COMPARATIVE GROWTH OF SALMONELLAE, COLIFORMS, AND OTHER MEMBERS OF THE MICROFLORA OF RAW AND RADURIZED GROUND BEEF[1]

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(Received for publication March 24, 1972)

ABSTRACT

The population of salmonellae in raw ground beef at 5 C was stable during 6 days while that of the total microflora increased markedly. Radurization reduced the numbers of salmonellae and of the total population. The buildup of numbers also was retarded by radurization so that the total count was $< 10^6$ per gram after 6 days while the number of salmonellae remained constant. Ground beef incubated at 25 C showed a rapid increase in numbers of salmonellae, coliforms, and in the total microflora. While radurization reduced the numbers of these microorganisms, it did change the general pattern of competitive growth. Highly contaminated samples contained some coliforms able to grow at 5 C. Unirradiated samples as well as samples immediately following irradiation showed the coliforms to consist predominately of *Escherichia coli*, but during storage there was an increase in the proportion of intermediate types and of *Aerobacter*.

Fresh ground meats are subjected to considerable delay between production and consumption as well as exposure through packing, transportation, reprocessing in the supermarket, and extended household storage. Contamination with food spoilage microorganisms and a few pathogens is inevitable. The microbial population increases with time and each processing operation.

Salmonellae have been found in retail meat products *(5, 6, 17, 18)* and their frequent presence is a cause for alarm to regulatory sanitarians. Several studies have been made on survival of salmonellae in various food products *(1, 3, 13)*. Goepfert and Chung *(7)* recently reported that salmonellae survived for more than 42 days in luncheon meats stored at 5 C, while they actually multiplied at room temperature (25 C). However, there has been no report on the relation between growth of salmonellae and the total microflora of ground beef.

Fresh ground meat in presently acceptable commercial form is not amenable to heat pasteurization, therefore indicating the need for an alternate process of microbial destruction. Low dose gamma-irradiation for radurization (radiation pasteurization) has potential for an acceptable process *(11, 16)*. However, radurization results in a yet unstudied microflora, which is of particular interest because of potential survival and growth of organisms of public health significance.

This investigation was undertaken to examine the behavior of salmonellae and coliforms as examples of *Enterobacteriaceae* in the presence of the natural microflora of raw and radurized ground beef. Both common refrigerated storage at 5 C and gross mishandling of room temperature storage (25 C) were considered.

MATERIALS AND METHODS

Source of ground beef

Machine-dispensed units of ground beef weighing approximately ¼ lb., as prepared for hamburgers, were obtained from a local commissary. This source was selected because it was known to provide a high quality, low microbial count product. For comparative purposes, samples were obtained from a local supermarket which consistently sold products of unusually high microbial count.

Cultures

Salmonella typhimurium, *Salmonella enteritidis*, and *Salmonella heidelberg* were obtained from the Center for Disease Control, Atlanta, Georgia. *Escherichia coli*, two strains, was from the departmental stock culture collection. Cultures were maintained on Plate Count Agar (PCA; Difco) slants. Organisms were subcultured twice in nutrient broth at 37 C to provide 18 hr cultures for experimental purposes.

Inoculation and radurization of meat

Dilutions of the culture were prepared in sterile buffered phosphate diluent *(2)* and inoculated into ¼ lb. meat to obtain approximately 10^2-10^3 salmonellae or *E. coli* cells per gram in the control samples. In the samples to be radurized, the inoculum was increased to obtain a population level after irradiation comparable to that of the control. At this level of inoculation, the number of inherent salmonellae or *E. coli*, if any, was rendered insignificant. The inoculated meat was passed five times through a sterile meat grinder to distribute uniformly the inoculated bacteria. Inoculation and mixing were at 5 C. The inoculated meat in ¼-lb. quantities was put into sterile polyethylene bags. Irradiation was at room temperature using a ^{60}Co source providing 17 Krad of gamma-radiation per minute. During this short exposure of irradiation the temperature of the meat did not increase more than 1 C. The physical facilities were essentially those reported by Teeny and Miyauchi *(14)*.

[1]Paper No. 3000 Journal Series, Nebraska Agricultural Experiment Station, Lincoln.

