J. Indian Inst. Sci., 64 (C), June 1983, Pp. 91–106

REVIEW PAPER

Biochemical mechanism of radiation-induced dormancy in potatoes

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Received on October 8, 1982 ; Revised on December 20, 1982.

Abstract

The studies described here will show that after gamma irradiation the dormant bud tissue of potato exhibits a transient metabolic activation. During this period of active metabolic state, the tissue is capable of synthesis of DNA, RNA and protein. Apart from this there is increased utilization of carbohydrate for the production of the energy source ATP, to meet the demand for these processes. The site of active protein synthesis during this transient phase of activation has been recognised as nuclei of the bud tissue and the synthesis of new proteins of the nuclei takes place within one to two hours after irradiation. The synthesis of nuclear acidic proteins was increased to about 3 to 5 fold during the activation period compared to unirradiated bud tissue. The increase in acidic protein synthesis lasted for 4½ hr. During the time there was no synthesis of histones. The synthesis of histones started only 7 hr after irradiation showing about 10 fold increase over the control bud tissue. The increase in the concentration of non-histone protein prior to active RNA synthetic phase (2 hr) is suggestive of their involvement in the metabolic activation ensured after gamma irradiation. Gamma irradiation adversely affected the IAA synthesising system and the production of IAA. Treatment with low concentrations of IAA within 6 hr after irradiation could restore the IAA synthesising capacity as well as reversal of sprout inhibition.

Key words : Gamma irradiation, bud tissue, potato, nuclear acidic proteins, metabolism.

1. Introduction

In India, potatoes represent a major food crop and the production per annum averages out to 9 million tonnes. The harvested tubers do not sprout and spoil for a long

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period of time although all the necessary external conditions like moisture, temperature and air exist for this process. This special character of potatoes, termed as dormancy, enables the equitable distribution in good physical condition for utilization gradually over a period till dormancy is over. The length of dormancy is controlled genetically and it varies from cultivar to cultivar. Once dormany is broken the tubers sprout and they become unfit for consumption. Out of the many methods used for controlling sprouting in tuber, extension of dormancy by exposing the tubers to ionizing irradiation has received considerable attention in recent years. Since the pioneering discovery of Sparrow and Christensen[1] on the efficacy of gamma irradiation to control sprouting a considerable amount of investigations has gone into the understanding of the biochemical changes brought about by gamma irradiation which culminated in the inhibition of sprouting of potatoes. Since no comprehensive review consolidating all these observations is available in literature, an attempt is made in this paper to compile all available information on this subject and propose a plausible hypothesis on the mechanism of radiation-induced dormancy in potatoes.

2. Metabolic changes

2.1. *Respiration and carbohydrate metabolism*

Sussman[2] was the first to study in detail the biochemical changes induced by gamma irradiation. He found that upon gamma irradiation, oxygen uptake as well as CO_2 evolution enhanced and reached maximum in 24 hr and this increased respiration rate was sustained for one or two weeks. A six-fold increase in the respiratory rate was observed over control in certain potato varieties. Thus he concluded that one of the primary changes caused by irradiation is this increased respiration rate. Irradiation was found to augment the accumulation of sugars, especially sucrose and an increase was observed in lactate dehydrogenase and polyphenol oxidase activities by Schwimmer *et al*[3]. They have also showed a 30% activation of starch phosphorylase over control tubers on storage at 21°C. Rubin and Metlitsky[4] have reported an increase in sugar concentration on gamma irradiation. Ussuf and Nair[5] have shown that the immediate effect discernible after irradiation at 10 krad is the increase in respiratory rate to about 2 to 3 fold at 24 hr which sustained for 3 days and declined to the level of control in *Kufri Chandramukhi* variety of potatoes stored at room temperature. The levels of reducing sugars also showed an increase. Gamma irradiation also caused 25% increase in starch phosphorylase activity which persists at 24 hr. The increase in sugar was due to the activation of phosphorylase and corresponding increase in phosphoglucomutase, which supplied glucose-6-phosphate in abundance for utilization through glycolytic or pentose-phosphate pathway. A number of investigators have confirmed the operation of Emden-Mayerhoff pathway as well as pentose-phosphate pathway in potato[6-8]. The increased respiration rate observed may be due to accelerated functioning of the tricarboxylic acid cycle which can satisfy the requirements of energy for the recovery of tissue from radiation damage.

An enhanced functioning of Kreb's cycle is evident from the observation of Ussuf and Nair[5] that 2^{14}C-acetate was more efficiently incorporated into major organic acids of potato, namely, citric and malic acids. A rapid turnover of the label was found in 24 hr after irradiation. According to Rubin and Mikkeva[9] irradiation resulted in increased cytochrome oxidase activity of the mitochondria but no increase in polyphenol oxidase or peroxidase activities was observed. Studies by Jaarma[10] on the effect of gamma irradiation and the capacity of potato mitochondria for oxidative phosphorylation showed that mitochondria isolated fom 14-15 krad irradiated potatoes exhibited no uncoupling. The P/O ratio with succinate was 2 and with malate was 3. Hence it can be surmised that an increase in respiration observed at 10 krad irradiation is due to an increased rate of utilization of respiratory substrates coupled to phosphorylation of ADP.

