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Title: THE IRRADIATION RESISTANCE OF FOOD PATHOGENS CONTAMINATING SEAFOODS

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Studies have been made to determine the limits of tolerance to gamma radiation for 15 cultures of the following bacterial species both in culture media and in irradiation-sterilized seafoods (crab-meat, halibut and shrimp) inoculated with Salmonella (seven species), Shigella (three species), and one species each of Neisseria, Mycobacterium, Escherichia, Proteus and Streptococcus.

It was determined that the resistance dosages for the above mentioned bacterial species ranged from 2.0×10^5 to 5.0×10^5 rads. Salmonella schottmuelleri, Salmonella wichita, Salmonella typhosa and Escherichia coli were found to be the least sensitive to radiation, and therefore required the highest dosage. Shigella dysenteriae, Shigella paradysenteriae and Proteus vulgaris, which were most sensitive, required the lowest dosage. The rest of the cultures required dosages, in varying degree, between those of the foremention ed.

It was found that radiation dosage for each of the bacterial

species in culture media corresponded to that in seafoods upon irradiation.

Attempts were made to use vitamin K_5 as a radiation sensitizing agent for effective food preservation. The results did not indicate any obvious sensitizing effect of vitamin K_5 on Salmonella typhosa.

THE IRRADIATION RESISTANCE OF FOOD PATHOGENS CONTAMINATING SEAFOODS

by

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INTRODUCTION

Since the world population is increasing at a great rate, seafoods have unquestionably become more important sources of food for human consumption. Seafoods which are of commercial importance are, to name a few, crab, fish and shrimp, most of which are sold fresh or frozen.

The storage of seafoods has, unfortunately, been a tremendous problem because of the presence of various microorganisms (51, p. 752). This often results in spoilage of tissues due to biochemical and physiological changes, which subsequently cause undesirable odors and changes in flavor and texture, thereby shortening storage life. Pathogenic microorganisms, if any, are known to contaminate seafoods from natural sources, as well as from handling and processing. The elimination of microorganisms by physical and chemical methods would, undoubtedly, provide means of greatly extending storage life of seafoods.

Recently, research workers in food technology have devoted special attention to the application of ionizing radiation to the problem of food preservation. This method is highly desirable because of its potential applied value. However, there appears to be a lack of information on the conclusive practicability of this method, as

most of the work done so far is still in the experimental stage.

This study has been made in order to determine the levels of radiation doses which would destroy pathogenic microorganisms representative of various genera and species of public health significance in food. The pure cultures of each in growth media and in seafoods were irradiated at different levels to determine the relative numbers of surviving microorganisms as well as lethal doses. Vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride) was used for the purpose of radiation sensitization.

LITERATURE REVIEW

In spite of intense research work in the field of food preservation by ionizing radiation, the discussions about the value and economic status of the new processing method, as well as about the wholeness of the irradiated foods, are still going on. Food preservation by irradiation is already possible on a commercial scale; the cost of irradiation can be estimated, for the time being, only approximately, but it seems that the application of ionizing radiation to single products will be of economic interest. More work must be done to support the conclusions drawn from the results of feeding experiments on wholesomeness of irradiated foodstuffs. Up to now only in single cases has it been possible for one to eliminate or avoid off-flavors and other deleterious effects in irradiated produce. Intensive research on both problems is being carried out (69).

The first reports on a study of the effects of X-rays on bacteria was published in 1896 by Minch (57, p. 367). Pacinotti and Porcelli (92, p. 188) were among the early investigators in 1898.

Prescott, in 1904 (96, p. 246-248) reported the effect of radium rays on the colon bacillus, diptheria and yeast. The use of high energy radiation for food processing was issued a French patent in 1930. English workers reported in 1936 and 1937 on their studies on sterilization with ionizing radiations (53, p. 1). In 1943,

Proctor, van der Graaf and Fram showed that X-rays could destroy microorganisms in highly contaminated food material (104, p. 927).

The application of ionizing radiation to food preservation was not started until 1945 (89, p. 554); the studies concerning the use of this energy for cold sterilization were begun in the Food Technology Laboratories of the Massachusetts Institute of Technology.

Nickerson, Proctor and Goldblith (89, p. 556) reported, in 1950, that the species of microorganisms found is of far greater importance than the number of microorganisms present.

In 1953, Tarr (128, p. 3) found that microorganisms of the genera Corynebacterium and Mycoplana occurred in variable ratios. He said that the external slime and digestive tracts of feeding fish supported a variable microflora both qualitatively and quantitatively. In the same year, Shewan (116, p. II 1-7) concluded that the chief bacterial genera associated with fish were Micrococcus, Serratia, Flavobacterium, Pseudomonas, Achromobacter, Proteus and Bacillus.

Bellamy et al., in 1955 (10, p. 266) said that a variety of conditions may modify the radiation sensitivity of microorganisms and influence the effects of a given dose of irradiation.

Anderson et al., in 1956 (4, p. 577) isolated a non-spore-forming, non-toxic bacterium Micrococcus radiodurans from ground beef which has a higher resistance to radiation than any other bacterium tested.

Morgan (81, p. 423; 33, p. 18) reported that bacteria of the genus Pseudomonas are quite sensitive to radiation, less than 100,000 reps prolonging shelf life under refrigeration without organoleptic changes.

The spore forming bacillus, Clostridium botulinum, appears to be the most radiation-resistant microorganism involved in food processing. Morgan (80, p. 24) indicated that the minimum dosage of 4.5×10^6 rads would be required to ensure radiation sterilization for food processing.

Shewan and Georgola in 1957 (118, p. 163) reported that the results of several studies indicated that coliform bacteria, Shigella, Salmonella and catalase positive Staphylococcus, from fish were never encountered; only a small number of fecal coliform bacteria were found during the filleting process.

Coleby, in 1958 (25, p. 877), found that the amount of irradiation required to eliminate all food microorganisms was high. However, the associated chemical changes, which may be small in quantity, would have an adverse effect upon the quality of the food and may result in changes in the odor, flavor, and texture of the product.

Cain et al. (19, p. 537) reported that the increase in the number of exposures resulted in a decrease in survivors and that the bacterial survival was dependent on both radiation dosage and

temperature.

In 1959, Coleby (26, p. 116) stated that there appears to be general agreement that meat stored at 0-5°C after irradiation at doses in the range of 5×10^4 to 1×10^6 rads can be held five to ten times longer than unirradiated meat before microbial spoilage terminates the shelf life of the product.

Eukel and Huber in 1960 (42, p. 200) reported that with salmon steaks irradiated at 2.5×10^5 rads, with proper care in selecting the fish and with proper handling techniques, the shelf life of the fish may be extended to about 20 days at a storage temperature of 5.5°C. The refrigerated shelf life extension for cooked crabmeat and shrimp could also be obtained with the same dose.

MacLean and Welander (74, p. 251-252) and Miyauchi (79, p. 382) compared bacterial counts of unirradiated cod fillets stored in ice at 0°C with cod fillets irradiated at levels from 7×10^4 - 2×10^6 rads and stored at 0°C. A pronounced difference was evident between results obtained at 5.0×10^5 and 1.0×10^6 rads and higher levels of irradiation where counts remained quite low over the entire storage period.

Sinnhuber et al. (122) found that 2.5×10^5 rads inhibited spoilage in crabmeat for 20 days at 7°C, whereas 5.0×10^5 rads preserved the crab for 60 days at the same temperature. Shrimp irradiated with 5.0 and 7.5×10^5 rads were not spoiled

after 180 days at $7^{\circ}C$, and shrimp irradiated with 2.5×10^{5} rads remain unspoiled for 60 days.

Radiation Preservation of Food

Meinke in 1954 (78, p. 37) found that induced radioactivity does not occur at the levels used in the irradiation of food. Shewan (117, p. 143) found that the sterilization of the more common varieties of sea foods by irradiation required doses which almost invariably produce undesirable and unacceptable organoleptic changes of these products.

As indicated by Hannan (51, p. 752), radiation preservation presents a novel approach to the inactivation of food spoilage organisms without heat treatment or addition of chemical preservatives. The possibility of combining radiation with other methods of processing has yet to be fully explored. The cost of radiation sterilization is acceptable for many foods, although initially too high for treatment of food of low cost per unit weight. The cost of pasteurization should be acceptable for most products (52, p. 179-209).

Clifcorn in 1955 (23, p. 39) pointed out that the use of radiation pasteurization may alter the storage and distribution patterns of food products thus allowing areas of production greater freedom of marketing.

In 1958, Shewan and Liston (119, p. 377) indicated that toxic products might be produced in minute but significant amounts, and subsequently chemical changes may occur in odor, taste, color and texture.

Niven (91, p. 518) mentioned that even though the predominant organisms are destroyed by this process, less numerous but more resistant bacterial species survive and ultimately cause spoilage.

Ingram (62, p. 106) reported that pathogenic bacteria do not develop below 4.4°C, so that radiation pasteurization in combination with low temperature storage is a feasible process.

Laser (70, p. 96-99) indicated that food preservation by ionizing radiations is dependent on our knowledge of the basic effects of these radiations on microorganisms, the growth of which is intended to be suppressed. The effects of radiation on components of the biological material itself, including the variations in oxygen tension which affect radiosensitivity prior to or during irradiation, also must be known.

Erdman, in 1961 (41, p. 199-205), used 21 cultures representative of the bacterial species of public health significance in food. Coliform organisms were more sensitive to irradiation than the staphylococci and Streptococcus faecalis. He also indicated that the destruction of coliform bacteria in irradiated food cannot be used as an index of adequate pasteurization.

Type of Ionizing Radiation

Current radiomicrobiological interest reported by Morton and Byrne in 1957 (84, p. 208) has centered chiefly on X-rays, gamma rays and cathode rays. Sources of these radiations at fairly high intensity have been generally available for use in bacteriological investigations.

The comparative effects of high and low intensity radiation were studied on organoleptic changes in foods, bactericidal efficiency and nutrient destruction. According to Taimuty and De La Rue in 1957 (125), a cobalt-60 gamma source was used for the low intensity irradiations, and a 6- to 9- Mev linear electron accelerator was used for the high intensity irradiations. The effects due to type of radiation and rate of irradiation do not play an important role in the effects of radiation on foods, bacteria and nutrients.

Goldblith et al. (48, p. 659-677) indicated that cathode rays, X-rays and gamma rays were essentially of the same bactericidal effectiveness when differences in uniformity of dose and dosimetery corrections were performed.

Units of Dose

Schinz and Winderace (109, p. 313-320) discussed the definitions of units in radiation measurement for the purpose of

determining clear definitions; the adsorption of the radiation in free air is ignored and the concept of dose is therefore limited only to the adsorbing body. The practical unit of measurement for radiometry is the "roentgen" which corresponds to the radiation density of the field. The unit employed in food irradiation dosimetry is the "rad". The roentgen should not be used. The "rho" is an auxiliary unit used in ionization measurements from which the dose in rads is determined. Rho is used for the measurement and rad for the dosage of radiation.

A rad is the quantity of ionizing radiation which results in the absorption of 100 ergs per gram of irradiated material (56, p. 287).

Dose Rate of Ionizing Radiation

Brasch et al. (12, p. 246) showed that dose rate has a considerable influence on irradiation changes in foodstuffs. High dose rates are useful for protection of color and flavor in foodstuffs (61, p. 109-115).

