per 100 ml. of brei. These concentrations were employed in order to keep the readings within the range of the instrument. The sample was first blended with 50 to 70 per cent of the calculated volume of the mixed acid, and the lid and sides of the Blendor rinsed down with the remainder. Then approximately 1 g. of acid-washed Norite was added and the mixture agitated until thoroughly mixed. The whole process of comminution was performed as rapidly as possible (less than 2 minutes) to prevent undue oxidation of the vitamin.

In this procedure, metaphosphoric acid complexes any heavy metals which may be present, thereby preventing metal-catalyzed oxidation, and it also precipitates interfering substances such as protein, thereby facilitating subsequent clarification of the extract (Association of Vitamin Chemists, 1947). The Norite oxidizes ascorbic acid to dehydroascorbic acid and adsorbs any coloring matter such as chlorophyll from the mixture. The acetic acid prevents adsorption of the vitamin to Norite and the low pH affords a stable medium for the dehydroascorbic acid.

The mixture was filtered through a single No. 1 Whatman filter paper and returned to the filter until clear. Four-milliliter aliquots of the clear filtrate were pipetted into each of three 50-ml. test tubes; two drops of 10 per cent thiourea in 50 per cent ethyl

alcohol were added to each tube, and 1 ml. of 2 per cent 2,4-dinitrophenylhydrazine in 9N sulfuric acid was pipetted into two of the tubes. The third tube served as a blank. The tubes were incubated in a constant-temperature water bath at 37°C. for three hours. During this period dehydroascorbic acid couples with 2,4-dinitrophenylhydrazine to form a red pigment 2,3-bis-(2,4-dinitrophenylhydrazone). At the end of the three hour period, the tubes were placed in an ice bath and 5 ml. of 85 per cent sulfuric acid was pipetted dropwise (to prevent charring) into each tube. If the sulfuric acid is added too rapidly or the temperature is allowed to rise, a darkening of solutions containing sugars may result. One milliliter of the 2,4-dinitrophenylhydrazine reagent was pipetted into each of the blanks. The tubes were shaken vigorously in the ice bath to mix their contents. They were removed from the ice bath and allowed to stand at room temperature for one hour to permit complete color development.

Dehydroascorbic acid (Association of Vitamin Chemists, 1947) or one of its derivatives (probably 2,3-diketo-1-gulonic acid) (Penny and Silva, 1943) reacts with 2,4-dinitrophenylhydrazine to form an osazone, 2,3-bis-(2,4-dinitrophenylhydrazone), which is red in color. Acidification dissolves this osazone and a red solution results.

The solutions were transferred into matched colorimeter tubes and the color intensities read in a Bausch and Lomb colorimeter using a 505 m $\mu$  interference filter. The ascorbic acid values were read from a standard curve on semilogarithmic paper and the ascorbic acid content of the tissues was calculated on the basis of fresh weight.

### Determination of Respiration

Manometric techniques using the "direct method" of Warburg as described by Umbreit, Burris, and Stauffer (1947) were used for this investigation. During the determination, the oxygen uptake of the living tissues, which also liberate carbon dioxide, is measured by absorbing the carbon dioxide continuously in alkali. Warburg flasks of 90 to 100 ml. capacity were prepared by placing a Whatman filter of suitable size in the bottom of each flask and adding 0.4 ml. of distilled water to maintain a moist atmosphere. Two-tenths milliliter of 20 per cent potassium hydroxide solution was added to the side arm well and a folded wick of Whatman filter paper was inserted into each well and allowed to protrude into the main compartment of the flask to afford greater absorbing area for the carbon dioxide. Composite samples of plant material were obtained by cutting 12-mm. discs from the leaves of the potato plants

(fig. 2). Each sample generally contained twenty-seven leaf sections, three each from a top, midsection, and bottom leaf of each of three plants. The determinations were usually run in duplicate. The volume of the leaf sections was determined by the water-displacement method. After gently drying adhering moisture from the discs on a towel, they were placed in the main compartment of the flask. The flask was attached to the manometer and placed in a constant-temperature bath of a circular type respirometer which was preheated and maintained at 28°C. After all the flasks had been prepared, the respirometer was shaken at the rate of 130 cycles per minute with a 5-cm. arc. The stopcocks on the manometers remained open during a 45-minute equilibration period, after which the manometer fluid was leveled, the stopcocks closed, and original readings taken. The readings were recorded three times at twenty-minute intervals. Finally, the leaf discs were removed from the flasks and dried to constant weight.

The amount of oxygen consumed ( $Q_{O_2}$ ) was calculated and expressed in units of  $\mu\text{l.}/\text{mg. dry wt.}/\text{hr.}$  or in  $\mu\text{l.}/\text{leaf section}/\text{hr.}$

#### Preparation of Slides to Demonstrate Starch Content

During the process of macerating and straining the leaves and stems of the plants in the preparation of the brei to be

extracted for the riboflavin and thiamine assays a noticeable deposit of a fine, white powder was observed in the bottom of the beakers containing the strained liquid. This deposit or layer seemed to be markedly greater in those plants receiving the higher radiation doses. Upon treatment with iodine solution, a blue-black color resulted, indicating the presence of starch. Preliminary examination of cross sections of stems made by freehand sectioning indicated considerable variation in the starch content of plants irradiated at different dosage levels. Sections uniform in thickness were then cut using a Bausch and Lomb freezing-microtome. First a cross section of stem about 0.75 cm. long was cut from the midsection of a given plant with a razor blade. Then a drop of dilute acacia solution was placed upon the stage of the microtome and the plant section placed upon this and frozen in position by means of a stream of carbon dioxide under pressure. A cone of ice was built up around the section by alternate applications of acacia solution and carbon dioxide. When the plant tissue was firmly frozen it was sectioned by use of a rotary microtome. Initially, sections were cut 75 microns thick. Upon staining and mounting, the entire surface of the slide appeared to be covered with starch grains. After taking cognizance of the fact that the parenchyma cells of the pith and cortex measure on the average 170 microns in length and in some instances exceed 280

microns, and that the sectioning was in all probability cutting across most of these cells and allowing the starch to sift out, it was decided to make the thickest sections which were feasible in order to retain as much starch in the cells as possible. Accordingly, the sections were cut 150 microns thick. These were washed in water, then fixed and stored in formalin-aceto-alcohol (FAA) solution (Johansen, 1940) until they were to be used. After washing in water, the fixed sections were stained with weak iodine solution and temporary mounts were made and photographed. Although many cells were still cut, it was possible to obtain enough cells containing starch to show the relative content at the various irradiation levels.

## EXPERIMENTAL RESULTS

A series of preliminary experiments were conducted to test culture methods, to develop laboratory techniques, and to determine on the basis of early results whether or not further investigation on the effects of gamma irradiation on potato plants was warranted.

Potato plants were grown and irradiated at the Phoenix Memorial Laboratory as previously outlined. Experimental data on riboflavin and thiamine content of irradiated plants from eight of these preliminary experiments were picked at random and, together with data on nonirradiated controls, were submitted to Miss E. B. Schaeffer of the Statistics Research Laboratory of the University of Michigan for statistical analysis. This analysis showed significantly large increases in the riboflavin content of stems (at 0.1 per cent and 1.0 per cent levels), particularly in those plants receiving doses of irradiation in excess of 15 kilorep. Changes in the riboflavin content of leaves were not generally significant. Significant increases in thiamine content of stems and leaves occurred in a large number of cases at the higher irradiation levels, but, in a few instances at the 5- and 10-kilorep dosage levels, there were

significant decreases. The results of this analysis indicated that further investigation was justifiable.

Gamma irradiation of potato plants at the dosage levels administered in the present investigation produced no effects which were immediately visible. Within a period of a week, however, changes could be observed in the general appearance of plants receiving more than 10 kilorep. Abnormalities became more striking with increase in length of time after irradiation. The first noticeable difference was the change in leaf color of plants irradiated at the higher dosage levels. The normally bright green color faded to a yellow green and/or mottling occurred. The apices of these plants often withered and died within a short time, and in many instances axillary growth in these regions was subsequently initiated. As the leaves aged, they assumed a whitish or gray-green color and they became thickened and leathery. The leaf blades rolled upward and curled in (figs. 4, 5). When the plants were shaken or jarred, their leaves rattled like the dry leaves on a shrub or tree do when rustled by the wind. Leaves on plants irradiated at the 15- and 25-kilorep dosage levels became mottled, their texture changed, and they became somewhat thicker and more leathery but not to as great a degree as those receiving the higher doses. Leaves on

Fig. 4. Effects of gamma irradiation on plant habit. Treatments from left to right--nonirradiated control, 5 kilorep, 10 kilorep, and 15 kilorep, respectively. Mottling and abnormal coloration observed at the 15 kilorep level are not apparent in black-and-white photographs.

Fig. 5. Effects of gamma irradiation on plant habit. Treatments from left to right--nonirradiated control, 25 kilorep, 50 kilorep, and 100 kilorep, respectively. Abnormal coloration which can be observed in irradiated plants is not apparent in black-and-white photographs. Thickening and curling of leaves at the higher levels can be discerned, however.

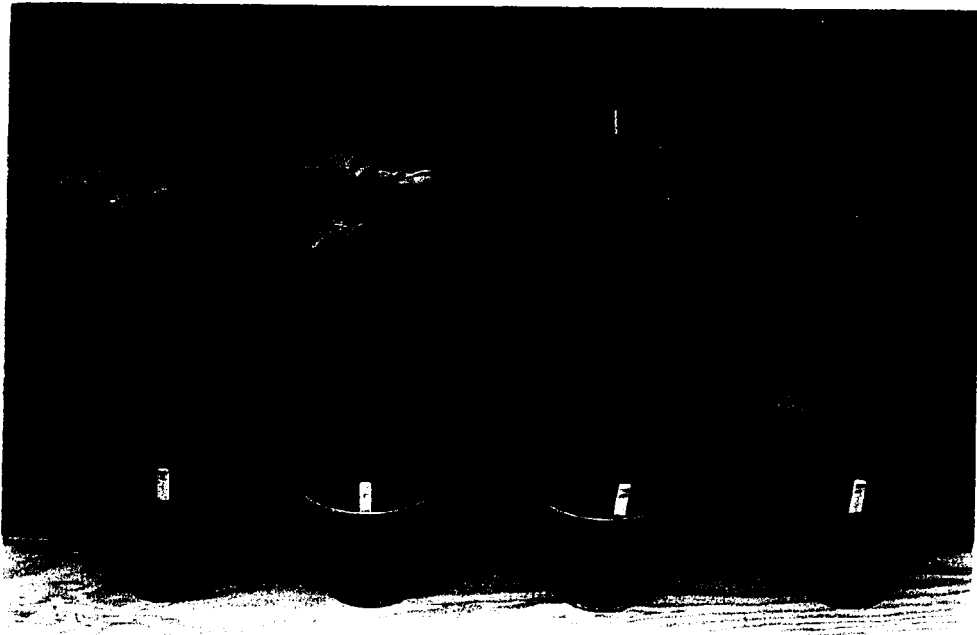


FIGURE 4.

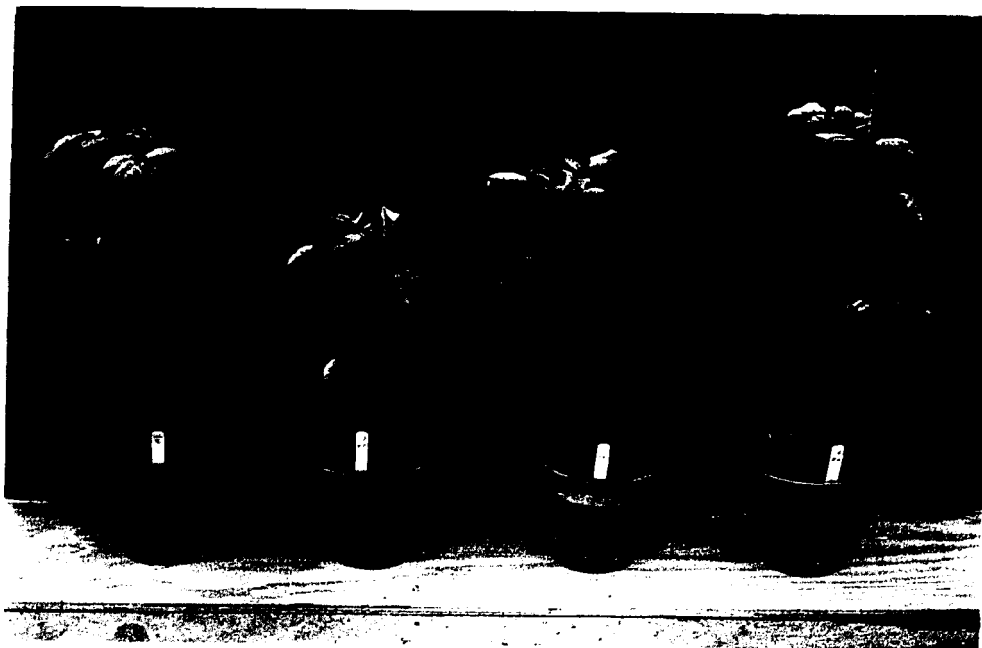


FIGURE 5.

plants irradiated at the 5- and 10-kilorep levels did not differ noticeably from the controls.

Leaves which appeared subsequent to irradiation showed various forms and degrees of modification. Some had rough, pebbly surfaces, some were densely hairy, some were thick with wavy margins, others had irregularities in form, with the blade narrow on one side of the midrib, and still others had their leaflets fused, especially the lower ones on a leaf.

Most leaves on these irradiated plants did not absciss but persisted until the plants died. The stems and petioles became thickened and the stems remained green for some time after the leaves had died. In one lot of plants which were not harvested for assay but were allowed to stand, aerial tubers were formed in the axils of the leaves toward the top of the plant about eight weeks after irradiation (fig. 6). Four out of this group of six plants which had been treated with 15 kilorep of gamma irradiation produced aerial tubers.

There was little difference in the over-all appearance of control plants and of those receiving doses of 5 and 10 kilorep. In some instances the treated plants appeared stockier and more sturdy than the controls. This observation was borne out when

Fig. 6. Aerial tubers on potato plant which had received 15 kilorep of gamma irradiation.

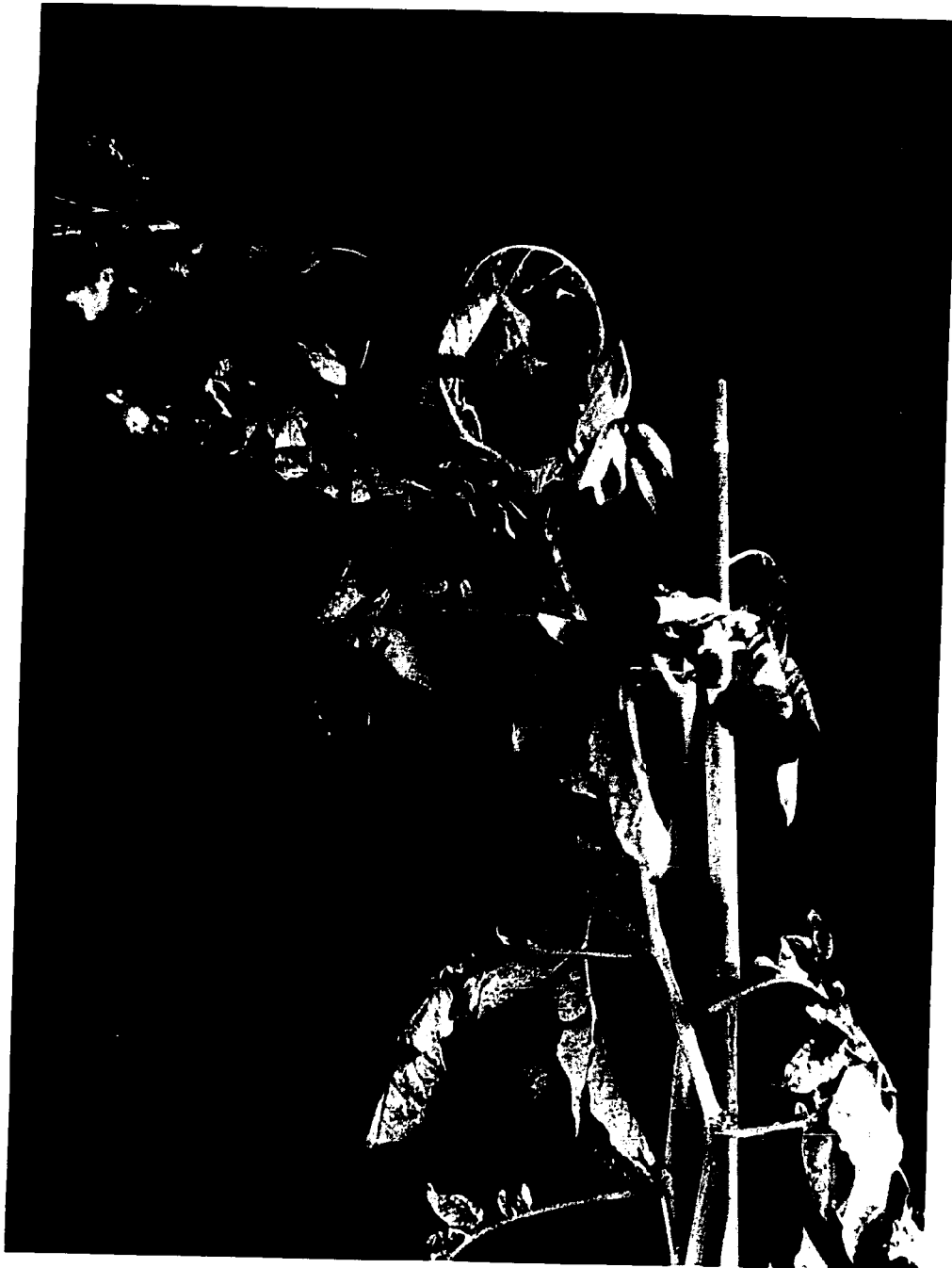


FIGURE 6.

dry-weight determinations were made and will be discussed in that connection.