2.2. *Amino acid metabolism*

The nitrogen metabolism of potatoes is of utmost importance since it has a close bearing on the development, differentiation and breaking of dormancy. The free amino acids of potato contribute the major portion of nitrogenous constituents and their concentration changed at various stages of development and maturation[11–13]. There are only very few reports on the effect of gamma irradiation on nitrogen metabolism in potatoes. Studies by Jaarma[14] on the relation between proline content and sprouting of potatoes showed a translocation of proline to the site of the buds as the tubers began to sprout. On irradiation with gamma rays the proline content decreased in the tuber. The finding prompted her to suggest that irradiation-induced extension of dormancy may be due to curtailment of proline synthesis or depletion by irradiation. She also observed[15] a decrease in glutamic acid and corresponding increase in γ-aminobutyric acid in 10 krad irradiated potatoes. The effect of gamma irradiation on the free amino acid content was examined by Fujimaki *et al*[16] who found that the content of almost all free amino acids in potatoes changed and after 105 days of storage the differences were normalized. The studies by Ussuf and Nair[5] on the level of free amino acids showed an interesting pattern. Twenty-four hours after 10 krad irradiation there was an increase in the concentration of aspartic acid (45%) asparagine (24%), threonine and serine (6%), leucine (50%), lysine (180%) and arginine (170%) glutamic acid decreased to about 20%. Apart from glutamic acid a decrease in the concentration of proline (50%), methionine (45%) and phenylalanine (63%) were also observed. Amount of proline and valine also showed recovery, glutamic acid concentration decreased still further. The studies on the incorporation of 2^{14}C-acetate into amino acids established the pattern observed by chemical examination (fig. 1). The increase was observed in all amino acids derived from Kreb's cycle intermediates except in glutamic acid.

2.3. *Arginine and lysine bio-synthesis*

The increase in free amino acids can be either due to the breakdown of potato protein by gamma irradiation or due to their synthesis from their precursors. Radio-

