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RNA Synthesis in 2,4,5-T-induced Tumors in Bean Embryos

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The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has proven to be a highly effective agent in the control of broad-leaf species in rice fields, grasslands, and forests. It has been investigated for teratogenicity and fetotoxicity in animals. Exposure of 2,4,5-T on mice during pregnancy created congenital malformation (COURTNEY & MOORE 1971; HOOD et al. 1979). Studies in rats (SPARSCHU et al. 1971) and monkey (DOUGHERTY et al. 1976) also indicated that 2,4,5-T is teratogenic.

The extensive usage of 2,4,5-T in agricultural and managed forest lands, and its potential health hazard lead us to study the biochemical effects on the ribonucleic acid (RNA) synthesis in bean embryos cultured in vitro.

MATERIALS and METHODS

Bean (Phaseolus vulgaris L. var Romano) seeds were purchased from Burpee Seed Company, Philadelphia, PA. 2,4,5-T was purchased from Carolina Biological Supply, Burlington, NC. All other chemicals were obtained from Sigma, St. Louis, MO. ^{3}H-uridine, Protosol and Omnifluor were purchased from New England Nuclear, Boston, MA. Radioactivity of samples was measured in Ready Solv HP scintillation cocktail and counted by a Beckman LS-7500 liquid scintillation counter.

The bean seeds were surface-sterilized, and embryonic axes were excised asceptically. They were placed in petri plates containing Hildebrandt's medium (HILDEBRANDT et al. 1946) supplemented with 2,4,5-T (4 mg/L) or non-supplemented medium. The concentration of agar was 0.7%. The 2,4,5-T was added to the medium before autoclaving. The culture plates were kept in the dark at 25 C. Samples were removed from these cultures at appropriate times of incubation for RNA synthesis studies.

The rate of RNA synthesis was determined by following the incorporation of ^{3}H-uridine into trichloroacetic acid (TCA)-insoluble material as described by MINOCHA (1979). After an appropriate incubation period in the growth media, batches of 10 embryo axes were placed in 20 mL Hildebrandt's liquid medium containing 20 uCi ^{3}H-uridine (Sp. Act. 40-50 Ci/mmole) for 20 min. This was followed by a 15 min chase in a similar liquid medium containing 3 x 10^{-4} M non-radioactive uridine. After the chase, batches of 10 embryo

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axes were placed in ice-cold 85% methanol for 5 min before repeated ethanol-diethyl ether washing steps. A modified method of HOLDGATE & GOODWIN (1965) was used to wash the embryos in order to remove lipids and nucleotides (FRASER & LOENING 1974). Embryos were washed 5 min each with the following solutions: 5% TCA (x2), 0.05 N formic acid in 85% methanol (x3), 80% ethanol, absolute ethanol, absolute ethanol: diethyl ether (1:1), and 100% diethyl ether. All washing was performed at 4 C. Ether washed embryos were air-dried and hydrolyzed in 6 mL of 0.5 N perchloric acid for 2 h at 70 C. Radioactivity was determined by counting 0.5 mL of perchloric acid extract directly in 10 mL of Ready Solv HP (Beckman) cocktail.

For uptake of ^{3}H-uridine studies, batches of 3 embryos were incubated in pulse medium (^{3}H-uridine, 20 uCi/10 mL) for 20 min and then washed twice in distilled deionized water for 5 min. The tissue was homogenized in 5 mL of 70% ethanol, and 0.5 mL aliquots of this homogenate were incubated in glass vials with 1 mL of Protosol each for 4 h at 50 C. After incubation the homogenate was mixed with 10 mL of a toluene based cocktail containing Omnifluor (4 g/L) and counted.

RESULTS and DISCUSSION

The 2,4,5-T produced tumors in bean embryo axes. The whole embryonic axes were transformed into a mass of disorganized callus. The hypocotyl and epicotyl portions of the embryonic axes were affected most. The first pair of leaves were not affected as far as callus formation is concern but the normal growth was inhibited. This tumorous growth in bean embryos demonstrate clearly that 2,4,5-T is very sensitive to differentiating tissue. Furthermore, it caused an active proliferation of cells and inhibited root and shoot elongation. It also increased the water uptake and permeability.

The rate of ^{3}H-uridine incorporation in TCA-insoluble material by the embryos at different periods of incubation is shown in Fig. 1. The control embryos showed one distinct peak of RNA synthesis at 80 h whereas a small peak of RNA synthesis was noted at 56 h. The tumors showed two distinct peaks at 56 and 80 h. The rate of RNA synthesis in tumors showed 16% increase over control at 56 h. However, at 80 h the rate of RNA synthesis was almost the same in control and tumor tissue.

