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I am submitting herewith a thesis written by Cynthia G. Clemen entitled "Recovery of Injured Organisms Inoculated into Ground Beef at Various Cooking Temperatures." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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We have read this thesis
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RECOVERY OF INJURED ORGANISMS INOCULATED INTO GROUND BEEF AT VARIOUS COOKING TEMPERATURES

A Thesis

Presented for the

Master of Science

Degree

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Cynthia G. Clemen

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ABSTRACT

The objective of this study was to determine whether sublethally injured organisms could be recovered from a substrate of ground beef heated to three different temperatures.

Three organisms, Escherichia coli, Staphylococcus aureus, and Salmonella enteritidis, were injected uniformly into lots of ground beef and allowed to propagate at 4°C over three days of storage. Each day samples were procured and subjected to one of the three cooking temperatures, 60.0°C, 65.6°C or 71.1°C. After each heat treatment the patties were analyzed for total viable population (injured and uninjured organisms), injured organisms, and aerobic plate count. In addition, counts were obtained on the raw ground beef over the three days of storage at 4°C.

The data obtained were subjected to analysis of variance. Recovery of sublethally injured Escherichia coli and Staphylococcus aureus occurred at all three temperatures, but the variation was insignificant. Recovery of sublethally injured Salmonella enteritidis showed significance in storage-temperature interaction. Survival of total bacterial population, after heat exposure, showed only significance in replication. With the third set of samples, storage time had a significant effect, as well as temperature. Over three days storage, the number of Escherichia coli and Staphylococcus aureus did not change significantly. The counts of Salmonella enteritidis decreased throughout storage. The percentages of Escherichia coli and Salmonella

enteritidis killed continually decreased as the cooking temperature increased. The percentage of Staphylococcus aureus appeared to follow no obvious trend.

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CHAPTER I

INTRODUCTION

A variety of processing techniques are used today to formulate, manufacture and preserve food products. During such treatments microorganisms are subjected to stress or injury, and are hopefully killed (Hobbs and Olson, Jr., 1971).

Heat treatment of foods is one of the oldest and one of the most common methods of commercial sterilization as well as food preparation (Jackson and Woodbine, 1963). However, research has shown that heat can exert a "sublethal" effect, that is, a certain portion of the microbial populations are apparently only sublethally injured rather than killed. Hence, given the proper nutrients and time, this fraction of the cell population is able to repair the sublethal damage and will again reproduce, causing food spoilage problems as well as human health problems (Food Safety Task Force, 1974-1975).

Laboratory tests have been conducted on a number of problem causing organisms, among them Clostridium bolutinum, Escherichia coli, Salmonella and Staphylococcus aureus (Hobbs and Olson, Jr., 1971). However, these experiments were conducted under ideal laboratory conditions, utilizing only laboratory grade equipment and chemical compounds. Very little work has been done utilizing actual food products. The work herein reported sought to translate in vitro investigations into a food product, ground beef.

CHAPTER II

REVIEW OF LITERATURE

I. MICROBIOLOGY OF RAW GROUND BEEF

Because of their composition (carbohydrates, proteins, fats, minerals, water, vitamins, hormones, nucleotides, other unnamed constituents termed "growth factors" and a complex enzyme system) (Weiser et al., 1971), food products serve as an ideal growth medium for microorganisms. Thus one can conclude that there exists a close inter-relationship between food products and microorganisms.

Meat is an excellent source of the above compounds; thus microorganisms can reproduce quite rapidly and cause spoilage in a very short time. In fact, meat is quite often used as an ingredient in many of the culture media utilized for growing microorganisms (Weiser et al., 1971).

Meat is usually held under refrigeration. Thus, there is little chance for bacterial growth to occur, except for the psychrophiles, which normally grow at 0°C with an optimum at 20-30°C. If meat is held at 30°C, mesophiles, as well as psychrophiles, will grow producing a mixed flora that will reach high numbers within a few hours. The obligate and facultative anaerobes prefer the interior of a meat product or one that has been vacuumed packed in an oxygen impermeable film; both types possess a reduced oxygen supply. The surface of non-packaged meats allows aerobic microorganisms to grow. Thus it is possible to find microaerophilic bacteria growing within one millimeter or two of the fresh meat surface (Lechowich, 1971).

Generally the pH of fresh meat is between 5.3 and 6.5, depending on the feeding and handling of the cattle prior to slaughter. Most microorganisms can grow in this pH range. However, meat possessing a pH of 6.5 will spoil bacteriologically much more rapidly than meat with a pH of 5.3 (Lechowich, 1971).

Moisture is essential for microbial growth. Meat possesses an aqueous phase with many dissolved substances. These dissolved solids in the water determines the water activity (A_w), which in turn determines the growth of microorganisms. The optimum water activity for bacterial growth is 0.995 to 0.990. As this range decreases, so does bacterial growth. Fresh meat possesses a water activity of 0.99 or above, which is near optimum for many bacteria (Lechowich, 1971).

The manufacturing process of ground beef involves the grinding of cellular tissues (Duitschaever et al., 1973) twice through two plates of diameter one-fourth inch and one-eighth inch, respectively. In addition, the Meat and Poultry Inspection Regulations state that ground beef shall consist of

> Chopped fresh and/or frozen beef with or without seasoning and without the addition of beef fat as such, shall not contain more than 30 percent, and shall not contain added water, binders or extenders.

Bacteria normally found on the surface of meat was then distributed by this process throughout the entire product and ideal conditions for their multiplication are set up. In addition, the bacterial flora present in the final product is also dependent upon the sanitary conditions during processing, the temperature and storage time before sale (Foster et al., 1977). Rogers and McClesky (1957) found that the

bacterial counts of market samples of ground meat were often an indicator of the product's history.

When spoiled ground beef is examined only a few genera are found. The most important ones include Pseudomonas, Achromobacter, Bacillus, Flavobacter, Microbacterium, Micrococcus and Aeromonas, with Pseudomonas and Achromobacter being the predominant cause of spoilage (Ayres et al., 1950; Kirsch et al., 1952; Ayres, 1955; Brown and Weidemann, 1958; and Jay, 1967).

In 1971, Ingram and Dainty demonstrated the relationship between off odors and superficial bacterial numbers. Off odors developed when bacterial counts exceeded 10,000,000 per square centimeter. Kirsh et al., in 1952, and Barnes in 1957 found off odors developing when the counts reached 100,000,000 organisms per gram. In addition to the presence of off odors, Ayres et al. in 1950 noticed that along with a characteristic rancid, sweetly aromatic ester-like odor, there also developed a typical slime, composed of tiny drop-like colonies which coalesced to form a "wet," viscous slimy coating. The total numbers of bacteria corresponding to the presence of the slime and off odor was approximately 100,000,000 organisms per gram.

In 1977, Foster et al. conducted a study to determine what the bacterial flora was of raw ground beef. A basis for their study was that 50% of beef consumed in the United States was in the form of ground beef or hamburger, and much of this was consumed in a partially cooked state (Consumer Reports, 1971). In addition, recent epidemiological statistics indicated that this product was responsible for 3.6% of the

identified food borne outbreaks from 1967 to 1973. Ground beef has also been implicated in outbreaks of toxoplasmosis and salmonellosis from consumption of raw or grossly undercooked products (Fleming et al., 1973; and Lord et al., 1975). In this study 150 units of ground beef were obtained from a local retail store. Aerobic plate counts ranged from 6.9×10^4 to 8.3×10^7 organisms per gram. Of the 150 samples, 96.7% were positive for coliforms by the most probable number method, 94.7% for Escherichia coli, and 61.3% for Staphylococcus aureus. Using plate methods, 99.3% were positive for fecal streptococci and 56% were positive for Clostridium perfringens. The absence of Salmonella was explained by the fact that this organism was unable to proliferate in raw ground beef maintained under proper refrigeration, or that the Salmonella was unable to adequately compete with the coliforms present. However, they noted that Salmonella contamination does exist and this was most likely attributed to improper cooking, handling or storage and could prove to be hazardous in this product. Aerobic organisms isolated most frequently were identified as Escherichia coli, followed by organisms in the Klebsiella-Enterobacter group. The most frequently isolated anaerobe was Clostridum perfringens.

Karin (1977) conducted a study of 120 samples of raw ground beef. Staphylococcus species were present in 62% of the samples, with 37% of this percentage containing coagulase positive strains.

Goepfert (1975) injected five strains each of Escherichia coli, Salmonella, Enterococci, Staphylococcus, Bacillus cereus and Clostridium perfringens into ground beef, and subjected the samples to various

refrigeration temperatures. The results indicated only Escherichia coli, Salmonella and enterococci grew at 12.5 ± 0.5°C. This temperature was referred to as refrigeration abuse. They found that Staphylococcus aureus did not grow at all, because the storage temperatures were below the minimum growth range of the species and also that the organism could not compete with the normal flora. Clostridium perfringens also did not grow due to the sensitivity of the vegetative cells at the low temperature. Thus, it was concluded that the food poisoning microorganisms were not able to compete effectively with the natural flora of raw beef over a wide range of temperatures, and that growth, if it did occur, was abused by temperature times of refrigeration.

Duitschaever and his associates (1973) assayed 213 samples of various types of raw refrigerated ground beef from 51 different retail stores. Sixty-four percent of the samples possessed mesophilic and psychrophilic counts in excess of 10,000,000 organisms per gram. Packaged hamburger and hamburger sold in bulk accounted for the highest bacterial counts. Psychrophilic and mesophilic counts showed no difference between different types of meat, except hamburger sold in bulk showed psychrophilic counts twice as high as mesophilic counts. Average coliform counts ranged from 1400 to 19,000 organisms per gram, with some individual samples as high as 100,000 organisms per gram. Enterococcus counts ranged from less than 10 to 10,000 organisms per gram. All samples were positive for Staphylococcus with 98% containing greater than 1,000 organisms per gram. Cogulase positive Staphylococcus was found in 17% of the samples, ranging from five to 100% of the total Staphylococcus count.

Tiwari and Maxey (1971) sampled ground beef from six local supermarkets and found logarithmic total aerobic counts that averaged 7.54 organisms per gram and 5.11 for coliform organisms. After six days storage at 5°C, logarithmic total aerobic counts had increased to 9.6 organisms per gram and 7.9 for coliform organisms.

Rey and his associates (1970) sampled ground beef at the beginning and completion of seven days storage at 5°C. They found a logarithmic aerobic count of 6.3 organisms per gram at the beginning of storage and 8.6 organisms per gram at the conclusion of storage. In addition, enterococcus counts did not increase during storage but coliform counts did. Total aerobic organism counts increased from 5.5 at zero day to about 8.0 during five days of storage; coliforms increased from 4.5 to 6.75 organisms per gram. Coagulase positive Staphylococcus was observed 60% of the time.

