THE SPOILAGE MICROFLORA OF IRRADIATED BEEF

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ABSTRACT

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OF IRRADIATED BEEF

By

Cheryl P. Groesbeck

This study was initiated as an extension of previous work on phosphate-treated, vacuum-packaged, irradiated, and refrigeration-stored fresh beefsteaks (Giddings, 1969; Urbain et al., 1968, 1969; Urbain and Giddings, 1972). Because of the consideration being given this combination of treatments as a proposed process for the centralization of the preparation of fresh retail cuts of red meats, further study was needed on the microbiological outgrowth pattern.

By using methods for irradiated foods published by the International Atomic Energy Agency, representative isolates were chosen from countable plates for characterization. Special methods were employed to determine the presence of Clostridium perfringens, Salmonella, coagulase positive Staphylococcus aureus, coliforms, and fecal coliforms.

The major findings for this study were 1) the microflora of the nonirradiated, nonphosphated and the nonirradiated, phosphated beef samples consisted of primarily gram negative rods with a few Lactobacillus and gram positive, nonsporulating, catalase positive rods; 2) the microflora of the irradiated, nonphosphated and the irradiated, phosphated beef samples consisted of primarily Lactobacillus with a few gram positive, nonsporulating, catalase positive

rods and cocci, and gram negative rods; 3) coagulase positive Staphylococcus, coliforms and fecal coliforms do not appear to thrive and grow out in the irradiated, phosphated and nonphosphated samples, although they are prevalent in the nonirradiated samples; 4) no evaluation of Salmonella and Clostridium perfringens could be made as they were not found in any of the samples.

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By

Cheryl Pugh Groesbeck

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INTRODUCTION

The topic of centralized preparation of fresh meats including retail cuts has been discussed for some 20 years (Burroughs, 1972). Some centralized cutting and packaging is being done on a limited scale with the emphasis on the subprimal approach (Burroughs, 1972, Urbain and Giddings, 1972). Urbain et al. (1968) in a review of this topic revealed the many advantages to be gained from developing and utilizing a method of centralized cutting and prepackaging of fresh meats. A process which is technologically feasible for centralized preparation of fresh meats has been developed by Giddings (1969) and Urbain and Giddings (1972). The processing steps of this proposed process are summarized in Table 1.

As with any new process for food products, it is necessary to examine the effect of the process on the microbial ecology of the product to insure the safety of the product for the consumer. There are at least two questions which may be raised regarding the effect this process has on the microbial ecology of the fresh meat: 1) what microorganisms will grow and will the risk of foodborne disease be increased?, and 2) when the meat finally spoils, will it spoil in a way familiar to consumers? The USDA has expressed an interest in answers to both of these questions (Urbain[1], 1972). This study will not attempt to answer the second question, but will be directed towards answering

[1] Urbain, W. M. 1972. Private communication.

Table 1. The Process for a Centralized Operation for the Preparation of Retail Cuts of Fresh Meat. [a,b,c]

Sequence	Procedure	Purpose
1.	Dip or spray retail cuts with a sodium tripolyphosphate solution.	To control fluid exudation and to aid prevention of pigment oxidation.
2a.	Bulk vacuum package the retail cuts.	To retard pigment oxidation and aid in flavor retention.
OR		
b.	Wrap individual cuts in fresh meat film; then bulk vacuum package.	To have the cuts ready for immediate display at the retail outlet and same reason as 2a.
3a.	Irradiate the bulk vacuum package to 50 to 200 Krad. and store at refrigeration temperatures (ca. 35 to 45°F).	To delay onset of microbial spoilage and increase shelf life; meat can be stored up to 3 weeks at refrigeration temperatures.
OR		
b.	Store the bulk vacuum package at 28° to 40°F throughout the storage period.	Same reason as 3a.
4.	Remove the cuts from the bulk vacuum package and wrap in fresh meat film for retail display (under refrigeration); no need to rewrap cuts processed as in 2b above.	To expose the cuts to oxygen to allow the pigment to bloom for retail sale.

[a] Urbain et al., 1969.

[b] Giddings, 1969.

[c] Urbain and Giddings, 1972.

the first question. As an extension of the project begun by Urbain et al. (1968, 1969), this study is confined to microbiological analysis of fresh beef treated generally as described in Table 1; that is, the beef is reduced to a workable sample size, phosphate treated, vacuum-packaged, irradiated, stored for 3 weeks (in the vacuum package) at 40°F (4.4°C), and for an additional 5 days in an aerobic package at 40°F (to simulate retail display). The analysis consisted of characterization of representative isolates from the total microflora, and a search for coliforms and fecal coliforms, Salmonella, coagulase positive Staphylococcus, and Clostridium perfringens.

LITERATURE REVIEW

The spoilage microflora of fresh meat, poultry, and fish has been studied extensively by many investigators. The findings as reviewed by several workers (Jay, 1970; Ayres, 1960b; Wolin *et al*, 1957; Kraft, 1971; and Elliott and Michener, 1965) indicate that the organisms responsible for the aerobic spoilage of meat, poultry, and fish at refrigeration temperatures consist primarily of members of the genera *Pseudomonas* and *Achromobacter* (the taxonomy of the genus *Achromobacter* is being reconsidered with certain strains being assigned to *Pseudomonas* and others to *Alcaligenes* and *Acinetobacter* as noted by Ingram and Dainty in 1971). This review will be primarily limited to the microbiology of fresh beef, although the microbiology of fresh pork, lamb, poultry, and fish will be referred to when particularly applicable, recognizing that the microbiology of these products is similar (Jay, 1970).

The microflora of fresh beef can be quite varied, reflecting the environment in which it is processed beginning at the moment of slaughter. Some members of all of the following genera have been reported to be found: *Micrococcus*, *Proteus*, *Flavobacterium*, *Aeromonas*, *Streptococcus*, *Alcaligenes*, *Microbacterium*, *Escherichia*, *Aerobacter* (now referred to as *Enterobacter*), *Paracolobactrum*, *Serratia*, *Salmonella*, *Staphylococcus*, *Sarcina*, *Bacillus*, *Clostridium*, *Lactobacillus*, and *Leuconostoc*, as well as *Pseudomonas* and *Achromobacter*

as previously mentioned (Jay, 1970; Ayres, 1960a; King, 1967; Halleck et al., 1958; Pierson et al., 1970; and Jensen, 1954). There are some genera of fungi which have been reported to occur on beef as follows: Penicillium, Cladosporium, Thamnidium, Mucor, Rhizopus, Aspergillus, Sporotrichum, Torulopsis, Candida, and Rhodotorula (Ayres, 1960a; Jay, 1970; and Jensen, 1954).

There are many pathogenic microorganisms which potentially may infect man if present in meat. Jay (1970) has reproduced a table from The Safety of Foods (ed. H. D. Graham, 1968) in which 22 diseases are listed as being "transmissible to man through meat". These diseases include a wide variety of bacterial, viral, and parasitic agents.

When beef cuts spoil in air at refrigeration temperatures, the microorganisms which become predominate are the pseudomonads-achromobacter bacteria, as mentioned earlier in the text. In a recent paper Brown and Hoffman (1972) stated that packaging beef in oxygen-permeable films may retard the growth of pseudomonads, but that the pseudomonads remain the primary spoilage organisms. In their study, these workers established a spoilage index for fresh beef as a total count of $1x10^6$ organisms/gram. On beef knuckles wrapped in a polyvinyl chloride film of high oxygen-permeability and held at 34 to 36°F, microbial numbers reached the spoilage index before the fifth day. Ayres (1960b), in reviewing several investigators, reported a range of $3x10^6$ to $1x10^8$ organisms/gram for incipient spoilage on beef. The time required to reach these numbers would vary with temperature of storage and the initial load of bacteria as demonstrated by Ayres (1960a) with his results showing a correlation between off odor and counts of 10^7 organisms/gram at 10 days if held at 5°C and at 20 days

if held at 0°C. According to Urbain and Giddings (1972), the salable shelf life of a retail cut of beef is probably 3 days under refrigeration and possibly up to 5 or 6 days with strict control of sanitation and refrigeration. With the proposed process of a centralized operation for the cutting and packaging of fresh meats as outlined in Table 1, the shelf life of retail cuts could be increased to 3 weeks (Giddings, 1969, Urbain and Giddings, 1972).

In the list of bacteria reported on meats earlier in the text, not all of the bacteria would be considered harmless to man. In fact, there are bacteria on the list which can cause foodborne disease. An awareness of the fact that various details in the processing of meat can change the microbial flora (Niven, 1969) leads to a concern of the necessity of evaluating the public health significance of the potentially harmful bacteria on meat undergoing the proposed process. With respect to pathogens, this study has been limited to determining survival and outgrowth of the following organisms should they be present on the beef being examined: Clostridium perfringens, Salmonella, and coagulase positive Staphylococcus aureus, because they are often the cause of food poisoning from meat (Niven, 1969); and coliforms and fecal coliforms which are ubiquitous to the meat processing environment (Niven, 1969). Some of the incidence levels on meat reported in the literature for these bacteria are given in Table 2.

The data in Table 2 show a wide range of incidence levels for all of the bacteria. The data as given illustrate that fresh meats are a potential vector in getting food poisoning bacteria into homes, restaurants, and institutional eating facilities.

Table 2. The Incidence of *Clostridium perfringens*, *Salmonella*, Coagulase Positive *Staphylococcus aureus*, Coliforms, and Fecal Coliforms in Red Meats.

Product	No. and kind of samples	No. of positive found	% positive found	Refs. and remarks
	Clostridium perfringens			
Veal	17-raw, unprocessed	14	82	a retail packages obtained from supermarkets
Beef	50- "	35	70	a "
Lamb	27- "	14	52	a "
Pork	41- "	15	37	a "
Veal	291-boneless	31	10.7	b frozen and thawed
"	31- "	11	35.5	b "
Beef	35- "	6	17.1	b "
"	125- "	2	1.6	b "
"	12- "	1	8.3	b "
"	65- "	23	35.4	b "
Veal	10-carcass	0	0	b under refrigeration
Beef	37- "	0	0	b "
"	36- "	0	0	b "
"	67- "	2	3.0	b "
"	18- "	0	0	b "
Lamb	15- "	0	0	b "
"	4- "	1	25.0	b "
"	4- "	0	0	b "
Pork	4- "	0	0	b "
Market meats	not given	not given	60 to 70	f
Meat, poultry and fish	122	20	16.4	1

Table 2 (cont'd.)

Product	No. and kind of samples	No. of positive found	% positive found	Refs. and remarks
	. . . . .			
Beef	624-meat from the bloody neck area and trimmings	From these samples a total of 5,671 P.A. spores were isolated which is a mean of 3.0 P.A. spores per gram of beef sample.		n The P.A. spores included all mesophilic clostridial spores except C. botulinum type E.
Pork	656- "	From these samples a total of 5,963 P.A. spores were isolated which is a mean of 3.0 P.A. spores per gram of pork sample.		n "
		Salmonella		
Veal	300-boneless	54	18.0	b frozen and thawed
"	32- "	4	12.5	b "
Beef	35- "	0	0	b "
"	253- "	19	7.5	b "
"	12- "	0	0	b "
"	95- "	7	7.4	b "
Veal	10-carcass	1	10.0	b under refrigeration
Beef	39- "	0	0	b "
"	53- "	4	7.5	b "
"	91- "	3	3.3	b "
"	18- "	0	0	b "
"	45- "	4	8.9	b "
Lamb	35- "	0	0	b "
"	4- "	0	0	b "
Pork	5- "	1	20.0	b "
Beef	50- "	37	74.0	g

Table 2 (cont'd.)

Product	No. and kind of samples	No. of positive found	% positive found	Refs. and remarks
Pork	50-carcass	28	56.0	g
"	not given-carcasses	not given	6.0	h
Beef	512- "	not given	0.2	i
Pork	not given- "	not given	7.0	j
"	14- "	2	14.2	k
Veal-N. Zealand	60 to 584 boneless samples from 1962 to 1967	0 to 145	0 to 24.8	m
Veal-Australia	57 to 146 boneless samples from 1962 to 1967	1 to 16	1.9 to 10.9	m
Beef	470-steaks	14	3.0	o
"	60-fresh steaks	5	8.3	p
	coagulase positive Staphylococcus aureus			
Fresh retail cuts	from 28 markets hamburger, pork chops, beef liver, and round steak	21 markets had positive meats	75	c no breakdown given for each type of product
Beef	28-roundsteak	11	39	d fresh retail cut
"	33-hamburger	13	39	d "
"	26-liver	11	42	d "

Table 2 (cont'd.)

Product	No. and kind of samples	No. of positive found	% positive found	Refs. and remarks
Veal	8-steak	2	25	d fresh retail cut
Pork	30-chops	8	27	d "
Lamb	5-chops	1	20	d "
Chicken	11-whole and precut	11	100	d "
Fish	7	3	43	d "
Meat	236-samples of 17 varieties	70 only 11 varieties were positive	30	e Positive samples included chicken, ground beef, beef liver, porkchops, round beef steak, veal, pork liver, bovine lymph nodes, fish and some luncheon meats.
Beef	78-tissue	77	98.7	j
"	not given-steaks	not given	5.0	o
"	12-fresh steaks	11	92.0	p

Table 2 (cont'd.)

Product and type	No. of samples and organisms/g	No. of samples positive for: Coliforms	Fecal coliforms	Refs. and remarks
	Coliforms and Fecal Coliforms			
Veal-boneless	Present in 5 of 6 samples in 0.01 gm/sample	1	4	b total cts. ranged from 3.8×10^3 to 7.5×10^6/gm
" - "	Present in 3 of 5 samples in 0.1 gm for 2 samples and 0.01 gm for 1 sample	0	3	b total cts. ranged from 7×10^3 to 8.5×10^6/gm
Beef-boneless	Present in 2 of 3 samples in 0.1 gm for 1 sample and 0.01 gm for 1 sample	1	1	b total cts. ranged from 6×10^3 to 2.4×10^5/gm
" - "	Present in 1 of 2 samples in 0.01 gm for the 1 sample	0	1	b total cts. ranged from 3.5×10^4 to 3.5×10^7/gm
" -carcass	Present in 2 of 3 samples in 0.1 gm and 0.01 gm		2	b total cts. ranged from $<5.0 \times 10^2$ to 2.0×10^4/gm
" - "	Present in 3 of 4 samples in 0.01 gm	3	1	b total cts. ranged from 1×10^3 to 3.7×10^7/gm
" - "	Present in 4 of 6 samples in 0.01 gm	2	3	b total cts. ranged from 1.2×10^3 to 4.5×10^5/gm

Table 2 (cont'd.)

