AN ABSTRACT OF THE THESIS OF

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Title Survival Curves of Bacteria With Public Health Significance Irradiated in Crabmeat and in Hartsell's Broth

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Gamma irradiation survival curves of Salmonella enteriditis, Salmonella paratyphi A, Salmonella cholerasuis, Salmonella pullorum and Streptococcus pyogenes were determined in crabmeat and in Hartsell's broth. The survival pattern of Staphylococcus aureus in crabmeat was also determined.

A "tailing off" was found in the survival patterns of Salmonella paratyphi A, Salmonella pullorum, Salmonella enteriditis and Staphylococcus aureus when they were irradiated in crabmeat, but was not found when these organisms (excluding Staphylococcus aureus) were irradiated in Hartsell's broth. However, Salmonella cholerasuis and Streptococcus pyogenes showed a definite "tailing off" in the broth while only weakly, if any, in the crabmeat.

A comparison was made of the gamma irradiation recovery

of Salmonella cholerasuis in crabmeat assayed immediately following irradiation to that assayed after the crabmeat had been held seven days at 4°C. The refrigerated samples showed lower survival.

Staphylococcus aureus white mutants were observed at 1.0 Mrad and higher doses. Three mutants were isolated for further investigations. These mutants were stable and gave varied coagulase and hemolytic tests. In an examination of one mutant, no greater resistance to irradiation than parent culture was found.

SURVIVAL CURVES OF BACTERIA WITH PUBLIC HEALTH SIGNIFICANCE IRRADIATED IN CRABMEAT AND IN HARTSELL'S BROTH

by

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INTRODUCTION

Radiation pasteurization of low fat seafoods such as clams, shrimp and crab, have been found to extend the refrigerated storage life of these products and thereby indicates a potential commercial process (Hannan and Thornley, 1957; Nickerson, Goldblith and Masurovsky, 1962; Scholz, et al., 1962; Miyauchi et al., 1963; and Ingram and Rhodes, 1962). As was found with the initiation of heat processing of foods, problems have evolved with the use of ionizing radiation for food pasteurization and sterilization. One general area of problems has arisen with the influence of ionizing radiation on the contaminating, possibly pathogenic, flora. Recent works have shown that certain common food pathogens do not become inactivated in a simple exponential or sigmoidal manner when irradiated but rather show a "tailing off" at the higher doses of irradiation (Wheaton and Pratt, 1962; Anellis, Grecz and Berkowitz, 1965; and Dyer, 1965, p. 29-58). The possibility of a "tail" on survival curves would make any data extrapolated from lower doses meaningless. Thus, with this "tailing off" effect in mind, this thesis presents further studies on the survival patterns in crabmeat and Hartzell's broth of gamma irradiated bacteria with public health significance.

LITERATURE REVIEW

Irradiation Survival Curves

Exponential Survival Curves

It has been generally accepted that bacteria are inactivated in an exponential or sigmoidal manner when irradiated with ionizing radiation. It is for this reason that survival curves are usually graphed on semi-log paper, with percent survival on the logarithmic scale and radiation dose on the arithemetic scale (Lea, 1955).

Compositive Survival Curves

As studies progressed in the area of radiation inactivation of microorganisms, it became apparent that the inactivation of the microbe was not as clear cut as had been thought. Gunter and Kohn (1956) did a comparative study of the dose-survival curves of various bacteria and of yeast, in an attempt to correlate differences in survival curves to genetic constitution. Through these studies three types of curves illustrating ionizing radiation inactivation of microbes were found:

1. Exponential: a straight line relationship when plotted on a semi-log plot, and consistent with the 'Target Theory' (Lea, 1955).

2. Sigmoidal: this curve type indicated that more than 'one-hit' was needed to inactivate the cell; and
3. Composite: this survival pattern was found when Escherichia coli had been irradiated and indicated that two exponential curves were really involved.

Gunter and Kohn examined the survivors at the higher doses of the compositive curves and found that these organisms had a marked difference in radiation sensitivity when compared to the bacteria isolated in the upper part of the survival curve. They hypothesized that the Escherichia coli strain studied was composed of two kinds of cells: 66% irradiation sensitive (S) cells and 34% irradiation resistive (R) cells, with each type exhibiting an exponential curve.

"Tailing off" Survival Curves

Another type of compositive curve has been observed which demonstrates a "tailing off" segment from an initially straight line (exponential inactivation). Even though Bridges and Horne (1959) had emphasized that one must always entertain the possibility that irradiation survival curves may have a "tail" of resistant organisms, the "tailing" phenomenon had not been illustrated prior to 1962. This "tailing off" curve has been found in viruses (Hiatt, 1964), spores (Wheaton and Pratt, 1962; and Anellis, Grecz and Berkowitz, 1965) and in vegetative cells (Dyer, 1965, p. 29-58).

"Tailing off" Curves in Spores. Wheaton and Pratt (1962) found a "tailing off" phenomenon in the survial curves of Clostridium botulinum spores when they were irradiated in phosphate buffer and in pork-pea infusion. These Clostridium botulinum spores demonstrated a compositive-type curve when irradiated, which consisted of three distinct segments: a shoulder (sigmoidal), an exponentially declining segment (straight line), and a "tail" portion which began at 2.0 to 5.0 Mrads. This "tail" segment of the survival curves differed from the classical hit theory. It was also found that this "tailing" portion of the curve did not depend on the type of suspending medium nor did the initial concentration of the spores affect the "tail". The "tail" survivors were not put to any further studies to see if their resistance differed from the parent culture's. Wheaton and Pratt offered a possible explanation for the "tail" by suggesting that substances released during irradiation of the spores may have protected the remaining viable spores by either competing for active radicals or by assisting in the repair of cytoplasmic or nuclear damage. Anellis, Grecz and Berkowitz (1965) disagreed with this protective secretion theory; they found that substances released from spores during irradiation, such as amino acids, nucleic acids, etc. and the accumulation of inactivated spores had no apparent radioprotective effect. These workers further noted that from their observations it was not clear whether "tailing" represents a natural

phenomenon or an experimental artifact.

"Tailing off" Curves in Vegetative Cells. The first report of finding a "tail" in the survival patterns of vegetative cultures was presented by Dyer (1965, p. 29-58). He found that Salmonella typhosa, Salmonella paratyphi B and Salmonella wichita exhibited a "tailing off" effect when irradiated in solid crabmeat. By diluting this crabmeat with distilled water, he found that the "tail" segment in the survival curves could be eliminated. From this it was inferred that the suspending medium during irradiation may have introduced the "tailing off" portion in the survival curves.

Irradiation Recovery

Inactivation of microorganisms by irradiation has been found to be influenced by environmental conditions. Some environmental factors found to alter microbial inactivation via irradiation are: initial number of organisms, hydrogen ion concentration, availability of water of hydration, temperature effect, sensitizing agents, vegetative cells, spores, age of culture, etc., presence of gases and protective agents (Bridges and Horn, 1959). Only the effect of post-irradiation incubation at sub-optimal temperatures will be discussed in this review.