Clifcorn (23, p. 40) found that changes in color and flavor in foodstuffs are in direct proportion to the radiation dose applied and vary in degree from product to product. Irradiation doses of about one megarep were found to destroy all the microorganisms that cause food poisoning, except some of the more resistant spore formers (16).

Bacteria of the intestinal group were found to be killed by radiation doses of 4.0×10^5 to 6.0×10^5 rads. When spore-forming bacteria were contained in the material, sterilization was achieved by doses of 1.5 to 2.0×10^6 rads. A dose of 1.5×10^6 rads had a sterilizing effect, killing not only vegetative bacteria but spore formers as well. Irradiation with sterilizing doses did not reduce the nutrient properties of meat media used for growth of bacteria of the intestinal group (131).

In 1958, a study was made of the quality, antigenic and immunogenic properties, liability to retain Vi antigen and toxicity of vaccines and antigenic complexes prepared from irradiated dysentery and typhoid bacteria (130).

The inactivation of most species of microorganisms by ionizing radiation, according to Horne and Bridges (60, p. 100-104), is approximately an exponential process and doses suitable for sterilizing foodstuffs must be calculated with this fact in mind. It is shown that, assuming a standard sterility of 10^{-8} organisms per gram, a safe sterilizing dose will be of the order of 3.0 to 5.0×10^6 rads. The effect during irradiation of certain environmental factors such as pH, freezing, heating and additives is in relation to food processing.

Effect of Ionizing Radiation on Protein

Major chemical changes induced by radiation on proteins include denaturation, degradation and polymerization. The allergenicity of proteins has been shown to be particularly sensitive to irradiation (112, p. 76).

Proteins are condensation polymers composed of many amino acids linked together by peptide bonds. The nature of irradiation effects upon proteins is dependent upon the polymer chain length, amino acid composition and the chronological sequence of the amino acids. A protein molecule may behave in the presence of radiation both as a single molecular entity, such as when it is denatured, and as a composite of various amino acids with each acid displaying a specific radiation sensitivity (35, p. 156-158). It is evident that fragmentation as well as aggregation of protein occurs when they are subjected to ionizing irradiation. It is expected that some random cleavage of peptide chains occurs since carbon bonds are ruptured by both direct and indirect action (35, p. 149).

The physical changes in irradiated proteins were described by Hannan (53) as that of some form of denaturation with the character of changes varying with the nature of the free polymer and the irradiation conditions. It is most likely that the primary valency bonds are broken and reactive free radical fragments are formed.

McArdle and Desrosier (76, p. 527-532), studying the effects of cathode rays, found changes in the protein molecule of casein and egg albumin. The pattern of changes differed in these two proteins; the increase in free sulfhydryl group indicated that the sulfur linkages and hydrogen bonds were attacked and caused molecular rearrangement. Since these molecular changes did not result in any increase in amino nitrogen, it was believed that the peptide linkages were not attacked. The sulfur linkages were very definitely the site of a large share of the radiation effect. Hydrogen bond linkages also are undoubtedly broken.

Doty et al. (32, p. 424) reported that non-protein nitrogen compounds increase in ground lean beef after irradiation, but there was an appreciable reduction of soluble protein. The formation of methyl mercaptans and hydrogen sulfide increased, as well as the pH and the carbonyl compounds in meat, with increases in radiation dosage (7, p. 64). The studies conducted by Zender (137, p. 390) found a decrease in the glycine soluble protein content of beef muscle after irradiation.

According to Morgan (81, p. 425), the total carbonyl content of irradiated beef indicated that low molecular weight extractable carbonyls were obtained from the protein fraction. He also suggested that volatile bases such as methylamine and ethylamine could react with carbon dioxide to form low molecular weight volatile

compounds. This could explain the loss of carbon dioxide during irradiation.

Other investigators (34, p. 61-63; 81, p. 425) have also found that the amount of protein breakdown is related to the level of irradiation dosage.

Effect of Ionizing Radiation on Amino Acids

The deamination of amino acids is known to be characteristic of ionizing irradiations (63, p. 6-10; 100, p. 535-538). The principal products of deamination of amino acids are ammonia and the corresponding aldehydes. Drake and Giffee (35, p. 157-158) observed that the chemical changes in irradiated proteins include liberation of ammonia, oxidation of -SH groups and formation of peroxides.

The sulfur containing amino acids - methionine, cystine and cysteine, and the ring-containing amino acids - histidine, hydroxyproline, phenylalanine, proline and tryptophan, are the most sensitive to irradiation, (81, p. 426). According to Dale and Davies (28, p. 129-134) the irradiation of a sulfur-containing amino acid liberates hydrogen sulfide or oxidizes the -SH groups to disulfides together with sulfinic-SO_2H and sulfinic - SO_3 acid groupings.

Proctor and Bhatia (100, p. 535-540) reported from the findings of their studies that hydroxylation of the ring in aqueous

solutions of a cyclic compound was found in some of their experiments. The hydroxylation of phenylalanine to tyrosine and a second hydroxylation forming 3,4-dihydroxy phenylalanine gave solid evidence from which their conclusions were drawn. They also found that radiation caused no significant destruction of any of the ten amino acids in fish (99, p. 357-361).

Effect of Ionizing Radiation on Lipids

Many of the objectionable changes occuring in irradiated foods appear to originate in the lipid proteins. A number of research workers have shown the formation of peroxides from lipids both during and after irradiation (38, p. 605-606; 85, p. 589; 87, p. 84-88; 101, p. 119-189). Mukherjee (85, p. 589) noticed the peroxide formation in butterfat during, as well as after, irradiation. He also observed that butterfat was more susceptible to autoxidation, while irradiation of unsaturated fats did not produce this effect. Astrack et al. (5, p. 570-583) have examined by chemical and organoleptic means the effect of sterilizing doses of high intensity electron bursts upon various vegetable and fish oils. They found that the undesirable effects that occur in irradiated fats are largely oxidative in nature and involve reactions with free radicals (5, p. 570; 54, p. 152; 55, p. 1021). Hannan and Sheppard (55, p. 1021) demonstrated that the changes which occurred during the

irradiation of butterfat were followed by extensive changes after irradiation and both were affected by temperature.

Coleby (24, p. 71-75) reported that irradiation gives rise to off-flavors in lipids which, with some foods, are a serious disadvantage. The off-flavors show a resemblance to those encountered in oxidative rancidity, and experiments with model systems have shown that the course of reactions is somewhat similar in the two cases, i. e. hydroperoxides and carbonyl compounds are formed. The irradiation of fats stimulated oxidation processes, causing the peroxide number to increase. The irradiation in an atmosphere of nitrogen with the addition of an antioxidant inhibited the oxidation process. Fats stored at low temperature contained less peroxides (44).

Bernheim and Wilbur (11, p. 1-7) reported in 1962 the effect of ultraviolet and ionizing radiation on the oxidation of cell lipids. They found that both in vitro and in vivo radiation produced oxidation products of lipids that not only inhibited the activity of certain oxidative enzymes and depolymerized deoxyribonucleoprotein but also inhibited the division of marine eggs and retarded bacterial growth.

Effect of Ionizing Radiation on Enzymes

The effects of ionizing irradiations on enzyme systems have

been investigated by many research workers. Doty and Wachter (34, p. 61-63) showed that there was very little destruction of proteinase in beef irradiated at 5.0×10^5 rep, but at a higher dosage level, 1.6×10^6 rep, there was about a 50 percent loss in the apparent activity in some samples. The rate of inactivation of enzymes is most commonly described by an exponential decrease in activity with increasing irradiation dosage. This follows from the one-hit target theory for direct excitation described by Pollard (95, p. 99-109) and Setlow (114, p. 471-483).

Enzymes irradiated in dry states are inactivated directly by excitation but enzymes in solution are inactivated indirectly by -OH and $-OH_2$ radicals formed in the solvent, while H_2O_2 formed has a negligible influence (6, p. 188-201). A large amount of irradiation, possibly ten times as great as that necessary for bacteriological sterilization, was required to destroy or inactivate the enzymes in various foods (38, p. 605). Some peroxidase activity in milk irradiated at 1.0×10^7 reps was observed by Proctor and Goldblith (102, p. 376-379).

Dale (27, p. 1367) postulated that the enzyme molecules were not directly affected by ionizing radiation. Instead, the non-protein moiety of the enzyme or the prosthetic groups received the brunt of the irradiation dose. When acting upon the protein moiety, irradiation may destroy certain selective groups in the side chain which

are essential for enzyme activity or it may rupture the hydrogen bond and thus cause precipitation. It is evident that enzymes are considerably more resistant to irradiation than are the microorganisms. Conversely, microorganisms are less susceptible to heat treatment than are the enzymes. Morgan and Siu (83, p. 277) found that the application of heat remains the most practical method for inactivating enzymes. Drake and Giffee (36, p. 23) reported that the enzymes responsible for proteolysis in meat are inactivated by heating at 71°C for ten minutes.

Effect of Ionizing Radiation on Microorganisms

Hollaender (58, p. 562-563) suggested that the reaction mechanisms of ionizing radiations on microorganisms are influenced by atmospheric oxygen, nutritional state, enzyme systems, pH of the growth medium, addition of cysteamine, streptomycin treatment, radiation energy, radiation dose and radiation sensitivity. The radiation doses of 5.0×10^5 rads and 4.8×10^6 rads have been established as the dosage levels required for pasteurization and sterilization respectively (81, p. 423-427).

Dunn (37, p. 421) reported that some inorganic chlorides and sulfates enhanced the germicidal action of cathode rays toward Staphylococcus aureus, and that old cells of Staphylococcus aureus were the most sensitive. This microorganism was found to be most

sensitive at high and low pH values (53, p. 57).

Sarcina species appeared to have produced resistant strains to radiation due to its carotenoid pigment content (66, p. 207). Erdman et al. (41, p. 201) found Streptococcus faecalis to be more resistant than many non-spore-forming cultures in broth suspension. Rhodes (106, p. 10) reported in 1961 that the irradiation processing of frozen eggs for the elimination of Salmonella was simple and reliable. A slight odor and off-flavor of the product were the only disadvantages. Schweigert (113, p. 155) observed that doses from 1.25×10^5 to 6×10^5 rads destroyed Salmonella present in the eggs.

Members of the Salmonella group are capable of causing food poisoning, but according to reports by Brooks, Hannan and Hobbs in 1959, their position has been rendered more serious by the recent discovery of Salmonella schottmuelleri in imported egg products (15, p. 149-154). Having treated frozen whole eggs with 2 Mev cathode rays, they discovered that a dose of about 3.0×10^5 to 5.0×10^5 rads would destroy the Salmonella. The contaminants before irradiation were Salmonella schottmuelleri, Salmonella newport and Salmonella thompson.

Chekatilo (22), in his investigation of the effect of irradiation of mice on the virulence of typhoid bacilli, found that the mortality of mice infected with the bacterial cultures isolated from irradiated guinea pigs was higher than in mice infected with cultures isolated

from non-irradiated guinea pigs (54.5 percent in the experimental group, as against 38 percent in the control group). Also, it was revealed that the longer the organisms remained in the guinea pigs prior to inoculating the mice, the greater became their virulence. This was noted especially in organisms from irradiated guinea pigs.

Nakamura and Ramage (86, p. 1028) reported that a reduction of the surface tension of a suspending medium during exposure of Shigella sonnei to ultraviolet rays resulted in increasing survival of the irradiated cells. The addition of a metabolite after exposure promoted recovery of E. coli. The difference in viable plate count was determined to vary widely for irradiated E. coli B (3, p. 469). A catalase-negative E. coli was more sensitive than a catalase-positive strain, which was protected by added catalase (1, p. 451-458).

