Metabolic Changes Induced by Sprout Inhibiting Dose of γ-Irradiation in Potatoes

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The respiration studies after irradiating potatoes at doses of 0, 5, 10, 25 50, 100, 200, and 500 krad showed maximum CO_2 output at 10 krad except at higher doses like 200 and 500 krad. Respiratory quotient was 1, in all cases. A 25% increase in starch phosphorylase noted 2 hr after irradiation persisted even at 24 hr. But in the case of 500 krad, about 10% inhibition was observed at 24 hr. A 12% increase in reducing sugar content was obtained at 10 krad. Incorporation of 2-^{14}C-acetate into organic acids at 4 and 24 hr after irradiation revealed that the radioactivity in major organic acids, citric and malic, was much higher at 4 hr in irradiated sample, and at 24 hr the radioactivity was less than that in control. Studies of free amino acids showed an increase in aspartic acid, asparagine, threonine, serine, alanine, isoleucine, leucine, lysine, and arginine 24 hr after irradiation. A decrease was observed in the case of glutamic acid, proline, methionine, and phenylalanine. Radioautography of the free amino acids after 2-^{14}C-acetate incorporation confirmed the above observations.

The extension of dormancy in potato tubers by γ-irradiation has been known for more than two decades since the pioneering research by Sparrow and Christensen (1950). After that, there has been widespread interest in investigating the metabolic processes which bring about the sprout inhibition. Sussman (1953) was the first to describe that irradiation enhanced respiration in potatoes, which reached a maximum in 24 hr. Similar observations were made by Rubin and Metlitsky (1958) and Ogawa *et al.* (1969) on the respiration of irradiated potatoes. Schwimmer *et al.* (1958) observed that irradiation increased the accumulation of sucrose on storage at 4° C, and has also shown that starch phosphorylase was activated in irradiated potatoes stored at 21° C. Mukhin and Salkova (1961) found that oxidation processes proceeded at much the same rate in γ-irradiated and control potatoes. They concluded from their studies that γ-irradiation suppressed the primary phosphorylation of carbohydrates.

Information on nitrogen metabolism in irradiated potatoes is very scanty. Jaarma (1966) has studied the influence of γ-irradiation on proline content of potatoes. The results suggested that there is a correlation between proline content and sprouting. Fujimaki *et al.* (1968) and Jaarma (1969) independently have shown that exposure of the tubers to sprout inhibiting doses of γ-irradiation resulted in the decrease of glutamic acid content and a parallel increase in γ-amino butyric acid. Recently, Nair (1969) has observed that on irradiation of potatoes at sprout-inhibiting dose the activity of asparagine synthetase was enhanced to about sevenfold. In the present investigation studies on metabolic activity of irradiated potatoes, with special reference to changes in free amino acid content, have been undertaken.

EXPERIMENTAL

Freshly harvested potatoes (up-to-date variety) were used in these studies. During the experiments they were stored at 0–4° C in the dark, but not more than 2 weeks. This helped to extend the dormant period. Before γ-irradiation, the samples were tempered to room temperature (25° C). At a time 1 to 1.5 kg of potatoes, which were weighing an average of 100 g, were irradiated in a γ cell 220 (Atomic Energy of Canada Limited) at a dose rate of 2.29 krad/min. The overdose ratio was 30% and ferrous sulfate dosimetry was employed. After irradiation they were stored at 25° C and 50–60% Relative Humidity (R.H.).

Respiration Studies. The CO_2 liberated was determined according to the method of Loomis and Shull (1937) with necessary modifications as described by Thomas *et al.* (1971). The values are expressed as mg of CO_2 liberated per kg of potatoes per hr. The oxygen uptake was recorded using a Clark oxygen electrode and results were calculated as mg of oxygen uptake per kg of potatoes per hr. The respiratory quotient is defined as the ratio of the amount of CO_2 liberated to the amount of oxygen utilized. The respiration studies were carried out with potatoes irradiated at different doses, *viz.*, 0, 5, 10, 25, 50, 100, 200, and 500 krad for about 6 days.

Amino Acid Analysis. The free amino acids were isolated as follows. The potatoes were extracted in a Waring Blendor with cold 5% TCA in the rato 1:1 (weight/volume) and the extract was centrifuged. Clear supernatant (25 ml) was passed through a Dowex 50 H^+ column (30 × 1.5 cm) and washed three times with 200 ml of water. The elution of amino acids was carried out with 2 *N* NH_4OH until the eluate was ammoniacal. The eluate was then concentrated *in vacuo* at about 50° C. This procedure was repeated again with the concentrated amino acid mixture before putting it on Beckman Unichrom amino acid analyzer. The concentrated amino acid mixture was diluted with pH 2.2 sample dilutor buffer (Beckman amino acid analyzer manual) in such a way that the concentration should not exceed 0.5 μmol of amino acid by estimating N_2 by Nessler's method. The acidic and basic amino acids were quantitatively determined with the resin and operating condition used in Unichrom amino acid analyzer.