Pierson et al. (1970) found that the total bacterial counts of aerobically packaged beef increased until the tenth day of storage at 3.3°C, but only slightly thereafter with the final counts being about 10,000,000,000 organisms per square centimeter after 15 days of storage. Enterococcus was found to increase slowly or remain constant during anaerobic storage at 3.3°C.

Thus, the bacterial population in ground beef reflects the bacteriological quality of the meat used for grinding, the cleanliness of equipment, and the time and temperature of storage. However, ground beef is still a product with a large and varied microbial flora, which frequently can include a number of potentially pathogenic organisms.

II. FACTORS AFFECTING THE HEAT RESISTANCE OF NONSPORE FORMING ORGANISMS

Schmidt (1954) stated that

> The only single practical criterion of the death of microorganisms is the failure to reproduce when, as far as known, suitable conditions for reproduction are provided. This means that any organism which fails to show evidence of growth when placed under what are considered, in the light of our present knowledge of bacterial nutrition and growth requirements, adequate growth conditions is considered as dead.

Thus, death was considered as a loss of reproduction by the cell or spore, the end objective being the destruction of all life processes (Pflug, 1977).

The lethal agent in this review was heat or a

> . . . molecular energy state that is capable of producing changes in the cell that prevent the cell from reproducing either by direct effects on the reproductive mechanism or by disrupting cellular metabolic systems that provide energy and chemical intermediates for reproduction (Pflug, 1977).

However, research has demonstrated that the presence of certain environmental and growth conditions, such as pH, salt concentration, and age of culture, can influence the effect of heat. The organisms become resistant and survive.

Precht et al. (1955) demonstrated that cells underwent visible changes when exposed to heat. Gram negative organisms lost their stainability when heated in milk because of nucleic acid loss. This phenomenon did not appear to be the sole result of temperature and time exposure. Heating in distilled water or glycine or salt free protein solutions did not cause staining loss. However, heating in

sodium chloride and phosphate buffer did cause staining loss, which was again due to nucleic acid loss. Prech et al. (1955) showed by electron microscopy, that protoplasm granulation of young coli-aerogenes cells began at 40°C and continued to 55°C; between 56°and 60°C, the contents began to leach out.

The Effect of Water

Heat resistance of microbial cells has been shown to increase with decreasing humidity. Precht et al. (1955) noted that superheated steam acted like dry air at 140° to 150°C and possessed less killing effect than wet steam at 100°C. They assumed that the heat destruction of the cells was due to denaturation of nucleic acids, enzymes or other essential proteins. Proteins were more stable in a dry state and the effect of water on heat resistance was explained in relation to protein stability (Hansen and Riemann, 1963).

The water in close contact with the protein molecules determined the heat resistance. This water of hydration was attached to groups within or at the protein molecule surface when there were free charges and dipoles such as CO and NH_2 groups. When these "wet" proteins were heated, free sulhydral groups formed and water binding capacity increased The breakage of disulfide and hydrogen bonds was caused by thermal vibration. Peptide chains became flexible and established new bonds between themselves if water was present. By decreasing the water content, the number of water dipoles between polar groups of peptide chains became smaller; thus, the resulting interaction stabilized the protein in such a way that more energy was required to unwind the peptide

chains and gave rise to increased heat resistance (Precht et al., 1955).

The Effect of Oils and Fat

Non-heat resistant bacteria have sometimes been isolated from processed canned foods. The heat resistance of these bacteria was attributed to "fat protection," whose mechanism rests on the premise of a localized moisture absence. Bacteria trapped in fat were protected from heat injury and were prevented from reproducing (Yesair et al., 1946).

Jensen (1954) demonstrated with canned meats that it was necessary to impose higher temperatures or a longer subjection time to kill organisms contained in this fatty medium. His conclusion was that fat played a role in food preservation, but there were more factors involved such as water activity. Sugiyama (1951) showed that long chain fatty acids enhanced protection of serum albumin and increased the heat resistance of Clostridium bolutinum spores. However, knowledge of fat protection is very meager and warrants further investigation.

The Effect of Salt

Jensen (1954), working with micrococci, found that salt protected against heat. However, this protection was variable and depended on the type of salt, its concentration, suspending media and the test organism (Baumgartner and Hersom, 1956). The effect of salt was unpredictable and could include the following actions: 1) the regulation of pH may be affected by changing the transport of hydrogen

ions through the osmotic barrier; 2) depending on the concentration, the salt may establish a more favorable osmotic pressure difference between the interior of the cell and its suspending medium, and thus would decrease the leakage of the cell's essential constituents during heating (Mitchell, 1951). Sodium and potassium chloride affected protein hydration and therefore influenced protein and enzyme stability. The divalent cations of calcium and magnesium increased heat resistance by linking proteins together to form strong complexes (Precht et al., 1955). Soluble salts in high concentrations decreased the water activity and increased bacterial resistance due to a mechanism that was similar to drying (Hansen and Riemann, 1962).

The Effect of Carbohydrates

The addition of soluble carbohydrates in high concentrations increased the heat resistance of certain microorganisms by decreasing the water activity (Hansen and Riemann, 1963). This increase in resistance was attributed to partial dehydration of the protoplasm. This phenomenon has been demonstrated with yeasts and bacteria (Baumgartner and Wallace, 1934; Schmidt, 1954; and Baumgartner and Herson, 1956). However, this theory was somewhat questionable; Sugiyama (1951) found that carbohydrate protection was not proportional to molarity.

The Effect of pH

Protein denaturation by heat was increased in an acid or alkaline environment and hence would also cause a decrease in bacterial heat resistance. This heat resistance had an optimum within a narrow

pH range and decreased rapidly outside this range on either end (Hansen and Riemann, 1963). Generally resistance was at a maximum in the pH range of 6.0 to 8.0, and was affected by the particular suspending medium (buffer or food substrate), the techniques used and the subculture conditions (Pflug, 1977).

In 1963, White demonstrated that the maximum survival of heated Streptococcus faecalis occurred at pH 6.8 and decreased sharply on either side of this value. Further experimentation at 55° and 65°C gave the same results. Jordan and Jacobs (1948), working with Escherichia coli, found similar results at 51°C and also discovered an abnormal sensitivity at pH 6.4 and 7.0. Jyenger et al. (1951), working with lactic streptococci, found minimum heat resistance at pH 5.0 and maximum at pH 8.0. Bagger (1926) found enterococci heated in broth most resistant at pH values close to neutral. Anellis et al. (1954) reported that resistance of Salmonella strains in liquid eggs was a continuous function of pH between 6.1 and 8.5 with maximum resistance at pH 6.1.

The conflicting results that are seen could be attributed to the fact that the pH in the microbial cell may be different from that of the surrounding medium. When the pH value approached neutrality, the ampholytes in the cell possessed negative charges, attracted hydrogen ions and changed the pH in the vicinity of the cell proteins. In the presence of an electrolyte, such as sodium chloride, the pH was affected because the sodium ions displaced the hydrogen ions from the area of the negatively charged ampholytes (Hansen and Riemann, 1963).

The Effect of Proteins

The presence of proteins in the heating medium had a protective effect on microorganisms. But the mechanism has not been elucidated. White (1952) observed that the resistance of microbial cells in milk was comparatively higher than cells in quarter strength Ringer's solution. Precht et al. (1955) concluded that the resistance must be due to the protein concentration.

Hansen and Riemann (1963) observed that bacteria showed more resistance in cured hams than in phosphate buffer at the identical pH.

Precht et al. (1955) had postulated that proteins may increase enzyme and the other protein stability by a combination between protein molecules or between proteins and amino acids through electrostatic attraction, or a heat stable complex may be formed by combination between an enzyme and its substrate to form an enzyme complex. The latter has been demonstrated with a proteinase from pseudomonads.

Other Protective Substances

Precht et al. (1955) has shown that molecules other than proteins and amino acids may combine with proteins and provide protection. This has been shown with eosin and heparin. It was suggested that instead of protection against heat damage, the compounds involved may help in the reactivation of the damaged cell, since it was known that amino acids, hydrogen sulfide and other compounds could reactivate enzymes and other protein molecules. Further, if the damage was not too severe, such as breakage of disulfide and hydrogen bonds, the heat damaged protein molecules in the cell were brought back to almost

their original state. Reactivation could also be accomplished by adding certain metabolites, such as citric, maleic, oxaloacetic lactic and pyruvic acids, which stimulated synthesis of those enzymes damaged by heat (Heinmetz et al., 1954; Precht et al., 1955).

Inhibitory Compounds

If a lethal or inhibitory substance, such as sulfur dioxide, certain essential oils (garlic and onion), crude extracts from edible plants, and antibiotics, was present in the heating medium, it was thought that the heat resistance would be decreased. But this was not always true because these substances may interact with temperature and other factors. For example, Greenberg and Silliker (1961) found that 100 parts per million of nitrite had no effect on the numbers of surviving Streptococcus faecalis and Streptococcus faecium at 148.5°F, but at 155° and 158.5°F, there was a 100 to 500-fold decrease in survivors.

Demeter and Eisenreich (1937) found an unknown inhibitory compound or group of compounds present only in whole milk which reduced the time necessary to kill up to 70% of the bacteria and exerted its greatest effects at low temperatures. They postulated that the compound was possibly identical to a heat sensitive peroxidase.

Thus, some naturally occurring substances generally having little or no effect on normal microorganisms were postulated to have an inhibitory effect on heat damaged cells or increase the kill rate (Hansen and Riemann, 1963).

The Effect of Cell Concentration

With dense populations, an increased heat resistance has been found which was due to specific substances excreted by the cells. These perhaps behaved like proteins. These have been demonstrated in undiluted cultures after one to two days incubation, but disappeared after longer incubation (Hückel, 1926; Ørskov, 1926). And such an increase was expected because highly resistant cells, occurring at low frequencies, were present in higher numbers per unit volume (Vas and Proszt, 1957).

Sometimes one microbial strain has been shown to increase the resistance of another when the two grew together. This has been shown with certain sporeformers (Meyer and Lang, 1926; Prévot et al., 1951). Peppler and Frazier (1941), working with nonsporeformers, found that the resistance of lactobacilli was increased when grown in conjunction with Candida krusei.

The Effect of Age

The relationship between heat resistance and phase of growth has been known for a long time. Cells in the logarithmic phase are generally less resistant; Sherman and Albus (1923) were responsible for this determination and coined the term "physiological youth" to describe these young cells. Elliker and Frazier (1938), working with Escherichia coli, showed that the resistance decreased from the time of inoculation up to the stationary phase. Thus, as the rate of reproduction diminished approaching the end of logarithmic growth, a rise in heat resistance was initiated which peaked in the stationary phase.

