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TESTING FOR THE MUTAGENICITY OF IRRADIATED PAPYAS TO RATS IN A HOST-MEDIATED ASSAY WITH *Salmonella typhimurium* AS INDICATOR ORGANISM

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TESTING FOR THE MUTAGENICITY OF IRRADIATED PAPAYAS TO RATS IN A HOST-MEDIATED ASSAY WITH *Salmonella typhimurium* AS INDICATOR ORGANISM

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I. INTRODUCTION

Treatment of food with ionizing radiation can both decrease the economical losses associated with storage and transportation of food and lower the probability of spreading pests from one area to another. However, most foods are complex mixtures of substances that might undergo chemical changes during irradiation, leading to formation of toxic compounds, some of which might be mutagens or potential mutagens (Chopra et al., 1963; Holsten et al., 1965; Shaw and Hayes, 1966). Thus the large-scale use of irradiation to process food requires adequate scientific data to permit the evaluation of its safety for the consumer.

Assay systems for the detection of chemical mutagens utilize indicators ranging from microorganisms to mammals. Chromosomal damage is relatively easy to score in mammalian systems, e.g. human lymphocytes and any other mammalian tissue, both in vitro and in vivo; but it is difficult and time-consuming to score for gene mutations in any mammalian system in vivo (Russell, 1951, 1967). There are a number of microbial systems, however, in which it is possible to score for gene mutations quantitatively (de Serres and Osterbind, 1962; Ames, 1971).

A mammal can either detoxify a given mutagenic compound or form mutagenically active metabolites from nonmutagenic compounds. When the host-mediated assay (Gabridge and Legator, 1969) is used, in which an indicator organism is injected into the peritoneal cavity of rodents, the indicator organism is exposed not only to the original compound but also to its metabolites. When the indicator organism is recovered and checked for the presence of mutations, it is possible to determine whether any mutagenic compounds or metabolites were present during incubation of the indicator in the host animal.

We have used the induction of reverse mutation in a histidine-requiring mutant (G-46) of Salmonella typhimurium as an indicator in a host-mediated assay with rats to study whether irradiation of papaya causes formation of mutagenic compounds or substances which might form mutagenic metabolites. The rats were fed irradiated papayas for a period of 8 to 12 weeks prior to the injection of the bacteria. In such experiments it is important to have positive controls. We have used dimethylnitrosamine (DMN) as a positive control, because this compound was shown earlier to induce reversions in strain G-46 in the host-mediated assay (Gabridge and Legator, 1967) but not in vitro under nonhydroxylating conditions (Malling, 1971).

II. MATERIAL AND METHODS

Rat strain. — In all experiments Sprague-Dawley rats were used. They were furnished from Charles Rivers and were approximately 15 weeks old.

Indicator organism. — S. typhimurium strain G-46 is a histidine-requiring strain, and mutants were scored as reversions to histidine independence.

Preparation of cultures. — S. typhimurium stock cultures were maintained on nutrient agar slants at 4°C. The cultures used during experiments were grown in Trypticase Soy Broth with 1% yeast extract added.

Sixteen hours prior to the injection of the organism into the rats, a 100-ml flask containing 50 ml of Trypticase Soy Broth enriched with 1% yeast extract was inoculated with S. typhimurium G-46 taken from a nutrient agar slant. The culture was grown overnight at 37°C. Ninety minutes prior to rat inoculation, 5 ml of the overnight culture was injected into 50 ml of fresh Trypitcase Soy Broth at 37°C. This final 90 min of growth in fresh medium gave a logarithmic culture of the organism.

Injection of rats with bacteria cells. — Each rat was inoculated intraperitoneally with 5 ml of the S. typhimurium suspension taken from the logarithmic growth flasks. The site of injection (located on the abdomen close to the left hind leg) was saturated with a 70% ethanol solution. A 26-gauge needle was inserted through the skin and abdominal wall. After injection the animals were returned to their cages for 3 hr prior to the removal of the Salmonella.