When the tubers from plants used in these preliminary experiments were harvested, the yield decreased progressively with increasing doses of irradiation until at the 50- and 100-kilorep dosage levels, very few or no tubers were present.

Determinations of the vitamin content of potato tubers representative of those used for propagation were included in the preliminary experiments. Six typical samples were assayed. Each sample was composed of aliquot portions of longitudinal slices (including the epidermis) which were cut through the midsection of each of three tubers. These eighteen tubers showed an average riboflavin content of 0.179 microgram per gram of fresh weight (table 1), thiamine content averaged 0.302 microgram per gram of fresh weight (table 1), and ascorbic acid content averaged 9.57 milligrams per 100 grams of fresh weight (table 2).

Aerial portions of the plants were studied to determine the effect of gamma irradiation upon riboflavin, thiamine, and ascorbic acid content.

Table 1. Riboflavin and thiamine content of untreated potato tubers. These tubers were representative of those propagated for the plant material used in this investigation. Aliquot portions of longitudinal slices, including the epidermis, through the middle of each of three tubers were contained in each sample.

| Sample<br>No. | Micrograms per Gram<br>of Fresh Weight |                   |
|---------------|----------------------------------------|-------------------|
|               | Riboflavin                             | Thiamine          |
| 1             | 0.190                                  | 0.335             |
| 2             | 0.165                                  | 0.290             |
| 3             | 0.200                                  | 0.285             |
| 4             | 0.215                                  | 0.280             |
| 5             | 0.145                                  | 0.290             |
| 6             | 0.160                                  | 0.330             |
| Avg.          | $0.179 \pm 0.027$                      | $0.302 \pm 0.024$ |

Table 2. Total ascorbic acid content of untreated potato tubers. These tubers were representative of those propagated for plant material used in this investigation. Aliquot portions of longitudinal slices, including the epidermis, through the midsection of each of three tubers were contained in each sample.

| Sample<br>No. | Milligrams per 100 Grams<br>of Fresh Weight |
|---------------|---------------------------------------------|
|               | Total Ascorbic Acid                         |
| 1             | 12.4                                        |
| 2             | 10.2                                        |
| 3             | 9.0                                         |
| 4             | 9.8                                         |
| 5             | 7.2                                         |
| 6             | 8.8                                         |
| Avg.          | 9.57 $\pm$ 1.38                             |

Effect of Gamma Irradiation upon Riboflavin  
and Thiamine Content

The riboflavin and thiamine content of a group of plants receiving single, acute doses of irradiation was determined at five- and eight-week intervals after irradiation. The values recorded as micrograms per gram of fresh weight appear in table 3. These same values expressed as percentage of the control are presented graphically in figures 7, 8, 9, and 10. The histograms indicate that riboflavin content of leaves of irradiated plants was comparatively lower than that of the control in all cases, except at the 10-kilorep level, which was slightly higher at the end of five weeks, and which had dropped below the control at the end of eight weeks. The content of riboflavin in the stem was generally higher in irradiated plants than in controls. Concentration of thiamine in the leaf was higher than in the controls in all categories, except at the 5-kilorep level, at the end of five weeks after irradiation. At the end of eight weeks, increased concentration was again observed at all levels, except at the 5-kilorep dosage level. Thiamine content in the stem had increased markedly at all dosage levels above 10 kilorep at the end of five weeks. At the end of eight weeks, thiamine content of plants irradiated at the higher dosage levels remained

Table 3. The effect of acute doses of gamma irradiation upon the content of riboflavin and thiamine in potato leaves and stems. Determinations were made at five weeks and eight weeks after irradiation. Each value represents a composite of three plants.

| Dose<br>in<br>Kilo-<br>rep | Micrograms per Gram of Fresh Weight |       |       |       |
|----------------------------|-------------------------------------|-------|-------|-------|
|                            | Leaves                              |       | Stems |       |
|                            | 5 wk.                               | 8 wk. | 5 wk. | 8 wk. |
| <u>Riboflavin</u>          |                                     |       |       |       |
| 0                          | 1.220                               | 1.530 | 0.220 | 0.170 |
| 5                          | 1.135                               | 1.130 | 0.180 | 0.240 |
| 10                         | 1.285                               | 1.000 | 0.280 | 0.260 |
| 15                         | 0.985                               | 1.030 | 0.420 | 0.285 |
| 25                         | 0.940                               | 1.040 | 0.300 | 0.340 |
| 50                         | 0.860                               | 1.010 | 0.420 | 0.370 |
| 100                        | 1.025                               | 1.260 | 0.400 | 0.375 |
| <u>Thiamine</u>            |                                     |       |       |       |
| 0                          | 0.040                               | 0.060 | 0.040 | 0.075 |
| 5                          | 0.036                               | 0.040 | 0.056 | 0.040 |
| 10                         | 0.045                               | 0.170 | 0.120 | 0.700 |
| 15                         | 0.210                               | 0.570 | 1.470 | 1.970 |
| 25                         | 0.075                               | 0.490 | 1.350 | 2.185 |
| 50                         | 0.150                               | 0.530 | 1.685 | 2.280 |
| 100                        | 0.245                               | 0.580 | 1.830 | 2.255 |

Fig. 7. Effect of single, acute doses of gamma irradiation upon riboflavin content in potato leaves. Values represented as percentage of control.

Fig. 8. Effect of single, acute doses of gamma irradiation upon riboflavin content in potato stems. Values represented as percentage of control.

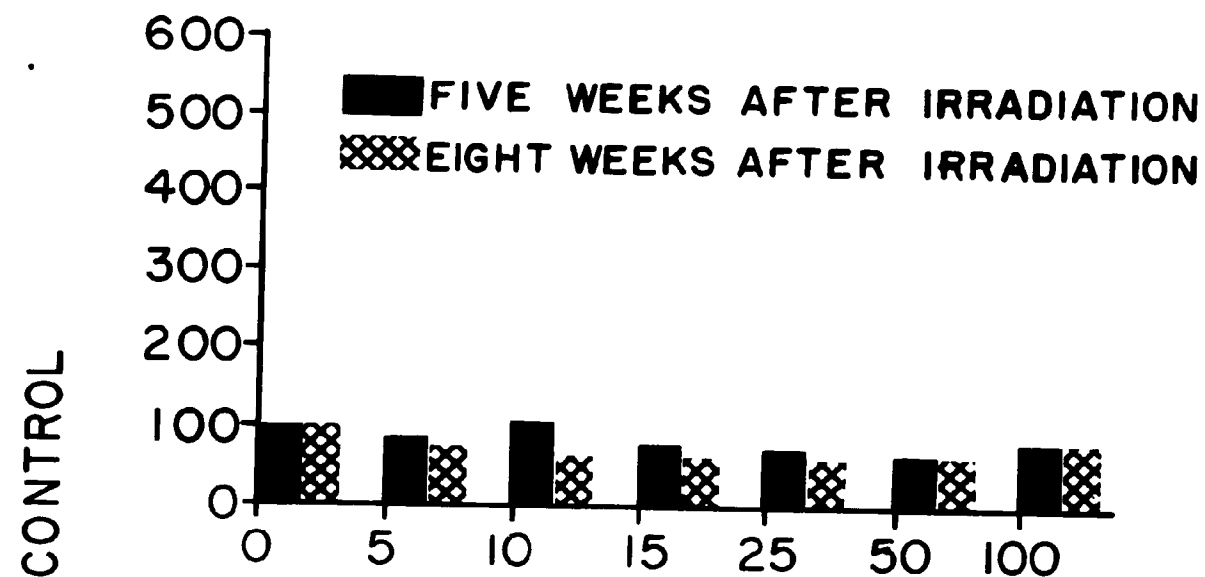


FIGURE 7. LEAF

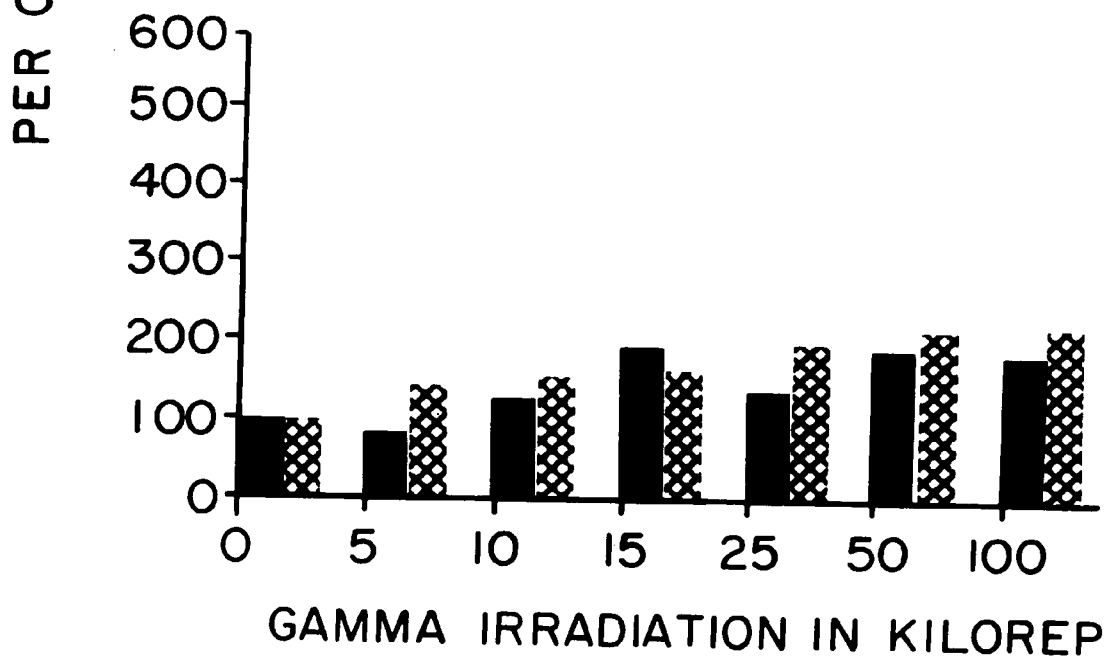
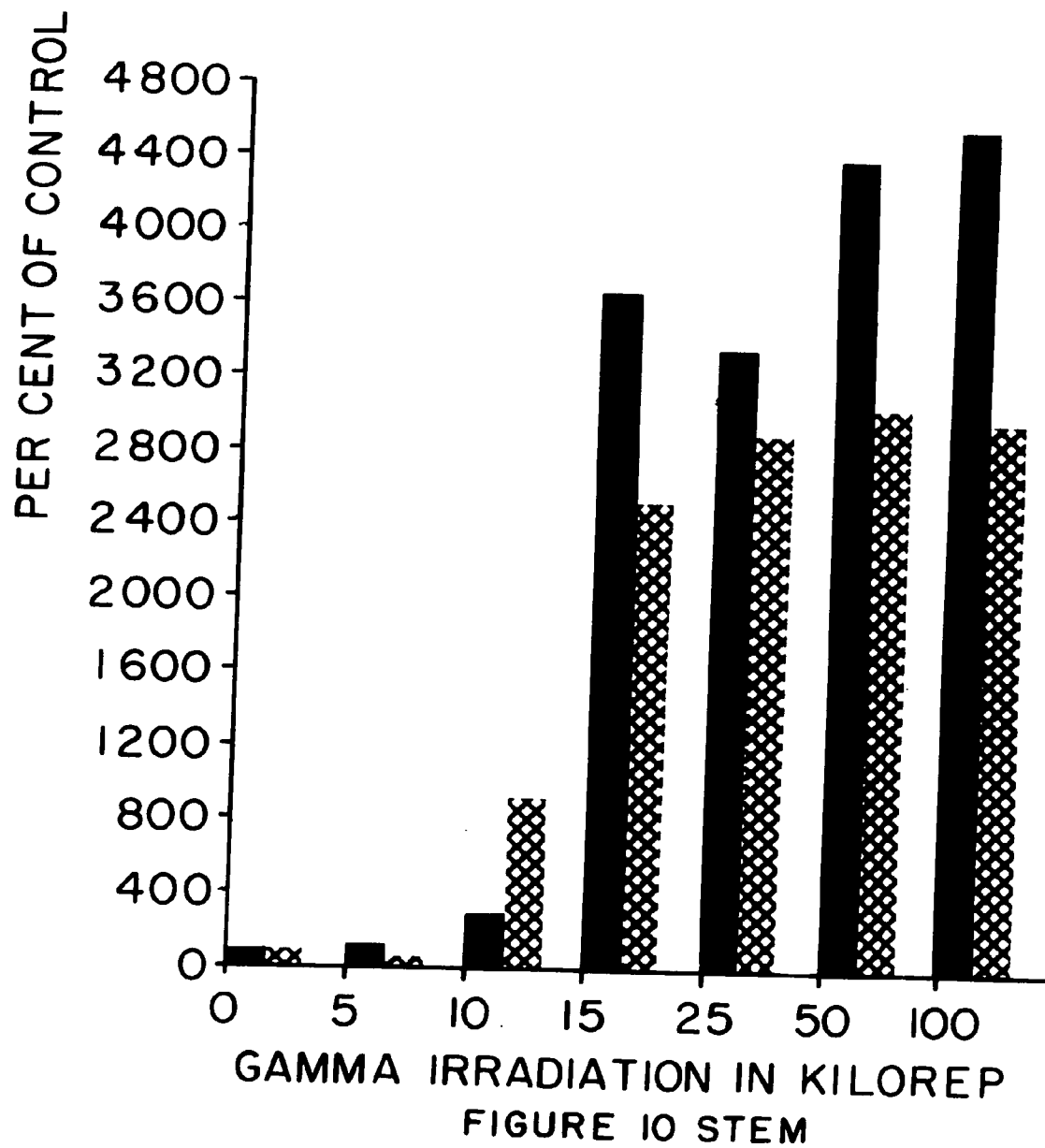
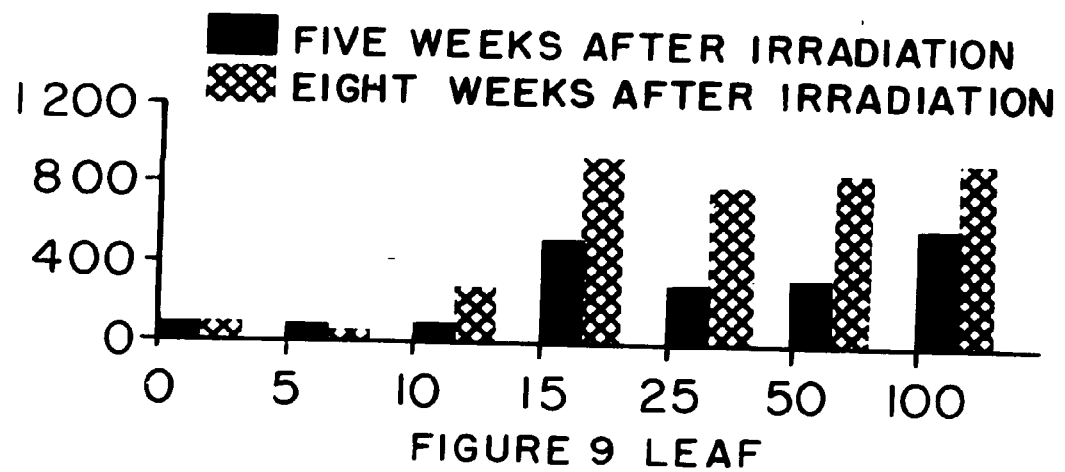


FIGURE 8. STEM

Fig. 9. Effect of single, acute doses of gamma irradiation upon thiamine content in potato leaves. Values represented as percentage of the non-irradiated control.

Fig. 10. Effect of single, acute doses of gamma irradiation upon thiamine content in potato stems. Values represented as percentage of the non-irradiated control.



relatively high, although somewhat lower than it was at the end of the five-week period.

A series of experiments was conducted which compared the effects of gamma irradiation upon riboflavin and thiamine content when the irradiation was administered in single, acute doses and when it was delivered in cumulative doses of equal magnitude. Numerical values for riboflavin and thiamine are given in table 4; percentage values based on the control appear in figures 11, 12, 13, and 14.

Plants treated with initial doses of 5 kilorep and 10 kilorep showed increased riboflavin content in leaf and stem twelve days after treatment (figures 11, 12). At the 15-kilorep dosage level, leaves showed slightly less riboflavin than the control and stems showed an increase above the control. In subsequent assays, eighteen and twenty-five days after irradiation, the leaves in all treated plants had a relatively lower concentration. At the end of thirty-nine days, leaves treated with 15 kilorep doses had a concentration of riboflavin equivalent to or in excess of untreated plants, irrespective of whether the irradiation was administered in single or cumulative doses. Stems of irradiated plants showed a definite increase in content, the most marked in plants receiving single, acute 15 kilorep doses.