However, when the culture was grown at 28°C, a transient and abrupt increase in the heat resistance occurred after incubation for two hours.

White in 1953, working with Streptococcus faecalis cultures of different ages, found that young cultures (cultures less than 30 minutes old) were much more resistant than the cells in the parent culture. A slight increase in resistance was discovered after the next half hour of incubation and then a steep decline set in as the culture began to multiply exponentially. The heat resistance then started to increase as the culture reached the stationary phase of growth. Similar results were obtained for Escherichia coli by Elliker and Frazier (1938). Lemcke and White (1959), working with Escherichia coli, demonstrated similar results. Heat resistance was high at the beginning of the lag phase, began to decrease and reached a minimum as the culture went into the exponential growth phase. Then an increase was noted as the culture went into the stationary growth phase.

Hansen and Riemann (1963) demonstrated a practical application of these factors with canned hams. They found that fecal streptococci in pasteurized canned hams went into the exponential growth phase when incubated for three hours at 30° to 42°C. During the come-up period, multiplication took place with a 100 to 200-fold increase and resistance decreased with the result that there was actually a smaller number of survivors after pasteurization. This indicated that it was useful to have a well defined come-up period before pasteurization and also to use a two-step cooking treatment, in which the food was preheated for a specific time at a lower temperature. In addition, the initial contamination must be low.

During cell division changes in the osmotic barrier might make growing organisms more susceptible than resting cells. Heat damage might also be attributed to a disturbance of the balance of metabolic reaction rates so that the cell cannot maintain the stable, steady conditions that correspond to viability (Mitchell, 1951).

Hansen and Riemann (1963), working with Streptococcus faecium and Streptococcus faecalis inoculated into pickle brine and heated to 60°C, showed that the destruction rate decreased during heating. Samples, taken after various times of heating, were repasteurized after storage for 48 hours at 5°C. Results showed a high rate of destruction at the beginning of the heating period followed by a decrease in the destruction rate. He assumed that the cells which were killed last during the heating do not permanently have a higher resistance than the majority of the cells in the population, but that the increased resistance was induced during heating.

Hansen and Riemann (1963), working with a pseudomonad, demonstrated that organisms may not only be inhibited but killed by keeping them at temperatures which fluctuate between 5° and 37°C. This was explained by an assumption that all cells would eventually go into the exponential growth phase because of the frequent exposure to growth temperatures and therefore become so heat sensitive that they would be killed by temperatures as low as 37°C.

The Effect of Temperature and Other Growth Conditions

The heat resistance of many bacteria and yeasts increases with an increase in the growth temperature. A possible explanation of this

is a change in the nature of the strains. Conversely, low temperatures might give high resistance. Sherman and Cameron (1934), working with Escherichia coli, found that young cells from slowly growing cultures exhibited greater resistance to various deleterious factors than cells from rapidly growing cultures. The growth rates were decreased by utilizing a low incubation temperature, dilute media or increased osmotic pressure. Anderson and Meanwell (1936), working with a thermoduric streptococcus, showed that in the lag and early logarithmic phases of growth, resistance to heat increased when the incubation temperature was reduced below the optimum. Claydon (1937) showed that Streptococcus lactis cultures grew more slowly at 10°C, but exhibited an increased thermal resistance compared to cells grown at a higher temperature.

Elliker and Frazier (1938) demonstrated that Escherichia coli in the stationary phase of growth showed a higher survival percentage when incubated at 38.5° and 40°C than at 28°, 30° or 30.5°C. The lowest heat resistance was found at 28°C. The previously mentioned trends in heat resistance for fecal streptococci by White (1963) were the same for incubation at 27°, 37° and 45°C with the resistance in the final stationary phase of the 45°C culture being the highest and being retained for at least 18 hours.

Other conditions of growth have been found to influence heat resistance. Peppler and Frazier (1941) showed that successive transfer every 12 to 24 hours resulted in more heat resistant cultures of Lactobacillus helveticus at 40° than 37°C.

Lembke (1937), working with Escherichia coli, demonstrated that there was no difference in heat resistance when the cells were grown initially on meat extract-peptone agar or lactose agar; but the time the cells stayed suspended in saline at 37°C before the heat treatment tests influenced the resistance. The resistance increased considerably during the first one to one and one-half hours, dropped again after four and one-half hours and in all cases, after 24 hours, was at the same level as the beginning.

Thus, there are many factors that influence heat resistance of microorganisms.

III. INJURY AND RECOVERY OF NONSPORING ORGANISMS

The finding of sublethally injured microorganisms has always been important for the interpretation of microbiological data. These injured organisms have important implications for each major area of food microbiology: food preservation and spoilage, in food safety and consumer protection and in food manufacture and culture propagation. Busta (1976) stated that

> Sublethal injury induced by exposure to environmental stress often is demonstrated as a loss by the microorganism of one or more abilities to function characteristically under conditions that are satisfactory for untreated cells.

Busta (1976) inferred that some essential considerations of injury might include use of damage to enhance lethal action of processing treatment, minimizing damage to preserve culture activity in food fermentations, predicting effects of product formula modification on subsequent microbial damage and survival, and eliminating inadequacies

within existing and proposed methodology for detecting and enumerating specific microorganisms.

Sublethal injury has been shown to be induced by many treatments used in food processing such as heat, cold, freezing and thawing, freeze-drying, moisture reduction, irradiation, and exposure to food environments, sanitizers or preservatives. This type of injury may be seen as an increased susceptibility to antimicrobial agents, leakage of intracellular material and modified metabolic activities (Busta, 1976).

The literature reveals that many investigators have identified certain media and cultural conditions that would enhance the growth of injured cells. Nelson (1943 and 1944) and Heinmetz et al. (1954) observed that coliforms, after heat exposure, would not grow very well on selective media, whereas organisms that were not heat treated would. Hartsell (1951) found similar results with freezing treatments. This cellular injury has been described as the formation of cultural inadequacies after exposure to environmental stress (Busta, 1976). Straka and Stokes (1959) found that pseudomonads exposed to freezing temperatures required complex components for growth. Busta and Jezeski (1961) found a salt tolerance loss by staphylococci after a sublethal heat treatment. Moss and Speck (1963) found that lactic streptococci, subjected to low temperatures, required the presence of certain peptides for growth.

In all of the above cases, the bacteria had lost the ability to grow under normal conditions. Thus Busta (1976) concludes that "injury is the inability of bacteria to form colonies on a defined

minimal medium while retaining the colony forming capability when complex nutrients are supplied."

Treatments Related to Food Processing That Induce Cell Injury

There are many processing techniques that are used to preserve food from microbial growth. Most of them will destroy the microorganisms. However, they also can just sublethally injure the cells.

Heat or the use of elevated temperatures is one of the most common ones and will sublethally injure microbial cells and spores. Temperatures used in pasteurization and subpasteurization were known to injure Staphylococcus aureus (Busta and Jezeski, 1963). In addition, temperatures utilized in concentration and dehydration, blanching and cleaning provided the same effect (Busta, 1976).

Sublethal injury has been reported in the following organisms: Staphylococcus aureus (Stiles and Witter, 1965); Streptococcus faecalis (Clark et al., 1968); Salmonella typhimurium (Clark et al., 1969); Escherichia coli (Russell and Harries, 1967); Enterobacter aerogenes (Strange and Schon, 1964); Pseudomonas fluorescens (Gray et al., 1973); Vibrio marinus (Haight and Morita, 1966); Bacillus subtilis (Miller and Ordal, 1972); and Clostridium bolutinum (Pierson et al., 1971). Thermal processing at normal and elevated temperatures damaged spores of Bacillus subtillis (Busta and Adams, 1972), Clostridium perfringens (Barach et al., 1974), and Clostridium botulinum (Alderton et al., 1974).

Refrigeration temperatures (above 0°C) used for the preservation of certain foods have been found to induce injury to Staphylococcus

aureus (Jackson, 1974) and Clostridium perfringens (Traci and Duncan, 1974).

Temperatures below 0°C are used in processes that include freezing to preserve food, concentration of liquids and production of frozen desserts. Freezing and subsequent thawing have been known to induce injury in Escherichia coli (Moss and Speck, 1973); Salmonella anatum (Janssen and Busta, 1973); Streptococcus lactis (Moss and Speck, 1963); Shigella sonnei (Nakamura and Dawson, 1962) and Pseudomonas fluorescens (Arpai, 1962). However, the characteristics of this particular type of injury have not been fully differentiated (Busta, 1963).

Freeze-drying, or the removal of moisture from foods or other biological materials in the frozen state and under vacuum has evolved as a common but gentle method of preservation. However, injury was induced by this method and with Salmonella anatum (Ray et al., 1971a), Escherichia coli (Sinskey and Silverman, 1970), and Staphylococcus aureus (Baird-Parker and Davenport, 1965). Thus food preserved by freeze-drying could contain injured organisms and cultures prepared by this method may contain damaged cells. But again the characteristics of this particular type of injury have not been differentiated (Busta, 1976).

Moisture reduction, involving removal of part or most of the available water, takes place during a variety of environmental conditions, which include elevated temperatures, low pressures and in dehydration equipment, such as agitators, evaporators, and spray and roller driers. Dried milk, for example, has been shown to contain

injured cells (Ray et al., 1972). Occasionally, dehydration during aspiration or chance drying on equipment also may induce injury (Stersky and Hedrick, 1972; Webb, 1969). But again these characteristics have not been well differentiated (Busta, 1976).

Injury can also occur when bacterial cells have been placed in a new environment. If a different substrate was used or the osmotic pressure was changed, injury occurred that might resemble dehydration or rehydration (Ray et al., 1972). When cells were placed in a fresh complex medium, the cells might respond to the selective or minimal media as if they were injured (Scheusner et al., 1971a). When cells were placed in a spent media, in which the cells were exposed to end products, such as acids, injury would occur (Minor and Marth, 1972). Sometimes exposure to the air could cause injury (Gomez and Sinskey, 1975). Injury might also occur if the cells were exposed to various diluents and distilled water (Hoadley and Cheng, 1974; MacLeod et al., 1967). If cells had been exposed to starvation conditions, Busta (1976) assumed that sublethal injury may occur.

When ultraviolet irradiation was used for sanitization or surfaces were irradiated by sunlight or gamma radiation, used for food preservation, there was a possibility of sublethal injury. This is seen in the production of radiation-resistant strains (Davies et al., 1973).

Chemicals used to sanitize equipment in the food industry have been known to induce injury if the sanitizers were used in concentrations and under specific conditions that do not completely destroy

the cells (Scheusner et al., 1971b). From the equipment, the cells could find a way into the food systems, producing misleading test results and potential problems of repair (Busta, 1963).