Product and type	No. of samples and organisms/g	No. of samples positive for: Coliforms	Fecal coliforms	Refs. and remarks
Beef-steaks	Present to about 1.0/cm^2 (log number/cm^2)	not given	not given	o steaks 5 days old
" "	Present to about 3.0/cm^2 (log number/cm^2)	not given	not given	p steaks 5 days old

[a]Hall and Angelotti, 1965.

[b]Hobbs and Wilson, 1959.

[c]Jay, 1961.

[d]Jay, 1962.

[e]Jay, 1963.

[f]Hall, 1962. (As cited according to U.S. Public Health Service Publication No. 1142, p. 52).

[g]Weissman and Carpenter, 1969.

[h]Cherry et al., 1943. (As cited by Weissman and Carpenter, 1969).

[i]Felsenfeld et al., 1950. (As cited by Weissman and Carpenter, 1969).

[j]Baer et al., 1971.

[k]Childers and Keahey, 1970.

[l]Strong et al., 1963. (As cited by Duncan, 1970).

[m]Hobbs and Gilbert, 1970.

[n]Greenberg et al., 1966.

[o]Rey et al., 1971.

[p]Rey et al., 1972.

The proposed process as outlined in Table 1 has parameters which will change the normal fresh meat flora. The first parameter to be considered is ionizing radiation.

The effect of a pasteurizing dose (from 50 to 500 Krad.) of ionizing radiation (Giddings, 1969) on meat, poultry, and fish has been studied by several investigators. Their results show that a pasteurizing dose of radiation inactivates most of the pseudomonads and many of the other organisms on fresh meat, poultry, and fish such that the shelf life of these products at refrigeration temperatures is increased (Wolin et al., 1957; Niven, 1963; Ingram and Thornley, 1959; Thornley et al., 1960; Miyauchi et al., 1963). The irradiation D_{10} values of several organisms are given in Table 3.

As seen in Table 3 the irradiation D_{10} values for Pseudomonas species are the smallest values given. It is no wonder then that low doses of irradiation on meats are so effective in reducing the population levels of the pseudomonads. Thus the aerobic spoilage of irradiation pasteurized meats, poultry, and fish is due to organisms which survive the irradiation and grow aerobically at refrigeration temperatures. These microorganisms include the following: Achromobacter, yeasts, lactobacilli, streptococci, micrococci, organisms resembling Microbacterium thermosphactum, and others which were not always identified (Wolin et al., 1957; Niven, 1963; Ingram and Thornley, 1959; Thornley et al., 1960; Miyauchi et al., 1963; Corlett et al., 1965b). Ingram and Thornley (1959) also report that some of their poultry samples were eventually spoiled by Pseudomonas species after irradiation and aerobic refrigeration storage. Tiwari and

Table 3. Irradiation D_{10} Values for Some of the Bacteria Common to Meat.

Bacteria	Irradiation medium	D_{10} in Krad.	Refs.
Pseudomonas spp.	PO_4 buffer	3-6	a
Ps. fluorescens	Nutrient broth	2	b
Ps. geniculata	"	5	b
Achromobacter spp.	Not given	10-60	g
Lactobacillus brevis NCDO 110	PO_4 buffer	120	c
Lactobacillus planterium NCDO 343	"	8	c
Micrococcus radiodurans	Raw beef	250	b
Clostridium perfringens	Aqueous suspension	120-200	b
C. perfringens (spores of strains of Type A--heat resistant, food poisoning)	"	260-340	d
Escherichia coli	Nutrient broth	10-20	b
Salmonella paratyphi B BL 179	Brain heart infusion broth and minced beef	27.0	e
Salmonella saintpaul BL 6	"	50.2	e
Staphylococcus aureus	PO_4 buffer	20	f
"	Nutrient broth	10	b
"	Dry	65	b

[a]Thornley, 1963. (As cited by Silverman and Sinsky, 1968).

[b]Internatl. Atomic Energy Agen., 1970b.

[c]Dupuy and Tremeau, 1961. (As cited by Silverman and Sinsky, 1968).

[d]Roberts, 1968.

[e]Tanasugarn, 1968.

[f]Bellamy and Lawton, 1955. (As cited by Silverman and Sinsky,

1968).

[g]Thornley, 1962.

Maxcy (1971, 1972) found a group of gram negative cocci in ground beef--both the nonirradiated and irradiated product--which they report are Moraxella-Acinetobacter. Vanderzant and Nickelson (1969) also reported finding Moraxella in low numbers in lamb muscle tissue and Acinetobacter anitratum (Herellea) in fresh ham tissue. The taxonomy of the gram negative cocci and coccoid rods found in foods is being reassessed (Tiwari and Maxcy, 1972; Ingram and Dainty, 1971; Thornley, 1967; and Thornley, 1968). Such organisms as Achromobacter, Alcaligenes, Moraxella, Herellea-Mima, Acinetobacter, and others are included in the reassessment which has yet to be resolved.

The effect of the phosphate treatment on the microflora of meat has been studied by a few investigators. Giddings (1969) observed that the total counts on phosphate treated beef (both nonirradiated and irradiated) were slightly greater than on beef samples which were not phosphate treated. In a study of vacuum packaged, irradiation pasteurized, phosphate treated fish by Spinelli et al. (1967), the authors reported that phosphate, irradiated vs. nonphosphated, irradiated vacuum packaged fillets has little effect on the resulting spoilage microflora. From the results of these two studies the use of sodium tripolyphosphate on meats causes little, if any, change in the microbiology of the meat.

The vacuum packaging of fresh meats has been investigated by several workers. In a study of ground beef, ground lamb, and ground

pork using various packaging films at storage temperatures of 34-38°F and 40-44°F for periods up to 5 weeks, Halleck et al. (1958) found that during the first 2 weeks of storage Pseudomonas-Achromobacter and lactobacilli are predominant and in the last 2 to 3 weeks of storage Ps. fluorescens is the primary spoilage organism. In exploring combinations of processes Ingram (1959) stated that meat treated by a pasteurizing dose of radiation (to delay microbial spoilage), vacuum packaged (to further delay spoilage by aerobic organisms), and held under 5°C (to prevent the growth of pathogenic organisms and to further delay spoilage) has an extended shelf life of several weeks. When Ingram and Thornley (1959) utilized this combination for minced chicken, the chicken was spoiled by microbacteria and fecal streptococci; thus, the investigators doubted the value of this combination of processes.

Jaye et al. (1962), in a study of the microflora on ground beef packaged in Saran (oxygen-impermeable) vs. cellophane (oxygen-permeable), found that the oxygen-permeability of the film and the temperature of storage affected the microflora of the meat. At 30°F and 38°F the Saran wrapped samples had lower total bacterial counts than the cellophane wrapped samples. At 30°F in the Saran wrapped samples the lactic organisms and fluorescent pseudomonads were present in about equal numbers, but in the cellophane wrapped samples the fluorescent pseudomonads greatly outnumbered the lactic organisms. At 38°F in the Saran wrapped samples the lactic organisms were present in much greater numbers than the fluorescent pseudomonads, whereas in the cellophane wrapped samples the fluorescent pseudomonads greatly outnumbered the lactic organisms. Ordal (1962) in reporting the same results, emphasized that anaerobic packaging and strict temperature

control (by retarding microbial growth and spoilage) could make central packaging of fresh meats feasible.

Silliker (1963) in reviewing some of the literature reported that the Pseudomonas-Achromobacter group is suppressed by a lack of oxygen in vacuum packaged meats. These vacuum packaged meats were found to support a larger population of lactic acid organisms (vs. pseudomonads) before spoilage becomes evident. Cavett (1968) in an extensive review of the literature reports on the work of several investigators which shows that vacuum packaging of meats in oxygen-impermeable films (held under refrigeration) causes the microaerophilic lactic acid bacteria to overgrow the aerobic pseudomonads. Cavett (1968) reports that the results of Halleck et al. (1958) are in contrast to those of Jaye et al. (1962) and that these results must be due to some unexplained factor in the medium or gas leakage of the packages. In a fairly recent study of vacuum packaged beef steak, Pierson et al. (1970) also found lactobacilli accounting for 90 to 95% of the total count; the fluorescent pseudomonads increased in numbers in the aerobic packages, but did not change numbers in the anaerobic packages. In a study by Baran et al. (1970) the results also illustrate that vacuum packaging of meats slows the growth of aerobic bacteria. A recent study by Brown and Hoffman (1972) of fresh beef packaged in oxygen-permeable vs. oxygen-impermeable films also shows an extension of shelf life in the vacuum packages over the aerobic packages and an increase in the numbers of lactic acid bacteria in the oxygen-impermeable film packages as compared to a predominance of pseudomonads in the oxygen-permeable film packages.

In studies of refrigerated irradiated fish--aerobic vs. anaerobic packaging--(Miyauchi et al., 1963; Technological Laboratory, 1964; Miyauchi et al., 1965; Pelroy and Eklund, 1966; Licciardello et al., 1967; Pelroy and Seaman, 1968; Spinelli et al., 1965) the results show an extension of the shelf life of the fish with the use of low levels of radiation growth of the more radiation resistant Achromobacter in irradiated, aerobically packaged samples, and growth of lactobacilli in irradiated, vacuum packaged samples. Also, the irradiated aerobically packaged fish had some fungi growing out, but the irradiated vacuum packaged samples had none (Miyauchi et al., 1963). In vacuum packaged fish fillets Pelroy and Seaman (1968) and Miyauchi et al. (1965) found coliforms growing when the unirradiated and the 0.1 Mrad. samples were held above 3.3°C (38°F) and when the 0.2 Mrad. samples were stored at and above 10.0°C (50°F). However, no coliforms were found in 0.2 Mrad. samples stored at or below 3.3°C (38°F). No coagulase positive Staphylococcus were isolated from irradiated samples, although some were found in the unirradiated samples.

In the study of vacuum packaged, phosphate treated, irradiated fish fillets mentioned earlier in the text (Spinelli et al., 1967; Miyauchi et al., 1966), the fish fillets were irradiated to a level of 0.2 Mrad. and stored at 33°F. When the fish spoiled after 36 days, Lactobacillus predominated in both the phosphate treated and nonphosphate treated samples. Thus the investigators concluded that the phosphate treatment does not alter the flora found after vacuum packaging and irradiation. When Giddings (1969) and Urbain et al. (1968, 1969) observed that readily detectable changes in the normal spoilage pattern of the meat occurred as a result of the proposed phosphate

dip-irradiation process for centrally prepared retail cuts of beef (see Table 1), the problem of characterizing this unfamiliar spoilage pattern needed to be elucidated. This study was initiated to further answer the question as to how the pattern changed and to investigate whether any potential public health problem might be introduced by the proposed process.

MATERIALS AND METHODS

A. Meat.

In all experiments in this study, the semitendinosus muscle (eye of the round) of US Good beef was used. The muscle, cut from a round of unknown history, was obtained from the MSU Food Store, which supplies the whole campus.

B. Reduction of meat to sample size units.

The muscle was chilled to firmness in a laboratory freezer set at $0^{o}F$ ($-17.7^{o}C$). The beef was removed from the freezer before it was frozen, and the muscle was trimmed of exterior fat. Then the muscle was cut across the grain of the muscle fibers into slices approximately 3/4" thick. The slices were packed into a 6" x 6" cutting board form, and cut into parallelepipeds weighing 12-13 grams, about 1" x 1" x 3/4". Two parallelepipeds, constituting a sample unit, were then selected at random.

C. Sample preparation.

1. Phosphate treatment.

Those beef samples to be treated with sodium tripolyphosphate (TPP) were dipped into a $38^{o}F$ ($3.3^{o}C$) 10% (by weight) solution of TPP for one minute and then drained on a wire screen for about 5 minutes.

2. Vacuum packaging.

While the phosphate treated samples were draining, the

remaining samples were put two to a package (two parallelepipeds comprised the sample unit) into a triple-laminated pouch. The gas-water vapor impermeable pouches consist of a Mylar base with a Saran layer as the outer surface and a polyethylene layer as the inner surface; they were supplied by the International Kenfield Distributing Company under the trade name "IKD Super All-Vak #13". After draining, the phosphate treated samples were also put into the impermeable pouches. Before sealing, all pouches were wiped with Kem Wipes at the top inner surface to remove moisture and fat traces to insure a proper seal. Vacuum packaging and sealing was accomplished by using a Kenfield flexible package sealer with a vacuum pump (to 27" of Hg) and gas flush attachments. All samples were returned to the laboratory freezer ($0^{o}F$, $-17.7^{o}C$) for rapid chilling, but removed from the freezer before freezing could occur. Those samples not getting further treatment were put in the laboratory refrigerator ($38^{o}F$, $3.3^{o}C$) temporarily.

3. Radiation treatment.

The samples receiving further treatment were taken to the ^{60}Co source (in the Food Science building) for irradiation. There the samples were irradiated to a dose of 100 Krad. at a rate of 200 Krad. per hour. The samples were not specially refrigerated during the 30 minute irradiation; however, the samples remained cool, having been thoroughly chilled before irradiation. After irradiation the samples were also put in the laboratory refrigerator ($38^{o}F$, $3.3^{o}C$).

Upon completion of all these procedures, the samples had been divided by the treatments described into four lots: a) unirradiated

and not phosphated, b) unirradiated and phosphate treated, c) irradiated and not phosphated, and d) irradiated and phosphate treated (all samples were vacuum packaged).