Stapleton, Billen and Hollaender (1953) found that the recovery of x-irradiated Escherichia coli was greater for those

bacteria held at sub-optimal incubation temperatures prior to incubation at the optimal temperature. Thus it appeared that E. coli can partially recover from the lethal effects of x-rays if incubated in the presence of available nutrients at temperatures below those which are normal for growth. Irradiation recovery was found to be neither a non-physiological decay of a radiation-produced toxic substance, nor a stimulation of early cell division. It was further found by the above workers that irradiated bacteria were not able to recover in a minimal medium. In another similar investigation on E. coli cells for post-irradiation temperature effect, it was found that after holding the irradiated bacteria at 4° - 5° C for six days more survivors were found than for those plated immediately following irradiation (Pratt, Moos and Eden, 1955).

Comparative Sensitivities of Specific Bacteria

Studies have been made on the comparative sensitivities to irradiation of specific bacteria with public health significance. Ingram and Rhodes (1962) found that Salmonella are most easily inactivated of the common food pathogens. Erdman, Thatcher and MacQueen (1961) found the following comparison of radiation sensitivities: Clostridium botulinum type A (most resistant) > Clostridium botulinum type E > Streptococcus faecalis > Staphylococcus > Salmonella > coliform > Mycobacterium tuberculosis (lowest

resistance). Escherichia coli and Staphylococci were more sensitive when irradiated in broth than meat, but the reverse was found for Streptococcus faecalis. Slabyj, Dollar and Liston (1965) found Staphylococcus aureus 's post-irradiation survival to vary with the type of medium used. Protection was highest with Brain-Heart-Infusion broth and least with phosphate buffer and fish extract. Fish homogenate and crabmeat gave intermediate levels of protection.

Ionizing Radiation Induced Mutations of Staphylococcus aureus

It is extremely important to investigate the possibility of development of radiation resistant mutants of such potential pathogens as Staphylococcus aureus in radiation pasteurized foods. Erdman, Thatcher and MacQueen (1961) induced radiation resistance in a hospital strain of S. aureus. This white mutant of S. aureus increased its resistance in a stepwise fashion during multiple irradiation.

METHODS AND MATERIALS

Microorganisms Studied

Six microorganisms were used in this study: Salmonella enteriditis, Salmonella pullorum, Salmonella paratyphi A, Salmonella cholerasuis, Staphylococcus aureus and Streptococcus pyogenes. Streptococcus pyogenes strain 624 was obtained from the American Type Culture Collection in Rockville, Maryland. A coagulase and hemolytic positive Staphylococcus aureus culture was obtained from the Good Samaritan Hospital, Corvallis, Oregon. The other organisms were obtained from the culture collection maintained in the Department of Microbiology, Oregon State University, Corvallis, Oregon. These cultures were stored on Hartsell's agar slants at $4^{\circ}C$ and transferred regularly to maintain activity. Cultures were routinely checked for contamination by following the procedure outlined in the seventh edition of Bergey's Manual.

Culture Medium

The cultures used in this experimentation were from vegetative cells cultivated in Hartsell's broth. This medium is composed of the following ingredients: 5.0 grams NaCl; 5.0 grams Bacto Proteose Peptone; 5.0 grams yeast extract (Difco); 100 ml veal infusion; distilled water q.s. to one liter. The pH was adjusted

to 7.2 (± 0.1) before autoclaving at 15 pounds pressure (121°C) for 20 minutes. Hartsell's agar was prepared by adding 20.0 grams of agar to one liter of broth.

Phosphate Buffer

Phosphate buffer was used to make all dilutions except the initial standardization of the cultures used in inoculating the crabmeat and the Hartsell's broth samples which were to be irradiated. This 0.067 M phosphate buffer was prepared from 5.8 grams of anhydrous dibasic phosphate and 4.6 grams of crystalline monobasic potassium phosphate plus the necessary volume of distilled water to make one liter. The buffer was adjusted to pH 7.0 and distributed to eight ounce oval bottles in amounts sufficient to give 99 ml after autoclaving at 15 pounds pressure (121°C) for 20 minutes.

Type of Seafood

Sterile Dungeness crabmeat (Cancer magister) was obtained from Fine Foods, Incorporated, a commerical corporation located in San Francisco, California. This crabmeat was processed with water, salt, and citric acid in number 1/2 flat tin cans.

Standardization of Cultures

The cultures used in this study were standardized in the following manner: a heavy inoculum from the pure culture was used to inoculate 15 ml of Hartsell's broth. After 24 hours incubation at 37°C, one ml of this culture was transferred into 99 ml of broth and again incubated for 24 hours. The cells were serially transferred twice more. All cultures used were in their 18th hour of growth. The number of viable bacteria for each 18 hour old culture was determined. The cells were not washed but were diluted with broth in amounts which would give an inoculum of one ml containing approximately 17×10^6 organisms. One ml inocula were used for the 17 gram crabmeat samples and the 17 ml Hartsell's broth samples. A three ml inoculum was used for each 50 gram crabmeat control. Thus a total load of 1×10^6 cells per gram or ml was inoculated to each sample.

Irradiation Source

The Cobalt-60 Irradiator, Model No. R-60124, used to irradiate the inoculated samples was designed and built by the Budd Company. January 1, 1964, the dosimetry was measured at the edge of the high flux chamber using the Fricke Ferrous Sulphate Method and found to be $8.13 \times 10^5 \pm 0.36$ rad per hour. The

exposure lengths were corrected during this study for radioactive decay to ascertain that the desired dosages were administered.

Irradiation Sample Holder

A vial holder was used which could hold 20 sample-filled glass vials (1.5 inches in diameter X 2.5 inches in height); this device fit inside the high flux chamber of the Cobalt-60 Irradiator.

Pre- and Post-Irradiation Treatment of Crabmeat Samples

Preparation of crabmeat samples. Crabmeat samples were prepared in a transfer chamber which utilized a germicidal ultra-violet lamp (G.E. '15 watt' No. G15T8). To prepare the samples, the canned crabmeat was opened and transferred aseptically to sterile baby food cans (202 X 202). The crabmeat was broken into a homogeneous mixture and distributed in 17 gram lots to sterile glass vials (1.5" in diameter X 2.5" in height). These samples were prepared the evening before irradiation and were stored at $4^{\circ}C$ until inoculated with one ml of broth containing approximately 1×10^{6} cells per gram of crabmeat. The inoculated samples were mixed with sterile glass rods to thoroughly distribute the bacteria throughout the crabmeat. Before and after the inoculation and mixing, the samples were maintained at a low temperature ($5^{\circ}C$).

Microbial examination of crabmeat samples. Crabmeat samples irradiated at low doses of radiation (0.01 to 0.2 Mrads) were transferred into 51 ml Hartsell's broth (1:4 dilution) and plated at appropriate dilutions in Hartsell's agar, incubated three days at 37°C and assayed for survivors. Crabmeat samples irradiated at higher doses or radiation (0.3 and above Mrads) were transferred to 99 ml of Hartsell's broth and incubated for at least ten days at 37°C. Tests were then made to check for survival. Bacto Salmonella-Shigella (SS) agar was used to determine the presence of Salmonella organisms studied; while Staphylococcal Medium 110 was used to detect the presence of Staphylococcus aureus. No differential medium was found for Streptococcus pyogenes. Therefore, any survivors found at the higher doses of irradiation were methylene blue stained to check for Streptococci.

