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GAMMA-RAY SPROUT INHIBITION OF POTATOES

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CONTRACT RESEARCH PROGRESS REPORT

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Title of Contract: Gamma-Ray Sprout Inhibition of Potatoes

SUMMARY

Reducing-sugar, sucrose, and starch analyses have been made on Sebago variety and on Russet Rural variety potatoes given nine different doses of gamma radiation and stored from eight to ten weeks at five different storage temperatures. The results show that there is little change in reducing sugar as a function of radiation dose, but that the sucrose content increases and the starch content decreases with increasing dosage of radiation. Both the reducing-sugar and sucrose contents are low in all potatoes given 15 kilorep radiation and stored at temperatures other than the lowest (35°F). Radiation lowered the starch content of Sebagos but made little change in the starch content of Russet Rurals.

Sebago variety potatoes given as little as 5,000 rep show no signs of sprouting, but Russet Rurals given 20,000 rep show evidence of arrested sprout development. Tiny sprouts (1 mm) form but apparently die. All nonirradiated potatoes except those stored at 35°F show definite sprout development. Most of the potatoes, irradiated and nonirradiated, except those stored at 35°F, show slight softening, usually confined to the skin. Weight loss is only slightly higher at higher radiation dosages than at low dosages, but increases progressively with increasing storage temperature. These results differ from those observed with tubers irradiated after long storage. Preliminary results on humidity studies at Michigan State University show that both Russet Rurals and Sebagos lose more weight at lower humidity of storage than was expected. Also the Sebagos lose more weight than Russet Rurals given the same treatment. As yet there appears to be no effect of radiation dosage on weight loss during storage at any humidity.

Preliminary observations indicate that 200 kilorep of radiation severely retard or prevent periderm formation, but that the reduction following the 15 kilorep treatment is much less pronounced.

Studies on rate of carbon dioxide evolution by whole tubers revealed that, compared to controls, the rate is decreased immediately after irradiation, but thereafter increases to a level roughly one and a half times that of the controls. Oxygen uptake by potato slices also increases as a result of irradiation, but the increase occurs immediately and continues for several weeks after irradiation.

The hormone content of the eyes of nonirradiated Sebago variety potatoes appears to have decreased by about one-fourth over a storage period of two and a half months. Gamma radiation has been found to decrease

the content of growth hormone in the eyes of Sebago and Russet-variety potatoes, but the magnitude of the effect was not found to be related to the dosage of radiation.

TECHNICAL OBJECTIVES

Low-dosage gamma irradiation of potatoes has been found to be very successful in preventing sprouting and spoilage of potatoes 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, by 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 25,000 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°, 60°, and 80°F with 85% relative humidity.

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.

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

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

1. total starch, sucrose, 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. As time allows, a limited study will be made on the effects of wound healing, with special emphasis on formation of cork cambium, cellular organization, and structure.

F. A quantitative respiration study will be conducted on at least a white-skinned variety and a selected Russet variety of potatoes.

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 made of the growth hormone and inhibitors in and around the eyes of irradiated and control potatoes to determine whether or not gamma-ray-induced inhibition of potato sprouting is caused by an increase in the quantity of sprout inhibitors.

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

J. Samples of potatoes described under (A) will be made available for acceptance testing by personnel of QMF and CI.

CARBOHYDRATE ANALYSES

A. IRRADIATION AND STORAGE OF POTATOES

The Sebago (white skinned) variety potatoes were received from Michigan State University Farm Crops Department on November 1, 1955. They were given the designated dosages of gamma radiation and placed in the 45°F storage room at the Food Service Building during the period

November 2-5. Those potatoes being stored at temperatures other than 45°F were transferred to the appropriate storage rooms on November 7. Zero-time determinations of reducing sugar and sucrose were made shortly thereafter. The dose of radiation was applied just prior to the time the determinations were made. The data to be presented for the values for reducing sugar, sucrose, and starch following the first storage period were obtained during the third week in January, 1956. The first storage period was thus ten weeks in duration.

The Russet Rural variety potatoes were received from Michigan State University Farm Crops Department on November 15, 1955. They were held between 35° and 45°F until further treatment. They were irradiated and placed in the 45°F storage room at Food Service during the period November 27 to December 1, 1955. Those potatoes which were to be stored at temperatures other than 45°F were transferred to the appropriate storage rooms on December 7, 1955. Zero-time determinations of reducing sugar and sucrose were made shortly thereafter. The dose of radiation was applied just prior to the time the determinations were made. The data to be presented for the values for reducing sugar, sucrose, and starch following the first storage period were obtained during the last week in January and the first week in February, 1956. The first storage period was thus eight weeks in duration for Russets.

B. METHODS

1. Reducing Sugar.—The method was reported in Progress Report No. 3, pp. 15-22. It was also the basis of the determination of sucrose and starch.

2. Sucrose.—The determination of sucrose is made on the same filtrate of the potato slurry as used for the reducing-sugar determination. A 40-ml aliquot of the filtrate is transferred to a 100-ml volumetric flask, and 2 ml of concentrated hydrochloric acid is added (giving a solution approximately 0.6 N in HCl). The flask is heated in a boiling water bath for 10 minutes. The flask is then removed and cooled to room temperature and nearly neutralized (to pH 6.0) with concentrated sodium hydroxide solution. The flask is made to volume with distilled water, and the solution filtered. The filtrate is titrated as in the case of reducing sugar. The value for reducing sugar prior to hydrolysis is then subtracted, and the difference is multiplied by 0.95 to give the value for sucrose.

An example of the validity of the method is shown by determining

the reducing sugar concentration of a mixture of sucrose and glucose (a reducing sugar) before and after hydrolysis. The data are shown in Table I:

TABLE I

DETERMINATION OF SUCROSE IN MIXTURES OF SUCROSE AND GLUCOSE

Sample	Aliquot Taken	Before Hydrolysis		After Hydrolysis			Percent Recovery
		Ceric Sulfate	Glucose	Ceric Sulfate	Red'g Sugar	Sucrose	
	ml	ml	mg	ml	mg	mg	
Glucose, 1.0 mg/ml	0.5	1.3	(0.5)				
	1.0	2.7	(1.0)				
Glucose, 0.90 mg/ml plus	0.5	1.25	0.46	1.67	0.62	0.16	107
Sucrose, 0.30 mg/ml	1.0	2.40	0.89	3.25	1.20	0.31	103

Further evidence of the specificity of the determination for sucrose is afforded by the use of the enzyme invertase whose only action is to hydrolyze sucrose. The following methods were tested:

(a) See method described above.

(b) Determination of sucrose by the official A.O.A.C. hydrolysis method (inversion at room temperature).

> For inversion at room temperature, transfer a 50-ml aliquot of clarified and deleaded solution to a 100-ml flask, and 10 ml of HCl (1 + 1) and allow to stand for 24 hours at room temperature (20°C or above). Neutralize with NaOH solution and make to 100 cc with H_2O. Determine percent of reducing sugar (by ceric sulfate method). Percent sucrose = (percent reducing sugar after inversion minus percent reducing sugar before inversion) x 0.95.

(c) Determination of sucrose by the action of invertase (inversion at room temperature).

Determine quantity of acetic acid necessary to make 50 ml of solution distinctly acid to methyl red indicator (pH = 4.6). Then to another 50 ml add this quantity of acetic acid and 5 ml of invertase solution (nutritional biochemicals). Fill flask almost to 100 cc and let stand overnight, preferably at not less than 20°C. Cool, neutralize, bring to 100 cc. Determine percent of reducing sugar, and use formula for sucrose as in Method (b).

(d) Determination of sucrose by invertase (rapid inversion).

To 50 cc of solution (clarified and deleaded) add the quantity of CH_3COOH (acetic acid) necessary to bring the pH to 4.6. Then add 10 ml of invertase solution, mix thoroughly, place flask in the bath at 55-60° for 15 minutes, with occasional shaking. Cool to room temperature, neutralize, dilute to 100 cc with H_2O.

Determine the percent of reducing sugar and use formula for sucrose as in Method (b).

The action of invertase is compared with that of hydrochloric acid by applying Method (b) and Method (c) to a standard sucrose solution. The results are given in Table II.

In order to compare the four methods and check the method of rapid conversion by HCl used in subsequent analyses, determinations of sucrose were made on the same sample by these four methods. The sample consisted of the clarified and deleaded solution after filtration of potato slurry. The data are shown in Table III.

The results indicate fair agreement. The rapid methods, (a) and (d), show better agreement between acid and enzymatic hydrolysis (1.52 and 1.57%, respectively) than do the "slow" methods, [(b) and (c), 1.46 and 1.61%, respectively]. These data show that reducing sugars (mainly glucose and fructose) and sucrose are the only carbohydrates of importance in the aqueous extract of potatoes.

TABLE II

COMPARISON OF INVERTASE AND HYDROCHLORIC ACID AS AGENTS FOR INVERTING SUCROSE

Sample	Method of Hydrolysis Used	Volume of Aliquot, cc	Ceric Sulfate cc	Percent Inversion
Glucose vol 100 mg/100 cc		1.0	2.8	
Sugar sol. 100 mg/100 cc	Method (b) (Official, hydrolysis by HCl method)	1.0	1.50	102
Sugar sol. 100 mg/100 cc	Method (c) (Official, with invertase at room temperature)	1.0	1.50	102

TABLE III

DETERMINATIONS OF SUCROSE BY FOUR DIFFERENT METHODS
ON THE SAME SAMPLE

Method of Inversion Used	Percent of Reducing Sugar Before Inversion	Percent of Reducing Sugar After Inversion	Percent Sucrose
Method (a) (Our method, rapid inversion by HCl)	0.54	2.74	1.52
Method (b) (Inversion of sucrose by official hydrolysis, HCl method, at room temp.)	0.54	2.18	1.46
Method (c) (Determination of sucrose by invertase at room temp.)	0.54	2.24	1.61
Method (d) (By invertase, rapid inversion)	0.54	2.20	1.57

3. Starch.—The determination for starch is made according to Method 22.4 of the Association of Official Agricultural Chemists, seventh edition, p. 348.[1] It is the official direct acid hydrolysis for materials such as raw starch, potatoes, etc. The method is quoted as follows: "Stir weighed sample, representing 2.5-3 g of the dry material, in a beaker with 50 ml of cold H_2O 1 hr. Transfer to filter and wash with 250 ml of cold H_2O. Heat insol. residue 2.5 hrs with 200 ml of H_2O and 20 ml HCl (sp. gr. 1.125) in flask provided with reflux condenser. Cool, and nearly neutralize with NaOH. Complete vol. to 250 ml, filter, and det. dextrose [glucose, or reducing sugar] in aliquot of filtrate. . . . Wt of dextrose obtained x 0.90 equals wt of starch." Dextrose was determined by Method (a), above.

The procedure is modified in that a 1:10 dilution of the 250-ml volume must be made prior to determination by the ceric sulfate method. This dilution is necessary to bring the glucose concentration within range of the ceric sulfate method. The filter paper as well as the insoluble residue is included in the hydrolysis for simplicity in transferring the residue. The acid treatment of the paper does not yield a detectable amount of reducing sugar. An iodine test on the filtrate revealed that no starch was lost from the residue through the filter paper. Ceric sulfate determinations on the filtrate after 10-minute hydrolysis and after prolonged hydrolysis with HCl showed no change, further indicating that nothing other than reducing sugar and sucrose is present in the filtrate to give a test for reducing sugar. Filtration is facilitated by the use of celite as a filter aid and by vacuum.

All determinations reported were made on a minimum of four potatoes per sample, and values for reducing sugar, sucrose, and starch for potatoes at a given treatment were made on the same four or more potatoes. Each potato in the sample was cut up, and pieces of each were used (a) for reducing sugar and sucrose determination, (b) for starch determination, and (c) for dry-weight determination. Tables IV, V, VI, and VII give the values for the three carbohydrates, as well as their sum, in terms of percentage of whole weight of tuber.