Food additives and the acid end products of food fermentations serve not only as preservatives for food, but also as inhibitors of unwanted growth of spoilage organisms. Staphylococcus aureus showed injury when exposed to acids combined with other stresses (Minor and Marth, 1972), and Clostridium perfringens spores were altered by alkali exposure (Duncan et al., 1972).

Some processing techniques, such as freeze-drying, are combinations of several individual treatments. However, injury investigation sometimes does not take into account interaction and combined effects. For example, low levels of sodium chloride in the heating medium reduced the amount of injury in Escherichia coli (Shibasaki and Tsuchido, 1973). Staphylococcus aureus was injured by utilizing a freezing treatment with a low pH (Minor and Marth, 1972).

Cellular Changes Induced by Stress

After sublethal injury, injured cells were usually identified by their inability to reproduce under normal conditions. This inability was manifested by lack of colony formation on or in solid media, absence of turbidity in broth media or low production of end products from specific substrates (Busta, 1963).

Injury was observed when the cell acquired an increased or new sensitivity to selective agents, antimicrobials or similar substances in the growth medium. Salt tolerance was lost by Staphylococcus aureus

(Busta and Jeseski, 1971); Bacillus subtilis (Miller and Ordal, 1972); Escherichia coli (Shibasaki and Tsuchido, 1973); and Salmonella typhimurium (Clark and Ordal, 1969). Injured Escherichia coli cells were inhibited by deoxycholate (Scheusner et al., 1971a). Actinomycin D affects injured Salmonella anatum (Ray et al., 1971c). Heat treated spores of Clostridium botulinim and Clostridium perfringens were very sensitive to the presence of lysozyme in the recovery media (Adams, 1974; Alderton et al., 1974). Busta (1963) puts forth the explanation that these sensitivities were due to cell membrane or cell envelope modifications that had taken place in the injured cell.

Injured cells lose some of their cellular material by leakage into the surrounding environment. Escherichia coli cells that were frozen released amino acids, small molecular weight ribonucleic acids and peptides (Ray and Speck, 1972). Sublethally injured Staphylococcus aureus released potassium, amino acids, and proteins and 260nm absorbing material (Iandolo and Ordal, 1966; Allwood and Russell, 1968). Heated Escherichia coli cells released lipopolysaccharide which was correlated with outer membrane damage. This preceded the death of the organism (Hitchner et al., 1975). Thus, if enough of the cellular constituents were lost through leakage, the cells would not reproduce, and hence be termed legally dead.

Macromolecules contained within the cell could be changed by environmental stress. Ribosomal ribonucleic acids (RNA) were degraded in heat treated cells of Staphylococcus aureus (Iandolo and Ordal, 1966; Allwood and Russell, 1968), and in Salmonella typhimurium (Tomlins and Ordal, 1971a). Degradation of deoxyribonucleic acid (DNA) has been

reported, but this was related to the death of the organism (Bridges et al., 1969; Woodcock and Grigg, 1972). Recently deoxyribonucleic acid degradation has been correlated to heat injury of Salmonella that reacted negatively to complex nutritional media (Gomez and Sinskey, 1973; Gomez et al., 1973). Thus, if these damaged components were not repaired, then the cell would not be able to reproduce and therefore would be considered dead.

Disruption of certain metabolic activities could be induced by stress. Staphylococcus aureus, sublethally injured by heat, possessed decreased catabolic capabilities and decreased activity of certain enzymes of glucose metabolism (Bluhm and Ordal, 1969). Injured Salmonella typhimurium that had undergone heat exposure was found to possess an altered glucose transport system (Pierson and Ordal, 1971). Streptococcus lactis, subjected to low temperature storage, had a membrane proteinase inactivated (Speck and Cowman, 1970).

Restoration of Lost Capabilities

Busta (1976) stated

> Cells are classified as injured rather than dead when they are damaged but have the capability to function in an unrestrictive environment and restore a normal physiological state concommitant with initiation of growth and cell division.

Thus injury damage disappeared when the cell divided to support the observation of injury and rule out mutational changes. Restoration of the damaged cell to its normal state was termed resuscitation because the cells were revived from an outward appearance of death (Busta, 1976). This recuscitation process was related to some or most of the

cellular changes that injured the cell. Many of the cellular changes were reversed or losses were restored to normalcy by incubating the damaged cells for a period of time (Busta, 1963). During this period degraded ribosomes were regenerated (Iandolo and Ordal, 1966; Sogin and Ordal, 1967). During the recovery period phospholipids were synthesized (Tomlins et al., 1972). Protein synthesis took place for recovery of some cells that were frozen, freeze-dried or heated (Ray and Speck, 1973; Sinskey and Silverman, 1970; Tomlins and Ordal, 1971b). The repair process was accompanied by the formation of adenosinetriphosphate (ATP) (Ray et al., 1971c and 1972; Ray and Speck, 1972). Single strand breaks were repaired to prevent the organism from expiring (Gomez and Sinskey, 1973). In addition, the synthetic activities of one strain were unique to that particular strain (Busta, 1976).

Several workers have recognized the importance of injured organisms as they influence enumeration and isolation techniques. Routinely, an "enrichment" step has been common practice in demonstrating Salmonella in foods, which could be likened to a resuscitation period for sublethally injured cells. A rapid method to test for Enterobacteriaceae in dried foods required a restoration step (Mossel and Ratto, 1970). Speck et al. (1975) published a plating procedure for injured coliforms which used a resuscitation period before exposure to the selective agents of bile salts and crystal violet. In other instances, injured organisms were able to grow on selective media with no identifiable antagonistic effect on colony formation and repair (Gray et al., 1974).

Factors That Influence Stress

Growth conditions, which normally affect the physiological state and composition of the cell or spore, can also affect the susceptibility of the microbial cell to injury. The recovery of heated Salmonella typhimurium has shown that growth media and its accompanying conditions influenced the cell's ability to recovery from injury, the expression of the injury and the type of repair implemented (Gomez et al., 1973). Specific nutrients, pH, redox potential, osmolality, water activity, ionic strength, surface tension, temperature, agitation, gaseous atmosphere, culture age and other factors involved in growth had an effect on the normal functions of the cell and it was not impossible for the above to interact and influence the injured cell (Busta, 1976). Factors that influence cell quality would also interact with the injured cell. The storage temperature and humidity were found to influence the amount of injury, when Salmonella typhimurium was freeze-dried (Sinskey et al., 1967). The type of suspending medium was an influence on injury; the type of damage to Salmonella anatum was influenced by the milk components contained in the medium (Janssen and Busta, 1973), as well as the type of damage that was incurred by Escherichia coli in various foods (Ray and Speck, 1973). Solutes containing phosphate buffers, magnesium ions and sucrose were shown to effect the thermally injured cells of Salmonella typhimurium (Lee and Goepfer, 1975). With Staphylococcus aureus, the damage increased with a low pH in combination with freeze-thaw, but this combination did not effect other bacteria (Minor and Marth, 1972). Staphylococcus aureus and its subsequent injury was influenced by the pH and sodium chloride

concentration in the heating medium (Bean and Roberts, 1974). In addition, if the above factors influenced injury then they could be assumed to affect resuscitation. The interaction of pH and sodium chloride was known to interact with the following: repair of heat injured Staphylococcus aureus (Iandolo and Ordal, 1966); repair of injury in freeze-dried Salmonella anatum (Ray et al., 1971c); and revival of heat treated Escherichia coli (Russel and Harries, 1968).

Again, antagonistic agents, such as metabolic end products or food additives, could further damage the injured cells, and this effect would be greater than on the uninjured cell (Busta, 1976).

CHAPTER III

MATERIALS AND METHODS

I. MATERIALS

Four rounds of beef from four different animals were obtained from East Tennessee Packing Company, Knoxville, Tennessee. In addition, two extra rounds were obtained from the University of Tennessee Meats Laboratory.

The cultures of Staphylococcus aureus and Escherichia coli were obtained from the laboratory of the Department of Food Technology and Science, University of Tennessee.

The culture of Salmonella enteritidis was obtained from the laboratory of Dr. O. J. Mundt, Professor of Microbiology, University of Tennessee, Knoxville.

II. CULTURE MAINTENANCE

Cultures of Staphylococcus aureus, Escherichia coli, and Salmonella enteritidis were maintained on Trypticase Soy Agar slants at 4°C. They were transferred to fresh slants at four week intervals. Prior to inoculation into the ground beef, the cultures were grown in Trypticase Soy Broth for 24 hours at 32°C for Staphylococcus aureus and Escherichia coli; 35°C for Salmonella enteritidis.

III. PREPARATION OF INOCULUM

To obtain an inoculum requirement of 3,000,000-4,000,000 organisms per gram, it was necessary to determine how many organisms existed in a 24 hour broth culture. This was accomplished by aseptically transferring one loopful of culture from the slant to five milliliters of Trypticase Soy Broth and incubated for 24 hours at 32°C; 35°C for Salmonella enteritidis. At the completion of the 24 hour period, standard plate counts were conducted to determine the number of organisms at 24 hours. Media used included Standard Plate Count Agar for the Escherichia coli and Staphylococcus aureus; Trypticase Soy Agar with yeast extract for the Salmonella enteritidis, and these counts were determined for two days.

Utilizing the above data, a specified dilution of the culture was aseptically made of the 24 hour broth to obtain the desired inoculum requirement at a final volume of 60 milliliters. Peptone water (0.1%) was used as the diluting fluid.

IV. PREPARATION OF GROUND BEEF AND INOCULUM

The four rounds of beef, at a total weight of 10,800 grams minimum, were cut at the University of Tennessee Meats Laboratory in a manner to minimize microbial contamination. Each of the four rounds were cut into small cubes, approximately 1" × 1" and divided into three lots (one for each test organism) of 2700 grams each. The lots were frozen at -20°C and held there until used. When pulled from stock for use, each lot was allowed to thaw for two days at 1.7°C.

Each 2700 gram lot of meat was then placed on a piece of sterile aluminum foil. One milliliter of the inoculum was injected into each beef cube with a 50 milliliter sterile syringe with an attached 21 gauge sterile needle. The inoculated beef cubes were then fed into a Kitchen Aid grinder that had been sanitized by submersing plastic parts in 200 parts per million chlorine and sterilized by autoclaving the metal and wood parts. This grinding procedure was repeated to insure maximum mixing of microorganisms and beef and then the meat was placed in a sterile polyethylene bag for storage at 4°C.

V. FOOD PRODUCT PREPARATION

Raw meat samples of 50 grams were procured to follow bacterial counts during the storage period. The food homogenate, consisting of 50 grams of beef and 450 milliliters of sterile 0.125 % phosphate buffer, was prepared according to the method described by Gilliland et al. (1976), with one modification. A three, instead of two, minute blending time was used because of the coarseness of the sample material. To aid uniformity of results, each sample was mixed quickly and aseptically prior to removal from the polyethylene bag for weighing.

