EFFECTS OF IONIZING RADIATION ON THE FORMATION OF RIBOFLAVIN IN SOYBEAN SPROUTS

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The effects of excessive ionizing radiation on biological systems involve mostly degradative results and changes in metabolic processes as well as eventual death. However, moderate doses of the radiation may stimulate the growth of living cells and enhance their metabolic activities especially in vegetative life.

In the present study irradiation of soybean with X-ray(300r, 600r, and 900r)was performed to observe the changes in its growth and its riboflavin content during its germination. Furthermore, the fractional determination of three forms of riboflavin, that is, FMN, FAD, and FR was carried out under a favorable dose(600r) of the radiation and control as well.

EXPERIMENTAL

1. Irradiation and sprouting: Soybeans (Glycine Max Merril) of even size were soaked in water for 12 to 15 houre at 25° C. A portion of the drained beans were irradiated with X ray, the other being the control. The dosages under study were as listed in Table I. The instrument used was X ray unit General Electric Co. Type 250III.

Table I Dosage of X ray

r	Kv	MA	Filter	Distance	Time of Irrad.
300	140	10	2mm-Al	50 Cm	7 min. 3 sec.
600	140	10	〃	〃	14 min. 6 sec.
900	140	10	〃	〃	21 min. 9 sec.

Both the treated and untreated (control) beans were placed in the sterilized containers with holes at bottom, covered with a black cloth, and allowed to germinate at room temperature (20-25°C). Wetering was made four times a day.

The weighed germinated beans(or cotyledons, hypocotyl, and tap roots separately) were soked in a warm water (80 C) for 3 to 5minutes, ground in a homogenizer, and transferred to a graduated vessel. The pH was adjusted to 4.5 with an acetic buffer. The solution was heated in a water bath(80°C)for 15-20 minutes, made up to 100 ml with distilled water, and thoroughly mixed. A

portion of the mixture (30 ml etc..) was centrifuged and the upper layer free of protein taken for the estimation of riboflavin.

One (or three) ml. of the respective solutions was placed in the tubes A and B. 1.0 ml. of a riboflavin standard solution (0.05 r/ml.) was added to the A tube and 1.0 ml of distlled water to the B tube followed by addition of 2.0 ml. N-NaOH to each tube. Each mixture was subjected to photolysis (40 minute exposure of a solution). To the resulting solutions in A and B O. 2 ml of the glacial acetic acid were added respectively. In the tube C 1.0 ml of the sample solutin, 1.0 ml of distilled water, 0.2 ml of glacial acetic acid and 2.0 ml of N-NaOH solution were pladec in this order. Then 6.0 ml of chloroform were added to A, B, and C respectively. After each tube was shaken in a cold water (over hundred times) 4.0 ml of the solution was taken in a cuvette for the fluorometry (photo volt-fluorometer: M 540).

The calculation was as follows.

$$0.05\times\frac{f_2-f_3}{f_1-f_2}\times\frac{b}{a}(\gamma/g)$$

$$0.05\times\frac{f_2-f_3}{f_1-f_2}\times\frac{b}{\text{number}}(\gamma/\text{grain})$$

Fractional determination: Various methods have been offered for the fractional estimation of riboflavins(1-7). In the present experiment a paperchromatographic procedure was employed for the purpose.

To the warm water extracted and deproteinized solution as described two ml of phenol were added and the solution was vigorously shaken and centrifuged. The flavin was shifted in the phenol layer. The phenol layer was removed by a pipet and the residue again extracted with phenol likewise. To the combined phenol 0.5 ml of distilled water was added, and the mixture was stirred. Ten to twenty ml of ether were added to it. The mixture was stoppered, cooled and vigorously shaken for one minute, and centrifuged. The water layer was separated in the bottom. The phenol-ether layer was discarded and again 5 ml of ether was added to the water layer. The mixture was stirred and centrifuged. Most of flavin was thus concentrated in the water layer.

The concetrate was submitted to the one dimensional paper chromatography in dark for 15—20 hours. The solvent was composed of n-butanol: acetic acid :water (4:1:5).

