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Sensitivity of Listeria to irradiation in raw ground meat, as affected by type of radiation, product temperature, packaging atmosphere, and recovery medium

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Sensitivity of *Listeria* to irradiation in raw ground meat, as affected by type of radiation, product temperature, packaging atmosphere, and recovery medium

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For the Graduate College

To Mercedes, my wonderful wife and companion,

and to our son, Daniel, and our future children

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The sensitivity of five strains of *Listeria* to electron beam irradiation in ground pork, as well as the extent of sublethal radiation injury exhibited by each, were investigated. Ground pork was inoculated with one of five strains of *Listeria* and irradiated from 0 to 1.25 kGy at 0.25 kGy intervals. *Listeria innocua* NADC 2841 was more radiation-resistant (D_{10} = 0.638 kGy) than *L. monocytogenes* NADC 2045 Scott A (D_{10} = 0.447 kGy), *L. monocytogenes* NADC 2783 (a hamburger isolate) (D_{10} = 0.424 kGy), *L. monocytogenes* ATCC 15313 (D_{10} = 0.445 kGy) and *L. ivanovii* NADC 3518 (D_{10} = 0.372 kGy), when recovered on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE). D_{10} values for *L. innocua*, *L. ivanovii* and *L. monocytogenes* ATCC 15313 were lower when cells were recovered on modified Oxford (MOX) medium. These three strains were susceptible to radiation-induced sublethal injury, with the numbers of injured organisms increasing with irradiation dose. The two pathogenic strains of *L. monocytogenes* were not injured significantly at the dose levels used. The results show that the dose range currently being considered by the Food and Drug Administration for the irradiation of beef and pork (1.5-4.5 kGy) is adequate for the elimination of *L. monocytogenes* from pork.

In a second study, the radiation sensitivity of *Listeria monocytogenes* in ground beef as affected by type of radiation (X-rays and electrons), product temperature (4, -20, and -78°C), packaging atmosphere (air, vacuum, and nitrogen) and recovery medium [trypticase soy agar + 0.6% yeast extract (TSAYE), modified Oxford medium (MOX)] was investigated. Irradiation doses were 0-2.0 kGy in 0.5 kGy intervals. Survival curves

shoulders were observed under vacuum and nitrogen, regardless of temperature. D_{10} values were independent of packaging atmosphere and increased with decreasing temperature (except for X-rays from -20 to -78°C). They were 0.417 to 0.486 kGy under air, 0.527 to 0.668 under vacuum, and 0.648 to 0.704 kGy and 0.833 to 0.885 kGy under nitrogen for X-rays and electrons, respectively. Sublethal injury increased at doses higher than 0.5 kGy and did so more slowly at -78°C than at -20 and 4°C.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Although *Listeria monocytogenes* was first recognized as a foodborne pathogen as early as 1929 (Broome, 1993), it wasn't until the early 1980s that the first confirmed outbreak of foodborne listeriosis was identified (Schlech, 1983). This was followed by several other major outbreaks of foodborne listeriosis within the next 5 years (Fleming et al., 1985; Linnan et al., 1988; Bille, 1990). As a result of these outbreaks, *L. monocytogenes* became a source of great concern for the food industry. This, however, prompted much research on ways to control the organism's growth and proliferation in foods.

Listeria monocytogenes possesses several characteristics that make it a particularly hardy foodborne pathogen. First, it is a psychrotroph, having been reported to grow between 3 and 45°C (Wilkins et al., 1972). This means refrigeration may not be enough to control its growth. It is also very thermotolerant. In fact, some studies have suggested that, under certain conditions, it may survive high-temperature short-time pasteurization of milk (Knabel et al., 1990; Farber and Peterkin, 1991). In hot dogs, heating to an internal temperature of 71°C reduced the numbers of the organism by approximately 3 log (Zaika et al., 1990). Of special significance to the meat processing industry is the halotolerance of *L. monocytogenes*. It is known to grow well in the presence of 10-12% sodium chloride (Bille and Doyle, 1991) and, in beef, the presence of curing salts has been found to increase its thermoresistance (Mackey et al., 1990).

Listeria monocytogenes has been found in a wide variety of meats and meat products

(Farber et al., 1989; Genigeorgis et al., 1989; Grau and Vanderlinde, 1992; MacGowan et al., 1994; Wang and Muriana, 1994), and many of these kinds of products have been implicated in sporadic cases of foodborne listeriosis (Schwartz et al., 1988; Wenger et al., 1991; Pinner et al., 1992).

The technology of food irradiation can effectively eliminate *L. monocytogenes* from meats. Currently in the United States, legal approval of ionizing radiation to treat foods of animal origin is limited to raw, packaged poultry at 1.5 to 3.0 kGy for the elimination of pathogens, and pork carcasses or fresh cuts at 0.3 to 1.0 kGy for the destruction of *Trichinella spiralis* (FDA, 1995). However, the Food and Drug Administration is currently reviewing a petition to allow the irradiation of raw red meat, at doses of 1.5 to 4.5 kGy if fresh or chilled and 2.5 to 7.5 kGy if frozen, to reduce microbial pathogens and parasites (FDA, 1994).

Since *L. monocytogenes* was recognized as a foodborne pathogen relatively recently, only a few studies have been published on the use of ionizing radiation to eliminate it from foods. These are discussed in the literature review. The research studies covered by this dissertation purposed to expand the current knowledge base on the effects of irradiation on *L. monocytogenes* (and other listeriae) by observing the effects that several factors have on the organism's radiosensitivity and on its recovery from radiation-induced sublethal injury.

Dissertation Organization

This dissertation is organized into a literature review (chapter 2), two papers (chapters 3 and 4) and general conclusions (chapter 5). The first paper, chapter 3, entitled "Survival

and injury of *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* in ground pork following electron beam irradiation", was co-authored with Dr. Elsa A. Murano and Dr. Dennis G. Olson and has been accepted for publication by the Journal of Food Protection. It was also presented in slightly modified form at the 41st International Congress of Meat Science and Technology, held in San Antonio, Texas on August 20-25, 1995. The second paper, chapter 4, entitled "Sensitivity of *Listeria monocytogenes* to irradiation in ground beef as affected by type of radiation (X-rays or electrons), product temperature, packaging atmosphere, and recovery medium", was co-authored with Dr. Olson, Dr. Murano and fellow graduate student Priya Sundaram, and will be submitted for publication to the Journal of Food Science. Except for chapter 3, reference citations throughout the dissertation were formatted following the Journal of Food Science Style Guide for Research Papers.

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CHAPTER 2. LITERATURE REVIEW

The application of radiation for the preservation of food promises, in the near future, to be one of the most important applications of atomic energy in the service of man.

Chadwick and Leenhouts, 1981

Brief History and Current Status of Food Irradiation

The use of ionizing radiation to treat foods was first conceived one hundred years ago, a year after the discovery of X-rays by Wilhelm Röntgen and the same year that Henri Becquerel discovered radioactivity, when Minck (1896) first suggested its use to kill microorganisms in food. Over the next three decades others would also come to make the same suggestion (Josephson, 1983). In 1905, patents which described the use of ionizing energy to destroy bacteria in foods were issued in the United States and Great Britain. In 1916 the effects of X-rays on the egg, larvae, pupae and adult forms of the tobacco beetle were described (Runner, 1916). High-energy electrons were shown to kill microbes in 1919 (Grober and Pauli, 1919) and in 1921 a United States patent was issued for the use of X-rays to kill *Trichinella spiralis* in fresh meat (Schwartz, 1921). Several years later, in 1930, a French patent was issued for the use of X-rays to kill all bacteria in all kinds of foods sealed in metallic containers (Wüst, 1931).

The practical application of food irradiation, however, would not become a reality until the 1940s. In 1943, Proctor et al. demonstrated that X-rays could be used to preserve hamburger meat. Despite the previously issued patents, this has been considered the first real and successful use of ionizing energy for preserving foods (Goldblith, 1966). It wasn't

until 1948, however, that the first scientific paper demonstrating the use of ionizing radiation for food preservation was published (Brasch and Huber, 1948). Before this time, the actual application of food irradiation had been limited primarily by insufficient quantities of radioactive isotopes and a lack of capable machine sources (Cleland and Beck, 1992; Josephson, 1983). X-rays were effective, but were too costly (Satin, 1993). It wasn't until after World War II, with the subsequent advent of the atomic age, that production of sufficient quantities of cobalt-60 and cesium-137 and the development of suitable machine sources were realized (Josephson, 1983; Satin, 1993; Urbain, 1986).

Food irradiation in the United States

Food irradiation research in the United States began at the Massachusetts Institute of Technology, at Electronized Chemicals Corporation, Brooklyn, NY, in partnership with the research laboratories of Swift and Company, and at the General Electric Research Laboratories, Schenectady, NY (Josephson, 1983). The U.S. government began sponsoring food irradiation research programs a few years later, through the Atomic Energy Commission in 1950 and the Army Quartermaster Corps in 1953. In 1953, President Dwight D. Eisenhower proposed the "Atoms for Peace" program, which led to the establishment of a National Food Irradiation Program and the formation, in 1956, of the Interdepartmental Committee on Radiation Preservation of Food (CAST, 1989).

Most of the work on the National Food Irradiation Program was initially conducted by the Army, which was later joined by the Atomic Energy Commission (AEC) in 1961. The Army's program, which up to 1962 had been contracted out by the Quartermaster Food and

Container Institute in Chicago to approximately 90 research groups representing universities, industry and government agencies, was concerned with radappertization (radiation sterilization) doses of greater than 10 kGy. The AEC's program, which was also contracted out to other institutions, studied food irradiation applications at lower doses. Wholesomeness studies were the responsibility of the Army Medical Department which, in 1955, began a 10-year program of long-term multigeneration animal feeding studies to assess the safety of 21 foods important in the diets of North Americans (CAST, 1986; Josephson, 1983).

The enactment, in 1958, of the Food Additives Amendment to the Federal Food, Drug and Cosmetic Act of 1938 had, and continues to have, an enormous impact on the commercial application of food irradiation, not just in the U.S., but worldwide. This law, which is administered by the Food and Drug Administration (FDA), legally defines ionizing radiation as a food additive and requires that all new food additives undergo extensive safety testing.

The first irradiated food product to be granted approval under this amendment was radappertized bacon in 1963. In the same year, the FDA approved the irradiation of wheat and wheat products at doses of 0.2-0.5 kGy for insect disinfestation and, in 1964, the irradiation of white potatoes at doses of 0.05-0.15 kGy for sprout inhibition (Josephson, 1983; Olson, 1995). In 1965, following completion of the Army's 10-year wholesomeness assessment of 21 irradiated foods, the Army Surgeon General concluded "that foods irradiated up to absorbed doses of 5.6 megarads with a cobalt 60 source of gamma radiation or with electrons with energies up to 10 million electron volts have been found to be

wholesome; i.e., safe, and nutritionally adequate." (Surgeon General, 1965).

In 1966, the Army petitioned the FDA for approval of radappertized ham. In the absence of long-term wholesomeness data for ham, the Army argued that approval should be granted based on the available wholesomeness data for cured bacon and uncured pork. By then, however, toxicity testing standards had become more demanding, and the FDA ruled that the wholesomeness data for pork and bacon was insufficient to prove the wholesomeness of irradiated ham. The Army then withdrew its petition, and the FDA proceeded to rescind its previous approval for bacon since the wholesomeness data previously submitted did not meet the new testing criteria (CAST, 1986; Josephson, 1983). These events caused a very significant slowdown of food irradiation activity in the U.S. and prompted many other countries to reassess their food irradiation programs (Josephson, 1983). Soon thereafter, in 1969, the AEC phased out its low-dose food irradiation program and, in 1971, the Army redirected its efforts towards the wholesomeness assessment of radappertized beef (CAST, 1986).

In 1980, the Army's contracts to assess the wholesomeness of radappertized beef, pork and ham were terminated, and the government's food irradiation program was transferred from the Army's Natick, Massachusetts laboratories to the U.S. Department of Agriculture's Eastern Regional Research Center in Philadelphia, PA (Josephson, 1983).

Since the mid 1980s, there has been a renewed interest in the commercial prospects of food irradiation in the United States. Since that time, the FDA has approved the irradiation of spices and dry or dehydrated vegetable seasonings at a maximum of 30 kGy for microbial decontamination (1983), of dry or dehydrated enzyme preparations at a maximum of 10

kGy for microbial decontamination (1985), of pork carcasses or fresh cuts at 0.3 to 1 kGy for the destruction of *Trichinella spiralis* (1985), of fresh foods at 1 kGy to delay growth and maturation (1986), of foods at 1 kGy for insect disinfestation (1986), and of raw, packaged poultry at 1.5 to 3 kGy for the elimination of pathogens (1992) (CAST, 1989; FDA, 1995). As of this writing, the agency is reviewing a petition to allow the irradiation of fresh or frozen raw red meat, at doses of 1.5 to 4.5 kGy and 2.5 to 7.5 kGy, respectively, to reduce microbial pathogens and parasites (FDA, 1994).

A major commercial development took place in 1992 when Vindicator Co. (now Food Technology Services), a gamma ray facility located in Mulberry, Florida, became the U.S.'s first commercial food irradiator. Vindicator's first commercial batch of irradiated strawberries was successfully marketed in January, 1992 (Marcotte, 1992), and its first batch of irradiated poultry was also marketed successfully in September of the following year (Pszczola, 1993). Early in 1993, the United States' first pilot food irradiator designed specifically for the irradiation of meat and poultry, an electron beam/X-ray facility, started operating at Iowa State University in Ames, Iowa.

Food irradiation worldwide

Several countries began their food irradiation research programs during the 1950s. By 1950, research work was underway at the Low Temperature Research Station in Cambridge, England, and by the end of that decade Belgium, Canada, the Netherlands, West Germany, Poland and the Soviet Union had established their own research programs (WHO, 1994). By 1972, 55 countries were engaged in food irradiation activities. In 1974 Japan became the

first country to commercialize an irradiated food product, with the commercial production of irradiated potatoes. By 1992, 26 countries had approved the commercial irradiation of at least one food or food ingredient (Loaharanu, 1994).

International cooperation

Under the auspices of the United Nations, the first International Conference on the Peaceful Uses of Atomic Energy was held in Geneva, Switzerland in 1955. In 1956, the Food and Agriculture Organization of the United Nations (FAO) established an Atomic Energy Branch for the purpose of aiding member nations in the application of ionizing energy to reduce losses due to food spoilage. In December, 1956 the first meeting of the European Contact Group on the Use of Isotopes and Radiation in Agricultural Research took place in Wageningen, the Netherlands. In 1960, the International Atomic Energy Agency (IAEA) established its Unit of Agriculture, which in 1964 was combined with FAO's Atomic Energy Branch into a Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture. Under this Joint Division, a Food Preservation Section was created and given responsibility for all food irradiation projects, including assistance to member countries, sponsoring fellowships, awarding contracts and processing equipment for research, convening panels of experts, organizing symposia and training courses and publishing the *Food Irradiation Newsletter* (CAST, 1989; Josephson, 1983).

Following the FDA's lack of approval for the sterilization of ham in 1968 and the recision of the prior approval for bacon, many countries decided to act on their own and not to look to the U.S. for leadership in the field of food irradiation. To this effect an agreement

was signed in 1970 to establish the International Project in the Field of Food Irradiation (IFIP) in Karlsruhe, West Germany. IFIP was sponsored by FAO, IAEA, and the Nuclear Energy Commission of the Organization for Economic Cooperation and Development, with WHO acting in an advisory capacity, and received support from 24 countries, including the U.S. Its mission was to conduct an international program of studies on the wholesomeness of foods treated with ionizing energy. In 1976 IFIP released a report entitled "Review on International Wholesomeness Testing of Irradiated Food and Feed from 1925 to the Present." This report, in published form (Barna, 1979), reviewed some 1,221 studies of wholesomeness of 278 irradiated foods and feeds. This report greatly aided the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food in the gathering of the wholesomeness data it would evaluate (CAST, 1989; Josephson, 1983).

In 1981, after extensively reviewing the accumulated data on the wholesomeness of irradiated foods, the Joint Expert Committee concluded that "irradiation of any food commodity up to an overall average dose of 10 kGy introduces no toxicological hazard; hence, toxicological testing of food so treated is no longer required" (WHO, 1981). In the same report the Committee also "considered that the irradiation of food up to an overall average dose of 10 kGy introduces no special nutritional or microbiological problems."

This Joint FAO/IAEA/WHO Expert Committee's recommendations and conclusions later became the basis of two international standards issued in 1983 by the Joint FAO/WHO Codex Alimentarius Commission (CAC) in consultation with its member countries (Loaharanu, 1994; WHO, 1994): the Codex General Standard for Irradiated Foods and the Recommended International Code of Practice for the Operation of Radiation Facilities Used

for the Treatment of Food (CAC, 1983, 1984). These provided the principles and control procedures for the proper irradiation of food up to an overall average dose of 10 kGy.

Technical Aspects of Food Irradiation

Objectives

Food irradiation is mainly a food preservation technology which consists of the treatment of a food with ionizing radiation, and is usually done in order to accomplish one or more of several objectives. These objectives may be (Urbain, 1986): microbial control, inhibition of sprouting, delay of ripening and senescence, decontamination, insect disinfestation and other quality improvements.

Microbial control

Food irradiation can be used to control or inhibit the growth and proliferation of those microorganisms that cause food spoilage (bacteria, yeasts and molds) and/or of those that cause food poisoning (primarily pathogenic bacteria). The following terms, first suggested in 1964 (Goresline et al., 1964), are commonly used today to refer to microbial destruction by food irradiation and have been defined as follows (WHO, 1977):

(1) Radappertization - *Treatment of food with a dose of ionizing radiation sufficient to reduce the number and/or activity of viable microorganisms to such a level that very few, if any, are detectable by any recognized bacteriological or mycological testing method applied to the treated food.*

These are essentially radiation-sterilization doses and are of the order of 10-50 kGy.

(2) Radicidation - *Treatment of food with a dose of ionizing radiation sufficient to reduce the number of viable specific non-spore-forming pathogenic bacteria to such a level that none is detectable in the treated food when it is examined by any recognized bacteriological testing method.*

Radicidation could be considered equivalent to pasteurization. It seeks to destroy pathogenic organisms and the doses used are in the 2-6.5 kGy range.

(3) Radurization - *Treatment of food with a dose of ionizing radiation sufficient to enhance its keeping quality by causing a substantial reduction in the numbers of viable specific spoilage microorganisms.*

The main purpose of radurization doses is to extend the shelf-life of the product by causing a reduction of the product's microbial population. Doses in the range of 1-5 kGy have been suggested to accomplish this.

Inhibition of sprouting

Doses needed to inhibit sprouting of different bulb, root and tuber crops (e.g., onions, garlic, carrots, yams, potatoes) are dependent on the product to be irradiated, and range from 0.01-0.5 kGy.

Delay of ripening and senescence

The ripening of climacteric fruits (i.e., those that undergo further ripening after harvesting) can be delayed by irradiation. Depending on the fruit, this involves doses of 0.1-3 kGy. Some fruits, however, suffer severe damage with even very low doses of radiation.

Therefore, the suitability of fruits for irradiation must be considered on an individual basis.

Decontamination

For certain food products, especially those used as ingredients (e.g., spices and seasonings), it may be desirable to use irradiation to reduce their microbial load. This can be accomplished by doses in the 3-30 kGy range.

Insect disinfestation

In certain agricultural commodities (e.g., cereal grains, legumes, fruits and vegetables, nuts, spices) insects can be a source of product loss, loss of nutritive value and consumer objection. Treatment of these products with doses of 0.1-1 kGy can destroy insects and prevent their spread.

Other quality improvements

Irradiation can be also used to alter one or more of the intrinsic characteristics of a food in order to enhance its quality. These altered characteristics can be sensory, such as texture, or related to a functional property of the food, and some of the resulting improvements might include (CAST, 1989): (a) increasing the rates of hydration of dehydrated vegetables (e.g., soup mixes), (b) increasing the yield of juice from grapes, (c) increasing the drying rate of fruits (e.g., prunes), (d) reducing the cooking time of certain foods (e.g., dried beans), (e) increasing the volume of loaves of bread, (f) reducing the flatulence-causing ability of beans, (g) reducing the amount of sodium nitrite required in cured meats, and (g)

tenderizing meat (Lee et al., 1996). These quality improvements usually involve depolymerization of macromolecules, such as proteins, or release of cell contents by injured cells. Doses to accomplish these effects vary widely (0.1-10 kGy), depending on the quality improvements sought.

Radiation basics

Definition and types of radiation

The term radiation refers to a physical phenomenon in which energy travels, or propagates, through space (Grosch and Hopwood, 1979; Urbain, 1986). Radiations have been classified in two basic types, based on their properties: (1) electromagnetic and (2) corpuscular or particulate. The former possess only energy, while the latter have both mass and energy.

Electromagnetic radiation. Electromagnetic radiation consists basically of self-propagating electric and magnetic disturbances (Urbain, 1986). Examples of electromagnetic radiation include visible light, radio waves, radar, ultraviolet (UV) rays, X-rays and gamma (γ) rays. The properties of electromagnetic radiation are best explained by considering their dual nature, i.e., they sometimes behave as waves and sometimes as particles. Electromagnetic waves are characterized by their velocity of propagation in a vacuum, $c = 3 \times 10^8$ m/sec, their wavelength, λ, and their frequency, ν (Barish, 1982). These three parameters are related by the following equation:

$$c = \lambda \nu$$

The particle nature of electromagnetic radiation is based on the fact that electromagnetic radiation travels through space in discrete bundles called photons, which, according to quantum theory, carry an energy, E, given by:

$$E = h\nu = \frac{hc}{\lambda}$$

where h is Planck's constant (6.63×10^{-34} J sec).

