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GAMMA-RAY SPROUT INHIBITION OF POTATOES

L. E. Brownell

Collaborators:

C. H. Burns
D. Islieb
L. L. Kempe
R. A. Martens

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

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Official Investigator: L. E. Brownell, Supervisor, Fission Products Laboratory

Collaborators: C. H. Burns, Biochemist, Fission Products Laboratory
D. Islieb, Assistant Professor of Farm Crops, Michigan State University
L. L. Kempe, Associate Professor of Bacteriology, The University of Michigan
R. A. Martens, Botanist and Research Assistant, Fission Products Laboratory

Tital of Contract: Gamma-Ray Sprout Inhibition of Potatoes

SUMMARY

One ton of Idaho seed potatoes from the 1954 crop was obtained in May, 1955. These potatoes were treated with gamma radiation and stored at various temperatures and humidities as described in the previous progress report.

After two months, all potatoes stored at temperatures of 50°F or above show marked loss of weight. The 10,000 rep irradiated potatoes lost only about one-half as much weight as nonirradiated potatoes stored at the same temperature. At storage temperatures

less than 50°F, only a small loss of weight was observed in all potatoes, with no outstanding difference in weight loss between irradiated and nonirradiated potatoes.

It was found, as would be expected, that potatoes lost less weight as the humidity of the air in which they were stored increased. It was also observed that irradiated potatoes lost less weight than nonirradiated potatoes when samples of each were stored at the same relative humidity.

Preliminary investigations into methods for sugar analysis of potato tissue suggest that the ceric sulfate titration proposed by Hassid[4] may be most satisfactory for this study. The results of the application of this method of analysis to potato samples which were frozen shortly after irradiation indicate an increase in the reducing-sugar concentration in potatoes which have received large doses of radiation. This increase does not appear until twenty-four to forty-eight hours after irradiation.

The unfortunate death of Professor C. D. LaRue on August 19, 1955, has interrupted the study for differences in plant physiology and metabolism. Arrangements are being made for someone else to continue these studies.

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I. TECHNICAL OBJECTIVES

Low-dosage gamma irradiation of potatoes has been found to be very successful in preventing sprouting and spoilage of potoes under storage without the development of undesirable changes. Northern-Grown potatoes are available only 8 or 9 months of the year. Because of sprouting followed by rapid deterioration, it usually is not possible to keep potatoes under storage for longer periods. It is believed that desirable types of potatoes can, by irradiation, be made available the year around. This treatment might be particularly useful in increasing the storage life of any type of potato shipped overseas for the armed services.

More specifically, the general technical objective is described below:

A. A study will be made on the effect of low dosages of gamma radiation (approximately 5,000 to 20,000 rep) on at least one white-skinned and one russet-variety potato with the object of determining the dosage needed to inhibit sprouting when stored at 35°, 40°, 50°, and 70°F with 50% relative humidity. Samples of these potatoes will be made available for acceptance testing by the QMF and CI.

B. An investigation will be made using doses of gamma radiation as high as 200,000 rep on the same types of potatoes as studied in (A) above to determine the effect of overdose. Evaluation will include sprout inhibition, general appearance, keeping quality at 40° to 50°F, and sugar and starch content during intervals of storage.

C. A study will be made of the effect of two different relative humidities and two different temperatures during storage on a white-skinned and a russet-variety potato.

D. An evaluation will be made at no less than four regularly scheduled intervals during the storage of the irradiated potatoes that have been stored. The said evaluation shall include:

1. total starch and reducing-sugar content,
2. sprouting and its inhibition,
3. general appearance and texture,
4. interior fleshy region of peeled and sliced potatoes for decay, black heart, blackening, and other manifestations of enzyme and/or microbial action, and

5. loss in weight, to be determined and subdivided into combined respiration and transpiration loss and loss due to sprouts.

E. A limited study will be made on the effects of wound healing with special emphasis on formation of special cork cambium, and a limited study will also be conducted on chlorophyll formation in stored potatoes.

F. A quantitative respiration study will be conducted on at least a white-skinned variety and a selected russet variety of potatoes. As time permits, this study may be extended to differentiate between the tuber respiration and microbial respiration.

G. The effect of gamma radiation on the activity of specific enzymes involved in potato respiration will be investigated. This will be aimed at understanding the inhibition of enzyme activity as reflected by changes in starch content, total and reducing-sugar content, and color change allowing for extended storage life of the potato.

H. A study will be undertaken to determine whether gamma-ray-induced inhibition of potato sprouting is caused directly by destruction of germinal cells or indirectly by interfering with the nutrition of these cells.

I. A study will be conducted to determine the susceptibility of irradiated potatoes to common storage rot. This will include innoculation storage studies utilizing common potato-rotting bacteria and fungi.

J. As time allows, limited studies will be undertaken to determine the effects of radiation on the internal structure of the potato. This will include studies on cell growth, cell size, nuclear condition, condition of plastids, starch, cell turgor, penetrometer tests, etc.

II. STORAGE TESTS ON IDAHO RUSSET SEED POTATOES

Because of the late date of activation of this research contract relative to the potato season, uniform potatoes of good quality and known history which were suitable for experimental studies were difficult to obtain. Twenty bags, 100 lb each, of Idaho certified seed potatoes of known history were obtained in May, 1955, irradiated as soon as possible, and placed in storage at The University of Michigan and Michigan State University.

A. STORAGE TESTS AT VARIOUS TEMPERATURES

On June 14, two weeks after irradiation, the potatoes were inspected and excess dirt was removed from the potatoes by brushing with a soft-bristle brush. Decayed potatoes were discarded and the sound potatoes were placed in tared cardboard drums and weighed. These weights were recorded and the size of the sprouts and the condition of the potatoes were noted. Every two weeks thereafter the potatoes were weighed, and the sprouts were measured, and the general condition of the potatoes was observed.

The potatoes were stored in The University of Michigan Food Service in rooms with controlled temperatures maintained at 35°, 40°, 45°, 50°, 55°, and 60°F. One lot of potatoes was kept in a room without temperature control. The temperature in this room is designated as "room temperature" in this report (the temperature varied appreciably, but averaged about 75°-80°F). All the weighings were made in the Food Service building.

Figure 1 shows a plot of the percentage of weight lost by lots of potatoes stored at various temperatures for a period of 61 days. There are "breaks" in the curves at or about 50°F for both the data on the 10,000-rep irradiated and nonirradiated potatoes. The data on both groups can be approximated by straight lines with different slopes above and below 50°F. There was only slight weight loss in all lots of potatoes stored below 50°F, but the loss in the irradiated potatoes stored below 50°F was less than that in the controls. At storage temperatures above 50°F, the weight loss increased rapidly with temperature and was much greater for the controls than for the 10,000-rep irradiated potatoes. The slope of the straight line drawn for the control potatoes stored above 50°F for 61 days shows an average weight loss of 1.20 wt % per °F of storage above 50°F. The irradiated potatoes stored above 50°F for 61 days show an average weight loss of 0.44 wt % per °F. Thus, at temperatures above 50°F, and for these particular potatoes stored for 61 days, the control potatoes lose weight about 2.7 times as fast as the 10,000-rep irradiated potatoes.