At least five and at the most ten, replicates were run for each dose level administered. In determining the minimal lethal dose, all replicates run for that sample had to show negative growth.

Crabmeat control preparation. Crabmeat controls were prepared for each experimentation as a check that the inocula used approximated 1×10^6 organisms per gram of crabmeat. Two fifty gram lots of crabmeat were stored at 4°C in number 202 X 202 cans in the same manner as were the samples to be irradiated. The

control crabmeat was transferred asceptically to a sterile blender, inoculated with three ml of the organisms being studied, diluted 1:4 with buffer and blended for one and a half minutes at a setting of 70 volts on the rheostat. The homogenate was further diluted with buffer to get readable plates and plated on Hartsell's agar. The plates were examined after three days incubation at $37^{o}C$.

Pre- and Post-Irradiation Treatment of Hartsell's Broth Samples

Preparation of Hartsell's broth samples. Hartsell's broth samples were prepared so that after autoclaving at 15 pounds pressure ($121^{o}C$) for 20 minutes, 17 ml of the broth would be left in the glass vials (1.5" in diameter X 2.5" in height). These broth samples were inoculated with one ml culture (17×10^{6} organisms per ml). The vials were shaken to distribute the bacteria.

Microbial examination of Hartsell's broth samples. Hartsell's broth samples were assayed for survivors by directly plating the broth, after making the necessary dilution, into Hartsell's agar. Higher doses (0.2 Mrads and above) were incubated for at least ten days before checking for survivors. Any survivors found were checked on the appropriate differential medium and gram stained for confirmation.

RESULTS AND DISCUSSION

One goal of the microbiologist working with radiation pasteurization of foods is to ascertain that all pathogenic organisms have been inactivated and no longer represent a health hazard. Thus, with this goal in mind, this work represents a continuation of a series of studies being conducted at Oregon State University to study the effects of gamma irradiation on the survival of bacteria with public health significance. Recent studies (Dyer, 1965, p. 29-58) have shown that gamma irradiation survival patterns of Salmonella typhosa, Salmonella paratyphi B and Salmonella wichita in crabmeat exhibit a "tailing off" after an initial exponential (straight line) inactivation. Dutiyabodhi (1964, p. 43-48) in an earlier study, using the same Salmonella strains, did not find any "tailing" in the survival patterns when these same organisms were irradiated in Hartsell's broth. The Salmonella cultures used in this study were also the same as those used by Dutiyabodhi (1964, p. 35).

Control of Environmental Conditions

The purpose of this study was to determine the survival patterns of specific Salmonella, Staphylococcus and Streptococcus organisms in crabmeat and in Hartsell's broth. And also, to see if there were any definite trends of these survival curves which

may be attributed to the specific media, i.e., the influence of crabmeat or of Hartsell's broth to the respective radiation sensitivities. In order to accomplish such a comparative study of the possible influence of the medium in which the bacteria were irradiated in, it was necessary to use standardized procedures. Essentially the same criteria as those listed by Dyer (1965, p. 29-30) were followed for establishing controlled conditions:

1. The same initial number of cells per ml for each organism was maintained throughout the study.
2. Contamination was rigidly controlled.
3. Experiments were all carried out under the same atmospheric conditions.
4. The bacteria were held at low temperatures throughout each experiment with only the irradiation of the samples taking place at room temperature.
5. All samples were selected on a random basis.
6. The same irradiator and techniques for irradiation were used throughout the study.
7. An enriched medium (Hartsell's broth) was used for viable plate counts (Freeman and Bridges, 1960).

Standardization of Cells

The initial concentration of cells per gram of crabmeat and/or ml of Hartsell's broth was standardized to 1 X 10^6 organisms. The volume of the inoculum per sample was also standardized to one ml. This was accomplished by following the standardization of cultures procedure previously given under Methods and Materials section. The viable counts thus obtained fell within a variation of $\pm$ 10% (except for Staphylococcus aureus).

Temperature Change in Samples

The samples, before and after inoculation, were held at 4°C throughout each experiment, except during the time of irradiation. Studies were made by Dyer (1965, p. 35-38) on the temperature increase of crabmeat samples during irradiation. Dyer found that after 85 minutes the crabmeat samples' temperatures changed from 4°C to 25.5°C. Temperature changes in crabmeat were determined beyond those studied by Dyer. After 144 minutes and 198 minutes of irradiation the temperature had changed from 4°C to 27°C. Since 37°C is the optimal temperature for the organisms studied, the possibility of a temperature effect will be ignored.

Radiation Inactivation Kinetics in Crabmeat

The recovery from gamma irradiation inactivation of Salmonella enteriditis, Salmonella cholerasuis, Salmonella paratyphi A, Salmonella pullorum, Staphylococcus aureus and Streptococcus pyogenes in crabmeat (assayed for survivors immediately following irradiation) are shown in Tables 1, 5, 6, 7, 8 and 9, respectively. The data shown for the irradiation survivors represent the mean value of three duplicate samples taken from a minimum of five replicate samples at each dose level.

Radiation Inactivation Kinetics in Hartsell's Broth

The percent survival from gamma irradiation inactivation of Salmonella enteriditis, Salmonella cholerasuis, and Streptococcus pyogenes were determined in Hartsell's broth and are shown in Tables 2, 4 and 9. The data obtained by Dutiyabodhi (1964, p. 43-48) for the percent survival of Salmonella paratyphi A and Salmonella pullorum in Hartsell's broth are included in Tables 6 and 7 respectively. The results tabulated in Tables 1-9 are shown in graphic form in Figures 1-6.

"Tailing Off" Phenomenon

A "tailing off" from an initial exponential (straight line) inactivation was found in the survival patterns of Salmonella

Table 1. Radiation inactivation kinetics of Salmonella enteriditis in crabmeat.

Dose (Mrad)	Survivors (no./gm)	Percent Survival
0.01	174,000.000	17.4000000
0.03	22,000.000	2.2000000
0.05	387.000	0.0387000
0.06	430.000	0.0430000
0.07	128.000	0.0128000
0.09	32.000	0.0032000
0.10	2.900	0.0002900
0.20	0.029	0.0000029
0.50	0.000	0.0000000
0.70	0.000	0.0000000
0.90	0.000	0.0000000
1.00	0..000	0.0000000

Table 2. Radiation inactivation kinetics of Salmonella enteriditis in Hartsell's broth.

