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Effects of Ionizing Radiation on Development of Invertase Activity, Nucleic Acids, and Respiratory Activity in Aging Potato Tuber Slices

by

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放射線 照射가 熟成시킨 감자 塊莖 薄片에서 Invertase, 核酸 및 呼吸作用의 發達에 미치는 影響

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Abstract

Mode of action study of irradiation was performed with potato tuber slices, 1 mm×1.5 cm, aged on moist filter paper under aseptic technique. The time courses of invertase activity, nucleic acids and respiratory activity were determined, and sensitivities of these three processes to ionizing radiation were measured. None of those processes was severely inhibited by the dosage suppressing cell division. The result of ^{3}H-thymidine incorporation suggests that the reaction site of ionizing radiation might be existent during mitosis or G_2 period.

Introduction

The potential of ionizing radiation for sprout control of potato tubers is well recongnized,[1] however, its sprout inhibiting mechanism is still ambiguous.[2] It is generally assumed that the mechanism inhibiting cell division which leads to potato sprout inhibition is the same as the mechanism inhibiting wound periderm formation in cut potato tubers.

Our previous report[3] indicated that optimum dosage of gamma irradiation for sprout inhibition was coincided with the dosage required for complete prevention of new cell formation in aging tuber slices. Since considerable study has been achieved on the metabolic processes which occur during aging of storage tissue slices, potato tuber slices provide a convenient system for studying effects of ionizing radiation on cell division and other aspects of metabolism.[4]

Excision of thin slices from dormant storage organs initiates immediate promotion of various metabolic processes in the tissue and these metabolic changes accompany reactivated growth.[5~10] It has been postulated that this phenomenon involves gene derepression induced by the slicing of the storage tissue.[6,10,11~15]

Invertase activity,[7,12,16~21] RNA synthesis[10,14,15,18] and respiratory activity[22~26] have been shown to increase with aging in slices of storage organs. Therefore

it appears interesting to investigate the effect of ionizing radiation on development of those metabolic activity in aging potato tuber slices.

Materials and Methods

All experiments were conducted with potato tuber slices 1 mm thick and 1.5 cm in diameter. 'Irish Cobbler' variety, newly harvested or stored at 5°C, was used for slicing.

Defect-free potato tubers were washed with tap water, and immersed in 10% calcium hypochlorite solution for 20 minutes. Then their apical and basal ends were removed with sterile knife. Slices were cut from central tuber tissue (mainly pith and storage parenchyma of inner phloem) employing a devise designed for this specific purpose.[3]

Slicing and aging were performed under aseptic condition. Glassware and slicer were sterilized by autoclaving at 120°C and 15 psi for 20 minutes. All slicing operations were proceeded in a glove box previously exposed to ultraviolet light. Cut slices were momentarily rinsed in sterile water to eliminate starch from the cut surfaces. Then they are placed on a sheet of filter paper supported by a single layer of 5 mm glass bead in 9×1.5 cm petri dishes containing distilled water.[11,25]

Immediately after cutting, slices were irradiated at a dose rate of 109 rad/sec by BNL shipboard irradiator (CO^{60}; 20,000 Ci). Five dosages of irradiation—0, 2, 4, 8 and 16 krad—were chosen for treatment. Slices were aged at 25°C in dark. All analytical data were expressed as quantities per gram of original fresh weight.

The invertase assay was based on the procedures described by Bacon, MacDonald and Knight[17] and Edelman and Hall.[12] To determine RNA and DNA content in tuber slices, the procedures described by Cherry[27] were followed. DNA content was estimated by the diphenylamine test,[28] and the amount of RNA was obtained by subtracting DNA content from total nucleic acids.

For studying the effect of irradiation on DNA synthesis, two day aged slices were employed. Unirradiated and 16 krad treated tuber slices were aseptically incubated with ^{3}H-thymidine (20 μCi/1.2 g of tissue) at 25°C for 2 hours. Incubation mixture contained 50 μg per ml of chloramphenicol.

Nucleic acids were extracted from tissue,[27] aliquots of the final dialyzed product were placed into scintillation solution containing 2,5-diphenyloxazole (PPO) 3 g, p-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) 50 mg, toluene 209 ml, ethanol 167 ml and dioxane 125 ml, and counted in Liquid Scintillation Counter (LSG-13, Shimadzu Seisakusho Ltd.).

Respiratory activity was measured by standard Warburg manometric techniques with 120 oscillations per minute.