C. DISCUSSION OF RESULTS

The effect of radiation dosage on the reducing sugar and sucrose contents of both Russet Rural and Sebago variety potatoes measured directly after irradiation is negligible. The values for reducing sugar in Sebagos for all doses of radiation have an average value of $0.78 \pm .11\%$, with no apparent pattern among the values; for sucrose the average value is

TABLE IV

CARBOHYDRATE ANALYSIS OF RUSSET RURAL POTATOES AS A FUNCTION OF RADIATION DOSAGE AND TIME

Dose (Krep) of Radiation	0 Time		End of First Storage Period				(8 weeks)
	% Red'g Sugar	% Sucrose	% Red'g Sugar	% Sucrose	% Starch	Total	% Dry Weight
0-Control	0.43	0.19	0.50	0.28	20.7	21.5	22.1
5	0.43	0.23	0.64	0.38	19.8	20.8	-
10	0.45	0.18	0.78	1.3	21.4	23.5	27.3
15	0.43	0.30	0.40	0.81	21.4	22.6	-
20	0.44	0.27	0.75	3.56	17.2	21.5	-
25	0.52	0.23	0.40	0.63	16.0	17.0	21.1
50	0.37	0.20	0.56	1.32	17.7	19.6	22.2
100	0.68	0.29	0.57	3.02	18.7	22.3	29.2
200	0.44	0.29	0.77	2.98	16.0	19.8	-

TABLE V

CARBOHYDRATE ANALYSIS OF RUSSET RURAL POTATOES AS A FUNCTION OF STORAGE TEMPERATURE AND TIME

Time in Weeks	Temperature	Control				Irradiated (15 Krep)			
		% Red'g Sugar	% Sucrose	% Starch	Total	% Red'g Sugar	% Sucrose	% Starch	Total
0	All temps.	0.43	0.19			0.43	0.30		
8	35	1.71	0.47	13.1	15.3	1.25	2.71	13.4	17.4
8	45	0.50	0.28	20.7	21.5	0.40	0.81	21.4	22.6
8	55	0.27	0.31	20.4	21.0	0.43	0.45	20.4	21.3
8	65	0.37	0.25	20.9	21.5	0.55	0.20	20.9	21.7
8	room	0.36	0.46	22.5	23.3	0.37	0.49	17.1	18.0

TABLE VI

CARBOHYDRATE ANALYSIS OF SEBAGO POTATOES AS A FUNCTION OF RADIATION DOSAGE AND TIME

Dose (Krep) of Radiation	0 Time		End of First Storage Period				(10 weeks)
	% Red'g Sugar	% Sucrose	% Red'g Sugar	% Sucrose	% Starch	Total	% Dry Weight
0-Control	0.95	0.33	0.90	0.43	17.1	18.4	21.7
5	0.75	0.21	1.00	0.78	15.5	17.3	-
10	0.92	0.36	1.06	1.51	13.32	15.9	18.4
15	0.89	0.11	1.11	1.19	15.6	17.9	-
20	0.72	0.17	1.00	1.37	12.87	15.2	-
25	0.80	0.09	0.91	2.08	14.94	17.9	18.7
50	0.64	0.24	0.75	2.14	14.94	17.8	18.1
100	0.59	0.15	1.10	2.47	14.94	18.5	19.4
200	0.80	0.09	0.97	3.8	12.69	17.5	17.5

TABLE VII

CARBOHYDRATE ANALYSIS OF SEBAGO POTATOES AS A FUNCTION OF STORAGE TEMPERATURE AND TIME

Time in Weeks	Temperature	Control				Irradiated (15 Krep)			
		% Red'g Sugar	% Sucrose	% Starch	Total	% Red'g Sugar	% Sucrose	% Starch	Total
0	All temps.	0.95	0.33			0.89	0.11		
10	35	1.61	0.75	18.1	20.5	2.3	2.19	13.4	17.9
10	45	0.90	0.43	17.1	18.4	1.11	1.19	15.6	17.9
10	55	0.47	0.41	19.3	20.2	0.61	0.24	13.7	14.6
10	65	0.28	0.68	17.7	18.7	0.26	1.92	11.8	14.0
10	room	0.48	0.26	13.4	14.1	0.51	0.53	12.3	13.3

0.19 ± .08%. The corresponding values for Russet Rurals at zero time are .47 ± .06% for reducing sugar and .24 ± .04% for sucrose, again with no pattern among the values with respect to dose of radiation.

At the end of the first storage period (10 weeks for Sebagos and 8 weeks for Russet Rurals), there is still no apparent effect of dose level of radiation on the reducing-sugar content. The values for reducing sugar in Sebagos for all levels of radiation have an average value of 0.98 ± .08%, only very slightly higher than the zero-time values. In Russet Rurals, the average value is .60 ± .12%, not significantly higher than the zero-time value.

Sucrose levels in both Russets and Sebagos following the first storage interval, however, do show a marked effect of dosage level of radiation. An increase in dose from 0 to 200 kilorep increases the sucrose level in Sebagos from 0.43 to 3.8% and in Russets from 0.28 to 2.98%. These values are shown graphically in Figs. 1 and 2 which also show the values for reducing sugar and starch for the first storage period. In the case of the Sebagos, the curve showing the change in sucrose levels progresses from low to high values with increasing radiation dosage. In the case of the Russets, there appears to be a more marked change in the slope of the curve producing a "hump" at about 10 kilorep. Except for this change in slope, there is a gradual increase in sucrose content with increase in radiation dosage comparable to that observed for the Sebagos. The change in slope may correspond to a related "dip" in the curve for starch or may simply be experimental deviation.

Values for starch content do not show the constancy or regularity of change with dose of radiation as do the other two carbohydrates. One explanation may be that as starch is a storage compound, only part of the total amount is responsive to metabolic processes. However, in the case of both the Russet and Sebago varieties, it appears that the starch content decreases abruptly with increasing dosage of radiation up to about 10-20 kilorep, then increases gradually to a second maximum after which it falls gradually with increasing dosage up to 200 kilorep. The decreases may bear some relation to the amount of sucrose formed, indicating that irradiation stimulates a conversion of starch to sucrose (or possibly inhibits the conversion of sucrose to starch), this effect being more apparent with the Russets than with the Sebagos.

The effect of temperature of storage for both irradiated (15 kilorep) and nonirradiated potatoes of both varieties for the first storage period is shown in Tables 5 and 7 , and is shown graphically in Figs. 3 and 4. For both the Russets and the Sebagos the lowest temperature

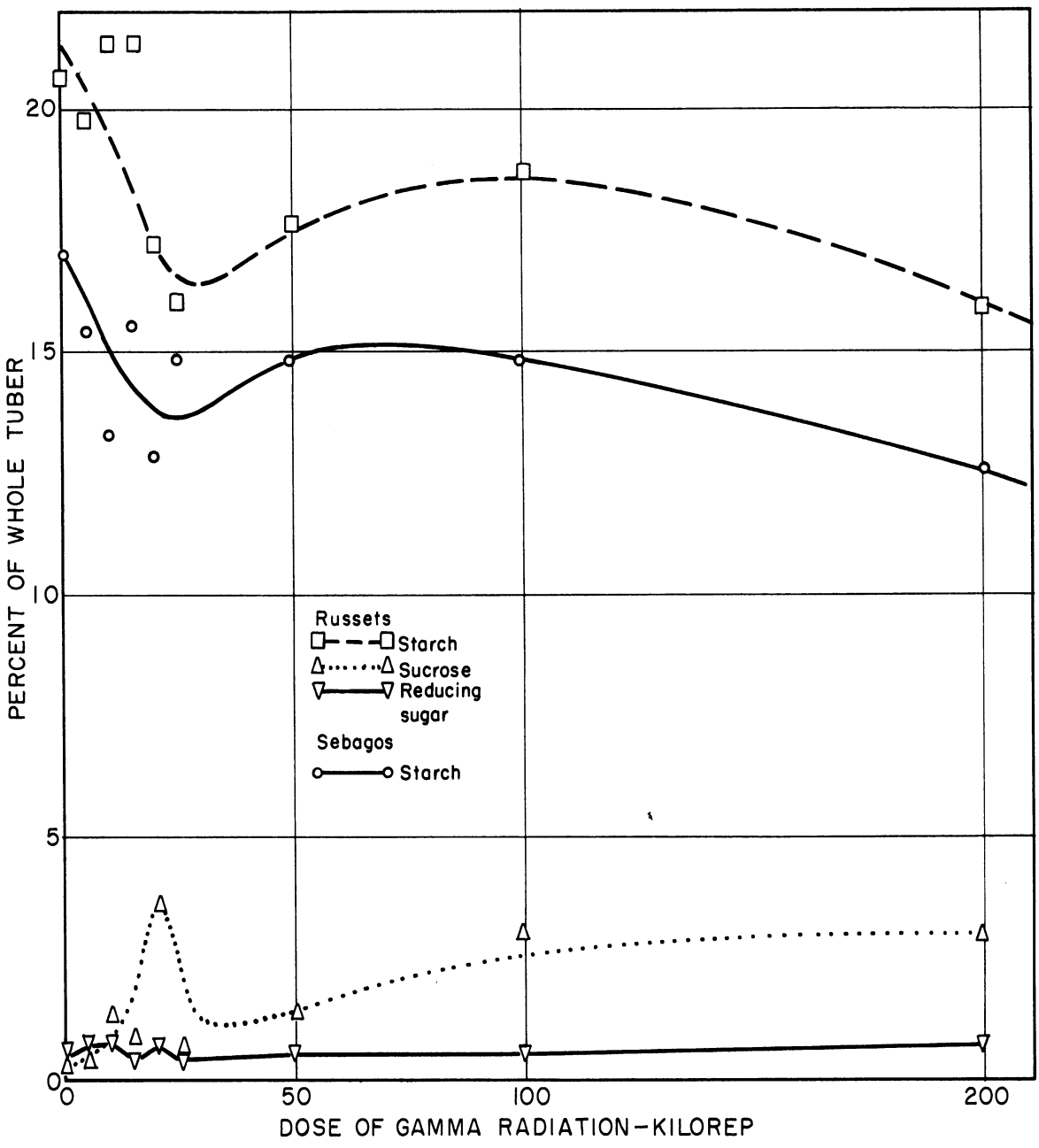

Fig. 1. Effect of radiation dosage on the reducing-sugar, sucrose, and starch content of Russet Rurals stored 8 weeks.

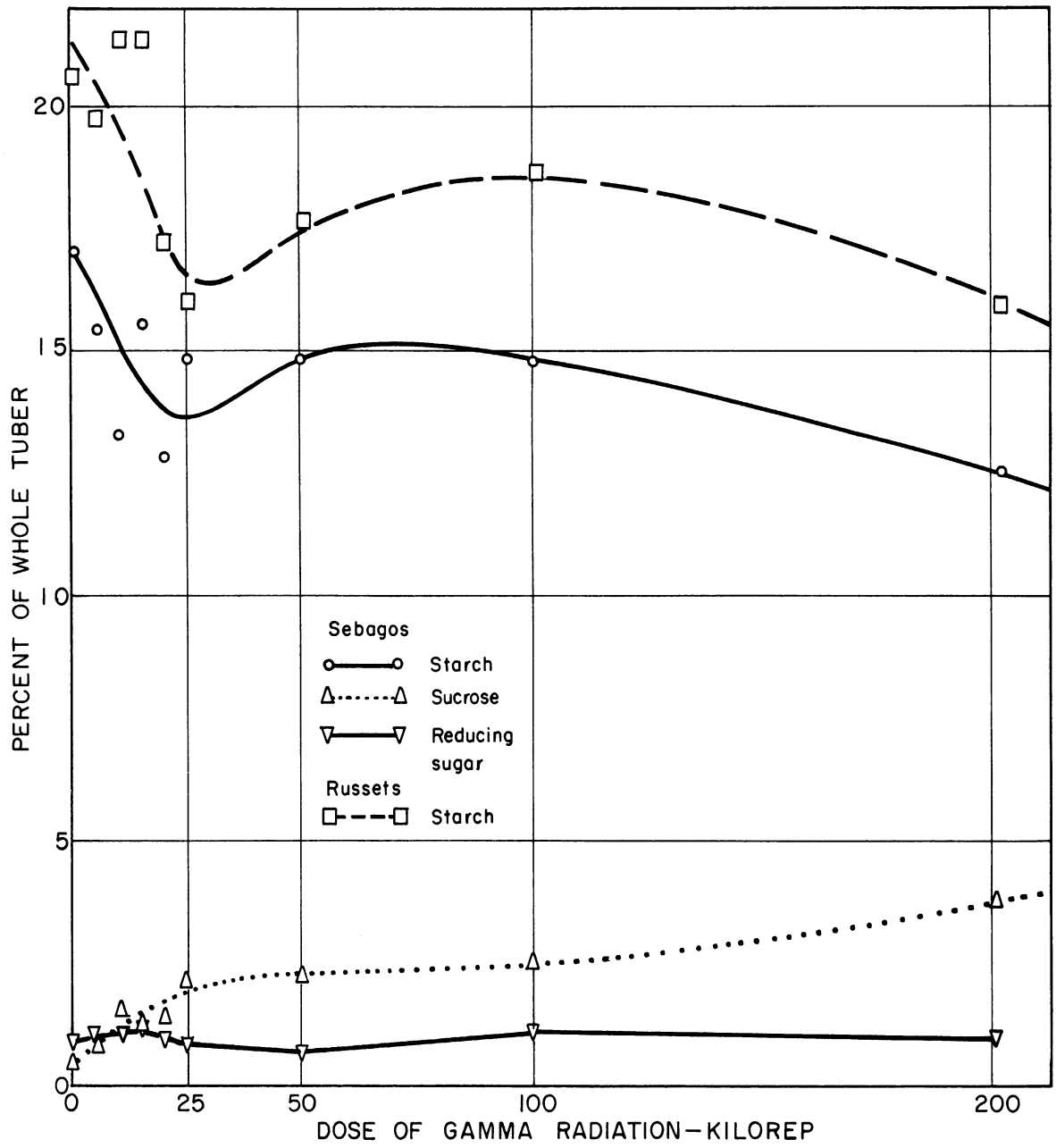

Fig. 2. Effect of radiation dosage on the reducing-sugar, sucrose, and starch content of Sebago potatoes stored 10 weeks.

