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Title GAMMA IRRADIATION INACTIVATION KINETICS OF MEDICALLY IMPORTANT BACTERIA CONTAMINATING SEAFOODS

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Gamma irradiation inactivation kinetics were studied using fifteen organisms naturally contaminating seafoods. Survival curves for seven Salmonella, three Shigella, two Streptococcus species and a Staphylococcus, Escherichia and Proteus species were determined using Hartsell's broth and fresh oysters. Also, the Shigella species and one species of Streptococcus, Escherichia and Proteus were studied in sterile crabmeat.

A "tailing effect" at high doses was noted with Salmonella choleraesuis, Salmonella paratyphi A, Salmonella wichita, Salmonella typhosa, Salmonella paratyphi B and Streptococcus faecalis. A similar effect was observed with Shigella dysenteriae and Streptococcus faecalis irradiated in all three substrates, Shigella sonnei irradiated in crabmeat, Escherichia coli irradiated in Hartsell's broth and Proteus vulgaris in oysters.

The organisms all demonstrated maximum resistance when irradiated in oysters with the exception of Shigella sonnei which was more resistant in crabmeat. The substrate offering the least protection was Hartsell's broth.

GAMMA IRRADIATION INACTIVATION KINETICS OF MEDICALLY IMPORTANT BACTERIA CONTAMINATING SEAFOODS

by

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GAMMA IRRADIATION INACTIVATION KINETICS OF MEDICALLY IMPORTANT BACTERIA CONTAMINATING SEAFOODS

INTRODUCTION

Supplying the population with food is becoming an increasingly urgent problem because of the expanding population and the changing food habits of underdeveloped nations. Because of this increasing demand, food scientists are constantly investigating innovations in food processing techniques. An ideal method would preserve the natural flavor, aroma and texture of the products and concurrently remove the dangerous pathogenic organisms as well as prevent spoilage.

Although still primarily in the experimental stages, food irradiation processing shows promise. However, the production of undesirable organoleptic changes frequently proves detrimental (Gardner and Watts, 1957). Investigations of possible additives to remove these undesirable characteristics of irradiated products are rapidly advancing (Drake, 1959; Thornley, Ingram and Barnes, 1960).

The primary concern of the microbiologist is the destruction of organisms capable of inducing public health hazards. Seafoods are extremely susceptible to contamination and spoilage from their

natural habitat. The enteric pathogens from polluted shore water are most commonly encountered in shell fish.

The purpose of this study is to determine the resistance of organisms potentially contaminating shell fish and to establish a standard working dose of irradiation that would destroy existing pathogens.

LITERATURE REVIEW

Types of Ionizing Radiations

Ionizing radiations are characterized by their ability to strip orbital electrons from inert atoms and convert them to highly energetic ions. The energy absorbed by the ions is greater than that required to produce a simple ionization; this surplus energy exists as kinetic energy in the two particles. As they impart their energy to surrounding atoms, the ions are capable of mediating chemical reactions. In the conversion, the initial ionization causes no chemical changes if the subsequent energy loss reactions could be averted. However, to maintain an equilibrium state changes must occur to stabilize the atoms involved.

Active radiations fall into two categories, particulate and electromagnetic. The particulate radiations consist of electrons (beta-rays), helium nuclei (alpha-rays), protons and neutrons. Of these, beta-rays impart the most energy to the surrounding material due to their relatively low mass and greater velocity while the heavier particles lack the ability to penetrate to a significant depth in matter.

Gamma rays and X-rays constitute the bulk of the electromagnetic radiations. These photons have high penetration power due

to lack of mass and consequently are effective bactericidal agents. Gamma rays and X-rays possess the same characteristics, the distinction between them being their origin.

Sources of Ionizing Radiations

Radioactive decay constitutes the only natural source of ionizing radiations. As radioactive atoms are spontaneously converted into stable atoms, they emit ionizing radiations which can be either particulate or electromagnetic depending upon the nuclide involved. This is the only source of gamma rays.

X-rays are artificially produced by bombarding a metallic target with high energy electrons. The fast-moving electron, entering the target, experiences random accelerations as it interacts with the target atoms. This acceleration of the charge results in electromagnetic energy known as bremsstrahlung or X-rays. These X-rays can then be focused on a material for irradiation.

Heavy charged particles are most commonly produced by bombardment of a stable atom with a light particle thus inducing radioactivity. As this atom decays, a heavy charged particle is often emitted which can also be focused at a target.

Cyclotrons are effective agents in the production of high energy particles. The method involves a rapid particle acceleration

by alternating and increasing the magnetic field as the particles traverse a circular orbit. The betatron operates on the same principle in acceleration of electrons.

Units of Dose

The standard unit of exposure dose is the Roentgen (R). It is defined as that quantity of X- or gamma radiation such that the associated corpuscular emission per cubic centimeter of air at standard temperature and pressure produces, in air, ions carrying one electrostatic unit of charge of either sign. This unit specifies that the absorbing material be air, thus the exposure dose is characteristic of the radiation beam and not of the absorber. The exact measurement of the Roentgen becomes meaningless at very high energies resulting from extremely energetic ions formed.

The unit of absorbed dose is the rad which is the deposition of 100 ergs per gram. The unit of rad is applicable to all radiations. Since no standard material is specified in the definition, the amount of energy absorbed is dependent upon the absorber. Exposure of air to one Roentgen results in an absorption of approximately 87.6 ergs per gram (0.876 rad).

The unit known as rep was derived for use with particulate radiations. It corresponds approximately to the amount of ionization

caused by a Roentgen. However, because of the variety of definitions that have been attached to this term, it has largely been discarded.

Because of the infinitesimal quantity represented by a rad and/or rep, the more desirable megarad (Mrad) or megarep (Mrep) representing one million units is used.

Food Preservation by Irradiation

General Effects

Extensive studies have dealt with the over-all acceptability of irradiated foods. The most common problems encountered are the characteristic changes in flavor, odor and appearance. Pratt and Ecklund (1956) believe that some but not all of these changes might be attributed to enzymatic action on the food.

The irradiation odor was investigated by Tausig and Drake (1959). By using activated carbon during irradiation, they were able to reduce the intensity of the odor produced. Pearson et al. (1959) demonstrated that hydrogen sulfide, methyl mercaptan and carbonyls were responsible for a considerable part of the poor acceptability of irradiated beef, pork and veal.

Wholesomeness

The irradiation sterilization of beef, beans, peas and milk has

not caused greater loss in the nutritive value of food protein than are caused by other food processing methods (Johnson and Metta, 1956).

Kraybill, Read and Friedmann (1956) obtained results indicating that radiation sterilized foods were not toxic when individually fed to rats for eight week periods. They also found that continued consumption of 100 per cent irradiated diet through two successive generations of rats showed no evidence of toxicity. Teply and Kline (1956) concluded that there was no clear evidence of the production of carcinogens from irradiation of food materials.

Effects of Ionizing Radiations on Water

The bacterial cell is composed of approximately 80 per cent water. Therefore, a photon of irradiation would be most likely to interact with a water molecule before encountering anything else within the cell. It has long been known that irradiation of water gives rise to hydrogen gas, oxygen gas and hydrogen peroxide (Allen, 1948).

Hart and Platzman (1961) clarified the events of decomposition of water and the reactions leading to formation of the products. The activated electron formed by the initial ionization combines with a stable water molecule to excite it. This causes the production of free radicals, H and OH, and an additional electron which

can propogate the reaction. These free radicals form various combinations to yield the final products.

Effects of Ionizing Radiations on Carbohydrates

Phillips (1954) presented a general model for the product formed upon irradiation of a hexose which was proposed to be a ring structure, uronic acid. Glucose products formed by irradiation with electrons and X-rays were studied by Bothner-By and Balazs (1957). They concluded that several products were formed when either alkaline or neutral solutions of glucose were irradiated. These products were not identified.

Identification of products of irradiated aqueous glucose solutions was done by Phillips and Moody (1959). By using paper chromotography and radioactive tracer methods, the products were found to be D-glucuronic acid, D-gluconic acid, glyoxal, D-arabinose, D-erythrose, formaldehyde, saccharic acid and 1, 3-dihydroxyacetone.

Phillips (1963) further investigated the products of glucose irradiation in vacuo. The following primary processes were recognized: (1) Oxidation at carbon one to give gluconic acid, (2) Attack at carbon two to give glucosone, (3) Ring scission to yield two- and three-carbon aldehyde fragments and

(4) Dimerization of radicals as an initial step in the formation of polymeric materials.

Effects of Ionizing Radiations on Vitamins

The effect of X-irradiation of niacin has been studied by Proctor and Goldblith (1948). It was observed that niacin in a concentration of 100 micrograms per milliliter was not destroyed by total doses between 0.125 and 0.85 MR. When the concentration was reduced to 50 micrograms per milliliter, partial destruction of the vitamin occurred at doses between 0.05 and 0.10 MR. The effect produced by a given total dosage of X-rays was independent of the rate of dosage up to at least five times the initial rate.

Goldblith and Proctor (1949) also investigated the sensitivity of riboflavin and carotene, finding that riboflavin was radiosensitive to both cathode rays and X-rays. A petroleum ether solution containing 100 micrograms per milliliter carotene was 50 per cent destroyed by 0.66 Mrep of cathode rays. The destruction of both riboflavin and carotene was greater the more dilute the solutions.

Thiamine sensitivity has been studied by Groninger and Tappel (1957). They reported that thiamine was destroyed in meats and in aqueous solutions by gamma irradiation. There was formation of ammonia and titratable acid and destruction of a pyrimidine

ring.

Richardson, Martin and Hart (1958) noted that choline was not destroyed by 2.79 megarad of gamma rays when irradiating a solution of 300 micrograms per milliliter choline chloride. Twenty-five to fifty per cent of the folic acid in a 50 microgram per milliliter solution was lost.

Ascorbic acid has been found to be rapidly destroyed by gamma-radiation in dilute solution (Rao, 1962). The main initial product formed was dehydroascorbic acid, which underwent further oxidation under irradiation. Destruction of ascorbic acid was enhanced in the presence of oxygen but dissolved carbon dioxide protected the vitamin.

Effects of Ionizing Radiations on Amino Acids

The initial reaction following X-irradiation of amino acids seems to be oxidative deamination. Glycine exposed to X-rays produced an equimolar formation of glyoxylic acid and ammonia (Barron, Ambrose and Johnson, 1955). Maxwell, Peterson and White (1955) concluded that upon exposure of aqueous solutions of glycine to X rays and cathode rays the initial radiation induced reactions were the same for both radiations.

A mechanism for the radiolysis of the glycine-water system

was developed by Weeks and Garrison (1958). It was proposed that the indirect action of radiation on glycine in oxygen-free solution results in formation of free radical intermediates, CH_2COOH and $NH_2CHCOOH$. Although most of these ultimately yield acetic acid and glyoxylic acid, it was concluded that some undergo radical-radical reactions to yield succinic acid, aspartic acid and diamino-succinic acid.

The action of ionizing radiation on aqueous alanine solutions gives rise to a variety of products (Sharpless, Blair and Maxwell, 1955). In the absence of oxygen, the principal products found were ammonia, pyruvic acid, acetaldehyde, propionic acid, carbon dioxide and ethylamine. In addition to the above products, acetic and formic acids were produced with large doses in the presence of oxygen.

Donde and Korgaonkar (1962) investigated the changes induced in tyrosine solutions by gamma irradiation. Marked changes in both the ultraviolet and infrared spectra were observed.

Proctor and Bhatia (1952) found that aqueous solutions of L-cystine, DL-phenylalanine, L-tyrosine and DL-tryptophane were decomposed upon beta irradiation and the decomposition was related exponentially to the dose. Evolution of hydrogen sulfide from the cystine solution indicated that cystine was probably decomposed at

the disulfide linkage.

It was established that formylkynurenine was one of the products formed when an aqueous solution of DL-tryptophane was irradiated with X-rays in the presence of molecular oxygen (Jayson, Scholes and Weiss, 1954). Marked changes in the ultraviolet and infrared spectra following gamma irradiation of DL-phenylalanine and DL-tryptophane were observed by Korgaonkar and Donde (1962).

Effects of Ionizing Radiations on Proteins

Marked physical and biological changes have been noted in proteins subjected to ionizing radiations (Schweigert, 1959). The major chemical changes included denaturation, degradation and polymerization. The allergenicity of proteins has been shown to be particularly sensitive to irradiation. The primary chemical change appeared to involve the sulfhydryl bond on the protein.

McArdle and Desrosier (1955) found that splitting of casein molecules into smaller fractions occurred when the purified protein was irradiated in aqueous solutions. Further irradiation caused association of these molecular fractions until complete coagulation took place. The effects were similar to those produced by heat.

Kraybill et al. (1960) investigated the alteration of milk proteins by gamma irradiation and reported that the changes

resulted in a decrease in anaphylactic response when the treated milk was injected into milk-sensitized guinea pigs. They also noted that gamma irradiation caused dosage-related increases in viscosity, sulfhydryl and disulfide content.

Upon gamma irradiation significant changes in molecular weights of lysozyme and insulin were noted by Desai and Korgaonkar (1964).

Effects of Ionizing Radiations on Enzymes

Okada and Gehrmann (1957) observed significant changes in the amino acid composition of deoxyribonuclease I (DNase) after exposure to irradiation. Radiation-induced deamination appeared to be the most important factor in the production of this change. DNase I was found to be inactivated completely by the destruction of one tryptophane residue at the active site (Okada and Fletcher, 1962).