D. Sample storage.

All of the samples were put into a special, constant temperature refrigerated storage chamber at 40°F (4.4°C) ± 1°F. The storage regime was 3 weeks in the vacuum packages, followed by 5 days in an aerobic package, both at 40°F. Samples for microbiological analysis were withdrawn from storage according to the following schedule: 1) 0 day--as soon as possible after irradiation, 2) 10 days, 3) 21 days, 4) 21 days plus 5 days in air--after 21 days in the vacuum packages, the samples were opened and repackaged in a high-gas and low-moisture permeable fresh meat film (plasticized stretch polyvinylchloride, Dow) and stored an additional 5 days at the same temperature to simulate retail display.

The entire experiment was repeated three times, each time using a different eye of the round from a different carcass.

E. Microbiological analysis.

The analysis consisted of total counts, characterization of representative isolates from the total count plates, and a determination of the presence of the organisms Salmonella, coagulase positive Staphylococcus aureus, coliforms, fecal coliforms, and Clostridium perfringens.

1. Preliminary tests.

Several agars were tested to compare total plate count recoveries and it was found that APT Agar (Difco Lot 536188) recovered as many or more organisms than Plate Count Agar or TPN Agar (Corlett et al., 1965a) with or without NaCl. It was also found the spread

plate technique of total counts resulted in easier recovery of representative isolates than did the pour plate technique; consequently, APT Agar (Difco Lot 536188) and the spread plate technique were employed for total counts.

Also cultures of Salmonella oranienburg (originally from ATCC), Staphylococcus aureus 265 (type A enterotoxin producer from Dr. E. Casman, FDA, Washington, D.C.), Escherichia coli (isolated from water), and Clostridium perfringens (ATCC 3624) were employed to test the procedures and media used to ensure that these types of organisms could be recovered should they be present on the experimental meat samples.

2. Procedure.

The procedure given in Microbiological Specifications and Testing Methods for Irradiated Foods (Tech. Report Series 104, International Atomic Energy Agency, Vienna, 1970a) were followed as closely as possible with modifications only where necessary.

The samples, 2 bags from each of the four treatments, were removed from the storage chamber and placed in the laboratory refrigerator at 40°F (4.4°C). The samples were always analysed in this order: 1) unirradiated, not phosphate treated (0-0), 2) unirradiated, phosphated (0-P), 3) irradiated, not phosphate treated (I-0), and 4) irradiated, phosphated (I-P). One of the bags of each treatment was reserved for the Salmonella enrichment, while the other bag of each treatment was used to prepare the dilution series for the remainder of the analysis.

The following is the IAEA procedure for preparation of a food homogenate:

"5.B. Procedure

a. Begin the examination as soon as possible after the sample is taken. Refrigerate the sample at 0-5°C whenever the examination cannot be started within one hour after sampling. If the sample is frozen, thaw it in its original container (or in the container in which it was received in the laboratory) in a refrigerator at 2-5°C and examine as soon as possible after thawing is complete or after sufficient thawing has occurred to permit suitable sub-samples to be taken. If the contents of the package are obviously not homogeneous, as for example, a frozen dinner, a sample should be taken from a macerate of the whole dinner, or each different food portion should be analysed separately, depending upon the purpose of the test.
b. Weigh into a tared blendor jar at least 10 g of sample, representative of the food specimen.
c. Add nine times as much dilution fluid (M 43) as sample. This provides a dilution of 10^{-1}.
d. Operate the blendor according to its speed for sufficient time to give a total number of 15,000 to 20,000 revolutions. Thus, even with the slowest blendor the duration of grinding will not exceed 2.5 min.
e. Allow the mixture to stand for 15 min at room temperature to permit resuscitation of the micro-organisms.
f. Mix the contents of the jar by shaking, and pipette duplicate portions of 1 ml each into separate tubes containing 9 ml of dilution fluid. Carry out steps g and h below on each of the diluted portions.
g. Mix the liquids carefully by aspirating ten times with a pipette.
h. Transfer with the same pipette 1.0 ml to another dilution tube containing 9 ml of dilution fluid, and mix with a fresh pipette.
i. Repeat steps g and h until the required number of dilutions is made. Each successive dilution will decrease the concentration tenfold."

Some modifications of this procedure were necessary. One bag of each lot was wiped with cotton dipped into 95% ethanol. A pair of scissors was dipped in 95% ethanol, flamed in a bunsen burner, and used to cut open the bag. The meat in the bag was dumped directly into a tared sterile stainless steel blendor jar (of 1 quart capacity). The weight of the meat was noted (usually 25 gram $\pm$ 2 gram) and sterile 0.2% peptone water was added to make

the 10^{-1} dilution. The mixture was blended for one minute on a Waring blendor (approximately 21,000 rpms). During the 15 minute resuscitation period, the other bag of each lot was opened in the previously described manner and dumped into the Salmonella enrichment medium, which will be described below. After the resuscitation period, the blendor jar and contents were shaken and serially diluted by transferring an 11 ml aliquot to a 99 ml solution of 0.2% peptone water in a milk dilution bottle. The 10^{-2} dilution was shaken at least 25 times and the dilution series continued until a sufficient number of dilutions had been prepared (depending on the age of the meat sample).

The following is the IAEA procedure for the mesophilic count:

"Procedure

a. Prepare agar plates for drying by adding 15 ml of melted cooled (45-60°C) Standard Methods Agar (M 60) to each Petri dish used and allow to solidify. Dry agar by placing the plates (i) in a convention-type oven or incubator at 50°C for 30 min with lids removed and agar surface downward; (ii) in an oven or incubator (preferably a forced-air type) for 2 hours at 50°C with lids on and agar surface upward; (iii) in a 35-37°C incubator for 4 hours with lids on and agar surface upward; or (iv) on a laboratory bench for about 16 hours at room temperature with lids on and agar surface upward. If prepared in advance the plates should not be kept longer than 24 hours at room temperature or 7 days in a refrigerator at 2-5°C.
b. Prepare food samples by procedures recommended in Part II, Section 5 on Preparation of a Food Homogenate.
c. Using only 1 pipette, transfer 0.1 ml of each of the dilutions tested (test at least 3, even if the approximate range of numbers of organisms in the food specimen is known) to the agar surface of each of two plates. Start with the highest dilution and proceed to the lowest, filling and emptying the pipette three times before transferring the 0.1 ml portion to the plate.
d. Spread the 0.1 ml portions, as quickly as possible, carefully on the surface of the agar plates using glass spreaders (use a separate spreader for each

plate). Allow the surfaces of the plates to dry for 15 minutes.

e. Incubate the plates inverted for 3 days at 30 ± 1°C.
f. Count all colonies on plates containing 30 to 300 colonies. If available use colony counter and tally register for convenience.
g. Compute the number of mesophilic aerobes per gram of specimen."

Some modifications of this procedure were necessary. As stated in the section on Preliminary tests, APT Agar (Difco Lot No. 536188) was chosen for the bacterial counts. The plates were poured the afternoon before plating and left on the bench top overnight to dry (about 16 hours). Two kinds of counts were needed --mesophilic and psychrophilic. Therefore duplicate plates for each count were prepared and incubated at 32°C (89.6°F) for 2 days to give the mesophilic count and at 7°C (44.6°F) for 10 days for the psychrophilic count (American Public Health Association, Inc., 1967).

The following is the IAEA procedure for the determination of the most probable number (MPN) for coliforms and fecal coliforms:

"I.A.2.(a).2. Procedure

a. Prepare food samples by the procedure recommended in the section on Preparation of a Food Homogenate (Part II, Section 5). All techniques of dilution should be the same. Suspensions remaining from the dilutions employed in the determination of plate count can be used.
Pipette 1 ml of each of the decimal dilutions of food homogenate to each of three separate tubes of Lauryl Sulphate Tryptose (LST) Broth (M 30).
Incubate tubes at 35 ± 1°C for 24 and 48 hours.
After 24 hours, record tubes showing gas production.
Return tubes not displaying gas to incubator for an additional 24 hours.
After 48 hours, record tubes showing gas production.
Select the highest dilution in which all three tubes are positive for gas production and the next two higher dilutions. If this is not possible because none of the dilutions yielded three positive tubes or

because further dilutions were not made beyond the one yielding three positive tubes, select the last three dilutions and record the number of positive tubes in each dilution. . . .
Confirm that the tubes of LST Broth selected in step f above are positive for coliform organisms by transferring a loopful of each to separate tubes of Brilliant Green Lactose-Bile Broth 2% (M 8). Incubate 24 and 48 hours at 35 ± 1°C and note gas production. The formation of gas confirms the presence of coliform organisms.
Record the number of tubes in each dilution that were confirmed, as positive for coliform organisms.
To obtain the most probable number (MPN), proceed as follows: determine, from each of the three selected dilutions, the number of tubes that provided a confirmed coliform result. Refer to the MPN in Table II and note the most probable number appropriate to the number of positive tubes for each dilution. . . . To obtain the MPN of coliform organisms per gram of food, use the following formula:

$$\frac{\text{MPN from Table II}}{100} \times \text{dilution factor of middle tube} = \text{MPN/g}. \ . \ .$$

I.A.2.(b).2. Procedure

a. Select tubes of Lauryl Sulphate Tryptose Broth (M 30) that are positive for gas production in Section I.A.2.(a).2. under Enumeration of coliforms.
b. Inoculate a loopful of broth from each of the selected cultures into a separate tube of E.C. Broth (M 14).
c. Incubate E.C. Broth tubes at 45.5 ± 0.2°C and read for gas production after 24 and 48 hours.
d. E.C. Broth tubes displaying gas production may be presumed to be positive for fecal coliform organisms."

One modification of this procedure was necessary. The MPN in the LST medium was begun by taking three 10 ml aliquots from the homogenate in the blendor and adding these to 3 tubes of double strength LST medium. This was done to start the MPN series at the 1:1 dilution level.

The following is the IAEA procedure for the determination of Staphylococcus aureus:

"Procedure

a. Prepare food samples as in the section on Preparation of a Food Homogenate (Part II, Section 5.B, steps b through i) using same technique and extent of dilution. Where food samples contain very high numbers of staphylococci, dilutions higher than 10^{-5} may be needed, since this method depends on at least one dilution giving negative results.
b. Inoculate single tubes of Trypticase Soy Broth (M 67) (10% sodium chloride) with 1-ml aliquots of decimal dilutions of food homogenate. Maximum dilution of sample must be sufficiently high to yield negative results for at least one dilution.
c. Incubate tube at 35-37°C for 48 hours.
d. Using a 3-mm loop, transfer one loopful from each inoculated tube to previously prepared Vogel-Johnson Agar plate (M 69) and streak in such a manner as to give isolated colonies.
e. Incubate plates at 35-37°C for 48 $\pm$ 2 hours.
f. Select at least one of each visibly different colony type, which has reduced tellurite, from all sample dilutions tested, and test these for coagulase production (for procedure, see Section I.A.5.(b) on Testing for Coagulase Production).
g. From highest dilution containing coagulase-positive staphylococci, estimate the number in the original specimen.

. . .

I.A.5.(b). Testing for coagulase production

. . .

Procedure

a. Subculture selected colonies in Brain Heart Infusion Broth (M 6) and incubate 20-24 hours at 35-37°C.
b. Add 0.1 ml of resulting cultures to 0.3 ml of Rabbit Plasma (M 51) in small tubes and incubate at 35-37°C.
c. Examine tubes for clotting after 4 hours and, if not positive, again after 24 hours. The formation of a distinct clot is evidence of coagulase activity."

Here again, the homogenate was used for the Staphylococcus determination and the inoculation for the Trypticase Soy Broth was begun at the 1:10 level of the dilution series and continued through several dilutions as determined by the age of the meat sample. Only coagulase positive cultures were recorded.

The following is the IAEA procedure for enumeration of

Clostridium perfringens:

"I.B.1.(b).2. Procedure for plate count and culture identification

a. Pipette aseptically 1 ml of each dilution of the food homogenate, prepared as described in the section on Preparation of Food Homogenate, to each of the appropriately marked duplicate culture dishes.
b. Pour 15 to 20 ml of Sulphite Polymixin-Sulphadiazine Agar (M 63) into each plate, rotate and tilt to mix inoculum and agar and allow to solidify.
c. Invert plates and place in Case-anaerob jar. Evacuate anaerob jar to 25 in. of vacuum and replace vacuum with the CO_2-N_2 gas mixture. Repeat procedure once. Place jar in a 35-37°C incubator and allow to incubate for 24 hours.
d. Following incubation, observe plates microscopically for evidence of growth and black colony (H_2S) production.
e. Select plates showing an estimated 30 to 300 black colonies; and using the Quebec colony counter with a piece of white tissue paper over the counting area, count colonies and calculate number of organisms per gram of food. This black colony is the total clostridial count, since clostridia other than Cl. perfringens may grow on this medium.
f. Select a representative number of colonies from the countable plates and inoculate a separate tube of Fluid Thioglycollate Medium (M 19) with cells from each.
g. Incubate the tube cultures in a water bath at 46 ± 0.5°C for 3 to 4 hours.
h. Check growth in fluid thioglycollate medium for purity by examining microscopically smears stained by Gram's method (R 7). Cells should appear as large, Gram-positive rods with blunt ends.
i. If cultures are pure, inoculate separate tubes of Nitrate Motility Medium (M 39), Sporulation Broth (M 59) and Cooked Meat Medium (M 11) with cells from the 3- or 4-hour-old Fluid Thioglycollate Cultures (M 19).
j. Incubate the media in a 37 ± 0.5°C water bath for 18 to 24 hours.
k. Examine tubes of Nitrate Motility Medium (M 39) by transmitted light for type of growth along stab. Non-motile organisms produce growth only in and along the line of stab. Motile organisms produce a diffuse growth out into the medium away from the stab.
l. Test Nitrate Motility Medium (M 39) for presence of nitrite by adding 0.5 to 1 ml of α-Naphthylamine Solution (R 2) and the same amount of Sulphanilic Acid Solution (R 16). The production of a pink or red

colour denotes the presence of nitrites. If no colour develops, mix reagents with upper third of medium by jabbing down into medium with sterile loop.
Note: The only known species of a sulphite-reducing Clostridium, in addition to Cl. perfringens, which is non-motile and produces nitrite from nitrate is Cl. filiforme, an extremely rare organism described only once [35].

m. When desirable for confirmatory purposes, examine the Sporulation Broth (M 59) for spores by Bartholomew and Mitlwer's 'cold' method [36]. Make a smear from sediment in tube, air-dry and heat-fix. Stain for 10 min with Malachite Green (R 15), wash with water, stain with Aqueous Safranin (R 3) for 15 sec, rinse, blot, dry, and examine microscopically. Spores will be stained green, vegetative cells red.

n. Pipette 2 ml of Sporulation Broth (M 59) into a sterile test tube and heat in an 80°C water bath for 10 min. Remove from bath and when cool add 1 ml to a tube of Fluid Thioglycollate Medium (M 19). Incubate 37 ± 0.5°C in a water bath for 18 to 24 hours.

o. Examine Fluid Thioglycollate Medium for evidence of growth, and observe microscopically for typical Gram-positive rods.

p. If growth is present, record that Sporulation Broth contained spores (step m above).

q. If no growth is seen, reincubate for 24 hours and examine once again. If no growth is evident after the second 24-hour incubation, record that Sporulation Broth did not contain spores.

r. The Cooked Meat Stock Cultures (see step i above) of those strains which: (1) produce black colonies in SPS Agar (M 63), (2) are non-motile and reduce nitrate, and (3) produce spores, are saved for further confirmatory tests, if necessary, for procedure for serological typing and carbohydrate fermentation. In routine work, evidence obtained from the tests described in steps d through r immediately above is sufficiently reliable to enable calculation of the plate count of Cl. perfringens to be made.

s. Calculate the total Cl. perfringens count from the percentage of the total black colony count (step e immediately above) that proved to be Cl. perfringens on the basis of the tests described in steps h through q above."