Table 4. Comparison of the effects of gamma irradiation delivered in single, acute doses and in cumulative doses upon the riboflavin and thiamine content in potato leaves and stems. Successive doses of irradiation were administered at seven-day intervals. Each value represents a composite of three plants.

| Dose<br>in<br>Kilo-<br>rep | Micrograms per Gram of Fresh Weight |       |       |       |       |       |       |       |
|----------------------------|-------------------------------------|-------|-------|-------|-------|-------|-------|-------|
|                            | Days after Initial Irradiation      |       |       |       |       |       |       |       |
|                            | 12                                  | 18    | 25    | 39    | 12    | 18    | 25    | 39    |
| <u>Riboflavin</u>          |                                     |       |       |       |       |       |       |       |
|                            | Leaves                              |       |       |       | Stems |       |       |       |
| 0                          | 0.930                               | 1.175 | 1.150 | 1.545 | 0.140 | 0.130 | 0.110 | 0.115 |
| 5                          | 1.005                               | 0.975 | 1.025 | 1.305 | 0.160 | 0.135 | 0.180 | 0.135 |
| 5+5                        |                                     | 0.940 | 0.955 | 1.425 |       | 0.180 | 0.190 | 0.195 |
| 5+5+5                      |                                     |       | 0.845 | 1.620 |       |       | 0.130 | 0.195 |
| 10+5                       | 1.045                               | 1.100 | 1.060 | 1.580 | 0.210 | 0.175 | 0.265 | 0.195 |
| 15                         | 0.890                               | 1.070 | 1.035 | 1.605 | 0.180 | 0.200 | 0.280 | 0.290 |
| <u>Thiamine</u>            |                                     |       |       |       |       |       |       |       |
|                            | Leaves                              |       |       |       | Stems |       |       |       |
| 0                          | 0.040                               | 0.105 | 0.105 | 0.095 | 0.045 | 0.075 | 0.050 | 0.040 |
| 5                          | 0.065                               | 0.115 | 0.135 | 0.105 | 0.040 | 0.020 | 0.045 | 0.020 |
| 5+5                        |                                     | 0.115 | 0.105 | 0.135 |       | 0.045 | 0.050 | 0.090 |
| 5+5+5                      |                                     |       | 0.070 | 0.120 |       |       |       | 0.195 |
| 10+5                       | 0.075                               | 0.075 | 0.105 | 0.140 | 0.075 | 0.205 | 0.240 | 0.530 |
| 15                         | 0.095                               | 0.140 | 0.125 | 0.260 | 0.115 | 0.255 | 0.475 | 0.790 |

Fig. 11. Comparative effects of acute and cumulative doses of gamma irradiation upon riboflavin content in potato leaves. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.

Fig. 12. Comparative effects of acute and cumulative doses of gamma irradiation upon riboflavin content in potato stems. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.

## TIME AFTER INITIAL IRRADIATION

■ 12 DAYS,

⋯ 28 DAYS,

⊗ 18 DAYS,

▨ 39 DAYS.

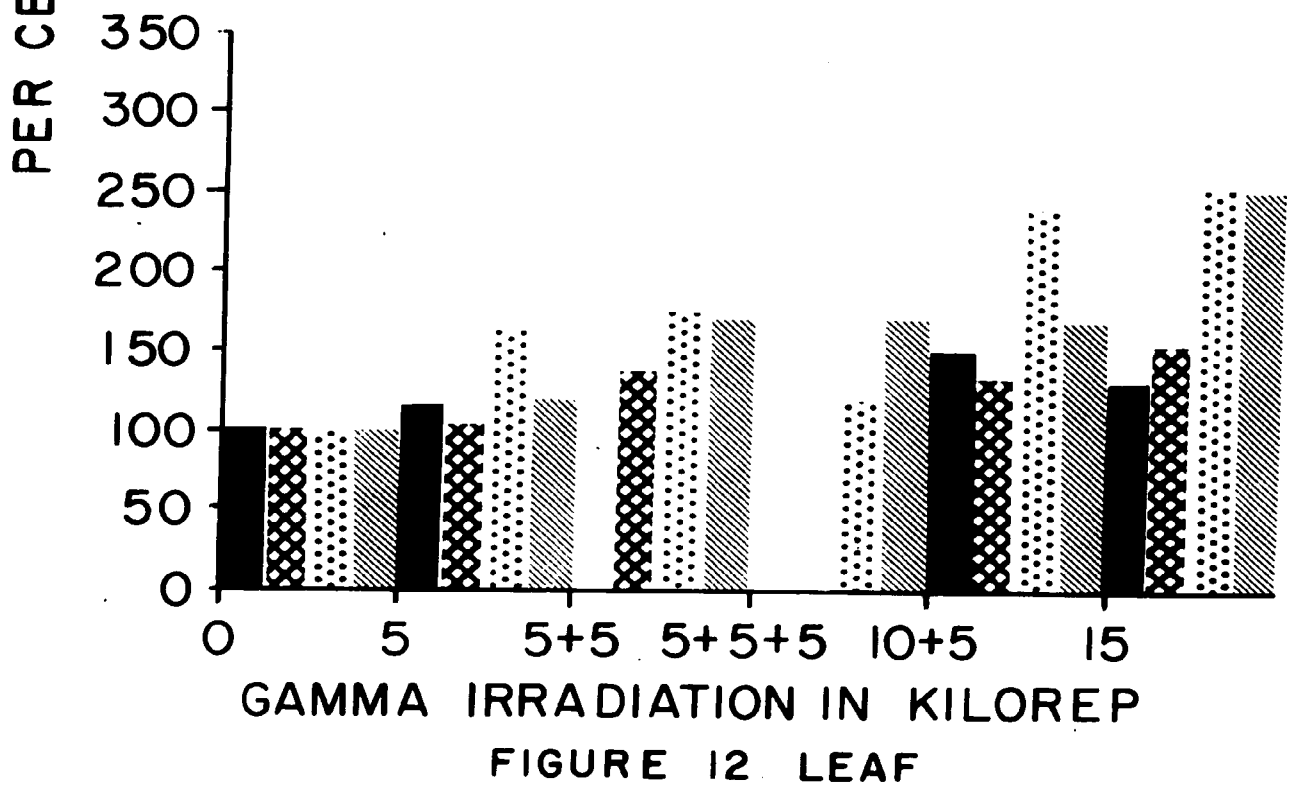
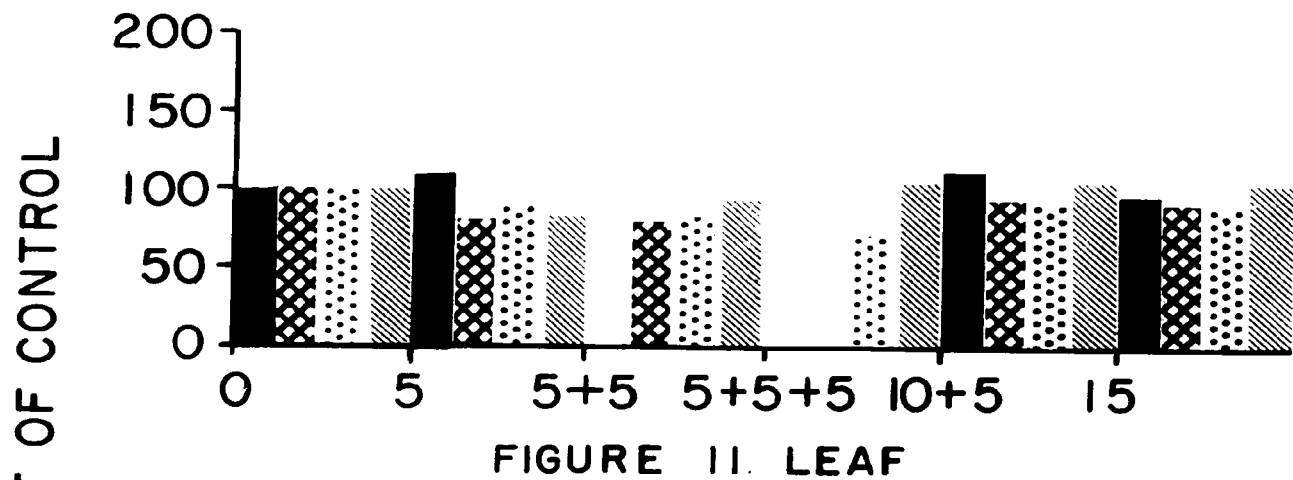
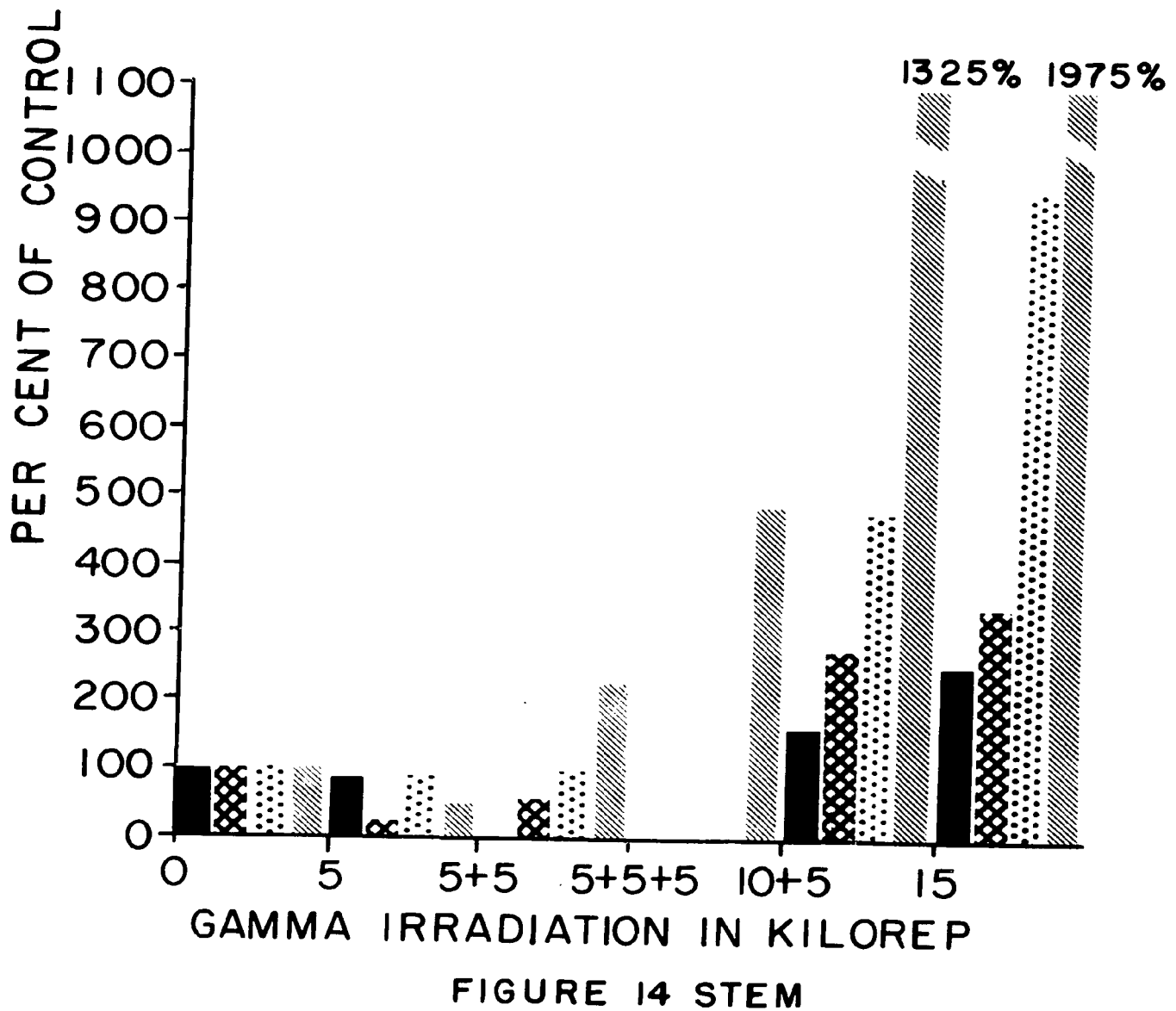
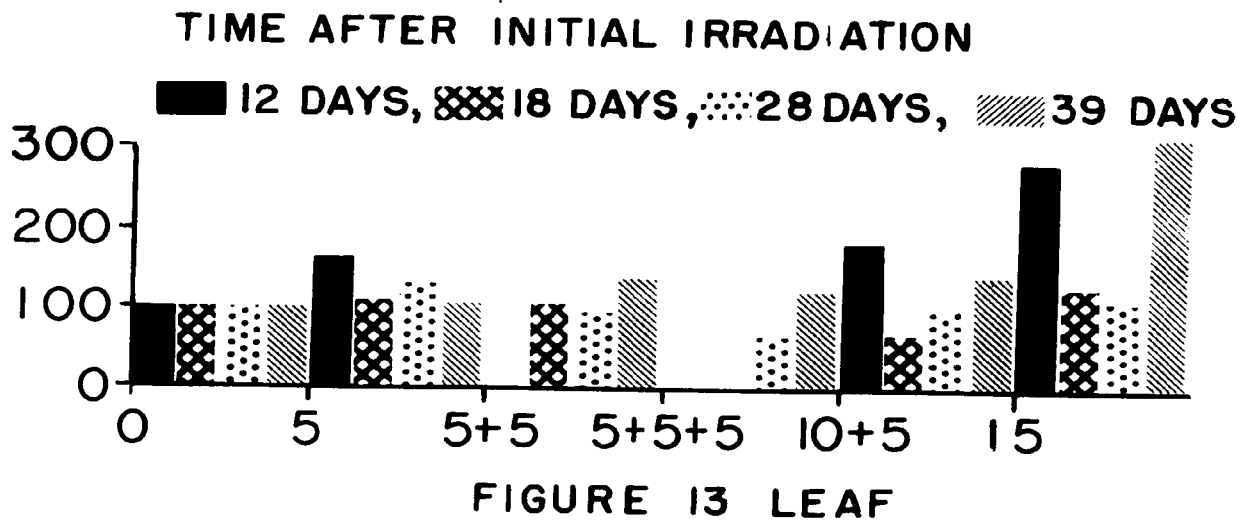


Fig. 13. Comparative effects of acute and cumulative doses of gamma irradiation upon thiamine content in potato leaves. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.

Fig. 14. Comparative effects of acute and cumulative doses of gamma irradiation upon thiamine content in potato stems. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.



The thiamine content of leaves from irradiated plants was higher than that of the control at the time of the first determination (figure 13). It had dropped below the levels of the first determination at the time of the second determination and thereafter the trend was toward increased accumulation with elapse of time. In the stems (fig. 14), plants irradiated with a single 5 kilorep dose and with a 5+5 kilorep dose were consistently low in thiamine. In all doses above this range, there was an increase in thiamine accumulation with increase in length of time after irradiation. The most marked increase occurred in plants receiving acute 15 kilorep doses.

Riboflavin and thiamine accumulation was greater in plants receiving single, acute 15 kilorep doses than in plants receiving an equivalent amount of irradiation in multiple doses.

#### Effect of Gamma Irradiation on Ascorbic Acid Content

Ascorbic acid determinations were made upon the same plants which were assayed for riboflavin and thiamine. Single, acute doses of irradiation produced a general increase in total ascorbic acid in leaves and in stems (table 5). The only exception occurred in leaves irradiated with 5 kilorep which, at the time of the initial assay five weeks after treatment, showed a lower concentration than in controls;

Table 5. The effect of acute doses of gamma irradiation upon the content of ascorbic acid in potato leaves and stems. Determinations were made at five- and eight-week intervals after irradiation. Each value represents a composite of three plants. Ascorbic acid expressed as milligrams per 100 grams of fresh weight.

| Dose<br>in<br>Kilo-<br>rep | Milligrams per 100 Grams of Fresh Weight |       |       |       |
|----------------------------|------------------------------------------|-------|-------|-------|
|                            | Leaves                                   |       | Stems |       |
|                            | 5 wk.                                    | 8 wk. | 5 wk. | 8 wk. |
| 0                          | 70.0                                     | 68.8  | 7.4   | 4.9   |
| 5                          | 60.0                                     | 79.6  | 11.9  | 13.1  |
| 10                         | 99.2                                     | 98.0  | 14.9  | 31.7  |
| 15                         | 116.8                                    | 101.6 | 35.6  | 35.8  |
| 25                         | 91.6                                     | 84.8  | 42.6  | 44.6  |
| 50                         | 91.2                                     | 84.4  | 35.8  | 40.6  |
| 100                        | 80.0                                     | 72.8  | 33.8  | 28.4  |

subsequently, they also showed greater values than the controls (fig. 15). In the stems, there was a general rise in the content of this vitamin with increase in time after treatment but the greatest concentration was observed at the 25-kilorep level where the quantity was in excess of five times that of the control at the end of five weeks, and over nine times that of the control three weeks later (fig. 16).

Determinations made to compare the effects of single dose versus repetitive dose administration of irradiation of the same magnitude revealed that, on the whole, single doses were more effective in increasing ascorbic acid content in the leaves and stems than were divided doses of equivalent value (table 6; figs. 17, 18). Initial administration of 10 kilorep followed by 5 kilorep seven days later was less effective than a single 15 kilorep dose, but more effective than 5 kilorep repetitive doses. These results are in accord with the findings of Russ (1919) and Arntzen and Krebs (1925), who observed that single full exposures of X rays produced a greater biological effect than the same amount of radiation given by small repeated doses. Russ believes that the process of repair can more easily cope with feeble radiation acting for a long time than with intense radiation acting for a short time. At the time of the first assay, fourteen days after the initial irradiation, leaves in the

Fig. 15. Effect of single, acute doses of gamma irradiation upon total ascorbic acid content in potato leaves. Values represented as percentage of the nonirradiated control.