When irradiated in the solid state, ribonuclease (RNase) appears resistant up to 45 MR to noticeable chemical covalent changes such as splitting of disulfide bonds or destruction of methionine, tyrosine or phenylalanine (Hayden and Friedberg, 1964).

Friedberg and Hayden (1965) found definite indications of structural unfolding of the RNase molecule in the solid state, concomitant with the loss in enzymatic activity. Disulfide interchange

did not occur to any appreciable extent.

The rennet and protease activities of dilute solutions of crystalline chymotrypsin were equally and simultaneously destroyed by X-irradiation (Moore and McDonald, 1955). It was found that the inactivation was an exponential function of the radiation dose. Mee (1964) observed that in solution, chymotrypsin was more sensitive when irradiated in the absence of oxygen; in the dry state, the enzyme was more sensitive when irradiated in the presence of oxygen.

The proteolytic activity of dilute solutions of crystalline trypsin was found by McDonald (1954) to be destroyed by X-rays. The amount of inactivation was an exponential function of the radiation dose.

The activity of pepsin exposed to radiation was found to depend upon the concentration of the enzyme during irradiation and the addition of organic compounds to the enzyme solution before radiation exposure (Loken et al., 1959).

Dale (1940) examined the effects of X-rays on crystalline carboxypeptidase and on partly purified polyphenoloxidase. He observed that the percentage inactivation of these enzymes was a function of the concentration of the enzyme for a given dose of radiation. Also, it was found that inactivation of carboxypeptidase did not take place when the enzyme was acting on its substrate

during irradiation, whereas the enzyme irradiated without its substrate was 85 per cent inactivated.

This phenomenon of enzyme protection by substrate was also observed by Okada (1957) who found that the addition of DNA to an aqueous solution of DNase afforded potent protection against inactivation of the enzyme. The substrate protection was also noted by Yost, Fitterer and Goldin (1958) with tyrosinase.

Effects of Ionizing Radiations on Lipids

Irradiation gives rise to off-flavors which, in some foods, are a serious disadvantage. The off-flavors show a resemblance to those encountered in oxidative rancidity (Coleby, 1959). Ionizing radiation accelerates the normal process of lipid oxidation. Because of the high concentration of radicals produced by the radiation, the numbers of chain reactions started will be greater than in ordinary auto-oxidation (Lea, 1959).

Effects of Ionizing Radiations on Nucleic Acids

It has been shown that some well-defined chemical changes take place when aqueous solutions of nucleic acids are X-irradiated. Fragmentation of the polynucleotides is accompanied by an increase in the number of titratable acid groups and by a liberation of inorganic phosphate and small amounts of free purine bases (Scholes and

Weiss, 1953).

Barron, Johnson and Cobure (1954) investigated the effects of X-rays on solutions of nucleic acids and their component bases. It was found that the ultraviolet absorption at 2600 Angstroms decreased in all cases. The decrease was proportional to the dose at all times.

The decreased absorption was also noted by Ranadive, Korgaonkar and Sahasrabudhe (1955). They observed that uracil was the most radiolabile and adenine the least.

Gels containing DNA with between 0 and 400 per cent water were irradiated by Lett and Alexander (1961). They demonstrated that degradation of the molecule due to breaks in both strands of the helix became increasingly more efficient as the water content was increased. This was attributed to the free radicals formed in water.

Effects of Ionizing Radiations on Microorganisms

Recovery of Irradiated Organisms

In experimentation such as that to be discussed, it is essential to detect every viable organism in the irradiated product. Irradiated organisms tend to survive better on a richer media (Freeman and Bridges, 1960). Hartsell (1951) found that a medium containing yeast extract and veal infusion would recover large numbers of damaged

bacteria. Apparently, this media gives a booster dose of metabolites needed.

Harris (1963) demonstrated that the viability of damaged organisms is often sensitive to environmental changes which do not influence that of undamaged cells.

Salmonella

The earliest investigation of irradiation of Salmonella was reported by Grainger (1946). He found that the lethal effect of X-rays on Salmonella typhosa resulted in a log order of death. However, some of the organisms were still motile but failed to grow on suitable laboratory media.

Proctor et al. (1953) irradiated Salmonella-inoculated whole egg magma with cathode rays. Complete destruction of Salmonella senftenberg was obtained with 0.125 Mrep. Salmonella paratyphi and Salmonella typhimurium required 0.3 Mrep for complete destruction.

Using cathode rays, Nickerson et al. (1957) noted that 0.7 Mrep was required for a seven-fold dilution kill of Salmonella typhimurium and Salmonella senftenberg in unsugared egg white solids. The dose requirements were less in reconstituted egg white and frozen egg white. Brogen et al. (1957) found that 0.45 Mrep of cathode rays was necessary for a seven-decimal destruction of

Salmonella typhimurium and Salmonella senftenberg in whole egg solids and 0.65 Mrep in egg yolk solids.

Comer, Anderson and Garrard (1963) observed a definite variation in sensitivity to gamma radiation among Salmonella species commonly occurring in frozen whole egg. The dose levels required for a seven-decimal reduction ranged from 0.36 Mrad to 0.54 Mrad.

Ley, Freeman and Hobbs (1963) estimated the amount of gamma radiation necessary to obtain a seven-decimal decrease in Salmonella typhimurium in frozen whole egg to be 0.5 Mrad.

Staphylococcus and Streptococcus

Christensen and Holm (1964) observed that about 1.0 Mrad of radiation was necessary to reduce a population of Staphylococcus aureus to 10^{-6}.

Influence of suspending media on survival of Staphylococcus and Streptococcus faecalis was noted by Erdman, Thatcher and MacQueen (1961). Comparing the sensitivity of the cultures suspended in culture broth or chopped beef at 0.2 Mrep, it was found that there was 0.11 per cent survival of Staphylococcus in broth compared to 0.27 per cent in the meat. The broth afforded a three-fold increase in survival of Streptococcus faecalis at 0.2 Mrep.

Slabyj, Dollar and Liston (1965) also noted the protection. Following irradiation of Staphylococcus aureus with 0.05 Mrad of gamma rays, they recovered about 8 per cent survivors in crabmeat, 2 per cent in fish homogenate and 0.6 per cent in buffer.

Escherichia coli

Lea, Haines and Bretscher (1941) reported that the survival curves of Escherichia coli after X-ray exposure were exponential and the lethal dose was independent of the intensity of the radiation. A 99.99 per cent reduction of Escherichia coli was found to occur following exposure to 0.149 Mrep of gamma radiation (Lawrence, Brownell and Graikoski, 1953). Erdman, Thatcher and MacQueen (1961) observed that 0.15 Mrep of gamma radiation resulted in 0.0047 per cent survival in chopped meat and 0.00063 per cent survival in culture broth.

Many investigators have noted that irradiated cells continue to elongate but fail to divide. Shekhtman, Plokhoi and Filippova (1958) noted that cells of Escherichia coli were 2.6 times as long after irradiation as normal. Spoerl et al. (1954) also reported the division inhibition of irradiated Escherichia coli cells.

Adler and Hardigree (1965) further investigated this and noted that cells of certain strains of Escherichia coli grew into long multinucleate filaments after exposure to irradiation. Deoxyribonucleic acid, ribonucleic acid and protein synthesis proceeded but

cell division did not occur. Cross-septation and cell division could be initiated by pantoyl lactone. Adler et al. (1966) observed that filaments of Escherichia coli could be induced to divide by a substance donated by neighboring cells. This substance has not been identified.

Spores

Under arbitrarily standardized conditions, spores of Clostridium botulinum were more resistant to gamma radiation than spores of other food spoilage organisms investigated (Morgan and Reed, 1954). Denny et al. (1959) observed that the D-value (90 per cent destruction) was dependent upon the initial concentration of spores. This was found to vary from 0.18 Mrad with 4.6×10^4 spores per milliliter to 0.38 Mrad when the inoculum consisted of 8.2×10^7 spores per milliliter.

Roberts and Ingram (1965) noted that pasteurizing doses of radiation are unlikely to reduce the clostridial count by more than a factor of ten, and not until about one Mrad might a hundred-fold reduction be anticipated.

Radiation resistance of Bacillus coagulans spores was investigated by Anellis, Cichon and Rayman (1960). A D-value was determined in phosphate buffer to be 0.129 Mrad when irradiated in air. Matsuyama, Thornley and Ingram (1964) calculated the D-value for

Bacillus pumilus spores irradiated at room temperature aerobically to be 0.172 Mrad.

Radiation Resistant Microorganisms

Anderson et al. (1956) isolated a radio-resistant micrococcus resembling Micrococcus roseus and Micrococcus rubens from samples of ground beef and pork following gamma-radiation dosages of two to three Mrep. Pure cultures of the isolated organism survived extreme exposures of six Mrep on agar slopes. Raj et al. (1960) investigated the utilization of carbohydrates and amino acids by this organism (Micrococcus radiodurans). It was found that methionine, the only amino acid shown to be essential in a chemically defined medium, appeared to be rapidly incorporated into the cells. The operation of the tricarboxylic acid cycle was suggested by the oxidation of certain TCA intermediates. Observations by Duggan, Anderson and Elliker (1963) suggested that Micrococcus radiodurans R_1 was more resistant to radiation than spore-forming bacteria.

An ecological study of Micrococcus radiodurans by Krabbenhoft, Anderson and Elliker (1965) indicated that microorganisms possessing the same morphological and radiation-resistant characteristics as that organism could be isolated from ground beef and pork sausage. It was also shown that such organisms could be isolated from beef hides and from water from a creek adjacent to the packing plant

from which the meat samples were obtained.

Effects of Ionizing Radiations on Seafoods

Storage life of crabmeat irradiated at 0.25 Mrad was extended three to four weeks, compared with the one week for unirradiated samples held at the same temperature (Sholz et al., 1962). A dose of 0.5 Mrad extended the shelf life five weeks. Taste panel tests of the irradiated crab indicated that irradiation flavor declined during storage.

Doses of 0.2 and 0.4 Mrad to King crab meat were judged by Miyauchi et al. (1964) to be the most suitable for storage life studies. At 33°F, the storage life of five different lots of irradiated crabmeat varied from four to more than six weeks, whereas the unirradiated control samples had a storage life of about one week. Spinelli, Eklund and Miyauchi (1964) observed that three weeks of shelf life could be confidently predicted following irradiation of King crab at 0.2 Mrad and storage at 33°F. The shelf life was increased to six weeks after the product was irradiated at 0.4 Mrad and stored under the same conditions.

Gardner and Watts (1957) observed a "grassy" odor following irradiation of raw oysters with doses of 3.5 Mrep. Neither free sulfhydryl groups nor catalase activity were noticeably reduced.

Novak et al. (1966) irradiated Gulf Coast oysters at 0.15, 0.2 and 0.3 Mrad and determined that 0.2 Mrad was the maximum level tolerated without producing adverse organoleptic changes. Oysters irradiated at 0.2 Mrad and stored in crushed ice up to 21 days were still acceptable as judged by flavor, odor and appearance.

Combination Processes

Heat

Pre-irradiation with gamma rays sensitized the spores of Clostridium botulinum to the subsequent lethal action of heat, but preliminary heat shocking at 99°C did not affect the lethal action of subsequent gamma irradiation (Kempe, 1955). Kan, Goldblith and Proctor (1957) also observed this effect with a putrefactive anaerobe and Bacillus cereus spores.

Licciardello and Nickerson (1962) noted that pre-irradiation with gamma rays sensitized Clostridium sporogenes spores to heat and spores irradiated either in air or under vacuum were heat-sensitized to the same extent.

Antibiotics

Results obtained by Cain, Anderson and Malaspina (1958) indicated that a combination of antibiotics and radiation on fresh meat would result in a product with high keeping qualities. Thornley,

Ingram and Barnes (1960) noted that a combination treatment resulted in an extension of shelf life which was approximately equal to the sum of the two treatments separately.

Lerke, Farber and Huber (1961) found that low doses of beta radiation, as low as 0.0118 Mrad, had a definite though limited inhibitory action on spoilage rate, with inhibition being further increased by chlortetracycline.

Other Additives

Spores of Bacillus subtilis var. niger and Bacillus stearothermophilus irradiated in nitrogen were killed in greater numbers in the presence of vitamin K_5 than when irradiated without the chemical (Silverman, Davis and Goldblith, 1963). When irradiation was performed in air the chemical was ineffective and protective.

Six compounds structurally related to and including vitamin K_5 were found to increase the radio-sensitivity of Escherichia coli and Streptococcus faecalis (Silverman, Shehata and Goldblith, 1962). They were all found to be more effective against Escherichia coli in the absence of oxygen.

Lee, Shiflett and Sinnhuber (1965) noted that microbial spoilage of Dover sole stored at 42-44°F was retarded when the fish had been treated with 0.1 per cent sodium benzoate and was given pasteurizing doses of gamma-irradiation. The benzoate by itself had no

microbicidal effects.

Radiation Protection

Protection of organisms by irradiation in the presence of nitrogen was observed by Hollaender, Stapleton and Martin (1951). Irradiating Escherichia coli in the presence of nitrogen, a D-value of 0.05 Mrad was obtained compared to 0.015 Mrad following irradiation with oxygen. Dewey and Boag (1959) also noted this effect with Serratia marcescens.