The lots of potatoes stored at "room temperature" at an average temperature of about 75°F lost weight during storage as

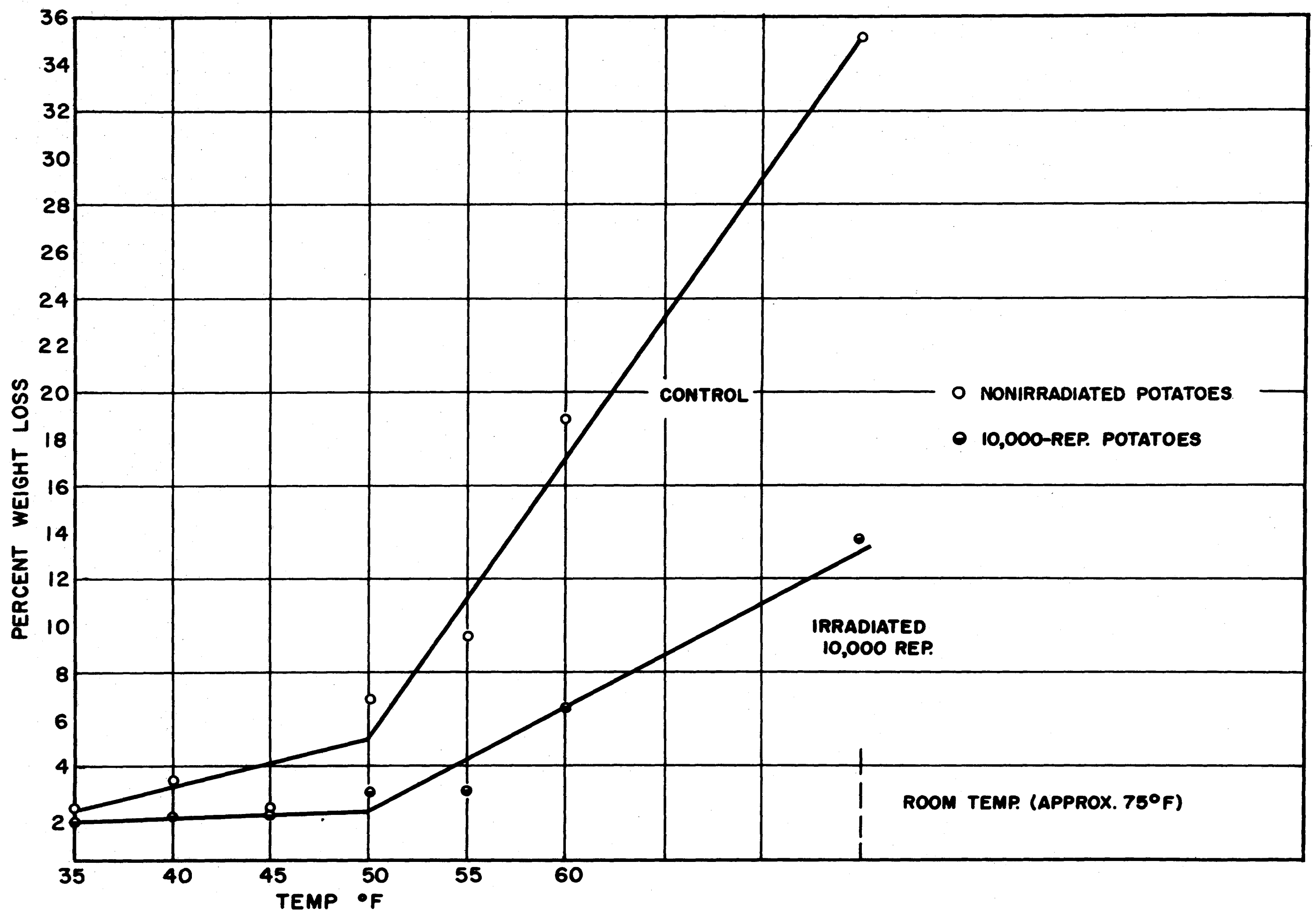

Fig. 1. Percentage weight loss of 10,000-rep irradiated and nonirradiated potatoes after 61 days of storage at various temperatures

shown in Fig. 2. The weight losses for both the control and 10,000-rep irradiated potatoes can each be approximated by a straight line. The slope of the lines indicates an average weight loss for the controls of 0.58 wt % per day and for the 10,000-rep irradiated potatoes an average weight loss of 0.225 wt % per day. On this basis, the controls are losing weight at a rate of about 2.6 times that of the 10,000-rep irradiated potatoes.

After 61 days, weight losses of potatoes stored at a temperature of 45°F or less were, as a rule, less than 2.0% in both irradiated and nonirradiated potatoes (most of the percentage losses varied between 1.5 and 2.5%). At all these lower temperatures the irradiated potatoes lost a slightly lower percentage of weight than did the nonirradiated potatoes. Usually this difference amounted to only 0.2-0.4%.

The samples which were treated with doses of irradiation other than the 10,000 rep discussed above (i.e., 5,000, 15,000, 50,000, 100,000, and 200,000 rep) were all stored at 45°F. Of these lots, only the ones treated with 5,000 and 50,000 rep showed percentage weight losses that were less than those of the controls. The remaining samples exhibited percentage weight losses approximately the same as the controls. Storage at 45°F has not been of sufficient duration as yet to show any significant effects of differences in radiation dosage.

The sprout length was measured every two weeks. It was found that nonirradiated potatoes stored at less than 45°F did not produce sprouts over 3/4 in. long over the 67-day observation period, and hence, they are not included in Fig. 3 with the growth curves of sprouts for potatoes stored at higher temperatures. The small original sprouts of those potatoes which received irradiation doses of 10,000 rep or more soon withered and died, as did the sprouts of the irradiated potatoes with such dosages and stored at 35° and 40°F.

The nonirradiated potatoes stored at "room temperature" behaved differently from all the other potatoes. In this instance, the sprouts quickly grew to a length of one inch or slightly more, but they were greatly thickened and soon stopped growing. The growth of the sprouts seemed to be inhibited by the higher temperatures.

