Report of

AN EVALUATION IN HUMAN BEINGS OF THE ACCEPTABILITY, DIGESTIBILITY AND TOXICITY OF PORK STERILIZED BY GAMMA RADIATION AND STORED AT ROOM TEMPERATURE

by

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OBJECT:

To assess in human beings the acceptability, digestibility and toxicity of pork sterilized by gamma radiation and stored at room temperature.

SUMMARY:

1. Ground pork, packed in enameled cans, irradiated at 3 million rep by a gamma ray source, and stored at room temperature for approximately a year, was found to be bacteriologically sterile and contained no exotoxins for mice or rats.

2. Such irradiated pork when fed to human subjects at a level of 32% of the dietary calories for a short period of time (15 days) produced no discernable toxic effects.

3. Such irradiated pork had sufficient changes in color, texture, odor, and flavor to make it unacceptable to the test subjects.

4. No differences were observed in the apparent digestibility of protein, carbohydrate or fat of the irradiated or control diets, or of the ability of the two diets to maintain nitrogen equilibrium.

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INTRODUCTION

The Quartermaster General and The Surgeon General of the United States Army have undertaken an elaborate program to explore the potentialities of the use of ionizing radiation in food preservation. This Laboratory has already completed four studies in human beings of the acceptability, digestibility, available metabolizable energy, and toxicity of some 42 different foods sterilized by gamma radiation and then stored in the frozen state until use (7,8,10). Such food had no toxic effects. There were slight decreases in acceptability of some of the food items, and there were a few unimportant differences between irradiated and control items in digestibility and available metabolizable energy. The present report describes the results of the first test that has been conducted in human beings of the safety, acceptability, and digestibility of an irradiated item of food that has been stored for a considerable length of time at room temperature since sterilization.

METHODS

Test Subjects. Ten young men served as test subjects for this study. They were conscientious objectors who had volunteered for this program as a means of fulfilling their Selective Service obligation. They were between 18 and 22 years of age and had passed a careful medical examination. During the study the men lived on the Metabolic Ward.

Irradiated Pork. The only food tested in this study was pork loin which had been ground, packed in No. 2 cans, and subjected to gamma radiation at a dosage of 3 million rep. Part of this lot of pork had previously been used for a study of its nutritional adequacy in animals (9). The results of this test were satisfactory although the investigator (9) had noticed some discoloration of the meat as well as an off-odor. The cans of pork were divided into five separate groups on the basis of the dates of irradiation. The first group of cans had not been enameled and a considerable number of them were slightly swollen or rusted on the outside. These cans were not used for human feeding although they were subjected to bacteriologic and animal toxicity tests.

Bacteriologic and Animal Toxicity Tests. Thirty-six individual cans, a 20% sample of each of the five groups, were tested separately. All cans were opened and sampled under sterile conditions. In addition, three composite samples were tested. The first two composites were prepared by mixing under sterile conditions the contents of all of the cans of groups 2 and 3; the third was taken from a composite of the cans of groups 4 and 5. Samples from individual cans weighed approximately 25 grams; those from the composite samples approximately 100 grams.

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. A diphtheroid organism was cultured from two separate cans from group one, the unenameled cans. All other 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 the meat samples. In addition, samples of the first and third composites were fed as the only source of food for five days to two groups of five rats each which had previously been starved for a period of three days. No toxic effects were observed in any of the rats fed the irradiated pork. With the results described here, the irradiated pork from the four groups of enameled cans was considered safe for human feeding.

General Plan of the Study. The study consisted of two 15-day periods separated by a rest period of five days. During the first 15-day period, five of the subjects received the diet containing irradiated pork, while the other five received a similar diet containing non-irradiated pork. During the second 15-day period those subjects who had first received the irradiated food received control food, and vice versa. The diets were of constant composition and the subjects were required to consume them completely. During the central five days of each 15-day period, all feces and urine were collected for analysis in order to measure digestibility and nitrogen balance. Throughout both 15-day periods, a series of clinical examinations and clinical laboratory procedures were carried out on each subject as a check against any possible toxic effects from the food.

Preparation of Diets. Two constant metabolic diets were prepared which were identical except that one contained irradiated pork and the other non-irradiated pork. A single menu was prepared (see table 1). After the cans of irradiated pork were opened for bacteriologic examination and mixed, samples were analyzed chemically. The remainder of the irradiated pork was made up into weighed patties and stored frozen until use. On the basis of the chemical examination of this irradiated pork, an attempt was made to prepare from pork loin obtained at the Post commissary a ground pork product similar in composition to the irradiated pork. The results of the chemical analyses of the irradiated pork and of this control pork are given in table 2. All other foods used in
preparing the diets were obtained from the Post commissary. Wherever possible, foods from the same packing lots were used. The diet was prepared to provide approximately 2700 Calories of which 32% of the calories were to be supplied by the ground pork. Composites of the diet excluding pork were also analyzed chemically, with results also shown in Table 2. In addition, the subjects received small amounts of nitrogen in tea or coffee; the amounts were the same each day for an individual and were added to the diet figures in calculating individual nitrogen intake. Except as noted, all analytical methods were those of the A.O.A.C.

Because the irradiated pork was somewhat discolored and had an abnormal odor, samples of both irradiated and control pork were tested chemically by the thiobarbituric acid reaction which may be considered a measure of rancidity. The method was essentially that of Biggs and Bryant (3). The meat samples were ground to a paste consistency and refluxed one hour with 2-thiobarbituric acid at a temperature of 65°C, under an atmosphere of nitrogen. The orange-red color produced was measured spectrophotometrically at 535 millimicrons. The color reaction measures the aldehydes formed by oxidation, primarily of certain lipids. Since the fat content of these samples varied somewhat the results were expressed in the amount of color produced per unit weight of lipid in the sample. The results are listed in Table 3.