Kohn and Gunter (1959) investigated protection of Escherichia coli by cysteine. It was found that cysteine protection was the end result of events in two sequential periods, a temperature-dependent reaction period followed by a temperature-independent period. This investigation was continued by Kohn and Gunter (1960) to determine the effect of oxygen on the protection. It was observed that L-cysteine afforded significant protection with 0 to 100 per cent oxygen.

"Tailing Effect"

The "tailing effect" caused by survival of low numbers of organisms at higher doses has not been investigated extensively. Wheaton and Pratt (1962) observed this effect with Clostridium botulinum

spores. Originally it was attributed to highly resistant spores being present in the headspace foam. This explanation was discarded when the dry spores were found to be relatively radiosensitive.

Dyer, Anderson and Dutiyabodhi (1966) noted the "tailing effect" when Salmonella paratyphi B, Salmonella typhi and Salmonella wichita were irradiated in crabmeat. The effect diminished as the moisture content was increased.

METHODS AND MATERIALS

Microorganisms Studied

The microorganisms studied in this survey include those most often associated with food intoxication and infection outbreaks. Most are enteric pathogens which can be present in polluted water. The common specialty seafoods used are found in or near estuaries and can readily be contaminated by organisms present in the water.

All cultures used were obtained from the Oregon State University Microbiology Department stock culture collection.

Salmonella Species

The Salmonella species are responsible for most of the food poisoning associated with seafoods. The organisms of this genus studied were Salmonella pullorum, Salmonella wichita, Salmonella enteritidis, Salmonella choleraesuis, Salmonella paratyphi A, Salmonella typhosa and Salmonella paratyphi B.

Shigella Species

The Shigella species are another group associated with enteric infections. The organisms studied were Shigella dysenteriae, Shigella paradysenteriae and Shigella sonnei.

Streptococcus Species

The streptococci are associated with many diseases. The most important is Streptococcus pyogenes which is hemolytic and is often the causative agent in wound infections and strep throat. Streptococcus faecalis is used in some countries as an index of fecal contamination in water since it is usually part of the flora of the intestinal tract.

Staphylococcus Species

Staphylococcus aureus is the most prevalent organism of this genus. It is a common cause of food intoxication and is usually introduced into the food by infected food handlers.

Proteus Species

Some food poisoning outbreaks have been ascribed to Proteus vulgaris. It is also believed to be the causative agent in some infant diarrhea cases. Members of this genus are common food spoilage microorganisms.

Escherichia Species

The organism of significance of this genus is Escherichia coli and is commonly used to determine the presence of fecal pollution. It has been associated with outbreaks of infant diarrhea.

Culture Media

The medium used for propagation of these cultures was Hartsell's broth which consists of 5 grams proteose-peptone, 5 grams tryptone, 5 grams sodium chloride, 5 grams yeast extract, 2.5 grams veal infusion medium and 1000 milliliters distilled water. The pH was adjusted with potassium hydroxide so that after autoclaving at 15 pounds pressure (121°C) for 20 minutes it would be 7.2. When used as solid media, 20 grams agar was added. This medium has been found to be very effective in recovering damaged cells and supports growth of all organisms studied very well.

Peptone water was used for the dilutions necessary to determine the number of survivors present when plated. It consists of one gram proteose-peptone and 1000 milliliters distilled water. This was distributed in eight ounce bottles in amounts sufficient to give 99 milliliters after autoclaving at 15 pounds pressure (121°C) for 20 minutes.

For recovery of survivors after irradiation, differential media were used when the organisms were irradiated in fresh seafoods. The *Salmonella*, *Shigella* and *Proteus* were recovered with Difco *Salmonella-Shigella* agar. Difco *Staphylococcus* 110 medium was used for plating the *Staphylococcus aureus*. *Escherichia coli* was detected with Difco Eosin Methylene Blue (EMB) agar.

Streptococcus faecalis was recovered with Difco Buffered Azide Glucose Glycerol (BAGG) broth with 1.5 per cent Bacto-agar added. Hartsell's agar plus five per cent sterile human blood was the medium used for detection of Streptococcus pyogenes. The differential media were prepared according to directions supplied by the manufacturer.

The detection of survivors after irradiation in sterile seafoods or Hartsell's broth was with Hartsell's agar.

Seafoods

The seafoods used were crabmeat and oysters. The sterile crabmeat, Cancer magister, was packed by S & W Foods with water, salt and citric acid in number one-half flat tin cans. Fresh unprocessed oysters, Crassostrea gigas, were obtained from Rose City Oyster Company in Portland.

Preparation of Culture

Initial culture was started from stock culture slants which were maintained on Hartsell's agar at 5°C. A transfer was made from this to 15 ml of Hartsell's broth and incubated 24 hours at 35°C. One ml of this culture was transferred to 100 ml Hartsell's broth and incubated 24 hours at 35°C. This was repeated for three days until the culture was fully activated and was Gram stained at

each transfer to ascertain the purity. The irradiation test culture was 18 hours old in all cases and was diluted with sterile Hartsell's broth until the final concentration was approximately 17 x 10^6 cells/ml. One ml of this dilution was added to 17 grams of seafood or 17 ml of sterile Hartsell's broth for irradiation.

Preparation of Seafoods

The crabmeat samples were prepared in a transfer chamber equipped with a germicidal ultraviolet lamp. The cans were opened and the contents were transferred aseptically to number 202 cans. The crabmeat was broken into small pieces and transferred to sterile glass vials (1.5 inches in diameter and 2.5 inches in height) each vial containing 17 grams of crabmeat. One ml of 17 x 10^6 cells/ml was added and mixed with sterile glass rods to distribute the cells throughout the crabmeat. The samples were maintained at $5^{\circ}C$ until irradiation.

The fresh oysters were homogenized in a Waring blender for five minutes. Seventeen grams were transferred to irradiation vials and maintained at $5^{\circ}C$ for inoculation and irradiation.

Irradiation Source and Sample Holder

The source was a Cobalt-60 irradiator, Model No. R-60124,

designed and built by the Budd Company and consisting of 12 rods with an original activity of 3600 Curies. The dosimetry was measured at the edge of the high flux chamber using the Fricke-ferrous sulphate method January 1, 1964 and found to be $8.13 \times 10^5 \pm 0.36$ rads/hour. Exposure times were corrected regularly to account for the natural decay of the isotope in order to maintain a constant exposure dose.

A sample holder was used which held 20 sample vials and fitted around the periphery inside the high flux chamber of the irradiator.

Irradiation

The inoculated samples were irradiated in the sample holder in the high flux chamber. Five samples were irradiated at each dose and the number of survivors was averaged to compensate for the non-uniformity of dose from the source.

Microbiological Examination

The irradiated Hartsell's broth samples were plated with Hartsell's agar using appropriate peptone water dilutions. The plates were incubated at $35^{\circ}C$ for 48 hours or until the colonies were easily countable with a Quebec colony counter.

The irradiated seafood samples were transferred aseptically in the transfer chamber to 51 ml of sterile Hartsell's broth. These were refrigerated until plating. Sterile peptone water dilutions were made and the plating was done in the appropriate media. The plates were incubated at 35°C for 48 hours or until colonies were readily visible. At high doses where no survivors were anticipated when plated from the seafood, samples were aseptically transferred to 100 ml of Hartsell's broth as an enrichment and incubated at 35°C for a minimum of five days. After incubation, a loopful was streaked on differential media for a qualitative determination of survivors.

Unirradiated controls of all samples were prepared as the others. These were used to determine the initial concentration of cells.

RESULTS AND DISCUSSION

When experiments of this nature are conducted, environmental conditions such as temperature, oxygen and cell concentration must be maintained constant, and procedures must be standardized for the consistency necessary for valid comparison of data. By utilizing the same procedure throughout the investigation, it is possible to compare the kinetics of various organisms as well as noting the protection exerted by the substrate.

The following controlled conditions were maintained throughout the investigations:

1. The same approximate initial number of cells per gram was maintained for each organism.

2. Contamination was carefully avoided throughout all phases of each experiment.

3. Normal atmospheric conditions were maintained throughout the investigation period.

4. The samples were maintained at low temperatures ($5^{o}C \pm 1^{o}$) at all times except during the irradiation period when they were at room temperature.

5. The same facility for irradiation was used throughout the study.

6. The methodology was maintained constant for the entire

investigation.

The elimination of salmonellae and other intoxicating and infecting organisms is a similar process to pasteurization since the doses used are in the same range. However, the aim is to remove one group of organisms which is particularly undesirable in the product. Vegetative bacteria are moderately sensitive to irradiation, and doses around 0.5 to 0.8 Mrad will affect a seven decimal reduction of most infectious and intoxicating non-spore forming microorganisms occurring in seafoods.

Most work to this date has been concerned with removal of Salmonella from non-perishable products (Ley, Freeman and Hobbs, 1963; Proctor et al., 1953; Nickerson et al., 1957; Brogle, 1957) in which no multiplication of survivors occurs. However, when one investigates a perishable product one must consider the survival of other pathogenic microorganisms which might be present. A dose which might inactivate salmonellae would be ineffective against spore-forming organisms which, upon storage under favorable conditions of temperature, could vegetate and lead to trouble.

Some idea of the magnitude of dose required may be obtained by irradiation of naturally contaminated material. However, since the number of pathogenic organisms is usually low, it has been found useful to inoculate the product with large numbers, about 10^6 per gram, from pure cultures to study their inactivation. These

organisms show an exponential death rate to a certain point in some substrates. However, in more complex food material, a "tailing effect" indicative of greater survival than anticipated from exponential death is found. Thus, the D-value, or dose required to inactivate 90 per cent of the bacteria, cannot be applied with as much assurance.

The $\log_{10}$ of the number of survivors is usually plotted against dose and a line is drawn between the points. This curve is usually used to obtain the D-value if the "tailing effect" does not occur. However, due to this "tailing effect", it is impractical to extrapolate directly to obtain a value which would completely inactivate the organisms present.

In this work it was found impractical to incubate the organisms directly in the food itself. Preliminary experiments have shown that organisms inoculated directly into irradiated food resulted in a one-log lower recovery than if the same organisms were inoculated into an unirradiated product (Personal observation).

Because of the effect of the irradiated media on organisms, it was found necessary to transfer the samples to artificial media in order to recover cells able to multiply after irradiation. Each result shown in the tables is the observation made on five replications and with each replicate being plated in triplicate. The lower

dilutions were plated in six inch plates instead of the usual four inch recommended and used in usual bacteriological techniques.

Inactivation Kinetics of Shigella

Three species of Shigella were studied, Shigella dysenteriae, Shigella paradysenteriae and Shigella sonnei. Of the three, Shigella dysenteriae exhibited the highest level of resistance to gamma radiation as tabulated in Tables 1, 2 and 3. Shigella paradysenteriae and Shigella sonnei demonstrated almost the same degree of resistance in all three substrates. The resistance of Shigella dysenteriae and Shigella paradysenteriae was greater in oysters, but Shigella sonnei was more resistant in crabmeat (Figures 1, 2 and 3). In all three cases, the least resistance was encountered in Hartsell's broth. Shigella sonnei in crabmeat and Shigella dysenteriae in all three substrates exhibited the "tailing effect".

Inactivation Kinetics of Escherichia and Proteus

Escherichia coli demonstrated much greater resistance in oysters than in Hartsell's broth or crabmeat (Table 4). However, the "tailing effect" was observed in broth and crabmeat but not in oysters (Figure 4).

Table 5 shows that the protection of Proteus vulgaris afforded by Hartsell's broth and crabmeat appears to be similar. However,

the protection provided by oysters was much greater, (Figure 5) a phenomenon which appeared consistent throughout the investigation.

Inactivation Kinetics of Staphylococcus and Streptococcus

Of the two streptococci, Streptococcus faecalis was more resistant in Hartsell's broth than Streptococcus pyogenes (Tables 6 and 7). Disregarding the "tail" of the curve at high doses, Figures 6 and 7 show that it was also more resistant in oysters. Both organisms demonstrated the "tailing effect".

The protection afforded Staphylococcus aureus was greater in oysters than in Hartsell's broth as shown by Table 8. No "tailing effect" was noted with this organism even at higher doses (Figure 8).

The "hump" near the top of the curves is probably due to the clumping of these organisms resulting in multiple targets. This has been noted in Clostridium botulinum by Wheaton and Pratt (1962).

Inactivation Kinetics of Salmonella

In all cases, greater protection of Salmonella was provided by oysters than Hartsell's broth (Tables 9-15). The "tailing effect" is noted with all Salmonella except Salmonella pullorum and Salmonella enteritidis. This occurs when irradiated in either Hartsell's broth or oysters (Figures 9-15).

The following order of resistance can be determined in Hartsell's broth:

Salmonella pullorum < Salmonella enteritidis < Salmonella paratyphi A < Salmonella typhosa < Salmonella choleraesuis < Salmonella wichita < Salmonella paratyphi B. If the tail of the curve for Salmonella paratyphi A is extended, the order in oysters is as follows: Salmonella pullorum < Salmonella enteritidis < Salmonella typhosa < Salmonella choleraesuis < Salmonella wichita < Salmonella partyphi B < Salmonella paratyphi A.

D-value

The amount of irradiation necessary to obtain 90 per cent destruction of the organisms (D-value is tabulated in Table 16. However, due to the "tailing effect" observed in most cases, it is not possible to extrapolate from the D-value to obtain a level of irradiation sufficient for pasteurization. Since the "tailing effect" is not uniform, no factor can be applied to compensate for this effect.