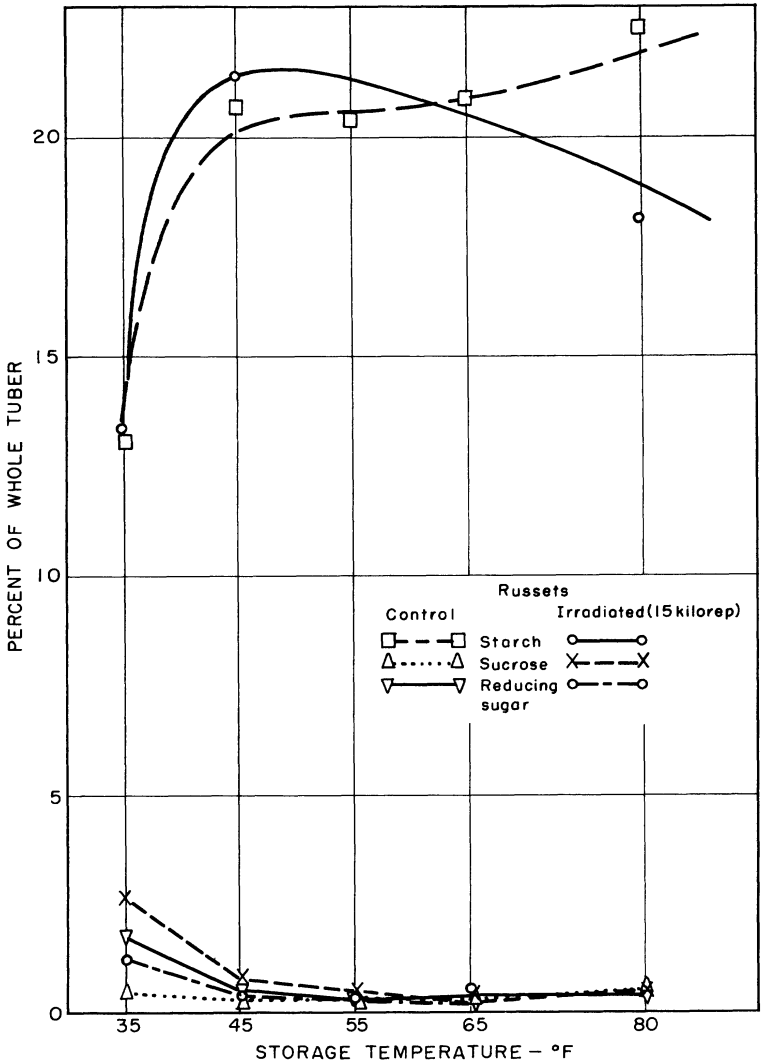

Fig. 3. Effect of storage temperature on the reducing-sugar, sucrose, and starch content of Russet Rural potatoes stored 8 weeks.

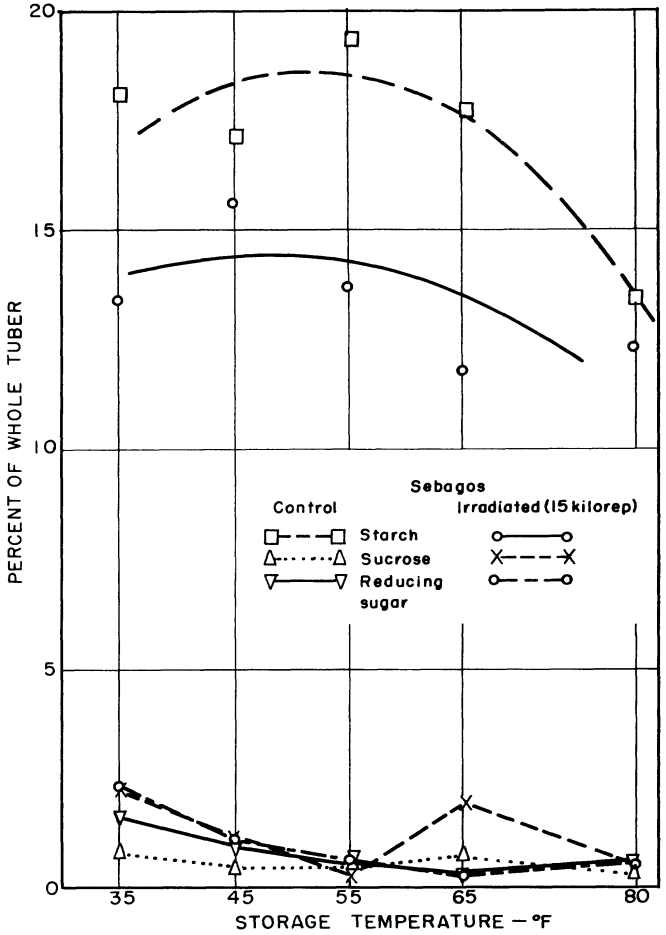

Fig. 4. Effect of storage temperature on the reducing-sugar, sucrose, and starch content of Sebago potatoes stored 10 weeks.

level results in elevated sucrose and reducing-sugar contents, but all other storage temperatures result in low levels.

The values for starch show a somewhat irregular pattern with respect to storage temperature. The curves drawn through these points suggest that there is probably little effect of storage temperature until room temperature is reached, which results in a lowering of the starch content.

OBSERVATIONS ON POTATOES IN STORAGE AT VARIOUS TEMPERATURES (at The University of Michigan Food Service Building)

A. SPROUT FORMATION

All nonirradiated Russet Rurals held at 45°F uniformly show development of sprouts up to 5 mm in length as of February 3, 1956. About half of the Russets given a 5 kilorep dose of gamma radiation show development of sprouts up to 2 mm in length, the other half show no signs of sprouting. Russets given radiation doses of 10 and 15 kilorep and stored at 45°F show very few and very tiny sprouts.

At 20 kilorep, very tiny sprouts having a fresh pale green color are apparent. Where there is evidence of larger sprouts having formed, their color is a brownish green, and still larger sprouts have "burned" tips and are dead. This suggests that sprouts that started to develop prior to irradiation were arrested by the irradiation, but that sprouts could still form from eyes that were completely dormant at the time of irradiation.

No sprout formation whatsoever has occurred as of February 3 on any Russet Rural potato given a radiation dose of 25 kilorep or higher.

Russet Rurals given a 15 kilorep dose and stored for 8 weeks at temperatures varying from 35° to 80°F show a slight increase in sprout formation with increasing temperature. No sprouting occurred at 35°F. The slight extent of sprouting of potatoes stored at 45°F has already been mentioned. With storage at 55°F, tiny sprouts having a length up to 1 mm appear to be alive on most potatoes, but sprouts of greater length appear not to be living. With storage at 65°F, tiny sprouts of 1-2 mm occur uniformly over most of the potatoes; the sprouts are small,

whitish "buds." With storage at 80°F, the extent and appearance of the sprouts were the same as observed with storage at 65°F.

In the case of the Sebagos, most but not all, the nonirradiated potatoes held at 45°F have sprouted as of February 3, 1956. However, none of the irradiated potatoes held at 45°F have sprouted, not even those given only a 5 kilorep dose of gamma radiation.

Those potatoes given a 15 kilorep radiation dose and stored at temperatures from 35° to 55°F showed no sprout development after 10 weeks. Those stored at 65°F, however, do show very tiny sprouts on most of the potatoes. Those stored at 80°F show only a few tiny sprouts. This indicates that a 15-kilorep dose does not destroy the ability of the tubers to begin to sprout. The eyes of the tubers may germinate after the radiation treatment, but sprout development may continue in irradiated tubers.

At 8 or 10 weeks after irradiation there appears to be a marked difference in resistance of the two varieties of potatoes to the sprout-inhibiting effects of irradiation, with the Russet Rurals showing greater resistance. Varietal differences in resistance to the sprout-inhibiting effects of gamma irradiation were also observed with onions.[2]

During the present series of tests with Sebago and Russet Rural varieties of potatoes, post-irradiation sprouting of potatoes has been observed for the first time. It should be stressed, however, that the sprouts are tiny and probably will not continue to grow if the behavior parallels that previously observed with onions[2] and walnut seeds.[3] In the case of onions and walnuts, initial sprouting was actually stimulated by radiation doses up to 50,000 rep but the initial sprouts on the irradiated samples grew only a fraction of an inch, then withered and died, whereas the nonirradiated samples sprouted later but developed normally. This initial limited sprouting of irradiated potatoes was not observed with the Idaho Russet seed potatoes irradiated in May, 1955, nor with the Idaho potatoes irradiated in May, 1953, probably because in each of these cases the tubers were near the end of the normal storage period and already possessed minute sprouts.

B. TEXTURE AND APPEARANCE

1. Sebagos.—Most of the Sebago potatoes given radiation doses of 0, 5, and 10 kilorep and held at 45°F for 10 weeks appear fairly firm and show no signs of decay, softening, or rot. Potatoes given 15 kilorep begin to exhibit a mottled appearance of their skin. The skin is slightly

soft and pocked with very slight indentations over the surface. The surface feels slightly bumpy. This mottling is apparent at all doses above 15 kilorep. The skins are soft, but the softness does not increase with higher dosages of radiation. The softness seems confined to the outside 1/4-1/2 in. layer, but the body of the potato feels firm. Molding and rotting has occurred only with potatoes given the highest dose of irradiation.

The Sebagos given 15 kilorep doses and stored at 35°F are firm and of good appearance, as are also those stored at 55°F. The latter, however, show some tendency to green, even though they are not exposed to light. All the potatoes stored at 65° and 80°F, however, show varying degrees of softness, from superficial to thorough. This softness does not appear due to dehydration. There is no mold or rot among these potatoes.

2. Russets.—The Russets given irradiation doses from 0 to 200 kilorep and held at 45°F for two months show some tendency to soften near the surface, but all seem to have firm interiors. At the higher doses (50 to 200 kilorep) the skins take on a wrinkled appearance and a leathery feel. Mold has occurred only on a few potatoes of those held at the two highest doses (100 and 200 kilorep).

Russets stored at 35° and 45°F are fairly firm. Only a few slightly soft ones were found with storage at 45°F, and most of those stored at 35°F were quite hard. At 55°F storage, about a fourth of the potatoes have soft, leathery skins, and most of those stored at 65° and 80°F also have a similar appearance, but extensive softness is not as apparent as it is with the Sebagos stored at the higher temperatures. The nonirradiated Russets stored at these temperatures are practically indistinguishable from those that received 15 kilorep doses of radiation.

C. INTERIOR APPEARANCE

Each treatment of each variety of potato is held in two adjacent compartments, from one of which potatoes are periodically withdrawn for carbohydrate analyses. Those in the other compartment are left intact and undisturbed except when being weighed and used for observations on sprout development, appearance, and texture. These are the potatoes which, at the end of the storage period (about June), will be cut open to make measurement of the extent of decay, black heart, blackening, and other manifestations of enzyme and/or microbial action. However, on potatoes used for

carbohydrate analysis, there has been little or no evidence of deterioration of the interior.

D. WEIGHT CHANGES

Tables VIII, IX, X, and XI show the weight in kilograms of the thirty-four samples of potatoes in storage at the Food Service Building and in the Fission Products Laboratory. Two varieties, nine radiation dosages, and five storage temperatures are represented. The weight data were taken on December 7 and February 2 for all the samples and, in addition, on January 16, for the potatoes stored at 45°F.

Both Sebago and Russet variety tubers given radiation doses from 0 to 200 kilorep and stored at one temperature (45°F) show a general tendency to lose more weight when treated with the higher radiation doses. It was also observed that the Sebago variety shows higher weight losses than does the Russet variety for every treatment used.

The effect of temperature of storage is very marked. Both Sebago and Russet varieties and both the irradiated and nonirradiated potatoes show progressive decrease in weight with increasing temperature of storage. All irradiated Sebago tubers show slightly greater weight losses than do their corresponding controls, and this was also true of the Russets except at the highest storage temperature.