Corpuscular radiation. Corpuscular, or particulate, radiation, consists of particles which have a mass. Examples of corpuscular radiation include electrons, protons, neutrons and alpha (α) particles. When in motion, these particles possess kinetic energy, which depends on their velocity and their mass according to the following equation:

$$E = \frac{1}{2} \frac{m_o v^2}{\sqrt{1 - v^2 / c^2}}$$

where m_o is the particle's rest mass, v its velocity and c the velocity of electromagnetic energy in a vacuum (3×10^8 m/sec). As can be seen from this equation, unless v is very large, the term $\sqrt{1 - v^2 / c^2}$ approaches unity; in this case E would then approach the term for kinetic energy of classical physics, $\frac{1}{2} m_o v^2$. As v becomes larger, i.e., as it approaches c, the term $\sqrt{1 - v^2 / c^2}$ approaches zero, thus causing a very large increase in the particle mass, m, and, consequently, in the particle's kinetic energy, E (Urbain, 1986). This is the principle behind the acceleration of electrons.

Ionizing radiation

Radiations lose their energy as they pass through materials. This lost energy is actually absorbed by the atoms which make up the material and, if high enough, can have a significant effect on the stability of these atoms. Some types of radiation may only have enough energy to simply displace electrons from one orbital to another of a higher energy. These electrons are called "excited electrons", and the radiation is classified as an exciting radiation (e.g., UV light) (Grosch and Hopwood, 1979). Other types of radiation have energy levels so high that they are able to eject orbital electrons from their parent atom, leading to the formation of one or more unpaired electrons, and a positively charged ion, i.e., a cation (Urbain, 1986). Hence they are given the name ionizing radiation, or ionizing energy. In order to remove an electron from an atom in its ground state, a minimum energy content, the ionization energy, is necessary (Chang, 1984). Depending on the particular atom, ionization energies of valence, or outer, electrons are around 4-20 eV. The removal of additional electrons requires even higher ionization energies (Chang, 1984); for inner electrons, these can be of several thousand electron volts (Urbain, 1986). If an electron is hit with more energy than required for ionization, the excess energy becomes kinetic energy and enables the electron to travel away form its parent atom and interact with another atom, making the latter a negative ion, i.e., an anion. With ionizing radiation, each photon or particle has levels of energy so large that it can excite or ionize more than one atom (Urbain, 1986).

Absorbed dose, dose rate and energy level

The absorbed dose is a measure of the radiation energy absorbed per unit mass of a substance (e.g., a food), which we shall generically call the absorber. The SI unit of absorbed dose is the gray (Gy), which is equivalent to the absorption of 1 joule (J) of energy by 1 kg of material. Prior to 1986, the official unit of absorbed dose was the rad, which is equal to the absorption of 100 erg/g. Since 1 J = 10^7 erg, then 1 Gy = 10^7 erg/kg = 10^4 erg/g = 100 rad, which means that 1 kGy =100 krad = 0.1 Mrad (Cleland and Beck, 1992; Urbain, 1986). In the older literature in the field the rad was always used as the unit of absorbed dose.

The rate at which radiation energy is absorbed by an absorber must be considered, since it may have an effect on a cell's biological response to a given dose. However, for reasons that will be discussed later, this effect is not likely to be significant in food irradiation.

Another parameter to consider is the energy of the individual particles or photons, which is commonly expressed in electron volts (eV), where 1 eV = 1.60×10^{-19} J. This energy determines the penetration of the radiation and, therefore, affects the dose distribution within the absorber (Cleland and Beck, 1992; Urbain, 1986).

Radiations used to treat foods

Electrons

Of all the atomic and sub-atomic particles that could be given energy, only the electron has been found to be applicable for food irradiation. Electrons offer advantages because they can (1) be easily generated in large quantities, and (2) are electrically charged, which

allows them to be directed and focused magnetically and their energy to be controlled by electrostatic fields (Urbain, 1986). Electron beam generators or electron accelerators take advantage of these properties. The heavier particles, primarily α particles, protons, neutrons, and deuterons, which have masses thousands of times greater than that of the electron, are capable of inducing radioactivity even at low energy levels; therefore, they are not used for food irradiation (Urbain, 1986).

Radiation used for food irradiation must be able to penetrate a useful depth of the food to be treated. Because electrons have a mass, they do not have very much penetrating power. As previously discussed, however, when electrons are accelerated they acquire very high amounts of kinetic energy, which allows them to penetrate foods enough to be useful.

Sources of electrons. Electrons for industrial food irradiation processing are generated by electron accelerators. Electron accelerators are highly complex machines, the details of which are beyond the scope of this review. However, those considerations that are deemed most important in terms of practical food irradiation processing are presented here. In very simple terms, an electron accelerator consists of a hot filament that emits electrons into an evacuated chamber. A high positive electric potential then attracts these electrons and focuses them into a narrow beam. The final step involves passage of the beam through the poles of an electromagnet with a constantly changing magnetic field that causes the electron beam to sweep from side to side (CAST, 1989). This sweeping beam is then directed at the food.

Electron accelerators can be classified into two broad groups, according to the method

of acceleration: direct (or DC) or indirect (or RF) (Cleland and Beck, 1992; Ramler, 1982). Generally, direct accelerators are suitable for applications requiring energies up to 5 MeV and power ratings up to 300 kW, while indirect accelerators are more suitable for those applications, such as food irradiation, that require electron beam energies above 5 MeV and power ratings up to 50 kW (Cleland and Beck, 1992). Linear accelerators, or linacs, are indirect accelerators, and hold the most promise for food irradiation applications.

In electron beam food irradiation, the critical process parameters are those that directly affect the magnitude, or the distribution, of the applied dose. These parameters, which require close monitoring, include the electron energy, the beam current, the beam distribution across the conveyor, the conveyor speed, and the configuration of the product on the conveyor (Cleland and Beck, 1992).

Interactions with matter. Accelerated electrons act mainly through coulombic (electrical) forces between them and the electrons of absorber atoms. When they collide with orbital electrons, these repulsive forces cause the latter to be ejected from their parent atoms and the former to be deflected from their paths. As a result of these deflections, the path of an electron is difficult to predict and its penetrating depth is less than its true path length (Coggle, 1973). In these types of collisions, energy is transferred from the incident electron to the atoms they strike (Wang and Brynjolfsson, 1982). This process of energy transfer happens until all the kinetic energy of the incident electron is dissipated and the electron is captured by a cation (Urbain, 1986).

Another way in which electrons can lose energy is by X-ray production due to

coulombic interactions with the nuclei of the absorber (Barish, 1982). These X-rays, known as bremstrahlung (from the German for "braking radiation"), result from the conversion of some of the electron's kinetic energy to electromagnetic energy that takes place when the electrons interact with and are decelerated by the nuclei. The amount of bremstrahlung produced increases with the electron's kinetic energy and with the atomic number of the absorber atoms. Although its production in foods is basically insignificant, it may induce radioactivity at very high electron energy levels (usually over 10.5 MeV). Therefore, the energy of electron beams used for food irradiation has been limited to 10 MeV (Urbain, 1986).

Electromagnetic radiation

The use of electromagnetic radiation for food irradiation is limited to γ rays and X-rays. Since photons carry no electric charge and have no mass, they are able to penetrate deeper into foods than electrons.

Sources of electromagnetic radiation. X-rays are emitted when high-energy electrons strike a material, commonly a metal target. Therefore, electron accelerators are commonly used to generate X-rays for food irradiation processing. The conversion of electron kinetic energy to X-rays is quite inefficient, about 8.2 and 14.4%, respectively, for 5 and 10 MeV electrons when using an iron (Z=26) target. A target of higher atomic number, such as tungsten (Z=74), can increase the conversion efficiency to 21.2 and 31.8%, respectively (Urbain, 1986). The Iowa State University Linear Accelerator Facility, which uses a

stainless steel target, can convert electrons to X-rays with an efficiency of about 6%.

γ rays are a form of electromagnetic radiation that results form radioactive decay. Of the many radionuclides that emit γ rays, only two, cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs), produce γ rays suitable for food irradiation and are obtainable in high enough amounts. ^{60}Co is obtained by activating ^{59}Co with neutrons in a nuclear reactor. ^{137}Cs is a by-product of the nuclear fission of uranium and is usually available in the form of cesium chloride. To prevent environmental contamination, these radionuclide sources are usually doubly encapsulated in stainless steel (CAST, 1989; Urbain, 1986). Because these sources are constantly undergoing radioactive decay, they are usually stored under water when not in use to permit the safe entry of plant personnel into the processing area.

Interactions with matter. X-rays and γ rays can transfer their energy to matter by four different mechanisms:

(a) The photoelectric effect - In the photoelectric effect, first described in 1905 by Albert Einstein, a photon (or quantum) of electromagnetic energy strikes an atom and ejects an orbital electron from it, while losing all its energy in the process. Some of the photon's energy is used to eject the electron, and the remainder becomes kinetic energy which is acquired by the ejected electron and the residual cation (Barish, 1982; Urbain, 1986). The photoelectric effect is the predominant mechanism of absorption of photons with energy levels below 0.5 MeV (Coggle, 1973).

(b) Compton scattering - If the energy of the photon is much greater than the ionization energy of the electron with which it collides, it may use only part of its energy to eject the

electron from its atom and the remainder to interact with another atom. This event, therefore, results in an ejected electron and a scattered "secondary" photon of lower energy. The Compton effect is the dominant interaction of electromagnetic radiation with biological materials over the 50 keV to 20 MeV range (Barish, 1982) and is, therefore, the most important mechanism of electromagnetic energy transfer in food irradiation (Urbain, 1986).

(c) Pair production - When a photon passes sufficiently close to a nucleus, it can be converted into two particles: an electron and a positron (a particle having the same mass as an electron but a positive charge). For this to take place, the photon must have an energy of at least 1.02 MeV (since the energy equivalency of each particle is 0.51 MeV). Any excess energy is shared by the two particles as kinetic energy, which allows them to travel through the absorber and, if their energy is sufficiently high, to excite or ionize surrounding atoms. Eventually these particles slow down, recombine and yield two photons of 0.51 MeV each, which travel in opposite directions and interact with the atoms of the absorber in accordance with their energy content. (Barish, 1982; Urbain, 1986; Wang and Brynjolfsson, 1982). Pair production is not a very important mechanism of energy transfer in photons of less than 10 MeV of energy; hence it is not very important in food irradiation (Urbain, 1986).

(d) Photodisintegration - Photodisintegration occurs when a high energy photon excites the nucleus of an atom and causes it to emit any of a number of nuclear particles (neutrons or protons) or α particles (Barish, 1982). These disrupted atoms may then undergo radioactive decay, with the production of other particles or γ rays. This is termed induced radioactivity, and is determined primarily by the energy of the original photon in relation to the atomic number of the atom it interacts with. In order for particles to be expelled from

the nucleus, the energy of the photon must be higher than the nuclear binding energy of the last nucleon. Only three nuclides with binding energies below 5 MeV occur naturally in foods. These are ^{2}H (2.23 MeV), ^{17}O (4.14 MeV), and ^{13}C (4.95 MeV). These react to form an isotope and a γ ray but, fortunately, the resulting isotopes, ^{1}H, ^{16}O and ^{12}C, respectively, are stable (Urbain, 1986). For most other light elements nuclear binding energies are of the order of 10-15 MeV and for heavy elements they are about 7 MeV (Barish, 1982). These considerations, coupled with actual measurements for induced radioactivity in irradiated foods, have led to the establishment of an energy level of 5 MeV as the upper limit for electromagnetic radiation to be used for food irradiation. The two γ rays emitted by ^{60}Co have energies of 1.17 and 1.33 MeV, while the sole γ ray emitted by ^{137}Cs has an energy of 0.66 MeV (Urbain, 1986).

Physicochemical effects of ionizing radiation

Most of the effects of ionizing radiation on foods are caused by high-energy electrons (CAST, 1989), whether they are generated externally by a machine source or are produced within the food by X-rays or γ rays (through Compton and photoelectric interactions). These so-called primary electrons lose their energy through ionizations and excitations that occur over very short time periods (10^{-18}-10^{-15} sec). These interactions lead to a change in the electron energy distribution, which now includes degraded (and, hence, less energetic) primary electrons and electrons ejected during ionization (secondary, tertiary, etc., electrons). Eventually, electron energies drop below the ionization energies of the absorber molecules, at which point additional degradation occurs solely by electronic, vibrational and

rotational excitation (Taub, 1983; Taub and Halliday, 1979).

Direct or primary effects

The direct or primary effects of radiation result from the physical absorption of energy by the absorber and involve the interaction of primary electrons with the molecules of the absorber (Okada, 1970). As has been previously mentioned, these interactions produce, in addition to free electrons, ionized and excited molecules. These molecules, being unstable, seek to stabilize themselves in one of several ways. Excited molecules usually retain their extra energy for about 10^{-8} sec, after which they lose it by emitting a photon, by conversion to heat, by transferring it to another molecule or by chemical reactions. These chemical reactions may involve isomerization or dissociation of the excited molecule or may involve a second molecule (electron transfer, abstraction of hydrogen or addition). When these latter types of reactions take place, their products may be stable molecules or reactive molecules such as free radicals (Urbain, 1986). Free radicals are atoms or molecules having an unpaired electron and are usually very short-lived and reactive (Chang, 1984), since they have the tendency to either pair their odd electron with one from another radical or eliminate it by an electron transfer reaction. (Coggle, 1973).

Ionized molecules, or ions, normally seek stability by reacting with another ion of opposite charge. They may also, however, undergo reactions similar to those of excited molecules, some of which may yield free radicals. The ionizations and excitations caused by radiation occur over a very short period of time, 10^{-17} to 10^{-15} sec (Coggle, 1973).

Indirect or secondary effects

Indirect or secondary effects of radiation involve the reactions of primary radicals with either the solutes or the solvent of the absorber (Okada, 1970; Taub, 1983). As opposed to the direct effects, which are essentially independent of external factors, indirect effects can be affected by certain conditions. One of these is the composition of the absorber. In order for indirect effects to occur, there must be free movement of the reactants involved. In foods this free movement is usually facilitated by water. Therefore, in foods devoid of significant amounts of water (e.g., dehydrated products), or in those where the water has been immobilized (e.g., by freezing), the movement of these reactants is restricted and, consequently, indirect effects become less significant.

Since water is a major component of many foods, most of the indirect effects in food irradiation involve reactive species derived from water molecules (Urbain, 1986). Therefore, the radiation chemistry of water is very important in food irradiation.

When pure water is irradiated it is ionized, yielding a free electron and a positively charged water ion:

$$H_2O \xrightarrow{\text{radiation}} \cdot H_2O^+ + e^-$$

This electron can then react with another water molecule to yield a negatively charged water ion, or it can become hydrated:

$$e^- + H_2O \rightarrow \cdot H_2O^-$$

$$e^- + nH_2O \rightarrow e^-_{aq}$$

The hydrated, or aqueous, electron (e^-_{aq}) is formed when an electron, after having lost most of its kinetic energy by collisions, attracts several water molecules and becomes surrounded

by them, with the electric dipole moments of the water molecules oriented towards the electron's negative charge (Coggle, 1973; Okada, 1970). Hydrated electrons have impaired mobility and are more stable than free electrons. Nevertheless, they are still very reactive (Urbain, 1986) and behave very much like •H radicals (Okada, 1970).

Neither of the two water ions produced is very stable, so they both dissociate into an ion and a free radical:

$$\bullet H_2O^+ \rightarrow H^+ + \bullet OH$$

$$\bullet H_2O^- \rightarrow \bullet H + OH^-$$

Some excited water molecules can also yield free radicals:

$$H_2O^* \rightarrow \bullet OH + \bullet H$$

While some •H, •OH and e^-_{aq} radicals can react with solute molecules, they can also recombine with each other:

$$\bullet H + \bullet H \rightarrow H_2$$

$$\bullet OH + \bullet OH \rightarrow H_2O_2$$

$$\bullet H + \bullet OH \rightarrow H_2O$$

All these reactions can be pooled together and their products shown in a more simplified equation for the irradiation of pure water (Okada, 1970, Urbain, 1986):

$$H_2O \xrightarrow{\text{radiation}} \bullet H + \bullet OH + e^-_{aq} + H_2 + H_2O_2 + H_3O^+$$

In the presence of molecular oxygen, reactions can occur between it and the e^-_{aq} and •H radicals (Okada, 1970):

$$e^-_{aq} + O_2 \rightarrow \bullet O_2^-$$

$$\bullet H + O_2 \rightarrow \bullet HO_2 \rightarrow H^+ + O_2^-$$

where $\bullet O_2^-$ is the superoxide anion and $\bullet HO_2$ is the hydroperoxide radical.

The free radicals from water can also react with their own reaction products to yield free radicals:

$$H_2O_2 + \bullet OH \rightarrow H_2O + \bullet HO_2$$

or they can also react with surrounding organic molecules to yield secondary free radicals by hydrogen abstraction (CAST, 1989; Coggle, 1973; Okada, 1970):

$$\bullet H + RH_2 \rightarrow \bullet RH + H_2$$

$$\bullet OH + RH_2 \rightarrow \bullet RH + H_2O$$

where RH represents an organic molecule, such as a protein, lipid, or carbohydrate. Secondary radicals are those formed by the reaction of a primary radical from water and a molecule that is not a radical (CAST, 1989). It must be pointed out, however, that similar free radicals can also be formed by direct action of radiation (Coggle, 1973):

$$RH_2 \xrightarrow{\text{radiation}} \bullet RH_2^+ + e^-$$

$$\downarrow$$

$$\bullet RH + H^+$$

In the presence of molecular oxygen, these organic free radicals, be they primary or secondary, can react with it to yield a variety of peroxide radicals (CAST, 1989):

$$\bullet RH + O_2 \rightarrow \bullet OORH$$

These peroxide radicals can then abstract hydrogen to form hydroperoxides (HOORH) which can, in turn, yield oxidized products. Therefore, these free radical reactions that occur in the presence of oxygen are very significant from the standpoint of food quality, since

their products will usually contribute to rancidity.

Free radicals finally become stabilized by either reacting with each other or with solute molecules. Because they are so reactive and short-lived, their reactions, from the time they are formed to the time they become stable, last 10^{-14} to 10^{-3} sec (Coggle, 1973).

As primary and secondary free radicals seek to form stable products, they can also react with biologically important molecules and cause radiation damage (Coggle, 1973). These molecules can be those of the food as well as those of its contaminants (i.e., bacteria, parasites, viruses, etc.). Radiobiological damage can be the result of both direct and indirect effects. However, since many foods contain high amounts of water, it seems likely that much of this damage is caused by indirect effects.

Biological effects of ionizing radiation

Although the effects of ionizing radiation on living cells have been the subject of much research over the years, the processes by which they cause cell death are not yet completely understood. What is certain is that ionizing radiation affects living cells and, at high enough doses, is lethal to all living organisms (Urbain, 1986).

Target theory and DNA damage

Of the many theories that have attempted to explain the processes responsible for cell inactivation by ionizing radiation, the target theory, proposed in 1946 by Lea (1946), has been the most influential (McNally, 1982). This theory is based on the assumption that the observed biological effect (i.e., cell death or injury) is the result of a random "hit" (an

energy absorption event) on a "target" in a living cell (McNally, 1982; Okada, 1970). This random hit is usually an ionization event, while the target may consist of a molecule or an organelle.

For several years after the formulation of the target theory, proteins were widely considered to be the essential biological targets of ionizing radiation. However, this all changed with the discovery in 1953 of the double helical structure of deoxyribonucleic acid (DNA) (Watson and Crick, 1953a) and the subsequent recognition of the central role it plays in the cell (Watson and Crick, 1953b). Since then, DNA has been accepted, and confirmed by numerous studies, as the essential biological target of radiation (Grecz et al., 1983; Okada, 1970). Much of this evidence has come from numerous studies in which radiation-sensitive mutants of wild-type bacteria have been observed to be defective in their ability to repair radiation-induced DNA damage (Moseley, 1990). Although other cellular components have also been proposed as radiation targets, most of the evidence overwhelmingly points to effects on DNA as the major cause of cellular damage due to ionizing radiation.

DNA damage can be caused by both the direct and indirect effects of radiation (Grecz et al., 1983; Korystov, 1992). As mentioned previously, the indirect effects of radiation in most biological systems involve primarily the reactive species formed from water. Johansen and Howard-Flanders (1965), working with *Escherichia coli*, demonstrated that of the three major free radical species formed from the radiolysis of water (•H, •OH, e^-_{aq}), the hydroxyl radical (•OH) is responsible for most of the cell killing. This important role of the •OH radical has been confirmed by more recent studies and is currently widely recognized

(Chadwick and Leenhouts, 1981; Moseley, 1990; Ward, 1975).

Nature of radiation injury

DNA strand breaks. Molecular fragmentation was observed in the earliest studies of DNA damage caused by radiation (Scholes et al., 1949). Ionizing energy primarily causes single- and double-strand breaks in the DNA molecule, thus disrupting DNA replication (Grecz et al., 1983; Pollard, 1983). It has been estimated that in the DNA of irradiated bacteria the number of single-strand breaks is 10 to 20 times greater than the number of double-strand breaks (Moseley, 1984).