Radioautography. 50 μCi of 2-^{14}C-Na acetate (sp. act. 7.3 mCi/m*M*) was injected into a slot 2 mm in diameter and 2 mm deep and was allowed to infiltrate into control and 10 krad of irradiated potatoes immediately after irradiation. The samples were incubated for 24 hr at 25° C and 50–60% R.H. The free amino acids were extracted as described above. Concentrated extract (25 μl) was spotted on 10-in. × 10-in. Whatman No. 1 paper and chromatogram was subjected to two-dimensional chromatography, using 1-butanol:acetic acid (glacial):water (120:30:50) as the first solvent, and phenol:NH_3:water [Ph solvent, 200:NH_3 (0.880) 1]. The chromatograms were dried and kept in contact with super Vido HS 11 X-ray film (Veb Foto Chemische Werke, Berlin) for 20 days, and radioautograms were developed.

Estimation of Organic Acids. The organic acids were

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extracted from potatoes after infiltrating 50 μCi of 2-^{14}C-Na acetate and incubating for 4 hr and 24 hr after irradiation by using boiling ethanol according to the method described by Schwartz *et al.* (1962). For 50 g of tissue 100 ml of alcohol was used. The residue obtained after first extraction was extracted twice with ethanol and the extracts were pooled. The alcohol was removed under vacuum and the aqueous solution was first passed through a column of Dowex 50 H^+ to remove the amino acids. The filtrate and washings were then passed through Dowex 1 anion exchanger in Na^+ form. The column was washed several times with water, and organic acids were eluted with 4 *N* formic acid. The eluate was concentrated to dryness. The organic acids were separated by descending paper chromatography on Whatman No. 3 filter paper, using 1-propanol (50), Eucalyptol (50), formic acid 98% (20), and water as solvent. The spots were detected by spraying with bromocresol green. The spots were cut and radioactivity was determined in Beckman liquid scintillation counter after applying a quenching correction.

Estimation of Reducing Sugars. Reducing sugars were extracted with 80% ethanol from control and samples irradiated at different doses, *viz.*, 10, 20, 50, 100, 200, and 500 krad. They were estimated by the arsenomolybdate method (Nelson, 1944).

Starch Phosphorylase Assay. Phosphorylase activity of control and irradiated potatoes was determined according to McCready and Hassid (1957). Crude extracts were used as the enzyme preparation and activity was expressed as μmoles of labile Pi per mg of protein. The reaction mixture contained 2 ml of 0.2 *M* Na phosphate buffer (pH 6.8), 2 ml of enzyme, and 1 ml of starch (20 g in 500 ml). After incubation for 3 hr at 37° C, the reaction was stopped by heating the reaction mixture in a boiling water bath for 5 min. The precipitated proteins were filtered out and 1 ml of this filtrate was added to about 15 ml of water. Then 2.5 ml of magnesium ammonium chloride solution (55 g of $MgCl_2$-$6H_2O$ and 70 g of NH_4Cl dissolved in 650 ml of water and added to 350 ml of 10% NH_4OH), and 1.5 ml of NH_4OH (25% w/v) were also added. The volume was made up to 25 ml, and the precipitate removed by filtration. A 5-ml aliquot was hydrolyzed with 2 ml of perchloric acid for 10 min at 100° C and the Pi liberated was estimated (Fiske and Subbarao, 1929). Suitable controls were always included.

Preparation of Enzyme Extract. 20 grams of deskinned potato was ground with sand in a cooled pestle and mortar at 5° C and the enzyme was extracted with 20 ml of distilled water. The slurry was filtered through a double layer of cheese cloth and centrifuged at 20,000 × *g* for 15 min. The clear supernatant was used as the enzyme preparation.

Protein was determined by the biuret method (Gornall *et al.*, 1949).