Protection by Seafoods

As has been demonstrated, all organisms studied exhibited greater resistance when suspended in the seafood for irradiation than when irradiated in culture media. This will necessitate studies with

each product before attempting irradiation food processing commercially. Without preliminary investigations, it would be impossible to predict a safe working dose that would be applicable to the product involved. The protection exerted by the products is not consistent so a standard dose cannot be used for all products.

"Tailing Effect"

The experiments which resulted in the "tailing effect" are tabulated in Table 17. This is not a consistent phenomenon but occasionally occurs in one substrate but not in another for the same organism. The results of Salmonella irradiation demonstrated substrate consistency but the effect was not observed with all members of the Salmonella genus.

Recommendations for Further Study

The incubation temperature used in all of the studies was 35°C. This is not the optimum temperature for all the organisms investigated. In future studies, the incubation temperature should be adjusted to the optimum for each organism.

To obtain an exact determination of the amount of protection exerted by the seafood, it is suggested that the cells be centrifuged and resuspended in buffer or sterile water to remove all adhering nutrients.

The study should be continued and expanded to investigate the protection of other seafoods such as shrimp and sole.

SUMMARY

Gamma irradiation inactivation kinetics were studied for seven Salmonella species, three Shigella species, two Streptococcus species and one each of Staphylococcus, Escherichia and Proteus in Hartsell's broth and seafoods. "Tailing effect" at the higher doses was observed with Escherichia coli in Hartsell's broth and crabmeat, Proteus vulgaris in oysters, Shigella sonnei in crabmeat, Shigella dysenteriae and Streptococcus faecalis in all three substrates, Streptococcus pyogenes, Salmonella choleraesuis, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella wichita and Salmonella typhosa in Hartsell's broth and oysters.

Disregarding the "tailing effect," Staphylococcus aureus was found to be the most resistant organism followed by the streptococci. Proteus vulgaris and Escherichia coli were the least resistant.

Because of the low resistance of Escherichia coli, it will no longer be practical to use this organism as the index of fecal contamination in radiation processed foods.

Oysters provided the most protection except for Shigella sonnei which was more resistant in crabmeat.

Table 1. Radiation inactivation kinetics of Shigella dysenteriae

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	6.33132	12.15418	18.34192
0.05	0.29594	2.90823	7.16042
0.07	0.09349	0.44231	1.37291
0.09	0.01183	0.05923	0.12000
0.10	0.01331	0.02030	0.04251
0.20	0.00091	0.000785	0.00234
0.30	0.00000474	$\geq$0.000000454	

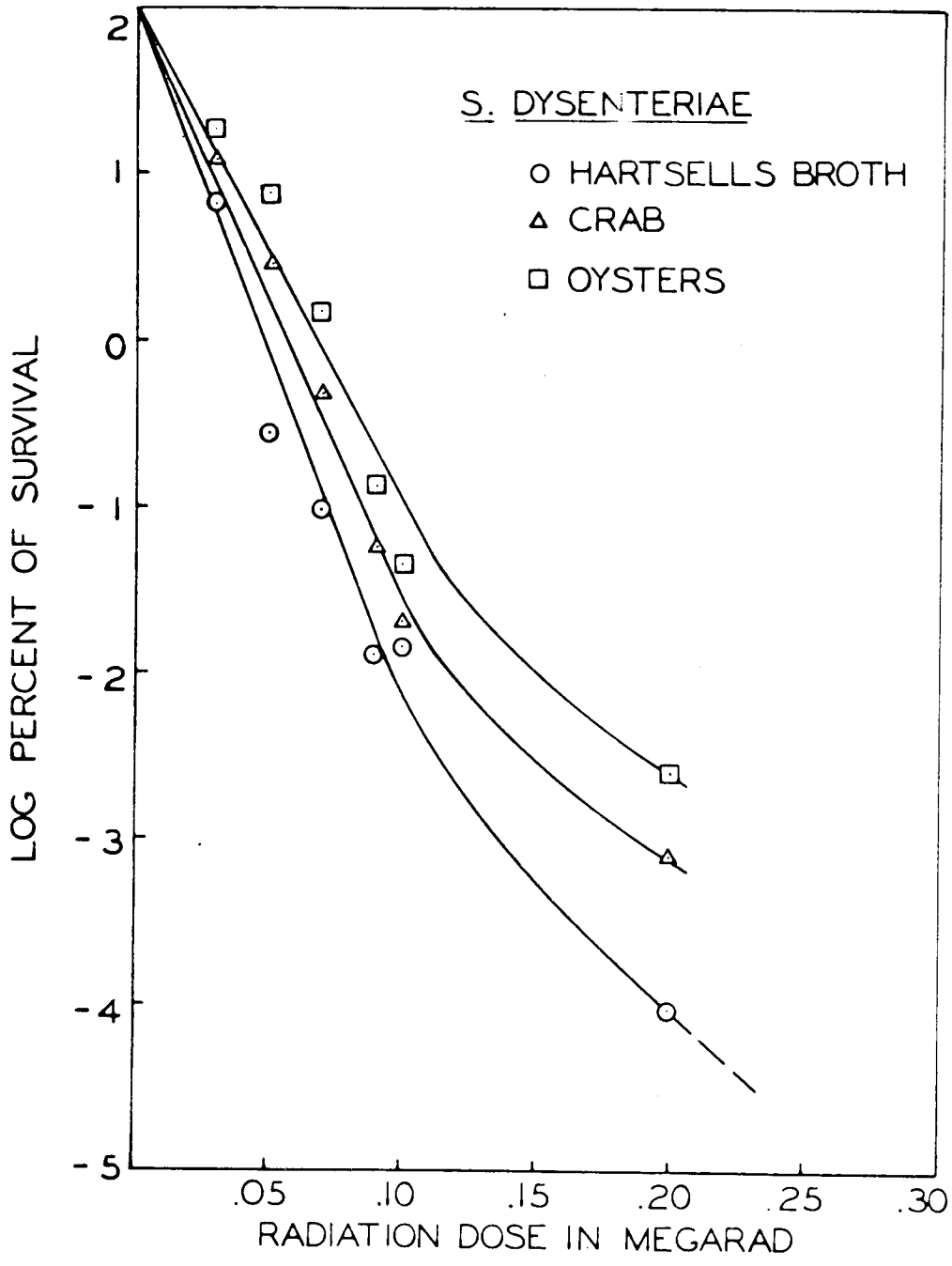

Figure 1. Radiation survival curves of Shigella dysenteriae

Table 2. Radiation inactivation kinetics of Shigella paradysenteriae.

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	3.20643	8.75000	6.85312
0.05	0.47953	0.53133	0.72502
0.07	0.003567	0.05117	0.08000
0.09	0.004912	0.003203	0.03152
0.10	0.000695	0.001336	0.004218
0.20	0.0000007	$\geq$0.00000184	

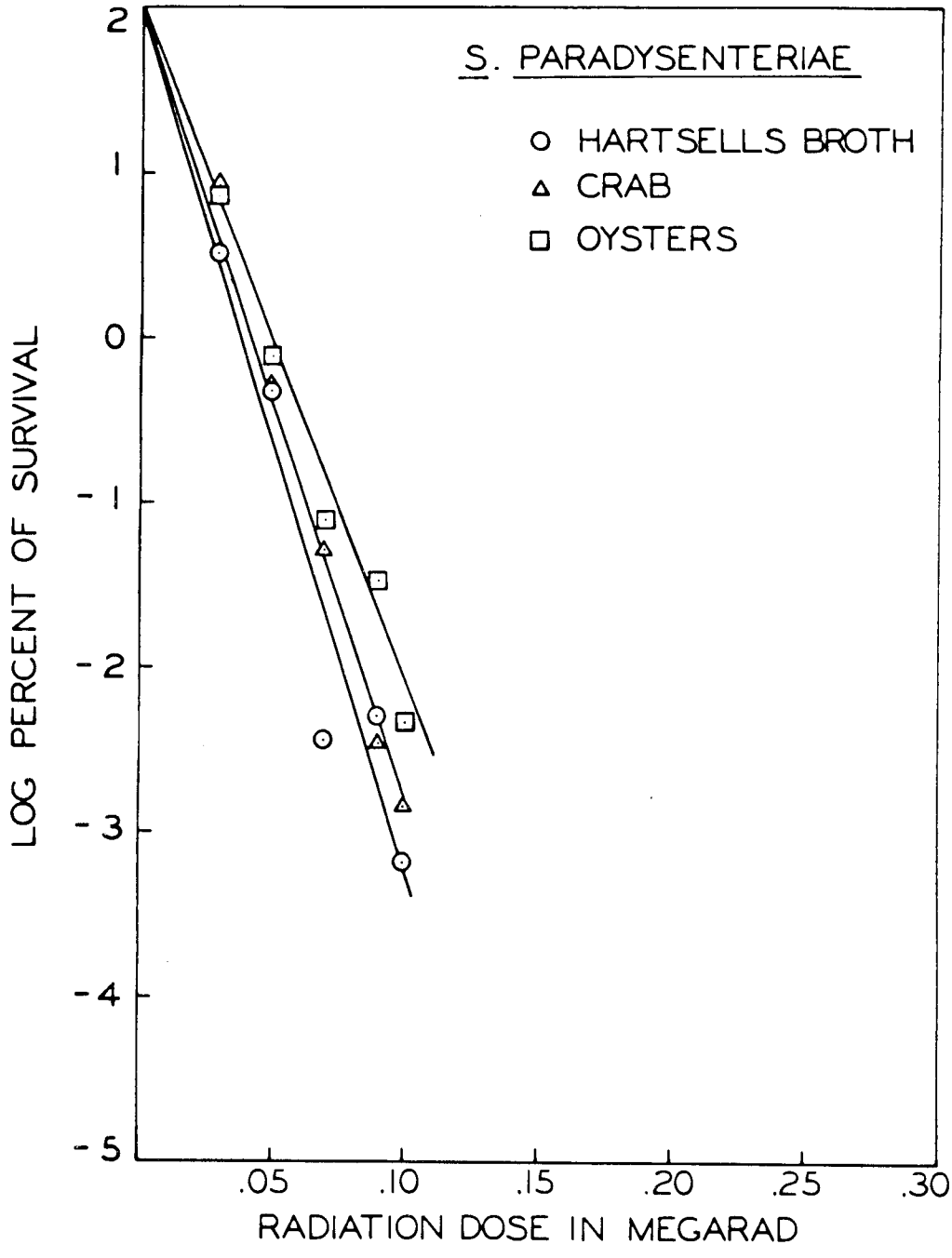

Figure 2. Radiation survival curves of Shigella paradysenteriae

Table 3. Radiation inactivation kinetics of Shigella sonnei

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	6.00000	4.65116	2.96152
0.05	0.87586	1.23260	0.62851
0.07	0.00872	0.16279	0.12903
0.09	0.00600	0.03256	0.02352
0.10	0.000689	0.02791	0.011902
0.20		0.00008	
0.30		0.000000745	

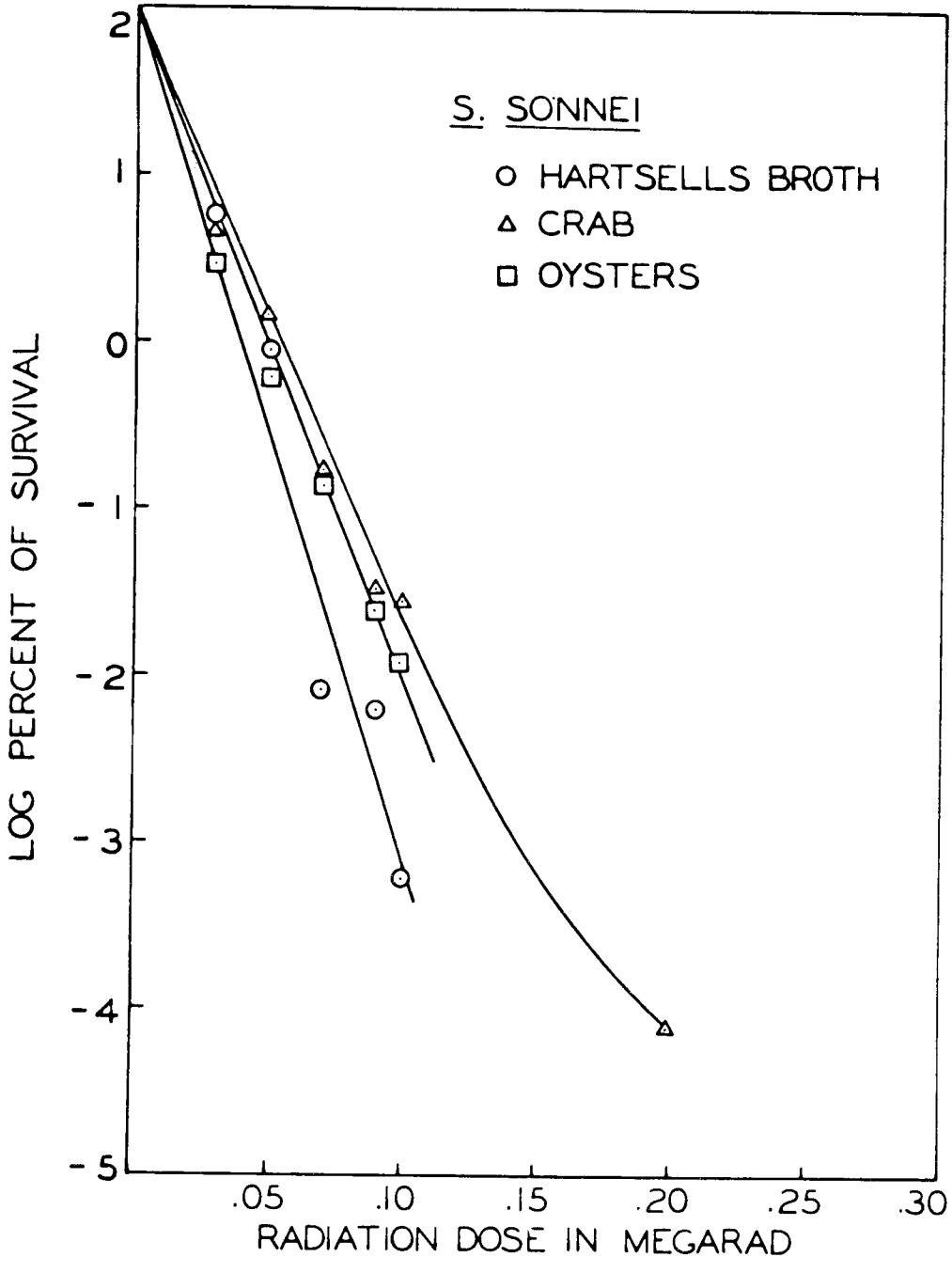

Figure 3. Radiation survival curves of Shigella sonnei.