Host-mediated assay with DMN (positive control). — A positive DMN control was included in each experiment to demonstrate that the assay system to detect

mutagenicity was functioning properly. The animals in this test group were treated exactly like the other animals, except that each received 100 mg/kg DMN by an intramuscular injection in the left hind leg of 0.1 ml of a stock solution of DMN in water. Immediately afterwards, the bacterial cells were injected into the peritoneal cavity.

Recovery of Salmonella from rats. — Prior to the recovery of the Salmonella, each rat was suffocated with carbon dioxide and its abdominal area was saturated with 70% ethyl alcohol. The cavity was then exposed by cutting through the outer skin and peritoneal membrane. The peritoneal fluid was removed from the cavity using a 1-ml T.B. syringe without a needle. Approximately 2 ml of fluid was collected in sterile 13- X 100-mm test tubes and placed in an ice bath.

Plating of the samples. — The revertants and survivors were scored according to the protocol of Ames (1971). In brief, tenfold serial dilutions of the samples were made in saline. For detection of reversions, 0.4 ml of the undiluted sample was added to 2 ml of soft molten agar containing no histidine. This agar was then placed on the surface of a 15 X 100-mm plastic petri plate containing 20 ml of minimal medium. The total number of viable bacteria was counted by adding 0.2 ml of the 10^{-6} dilution to 2 ml of soft molten agar containing histidine (39 mg/liter). This agar was then poured on the surface of a petri plate containing minimal medium. All plating procedures were done in duplicate. The plates were incubated at 37°C for 48 hr before the colonies were counted.

Preparation of the papayas. — Irradiation, preparation, and freezing of the fresh papayas used in these experiments have been described in the report

"Two Year Chronic Oral Toxicity Study with Low-Dose Irradiated Papayas in Albino Rats " [final report of AEC Contract No. At (11-1)-2014]. Fresh papayas were mashed and frozen until needed to ensure a continuous supply. The purée was not dried, cooked, or otherwise changed. It should be emphasized that this handling differs somewhat from the way irradiated papayas normally would be used for human consumption.

Feeding protocol for rats. — The amount of frozen papayas mixed into the rat food and the handling of the rats is described in the report mentioned above. In brief, the papaya diet contained 15% frozen papayas based on net weight. The animals were cared for and fed in the Industrial Bio-Test Laboratories, Inc., 1810 Frontage Rd., Northbrook, Ill. 60062

III. RESULTS

Four groups of rats were set up with differing diets as shown in Table 1. The animals were fed the prescribed diets for 8 to 12 weeks prior to the host-mediated assay. For each experiment, five test groups were set up according to Table 2. All animals were maintained under the same conditions, with their diets being the only variant.

In Table 3 the results of the first experiment are given. The number of reversions per 10^6 survivors in the positive DMN control was 39 times greater than the number of reversions in the normal food control. In the other three treatments—normal food + nonirradiated papayas, normal food + irradiated (75 kr) papayas, normal food + irradiated (210 kr) papayas—the mutation

frequency was not significantly different from the mutation frequency from normal food.

Table 3 also shows the results of the second experiment, in which the procedures of Experiment 1 were duplicated; the positive DMN control displayed a mutation frequency 30 times greater than the normal food control. As in Experiment 1, the diets containing irradiated and nonirradiated papayas produced mutation frequencies within a similar range, which did not differ significantly from the frequency found among bacteria injected into rats that were fed normal food and did not receive the injection of DMN.

IV. DISCUSSION

There have been numerous reports in the published literature dealing with the adverse biological effects of irradiated food products. Cytotoxic effects have been observed in pineapple juice immediately after gamma-irradiation with doses up to 500 krad (Mäkinen, Upadhya, and Brewbaker, 1967). Rinehart and Ratty (1967) have demonstrated a small but consistent increase in the sex-linked recessive lethality in *Drosophila* grown on food irradiated with heavy doses of X-rays.

Genetic tests for mutagenicity of irradiated food have given both negative and positive results (Scarascia-Mugnozza *et al.*, 1965). The discrepancies between the results may be explained on the basis of the complex biochemical nature of the interactions of the products involved, or the nature of the testing systems for mutagenicity.