B. STORAGE TESTS AT 70, 80, AND 90% RELATIVE HUMIDITY AND 50°F

Samples of potatoes from each of the different dosage lots

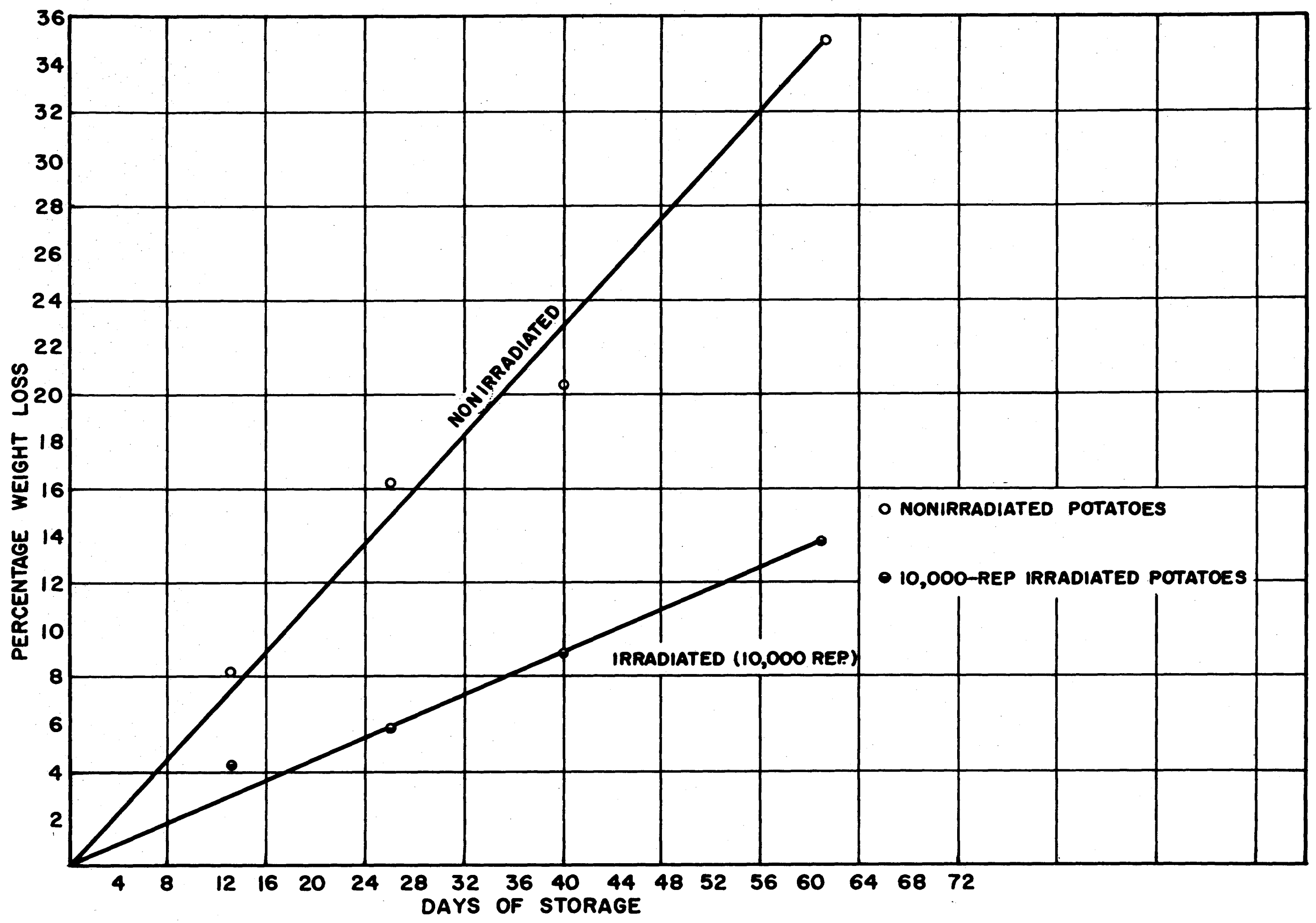

Fig. 2. Percentage weight loss of 10,000-rep irradiated and nonirradiated potatoes stored at "room temperature" (approx. 70°F average) over a 61-day period

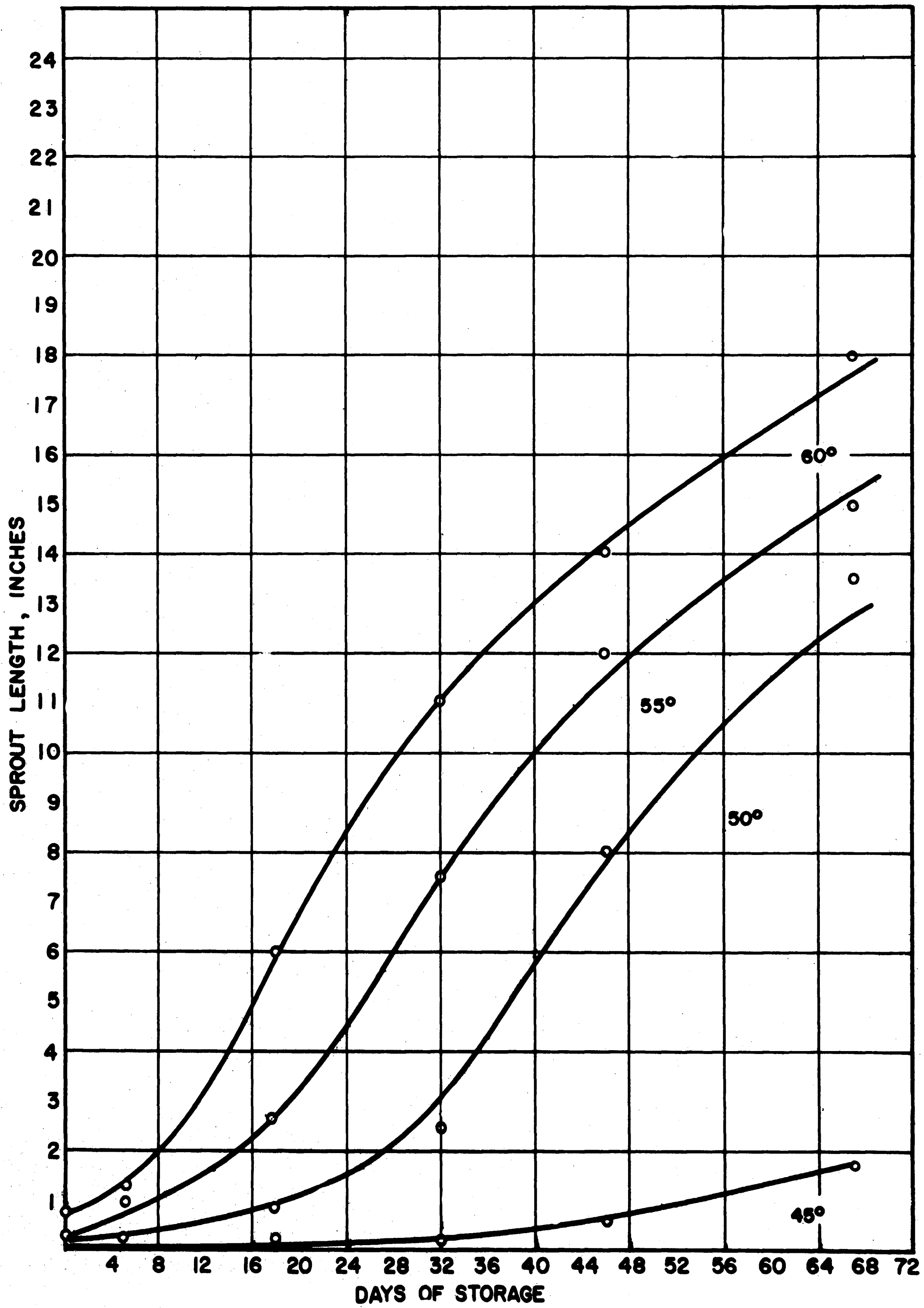

Fig. 3. Length of sprouts of nonirradiated potatoes as a function of duration of storage at various temperatures

were put in storage at Michigan State University at 50°F and three controlled humidities; 70, 80, and 90%. The data from these tests are given in Table I and portions of these data are plotted in Figs. 4, 5, and 6. Table I shows that weight-loss changes decrease rapidly with increasing dosage up to 15,000 rep, but that at higher dosages there appears to be no significant decrease in weight loss. Therefore, in Figs. 4, 5, and 6 only the data for the control, 5,000-, 10,000-, and 15,000-rep samples are reported.

