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Report of

SHORT-TERM HUMAN FEEDING STUDIES OF FOODS STERILIZED BY GAMMA RADIATION AND STORED AT ROOM TEMPERATURE

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OBJECT:

To determine the nutrient content and to test for the appearance of decomposition products in foods which have been treated with ionizing radiations and stored 3 months at room temperature; and to test the acceptability and possible acute toxicity of the foods in human subjects.

SUMMARY AND CONCLUSIONS:

Eighteen foods, including 5 meats and meat products, 6 vegetables, 6 fruits and fruit products, and one cereal, were frozen, irradiated at the following levels (rep), and subsequently stored 3 months at room temperature: potatoes, 10,000; flour, 75,000 - 80,000; oranges, 150,000; all others, 2,500,000 - 4,000,000. The control foods were similarly frozen, but were stored in the deep-freeze. Three alternate diets were prepared in which the irradiated (or the identical control) foods constituted 80% of the total calories; they were fed to 13 volunteer human subjects during 15 days of control diets and 15 days of irradiated food diets. The foods contained no soluble substances toxic to mice; no clinical abnormalities were noted in the human subjects. Irradiation decreased the thiamine and ascorbic acid content and increased the "browning reaction" derivatives, fat soluble carbonyl compounds, and thiobarbituric acid reactants. Except for string beans and orange juice, no significant differences in acceptability were noted between the irradiated and control foods.

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INTRODUCTION

As part of a large scale program initiated to evaluate the use of radiation in food preservation, human feeding studies of the nutritional aspects and possible toxic effects of irradiated foods have been conducted periodically on the Metabolic Research Ward of the U. S. Army Medical Nutrition Laboratory (1,2,3,4). To date, four human feeding studies of the acceptability, digestibility, available metabolizable energy, and toxicity of some 42 different foods sterilized by gamma radiation and stored frozen until use have been completed (1). In addition, one irradiated food (pork), stored at room temperature for approximately one year, was evaluated in a short-term feeding study (4). Thus far there have been no discernible toxic effects attributable to irradiated foods, whether stored frozen or at ambient temperature. Differences in acceptability of some of the food items have been noted in previous reports (3,4).

The present report describes the results of the sixth and seventh short-term human feeding studies of irradiated food items. Eighteen different foods were tested in these studies, all stored at room temperature for approximately three months after irradiation. This brings the number of food items that have been irradiated and stored at room temperature prior to testing to a total of nineteen. All of these foods were found to be bacteriologically sterile and nontoxic in animals prior to use.

METHODS

Test Subjects

Thirteen young men served as test subjects in the present studies; six of them participated in both tests. They were conscientious objectors who had volunteered for this program as a means of fulfilling their Selective Service obligation. All of the men were between 20 and 23 years of age and had passed a careful medical examination prior to admission. During the study, the men lived on the Metabolic Ward.

General Plan

Each study was approximately 30 days in length. During November 1957, each of ten subjects received an irradiated food diet for two 7-day periods alternating with two control diet periods. Half of the group was on the irradiated diet during any one period while the other subjects were receiving the control diet.

During March 1958, each of 9 subjects received an irradiated food diet for one 15-day period and a control diet during the other period. Again, the subjects were divided into two groups, each group received irradiated food for one of these periods while the other received the control diet.

Thus, each subject was given irradiated food for 15 days and served as his own control for the other 15 days.

Foods

Test foods, both irradiated and control, were received through the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois. Most of the food items were canned; half of each lot was subjected to varying doses of gamma radiation at either Idaho Falls or Dugway, Utah, and subsequently stored at room temperature approximately 3 months prior to testing.

During November 1957, 8 different food items were tested (Table 1). All test foods, both irradiated and control, came from the same lot, except control potatoes which were procured locally. Three rotating menus were used, which supplied approximately 80% of the calories from irradiated foods (Table 2).

During March 1958, 14 different food items were tested, four of which were also fed during the previous study (Table 1). All test foods, both irradiated and control, came from the same lot, except control potatoes and cabbage (for coleslaw) which were procured locally. Again, three rotating menus were used, with approximately 80% of the calories being derived from irradiated foods (Table 3). Non-test foods, which were used to supply the balance of calories in both studies, were procured locally.

Since these were not balance studies, every attempt was made to serve the food items in forms that would be most acceptable to the subjects.