The fluorescent chromatograms were cut off, washed with ether and extracted with hot (80°C) water. The extracted were subjected to the determination of FAD, FMN, and FR. by the Lumiflavin fluorescent method. The calculation was:

$$FAD : FMN : FR = a : b : c$$

$$FAD = \text{Total}(\gamma/\text{grain}) \times \frac{a}{a+b+c}$$

$$FMN = \text{Total}(\gamma/\text{grain}) \times \frac{c}{a+b+c}$$

$$FR = \text{Total}(\gamma/\text{grainc}) \times \frac{c}{a+b+c}$$

RESULTS AND DISCUSSION

(1) **Growth and Total Riboflavin**: The daily changes in the riboflavin content of soybean on sprouting for seven days as well as their growth were observed as shown in Fig. 1 and 2. The growth rate was highest for the group of

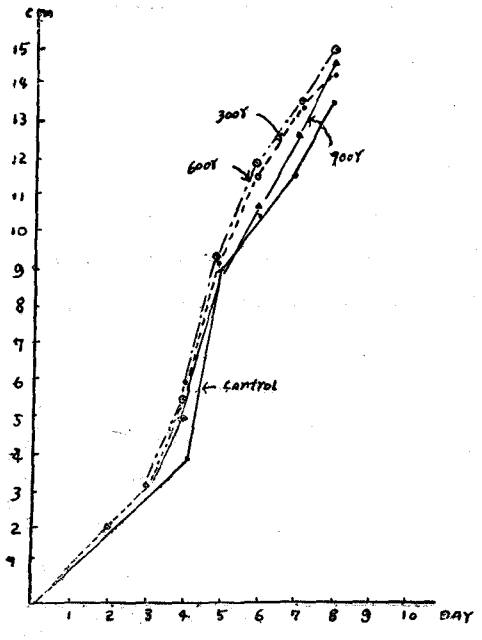

Fig. 1 Growth of Irradiated Saybean

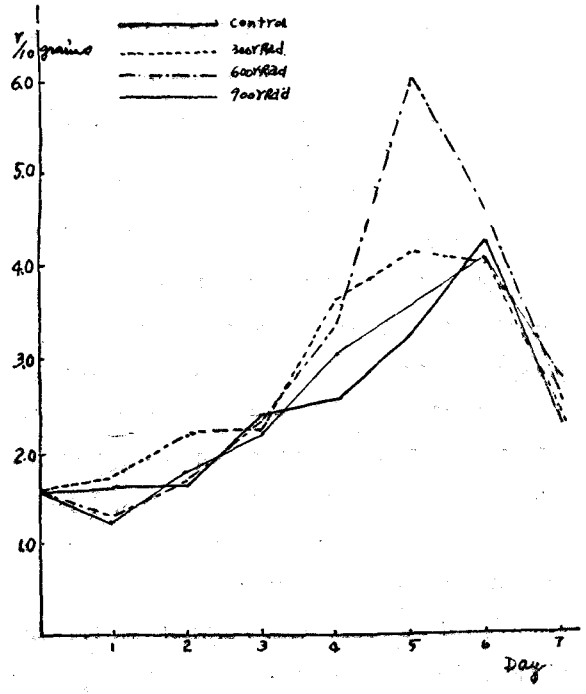

Fig. 2 Total Riboflavin Contents

300 r, those of 600 r and 900 r being the next in this order up to the first four days. After five days the growth rate of the group 600 r was marked. In general, the flavin contents increased with the growth of the seedlings, the control and the 900 r group showing their maximun values on the fifth day and the groups of 300 r and 600 r the sixth day.

The maximun flavin content was highest for the group 600 r about one and half times of others. The first day contents of riboflavin revealed that the higher does of X ray radiation inhibited the growth somewhat at the earlier stage. From the second day the gradual increase in the flavin contenti was

indicated for all groups up to the thsrd day when the contents were about the same. The flavin content after sixth day indicated a sudden decrease for all tbe samples.