Dose (Mrad)	Survivors (no./gm)	Percent Survival
0.03	28,000.000	2.8000000
0.06	63.500	0.0063500
0.09	4.340	0.0004340
0.10	49.000	0.0040000
0.20	0.224	0.0000224
0.50	0.000	0.0000000
0.70	0.000	0.0000000
0.90	0.000	0.0000000
1.00	0.000	0.0000000

Table 3. Radiation inactivation kinetics of Salmonella cholerasuis in crabmeat (refrigerated at 4°C. seven days before assaying for survivors)

Dose (Mrad)	Survivors (no./gm)	Percent Survival
0.03	5,972.000	0.5972000
0.05	2,100.000	0.2100000
0.07	454.000	0.0454000
0.09	46.000	0.0046000
0.20	0.045	0.0000045
0.50	0.000	0.0000000
0.70	0.000	0.0000000
0.99	0.000	0.0000000

Table 4. Radiation inactivation kinetics of Salmonella cholerasuis in Hartsell's broth.

Dose (Mrad)	Survivors (no./gm)	Percent Survivals
0.03	7,000.000	0.7000000
0.05	198.000	0.0198000
0.07	81.000	0.0081000
0.09	25.000	0.0025000
0.10	16.000	0.0016000
0.2	7.900	0.0007900
0.3	0.140	0.0000140
0.5	0.012	0.0000012
0.7	0.000	0.0000000
0.9	0.000	0.0000000
1.0	0.000	0.0000000
1.1	0.000	0.0000000
1.2	0.000	0.0000000
1.3	0.000	0.0000000

Table 5. Radiation inactivation kinetics of Salmonella cholerasuis in crabmeat (assayed immediately after irradiation)

Dose (Mrad)	Survivors (no./gm)	Percent Survival
0.03	35,000.0	3.5000
0.05	5,280.0	0.5280
0.07	2,084.0	0.2084
0.09	161.0	0.0161
0.10	31.2	0.0031
0.20	0.0	0,0000
0.30	0.0	0.0000
0.50	0.0	0.0000
0.70	0.0	0.0000
0.9	0.0	0.0000
1.0	0.0	0.0000
1.1	0.0	0.0000
1.2	0.0	0.0000
1.3	0.0	0.0000

Table 6. Radiation inactivation kinetics of Salmonella paratyphi A in crabmeat.

Dose (Mrad)	Survivors (no./gm)	Percent Survival	Percent Survival (broth)*
0.03	30,700.000	3.0700000	-
0.07	8,840.000	0.8840000	-
0.09	2,220.000	0.2220000	-
0.10	-	-	0.75000
0.20	7.900	0.0007900	0.01900
0.30	2.600	0.0002600	0.00015
0.40	-	-	0.00000
0.50	≥ 0.059	≥ 0.0000059	0.00000
0.60	-	-	0.00000
0.70	≥ 0.018	≥ 0.0000018	-
0.90	≥ 0.030	≥ 0.0000030	-
1.00	0.000	0.0000000	-
1.10	0.000	0.0000000	-
1.20	0.000	0.0000000	-
1.30	0.000	0.0000000	-

* See (Dutiyabodhi, 1964, p. 47) in Bibliography

Table 7. Radiation inactivation kinetics of Salmonella pullorum in crabmeat.

Dose (Mrad)	Survivors (no./gm)	Percent Survival	Percent Survival (broth)*
0.03	68,000.0000	6.80000000	-
0.05	5,200.0000	0.52000000	-
0.07	1,000.0000	0.10000000	-
0.09	128.0000	0.01280000	-
0.10	19.4000	0.00194000	0.4000
0.20	0.5600	0.00005600	0.0018
0.30	-	-	0.0000
0.40	-	-	0.0000
0.50	0.0560	0.00000560	0.0000
0.60	-	-	0.0000
0.70	≥ 0.0170	≥ 0.00000170	-
0.90	≥ 0.0084	≥ 0.00000084	-
1.00	≥ 0.0100	≥ 0.00000100	-
1.10	0.0000	0.00000000	-
1.20	0.0000	0.00000000	-
1.30	0.0000	0.00000000	-

*See (Dutiyabodhi, 1964, p. 47) in Bibliography

Table 8. Radiation inactivation kinetics of Staphylococcus aureus in crabmeat

Dose (Mrad)	Survivors (no./gm)	Percent Survival
0.05	823,000.000	82.3000000
0.08	151,000.000	15.1000000
0.10	89,800.000	8.9800000
0.20	2,090.000	0.2090000
0.30	65.300	0.0065300
0.40	19.500	0.0019500
0.70	≥ 0.059	≥ 0.0000059
0.80	≥ 0.059	≥ 0.0000059
0.90	≥ 0.039	≥ 0.0000039
1.00	≥ 0.059	≥ 0.0000059
1.10	≥ 0.059	≥ 0.0000059
1.20	≥ 0.059	≥ 0.0000059
1.30	≥ 0.059	≥ 0.0000059
1.40	≥ 0.048	≥ 0.0000048
1.50	≥ 0.059	≥ 0.0000059
1.60	≥ 0.059	≥ 0.0000059
1.80	≥ 0.059	≥ 0.0000059
2.00	0.000	0.0000000
2.20	0.000	0.0000000
2.30	0.000	0.0000000
2.50	0.000	0.0000000
2.70	0.000	0.0000000
3.00	0.000	0.0000000

Table 9. Radiation inactivation kinetics of Streptococcus pyogenes in crabmeat and in Hartsell's broth.

Dose (Mrad)	Survivors (no./gm crabmeat)	Percent Survival (crabmeat)	Percent Survival (broth)*
0.03	74,960.00	7.500000	40.0000
0.05	6,824.00	0.682400	4.4000
0.07	1,382.00	0.138200	0.5600
0.09	2,083.00	0.208300	0.1120
0.10	356.00	0.025600	0.0450
0.15	36.00	0.003600	-
0.20	0.88	0.000088	0.0057
0.30	0.00	0.000000	0.0000
0.50	0.00	0.000000	0.0000
0.70	0.00	0.000000	0.0000
0.90	0.00	0.000000	0.0000

* See (Quinn, 1965) in Bibliography

Table 10. D-values of specific bacteria in crabmeat and in Hartsell's broth

Organism	D-values (in Mrad) crabmeat	Hartsell's broth
Salmonella enteriditis	0.016	0.020
Salmonella cholerasuis	0.020, 0.01**	0.020
Salmonella paratyphi A	0.030	0.050*
Salmonella pullorum	0.025	0.040*
Staphylococcus aureus	0.080	-
Streptococcus pyogenes	0.024	0.038

** Survivors were assayed after seven days of refrigeration

* See (Dutiyabodhi, 1963, p. 47)

enteriditis, Salmonella paratyphi A, Salmonella pullorum and Staphylococcus aureus (Figures 1, 3, 4 and 5, respectively) when they were irradiated in crabmeat, but was not shown when these organisms (excluding Staphylococcus aureus) were irradiated in Hartsell's broth. The opposite trend was found with Salmonella cholerasuis and Streptococcus pyogenes (Figures 2 and 6, respectively) for these bacteria produced definite "tailing off" patterns in the broth while only weakly, if any, in the crabmeat.

Post-Irradiation Sub-Optimal Incubation

A comparison was made of the gamma-irradiation survival of Salmonella cholerasuis in crabmeat assayed as usual, immediately following irradiation, to that assayed after the irradiated crabmeat samples (in irradiation vials) had been held at $4^{\circ}C$ for seven days (Tables 4 and 3, respectively). Figure 2 graphically illustrates the data. Survival was greater in samples assayed immediately after irradiation.