Table 4. Radiation inactivation kinetics of Escherichia coli

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	0.22741	1.08812	
0.05	0.00323	0.00282	6.00000
0.07	0.00139	0.00094	1.00000
0.09	0.000984	0.000268	0.16281
0.10	0.000194	0.000097	
0.20	0.0000032	0.000022	0.0000106
0.30			$\geq$0.000000052

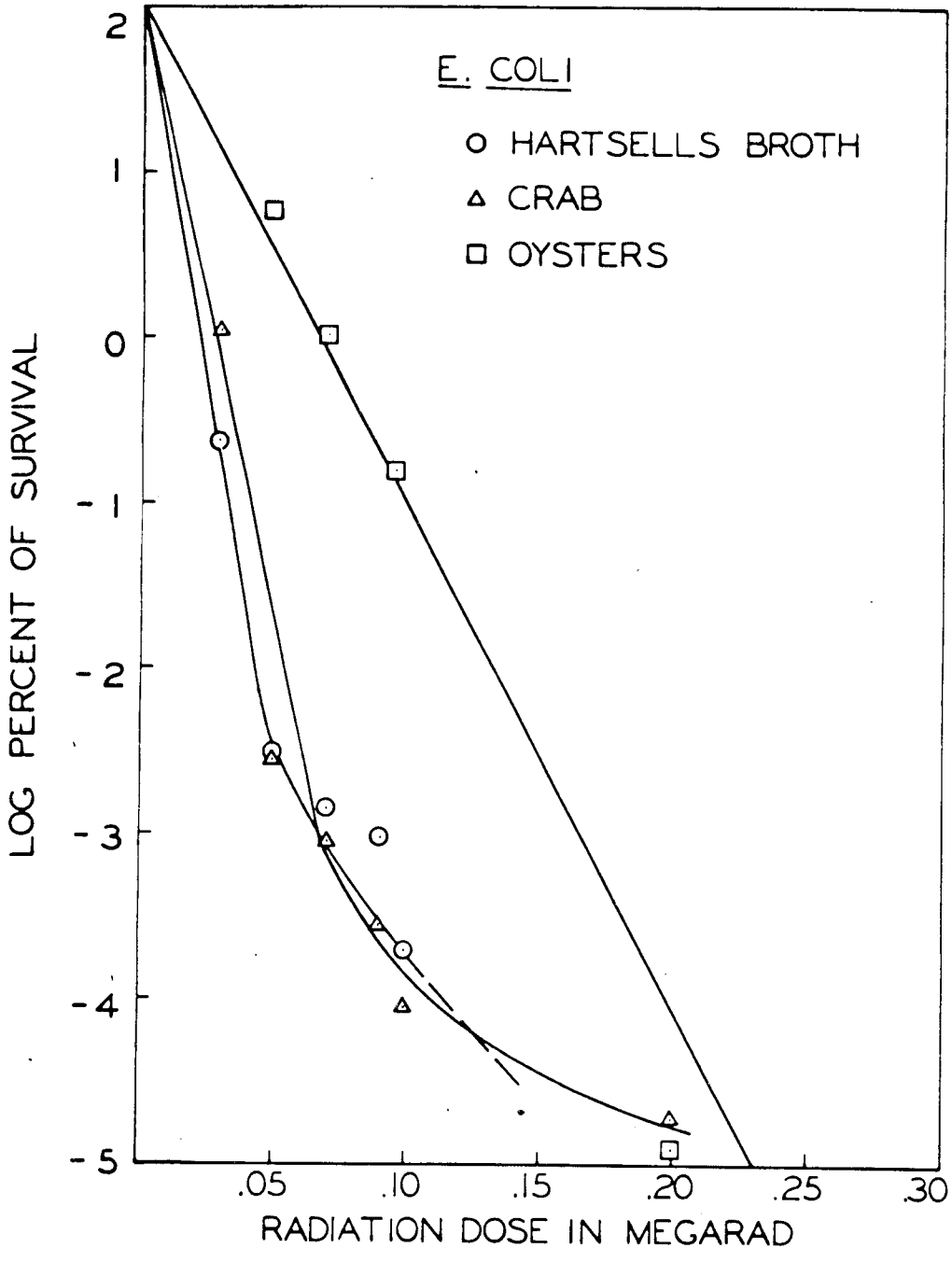

Figure 4. Radiation survival curves of Escherichia coli

Table 5. Radiation inactivation kinetics of *Proteus vulgaris*

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	0.35853	0.10256	0.86621
0.05	0.000229	0.00062	
0.07		0.000045	0.2120
0.09		0.0000094	0.00862
0.10		$\geq$0.00000126	0.00809

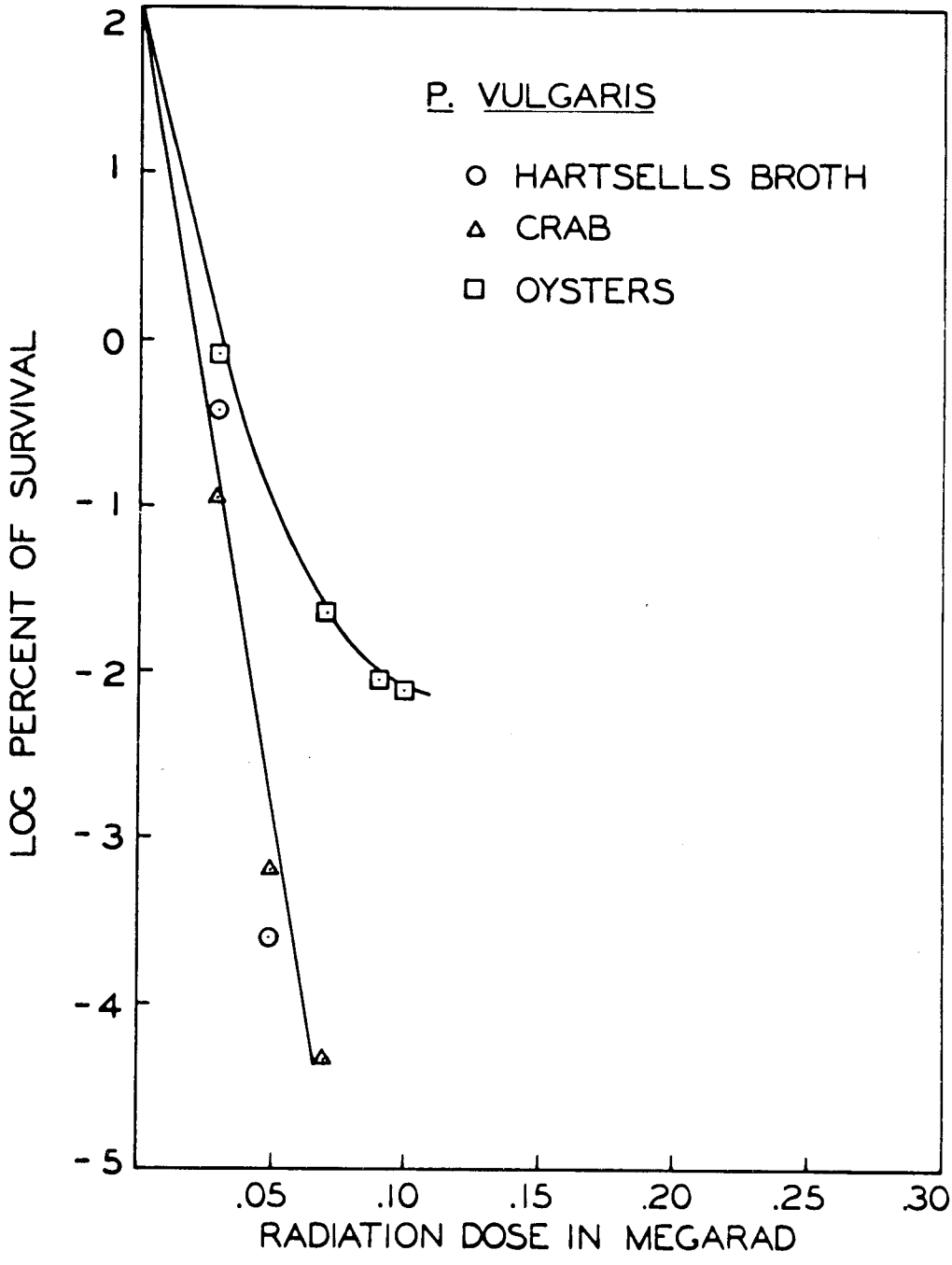

Figure 5. Radiation survival curves of Proteus vulgaris

Table 6. Radiation inactivation kinetics of Streptococcus faecalis.

Dose (Mrad)	Per cent survival		
	Hartsell's broth	Crabmeat	Oysters
0.03	47.74501	38.57142	76.30125
0.05	10.84502	17.14238	42.50510
0.07	1.63388	4.85741	30.04182
0.09	0.67890	1.30950	
0.10	0.36482	0.74285	
0.20	0.00161	0.02190	0.14415
0.30	0.00029	0.00143	0.01331
0.50	0.00018	0.00000071	
0.70	0.00003		

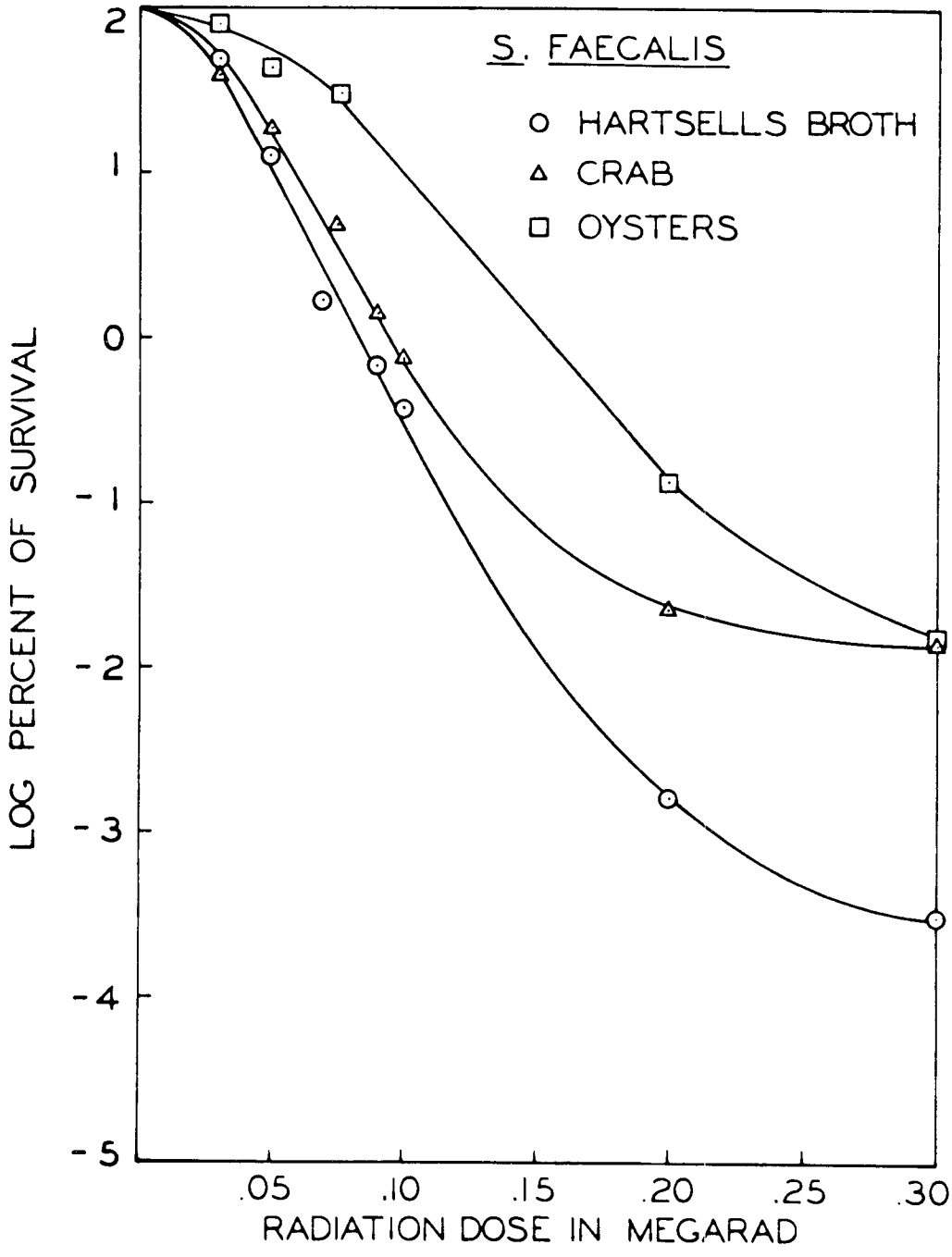

Figure 6. Radiation survival curves of Streptococcus faecalis

Table 7. Radiation inactivation kinetics of *Streptococcus pyogenes*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	36.28300	72.19512
0.05	3.89381	20.12510
0.07	0.49562	10.04222
0.09	0.09734	0.77138
0.10	0.03896	1.19428
0.20	0.00005	0.06142
0.30		0.05802