Fig. 16. Effect of single, acute doses of gamma irradiation upon total ascorbic acid content in potato stems. Values represented as percentage of the nonirradiated control.

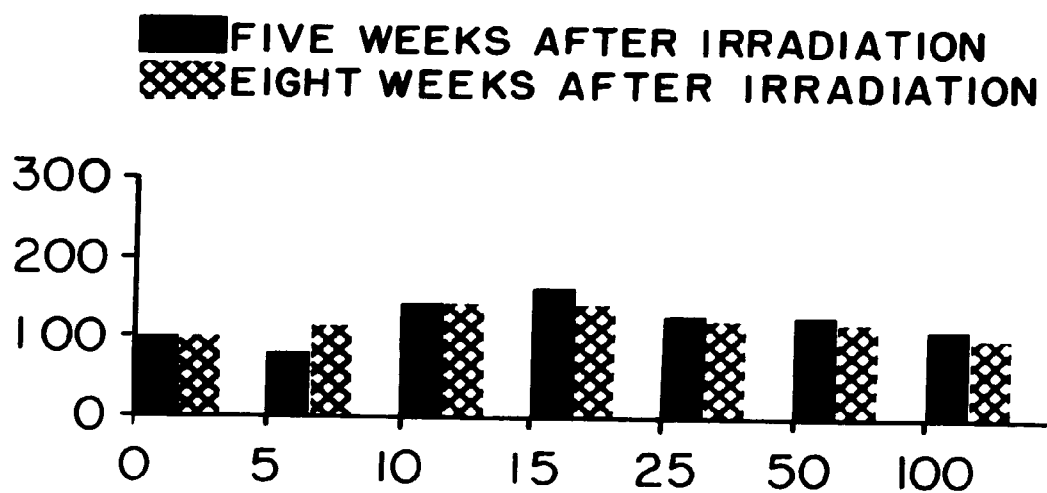


FIGURE 15 LEAF

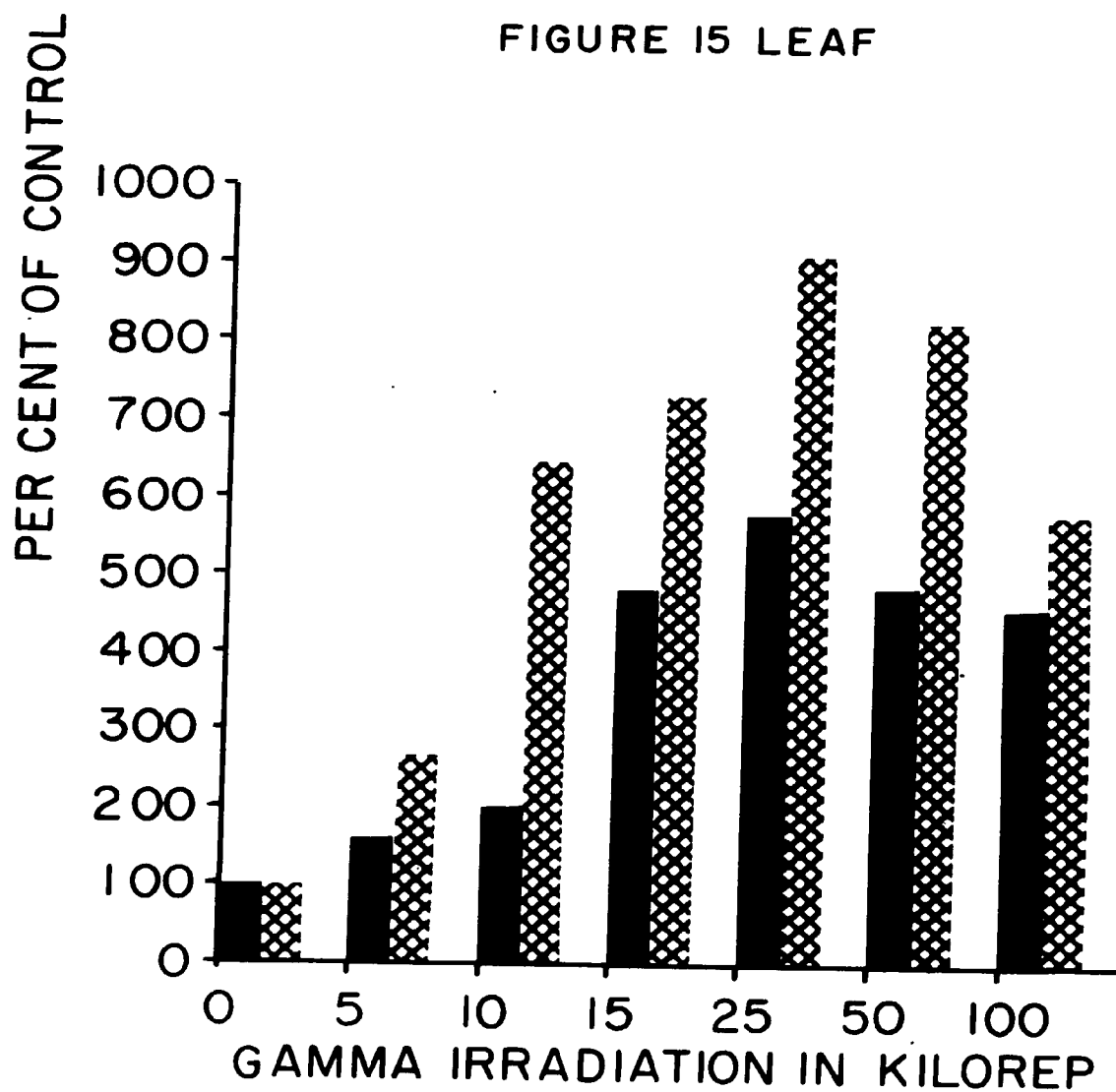


FIGURE 16. STEM

Table 6. A comparison of the effects of single doses and of cumulative doses of gamma irradiation upon the total ascorbic acid content of potato leaves and stems. Each value represents a composite of three plants. Ascorbic acid expressed as milligrams per 100 grams of fresh weight.

| Dose<br>in<br>Kilo-<br>rep | Milligrams per 100 Grams of Fresh Weight |                 |                 |                 |
|----------------------------|------------------------------------------|-----------------|-----------------|-----------------|
|                            | Days after Initial Irradiation           |                 |                 |                 |
|                            | 14 <sup>a</sup>                          | 20 <sup>b</sup> | 27 <sup>c</sup> | 41 <sup>d</sup> |
|                            | <u>Leaves</u>                            |                 |                 |                 |
| 0                          | 60.0                                     | 40.4            | 62.8            | 54.0            |
| 5                          | 55.6                                     | 38.0            | 62.4            | 62.0            |
| 5+5                        |                                          | 50.4            | 50.4            | 58.4            |
| 5+5+5                      |                                          |                 | 66.8            | 68.8            |
| 10+5                       | 55.2                                     | 45.6            | 69.2            | 74.8            |
| 15                         | 61.2                                     | 58.4            | 73.2            | 73.6            |
|                            | <u>Stems</u>                             |                 |                 |                 |
| 0                          | 4.5                                      | 6.1             | 8.3             | 7.9             |
| 5                          | 4.3                                      | 8.5             | 10.7            | 9.7             |
| 5+5                        |                                          | 7.5             | 10.3            | 10.0            |
| 5+5+5                      |                                          |                 | 13.1            | 18.6            |
| 10+5                       | 15.8                                     | 16.7            | 29.7            | 35.2            |
| 15                         | 12.2                                     | 25.8            | 39.2            | 38.6            |

<sup>a</sup>Seven days after the second irradiation.

<sup>b</sup>Thirteen days after the second irradiation, six days after the third irradiation.

<sup>c</sup>Twenty days after the second irradiation, thirteen days after the third irradiation.

<sup>d</sup>Thirty-four days after the second irradiation, twenty-seven days after the third irradiation.

Fig. 17. Comparative effects of acute and cumulative doses of gamma irradiation upon total ascorbic acid content in potato leaves. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.

Fig. 18. Comparative effects of acute and cumulative doses of gamma irradiation upon total ascorbic acid content in potato stems. Cumulative doses were administered at seven-day intervals. Values are represented as percentage of the nonirradiated control.

## TIME AFTER INITIAL IRRADIATION

■ 12 DAYS,  
 ▤ 28 DAYS,

⊗ 18 DAYS,  
 ▨ 39 DAYS.

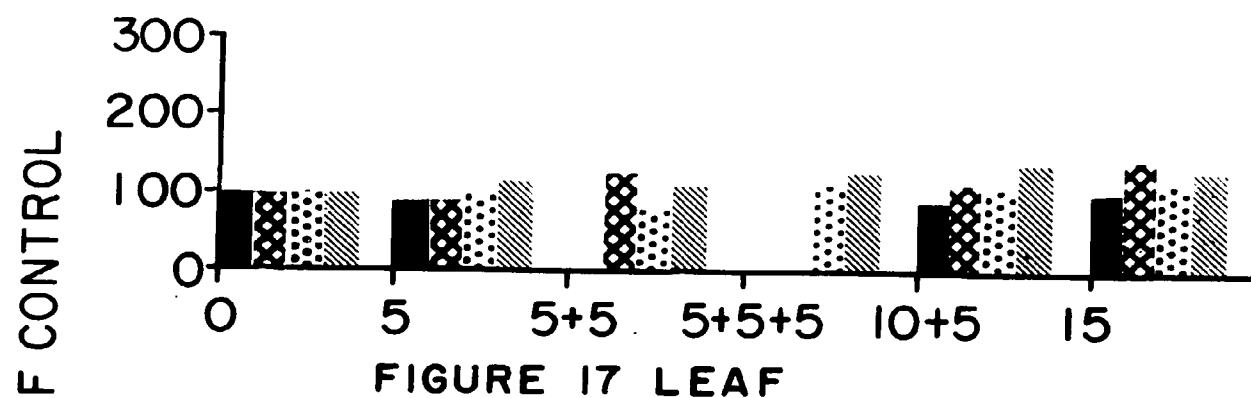


FIGURE 17 LEAF

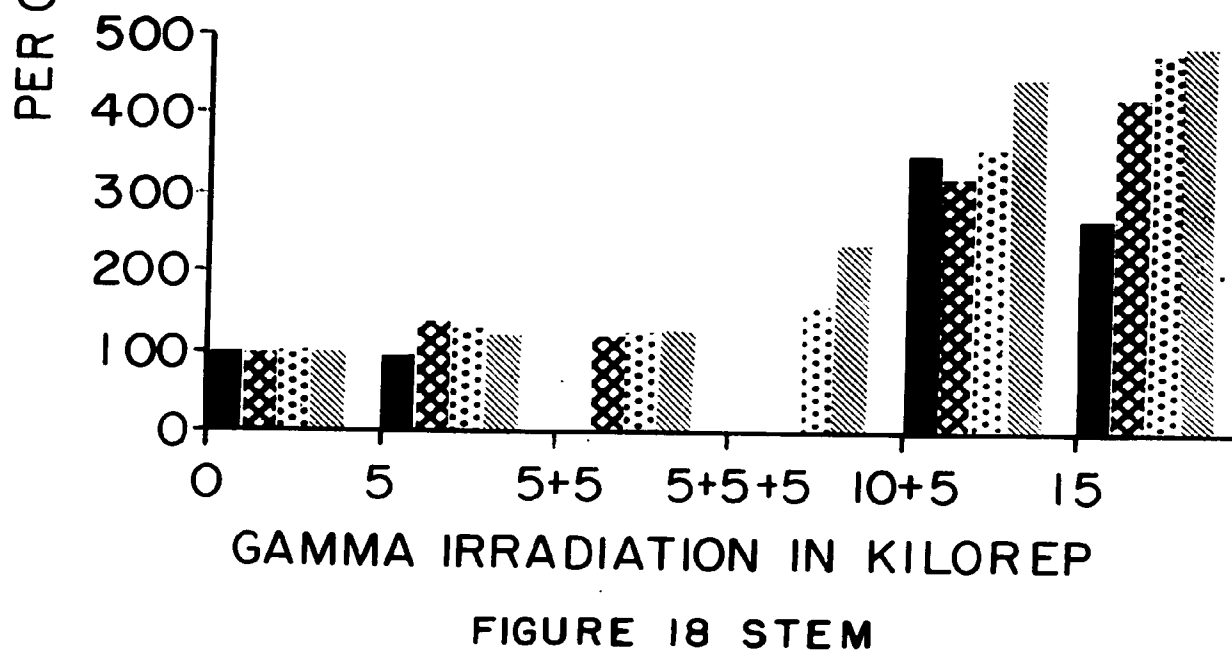


FIGURE 18 STEM

5-kilorep level had a lower ascorbic acid content than the control. Leaves exposed to a 10 kilorep dose originally and to an additional 5 kilorep seven days later were also lower in the vitamin content than the control. With 15-kilorep exposures, content was slightly higher than in the control. At the time of the second assay, all values were relatively lower than previously. Thereafter, increasing amounts of ascorbic acid were observed at all levels of treatment, but with the most marked increases appearing in leaves exposed to single 15 kilorep doses.

In the stems, the content of ascorbic acid increased with elapse of time after irradiation and was greater with larger doses. The single 15 kilorep dose was more effective than an equivalent amount delivered in divided doses. While the relative increase in ascorbic acid in the stems was several times that of the controls, the actual value at the highest concentration was roughly only from one-half to two-thirds of the quantity contained in the leaves.

Gamma irradiation, whether administered in single or cumulative doses, resulted in an increased concentration of ascorbic acid, which was more pronounced in stems than in leaves. Large single doses effected greater increases in vitamin content than did equivalent amounts delivered in divided doses.

### Effect of Gamma Irradiation on Respiration

Since respiration is one of the essential metabolic processes of all living organisms, a study of the effect of gamma irradiation upon respiration was included in this investigation. Determination of respiration was made upon leaf samples from the same lots of plants that were used for riboflavin, thiamine, and ascorbic acid assays.

Plants receiving single, acute doses of gamma irradiation ranging from 0 through 100 kilorep were sampled thirty-three and forty-six days after treatment and their respiratory rates determined. Oxygen consumption in microliters per milligram of dry weight per hour for one of these experiments is recorded in table 7. The same results calculated on a percentage basis are presented in the form of a histogram (fig. 19). Respiration at all dosage levels was less than that of the control at the time of the first determination. In most instances, the comparative rates based on the control were even lower at the time of the second determination. The value for the 50-kilorep dosage level in the first determination appears inconsistent with the values observed at the other dosage levels. No explanation or reason can be given for this apparent inconsistency.

Table 7. Effect of acute gamma irradiation upon respiration of potato leaves. Each sample consisted of nine 12-mm. leaf discs from each of three plants. Each value represents the average of duplicate determinations. Oxygen consumption is recorded as microliters per milligram dry weight per hour.

| Days<br>after<br>Irra-<br>diation | Oxygen Consumption in Microliters per<br>Milligram Dry Weight per Hour |      |      |      |      |      |      |
|-----------------------------------|------------------------------------------------------------------------|------|------|------|------|------|------|
|                                   | Gamma Irradiation in Kilorep                                           |      |      |      |      |      |      |
|                                   | 0                                                                      | 5    | 10   | 15   | 25   | 50   | 100  |
| 33                                | 2.16                                                                   | 2.10 | 1.95 | 1.78 | 1.92 | 1.10 | 1.87 |
| 46                                | 2.61                                                                   | 2.19 | 1.81 | 1.59 | 2.02 | 1.96 | 2.01 |

Fig. 19. Effect of acute gamma irradiation upon the respiration of potato leaf discs. Values calculated as microliters of oxygen per milligram dry weight per hour, and expressed as percentage of the control.

Fig. 20. Comparative effects of acute and cumulative doses of gamma irradiation upon the respiration of potato leaf discs. Values calculated as microliters of oxygen per milligram dry weight per hour, and expressed as percentage of the control.

■ THIRTY-TWO DAYS AFTER IRRADIATION  
 ▤ FORTY-FOUR DAYS AFTER IRRADIATION

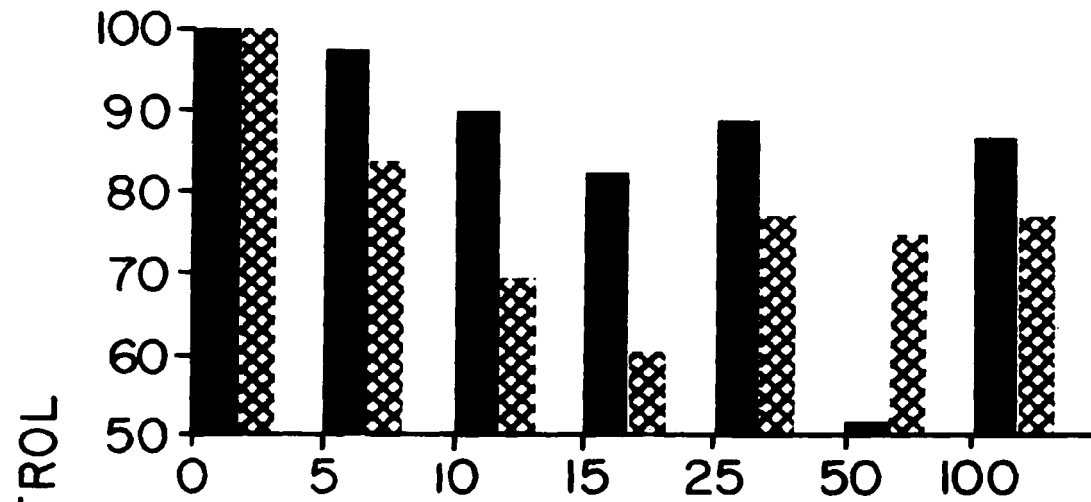


FIGURE 19.