Modifications of this procedure were few. The homogenate was used for the plating with the dilution series beginning at the 1:10 level. The SPS Agar plates were overlaid with 5 to 10 ml of

SPS Agar after the first layer had solidified. Then the plates were put into a vacuum incubator at 37°C (98°F). The incubator was evacuated twice and then flushed with N_2 gas. Then the incubator was evacuated once more and filled with N_2 gas and CO_2 gas at 90% and 10% respectively. After 24 hours the plates were removed and examined for the presence of black colonies.

The following is the IAEA procedure for the isolation of salmonellae:

"I.A.4.(a).3. Procedure for Type II, raw meat and poultry

a. Non-selective enrichment is not required for these types of foods
b. Procedure for selective enrichment
   (1) If food is frozen, thaw samples overnight at approximately 5-10°C and temper to 35°C before weighing.
   (2) Weigh 25 g of samples into each of two tared sterile jars (capacity approx. 500 ml), cut sample into small pieces with scissors and add 225 ml of one of the following broths to each jar: Selenite Cystine Broth (M 57) or Tetrathionate Brilliant Green Broth (M 64).
   (3) Incubate one jar for 24 hours at 43 ± 0.2°C and the duplicate jar for 24 hours at 35-37°C.

Plating on selective agar media

Follow procedures described under I.A.4.(a).2.c above, steps (1) through (6), using Brilliant Green Sulphadiazine Agar (M 9) and Bismuth Sulphite Agar (M 2). Incubate at 35-37°C, the former agar plates for 24 hours, the latter agar plates for 48 hours.

. . .

I.A.4.(a).2.c. Plating on selective agar media

(1) Prepare dried plates of two selective agar media: Brilliant Green Agar (M 7) and Bismuth Sulphite Agar (M 2).
(2) Transfer a 5-mm loopful of each enrichment broth culture to the surface of one plate each of the two selective agar media, and spread in a manner to obtain isolated colonies.
(3) Incubate plates inverted at 35-37°C. Examine

Brilliant Green Agar after 24 hours and Bismuth Sulphite Agar after 48 hours.

(a) Typical Salmonella colonies on Brilliant Green Agar appear colourless, pink to fuchsia or translucent to opaque with the surrounding medium pink to red. Some salmonellae appear as translucent green colonies when surrounded by lactose or sucrose fermenting organisms which produce colonies that are yellow-green or green in colour.

(b) Typical Salmonella colonies on Bismuth Sulphite Agar appear brown, grey, to black, sometimes with metallic sheen. The medium surrounding the colony is usually brown at first, then turns black as incubation time increases. Some strains produce green colonies with little or no darkening of the surrounding medium.

(4) Select several suspect colonies from each selective agar medium used for the identification tests described in Section I.A.4.(b) on Identification of Salmonellae. If the purpose of the examination is to determine the number and the relative proportion of the different serotypes present in the specimen, then as many as twelve (six from each selective agar) colonies should be selected. If the object is simply to determine the presence or absence of salmonellae, then only two typical colonies from each agar medium need be used in identification tests.

(5) If the agar plates are crowded with coliform organisms, streak new plates of the chosen selective agar media using a 1:1000 dilution of the enrichment cultures. The enrichment cultures can be held at room temperature or in the refrigerator at 5-8°C during the time the original set of streaked plates are incubating.

(6) Selective agar plates containing typical Salmonella colonies should be held at 5-8°C until identification tests with chosen colonies are completed.

. . .

I.A.4.(b).1. Biochemical screening tests for salmonellae

. . .

Test Series B

a. Purify suspect colonies from selective agar plates as described in item a of Test Series A above. For practical purposes, if time is limited, this step may be by-passed at this point and performed, if necessary, from the differential sugar medium after b, iii below.

b. Determine presumptive salmonellae as follows:
   i. Inoculate one tube each of Triple Sugar Iron Agar (M 65) and Lysine Iron Agar (M 33) with 24-hour-old purified culture from Nutrient Agar (M 40) slants or directly from a single suspected colony on selective agar plates (see Section I.A.4.(a).2.c, item (4), of section on Isolation of Salmonellae above, for comment on number of colonies to pick). Inoculate Triple Sugar Iron Agar (M 65) and Lysine Iron Agar (M 33) with needle by streaking the slant and stabbing the butt.
   ii. Incubate the cultures overnight at 35-37°C.
   iii. Discard cultures that do not give reactions typical of salmonellae in the test media. Typical reactions in TSI Agar (M 65) are indicated by a red slant (alkaline reaction) and a yellow butt (acid; glucose fermentation), with or without production of H_2S (and gas H_2S indicated by blackening of the medium). Typical reactions of salmonellae and Arizona species on Lysine Iron Agar are indicated by a light purple slant and butt (alkaline reaction) with production of H_2S and sometimes gas. Cultures that have not been purified should be streaked onto MacConkey Agar (M 34) plates as described above in item a of Test Series A.
c. If all cultures are eliminated as a result of their action on Triple Sugar Iron Agar or Lysine Iron Agar, pick additional colonies from selective agar plates and repeat steps a and b above.
d. Submit presumptive Salmonella cultures to serological tests described in Section I.A.4.(b).2."

Some modifications of this procedure were necessary. Tetrathionate Brilliant Green Broth was chosen as the selective enrichment medium. The meat in the 2nd bag of each treatment was dumped into separate flasks of medium and incubated for 24 and 48 hours. Plates of Brilliant Green Sulphadiazine Agar and XLD Agar (U.S. Dept. Agr., 1969) were streaked at 24 and 48 hours from the selective enrichment media. Suspect colonies were picked and re-streaked for further isolation. Then they were transferred to Triple Sugar Iron Agar, Lysine Iron Agar slants, and to Nutrient Agar slants, the last for storage. Results of the TSI and LI media were recorded, and the cultures still of suspect were run through

the IMVIC test for further information (Galton et al., 1968).

For the characterization of the flora, isolates from the countable dilutions (between 30 and 300 colonies per plate) of both the mesophilic and psychrophilic plates were chosen in the following way: the plates were examined visually; in the first replicate, one or two colonies of each clearly different morphological type were picked for isolation; these cultures were recorded as to treatment, replicate, sample day, mesophilic or psychrophilic plate, dilution level, total number of colonies present on the plate, and number of other colonies present on the plate having identical morphology to the colony picked. Before the final analysis of the first replicate was completed, it was discovered that several of the colony types were reoccurring in subsequent replications. Therefore, these colonies in the second and third replicates were simply recorded as having the identical morphology of the corresponding previously picked colony. After isolation the cultures were streaked and examined in wet mount by phase contrast microscopy to ascertain culture purity. Gram staining, MacConkey Agar, and Phenyl Ethyl Agar were sometimes used to aid in culture isolation and purification as well as determine the gram reaction. After the gram reaction and the bacterial cell morphology were determined, the cultures were further characterized with the aid of two simplified keys--Harrigan and McCance, 1966, Laboratory Methods in Microbiology; and Jay, 1970, Modern Food Microbiology. Bergey's Manual (1957) and Skerman (1967) were also used when necessary. Characterized cultures were then recorded as the percentage of the total count which they represented.

RESULTS AND DISCUSSION

A. Total counts and microbial characterization.

Each tabular entry for total bacterial counts given in Table 4 is an average of duplicate plates. Irradiation with 100 Krad. lowers the total count by $1\frac{1}{2}$ to 2 log cycles as seen by comparing the counts of 0-0 and 0-P with I-0 and I-P samples. From the 0 day sampling until the 10 day sampling, the 0-0 and 0-P samples greatly increase in total counts, while the I-0 and I-P samples, having lower numbers after irradiation, do not reach the population levels that the unirradiated samples do. Between the 10 day sampling and the 21 day sampling, the growth rate of the microorganisms on the 0-0 and 0-P samples has slowed considerably, whereas the bacteria on the I-0 and I-P samples are still increasing fairly rapidly. On repackaging the vacuum-packaged samples in aerobic packages on the 21st day, the bacteria on the 0-0 and 0-P samples increased again, and the bacterial counts on the I-0 and I-P samples also increased, but remained $\frac{1}{2}$ to 2 log cycles behind the 0-0 and 0-P samples. Thus, over a period of $3\frac{1}{2}$ weeks, irradiation, vacuum-packaging and refrigeration do retard bacterial growth. The total counts found on the samples of all the treatments--0-0, 0-P, I-0, and I-P--agree with the total counts reported by Giddings (1969) in his study of these four treatments (storage was also at 40°F in his study).

The microorganisms isolated from the total plate counts and subsequently characterized are presented in Table 5. These figures are

Table 4. The Total Bacterial Counts/Gram--Mesophilic (32°C for 2 days) and Psychrophilic (7°C for 10 days)--of the 25 Gram Beef Samples.[a]

Count type	Ref. no.	0-0[b]	0-P[b]	I-0[b]	I-P[b]
		0 Day Samples			
Mesophiles	1	1.2×10^4	5.2×10^3	7.5×10^1	2.2×10^2
"	2	1.2×10^5	2.4×10^5	1.3×10^3	8.1×10^3
"	3	3.0×10^4	1.7×10^4	1.4×10^3	1.0×10^3
Psychrophiles	1	1.6×10^3	5.8×10^2	$<5.0 \times 10^1$	2.5×10^1
"	2	1.1×10^4	1.3×10^5	6.4×10^2	1.4×10^3
"	3	1.3×10^4	1.2×10^4	1.4×10^2	1.3×10^2
		10 Day Samples			
Mesophiles	1	8.7×10^{7c}	1.1×10^7	2.0×10^2	4.0×10^3
"	2	2.9×10^7	3.5×10^7	1.5×10^5	1.2×10^5
"	3	1.1×10^7	3.4×10^7	3.2×10^3	4.2×10^5
Psychrophiles	1	9.1×10^{7c}	1.2×10^7	$<1.0 \times 10^2$	5.8×10^3
"	2	3.2×10^7	3.9×10^7	4.6×10^4	5.6×10^5
"	3	1.6×10^7	3.1×10^7	4.8×10^3	7.7×10^5
		21 Day Samples			
Mesophiles	1	2.1×10^8	1.6×10^8	2.7×10^6	6.3×10^6
"	2	1.8×10^8	2.7×10^8	1.1×10^7	3.0×10^7
"	3	1.1×10^8	1.3×10^8	3.2×10^4	2.7×10^7
Psychrophiles	1	1.8×10^8	1.8×10^8	1.2×10^7	1.3×10^7
"	2	1.8×10^8	4.6×10^8	1.2×10^7	5.8×10^7
"	3	1.3×10^8	1.3×10^8	3.5×10^4	2.6×10^7
		26[d] Day Samples			
Mesophiles	1	1.3×10^{10c}	9.7×10^{9c}	1.5×10^8	1.0×10^8
"	2	5.0×10^9	2.3×10^9	1.0×10^9	5.6×10^8
"	3	7.6×10^9	9.0×10^9	1.6×10^6	5.4×10^8
Psychrophiles	1	1.2×10^{10c}	1.2×10^{10c}	1.8×10^8	1.0×10^{9c}
"	2	6.8×10^9	2.6×10^9	1.9×10^9	2.2×10^9
"	3	5.8×10^9	8.8×10^9	1.8×10^6	9.6×10^8

[a]The counts as given are the average of duplicate plates.

[b]The code for the samples is as follows:
0-0--no radiation, no phosphate dip;
0-P--no radiation, 60 sec. dip in 10% sodium tripolyphosphate;
I-0--100 Krad., no phosphate dip; and
I-P--100 Krad., 60 sec. dip in 10% sodium tripolyphosphate.

[c]These counts were estimated from plates too numerous to count by counting 5 squares on the Quebec colony counter at random and multiplying by 13 to give an estimated colony count for the plate.

[d]The 26 day sample is 21 days in vacuum plus 5 days in air.

percentages of the total microflora, from the countable dilutions, recovered on plates incubated at a temperature suitable for mesophiles and plates incubated at a temperature suitable for psychrophiles. Throughout all of the data in Tables 4 and 5, there appears to be more variability among the three replicates than between the plates incubated at 32°C and plates incubated at 7°C of each replicate. Because each replicate is from a separate muscle from different carcasses of unknown history, and as the initial inoculum of microorganisms on the meat is variable, some variability from replicate to replicate is to be expected. Comparison of the numbers of microorganisms found at 32°C vs. 7°C (in Tables 4 and 5) reveals little difference between those which were expected to be either mesophiles or psychrophiles. It is possible that these bacteria are all the same kinds of bacteria on both sets of counts. Brown and Hoffman (1972) have recently reported finding little difference in the counts or generic distribution of flora from beef for plates held at 20°C and 35°C during incubation.