RESULTS AND DISCUSSION

Effects of γ-Irradiation. RESPIRATION OF POTATOES. One of the immediate results of γ-irradiation of fruits and vegetables is the increased respiration (Romani, 1966) which is sustained for a number of days and then falls back to the level of control. Potatoes also showed a similar response when they were irradiated at different doses, *viz.*, 0, 5, 10, 25, 50, 100, 200, and 500 krad. In these studies we observed certain differences in CO_2 evolution of potatoes at various time intervals after irradiation. The data presented in Figure 1 show that a 10-krad sample showed maximum respiration in comparison with other doses, except at very high doses like 200 and 500 krad. In the case of doses above 10

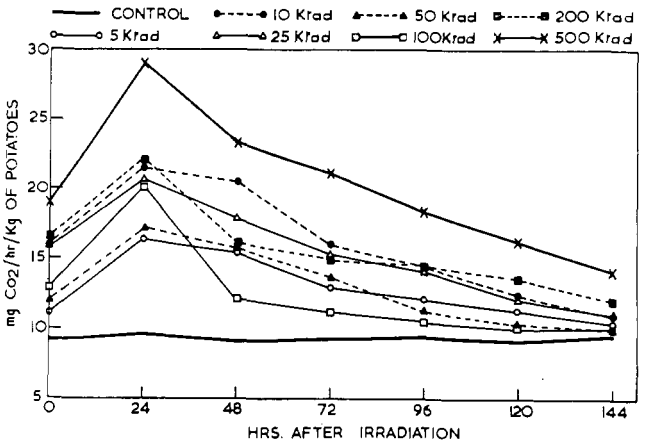

Figure 1. Respiration of potatoes irradiated at different dose levels measured as carbon dioxide evolved

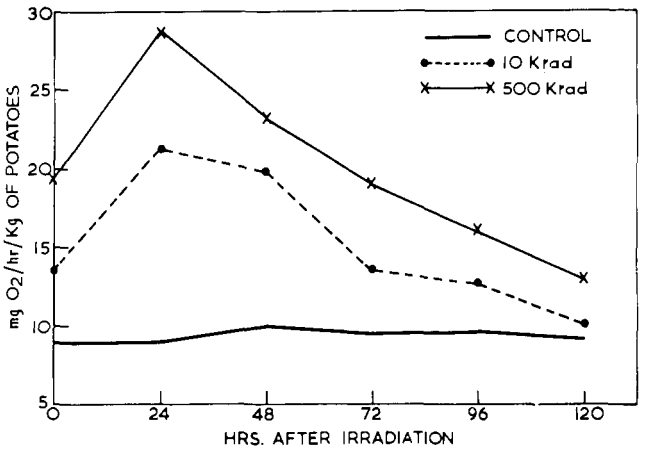

Figure 2. A comparison of oxygen uptake of potatoes irradiated at 0, 10, 500 krad at varying time intervals after irradiation

krad, the rate of fall after reaching a maximum in 24 hr was much faster than 10 krad. Until 48 hr, the 10 krad sample maintained the respiration rate almost at the same level as at 24 hr. This behavior of the 10 krad dose was also observed by Sussman (1953) in his studies with irradiated potatoes. There was an initial increase in the respiration rate, which steadily decreased after the third day and reached its original level in about a week of storage. Oxygen uptake studies with 10 and 500 krad irradiated potatoes showed an increase of similar pattern as CO_2 evolution (Figure 2). This excluded the possibility of CO_2 being evolved from decarboxylation. The other possibility is the radiation induced uncoupling of phosphorylation which, in turn, increases the respiratory rate. Romani (1966) has suggested that damage of mitochondria would not be very extensive except in the case of doses above 300 krad. He found (Romani, 1964) that pear fruit mitochondria showed good respiratory control even at 250 krad, although the overall yield of the mitochondria was decreased. At low doses there was partial recovery in the yield. Jaarma (1967) has studied the effect of γ-irradiation on the oxidative phosphorylation in potato. Mitochondria isolated from 14–15 krad irradiated potatoes showed no uncoupling. The P/O ratio with succinate was 2, and with malate was 3.0. Hence, it is likely that increased respiration observed at 10 krad irradiation is due to an acceleration of oxidation of respiratory substrates coupled to phosphorylation of ADP. There may be uncoupling of phosphorylation at higher doses

Table I. Effect of γ-Irradiation on Starch Phosphorylase Activity in Potato

Dose, krad	Specific activity μmol of Pi/mg of protein 0 hr	24 hr	Total activity μmol of Pi 0 hr	24 hr
0	7.0	7.0	470	470
10	8.5	8.8	573	660
500	6.8	6.4	460	440

Assay methods are described under Methods and Materials.