D-Values

The D-value, or that radiation dose which shows 10% survival (i.e. 90% inactivation) of the initial bacterial concentration was found for each organism from their survival curves. The D-values thus obtained are shown in Table 10. Since D-values depend

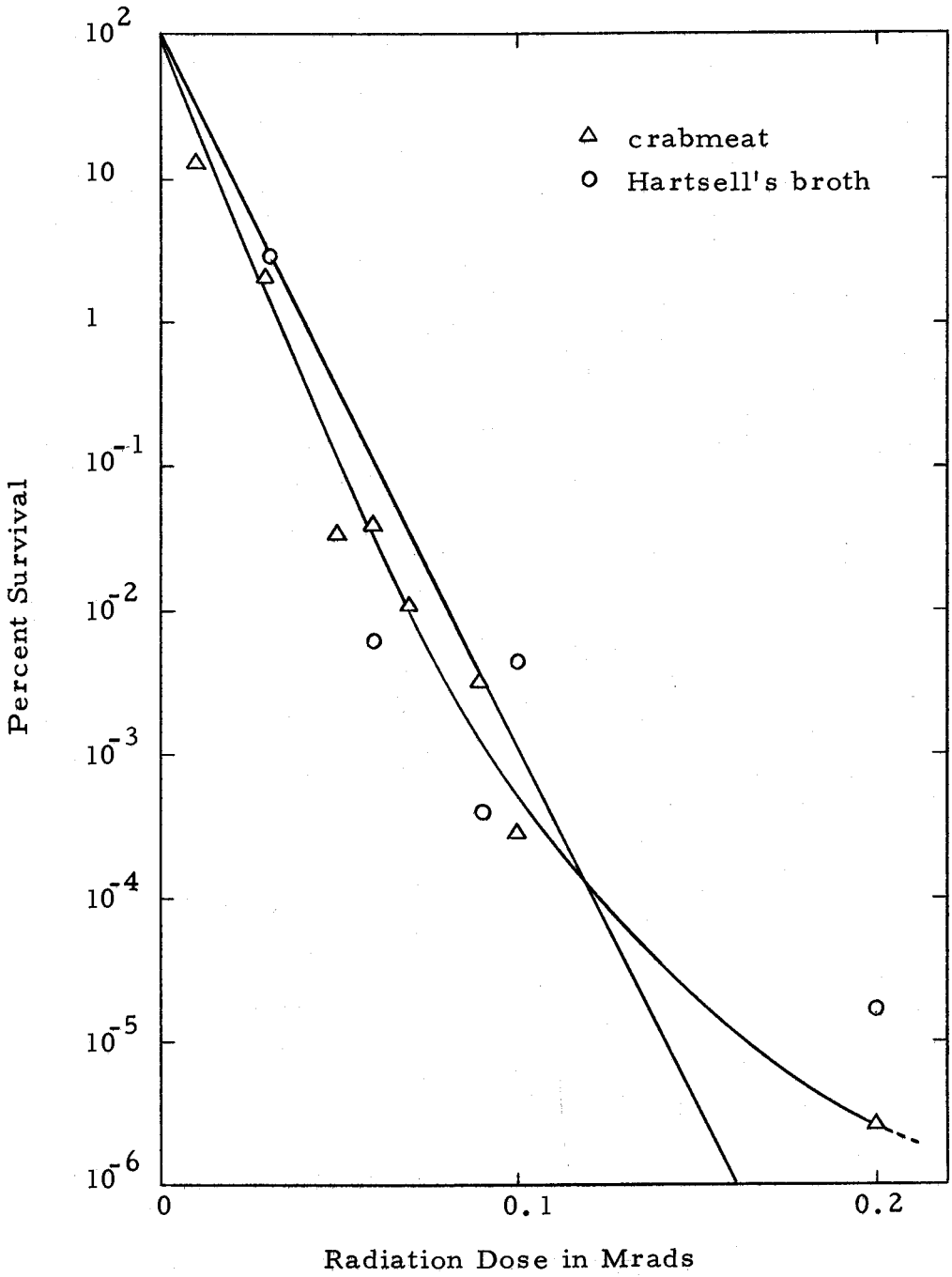

Figure 1. Radiation survival curves of Salmonella enteriditis in crabmeat and in Hartsell's broth.