The microorganisms in Table 5 have been classified into 7 groups. The gram positive, catalase positive cocci include such genera as Micrococcus, Staphylococcus, and Sarcina. The gram positive, catalase

Table 5. Microorganisms Characterized from Representative Isolates from the Mesophilic or Psychrophilic Plate Counts in Percentages of the Total Flora which the Isolates Represent.

Sample identification		Gram positive				Yeasts	Gram negative Rods	Unknown
		Cocci		Rods, nonsporulating				
		Catalase		Catalase	Lactobacillus			
Day and type	Rep. no.	Pos.	Neg.	Pos.				
					0-0[a]			
0 day Meso-	1	36.4	-[c]	-	2.5	0.0	43.0	18.2
philes	2	6.6	0.0	0.0	0.0	0.0	93.4	0.0
	3	6.7	-	-	-	0.0	-	93.3
Psy-	1	8.3	-	-	2.7	0.0	36.1	52.8
chro-	2	25.0	0.0	0.0	0.0	0.0	75.0	0.0
philes	3	0.0	0.0	0.8	0.0	0.0	99.3	0.0
10 day Meso-	1	0.0	0.0	0.0	1.8	0.0	98.2	0.0
philes	2	-	-	-	-	0.0	66.7	33.3
	3	-	-	0.8	1.6	0.0	95.9	1.6
Psy-	1	0.0	15.4	0.0	0.0	0.0	84.6	0.0
chro-	2	0.0	0.0	0.0	0.0	0.0	100.0	0.0
philes	3	-	7.5	-	-	0.0	90.1	2.5
21 day Meso-	1	-	-	-	6.8	0.0	77.2	16.0
philes	2	-	-	3.3	6.7	0.0	83.3	6.7
	3	24.8	-	-	2.5	0.0	69.4	3.3
Psy-	1	0.0	0.0	0.0	26.7	0.0	73.3	0.0
chro-	2	-	-	-	12.2	0.0	82.2	5.6
philes	3	28.7	0.0	0.0	15.8	0.0	55.5	0.0
26 day[b] Meso-	1	-	-	-	1.0	0.0	87.5	11.5
philes	2	0.0	0.0	6.4	0.0	0.0	93.6	0.0
	3	0.0	0.0	3.8	0.0	0.0	96.2	0.0
Psy-	1	-	-	-	0.5	0.0	99.1	0.3
chro-	2	7.2	-	-	-	0.0	75.9	16.9
philes	3	-	8.2	-	-	0.0	24.6	67.2

. . . . . .

Table 5 (cont'd.)

Sample identification		Gram positive				Yeasts	Gram negative Rods	Unknown
		Cocci		Rods, nonsporulating				
		Catalase		Catalase	Lactobacillus			
Day and type	Rep. no.	Pos.	Neg.	Pos.				
					O-P[a]			
0 day Meso-	1	6.8	-[c]	-	-	0.0	52.5	40.7
	2	6.2	-	-	-	0.0	78.1	15.6
philes	3	4.8	0.6	0.6	-	0.0	1.2	92.8
Psy-	1	-	-	-	-	0.0	66.7	33.3
chro-	2	-	-	-	0.8	0.0	45.4	53.8
philes	3	4.2	-	2.9	1.4	1.4	87.5	2.9
10 day Meso-	1	0.0	0.0	0.0	16.3	0.0	83.7	0.0
	2	3.2	-	19.4	6.5	0.0	32.3	38.7
philes	3	0.0	0.0	47.2	2.8	0.0	50.0	0.0
Psy-	1	-	-	-	26.3	0.0	64.9	8.8
chro-	2	0.0	0.0	0.0	15.2	0.0	84.8	0.0
philes	3	0.0	14.6	4.9	24.4	0.0	56.1	0.0
21 day Meso-	1	-	-	-	11.4	0.0	82.9	5.7
	2	-	-	7.7	7.7	0.0	74.4	10.3
philes	3	-	-	3.5	27.0	0.0	68.1	1.4
Psy-	1	0.0	0.0	0.0	11.9	0.0	88.1	0.0
chro-	2	0.0	0.0	0.0	26.8	0.0	73.2	0.0
philes	3	5.9	-	-	24.4	0.0	64.5	5.3
26 day[b] Meso-	1	-	-	-	1.0	0.0	91.2	7.8
	2	0.0	0.0	4.0	0.0	0.0	96.0	0.0
philes	3	-	-	-	6.1	0.0	91.9	2.0
Psy-	1	0.0	0.0	0.0	2.7	0.0	91.3	0.0
chro-	2	9.1	0.0	0.0	3.0	0.0	87.9	0.0
philes	3	0.0	0.0	0.0	11.6	0.0	88.4	0.0
					I-O[a]			
0 day Meso-	1	66.7	0.0	33.3	0.0	0.0	0.0	0.0
	2	63.6	-[c]	-	9.1	0.0	18.2	9.1
philes	3	0.0	0.0	97.1	0.0	2.9	0.0	0.0

Table 5 (cont'd.)

Sample identification		Gram positive				Yeasts	Gram negative Rods	Unknown
		Cocci		Rods, nonsporulating				
		Catalase		Catalase	Lactobacillus			
Day and type	Rep. no.	Pos.	Neg.	Pos.				
Psy-	1[d]	-	-	-	-	-	-	-
chro-	2	-	-	-	-	0.0	62.5	37.5
philes	3	0.0	0.0	0.0	7.7	0.0	92.3	0.0
10 day	1	25.0	0.0	0.0	50.0	25.0	0.0	0.0
Meso-	2	0.0	0.0	0.0	100.0	0.0	0.0	0.0
philes	3	2.6	0.0	0.0	97.4	0.0	0.0	0.0
Psy-	1[d]	-	-	-	-	-	-	-
chro-	2	0.0	0.0	0.0	98.0	0.0	2.0	0.0
philes	3	0.0	0.0	0.0	98.0	2.0	0.0	0.0
21 day	1	0.0	0.0	0.0	97.2	2.8	0.0	0.0
Meso-	2	0.0	0.0	16.0	84.0	0.0	0.0	0.0
philes	3	0.0	0.0	0.0	99.3	0.7	0.0	0.0
Psy-	1	0.0	0.0	0.0	95.4	4.6	0.0	0.0
chro-	2	0.0	0.0	0.0	99.1	0.0	0.9	0.0
philes	3	0.0	0.0	0.0	83.3	16.7	0.0	0.0
26 day[b]	1	0.0	0.0	0.0	99.2	0.8	0.0	0.0
Meso-	2	-	-	26.7	25.7	0.0	-	47.6
philes	3	0.0	0.0	1.2	96.5	2.3	0.0	0.0
Psy-	1	0.0	0.0	0.0	98.4	1.6	0.0	0.0
chro-	2	32.7	0.0	0.0	66.7	0.0	0.7	0.0
philes	3	0.0	0.0	0.0	98.5	1.5	0.0	0.0
					I-P[a]			
0 day	1	60.0	0.0	0.0	0.0	0.0	40.0	0.0
Meso-	2	58.7	-[c]	-	-	0.0	4.0	37.3
philes	3	0.0	0.0	78.6	7.1	14.3	0.0	0.0
Psy-	1	0.0	0.0	100.0	0.0	0.0	0.0	0.0
chro-	2	-	-	43.8	-	0.0	-	56.2
philes	3	0.0	0.0	52.9	23.5	0.0	23.5	0.0

Table 5 (cont'd.)

Sample identification		Gram positive				Yeasts	Gram negative Rods	Unknown
		Cocci		Rods, nonsporulating				
		Catalase		Catalase	Lactobacillus			
Day and type	Rep. no.	Pos.	Neg.	Pos.				
10 day Meso-	1	-	-	23.4	-	0.0	-	76.6
philes	2	2.8	-	1.4	95.1	0.0	-	0.7
	3	0.0	0.0	80.0	20.0	0.0	0.0	0.0
Psy-	1	-	-	7.1	14.3	0.0	-	78.6
chro-	2	67.9	0.0	8.9	5.4	0.0	17.9	0.0
philes	3	0.0	0.0	0.0	100.0	0.0	0.0	0.0
21 day Meso-	1	0.0	0.0	0.0	100.0	0.0	0.0	0.0
philes	2	0.0	0.0	20.6	79.4	0.0	0.0	0.0
	3	0.4	0.0	0.0	99.2	0.4	0.0	0.0
Psy-	1	-	-	-	99.0	0.0	-	1.0
chro-	2	28.3	0.0	0.0	56.6	0.0	15.1	0.0
philes	3	-	-	21.7	76.0	0.0	-	2.2
26 day[b] Meso-	1	0.0	0.0	3.9	96.1	0.0	0.0	0.0
philes	2	0.0	0.0	0.0	82.8	0.0	17.2	0.0
	3	0.0	0.0	85.2	14.8	0.0	0.0	0.0
Psy-	1	60.9	0.0	0.0	39.1	0.0	0.0	0.0
chro-	2	15.7	0.0	0.0	82.7	0.0	1.6	0.0
philes	3	0.0	0.0	0.0	100.0	0.0	0.0	0.0

[a]The code for the samples is as follows:
0-0--no radiation, no phosphate dip;
0-P--no radiation, 60 sec. dip in 10% sodium tripolyphosphate;
I-0--100 Krad., no phosphate dip; and
I-P--100 Krad., 60 sec. dip in 10% sodium tripolyphosphate.

[b]The 26 day sample is 21 days in vacuum plus 5 days in air.

[c]The lines fill places where 0.0 cannot be put due to the presence of unknowns.

[d]These samples had no colonies on the plates, thus no cultures could be isolated.

negative cocci include such genera as Streptococcus, Pediococcus, and Leuconostoc, many of which can produce lactic acid. All of the gram positive rods found were nonsporulating, so they cannot have been Bacillus and Clostridium species. The gram positive rods were further divided into catalase positive organisms or into Lactobacillus species. The gram positive, nonsporulating, catalase positive rods include several genera some of which are Kurthia, Microbacterium, and Corynebacterium. Microbacterium thermosphactum has been reported to be found in red meats and produces some lactic acid (McLean and Sulzbacher, 1953; Wolin et al., 1957; Jaye et al., 1962; Ayres, 1960a; Pierson et al., 1970). Lactobacillus species also produce lactic acid and are usually termed facultatively anaerobic. Those cultures identified as yeasts were not characterized further. All gram negative rods were left simply as gram negative rods. As explained in the literature review, the usual spoilage organisms of fresh beef, poultry, and fish are the psychrophilic pseudomonad-achromobacter bacteria, which are gram negative rods. The bacteria labeled unknown in this study consist of cultures which either could not be purified after isolation, or gram stained satisfactorily, or else consisted of such pleomorphic bacteria that they could not be classified morphologically. No gram negative cocci were isolated from any of the samples. Some of the unidentified bacteria could be in this category.

Gram negative cocci have been reported to be found in irradiated ground beef (Tiwari and Maxcy, 1971 and 1972, Maxcy and Tiwari, in press); however, since these bacteria are reported to be aerobic and are found in aerobically packaged beef, the vacuum packaging used in this experiment could suppress their growth and account for the failure

to discover their presence.

Examination of the data in Table 5 reveals several findings. In the 0-0 samples the initial flora consists primarily of gram negative rods with some gram positive, catalase positive cocci also present. In one replicate a very few Lactobacillus were found. Not until the 21st day have the Lactobacillus increased in numbers enough to be found in all the replicates, although the gram negative rods still outnumber them. When the meat is re-exposed to oxygen, the gram negative organisms become more dominant with just a very few Lactobacillus, gram positive, nonsporulating, catalase positive rods, and gram positive cocci being present. In the 0-P samples the situation is much the same. The initial flora consists primarily of gram negative bacteria with a few gram positive cocci and rods, Lactobacillus, and yeasts present. At the 10 day sampling, the 0-P had more Lactobacillus and gram positive, nonsporulating, catalase positive rods than did the 0-0 samples. By the 21st day these gram positive rod types were still present in about the same proportions as at the 10 day sampling period; on the whole these numbers are higher than those of the 0-0 samples for this same period. The gram negative flora still comprise the majority of the flora after 10 days. In the 0-P samples, as in the 0-0 samples, the gram negative flora increases its proportion of the total flora on the re-exposure of the meat to oxygen. However, there appear to be slightly more Lactobacillus organisms isolated from the 26 day samples of 0-P than from the 0-0 samples.

In the I-0 samples initially the flora consisted of mostly gram positive, catalase positive cocci, gram positive, nonsporulating, catalase positive rods, and gram negative rods with a few Lactobacillus

and yeasts. There is a lot of variability among replicates in this 0 day sampling period. The gram negative rods showing up in this initial sampling period are most likely achromobacter type organisms, which have been reported in the literature as being more radiation resistant than the pseudomonads. In the 10 day and 21 day sampling period the flora is predominantly Lactobacillus with occasional gram positive, catalase positive cocci and yeasts appearing. On re-exposure of the meat to oxygen, the types of bacteria present did not show any great shifts, but remained essentially the same with the appearance of occasional gram positive, nonsporulating, catalase positive rods. The I-P samples were very similar to the I-0 samples. The 0 day sampling yielded gram positive, catalase positive cocci and rods (nonsporulating) with a few Lactobacillus, yeasts, and gram negative rods being isolated. On the whole undoubtedly due to the irradiation, the I-0 and I-P samples had many fewer gram negative rods present than did the 0-0 and 0-P samples. In the 10 day and 21 day sampling periods the I-P samples had primarily Lactobacillus organisms with many gram positive, nonsporulating, catalase positive rods and a few gram positive, catalase positive cocci present, as did the I-0 samples. These results are consistent with those reported in the literature for the irradiated, vacuum-packaged, phosphate treated and nonphosphate treated fish. The 26 day sampling of I-P yielded much the same results as the I-0 samples; that is, a predominance of Lactobacillus with some gram positive, catalase positive cocci and rods (nonsporulating).