Table II. Effect of γ-Irradiation on Reducing Sugar Content in Potatoes

Dose, krad	μg of reducing sugar/g of potatoes Cold stored	Fresh
0	2992 ± 74.4	25 ± 1.7
10	3573 ± 62.3	30 ± 2.6
25	3500 ± 100.7	40 ± 1.8
50	2734 ± 51.3	27 ± 2.1
100	2850 ± 86.3	35 ± 3.0
200	2993 ± 44.6	33 ± 3.1
500	2534 ± 73.3	32 ± 2.9

Values given are mean ± SE.

like 200 and 500 krad. However, this has to be verified. Nair and Sreenivasan (1969) have shown that at this state of accelerated metabolism 10 krad irradiated potatoes are capable of performing energy-requiring reactions and also protein and nucleic acid synthesis.

Starch Phosphorylase of Potato. The increase in respiration observed with 10 krad irradiated potato can be due to the mobilization of reducing sugars from storage starch. Studies on starch phosphorylase (Table I) showed above 25% increase in starch phosphorylase within 2 hr after irradiation and this increase was observed even after 24 hr. But with 500 krad doses initially there was about a 5% decrease in activity, which decreased further to 10% in 24 hr. This observation is in agreement with that of Schwimmer *et al.* (1958). Rubin and Metlitsky (1958) suggested an increased decomposition of starch by phosphorylase with simultaneous increase in phosphoglucomutase activity. Thus, increased utilization of G-1-P is facilitated either *via* glycolytic or pentose phosphate pathway. An estimation of reducing sugars revealed (Table II) that on irradiation there is an increase in the reducing sugar concentration. Potatoes stored in the cold for about 1 month also showed an increase on irradiation, although they contained a much higher concentration of reducing sugars.

Organic Acids of Potato. According to Schwartz *et al.* (1962) the major organc acids present in potato are citric, malic, and oxalic. In order to evaluate the functioning of Kreb cycle, incorporation 2-^{14}C-Na acetate into these acids was studied. The data given in Table III show an increased incorporation into major organic acids within 4 hr after irradiation. But after 24 hr there was a decrease in the radioactivity in these organic acids compared to control, suggesting that they are turning over at a faster rate than in control sample.

Free Amino Acids of Potato. The effect of γ-irradiation on the free amino acids content of potato was studied. The results obtained are given in Table IV, which showed that at 24 hr after a 10 krad dose of γ-irradiation there was an in-

Table III. Incorporation of 2-^{14}C Acetate into Organic Acid of Varying Time Intervals

	Cpm per g of potatoes Malic 4 hr	Malic 24 hr	Citric 4 hr	Citric 24 hr	Ratio = irradiated/control Malic 4 hr	Malic 24 hr	Citric 4 hr	Citric 24 hr
0	1316	110572	926	36901				
					4.8	0.9	1.8	0.12
10	6197	101706	1685	4596				

Organic acids were extracted with hot ethanol and purified on Dowex-1 anion exchanger in Na^+ form. They were separated by paper chromatography in Whatman No. 3 filter paper using 1-propanol (50), eucalyptol (50), formic acid 98% (20), and water as solvent. The spots were detected by spraying with Bromocresol green. Radioactivity determined in Beckman liquid scintillation counter.

Table IV. Effect of γ-Irradiation on Free Amino Acid Content of Potatoes

Amino acids	Control	10 krad 24 hr	10 krad 1 week	10 krad 1 month	500 krad 24 hr	500 krad 1 week
1) Aspartic acid	672	972	800	2619	412	642
2) Asparagine[a]	468	572				
3) Threonine + 4) Serine	899	958	600	210	374	500
5) Glutamic acid	1250	981	570	1016	726	697
6) Proline	36	10	22	62	5	31
7) Glycine	12	12	7	26	19	15
8) Alanine	58	81	25	33	13	42
9) Valine	159	139	160	182	99	152
10) Methionine	31	17	17	36	4	17
11) Isoleucine	49	52	50	63	34	48
12) Leucine	16	24	20	27	11	20
13) Tyrosine	48	44	60	41	34	50
14) Phenylalanine	54	20	34	42	14	59
15) Lysine	20	57	120	60	10	14
16) Histidine	20	21	17	47	9	21
17) Arginine	105	286	117	293	90	139

The values are mean of six to eight independent determinations. The concentration is expressed as μg of amino acid per g of potato.
[a] Asparagine was determined after separation by paper chromatography using phenol:NH_3:water solvent and eluting the ninhydrin spot with alcohol containing 0.5% $CuSO_4$.