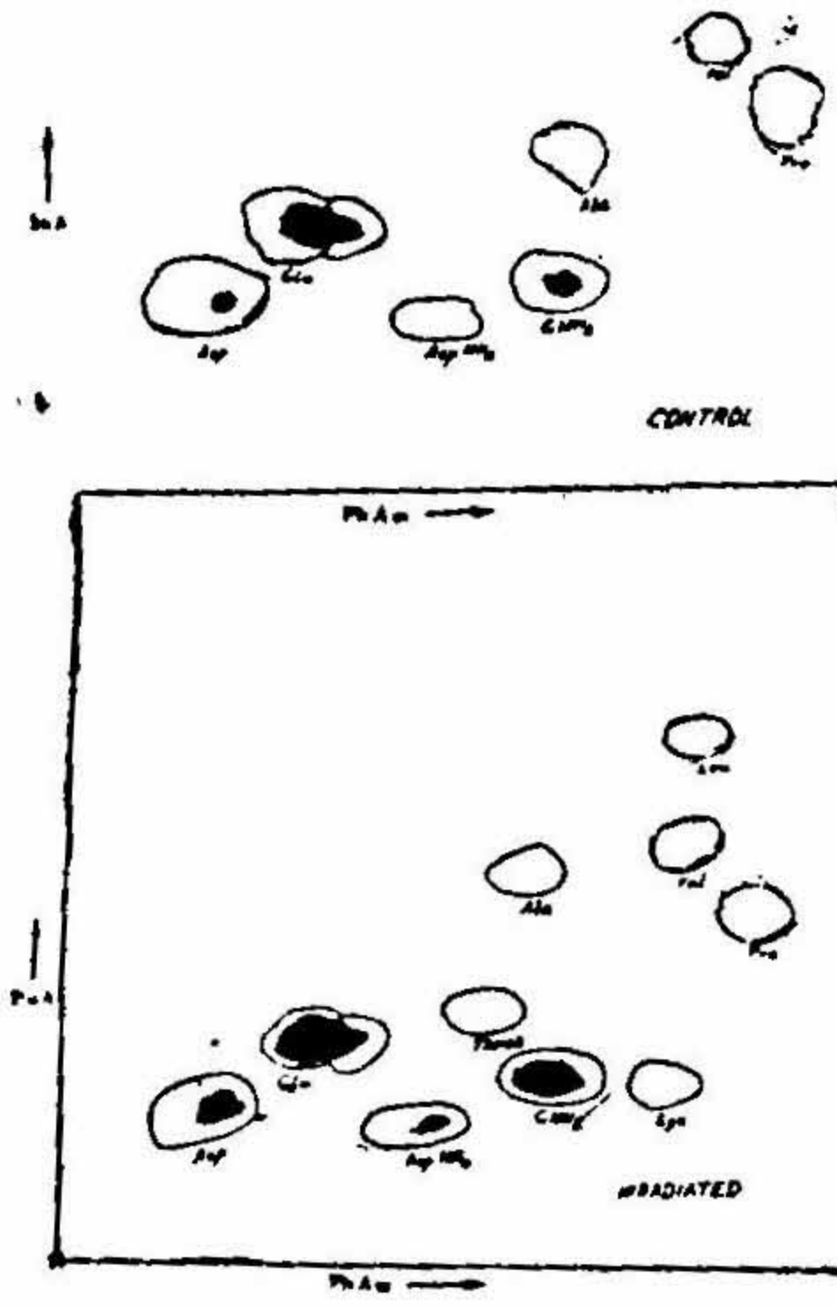


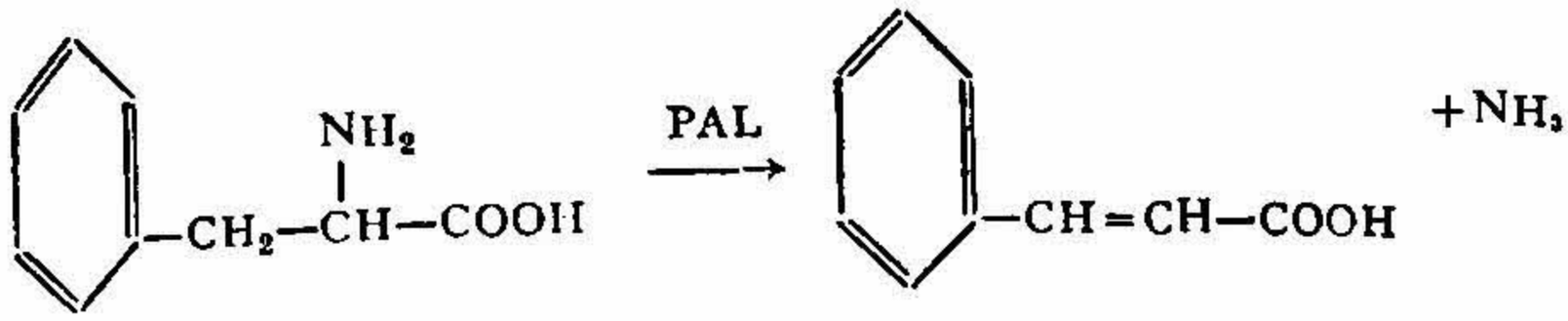
FIG. 1. Radioautographic study of the incorporation of 2 ^{14}C-acetate into various free amino acids of potatoes 24 hr after irradiation compared with control sample. Identification of the spots was done after preparing a two-dimensional chromatogram of standard amino acid mixture under identical conditions.

autographic studies[5] after incorporating 2^{14}C-acetate into free amino acids showed that there is incorporation of acetate into lysine. Thus there is a possibility of *de novo* synthesis of these amino acids from their precursors formed as a result of the alteration in the metabolic activity in gamma-irradiated potatoes. The existence of arginine synthetic pathway involving Krebs–Henseleit cycle enzymes was inferred from the findings on the incorporation of $NaH^{14}CO_3$ into arginine using a cell-free system. Gamma irradiation activated the arginine synthesis to about 4 fold over the control and the maximum was at 6 hr after irradiation. The activation was dose-dependent showing maximum at 25 krad.

The major pathway for the biosynthesis of lysine in higher plants has been recognised as diamino-pimelic acid pathway. The presence of the enzyme catalysing the final step in lysine biosynthesis, namely, diamino-pimelic acid decarboxylase has not been demonstrated in potato tissue. The existence of the enzyme activity and its activation by gamma radiation have been demonstrated in the potato bud tissue. Gamma irradiation enhanced the enzyme activity to about 4 fold and the activation was time-dependent because the activity increased linearly up to 18 hr to reach a maximum level. The activation was also dose-dependent and maximum activation was observed between 10–25 krad which was the sprout inhibiting dose range. The activation of the enzymes involved in the biosynthesis of these amino acids corroborated the irradiation-induced increase in the amino acids as biosynthesis.

2.4. *Phenylalanine ammonia lyase synthesis and metabolism of phenols and phenolic acid*

Phenylalanine ammonia lyase (PAL) catalysing the reaction has a central role in the metabolism of phenolic acids[17,18]. This is the first enzyme in the pathway for the production of phenolic acids and phytoalexins which are responsible for the healthy growth of plant tissues.



The observation that there is a decrease in phenylalanine content (63%) as a result of irradiation hinted at the activation of PAL. Studies by Pendharkar and Nair[19] have demonstrated the activation of PAL as a result of irradiation of whole potatoes. Unirradiated potatoes showed negligible PAL activity. However, as a result of irradiation the activity was found to be induced in whole potatoes. This induction of activity was not uniform throughout the tuber but was mainly in the cortex tissue, while parenchyma tissue did not show any induction at all. In the cortex tissue the induction was more in bud tissue (Table I). Potatoes on irradiation at 10 krad dose exhibited the maximum PAL activity at 3 hr after irradiation when stored at ambient temperature. On further storage, the activity was found to decrease rapidly in the first few days followed by a very slow decrease during subsequent storage period up to 6 months. The maximum activity observed at 3 hr was about 20 fold of the original activity before irradiation which declined to 3·5 fold after a week. The formation of free NH_3 in irradiated potatoes corresponded well with the increase in PAL activity. It

Table I

Induction pattern of PAL in various tissues of gamma-irradiated potato tuber

Tissue	PAL activity per g tissue	
	Unirradiated	Irradiated
1. Buds (cortex tissue)	18	360
2. Cortex tissue (but away from buds)	18	155
3. Central pulp (Parenchymatous tissue)	36	14

The tissue samples were excised 3 hr after irradiation. PAL was isolated and estimated as described by Pendharkar and Nair[19] and PAL activity is expressed as nmoles of transcinnamic acid formed per hour under standard assay conditions.

is also established that the increase in PAL activity by gamma irradiation was partly due to activation ol the existing enzyme and the rest of it by *de novo* synthesis.

The induction of PAL activity caused variation in the steady state levels of caffeic and chlorogenic acids in irradiated potatoes. The concentrations of the two phenolic acids increased to about as 50–60% within 24 hr after irradiation. There was a gradual fall and within a week the concentrations were 60–80% of that of unirradiated potatoes (fig. 2). This level was maintained on further storage for six months. The increased turnover of chlorogenic acid may be due either to accelerated utilization for the formation of an insoluble lignin polymer which plays an important role in disease resistance of potatoes and/or its accelerated oxidation by polyphenol oxidase which is activated by irradiation.

The studies on the changes happening in phenolase enzymes in irradiated potatoes at sprout inhibiting dose conducted by Pendhaikar and Nair[21] showed that there is 22% activation of chlorogenic acid oxidase activity. The increase in chlorogenic acid oxidase may be responsible for the rapid decrease in chlorogenic and caffeic acid content in irradiated potatoes.