The study of radiation-induced DNA single-strand breaks was greatly aided by the development by McGrath and Williams (1966) of a method that permitted the direct isolation and sedimentation of single-stranded pieces of bacterial DNA by lysing the cells on top of an alkaline sucrose gradient and measuring their sedimentation velocity. The yield of single-strand breaks in the DNA of *E. coli* K12 irradiated with 4 MeV electrons under oxygen has been calculated to be 5×10^{-12} breaks per rad per dalton (i.e., 5×10^{-7} breaks/kGy dalton); the yield of strand breaks under nitrogen was 1.2×10^{-12}/rad dalton, about four times smaller (Johansen, 1975). Since the DNA of a "typical" *E. coli* cell (strain B/r) has a molecular weight of 2.5×10^9 and a chain length of about 4,720 kbp (Neidhardt et al., 1990), the total number of single-strand breaks induced by the absorption of a dose of 1 kGy would be about 1,250 per DNA molecule (i.e., one for every 3.8 kbp) under oxygen, and about 240 per molecule (i.e., one for every 20 kbp) under nitrogen.

Most single-strand breaks are caused by the breakage of phosphodiester bonds in one of

the DNA chains. This break is primarily the result of damage produced in the deoxyribose moiety by hydroxyl radicals. An •OH radical abstracts a hydrogen atom from one of the five carbons of the deoxyribose moiety, leading to hydrolysis of the 3' phosphodiester bond by β elimination (Friedberg, 1985), and yielding a 5'-PO_4 and a 3'-OH terminus (Moseley, 1990; Ward; 1975). In half of these breaks, the damaged 3' nucleoside will split off to yield a 3'-PO_4 terminus. Therefore, there is a 3:1 ratio of 5'-PO_4 termini to either 3'-PO_4 or 3'-OH termini (Moseley, 1990). It is estimated that approximately 20% of all hydroxyl radicals are involved in strand breakage (Moseley, 1990; Ward, 1975).

DNA double-strand breaks are formed when the breaks in the double helix occur just opposite each other or so close to each other that hydrogen bonding is unable to maintain the molecule's structure (Moseley, 1990). They may be the result of a single event affecting both strands, or of two independent single-strand breaks occurring in close proximity to each other (Chadwick and Leenhouts, 1981; Friedberg, 1985). The number of double-strand breaks is determined by measuring the sedimentation velocity of DNA fragments from irradiated bacteria in neutral, rather than alkaline, sucrose gradients (Kaplan, 1966). Double-strand breaks in *E. coli* have been found to be lethal, even in the presence of properly functioning DNA repair mechanisms (Bonura and Smith, 1976; Kaplan, 1966), although it was later found that the organism can survive up to 4 double-strand breaks in the presence of a homologous chromosome (Krasin and Hutchinson, 1977). Repair of double-strand breaks has also been observed in *Bacillus subtilis* (Hariharan and Hutchinson, 1973). On the other hand, however, the very radiation-resistant bacterium *Deinococcus radiodurans* has been reported to survive a large number of double-strand breaks, thus

suggesting that it possesses an effective mechanism for their repair (Burrell and Dean, 1975; Dean et al., 1966; Kitayama and Matsuyama, 1968).

Although single-strand breaks can lead to cell death, they are more easily repairable and, therefore, don't correlate as well with cell lethality as double-strand breaks (Bonura and Smith, 1976; Youngs and Smith, 1976). This higher degree of lethality of double-strand breaks is believed to be due to a physical separation of the two free ends of the DNA molecule, which would make its contiguous repair difficult, if not impossible (Moseley, 1984).

Base damage. Scholes et al. (1956) first detected hydroperoxides in irradiated DNA. These were later shown (Daniels et al., 1957) to be formed by saturation of the 5,6 double bond of pyrimidine bases following addition of •OH radicals. The pyrimidines, especially thymine, have been found to be more radiosensitive than the purines. Of all the radiolysis products of DNA bases, the hydroperoxide 5,6 dihydroxydihydrothymine, first recognized as radiation-induced in *D. radiodurans* (Hariharan and Cerutti, 1971, 1972), has been found to be the most abundant. Radiation-induced DNA base damage is very unstable; therefore, it is likely that a lot of it has not yet been characterized (Friedberg, 1985).

Repair of radiation injury

The repair of DNA single-strand breaks following irradiation was first reported by McGrath and Williams (1966), who found that, following X-ray irradiation, *E. coli* B/r was able to repair its radiation-induced DNA single-strand breaks within 40 min of incubation,

as opposed to a radiosensitive mutant, *E. coli* B_{s-1}, which could not. The repair of DNA single-strand breaks involves one or more of four basic DNA repair mechanisms: direct rejoining, excision repair, recombinational repair, and induced error-prone repair (a.k.a. SOS response). The mechanism of direct rejoining of DNA strand breaks (Friedberg, 1985) involves a single enzyme, polynucleotide (DNA) ligase (Dean and Pauling, 1970; Grecz et al., 1983; Jacobs et al., 1972), which directly rejoins the 5'-PO_4 and 3'-OH termini of a broken strand (Lewin, 1983). This rejoining appears to occur very shortly (30-40 sec) after introduction of the break and is impaired in the presence of oxygen (Grecz et al., 1983). It is likely to be the cell's first attempt at repairing DNA strand breaks, and its failure would cause the cell to rely on one or more of the more complex repair mechanisms.

At the present time, the way in which the remaining three mechanisms of DNA damage repair actually repair single-strand breaks is somewhat unclear. These repair mechanisms are normally involved in the repair of DNA base damage (e.g., pyrimidine dimers). However, evidence that they may be involved in the repair of single-strand breaks has come from studies where bacterial mutants deficient in one or more of the gene products required by these mechanisms (e.g., *recA*, *lex*, *recB*, *recC*, and *polA*) have been shown to be more radiosensitive (Pollard, 1983) and to yield more DNA strand breaks following irradiation than corresponding wild type cells (Moseley, 1984).

Excision repair, a relatively well-understood mechanism of DNA repair (Grecz et al., 1983), is generally used by the cell to replace portions of DNA where base damage has occurred. It involves the action of several enzymes: an endonuclease to recognize and cleave to the damaged site, a 5'→3' exonuclease to remove a stretch of the strand,

(extending several bases past the site of damage), a DNA polymerase to synthesize a replacement for the excised region, using the intact strand as a template, and DNA ligase to join the 3' end of the new stretch to the old portion of the strand. DNA polymerase I, however, has the ability to promote DNA replication at a nick in one of the strands; the 5'→3' exonuclease activity of *E. coli* DNA polymerase I allows it to catalyze the degradation of DNA in the absence of base damage, as long as the nick to be repaired contains a 3'-OH terminus (Friedberg, 1985). Town et al. (1971) observed that a *polA* mutant (i.e., polymerase I-deficient) of *E. coli* was three to five times more sensitive to X-rays and had more DNA single-strand breaks than the wild type.

Recombinational, or postreplication, repair is a somewhat complex DNA repair mechanism used by actively growing cells to prevent base damage from blocking DNA replication. In this mechanism, DNA replication proceeds normally until it encounters a non-coding lesion. A gap is then left opposite this lesion and replication continues some 800-1,000 bases downstream. The other parent strand, however, is replicated normally in this region. The gap left in one of the daughter strands is then filled in by recombinational insertion of the corresponding region from the parent DNA of the sister duplex. The gap now left in the sister duplex, which faces a normal strand, is filled in by repair replication. Since the original damage is left intact, this process must be repeated every time it encounters a replication fork until the damage is removed by excision repair (Lewin, 1983; Moseley, 1984). In *E. coli*, this mechanism requires the recA protein (coded by the *recA* gene) (Watson, et al., 1987), as well as the recBC enzyme (coded by the *recB* and *recC* genes) (Moseley, 1984). *recA* and *recBC* mutants of *E. coli* have been found to be more

sensitive to X-irradiation (Ganesan and Smith, 1972).

The SOS response, or induced error-prone repair, is a very complex mechanism that involves many gene products and many repair functions. In *E. coli*, basically, the lexA protein represses its own production and that of the enzymes coded by the *recA*, *uvrA*, and *uvrB* genes. Upon encountering a gap in a DNA strand (such as that produced during recombinational repair), recA molecules already present bind to it and cleave the repressor lexA protein, causing the activation of the *recA*, *uvrA*, and *uvrB* genes. This process is very prone to causing mutations, by mechanisms that are not yet completely understood (Moseley, 1984). As previously mentioned, the *lex* gene has also been found to be necessary for proper repair of DNA single-strand breaks in irradiated bacteria (Moseley, 1984).

It is not currently known how double-strand breaks are repaired, although there is some evidence that a recombinational mechanism may be involved (Billen, 1987; Krasin and Hutchinson, 1977). What is known is that most bacteria cannot tolerate very many double-strand breaks. *Deinococcus radiodurans*, being able to survive doses that induce up to 240 double-strand breaks per genome, is an exception (Moseley, 1983).

Survival curves

When a population of cells, bacterial, yeast or mammalian, is irradiated, it experiences a dose-dependent reduction of its surviving fraction. Based on target theory, cell inactivation is caused when an energy deposition event, such as an ionization, hits a target molecule. At low doses, a few cells will receive hits and, therefore, be inactivated. As the dose is increased, some targets will be hit more than once, and so the number of targets hit

will only be a portion of the total present. Therefore, the number of targets affected would be less than the number of potentially lethal hits and the fraction of survivors decreases in a geometrical progression with successive increments in dose (Grosch and Hopwood, 1979; Urbain, 1986). Since the relationship between dose and surviving fraction is exponential, a plot of the surviving fraction (on a logarithmic scale) as a function of dose will give a straight line called a survival, or dose-response, curve (Fig. 1). This relationship is mathematically expressed as:

$$\log_{10}\frac{N}{N_o} = -\frac{D}{D_{10}}$$

where N_o is the initial number of microorganisms, N is the number of organisms surviving after a dose D, and D_{10} is the dose that will inactivate 90% of the population (i.e., reduce it to 10% of N_o). N/N_o is referred to as the surviving fraction. Close examination of this equation reveals that the slope of the curve is $-1/D_{10}$.

Instead of the D_{10} value, a similar term, the D_{37}, is used more widely in the field of radiobiology. The D_{37} represents the dose necessary for 37% survival of an organism, and is based on target theory. The D_{37} and, hence, the D_{10}, assumes (1) that only direct action of radiation is involved, (2) that a living organism has one target and (3) that one hit in the target kills the organism (Okada, 1970). This very simplistic one-hit, one-target model can be described as follows:

$$N/N_o = e^{-D/D_o} = e^{-1} = 0.37$$

where D_o is the dose required to achieve one hit per cell. When $D = D_o$ all available targets should each receive one lethal hit. However, according to this model, 37% of them would

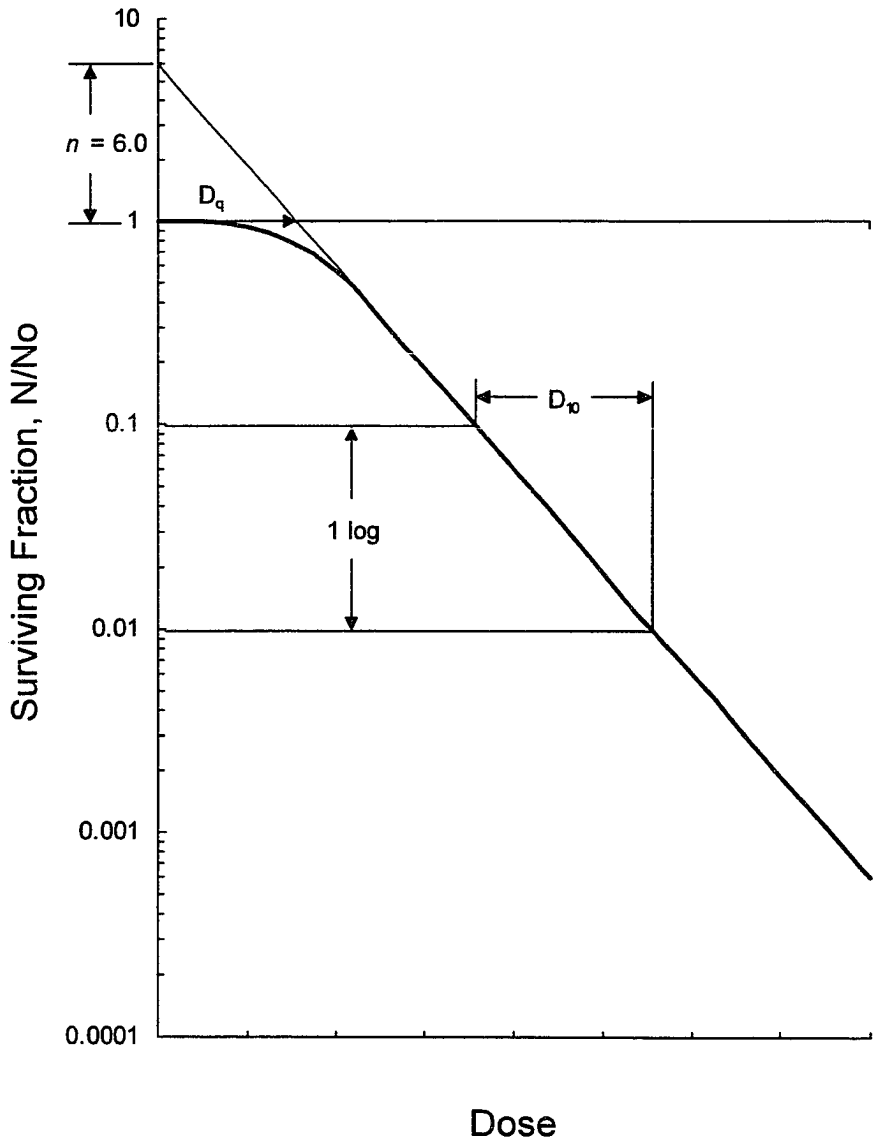

Fig. 1—A typical radiation dose-response survival curve, illustrating the decimal reduction value, D_{10}, the quasi-threshold value, D_q, and the extrapolation number, *n*.

escape without being hit, due to duplicating hits in other targets (Grosch and Hopwood, 1979). The D_{37} is, therefore, considered to be the dose necessary to inactivate one cell of the organism. It can be shown mathematically that:

$$D_{37} = \log e \, D_{10} = 0.434 \, D_{10}$$

Both D_{10} and D_{37} are indices of the radiation sensitivity of a cell population. Therefore, they are characteristics of the organism and permit the comparison of the radiosensitivities of different organism (Urbain, 1986). Since they are mathematically related in a linear fashion, it becomes unnecessary to report both values. In the field of food microbiology it is customary to report radiosensitivities as D_{10} values.

When organisms are irradiated, they sometimes exhibit deviations from simple exponential kinetics. At very low doses, survival curves often exhibit a "shoulder", i.e., a region in which there is very little or no cell inactivation. After a certain point cell inactivation again becomes exponential, with a slope of $-1/D_{10}$. Shouldered regions in survival curves suggest an ability by the cells to accumulate, and possibly repair, sublethal damage; at some point this damage becomes saturating and any further dosage becomes lethal (Bedford, 1982; Grosch and Hopwood, 1979). Sublethal damage is defined in radiation biology as damage which by itself is not lethal to the cell, whether or not it is repaired or modified (Billen, 1987). In terms of target theory, survival curve shoulders suggest that, for cell death to occur, either several targets in the cell must be inactivated (i.e., a one-hit, multitarget model) or the targets must be hit more than once (a multihit, one-target model) (Grosch and Hopwood, 1979; Okada, 1970).

Another theory that attempts to explain this phenomenon, the repair theory, has also

received considerable attention. According to this theory, one damaging event can kill a cell if it is not repaired, or if it is fixed such that it cannot be repaired, within a certain time. A limited repair system would be able to handle most of the damage that occurs at relatively low doses, but as the dose increases this repair system would become saturated and would not be able to repair the damage before it becomes irreversibly fixed. At this point, cells would start dying exponentially. In repair theory, the size of the shoulder is determined by the capacity of the repair system (Bedford, 1982).

Two parameters have been calculated in attempts to quantify survival curve shoulders: the extrapolation number, *n*, and the quasi-threshold value, D_q, both of which are illustrated in Fig. 1. The extrapolation number was first described and named by Alper et al. (1960), and has been used widely. It represents the intercept of the exponential portion of the curve on the survival axis (expressed as the surviving fraction, N/N_o) at zero dose (Bedford, 1982).

D_q corresponds to the dose at which the exponential portion of the curve intercepts 100% survival ($N/N_o = 1.0$) (Bedford, 1982). D_q is a more convenient number, since it measures shoulder width in units of dose (as opposed to the unitless extrapolation number) and thus can be used, along with the D_{10} value, to calculate the true lethal dose under a given set of conditions. These two parameters are related to the D_{10} value as follows:

$$D_q = D_{10} \log n$$

Although the D_{10} value is a very useful index of an organism's radiosensitivity, it can be observed that, when survival curves exhibit shoulders, it is not a true measure of the total dose necessary to inactivate a specific number of organisms, since it only applies to a

portion of the curve. In these cases ignoring the shoulder would lead to an underestimate of the required dose. The best estimate of the true dose required to inactivate a specific number of organisms would be:

$$D = D_q + D_{10} \log \frac{N_o}{N}$$

Factors that affect the biological effects of ionizing radiation

The biological effects of ionizing radiation and, hence, the radiosensitivities of affected organisms, are dependent on certain intrinsic (absorber-related) and extrinsic (environmental) conditions. Some of the more important, as they relate to food irradiation, include:

Temperature and water content

Temperature effects are mostly related to the indirect effects of radiation and are, therefore, related to water content. As water freezes, the mobility, and, consequently, the reactivity, of free radicals is decreased (Urbain, 1986). This, therefore, results in a decrease in the radiosensitivity of an irradiated organism. It has been reported that at the temperature of liquid helium free radicals are basically immobile and can persist for days. They remain relatively immobile up to a temperature of about -80°C (solid CO_2), but as the temperature increases past this point, they disappear more rapidly (CAST, 1989). However, free radicals in myosin at -10°C may still remain for about 24 h (Taub et al., 1978). Once the temperature is raised above freezing, free radicals disappear within seconds (CAST, 1989). In dehydrated foods free radicals are also relatively immobile. Their disappearance, however,

has been observed to be greatly accelerated by the presence of even small amounts of water (Diehl, 1972).

Because free radicals become mobile and free to react with the target during the thawing required to measure radiosensitivity, some investigators have argued that their immobilization alone cannot account for the observed decrease in indirect effects. An alternative theory states that the so-called temperature effect is actually due to cell dehydration that occurs during freezing (Korystov, 1992). Microscopic and electron microscopic studies have shown that, during the slow freezing of cells in suspension or as tissue components, ice crystals are formed exclusively outside the cells. As these crystals grow in size, the extracellular osmotic pressure increases and causes water to diffuse out of the cells (Meryman, 1956). Rapid freezing, on the other hand, leads to intracellular crystallization (Smith, 1961). The biological effectiveness of the free radicals formed in the ice would then be dependent on the position of these radicals, with respect to the radiation target, during thawing.

Evidence that the reduction in the indirect effects upon freezing may be due to cell dehydration has been obtained by Wood and Taylor (1957), who observed that the radiosensitivity of X-irradiated suspensions of the yeast *Saccharomyces cerevisiae* decreased from 0 to -33°C but actually increased from -33 to -72°C. When the suspensions were frozen to -72°C in a slower, stepwise manner, no change in radiosensitivity was observed between -33 and -72°C. They concluded that, in their study, freezing to -33°C was slow and thus lead to cell dehydration, while freezing directly to -72°C occurred at a rate faster than the rate of dehydration and allowed a significant amount of cellular water to be

frozen inside the cell. Slow freezing to -72°C presumably allowed for dehydration prior to freezing of the intracellular water. The rate of water loss by a cell is directly proportional to it's surface-to-volume ratio. Since bacterial cells have a relatively large surface-to-volume ratio, they will become dehydrated even during fast freezing (Korystov, 1992).

The radiosensitivity of bacteria has been found to be highly dependent on irradiation temperature. *Deinococcus radiodurans* D_{10} values have been reported to increase from 0.8 Mrad at ambient temperature to 4.5 Mrad at -30°C in ground beef; even greater radiation resistance was observed when cultures were lyophilized (Bruns and Maxcy, 1979). More recently, D_{10} values of *E. coli* O157:H7 have been observed to increase from 0.28 kGy at 5°C to 0.44 kGy at -5°C in vacuum-packaged mechanically-deboned chicken meat (Thayer and Boyd, 1993). In the same study, the radioresistance of the organism to a dose of 1.5 kGy in vacuum-packaged ground beef was found to increase sharply between 0 and -15°C; from -15 to -60°C the increases in resistance were very small. Similar temperature effects have also been reported for *Campylobacter jejuni* (Lambert and Maxcy, 1984), *Yersinia enterocolitica* (El-Zawahry and Rowley, 1979) and *Listeria monocytogenes* (Thayer and Boyd, 1995), among others.