TIME AFTER INITIAL IRRADIATION  
 ■ 8 DAYS, ▤ 18 DAYS, ▥ 22 DAYS, ▦ 36 DAYS



FIGURE 20.

The respiratory rates of plants which were treated with single doses of gamma irradiation and of those exposed to equivalent amounts delivered in repetitive doses were compared (table 8; fig. 20). During the initial irradiation period, all single, acute doses were given, and the first portion of the cumulative doses. Successive portions of the cumulative doses were administered at seven-day intervals. The first determination was made one week after the initial irradiation. At that time, oxygen consumption at the 5-kilorep level was significantly greater than in the control. Eighteen days after irradiation, the rate at this level had decreased in comparison to the rate observed after seven days. However, respiration in all of the irradiated plants which were observed at the eighteen-day period was greater than that of the controls. Observations on all categories were recorded at twenty-two and thirty-six day intervals after the original irradiation and showed consistent decreases in rate with elapse of time. Plants which received a single 5 kilorep dose and those which received one additional 5 kilorep dose showed essentially the same respiratory rates on any given day throughout the experiment. Plants exposed to three 5 kilorep doses and those exposed to 10+5 kilorep showed lower oxygen consumption at the time of the last determination than plants in other categories. Plants irradiated with 15 kilorep delivered in a single exposure

Table 8. Effect of single and cumulative doses of gamma irradiation upon the respiration of potato leaves. Each sample consisted of nine 12-mm. discs from each of three plants. Cumulative doses were administered at seven-day intervals. Oxygen consumption is recorded as microliters per milligram dry weight per hour.

| Days<br>after<br>Initial<br>Irra-<br>diation | Oxygen Consumption in Microliters per<br>Milligram Dry Weight per Hour |      |                   |                   |                   |      |
|----------------------------------------------|------------------------------------------------------------------------|------|-------------------|-------------------|-------------------|------|
|                                              | Gamma Irradiation in Kilorep                                           |      |                   |                   |                   |      |
|                                              | 0                                                                      | 5    | 5+5               | 5+5+5             | 10+5              | 15   |
| 7                                            | 3.36                                                                   | 3.92 |                   |                   | 3.37              | 3.15 |
| 18                                           | 2.50                                                                   | 2.74 | 2.72 <sup>a</sup> |                   | 2.96 <sup>a</sup> | 2.55 |
| 22                                           | 3.88                                                                   | 4.00 | 3.59 <sup>b</sup> | 3.42 <sup>b</sup> | 3.31 <sup>b</sup> | 3.41 |
| 36                                           | 3.49                                                                   | 2.73 | 2.85 <sup>c</sup> | 2.34 <sup>c</sup> | 2.29 <sup>c</sup> | 2.89 |

<sup>a</sup>Eleven days after the second irradiation.

<sup>b</sup>Fifteen days after the second irradiation, eight days after the third irradiation.

<sup>c</sup>Twenty-nine days after the second irradiation, twenty-two days after the third irradiation.

exhibited the greatest initial decrease in respiration; after eighteen days, respiration increased, and thereafter it decreased with elapsing time. At the time of the last determination, respiration in this category did not vary as greatly from the controls as did the respiration of groups exposed to cumulative doses of the same magnitude.

A third series of experiments which was designed to follow the respiratory rate more frequently was run. Oxygen consumption was determined every second day for a period of approximately one month. Duplicate determinations were made, one in the morning and the other in the afternoon, on leaf discs cut from the same leaflets on the selected plants. The entire lot of plants was first divided into three groups as uniform as possible. Then, two sets of leaves, consisting of three leaves each, were tagged on each plant. The first two duplicate determinations were made four days after irradiation on one set of the tagged leaves on the first group of plants. Two days later, leaf sections were cut from the same leaves for the second duplicate determinations. On the sixth and eighth days after irradiation, additional assays were made on the second set of tagged leaves from these same plants. The second group of the lot of plants was sampled in a similar manner for assay on the tenth, twelfth, fourteenth, and sixteenth days; and the third group was utilized for the last four assays. Results are

shown in table 9. The same results represented as percentage of the control appear in the form of a graph (fig. 21). This graph indicates increased respiratory rates at all dosage levels on the fourth day after irradiation as compared to the untreated controls. The higher doses produced greater percentages of increase. There was a downward trend in respiration in all categories until the fourteenth day after irradiation when another peak was reached. A subsequent decrease followed and a third peak appeared at the twenty-five day interval.

Two of the factors involved in the calculation of the respiratory rate are the fresh volume and the dry weight of the test material. During the course of this study it was observed that the dry weight of leaf discs from plants which had received high doses of irradiation, especially at the 25- and 50-kilorep level, increased with elapse of time after irradiation (table 10). Examination of the volumes of the fresh leaf discs (table 11) showed that, while the average volume of discs from irradiated plants was also higher, there was no consistent correlation between the dry weights and the volumes. In some instances, for example, the dry weight would double while the leaf volume would increase by only 25 per cent. Results from other phases of this investigation showed accumulation of large amounts of starch in the leaf and stem tissues.

Table 9. Effect of acute gamma irradiation upon the respiration of potato leaves. Each sample contained nine 12-mm. leaf discs from each of three plants. Each value represents the average of duplicate determinations. Oxygen consumption is recorded in microliters per milligram of dry weight per hour.

| Days<br>after<br>Irra-<br>diation | Oxygen Consumption in Microliters per<br>Milligram Dry Weight per Hour |       |       |       |       |       |       |
|-----------------------------------|------------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|
|                                   | Gamma Irradiation in Kilorep                                           |       |       |       |       |       |       |
|                                   | 0                                                                      | 5     | 10    | 15    | 25    | 50    | 100   |
| 4                                 | 2.418                                                                  | 2.528 | 2.440 | 2.627 | 2.816 | 2.974 | 2.833 |
| 6                                 | 3.221                                                                  | 2.463 | 2.650 | 2.749 | 3.519 | 3.304 | 3.213 |
| 8                                 | 3.083                                                                  | 3.136 | 2.674 | 2.885 | 2.754 | 3.004 | 2.954 |
| 10                                | 2.891                                                                  | 2.634 | 2.283 | 2.151 | 2.500 | 2.798 | 2.522 |
| 12                                | 3.705                                                                  | 3.005 | 3.040 | 2.965 | 2.969 | 2.893 | 3.031 |
| 14                                | 1.987                                                                  | 1.837 | 2.118 | 2.045 | 2.223 | 2.343 | 2.350 |
| 16                                | 2.495                                                                  | 2.150 | 2.379 | 2.292 | 2.417 | 2.126 | 2.468 |
| 18                                | 3.046                                                                  | 2.198 | 2.469 | 2.485 | 2.638 | 2.164 | 2.574 |
| 20                                | 2.519                                                                  | 2.470 | 2.588 | 2.554 | 2.638 | 2.722 | 2.961 |
| 22                                | 2.387                                                                  | 2.029 | 2.163 | 2.002 | 2.232 | 2.133 | 2.449 |
| 29                                | 2.937                                                                  | 2.499 | 2.026 | 1.967 | 2.613 | 2.441 | 1.967 |
| 31                                | 3.022                                                                  | 2.506 | 2.170 | 2.086 | 2.696 | 2.396 | 2.546 |

Fig. 21. Effects of acute gamma irradiation upon oxygen consumption of potato leaf discs. Values appear in percentage based on the control.

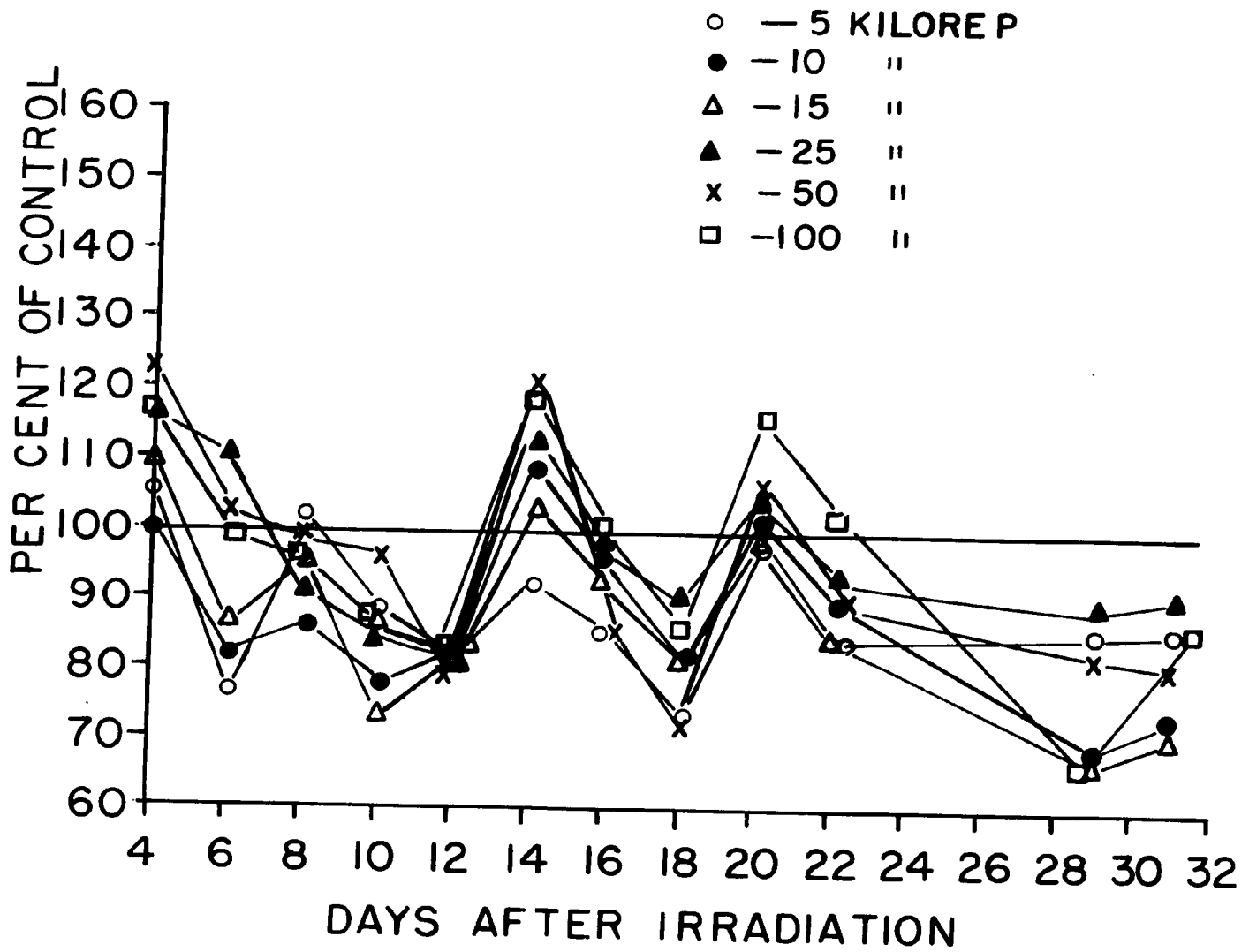


FIGURE 21

Table 10. Influence of gamma irradiation upon the dry weight of leaf discs. Each sample contained nine 12-mm. leaf discs from each of three plants. Each value represents the average of duplicate determinations. Dry weight is recorded in milligrams.

| Days<br>after<br>Irra-<br>diation | Dry Weight in Milligrams of Twenty-seven<br>12-mm. Leaf Discs |       |       |       |                            |               |       |
|-----------------------------------|---------------------------------------------------------------|-------|-------|-------|----------------------------|---------------|-------|
|                                   | Gamma Irradiation in Kilorep                                  |       |       |       |                            |               |       |
|                                   | 0                                                             | 5     | 10    | 15    | 25                         | 50            | 100   |
| 4 <sup>a</sup>                    | 57.65                                                         | 62.90 | 72.70 | 64.85 | 69.70                      | 70.15         | 60.15 |
| 6                                 | 60.25                                                         | 71.65 | 72.65 | 66.35 | 64.20                      | 73.90         | 61.10 |
| 8                                 | 65.90                                                         | 66.55 | 77.75 | 70.40 | 86.05                      | 87.05         | 72.20 |
| 10                                | 59.65                                                         | 55.75 | 60.65 | 59.05 | 74.25                      | 76.85         | 66.80 |
| 12 <sup>b</sup>                   | 54.40                                                         | 71.25 | 70.25 | 77.85 | 82.55                      | 88.50         | 79.40 |
| 14                                | 50.90                                                         | 68.35 | 62.55 | 74.85 | 83.55                      | 86.30         | 77.90 |
| 16                                | 57.25                                                         | 74.35 | 66.10 | 71.35 | 85.25                      | 88.00         | 82.35 |
| 18                                | 57.75                                                         | 73.25 | 67.15 | 74.50 | 94.55                      | 99.00         | 88.10 |
| 20 <sup>c</sup>                   | 64.90                                                         | 63.75 | 75.50 | 90.70 | <u>102.45</u> <sup>d</sup> | <u>109.65</u> | 86.20 |
| 22                                | 59.75                                                         | 59.65 | 73.35 | 82.65 | <u>104.20</u>              | <u>100.95</u> | 89.35 |
| 29                                | 55.90                                                         | 57.05 | 71.90 | 82.35 | <u>106.30</u>              | <u>101.75</u> | 80.80 |
| 31                                | 52.30                                                         | 56.80 | 63.10 | 74.95 | <u>91.05</u>               | <u>87.45</u>  | 82.35 |
| Avg.                              | 58.05                                                         | 65.11 | 69.47 | 74.15 | 87.01                      | 89.13         | 77.23 |

<sup>a</sup> Discs for determination on the fourth, sixth, eighth, and tenth days were cut from the same plants of group I.

<sup>b</sup> Discs for determinations on the twelfth, fourteenth, sixteenth, and eighteenth days were cut from the same plants of group II.

<sup>c</sup> Discs for determinations on the twentieth, twenty-second, twenty-ninth, and thirty-first days were cut from the same plants of group III.

<sup>d</sup> Underlined figures represent the high values.

Table 11. Volumes of leaf discs used in respiration studies. Each value represents the average volume in milliliters of two samples each consisting of twenty-seven 12-mm. discs.

| Days<br>after<br>Irra-<br>diation | Volume in Milliliters of Twenty-seven<br>12-mm. Leaf Discs |      |      |      |      |      |      |
|-----------------------------------|------------------------------------------------------------|------|------|------|------|------|------|
|                                   | Gamma Irradiation in Kilorep                               |      |      |      |      |      |      |
|                                   | 0                                                          | 5    | 10   | 15   | 25   | 50   | 100  |
| 4                                 | 1.05                                                       | 1.20 | 1.10 | 1.05 | 0.95 | 1.00 | 0.95 |
| 6                                 | 1.05                                                       | 1.15 | 1.10 | 1.00 | 1.10 | 1.20 | 1.15 |
| 8                                 | 1.20                                                       | 1.15 | 1.10 | 1.15 | 1.15 | 1.10 | 1.05 |
| 10                                | 1.15                                                       | 1.05 | 1.15 | 1.20 | 1.10 | 1.20 | 1.25 |
| 12                                | 1.10                                                       | 1.25 | 1.05 | 1.05 | 1.25 | 1.30 | 1.15 |
| 14                                | 1.15                                                       | 1.25 | 1.20 | 1.10 | 1.15 | 1.15 | 1.15 |
| 16                                | 1.10                                                       | 1.20 | 1.00 | 1.00 | 1.15 | 1.10 | 1.05 |
| 18                                | 1.05                                                       | 1.10 | 1.15 | 1.00 | 1.05 | 1.20 | 1.05 |
| 20                                | 1.20                                                       | 1.15 | 1.35 | 1.25 | 1.25 | 1.25 | 1.20 |
| 22                                | 1.20                                                       | 1.20 | 1.15 | 1.20 | 1.25 | 1.25 | 1.15 |
| 29                                | 1.00                                                       | 1.00 | 1.15 | 1.20 | 1.20 | 1.20 | 1.05 |
| 31                                | 1.00                                                       | 1.20 | 1.10 | 1.15 | 1.20 | 1.25 | 1.15 |
| Avg.                              | 1.10                                                       | 1.16 | 1.13 | 1.11 | 1.15 | 1.18 | 1.11 |

This suggested the idea that perhaps these large amounts of starch were increasing the weight of the leaf sections and thereby lowering the apparent rate of respiration. Accordingly, respiration has been calculated as oxygen consumption per leaf disc per hour and graphed as percentage of control (fig. 22). On this basis, irradiated plants tended to show a higher respiratory rate than did controls; those plants treated with the higher doses had the higher respiratory rates. Plants exposed to 5, 10, and 15 kilorep doses did not vary greatly from the untreated plants. Here again, respiration appeared to change rhythmically, an initial rise being followed by a decrease in the rate, and a second peak occurring about two weeks after irradiation.

#### Effect of Gamma Irradiation upon Starch Content

As was stated earlier, appreciable quantities of starch were observed to settle out during the preparation and extraction of brei of irradiated potato leaves and stems which were used for vitamin determinations. Preliminary examination of freehand cross sections of the stems showed a greater amount of starch in plants which were irradiated with 25, 50, and 100 kilorep than in nonirradiated plants. Microscopic examination of thick cross sections revealed

Fig. 22. Effects of gamma irradiation upon respiration. Values calculated as microliters of oxygen per disc per hour, and expressed as percentage of control.