In general, the curves show a decrease in weight loss with both increase in humidity and increase in dosage. However, there is one inconsistency in Fig. 4. In this figure the 15,000-rep and the 10,000-rep curves have positions reversed from that expected from the general trends. This may be the result of an error in dosage or a mix-up in samples or simply the result of statistical variation.

Figure 7 is an interesting curve in that it clearly shows the general trend of the effect of increasing radiation dosage on the reduction of weight loss.

The potatoes lose weight for a number of reasons, but for simplicity the losses may be divided into two groups, (a) "physical" and (b) "biological."

Regardless of whether or not the potato tubers are living or dead, they will lose weight when stored in an atmosphere not saturated with water vapor. This is true because the skin of the potato tuber behaves as a membrane through which water, oxygen, carbon dioxide, etc., may diffuse. The "physical" weight loss is largely the result of the evaporation of water which depends in part upon the diffusivity of water through the skin, the partial pressure of water vapor in the storage air, and the velocity of air circulation around the stored tubers. These and other variables which influence the mass transfer coefficients will have some effect upon this "physical" loss in weight.

Potato tubers with living cells and/or with active enzyme systems may lose weight as a result of biological processes such as sprouting and respiration. This loss will be in addition to that lost by evaporation and will be influenced by the biological activity of the tubers.

To explore the nature of the loss in weight, an average loss from "physical" evaporation may be subtracted from the total weight loss to give a weight loss which can be attributed primarily to "biological" factors. Some damage to the skin of the tubers

TABLE I

WEIGHT LOSS IN PERCENT OF ORIGINAL WEIGHT OF POTATO TUBERS

STORED AT 50°F AND DIFFERENT RELATIVE HUMIDITIES

Relative Humidity	Dosage rep x 10^3	Date of Observation				
		Initial 6/14	7/1	7/22	8/11	8/30
70% d.p.d. = 2.30 mm Hg	0	0	2.3	3.7	12.5	18.5
	5	0	2.2	4.5	10.4	14.6
	10	0	1.1	2.5	4.7	6.8
	15	0	1.4	3.2	5.6	8.8
	50	0	1.2	2.8	5.1	7.8
	100	0	1.1	2.4	4.3	6.0
	200	0	1.3	2.9	5.2	8.0
80% d.p.d. = 1.84 mm Hg	0	0	1.9	5.0	10.3	14.6
	5	0	1.4	3.7	3.9	10.5
	10	0	1.3	3.3	5.9	7.6
	15	0	1.2	2.6	3.3	6.5
	50	0	1.8	2.9	4.9	5.1
	100	0	0.8	2.0	3.6	5.1
	200	0	0.9	2.1	4.2	6.1
90% d.p.d. = 1.38 mm Hg	0	0	1.2	3.3	9.4	13.4
	5	0	0.8	2.6	6.4	9.1
	10	0	0.7	2.4	4.9	5.8
	15	0	0.8	2.2	4.3	5.5
	50	0	0.4	1.6	3.7	4.8
	100	0	0.5	1.3	3.6	4.9
	200	0	0.5	1.4	3.0	3.7