Ultraviolet irradiation of Vibrio cholerae seemed to immediately interfere with the ability of the cells to form deoxyribonucleic acid, followed by the lysis and the breakdown of the cellular nucleic acid. Sagar (108, p. 166) found that the growth from irradiated cultures resulted in abnormally long cells.

Dewey (31, p. 1008), who studied radiation protective effect of glycerine on Serratia marcescens, concluded that the effect was independent of the changes in oxygen concentration by a factor of 100 and was also independent of temperature changes.

Pseudomonas geniculata was more resistant in meat than in

broth (133, p. 684). Thornley et al. (129, p. 487-498) reported that Pseudomonas was more sensitive to radiation, but Achromobacter was more sensitive to tetracyclines. The combined effects of chlortetracycline and irradiation prolonged the storage life of food approximately two times.

Taplin et al. (126, p. 771-773) exposed lyophilized Brucella abortus 19 and Mycobacterium tuberculosis H37RV to various doses of gamma irradiation from a cobalt-60 source and tested for oxygen uptake on suitable substrates. They found that cells exposed to 7.5×10^5 to 8.0×10^5 rads failed to grow on appropriate culture media.

The survival curves of Bacillus anthracis and Bacillus cereus spores after exposure to gamma radiation were of the sigmoid type. The dosage of gamma rays was 1.8×10^6 rads in Bacillus anthracis and that of Bacillus cereus was 2.1×10^6 rads. This killing effect was dependent upon the dose rate which showed a tendency to be less at higher dose rates. The resistance of this organism to gamma rays was increased by successive irradiations. The lethal dose for the 50th generation was found to be at least 3.0×10^6 rads. Generally, in resistant strains, protein hydrolysis and capsule forming ability were reduced and the virulence of a virulent strain decreased (136, p. 207-215).

Morgan (82, p. 357) stated that the killing dose against spoilage microorganisms is about 3.0×10^6 to 4.0×10^6 rads, but

a dose of 4.5×10^6 rads is required to ensure safety from the more resistant spores of Clostridium botulinum in foods with a pH above 4.5. Fuld, Proctor and Goldblith (47, p. 35-43) found Bacillus subtilis spores to be more resistant in the non-frozen state but spores of Bacillus thermoacidurans and Clostridium sporogenes showed no such difference.

Morgan and Reed (82, p. 360-361) found that the temperature during growth affected the resistance of spores of Bacillus coagulans. Darmady et al. (29, p. 112-115) indicated that 2.5×10^6 rads gives a high degree of sterility. Spores of Bacillus pumilus (E601) showed such resistance that it might be a suitable test organism for determining the efficiency of a radiation treatment.

Radiation Induced Changes in Quality

It is known that side effects of radiation include changes in the flavor, color and texture of food. The changes brought about in food products by the ionizing treatments are minute and in many cases beyond the sensitivity of the usual chemical and physical methods of measurement (42, p. 198). Robinson (107, p. 192) mentioned that the changes in color and flavor are probably due to radicals produced by the effect of the ionizing radiations on water. These ions are either reducing or oxidizing substances which react with components of the food being irradiated.

Hannan and Sheppard(56, p. 286) observed that after slight cooking, a dose in excess of 2.5×10^5 rads was necessary before discrimination of irradiated flavor could be made in minced chicken. A dose of 8.0×10^5 rads given to roasted whole chickens as reported by Coleby (26, p. 116-117), produced scarcely perceptible flavor changes. The irradiation odor in foods is due to the formation of complex mixtures of volatile compounds including hydrogen sulfide and other sulfur containing compounds, soluble proteins and amino acids, and carbonyl compounds (56, p. 287). Water soluble proteins have been implicated in studies by Morgan (81, p. 426) as major contributors to odors in meat after irradiation. Radiation treatment tends to decrease the level of vitamins below that of fresh foods. Vitamins which are quite stable to radiation are D, K, riboflavin, niacin, folic acid and B_{12}; those which are unstable to irradiation are vitamins A, E, C, and thiamine.

Microbiological methods may provide a fairly good picture of the sanitary history and handling of the product, but a high bacterial count does not always mean that the product is spoiled (120, p. 892). Microbial action on fish gives rise to volatile breakdown products which are responsible for marked odors that develop during spoilage (43, p. 319). Many chemical tests are available for evaluating the quality of fish products, but none are considered accurate (120, p. 893; 123, p. 260). Beatty and Gibbons (9, p. 91)

observed that the increase in trimethylamine in the muscle of marine fish closely paralleled the degree of decomposition. At a concentration of four to six milligrams of trimethylamine nitrogen per hundred grams of fish, off-odors began to appear and at ten milligrams they were definite. Variations were found among species according to the initial content of trimethylamine.

Watson (132, p. 266) observed that trimethylamine oxide, which functions as a hydrogen acceptor during the decomposition of the flesh, is reduced to trimethylamine during the fermentation of lactic acid by certain facultatively anaerobic bacteria. Reay and Shewan (105, p. 383) concluded that of all the objective tests, the most useful and reliable for routine checking and grading of quality appeared at the time to be the measurement of trimethylamine production.

Beatty and Collins (8, p. 412-413) reported that the fermentation of carbohydrates and carbohydrate derivatives accompanied by production of trimethylamine constitutes the first phase of spoilage, the second phase being the degradation of proteins and amino acids, which does not begin until the trimethylamine has reached a fairly high concentration. The development of ammonia is not as good an index of spoilage as is trimethylamine (9, p. 91).

Volatile acid content and the indole test also proved to be useful indications of spoilage of fish and fish products (43, p. 323;

120, p. 892). pH can be used for grading fish (21, p. 32). The surface pH measurements have been made by placing a glass electrode on the moist surface of the tissue (39, p. 183-184), a pH value of 6.4 indicating fresh fish, while that of 8.4 indicating spoilage.

The upper limit of storage temperature was determined by Sigurdsson (120, p. 899) to be 0°C, below which the development of volatile acids and trimethylamine was largely inhibited.

Stansby (123, p. 261) suggested that it is desirable to determine which chemical and which test, when run in conjunction with one another, will give one the best overall criteria as to the deterioration that has taken place, bearing in mind that the development of a single reliable test is not feasible.

Irradiation of Shrimp and Crabmeat

Radiation-induced chemical changes were found largely in the protein constituents of crabmeat and shrimp. Jacobs (64) mentioned that shrimp contains approximately 25 percent protein which contain the amino acids arginine, lysine, histidine and tryptophan. Crabmeat is about 17 percent protein, having similar amino acids to shrimp, but including significant amounts of cystine, a sulfur-containing amino acid, which is very sensitive to irradiation effects.

The studies of Morgan and Siu (83, p. 277) showed that the

bacterial count of crabmeat was decreased from 3.0×10^6 to 7.8×10^5 by dosages of 1.2×10^5 reps. The crab was still good in quality and had no off-odor.

Tappel et al. (127, p. 274) found that the color and taste of shrimp were normal at radiation dosages up to 5.0×10^5 reps. The stability of color in crab and shrimp is due to the carotenoid pigment, astaxanthin, associated with the protein, but the same pigment when associated with lipids is very susceptible to degradation by radiation (81, p. 424).

Sinnhuber et al. (122) found that 2.5×10^5 rads inhibited spoilage in crabmeat for 20 days at 7°C whereas 5.0×10^5 rads preserved the crab for 60 days at the same temperature. Shrimp irradiated with 5.0 and 7.5×10^5 rads were not spoiled after 180 days at 7°C, and shrimp irradiated with 2.5×10^5 rads remained unspoiled for 60 days. A loss of irradiated flavor during the storage of the shrimp and crabmeat at 7°C was observed.

Scholz et al. (110, p. 118-120) found no adverse changes in quality of irradiated shrimp and crab in their investigation.

Many research workers have studied the nutritive value of gamma irradiated proteins of crabmeat and shrimp. Engel and Watson (40) investigated long-term dog-feeding of irradiated shrimp and carrots to find the nutritive value. They found no deleterious effect on reproduction, lactation and hematological findings in dogs.

Phillips (93, 94) observed no adverse affect on growth or reproduction on long-term feeding of irradiated shrimp, peeled and whole oranges in rats. Gross pathology was essentially negative in all cases. Blood values and longevity were normal through the second generation.

Irradiation of Fish

Nickerson, Goldblith and Masurosky (88, p. 33) investigated the maximum counts of aerobic and anaerobic bacteria in fresh and irradiated shucked, soft shelled clams and haddock fillets. Organoleptic tests indicated that air packed samples were not significantly different from the controls, but those vacuum packed became significantly different after 14 days storage at $0^{\circ}C$.

Carver and Steinberg(20, p. 1-6), in their studies on storage life and acceptability of some North Atlantic fish, found that fish irradiated at levels of 4.65×10^{5} rads or lower have greater acceptability. Storage life increased as the dosage level increased. Deep-fat frying increased considerably the acceptability of raw, irradiated cod and pollock. Autolysis accounts for a small part of fish decomposition (120, p. 892). The spoilage pattern varies widely from one fish to another, but is usually a combination of loss of flavor and texture, and reduction of keeping qualities (123, p. 261).

The nutritive value of irradiated fish has been investigated

extensively. McNamara et al. (77, p. 58) indicated the studies in laboratory tests and in feeding trials showed no toxic substances produced by the irradiation process were found.

Reports were circulated that suggested that irradiated food fed to animals gave rise to bleeding disorders, blindness, heart defect or auricular rupture in mice and sterility or infertility in dogs. Kraybill (68, p. 114-115) stated that no evidence of any toxicity in irradiated foods for either man or animals has been found.

Food Radiation Preservation in Industry

The present status of the technology of the preservation of marine products based on exploratory studies over a six year period was reported by Proctor et al. (98) in 1960. They concluded that low dose substerilization radiation processing of certain selected seafoods can be advantageous for producer, processor, distributor and consumer of edible marine products. They also recommended a comprehensive, synchronized government-industry program be initiated for the development of substerilized, radiation processed, marine products.

Clifcorn (23, p. 39) stated that the use of radiation pasteurization may alter the storage and distribution patterns of food products, thus allowing greater freedom of marketing. The storage

and distribution life of fresh food can be extended by a factor of five to eleven and the costs for irradiation at these lower levels can be reduced by one-fifth of that of sterilizing doses.

Robinson (107, p. 191-194) said that the cost of radiation sterilization might compare favorably with that of heat sterilization for certain products. Huber et al. (61, p. 109) have discussed a number of methods applicable to prevent undesirable changes in foodstuffs. These methods include freezing, vacuum packing, stripping with inert gas, regulation of loose gas, regulation of dose rate and addition of chemical protectors.

Mason (75, p. 704) also reported that major economic benefits would be obtained by the farmer and the consumer by reducing food spoilage and costs due to transportation, storage and in-store marketing in spite of the necessary capital outlay for installation and maintenance of radiation sources.

Besides the advantages of gamma irradiation sterilization in the food preservation industry, there are advantages in other fields, especially for medical purposes, the first cobalt-60 plant having been designed in 1963 (49, p. 49-53).

Combination Processes

Combination processes are regarded as those whose influence exists primarily during the period after irradiation. Ingram

(62, p. 105-109) has stated that refrigeration, vacuum-packing, addition of antibiotic curing and heating are very useful. He noted that the first three do not change the nature of raw foods.