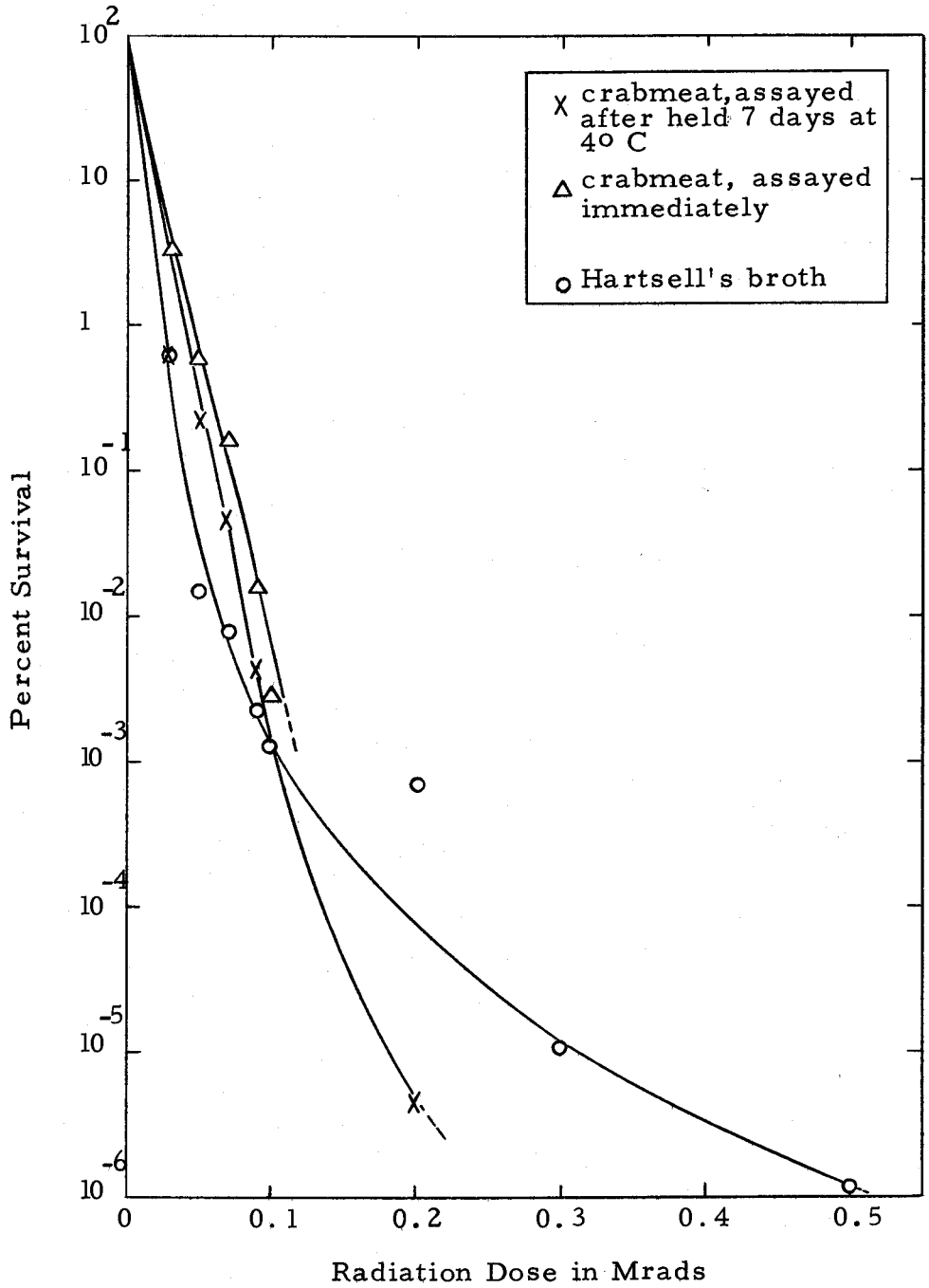

Figure 2. Radiation survival curves of Salmonella cholerasuis in crabmeat and in Hartsell's broth.

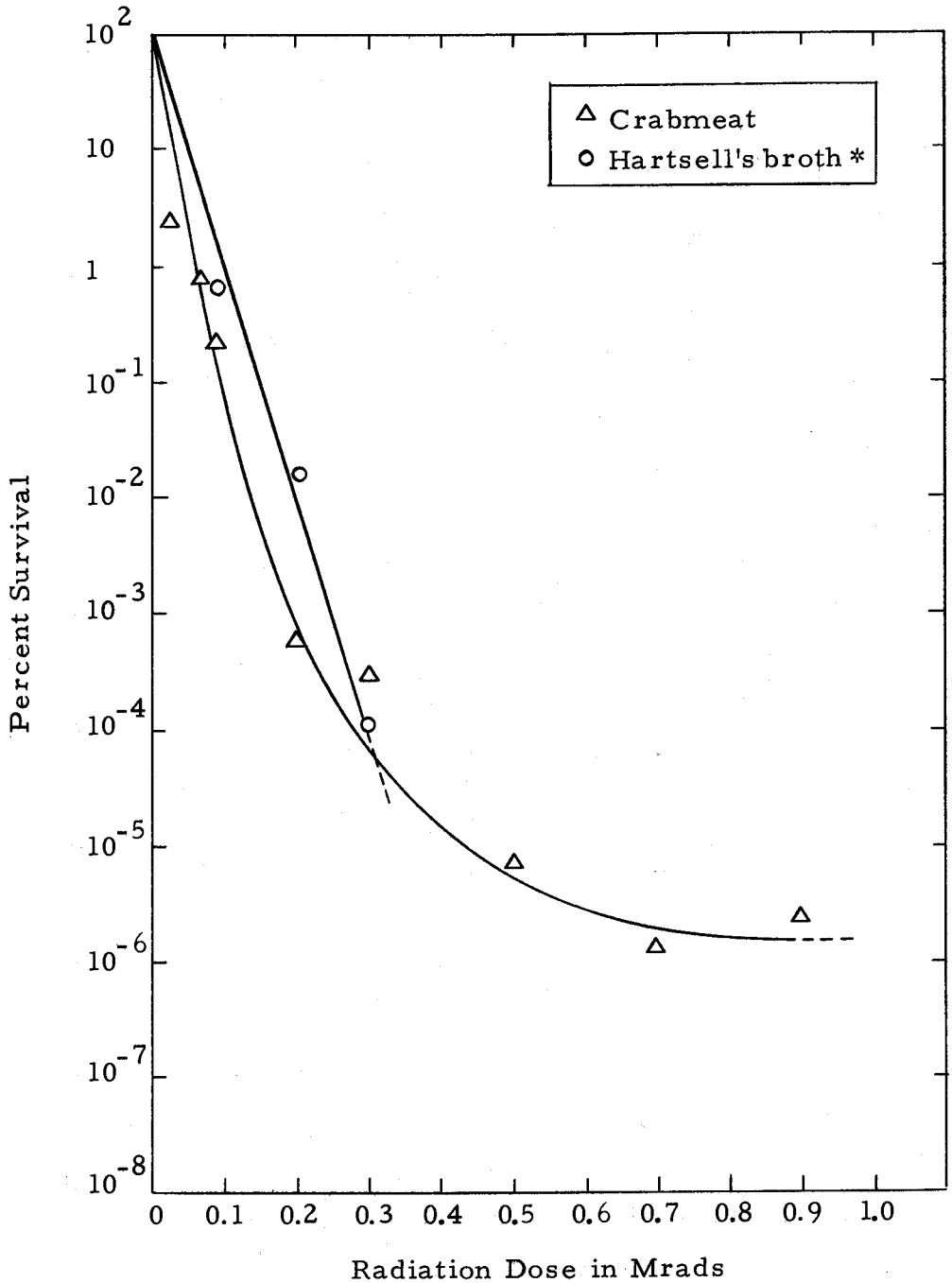

Figure 3. Radiation survival curves of Salmonella paratyphi A in crabmeat and in Hartsell's broth.