For the major microfloral groups present on the treated beef samples, the Figures 1 through 4 illustrate the relationship of the total counts and the percentage of the total count which each group of

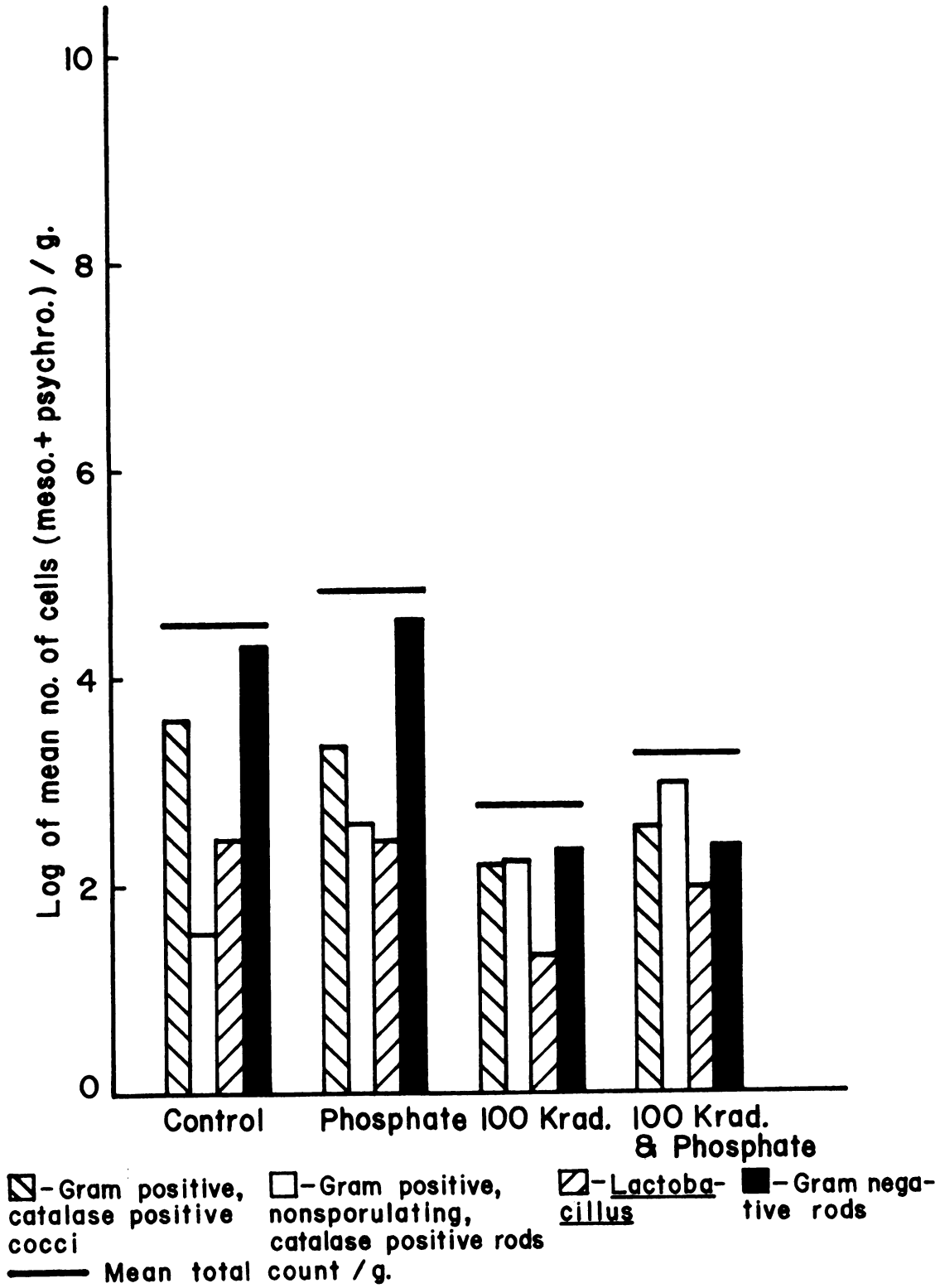


Figure 1. Initial Populations of the Major Microfloral Groups on Beef which Received the Four Treatments.

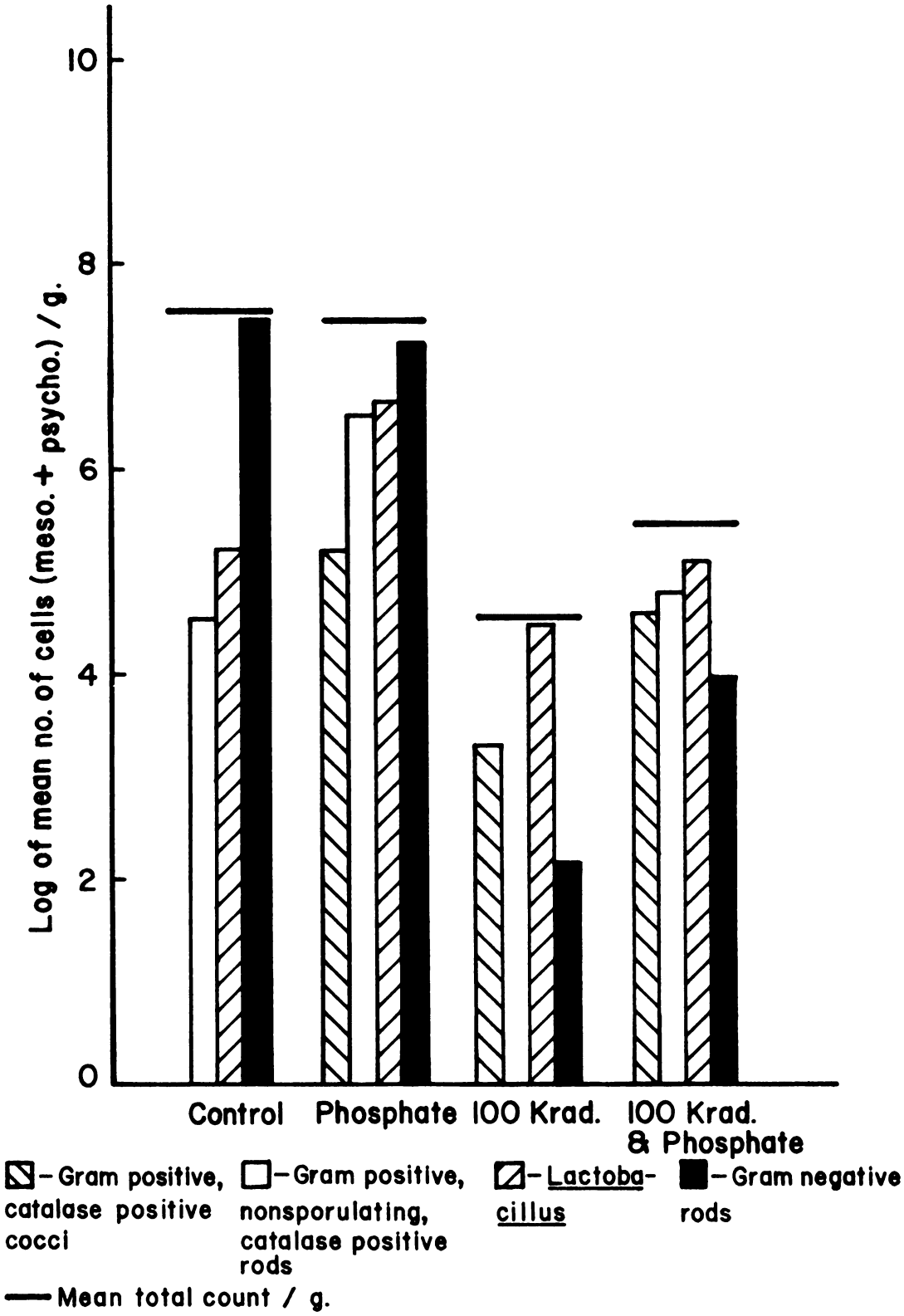


Figure 2. Populations of the Major Microfloral Groups after 10 Days in Vacuum at 40°F (4.4°C) on Beef which Received the Four Treatments.

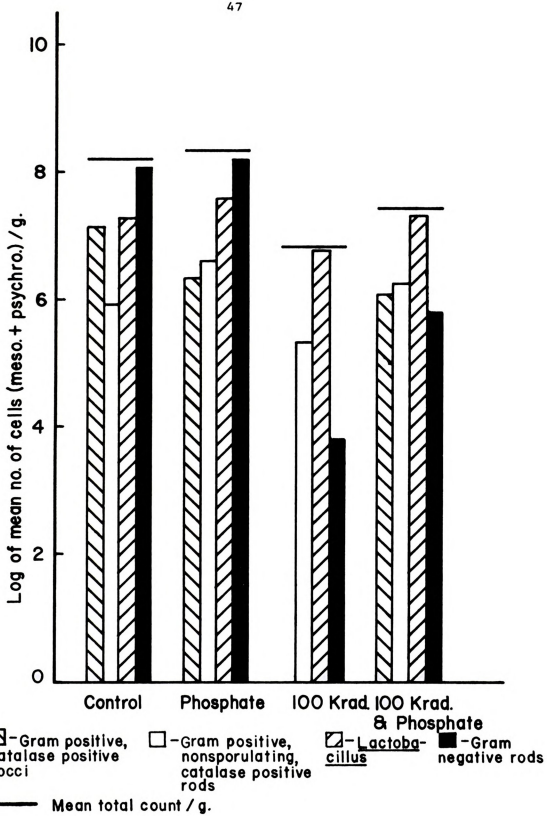


Figure 3. Populations of the Major Microfloral Groups after 21 Days in Vacuum at 40°F (4.4°C) on Beef which Received the Four Treatments.

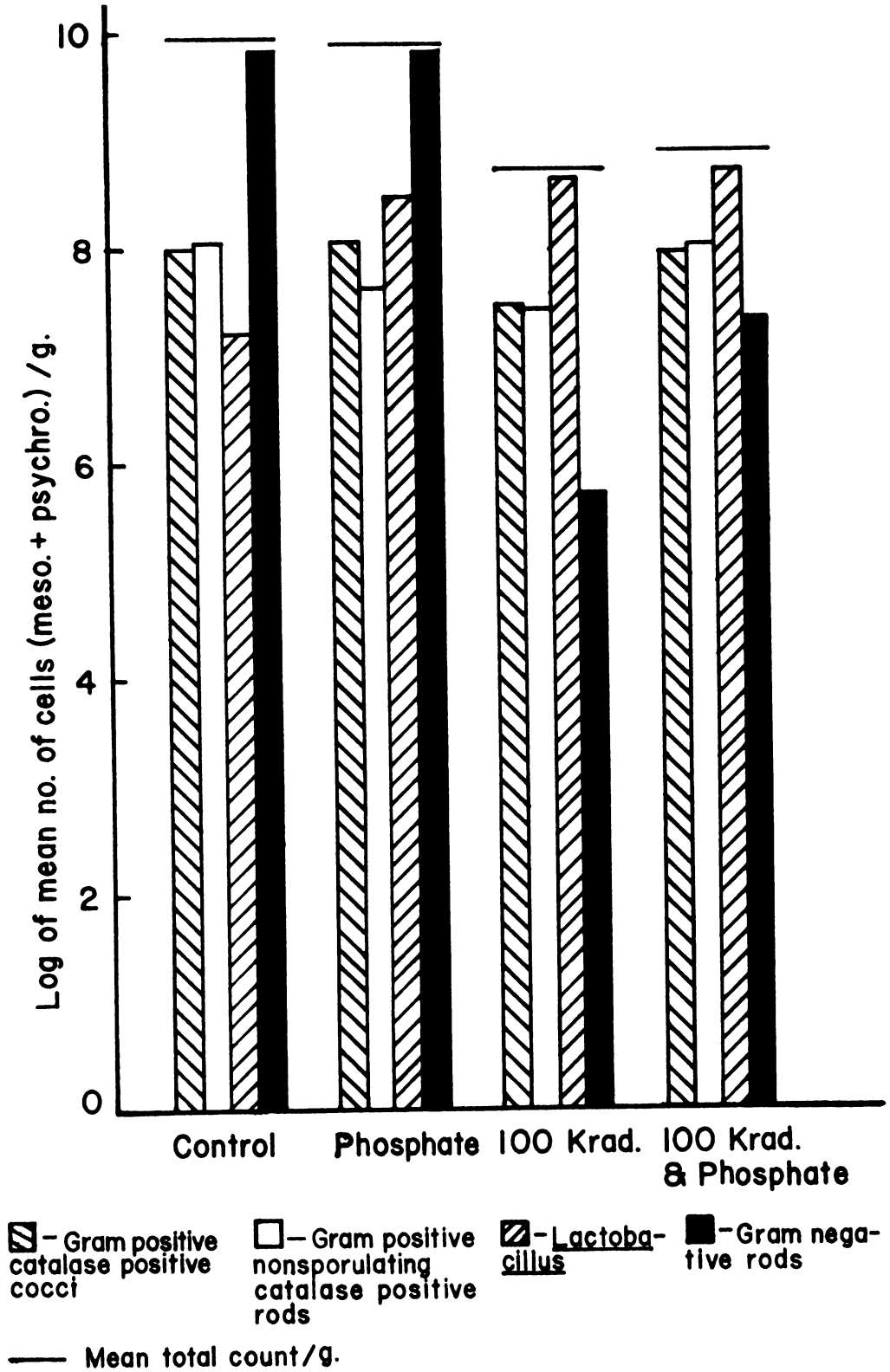


Figure 4. Populations of the Major Microfloral Groups after 21 Days in Vacuum Plus 5 Days in Air at 40°F (4.4°C) on Beef which Received the Four Treatments.

organisms represents. Three of the groups shown on Table 5 are not represented in Figures 1 through 4, because 2 of these groups--the gram positive, catalase negative cocci and the yeasts--are present in only a very few of the samples and as such do not constitute a major portion of the microflora. The third group, the unknown category, was eliminated simply because the organisms within this category were not classified. The groups which are represented in Figures 1 through 4 are as follows: the gram positive, catalase positive cocci; the gram positive, nonsporulating, catalase positive rods; Lactobacillus; and the gram negative rods. These 4 groups of organisms are the bacterial groups which appear most consistently in large numbers on the beef samples; thus, the phrase "major microfloral groups" is used in reference to them. The mean values from which these Figures are plotted are given in Tables 7 and 8 in the Appendix, with the mesophiles and psychrophiles being averaged together on the ground that there appeared to be little difference in the flora and counts of the 2 groups and, therefore, they are the same types of bacteria.