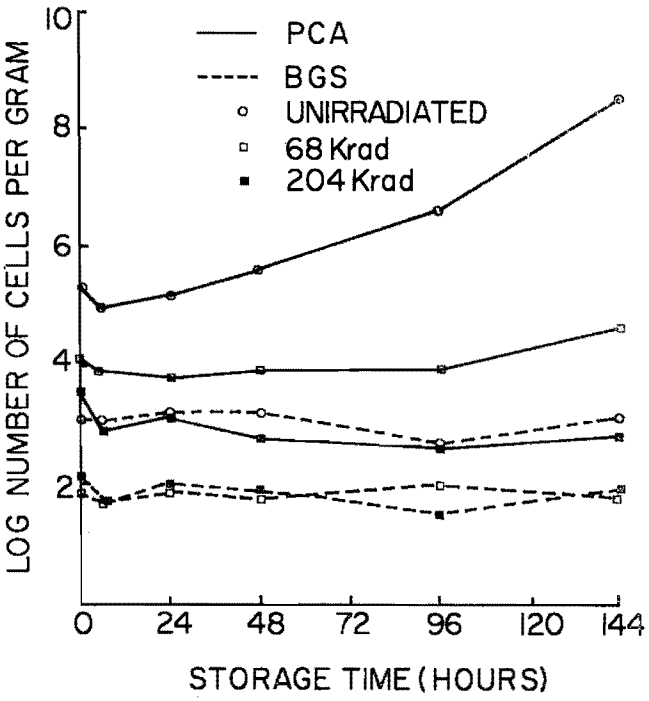

Figure 1. Comparative behavior of S. *typhimurium* and other members of the flora in raw and radurized ground beef at 5 C.

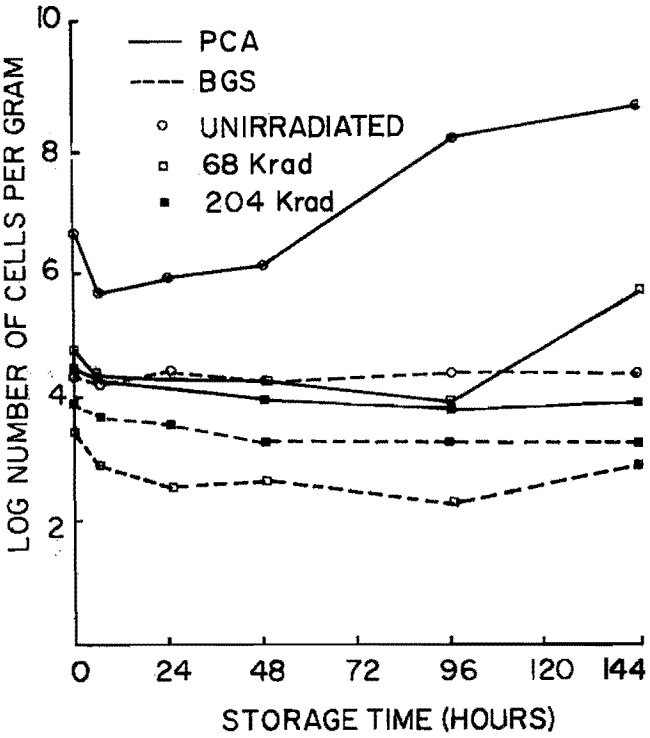

Figure 2. Comparative behavior of S. *enteritidis* and other members of the flora in raw and in radurized ground beef at 5 C.

Bacterial counts

Dilutions were prepared in sterile buffered diluent *(2)* using a Waring Blendor. For total counts, PCA was used with incubation at 32 C for 48 hr. Coliform or *E. coli* counts were made using Violet Red Bile Agar (VRBA; Difco) with incubation for 24 hr at 35 C. Salmonellae were determined by pour plating with Brilliant Green Sulfa Agar (BGS) (BBL) and incubated at 37 C for 48 hr. The counting involved differentiation of salmonellae from greenish-yellow coliform colonies. To differentiate *Salmonella* colonies from pseudomonads and *Proteus*, colonies were transferred to Triple Sugar Iron Agar *(12)*. Black colonies, which represented more than 99% of the colonies picked from BGS agar, were transferred into Urea Broth (Difco). Negative urease test confirmed the colonies of *Salmonella*.