Bacteriologic and Animal Toxicity Tests

All cans of processed food, both control and irradiated, were tested. The raw foods, white potatoes and oranges, were sampled at random. The irradiated coleslaw was sampled by random selection of several polyethylene bags. The control coleslaw was prepared from cabbage purchased locally and was not tested bacteriologically. The cans of flour, both control and irradiated were tested by random samplings.

Each sample was tested for sterility by the following procedures: After mixing, approximately 1 gram was placed in each of four tubes of fluid thioglycolate medium. Two of the tubes were placed in a water bath at 70°C. for 30 minutes to destroy vegetative bacterial cells, but not spores. All tubes were then incubated at 37°C. and observed for eight days. On the 4th day of incubation all tubes were subcultured to fresh thioglycolate medium, and smears were prepared. On the 8th day of incubation samples of both original cultures and subcultures were again examined by Gram stained smears and subcultured on 5% blood agar plates. All smears and cultures were negative.

Exotoxin assays were performed in the following manner: To 10 grams of each food sample was added 10 to 15 ml. of sterile distilled water, and the material shaken for 10 minutes. One-half ml. of the supernatant was then injected intraperitoneally into each of five CF-1 white mice

weighing 20 to 25 grams. All mice were observed for an eight-day period, and were then again injected intraperitoneally with 0.2 ml. of the thioglycolate cultures and observed for another period of four days. None of the mice suffered any ill effects from the injections of extracts or cultures from irradiated food samples.

Chemical Analyses

Proximate chemical analyses were performed on random samples of the individual test foods, both control and irradiated. Analytical methods were those of the A.O.A.C. (5) as utilized by this laboratory. Bomb calorimetric studies were done by a standard method as modified by Cleland and Harding (6). In addition, determinations of thiobarbituric acid reactants (7), carbonyl containing compounds (8), non-protein nitrogen (9,10), and the content of several vitamins (11,12), were performed on these samples.

Clinical Examinations

Each man received a physical examination, electrocardiogram and chest x-ray before and at intervals after each irradiated food study. The subjects were weighed each morning upon arising. Blood pressure, pulse, repsiration and body temperature were measured several times daily. Each man was encouraged to report any symptoms which might have bearing on the tests.

Clinical Laboratory Tests

Before, during and at intervals after feeding of irradiated food, the following clinical laboratory tests were carried out:

Hematology: hemoglobin (13), hematocrit, total and differential white blood cell count (14).

Liver function: 45 minute bromsulfalein retention (15), both total and one-minute serum bilirubin (16), thymol turbidity (14), alkaline phosphatase (17), and prothrombin time (18).

Renal function: complete urine analysis and phenolsulfonphthalein excretion tests (14).

Urinary Excretion Tests

In an effort to further assess possible toxic effects of irradiated foods on humans, several urinary excretion studies were performed. Since many foreign substances are frequently detoxified by the liver and excreted in combination with glucuronic acid, urine glucuronides were measured (19). In addition, determinations of the urinary excretion of total ketones (20), nitroprusside-reacting substances (21), and indophenol reducing substances (22,23) were performed. Total urine organic acidity

was measured by a modification of the method of Van Slyke (24).

Acceptability

Acceptability data on irradiated foods were obtained in two ways. Several times during each feeding period, the subjects noted their reaction to each individual food in the diet on the five-point hedonic scale developed by the Quartermaster Food and Container Institute (Table 4). In addition, each man was interviewed at the end of the study in order to obtain his impressions about any differences noted in color, texture, odor and flavor of the food items, during the feeding periods.

RESULTS

Clinical Examinations

Physical examinations revealed no changes that could be attributed to the ingestion of irradiated food. Chest x-rays and electrocardiograms remained essentially unchanged from normal controls obtained prior to testing. Fluctuations in weight, blood pressure, pulse, respiration, and body temperature remained within normal limits except during those periods when the subjects had seasonal respiratory tract infections, influenza and other unrelated disorders.

Clinical Laboratory Tests

The series of tests that were performed at various intervals before, during, and after the feeding of irradiated food showed no significant changes in any of the subjects (Appendix). Although there were occasional results that might be considered outside the normal range, these variations did not appear to be of clinical significance and could not be related to the ingestion of irradiated food.