Refrigeration. The value of refrigeration, besides the general slowing down of enzyme, chemical and microbiological action, is that pathogenic bacteria do not develop below 4°C so pasteurization radiation in combination with low temperature storage is a very feasible process(62, p. 106). Proctor and Goldblith (97, p. 237-242; 101, p. 119-189) have shown that the side effects of radiation may be minimized in the frozen state.

Heat. The combination of heating and radiation for sterilization is interesting because irradiation might replace part of the often excessive heat treatments given to canned foods, as suggested by Ingram (62, p. 105-109).

Lück and Kühn (73, p. 37-45) discussed the physical character of corpuscular and electromagnetic radiation and their effect on foods and microorganisms. They reported that one of the great economic advantages of irradiation over thermal preservation is better utilized energy. To heat one kilogram of food from 20° to 100°C and keep it at this temperature until complete sterilization is achieved requires about 10 to 15 times more energy than is necessary to achieve sterilization by gamma radiation.

Schultz (111) reported on enzyme inactivation studies were made on ground codfish cakes by preheating to internal temperatures

of 65°, 71° and 76°C and holding for 15, 60, 180 and 300 seconds prior to irradiation at 4.5×10^6 rads. It required a minimum of 300 seconds at 65° or 71°C for 15 seconds to inactivate the autolytic enzyme present according to chemical tests for free amino nitrogen.

Kempe (65, p. 108-113) suggested that heat and radiation used together may be more effective for food preservation than either one alone. Each of these agents can be somewhat damaging: overcooked food sometimes reduces its nutritive value; radiation can produce off-odors, off-flavors and degraded textures. Results showed that a radiation dose that is about one third of the sterilization dose reduces the heat treatment required to sterilize to about one fourth of what is necessary without radiation.

Antibiotics. Cain et al. (18, p. 582) investigated the stability of tetracyclines at different levels of irradiation to see if appreciable amounts of antibiotics remained in meat after irradiation to offer antibacterial protection during storage. They found that antibiotics when incorporated in meat or water and subjected to ionizing radiations were destroyed with increasing radiation dosage. At sterilization doses of 3.0×10^6 rads the antibiotics were almost or completely destroyed, while at pasteurization levels sufficient antibiotics still remained.

Shewan (117, p. 143) reported on the combined treatment of gamma radiation and chlortetracycline on cod fillets, and

Lerke et al. (72, p. 145) reported on shell fish. They found that the addition of antibiotics decreased the radiation dose requirement for pasteurization and increased the storage life of the product.

Deeney (30, p. 58), studying the effect of antibiotics on Micrococcus radiodurans, found that the radiation-resistant microorganism is sensitive to antibiotics, and antibiotics plus radiation showed that antibiotics are destroyed in a direct proportion to the increases in radiation dosage.

Other additives. Proctor and Goldblith (103, p. 65) eliminated off-flavors in several foods by using ascorbic acid, d-isoascorbic acid, and their salts. The addition of chemical protectors to prevent the irradiation induced chain reactions due to free radicals, has been reported by Proctor et al. (97, p. 237-242). The addition of methionine in minute amounts to irradiated milk increased off-flavor, while the addition of ascorbic acid did not significantly reduce it (2, p. 424).

The effect of spices plus irradiation was investigated by Deeney (30, p. 58-59) in concentrations used in foods. Results showed that garlic, which contains a sulfhydryl group, protected the growth of Micrococcus radiodurans from the effects of radiation.

Packing. The advantages of vacuum and nitrogen packing on checking the development of rancidity in various fish oils have been reported by Astrack et al.(5, p. 570-583). Nickerson (90, p. 311) described

in detail nitrogen packing, vacuum packing, freezing and vacuum freezing.

Ingram (62, p. 105-109) described vacuum packing and antibiotics. Although these methods further prolong storage life of irradiated foods, they are unreliable in inhibiting pathogens, so that refrigeration is desirable when using these processes.

Radiation Sensitivity

Radiation sensitivity of microorganisms appears to be dependent on many factors, as the nature and degree of cellular responses, chemical bonds such as sulfhydryl linkages, growth phase, physical and chemical influences. Bridges and Horne (14, p. 105) defined a sensitizing agent as a substance which enhances the effect of radiation without itself being toxic: or if it is toxic, the resulting effect of radiation and sensitizer should be more than the sum of the radiation effect and the agent's effect separately.

Vitamin K_5 has been used as a food preservative (134, p. 109-111; 135, p. 501-504). However, enhancement of radiation damage to tumors with vitamin K_5 and its analogs has drawn the attention of many research workers for further investigation in the field of microbiology. No significant sensitizing effect was found when applied to suspensions of some species of bacteria during irradiation. However Shehata (115, p. 78-85) and Silverman (121, p. 432-440)

reported that vitamin K_5 appears to increase the radiosensitivity of Escherichia coli, Micrococcus radiodurans, Pseudomonas fragi, and Torula rosae. The magnitude of the sensitizing effect and the influence of oxygen varied with different microorganisms. The sensitizing action could be demonstrated on these bacteria in the presence of nitrogen, but for yeast, it was demonstrable only under aerobic conditions.

Bridges (13, p. 467-472) proposed that radiation produces more reactive sulfhydryl groups in the cell and they react with the binding agent. He observed that highly reactive sulfhydryl binding agents were the most efficient sensitizers.

Lee (71, p. 62) found on studying the action of iodoacetic acid at a non-toxic level of 100 μM, a reduction of 1000 fold at all levels of radiation for Micrococcus radiodurans.

EXPERIMENTAL STUDIES

A. Microorganisms used

Representatives of various genera and species of pathogenic bacteria were selected for use as the inocula in seafoods. All of them are aerobic, non-spore-forming, mesophiles, which give good growth in ordinary media.

1. Most pathogenic microorganisms which are frequently found to be the causes of epidemic diseases from marine products are classified in the genus Salmonella. The following seven species of the genus Salmonella were used: Salmonella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Salmonella choleraesuis, Salmonella enteritidis, Salmonella pullorum and Salmonella wichita.

2. According to the natural sources of seafoods, polluted water may play a part in some outbreaks of epidemic diseases. It is apparently not nearly so important a factor in dysentery as it is in typhoid fever. Contamination of food is frequently caused by flies in the spread of bacillary dysentery (17, p. 497-500). The three species of microorganisms used in the genus Shigella were: Shigella dysenteriae, Shigella paradysenteriae and Shigella sonnei.

3. Escherichia coli was used as the representative of the genus Escherichia, as it is a constant inhabitant of the human intestinal tract. Its presence in water is a good index of the source of fecal

contamination (124, p. 430). It is interesting to note that some serotypes of Escherichia coli cause diarrheal diseases in infants (17, p. 463).

4. Among the fecal streptococci, Streptococcus faecalis is normal in the intestinal flora of man. The presence of enterococci is a useful biological index of the fecal contamination of water. In European countries, enterococci are commonly looked for in the sanitary analysis of water supplies (124, p. 437). Streptococcus faecalis is used as the representative of this genus in radiation studies.

5. Members of the Proteus group are found in feces, water, sewage and decayed matter. Some strains of Proteus may cause diseases of the genitourinary and gastrointestinal tracts in man. Certain food-poisoning epidemics have been ascribed to Proteus (17, p. 469). Proteus vulgaris was, in this case, used as the representative of this group.

6. Neisseria catarrhalis, a gram-negative diplococcus, is used as representative of the genus Neisseria in radiation studies. It is commonly found in the nasopharynx of healthy individuals as well as in persons suffering from colds and other respiratory infections. Different strains vary in their pathogenicity. In man they appear at time to excite catarrhal inflammation, and some Neisseria cause pneumonia and meningitis (17, p. 454).

7. Mycobacterium smegmatis is often difficult to distinguish morphologically from the tubercle bacillus. It may be noted that it is also found in the urine and may contaminate fecal specimens. The saprophytic bacilli grow much more rapidly than the tubercle bacilli, and neither they nor the bacilli isolated from cold-blooded animals are pathogenic for guinea pigs and rabbits, or at best only feebly so. Some chromogenic (yellow) strains have, however, been associated with human disease on occasion (17, p. 650). This species was used as the representative of genus Mycobacterium.

B. Cultures

All cultures used in this investigation were obtained from the Department of Microbiology, Oregon State University, Corvallis, Oregon. They were maintained in Hartsell's medium sealed with vaspar. Cultures were transferred into Hartsell's agar slant media. Checking for culture purity was routinely performed throughout the course of this study, following the procedure outlined in the seventh edition of Bergey's manual.

C. Preparation of Culture Media

1. Growth. Cultures used in all experiments were from the growth of vegetative cells cultivated in the Hartsell's broth of the following composition: 5.0 grams yeast extract; 5.0 grams tryptone;

5.0 grams preteose peptone; 5.0 grams sodium chloride; 100.0 milliliter veal infusion, distilled water q. s. ad one liter. The pH was adjusted so that after autoclaving at 121° C for 20 minutes it would be 7.2. Harsell's agar culture medium was prepared by adding 20.0 grams of agar to one liter of broth, dispensed into bottles, flasks, or tubes as desired, and autoclaved at 121° C for 20 minutes.

The medium was dispensed in 100 ml aliquots into 240 ml screw cap bottles, sterilized at 15 pounds per square inch for 20 minutes. The sterile medium was then inoculated with 0.1 ml of a 24 hour Harsell's broth culture. Cultures incubated at 34°C for 24 hours were used for irradiation purposes. The same strain was also inoculated on Hartsell's agar slants in a quantity of three milliliters or five milliters in test tubes of 1.0 x 5.0 or 2.0 x 5.0 cm, respectively, incubated at the same time and held at the same temperature as the broth.

2. Growth Measurement. The growth of cells in broth was measured by optical density, the determination being carried out with a Bausch and Lomb colorimeter, at a wave length of 550 mμ. The optical density of each culture was adjusted to the same concentration with phosphate buffer of pH 7.0 before irradiation.

D. Preparation of Seafoods

1. Raw materials. The seafoods used for this study were crabmeat (Cancer magister) and shrimp (Pandalus jardoni) which were cooked at 82°C in mild brine, and packed and frozen in five pound packages. Fish used for this experiment was halibut (Hippoglossus stenotepis Schmidt 1904) with skin and bone removed. They were obtained from a commercial firm in Portland, Oregon.

All seafoods were ground separately and five grams weighed into screw cap test tubes (2.0 x 7.5 cm). Test tubes of ground seafoods were packed into No. 2 cans, properly labeled and hermatically sealed.

2. Irradiation. Prepared seafoods in cans were frozen immediately and shipped in dry ice by Railway Express to the Materials Testing Reactor, Idaho Falls, Idaho. Samples were irradiated under water for three hours, at a water temperature of approximately 12°C, and a dose rate of 1.42×10^6 rads per hours $\pm$ 5 percent. Total exposure was 4.3×10^6 rads. This was the dose required for radiation sterilization. After irradiation the samples were returned to Oregon State University laboratories and kept at 3 - 4°C during the storage period prior to microbiological testing.

E. Preparation of Cultures for Irradiation

1. Preparation of cultures in culture media. The 24-hour broth culture, prepared as above (C.1.) was transferred into sterile screw cap test tubes, 1.0 x 5.0 or 2.0 x 5.0 cm in quantities of three milliliters and five milliliters respectively. Cultures of the same strains on agar slants, prepared as above (C. 1.) were also ready to be exposed to ionizing radiation.