The results differ from those observed in the earlier study using Idaho seed potatoes irradiated in May, 1955. However, the Idaho-grown potatoes were at the end of their normal storage period when irradiated, whereas the Michigan-grown Sebago and Russet-variety potatoes were irradiated shortly after harvest. Although some of the results observed may be caused by varietal differences or differences in growing area, it is believed that most of these differences are the result of different storage periods prior to irradiation. These results will be further discussed in subsequent reports after additional data have been obtained.

TABLE VIII

WEIGHT OF RUSSET RURAL POTATOES GIVEN NINE DIFFERENT RADIATION DOSAGES AND STORED AT 45°F

Dose (rep)	Weight (kilograms)			Percent Weight Loss	
	Dec. 7	Jan. 16	Feb. 2	Dec. 7 to Jan. 16	Dec. 7 to Feb. 2
0	9.04	8.79	8.68	1.6	3.1
5,000	9.02	8.79	8.25	2.5	3.1
10,000	8.93	8.68	8.56	2.9	5.1
15,000	8.99	8.62	8.71	4.1	5.7
20,000	8.96	8.62	8.48	3.8	6.3
25,000	9.02	8.68	8.56	3.8	5.0
50,000	8.90	8.56	8.48	3.8	6.1
100,000	8.96	8.62	8.71	3.8	4.4
200,000	8.90	8.62	8.45	3.2	7.0

TABLE IX

WEIGHT OF RUSSET RURAL POTATOES GIVEN 15 KILOREP AND STORED AT DIFFERENT TEMPERATURES FOR 8 WEEKS

Storage Temp. °F	Weight (kilograms) Dec. 7		Weight (kilograms) Feb. 2		Percent Weight Loss Dec. 7 to Feb. 2	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
35	9.13	9.13	8.76	8.34	4.0	3.7
45	9.04	8.99	8.68	8.71	3.1	5.7
55	9.07	6.75	8.42	6.23	6.6	7.6
65	9.07	9.07	8.45	8.19	6.8	9.7
Room Temp.	9.13	9.13	7.94	8.19	13.0	10.3

TABLE X

WEIGHT OF SEBAGO POTATOES GIVEN NINE DIFFERENT RADIATION DOSAGES AND STORED AT 45°F

Dose (rep)	Weight (kilograms)			Percent Weight Loss	
	Dec. 7	Jan. 16	Feb. 2	Dec. 7 to Jan. 16	Dec. 7 to Feb. 2
0	8.96	8.56	8.42	4.4	6.0
5,000	8.99	8.71	8.36	5.4	7.6
10,000	9.02	8.56	8.42	5.0	6.6
15,000	9.02	8.45	8.19	6.3	9.1
20,000	9.04	8.71	8.31	6.0	8.2
25,000	9.02	8.42	8.22	6.6	8.8
50,000	9.02	8.39	8.22	6.9	8.8
100,000	8.90	8.28	8.05	7.0	9.6
200,000	9.07	8.45	8.16	6.9	10.0

TABLE XI

WEIGHT OF SEBAGO POTATOES GIVEN 15 KILOREP AND STORED AT DIFFERENT TEMPERATURES FOR 8 WEEKS

Storage Temp. °F	Weight (kilograms) Dec. 7		Weight (kilograms) Feb. 2		Percent Weight Loss Dec. 7 to Feb. 2	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
35	9.07	9.07	8.42	8.34	7.2	8.1
45	8.96	9.02	8.42	8.19	6.0	9.1
55	9.07	9.07	8.25	8.16	9.1	10.1
65	9.07	9.13	8.39	7.85	12.5	14.0
Room Temp.	9.13	9.10	7.64	7.44	16.3	18.2

STORAGE STUDIES AT DIFFERENT HUMIDITIES
(at Michigan State University)

The second series of irradiated samples of Russet Rural and Sebago potatoes have been in storage for about two months at controlled relative humidities of 95, 75, and 55%. Considerable difficulty has been experienced with the 55% relative-humidity chamber, so that the early data from this group cannot be considered very reliable. This difficulty has since been corrected. The temperature of these storage chambers varies between 40° and 45°F, and has been automatically recorded since the inception of this trial. The experience of a previous similar experiment indicates that the initial weight losses are not the most representative of the data, but summary data of weight losses incurred thus far are given in Tables XII and XIII. Considerable care was taken in selecting individual tubers for this trial in an attempt to reduce loss through decay, but abnormally high loss figures in one or two cases would seem to indicate some difficulty resulting from this problem. The decay losses assume an increased importance when the sample size is relatively small, as is the case in the current experiment.

TABLE XII

AVERAGE PERCENT WEIGHT LOSS (FOR ALL DOSES) OF POTATO SAMPLES (TWO VARIETIES) STORED FOR 58 DAYS AT 40°-45°F AND THREE RELATIVE HUMIDITIES

Relative Humidity, Percent	Variety	
	Sebago	Russet Rural
55	4.2	1.6
75	2.8	1.5
95	2.7	1.0

From the data in Table XII it is apparent that weight is lost more slowly by the Russet Rural variety than by the Sebago variety. Whether this is caused by the extra thickness of the Russet Rural skin, or by a differential permeability of the skins of the two varieties to water because of some factor other than thickness, or by a difference in metabolic activity between the two varieties is not known.

TABLE XIII

AVERAGE PERCENT WEIGHT LOSS (FOR ALL HUMIDITIES) OF POTATO SAMPLES (TWO VARIETIES) STORED 58 DAYS AT 40°-45°F AND THREE RELATIVE HUMIDITIES

Dosage, Kilorep	Variety	
	Sebago	Russet Rural
0	6.4	1.0
5	1.5	1.6
10	1.7	1.4
15	1.6	1.3
50	4.7	1.2
100	2.6	1.4
200	4.1	1.8

The errors involved in measuring small weight changes such as are reported in this table make it difficult to attach much importance to these data.

WOUND HEALING
(at Michigan State University)

Considerable work has been completed in the area of wound-healing investigations. One complete series of paraffin-imbedded sections has progressed through all operations up to staining. Photomicrographs of this material will be submitted in the next report. Preliminary observations indicate that 200 kilorep irradiation severely retards or prevents periderm formation, but that the reduction following a 15-kilorep treatment is much less pronounced. These observations are in agreement with the recently-published work of Waggoner[4] who reports reduction in periderm formation following 20 and 80 kilorep radiation doses.

Work is continuing on both phases of this project.

RESPIRATION STUDIES

A. INTRODUCTION

One of the fundamental processes characteristic of all living organisms, plant or animal, is respiration. This is the chemical process whereby living organisms obtain energy from organic compounds. Physiologists usually write an equation for the overall reaction as follows:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \text{energy}$$

glucose + oxygen = carbon dioxide + water + energy.

The energy derived is used in growth and the multitude of activities going on in a living organism. The carbon dioxide is given off as a gas which passes into the atmosphere and becomes available for the photosynthetic activity of green plants.

Two quite different experiments have been set up to determine the rate of respiration quantitatively. In one, the rate of carbon dioxide production by whole tubers is determined. In the other the amount of oxygen consumed by thin slices of potato is determined.

In the respiration of stored potatoes there is a consumption of food material by the tuber. Some of the starch and sugar in the potato is used to maintain the life processes of the potato. As this respiration proceeds, the food stored in the potato becomes depleted. It would therefore be an advantage if ionizing radiation could be used to depress the rate of respiration and reduce the loss of food material. From a theoretical point of view it would be of interest to know what ionizing radiation does to the potato to inhibit sprouting. It is hoped that some clues to the answer to this question may be found through respiration studies. Such studies should provide some information on the nature of the effect of irradiation. If there is an increase in respiration as a result of irradiation, it might be assumed that some fundamental change in the metabolism of the tubers has been caused by irradiation.

The equation for the process of respiration shown previously indicates that in aerobic respiration the oxygen and carbon dioxide are respectively consumed and produced in equal amounts. Both are gases and

can readily be determined quantitatively. Thus respiration rate may be studied either by determining the rate of uptake of oxygen or the rate of production of carbon dioxide.

B. RESPIRATION OF WHOLE TUBERS

1. Methods.—In the studies on the respiration of whole potatoes the output of carbon dioxide was used as the indicator of respiration rate. A stream of carbon dioxide free air was drawn through an enclosed chamber containing the tubers and thence drawn through long tubes containing a known volume of barium hydroxide solution (standardized 0.1N). The solution was titrated with 0.1N oxalic acid to determine the amount of $Ba(OH)_2$ remaining after carbon dioxide absorption. The amount of $Ba(OH)_2$ lost is equivalent to the amount of CO_2 absorbed.

As shown in Fig. 5, air was drawn by an aspirator through granular soda lime in three 12-in. towers and then was bubbled through a solution of barium hydroxide as an added guarantee of absence of carbon dioxide in the air stream.

Experiments were first run in the last part of November, and in early December, 1955, with the setup shown in Fig. 5. However, this setup was found to be quite unsatisfactory as a result of wide variations in room temperature in the laboratory. As a result of the high temperatures, the potatoes became covered with molds in a few days. The variations in room temperatures also caused wide variations in respiration rates so that no valid comparisons could be made.

Toward the end of December, a refrigerator was installed (see Fig. 6) and adapted so that the potatoes could be kept inside at a temperature comparable to the one at which they had been stored (45°F). It was hoped that this procedure would give the approximate rate of respiration that was occurring in the storage rooms held at 45°F.

The barium hydroxide solution was placed inside the refrigerator beside the jars in which the potatoes were kept. Then the CO_2 free air was passed from the $Ba(OH)_2$ solution into the jars containing the potatoes through a tube which led to the bottom of each jar. The gas was removed from the top of the jar as shown in Fig. 6. This arrangement of tubes was used in an effort to insure complete gas displacement in the jars.

The gas from the jars was then drawn from the refrigerator by

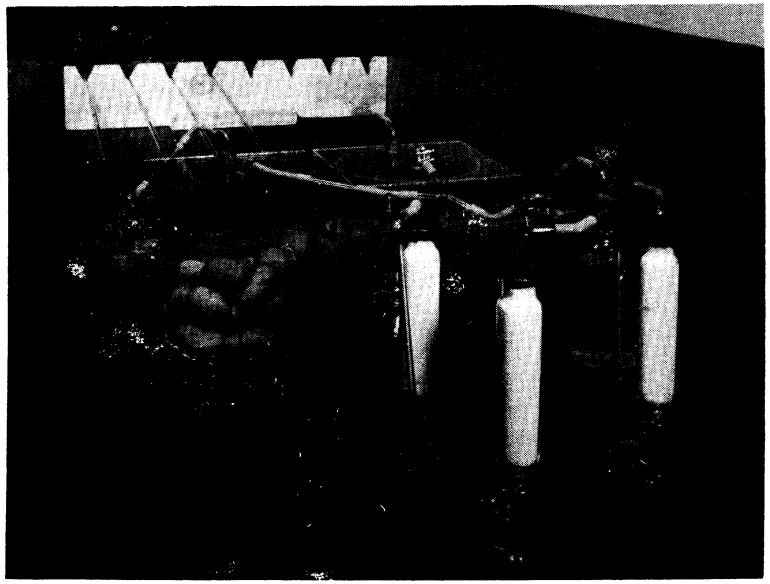

Fig. 5. View of original gas train for whole tuber respiration studies showing carbon dioxide absorption towers for producing carbon dioxide free air.

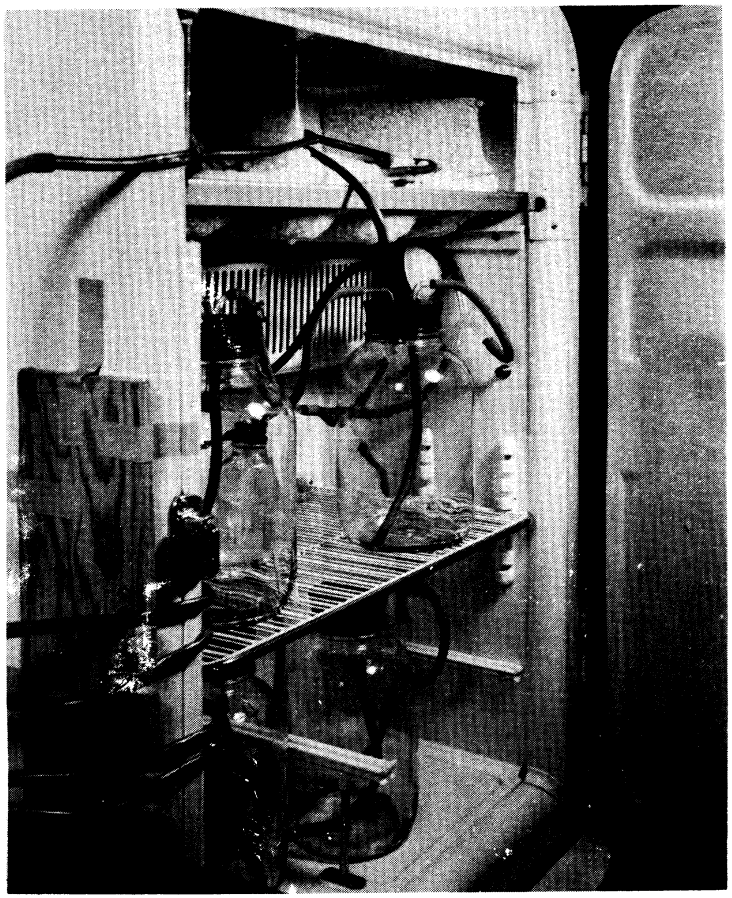

Fig. 6. View of refrigerator and containers for potatoes used in the current studies of whole tuber respiration at 45°F.

copper tubing and rubber tubing which was connected to the absorption tubes as shown in Fig. 7. The gas enters the absorption tubes via a short piece of glass tubing which has been drawn to a fine tip. This small tip aids in producing small bubbles which expose the gas more effectively than large bubbles to the barium hydroxide. A schematic diagram of the gas train is shown in Fig. 8.