Atmosphere

The effects of atmosphere are mostly related to the presence or absence of oxygen. It has been observed in many biological systems, including bacteria, that radiation damage is enhanced in the presence of oxygen (Okada, 1970), generally by a factor of 2 to 4 (CAST, 1989). This phenomenon has been termed the oxygen effect and was first described by

Alper and Howard-Flanders (1956) and Howard-Flanders and Alper (1957) using *E. coli*. The mechanism of the oxygen effect appears to involve both the addition of oxygen onto free radicals to yield peroxy radicals (CAST, 1989; McNally, 1982; Ward, 1975):

$$\cdot RH + O_2 \rightarrow \cdot OORH$$

and electron transfer to yield superoxide radicals (CAST, 1989; Ward, 1975):

$$\cdot RH + \cdot O_2 \rightarrow RH^+ + O_2^-$$

This would prevent radical recombination and dimerization and, therefore, "fix" the radiation damage. Peroxy radicals usually abstract hydrogen atoms from other molecules to form hydroperoxides (HROOH), which are unstable and lead to oxidized products (CAST; 1989).

Food composition

Besides water, other components of the medium in which an organism is irradiated have been found to have an effect on radiosensitivity. Generally, more complex irradiation media have been found to exert a protective effect on microorganisms, thus increasing their D_{10} values. Patterson (1989) reported higher D_{10} values for *Listeria monocytogenes* in poultry meat than in phosphate-buffered saline. El-Shenawy et al. (1989) found the same organism to be more resistant in ground beef than in broth. This protective effect of more complex media has been attributed to competition between the food components and the organisms for interaction with the radiolytic products of water (Urbain, 1986) and underscores the importance of determining D_{10} values for particular foods.

Dose rate

In radiobiology, the dose rate is one of the main factors that determines the effectiveness of the radiation used (McNally, 1982). Four important dose rates have been identified (Hall, 1972): Ultrahigh (exposures in fractions of a second), high (exposures of a few minutes), low (exposures of hours or days), and very low (continuous exposure over weeks). Dose rate effects are generally not observed at high dose rates (0.001-10 kGy/min), which is where most food irradiation dose rates fall. However, dose rates obtained with machine sources are near the high end of this region, so under certain conditions there could be a dose-rate effect in the presence of oxygen (Urbain, 1986). In these cases the oxygen of the system would be depleted faster than it can be replaced by diffusion into the food (Dewey and Boag, 1959). This would reduce the lethal effect of the radiation (Urbain, 1986).

Postirradiation incubation

Postirradiation incubation of microorganisms under both optimal and suboptimal growth conditions can affect the repair and, hence, the expression of radiation-induced damage. Presumably, cells incubated under both conditions have undergone the same amount of damage, some of which is lethal under any incubation condition, and the remainder of which is potentially lethal, but repairable (Bedford, 1982). This concept of potentially lethal damage (PLD) used in radiation biology is analogous to that of sublethal injury used in microbiology. The incubation condition used the most for estimating the extent of potentially lethal damage (PLD) experienced by a cell population is the

composition of the growth medium. A growth medium containing selective agents inhibits the repair of PLD and, hence, the growth of sublethally injured cells, while allowing the growth of uninjured, healthy cells. The use of a nonselective growth medium, on the other hand, permits the repair of PLD and, therefore, the growth of both uninjured and sublethally injured cells. Lethally injured cells are unrecoverable with either medium. The difference between the numbers of colonies that grow on both media corresponds to the number of sublethally injured cells (Jay, 1992).

Full repair of sublethal injury should result in an increased D_{10} value, with no change in the survival curve shoulder (Bedford, 1982). Patterson (1989) and El-Shenawy et al. (1989) have both reported D_{10} values for irradiated *L. monocytogenes* to be higher with recovery on nonselective media than with recovery on selective media. El-Shenawy et al. (1989) found D_{10} values of irradiated *L. monocytogenes* recovered on tryptic soy agar to be lower when the medium was supplemented with 5.5% salt.

Listeria monocytogenes and the genus *Listeria*

Brief history

Listeria monocytogenes was the first species of the present-day genus *Listeria* to be identified and classified, and was also the genus' only member up until 1961 (Rocourt et al., 1982). It was first described in 1924 by Murray et al. (1926) after they isolated it from the blood of infected rabbits, in which a typical monocytosis was observed. The organism was, therefore, named *Bacterium monocytogenes*. It is believed, however, that *L. monocytogenes* had been isolated several times prior to 1924, even as early as 1891 (Gray and Killinger,

1966). The oldest known strain of *L. monocytogenes* was isolated from cerebrospinal fluid in 1918 (Dumont and Cotoni, 1921) and identified as *L. monocytogenes* in 1940 (Paterson, 1940). In 1927, the organism was isolated from a gerbille in South Africa and named *Listerella hepatolytica*. After recognizing that it was the same as *B. monocytogenes*, its name was changed to *Listerella monocytogenes*, and, finally, in 1940, to *Listeria monocytogenes* (Pirie, 1940).

Characteristics

Microbiology

Members of the genus are gram-positive regular, short rods (0.4-0.5 μm in diameter by 0.5-2 μm in length) with rounded ends. They are aerobic and facultatively anaerobic, mesophilic, nonsporeforming and motile by a few peritrichous flagella at 20-25°C. While their optimum growth temperature is between 30 and 37°C, they are able to grow between 1 and 45°C (Seeliger and Jones, 1986). Their optimum growth pH is between 6 and 9, but they have been reported to grow at pH<5.5. They are also halotolerant, and can grow in the presence of sodium chloride concentrations up to 10-12% (Bille and Doyle, 1991). All listeriae are catalase-positive, oxidase-negative, and ferment glucose with the production of L(+)-lactic acid. They are methyl red positive, Voges-Proskauer positive, do not utilize citrate, hydrolyze esculin and sodium hippurate, do not hydrolyze urea, and do not produce indole (Seeliger and Jones, 1986).

Classification

Based on biochemical, genomic, and serological data, the genus *Listeria* has been divided into seven species: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. murrayi* and *L. grayi* (Rocourt et al., 1982; Seeliger and Jones, 1982). Three of these, *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*, exhibit β-hemolysis on blood agar and only two, *L. monocytogenes* and, very rarely, *L. ivanovii* (Busch, 1971; Hof and Hefner, 1988; Rocourt and Seeliger, 1985), have been found to be human pathogens (Bille and Doyle, 1991). *Listeria monocytogenes* is such an important human pathogen that it deserves special attention.

Typing

Serotyping. The serotyping scheme for members of the genus *Listeria* is based on 5 heat-labile flagellar antigens and 14 heat-stable somatic antigens (Seeliger and Hohne, 1979). This scheme distinguishes 16 different serovars, none of which, except for serovar 5 (which includes only *L. ivanovii*), is species-specific (Bille and Doyle, 1991). Of the 13 serotypes of *L. monocytogenes* that have been identified, three, 1/2a, 1/2b, and 4b, are responsible for most cases of human listeriosis (Belle and Doyle, 1991; Farber and Peterkin, 1991).

Other typing schemes. Other schemes that have been used to type the listeriae include phage typing, isoenzyme typing (i.e., multilocus enzyme electrophoresis), DNA fingerprinting (i.e., restriction enzyme analysis), rRNA typing, plasmid typing and

monocine typing. Of these, phage typing (Audurier et al., 1984) has proven to be a valuable tool in epidemiological investigations of listeriosis outbreaks (Bille and Doyle, 1991; Farber and Peterkin, 1991).

Clinical listeriosis in humans

Listeriosis in nonpregnant adults

Most cases of human listeriosis occur in individuals suffering from underlying conditions that may lead to a suppression of their T-cell-mediated immunity (Lorber, 1990). The clinical syndromes of listeriosis in nonpregnant adults include primary bacteremia or central nervous system infections, such as meningitis and encephalitis (McLauchlin, 1990a; Nieman and Lorber, 1980). It can also cause endocarditis or affect other parts of the body (Farber and Peterkin, 1991). Meningitis is usually associated with the elderly or immunocompromised (Lorber, 1990). The focal signs of meningitis and encephalitis are usually preceded by prodromal symptoms, such as vomiting, fever, headache, and malaise (Farber and Peterkin, 1991).

Predisposing conditions that have been associated with listeriosis include neoplastic disease, immunosuppression, pregnancy, extremes of age (i.e., neonates, people over 60), diabetes mellitus, alcoholism, cardiovascular and renal collagen diseases, hemodyalisis failure (Nieman and Lorber, 1980), and decreased gastric acidity (Ho, et al., 1986). However, healthy individuals with no known predisposing conditions have also become ill with listeriosis (Schlech, 1983).

Perinatal listeriosis

Most perinatal listerial infections are detected during the third trimester. Infected pregnant women may experience a mild flu-like illness, with fever, headaches, myalgia and, sometimes, gastrointestinal symptoms. This prodrome occurs in approximately two-thirds of the cases and represents a bacteremic period when blood cultures should be taken for diagnosis (Gellin and Broome, 1989; Schuchat et al., 1991). It is also presumed to be the point at which intrauterine infection is initiated (Gellin and Broome, 1989). Intrauterine infection of the fetus is thought to result from transplacental transmission following maternal bacteremia and can lead to amnionitis, preterm labor, spontaneous abortion, stillbirth, or early-onset neonatal infection (Schuchat et al., 1991). Perinatal listeriosis is rarely life-threatening to the mother (Gillen and Broome, 1989).

Neonatal listeriosis

Early-onset neonatal listeriosis. Two clinical forms of neonatal listeriosis have been identified: early-onset and late-onset. Early-onset listeriosis occurs in infants that have been infected in utero. The onset of disease can be at birth or shortly (hours or a few days) thereafter, with the mean time for the onset of symptoms being 1.5 days. Clinical symptoms include sepsis and, less frequently, granulomatosis infantisepticum (Gillen and Broome, 1989; Schuchat et al., 1991). In this syndrome, the organism is so widely disseminated throughout the body that lesions appear, mainly in the liver and the placenta, and sometimes in the brain, adrenal glands, kidney, spleen, lungs and the gastrointestinal tract (Farber and Peterkin, 1991; Gillen and Broome, 1989). The prognosis for infants in the early-onset

group is usually very poor (Farber and Peterkin, 1991). A fatality rate of 38% has been reported (McLauchlin, 1990b).

Late-onset neonatal listeriosis. Symptoms of late-onset neonatal listeriosis may present themselves several days to weeks after birth. The mean time for the onset of symptoms is 14.3 days. Infants in this group are usually healthy at birth and are associated with uncomplicated pregnancies (Farber and Peterkin, 1991; Gillen and Broome, 1989). The source of the organism in this case is unclear. It could be acquired from the mother's genital tract at the moment of birth, although it can sometimes occur following cesarian delivery (Schuchat et al., 1991). The primary manifestation of neonatal listeriosis of the late-onset type is meningitis. In a British study, a 25% fatality rate was reported (McLauchlin, 1990b).

Pathogenesis of *Listeria monocytogenes*

Virulence mechanisms

L. monocytogenes has been known to be an intracellular pathogen since Mackaness (1962) observed its growth inside nonimmune phagocytes. Therefore, for it to become invasive, it must first penetrate the host's intestinal wall (Berche et al., 1988) and then its macrophages. This is accomplished by parasite-directed endocytosis (Rácz et al., 1972) and does not appear to depend on listeriolysin O (Kuhn et al., 1988). Instead, the organism's entry into mammalian cells has been associated with the production of a 60 kDa extracellular protein (Kuhn and Goebel, 1989), while listeriolysin O has been shown to be

required for intracellular growth (Portnoy et al., 1988).

Once the organism is inside the macrophage and encapsulated by a phagosome, the phagosomal membrane undergoes listeriolysin-mediated cytolysis, which allows the bacterium to escape the phagosome and grow in the more favorable conditions of the cytoplasm (Kathariou et al., 1987; Gaillard et al., 1986; Portnoy et al., 1988). Within 2 h of infection, the cells of *L. monocytogenes* become coated by actin filaments, which presumably allow them to move intracellularly and to spread to other cells (Tilney and Portnoy, 1989). Although these actin filaments are assembled by the host cell, their formation has been shown to be induced by *L. monocytogenes* and not by the macrophage itself (Tilney et al., 1990). In this way, *L. monocytogenes* is able to invade macrophages, grow inside them and escape them to invade adjacent cells.

Most cases of listeriosis are known to occur in patient's suffering from one or more underlying conditions that interfere with T-cell-mediated immunity. Some of these conditions could be disease (e.g., malignancy), immunosuppressive drugs (e.g., steroids) and physiological states (e.g., extremes of age) (Lorber, 1990). Upon exposure to *L. monocytogenes*, T cells become sensitized and apparently attract, focus and activate macrophages at the site of infection (Mielke et al, 1988), presumably by producing lymphokines, including gamma interferon. When macrophages are activated, they become much more lethal to invaders, such as *L. monocytogenes*. In fact, *L. monocytogenes* has been observed to be unable to survive inside activated macrophages (Kaufmann, 1988). This explains why T-cell-mediated immunity plays such a critical role in the host's defense against *L. monocytogenes* and why a healthy immune system is so important to combat this

organism.

Virulence factors

Most of the experimental evidence to date indicates that the virulence of *L. monocytogenes* is multifactorial (Farber and Peterkin, 1991). Many virulence factors have been proposed and studied (Chakraborty and Goebel, 1988). Among these are iron compounds, catalase and superoxide dismutase, cell wall components, hemolysin and the 60 kDa extracellular protein.

It has been demonstrated that iron deprivation stimulates the secretion of listeriolysin (Sword, 1966). Since the phagosome is an iron-deprived environment, production of listeriolysin would be stimulated inside it. Once the phagosomal membrane has been disrupted, the organism can utilize the iron available in the cytoplasm.

Cell wall components, such as peptidoglycan, teichoic and lipoteichoic acids, have been identified as virulence factors of *L. monocytogenes* (Fiedler, 1988). Peptidoglycan, for example, confers resistance to lysozyme, and thus helps the organism survive inside macrophages (Galsworthy, 1987).

The hemolysin produced by *L. monocytogenes*, termed listeriolysin O (LLO), is a 58-kDa thiol-activated pore-forming hemolysin (cytolysin), which shares genetic and antigenic homology with the streptolysin O (SLO) of *Streptococcus pyogenes* (Braun and Focareta, 1991). Although its levels do not always correlate directly with degree of virulence in mice (Kathariou et al., 1988), it has been found to be critical for the expression of virulence in *L. monocytogenes* (Kathariou et al. 1987; Kathariou and Pine, 1990; Mengaud et al., 1990) and

is also phenotypically related to virulence.

Epidemiology of foodborne listeriosis

Listeria monocytogenes has long been recognized as an animal pathogen (Gray and Killinger, 1966), with approximately 90% of animal listeriosis cases attributed to it (Ryser and Marth, 1991). Direct transmission of the organism from animals to veterinarians and farm workers has been well documented (Farber and Peterkin, 1991), as well as its isolation from the gastrointestinal tract of healthy individuals (Kampelmacher and van Noorle Jansen, 1969, 1972; Lamont and Postlethwaite, 1986). However, it wasn't until the investigation of recent large outbreaks that enough epidemiologic and clinical data was obtained to prove that listeriosis is indeed a foodborne disease (Schuchat et al., 1991).

Hospital data from 1987 through 1990 have shown a yearly average of 1,089 cases of listeriosis, with an average hospital stay of 13.9 days, and average annual hospital costs of $10.4 million (CAST, 1994). Considering that most epidemiologists consider reported disease data to be just a small fraction of the total, Todd (1989), among others, has estimated the annual incidence of listeriosis in the United States at about 25,000 cases, with 67.9 deaths.

Epidemic foodborne listeriosis

Foodborne transmission of listeriosis was conclusively documented in 1981, following an outbreak in the maritime provinces of Canada (Schlech et al., 1983). Between March and September of 1981, 7 adult cases and 34 perinatal cases (including 5 abortions, 4 stillbirths

and 23 infants born seriously ill) occurred. The epidemic strain was *L. monocytogenes* 4b. The implicated vehicle of transmission, according to case-control studies, was coleslaw that had been grown by a farmer who also had a flock of sheep, two of which had died of listeriosis. Composted and raw manure from this flock had been used to fertilize the field in which the cabbage had been grown. Although *L. monocytogenes* was never recovered from the manure, it is still presumed to have been the source of contamination.

An earlier foodborne outbreak may have occurred in eight Boston hospitals in 1979 (Ho et al., 1986). There were 20 cases of listeriosis, 10 of which were among patients who were immunosuppressed due to steroid or chemotherapy treatment; 3 of them died. Foods identified as the possible vehicle of transmission were tuna fish, chicken salad and cheese, all of which were served with raw tomatoes, celery and lettuce. This outbreak was reported 7 years after it occurred, and its source was never confirmed. Its investigation, however, suggested that an individual's risk for developing listeriosis my be somewhat increased by drugs that lower gastric acidity (Schuchat et al., 1991).

Another outbreak occurred in Massachusetts in 1983 (Fleming et al., 1985). There were 42 adult and 7 perinatal cases, with a death rate of 29%. All 42 adult cases were among immunocompromised individuals. *Listeria monocytogenes* 4b was identified as the epidemic strain. Case-control studies associated the outbreak with a specific brand of pasteurized milk. The raw milk used for the pasteurized product had come from a group of farms where listeriosis had occurred at the time of the outbreak. *Listeria monocytogenes* was never cultured form the pasteurized milk, but it was found in the raw milk. Inspections by the FDA and the Massachusetts Department of Public Health did not find any evidence

of improper pasteurization or of contamination after pasteurization. This report was the first to suggest the possibility that proper pasteurization procedures may not be sufficient to completely destroy *L. monocytogenes*.

The largest outbreak of foodborne listeriosis to date occurred in Los Angeles County, California in 1985 (Linnan et al., 1988). There were a total of 142 cases, 93 in pregnant women or their offspring and 49 in nonpregnant adults, in an eight-month period. Of the 48 deaths, 20 were fetuses, 10 were neonates, and 18 were nonpregnant adults. Of the nonpregnant adults 98% were immunocompromised or had a predisposing condition. Case-control studies implicated a particular brand of Mexican-style cheese as the vehicle of transmission. *Listeria monocytogenes* 4b of the same phage type as the epidemic strain was cultured from unopened packages of cheese and is thought to have been introduced into the cheese by the addition of contaminated raw milk. This was the first listeriosis outbreak in which the food that caused it was identified and recalled during the outbreak.

Another major outbreak occurred in Switzerland between 1983 and 1987 (Bille, 1990). There were 122 cases with 31 deaths. The vehicle of transmission was found to be a Vacherin Mont d'Or soft cheese. Eighty-five percent of the strains isolated during the outbreak were *L. monocytogenes* 4b of the same phage type.

Sporadic foodborne listeriosis

In spite of these epidemic outbreaks, human listeriosis typically occurs sporadically (Gellin and Broome, 1989). In a case-control study of dietary risk factors, Schuchat et al. (1992) estimated the annual incidence of sporadic listeriosis in the United States at 7.4

cases per 1,000,000 population, with a fatality rate of 23%. These figures, projected to the national population, suggested a yearly occurrence of 1,850 cases and 425 deaths due to listeriosis. It was also estimated that 32% of cases could be attributed to the consumption of soft cheeses or foods purchased from store delicatessen counters. A previous case-control study (Schwartz et al., 1988) found a link between sporadic listeriosis and the consumption of undercooked chicken and unheated hot dogs. In the study of Schuchat el al. (1992) 69% of cases in men and nonpregnant women occurred in cancer, AIDS, organ transplant, or corticosteroid therapy patients. In fact, 98% of cases in this group occurred in people with at least one underlying disease. For these immunocompromised people, eating undercooked chicken represented an additional risk factor.

Pinner et al. (1992) conducted a microbiological and epidemiological investigation of the foods collected from the refrigerators of 123 patients with sporadic cases of listeriosis. They isolated *L. monocytogenes* from at least one food sample in the refrigerators of 79 (64%) of the patients. In 26 (33%) of these 79 refrigerators they found at least one food isolate of *L. monocytogenes* of the same strain as in the corresponding patient. This patient-matching was not observed for all food types. For example, 36% of beef samples contained *L. monocytogenes*, but only 2 of 50 beef isolates were patient-matching strains. Better patient-matching was observed in dairy foods and vegetables. Although only 2% of dairy samples contained the organism, 6 of 9 dairy isolates were patient-matching. For 23 cases, food specimens just like those found in the patients' refrigerators were purchased. Of these, 4 (one each of turkey franks, sliced turkey ham, pork sausage and ground beef) contained *L. monocytogenes* of the same type as the patient. Purchased turkey franks had less than 1

CFU/g of the organism, compared to 1100 CFU/g in the same product from the patient's refrigerator (Wenger et al., 1991). Overall, most of the isolates obtained from both patients and foods belonged to serotypes 4b, 1/2a and 1/2b.

Listeria monocytogenes in foods and the environment

Listeria monocytogenes in meats and poultry

In meats and poultry *L. monocytogenes* has been found in raw as well as processed products (Carosella, 1990). Farber et al. (1989) isolated *L. monocytogenes* from 86.4% of ground meats (beef, pork and veal) and 20% of fermented sausages. In Canada, Tiwari and Aldenrath (1990) found the organism in 44.4% of raw meat cuts, 21% of packaged wieners, 13% of packaged luncheon meats, and 14% of packaged sliced meats. In Australia, Grau and Vanderlinde (1992) found *L. monocytogenes* in 72% of corned beef samples, 34% of ham samples, 15% of luncheon meats and none in salami. USDA/FSIS national monitoring programs for *L. monocytogenes* in meat and poultry products, conducted between 1987 and 1990, found the organism in 7.1% of domestically produced raw beef, with the highest percentage of positive samples associated with beef from cows (9.6%) and bulls (7.6%) (Carosella, 1990; Ryser and Marth, 1991). This program also found the organism present in cooked beef/roast beef/cooked corned beef (2.7%), sliced canned ham/sliced canned luncheon meat (0.36%), Prosciutto ham (2.2%) and cooked large- and small-diameter sausage (0.87%).