The sectional distribution of riboflavin was found as indicated in Figures 3, 4, and 5. It seems apparent that riboflavin concentrated in the part of the cotyledon. It also suggests that the formation of riboflavin was performed mostly in cotyledon and transfered to the lower part of the sprout. The amount of riboflavin in the irradiated cotyledon especially those with 300 r and 600 r showed a rapid increase starting from about the third day and reached their maximal points followed by a rapid decrease. The does of 900 r afforded slow increment of riboflavin untill the sixth day just as the control. This fact explains that a moderate irradiation with X ray may provide an elevation of the riboflavin level in the cotyledon at the earlier stage. Both the hypocotyls and the tap roots whether the irradiated

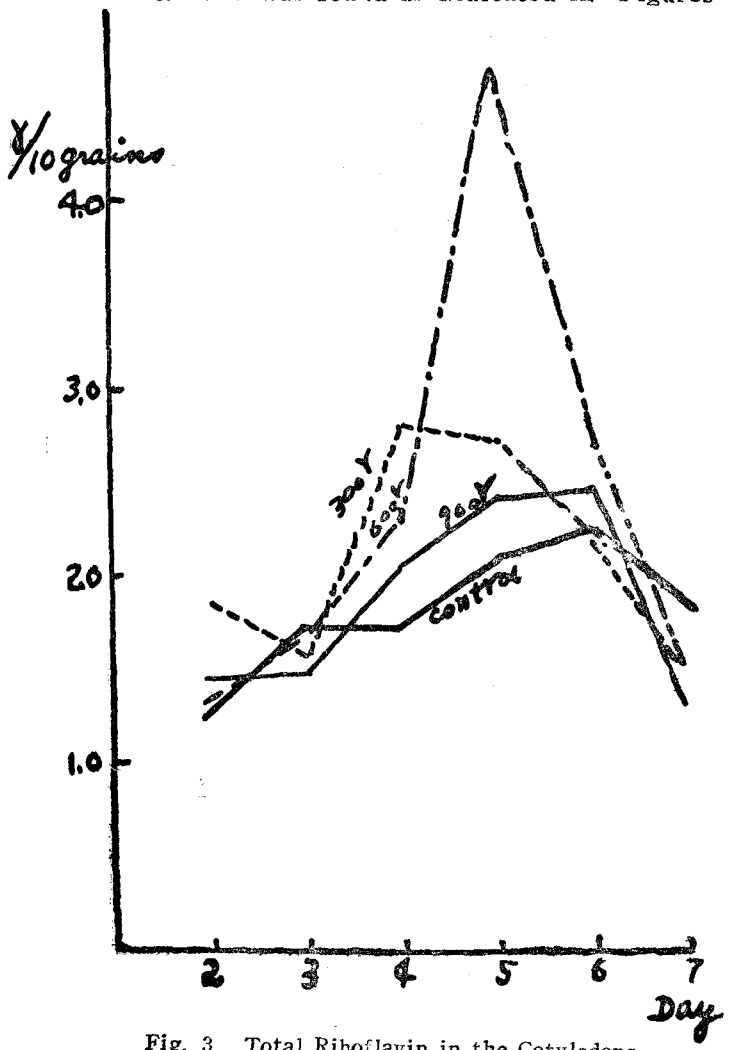

Fig. 3 Total Riboflavin in the Cotyledons

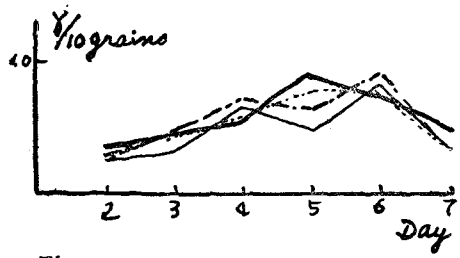

Fig. 4 Total Riboflavin in the hypocotyl

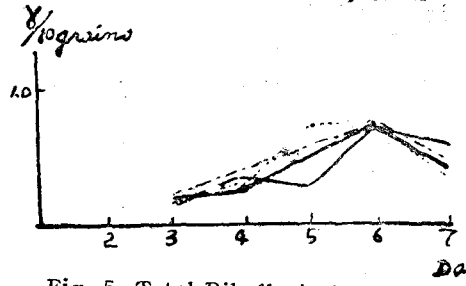

Fig. 5 Total Riboflavin in the tap roots

or the control exhibited no significant change in the flavin content during the seven day period of germination.

(2) **Determination of Separate Forms of Riboflavin**; In this study the dose of 600 r was taken to observe the quantiative changes of three forms of riboflavin in soybean on sprouting. The control gave the following result (Fig 6). As clearly indicated the major part of total riboflavin in the sprout comprises FAD and therefore the changes in the amount of total riboflavin in the sprout must follow the pattern of FAD change therein. Practically the amounts and changes of both FMN and FR are insignificant as shown in the Fig. 7.