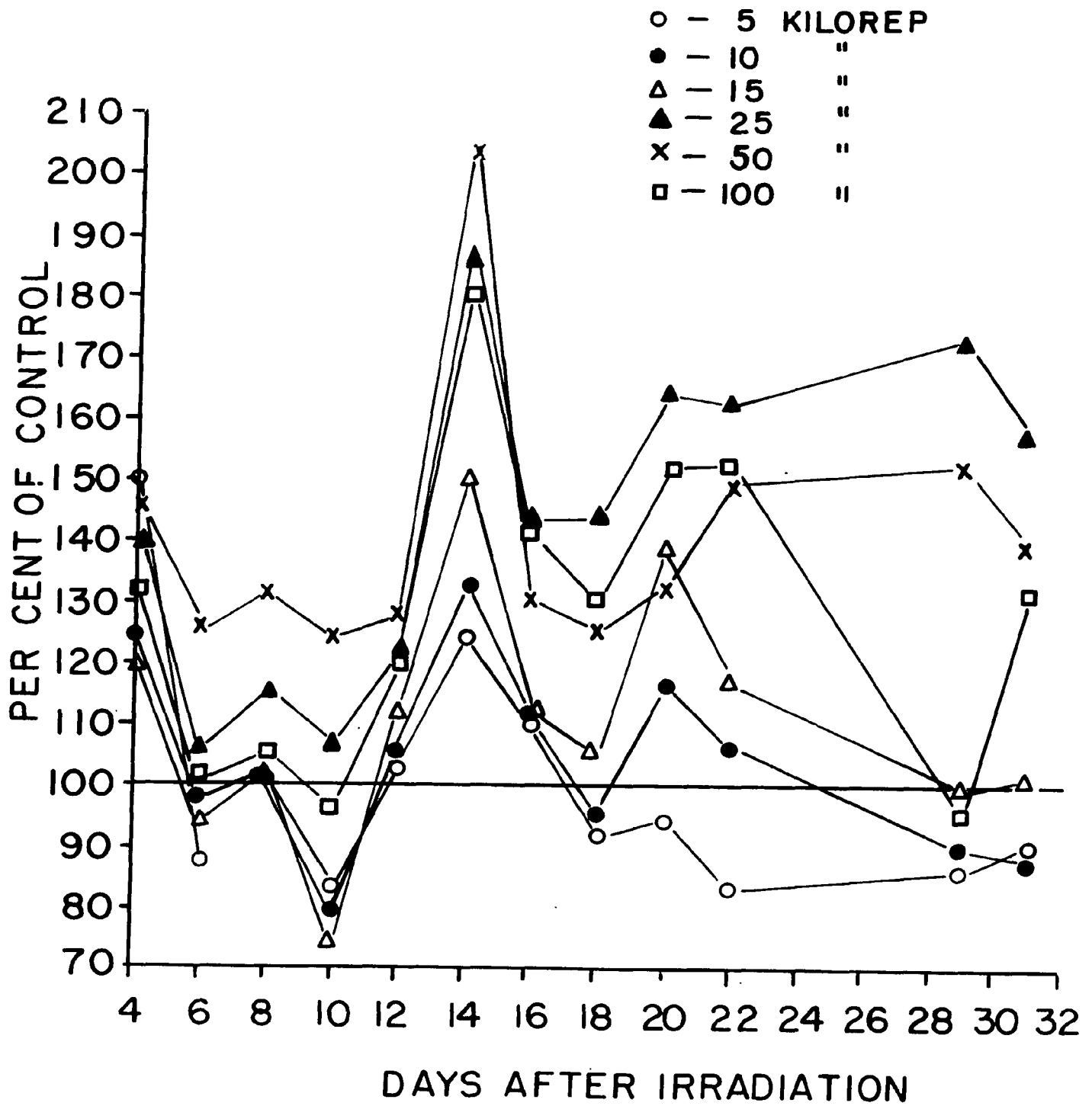


FIGURE 22

that there were many intact cells which contained large amounts of starch. In the preparation of slides for this study, the sectioning process cut through the large parenchyma cells in the pith, and that portion of the cell which was contained in the section was devoid of starch in many instances. Since a similar condition prevailed in all sections, a comparison of the starch content at the various irradiation levels was possible.

Cross sections of the stems were made during the third week after irradiation. They were stained with iodine preparatory to photographing. In freshly stained sections the xylem had a yellow-orange color, the phloem a light yellow color, and the starch appeared as black dots or masses, but these contrasts do not appear in black-and-white photographs. Figures 23 and 24 demonstrate the relative starch content. Little difference in starch content can be noted in the stems of controls and of those plants receiving 5 and 10 kilorep doses. At the 15-kilorep dosage level, starch grains were scattered throughout the pith. At this level, freshly stained sections exhibited a deposit of starch in the endodermis but this is not visible in the photographs. Twenty-five, 50, and 100 kilorep doses produced progressive increases in starch content in the pith. With the higher doses there were also deposits visible in the cortex,

Fig. 23. Effect of gamma irradiation on content of starch in stems. Treatments from left to right--top row, nonirradiated control and 5 kilorep, respectively; bottom row, 10 kilorep and 15 kilorep, respectively. Three weeks after irradiation.

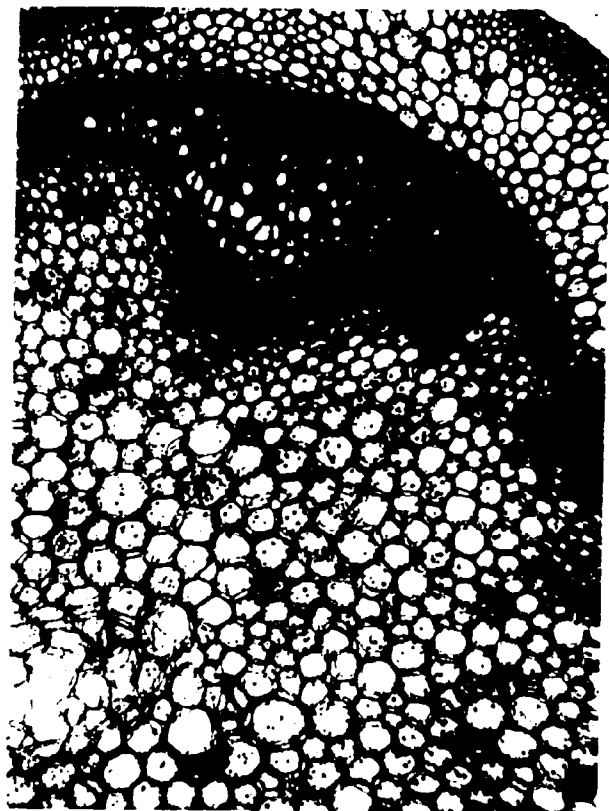
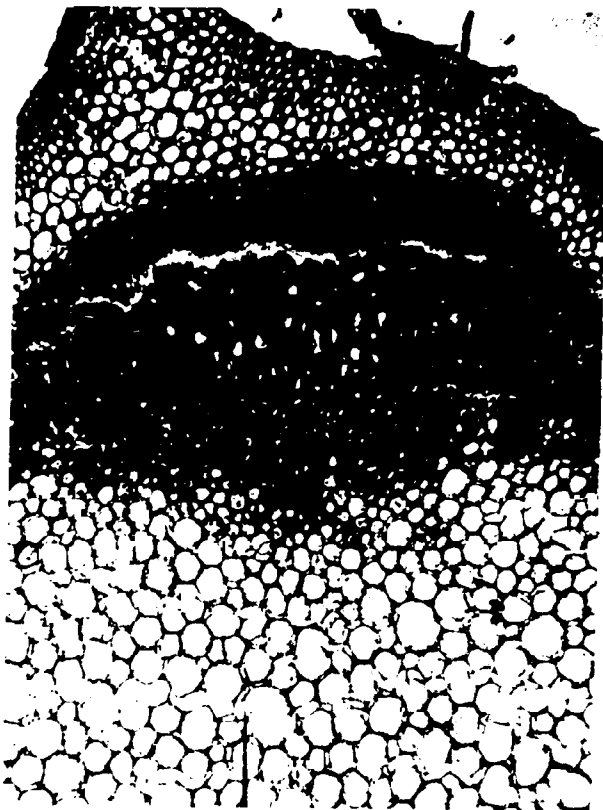
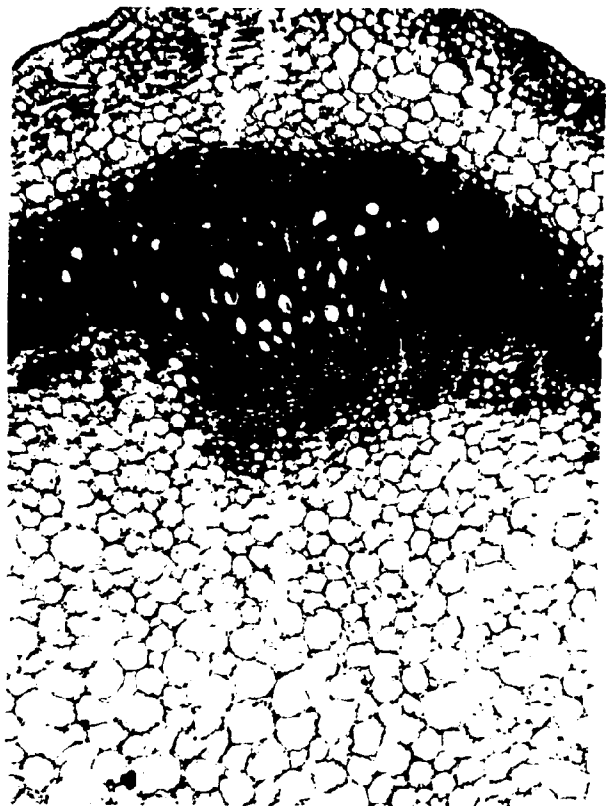
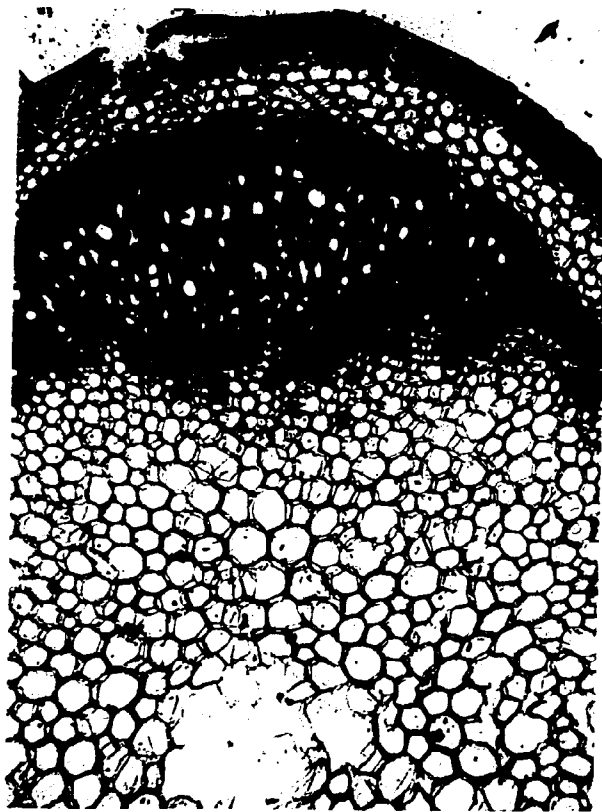


FIGURE 23.

Fig. 24. Effect of gamma irradiation on content of starch in stems. Treatments from left to right--top row, nonirradiated control and 25 kilorep, respectively; bottom row, 50 kilorep and 100 kilorep, respectively. Three weeks after irradiation.

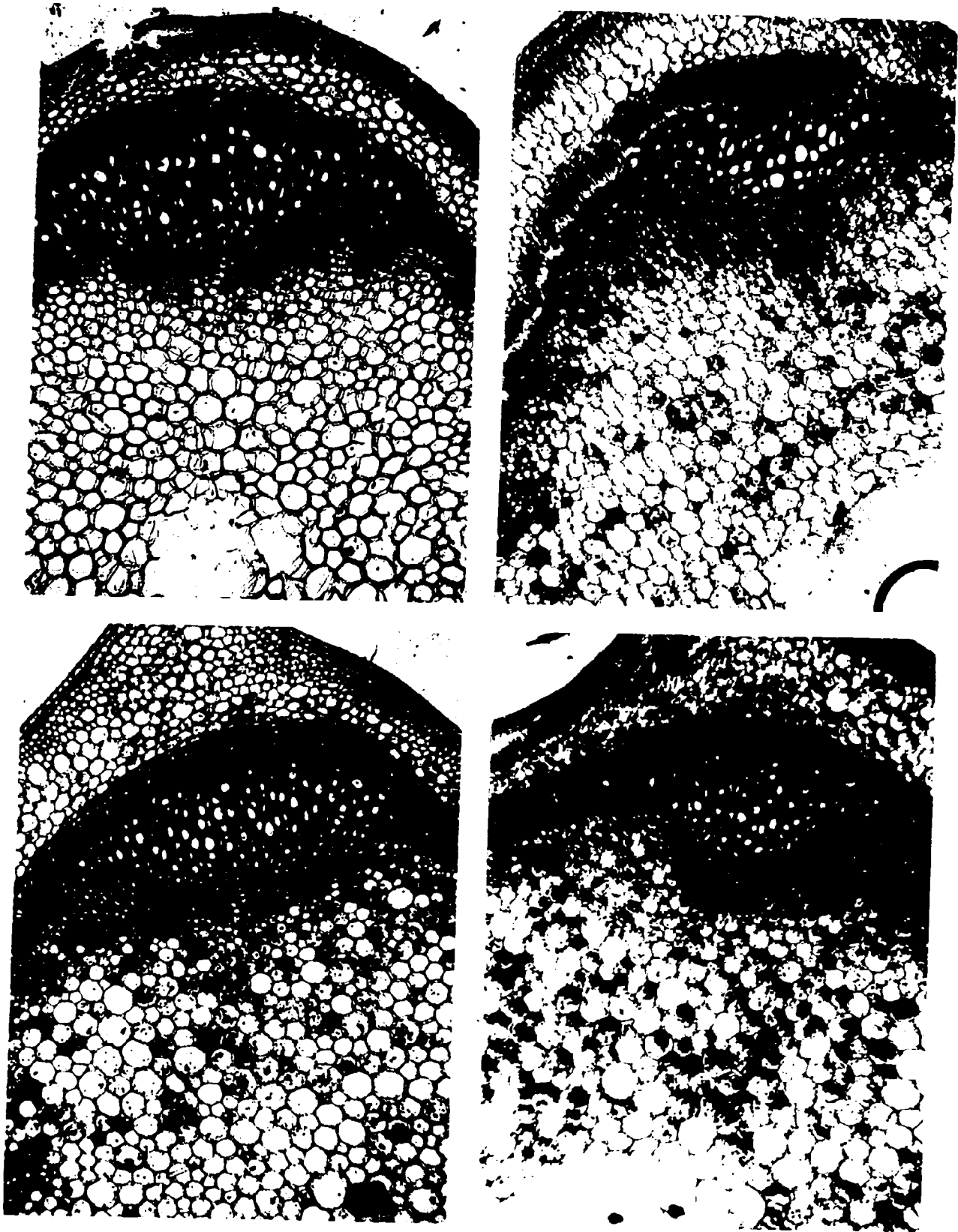


FIGURE 24.

the phloem, and, occasionally in freshly stained slides, some grains could be seen in the ray cells of the xylem.

Photomicrographs of sections which were cut approximately ten weeks after irradiation appear in figure 25. They demonstrate the increased content of starch in the stem as the time after irradiation increased. The photographs at the 10- and 15-kilorep dosage level indicate appreciable starch content which is comparable in amount to that found in stems which received 50 and 100 kilorep, after a three-week period.

#### Effect of Shielding the Root System

Examination of the root systems of the plants which were harvested for vitamin assays revealed that the extent and size of the root system and tubers appeared to be related inversely to the size of the dose of irradiation which had been administered. Tubers were entirely lacking at the 50- and 100-kilorep dosage levels in many instances. At the same time, starch content in stems increased at the higher dosage levels, as has been recorded. These phenomena conceivably could have resulted from direct irradiation damage to enzymes of the leaf or stem which are involved in the translocation of starch; or from injury to the phloem tissue with a resultant blockage. The possibility also existed that, although the

Fig. 25. Effect of gamma irradiation on content of starch in stems. Treatment from left to right--top row, nonirradiated control and 5 kilorep, respectively; bottom row, 10 kilorep and 15 kilorep, respectively. Ten weeks after irradiation.