Determination of the nature of coliform bacteria in retail ground beef

Twenty colonies from the countable VRBA plate were picked by random design into nutrient broth. Growth from nutrient broth was streaked on EMB plates and was also inoculated into another nutrient broth tube. The latter nutrient broth tube was incubated in a water bath at 44.5 C for 24 hr and observed for growth. The isolates were also subjected to indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests *(8)*.

RESULTS

The fate of salmonellae and other members of the microflora of ground beef at 5 C.

An inoculum of S. *typhimurium* of approximately 10^3 cells per gram in ground beef, as determined by plating on BGS agar, showed no change in population density for 6 days at 5 C. Other members of the microflora grew to increase the PCA count which was 1.9×10^5 - 2.9×10^8. When samples were inoculated with S. *typhimurium* before radurization to provide

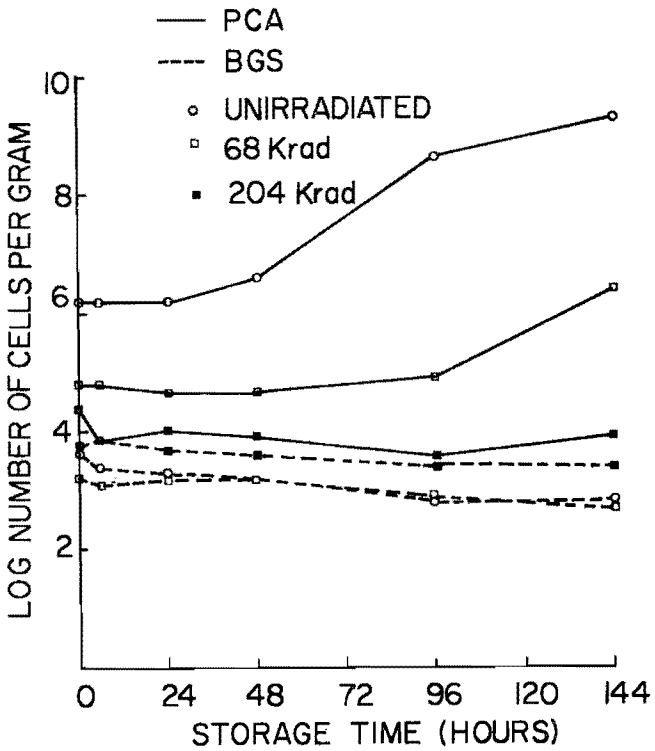

Figure 3. Comparative behavior of S. *heidelberg* and other members of the flora in raw and in radurized ground beef at 5 C.

TABLE 1. THE EFFECT OF STORAGE AT 5 C ON THE DISTRIBUTION OF *Aerobacter*, *E. coli*, AND INTERMEDIATES IN RAW AND RADURIZED GROUND BEEF FROM A RETAIL STORE.

Irradiation dose	Without storage: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	3.7×10^7	3.8×10^4	100	77	16	7
68 Krads	2.2×10^6	1.4×10^3	89	82	12	6
136 Krads	1.2×10^6	70	26	96	4	0

Irradiation dose	After storage for 144 hr: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	5.3×10^9	7.8×10^4	94	44	52	4
68 Krads	2.3×10^8	2.8×10^2	73	74	23	3
136 Krads	3.3×10^6	<10	10	100	0	0

TABLE 2. THE EFFECT OF STORAGE AT 25 C ON THE DISTRIBUTION OF *Aerobacter*, *E. coli*, AND INTERMEDIATES IN RAW AND RADURIZED GROUND BEEF FROM A RETAIL STORE.

Irradiation dose	Without storage: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	7.6×10^6	7.2×10^3	60	70	30	0
68 Krads	7.4×10^5	1.7×10^2	57	67	33	0
136 Krads	2.2×10^5	6.7×10^1	23	91	9	0

Irradiation dose	After storage for 24 hr: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	1.8×10^9	2.5×10^8	60	97	3	0
68 Krads	6.2×10^8	8.2×10^7	60	93	7	0
136 Krads	4.6×10^8	5.7×10^7	60	92	8	0

10^2 - 10^3 cells per gram after radurization at 68, 136, or 204 Krads, the counts on BGS showed no change during 6 days at 5 C. The PCA count increased slightly during 6 days at 5 C in the samples radurized at 68 Krad. Comparative growth is shown in Fig. 1. There was no apparent increase in count in samples radurized at 136 or 204 Krad. The results with 136 Krad were similar to those with 204 Krad and for the sake of simplicity were not included in Fig. 1.