2. Preparation of cultures in seafoods. Radiation sterilized seafoods - crabmeat, shrimp and halibut prepared as in procedure D. 2., served as the media for each pure culture of microorganisms to be tested. The frozen seafoods were thawed at least three hours before inoculation. The inoculum was prepared from 24-hour cultures in Hartsell's broth. The concentration was adjusted with phosphate buffer, so as to contain a population of bacteria giving a final concentration of 10^6 cells per gram in the sampled product. One milliliter of inoculum was added to each tube of sterile seafood, which was then mixed thoroughly on a vibrating machine and incubated at $34^{\circ}C$ for 24 hours.

F. Irradiation

The cultures of pathogenic bacteria inoculated into broth, agar slants, crabmeat, shrimp and halibut were prepared for radiation

by packing and sealing in labeled No. 2 cans. The radiation doses used were 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 rads x 10^5, except as indicated otherwise. All samples of the same strain were replicated from three to eight times, as indicated under Results and Discussion.

G. Microbiological Examination

The irradiated samples were examined immediately after irradiation or as soon thereafter as possible.

1. Positive and negative test for growth. Irradiated broth and agar cultures were transferred into corresponding sterile media. This examination was in duplicate. The subcultured samples were incubated at 34°C for observation of visible growth, on agar slants and in broth, after 24 and 48 hours. For doubtful results, the observation was continued for 72 hours or longer to ensure the precise dosage of irradiation which would inhibit or kill the micro-organisms.

2. Determination of viable count. Besides the observation of growth in broth cultures after irradiation, as indicated in procedure G. 1., the irradiated broth cultures from each dose were examined for the number of viable bacteria. Dilutions of each irradiated broth culture in suitable concentrations were prepared from each irradiation dose, a solution of phosphate buffer was used as the

diluent. Plate count agar media used was Tryptone Glucose Yeast Extract agar; the incubation temperature was 34°C. Plate counts were made after 40 to 48 hours. The viable count was always run in triplicate, from which average results were taken.

The determination of the viable count from irradiated seafoods was done by the same method as for irradiated broth, such as suspending the seafood with ten milliliters of phosphate buffer to each tube, and shaking thoroughly with a vibrating machine. The dilutions were prepared in suitable concentrations corresponding to the dosages used.

H. Radiation Sensitivity

According to some investigators, vitamin K_5 was found to have effect on the action of radiation to microorganisms. In this experiment, attempts were made to use vitamin K_5 as a sensitizing agent for effective food preservation on some species of microorganisms. Vitamin K_5 in phosphate buffer was used in dilutions of 10 ppm to 100 ppm in Hartsell's broth cultures.

Bacterial counts in the presence of vitamin K_5 were examined with and without irradiation at various dilutions. Irradiation dosages used were 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 rads x 10^5. Cells were diluted in phosphate buffer, while Tryptone Glucose Yeast Extract agar was used as the plate count agar medium.

RESULTS AND DISCUSSION

The microorganisms used in this study included non-pathogens and some pathogens having a public health significance in foods. Most pathogens are quite interesting to study, but under the circumstances and techniques of this study, they were quite difficult to handle--some require special media, some are quite dangerous, some take a long time to grow and others fail to grow on the experimental seafoods used.

Fish have been found to contain many different types of microorganisms, both in the slime of the outer surface of the fish and in the intestines. It is believed that the bacteria present in fish enter by way of the gills. Oysters and other shellfish that pass large amounts of water through their tissues are believed to become contaminated in this manner. Shrimp, crabs, lobsters and similar seafood have slime bacteria on their surfaces, which are probably similar to those found on the surface of fish (45, p. 74).

Seafood may become contaminated by handling and by equipment with which they come in contact, and contaminants may build up in individual specimens so as to seed the entire supply of seafood.

1. Effect of Radiation on Salmonella

The effect of radiation on seven species of Salmonella was

tested in preliminary studies of the sensitivity to gamma radiation of non-spore-forming mesophiles in broth cultures. Results were obtained from averages of eight determinations. Salmonella typhosa, Salmonella schottmuelleri and Salmonella wichita were found to be more resistant to radiation than were Salmonella choleraesuis, Salmonella paratyphi, Salmonella enteritidis and Salmonella pullorum. Data is shown in Table I.

The initial bacterial population of each culture was 2.5×10^8 to 5.0×10^8 cells per milliliter, which appeared to be too high a concentration. The concentration of cells affects the dose required for sterilization. Koh, Morehouse and Chandler (67, p. 145) studying the relative resistance of non-spore-forming bacteria, found an apparent protective effect as concentrations of E. coli increased above a certain level. High concentrations of bacteria in a menstruum may lower oxygen content by normal metabolic processes and thus appear to affect resistance to radiation. Gunter and Kohn (50, p. 422-428) demonstrated that decreases in sensitivity were eliminated by shaking in contact with air.

The media used in this experiment, Hartsell's medium, is a very rich nutrient for bacteria. Freeman and Bridges (46, p. 136) reported that survival was greatest in the richest medium.

Table I. Effect of Radiation on *Salmonella* in Culture Media.

Organisms	Rads x 10^5										
	1.00	2.00	2.50	2.75	3.00	3.50	4.00	4.50	5.00	5.25	5.50
S. typhosa	+	+	+	+	+	+	+	+	+	-	-
S. paratyphi	+	+	+	-	-	-	-	-	-	-	-
S. schottmuelleri	+	+	+	+	+	+	+	+	+	-	-
S. choleraesuis	+	+	+	+	-	-	-	-	-	-	-
S. enteritidis	+	+	+	-	-	-	-	-	-	-	-
S. pullorum	+	+	+	-	-	-	-	-	-	-	-
S. wichita	+	+	+	+	+	+	+	+	+	-	-

+ = growth
- = no growth

2. Effect of Radiation on Salmonella in Culture Media

Seven species of Salmonella were studied for radiation resistance. The survival of microorganisms in percent was noted after irradiation in doses of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 rads x 10^5. The initial numbers of microorganisms were 8 x 10^6 to 10 x 10^6 cells per milliliter. Salmonella typhosa, Salmonella schottmuelleri and Salmonella wichita were found to resist radiation more than did Salmonella choleraesuis, Salmonella paratyphi and Salmonella enteritidis. Salmonella pullorum was the least resistant strain among them, as no growth was observed at 3.0 x 10^5 rads. Data for the effect of radiation on Salmonella in culture media are presented in Table II. The results were averages of three determinations.

It was expected that all the curves in Figure 1 should fall on the same straight line because it has been established that the bacteria of the same species would have almost the same resistance. Nevertheless, they appeared to be slightly displaced from one another. This is due perhaps to an error in measurement in the experiment.

The graphic representation for percent survival in log scale, versus radiation dose in arithmetic scale, is shown in Figure 1.

Table II. Effect of Radiation on Salmonella in Culture Media

Microorganisms	% Survival					
	1.0*	2.0*	3.0*	4.0*	5.0*	6.0*
S. typhosa	3.1	.045	.0019	.00001	0	0
S. schottmuelleri	6.5	.18	.004	.00016	0	0
S. wichita	4.5	.08	.0018	.000032	0	0
S. choleraesuis	2.5	.075	.0005	0	0	0
S. paratyphi	0.75	.019	.00015	0	0	0
S. enteritidis	0.55	.008	.000075	0	0	0
S. pullorum	0.4	.0018	0	0	0	0

*Rads x 10^5

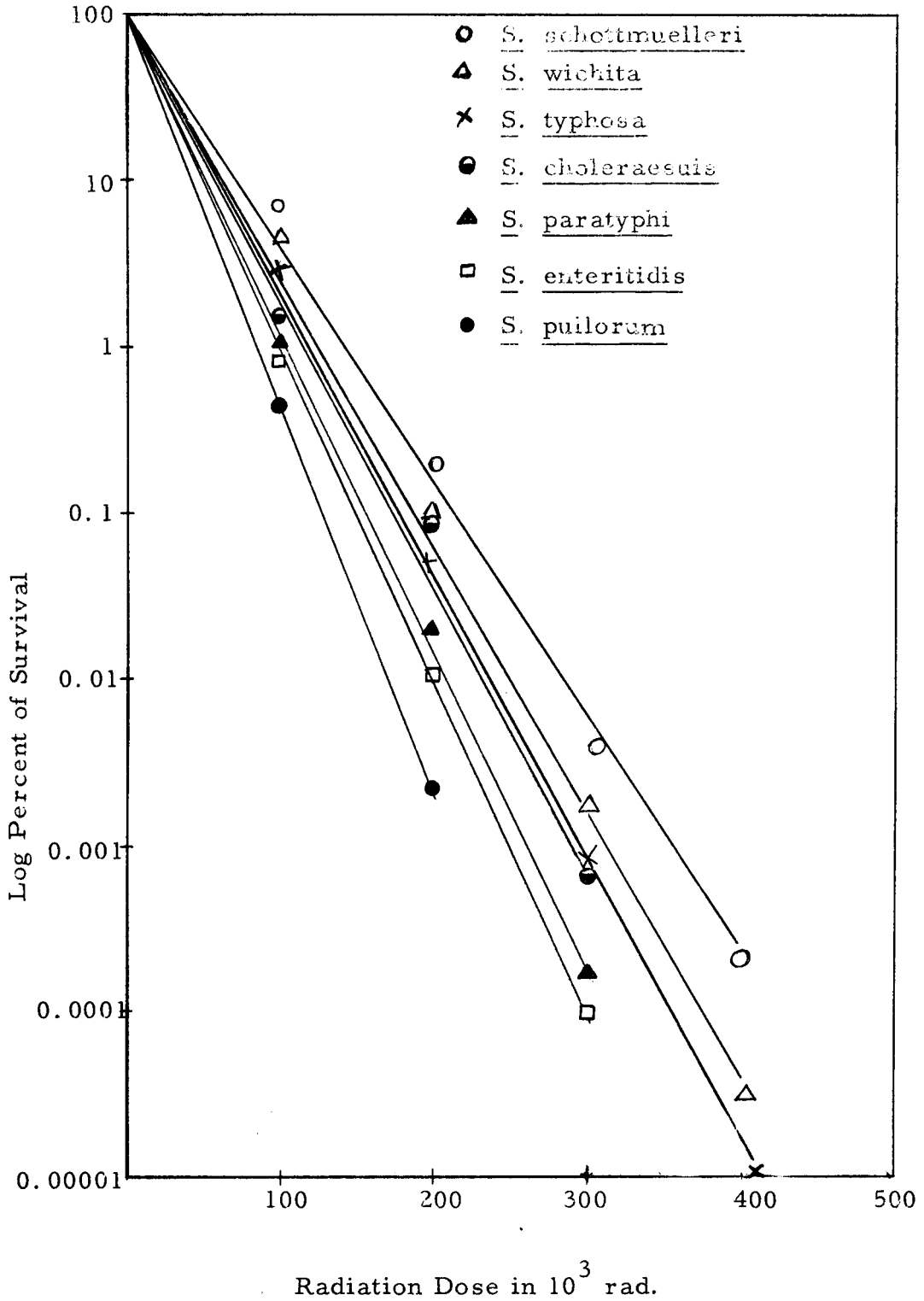

Figure 1. Effect of Radiation on Salmonella (7 species) in Culture Media.