The Figures 1 through 4 allow a direct comparison of the major microfloral groups among the treatments for each of the four sampling periods. First, the Figures illustrate the rise in total count and relative increase in count for the major microfloral groups. The effect of irradiation upon the population levels and the resultant microfloral shifts are easily visualized from Figures 1 through 4. Note that the population levels of the two irradiated (I-0 and I-P) samples continue to lag behind the unirradiated (0-0 and 0-P) samples throughout the 26 day storage period. Also, by transforming the percentages of Table 5 into numbers of organisms/gram on the Figures 1 through 4, the

observation can be made that even though a group may be only 1 or 2 percent of the total flora, when the counts reach 10^7 through 10^9 cells/gram, we are faced with a sizable number of bacteria. This can be seen on treatment I-P, for example, at the 21 day and 26 day sampling periods where the gram negative rods only account for 2.5 to 3 percent (average values from Table 8) of the total flora, yet in terms of numbers/gram this equals 10^6 and 10^7 organisms/gram--too many to be considered insignificant. The effect of the phosphate as observed on Figures 1 through 4 can be interpreted as a slight enhancement of the microflora levels at the 0 day, 10 day, and 21 day sampling periods; but by the 26th day the differences are practically nonexistent, except for the gram negative rods on the I-0 samples being lower than the levels on the I-P samples. These results agree with those reported in the literature (Giddings, 1969; Spinelli et al., 1967; Miyauchi et al., 1966).

Figures 5 and 6 illustrate the effects of the proposed treatment upon the growth of Lactobacillus and the gram negative rods, respectively. These figures show even more clearly that irradiation kills many more gram negative rods than Lactobacillus organisms. It is evident from Figures 2 and 6 that the gram negative rods reach the level of 10^7 before the 10th day and the level of 10^8 before the 21st day (Figures 3 and 6) in the 0-0 and 0-P samples, whereas in the I-P samples the gram negative rods do not reach the 10^7 level until after the 21st day (Figures 3, 4 and 6). A range of 10^6 to 10^8 organisms/gram has been reported in the literature (Ayres, 1960a, 1960b; Brown and Hoffman, 1972; Maxcy and Tiwari, in press) to be the spoilage level of aerobically stored beef steak and ground beef. In Figures 2, 3, 4, 5,

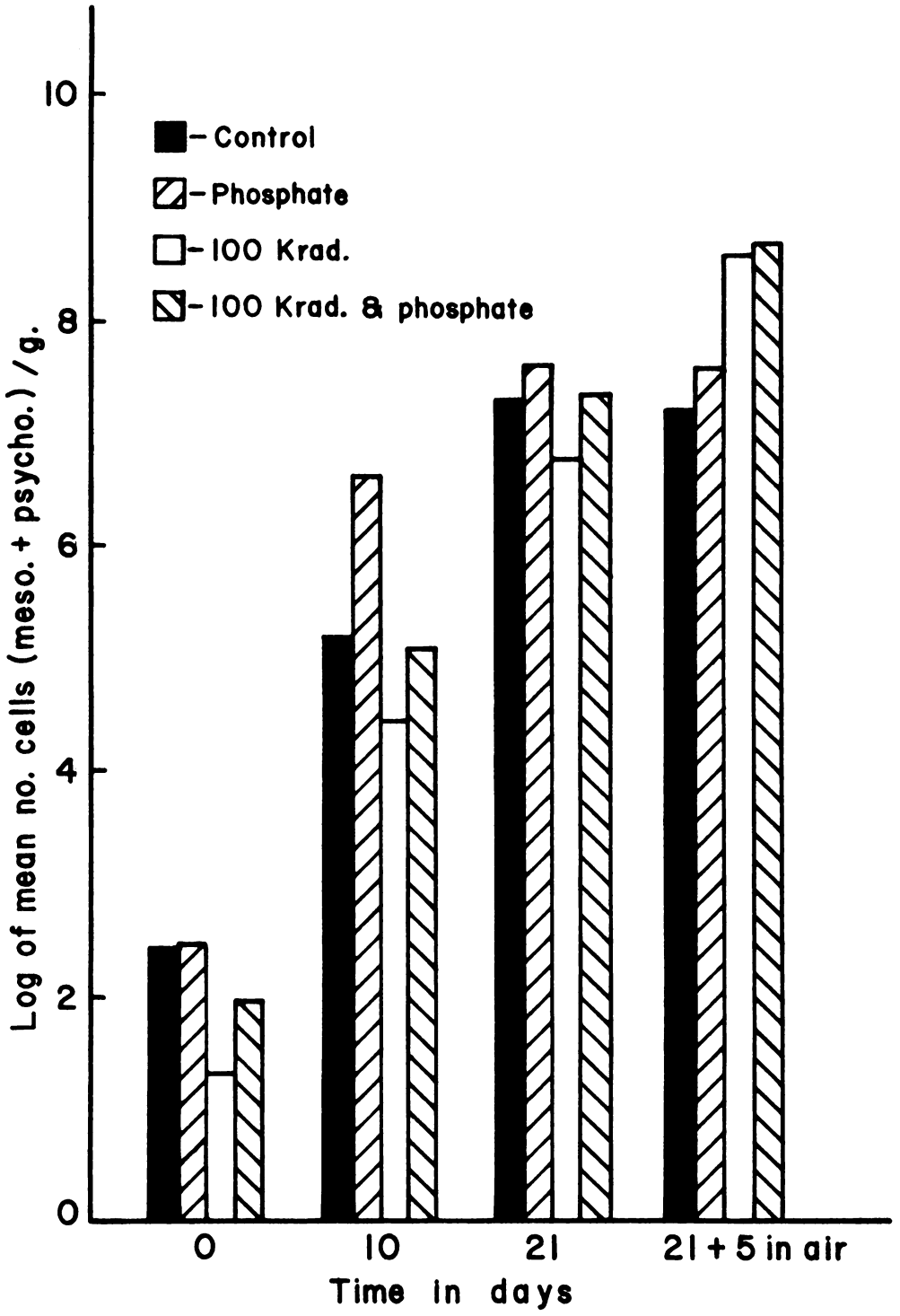
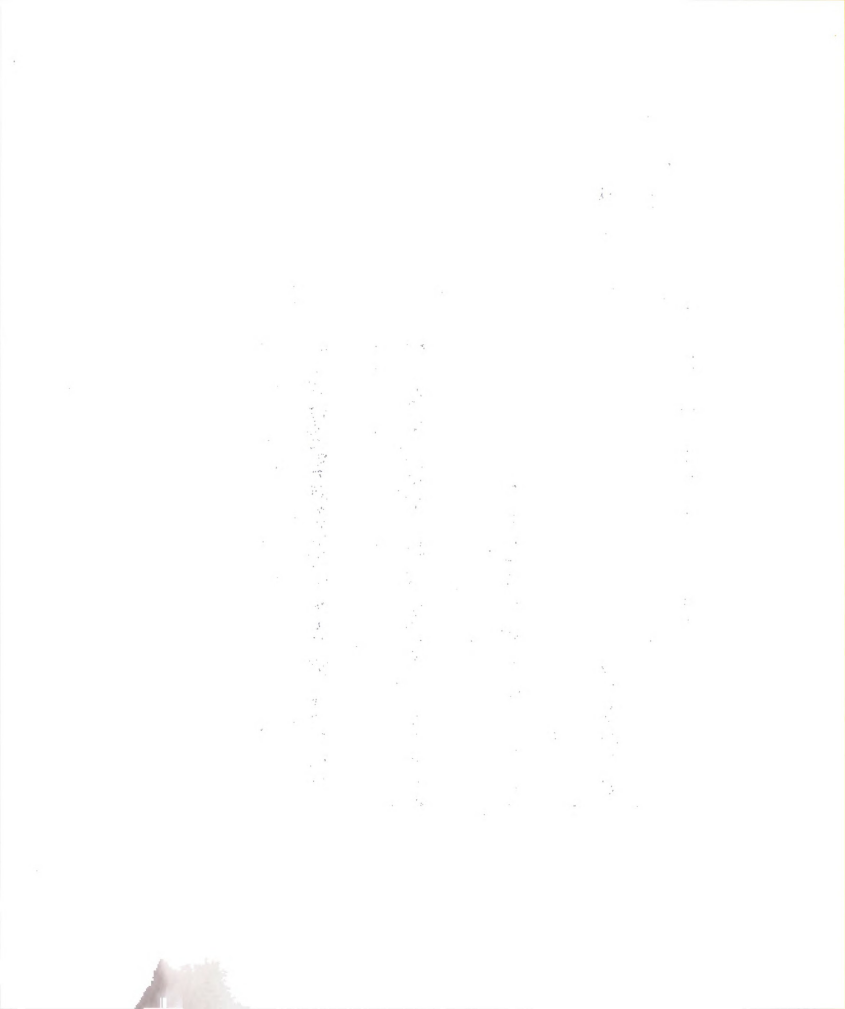


Figure 5. The Growth of Lactobacillus on Vacuum-packaged Beef as Influenced by the Proposed Process at 40°F (4.4°C).



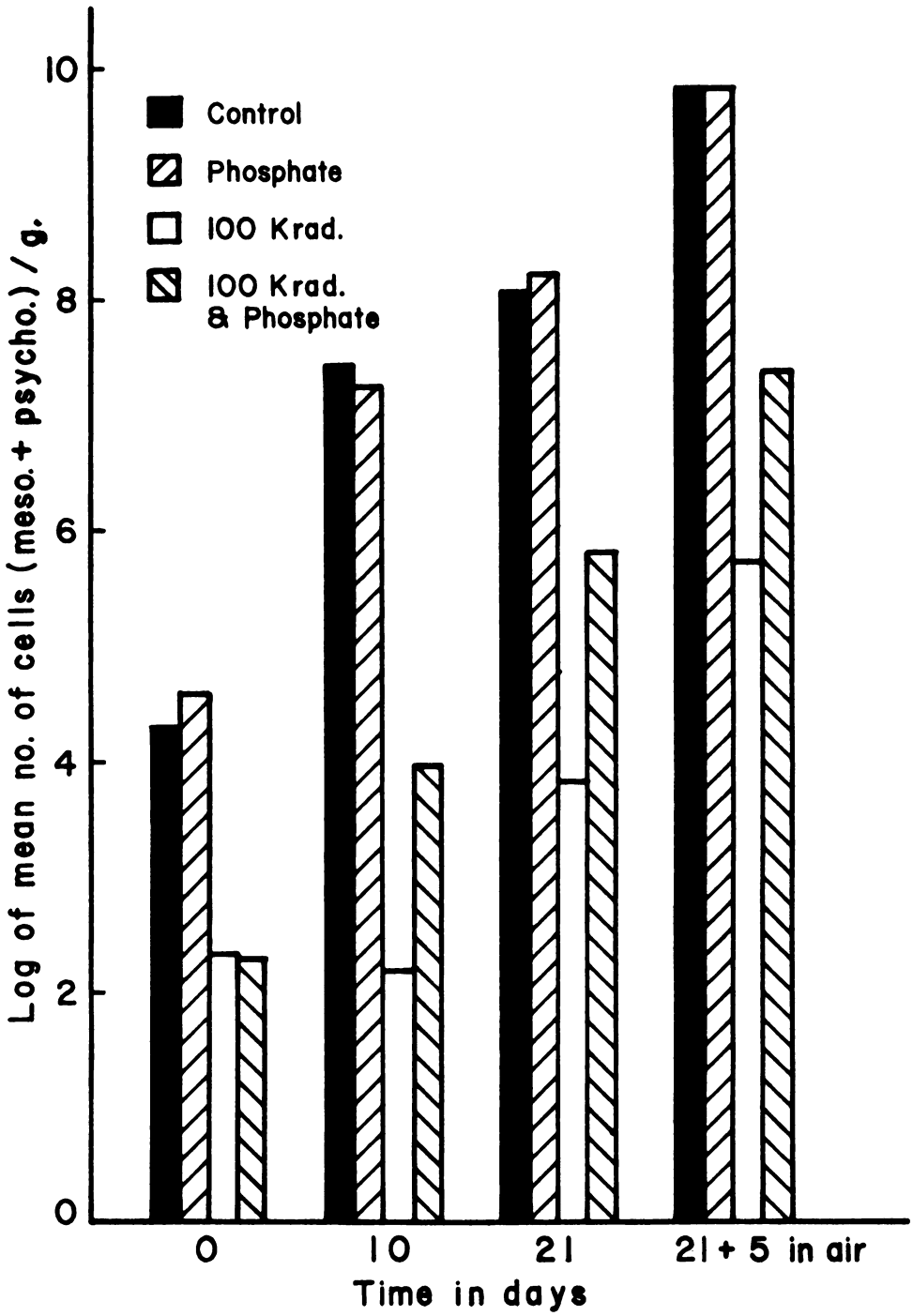


Figure 6. The Growth of Gram Negative Rods on Vacuum-packaged Beef as Influenced by the Proposed Process at 40°F (4.4°C).

and 6 it can be seen that Lactobacillus is the predominant organism on the irradiated samples and reaches levels of 10^6 and 10^7 by the 21st day and levels of 10^8 by the 26th day. Because the beef samples were held in vacuum packages for the major portion of the storage in this study, the microflora are different from the microflora on aerobically held beef, although the total counts reach approximately the same level by the 26th day as the aerobically stored beef. Therefore, the usual criteria of spoilage need not apply. Another study has been made which evaluates the consumer's response as to spoilage of the irradiated beef at this period of storage.[2]

B. Food poisoning microorganisms, coliforms and fecal coliforms.

The data for the enumeration of coagulase positive staphylococci and the most probable numbers of coliforms and fecal coliforms are given in Table 6. As seen from Table 6 there are a few samples of beef which had low numbers of coagulase positive Staphylococcus. They are even present in low numbers in some of the irradiated samples; however, no outgrowth appears to be occurring in the irradiated samples. In the 0-0 and 0-P samples at the 10 day and 21 day sampling periods the Staphylococcus seem to persist and possibly even increase slightly. In the 26 day samples, however, practically none is detected. From the data it would seem as though irradiation and the resulting shift in flora controls their persistence and possible outgrowth. The refrigeration storage is also a primary factor in preventing outgrowth of these organisms.