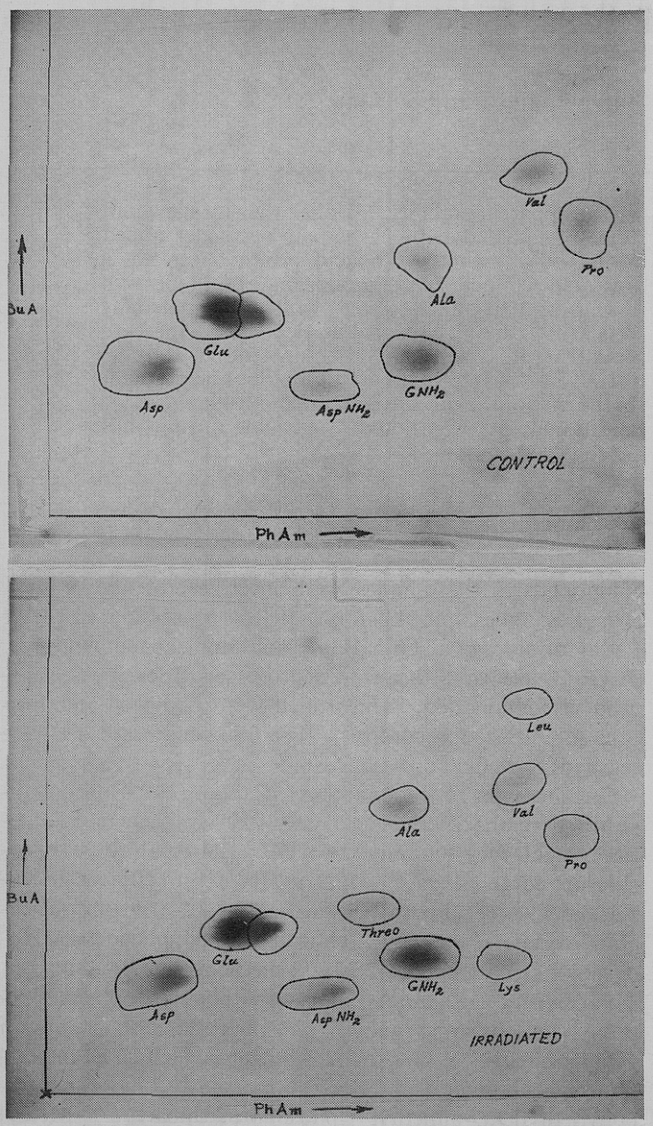

Figure 3. Radioautogram of free amino acid pattern 24 hr after administration of 2-^{14}C-Na acetate. Identification of the spots was made after preparing a two dimensional chromatogram of a standard amino acid mixture under identical conditions. a. control. b. irradiated

crease in the concentration of aspartic acid (45%), asparagine (24%), threonine and serine (6%), alanine (40%), isoleucine (6%), leucine (50%), lysine (180%), and arginine (170%). Glutamic acid was decreased to about 20%. A similar observation was made by Fujimaki *et al.* (1968) and Jaarma (1969). Glutamic acid concentration was decreased in irradiated sample due to its decarboxylation to γ-amino butyric acid. Its concentration was recovered after 105 days' storage. Apart from glutamic acid, a decrease in the concentration of proline (56%), methionine (45%), and phenylalanine (63%) was also observed. When potatoes were irradiated at 500 krad the concentration of all amino acids decreased invariably. A striking feature observed on 1 week of storage of the 10 krad irradiated sample was the increase in the concentration of lysine and decrease in the concentration of arginine. The amount of proline and valine also showed a recovery after a week's storage. Glutamic acid concentration was decreased still further, suggesting that it is taking part in reactions such as the synthesis of proline and also in transamination reactions. The increase in asparagine was confirmed by the observation (Nair, 1969) that irradiation caused an increase in asparagine synthetase. An increase in lysine, which may be derived either from the breakdown of proteins or by synthesis, makes the irradiated potatoes a good source for this essential amino acid. However, the variations in the content of this amino acid on longer storage periods have to be studied.

In order to investigate whether the increase observed in certain free amino acids is due to an increase in synthesis or due to protein breakdown, incorporation of 2-^{14}C-Na acetate into free amino acids 24 hr after irradiation was studied. A comparison of the radioautogram on free amino acid pattern for control and 10 krad irradiated potatoes (Figure 3a,b) reveals that there was a definite increase in the concentration of aspartic acid, asparagine, glutamine, and alanine. Lysine and leucine showed only slight increase. All these amino acids are derived from acetate or Kreb cycle intermediates. The decrease in glutamic acid, proline, and valine confirmed the data observed on the amino acid determination. The results presented in this paper supported the contention that the potatoes irradiated at sprout-inhibition dose are at a transient, higher level of metabolism, which is required for the recovery of the tissue from radiation damage and also to prepare the conditions for the extension of dormancy. Further investigation on this line is needed to understand the significance of these findings in relation to the sprout-inhibiting mechanism.

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