Results and Discussion

The number of newly divided cells in aging potato tuber slices treated with various dosages of gamma ray is presented in Table 1. Twenty of new cells were formed per mm^2 area in the unirradiated slices, however, only one third in 2 krad treated slices. Slightly fewer new cells were respectively formed in 4 and 8 krad treated slices than in 2 krad treated ones. No cell division occurred in tuber slices treated with 16 krad, which is a dosage for complete inhibition of sprout growth of potato tubers.[1] Accordingly it became interesting to determine whether various metabolic processes in aging tuber slices would be affected at a dosage inhibiting cell division.

The time course of invertase development in potato tuber slices is shown in Fig. 1. Enzyme activity was very low in freshly cut slices. However, the enzyme activity was somewhat increased in 3 hours and showed continuously rapid development up to 24 hour. Then very slight increase was obtained at 48 and 96 hour. The overall pattern was similar to the ones reported by previous workers.[4,12,16,17,20]

Effect of various dosages of gamma ray on invertase development was investigated with 1 day aged slices. As shown in Fig. 2, enzyme activity didn't appear to be affected by 4 krad treatment, however, somewhat decreased by 8 and 16 krad treatments. No statistcally significant differences were existent between control and irradiated slices.

Fig. 3 shows nucleic acid development of potato tuber slices during aging. RNA content was gradually increased up to 2 days and somewhat declined at 4 days. However, DNA content tended to be increased continuously throughout 4 day aging period, even

Table 1. Effect of various dosages of gamma ray on cell division[x]

Dosage (krad)	New cells divided (No. /mm² slice)
0	20.2 a
2	7.2 b
4	4.1 b
8	2.5 b
16	0 b

x : Any two means followed by a common letter are not significantly different at 5% level by the honestly significant difference (hsd) of Tukey's procedure.

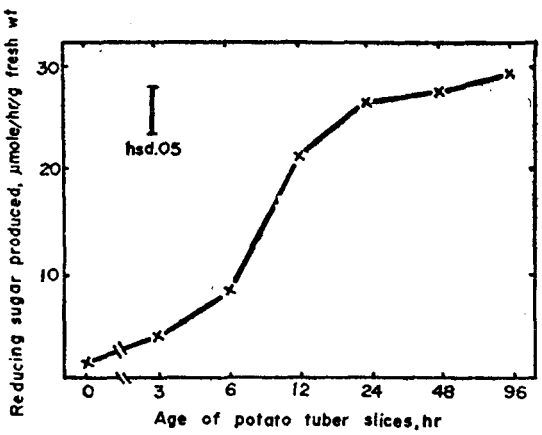

Fig. 1. Invertase development of potato tuber slices during aging

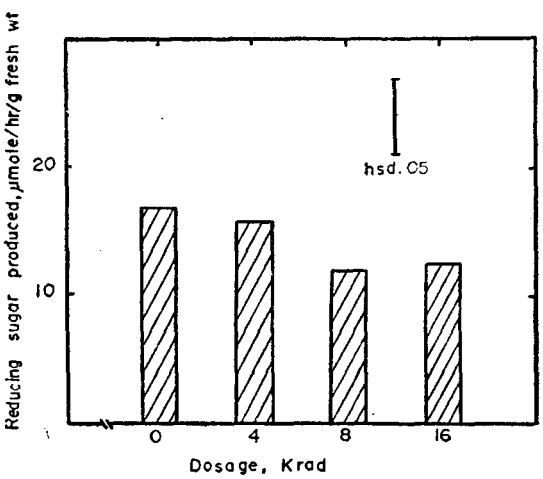

Fig. 2. Effect of various dosages of gamma ray on invertase development of potato tuber slices aged 1 day

though there was some fluctuation.

Effects of various dosages of gamma ray on RNA and DNA content were investigated with 2 day aged slices. RNA content tended to be gradually suppressed with increasing dosages of gamma ray, although no statistically significant differences were found among various dosages of gamma ray treatment. DNA content of potato tuber slices treated with various dosages of gamma ray was found to have considerable fluctuation, however, it appeared that DNA content was not affected by various dosages of gamma ray treatments. (Table 2.)

Fig. 4 shows susceptibility of cell division, invertase and total nucleic acid development to various dosages of gamma ray. It is apparent that cell division is very sensitive to gamma irradiation, while invertase and total nucleic acid development is insensitive.