In poultry, *L. monocytogenes* has been found in 5% of fresh chicken wings, 8% of fresh and 1% of frozen chicken legs, 6% of fresh and 1% of frozen chicken livers (Genigeorgis et

al. 1989), 12% of fresh turkey wings, 8% of fresh turkey legs and 7% of fresh turkey tails (Genigeorgis et al., 1990). Farber et al. (1989) isolated it form 56.3% of fresh chicken legs. It has also been isolated from raw liquid whole egg (Leasor and Foegeding, 1989).

Inoculation studies have shed some light on the behavior of *L. monocytogenes* in meat and poultry products under various conditions. Grau and Vanderlinde (1990) have shown that *L. monocytogenes* is capable of growth in both high (6.0-6.1) and low (5.5-5.7) pH vacuum-packaged beef at 0°C. Growth was considerably faster in the high pH meat. Working with processed meat products, Glass and Doyle (1989) found that *L. monocytogenes* grows well at 4.4°C on frankfurters, ham and bologna.

Listeria monocytogenes in other foods

Among dairy products, *L. monocytogenes* has been found in raw milk (Lovett, et al., 1987), various types of cheese (Farber et al., 1987; Linnan et al., 1988), ice cream and ice milk (Ryser and Marth, 1991). It has also been found in seafood (Ryser and Marth, 1991) and in certain vegetables (Heisick et al., 1989).

Other *Listeria* species

Other species of *Listeria*, particularly *L. innocua*, *L. welshimeri* and *L. ivanovii* have also been isolated from various foods (Farber et al., 1989; Tiwari and Aldenrath, 1990). The nonpathogenic *L. innocua* is often isolated from foods that also contain *L. monocytogenes* (Farber et al., 1989; Grau and Vanderlinde, 1992; Genigeorgis et al., 1989, 1990; Leasor and Foegeding, 1989; Varabioff, 1992), and sometimes in even greater numbers

(Genigeorgis et al., 1989). It is, therefore, very important that food isolates of *Listeria* be positively identified to the species level.

Listeria monocytogenes in the environment

Listeria monocytogenes is considered ubiquitous in the natural environment. It has been isolated from soil, vegetation, sewage, surface fresh waters, fecal material of diseased as well as healthy sheep, cattle, goats, pigs, chickens, turkeys and pheasants (McCarthy, 1990; Ryser and Marth, 1991) and even the gastrointestinal tract of healthy humans (Kampelmacher and van Noorle Jansen, 1969, 1972; Lamont and Postlethwaite, 1986).

Elimination of *Listeria monocytogenes* by Food Irradiation

Since *L. monocytogenes* was confirmed as a foodborne pathogen relatively recently (Schlech et al., 1983), all of the food irradiation work involving this organism dates since 1988.

In one of the first reports on the sensitivity of *L. monocytogenes* to gamma radiation, El-Shenawy et al. (1988) inoculated ground beef or tryptic soy broth with *L. monocytogenes* strains V7, Scott A or California, and irradiated it under air at 20-21°C with ^{60}Co gamma rays at doses ranging from 0.75 to 4.5 kGy. Strain V7 was found the most radioresistant and Scott A the least resistant in both irradiation media. Overall, D_{10} values were smaller in broth (D_{10} = 0.34 to 0.5 kGy) than in ground beef (D_{10} = 0.51 to 1.0 kGy) and were also smaller when the organism was recovered on a selective medium, indicating a certain degree of injury. The authors concluded that the radiosensitivity of *L. monocytogenes*

depended on the food menstruum and the strain of the organism.

Huhtanen et al. (1989) irradiated seven strains of the organism in BNT medium (a mixture of 0.4% nutrient broth and 1.5% trypticase soy broth with glucose) and in mechanically deboned chicken meat (MDCM) under air at 2-4°C. D_{10} values were calculated for three different dose intervals (0-0.50, 1.0-2.0, and 0-2.0 kGy), presumably to observe whether survival curves deviated from linearity. D_{10} values were higher in MDCM than in BNT medium for all seven strains. Average D_{10} values in MDCM were 0.27 kGy from the 0-0.50 kGy interval, 0.77 kGy from the 1.0-2.0 kGy interval, and 0.46 kGy from the 0-2.0 kGy interval. Since data points for only two doses were used to determine the D_{10} values from the first two intervals, it becomes difficult to assign a significant meaning to these numbers. However, it is evident that none of the survival curves, either in MDCM or BNT medium, showed a shoulder. Cells at different stages of growth (1.5, 2.5, 5, and 18 h cultures) were also irradiated in BNT medium. Survival curves for the fully grown cultures (5 and 18 h) were linear, while those of the growing cells were slightly convex with highly significant quadratic trends. The authors concluded that a dose of 2.0 kGy would be sufficient to destroy 4 log of *Listeria.*

Patterson (1989) irradiated four strains of *L. monocytogenes* in sterile minced chicken meat and in phosphate-buffered saline (PBS) under air at an unspecified temperature, and isolated them in three different culture media: tryptone soya agar containing 0.3% yeast extract (TSYEA), Listeria selective agar (LSA) and McBride agar containing 50 ml/L horse blood (MBA). Overall, D_{10} values were higher in chicken (0.417-0.553 kGy) than in PBS (0.318-0.494 kGy). Plating medium was found to make a significant difference, with

TSYEA and MBA yielding higher D_{10} values than the more selective LSA. The author concluded that, since these D_{10} values were similar to those previously reported for *Salmonella* spp., irradiation doses of 2.5-7 kGy suggested to eliminate salmonellae would also eliminate *L. monocytogenes*.

Hashisaka et al. (1989) tested the survival of *L. monocytogenes* inoculated in ice cream and mozzarella cheese. Samples were kept at -78°C and gamma irradiated with doses of 2, 4, 8, 16, and 32 kGy. D_{10} values were calculated to be 1.4 kGy for mozzarella cheese and 2.0 kGy for ice cream.

Farag et al. (1990) investigated the radiosensitivity of three strains of *L. monocytogenes* inoculated into phosphate buffer, trypticase soy broth containing 0.6% yeast extract (TSB-YE) and poultry feed. Irradiation was done under air at 0-0.5°C. D_{10} values of 0.18, 0.21 and 0.44 were determined for *L. monocytogenes* strain CFPDC when gamma irradiated in phosphate buffer, TSB-YE and poultry feed, respectively. The higher D_{10} value of the organism in the feed was attributed to the feed's low water content (10.4%). Differences in radiation sensitivity were found across the three strains irradiated in TSB-YE, with strain 81-861 (D_{10} = 0.46 kGy) being more radioresistant than strains CFPDC (D_{10} = 0.21 kGy) and Scott A (D_{10} = 0.25 kGy). Also, D_{10} values for *L. monocytogenes* CFPDC in poultry feed were found to be nearly the same, regardless of mode of irradiation (electron beam vs. ^{60}Co gamma rays).

Varabioff et al. (1992) inoculated raw whole chickens with a total of 2×10^6 CFU of *L. monocytogenes* UQM 3172 (serotype 4b), packaged them under air or under vacuum, irradiated them at 2.5 kGy with ^{60}Co gamma rays at room temperature, and stored them at

4°C for 15 d. *Listeria monocytogenes* was only recovered from the vacuum-packaged chickens after 7 d of storage.

Shamsuzzaman et al. (1992) investigated the effects of electron beam irradiation (up to 2.9 kGy) combined with mild cooking (65.6°C) of vacuum-packaged (sous vide) chicken breasts on the survival of *L. monocytogenes* 81-861 during 2°C storage. Sous vide treatment alone was not effective in reducing the numbers of *L. monocytogenes*. However, when sous vide treatment was combined with 2.9 kGy irradiation, the organism remained undetectable during the entire 8-week storage period.

Grant and Patterson (1992) inoculated several foodborne pathogens, including *L. monocytogenes* P(10)4 and CRA 711, into each of the components of a roast beef meal (beef, gravy, cauliflower, roast potato and mashed potato) and irradiated them under air with ^{60}Co gamma rays (irradiation temperature was not specified). D_{10} values for *L. monocytogenes* P(10)4 ranged from 0.532 to 0.648 kGy when recovered on tryptone soy-yeast extract agar, and from 0.386-0.598 kGy when a more selective medium (Oxford agar) was used for recovery.

Fu (1994) has recently reported that an electron beam irradiation dose of 1.5 kGy reduced the population of *L. monocytogenes* Scott A in beef steaks by 3 log cycles, while a dose of 2.0 kGy reduced the population by 5 log cycles in ground beef. *Listeria monocytogenes* Scott A was even more sensitive to electron beam irradiation when inoculated into pork chops and hams (Fu et al., 1995), where doses of 1.8-2.0 kGy caused reductions in cell numbers of greater than 4 log cycles. However, after a 7-day period of refrigerated storage followed by a 2-day period of temperature abuse, *L. monocytogenes*

recovered better in the pork products (chops and hams) than in the beef products (steaks and ground beef).

To date the only two studies that have specifically investigated the effects of irradiation temperature on the radiosensitivity of *L. monocytogenes* are those of Monk et al. (1994) and Thayer and Boyd (1995). Monk et al. (1994) inoculated ground beef of two different fat levels (11.1-13.9% and 27.1-27.9%) with a mixture of five strains of *L. monocytogenes*. After inoculation the meat was formed into patties and stored at -16 or 4°C. Samples were gamma irradiated up to a dose of 2.06 kGy. D_{10} values ranged from 0.507 to 0.610 kGy, but it was concluded that neither the temperature nor the fat content had an effect on the radiosensitivity of the organism. Results of this study could have been affected by the transport time between the laboratory (Athens, GA) and the irradiation facility (Mulberry, FL).

Thayer and Boyd (1995) inoculated ground beef with a mixture of four strains of *L. monocytogenes* and irradiated it under vacuum with ^{137}Cs gamma rays at 15, 10, 5, 0, -5, -10, -15, -20, -30, -40, -50, and -60°C. D_{10} values were determined for the -20 to 5°C temperature interval and were found to be significantly affected by it. D_{10} was 0.45 kGy at 0°C, 0.77 kGy at -5°C and 1.21 kGy at -20°C. The log surviving fraction ($\log N/N_o$) of the organism was determined for the entire temperature range (15 to -60°C) for a dose of 2.0 kGy. No change occurred in the surviving fraction between 15 and 0°C; however, a very high increase in resistance occurred between 0 and -5°C and continued to increase, though not as sharply, until the temperature reached -40°C.

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CHAPTER 3. SURVIVAL AND INJURY OF *LISTERIA MONOCYTOGENES, LISTERIA INNOCUA* AND *LISTERIA IVANOVII* IN GROUND PORK FOLLOWING ELECTRON BEAM IRRADIATION[1]

A paper published in the Journal of Food Protection[2]

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ABSTRACT

The sensitivity of five strains of *Listeria* to electron beam irradiation in ground pork as well as the extent of sublethal radiation injury exhibited by each, were investigated. Ground pork was inoculated with one of five strains of *Listeria* and irradiated with from 0 to 1.25 kGy at 0.25 kGy intervals. *Listeria innocua* NADC 2841 was more radiation-resistant (D_{10} = 0.638 kGy) than *L. monocytogenes* NADC 2045 Scott A (D_{10} = 0.447 kGy), *L. monocytogenes* NADC 2783 (a hamburger isolate) (D_{10} = 0.424 kGy), *L. monocytogenes*

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ATCC 15313 (D_{10} = 0.445 kGy) and *L. ivanovii* NADC 3518 (D_{10} = 0.372 kGy), when recovered on tryptic soy agar supplemented with 0.6% yeast extract. D_{10} values for *L. innocua*, *L. ivanovii* and *L. monocytogenes* ATCC 15313 were lower when cells were recovered on modified Oxford medium. These three strains were susceptible to radiation-induced sublethal injury, with the numbers of injured organisms increasing with irradiation dose. The two pathogenic strains of *L. monocytogenes* were not injured significantly at the dose levels used. The results show that the dose range currently being considered by the Food and Drug Administration for the irradiation of beef and pork (1.5 to 4.5 kGy) is adequate for the elimination of *L. monocytogenes* from pork.

INTRODUCTION

Although listeriosis does not afflict the majority of the healthy population, its causative organism, *Listeria monocytogenes*, is considered a pathogen of public health significance. This is due to its ability to affect immunocompromised individuals *(6)* and to reports that it is found in high incidence in a wide variety of meats and meat products in the United States and Europe *(7, 11, 15, 22, 36)*.

The technology of food irradiation has been researched for many decades *(21)*, and it has the potential to eliminate bacterial pathogens, including *L. monocytogenes (10, 29, 32, 34)*. In the United States, legal approval of ionizing radiation to treat foods of animal origin is limited to raw, packaged poultry at 1.5 to 3.0 kGy for the elimination of pathogens *(35)* and pork carcasses or fresh cuts at 0.3 to 1.0 kGy for the destruction of *Trichinella spiralis (8)*. However, the Food and Drug Administration is currently reviewing a petition to allow

the irradiation of fresh or frozen raw meat, at doses of 1.5 to 4.5 kGy, to reduce microbial pathogens and parasites and extend product shelf life *(9)*.

Most food preservation methods, such as heating, freezing, or irradiation, can result in sublethal injury to microorganisms. Injured cells may be unrecoverable with conventional methods that involve the use of selective media *(19)*, thus raising the potential for error in reporting mistakenly low numbers of survivors. Under favorable environmental conditions, sublethally injured cells could repair and grow in the food *(27)*.

This study was conducted (i) to determine the effect of electron beam irradiation on the survival of five strains of *Listeria* in fresh ground pork, and (ii) to evaluate radiation-induced sublethal injury of the five strains by recovering them on both selective and nonselective plating media.

MATERIALS AND METHODS

Cultures

The organisms studied are shown in Table 1. Cultures were maintained by incubating in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) for 24 h at 37°C, followed by storage at 4°C. When needed, cultures were grown in 10 ml of BHI broth for 18 h at 37°C; 0.1 ml of the 18-h cultures was then transferred to 10-ml volumes of BHI broth and grown at 37°C to early stationary phase (12 h for *L. monocytogenes* ATCC 15313; 8 h for all others). Cultures reached densities of 1 to 3 x 10^9 cells per ml.

TABLE 1. *Strains of* Listeria *used in the study.*

Organism	Culture designation[a]	Serotype	β-hemolysis	Source of isolation
L. monocytogenes Scott A	NADC 2045	4b	+	Human cerebrospinal fluid
L. monocytogenes	NADC 2783	4b	+	Hamburger
L. monocytogenes	ATCC 15313	NT[b]	-	Rabbit; type strain
L. innocua	NADC 2841	6a	-	Cow brain; type strain
L. ivanovii	NADC 3518	5	+	Sheep; type strain

[a] NADC: National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA; ATCC: American Type Culture Collection, Rockville, MD.

[b] NT: Not serotyped.

Test sample preparation

Ground lean pork (10.5 to 13% fat) was obtained from the Iowa State University Meat Laboratory, frozen to -30°C and vacuum packaged. It was then irradiated at 30 kGy to achieve sterility and kept frozen at -30°C until needed (not longer than 3 mo.).

Sterile ground pork was thawed at room temperature for 8 h prior to inoculation. An 8-h bacterial suspension in BHI broth was diluted in 0.1% peptone diluent to approx. 2×10^8 cells per ml. Three milliliters of the diluted suspension were added to 300 g of the sterile ground pork (for an inoculum concentration of approx. 2×10^6 cells per g) and mixed in a KitchenAid bowl mixer (KitchenAid, Inc., St. Joseph, MI) with a flat beater attachment for 2 min. Portions of inoculated meat (25 g) were packed into sterile 60 by 15 mm polystyrene petri dishes (Fisher Scientific Co., Pittsburgh, PA); lids were placed and held with strips of

Parafilm laboratory film (American Can Co., Neenah, WI) wrapped around the periphery of the plates. Samples were kept refrigerated at 4°C overnight (15 to 16 h) prior to irradiation.

Irradiation and dosimetry

Samples were irradiated at the Iowa State University Linear Accelerator Facility, which is equipped with an MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France). Samples were irradiated in duplicate at six target average dose levels (0, 0.25, 0.50, 0.75, 1.00, and 1.25 kGy) in electron beam mode at an energy level of 10 MeV and dose rates of 17 kGy/min (0.25 kGy) and 29 kGy/min (0.50-1.25 kGy). Dose rates differed due to the need to operate the linear accelerator at a low power level to obtain the lower dose. Target average doses represent an arithmetic average of the top and bottom surface doses of the test samples.

Absorbed radiation doses were determined by the use of 5 mm (dia.) by 5 mm (length) dosimeter alanine pellets (Bruker Analytische Messtechnik, Rheinstetten, Germany) placed on the top and bottom surfaces of one of the duplicate petri dishes. Immediately following irradiation, absorbed doses were determined by electron paramagnetic resonance on a Bruker EMS 104 EPR Analyzer. The arithmetic average of the top and bottom surface readings was taken as the average absorbed dose.

Determination of survivors

All samples were analyzed within 3 h following irradiation. Samples were diluted 1:10 in 0.1% peptone diluent and homogenized in a Stomacher 400 Lab Blender (Seward

Medical, London, UK) for 2 min. Serial dilutions in 0.1% peptone were then prepared and 0.1-ml volumes of the appropriate dilution were surface plated in duplicate onto plates of tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) (Difco) for the enumeration of uninjured and sublethally injured cells and on modified Oxford medium (MOX) (Difco) for the enumeration of uninjured cells. Plates were incubated aerobically at 37°C for 48 h (TSAYE) or 72 h (MOX). After incubation, colonies on plates were counted and recorded as colony count per gram of sample.

Calculation of D_{10} values

D_{10} values were determined separately for the TSAYE and MOX plate counts by plotting the log CFU/g as a function of irradiation dose (in kGy) to obtain a regression curve, where the D_{10} value is the reciprocal of the absolute value of the slope of the curve.

Determination of sublethal injury

For each replication, linear regression equations were used to determine the number of cells recovered on each medium. Percent sublethal injury for each strain at each dose used (0 to 1.25 kGy) was then calculated as:

$$\frac{\text{(CFU/g on TSAYE) - (CFU/g on MOX)}}{\text{CFU/g on TSAYE}} \times 100$$

Statistical analysis

D_{10} value data were analyzed as a split-plot design with whole units arranged in a completely randomized fashion *(3, 31)*, with strain of *Listeria* as a whole-plot factor and plating medium as a sub-plot factor. Sublethal injury data were analyzed as a 5 by 6 factorial (5 strains by 6 doses). The analyses were done using release 6.07 of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC). The ANOVA procedure was used to obtain the analyses of variance and, when these showed significant treatment effects ($P < 0.05$), least significant differences (LSD) were calculated to identify significant differences ($P = 0.05$) among treatment means *(31)*. The entire experiment was replicated three times.