Salmonella enteritidis and S. *heidelberg* gave similar results to those obtained with S. *typhimurium* (Fig. 2-3).

Growth of salmonellae and other members of the microflora of ground beef at 25 C.

Salmonella typhimurium was inoculated into raw ground beef to obtain a population of approximately 10^3 cells per gram. When incubated at 25 C for up to 24 hr, the BGS count increased at near the same rate as did the numbers of other members of the microflora as measured by PCA count (Fig. 4). Samples inoculated with S. *typhimurium* before radurization to provide 10^2 - 10^3 cells per gram after radurization at 68, 136, or 204 Krad showed a similar pattern of growth to that obtained with inoculated unirradiated product. However, the growth lag was less in unirradiated samples than in radurized samples. Results from only two dose levels were included in Fig. 4 to show the general trend and to avoid excess data in one figure.

Repetition of the above experiments but with S. *enteritidis* or S. *heidelberg* gave similar results to those obtained with S. *typhimurium* (Fig. 5-6).

The fate of coliform organisms and other members of the microflora of ground beef at 5 C

For the observation on raw product an inoculum of *E. coli* was added to obtain a coliform count

(VRBA count) of approximately 0.2% of the total population (PCA count). During storage at 5 C, the PCA count increased, but the coliform count remained approximately the same (Fig. 7). Since *E. coli* was more sensitive to radiation than many other members of the microflora, it was necessary to adjust the inoculum for the samples to be irradiated. The inoculum of *E. coli* was increased to provide approximately 10^2 - 10^3 cells per gram. After radurization at 68 or 136 Krad, the coliform count showed a reduction during 6 days at 5 C. The reduction was greater in radurized samples at 136 Krad. Two strains of *E. coli* gave similar results.

Growth of coliform bacteria and other members of the microflora of ground beef at 25 C

Coliform organisms in the raw and the radurized product grew rapidly at 25 C and approximately at the same rate as other members of the microflora (Fig. 8).

Nature of coliform bacteria in retail ground beef

The above data were obtained using ground beef from a central processing operation. These samples consistently had a low total and coliform count. Somewhat different results were obtained when ground beef was obtained from an atypical retail store *(15)* (two years of unreported results), which continually supplied a product with a high total and

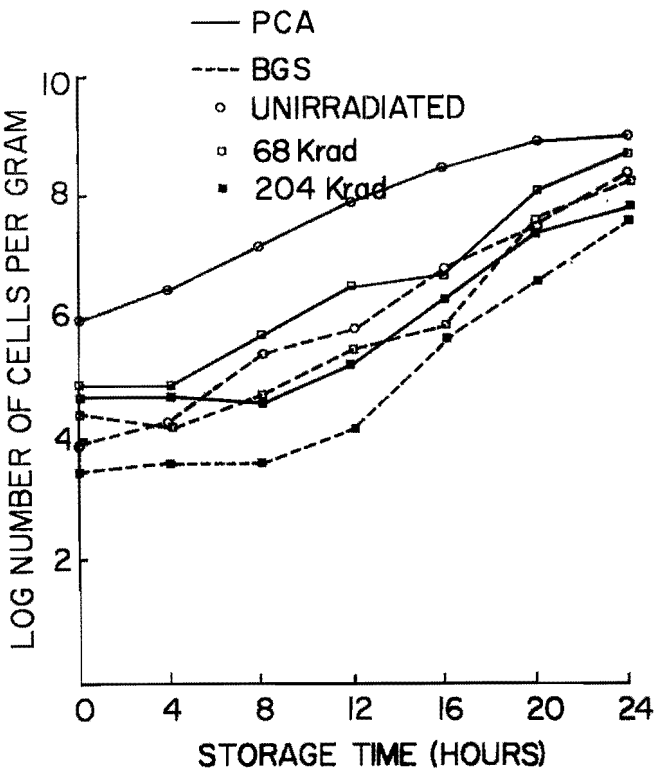

Figure 5. Comparative growth of S. *enterttidis* and other members of the flora in raw and in radurized ground beef at 25 C.