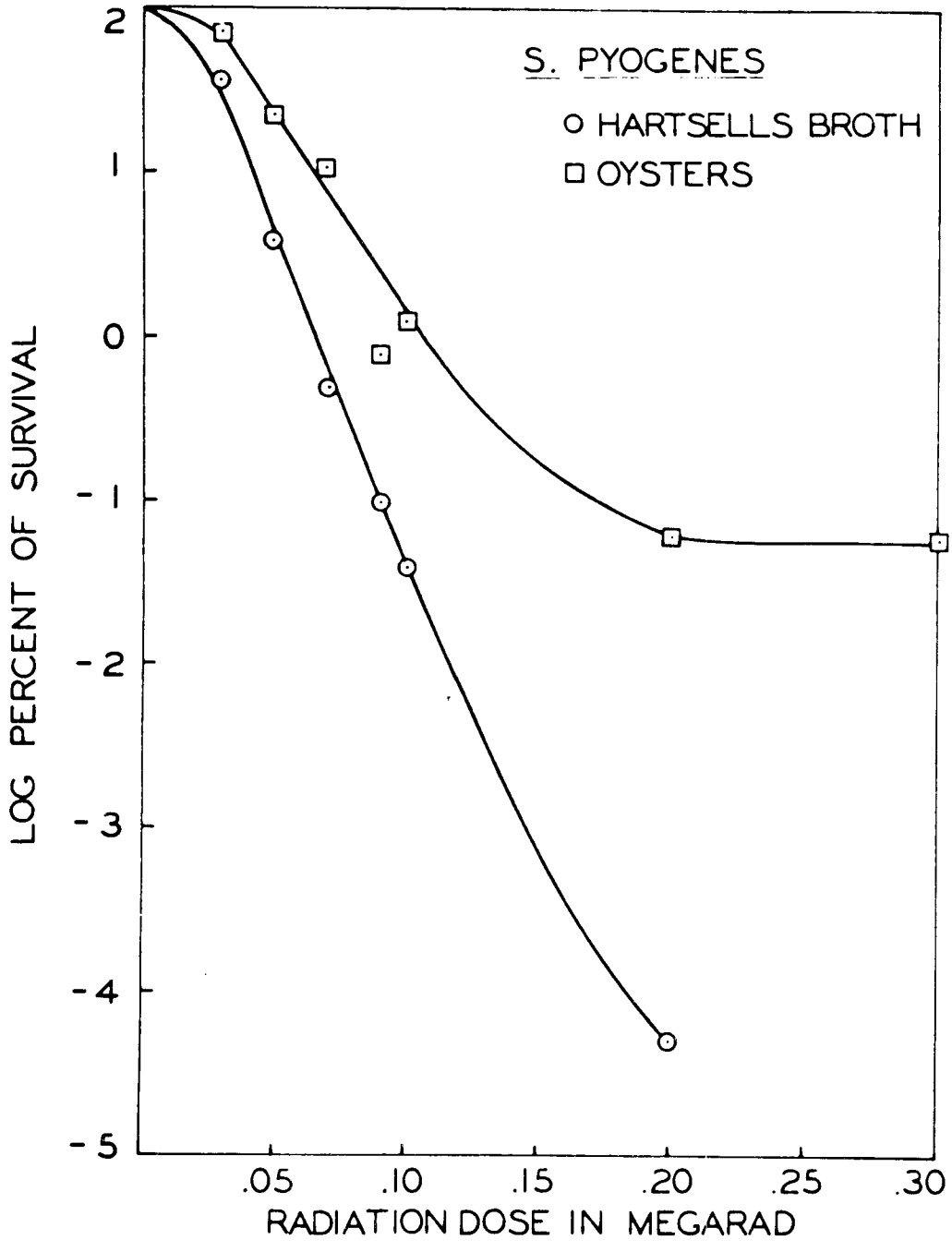

Figure 7. Radiation survival curves of Streptococcus pyogenes

Table 8. Radiation inactivation kinetics of *Staphylococcus aureus*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	68.91340	91.14182
0.05	27.75412	63.47387
0.07	7.57420	32.64151
0.09	6.90413	30.47112
0.10	1.49942	19.26452
0.20	0.12983	
0.30	0.00334	0.13628
0.50		0.00072

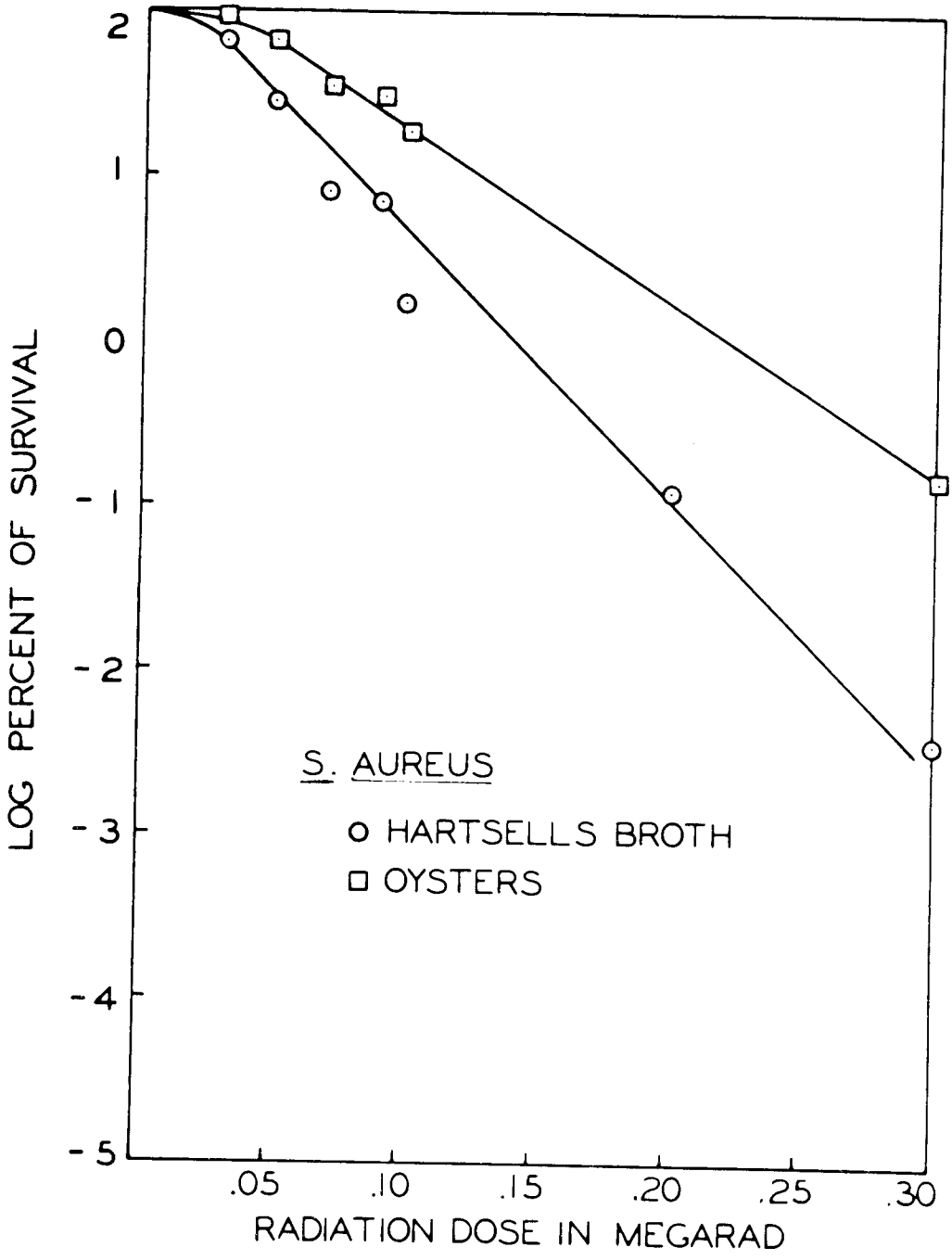

Figure 8. Radiation survival curves of Staphylococcus aureus

Table 9. Radiation inactivation kinetics of *Salmonella choleraesuis*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	2.56812	0.56615
0.05	0.45738	0.01574
0.07	0.05683	2.43815
0.09	0.02737	0.05195
0.10	0.01208	0.03300
0.20	0.0004957	0.002124
0.30	0.0000254	

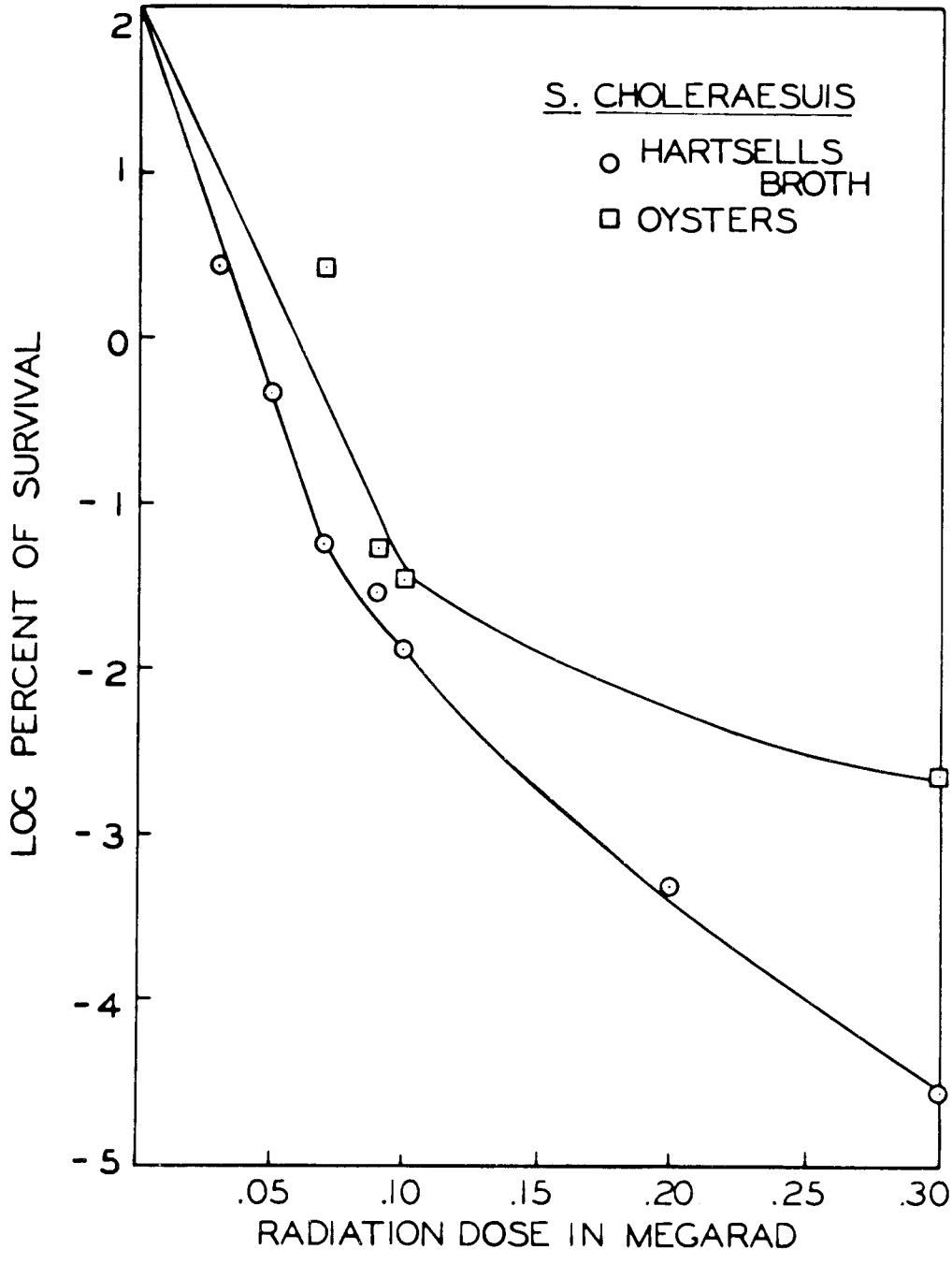

Figure 9. Radiation survival curves of Salmonella choleraesuis

Table 10. Radiation inactivation kinetics of *Salmonella paratyphi A*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	3.04161	
0.05	1.22351	
0.07	0.04205	1.83156
0.09	0.01316	1.43386
0.10	0.00003	0.91425
0.20		0.18142