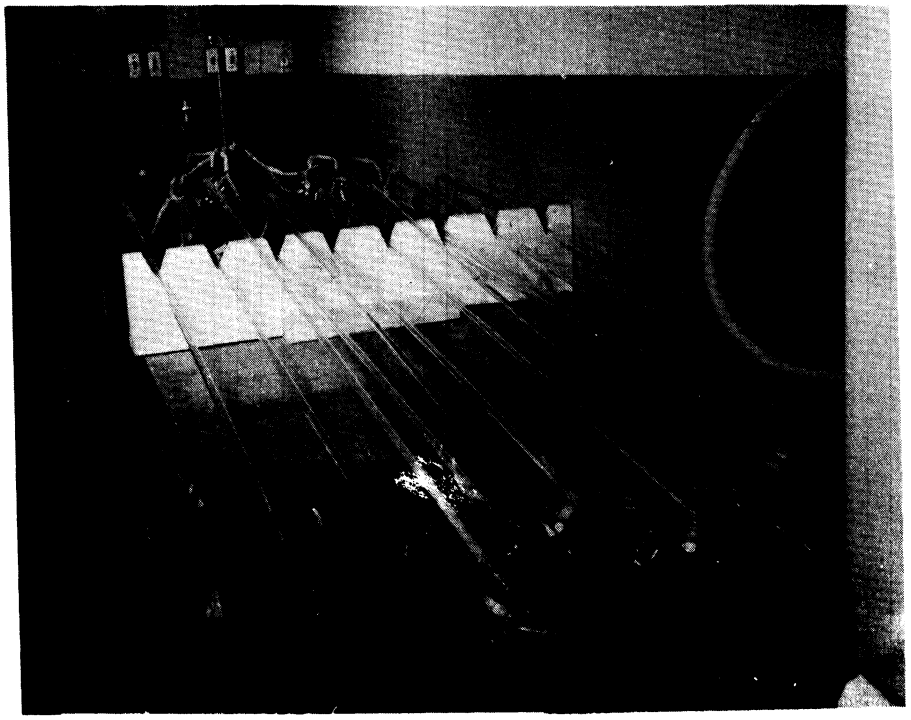

Fig. 7. View of the absorption tubes, indicator tubes, and pressure-regulating device in whole-tuber respiration studies.

The gas flow is regulated so that the rate of flow is rapid without the bubbles becoming too large and merging with one another as they pass through the tubes. The tubes are about 6 ft long and can hold about 100 cc of liquid. In this experiment, however, only 80 ml of the $Ba(OH)_2$ is added to each tube. The occurrence of foaming necessitates leaving a free space at the top of the tube. The gas emerging from the tube is passed through test tubes containing phenolphthalein in a slightly alkaline solution to test for unabsorbed CO_2. The gas is drawn from the absorption tubes through a jar of mercury which serves as a vacuum regulator and then to the aspirator.

After the collecting system has run for the desired length of time, the barium hydroxide solution is carefully removed from the tubes.

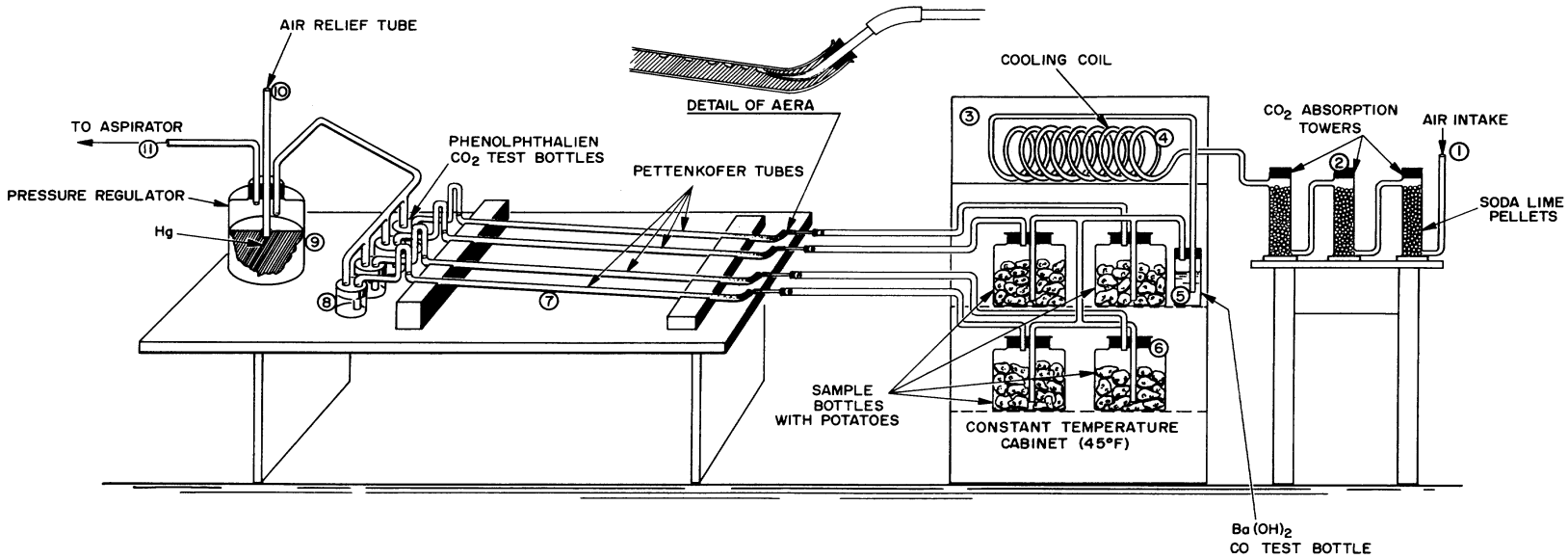

Fig. 8. Schematic view of gas train currently being used in whole-tuber respiration studies.

(It can be noted that the carbon dioxide absorbed reacts with barium hydroxide to yield barium carbonate, a white precipitate which is insoluble in this solution.) A 20-ml sample of this solution is placed in an Erlenmeyer flask, and a drop of 0.1% phenolphthalein solution is added, which turns the solution red. Then 0.1 N oxalic acid is added until the solution remains colorless for about 30 seconds.

Oxalic acid is used because it is a weak acid and will not react with $BaCO_3$ to release CO_2. Hence, the procedure used is a titration of the $Ba(OH)_2$ that was not consumed by the CO_2 to produce $BaCO_3$. The oxalic acid also forms a white precipitate with the barium hydroxide in this titration, which gives a good background for judging the color change of the phenolphthalein.

Routine followed: on the morning of the day an experiment was to be started the samples of potatoes were brought from the Food Service and irradiated immediately. They were then weighed and the weight recorded at the same temperature as the room in which they had been stored (45°F). Usually seven or eight potatoes were chosen which together weighed just over a kilo. At this time the carbon dioxide free air was allowed to flow through the voids between the potatoes. The system was equilibrated by drawing the air through tubes containing distilled water for the first two hours after the potatoes were placed in the refrigerator. After two hours the gas stream was diverted to the absorption tubes containing the $Ba(OH)_2$ and allowed to run for 2-1/2 to 3 hours. Then the $Ba(OH)_2$ was collected and titrated.

Determinations were made on the same potatoes during the first two days, again after two weeks, and will be tested again at a later date.

2. Results and Discussion.—Sebago potatoes exposed to doses of 5, 15, 25, 50, 100, and 200 kilorep were used in the first experiments. The results are given in Tables XIV and XV.

When experiments were run the day the potatoes were irradiated, there seemed to be an actual decrease in the amount of CO_2 produced by the irradiated potatoes compared with the controls, at least at higher irradiation dosages. During the first day after irradiation the irradiated potatoes showed a decrease of CO_2 production to as low as 84% of that of the controls. During the second day, CO_2 production increased in the irradiated potatoes until it was about 150% of the controls. This percentage was, however, less at lower irradiation levels, although there seemed to be little consistent relationship between the amount of irradiation and the amount of CO_2 produced. In other words, if a potato was irradiated it produced half

TABLE XIV

RATE OF PRODUCTION OF CARBON DIOXIDE BY IRRADIATED AND NONIRRADIATED POTATOES AT 45°F

Irradiation Dose, rep	Milliliters CO_2 Produced/Hour/Kilo Potatoes at 45°F			
	Same Day	Second Day	Second Week	Fourth Week
Control	(0.98)* and 1.69	1.89	2.71	2.78 - 2.98
5,000	(3.19)* and 3.19	1.48	3.97	2.71 - 2.91
15,000	(1.35)* and 1.94	3.33	3.79	3.03 - 3.39
25,000	(0.79)* and 1.99	2.16	3.76	3.43 - 3.78
Control	2.75	4.05 - 3.38	2.90 - 3.06	
50,000	2.67	5.22 - 4.55	4.03 - 3.77	
100,000	2.41	6.34 - 5.62	3.66 - 5.14	
200,000	2.30	6.22 - 4.74	4.27 - 4.32	

*Not used in calculating percentages.

TABLE XV

CHANGE IN RATE OF CARBON DIOXIDE PRODUCTION FROM IRRADIATED POTATOES AS PERCENT OF THAT FROM NONIRRADIATED POTATOES

Irradiation Dose, rep	CO_2 Production as Percent of Control			
	Same Day, %	After One Day, %	After Two Weeks, %	After Four Weeks, %
5,000	(189)*	(88)*	146	97, 98 Avg = 98
15,000	115	(176)*	140	109, 114 Avg = 112
25,000	118	114	139	123, 127 Avg = 125
50,000	97	135, 129 Avg = 132	139, 123 Avg = 131	
100,000	88	157, 166 Avg = 162	126, 168 Avg = 147	
200,000	84	154, 140 Avg = 147	144, 141 Avg = 142	

*Data not very reliable

again as much CO_2 as the nonirradiated potatoes regardless of the amount of irradiation it received.

After two weeks when the sample potatoes were again tested for CO_2 production all irradiated potatoes were found to produce between 1.3 and 1.5 times as much CO_2 as the control potatoes.

Some difficulty in interpretation of the results is caused by the fact that the nonirradiated potatoes were starting to sprout after two weeks. This increased their rate of CO_2 output, as was observed.

It is proposed that some experiments be performed in which CO_2 is collected continuously from the time of irradiation through the first 48 hours after irradiation. This would provide valuable information concerning the apparent early reduction in CO_2 output and the subsequent acceleration of CO_2 production. The results obtained in the experiments described should not be considered conclusive or complete. It is also planned to determine the amount of CO_2 produced about one week after irradiation.

C. DETERMINATION OF OXYGEN CONSUMED IN THE RESPIRATION OF TISSUE SLICES BY THE WARBURG RESPIROMETER

1. Methods.—In this method the disks are placed in a small flask, which is attached to a manometer as shown in Fig. 9. A small volume of potassium hydroxide is held in the side arm of the flask. This alkaline solution absorbs the carbon dioxide produced by the slices. By manipulating the manometer the decrease in the volume of the enclosed gas is determined. This decrease is caused by the consumption of oxygen. The flask and the manometer have been previously calibrated so that with the aid of a few constants the amount of oxygen used per gram per hour can quickly be determined.