Clinical Examinations. Before the test and at the end of each 15-day period, each man received a careful physical examination, an electrocardiogram and a chest X-ray. The subjects were weighed each morning upon arising. Blood pressure, pulse, respiration and body temperature were measured several times daily. Each man kept a diary in which he recorded any symptoms which might have bearing on the test.

Clinical Laboratory Tests. Before, during and after eating of irradiated food, the following clinical laboratory tests were carried out:

Hematology: hemoglobin (4), hematocrit, total and differential white blood cell count.

Liver function: 45-minute bromsulfalein retention, both total and one-minute serum bilirubin (6), thymol turbidity, alkaline phosphatase (12), and prothrombin time.

Renal function: complete urine analysis and phenolsulfonphthalein excretion test.

Digestibility and Nitrogen Balance. During the central five days of each 15-day period, complete collections were made of urine and of feces on each subject. Urine was analyzed for nitrogen, and feces were analyzed for moisture, ash, fat and nitrogen. From these data and the results of analyses of food composites, the apparent digestibilities of protein, fat and carbohydrate were calculated, as was nitrogen balance.
Acceptability. Food acceptability data on the irradiated pork were obtained in two ways. Three times during each 15-day period, each subject noted his reaction to each individual food in the diet on the nine-point hedonic scale developed by the Quartermaster Food and Container Institute. 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. All items on the menu were discussed.

RESULTS

Clinical Examinations. The various clinical examinations showed no changes that could be attributed to the ingestion of irradiated food. Some of the men lost small amounts of weight during the period of feeding; blood pressure, pulse rate, respiration and temperature remained within normal limits. Chest X-rays and electrocardiograms showed no changes from the control normal records.

Clinical Laboratory Tests. The series of clinical laboratory 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. Although there were occasional results that might be considered outside the range of normal, these variations did not appear to be of significance. In particular, there were no changes correlated with the feeding of irradiated food. The results are listed in the appendix.

Digestibility and Nitrogen Balance. The values of apparent digestibility of protein, fat and carbohydrate and of nitrogen balance calculated from the results of analysis of diets, feces and urine are given in table 4. Mean values of the digestibility and nitrogen balance figures did not differ significantly from control to irradiated diets.

Acceptability. The acceptability rating data showed a marked difference in acceptability between the irradiated and control pork. On the nine-point hedonic scale in which 1 is "like extremely" and 9 is "dislike extremely" the irradiated pork was given an average rating of 7.9, whereas the control pork received an average rating of 4.4. Although one subject found the irradiated pork more acceptable than the control, nine of the ten men much preferred the control to irradiated pork. The difference in acceptability rating of the two meats was statistically significant at the 1% level. Analysis of variance of the data revealed a statistically significant difference between ratings on the first and second 15-day periods. Control pork was rated less favorably during the second period than during the first. Irradiated pork was rated uniformly unfavorably throughout the study. The differences in color, texture, odor and flavor between the two meats which were observed by the subjects are listed in table 5. All the subjects noted the darker color of irradiated pork as compared to the control pork. Subjects noted a flavor difference and a majority noted a difference in odor.
Most of the subjects also noted a difference in texture; irradiated pork was described as softer in texture than the control pork; it "fell apart more readily."

**DISCUSSION**

Irradiated pork used in this study was off-color, and the texture, odor and flavor were considerably altered. The cause of these changes in the food is not known. Possibly the method of packing may have had some effect upon the food. Certainly it was noted that pork packed in unenameled cans had deteriorated more than that in enameled cans. The only cans which were not bacteriologically sterile had not been enameled. However, only food from enameled cans was used in the present test. Although the dosage of radiation used on the food in the present test, 3 million rep, has not always been sufficient to destroy all bacteria (1), it apparently did so in the present instance. Such doses of radiation, however, are known to be insufficient to inactivate enzymes in the food (5). Such enzymes may act to cause breakdown of protein, fat and carbohydrate during room temperature storage of the canned product. There is some evidence that this actually did take place from the high thiobarbituric acid reaction values obtained on samples of the irradiated pork. It should be noted, however, that the control pork in this study did not come from the same batch as the irradiated pork. This fact is a major defect in this whole study and tends to vitiate all comparisons of differences between the irradiated and control pork.

Although irradiated ground pork was certainly unacceptable, it had no toxic effects when fed to the test subjects as far as could be determined. In this regard the room temperature stored irradiated pork was a perfectly satisfactory product. These results, however, do not preclude the possible development of toxic effects from such a product when it is fed over a much more prolonged period of time. Although most of the subjects lost some weight while consuming the diets, the weight loss was no greater on the irradiated diet than on the control diet. The cause of the weight loss presumably the fact that the total caloric intake was only 2700 Calories, whereas previous experience with such subjects indicates that the usual daily caloric requirement is about 3000 Calories.

The apparent digestibility of protein, carbohydrate and fat in the two diets, and the ability of the two diets to maintain nitrogen balance, were almost exactly the same. It is apparent, however, that there may very well be differences in the digestibility of the foodstuffs from irradiated or non-irradiated meat and in the ability of protein in irradiated and non-irradiated meat to maintain nitrogen balance. Since the irradiated meat provided only 32% of the calories of the diet, non-test foodstuffs could have masked any differences.
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