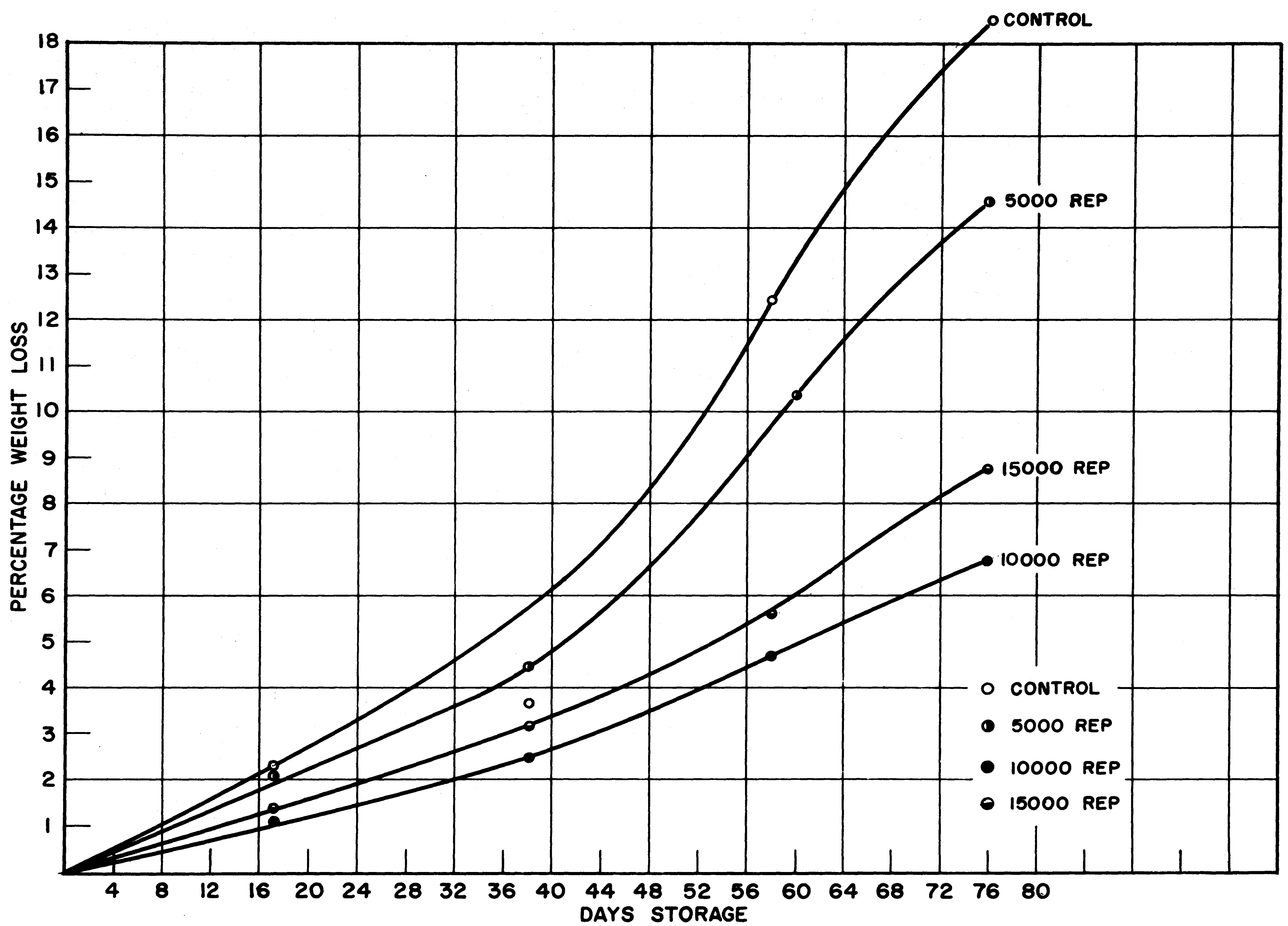

Fig. 4. Weight loss for potatoes stored at 70% relative humidity

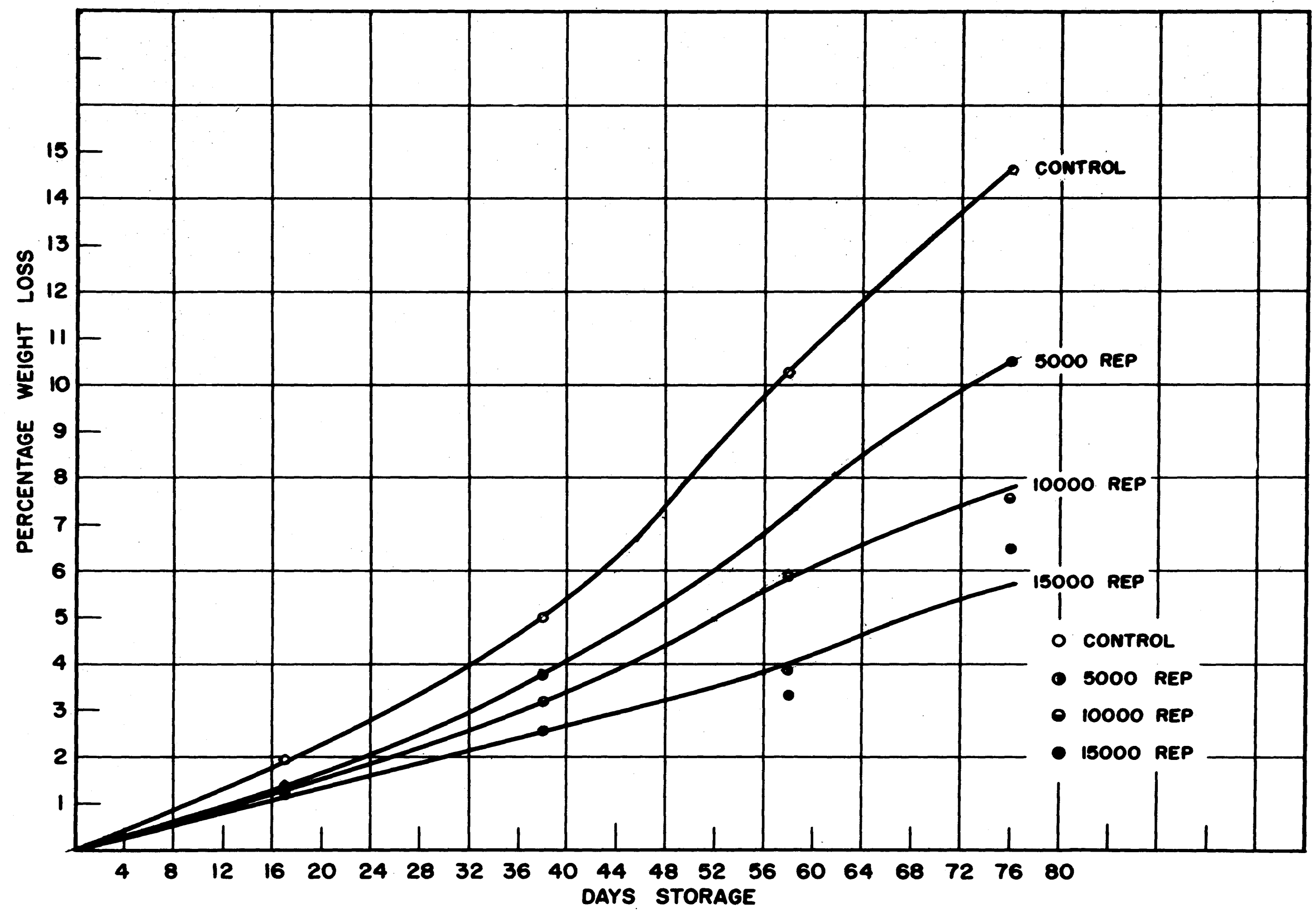

Fig. 5. Weight loss for potatoes stored at 80% relative humidity

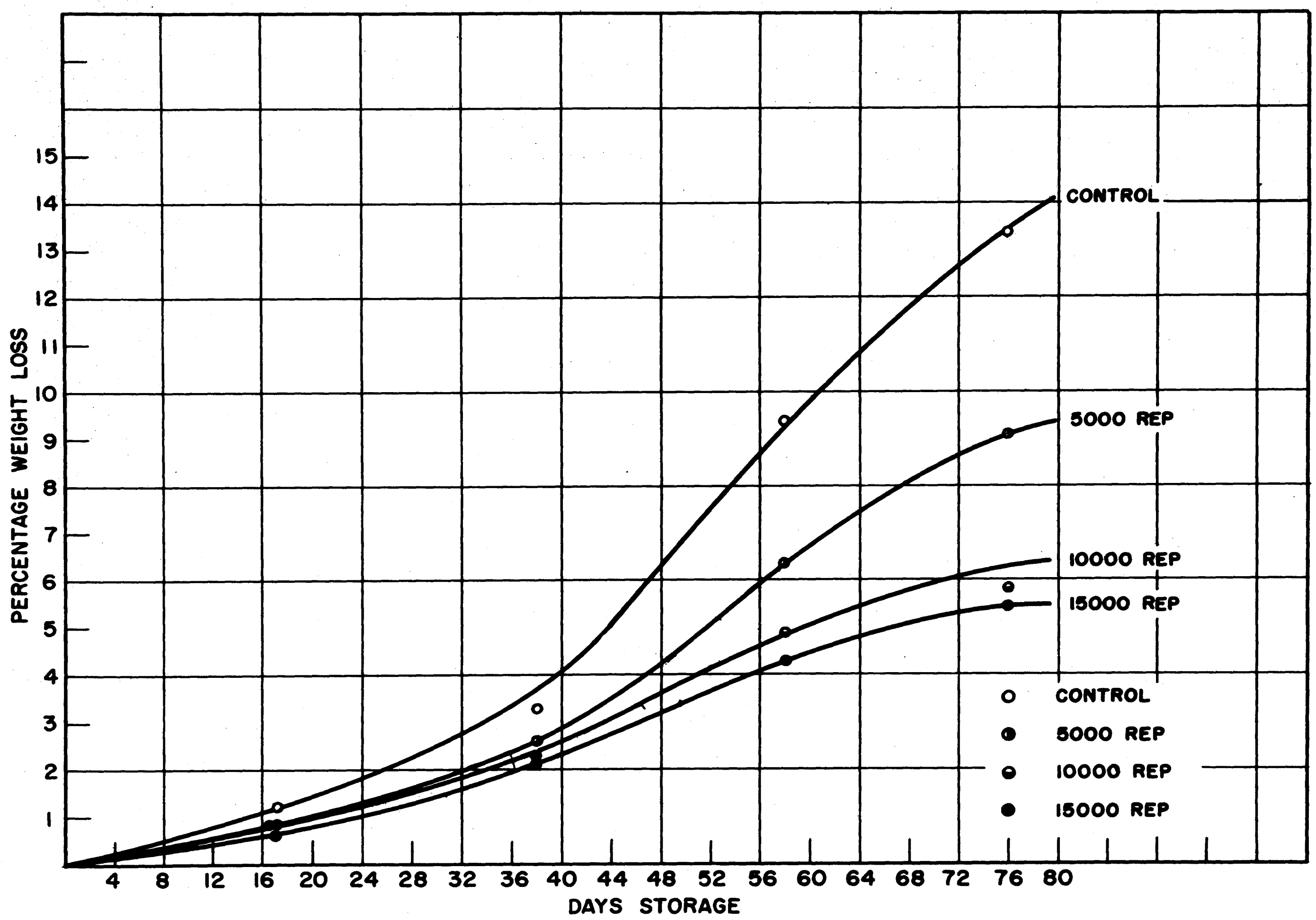

Fig. 6. Weight loss for potatoes stored at 90% relative humidity

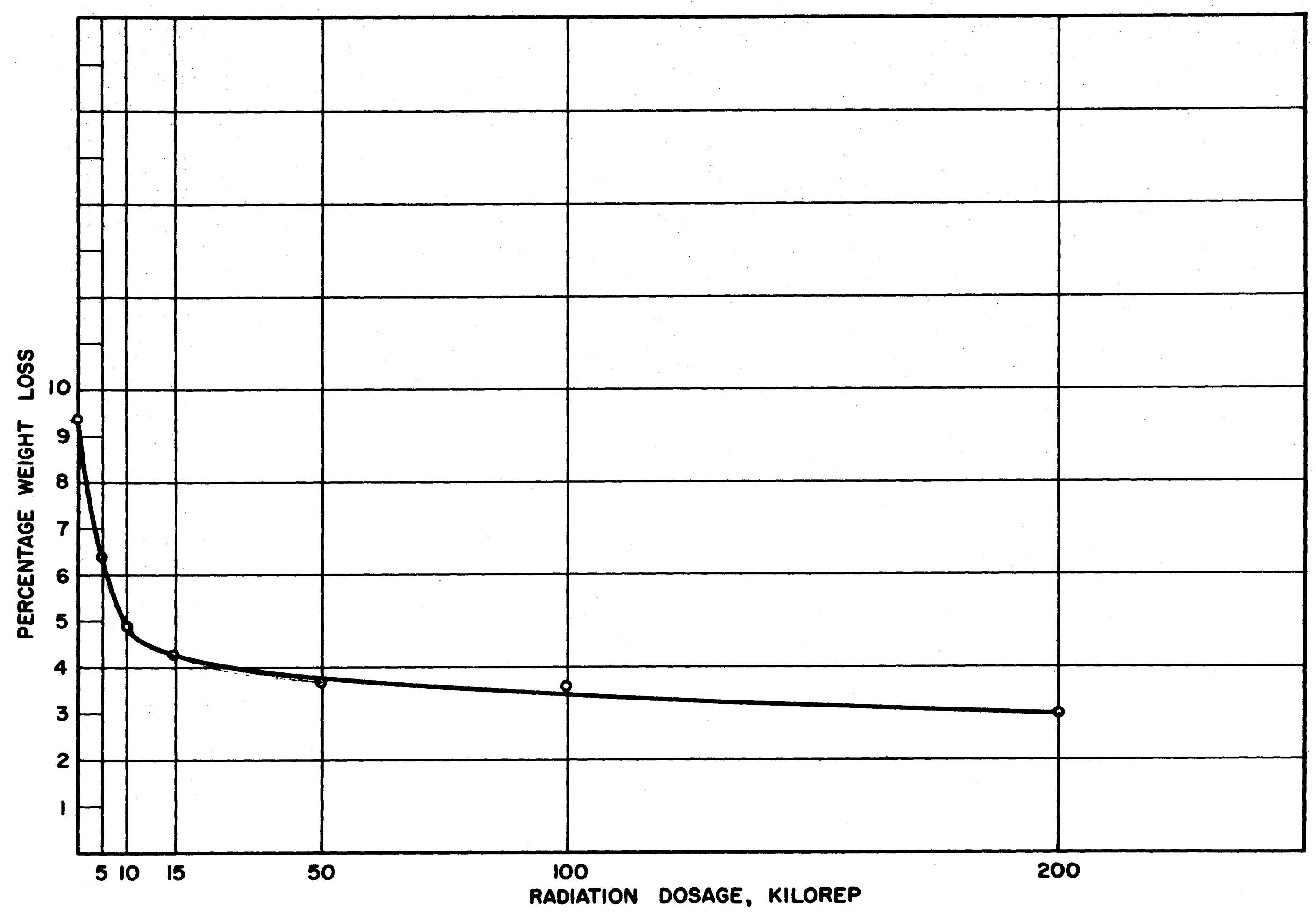

Fig. 7. Percentage weight loss of irradiated potatoes as a function of radiation dosage after 58-days storage at 50°F and 90% relative humidity

was observed at the higher doses. This damage consisted of a weakening of the skin as indicated by cracking of the skin when stretched. The higher doses may introduce a modification of the skin of the tuber and, therefore, increase weight loss for another reason. For this reason, and because of the extreme sensitivity of the "biological" weight loss when the total weight loss nearly equals the "physical" weight loss, only the data from zero to 50,000-rep dose were used in this analysis.

Such an analysis was prepared by subtracting 3.69% weight loss from the weight losses of potatoes receiving from zero through 50,000-rep doses and replotting the remainder on semilogarithmetic paper as shown in Fig. 8. The fact that the first five points of Fig. 7 can be placed on a straight line semilogarithmetic paper in Fig. 8 is of interest because it shows a possible similarity between the effects of ionizing radiation on microorganisms and on tubers. Thus, the "target" theory, which sometimes is used to explain the destruction of vegetative cells of microorganisms by ionizing radiation, may have some application in explaining the effect of ionizing radiation on tubers. Such a theory is not proposed, based on these limited data, but will be considered again after more data have been obtained.

III. BIOCHEMICAL CHANGES IN IRRADIATED POTATOES

This section is concerned with the relationship between such changes in reducing sugars and sucrose and changes in content of starch, pentoses, and other carbohydrates, compounds that may be involved in oxidation-reduction reaction (such as ascorbic acid, sulfhydryl compounds, phenolic derivatives, etc.), enzyme systems involved in carbohydrate metabolism, and other specific compounds more directly related to the primary effect of gamma radiation. The importance of measurements of reducing sugars and sucrose in irradiated potatoes needs emphasis. From a practical standpoint, the increase in reducing sugars that follows irradiation is of paramount importance as it usually leads to an undesirable product in processed potatoes.