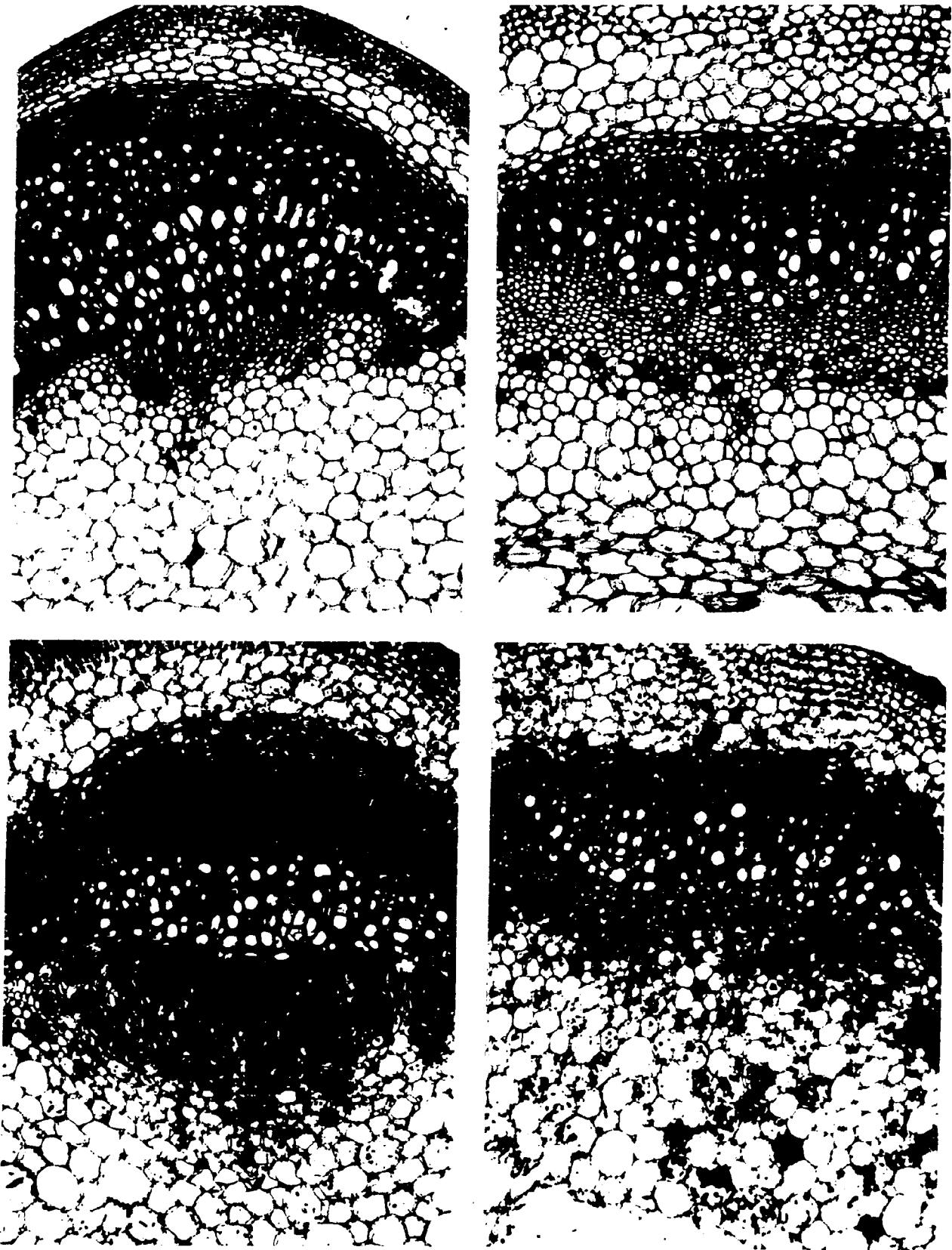


FIGURE 25.

underground system was shielded by soil and clay pot, an unusual susceptibility of the rhizome and tuber to irradiation could have produced these phenomena simply by inhibiting the initiation or development of the tuber which serves as a storage organ for starch. An experiment was devised to investigate the latter possibility and to determine whether or not irradiation effects could be prevented or minimized by shielding the underground systems of the plants.

A uniform lot of plants was divided into two groups which were irradiated simultaneously. One group received the customary treatment in which the entire plant and the pot in which it was growing was exposed to gamma irradiation. The root system of plants in a second group was shielded by two-inch lead bricks. For the sake of brevity, this latter group of plants will be referred to hereafter as "shielded" plants, and irradiation doses for this group will be designated by an "S"; e.g., 15-S kilorep.

Representative samples of shielded and irradiated plants were harvested sixteen days after treatment. At that time all of the plants had small tubers of approximately the same size. Tubers on control plants, on plants irradiated with 5 and 10 kilorep doses, and on shielded plants in the same dosage ranges were creamy-white in color. Tubers on plants in the remaining categories which had been shielded were somewhat darker in color, but their general

appearance was good. Tubers from plants in the 15-kilorep range had some brown spots and a few small fissures appeared in the epidermis. With a 25 kilorep dose fissures developed at the eyes. Fifty kilorep produced a general brown coloration and occasional dry, scaly spots on the tubers, and fissures appeared at the eyes. Tubers from plants irradiated with 100 kilorep had extensive fissuring at the eyes and elsewhere on the surface. These fissures measured up to 5 mm. in width and were nearly as deep. Dark, soft spots appeared around the eyes.

The rhizomes on these plants were also examined. New rhizomes appeared on the control and on all shielded plants. In the 5- and 10-kilorep ranges, some new rhizomes and tubers were apparent. With higher doses, no new rhizomes were produced, and those that were already present were damaged and not growing. In many instances the propagation tuber remained intact after irradiation at the higher dosage levels, but it was disintegrating in the pots which had been shielded.

Approximately two months after irradiation the remainder of the plants were harvested. The leaves on all plants showed signs of maturity and/or aging. The leaves were dry or had abscised on the control and shielded plants, and the stems were brown or had lost their color. Most of the leaves on plants receiving total

irradiation were dry or had dry spots on them, but the stems were still green.

Shortly after irradiation, some of the plants treated with 50-S and 100-S kilorep doses developed new aerial shoots which originated immediately below the surface of the soil where the underground portion of the stem had been shielded. These shoots grew to a height of eight to ten inches and then matured.

Examination of stem sections in this experiment showed large quantities of starch in plants which received doses of 15 kilorep or over. A negligible amount or no starch was observed in the stems of plants which received 5 and 10 kilorep doses and in those plants which had been shielded.

The underground portions of irradiated and shielded plants showed considerable difference in the extent of their root systems, and in the number and size of the tubers which they bore. Comparative effects are shown in figures 26 and 27.

The root systems of the controls and of plants irradiated with 5, 5-S, 10-S, and 15-S kilorep doses were fairly uniform. The main roots, however, appeared somewhat thicker and more fibrous in irradiated plants. With doses of 10 kilorep and above, unshielded root systems were less extensive and clearly showed evidence of damage and stunting. The root tips were brown and disintegrating

Fig. 26. Comparison of root and tuber formation in totally irradiated plants and in plants whose underground portions had been shielded with two-inch lead bricks. Treatments from left to right--top row, control; middle row, 5, 10, and 15 kilorep doses, respectively; bottom row, 5-S, 10-S, and 15-S kilorep doses, respectively.

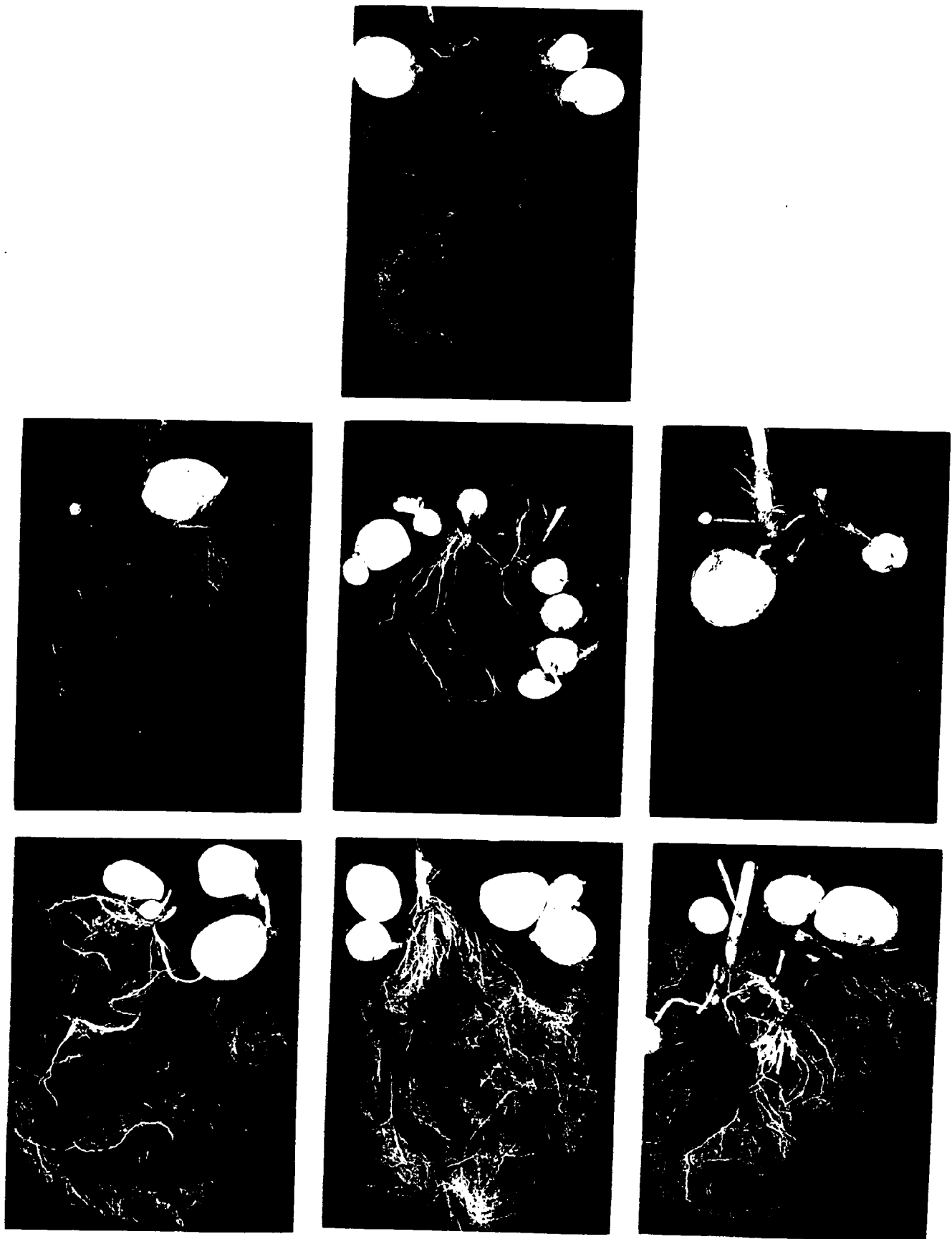


FIGURE 26.

Fig. 27. Comparison of root and tuber formation in totally irradiated plants and in plants whose underground portions had been shielded with two-inch lead bricks. Treatments from left to right--top row, control; middle row, 25, 50, and 100 kilorep doses, respectively; bottom row, 25-S, 50-S, and 100-S kilorep, respectively.

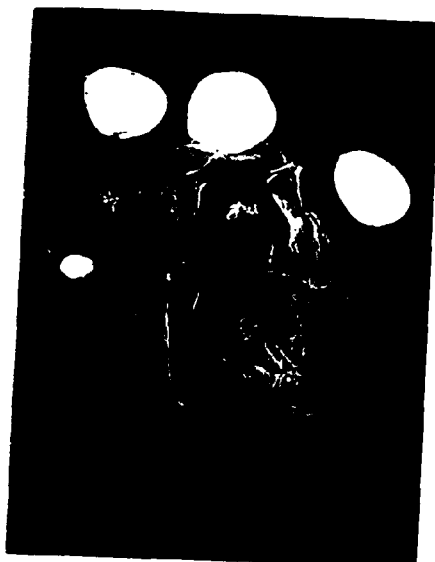
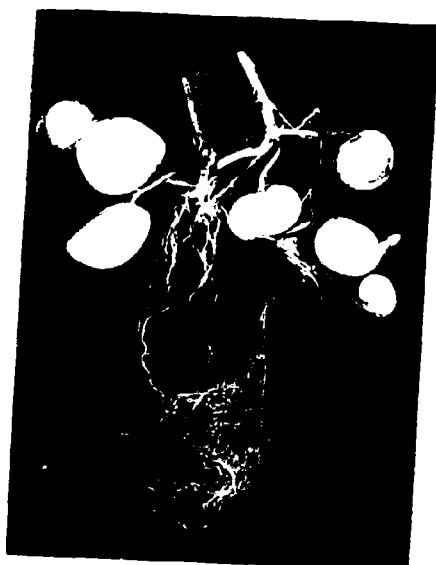
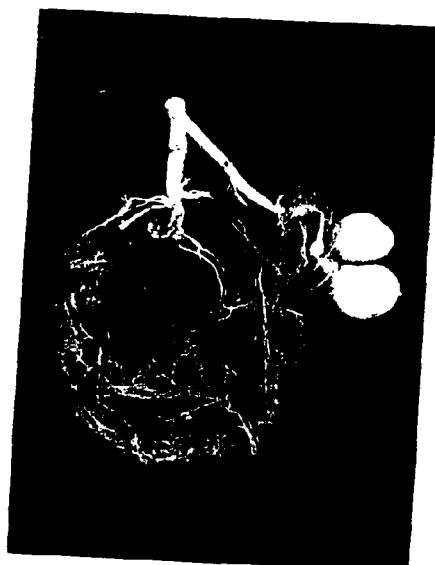


FIGURE 27.

in many instances. Shielded root systems treated with 25-S, 50-S, and 100-S kilorep doses were not as extensive as those at the lower dose rates.

Tubers were produced on all plants which had been shielded, but there was a decrease at the higher levels of irradiation. Plants with nonshielded underground systems also bore progressively smaller yields with increasing doses until at 50- and 100-kilorep dosage levels tubers were produced only rarely. Any tubers which might have been present on the plant at the time of irradiation with 50 or 100 kilorep doses had disintegrated completely or were rotting at the time of the harvest.

The aerial portions of totally irradiated plants differed noticeably from those with shielded root systems. Figure 28 illustrates typical examples of plants thirty-five days after irradiation with 100 and 100-S kilorep doses. Leaves on the shielded plant at the left were not appreciably thickened or curled; however, they were quite yellow, which is not perceptible in the picture, and the lower leaves were dying.

#### Effect of Gamma Irradiation on Weight

During the course of this investigation, observations were made from time to time on the number and dry weight of the

Fig. 28. Comparison of the aerial portions of irradiated plants with shielded and nonshielded root systems. Leaves on the plant at the left whose root system had been shielded were yellow in color, which is not apparent in the picture.



FIGURE 28.

tubers produced by plants irradiated at the various dosage levels.

There was a progressive decrease in yield with increased doses of irradiation.

The dry weights of leaf discs were determined in conjunction with respiration studies (table 10). In leaf tissue the dry weight increased at the higher levels of irradiation with elapse of time after the treatment. This increase was appreciably greater at the 25- and 50-kilorep dosage levels.

Dry-weight determinations were made in connection with the series of experiments which contrasted the effects of irradiation upon plants with shielded and nonshielded root systems. Seventeen days after irradiation, a representative plant from each category was harvested and the dry weights of the tubers were determined (table 12). At this time, the differences in weight throughout the entire series were not great, so the remainder of the plants were allowed to develop for a longer period of time. At the end of sixty-five days, the dry weight of tubers from three plants in each category was determined. The average values appear in table 12. Without exception, plants with shielded root systems produced greater yields of tubers than totally irradiated plants receiving equivalent doses of irradiation. Doses of 5-S, 10-S, and 15-S kilorep produced high yields, even exceeding the control in the

Table 12. Comparison of the dry weight in grams of tubers produced by plants with shielded and with irradiated root systems.

| Dose in<br>Kilorep | Dry Weight in Grams                 |                 |
|--------------------|-------------------------------------|-----------------|
|                    | Number of Days<br>after Irradiation |                 |
|                    | 17 <sup>a</sup>                     | 65 <sup>b</sup> |
| 0                  | 4.05                                | 14.55           |
| 5                  | 7.05                                | 9.24            |
| 5-S                | 6.50                                | 17.68           |
| 10                 | 5.30                                | 12.43           |
| 10-S               | 7.35                                | 12.96           |
| 15                 | 5.00                                | 9.87            |
| 15-S               | 6.75                                | 15.10           |
| 25                 | 4.00                                | 5.12            |
| 25-S               | 4.15                                | 10.20           |
| 50                 | 4.30                                | --              |
| 50-S               | 7.75                                | 10.20           |
| 100                | 7.35                                | 0.56            |
| 100-S              | 6.95                                | 6.40            |

<sup>a</sup>Values for single plant.

<sup>b</sup>Values represent the average of three plants.

5-S and 15-S dosage range. At 25-S, 50-S, and 100-S kilorep, the yields were less than the control. The dry weight of tubers produced by totally irradiated plants was less than that of the controls in all instances, the decreases being greatest at the high dosage levels.

A more comprehensive experiment was run for the purpose of comparing the total weights of treated and control plants. The experiment was broken down to include determinations of fresh weight, dry weight, and percentage dry weight of leaves, stems, tubers, and roots (table 13).

Weight determinations of leaves and stems substantiated earlier observations that irradiated plants apparently had thicker and heavier stems and leaves than the controls. Fresh weight, dry weight, and percentage dry weight increased progressively toward the higher dosage levels. The maximum increase in dry weight of leaves occurred in the 25- and 100-kilorep range. In stems, the increases were most pronounced at the 15-kilorep level and above.