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[2]Urbain, W. M. 1973. Private communication.

Table 6. Levels/Gram of Coagulase Positive Staphylococcus aureus, Coliforms, and Fecal Coliforms. All Results Based on a Most Probable Number Method.

Sample day	Rep. no.	0-0[a]	0-P[a]	I-0[a]	I-P[a]
		Coagulase Positive Staphylococcus aureus			
0 day	1	>100 and <1,000	>10 and <100	>5 and <10	>10 and <100
	2	<10	<10	<10	<10
	3	<10	<10	<10	<10
10 day	1	>100 and <1,000	<10	>10 and <100	<10
	2	<10	>100 and <1,000	>10 and <100	<10
	3	<10	<10	<10	<10
21 day	1	>1,000 and <10,000	>10 and <100	>100 and <1,000	<10
	2	>100 and <1,000	>100 and <1,000	<10	>10 and <100
	3	<10	<10	<10	<10
26[b] day	1	<10	>10 and <100	>10 and <100	<10
	2	<10	<10	<10	<10
	3	<10	<10	<10	<10
		Coliforms			
0 day	1	9.3	0.4	<0.3	<0.3
	2	4.3	46	<0.3	<0.3
	3	9.3	24	<0.3	<0.3

Table 6 (cont'd.)

Sample day	Rep. no.	0-0[a]	0-P[a]	I-0[a]	I-P[a]
10 day	1	11,000	11,000	<0.3	<0.3
	2	0.9	0.4	<0.3	<0.3
	3	46	9.3	<0.3	<0.4
21 day	1	>110,000	>110,000	<0.3	<0.3
	2	0.7	46,000	<0.3	<0.3
	3	2.3	700	<0.3	>1,100
26[b] day	1	1,500,000	>11,000,000	15	<0.3
	2	0.4	1,500	<0.3	<0.3
	3	2.3	>1,100,000	<0.3	<0.3
		Fecal Coliforms			
0 day	1	4.3	<0.3	<0.3	<0.3
	2	<0.3	<0.3	<0.3	<0.3
	3	4.3	2.3	<0.3	<0.3
10 day	1	0.7	0.4	<0.3	<0.3
	2	<0.3	<0.3	<0.3	<0.3
	3	15.0	4.3	<0.3	<0.3
21 day	1	1.4	0.4	<0.3	<0.3
	2	<0.3	<0.3	<0.3	<0.3
	3	0.9	2.3	<0.3	43.0

Table 6 (cont'd.)

Sample day	Rep. no.	0-0[a]	0-P[a]	I-0[a]	I-P[a]
26[b] day	1	<0.3	<0.3	<0.3	<0.3
	2	<0.3	<0.3	<0.3	<0.3
	3	0.4	0.4	<0.3	<0.3

[a]The code for the samples is as follows:
0-0--no radiation, no phosphate dip;
0-P--no radiation, 60 sec. dip in 10% sodium tripolyphosphate;
I-0--100 Krad., no phosphate dip;
I-P--100 Krad., 60 sec. dip in 10% sodium tripolyphosphate.

[b]The 26 day sample is 21 days in vacuum plus 5 days in air.

Examination of the data in Table 6 on the coliforms and fecal coliforms reveals that with the exception of one sample (I-P, 21 day, rep. 3) irradiation has eliminated many of the coliforms. In any case, the presence of coliforms (and fecal coliforms) is not proof of fecal contamination (Niven, 1969); moreover, the levels found in this study were low. The 0-P samples seem to have an outgrowth of coliforms in all three replicates. This could possibly be a result of the pH rise in the meat due to the phosphate treatment (Giddings, 1969).

The results of the Salmonella and Clostridium perfringens evaluations have not been put in tabular form, but are summarized here. No Salmonella organisms were detected in any of the samples, thus, no evaluation of their reaction to the proposed process can be made. In the smallest dilutions for Clostridium perfringens none was found; thus, all samples had less than 10/gram based on finding zero Cl. perfringens in the duplicate 1:10 SPS agar plates.

SUMMARY AND RECOMMENDATIONS

In this study of phosphate treated, vacuum packaged, irradiated, refrigeration stored beef the flora which survives irradiation and thrives in the vacuum packaging is primarily Lactobacillus, with some gram positive, catalase positive cocci and rods (nonsporulating) and gram negative rods persisting in smaller numbers. These results agree with those reported for phosphate treated, vacuum packaged, irradiated fish (Spinelli et al., 1967, Miyauchi et al., 1966). The flora on the irradiated, nonphosphated samples was very similar to the irradiated, phosphate treated samples. Whereas, the unirradiated, nonphosphated and the unirradiated, phosphate treated samples had a completely different bacterial flora. These samples have primarily gram negative rods with a very few gram positive, nonsporulating, catalase positive rods and Lactobacillus. The total bacterial counts as found in this study are similar to those reported by Giddings (1969) and Urbain et al. (1968, 1969).

Based on this study there is no increased threat to public health from coagulase positive Staphylococcus aureus, coliforms, or fecal coliforms due to the proposed phosphate dip, vacuum package, irradiation process. No evaluation can be made for Salmonella or Clostridium perfringens as none was detected in any of the samples.

On the whole, this study confirms the shelf life extension of the beef as a result of the irradiation in the proposed process as reported

by Giddings (1969), Urbain *et al.* (1968, 1969), and Urbain and Giddings (1972).

Further study of the microbiological implications of the proposed process as outlined in Table 1 could include inoculated pack studies with *Salmonella* and *Clostridium perfringens* to evaluate their behavior in the proposed process.

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APPENDIX

Table 7. Means of the Total Counts/Gram Given in Table 4.

Sample day	Mesophile mean	Psychrophile mean	Mesophile plus psychrophile mean
		0-0[a]	
0 day	5.4×10^4	8.5×10^3	3.3×10^4
10 day	4.2×10^7	4.6×10^7	4.4×10^7
21 day	1.7×10^8	1.6×10^8	1.6×10^8
26 day[b]	8.5×10^9	8.2×10^9	8.4×10^9
		0-P[a]	
0 day	8.7×10^4	4.8×10^4	6.7×10^4
10 day	2.7×10^7	2.7×10^7	2.7×10^7
21 day	1.9×10^8	2.6×10^8	2.2×10^8
26 day[b]	7.0×10^9	7.8×10^9	7.4×10^9
		I-0[a]	
0 day	9.2×10^2	2.8×10^2	6.0×10^2
10 day	5.1×10^4	1.7×10^4	3.4×10^4
21 day	4.6×10^6	8.0×10^6	6.3×10^6
26 day[b]	3.8×10^8	6.9×10^8	5.4×10^8
		I-P[a]	
0 day	3.1×10^3	5.2×10^2	1.8×10^3
10 day	1.8×10^5	4.5×10^5	3.1×10^5
21 day	2.1×10^7	3.2×10^7	2.6×10^7
26 day[b]	4.0×10^8	1.4×10^9	7.4×10^8

[a]The code for the samples is as follows:
0-0--no radiation, no phosphate dip;
0-P--no radiation, 60 sec. dip in 10% sodium tripolyphosphate;
I-0--100 Krad., no phosphate dip; and
I-P--100 Krad., 60 sec. dip in 10% sodium tripolyphosphate.

[b]The 26 day sample is 21 days in vacuum plus 5 days in air.

Table 8. Means of the Percentages of the Bacteria in Table 5.[a]

Sample identification	Gram positive: Cocci: Catalase: Pos.	Gram positive: Cocci: Catalase: Neg.	Gram positive: Rods, nonsporulating: Catalase: Pos.	Gram positive: Rods, nonsporulating: Lactobacillus	Yeasts	Gram negative: Rods	Unknown
			0-0[b]				
			0 day				
Mesophiles	16.5	0.0	0.0	0.8	0.0	45.4	37.1
Psychrophiles	11.1	0.0	0.2	0.9	0.0	70.1	17.6
Meso + psy	12.1	0.0	0.1	0.8	0.0	57.8	27.3
			10 day				
Mesophiles	0.0	0.0	0.2	1.1	0.0	74.3	11.6
Psychrophiles	0.0	7.6	0.0	0.0	0.0	91.5	0.8
Meso + psy	0.0	3.8	0.1	0.5	0.0	82.9	6.2
			21 day				
Mesophiles	8.2	0.0	1.1	5.3	0.0	76.6	8.6
Psychrophiles	9.5	0.0	0.0	18.2	0.0	70.3	1.8
Meso + psy	8.9	0.0	0.5	11.8	0.0	73.5	5.2
			26 day[c]				
Mesophiles	0.0	0.0	3.4	0.3	0.0	92.4	3.8
Psychrophiles	2.4	2.7	0.0	0.1	0.0	66.5	28.1
Meso + psy	1.2	1.3	1.7	0.2	0.0	79.5	15.9
			0-P[b]				
			0 day				
Mesophiles	5.9	0.2	0.2	0.0	0.0	43.9	49.7
Psychrophiles	1.4	0.0	1.0	0.7	0.5	66.5	30.0
Meso + psy	3.6	0.1	0.6	0.4	0.3	55.2	39.8
			10 day				
Mesophiles	1.1	0.0	22.2	8.5	0.0	55.3	12.9
Psychrophiles	0.0	4.9	1.6	22.0	0.0	68.6	2.9
Meso + psy	0.6	2.4	11.9	15.2	0.0	62.0	7.9

Table 8 (cont'd.)

Sample identification	Gram positive				Yeasts	Gram negative	Unknown
	Cocci		Rods, nonsporulating			Rods	
	Catalase		Catalase	Lactobacillus			
	Pos.	Neg.	Pos.				
			21 day				
Mesophiles	0.0	0.0	3.7	15.4	0.0	75.1	5.8
Psychrophiles	2.0	0.0	0.0	21.0	0.0	75.3	1.8
Meso + psy	1.0	0.0	1.8	18.2	0.0	75.2	2.8
			26 day[c]				
Mesophiles	0.0	0.0	1.3	2.4	0.0	93.0	3.3
Psychrophiles	3.0	0.0	0.0	5.8	0.0	89.2	0.0
Meso + psy	1.5	0.0	0.6	4.1	0.0	91.1	1.6
			I-0[b]				
			0 day				
Mesophiles	43.1	0.0	43.4	3.0	0.9	6.0	3.0
Psychrophiles	0.0	0.0	0.0	3.8	0.0	77.4	18.7
Meso + psy	25.8	0.0	26.0	3.3	0.5	34.6	9.3
			10 day				
Mesophiles	9.2	0.0	0.0	82.4	8.3	0.0	0.0
Psychrophiles	0.0	0.0	0.0	98.0	1.0	0.6	0.0
Meso + psy	5.5	0.0	0.0	88.6	5.4	0.4	0.0
			21 day				
Mesophiles	0.0	0.0	5.3	93.5	1.1	0.0	0.0
Psychrophiles	0.0	0.0	0.0	92.6	7.1	0.3	0.0
Meso + psy	0.0	0.0	3.3	93.0	4.1	0.1	0.0
			26 day[c]				
Mesophiles	0.0	0.0	9.3	73.8	1.0	0.0	15.8
Psychrophiles	10.9	0.0	0.0	87.8	1.0	0.2	0.0
Meso + psy	5.4	0.0	4.6	80.8	1.0	0.1	7.9

Table 8 (cont'd.)

Sample identification	Gram positive				Yeasts	Gram negative	Unknown
	Cocci		Rods, nonsporulating			Rods	
	Catalase		Catalase	Lactobacillus			
	Pos.	Neg.	Pos.				
			I-P[b]				
			0 day				
Mesophiles	39.5	0.0	26.2	2.3	4.7	14.6	12.4
Psychrophiles	0.0	0.0	65.5	7.8	0.0	7.8	18.7
Meso + psy	19.7	0.0	52.5	5.1	2.3	11.2	15.5
			10 day				
Mesophiles	0.9	0.0	34.9	38.3	0.0	0.0	25.5
Psychrophiles	22.6	0.0	5.3	39.9	0.0	5.9	26.2
Meso + psy	11.7	0.0	20.1	39.1	0.0	2.9	25.8
			21 day				
Mesophiles	0.1	0.0	6.8	92.8	0.1	0.0	0.0
Psychrophiles	9.4	0.0	7.2	77.2	0.0	5.0	1.0
Meso + psy	4.7	0.0	7.0	85.0	0.0	2.5	0.5
			26 day[c]				
Mesophiles	0.0	0.0	29.7	64.5	0.0	5.7	0.0
Psychrophiles	25.5	0.0	0.0	73.9	0.0	0.5	0.0
Meso + psy	12.7	0.0	14.8	69.2	0.0	3.1	0.0

[a]The blank spaces on Table 5 have been filled in with 0.0 here for determining the means.

[b]The code for the samples is as follows:
0-0--no radiation, no phosphate dip;
0-P--no radiation, 60 sec. dip in 10% sodiun tripolyphosphate;
I-0--100 Krad., no phosphate dip; and
I-P--100 Krad., 60 sec. dip in 10% sodium tripolyphosphate.

[c]The 26 day sample is 21 days in vacuum plus 5 days in air.