The measurement of reducing sugars and sucrose is likewise of importance in fundamental studies as it affords a starting point from which the primary effect of radiation can be explored. Not only do carbohydrates make up the bulk of potato solids, but these are intimately linked with metabolic systems that are probably

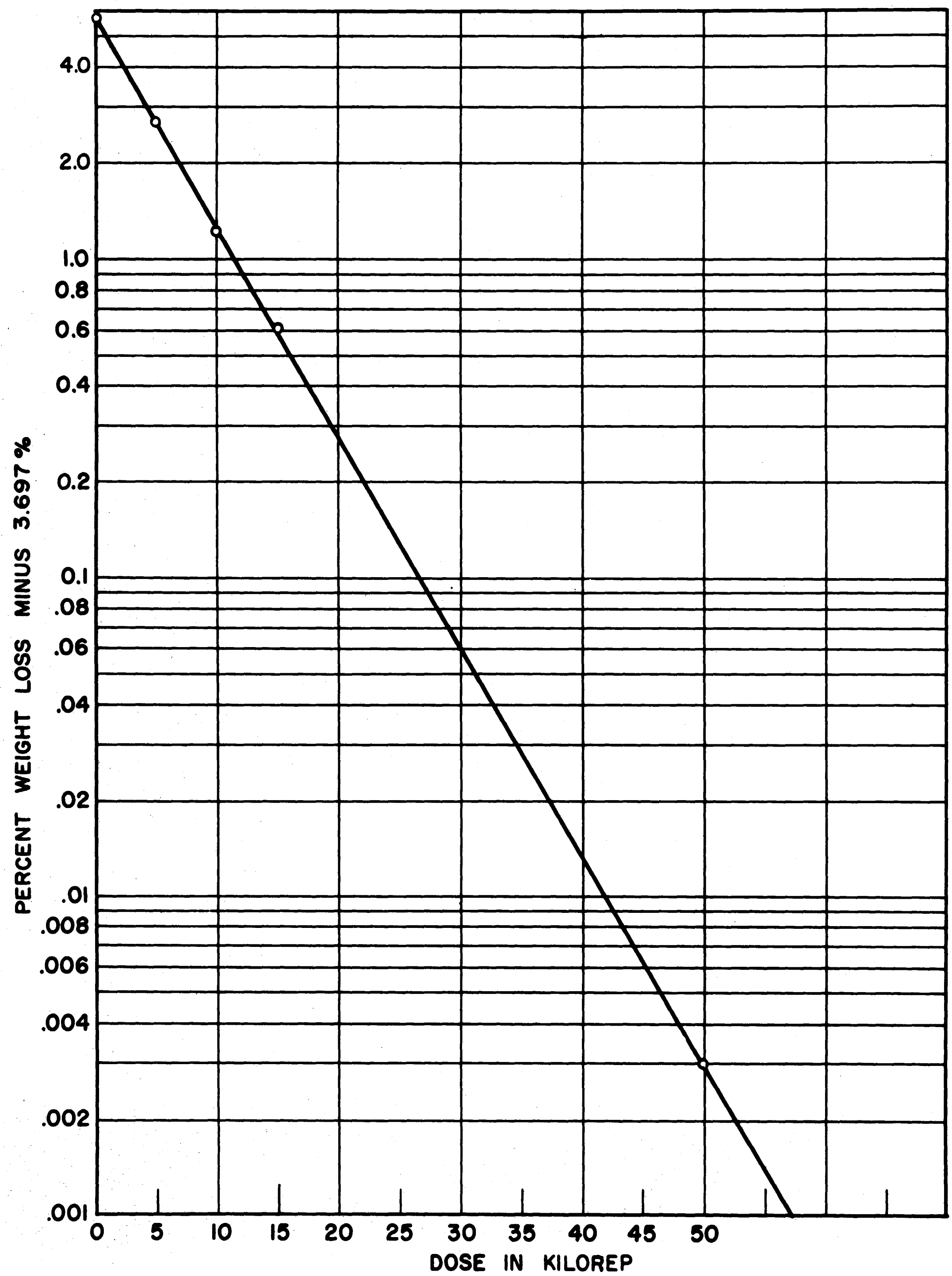

Fig. 8. "Biological" weight loss vs radiation doses

more vulnerable to radiation effects. The results of investigations directed toward determining whether or not such processes as cell destruction, enzyme stimulation, or oxidation of specific compounds are the actual target of the gamma photon may suggest means of controlling the reducing-sugar content that are unknown at the present time.

A. EXPERIMENTAL PROCEDURE

Considerable emphasis is being placed at the present time on methods of analysis for reducing sugar. This program consists of four steps: first, selection and perfection of a rapid means of determining reducing sugar; second, simplification of previous extraction procedures with a view toward facilitating measurement of other than carbohydrate compounds; third, investigation of a procedure for proper sampling of a large number of potatoes; fourth, investigation of some basis other than dry weight to relate the reducing-sugar content.

A rapid means of determining reducing sugars is necessary because the inevitable potato-to-potato variation requires determinations on a large number of potatoes receiving any one of the many treatments. It is also indispensable because the reducing-sugar content is believed to be the correlating factor for other biochemical substances. The "official" methods,[1] besides involving exhaustive extractions, involve a tedious gravimetric method, but are available for establishing the usefulness of newer methods.

With regard to the rapid measurement of reducing sugar, four methods were considered: (a) optical methods; (b) a colorimetric method utilizing the reaction of carbohydrates with anthrone;[2] (c) a chromatographic method involving the separation on Dowex-50 of the borate complexes of the carbohydrate compounds followed by spectrophotometric analysis;[3] (d) a direct titration method involving the reduction of ferric to ferrous ion by the reducing sugars and the titration of the ferrous ion formed with ceric ion.[4]

Optical methods are very rapid but may not be sufficiently sensitive. The first optical method investigated for the analysis of sugars in potatoes under investigation was the refractometric method. The refractive indices of sucrose and d-glucose are almost the same for sugar solutions with concentrations below 10%. Consequently, d-glucose and l-fructose, in an aliquot of potato extract, could be determined by use of a refractometer.

A Bausch and Lomb Precision Refractometer, made available by the Department of Chemical Engeering, The University of Michigan, was used for preliminary checks. A solution of sucrose was prepared and divided into two portions. To one portion was added a few drops of invertase and left at room temperatures for several hours. The indices of both portions of sugar solution were taken and compared. It was found that they agreed quite satisfactorily.

The use of this method, however, was hindered by the very low concentration of sugars in potato extract. The refractive index of a solution containing 0.001 gram glucose per liter was the same as that of distilled water, i.e., 0.3 to 27.5°C on the Precision Refractometer.

A second optical method studied involved the establishment of a spectrophotometric calibration for potassium borate solution containing various sugar solutions.[3] These sugar solutions are obtained as effluent from a chromatographic column. The column will also be used to separate the various sugars from one another in other methods of analysis. A Cambridge-type spectrophotometer was used in the first spectrophotometer tests. A wave-length of 620 microns was employed, and a blank borate solution was used as the reference solvent. The percent light transmittancy of a 0.01 M borate solution and that of such solution containing 0.01 gram glucose per liter are 100 and 102%, respectively. As these concentrations are what could ordinarily be expected in this analytical method, it was decided that the spectrophotometric readings with this instrument would not be sufficiently accurate. Further study of this method will be undertaken with the use of a Beckman-type spectrophotometer, which is equipped with an ultraviolet-light source.