A number of manometers are available so that the effect of several dosages can be studied at one time. The manometers are held in a rack of the respirometer (Fig. 10) in such a way that the flasks are in a water bath at a constant temperature, while the manometer is outside where it can easily be read. The rack is attached to a shaking device so that the flasks are continuously agitated, and the CO_2 absorbed as it is formed and fresh oxygen supplied to the potato slices. The manometric method of measuring respiration is the most accurate of those now in common use.[5]

During December, 1955, seven lots of carefully selected Sebago

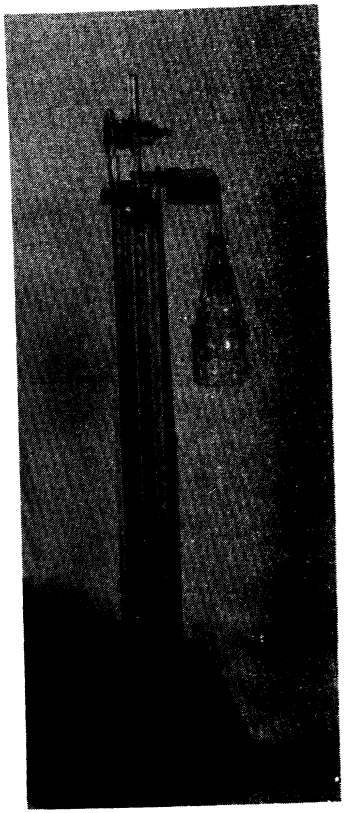

Fig. 9. Warburg manometer and attached flask for studying oxygen consumption of potato slices.

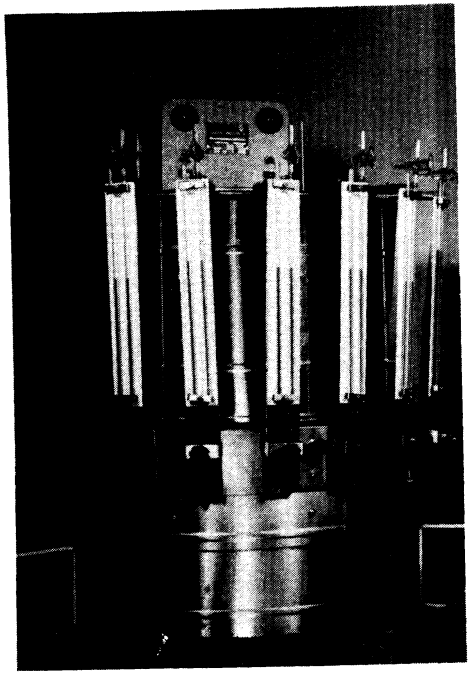

Fig. 10. Individual manometers mounted on the Warburg respirometer. The circular tank is a constant-temperature water bath. The manometers are made to move outward and inward in short strokes as a means of agitating the medium contained in the manometer flasks.

tubers were prepared and kept stored at 45°F. The tubers in these lots were irradiated at dosages of 5, 15, 25, 50, 100, and 200 kilorep and put back into storage. The irradiations were made at such intervals that respirations could be made 3 hours, 27 hours, 1, 3, 5, and 8 weeks, and perhaps, 5 months later. If time is available, experiments will be performed also on tubers stored at temperatures other than 45°F.

When an experiment is to be performed, 3 tubers from the desired lots are taken from the Food Service Building to the laboratory where sections 0.8 mm thick and 12 mm in diameter are cut by a microtome. Determinations are made in duplicate. Each respiration flask contains 21 disks. The disks are made from cylinders centered around an eye. Seven disks are cut from each cylinder beginning with the peel. Thus, each flask contains three eyes, two of which were obtained from one tuber and the third from another. The sections are washed and used within an hour after being cut. The 21 slices are placed in the flask in a 0.01 M phosphate buffer solution of pH 6.0. It is very necessary that the gas volume of the flask be known and for that reason the volume of the potato-buffer solution is always kept at 10 ml and the volume of the solution (20% KOH) in the side used to absorb the CO_2 is kept at 0.2 ml. As soon as a flask is loaded, it is connected to the appropriate manometer and set in the rack with the flask in the bath which is maintained at 28°C. When all the flasks have been loaded the shaker is set in motion. The equilibration time is 45 minutes. At the end of that time all stopcocks are closed and the manometer readings adjusted. Three readings are taken, 20 minutes apart. Thus, the oxygen consumption is determined for a one-hour period. At the end of this period the disks are removed from the flasks and dried in an oven at 95°C.

2. Results and Discussion.—The results obtained so far are recorded in Table XVI. The results as obtained with whole tubers, where the CO_2 produced was measured, and with slices, where oxygen consumption was determined, are essentially the same. Irradiation with all dosages used produced an increase in respiration activity after a day. This increase is, however, not very uniform. Irradiation with gamma rays then causes an increase in metabolic activity rather than a decrease, as might have been expected from the fact that sprouting is inhibited.

ENZYME STUDIES

That the exposure of potatoes to ionizing radiation causes alteration in the level of carbohydrates in the potato has been observed by

TABLE XVI

RESPIRATION OF IRRADIATED SEBAGO POTATOES, AS DETERMINED BY THE WARBURG RESPIROMETER. THE FIGURES REPRESENT THE AVERAGE OF THE TWO READINGS FOR EACH PERIOD OF RESPIRATION, AND THE FIGURES IN PARENTHESES ARE THE PERCENT DECREASE OR INCREASE OF THE IRRADIATED MATERIAL OVER THAT OF THE CONTROL

Date	Time After Irradiation	Control (0 reps)	Dosages in kilorep					
			5	15	25	50	10	200
12/20	3 hours	.661			.799 (+20.9%)	.688 (+ 4.1%)		
12/21	27 hours	.752			.993 (+32.0%)	1.204 (+60.1%)		
12/22	3 hours	.798					.895 (+12.2%)	.951 (+19.2%)
12/23	27 hours	.968					1.124 (+16.1%)	1.029 (+ 6.3%)
12/27	3 hours	.896	.856 --	1.097 (+22.4%)				
12/28	27 hours	.730	1.10 (+50.7%)	1.08 (+48. %)				
1/5	1 week	.60	.56 (- 6.6%)	.48 (-20.0%)	.65 (+ 8.3%)			
1/12	1 week	.73				.84 (+15.0%)	.80 (+ 9.6%)	.98 (+34.3%)
1/19	3 weeks	.651	.851 (+30.7%)	.778 (+19.5%)	.858 (-31.8%)			
1/26	3 weeks	.558				.689 (+23.5%)	.821 (+47.2%)	.813 (+45.7%)

several investigators. The present studies show that the level of sucrose increases with increasing doses of irradiation in stored potatoes and that the starch level declines. There is a suggestion in the data that this effect is irregular, i.e., two different dosages can each raise the sucrose and lower the starch contents. In potatoes stored at low temperatures, irradiation has a more marked effect on the sucrose than on reducing sugar, but at higher temperatures of storage both carbohydrates are at low levels.

When such biochemical changes are established, it is possible to proceed with investigations into the links which connect the radiation with increase in sucrose content and the other biochemical changes. Since the formation and disappearance of sucrose in living tissue is almost entirely mediated by enzymes, the activity of the enzymes involved becomes the usual point of departure. Changes in enzyme activity in vivo may be due to (1) changes in enzyme amount, (2) changes in the conditions, such as pH, oxidation-reduction potential, temperature, presence or absence of inhibitors, and other factors under which a particular enzyme operates, or (3) changes in the level of certain substrates, such as carbon dioxide, not brought about enzymatically. Since there are scores of enzymes in plant tissues which in one way or another affect the sucrose content, it is necessary first to "characterize" thoroughly the manner by which radiation brings about the changes in the carbohydrate levels.

Toward this end the first step will be to find means of accelerating the rate of increase of sucrose level with radiation. The effect of higher irradiation doses and several storage temperatures on sucrose content will be tested on whole tubers, thin slices, tissue including and surrounding the eyes, and on skin and layers just beneath skin in contrast to tissue in the interior.

If the effect of irradiation on sucrose content can be intensified and is found in tissue slices, two important advantages are gained besides a saving in time. The use of thin slices (1) automatically furnishes necessary controls consisting of adjacent slices from the same potato, and (2) allows one to approach control of the environment of individual intact cells. The next step will thus be to study the effect of the following on the rate of change of sucrose levels with irradiation dosage: (a) high-carbon dioxide, high oxygen, and 100% nitrogen atmospheres, (b) heat treatments prior or during irradiation, (c) bathing slices in solutions of varying pH and in solutions of various compounds playing a possible role in sucrose synthesis, such as ascorbic acid, glutathione, glucose, fructose, phosphate, etc.

The information derived from these short experiments should

enable one to speculate as to specific enzymes involved in the radiation effect on potatoes. It will then be feasible to study these enzymes independently of the intact cell in order to decide whether there have been changes in enzyme amount, as determined by standard assays, or changes of the conditions under which a given enzyme system operates. If radiation effects on enzymes can be found independently of the intact cell, one can then advance ideas regarding means for controlling undesirable radiation effects on plant and animal tissue.

STUDIES ON POTATO "HORMONES"
(at The University of Michigan)

A. INTRODUCTION

The observation that plants tend to grow toward the source of light is very old. That this phototropic action is related to definite chemical compounds was first clearly indicated in the classical investigations of Boysen-Jensen[6] on the coleoptile of Avena. Naturally occurring chemical compounds possessing the property of being able to regulate plant growth were given the name "hormone." Any compound, either synthetic or naturally occurring that has this property is termed auxin. A large number of scientific articles have appeared relating to auxins.[7]

The effects of x-rays on living matter have been studied since the 1890's. Investigators studied the effects on plant growth in the 1920's; but Skoog,[8] in 1935, was probably the first to study the effects on an auxin. Using a high-voltage tube capable of being operated at 900 kilovolts and 3 to 4 milliamperes, Skoog demonstrated the inactivating effect of x-rays on an auxin (indoleacetic acid) as well as on auxin extracts from plants. He showed this inactivation to be the result of an oxidation, on the basis of experiments in air and in nitrogen atmosphere.

In general, if there is no auxin present, there will be no growth, but a supra-optimal concentration of auxin will also prevent growth. Only minimal quantities are required for plant growth, and the application of an excess will retard or stop growth. By the use of chemicals it is possible to prolong artificially the rest period of potato tubers. On the other hand, the normal resting period can also be shortened by using ethylene chlorohydrin. The belief prevails that the content of auxin not only varies in quantity during storage, but that the concentration also varies in the different parts of the potato. Prior to sprouting of the potatoes

in the spring an increase in auxin has been observed in the fleshy part of the potato, while later the auxin, or its precursor, increases in the potato peel. The change of the precursors into auxin is generally assumed to be enzymatic in origin. One of the chief precursors may be presumed to be the amino acid tryptophane, because it has been shown that the amount of biosynthesis of auxin in a medium depends on the content of tryptophane in the medium.

One of the objectives of this research project is to increase the storage life of potatoes as a result of gamma irradiation. Gamma irradiation has been shown to slow down or halt the sprouting of the potato. Therefore, a study of the concentration of the growth-regulating substances which control sprouting appears advisable. This study involved a comparison of concentrations of phytohormones in irradiated and nonirradiated potatoes under varying storage conditions. The indole ring which occurs in most phytohormones is believed to be affected by irradiation which may be a possible explanation of some of the phenomena of sprout inhibition.

The term auxin is specifically defined as: an organic substance which promotes growth along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as practical from their own inherent growth-promoting substance.

The term phytohormone is defined as: an organic substance produced naturally in plants, controlling growth or other physiological functions at a point other than that of production. It is active in small quantities.

In this research study an extract of the "eyes" and a small amount of surrounding tissue of potatoes was made with freshly distilled ether which is completely free of hormone-destroying hydrogen peroxide. The various growth regulating substances were then separated by paper chromatography.

B. PREPARATION OF SAMPLE

1. Extraction.—The sample consists of the eyes of several potatoes including a small portion of the flesh around each eye. The eyes at the apical end are not used because they seem quite variable. Care is taken to sample uniform material. About 10 grams of this material is collected and covered with peroxide-free ether. (The ether is rendered peroxide-free by distilling it from a 50-50 mixture of calcium oxide and ferrous sulfate just prior to use.) The ether and eye tissue are kept in the refrigerator

(about 40°F) for a period of 20 hours. The ether is then decanted and evaporated to dryness. The residue is taken up in about 0.1 ml of alcohol, and this solution is subjected to paper chromatography.

2. Chromatography.—Chromatography had its origin with the Russian botanist M. Tswett,[9] who was able to separate chlorophyll pigments on adsorption columns. Chromatography is a method of separating similar compounds on the basis of differences in their adsorption coefficients, in the case of adsorption chromatography; or on the basis of differences in their partition coefficients, in the case of partition chromatography. A very high degree of resolution can be achieved. The method is applicable to the minutest amounts of mixtures, and, in fact, works best only when small amounts are involved.

In adsorption chromatography, a solvent is allowed to flow slowly down through a column of finely divided adsorbent. The components of the mixture, layered out at the top of the column, are carried down at different rates, each component appearing as a separate "band" down the length of the column. If a second solvent, immiscible with the moving solvent, is first adsorbed and held stationary on the adsorbent, the components of the mixture are distributed between the two liquid phases. This becomes partition chromatography and is analogous to counter-current distribution.