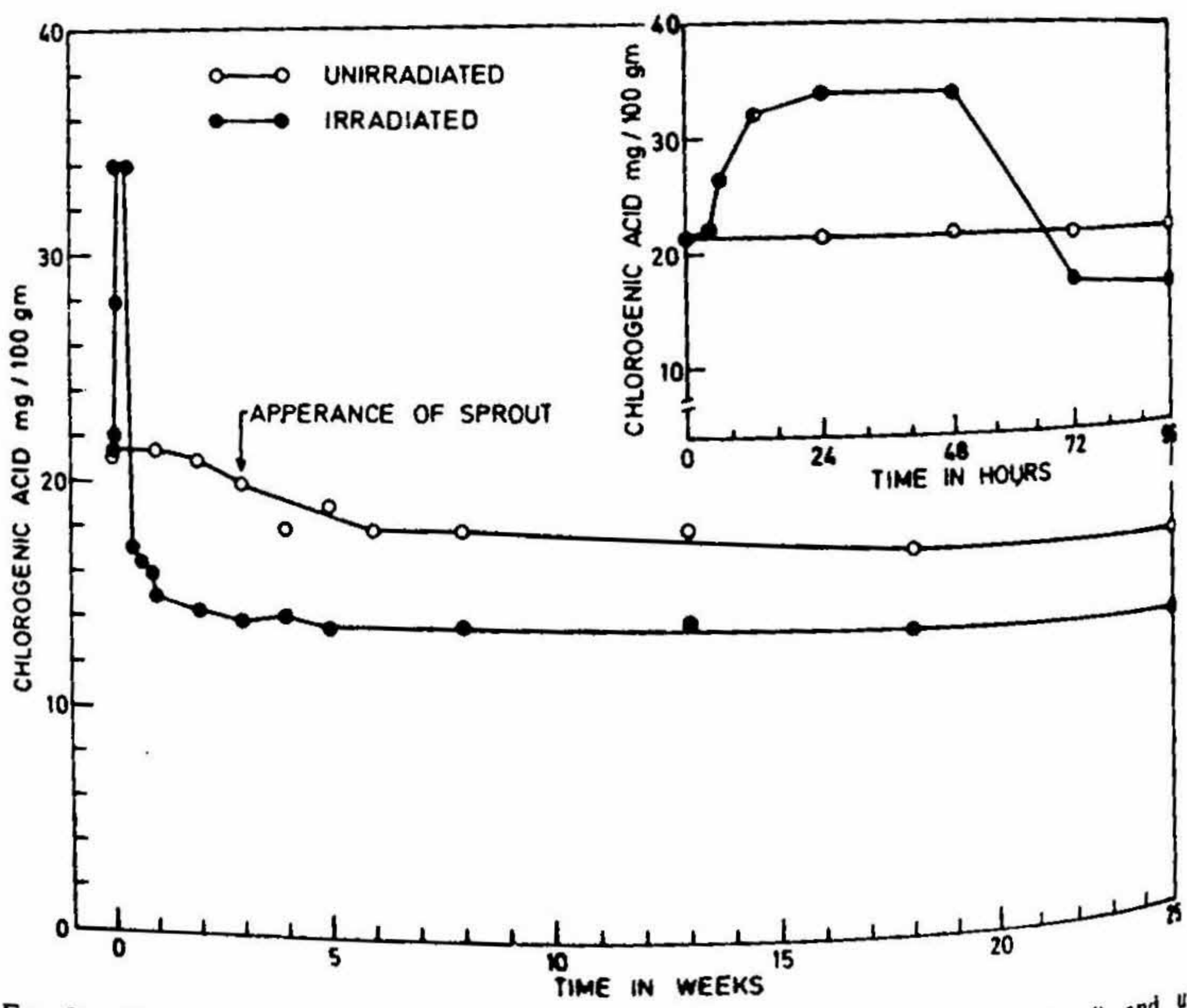


FIG. 2. Variation in chlorogenic acid content in gamma-irradiated (10 krad) and unirradiated potatoes during storage.

3. Transient metabolic activation and evidence for mRNA and new protein synthesis

3.1. *Irradiation-induced synthesis of mRNA*

Termination of bud rest in potatoes is associated with the increased capacity of the tuber to synthesize DNA and corresponding increase in RNA synthesis. During dormancy the genetic material is found to be repressed[22]. Irradiation interferes with the derepression process and as a result the genome is permanently repressed with in 6 hr after irradiation[23]. The dormant potatoes have limited capacity of DNA replication. Irradiation at 10 krad dose enabled them to synthesize both DNA and RNA[24]. This RNA synthesis was sensitive to actinomycin D, which means that RNA synthesized in potato buds, due to gamma irradiation represented transcription of the genetic material. On the other hand, it is known that the function of irradiation is to keep the tissue in a quiescent state for a longer period. The radiation-induced RNA synthesis was observed only for a short time after irradiation. The capacity for RNA synthesis was lost completely in the bud tissue excised from irradiated potatoes after 10 to 12 hr. An increase in RNA synthesis might be attributed to a number of factors like increase in RNA polymerase activity, increase in pool size of nucleotides or derepression of the genetic material by irradiation. Further studies on this line demonstrated a transient activation of template activity of potato bud chromatin by gamma irradiation[24]. These observations supported the idea that some of the RNA synthesized are of messenger type. The presence of poly (A) in mRNA facilitated the detection of newly synthesized mRNA using affinity chromatography on poly (U) Sepharose column[25]. Using this method poly (A) containing mRNA formed as a result of irradiations was separated from other RNA. Among the new RNA synthesized poly (A) RNA was about 1% in potato bud tissue[26]. Poly (A) RNA synthesized as a result of irradiation of potato bud sedimented between 12 and 4S which is characteristic of functional mRNA[27]. Poly (A) segment isolated from poly (A) RNA showed sedimentation value between 4 and 5S indicating the size of poly (A) to be about 50 AMP residue which is the same as that found in all plant functional mRNA[28]. The poly (A) RNA also showed transient existence.