Urine Excretion Tests

Determinations of urinary excretion products revealed no significant differences between the average values for the control and irradiated food periods in the case of total glucuronides, total ketones, total organic acidity, and nitroprusside-reacting substances (Figures 1 and 2). The excretion of indophenol-reducing substances was significantly increased ($p < .005$) during the irradiated food periods of the November study, but, on the other hand, was significantly decreased ($p < .001$) during the feeding of irradiated food in the March study.

Chemical Analyses

Proximate analysis of irradiated and control food samples revealed some minor differences in nutrient composition, but no consistent alterations were apparent (Tables 5 and 6). The gross caloric content of the foods appeared essentially unchanged as a result of irradiation.

The content of several vitamins, particularly thiamin and ascorbic acid, appeared to be somewhat reduced in many irradiated foods (Table 7). Thiobarbituric acid reaction values, presumably an index of aldehyde formation from certain lipids, were frequently elevated in irradiated foods. Compounds containing carbonyl groups were markedly increased in irradiated foods containing a relatively high proportion of fat. No increase in the non-protein nitrogen content of irradiated foods was observed (Table 7).

Acceptability

Statistical analysis of acceptability ratings for irradiated and control foods revealed significant differences in the average values of string beans and orange juice (Table 8). In both of these instances the control food was preferred. In general, most vegetables and fish items received the higher ratings, whether control or irradiated, indicating a dislike for these foods on the part of this group of subjects.

Individual interviews with the subjects revealed that they had no difficulty in distinguishing the feeding periods of control and irradiated food. Observations of the subjects concerning differences between irradiated and control food items are summarized in Tables 9 and 10. It is apparent that the majority of subjects noted differences in color, flavor, texture, and odor of all foods except items made with potatoes and flour. These differences were more consistently obvious in the case of green beans, corn and carrots where alterations in odor were noted.

DISCUSSION

Irradiated food produced no evident clinical abnormalities when fed to the test subjects in this study. Similar results have been reported in the previous short-term human feeding studies (1,4). Failure to produce toxicity was further evidenced by the lack of any consistent changes in the excretion of certain urinary constituents which may reflect alterations in ingested foodstuffs.

It is of interest that irradiation produced few changes in nutrient and caloric composition of the foods used in this study. In previous balance studies, the apparent digestibility of protein, carbohydrate and fat in irradiated and control diets were similar (2,4). In addition, the ability of the two diets to maintain nitrogen balance was shown to be essentially unchanged. The decreased thiamin and ascorbic acid content of certain irradiated foods used here is in accord with observations of other investigators. Proctor and Goldblith have reported that in solutions of various vitamins, ascorbic acid and thiamin are the most radiosensitive (25). Ziporin and coworkers (26) and Alexander, et al. (27) have shown that of the vitamins in several foods, thiamin is the most easily destroyed as a result of irradiation. The degree of radiosensitivity of vitamins apparently varies greatly among different food items.

Although there is evidence that irradiation of carbohydrates, proteins, and fats produces certain degradation products, no untoward effects were detected as a result of the ingestion of these products. It has been shown that the irradiation of carbohydrate produces some polymerization of breakdown products, leading to the formation of products typical of browning reactions (28). The urinary excretion of nitroprusside reacting substances, indophenol reducing substances, and ketones, which seem to reflect ingestion of these products of browning reactions (23), were not significantly increased in the test subjects during the irradiated food periods.

Fat soluble carbonyl compounds and thiobarbituric acid reactants are generally increased in foods subjected to irradiation (28). Results of analyses of the foods used in this study are in harmony with these observations. Alterations in proteins are known to occur as a result of irradiation, but significant protein breakdown, as indicated by non-protein nitrogen values, was not apparent in the foods tested. Thus an increase in products of proteolysis could not be detected in these room temperature stored foods following irradiation, although the doses of radiation used are known to be insufficient to inactivate enzymes (29).

In general, most of the irradiated foods tested were acceptable to the subjects. Alterations in several vegetables resulting from irradiation were profound, leading to a preference for the control food. On the other hand, foods such as potatoes and flour, subjected to lower radiation doses, were not detectably different following irradiation. Limitations affecting the interpretation and application of these acceptability data have been discussed in a previous report (3).

These results do not preclude the possible development of toxic effects from irradiated foods when fed to test subjects over a more prolonged period of time. In this regard, a long-term human feeding study of irradiated food is planned.

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