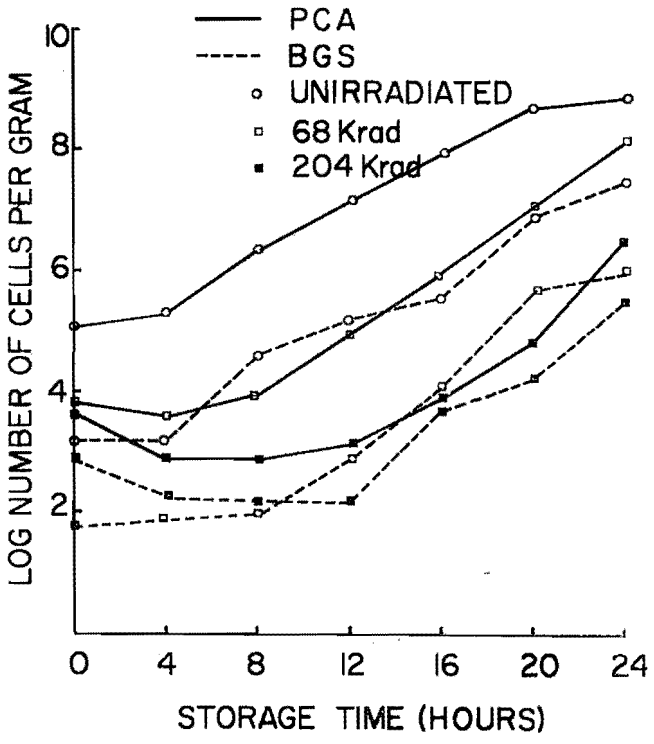

Figure 4. Comparative growth of S. *typhimurium* and other members of the microflora in raw and in radurized ground beef at 25 C.

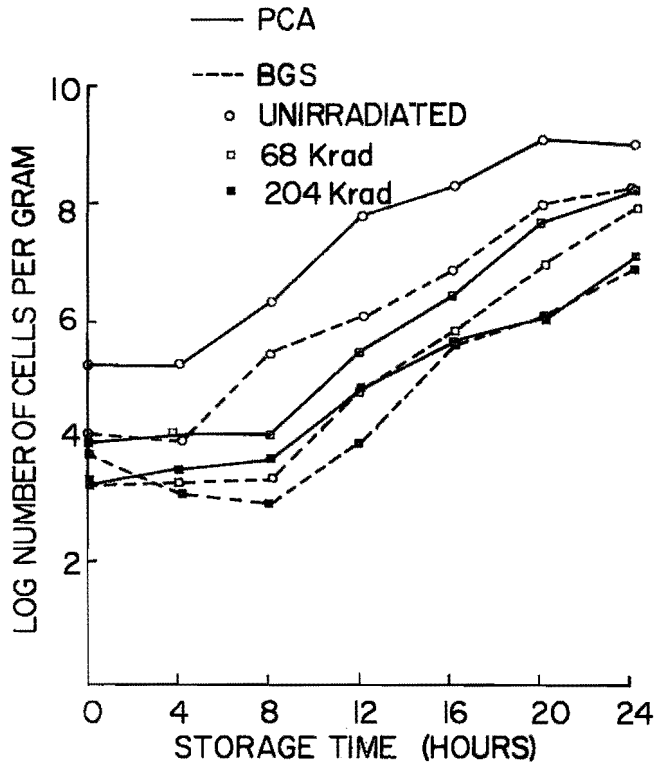

Figure 6. Comparative growth of S. *heidelberg* and other members of the flora in raw and in radurized ground beef at 25 C.