The anthrone method does not distinguish between reducing and nonreducing sugars and also requires a colorimeter, which is not on hand; however, it may be the most rapid means of estimating total carbohydrate. The chromatographic method makes possible the separation of the two principal reducing sugars in potatoes, glucose and fructose, as well as sucrose and other compounds, but requires many hours for operation. The ceric sulfate method appeared to satisfy the requirements for the present studies and has been chosen for the initial determinations. This method was verified by Williams _et al._,[5] using two other mthods; the cuprous oxide gravimetric method of the A. O. A. C. and the Somogyi method, involving the reduction of iodate to iodine by the cuprous oxide formed from the reducing sugars and titration of the iodine with thiosulfate.[5] These authors found it necessary to use ion-exchange resins in place of lead acetate or carbon as a means of clarifying the extracts for ceric sulfate titrations.

In regard to preparation of extract, there appears to be no study showing the most efficient manner of extracting reducing sugar from the potato solids. The exhaustive alcoholic extraction used by Williams et al.[5] required the removal of alcohol before non-sugar-reducing substances can be removed by ion-exchange resins. Ideally, the preferred method would consist of a hot-water extraction aided by a mechanical blender without any use of alcohol. The slurry can be filtered and the filtrate passed directly into a cation-resin and an anionic-resin bed in series, as the use of ion-exchange resins to remove nonsugar-reducing compounds was shown by Williams et al. to be superior to the use of lead acetate or carbon for this purpose. Also, the use of ion-exchange resins offers the advantage of isolating all cationic and anionic materials (e.g., sugar phosphates) as a single group.

Experiments are presently under way in which the hot-water-extraction procedure is being compared for efficiency of extraction with the exhaustive alcohol-extraction procedure used by previous investigators. This problem is being undertaken in the Fission Products Laboratory by Dr. Harry Yang whose recent doctoral dissertation involved extensive work in beet-sugar extraction. An attempt will be made to devise a routine extraction procedure that can be set up as a battery operation.

Individual potatoes vary considerably as to their normal reducing-sugar content and their response to irradiation and storage. Therefore, it is necessary to give a large number of potatoes any one of the desired external treatments and to analyze each potato individually for reducing sugars. To achieve the same result with fewer analyses, the possibility is being investigated as to whether or not a small portion of any given potato is reasonably representative of the whole potato, and whether or not such small portions from each potato receiving a given treatment can be combined and analyzed as a single sample. This is being done simply by determining reducing-sugar contents on several small portions of each of several potatoes at random.

In regard to the fourth step in the program, the search for some basis other than dry weight to be used to make significant comparisons of reducing sugar in potatoes receiving different treatments will begin shortly. The sugar phosphates that are trapped by the anion resin in the clarification of potato extracts will be measured. The possibility of using desoxyribonucleic acid measurements has been considered as providing a rough index to cell number, as changes in reducing-sugar content may be more closely related to number of functioning cells than their total mass. Measurement of cytochrom "C" and of protein-bound phosphate has been suggested as another possible "anchor."[6] In some respects, this investigation borders on the fundamental studies which are as yet not completely planned.

B. EXPERIMENTAL RESULTS

A comprehensive study on biochemical changes in irradiated potatoes should begin at the time of harvest and continue throughout the storage life of the potatoes in order to study the effect of irradiation at different times after storage in addition to knowing the detailed history of the potatoes. The only northern-grown potatoes of known history available at the beginning of this study were those from last year's crop and which had been stored for several months. However, short-term investigations with these potatoes can serve as a guide in the planning of the more comprehensive study planned for potatoes from the 1955 crop and can provide a means of testing the methods to be used. Data on these potatoes would be of additional significance inasmuch as commercial irradiation may frequently be carried out on stored potatoes such as these which had just begun to sprout.

QMC Progress Report No. 1[7] described the irradiation and storage schedule used to investigate the effect of a wide range of dose (0 to 200,000 rep) on the reducing-sugar content of potatoes from zero to 48 hours at room temperature following irradiation. The purpose of this study was to determine what biochemical changes, if any, occurred during or directly following irradiation. It is, of course, desirable when looking for the primary biochemical change to avoid complications from biochemical changes occurring later.

In the present studies, potatoes were placed in a deep-freeze at the desired time after irradiation and kept there until facilities had been established for making the reducing-sugar analyses. Recently a number of analyses have been made using the ceric sulfate titration procedure. Clarification of the aqueous extract was made with lead acetate and not with ion-exchange resins. The analyses were made on a small portion of each of several potatoes receiving a given combination of irradiation dose and storage time. The results are shown in Table II.

TABLE II

RESULTS OF PRELIMINARY SUGAR DETERMINATIONS BY CERIC TITRATION

Dose rep	Percentage Sugar							
	Time (hrs)							
	0	1	2	4	6	12	24	48
0			2.00		2.09		1.84	1.74
5,000							1.99	1.86
10,000			1.82		2.08		1.64	2.19
15,000								1.70
50,000			2.15		2.09		1.87	2.53
100,000			1.90		1.33			2.64
200,000			2.41		2.43		3.05	2.46

These results show only a slight effect of irradiation, and this is at the higher doses. Also, this increase at the higher doses appears to occur only after the potatoes are stored twenty-four hours or longer.

These results show the same trend but a different magnitude from those reported by Brownell, Pederson, et al.[8] There the increase in reducing sugars was more marked by increase at the low dose of 10,000 rep. Analyses are continuing with this batch. Both improvements in method (use of ion-exchange resins in place of lead acetate as a means of clarification) and in sampling (a larger number of potatoes will be analyzed by pooling representative portions from several potatoes receiving the same treatment) have been made.

Some analyses of several portions of individual potatoes have been analyzed to determine whether or not the reducing-sugar content was the same throughout the potato. The method was the same as that used above. The results on a number of portions of individual potatoes are shown below.

A. 1.37% reducing sugar
B. 1.79% " "
C. 1.81% " "
D. 1.49% " "

Sample A was taken from immediately beneath the skin of the potato. Samples B and C were taken at random from the tuber and show good agreement. Sample D was selected in the same manner but lost considerable cell fluid as it thawed before it could be made into a slurry. This fluid probably contained some reducing sugar, and this could account for the observation that the reducing-sugar content in this sample was lower than in samples B and C.

These results indicate that, while many potatoes may be needed to determine the effect of a single treatment, a relatively smaller number of reducing-sugar analyses need be performed to obtain reliable results.

IV. THE EFFECTS OF IRRADIATION DOSAGE ON ACCEPTABILITY

No report on the acceptability studies made at QMC has yet been made available to this laboratory.

V. THE EFFECTS OF IRRADIATION DOSE AND STORAGE TEMPERATURE ON PLANT PHYSIOLOGY AND METABOLISM

The unfortunate death of Professor C. D. LaRue on August 20, 1955, has interrupted the study for differences in plant physiology and metabolism. Arrangements are being made for someone else to continue these studies.

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