Three samples of 100 grams each were obtained and each was made into a standard patty, placed on a preheated griddle with a Yellow Springs Telethermometer inserted so as to measure internal temperature. The griddle was closed and the patty was cooked until the designated internal temperature was reached. The patty was then removed as quickly as possible to a sterile blender jar, and 900 milliliters of 0.1% peptone water for Escherichia coli, Trypticase Soy Broth for Staphylococcus

aureus, and Lactose Broth for Salmonella enteritidis, was added aseptically and blended for three minutes. The first two organisms were tested at three cooking temperatures (60.0°C, 65.6°C and 71.1°C) and three storage times (zero day, one day, two days, and three days).

For the Salmonella enteritidis, the patties were cooked until the internal temperature of the patty reached 60.0°, 65.6° and 71.1°C. The following times (60.0°C, three minutes, 16 seconds; 65.6°C, three minutes, 41 seconds; and 71.1°C, four minutes, six seconds) were predetermined utilizing ground beef patties. The storage times remained the same.

VI. BACTERIOLOGICAL ASSAYS

On all raw beef samples, the aerobic count assay was determined according to the described methods (Gilliland et al., 1976). The procedure for determining the assays of each organism individually (Escherichia coli, Staphylococcus aureus, Salmonella enteritidis) was identical to that used for the aerobic count assay with the exception of the media used. Baird Parker Agar was used for Staphylococcus aureus, Violet Red Bile Agar for Escherichia coli and Brilliant Green Agar for Salmonella enteritidis. The plates were then incubated for 48 hours at 35°C with the exception of the Escherichia coli, which was incubated for only 24 hours. The dark red colonies (0.5 millimeter in diameter or larger and surrounded with a reddish zone of precipitated bile) were counted and reported as the number of Escherichia coli per gram. The shiny, black colonies (with or without grey edges and exhibiting a clear zone extending into the surrounding agar) were

counted and reported as the number of Staphylococcus aureus per gram. The colorless, pink to fuschia, or translucent to opaque (with the surrounding medium pink to red) colonies were counted and reported as the number of Salmonella enteritidis per gram.

Recovery Determination--Escherichia coli

The cooked 100 gram samples, homogenized with 900 milliliters of 0.1% peptone water, were serially diluted from 1:1,000 through 1:1,000,000 and plated on both Standard Plate Count Agar and Violet Red Bile Agar, and incubated at 35°C. The two above assays represent the viable count or the number of organisms that survived the specified cooking temperatures.

The procedure for recovery was based on a method developed by Ordal and others (1976), and consisted of serially diluting the patty homogenate from 1:10 through 1:10,000 and pour plating with five milliliters of Trypticase Soy Agar with added yeast extract. The plates were then incubated at 35°C for two hours to effect repair. The plates were then overlaid with 10 milliliters of Violet Red Bile Agar, tempered to 45°C, and replaced back in the 35°C incubator for an additional 24 hours. The difference in counts between the Trypticase Soy Agar with added yeast and the Violet Red Bile Agar overlay and the Violet Red Bile Agar plates provided a measure of the number of sub-lethally injured organisms.

Recovery Determination--*Staphylococcus aureus*

The cooked 100 gram samples, homogenized with 900 milliliters Trypticase Soy Broth were serially diluted from 1:100 through 1:100,000 and plated on both Standard Plate Count Agar and Baird Parker Agar and incubated at 35°C for 48 hours. Again these two assays represented the viable count.

The procedure for recovery was based on a method developed by Ordal and others (1976) and consisted of incubating the homogenate of beef and Trypticase Soy Broth at 35°C for two hours. Of the homogenate 450 milliliters were aseptically transferred to a sterile 500 milliliter flask and placed in the incubator to effect repair. Serial dilutions of 1:10 through 1:10,000 were pour plated with Baird Parker Agar. These plates were then placed in the 35°C incubator for 48 hours. The difference in counts between the two sets of Baird Parker Agar plates provided a measure of the number of sublethally injured organisms.

Recovery Determination--*Salmonella enteritidis*

The cooked 100 gram samples, homogenized with 900 milliliters sterile 0.5% Lactose Broth, were serially diluted from 1:10 through 1:10,000 and plated on Brilliant Green Agar and Standard Plate Agar, and incubated at 35°C for 48 hours. Again these two assays represented the viable count.

The procedure for recovery was based on a method developed by Poelma and Silliker (1976), and consisted of incubating the homogenate

of beef and Lactose Broth with 4.4 milliliters Tergitol #7 at 35°C for 60 minutes. Of the homogenate, 450 milliliters were aseptically transferred to a sterile 500 milliliter flask and placed in the incubator to effect repair. Serial dilutions of 1:10 through 1:10,000 were pour plated with Brilliant Green Agar and incubated at 35°C for 48 hours. The difference in counts between the two sets of Brilliant Green Agar plates provided a measure of the number of sublethally injured organisms.

CHAPTER IV

RESULTS AND DISCUSSION

In enumerating sublethally injured organisms, media was utilized which provided growth conditions for these bacteria as well as supplying a suitable environment for those bacteria "uninjured" by the heat. From this information, the sublethally injured organisms could be determined by taking the difference between the count of cells propagating in enhancement media and the count of those cells propagating in normal selective media. However, in some instances, higher counts were obtained in the normal selective media than in the enhancement media. When the difference was taken, a paradox of negative counts of sublethally injured cells resulted.

Regardless of the results, relative numbers of injured and uninjured cells always occurred. So it seemed logical to work with a ratio of the two counts. In addition, to stabilize the variances encountered with numbers, the counts were transformed to logarithms to the base 10. Thus the relationship of injured and uninjured organisms could be expressed as a ratio, A/B, where A was the count in enhancement media (total viable population), and B was the count in the normal selective media. Transformation to $\log_{10}$ gave log A/B, or log A - log B. Thus, a mathematical concept of a difference in logarithms of counts, instead of a difference in numeric counts, was formed and applied in the analyses of variance.

Tables 1 and 2 illustrate pertinent data on the recovery of sublethally injured Escherichia coli. Table 1 summarizes the relative numbers of injured organisms recovered after the three heat treatments. Table 2 summarizes the analysis of variance of the data. There appeared to be a decrease of injured organisms as the heating temperature increased, even though heat, storage, and replication were not significant. This could be attributed to a number of factors. If the organisms, propagating in the ground beef, were in the log or exponential phase of growth, heat susceptibility would increase, and the organisms would succumb to the stress. If the pH of the ground beef was outside neutrality, heat susceptibility would also increase and the same results would occur. In addition, if the protein concentration was low, heat susceptibilty would increase and the same results would occur. But this is just a supposition of what may have happened.

Tables 3 and 4 illustrate pertinent data on the recovery of sublethally injured Staphylococcus aureus. Table 3, which summarizes the relative numbers of injured organisms after heat exposure, shows no predictable recovery of this organism, although the same factors discussed in the preceding paragraph might apply here, and explain the decrease of injured organisms at 65.6°C and the increase in injured organisms at 71.1°C. The analysis of variance, shown in Table 4, showed significance of replication only at the 1% level. Studying this data and that obtained with the Escherichia coli, it was decided that the method of cooking or heat treatment could not be controlled adequately.

TABLE 1

RELATIVE NUMBERS OF INJURED AND UNINJURED *ESCHERICHIA COLI* AS A FUNCTION OF HEATING TEMPERATURE

	Mean Counts[1] Per Gram		
Parameter	at 60.0°C	at 65.6°C	at 71.1°C
Total viable	492,000	483,000	374,000
Uninjured	327,000	323,000	462,000
Injured	165,000	160,000	0

[1]Means of four storage times and seven replications.

TABLE 2

ANALYSIS OF VARIANCE OF THE RELATIONSHIP BETWEEN TOTAL VIABLE AND UNINJURED *ESCHERICHIA COLI*[1]

Source	Degrees of Freedom	Mean Squares	F value
Storage	3	0.0688	1.53
Replication	6	0.0332	0.72
Storage × Replication	17	0.0542	1.20
Temperature	2	0.1155	2.56
Storage × Temperature	6	0.0412	0.92

[1]Data used was the logarithm A/B, where A was total viable count per gram, and B was the count of uninjured *Escherichia coli* per gram.

TABLE 3

RELATIVE NUMBERS OF INJURED AND UNINJURED STAPHYLOCOCCUS AUREUS AS A FUNCTION OF HEATING TEMPERATURE

	Mean Counts[1] Per Gram		
Parameter	at 60.0°C	at 65.6°C	at 71.1°C
Total viable	703,500	806,000	1,450,000
Uninjured	331,000	514,000	629,000
Injured	372,500	292,000	821,000

[1]Means of four storage times and four replications.

TABLE 4

ANALYSIS OF VARIANCE OF THE RELATIONSHIP BETWEEN TOTAL VIABLE AND UNINJURED *STAPHYLOCOCCUS AUREUS*[1]

Source	Degrees of Freedom	Mean Squares	F Value
Storage	3	0.0229	0.96
Replication	3	0.3137	13.10**
Storage × Replication	9	0.0380	1.59
Temperature	2	0.0265	1.11
Storage × Temperature	6	0.0400	1.67

**$P < 0.01$.

[1]Data used was the logarithm A/B, where A was the total viable count per gram, and B was the count of uninjured *Staphylococcus aureus* per gram.

Repeated trials were run with ground beef patties to determine the time needed to reach the following temperatures: 60.0°C; 65.6°C and 71.1°C. These times were 60.0°C, three minutes, 16 seconds; 65.6°C, three minutes, 41 seconds; and 71.1°C, four minutes and six seconds, with a fixed setting on the heating grill.

Tables 5 and 6 illustrate pertinent data on the recovery of sublethally injured Salmonella enteritidis under the new cooking conditions. Table 5, summarizing the relative numbers of injured organisms, showed no recovery at 65.6°C. Again the same factors discussed previously may apply here, such as age of culture, pH of medium, and protein concentration. Table 6, illustrating the analysis of variance, showed significance at the 5% level for replication and the storage-temperature interaction effects on the ratio of total viable to uninjured cells.

The significance in replication indicates that the change of the heating method did not give a foolproof system of heating. Further work would be needed to insure a repeatable heat treatment. The storage-temperature interaction resulted in the following effects. Utilizing a heat treatment at 60.0°C, there was no recovery at day zero and day two and there was recovery at days one and three; with a heat treatment of 65.6°C recovery of sublethally injured organisms occurred at days zero and one and no recovery at days two and three; with a heat treatment at 71.1°C recovery of sublethally injured organisms occurred at days zero, one and two and did not occur at day three. The most plausible explanation of this pattern would be the age of the culture and the pH

TABLE 5

RELATIVE NUMBERS OF INJURED AND UNINJURED *SALMONELLA ENTERITIDIS* AS A FUNCTION OF HEATING TEMPERATURE

	Mean Counts[1] Per Gram		
Parameter	at 60.0°C	at 65.6°C	at 71.1°C
Total viable	171,000	1500	482
Uninjured	4,900	1500	114
Injured	166,100	0	368

[1]Means of four storage times and four replications.