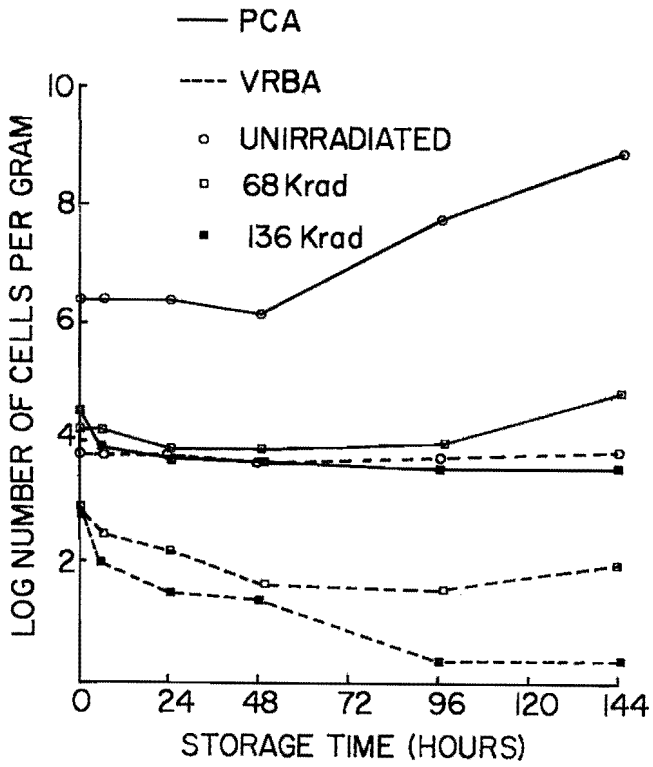

Figure 7. Comparative growth of *E. coli* and other members of the microflora in raw and in radurized ground beef at 5 C.

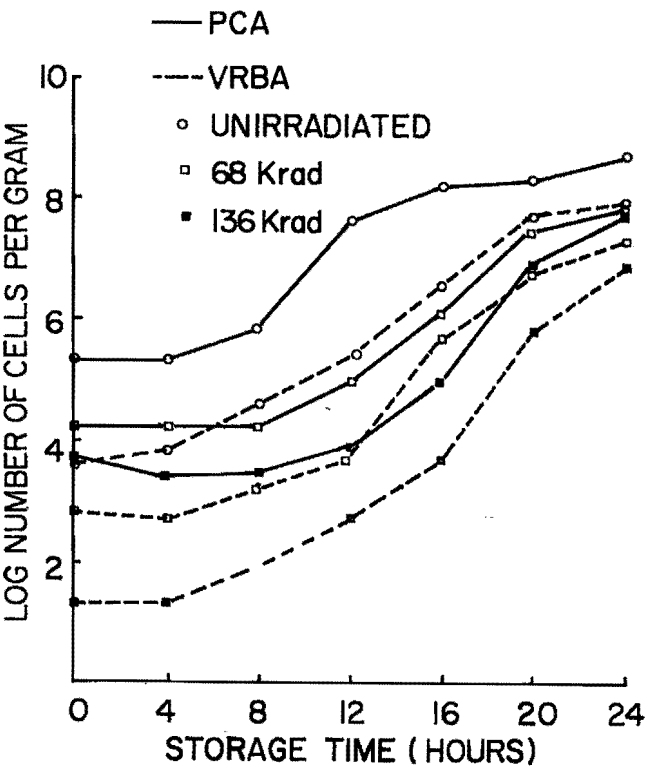

Figure 8. Comparative growth of *E. coli* and other members of the microflora in raw and in radurized ground beef at 25 C.

coliform count. Certain of the natural coliform contaminants were able to grow at 5 C in unirradiated samples, but no growth was observed in six separate experiments with radurized samples by the end of the 6-day test period. Average results are given in Fig. 9. The coliform count of radurized samples decreased slightly during storage at 5 C.

Coliforms in raw and radurized ground beef from the supermarket were characterized. The distribution of isolates is shown in Tables 1 and 2. The *Aerobacter* isolates are reported with the tacit assumption that *Klebsiella pneumoniae*, if present, would not contribute significantly to the numbers. The intermediates included those coliform bacteria not conforming to the traditional patterns of *Aerobacter* and *Escherichia* for indole, methyl red, Voges-Proskauer, and citrate tests. In the raw product *E. coli* constituted 70-77% of the coliforms. *Escherichia coli* was the most resistant to radiation of the three groups. In the raw samples multiplication of coliforms at 5 C was attributed to the intermediates. At 25 C *E. coli* grew most rapidly of the three groups.