3.2. De novo *synthesis of protein*

There was marked difference in protein synthesis estimated by the incorporation of ^{14}C-leucine into soluble protein in unirradiated and irradiated buds[29]. In irradiated buds there was rapid synthesis of protein in 3 to 5 hr (fig. 3) and the rate of synthesis at 5 hr was four times compared to that at one hour. Asparagine synthetase activity induced by gamma irradiation in the bud tissue also showed a similar increase in that period. The *de novo* synthesis of asparagine synthetase was a fast reaction initiated 3 hr after irradiation and reached a maximum in 5 hr and the bulk of the radio-activity incorporated was found to be associated with asparagine synthetase.

The studies on the rate of the synthesis of this protein in various cellular fractions showed that it is localized in the nucleus[30]. The nuclear fraction from irradiated bud

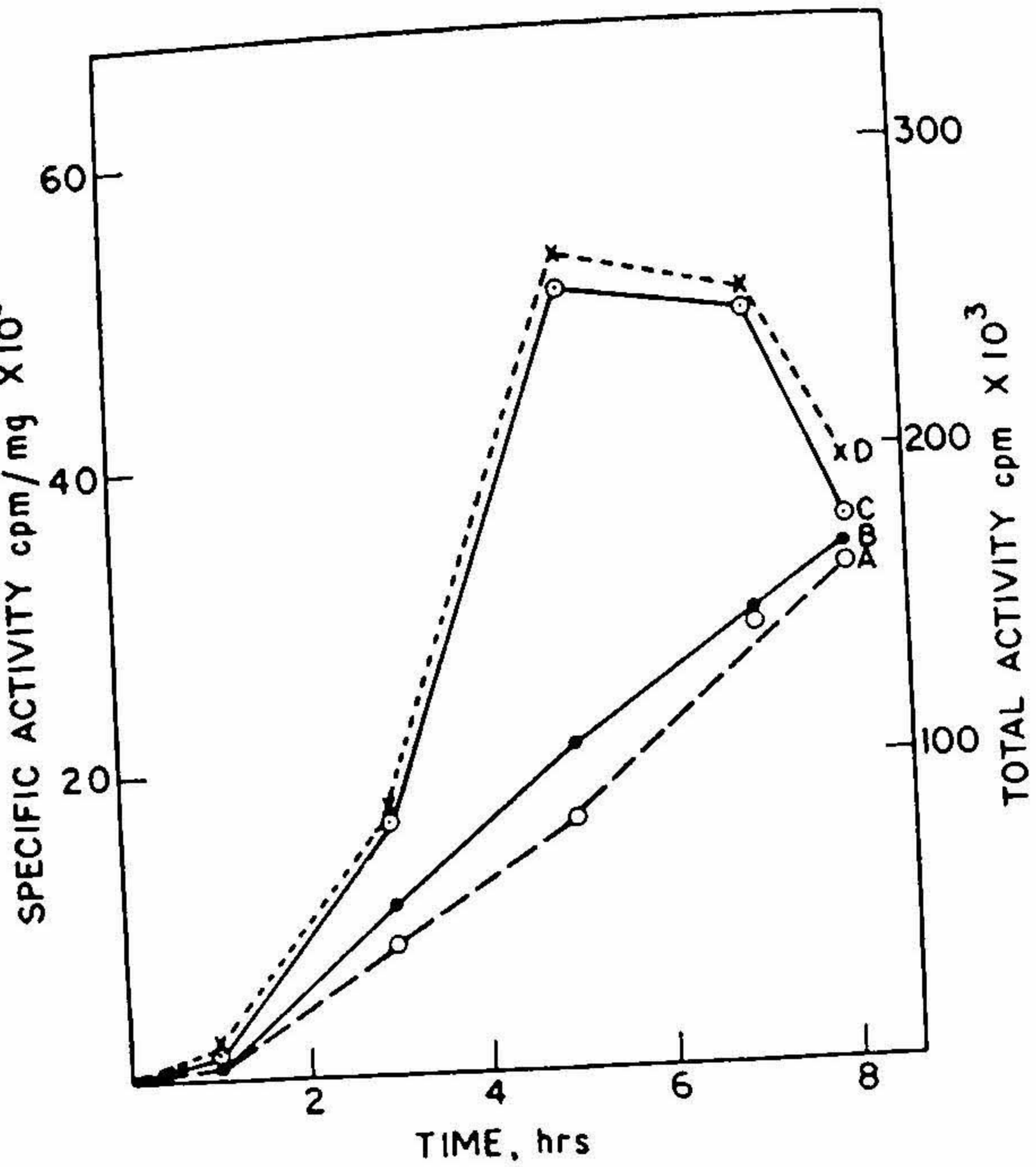


FIG. 3. Incorporation of ^{14}C-leucine into TCA insoluble protein of unirradiated and irradiated buds at different time intervals. Experimental details are described in ref. 29. Curve A: specific activity (unirradiated), Curve B: total activty (unirradiated), Curve C: total activity (irradiated), Curve D: specific activity (irradiated).

tissue was capable of efficient synthesis of protein and also showed an increase in asparagine synthetase on incubation for 5 hr. But this was not true with the nuclear fraction from unirradiated potato buds. Supplementation of amino acid incorporating system in the reaction mixture along with irradiated bud nuclear fraction resulted in an increased incorporation and concomitant increase in enzyme activity.