As a result of investigating mode of action of CIPC, chemical sprout inhibitor of potato tubers, Lee[4] also discovered that invertase development was not greatly

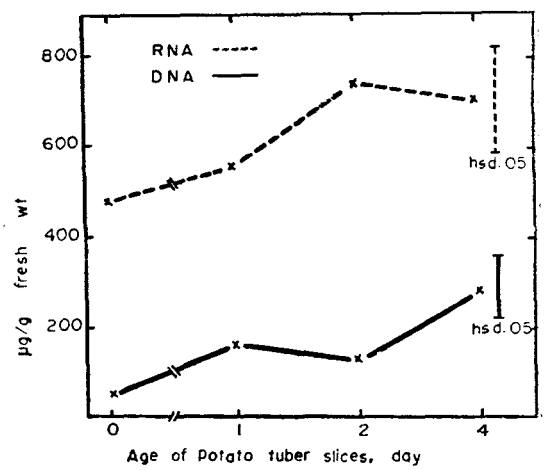

Fig. 3. Nucleic acid development of Potato tuber slices during aging

Table 2. Eeffects of various dosages of gamma ray on nucleic acid content of potato tuber slices aged 2 days [x]

Dosage (krad)	RNA (μg/g fresh wt)	DNA (μg/g fresh wt)
0	726.8 a	149.8 a
4	678.2 a	113.3 a
8	611.0 a	89.5 a
16	555.5 a	160.5 a

x : See footnote x, Table I.

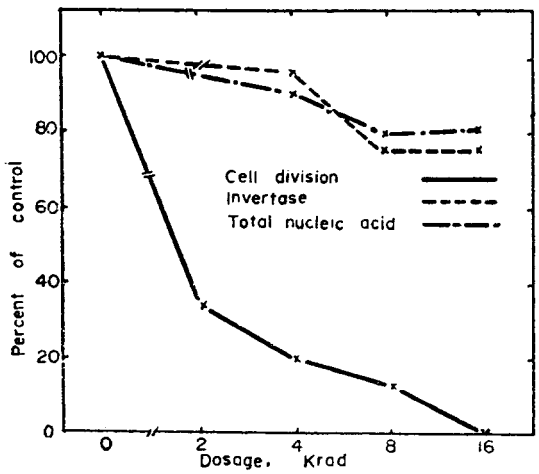

Fig. 4. Susceptibility of cell division, invertase and total nucleic acid development to various dosages of gamma ray

affected by CIPC concentration inhibiting cell division in aging potato slices. The physiological role on the development of invertase activity is not clearly understood; however, an increased invertase activity in sliced storage tissue appears to be an expression of protein synthesis,[12,15] since known inhibitors of protein synthesis suppresses this enzyme development. The fact that invertase development is insensitive to gamma irradiation suggests that protein synthesis might not be interfered with the dosage of gamma ray inhibiting cell division in aging potato tuber slices.

It is expected that suppression of cell division accompanies decreased DNA development in aging potato slices. However, as shown in Table 2, either RNA or DNA development was not affected by various deosages of gamma ray. Since diphenylamine test for DNA determination was not very reliable under the present experimental condition, effect of gamma irradiadiation on *in vivo* incorporation of ^{3}H-thymidine into nucleic acids was investigated with freshly cut or 2 day aged slices. (Table 3)

DNA synthesis was abruptly increased due to aging, however, it is worth noting that DNA synthesis was only slightly suppressed at the dosage of gamma ray completely inhibiting cell division.

According to Lee,[4] CIPC concentration suppressing cell division did not either influence DNA synthesis in aging potato tuber slices. She suggested that CIPC might exert its effect after S period during which DNA

Table 3. Effect of gamma irradiation on *in vivo* incorporation of ^{3}H-thymidine into nucleic acids[x]

Treatments	Incorporation (counts/min/g x 10^{-1})
Aging, day	
0	1,755 a
2	3,822 b
Dosage, krad	
0	3,111 a
16	2,466 a
Interaction	n.s.

[x] : See footnote [x]. Table 1.
[y] : This experiment was designed as 2 (aging) X 2 (dosage) factorial arrangement. Accordingly, main effect of one factor reveals the mean value of all levels of other.

synthesis occurs in mitotic cycle. It is likely that the attacking point of CIPC lies in mitosis itself or in the G_2 period, and this same explanation might be applicable to the gamma irradiation.