RESULTS

Mean radiation survivor curves for four of the five strains of *Listeria* tested, as recovered on TSAYE or MOX medium, are shown in Fig. 1. Determination coefficients of the curves used to construct the mean curves were consistently high ($r^2 \geq 0.96$), indicating that, in all cases, most of the variation in numbers of survivors is attributable to the irradiation dose. D_{10} values among the five strains, recovered on TSAYE or MOX, differed (Fig. 2). The analysis of variance of these data revealed that the main effects of both factors (strain, recovery medium), as well as the interaction between them, were significant ($P < 0.01$). *Listeria innocua* (D_{10} = 0.638 kGy) was significantly more radiation-resistant (LSD at $P = 0.05$) than all three strains of *L. monocytogenes* (D_{10} = 0.424-0.447 kGy) and *L. ivanovii* (D_{10} = 0.378 kGy), as recovered on TSAYE. D_{10} values were generally lower when

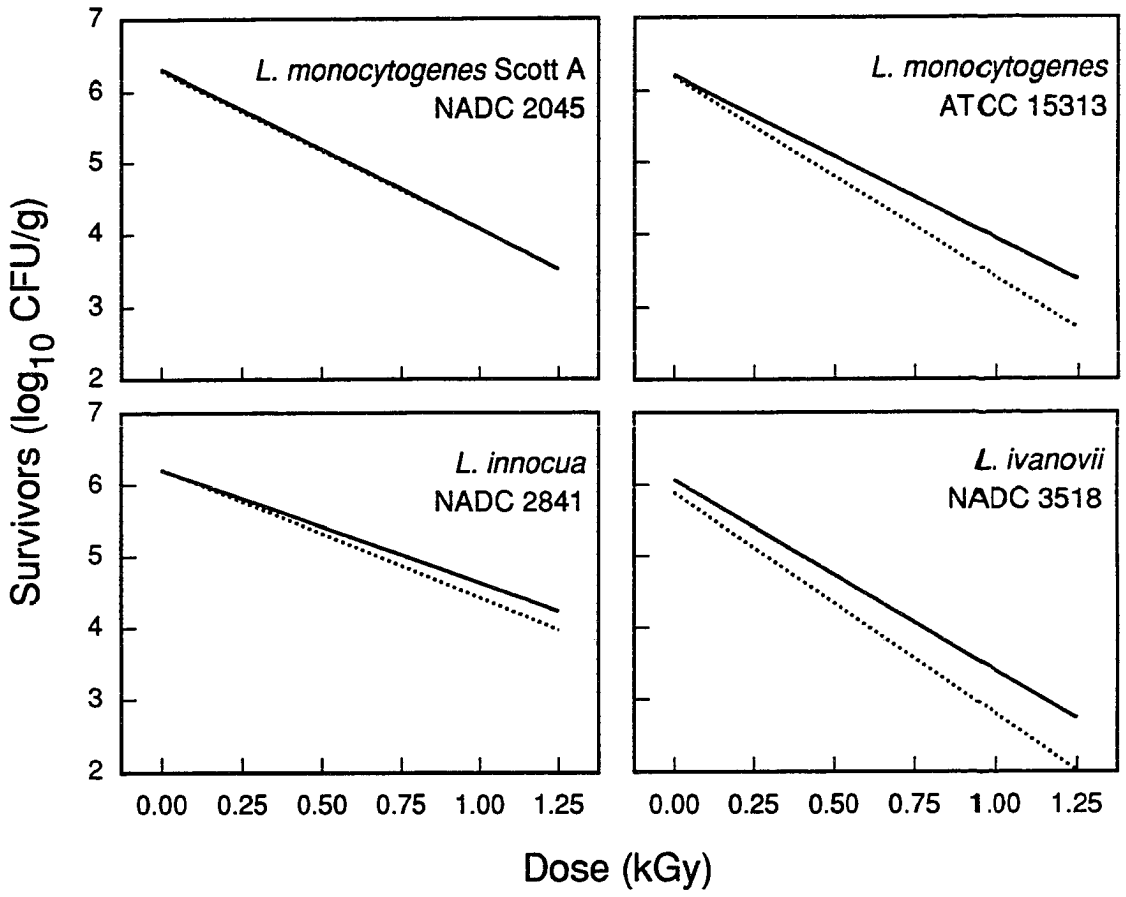

FIGURE 1. *Mean survivor curves of four strains of* Listeria *in ground pork, recovered on TSAYE (solid lines) and MOX medium (dashed lines) following electron beam irradiation. Curves represent averages of three replications.*

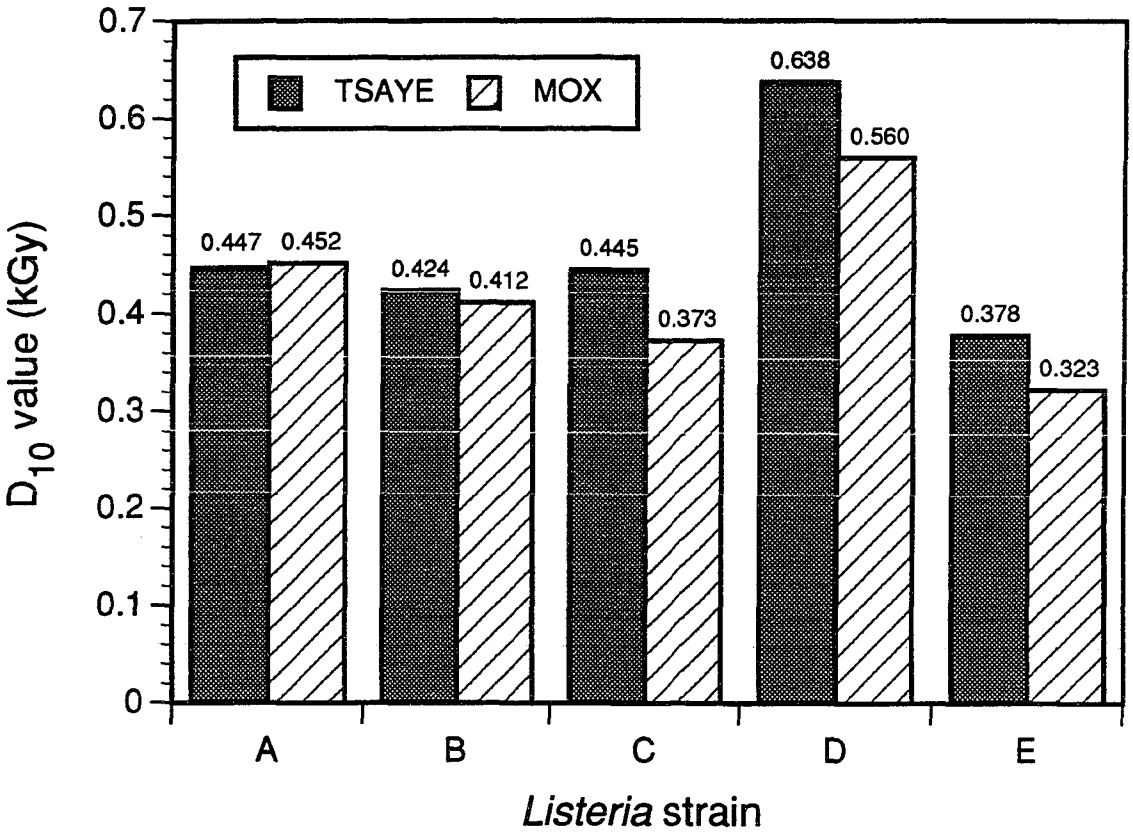

FIGURE 2. *D_{10} values of five strains of* Listeria *in ground pork following electron beam irradiation, as affected by recovery medium (TSAYE or MOX). $LSD_{(0.05)}$ = 0.093 kGy (across strains for one medium) and 0.031 kGy (within strains). A:* L. monocytogenes *NADC 2045 Scott A, B:* L. monocytogenes *NADC 2783 (hamburger isolate), C:* L. monocytogenes *ATCC 15313, D:* L. innocua *NADC 2841, E:* L. ivanovii *NADC 3518.*

cells were recovered on MOX medium, although this difference was significant (LSD at $P = 0.05$) only for *L. innocua*, *L. ivanovii*, and *L. monocytogenes* ATCC 15313 (Fig. 2).

The analysis of variance of the sublethal injury data revealed that the main effects due to strain and dose, as well as the strain-dose interaction, were significant ($P < 0.01$). *Listeria monocytogenes* ATCC 15313, *L. innocua* and *L. ivanovii* were susceptible to radiation-induced sublethal injury (LSD at $P = 0.05$), whereas the two hemolytic strains of *L. monocytogenes* were not (Fig. 3). Unirradiated (0 kGy) cells of *L. ivanovii* showed significant ($P < 0.05$) sublethal injury. Although *L. monocytogenes* ATCC 15313 did not exhibit this result, it did, upon irradiation, reach levels of sublethal injury which eventually equaled those of *L. ivanovii* (77.4% and 78.9%, respectively, at 1.25 kGy).

DISCUSSION

The D_{10} values determined in this study for *L. monocytogenes* in irradiated ground pork (Fig. 2) are consistent with those reported elsewhere *(4, 5, 13, 14, 18, 23, 24, 30)*. However, these D_{10} values appear to be affected by the type of food medium as well as the specific strain of *L. monocytogenes* evaluated. D_{10} values of 0.4 to 1.0 kGy have been reported for various strains of *L. monocytogenes* irradiated in various food media *(4, 13, 14, 18, 23, 24, 30)*. Irradiation in all of these studies was done by either gamma rays from ^{60}Co or electron beam. Despite the fundamental differences between these two types of ionizing energy, their lethal effects on microorganisms are considered to be similar *(5)*. The D_{10} values for *L. monocytogenes* determined in this study (0.424 to 0.447 kGy) indicate that the irradiation dose range currently allowed for the destruction of *Trichinella spiralis* in pork (0.3 to 1

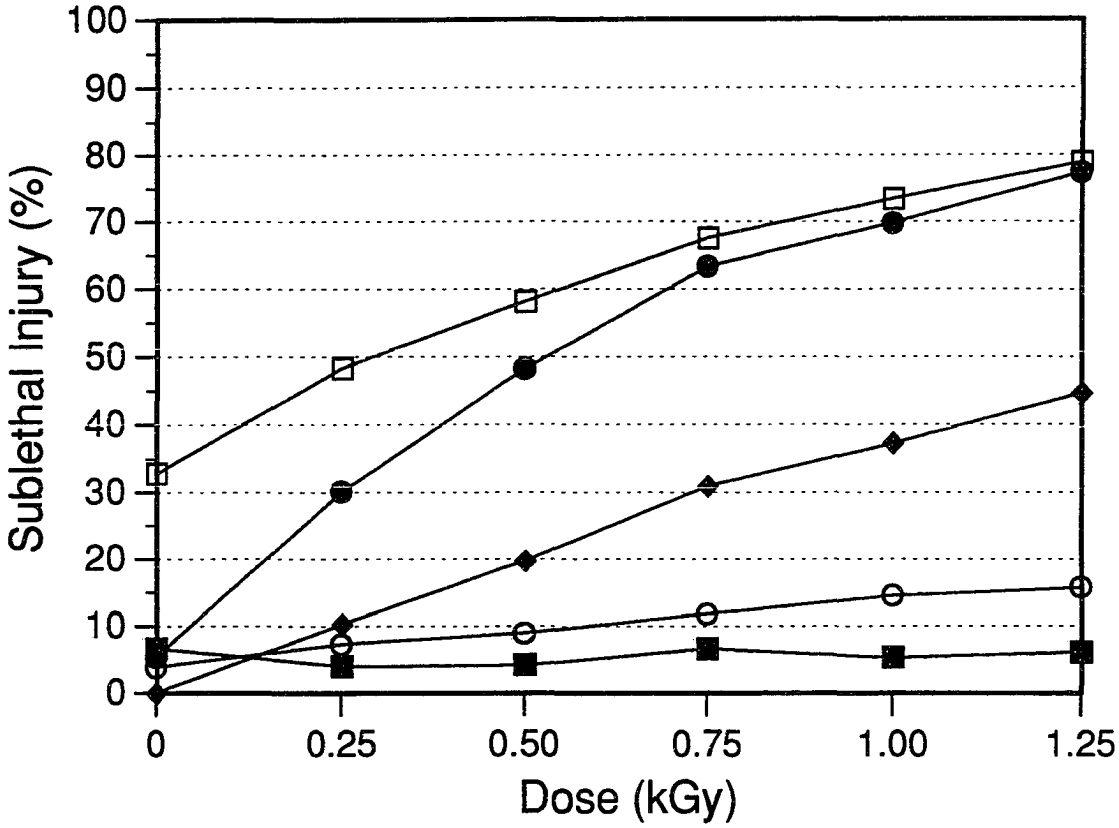

FIGURE 3. *Sublethal injury of five strains of* Listeria *in ground pork following electron beam irradiation.* $LSD_{(0.05)}$ *= 15.6 %.* ■ L. monocytogenes *NADC 2045 Scott A,* ○ L. monocytogenes *NADC 2783 (hamburger isolate),* ● L. monocytogenes *ATCC 15313,* ◆ L. innocua *NADC 2841,* □ L. ivanovii *NADC 3518.*

kGy) is inadequate for the complete inactivation of *L. monocytogenes*, since it would reduce the numbers by only 0.7 to 2.2 log units. Although this dose range would achieve some destruction, it does not provide an adequate safety margin, since meats contaminated with *L. monocytogenes* could contain higher numbers of the organism than would be inactivated even at 1 kGy *(29)*. However, the dose range in a petition to the FDA *(9)* for the irradiation of both beef and pork (1.5 to 4.5 kGy) would be adequate for the removal of the pathogen from pork, as it would achieve reductions of 3.4 to 10.1 log units, thus providing an acceptable margin of safety.

Nonpathogenic *L. innocua*, which has been shown in some surveys to be more prevalent in meats and meat products than *L. monocytogenes (7, 15, 29, 33)*, has a greater radiation resistance (D_{10} = 0.638 kGy). It has also been shown that *L. innocua* can sometimes outgrow *L. monocytogenes* and make its recovery more difficult *(25)*. It is therefore important that any listeriae isolated from irradiated foods be identified to the species level, in order to avoid losses due to unnecessary recalls and/or remedial actions.

Although it is not as prevalent in meats as *L. monocytogenes* and *L. innocua*, *L. ivanovii* is important, since it has been recognized as an occasional human pathogen *(2, 17, 28)*. Results in this study indicate that *L. ivanovii* NADC 3518 (D_{10} = 0.378 kGy) is more sensitive to electron beam irradiation than *L. innocua* NADC 2841 (D_{10} = 0.638 kGy), but not more sensitive than the three strains of *L. monocytogenes*. It is concluded that irradiation doses that would eliminate *L. monocytogenes* would also be adequate for the destruction of *L. ivanovii*.

When bacterial cells are exposed to a sublethal treatment, such as heating, freezing, or

irradiation, they may experience sublethal metabolic injury, which may prevent their growth and recovery on selective media on which they would normally grow *(19)*. This failure of the cells to repair sublethal damage could, therefore, lead to mistakenly low cell numbers. Previous studies have evaluated the efficacy of selective plating media for the isolation of *L. monocytogenes* following irradiation. Patterson *(24)* reported that D_{10} values for *L. monocytogenes* in poultry mince and phosphate-buffered saline were lower when Listeria selective (Oxford) agar was used as the recovery medium instead of tryptone soya-yeast extract agar. In the above-mentioned studies of Grant and Patterson *(13, 14)*, D_{10} values were significantly lower when the more selective Oxford agar was used for recovery. In this study D_{10} values were higher on TSAYE than on MOX medium only for the nonhemolytic *L. monocytogenes* ATCC 15313, *L. innocua*, and *L. ivanovii*, indicating that these cells experienced radiation-induced sublethal injury. This was not the case, however, for the two pathogenic strains of *L. monocytogenes*. Therefore, it is concluded that MOX medium is appropriate for the isolation of the latter. However, since the ground pork used in this study was pre-sterilized and inoculated with high numbers of listeriae, this finding does not obviate the need for selective enrichment prior to plating for *L. monocytogenes*. The presence of a competing microflora, coupled with low numbers of *L. monocytogenes*, could complicate the recovery of the organism and make direct plating ineffective *(12)*. Since the normal hemolytic strains of *L. monocytogenes* used in this study were not susceptible to sublethal radiation injury (Fig. 3), the results of this study suggest that the use of presently accepted methods for the recovery of *L. monocytogenes* may be just as acceptable for irradiated ground pork than they would be for other foods. It is recommended, however, that

other strains of *L. monocytogenes*, as well as different selective plating media, be tested before widespread recommendations are made.

The plot of sublethal injury versus irradiation dose (Fig. 3) shows the actual levels of sublethal radiation injury of the surviving populations at each absorbed dose. Cells of *L. ivanovii* NADC 3518 showed significant sublethal injury at every dose, even prior to irradiation (32.8% at 0 kGy). This sublethal injury must have occurred upon their addition to the meat and suggests that they are highly sensitive to relatively minor environmental changes, which could partly explain their greater degree of radiation-induced sublethal injury. *Listeria monocytogenes* ATCC 15313 and *L. innocua* showed significant levels of sublethal injury upon irradiation, the extent of which was dose-dependent, with greater injury at higher doses. Only the two hemolytic strains of *L. monocytogenes* did not show sublethal radiation injury within the study's dose range.

Deoxyribonucleic acid (DNA) is considered the most important cellular target for the inactivation of microorganisms by ionizing radiation *(16)*. Ionizing energy primarily causes single- and double-strand breaks in the DNA molecule, thus disrupting DNA replication *(16, 26)*. In addition, the indirect effects of radiation-induced radicals may affect DNA, as well as other cell components *(16)*. The first mechanism used by bacterial cells to repair damaged DNA is believed to be the direct rejoining of single-strand breaks by DNA ligase *(16)*. Previous studies have shown that single-strand breaks can be repaired very quickly, maybe as soon as 30 to 40 s after irradiation *(20)*. While not considered lethal for radiation-resistant bacteria, these breaks may be lethal for radiation-sensitive cells that lack appropriate repair systems *(16)*. Should direct rejoining of single-strand breaks fail, the cell

must resort to other more complex repair processes, such as excision repair, induced repair (or SOS response), recombinational repair or some other mechanism *(1, 16, 26)*. Under the reasonable assumption that the mechanisms and extent of radiation damage were similar for all five strains of *Listeria* used in this study (due to their close relatedness), the higher D_{10} value on TSAYE of *L. innocua* (Fig. 2) indicates that this strain underwent less radiation-induced lethal injury than the other four strains. When recovered on MOX medium, the D_{10} value of *L. innocua* was once again higher than those of the other four strains (Fig. 2). Since cells recovered on MOX medium are considered normal (i.e., uninjured) at the time of analysis, this suggests that *L. innocua* may possess a superior mechanism for the immediate and complete repair of damaged DNA.

In this study, sublethally injured cells are those that, at the time of analysis, have lost the ability to multiply in MOX medium but not in TSAYE. These cells have obviously undergone more severe damage, which probably requires more complex repair mechanisms and hence cannot be repaired until environmental conditions become more favorable. The two virulent strains of *L. monocytogenes* obviously possess very efficient mechanisms for the repair of their sublethal damage, given the fact that they showed virtually no sublethal injury at the time of analysis as opposed to the other three strains (Fig. 3). Direct comparisons of the levels of sublethal injury of different strains must be done carefully, however, since the expression (lethal versus sublethal) of radiation-induced DNA damage is greatly dependent on the effectiveness of the organism's repair systems *(1)*. For example, since *L. innocua* has the most efficient overall repair mechanisms of all strains tested, some or all of the damage expressed in it as sublethal could very well have been expressed as

lethal in the other more radiosensitive strains.

Although our results do not identify mechanisms of radiation injury and subsequent repair, the observed differences in radiosensitivity of several strains of *Listeria* indicate differences in the effectiveness of their postirradiation repair mechanisms following irradiation in raw ground pork. It is important to stress that all the factors and molecular mechanisms involved in the response of an organism to ionizing radiation, as well as how they interact with each other, are not known. Further studies on the nature of radiation injury of *Listeria* species are suggested to better understand this phenomenon at the cellular and molecular level.

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CHAPTER 4. SENSITIVITY OF *LISTERIA MONOCYTOGENES* TO IRRADIATION IN GROUND BEEF AS AFFECTED BY TYPE OF RADIATION (X-RAYS OR ELECTRONS), PRODUCT TEMPERATURE, PACKAGING ATMOSPHERE, AND RECOVERY MEDIUM

A paper to be submitted to the Journal of Food Science

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ABSTRACT

The effects of type of radiation (X-rays and electrons), product temperature (4, -20, and -78°C), packaging atmosphere (air, vacuum, and nitrogen), and recovery medium [tryptic soy agar + 0.6% yeast extract (TSAYE), modified Oxford medium (MOX)] on the radiation sensitivity of a mixture of strains of *Listeria monocytogenes* (Scott A, Murray B, and V7) in ground beef were investigated. Irradiation doses ranged from 0 to 2.0 kGy in 0.5 kGy intervals. Survival curves shoulders were observed under vacuum and nitrogen, regardless of temperature. D_{10} values were independent of packaging atmosphere and increased with

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decreasing temperature (except for X-rays from -20 to -78°C). They were 0.417 to 0.486 kGy at 4°C, 0.527 to 0.668 kGy at -20°C, and 0.648 to 0.704 kGy and 0.833 to 0.885 kGy at -78°C for X-rays and electrons, respectively. TSAYE yielded higher D_{10} values than MOX medium regardless of irradiation conditions. Sublethal injury increased at doses higher than 0.5 kGy and did so more slowly at -78°C than at -20 and 4°C.

INTRODUCTION

Although most outbreaks of foodborne listeriosis have been associated with non-meat foods such as coleslaw (Schlech et al., 1983), milk (Fleming et al., 1985), and cheese (Linnan et al., 1988; Bille, 1990), meat products such as undercooked chicken, unheated hot dogs, turkey frankfurters, sliced turkey ham, pork sausage and ground beef have been clearly implicated in sporadic cases of the disease (Schwartz et al., 1988; Wenger et al., 1991; Pinner et al., 1992). The causative organism, *Listeria monocytogenes*, has been isolated from a variety of commercial meats, poultry and their products (Farber et al., 1989; Genigeorgis et al., 1989; Grau and Vanderlinde, 1992; MacGowan et al., 1994; Wang and Muriana, 1994).

Several studies have shown that gamma or electron beam irradiation can effectively eliminate *L. monocytogenes* from foods (El-Shenawy et al., 1988; Huhtanen et al., 1989; Patterson, 1989; Farag et al., 1990; Grant and Patterson, 1992; Shamsuzzaman et al., 1992; Varabioff et al., 1992; Monk et al., 1994; Fu et al., 1995; Thayer and Boyd, 1995). Several factors, such as the temperature of the product during irradiation, the packaging atmosphere and the plating medium used for isolation, can affect the response of microorganisms to

ionizing radiation (Urbain, 1986). Varabioff et al. (1992) observed that irradiation of raw whole chickens under vacuum exerted a protective effect on *L. monocytogenes*. El-Shenawy et al (1988), Patterson (1989) and Grant and Patterson (1992) found D_{10} values of *L. monocytogenes* to be higher in nonselective than in selective media. To date, only two studies (Monk et al., 1994; Thayer and Boyd, 1995) have specifically investigated the response of *L. monocytogenes* to irradiation at various temperatures. Monk et al. (1994) irradiated ground beef at -17 to -15°C and at 3 to 5°C and did not find a significant effect of temperature on the radiosensitivity of the organism. On the other hand, Thayer and Boyd (1995), also working with ground beef, found the response of *L. monocytogenes* to be temperature-dependent in the range of 0 to -40°C, with increasing radioresistance as the temperature was decreased.

This study was done to determine the extent to which the radiation sensitivity and radiation-induced injury of *L. monocytogenes* in raw ground beef are affected by type of radiation, irradiation temperature, packaging atmosphere or recovery medium.