The newly synthesized RNA at 56 h in tumor tissue showed a rapid degradation at 64 h, which indicates that ribonucleases are very active at this period of growth. This could be interpreted as a rapid synthesis of ribonucleases or a synthesis of a short-lived RNA. However, after 64 h the tumor tissue showed tremendous burst of RNA synthesis at a steady rate of 72 and 80 h. This could be interpreted as enhanced activities of RNA polymerases and an inhibition of ribonucleases activities, or stabilization of RNA. On the other hand, the control bean embryos showed a steady rate of degradation of RNA until 72 h since the peak of RNA synthesis at

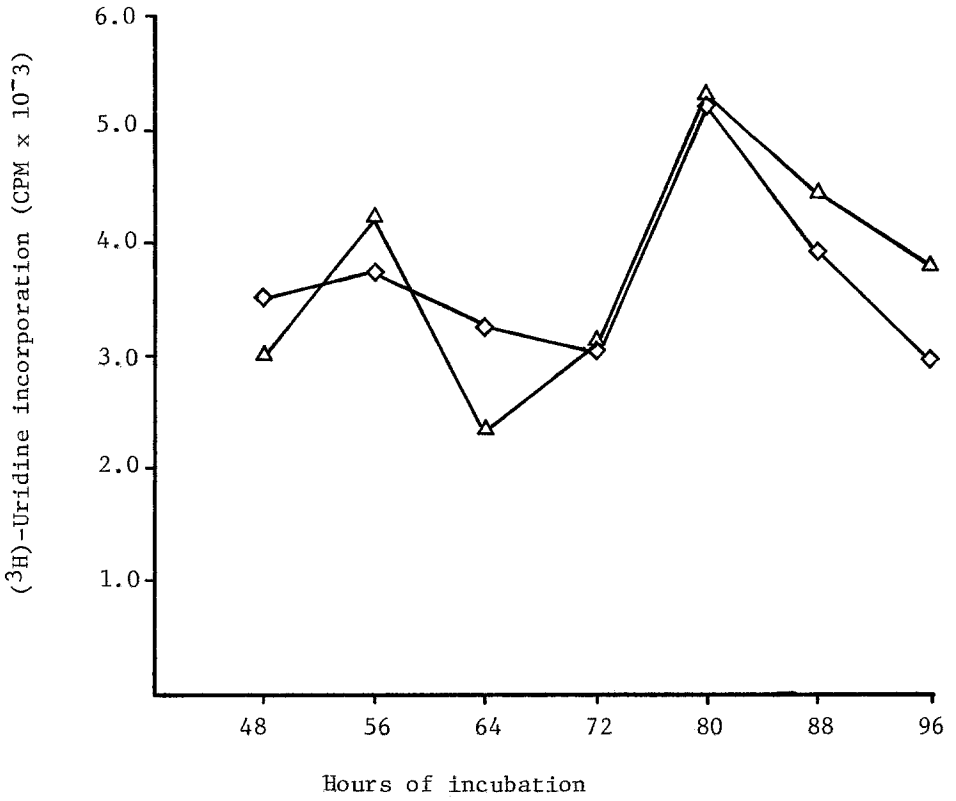

Fig. 1. Effect of 2,4,5-T on the rates of incorporation of ^{3}H-uridine into TCA-insoluble material in Phaseolus vulgaris embryo axes at various times of incubation. Pulse and Chase incubations were for 20 and 15 min, respectively. Each point represents the means ± S.E. of at least 3 replicates containing 10 embryos each. Control (—◇—) and 2,4,5-T (—△—).

56 h. After 72 h the rate of RNA synthesis increased 67% over at 80 h and then dropped sharply at 96 h where it reached almost at the same point as 72 h.

Uptake of ^{3}H-uridine studies showed a tremendous capacity for tumors to adsorb labeled precursor (Table 1). As the surface area increased due to growth in tumor and control tissue, the rate of uptake was also increased. The tumor showed a greater adsorptive capacity over control tissue. However, with ethanol-diethyl ether series of washings, 99% of the radioactivity was lost indicating that radioactivity was adsorbed on the surface of the cell walls rather than incorporated inside the cell. Thus, the results obtained in the rates of ^{3}H-uridine incorporation were a true reflection of labeled precursor incorporated in RNA.

Table 1. Effect of washing on the uptake of ^{3}H-uridine. The embryos were incubated in ^{3}H-uridine for 20 min and washed with sterile distilled water or perchloric acid as described in text. Embryos were then homogenized in 70% ethanol and a 0.5 mL aliquot from each batch was incubated in 1 mL of Protosol for 4 h at 50 C, and counted. Each value represents CPM/3 embryos.

	Age of tissue			
	48 h		72 h	
Treatment	Water Wash	Perchloric acid Wash	Water Wash	Perchloric acid Wash
None	7798	51	19,931	63
2,4,5-T	6998	68	26,418	69

2,4-Dichlorophenoxy acetic acid has been utilized extensively in plant tissue culture work as a substitute for indoleacetic acid (IAA) in promoting growth. Many reports have been published where 2,4-D produced an increase in various species of RNA (KEY & HANSON 1961; KEY et al. 1967; HARDIN & CHERRY 1972). It also enhanced the chromatin-bound RNA-polymerase activity (GUILFOYLE et al. 1975; GUILFOYLE & HANSON 1974). Recently, a cell-free translation technique demonstrated clearly that auxin increased the level of a specific messenger RNA (m-RNA), (VERMA et al. 1975). However, very little is known about the mode of action of 2,4,5-T on the plant tissue, particularly on the embryonic tissue.

Very recently, KUNG (1981) hypothesized that an imbalanced amount of cytokinin and auxin could trigger tumor formation. This hormone imbalance enhanced the synthesis of a specific group of proteins which specifically promote cell division but not differentiation. This 2,4,5-T induced tumor could be an excellent system to test this hypothesis. Furthermore, the tumorigenic properties of 2,4,5-T deserve more extensive investigation before the herbicide is considered safe for use.

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