Where minute amounts of material are involved, as in the case of hormones, or other materials isolated from small amounts of living matter, a sheet of paper is substituted for the cylindrical column of absorbent. This has the advantage of exposing the entire amount of each component to view and also facilitates the process of recovering each isolated component. The disadvantage is that the paper must be hung in a closed chamber to insure complete equilibration of the liquid phases with the atmosphere. Both adsorption chromatography and partition chromatography can be performed with paper. In the former method, the mobile solvent is allowed to flow down dry paper (in equilibrium with the atmosphere), at the top of which the mixture has been applied as a tiny spot; in the latter, the paper is first dampened or wetted with the other, immiscible, stationary phase. The principles and many of the details of paper chromatography were worked out by Martin[10] and Synge.[11]

3. Chromatography Procedure.—The residue from the ether extraction is dissolved in the minimum amount of absolute alcohol. This solution is drawn into a micropipette and applied to the chromatograph paper in the smallest spot possible. The paper is cut in vertical strips with a common top edge, and the spot is applied at the top of each separate strip. A view of the chromatograph chamber and the paper inside is shown in Fig. 11.

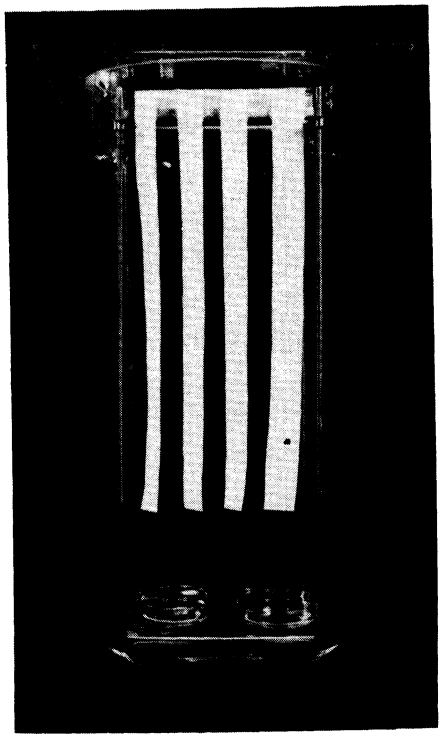

Fig. 11. Chromatograph chamber showing paper hanging from trough of solvent. To obtain complete equilibration of solvent between atmosphere and paper, it is necessary to provide more surface area for solvent than shown here. This is done by placing a layer of solvent in the bottom of the jar and lining the inside of the jar with paper which sits in the solvent.

A household hairdryer is used to direct a current of warm air at the paper as the spot is being applied so as to hasten the evaporation of the solvent before it spreads by capillarity and hence spreads the area of the spot. The smaller the spot, the smaller the area of overlap of constituents of the extract in the finished chromatogram. The extracts of potatoes treated in different ways can each be spotted at the head of one of the strips and subjected to the same conditions as the others.

After drying, the paper is hung from the trough at the top of the chromatograph chamber, shown also in Fig. 11. No solvent is placed in the trough for 24 hours, but solvent is placed in the bottom of the chamber during this period to insure complete equilibration of solvent with the atmosphere and the paper. After 24 hours, a stopper is removed in the top lid and solvent is admitted to the trough. As the solvent front moves down the paper and over the spots, the chromatogram begins to form. The solvent is allowed to flow down the paper for about 24 hours. The paper is then removed and allowed to dry.

Since the percent of growth hormone in the tissues is very low, a modified technique similar to that used by other investigators is employed.[12,13,14] This involves cutting strips of the dried chromatographic paper containing the adsorbed extract into five equal pieces. Each piece is placed in a separate flask and eluted with peroxide-free ether. After 12 hours extraction in the cold, the ether is decanted and the strips rinsed with a small portion of fresh ether. The ether is evaporated to dryness, and the residue analyzed for growth hormone activity by the standard Avena assay method. Growth activity is then plotted against the R_f, or percent the chemical has traveled with respect to the rate which the solvent has traveled. Peaks of activity may be taken as evidence of the presence of the hormone. These peaks are then correlated with the position of known compounds.

C. AVENA TECHNIQUE

The auxins thus separated were assayed by the Avena (oat) technique. In this procedure 72-hour old plants which have been grown in the dark at a relative humidity of 85% and at a temperature of 25°-26°C are used. Plants of this age and grown under these conditions are very reactive. This assay procedure was originally developed by Went,[15] but it has been variously modified by other investigators. It may be briefly described as follows:

Procedure for Avena Technique

(1) Seeds of a pure line of Siegeshafer oats are husked and soaked from two to three hours in water.

(2) The soaked seeds are placed on glass strips covered with strips of paper toweling which are then placed in a damp chamber (i.e., a glass refrigerator-type jar, which contains a small amount of water sufficient to cover the bottom of the jar).

In this step the seeds are placed with the embryo side up and overhanging the edge of the glass strips previously mentioned (see Fig. 12.). Placing the embryo end of the seed over the edge of the strip permits the roots of the young seedlings to grow straight downwards. This root orientation allows the seedlings to secure water when they are placed in the holders at a later stage of the experiment. After this point all work must be done in red light because other wavelengths cause phototropic curvature and a decrease in the sensitivity of the plants. The damp chamber is placed in a weak red light in a room which is held at a temperature of 25°-26°C.

(3) Thirty hours later the rooted seedlings are placed in special glass holders. The roots dip into water in a zinc trough coated on the inside with paraffin and the coleoptile (young oat sprout) grows vertically upward through a guide (see Fig. 13). The holders are held in brass clips in rows of twelve (see Fig. 14). The holder can be rotated in the clip and the clip can be moved back and forth so that the seedling can be made to stand strictly perpendicular. The seedlings are allowed to grow in the dark for about 40 more hours at a temperature of 25°-26°C and a relative humidity of 85-90%.

(4) After 40 hours, seedlings that are straight and of the same height are selected and about two millimeters of the tip of the coleoptile is removed with a sharp razor blade (see Fig. 15a and 15b).

(5) Meanwhile, agar blocks are made by mixing equal quantities of three percent agar and a standard solution of auxin. These are poured hot into a mould and then allowed to solidify. The large blocks produced in this manner are cut into 12 small blocks each containing a volume of about 10 mm^3. The actual size is not very important because the curvature is dependent upon the concentration of auxin in the blocks rather than upon the volume.

(6) Three hours after the first decapitation a second decapitation is made (see Fig. 15d and e). In this cut about 1 to 1-1/2 millimeters are removed from the top of the coleoptile. This is performed with a special pair of scissors which cut the coleoptile but not the leaf which it surrounds (see Fig. 15e). The leaf is then pulled upward with forceps until it breaks off deep inside the coleoptile (see Fig. 15f). The leaf is not actually attached to the plant but is only held by the coleoptile and used as a support for the agar block. The purpose of the two decapitations is to remove the region of hormone synthesis and produce a plant low in hormone, which will respond to the added auxins.

(7) Immediately after rupture of the leaf the small agar blocks are placed on the cut end of the coleoptile with one edge against the leaf (see Fig. 15g). The blocks must be placed precisely perpendicular to the plane of the light source to be used in photographing the seedlings, otherwise the full extent of the curvature will not be recorded. Figure 16 shows sprouts with attached agar blocks. The auxin diffuses down into the coleoptile and stimulates growth. Since the agar block is located on one side of the coleoptile, growth is more rapid on one side than the other. This results in curvature away from the agar block.

(8) The plant is allowed to stand thus for ninety minutes and then a shadowgraph is taken as shown in Fig. 17. If left more than ninety

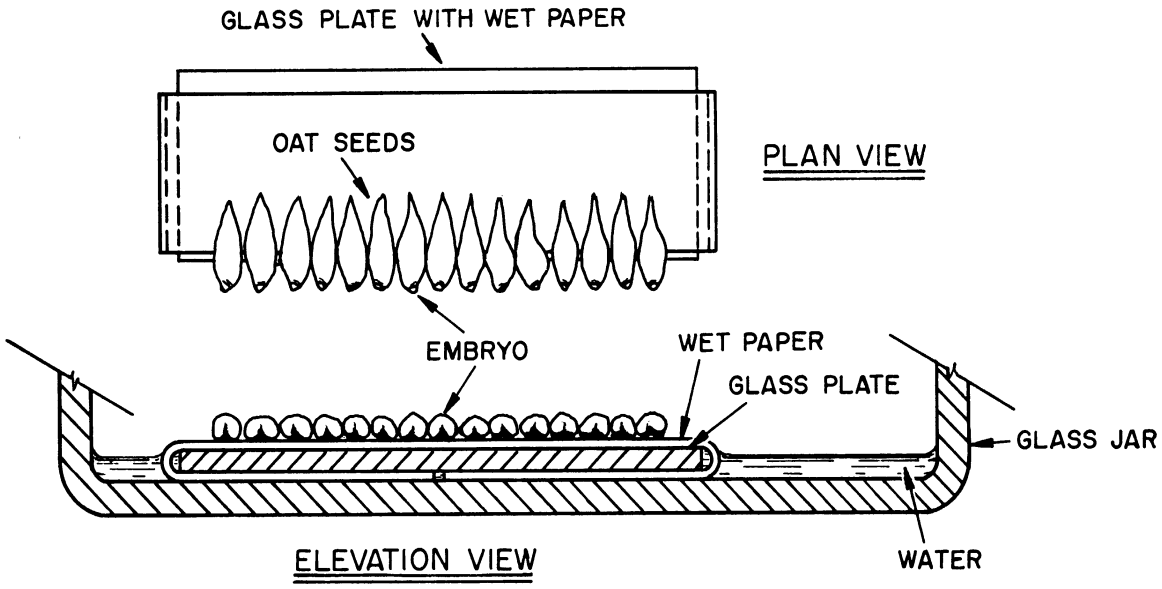

Fig. 12. Device for accommodating oat seeds in order to cause sprouting downward.

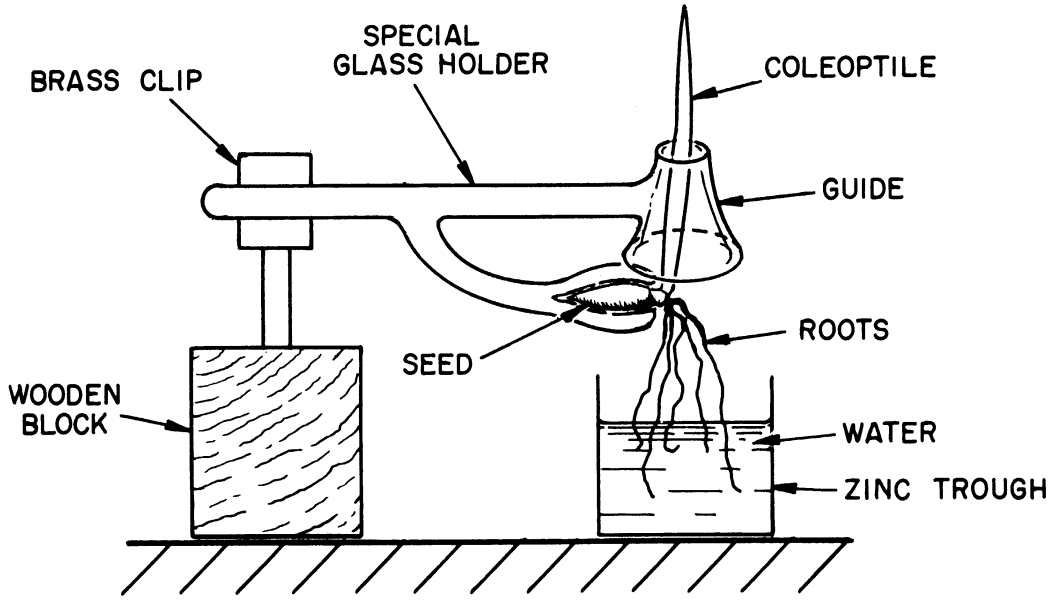

Fig. 13. Diagram of device for guiding upward the growth of the young oat sprout.