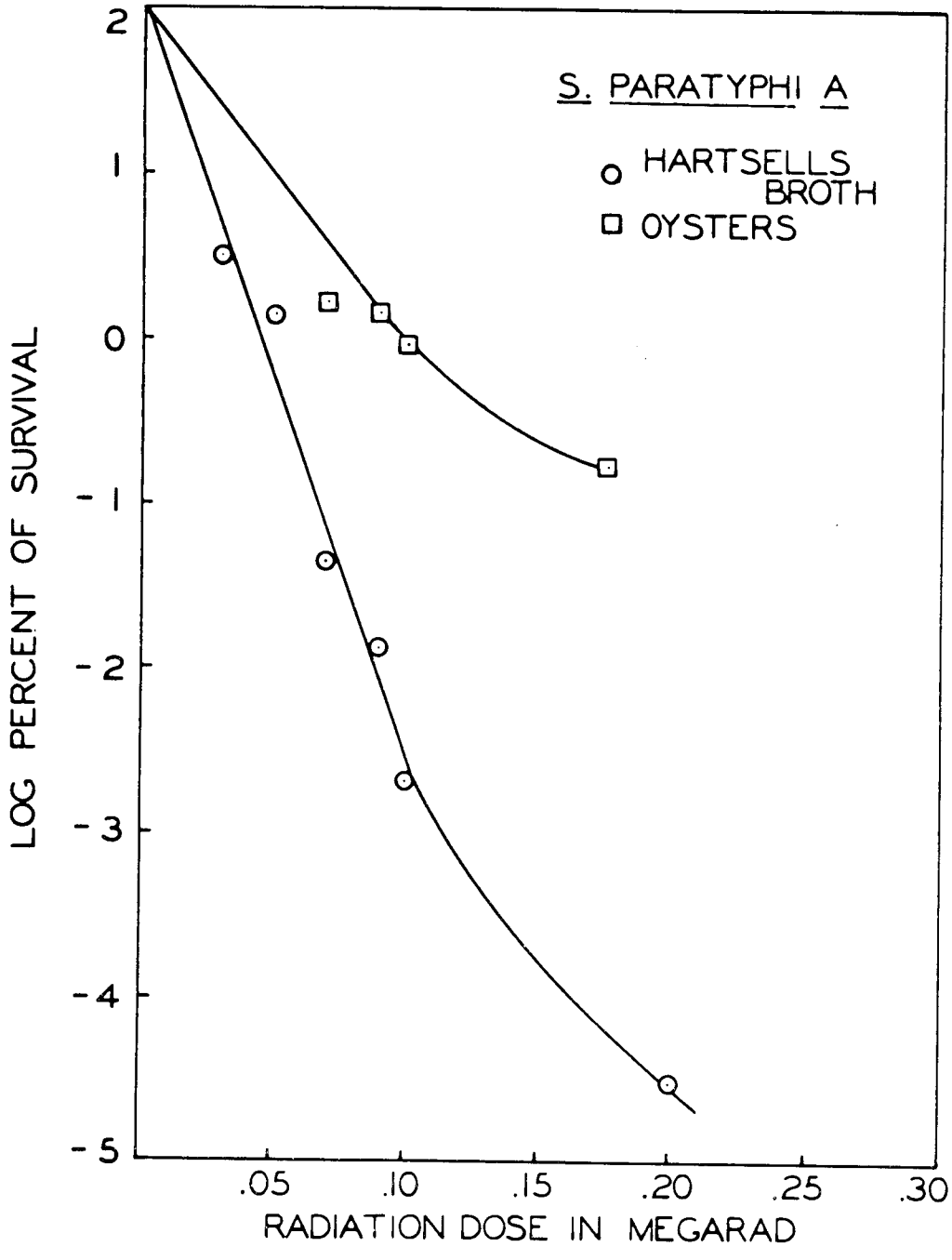

Figure 10. Radiation survival curves of Salmonella paratyphi A

Table 11. Radiation inactivation kinetics of Salmonella pullorum

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	0.60315	0.72968
0.05	0.02705	0.25032
0.07	0.02354	0.04598
0.09	0.00094	0.01891
0.10	0.00027	
0.20	0.0000054	0.00180
0.30		0.0000005

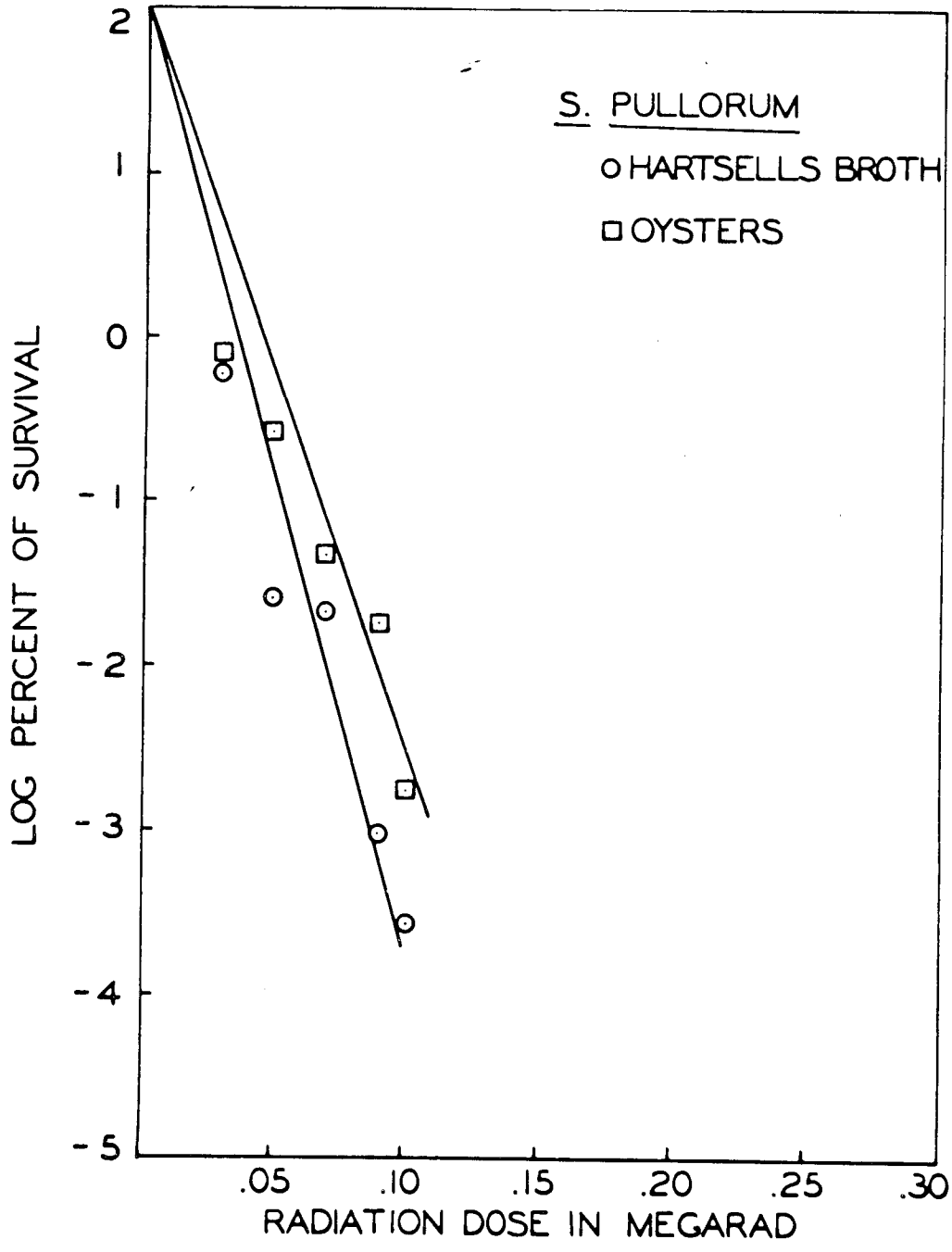

Figure 11. Radiation survival curves of Salmonella pullorum

Table 12. Radiation inactivation kinetics of *Salmonella wichita*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	12.29130	3.20313
0.05	2.59313	2.29441
0.07	0.29418	0.04901
0.09	0.09301	0.13074
0.10	0.05934	0.30592
0.20	0.001142	0.05921
0.30	0.000024	0.00432

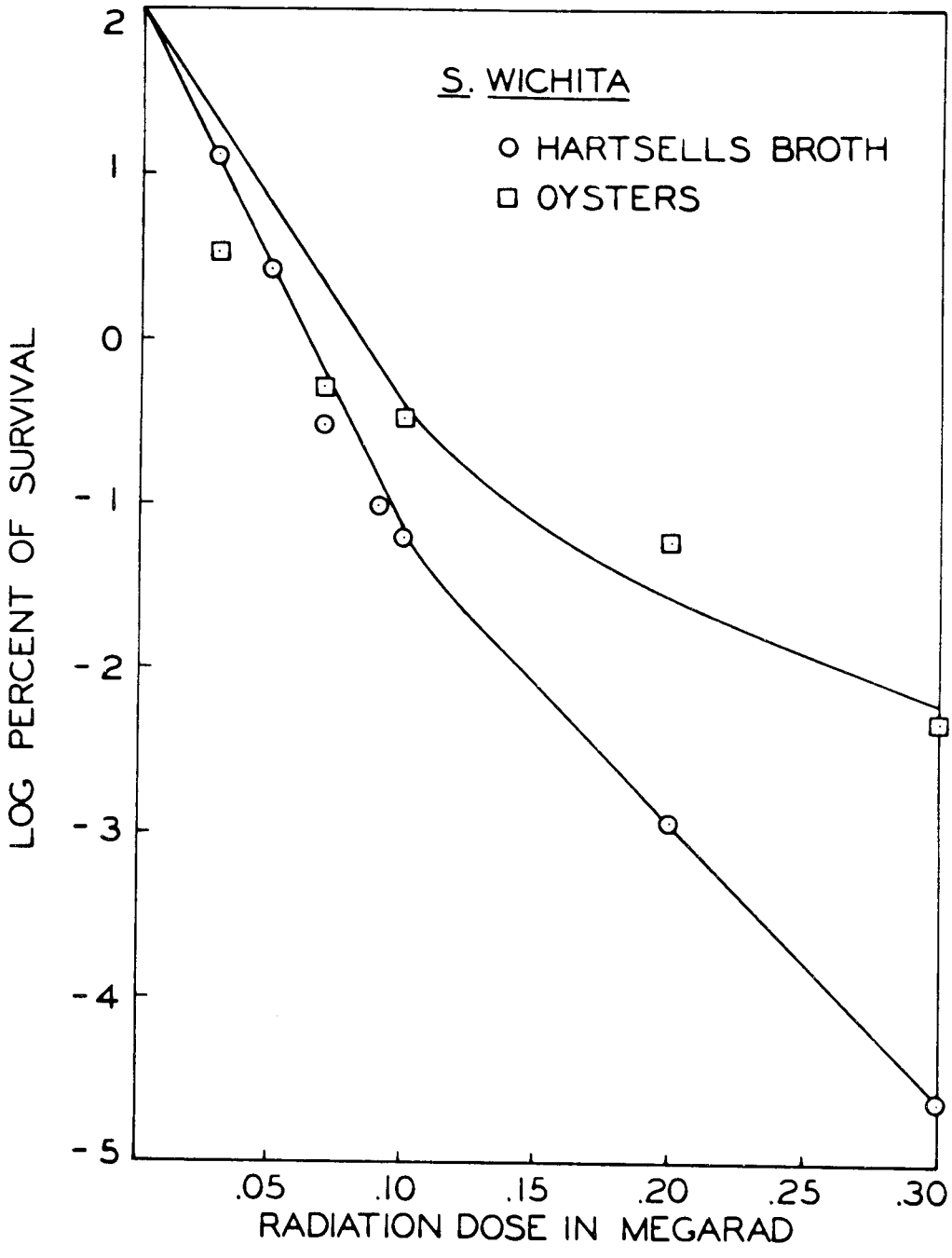

Figure 12. Radiation survival curves of Salmonella wichita

Table 13. Radiation inactivation kinetics of Salmonella enteritidis

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	0.72134	30.00000
0.05	0.04731	10.50000
0.07	0.00426	3.94162
0.09	0.00124	
0.10	0.000205	0.42413