* see (Dutiyabodhi, 1964, p. 47) in Bibliography

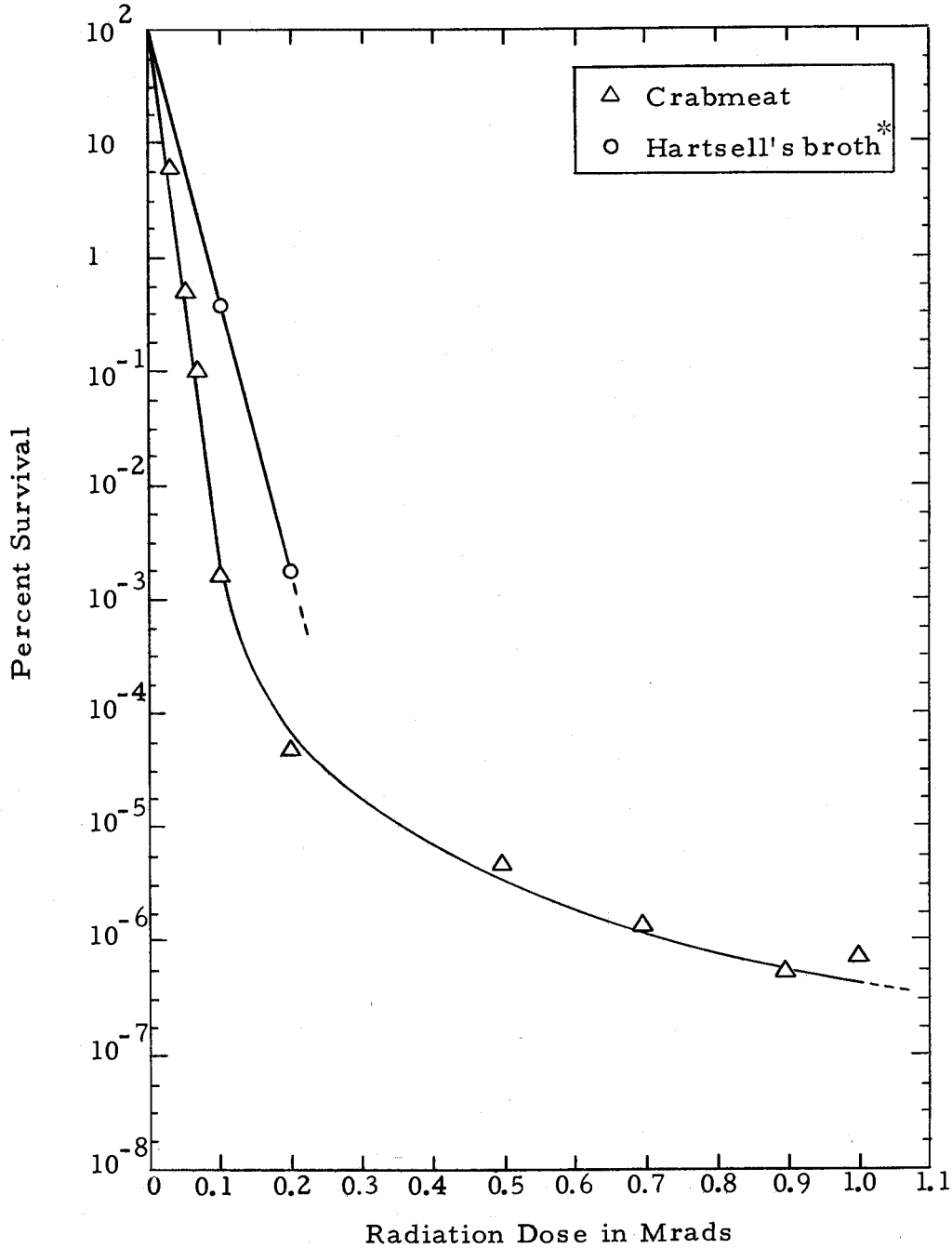

Figure 4. Radiation survival curves of Salmonella pullorum in crabmeat and in Hartsell's broth.

* see (Dutiyabodhi, 1964, p. 47) in Bibliography

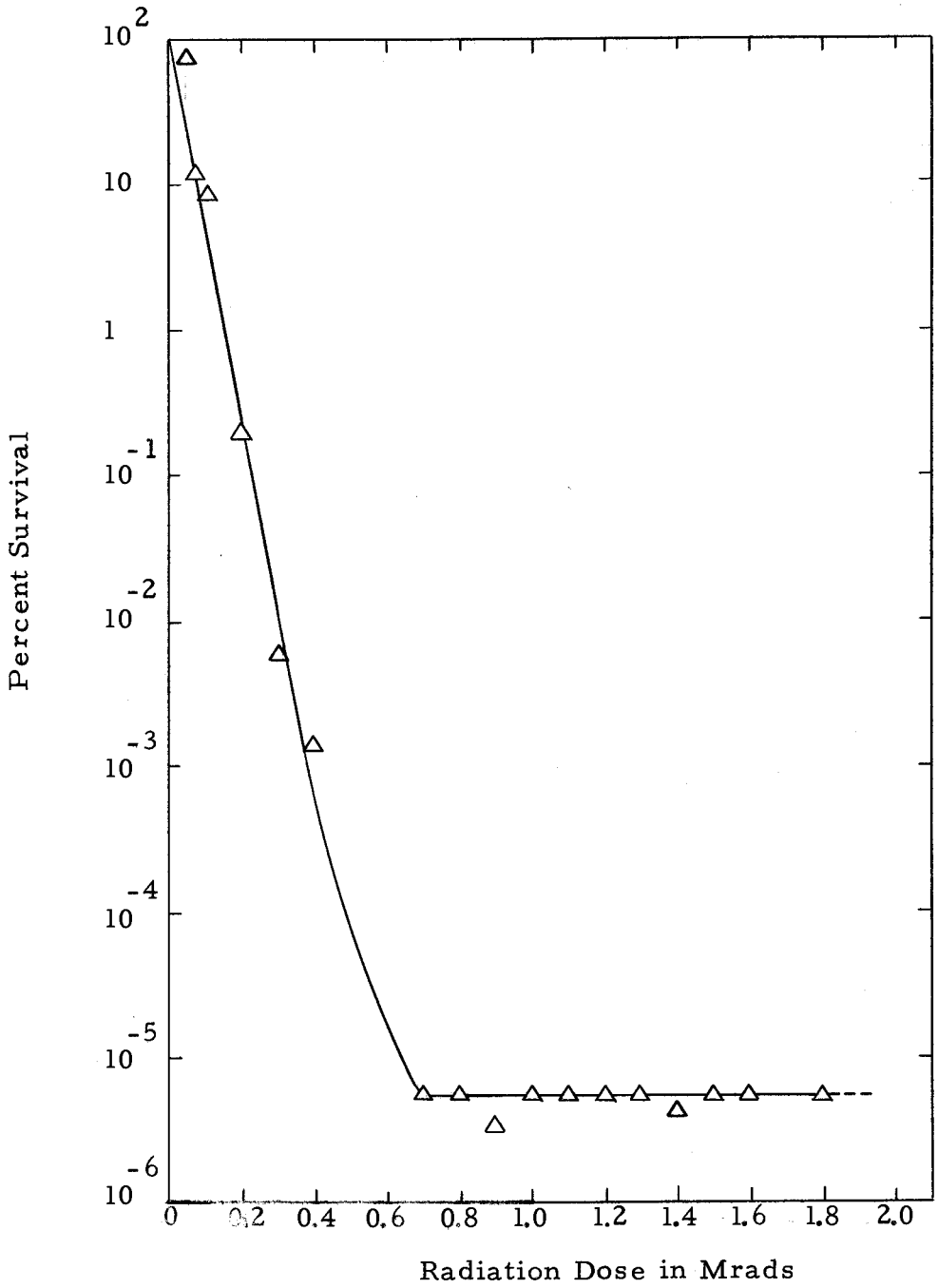

Figure 5. Radiation survival curve of Staphylococcus aureus in crabmeat.