MATERIALS AND METHODS

Cultures

The strains of *L. monocytogenes* studied are described in Table 1. Cultures were maintained by incubating in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) for 24 h at 37°C, followed by storage at 4°C. When needed, all three cultures were grown separately in 10 ml of BHI broth for 18 h at 37°C; 0.1 ml of each 18-h culture was then transferred to a 10-ml volume of BHI broth and grown at 37°C to early stationary

Table 1—Strains of *Listeria monocytogenes* used in the study[a]

Strain	Culture designation	Serotype	Source of isolation
Scott A	NADC 2045	4b	Human cerebrospinal fluid
Murray B	NADC 2099	4b	Human
V7	NADC 2100	4b	Milk

[a] NADC: National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA.

phase (8 h). Cultures reached densities of 1 to 3 x 10^9 cells per ml. The contents of all three BHI tubes were then mixed together prior to inoculation of the meat.

Test sample preparation

Ground lean beef (66% moisture, 21% protein, 13% fat) was obtained from the Iowa State University Meat Laboratory, frozen to -30°C and vacuum packaged. It was then irradiated at 40 kGy to achieve sterility and kept frozen at -30°C until needed (not longer than 3 mo.).

Sterile ground beef was thawed at room temperature for 8 h prior to inoculation. Three ml of the mixed culture of *L. monocytogenes* were then added to 300 g of the sterile ground beef (for an inoculum concentration of approx. 2 x 10^7 cells per g) and mixed in a Stomacher 400 Lab Blender (Seward Medical, London, UK) for 2 min. Ten-gram portions of inoculated meat were then packed into each of 30 sterile 50 by 9 mm polystyrene petri

dishes (Gelman Sciences, Inc., Ann Arbor, MI); on the edges of which four equidistant slits had been previously cut with a hot knife to allow for proper air evacuation during packaging. Each of these plates (without its lid) was then placed inside a 6" x 8" vacuum packaging pouch (861 material grade, Weldon, Inc., Oshkosh, WI). Ten of these pouches were then vacuum packaged, 10 were packaged in a 100% nitrogen atmosphere and the remaining 10 were heat-sealed under air. Packaging and heat-sealing were done with a CVP Model A300 Fresh Vac modified atmosphere packaging machine (CVP Systems, Inc., Downers Grove, IL). Each of the three groups of 10 pouches was then subdivided into two subgroups of five pouches, each of which was stored overnight (15 to 16 h) at a specific temperature. Storage temperatures were 4, -20 and -78°C.

Irradiation and dosimetry

Samples were irradiated at the Iowa State University Linear Accelerator Facility, which is equipped with an MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France). To minimize temperature changes during irradiation, all samples were irradiated in styrofoam chests containing ice (4°C samples), ice on top of dry ice (-20°C samples) or dry ice (-78°C samples). Samples were irradiated at five target average dose levels (0, 0.5, 1.0, 1.5, and 2.0 kGy) using accelerated electrons (7.5 MeV, 3.5 kW, 37 kGy/min) or X-rays (5 MeV, 12 kW, 0.069 kGy/min). X-rays were generated by placing a stainless steel target in the path of the electron beam. Target average doses represent an arithmetic average of the top and bottom surface doses of the test samples.

Absorbed radiation doses were determined by the use of 5 mm (dia.) by 5 mm (length)

dosimeter alanine pellets (Bruker Analytische Messtechnik, Rheinstetten, Germany) placed on the top and bottom surfaces of one of the petri dishes (a dummy sample kept at room temperature to avoid reading incorrect absorbed doses due to extremely cold temperatures). Immediately following irradiation, absorbed doses were determined by electron paramagnetic resonance on a Bruker EMS 104 EPR Analyzer. The arithmetic average of the top and bottom surface readings was taken as the average absorbed dose.

Immediately following irradiation, samples were stored under refrigeration and transported to the laboratory for analysis.

Determination of survivors

All samples were analyzed within 3 h following irradiation. Samples were diluted 1:5 in 0.1% peptone diluent and homogenized in a Stomacher 400 Lab Blender for 2 min. Serial dilutions in 0.1% peptone were then prepared and 0.1-ml volumes of the appropriate dilution were surface plated in duplicate onto plates of tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) (Difco) for the enumeration of uninjured and sublethally injured cells and on modified Oxford medium (MOX) (Difco) for the enumeration of uninjured cells. Plates were incubated aerobically at 37°C for 48 h (TSAYE) or 72 h (MOX). After incubation, colonies on plates were counted and recorded as colony count per gram of sample.

Survival curves and calculation of D_{10} values

D_{10} values were determined separately for the TSAYE and MOX plate counts by plotting the log CFU/g as a function of irradiation dose (in kGy) to obtain a regression curve, where the D_{10} value is the reciprocal of the absolute value of the slope of the curve. A second set of D_{10} values was calculated for the target dose interval of 0.5 to 2.0 kGy (i.e., ignoring the 0 kGy dose), in order to more accurately account for survival curve shoulders.

Calculation of extrapolation numbers and quasi-threshold values

The extrapolation number, *n*, originally described by Alper et al. (1960), and the quasi-threshold value, D_q (Bedford, 1982; Grosch and Hopwood, 1979), are mathematical descriptions of survival curve shoulders. Therefore, they were calculated based on the survival curves obtained for the 0.5 to 2.0 kGy interval. For a given curve, *n* is the intercept of the exponential portion of the curve on the survival axis (expressed as the surviving fraction, N/N_0) at zero dose (Bedford, 1982). D_q corresponds to the dose at which the exponential portion of the curve intercepts 100% survival (surviving fraction of 1.0). Both of these terms are graphically illustrated in Fig. 1.

Determination of sublethal injury

For each replication, linear regression equations for the 0.5 to 2.0 kGy interval were used to determine the number of cells recovered (as CFU) on each medium at each target dose (0.5, 1.0, 1.5, 2.0 kGy). Percent sublethal injury at each target dose was then calculated as:

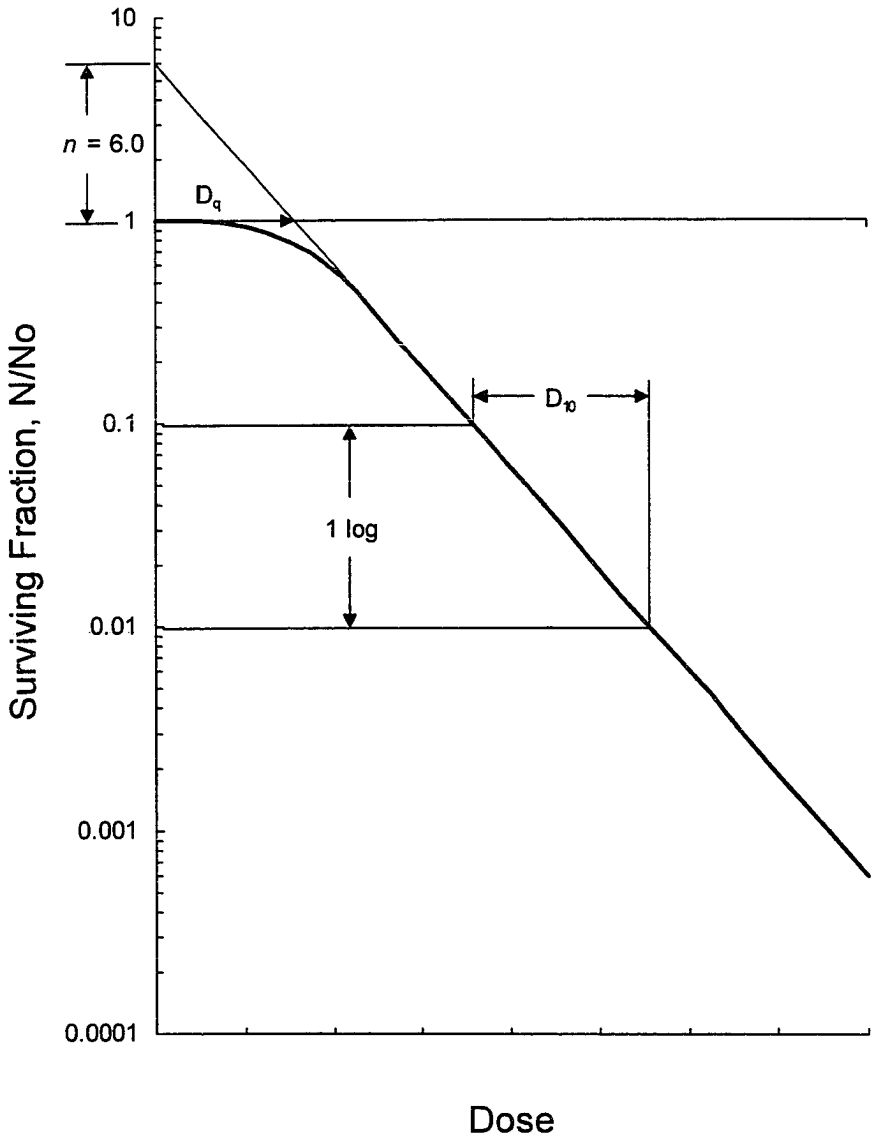

Fig. 1—A typical radiation dose-response survival curve, illustrating the decimal reduction value, D_{10}, the quasi-threshold value, D_q, and the extrapolation number, *n*.

$$\frac{\text{(CFU/g on TSAYE) - (CFU/g on MOX)}}{\text{CFU/g on TSAYE}} \times 100$$

Sublethal injury at 0 kGy was calculated similarly, but using the actual CFUs obtained.

Statistical analysis

Data for D_{10} values (separately for 0 to 2.0 kGy and 0.5 to 2.0 kGy intervals), extrapolation numbers and quasi-threshold values were analyzed as randomized block, split-plot designs (Damon and Harvey, 1987), with radiation type (2 levels: X- rays, electrons), packaging atmosphere (3 levels: air, vacuum, nitrogen) and product temperature (3 levels: 4, -20, -78°C) as whole plot factors, plating medium (2 levels: TSAYE, MOX) as a subplot factor, and replications as blocks (*3, 31*). Extrapolation numbers, being logarithmic, were linearized prior to statistical analysis as described below. Sublethal injury data were analyzed as a 3^2 by 2 by 5 factorial (3 levels of atmosphere, 3 levels of temperature, 2 levels of type of radiation, 5 levels of dose) arranged in a completely randomized block design, with replications as blocks. The analyses were done using release 6.09 of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC). The ANOVA procedure was used to obtain the analyses of variance and, when these showed significant treatment effects ($P < 0.05$), least significant differences (LSD) were calculated to identify significant differences ($P = 0.05$) among treatment means (*31*). The entire experiment was replicated three times.

Treatment of extrapolation numbers

Prior to statistical analysis, extrapolation number (n) values were linearized by logarithmic transformation. Since some of the values were small, 1 was added to each number prior to taking the logarithm, as suggested by Steel and Torrie (1980). The analysis of variance and LSDs were then calculated using the transformed (log [n+1]) data. Means were calculated using log n values and were transformed back to the original scale by calculating the antilogarithms of the corresponding log n means. The LSDs obtained with the transformed data were the equivalent of least significant ratios (LSR) of means that were transformed back to the original scale (Steel and Torrie, 1980).

RESULTS

D_{10} values

The determination coefficients (r^2) of the calculated regression curves were consistently higher than 0.94 (95% of the time) and never lower than 0.88. The analysis of variance (ANOVA) (not shown) of D_{10} values for the 0 to 2.0 kGy interval (Table 2) indicated that the main effects of the three whole plot factors (radiation type, temperature and packaging atmosphere) and of the subplot factor (plating medium) were significant, as well as the radiation type x temperature interaction. When D_{10} values were calculated for the 0.5 to 2.0 kGy interval (Table 3), ANOVA results were similar except that there was no longer a significant ($P > 0.05$) main effect of packaging atmosphere. Overall, D_{10} values were higher ($P < 0.05$) with recovery on TSAYE as opposed to MOX medium.

Further examination of the significant radiation type x temperature interaction (for

Table 2—D_{10} values (in kGy) of *Listeria monocytogenes* in irradiated ground beef as affected by type of radiation, temperature, packaging atmosphere and plating medium. Calculated for 0 to 2.0 kGy interval[a,b]

		TSAYE			MOX		
		Packaging Atmosphere			Packaging Atmosphere		
Radiation type	Temperature (°C)	Air	Vacuum	Nitrogen	Air	Vacuum	Nitrogen
X-ray	4	0.405	0.540	0.498	0.362	0.481	0.454
	-20	0.582	0.673	0.596	0.506	0.613	0.554
	-78	0.707	0.711	0.753	0.683	0.692	0.708
Electron beam	4	0.388	0.510	0.475	0.364	0.484	0.456
	-20	0.634	0.641	0.669	0.578	0.586	0.618
	-78	0.857	0.922	0.876	0.821	0.848	0.822

[a] Means are averages of three replications.

[b] $LSD_{(0.05)}$ = 0.128 kGy (within the same medium); 0.031 kGy (across media).

Table 3—D_{10} values (in kGy) of *Listeria monocytogenes* in irradiated ground beef as affected by type of radiation, temperature, packaging atmosphere and plating medium. Calculated for 0.5 to 2.0 kGy interval[a,b]

		Plating Medium					
		TSAYE			MOX		
		Packaging Atmosphere			Packaging Atmosphere		
Radiation type	Temperature (°C)	Air	Vacuum	Nitrogen	Air	Vacuum	Nitrogen
X-ray	4	0.425	0.486	0.446	0.366	0.432	0.400
	-20	0.594	0.661	0.527	0.502	0.599	0.486
	-78	0.652	0.648	0.704	0.621	0.623	0.664
Electron beam	4	0.417	0.452	0.435	0.380	0.426	0.409
	-20	0.668	0.600	0.612	0.585	0.538	0.563
	-78	0.876	0.885	0.833	0.848	0.788	0.760

[a] Means are averages of three replications.

[b] $LSD_{(0.05)}$ = 0.134 kGy (within the same medium); 0.039 kGy (across media).

both dose intervals) revealed that, averaged over atmosphere and plating media, D_{10} values were significantly ($P < 0.05$) higher for electrons than for X-rays at -78°C, but not at -20 or 4°C (Fig. 2). The same analysis also showed that, for electrons, the observed increase in D_{10} value associated with decreasing temperature was significant ($P < 0.05$) at every temperature. For X-rays, however, there was a significant ($P < 0.05$) increase between 4 and -20°C, but not between -20 and -78°C.

Survival curve shouldering

The ANOVA of log (n+1) (not shown) revealed that the main effects of packaging atmosphere and plating medium and the packaging atmosphere x temperature interaction were significant ($P < 0.05$). Overall, extrapolation numbers (Table 4) of *L. monocytogenes* survival curves were significantly higher ($P < 0.05$) when cells were recovered on MOX medium, as opposed to TSAYE. When averaged over radiation type and plating medium, extrapolation numbers under air were significantly ($P < 0.05$) lower than under vacuum and nitrogen at 4°C and lower than under nitrogen at -20°C (Fig. 3). No significant differences were observed at -78°C. Extrapolation numbers were also significantly ($P < 0.05$) lower at -78°C than at 4°C with irradiation under both vacuum and nitrogen (Fig. 3). The clear tendency, therefore, is for extrapolation numbers to decrease with decreasing temperature when irradiation is done under vacuum or nitrogen.

The ANOVA of D_q values showed significant ($P < 0.05$) main effects of packaging atmosphere and plating medium only. D_q values (Table 5) under nitrogen and vacuum were higher ($P < 0.05$) than under air, and were also, overall, higher ($P < 0.05$) with MOX

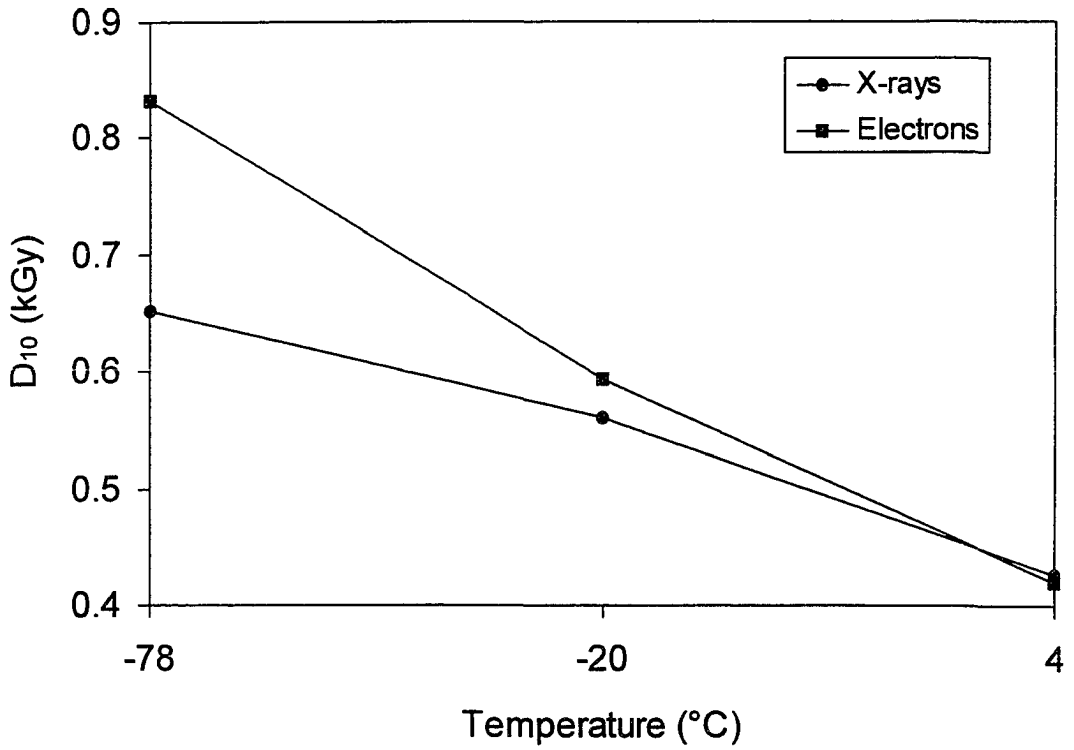

Fig. 2—Examination of significant radiation type x temperature interaction for D_{10} values. Values were calculated for the 0.5 to 2.0 kGy dose interval, and averaged over packaging atmospheres and plating media.

Table 4—Extrapolation numbers (n) of survival curves of *Listeria monocytogenes* in irradiated ground beef as affected by type of radiation, temperature, packaging atmosphere and plating medium. Calculated for 0.5 to 2.0 kGy interval[a,b]

		Plating Medium					
		TSAYE			MOX		
		Packaging Atmosphere			Packaging Atmosphere		
Radiation type	Temperature (°C)	Air	Vacuum	Nitrogen	Air	Vacuum	Nitrogen
X-ray	4	0.58	2.63	3.16	0.95	3.09	4.17
	-20	0.78	1.41	3.72	1.07	1.66	4.68
	-78	1.70	1.95	1.66	1.86	2.14	1.70
Electron beam	4	0.49	4.17	2.75	0.65	4.57	4.07
	-20	0.76	1.86	2.00	0.93	2.40	2.34
	-78	0.87	1.29	1.41	0.79	1.62	1.74

[a] $LSR_{(0.05)} = 1.91$ (within the same medium); 1.16 (across media).

[b] n means are the antilogarithms of the corresponding log n means, and represent geometric means.

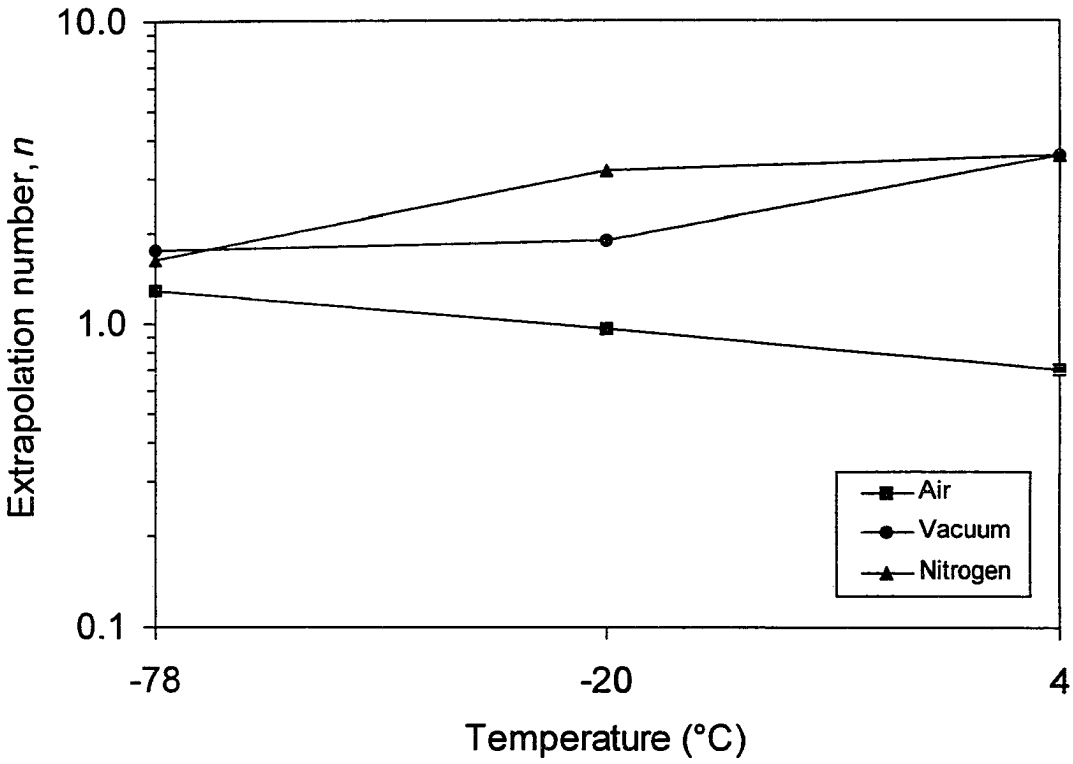

Fig. 3—Examination of significant packaging atmosphere x temperature interaction for extrapolation numbers. Means averaged over radiation type and plating medium.