3.3. *Nuclear protein synthesis*

The unusual kinetics and the site of synthesis of asparagine synthetase enzyme suggested that the induction of this enzyme could have some physiological function other than mere synthesis of asparagine. As a possible alternative, amidation of acidic amino acid residues of certain proteins were tested under the same conditions used for asparagine synthesis[31] (Table II). Among the different proteins tested ribonuclease was found to be the best substrate. The amidating enzyme exhibited the same kinetics as asparagine synthetase. For about 2 hr after irradiation no activity developed and

Table II

Amidation of different protein by the amidating enzyme present in nuclear fraction from gamma-irradiated potato bud tissue

Protein substrate	Enzyme activity μ moles of NH_3 formed
Bovine pancreatic ribonuclease	1·9
Bovine serum albumin	1·43
Hen egg white lysozyme	1·25
Non-histone acidic protein from irradiated potato bud nuclei	2·5

The concentration of protein in the reaction mixture was 3 mg/ml except in the case of non-histone protein which was 1 mg/ml. The experimental details for the isolation in nuclei and assay of enzyme activity are described in ref. 31.

then linearly increased to a maximum in 4½ hr, followed by a decline to no activity at 7 hr.

The demonstration of the synthesis of a specific protein namely the amidating enzyme in nuclear fraction lead to the characterization of its mRNA and isolation of cell-free system from nuclei[32]. Unirradiated dormant bud tissue did not have the mRNA to code for the synthesis of amidating enzyme in cell-free system. The level of functional mRNA for the synthesis of the enzyme was raised after 2 hr irradiation in the nuclear fraction and its synthesis was exclusively in the nuclei. The mRNA of this enzyme appeared to be of very transient nature. The mRNA is capable of coding for the synthesis of amidating enzyme in the presence of cell-free protein synthesizing system isolated from irradiated bud nuclear fraction as well as wheat germ system. The failure to obtain an active protein synthesizing system from unirradiated potato bud nuclei as well as from irradiated potatoes after attaining quiescent state showed that there is a possibility that translational mechanism of induction may have to be stimulated to translate the available mRNA.

3.4. *Kinetics of nuclear protein synthesis*

In eukaryotic cells, chromosome is a complex structure which in addition to DNA contains large amount of histone, non-histone protein and a small quantity of RNA[33]. Very little is known on the functional role of chromatin proteins, but recent studies

indicate that molecules responsible for specific gene regulation are to be found among chromosomal protein. The possibility of these proteins being regulators of gene expression in eukaryotic cells was expressed by many workers[34-36] using chromatin reconstitution technique. In the tissue where the gene activity is stimulated, increased rate of incorporation of labelled amino acids into NHC proteins prior to its function was observed[37,38]. A kinetic study on the synthesis of nuclear protein, non-histone acidic protein and histone in irradiated bud nuclei showed that at 1 hr after irradiation a 4-fold increase in the incorporation into acidic protein was found (Table III). Except histone all other fractions showed increase. A ten-fold increase was seen in total nuclear incorporation at 4½ hr; however, there was no incorporation in histone fraction. But at 7 hr there was 14-fold increase in histone synthesis. The increase was supported by the observation that the synthesis of basic amino acids like arginine and lysine had also increased. The transient increase in the synthesis of nuclear protein is required for the extension of dormancy.

4. Involvement of auxin in radiation-induced dormancy and the mechanism of sprout inhibition

4.1. *Studies with whole potatoes*

The growth regulators such as indoleacetic acid (IAA) or gibberellic acid (GA) can reverse the radiation-induced dormancy of potatoes[23,39]. IAA was effective only when low concentration of its solution was employed[23]. The effect of IAA is unique in the sense that the optimum concentration which brings about the reversal of sprout inhibition was not able to accelerate the sprouting in control tubers. But on treatment with 50 ppm GA the control potatoes showed accelerated sprouting. For complete reversal of sprout inhibition the optimum concentration of GA required was much more than that of IAA[23]. The mode of action of IAA in the reversal of sprout inhibition seems to be distinctly different from that of GA. Another notable characteristic of IAA is that the reversal of sprout inhibition was not discernible when treated with IAA after a time lapse. Treatment with 20 ppm IAA immediately or within 6 hr after irradiation only reversed the radiation-induced sprout inhibition[39].

IAA in the tissue is labile to radiation[41] and complete destruction occurred when potato were irradiated at 10 krad[40]. IAA in the tissue is known to be in a dynamic pool maintained as a steady state system with concomitant biosynthesis and depletion. While the free auxin is destroyed by radiation, studies on the status of IAA synthesizing system suggested that irradiation interferes with the synthesis of the enzyme involved in the conversion of tryptophan to IAA[40]. When IAA is exogenously supplied within 6 hr after irradiation it somehow triggers the machinery for the synthesis of IAA synthesizing system. A possible explanation for this finding is that the irradiation hinders the synthesis of IAA synthesizing system and treatment with IAA within 6 hr triggers some mechanism which restores it.

Table III

Incorporation of ^{14}C-leucine into various nuclear proteins at different time intervals after irradiation

Fractions	Specific activity at different time intervals (hr)					
	1½		4½		7	
	Control	Irr	Control	Irr	Control	Irr
Supernatant	1400	2600	3580	8000	7866	16400
Nuclear fraction	3320	6440	8600	64500	11500	63000
Tris wash of nuclear fraction	1254	3580	4400	10800	14900	36600
0·15 M NaCl wash	3100	10150	5200	8220	13100	33000
Histone fraction	2620	2580	10000	14700	7840	109000
Acidic protein (in NaOH soluble)	6100	23650	14650	99250	43200	304000

The bud tissue was incubated with 5 μCi of DL-leucine- ^{14}C for different time intervals mentioned above. The nuclear fraction was isolated for these buds after the incubation and the nuclear fraction was washed successively with 0·1 M Tris containing 0·01 M EDTA pH 6·5, 0·15 M NaCl, 0·25 N H_2SO_4, and finally the residue was dissolved in 1 N NaOH. Histones were precipitated with alcohol and dissolved in water and this was taken for protein estimation and counting. Protein and radioactivity incorporation into each fraction were tested after precipitation with cold 10% TCA followed by washing with 2% TCA twice, alcohol, ether mixture (1·1) twice. The precipitate was dissolved in known volume of 1 N NaOH and an aliquot was taken for determination of radioactivity and protein. Specific activity is defined as counts per mg protein.