TABLE 6

ANALYSIS OF VARIANCE OF THE RELATIONSHIP BETWEEN TOTAL VIABLE AND UNINJURED *SALMONELLA ENTERITIDIS*[1]

Source	Degrees of Freedom	Mean Squares	F Value
Storage	3	0.8627	1.61
Replication	3	1.6873	3.15*
Storage × Replication	9	0.2855	0.53
Temperature	2	0.0267	0.05
Storage × Temperature	6	1.4773	2.76*

*$P < 0.05$

[1]Data used was the logarithm A/B, where A was the total viable count per gram, and B was the count of uninjured *Salmonella enteritidis* per gram.

of the medium. As stated previously, if the organisms are in the log or exponential phase of growth, they are not very resistant to heat; however, once they reach the stationary phase of growth, they become very resistant to heat and most likely will survive. Also, if the pH range is close to neutrality, heat resistance will increase and the organisms will most likely survive the stress.

Tables 7 and 8 illustrate pertinent data on the survival of the total bacterial population contained in the ground beef with the three organisms added. For the first two sets of aerobic plate counts, the only significant data obtained was that of replication, which again indicated that a foolproof heating system was not available. With the third set of aerobic plate counts, which included the Salmonella enteritidis, there was significance for storage, temperature, replication and storage-replication interaction at the one percent level. Over storage the counts showed a definite trend. After one day the counts increased and then for the remainder of storage period they decreased. Replication significance indicated again a lack of stable heat treatments, as well as possible variation in organisms in the population. Since replication and storage were both significant, it follows that the combination would be significant too, which it was. Temperature showed significant trends, too. At zero day storage, the counts decreased with increasing temperature; at one day storage the counts decreased at temperature 65.6°C, but rose slightly at temperature 71.1°C; at two and three days storage, the counts progressively decreased. Again, this could be explained by the age of the culture and maybe the pH of the medium.

TABLE 7

LOGARITHMIC AEROBIC PLATE COUNTS OF MEAT INOCULATED WITH THREE ORGANISMS AS A FUNCTION OF STORAGE AND HEATING[1]

	Organism	Storage (days)	Mean Counts[1] at 60.0°C	at 65.6°C	at 71.1°C
			- - - - - - - $\log_{10}$ - - - - - - -		
I.	Escherichia coli	0	5.4050	5.1355	5.3095
		1	5.5143	5.5277	5.2232
		2	5.5473	5.0962	5.4437
		3	5.6198	5.5251	5.7510
II.	Staphylococcus aureus	0	5.8385	5.4667	5.4364
		1	5.6973	5.5991	5.8348
		2	5.9225	5.7205	6.0488
		3	5.4414	5.8108	6.2545
III.	Salmonella enteritidis	0	4.9515	4.6009	4.5755
		1	5.9578	5.1324	5.2549
		2	5.1559	4.9184	4.7030
		3	4.9417	4.6097	4.0646

[1]Means of seven replications for I; four for II and III.

TABLE 8

MEAN SQUARES OF LOGARITHMIC AEROBIC PLATE COUNTS OF MEAT INOCULATED WITH THREE ORGANISMS

Organism	Mean Squares
I. Escherichia coli	
Storage	0.3960
Replication	2.6837**
Storage × Replication	0.5416*
Temperature	0.2510
Storage × Temperature	0.1537
II. Staphylococcus aureus	
Storage	0.2366
Replication	1.9163*
Storage × Replication	0.4766
Temperature	0.2504
Storage × Temperature	0.2596
III. Salmonella enteritidis	
Storage	1.8728**
Replication	1.3410**
Storage × Replication	2.2485**
Temperature	1.5482**
Storage × Temperature	0.1372

*$P < 0.05$; **$P < 0.01$.

Tables 9 and 10 illustrate pertinent data on the counts obtained in the raw ground beef over three days of storage at 4°C. Table 9 summarizes the logarithmic counts and Table 10 the analysis of variance. There was no significant change in the counts of Escherichia coli, Staphylococcus aureus, and the corresponding aerobic plate counts over four days of storage. The counts appeared to stay the same throughout the storage period. Between day zero and one, both Escherichia coli and Staphylococcus aureus experienced a slight increase, which then remained constant through the second day of storage. The counts then appeared to decrease at the third day of storage. The total standard plate counts increased slightly from day zero to day one and they stayed relatively constant through day two, and then appeared to decrease with the third day of storage. The counts of Salmonella enteritidis showed significance at the 5% level for storage. The counts continually decreased throughout the storage period, indicating these organisms gradually die at 4°C.

In addition to the data obtained in this study, it was interesting to note that the percentages killed of each test organism and total bacterial population could be calculated. Trends were quite obvious. Table 11 illustrates these percentages at the three cooking temperatures. The percentage of Escherichia coli killed continued to increase as the cooking temperature increased; the same was noted for the total aerobic plate counts. The percentage of Staphylococcus aureus killed appeared to decrease as the temperature increased.

TABLE 9

LOGARITHMIC COUNTS OF ORGANISMS CONTAINED IN THE RAW GROUND BEEF OVER THREE DAYS STORAGE AT 4°C

	Organism	Mean Counts[1] Zero day	One day	Two day	Three day
		- - - - - - - - $\log_{10}$ - - - - - - - -			
I.	Escherichia coli	6.3098	6.4086	6.4107	6.3520
	Total count	6.3553	6.4241	6.4133	6.4497
II.	Staphylococcus aureus	5.9983	6.1552	6.1561	6.0305
	Total count	6.4762	6.5782	6.4939	6.3336
III.	Salmonella enteritidis	6.6783	6.5431	6.3663	5.9515
	Total count	6.9983	6.8226	6.6662	6.6085

[1]Means of seven replications for I; four replications for II and III.

TABLE 10

MEAN SQUARES OF LOGARITHMIC COUNTS OF THREE ORGANISMS OVER THREE DAYS STORAGE AT 4°C

Organism	Mean Squares
I. Escherichia coli	
Storage	0.0188
Replication	0.0209
Error	0.0175
II. Staphylococcus aureus	
Storage	0.0273
Replication	0.3584
Error	0.0141
III. Salmonella enteritidis	
Storage	0.3991*
Replication	0.1533
Error	0.0786

*$P < 0.05$.

TABLE 11

ORGANISMS KILLED BY HEAT TREATMENT AS PERCENTAGES OF INITIAL COUNT[1]

	Organism	Temperature (°C) 60.0	65.6	71.1
		- - - - - - Percentage - - - - - -		
I.	Escherichia coli	86.09	86.26	80.34
	Total count	86.85	99.09	98.82
II.	Staphylococcus aureus	72.87	57.87	48.44
	Total count	80.57	80.91	89.46
III.	Salmonella enteritidis	99.80	99.94	99.99
	Total count	94.84	98.74	98.82

[1]Percentages were calculated from overall means for the three temperatures.

These results do not follow an expected pattern; thus these results reiterate the poor control of heating used to generate the data. The percentage of Salmonella enteritidis killed continually increased as the cooking temperature increased, so that at 71.1°C, almost 99.99% of the organisms were dead.

Thus, although there was a problem with heat control, it was quite evident that all three organisms were able to survive. Survivors included uninjured organisms as well as injured organisms (those with some type of impairment of metabolic activity). The latter, when given the opportunity were able to repair the damage, and hence could add to spoilage or contamination problems as well as public health problems, too. This must be taken into consideration when enumerating microorganisms or the results could lead to mistaken conclusions.

CHAPTER V

SUMMARY

The objective of this study was to determine whether sublethally injured organisms could be recovered from a substrate of ground beef heated to three different temperatures.

Three organisms, Escherichia coli, Staphylococcus aureus, and Salmonella enteritidis, were injected uniformly into lots of ground beef and allowed to propagate at 4°C over three days of storage. Each day, three 100g samples were procured and subjected to one of three cooking temperatures, 60.0°C, 65.6°C or 71.1°C. After each heat treatment the patties were analyzed for total viable population (injured and uninjured organisms), injured organisms, and the aerobic plate count. The Salmonella enteritidis was subjected to a heat treatment, where each patty was cooked for a predetermined time needed to reach an internal temperature of 60.0°C, 65.6°C or 71.1°C. In addition, counts were obtained on the raw ground beef over the three days of storage at 4°C to determine the growth of each individual organism and the overall total bacterial population.

The data obtained were subjected to analysis of variance. Recovery of sublethally injured Escherichia coli appeared to decrease as the heating temperature increased; however, there was no significant heat effect. Recovery of sublethally injured Staphylococcus aureus showed no apparent trend, with only significance in replication, indicating a lack of stable heat control. Recovery of sublethally

injured Salmonella enteritidis showed a complete lack of recovery at 65.6°C with significance in replication and storage-temperature interaction. This most likely can be attributed to the age of the culture in the substrate.

Survival of total bacterial population, after heat exposure, showed only significance in replication, which indicated that a stable heat treatment had not been attained. However, with the third set of samples, storage time had a significant effect. The counts increased after one day of storage and then continually decreased for the remainder of the storage time. In addition, temperature showed a significant trend. At one day storage the counts decreased at 65.6°C but rose slightly at 71.1°C; at two and three days storage, the counts progressively decreased as heating temperature increased.

Over three days storage, the number of Escherichia coli and Staphylococcus aureus did not change significantly. The standard plate counts exhibited a slight rise from zero to one day and then remained relatively constant throughout the second day and then appeared to decrease during the third day of storage. The counts of Salmonella enteritidis decreased throughout storage.

The percentage of organisms killed at the three cooking temperatures showed obvious trends. Counts of Escherichia coli and Salmonella enteritidis continually decreased as the cooking temperature increased. The counts of Staphylococcus aureus appeared to follow no obvious pattern; the percentage killed decreased as the cooking temperature increased.

Although mechanical control of heating treatment was inadequate to produce significant differences as heat was varied, it was evident that some of the three test organisms survived. Some survived without impairment of metabolic ability, and others survived after "sublethal" injury. The latter, if given opportunity to recover, may present an added onus of contamination and may complicate apparent numbers of survivors enumerated with conventional techniques and media. Further work needs to be done in this area, especially in establishing a more stable heat subjection.

LITERATURE CITED

LITERATURE CITED

Adams, D. M. 1974. Requirement for and sensitivity to lysozyme by Clostridium perfringens spores heated at ultrahigh temperatures. Appl. Microbiol. 27: 797-801.