In the present experiments, the host-mediated assay showed no increase in the frequency of histidine revertants of S. typhimurium (G-46) incubated in the peritoneal cavity of rats fed diets containing irradiated papayas over nonirradiated controls.

Host-mediated tests similar to the one used in this work are quick, simple, and highly suitable for screening purposes. They detect not only mutagenic compounds formed during the irradiation of the food but also any mutagenic breakdown products which might be formed during the metabolism of the food by the mammalian host. The tests are flexible and applicable to a wide range of animal species and microbial indicators. However, the particular strain of S. typhimurium (G-46) used here can revert either by direct mutation or by a variety of suppressor mutations. It can be reverted by a variety of alkylating agents and by 2-aminopurine (which causes only transitions) (Ames, 1971). G-46 will not, however, revert by insertion or deletion of base-pairs (Ames, 1971). If irradiation of papayas produces compounds which are able to induce base-pair substitutions, the presence of such compounds is likely to be detected by G-46. On the other hand, if radiolytic products are formed which induce only base-pair insertions and deletions, they would not be detected by using this strain.

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TABLE 1. Diet schedule for 12 weeks prior to host-mediated experiments

Diet	Number of rats
Normal food	20
Normal food + nonirradiated papayas	20
Normal food + irradiated (75 kr) papayas	20
Normal food + irradiated (210 kr) papayas	20

TABLE 2. Treatment schedule showing positive DMN* control used during experiments

Treatment	Number of rats
Normal food + 100 mg/kg DMN†	3
Normal food	3
Normal food + nonirradiated papayas	3
Normal food + irradiated (75 kr) papayas	3
Normal food + irradiated (210 kr) papayas	3

* DMN = dimethylnitrosamine.

† Intramuscular injection.

TABLE 3. Effect of feeding diets containing unirradiated and irradiated papayas to rats on the hisitidine reversion frequency among cells of *Salmonella typhimurium* (G-46) injected into rats*

Experiment	Treatment		DMN† (mg/kg)	Surviving colonies observed (dilution, 4×10^{-7})	Number of revertants observed	Reversions per 10^8 survivors
1	Normal food	Rat 1	0	259	0	0
		Rat 2	0	606	4	0.33
		Rat 3	0	411	3	0.36
	Normal food + nonirradiated papayas	Rat 1	0	583	4	0.34
		Rat 2	0	313	3	0.47
		Rat 3	0	554	1	0.09
	Normal food + irradiated (75 kr) papayas	Rat 1	0	261	2	0.38
		Rat 2	0	448	3	0.33
		Rat 3	0	330	1	0.15
	Normal food + irradiated (210 kr) papayas	Rat 1	0	440	0	0
		Rat 2	0	829	3	0.18
		Rat 3	0	1142	5	0.21
	Normal food	Rat 1	100	227	45	9.9
		Rat 2	100	347	55	7.9
		Rat 3	100	430	77	8.9

Table 3 (Continued)

Experiment	Treatment		DMN† (mg/kg)	Surviving colonies observed (dilution, 4 X 10^{-7})	Number of revertants observed	Reversions per 10^8 survivors
2	Normal food	Rat 1	0	640	4	0.31
		Rat 2	0	483	3	0.31
		Rat 3	0	902	3	0.17
	Normal food + nonirradiated papayas	Rat 1	0	330	2	0.30
		Rat 2	0	518	3	0.29
		Rat 3	0	479	4	0.42
	Normal food + irradiated (75 kr) papayas	Rat 1	0	527	4	0.38
		Rat 2	0	683	3	0.22
		Rat 3	0	678	5	0.37
	Normal food + irradiated (210 kr) papayas	Rat 1	0	801	5	0.31
		Rat 2	0	704	5	0.36
		Rat 3	0	659	3	0.23
	Normal food	Rat 1	100	793	135	8.5
		Rat 2	100	1028	164	8.0
		Rat 3	100	752	121	8.1

*Experiment 1, 10 weeks' feeding; Experiment 2, 12 weeks' feeding.

†DMN = Dimethylnitrosamine

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