4.2. *Studies with excised bud tissues*

Using the bud tissue instead of whole potato the same pattern of depletion and reactivation of IAA synthesizing system by irradiation and subsequent treatment with IAA could be demonstrated[12] (fig. 4). With these studies it is established that irradiation has interfered with the synthesis of the enzymes involved in the formation of IAA from tryptophan. Treatment with IAA immediately after irradiation triggered the resumption of synthesis of protein. This fact is evident from the studies using protein synthesis inhibitors and actinomycin D. After impairing the synthesis of the enzyme, irradiation has also accelerated the degradation of existing enzyme by activating protease activity which increases 6 hr after irradiation. IAA produced by the action of IAA synthesizing system after irradiation will be destroyed by the IAA oxidase.

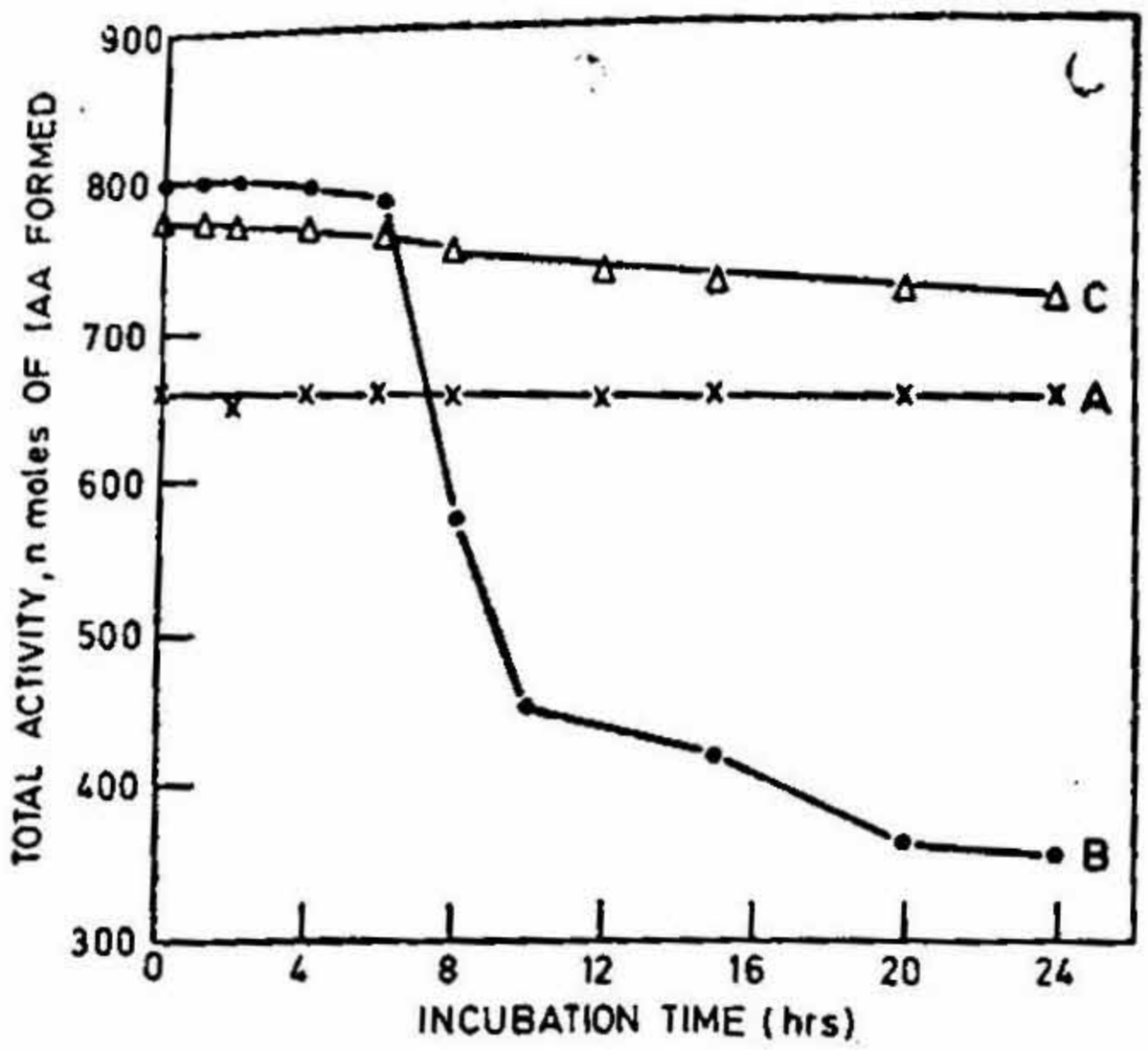


FIG. 4. Effect of irradiation and treatment with 10^{-3} M Indoleacetic acid on the IAA synthesizing system in excised potato bud tissue. Curve A: unirradiated; Curve B: irradiated ; Curve C: irradiated and treated with 10^{-3} M IAA.