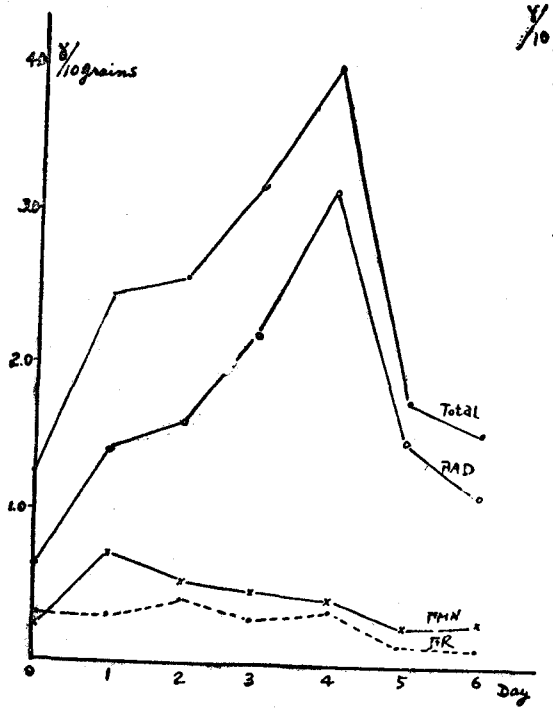

Fig. 6 Variations Riboflavin in Soybean Sprouts (Controls)

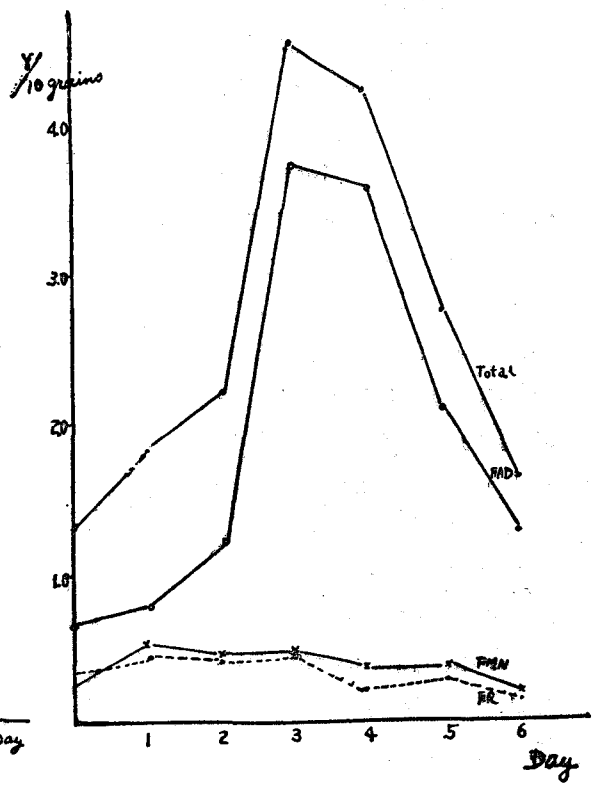

Fig. 7 Variations of Riboflavin in 600 r IrradiatedSoybean Sprouts

The irradiation (600r) also gave a similar result except its rapid and somewhat more increase in the FAD content as shown in Fig. 7. The irradiation considered to give effect mainly to the FAD content in the sprout during its germination.

For comparison results of analysis of FAD, FMN, and FR in soybean (before being soaked) was given below.

FAD	FMN	FR	Total
0.65(44%)	0.58(33%)	0.34(23%)	1.47r/10 grains

Ratio of three forms of riboflavin in soybean itself is not so notable upon germination; however, the increment of FAD is remarkable as well as the decrease, while the amounts and changes of both FMN and FR are rather in steady state.

Summary

1) The variations of flavin adenine dinucleotide(FAD), flavin monomucleotide (FMN) and free riboflavin in controll and 600r X-ray irradiated soybean sprout were estimated by the paper chromatographic method.
2) It was found that the formation of riboflavin in soybean sprout was mainly in FAD.
3) There were no great variation of FMN and free riboflavin in soybean sprout during the germination.
4) The moderate amount (600r) of X-ray irradiation accerelated the formation of FAD in soybean sprout during the germination.

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