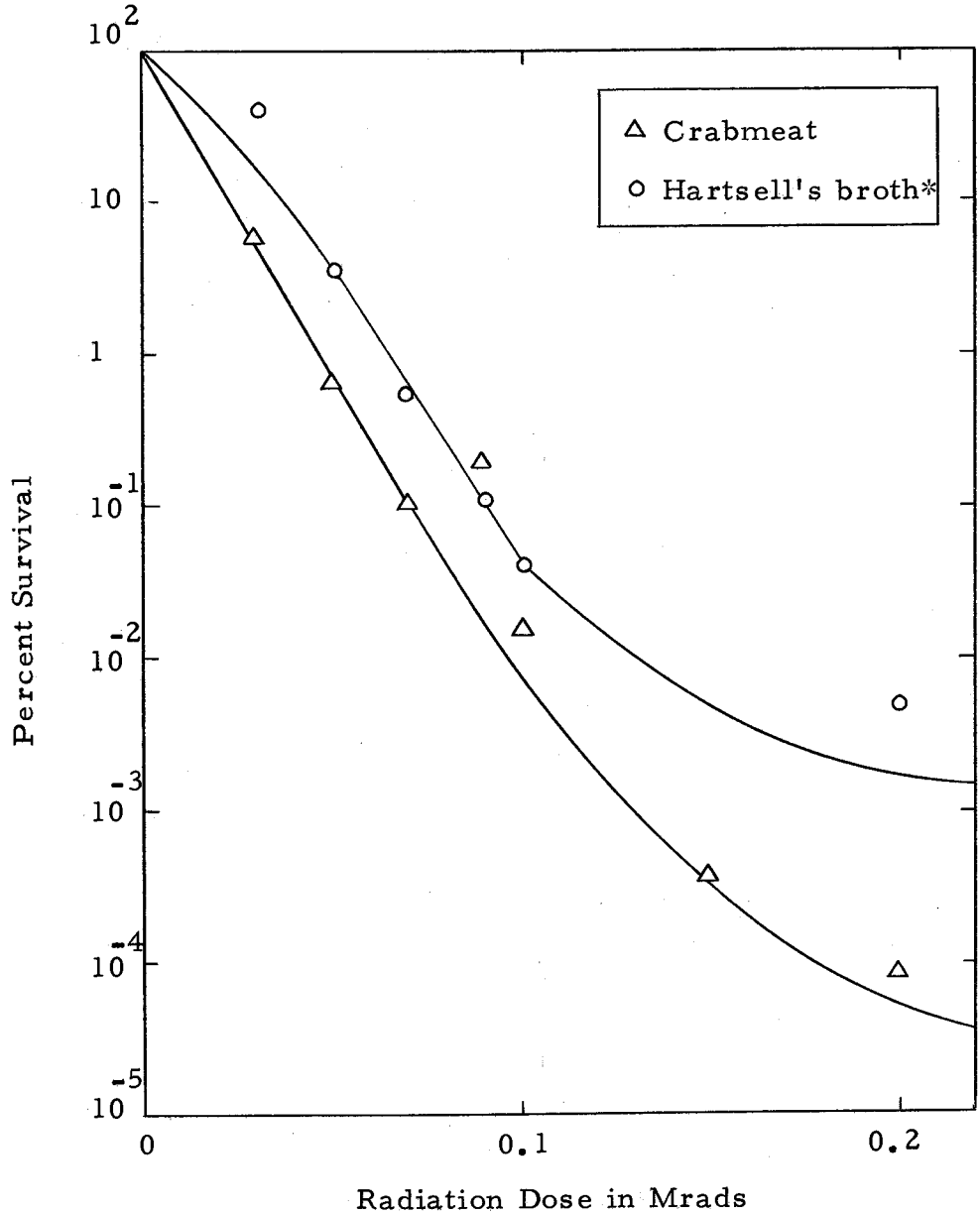

Figure 6. Survival curves of Streptococcus pyogenes in crabmeat and in Hartsell's broth.

* see (Quinn, 1965) in Bibliography

on exponential or sigmoidal shaped survival curves, it can be seen that these values are not very dependable for determining the sensitivity of any pathogen in foods.

Minimal Lethal Doses (MLD)

The MLD or sterilization dose of gamma radiation is shown in Tables 1-9 for each of the six organisms studied in crabmeat and/or Hartsell's broth. Like the previous study conducted (Dyer, 1965, p. 55) the sterilization dose in this case is synonymous with the pasteurization dose since this study was conducted with pure, potentially pathogenic cultures which would be eliminated through irradiation processing. The data in these tables represent a minimum of five replicate samples per radiation dose. Higher irradiation doses (0.2 Mrads and above) were incubated at least ten days before checking for survival.

Irradiation of Staphylococcus aureus in Crabmeat

Staphylococcus aureus was irradiated in crabmeat; Table 5 and Figure 5 show the survival data and graphic illustration. This organism gave the greatest "tailing off" which may be partly attributed to its clumping nature. This organisms, also by its clumping nature, did not give standardized viable counts of 1×10^6 ($\pm$ 10%) cells per gram of crabmeat. An improved standardization

technique such as the one developed by Hinds and Peterson (1963) should be used in further studies.

Induction of Staphylococcus aureus Mutants

At high doses (1.0 Mrad and above) of irradiation Staphylococcus aureus white mutants were found when plating on the differential Staphylococcal - Medium 110 (Difco). Three mutants were selected and serially transferred for two weeks in Hartsell's broth. No reversion of the mutants was noted. The selected mutants were checked for hemolysis and coagulase; two gave negative results for both tests while the other mutant was both coagulase and hemolytic positive. A hemolytic and coagulase negative mutant was irradiated simultaneously with the parent culture from 1.6 to 2.0 Mrads. The mutant did not show any greater radiation resistance than parent culture.

Limitations

As previously mentioned, standardization of Staphylococcus cultures should be accomplished by using a procedure such as the one suggested by Hinds and Peterson (1963) where the broth culture was mechanically shaken for 30 minutes to break up cell clumps. It is also suggested that in further studies the cultures used be washed free from the initial medium and standardized in buffer.

SUMMARY

A comparison was made of the sensitivities to gamma irradiation of specific bacteria with public health significance. The survival curves of the following bacteria were determined in crabmeat and/or in Hartsell's broth: Salmonella enteriditis, Salmonella paratyphi A, Salmonella cholerasuis, Salmonella pullorum, Streptococcus pyogenes and Staphylococcus aureus. The Salmonella species were found most sensitive to irradiation, while Staphylococcus aureus proved to be least sensitive. The Streptococcus specie, S. pyogenes, was more resistant to irradiation than the Salmonella species but less resistant than Staphylococcus aureus.

A "tailing off" effect was shown when Salmonella paratyphi A, Salmonella pullorum, Salmonella enteriditis and Staphylococcus aureus were irradiated in crabmeat, but was not noted when these organisms (excluding Staphylococcus aureus) were irradiated in Hartsell's broth. However, just the opposite results were shown with Salmonella cholerasuis and Streptococcus pyogenes. These bacteria produced a very definite "tailing" in the broth while only weakly, if any, in the crabmeat. Thus, this "tailing off" phenomenon cannot be explained away as a mere media effect, but rather indicates a much more complex situation. Further studies must be made on the "tailing" phenomenon of survival curves.

A comparison was made of the irradiation recovery of Salmonella cholerasuis in crabmeat which had been incubated immediately at optimum growth temperature (37°C) to that which had been held for seven days at 4°C (in irradiation vials) before assaying for survivors. The samples assayed immediately and incubated at 37°C produced more survivors.

White mutants of Staphylococcus aureus were induced at 1.0 Mrad and greater doses. These mutants were stable and gave varied coagulase and hemolytic tests. In an examination of one mutant, no greater resistance to irradiation than the parent culture was shown.

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