Table 5—D_q values (in kGy) of *Listeria monocytogenes* in irradiated ground beef as affected by type of radiation, temperature, packaging atmosphere and plating medium. Calculated for 0.5 to 2.0 kGy interval[a,b]

		Plating Medium					
		TSAYE			MOX		
		Packaging Atmosphere			Packaging Atmosphere		
Radiation type	Temperature (°C)	Air	Vacuum	Nitrogen	Air	Vacuum	Nitrogen
X-ray	4	-0.109	0.203	0.223	-0.017	0.207	0.247
	-20	-0.060	0.061	0.284	0.021	0.086	0.298
	-78	0.155	0.186	0.151	0.168	0.202	0.144
Electron beam	4	-0.137	0.277	0.187	-0.074	0.283	0.240
	-20	-0.112	0.158	0.185	-0.025	0.200	0.209
	-78	-0.055	0.087	0.116	-0.083	0.158	0.178

[a] Means are averages of three replications.

[b] $LSD_{(0.05)}$ = 0.211 kGy (within the same medium); 0.077 kGy (across media).

medium than with TSAYE.

Sublethal injury

Overall, sublethal injury results (Fig. 4) showed a lag between 0 and 0.5 kGy, after which values became increasingly higher with radiation dose. The ANOVA (not shown) showed significant ($P < 0.05$) radiation type x temperature and radiation dose x temperature interactions. Further analysis of the radiation type x temperature interaction showed that, for X-rays, sublethal injury was significantly ($P < 0.05$) lower at -78°C than at -20 and 4°C. For the significant radiation dose x temperature interaction, significant ($P < 0.05$) simple effects of temperature were found at doses of 1.5 and 2.0 kGy, with sublethal injury being lower at -78°C than at -20 and 4°C at both doses. There was also a significant ($P < 0.05$) main effect of packaging atmosphere, with sublethal injury being higher, overall, under air than under vacuum or nitrogen.

DISCUSSION

D_{10} values of *Listeria monocytogenes* in irradiated ground beef were calculated for two dose intervals (0 to 2.0 kGy and 0.5 to 2.0 kGy) to observe whether survival curve shoulders existed and whether these differed depending on irradiation conditions. Results obtained from the analyses of n and D_q (discussed below) confirmed that there were differences in the shoulder regions of the survival curves of *L. monocytogenes*. Therefore, D_{10} values calculated for the 0.5 to 2.0 kGy dose interval were considered better descriptors of the exponential portion of these curves. These D_{10} values increased with decreasing irradiation temperature (Table 3), thus confirming that indirect effects are a major cause of

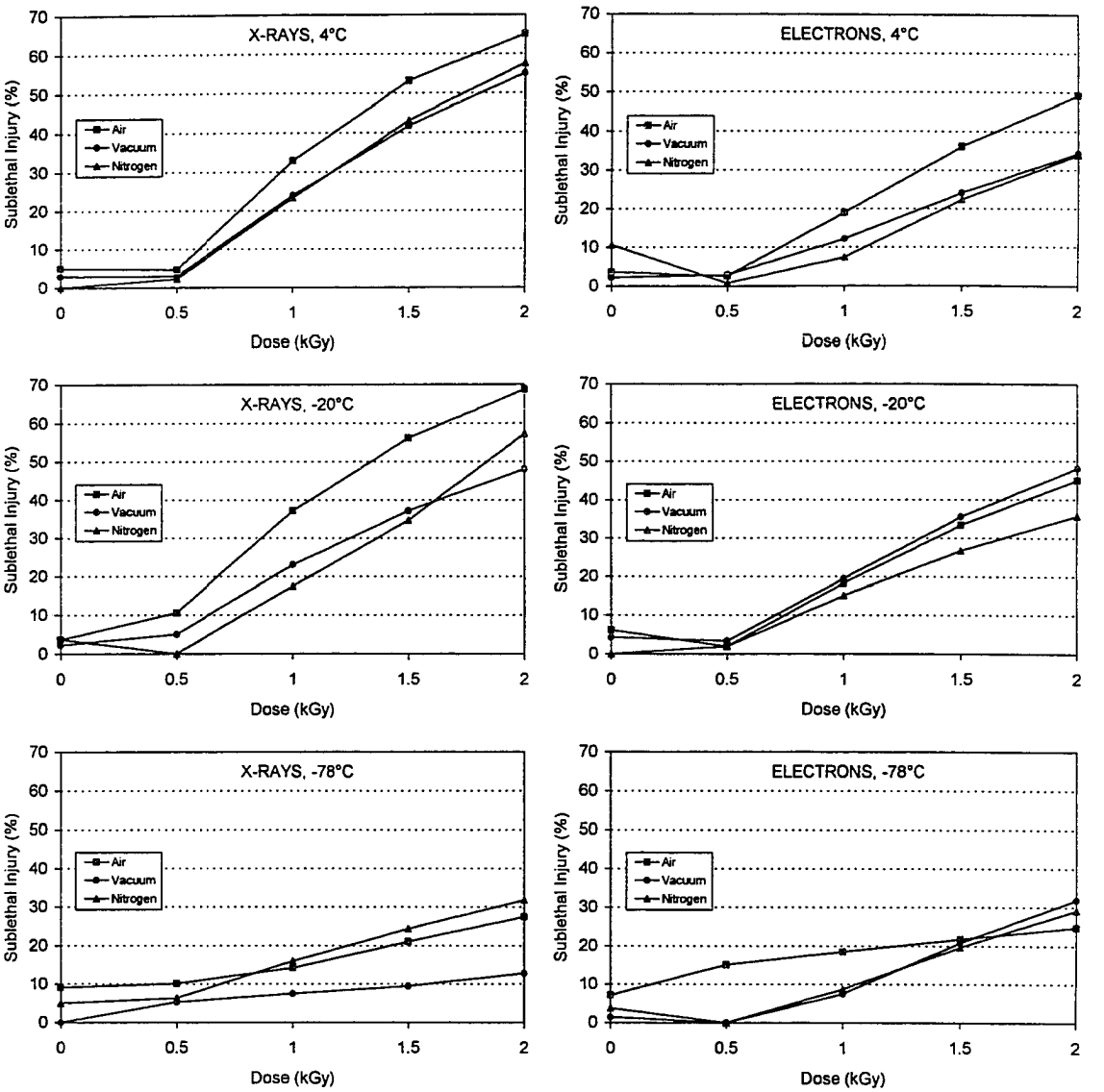

Fig. 4—Sublethal injury of *Listeria monocytogenes* in ground beef, as affected by type of radiation, temperature, and packaging atmosphere. $LSD_{(0.05)} = 21.9\%$.

radiation- induced cell death. The indirect effects of radiation primarily involve reactions of the free radicals that are formed when absorber molecules are hit directly by the radiation. Because water is a major component of many foods, most of the damage caused by indirect effects is caused by reactive species derived from water (Urbain, 1986), primarily the •OH radical (Johansen and Howard-Flanders, 1965; Moseley, 1990). In order for these reactants to react with other molecules, they must diffuse through the water medium (Okada, 1970); therefore, any treatment that immobilizes or removes water (e.g., freezing or drying) will increase the organism's radioresistance (Okada, 1970; Urbain, 1986).

Radiation-induced free radicals have been shown to decrease in mobility as temperature decreases to around -80°C (CAST, 1989). This would explain the higher D_{10} values observed at -78°C compared to -20°C, even though both temperatures are below freezing. Thayer and Boyd (1995) observed a continuous increase in the radioresistance of *L. monocytogenes* in ground beef between 0 and -40°C. Therefore, the radiation inactivation of bacteria at subfreezing temperatures is still temperature-dependent.

At -78°C, D_{10} values were higher for electrons than for X-rays (Table 3), regardless of packaging atmosphere. This was because D_{10} values for X-rays did not increase significantly between -20 and -78°C. At a temperature of -78°C most of the observed effects of radiation would be of the direct kind (Okada, 1970), because freezing would immobilize a great number of the free radicals involved in indirect effects (Urbain, 1986). Therefore, our results suggest that the contribution of direct effects to inactivation of *L. monocytogenes* is greater for X-rays than for electrons, i.e., electrons rely more on indirect effects than X-rays to exert their radiobiological effect. Hence, D_{10} values for X-rays were not as

responsive to the temperature decrease as those for electrons.

Our results clearly indicate that D_{10} values are independent of packaging atmosphere, regardless of the temperature of the product during irradiation. Other investigators have reported similar results. Thayer et al. (1990) reported that the presence or absence of air did not have a significant effect on the radiation D_{10} values of *Salmonella typhimurium* and *S. enteritidis* in mechanically deboned chicken meat. Patterson (1988) tested the radiosensitivities of several foodborne bacteria in poultry meat under various atmospheres (100% air, 100% CO_2, 100% nitrogen, and vacuum) and found that the D_{10} values of *Staphylococcus aureus* and *Streptococcus faecalis* were unaffected by any of them. However, the same study also found *Salmonella typhimurium* to be more resistant under nitrogen than under air or CO_2. A *Lactobacillus* sp., *Moraxella phenylpyruvica*, *Escherichia coli*, and *Pseudomonas putida* were generally equally or more resistant under air than under any of the anaerobic atmospheres. It is evident from these results and our own that different bacteria respond differently to different atmospheres (including oxygen) during irradiation.

The use of the D_{10} value as the only indicator of radiosensitivity may not be appropriate under certain circumstances. This would be the case when the survival curve is not exponential over the entire dose range. Shouldered regions in survival curves suggest an ability by the cells to accumulate, and possibly repair (Bedford, 1982), sublethal damage (SD) before higher doses cause this damage to become lethal (Grosch and Hopwood, 1979). Sublethal damage is defined in radiation biology as damage which by itself is not lethal to the cell, whether or not it is repaired or modified (Billen, 1987). To numerically evaluate these shoulders, extrapolation numbers (n) and quasi-threshold values (D_q) were calculated,

both of which are illustrated in Fig. 1. The extrapolation number was first described and named by Alper et al. (1960), and has been used widely. The D_q, which is another attempt to measure the same phenomenon, is a more convenient number, because it measures shoulder width in units of dose (as opposed to the unitless extrapolation number) and thus can be used, along with the D_{10} value, to calculate the true lethal dose under a given set of conditions. These two parameters are mathematically related to the D_{10} value as follows:

$$D_q = D_{10} \log n$$

In our study, D_q was negative (and n was below 1) when samples were irradiated under air regardless of radiation type or temperature, except for X-rays at -78°C (Tables 4 and 5). These negative D_q values (and values of n below 1) indicate a "negative" shoulder, i.e., a region in which the cells are initially more sensitive. This suggests that, while oxygen had no effect on the exponential portion of the survival curves (Table 3), it did inhibit the ability of *L. monocytogenes* to accumulate sublethal damage at the lower doses. This effect, therefore, must account for the significant effect of packaging atmosphere for D_{10} values calculated for the 0 to 2.0 kGy. When shoulders were taken into account, D_{10} values for irradiation under both vacuum and nitrogen decreased and were no longer different than those under air (Tables 2 and 3). Therefore, failure to consider the possibility of survival curve shoulders when they are present will lead to the calculation of higher D_{10} values that do not accurately describe the exponential portion of the curve.

The absence of a survival curve shoulder for electrons under oxygen at -78°C suggests that, even at this low temperature, indirect effects may still be somewhat responsible for the inhibition of sublethal damage accumulation by *L. monocytogenes*. The oxygen effect in

food irradiation involves the reaction of oxygen with free radicals (CAST, 1989; Ward, 1975) and is, therefore, associated with the indirect effects of radiation. Because freezing impairs the mobility of these free radicals, indirect effects, and, hence, an oxygen effect, would not be observed at a temperature low enough to completely immobilize them. It has been reported that free radicals are relatively immobile at the temperature of liquid nitrogen (-196°C) and that their disappearance rate increases greatly above -80°C (CAST, 1989). Because freezing to -78°C does not immobilize free radicals completely, an oxygen effect, albeit diminished, would still be observed at this temperature. Since the ratio of indirect to direct effects responsible for cell inactivation at a given dose is greater for electrons than for X-rays, as the temperature decreases this diminished oxygen effect would be expected to become increasingly more significant for electron beam irradiation. We postulate that electron beam irradiation at even lower temperatures would further reduce this oxygen effect and allow cells of *L. monocytogenes* to accumulate sublethal damage.

In cases where survival curve shoulders are present, the D_{10} value does not correspond to the total dose necessary to inactivate a given number of cells. In the absence of shoulder effects the dose required to reduce the population from an initial number, N_o, to a number N would be:

$$D = D_{10} \log \frac{N_o}{N}$$

When the curve possesses a shoulder, the shoulder width (D_q) must be taken into account, as it is also part of the description of the organism's radiosensitivity. The dose necessary to achieve the same destruction would then be:

$$D = D_q + D_{10} \log \frac{N_o}{N}$$

Therefore, when a survival curve shoulder is present, ignoring it leads to an underestimate of the total dose required to achieve a certain degree of bacterial inactivation. Table 6 has been tabulated based on the above formula to show the radiation doses required to achieve a 1 log reduction in the population of *L. monocytogenes* in ground beef. Our results underscore the importance of investigating and reporting the presence of survival curve shoulders. In the case of *L. monocytogenes*, and maybe that of other organisms, this is especially critical when irradiation is done in the absence of oxygen.

Sublethal injury, as used in this study, is defined as that injury sustained by an organism which causes it to lose the ability to multiply in a selective growth medium, in this case, MOX medium, but not in a nonselective medium, such as TSAYE (Ray, 1993). Sublethal injury, therefore, is that which is repairable under optimum growth conditions and should not be confused with the sublethal damage (SD) that cells sometimes accumulate at low irradiation doses, since the latter does not render the cell injured. This concept of sublethal injury is analogous to that of potentially lethal damage (PLD), which is defined in radiation biology as damage to DNA which can be lethal if unrepaired (Billen, 1987).

The lag in radiation-induced sublethal injury we observed between 0 and 0.5 kGy (Fig. 4) revealed that at these low doses *L. monocytogenes* was able to repair PLD. The fact that this lag was independent of the presence or absence of survival curve shoulders also suggests that SD and PLD involve different types of damage, and, hence different repair mechanisms. The lower levels of sublethal injury observed for X-rays at -78°C are evidence

Table 6—Radiation doses (in kGy) necessary to reduce the initial population of *Listeria monocytogenes* in ground beef by 1 log, as affected by type of radiation, temperature, packaging atmosphere and plating medium

		Plating Medium					
		TSAYE			MOX		
		Packaging Atmosphere			Packaging Atmosphere		
Radiation type	Temperature (°C)	Air	Vacuum	Nitrogen	Air	Vacuum	Nitrogen
X-ray	4	0.316	0.689	0.669	0.349	0.639	0.647
	-20	0.534	0.722	0.811	0.523	0.685	0.784
	-78	0.807	0.834	0.855	0.789	0.825	0.808
Electron beam	4	0.280	0.729	0.622	0.306	0.709	0.649
	-20	0.556	0.758	0.796	0.560	0.738	0.772
	-78	0.821	0.972	0.949	0.765	0.946	0.938

that a significant portion of PLD is caused by indirect effects. Billen (1987) suggested that damage due to water radicals is responsible for as much as 90% of PLD in X-irradiated *Escherichia coli.*

CONCLUSIONS

Results showed that both X-ray and electron beam irradiation were effective for the inactivation of *L. monocytogenes* in ground beef. The radioresistance of the organism to electron beam irradiation, as expressed by D_{10} values, increased as product temperature decreased from 4 to -78°C. For X-ray irradiation, the organism's radioresistance increased from 4 to -20°C but was generally unaffected between -20 and -78°C. The radioresistance of the organism as expressed by D_{10} values was unaffected by packaging atmosphere, regardless of the type of radiation used. However, survival curve shoulders and, hence, accumulation of damage, were observed when irradiation was done in the absence of oxygen (vacuum or nitrogen). Sublethal injury only increased at doses higher than 0.5 kGy and did so more slowly at -78°C than at -20 and 4°C. These factors must be taken into consideration when designing food irradiation processes. From the standpoint of *L. monocytogenes* destruction, irradiation under air at nonfreezing temperatures is most effective. However, other factors unrelated to microbiology (e.g., lipid oxidation) may suggest irradiation at lower temperatures and in the absence of oxygen. In these cases, our results can be used to estimate the degree of *L. monocytogenes* inactivation that would be achieved under various conditions.

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CHAPTER 5. GENERAL CONCLUSIONS

General Summary

In chapter 3, different strains of *Listeria* differed in their radiosensitivity and in their ability to repair sublethal damage. *Listeria innocua* was more radioresistant than *L. monocytogenes* and *L. ivanovii*. Even within *L. monocytogenes*, two hemolytic strains were more radioresistant than a nonhemolytic strain. These two hemolytic strains also showed a better ability to repair sublethal damage after irradiation than the other three strains tested. However, given that *L. innocua* had higher D_{10} values in both TSAYE and MOX medium, it is possible that some of the damage that this strain was able to repair was expressed as lethal damage in the other strains.

In chapter 4, X-ray and electron beam irradiation of ground beef were both effective for the inactivation of a mixture of strains of *L. monocytogenes* (Scott A, Murray B, and V7). As product temperature decreased from 4 to -78°C, the organism became more radioresistant. Higher D_{10} values for electrons than for X-rays at -78°C indicate their greater dependence on indirect effects to inactivate *L. monocytogenes*. D_{10} values, at any temperature, were independent of packaging atmosphere. However, oxygen inhibited the accumulation of sublethal damage at low doses (except for X-rays at -78°C), and even tended to oversensitize the cells in the low-dose region. TSAYE yielded higher D_{10} values than MOX medium in every case. Sublethal injury only increased at doses higher than 0.5 kGy and did so more slowly at -78°C than at -20 and 4°C.

General Discussion

Our results revealed that different strains of *Listeria* exhibit different patterns of radiosensitivity. The higher radioresistance of the strain of *L. innocua* tested stresses the importance of identifying *Listeria* isolates to the species level to avoid unnecessary recalls and/or remedial action.

It was also evident that factors such as product temperature and packaging atmosphere must be considered when planning to use food irradiation for the purpose of eliminating *L. monocytogenes* and other pathogens from meats.

The temperature of the product during irradiation appears to be the most critical factor. From the standpoint of microbiological destruction, irradiation under air at nonfreezing temperatures is most effective. However, under these conditions irradiation will promote lipid oxidation, thus limiting its use to frozen products. Even under freezing conditions, the actual temperature must be considered, since free radicals are known to remain mobile and, therefore, reactive, down to around -196°C. This was evidenced by our observation of lower D_{10} values at -78°C than at -20°C for electron beam irradiation. For X-ray irradiation, however, D_{10} values did not change significantly in this temperature range, so its use could obviate the need for extremely cold temperatures.

Oxygen inhibited the accumulation of sublethal damage by *L. monocytogenes* at low doses, even at low temperatures (except for X-rays at -78°C), thus increasing the effectiveness of the process. Nevertheless, lipid oxidation may still occur at freezing temperatures and the survival curve shoulder introduced by removing oxygen may not be large enough to be meaningful at higher doses, especially if the doses applied are within

legal limits. In light of these considerations, low-temperature irradiation may still be best carried out in the absence of oxygen.

The use of the selective modified Oxford medium is not recommended for the direct isolation of *L. monocytogenes* from irradiated meats. It may, however, still be appropriate when used in conjunction with one or more enrichment steps.

According to our results, the doses proposed under the red meat irradiation petition currently being considered by the U. S. Food and Drug Administration, 1.5-4.5 kGy for fresh and comminuted meat and 2.5-7.5 kGy for frozen meat, would suffice to destroy approximately 3 to 9 and 3 to 8 log of *L. monocytogenes*, respectively, which, given that meats are not likely to contain very high numbers of the organism, should provide a more than adequate safety margin.

Recommendations for Future Research

1. The observation that D_{10} values were significantly lower for X-rays than for electrons at -78°C warrants further investigation, as this may mean that irradiation with X-rays instead of electrons would obviate the need for deep freezing of meats prior to and during irradiation.
2. The lack of a survival curve shoulder in the presence of oxygen should be further investigated, both in *L. monocytogenes* and in other bacterial pathogens.
3. It is known that different bacteria may differ greatly in their responses to irradiation. It is therefore important to investigate the effects of the factors studied (product temperature, packaging atmosphere) on the radiosensitivity of other foodborne bacteria.

4. Irradiation does not only affect microorganisms, but the entire food and its constituents. This may lead to quality changes, which may be beneficial (e.g., meat tenderization) or detrimental (e.g., lipid oxidation). A more thorough understanding of these changes, as affected by the factors studied, would be desirable.

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Finally, to all present and future meat science graduate students, a word of advice: if you write a really good thesis, a certain professor will not ask you any questions!

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