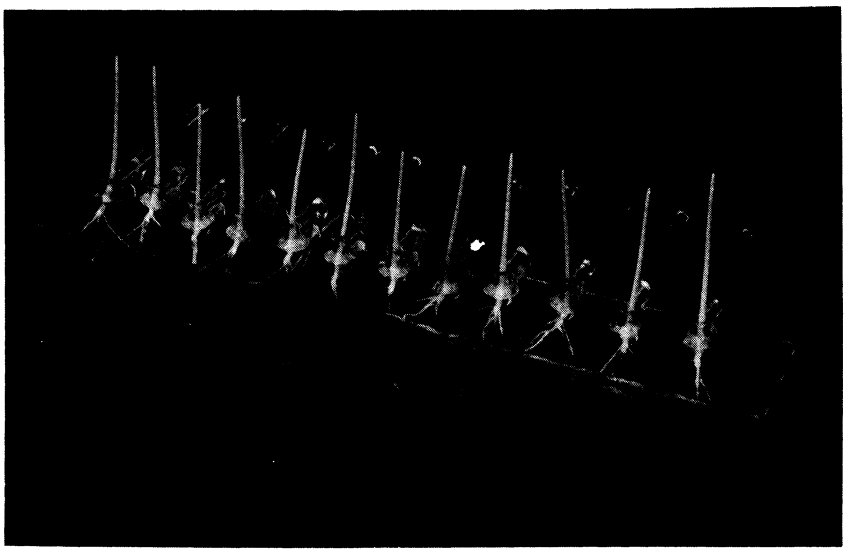

Fig. 14. Photograph of the actual apparatus used for growing the oat sprouts, showing the sprouts at a stage prior to cutting and applying the agar block containing the extract to be assayed.

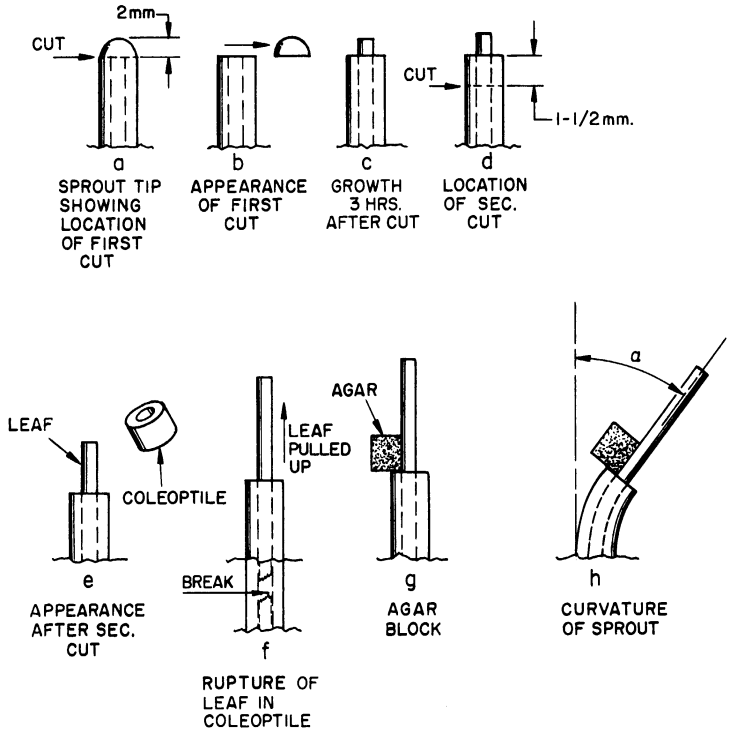

Fig. 15. Schematic views of an oat sprout in the process of being cut and manipulated in the Avena hormone assay technique.

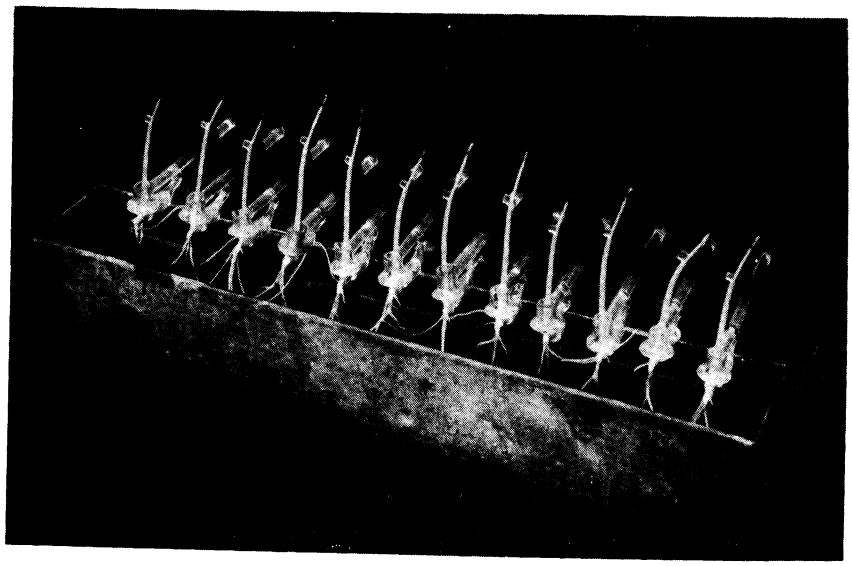

Fig. 16. Oat sprouts shown in Fig. 14 after cutting and applying the agar block.

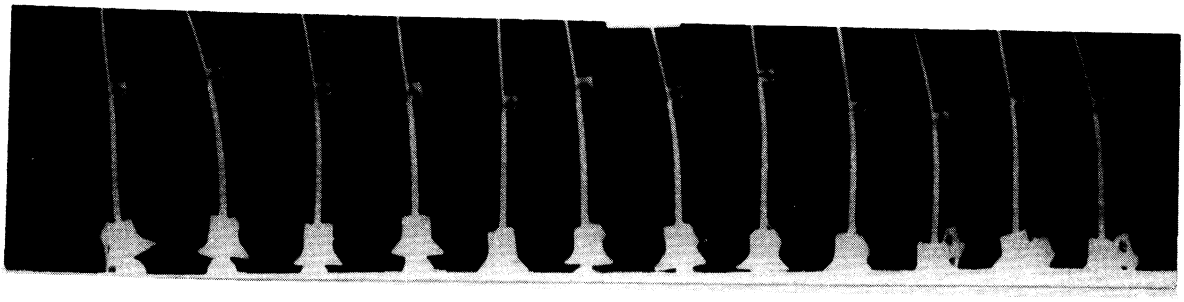

Fig. 17. Shadowgraph of oat sprouts showing curvature resulting from action of hormone or hormone inhibitor.

minutes, there is a "regeneration of the physiological tip," which produces auxin on both sides of the coleoptile so that the curvature is decreased.

The shadowgraph is measured with a special protractor to determine the number of degrees in the angle produced by the curvature (see Fig. 15h).

For a given range of auxin concentrations (e.g., 15-30 micrograms per liter of agar), the curvature is directly proportional to the amount of auxin present. Thus a standard solution can be made and used on one set of oat sprouts while at the same time and under the same conditions an extract is used on another set, and by comparison the amount of auxin extracted from a given amount of plant material can be determined.

D. PRELIMINARY RESULTS OF HORMONE STUDY OF IRRADIATED POTATOES

Nearly a dozen experiments have been conducted to date. The purpose of the first set of experiments was to test the Avena assay with varying amounts of whole extract (not chromatographed) of the eyes of nonirradiated tubers, using Sebagos for this purpose. The results showed that such extracts had pronounced growth hormone activity. The purpose of the second set of experiments (also with Sebagos) was to determine if the extract could be resolved by paper chromatography into separate components, and to determine the activity of each. In the early studies, two components were separated, one which moved slowly, by paper chromatography, and one which moved fast. The latter was found to consist of essentially all the growth-promoting activity of the original mixture (positive bending of the oat coleoptile to an approximate angle of 25°). The former, however, appeared to possess slight growth-hormone-inhibiting activity, as shown by a negative bending of the oat coleoptile. The extent of the negative bending was considerably less than 25°, indicating that the hormone inhibition, if any, was considerably weaker than the positive growth stimulating activity. Furthermore, the subsequent assays showed no evidence at all of the hormone-inhibitor activity in the eyes of nonirradiated as well as irradiated potatoes. This would be consistent with other studies which have shown the amount of growth-hormone inhibitor to decrease with cessation of dormancy.

The purpose of the next set of experiments was to determine the effect of irradiation of the potatoes on the hormone activity of the eye tissue directly after irradiation. Sebagos given 5, 15, 25, and 50 kilorep

were used in the first assay. It was found that all doses of irradiation caused a decrease in growth-hormone activity. As mentioned above, there was no hormone inhibition action in either irradiated or nonirradiated samples. Most of the activity appeared to come from one "spot" on the paper chromatogram, although this region of the chromatogram may contain several components only partially resolved. It was not possible to distinguish differences in the extent by which different doses of radiation caused a decrease in hormone activity with respect to controls. In a set of experiments with Russet Rurals given 5, 15, and 25-kilorep dosages of radiation, similar results were found. Since samples of Sebagos and Russets have not yet been assayed in the same experiment, it is not possible to compare the two varieties.

It has also been noted that throughout the course of these experiments, the amount of growth-hormone activity in the control (nonirradiated) Sebagos, which were used each time an experiment was conducted, has been slowly but steadily decreasing. Whereas the approximate average angle of bending was 25° in the first experiments, it has decreased to 18° in recent experiments (two and a half months later). There are no longer any freshly harvested Sebagos available. Thus, there is no way of determining whether these results were caused by a real change in hormone content or by some unnoticed change in the conditions under which the extract is prepared and assayed. Furthermore, the last two experiments, in which the effect of 100 and 200 kilorep was to be measured, resulted in the nonirradiated potatoes showing no hormone activity, whatsoever. This may be due to destruction of the hormones at some point during the preparation of the sample prior to assay. The current experiments are devoted to solving this difficulty.

STORAGE OF RING-ROT INFECTED POTATOES FOLLOWING GAMMA IRRADIATION
(Michigan State University)

Field-infected Sebago potatoes were obtained on December 5 from a grower near Howard City, Michigan. These tubers were uniformly sized ranging between 1-1/2 and 2 in. They had been graded twice, once over a 2 in. screen with the oversize removed and again over a 1-1/2 in. screen with the undersize removed.

The tubers were gamma irradiated in the Fission Products Laboratory on December 12-13 and were brought to East Lansing on December 19 in a heated truck. They were examined on December 22 (10 days after treat-

ment) for incidence of ring rot and other storage rots. The results of this inspection are given in Table XVII. In the inspection tubers suspected of being rotted were cut and those showing typical ring-rot symptoms were considered to have ring rot. Stain diagnosis was not made. Other rotted tubers lacking ring-rot symptoms were placed in a second grouping (see Table XVII).

TABLE XVII

INCIDENCE OF RING ROT AND STORAGE ROT IN POTATOES EXPOSED TO GAMMA RADIATION FROM COBALT-60 SOURCE

Gamma Irradiation Treatment, rep		Tubers with Ring Rot After Storage at 20°C			Tubers with Unidentified Rot After Storage		
		10 Days %	30 Days %	45 Days %	10 Days %	30 Days %	45 Days %
1.	0	2.9	5.1	7.7	0	0.6	1.6
2.	12,000	2.4	3.8	5.6	0	1.7	3.5
3.	32,000	1.3	3.2	5.5	0	1.6	6.5
4.	80,000	1.0	3.7	5.0	0	1.7	6.3
5.	200,000	0.3	2.1	3.5	0	6.2	17.6
6.	500,000	0?	0?	0?	0	92	100

It should be pointed out that some of the potatoes infected with ring rot in this lot had been discarded during harvesting operations and in grading operations before shipment. Thus, the amount of ring rot as shown (Table XVII) does not reflect the total amount of infection in the lot. There was no late blight in the shipment of tubers, which made possible rather accurate diagnosis of the tuber injury.

Tuber injury due to radiation was not evident following 10 days in storage. By the end of 30 days, the majority of tubers were rotted at 500,000 rep and a few had broken down at 200,000 rep. Positive diagnosis of ring rot in the 500,000-rep treatment could not be made due to inability to distinguish between radiation injury and ring rot.

Radiation injury resenbled severe freezing injury in many respects. Affected tubers when weighed were often somewhat cheesy in consistency, later breaking down into a soft rot. Affected tubers often had a fermented odor, and the general appearance was more suggestive of storage rots of sweet potatoes rather than that of Irish potatoes. The alcoholic type fermentation observed in the tubers receiving the higher doses of gamma radiation is consistent with the sucrose analyses reported previously.

Examination of tubers in cold storage is being made, but incidence of rot is at this date too low to indicate trends.

ACCEPTABILITY STUDIES
(QMF and CI)

Four hundred fifty pounds each of Sebago variety and Russet Rural variety potatoes from the same crop as those used for storage studies and carbohydrate analyses were irradiated and shipped to the Quartermaster Food and Container Institute, Chicago, for acceptance tests. Fifty pounds of each variety were given each of the irradiation doses used in these studies (0, 5, 10, 15, 20, 25, 50, 100, and 200 kilorep). Acceptance tests were made during the week of January 16-20, 1956. The indications were that potatoes given the highest irradiation dose were as acceptable as the nonirradiated potatoes. The elevated sucrose content of potatoes given the high radiation dose apparently did not result in a lowering of the acceptance.

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