Alderton, G., Chen, J. K. and Ito, K. A. 1974. Effect of lysozyme on the recovery of heated Clostridium botulinum spores. Appl. Microbiol. 27: 613-615.

Allwood, M. C., and Russell, A. D. 1968. Thermally induced ribonucleic acid degradation and leakage of substances from the metabolic pool of Staphylococcus aureus. J. Bacteriol. 95: 345-349.

Anderson, E. B. and Meanwell, L. J. 1936. Studies in the bacteriology of low temperature pasteurization. Part II. The heat resistance of a thermoduric streptococcus grown at different temperatures. J. Dairy Res. 7: 182.

Anellis, A., Lubas, J. and Rayman, M. M. 1954. Heat resistance in liquid eggs of some strains of the genus Salmonella. Food Res. 19: 377-395.

Arpai, J. 1962. Nonlethal freezing injury to metabolism and motility of Pseudomonas fluorescens and Escherichia coli. Appl. Microbiol. 10: 297-301.

Ayres, J. C. 1955. Microbiological implications in handling, slaughtering and dressing of meat animals. Adv. Food Res. 6: 109.

Ayres, J. C. 1960. The relationship of organisms of the genus Pseudomonas to the spoilage of meat, poultry and eggs. J. Appl. Bacteriol. 23: 471.

Ayres, J. C., Ogilvy, W. S. and Stewart, G. F. 1950. Postmortem changes in stored meats. I. Microorganisms associated with development of slime on eviscerated cut-up poultry. Food Technol. 4: 199.

Bagger, S. V. 1926. The enterococcus. J. Path. Bact. 29: 225.

Baird-Parker, A. C. and Davenport, E. 1965. The effect of recovery medium on the isolation of Staphylococcus aureus after heat treatment and after the storage of frozen or dried cells, J. Appl. Bacteriol. 28: 390-402.

Barach, J. T., Adams, D. M. and Speck, M. L. 1974. Recovery of heated Clostridium perfringens Type A spores on selective media. Appl. Microbiol. 28: 793-797.

Barnes, E. M. 1957. New Methods in food preservation: (a) Antibiotics, J. Poy. Soc. Health. 77: 446.

Baumgartner, J. G. and Hersom, A. C. 1956. "Canned Foods: An Introduction to their microbiology," 4th ed. J. and A. Churchill Ltd., London in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms, J. Appl. Bacteriol. 26(3): 314-333.

Baumgartner, J. G. and Wallace, M. D. 1934. The destruction of microorganisms in the presence of sugars. I. The role of sucrose in the commercial processing of canned fruits. J. Soc. Chem. Inc. 53: 294T.

Bluhm, L., and Ordal, Z. J. 1969. Effect of sublethal heat on the Metabolic activity of Staphylococcus aureus. J. Bacteriol. 97: 140-150.

Bridges, B. A., Ashwood-Smith, M. J., and Munson, R. J. 1969. Correlation of bacterial sensitivities to ionizing radiation and mild heating. J. Gen. Microbiol. 58: 115-124.

Brown, A. D., and Weidemann, J. F. 1958. The taxonomy of the psychrophilic meat-spoilage bacteria: A reassessment. J. Appl. Bacteriol. 21: 11.

Busta, F. F. 1976. Practical implications of injured microorganisms in food. J. Milk Food Technol. 39(2): 138-145.

Busta, F. F., and Adams, D. M. 1972. Identification of a germination system involved in the heat injury of Bacillus subtilis spores. Appl. Microbiol. 24: 412-417.

Busta, F. F., and Jezeski, J. J. 1961. Effect of sodium chloride concentration in an agar medium on growth of heat-shocked Staphylococcus aureus strain 196E. J. Dairy Sci. 44: 1160

Busta, F. F., and Jezeski, J. J. 1963. Effect of sodium chloride concentration in an agar medium on growth of heat-shocked Staphylococcus aureus. Appl. Microbiol. 11: 404-407.

Clark, C. W., and Ordal, Z. J. 1969. Thermal injury and recovery of Salmonella typhimurium and its effect on enumeration procedures. Appl. Microbiol. 18: 332-336.

Clark, C. W., Witter, L. D., and Ordal, Z. J. 1968. Thermal injury and recovery of Streptococcus faecalis. Appl. Microbiol. 16: 1764-1769.

Claydon, M. 1937. Uber die Bedingungen der Widerstandsfahigkeit von Bakterien gegen Erhitzung. Z. Hyg. 100: 388 in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Consumer Reports. 1971. A close look at hamburger. 36: 478-794.

Davies, R., Sinskey, A. J., and Botstein, D. 1973. Deoxyribonucleic acid repair in a highly radiation-resistant strain of Salmonella typhimurium. J. Bacteriol. 114: 357-366.

Demeter, K. J. and Eisenreich, L. 1937. Die Bedeutung einer in roher Milch vorkommenden resistenzverminderenden Substanz für die Hitzeabtötung von Molkereitechnisch wichtigen Bakterien, Wiss. Ber. 11, Milch. Weltkongr. 478, in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Duitschaever, C. L., Arnott, D. R., and Bullock, D. H. 1973. Bacteriological quality of raw refrigerated ground beef. J. Milk and Food Technol. 36: 375.

Duncan, C. L., Labbe, R. G. and Reich, R. 1972. Germination of heat- and alkali-altered spores of Clostridium perfringens Type A by lysozyme and an initiation protein. J. Bacteriol. 109: 550-559.

Elliker, P. R. and Frazier, W. C. 1938. Influence of time and temperature of incubation on heat resistance of Escherichia coli. J. Bact. 36: 83.

Fleming, D. S., Papra, J., Stoffels, M. A. and Havir, R. 1973. Salmonella typhimurium gastroenteritis, Minnesota Med. 56: 722-723.

Foster, J. F., Fowler, J. L. and Ladiges, W. C. 1977. A bacteriological survey of raw ground beef. J. Food Protection 40(11): 790-794.

Gilliland, S. E., Busta, F. F., Brinda, J. J. and Campbell, G. E. 1976. Aerobic plate count. In "Compendium of Methods for the Microbiological Examination of Foods," 107-131. American Public Health Assoc. Washington, D. C.

Goepfert, J. M. and Kim, H. U. 1975. Behavior of selected food borne pathogens in raw ground beef. J. Milk Food Technol. 38: 449-452.

Gomez, R. F., and Sinskey, A. J. 1973. Deoxyribonucleic acid breaks in heated Salmonella typhimurium LT-2 after exposure to nutritionally complex media. J. Bacteriol. 115: 522-528.

Gomez, R. F., and Sinskey, A. J. 1975. Effect of aeration on minimal medium recovery of heated Salmonella typhimurium. J. Bacteriol. 122: 106-109.

Gomez, R. F., Sinskey, A. J., Davies, R. and Labuza, T. P. 1973. Minimal medium recovery of heated Salmonella typhimurium LT-2. J. Gen. Microbiol. 74: 267-274.

Gray, R. J. H., Witter, L. D., and Ordal, Z. J. 1973. Characterization of mild thermal stress in Pseudomonas fluorescens and its repair. Appl. Microbiol. 26: 78-85.

Greenberg, R. A., and Silliker, J. H. 1961. Evidence for heat injury in enterococci. J. Fd. Sci. 26: 622.

Haight, R. D. and Morita, R. Y. 1966. Termally induced leakage from Vibrio marinus, an obligately psychrophilic marine bacterium. J. Bacteriol. 92: 1388-1393.

Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms, J. Appl. Bacteriol. 26(3): 314-333.

Hartsell, S. E. 1951. The longevity and behavior of pathogenic bacteria in frozen foods. The influence of plating media. Amer. J. Public Health. 41: 1072-1077.

Heinmetz, F., Taylor, W. W. and Lehman, J. J. 1954. The use of metabolities in the restoration of the viability of heat and chemically inactivated Escherichia coli. J. Bacteriol. 67: 5-12.

Hitchner, B. J., Grau, F. H., and Egan, A. F. 1975. Significance of the cell envelope in thermal death of Escherichia coli. Proc. Austral. Biochem. Soc. 8: 73.

Hoadley, A. W. and Cheng, C. M. 1974. The recovery of indicator bacteria on selective media. J. Appl. Bacteriol. 37: 45-57.

Hobbs, B. C. and Olson, Jr., J. C. 1971. Symposium on the restoration of sublethally impaired bacterial cells in foods. J. Milk Food Technol. 34(ii): 548-552.

Hückel, R. 1926. Uber die Abhängigkeit des Hitzeresistenz verschiedener Bakteriesuspensionen von ihrer Dichte. z. Hyg. 106, 730 in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Iandolo, J. J. and Ordal, Z. J. 1966. Repair of thermal injury of Staphylococcus aureus. J. Bacteriol. 91: 134-142.

Ingram, M., and Dainty, R. H. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bacteriol. 34: 21.

Jackson, H. 1974. Loss of viability and metabolic injury of Staphylococcus aureus resulting from storage at 5°C. J. Appl. Bacteriol. 37: 59-64.

Jackson, H., and Woodbine, M. 1963. The effect of sublethal heat treatment on the growth of Staphylococcus aureus. J. Appl. Bacteriol. 26(2): 152-158.

Janssen, D. W., and Busta, F. F. 1973. Repair of injury in Salmonella anatum cells after freezing and thawing in milk. Cryobiology 10: 386-392.

Jay, J. M. 1967. Nature, characteristics and proteolytic properties of beef spoilage bacteria at low and high temperatures. Appl. Microbiol. 15: 943.

Jensen, L. B. 1954. "Microbiology of Meats," 3rd ed. Garrard Press, Illinois in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Jordan, R. C. and Jacobs, S. E. 1944. Studies in the dynamics of disinfection. I. New data on the reaction between phenol and Bacterium coli using an improved technique together with an analysis of the distribution of resistance amongst the cells of the bacterial populations studies. J. Hyg. Camb. 43: 275.

Jordan, R. C., and Jacobs, S. E. 1948. Studies in the dynamics of disinfection. J. Hyg. Camb. 46: 136.

Karim, G. 1977. Bacteriological quality of raw and cooked hamburger at the retail level. J. Food Protection 40(8): 560-561.

Kirsch, L. H., Berry, F. E., Baldwin, C. L. and Foster, E.M. 1952. The bacteriology of refrigerated ground beef. Food Res. 17: 495.

Krishna Jyengar, M. K. Laxminarayana, H. and Iya, K. K. 1957. Studies on the heat resistance of some streptococci. Ind. J. Dairy Sci. 10: 90.

Lechowich, R. V. 1971. Microbiology of meat. In "The Science of Meat and Meat Products," 232-240. W. H. Freeman and Company, San Francisco.