DISCUSSION

Fresh ground beef constitutes one of the most

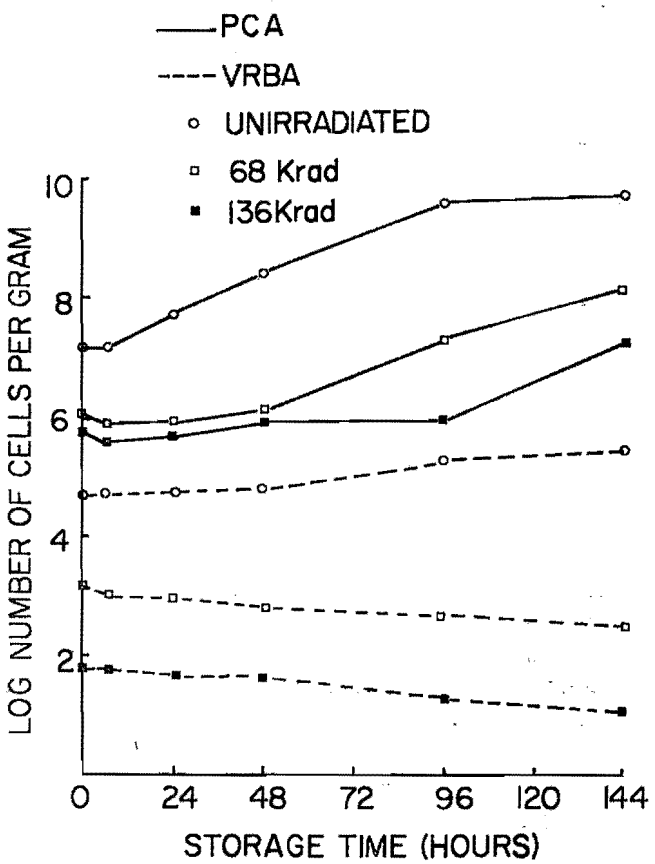

Figure 9. The fate of inherent coliform contaminants in raw and radurized ground beef at 5 C.

challenging of meat products for quality assurance and public health protection. Common reports of retail products with counts of well over 10^8 organisms per gram *(4, 9, 10, 15)* denote microbial contamination that should be reduced or eliminated. Contamination at this level indicates poor quality and potential hazards that persist or increase at common storage conditions of 5 C (Fig. 1, 2, 3, 7, 8). There is a marked increase in hazard with chance mishandling such as leaving the product at room temperature (Fig. 4, 5, 6, 9).

Our data indicate that radurization of ground beef reduced the incidence of salmonellae and coliform bacteria. It would be logical to expect that other members of the *Enterobacteriaceae* would be similarly affected.

Radurization of ground beef with dose levels used in this work reduced the total microflora and increased the lag in subsequent growth. Though our experiments were designed to emphasize the fate of the inoculated bacteria, it was apparent that the residual flora from the natural contaminants was predominant in subsequent growth at 5 C. A study of the nature and fate of surviving members of the microflora will be the subject of a later communication. Comparative growth of salmonellae, coliforms, and other members of the microflora arising from inherent contamination was similar at 25 C in raw and in radurized ground beef.

While the general pattern of growth was similar for the previously mentioned groups of bacteria, changes were apparent in the nature of the coliforms. At 5 C the inherent coliform contaminants grew in ground beef, which is in agreement with the work of Rey et al. *(12)*. The coliforms surviving radurization, however, did not grow. The growth in unirradiated samples was attributed to intermediates of *Escherichia-Aerobacter*.

Radurization may be used directly to eliminate contamination by destruction of microorganisms. An indirect and greater benefit, however, may be through shelf-life extension, which would allow central processing and packaging thereby reducing retail store contamination.

While radurization of red meat is not an accepted commercial process by United States regulatory agencies, because of the lack of proven safety of the process, ultimate use may be favored by public health protection. These potential benefits to society may offset the hazards, if any, from radurized products.

ACKNOWLEDGMENTS

This investigation was supported in part by Contract No. AT(11-1)-2038 from the U. S. Atomic Energy Commission. We appreciate the technical assistance of Mrs. Susan T. Skolnik.

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