3. Effect of Radiation on Shigella in culture media

Three species of Shigella were used for the effect of radiation. The initial numbers of microorganisms were adjusted to 8×10^6 to 10×10^6 cells per milliliter. The results were the averages of three determinations. Shigella sonnei was found to resist radiation doses up to 3.0×10^5 rads while Shigella dysenteriae and Shigella paradysenteriae were found to resist doses as high as 2.0×10^5 rads. Microorganisms in the genus Shigella were found to be more sensitive to radiation than were some species in the genus Salmonella.

Data for the effect of radiation on Shigella in culture media are shown in Table III. The graphic representation of the log of the percent survival and radiation doses is shown in Figure 2.

4. Effect of Radiation on Neisseria and Mycobacterium in Culture Media

The representatives of these genera used for study were Neisseria catarrhalis and Mycobacterium smegmatis. The average results of three determinations showed the radiation resistance to be 3.0×10^5 rads.

Data on the effect of radiation on Neisseria catarrhalis and Mycobacterium smegmatis are shown in Table IV.

The graphic representation of the log of the percent survival against radiation dose is shown in Figure 3.

Table III. Effect of Radiation on Shigella in Culture Media.

Microorganisms	% Survival					
	1.0*	2.0*	3.0*	4.0*	5.0*	6.0*
S. sonnei	1.0	.02	.0004	0	0	0
S. paradysenteriae	0.6	.0025	0	0	0	0
S. dysenteriae	0.185	.00009	0	0	0	0

* Rads x 10^5

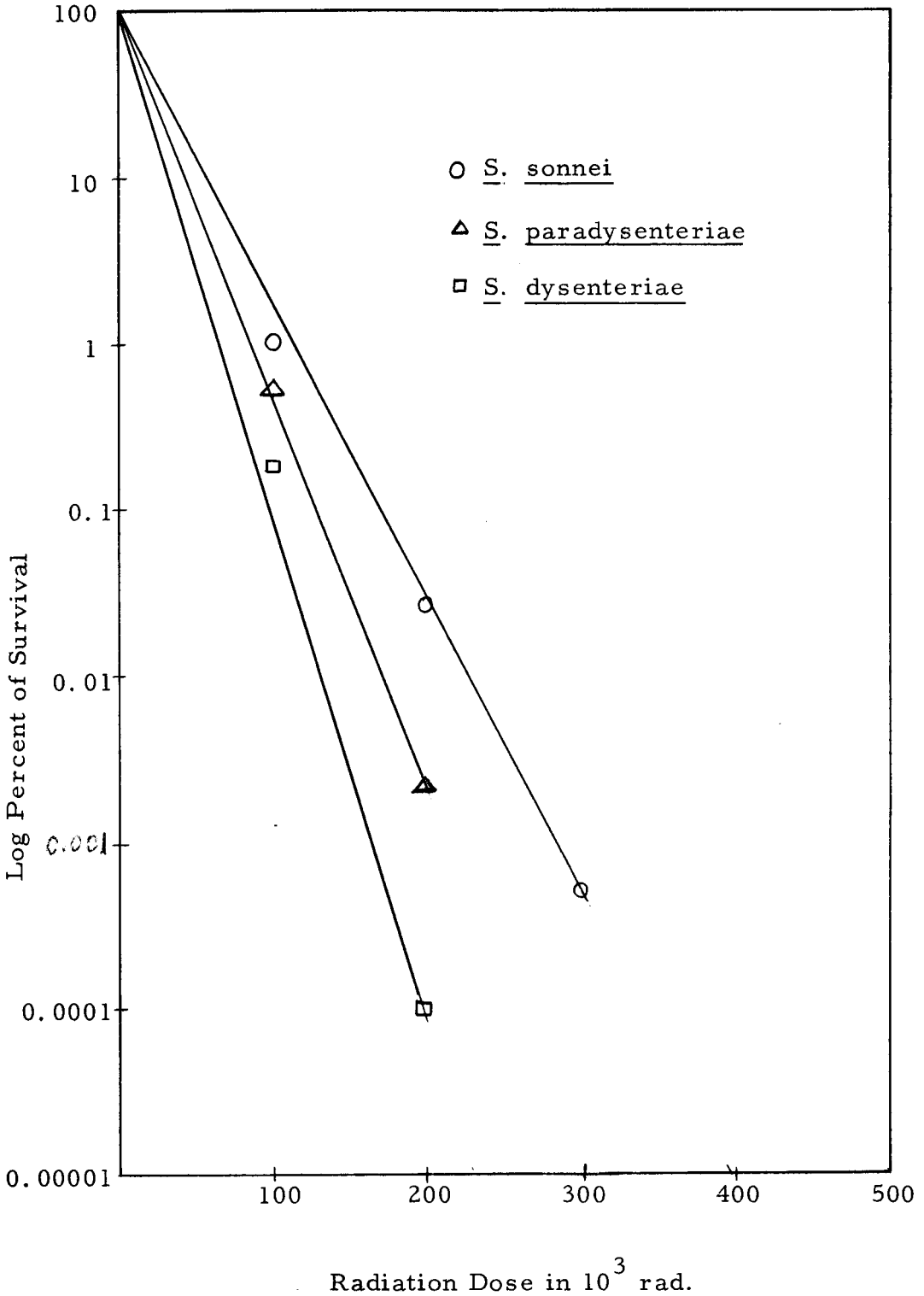

Figure 2. Effect of Radiation on Shigella (3 species) in Culture Media.

Table IV. Effect of Radiation on Neisseria and Mycobacterium in Culture Media

Microorganisms	% Survival					
	1.0*	2.0*	3.0*	4.0*	5.0*	6.0*
N. catarrhalis	2	.0175	.0001	0	0	0
M. smegmatis	.6	.008	.00001	0	0	0

* Rads x 10^5

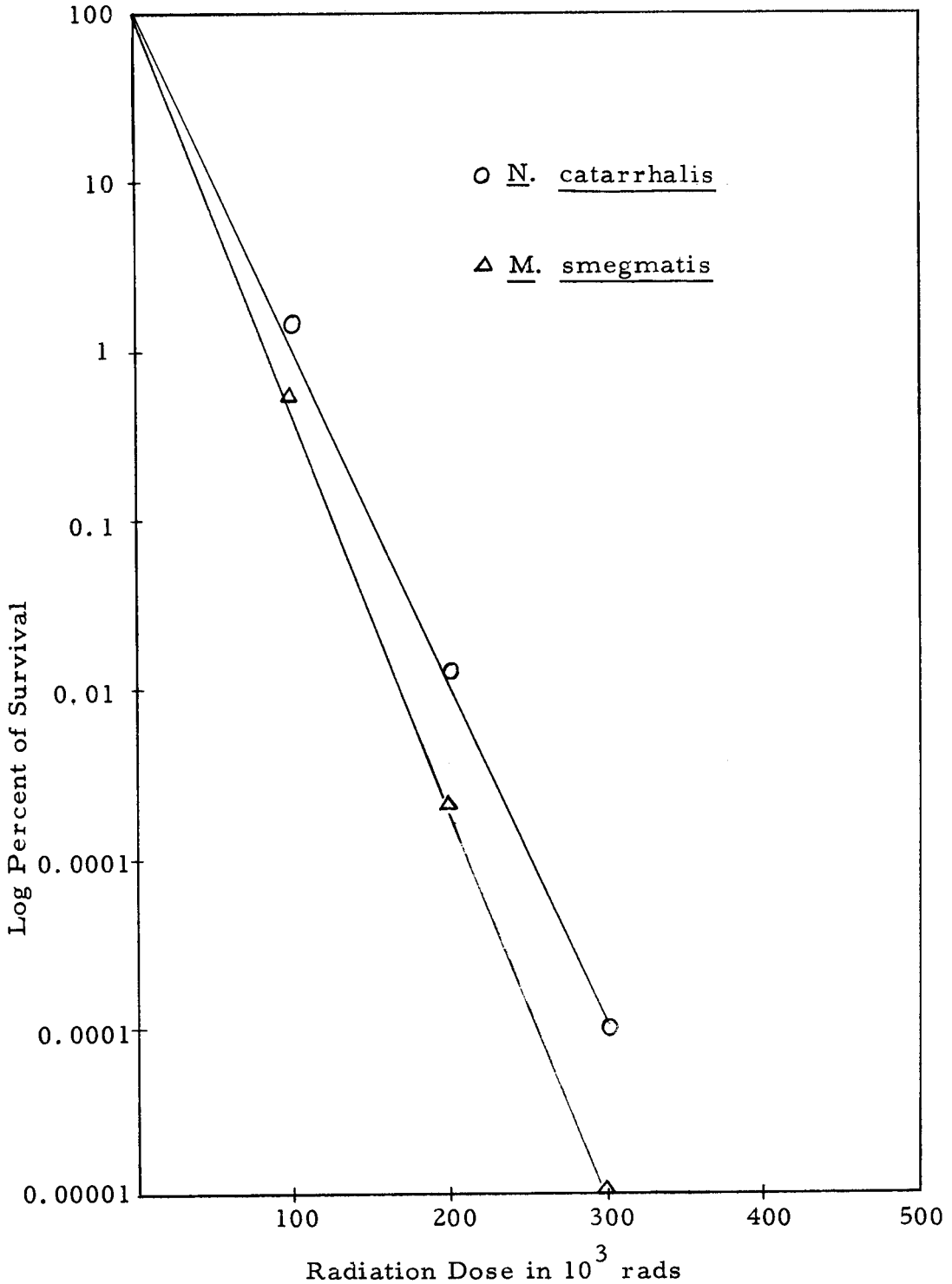

Figure 3. Effect of Radiation on Neisseria and Mycobacterium in Culture Media.

5. Effect of Radiation on Streptococcus, Escherichia and Proteus

Representatives of these genera were used to study the effect of radiation. Results were the averages of three determinations. Proteus vulgaris appeared to be the least resistant to radiation, with no growth after 2.0×10^5 rads, while Escherichia coli and Streptococcus faecalis resisted to 5.0×10^5 and 4.0×10^5 rads respectively. Streptococcus faecalis showed a sigmoidal curve which indicated a higher order reaction rate. It is believed to have more than one "sensitive" vital entity, so "multiple targets" must be "hit" to cause inactivation.

Streptococcus faecalis showed more resistance to radiation than did staphylococci, salmonellae, coliforms and Mycobacterium tuberculosis in that order. The low resistance of the last mentioned organism is in marked contrast to its heat resistance as compared to that of other Mycobacterium (41, p. 201).

Data of the effect of radiation of these three species are shown in Table V. The graph of the log of the percent survival versus radiation dose is plotted in Figure 4.