Lembke, A. 1937. Die Hitzewiderstandsfaghigkeit der Conbakterien und die Verwendmarkeit dieser Eigenschaft als Vergleichmasstab für die Beurteilung von Milcherhitzern. Zbl. Bakt. (abt. 2) 96, 92 in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Lemcke, R. M., and White, H. R. 1959. The heat resistance of Escherichia coli cells from cultures of different ages. J. Appl. Bacteriol. 22: 193.

Lord, W. G., Bonai, F., Bodek, A., Hilbert, R. W., Rosinn, R., and Clack, F. B. 1975. Toxoplasmosis-Pennsylvania. Morbidity and Mortality Weekly Report. 24: 285-286.

MacLeod, R. A., Kuo, S. C., and Gelinas, R. 1967. Metabolic injury to bacteria. II. Metabolic injury induced by distilled water or Cu^{++} in the plating diluent. J. Bacteriol. 93: 961-969.

Meyer, K. F. and Lang, O. W. 1926. A highly heat resistant sporulating anaerobic bacterium, Clostridium caloritolerans. J. Infec. Dis. 39: 321.

Miller, L. L., and Ordal, Z. J. 1972. Thermal injury and recovery of Bacillus subtilis. Appl. Microbiol. 24: 878-884.

Mitchell, P. 1951. Physical factors affecting growth and death. In "Bacterial Physiology." Academic Press, New York in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Minor, T. E. and Marth, R. Y. 1972. Loss of viability by Staphylococcus aureus in acidified media. II. Inactivation by acids in combination with sodium chloride, freezing, and heat. J. Milk Food Technol. 35: 548-555.

Moss, C. W. and Speck, M. L. 1963. Injury and death of Streptococcus lactis due to freezing and frozen storage. Appl. Microbiol. 11: 326-329.

Mossel, D. A. A., and Ratto, A. 1970. Rapid detection of sublethally impaired cells of Enterobacteriaceae in dried foods. Appl. Microbiol. 20: 273-275.

Nakamura, M., and Dawson, D. A. 1962. Role of suspending and recovery media in the survival of frozen Shigella sonnei. Appl. Microbiol. 10: 40-43.

Nelson, F. E. 1943. Factors which influence the growth of heat-treated bacteria. I. A comparison of four agar media. J. Bacteriol. 45: 395-403.

Nelson, F. E. 1944. Factors which influence the growth of heat-treated bacteria. II. Further studies on media. J. Bacteriol. 48: 473-477.

Ordal, Z. G., Iandola, J. J., Ray, B., and Sinskey, A. J. 1976. Detection and enumeration of injured microorganisms. In "Compendium of Methods for the Microbiological Examination Of Foods," 163-172. American Public Health Assoc. Washington, D. C.

Ørskov, S. L. 1926. Versuche uber Thermoresistenz. Thermoresistenz in verschiedenen Alters. Z. Hyg. 105, 317 in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Peppler, H. J., and Frazier, W. C. 1941. Influence of a film yeast, Candida krusei, on the heat resistance of certain lactic acid bacteria grown in symbiosis with it. J. Bact. 43: 181.

Pflug, I. J. and Holcomb, R. C. 1977. Principles of thermal destruction of microorganisms. In "Disinfection, Sterilization, and Preservation," 933-996. Lea and Febiger, Philadelphia, Pa.

Pierson, M. C., Collins-Thompson, D. L., and Ordal, F. J. 1970. Microbiological, sensory and pigment changes of aerobically packaged beef. Food Technol. 24: 129.

Pierson, M. D. and Ordal, Z. J. 1971. The transport of methyl-a-D-glucophyranoside by thermally stressed Salmonella typhimurium. Biochem. Biophys. Res. Comm. 43: 378-383.

Poelma, P. L., and Silliker, J. H. 1976. Salmonella. In "Compendium of Methods for the Microbiological Examination of Foods," 301-328. American Public Health Assoc.. Washington, D. C.

Precht, H., Christopherson, J., and Hensel, H. 1955. Temperatur und Leben. Berlin: Springer Verlag in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Prévot, A. R., Raynaud, M., and Tatki, H. 1951. Recherches sur la thermorésistance de Cl. sporogenes et le phénoméne d'entrainment des espèces peu résistantes. Ann. Inst. Pasteur 80, 553 in Hansen, N. H. and Riemann, H. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26(3): 314-333.

Ray, B., Janssen, D. W., and Busta, F. F. 1972. Characterization of the repair of injury induced by freezing Salmonella anatum. Appl. Microbiol. 23: 803-809.

Ray, B., Jezeski, J. J., and Busta, F. F. 1971a. Effect of rehydration on recovery, repair and growth of injured freeze-dried Salmonella anatum. Appl. Microbiol. 22: 184-189.

Ray, B., Jezeski, J. J., and Busta, F. F. 1971b. Isolation of Salmonella from naturally contaminated dried milk products. II. Influence of storage time on isolation of Salmonella. J. Milk Food Technol. 34: 423-427.

Ray, B., Jezeski, J. J., and Busta, F. F. 1971c. Repair of injury in freeze-dried Salmonella anatum. Appl. Microbiol. 22: 401-407.

Ray, B., Jezeski, J. J., and Busta, F. F. 1972. Isolation of Salmonella from naturally contaminated dried milk products. III. Influence of pre-enrichment conditions. J. Milk Food Technol. 35: 607-614.

Ray, B., and Speck, M. D. 1972. Metabolic process during the repair of freeze-injury in Escherichia coli. Appl. Microbiol. 24: 585-590.

Ray, B., and Speck, M. D. 1973a. Enumeration of Escherichia coli in frozen samples after recovery from injury. Appl. Microbiol. 25: 499-503.

Ray, B. and Speck, M. D. 1973b. Freeze-injury in bacteria. C. R. C. Critical Rev., Clinical Laboratory Sciences. 161-213.

Report of the Food Safety Task Force. 1974-1975. "Food Safety and Protection Research Needs in the Southern Region," 38.

Rey, C. R., Kraft, A. A., and Rust, R. E. 1971. Microbiology of beef shell frozen with liquid nitrogen. J. Food Sci. 36: 955.

Rey, C. R., Kraft, A. A., Walker, H. N., and Parrish, F. C., Jr. 1970. Microbial changes in meat during aging at elevated temperatures and later refrigerated storage. Food Technol. 24: 67.

Rogers, R. E., and McCleskey, C. S. 1957. Bacteriological quality of ground beef in retail markets. Food Technol. 11: 318.

Russell, A. D., and Harries, D. 1967. Some aspects of thermal injury in Escherichia coli. Appl. Microbiol. 15: 407-410.

Scheusner, D. L., Busta, F. F. and Speck, M. L. 1971a. Inhibition of injured Escherichia coli by several selective agents. Appl. Microbiol. 21: 46-49.

Scheusner, D. L., Busta, F. F. and Speck M. L. 1971b. Injury of bacteria by sanitizers. Appl. Microbiol. 21: 41-45.

Schmidt, C. F. 1954. Thermal resistance of microorganisms. In "Antiseptics, Disinfectants, Fungicides and Sterilization," 720-759. Lea and Febiger, Philadelphia, Pa.

Sherman, J. M. and Cameron, G. M. 1934. Rate of growth and viability in Bacterium coli. J. Bact. 27: 23.

Shibasaki, I., and Tsuchido, T. 1973. Enhancing effect of chemicals on the thermal injury of microorganisms. Acta Alimentaria 2: 327-349 in Busta, F. F. 1976. Practical implications of injured microorganisms in food. J. Milk Food Technol. 39(2): 138-145.

Sinskey, A. J., and Silverman, G. J. 1970. Characterization of injury incurred by Escherichia coli upon freeze-drying. J. Bacteriol. 101: 429-437.

Sogin, S. J., and Ordal, Z. J. 1967. Regeneration of ribosomes and ribosomal ribonucleic acid during repair of thermal injury to Staphylococcus aureus. J. Bacteriol. 94: 1082-1087.

Speck, M. L., Ray, B., and Read, R. B., Jr. 1975. Repair and enumeration of injured coliforms by a plating procedure. Appl. Microbiol. 29: 549-550.

Stersky, A. K. and Hedrick, T. I. 1972. Inhibition of growth of airborne coliforms and other bacteria on selective media. J. Milk Food Technol. 35: 156-162.

Stiles, M. E. and Witter, L. D. 1965. Thermal inactivation, heat injury, and recovery of Staphylococcus aureus. J. Dairy Sci. 48: 677-681.

Straka, R. P. and Stokes, J. L. 1959. Metabolic injury to bacteria at low temperatures. J. Bacteriol. 78: 181-185.

Strange, R. E. and Shon, M. 1964. Effects of thermal stress on viability and ribonucleic acid of Aerobacter aerogenes in aqueous suspensions. J. Gen. Microbiol. 34: 99-114.

Sugiyama, H. 1951. Studies on factors affecting the heat resistance of spores of Clostridium botulinum. J. Bact. 62:81.

Tiwari, N. P., and Maxey, R. B. 1971. Impact of low doses of gamma radiation and storage on the microflora of ground raw meat. J. Food Sci. 36: 833.

Tomlins, R. I., and Ordal, Z. J. 1971a. Precursor ribosomal ribonucleic acid and ribosome accumulation in vivo during the recovery of Salmonella typhimurium from thermal injury. J. Bacteriol. 107: 134-142.

Tomlins, R. I. and Ordal, Z. J. 1971b. Requirements of Salmonella typhimurium for recovery from thermal injury. J. Bacteriol. 105: 512-518.

Tomlins, R. I., Vaaler, G. L., and Ordal, Z. J. 1972. Lipid biosynthesis during the recovery of Salmonella typhimurium from thermal injury. Can. J. Microbiol. 18: 1015-1021.

Traci, P. A., and Duncan, C. L. 1974. Cold shock lethality and injury in Clostridium perfringens. Appl. Microbiol. 28: 815-821.

U.S.D.A. 1973. Meat and Poultry Inspection Regulations. Animal and Plant Health Inspection Service, Washington, D. C.

Vas, K., and Proszt, G. 1957. Observations on the heat destruction of spores of Bacillus cereus. J. Appl. Bacteriol. 20: 431.

Weiser, H. H., Mountney, G. J., and Gould, W. A. 1971. "Practical Food Microbiology and Technology." Avi Publishing Co., Westport, Conn.

White, H. R. 1963. The effect of variation in pH on the heat resistance of cultures of Streptococcus faecalis. J. Appl. Bact. 26: 91.

Woodcock, E. and Grigg, G. W. 1972. Repair of thermally induced DNA breakage in Escherichia coli. Nature New Biol. 237: 76-79.

Yesair, J., Bohrer, C. W., and Cameron, E. J. 1946. Effect of certain environmental conditions on heat resistance of micrococci. Food Res. 11: 327.

VITA

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