Previous workers [29,30] stated suppression or retardation of nucleic acid synthesis as one of the factors causing sprout inhibition of potato tubers. Brownell[31] suggested that sprout inhibition might be attributed to the disturbance of the dividing mechanism or the metabolic systems of the sprout cells by irradiation. However, the present experiment goes one step farther and shows that inhibition of cell division likely occurs during mitosis or G_2 period without affecting DNA synthesis.

The time course of respiratroy activity development of potato tuber slices is depicted in Fig. 5. Oxygen uptake was considerably low in freshly cut slices, and a somewhat increased amount of uptake was obtained in 6 hours. However, oxygen uptake revealed a sharp increase between 6 and 24 hours and no further increase occurred 48 hours after slicing.

Effect of various dosages of gamma ray on respiratory activity was investigated with 0 and 24 hour aged slices. It is well established that an increased respiration rate of aged potato tuber slices occurs in 2 successive stages. The initial increase occurs at the moment of slicing regardless of prior metabolic events. Secondary increase develops 24~48 hours after cutting, and this is recognized as induced respiration.

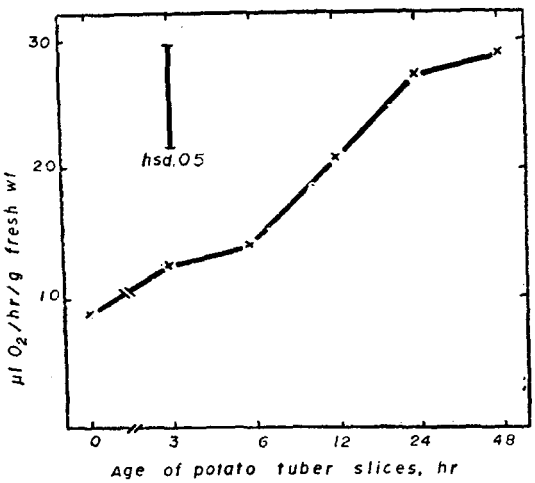

Fig. 5. Respiratory activity development of potato slices during aging

Table 4. Effect of various dosages of gamma ray on respiration of potato tuber slices aged for 0 and 24 hours when freshly cut slices or whole tubers are irradiated [x,y]

Treatments	O_2 uptake of potato tuber slices (μl/hr/g fresh wt)	
	When freshly cut slices are irradiated	When whole tubers are irradiated and sliced
Aging, hr		
0	9.5 a	8.9 a
24	22.1 b	29.6 b
Dosage, krad		
0	18.1 a	19.5 a
4	14.0 a	17.8 a
8	15.5 a	20.8 a
16	15.7 a	19.0 a
Interaction	n.s.	n.s.

x : See footnote x. Table 1.

y : This experiment was designed as 2 (aging) X 4 (dosage) factorial arrangement. Accordingly, main effect of one factor reveals mean value of all levels of other.

Therefore two series of treatments were established in the present experiment. In one series, tuber slices were irradiated with various dosages of gamma ray immediately after cutting and aged in dark at 25°C. Oxygen uptake was measured with fresh and 24 hour aged slices. In the second series, whole tubers were irradiated with various dosages of gamma ray, and immediately sliced and aged in dark at 25°C. Oxygen uptake was also measured with fresh and 24 hour aged slices.

In both series, the respiration rate was markedly increased due to aging, however, no difference in oxygen uptake was found among various dosages of gamma ray treatment.

As mentioned above, respiration rate sharply increases at the moment of slicing. If low dosage of irradiation immediately accelerates respiration of storage organs, as suggested by Park et al., [32] additive radiation effects should occur on oxygen uptake when whole tubers are irradiated and sliced or when newly cut slices are irradiated comparing with unirradiated slices. However, no additive effects were seen in the present experiment. Therefore, it is conceivable that respiratory activity is not disturbed at a dosage completely inhibiting cell division and that altered respiration is not directly concerned with sprout inhibiting mechanism.

요 약

無菌 상태하에서 습윤한 여지위에 熟成시킨 감자 塊莖 薄片(1 mm×1.5 cm)을 재료로 放射線의 作用機作연구를 행하였다.

Invertase, 핵산 및 호흡작용의 time course를 결정하고 이들 세작용의 放射線에 대한 민감도를 측정하였다. 세 작용중 어느것도 세포분열을 억제하는 선량에서 심하게 억제되지 않았다.

^{3}H-thymidine의 incorporation 결과는 放射線의 작용부위가 mitosis나 G_2 period에 존재할지도 모른다는 것을 암시한다.

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