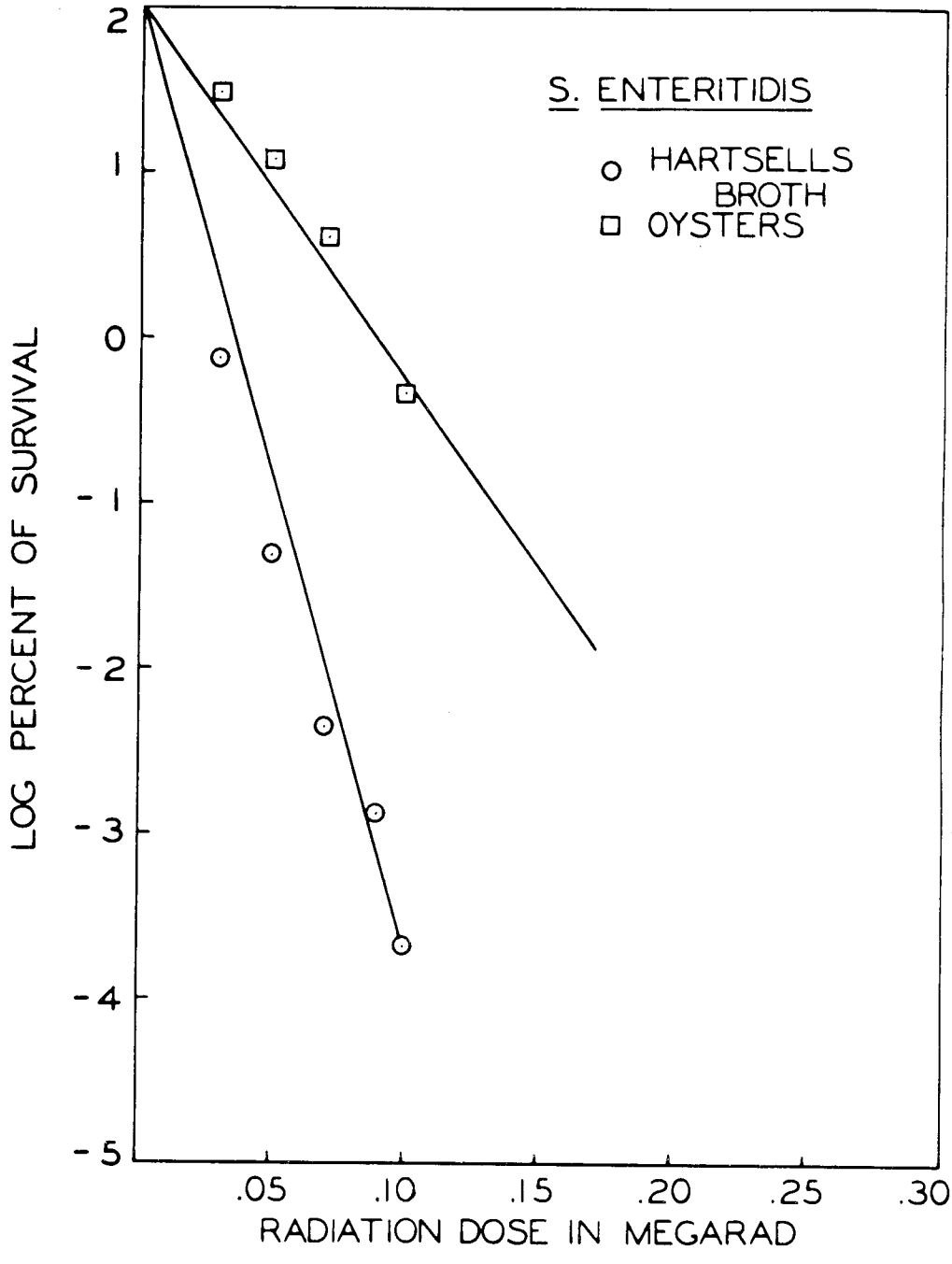

Figure 13. Radiation survival curves of Salmonella enteritidis

Table 14. Radiation inactivation kinetics of *Salmonella typhosa*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	2.67151	
0.05	0.12942	1.52613
0.07	0.01663	0.76464
0.09	0.00314	
0.10	0.00200	
0.20	0.00016	0.12405
0.30	0.0000124	0.000721

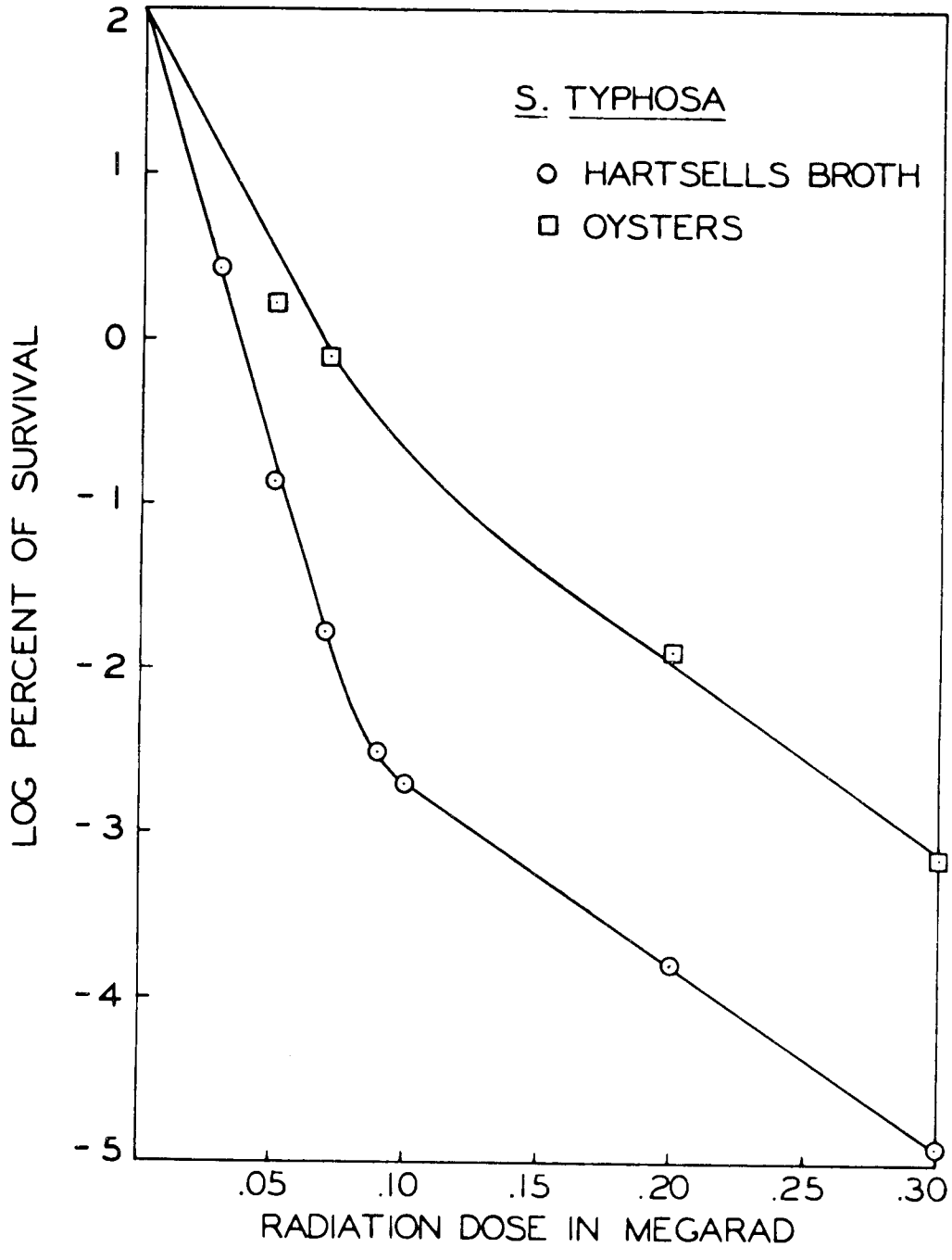

Figure 14. Radiation survival curves of Salmonella typhosa

Table 15. Radiation inactivation kinetics of *Salmonella paratyphi B*

Dose (Mrad)	Per cent survival	
	Hartsell's broth	Oysters
0.03	2.70371	7.63941
0.05	0.66667	3.50416
0.07	0.06827	1.26103
0.09	0.05272	0.18364
0.10	0.08987	0.05615
0.20	0.00406	0.05300
0.30	0.000189	0.00591
0.50	0.00000073	0.000713

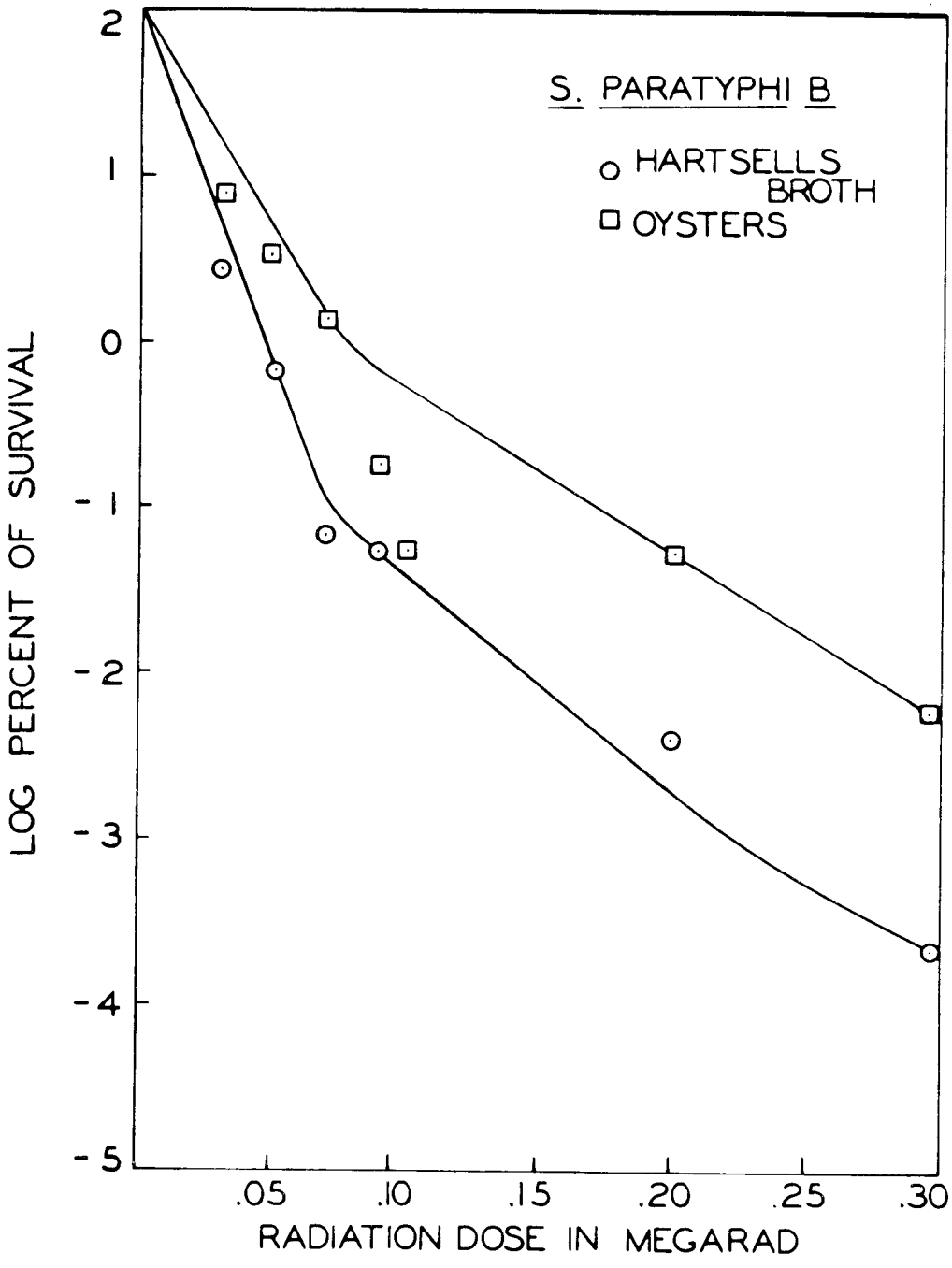

Figure 15. Radiation survival curves of Salmonella paratyphi B

Table 16. D-values of bacteria in seafoods and Hartsell's broth.

Organism	D-value (Mrad)		
	Hartsell's broth	Oysters	Crabmeat
E. coli	0.012	0.035	0.014
Pr. vulgaris	0.010	0.015	0.010
Sh. sonnei	0.020	0.025	0.027
Sh. paradysenteriae	0.020	0.026	0.022
Sh. dysenteriae	0.024	0.035	0.029
Strep. faecalis	0.050	0.100	0.059
Strep. pyogenes	0.043	0.065	
Staph. aureus	0.080	0.125	
Sal. pullorum	0.018	0.022	
Sal. choleraesuis	0.022	0.030	
Sal. enteritidis	0.018	0.045	
Sal. paratyphi A	0.023	0.050	
Sal. paratyphi B	0.025	0.038	
Sal. wichita	0.032	0.042	
Sal. typhosa	0.018	0.035	

Table 17. "Tailing effect"

Organism	Hartsell's broth	Oysters	Crabmeat
E. coli	+	-	+
Pr. vulgaris	-	+	-
Sh. sonnei	-	-	+
Sh. paradysenteriae	-	-	-
Sh. dysenteriae	+	+	+
Strep. faecalis	+	+	+
Strep. pyogenes	+	+	
Staph. aureus	-	-	
Sal. pullorum	-	-	
Sal. choleraesuis	+	+	
Sal. enteritidis	-	-	
Sal. paratyphi A	+	+	
Sal. paratyphi B	+	+	
Sal wichita	+	+	
Sal. typhosa	+	+	

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