6. Effect of Vitamin K_5 on Microorganisms in Broth Culture and in Seafood.

The effect of vitamin K_5 on microorganisms was determined by using various concentrations of vitamin K_5, and the numbers of

Table V. Effect of Radiation on Streptococcus, Escherichia and Proteus in Culture Media

Microorganisms	% Survival					
	1.0*	2.0*	3.0*	4.0*	5.0*	6.0*
S. faecalis	57.5	27	7.4	.005	0	0
E. coli	45	4.5	.3	.02	.0004	0
P. vulgaris	.4	.001	0	0	0	0

* Rads x 10^5

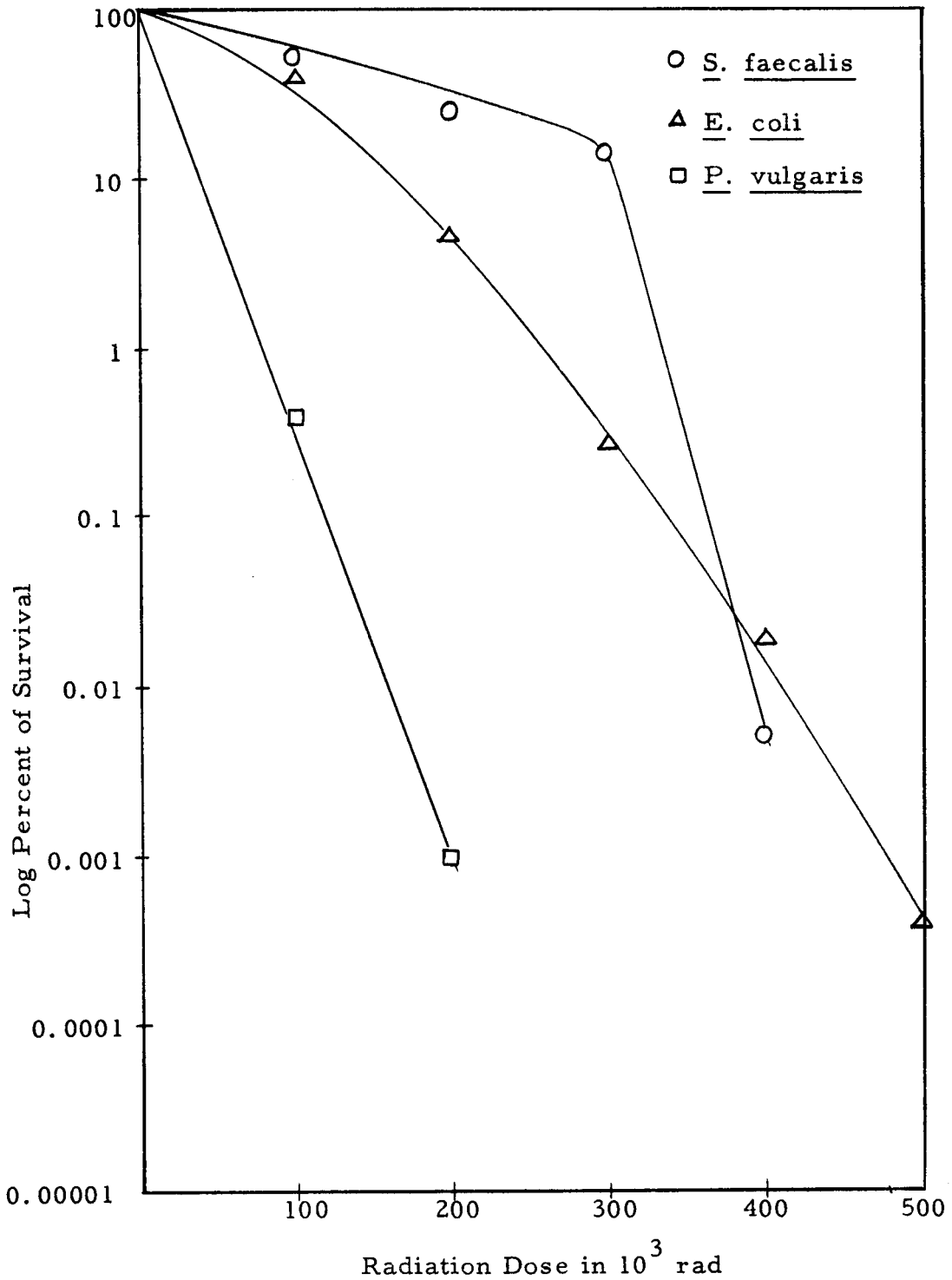

Figure 4. Effect of Radiation on Streptococcus, Escherichia and Proteus in Culture Media.

survival in percent were noted. The microorganism used was Salmonella typhosa in Hartsell's broth medium. The purpose was to find the bactericidal effects of vitamin K_5 with and without radiation. This experiment was run as a control, without radiation, to observe the numbers of survival under various concentrations of vitamin K_5.

The percent survival of Salmonella typhosa in shrimp was about eight times higher than in broth at 100 ppm of vitamin K_5. Data of the effect of vitamin K_5 on Salmonella in cultures and in shrimp are given in Tables VI and VII. The graphic representation of the log of the percent survival against radiation doses is shown in Figure 5.

7. Effect of Radiation on Salmonella typhosa and vitamin K_5

The dilutions of vitamin K_5 used in this study were 50 ppm and 100 ppm. The initial number of microorganisms in buffer was 8×10^6 to 10×10^6 cells per milliliter. The initial number of microorganisms in 50 ppm and 100 ppm vitamin K_5 were determined from the bactericidal effects of vitamin K_5 as shown in Table VI.

After irradiation, the number of survivors was converted to percent survival, as indicated in Table VIII. The graphic representation, Figure 6, of this study appears to be almost parallel straight lines with all concentrations of vitamin K_5 as well as with

Table VI. Effect of Vitamin K_5 on S. typhosa in Cultures.

Vitamin K_5 ppm	No. of survivors	No. of survivors in %
0	44.5×10^7	100
10	22.5×10^7	51.01
20	17.25×10^7	38.7
30	12.35×10^7	27.7
40	10.2×10^7	22.7
50	9.5×10^7	21.1
60	5.3×10^7	11.9
70	4.75×10^7	10.7
80	4.1×10^7	9.2
90	3.45×10^7	7.75
100	2.8×10^7	6.29

Table VII. Effect of Vitamin K_5 on S. typhosa in Shrimp.

Vitamin K_5 ppm	No. of survivors	No. of survivors in %
0	40.6×10^7	100
10	33.6×10^7	82.76
20	30.4×10^7	74.8
40	26.0×10^7	64.04
60	25.5×10^7	62.8
80	21.0×10^7	51.72
100	20.1×10^7	49.51

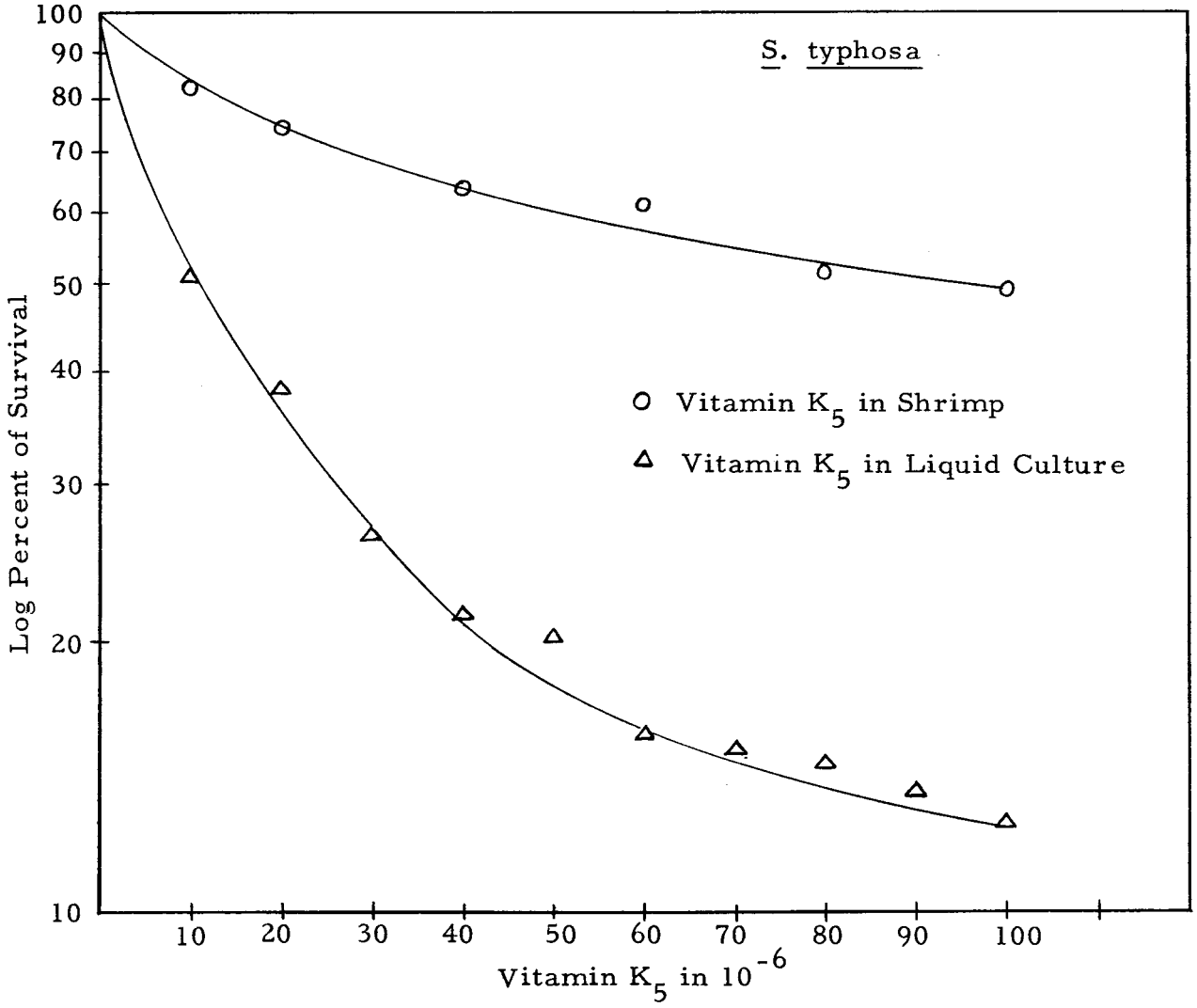

Figure 5. Effect of Vitamin K_5 on Salmonella typhosa in Culture Media and in Shrimp.