Irradiation has brought about a 2-fold increase in IAA oxidase activity, which is dependent on the availability of H_2O_2 within 24 hr after irradiation.

5. Conclusion

Unlike GA, IAA is not able to break the dormancy in potatoes. Therefore, once the genetic material is repressed completely, *i.e.*, 6 hr after irradiation, IAA has no effect either on restoration of IAA synthesizing system or the reversal of sprout inhibition. IAA is known to regulate both RNA[42,44] and protein [45,46] synthesis in plants and it can stimulate mRNA synthesis also[47,48]. The evidence presented here will show that during a short time interval, *i.e.*, 7–8 hr after irradiation there is a derepression of potato genome enhancing the synthesis of new DNA, followed by messenger RNA and proteins. Two of the proteins synthesized during this period are characterized. One of them is PAL whose synthesis takes place in 3 hr after irradiation in the cytoplasm and the second one is amidating enzyme synthesis which is localized in the nuclei in 3 to 5 hr after irradiation. Apart from these, there is active synthesis of non-histone protein during initial phase and synthesis of histone 7 hr after irradiation. The active synthesis of protein ensued after irradiation may also produce some proteins which will function as repressors for the synthesis of key enzymes involved in sprouting process thereby extending dormancy. This hypothesis is depicted in fig. 5 with time sequence of events taking place in the nuclear fraction. There is definite evidence that at least one enzyme, *i.e.*, IAA synthesizing system, is affected by irradiation and its synthesis can be resumed under certain specific condition on supplementation

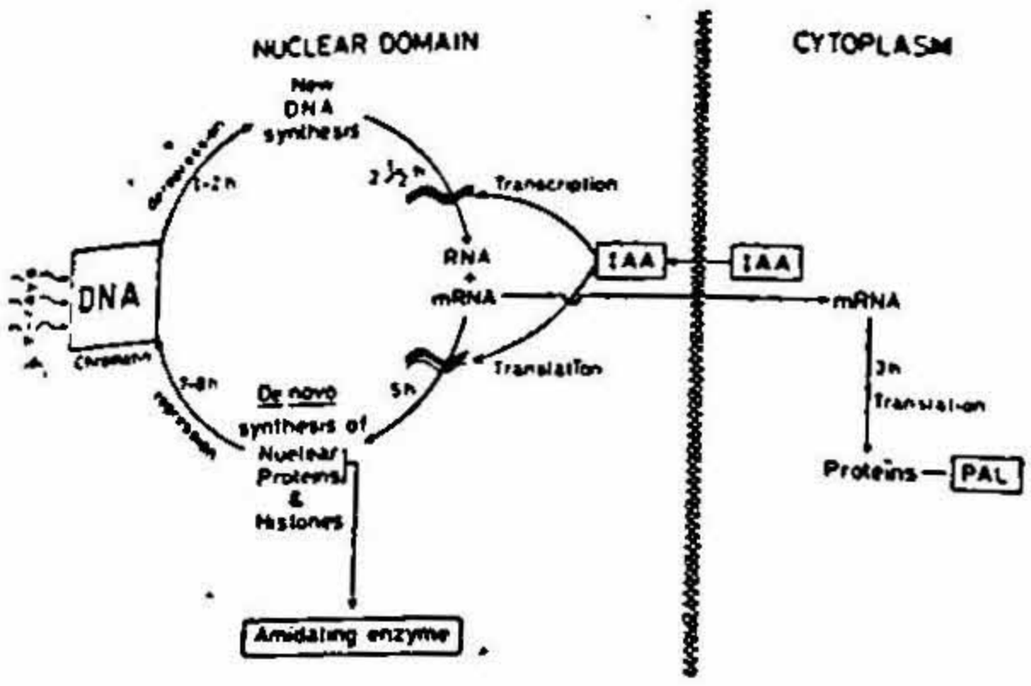


FIG. 5. Transient metabolic activation and sequence of events taking place in dormant potato bud nuclei after gamma irradiation culminating in permanent extension of dormancy. Treatment with indole acetic acid (IAA) within 6 hr after irradiation could reverse the sprout inhibition.

of IAA[12]. If the transient activation of the amidating enzyme in the nuclei has any metabolic significance in relation to the extension of dormancy in irradiated potatoes, the treatment with IAA should affect the appearance of enzyme activiy. This was found to be true[31]. Addition of IAA at the beginning of incubation inhibited the enzyme whereas a delay of 2½ hr produced no effect. This means that mRNA synthesis of this protein is altered by treatment with IAA. Therefore this observation indirectly supports the idea that the development of this enzyme has an important physiological function in sprout inhibition of the tuber by gamma rays.

It is possible that nuclear protein synthesis induced by gamma irradiation in potatoes may have a definite role in the regulation of dormancy. Recent evidence suggests that the molecules responsible for gene regulation are to be found among chromosomal proteins. Histone and non-histone proteins have significant regulatory role in transcription. Some kind of non-histone protein synthesis is associated with the induction of gene activity. The concentration of non-histone proteins was increased during active RNA synthetic phase ensued after irradiation (Table III). The changes in the type, quantities and various modifications of these proteins by phosphorylation and thiolation occur during the period of increased template activity for transcription. Ability of the amidating enzyme to amidate non-histone proteins isolated from irradiated potato bud efficiently attributes a definitie physiological function for this enzyme. Amidation may be considered as one of the methods for the modification of nuclear proteins. Thus it is quite conceivable that the amidation of nuclear proteins by amidating enzyme synthesized in the nuclei may have a definite role in the regulation of bud dormancy by repression of gene activity. The genes of the bud tissue may be permanently repressed by the histones whose synthesis is initiated 7 hr after irradiation, thereby extending dormancy indefinitely.

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