The number of tubers produced decreased above the 15 kilorep dose. The fresh weights and dry weights were greatly decreased with increase in magnitude of dose. The percentage dry weight also decreased, except at the 100-kilorep level, where the few tubers

Table 13. Comparison of the effects of gamma irradiation upon the fresh weight, dry weight, and percentage dry weight in grams of potato plants. Each number represents the average value for five plants. Plants were harvested thirty-four days after irradiation.

| Type of<br>Deter-<br>mination | Dry Weight in Grams          |       |       |       |       |       |       |
|-------------------------------|------------------------------|-------|-------|-------|-------|-------|-------|
|                               | Gamma Irradiation in Kilorep |       |       |       |       |       |       |
|                               | 0                            | 5     | 10    | 15    | 25    | 50    | 100   |
| <u>Leaves</u>                 |                              |       |       |       |       |       |       |
| Fresh wt.                     | 20.06                        | 20.71 | 22.27 | 25.28 | 25.96 | 24.88 | 23.96 |
| Dry wt.                       | 1.82                         | 1.70  | 1.78  | 2.57  | 3.48  | 3.09  | 3.36  |
| % dry wt.                     | 9.24                         | 8.30  | 8.02  | 10.22 | 14.30 | 12.36 | 14.50 |
| <u>Stems</u>                  |                              |       |       |       |       |       |       |
| Fresh wt.                     | 12.52                        | 11.70 | 10.72 | 13.09 | 16.59 | 15.26 | 18.15 |
| Dry wt.                       | 0.57                         | 0.61  | 0.81  | 1.90  | 2.22  | 2.09  | 2.13  |
| % dry wt.                     | 4.44                         | 5.32  | 7.80  | 15.10 | 14.20 | 14.30 | 12.30 |
| <u>Tubers</u>                 |                              |       |       |       |       |       |       |
| Number                        | 3.20                         | 3.60  | 2.60  | 3.60  | 2.40  | 1.80  | 1.60  |
| Fresh wt.                     | 55.61                        | 50.27 | 34.89 | 29.33 | 16.81 | 7.57  | 2.35  |
| Dry wt.                       | 10.84                        | 9.37  | 6.49  | 4.72  | 2.59  | 1.13  | 0.27  |
| % dry wt.                     | 19.06                        | 18.50 | 18.50 | 15.10 | 12.76 | 13.60 | 22.20 |
| <u>Roots</u>                  |                              |       |       |       |       |       |       |
| Fresh wt.                     | 1.77                         | 2.05  | 1.51  | 3.64  | 1.95  | 2.35  | 2.74  |
| Dry wt.                       | 0.04                         | 0.41  | 0.34  | 0.89  | 0.57  | 0.55  | 0.75  |
| % dry wt.                     | 34.92                        | 25.64 | 23.38 | 24.68 | 30.60 | 26.30 | 28.60 |
| <u>Total</u>                  |                              |       |       |       |       |       |       |
| Fresh wt.                     | 89.97                        | 84.73 | 69.40 | 71.34 | 61.31 | 50.06 | 47.20 |
| Dry wt.                       | 13.63                        | 12.10 | 9.42  | 10.08 | 8.87  | 6.86  | 6.50  |
| % dry wt.                     | 15.10                        | 14.30 | 13.60 | 14.10 | 14.50 | 13.70 | 13.80 |

which were present were already dry and shriveled at the time of harvest.

The fresh and dry weights of roots were generally higher with the larger dose of irradiation. As observed before, the main roots appeared thicker in treated plants. The percentage dry weight was greatest in untreated plants.

The values for total fresh and dry weights decreased noticeably with increasing doses of irradiation. The value for the control was roughly twice that of the 100-kilorep dose. Percentage total dry weight was highest in the control and generally tended to decrease with increased dosage of irradiation.

Determinations of fresh and dry weight have demonstrated increased values for the aerial portions of irradiated potato plants, and also for the roots. On the other hand, tuber yields were shown to be reduced materially. The total yield of irradiated plants decreased progressively with increasing dose until at the 100-kilorep level the total dry weight was only approximately half of that of the control, and this decrease was mainly in the tubers.

## DISCUSSION AND CONCLUSIONS

Medium and high doses of ionizing irradiation cause a variety of physiological, genetic, and metabolic disturbances in plants and animals (Nickson, 1952). These disturbances which are believed to be initiated at the molecular or submolecular levels become manifest as biochemical, physiological, or morphological responses (Gordon, 1957). Since specific information concerning normal plant metabolism is somewhat limited, it is often exceedingly difficult or impossible to relate induced abnormalities to any definite metabolic process or reaction. Accordingly, increased knowledge in regard to normal plant metabolism should provide a better basis for comparison of abnormal and induced effects. A few of the abnormalities induced by ionizing irradiation have been expressed quantitatively in terms of metabolic processes or products which are known at the present time. These include reduction in auxin levels (Skoog, 1935; Quastler and Baer, 1950), changes in enzymatic activity (Blinks, 1952), increase in ascorbic acid levels (Cooke, 1953), and inhibition of DNA synthesis (Pelc and Howard, 1952). These few examples must indeed represent an infinitesimal part of the metabolic

changes mediated by ionizing irradiation which eventually can be compared to a known standard of value.

Abnormal coloration in the leaves and apparent injury to the apices of the plants were the earliest symptoms of irradiation effects noted in the present study. As stated earlier, similar observations have been quite widely noted by various workers. Abnormal leaf coloration has been ascribed to uneven distribution of chlorophyll (Johnson, 1936b; Long and Kersten, 1937). Apical injury has been attributed to damage of the meristems, which in turn is thought to be caused by reduction in auxin levels, changes in enzyme activity, and inhibition of DNA synthesis.

Quastler and Baer (1950), working with Mung bean seedlings, found that the most striking effect produced by irradiation was the reduction of the capacity to initiate growth. They then attempted to ascertain the reasons for this reduction and to describe it in terms of known processes. The act of initiating growth was defined as an operation or series of operations in which building materials (organic molecules, salts, and water) are assimilated into the living structure in such a way that an increase in size is produced. They considered that this process could be interfered with in three ways: (1) by destroying the building material, (2) by preventing it from reaching the region where it is to be used, or (3) by interfering

with the mechanism of utilization. Radiation effects on the building material were not pertinent. Irradiation of the cotyledons, leaves, and hypocotyls where the organic molecules which served as building blocks were stored had no effect on the process, and if growth was not initiated the cotyledons were not depleted. The effects on salts and water were considered to be of short duration. The transport of building materials was not affected by irradiation. Salts and water were taken up by the root, even doses which caused severe stunting of the root failed to affect the growth of the stem. Materials from the cotyledons were found to be translocated to the growing tips where they were processed, modified, and assembled in the process of initiating growth. But, when the region where growth is initiated was irradiated, the building blocks were not processed, their flow stopped, and the cotyledons were not depleted but remained fleshy and did not shrivel as in normal plants. From these findings, the authors deduced that irradiation was affecting the mechanism of assimilation. They also found that administration of moderate or high doses of X rays stopped all visible growth for some time; the larger the dose, the longer the period of inhibition. Very large doses produced irreversible inhibition of growth. Skoog (1935) and Gordon (1957) have shown that auxin formation is inhibited by X-irradiation, and Quastler and Baer (1950) state that it is

possible that this effect is responsible for the temporary inhibition of growth. They observe that, "whether due to the destruction of auxin or some other process, the temporary inhibition of growth must operate through mechanisms which co-ordinate the events within and possibly among cells. Otherwise, it is hard to see how the effect could be complete after comparatively small doses."

Gordon (1957) has deduced from a series of experiments that irradiation inhibits auxin (IAA) production, which is due, in turn, to enzymatic failure in converting the precursor indoleacetaldehyde to indoleacetic acid. Again this does not answer the problem of how ionizing irradiation causes injury to apical meristems; it merely places the query in the preceding ontogenetic stage. The problem is now concerned with the enzyme or enzymes which are involved. The possibilities are that irradiation is affecting enzyme formation or function. Perhaps only a portion of the enzyme is being affected, either the coenzyme or the apoenzyme. Some chemical activator may be transformed into an unavailable form. Radiation could produce a toxic material in the system which is inactivating or inhibiting the enzyme. All of these possibilities need to be investigated in connection with a single enzyme, a very small part of a complex organic system.

A similar line of reasoning can be applied to the findings of Pelc and Howard (1952) that DNA synthesis is inhibited. These findings are also only a partial solution to a very intricate problem. Obviously, a myriad of problems remain to be solved and the results integrated before the effects of irradiation can be pinpointed on any specific site in an organism.

Examination of the data on vitamin experiments in this investigation showed that the riboflavin content of the leaves from irradiated plants did not differ greatly from that of the control, but that the values in most instances were somewhat smaller than those obtained from the control. However, some increase was observed in stems at the higher dosage levels. Thiamine and ascorbic acid showed a noticeable increase in concentration in both leaves and stems, particularly with higher doses. Other observations also showed that the content of starch in leaves and stems accrued progressively with increase in irradiation dose and with time. Why did thiamine, ascorbic acid, and starch content increase to so great a degree and riboflavin to a lesser extent? Consideration was given to the fact that these differences might be due to variation from the normal in rate of synthesis, in rate of utilization, in the rate of translocation, or to the lack of translocation. The literature was examined to find the site of synthesis of these substances and to

ascertain whether they were synthesized in the same region where they were utilized or stored, or whether they were synthesized in certain organs or tissues and then translocated to other parts of the plant.

Bonner and Bonner (1948) stated that the B vitamins, of which riboflavin and thiamine are representatives, are found in all species and all organs of higher plants. They are present in highest concentration in the young leaves of the vegetative plant with a general gradient of decreasing concentration toward the older portions. By the use of girdling techniques on tomato plants, Bonner (1942) demonstrated that thiamine is synthesized in the leaves of the plant and is transported through the stem toward the root. Thiamine formation occurs most vigorously in mature leaves from whence it is translocated through the phloem. Young leaves export little or no thiamine. Riboflavin, which is mainly synthesized by leaves and roots, appears to be synthesized by the other tissues also and is not translocated appreciably.

Mapson (1955) reported that ascorbic acid is continually formed in the green organs of plants, and that there is evidence which suggests that it may be translocated to other tissues. He also states that the literature suggests that this vitamin is synthesized from a hexose sugar both in plants and animals. Schwimmer

(1953) suggests the possibility of 3-glyceraldehyde phosphates as intermediates for the synthesis of ascorbic acid. Ogolevets (Nichiporovich, 1958) has attempted to demonstrate by the use of  $C^{14}O_2$  that ascorbic acid is a direct product of photosynthesis --the result of the divergence of an intermediary product of photosynthesis prior to the formation of carbohydrates. As a result of this experimental work, Nichiporovich considers ascorbic acid to be one of the direct products of photosynthesis.

From the findings of these authorities it can be concluded that thiamine is synthesized mainly in the leaves of the plant and is translocated through the phloem. Riboflavin is synthesized mainly by the leaves and roots and is not translocated to any appreciable extent. Ascorbic acid, whether it is produced in the green organs of plants as a direct product of photosynthesis as reported by Mapson and Ogolevets, or whether it is synthesized from a hexose sugar as suggested by Mapson and Schwimmer, depends upon translocation for its ubiquity.

The results obtained from the experiments in which the root systems were shielded with two-inch lead bricks indicated that the phloem was not blocked, and that carbohydrate was being translocated from the green parts of the plant to the site of tuber formation. Furthermore, there was no accumulation of starch in the

stems of irradiated plants the root system of which had been shielded. Lack of tuber formation in totally irradiated plants offered a probable reason for the increased thiamine, ascorbic acid, and starch content in stems and leaves. These materials which ordinarily were translocated from the site of synthesis had accumulated in the aerial organs either because of lack of utilization or lack of suitable storage organ.

The aerial tubers which were produced as a result of irradiation probably were formed because of lack of storage organ formation. Similar aerial tubers were produced experimentally as early as 1887 by Vöchting (Palladin, 1926), who cut a leafy shoot from a potato plant, and after carefully removing all buds from the basal portion of the cutting, rooted it in soil. In the absence of buds, no underground branches could develop and consequently no underground tubers. As the plant grew, starch that was formed in the leaves accumulated in the ordinary buds above the soil surface and developed into aerial tubers.

Recent observations by Plaisted (1957) on the growth of the potato tuber may explain the susceptibility of the developing tuber to irradiation. He found that stolon formation begins at the lower nodes near the propagating tuber and progresses upward. The first tubers are generally formed on the lower stolons, and tend

to become dominant. Tuber formation first becomes apparent as a thickening of the first internode back of the stolon apex. This thickening depends upon growth in the cortical, pericyclic, vascular, and pith parenchyma rather than upon a normal cambial development. The potato tuber is exceptional among plant tissues in its long continued meristematic development. Cell division and cell enlargement appear to be maintained at almost uniform rates in pith and cortical tissues during the entire period of growth from a 2-gram to a 200-gram tuber.

Plaisted states,

. . . the potato tuber, if not unique, is certainly unusual in its growth pattern. Here is the phenomenon of a maturing storage tissue, well filled with starch, which is still meristematic in its cell wall and protein characteristics, with its cells still dividing and enlarging. One might suspect that a growth of this sort is dependent upon unusual concentrations of growth materials and a liberal supply of growth hormones.

Since growing potato tubers are largely meristematic in character it is not surprising that they show much greater susceptibility to ionizing radiation. Irradiation damage to the tuber is apparent at the various stages of its development as shown by the destruction of the stolon apex, the fissuring and browning of the epidermis of the young tuber, and the partial or complete inhibition of growth depending upon the magnitude of the dose.

The response of respiration to ionizing radiation is a moot question. Some investigators find a transitory rise in respiration immediately following or shortly after irradiation (Sussman, 1953; Gustafson et al., 1957) which is ascribed to increased metabolic activity. This rise is followed by a gradual decrease in respiration. Working with irradiated barley seeds, Mikaelson and Halvorsen (1953) found that respiration appeared unaffected by a dose of 2500 r. Doses from 5000 to 15000 r did not affect the initial respiratory increase but after four or five days of germination at 20°C. the respiration of irradiated seeds proceeded markedly slower than in the control. They stated that "it is possible that radiation interferes more directly with the function of a particular respiratory system characterized by a threshold sensitivity somewhere between 2500 and 5000 r." Schjeide et al. (1956) observed that oxidative irradiation products which are formed in oxygenated aqueous systems react by conventional oxidation reactions. These products include hydrogen peroxide, free hydroperoxyl radicals, and the hydroxyl radical. In normal cell respiration, the cytochrome oxidase system forms identical substances in the cytoplasm. They believe that, "since these substances are normally present in respiratory systems, it is not surprising that the introduction of additional small amounts by radiation has little effect. Means for

utilizing them in normal metabolic processes are available, and antioxidants such as catalase are present to destroy any that escape normal metabolic routes." In the present study, an initial increase in respiration was observed at all dosage levels, followed by a decrease, and a second rise after approximately fourteen days, and thereafter rhythmic fluctuation. Respiration at the higher dosage levels deviated from the normal to a greater extent than at the lower levels. Respiration tended to be greater than the control in all treated plants.

Inadequacies in the sampling procedures may be responsible for some of the uncertainties about the effects on respiration. These uncertainties might be overcome by showing consistency in a normal, unexposed population first and then deviation from this in exposed material. The leaves of the latter seem to be variously affected so that such a population would have greater divergencies from the mean than the unexposed population.

Examination of data from total weight determinations reveals that, in terms of total yield, irradiation is detrimental at all dosage levels, with the yield decreasing progressively with increased magnitude of dose. In the case of the potato plant, this effect was produced primarily by damage to rhizomes and tubers, with few or no tubers being produced at the higher dosage levels.

## SUMMARY

1. The effects of gamma irradiation on the potato plant Solanum tuberosum L. have been studied. The plants were exposed to irradiation from Cobalt-60 in doses ranging from 5 to 100 kilorep.
2. Discoloration and/or mottling of the leaves was the first visible evidence of the effects of irradiation. Later, the leaves became leathery and thickened, and newly formed leaves showed various abnormalities as to form and texture. Aerial stems became thickened and tended to remain green longer than in the control plants.
3. Determinations were made of riboflavin, thiamine, and ascorbic acid concentration in the leaves and stems. Riboflavin content of the leaves of treated plants did not vary greatly from that of the control; however, in the stems there was some increase at higher dosage levels. Thiamine concentration of both leaves and stems tended to rise progressively with increase in magnitude of dose and with elapse of time after treatment, but to a greater degree in stems than in leaves. The trend in ascorbic acid content followed the same general pattern as that of thiamine, but the increases in the stem were larger.

4. Examination of cross sections of aerial stems revealed accumulations of starch which were first perceptible at doses ranging from 15 kilorep upward. The content increased with time after exposure and with greater dosage of irradiation.

5. Respiration showed an initial increase shortly after irradiation; thereafter, it fluctuated with a trend toward decreasing oxygen consumption. Plants exposed to the higher dosages had greater respiratory rates.

6. Tuber yield varied inversely with magnitude of dose. In most instances, few or no tubers were formed at the 50- and 100-kilorep dosage level.

7. Plants, the root system of which had been shielded with two-inch lead bricks produced tubers at all exposures; however, the yields were progressively less with increase in the dose. There was no accumulation of starch in the stems of these plants.

8. The dry weight of leaf sections increased with higher doses of irradiation, particularly with 25 and 50 kilorep doses.

9. Total dry weight determinations demonstrated progressive decreases with increased exposure. At the 100-kilorep dosage level, the total dry weight of the plants was only approximately one-half that of the control, and the decrease was due chiefly to lack of tuber

formation. These facts suggest that there was less photosynthesis as a result of irradiation.

10. It is concluded that gamma irradiation is deleterious to potato plants, particularly to the underground systems, and to a lesser degree to the aerial portions. Little variation from the normal was observed at the 5- and 10-kilorep dosage levels but with doses of 15 kilorep and above the effects were marked, rhizome and tuber formation being specifically inhibited with resultant accumulation of starch in leaves and stems.

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