Table VIII. Effect of Radiation on S. typhosa in Culture Media with Vitamin K_5

S. typhosa	% Survival					
	1.0*	2.0*	3.0*	4.0*	5.0*	6.0*
in buffer	8.1	.3	.02	0	0	0
+ vitamin K_5, 50 ppm	2	.065	.0035	0	0	0
+ vitamin K_5, 100 ppm	.27	.025	.00055	0	0	0

* Rads x 10^5

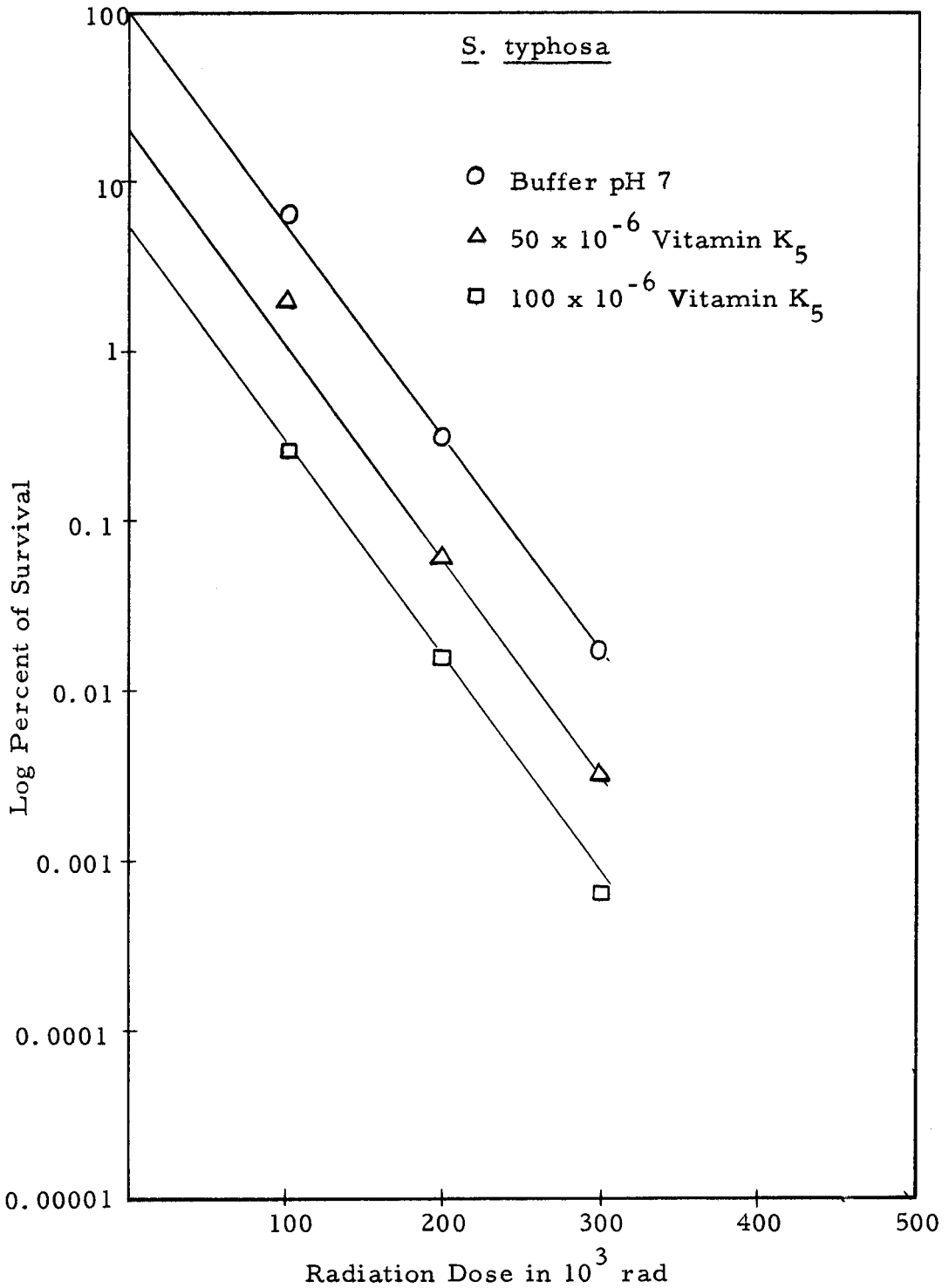

Figure 6. Effect of Radiation on Salmonella typhosa Cultures in the Presence of Vitamin K_5.

buffer which was used as control. It appears, though not conclusively, that there is no evidence of sensitizing to ionizing radiation with vitamin K_5 on Salmonella typhosa. Effect of radiation on micro organisms in seafoods with vitamin K_5 is shown in Table X, page 65.

8 Effect of Radiation on Microbial Cultures in Seafoods

The effect of radiation on microorganisms in seafood was determined with crabmeat, halibut and shrimp. The attempts to determine the number of survivors was a failure due to variations in the data obtained. The results have been tabulated as "growth" (+) and as "no growth" (-) of microorganisms irradiated in seafood and subcultured in broth and on agar slants. The results are the average of three determinations.

The results were comparable to results obtained with organisms irradiated in broth, except for Streptococcus faecalis and Escherichia coli, which showed less resistance in seafood than in broth.

The effect of radiation on various species of microorganisms in seafoods is shown in Table IX.

The results obtained differ with those obtained by other investigators. This could be due to environmental factors, as well as to species and strain differences of microorganisms (10, p. 266; 102, p. 376). Seven species of Salmonella had levels of radiation resistance varying from 2.0×10^5 to 5.0×10^5 rads.

Table IX. Effect of Radiation on Microorganisms in Seafoods (crab, halibut, shrimp)

Microorganisms	Radiation in 10^5 Rads					
	1.0	2.0	3.0	4.0	5.0	6.0
S. typhosa	+	+	+	+	-	-
S. schottmuelleri	+	+	+	+	-	-
S. wichita	+	+	+	+	-	-
S. choleraesuis	+	+	+	-	-	-
S. paratyphi	+	+	+	-	-	-
S. enteritidis	+	+	+	-	-	-
S. pullorum	+	+	+	-	-	-
S. sonnei	+	+	+	-	-	-
S. paradysenteriae	+	+	-	-	-	-
S. dysenteriae	+	+	-	-	-	-
N. catarrhalis	+	+	+	-	-	-
M. smegmatis	+	+	+	-	-	-
S. faecalis	+	+	+	+	-	-
E. coli	+	+	+	+	-	-
P. vulgaris	+	+	+	-	-	-

+ = growth

- = no growth

Table X. Effect of Radiation on Microorganisms in Seafoods (crab, halibut, shrimp) with Vitamin K_5.

Seafoods and Culture	Radiation in 10^5 Rads					
	1.0	2.0	3.0	4.0	5.0	6.0
with buffer	+	+	+	-	-	-
with vitamin K_5, 50 ppm	+	+	+	-	-	-
with vitamin K_5, 100 ppm	+	+	+	-	-	-

+ = Growth
- = No growth

Sweigert (113, p. 155) observed that the doses from 1.25 to 6.0×10^5 rads had completely eliminated Salmonella present in eggs. Brooks, Hannan and Hobbs (15, p. 149-154) treated frozen whole eggs and found that a dose of about 3.0×10^5 to 5.0×10^5 rads would destroy Salmonella.

Concentration of microorganisms also plays an important role on radiation resistance (102, p. 376). The results shown in Tables I and II, which represent the results of different concentrations of the initial number of microorganisms bear this out. The more concentrated the suspensions used, the higher the radiation dose required. However, the results did show a relationship. Hartsell's medium, which is a very rich medium, was used throughout the experiment as stock culture medium. The cultures in this medium have been found to have a greater percent survival (46, p. 136) than in a less rich medium. Also the rate of growth of some strains in rich media was found to be quite fast, which might be one of the factors that increased the radiation resistance.

Irradiation was applied to all samples in the presence of normal atmosphere. Hence, the results might be different from those of some other investigators, who had worked in the presence or absence of oxygen, nitrogen, nitrogen oxide or some other combination of gases (59, p. 77).

The cultivation of microorganisms on irradiation sterilized

seafoods - crabmeat, halibut and shrimp, provided a good growth but showed fluctuation in the numbers of survival among replicates. This might be due to the changes induced by irradiation on the constituents of seafood itself. For this reason some of the original nutrients might be lost. On the other hand, microorganisms were deposited and grew around the particles of seafood, where they multiplied in the surrounding moisture from foodstuff and in the broth from the inoculum.

These observations which were noted are further supported by the evidence of growth of microorganisms after varying doses of radiation of cultures, corresponding to the growth of microorganisms in crabmeat, halibut and shrimp, (Table 2).

Limitation of the Experiments

The microorganisms used in the experiment had not been washed, because they were highly virulent pathogens. The nature of the suspending medium can influence to a certain extent the relative radiation sensitivity of a specific culture (41, p. 204).

Incubation temperature was 34°C throughout the experiment because of the optimum temperature range of some microorganisms, but the experiments were run simultaneously for the sake of convenience. Optimum temperature for most pathogenic bacteris is 37°C. At 34°C the growth rate is somewhat slower but does not

show a significant difference in the population, as noted from the studies of the viable cell count.

Some pathogens fail to grow on ordinary media, therefore special media and longer incubation are needed. These microorganisms were not used, however, because of difficulties in comparing the results when complicated factors are involved.

SUMMARY AND CONCLUSION

This study has been made in order to determine the level of ionizing radiation which would eliminate pathogenic microorganisms contaminating seafoods, using gamma rays emitted by a cobalt-60 source. The levels of radiation doses ranged from 1.0×10^5 to 6.0×10^5 rads. No attempts have been made, however, to evaluate quality changes due to radiation effects, or chemical and organoleptic changes.

Crabmeat, halibut and shrimp were prepared from good quality frozen products, and used as samples for investigation. Crabmeat and shrimp were cooked in mild brine before use. Pathogenic microorganisms selected for use were virulent species of non-spore-forming mesophiles, which are frequently the cause of enteroepidemics all over the world.

The radiation resistance of Salmonella in broth culture varies with the species. Salmonella schottmuelleri, Salmonella wichita, and Salmonella typhosa resisted radiation doses up to 5.0×10^5 rads, as shown by positive and negative growth results. The initial population was from 2.5 to 5.0×10^8 cells in each case. When using the smaller initial population of 8 to 10×10^6, it was found that no growth was seen at dose rates higher than 4.0×10^5 rads. Evidently, the difference in radiation resistance was due to the initial

populations. Salmonella choleraesuis showed no growth above 3.0×10^5 rads, and the same was true for Salmonella paratyphi and Salmonella enteritidis. Salmonella pullorum did not survive dose rates above 2.0×10^5 rads when the initial population was between 8 to 10×10^6 cells. No growth was noted beyond 2.5×10^5 rads when the original population was 2.5 to 5.0×10^8 cells.

Shigella were found, on the average, to have less resistance to radiation than Salmonella. The highest level of radiation resisted by Shigella was found to be 3.0×10^5 rads for Shigella sonnei. It required only 2.0×10^5 rads to eliminate Shigella dysenteriae and Shigella paradysenteriae.

It was found that Neisseria catarrhalis maintained resistance to radiation up to 3.0×10^5 rads, as did Mycobacterium smegmatis.

Streptococcus faecalis showed resistance to 1.0 to 3.0×10^5 rads, which gradually decreased the viable population of cells. At a dose of 4.0×10^5 rads the number of bacteria rapidly declined, and no growth was observed beyond that level.

Escherichia coli showed gradual susceptibility to radiation and no growth was found beyond 5.0×10^5 rads.

It was noted that the bactericidal effect due to the action of various concentrations of vitamin K_5 (from 10 to 100 ppm) in liquid cultures of Salmonella typhosa was eight times higher than that on shrimp. This result indicated that the lower toxic effect on shrimp

was probably due to the poor solubility of vitamin K_5 in the moist shrimp tissues.

The effect of radiation on Salmonella typhosa in liquid culture in the presence of sensitizer was determined by using two dilutions of vitamin K_5, 50 and 100 ppm. The initial numbers of microorganisms were determined in the preceding experiment. The results did not indicate any obvious sensitizing effect of vitamin K_5 on Salmonella typhosa.

Radiation resistance of microorganisms contaminating crabmeat, halibut and shrimp was apparently interrelated among the kinds of seafoods and the species of bacteria when compared with those cultivated in liquid media.

Owing to tremendous fluctuation of survival counts of microorganisms after radiation, the positive and negative results, rather than viable counts, were used to determine radiation resistance.

RECOMMENDATION FOR FURTHER STUDIES

Representatives of bacterial species other than those used in this investigation may be used in order to find the correlation of radiation resistance among them.

It is suggested that the correct temperature be used for each pathogen for better experimental results.

Additives agents, such as antibiotics or radiation sensitizers, may be used in combinations and stored at the appropriate temperatures, whenever possible, for determination of the limits of tolerance to radiation for a specific bacterial species.

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