THE EFFECT OF GAMMA IRRADIATION ON *SALMONELLA* SPECIES IN FROZEN EGG PRODUCTS

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ABSTRACT

Salmonella give, Salmonella heidelberg, Salmonella senftenberg, Salmonella typhimurium, Salmonella worthington and Salmonella enteritidis were inoculated into frozen sterile whole egg, egg albumen and egg yolk and subjected to irradiation dosages ranging from 0.1 to 0.7 Mrads. From the resulting data 10^8 reduction values were obtained to determine the protective effects of the three egg products on each of the organisms during irradiation.

When the Salmonellae were suspended in the frozen sterile egg albumen it was found that Salmonella give displayed the highest 10^8 reduction value followed by Salmonella heidelberg, Salmonella enteritidis, Salmonella typhimurium and Salmonella senftenberg.

When the Salmonellae were suspended in frozen sterile egg yolk the highest 10^8 reduction value was displayed by Salmonella senftenberg, followed by Salmonella heidelberg, Salmonella typhimurium, Salmonella give, Salmonella worthington and Salmonella enteritidis.

When the Salmonellae were suspended in sterile frozen whole egg the highest 10^8 reduction value was displayed by Salmonella senftenberg followed by Salmonella give, Salmonella worthington, Salmonella heidelberg, Salmonella

typhimurium and Salmonella enteritidis.

Frozen egg yolk appeared to offer the most protection to the Salmonellae organisms during irradiation while frozen egg albumen appeared to offer the least, with frozen whole egg offering intermediate protection.

The presence of a natural flora in the frozen whole egg altered the 10^8 reduction values considerably. When irradiation was carried out on this product with the natural flora present the organism displaying the greatest 10^8 reduction value was Salmonella give followed by Salmonella senftenberg, Salmonella heidelberg, Salmonella typhimurium, Salmonella enteritidis and Salmonella worthington.

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INTRODUCTION

Food borne infection as a result of Salmonellae contamination of foods can be a serious problem for food processors. In the past many foods have been implicated as carriers of this organism, and eggs and egg products have been considered as one of the most common sources of the organism.

Outbreaks of food borne infection due to egg products contaminated with Salmonella have been reported in Canada and the United States. In December 1967, there were 48 cases of Salmonellosis traced to one flock of chickens and their eggs were contaminated with Salmonella infantis. During the week of December 26, 1967, Salmonella thompson, Salmonella siegburg, Salmonella oranienburg and Salmonella infantis were isolated from one lot of frozen egg whites. Furthermore, during the months of October, November and December 1967, there were a total of 58 isolations of various Salmonella species from frozen egg products.

The use of gamma irradiation as a means of destroying Salmonellae in frozen egg products has been reported and appears to hold much promise. The purpose of this study was to determine the effect of gamma irradiation on selected species of Salmonella in frozen egg products when a high

initial load was present. The destruction of Salmonellae both in the presence and absence of a natural egg flora in the egg products was investigated.

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REVIEW OF LITERATURE

Organism

According to Bergey's Manual (8), members of the genus Salmonella belong to the family Enterobactereceae and are similar in many respects to other genera such as Escherichia, Shigella, Aerobacter, Serratia and Klelesiella. Salmonellae are characterized as gram negative, asporogenous, facultative short rods which are usually motile by means of peritrichous flagella, although non-motile species of this organism have also been isolated. This group of organisms grow well on ordinary media and are known to produce acid (often gas) from glucose, mannitol, maltose and sorbitol. However, lactose, sucuese, salicin and adonital are not fermented. Salmonellae generally do not produce acetoin or hydrolyze urea, but are capable of producing nitrite from nitrate and may or may not produce hydrogen sulfide.

Salmonellae can grow over a fairly wide range of temperatures,but all have an optimum growth temperature of 37° C.

In view of the fact that it is not always possible to identify this group of organisms on the basis of their biochemical properties, serological techniques have been employed. Most members of this genus possess two antigens,

the somatic "O" antigen and the flagellar "H" antigen (6). To date, approximately 1200 serotypes of Salmonellae have been identified (8).

Pathogenicity

Salmonellae are pathogenic to man and animals and may cause an infection known as Salmonellosis. According to Thompson (48) there are three main types of Salmonellosis; enteric (typhoid) fever, gastroenteritis and septicemia. Generally, food-borne infections result in the seldom fatal gastroenteritis.

Salmonella gastroenteritis (48) is caused by the ingestion of large numbers of Salmonella organisms. Symptoms of the infection are characterized by nausea, vomiting, diarrhea, headache, fever and chills and will occur from 3 to 72 hours after ingestion of the contaminated foods and may persist for 2 to 7 days. Deaths attributable to this infection are normally confined to the very young, the very old or to those persons who are in a weak state of health due to some other illness. Salmonella typhosa and other typhoid serotypes may cause typoid fever and in severe cases death to the patient (48). However, this group of Salmonella organisms is not normally associated with foods, but is often found in polluted water supplies.

Septicemias (31) may be caused by members of the Salmonella genus. Symptoms of this disease are characterized

often by high fevers. The organisms may localize in any tissue of the body and may produce local abscesses.

Salmonellae in Egg Products

Salmonellae have been found in egg products and specifically frozen egg products by a number of investigators (32, 14, 42, 47, 46).

Newell et al (32) in 1955 reported two outbreaks of paratyphoid fever which were caused by ingestion of imitation cream cakes and other confectionery from specific commercial bakeries. A batch of frozen whole egg imported from China used by the bakeries was suspected of being responsible for the infection. This was later confirmed by epidemiological observations which located the same phage type of Salmonella paratyphi B as that isolated from unopened cans of the product as well as, from certain members of the bakery staff and from the infected patients.

In his extensive literature review, Edwards in 1957 (14) reported that domestic fowl probably constitute the largest single reservoir of Salmonellae among domestic animals. He also reported that Salmonellae may gain entrance to eggs either as a result of ovarian infection or by penetration of the egg shell. The frequent occurence of Salmonellae in processed eggs was attributed, according to Solowey et al (42), to contaminated eggshells. He suggested that as long as cracked and soiled eggs, which could not be

marketed as shell eggs were incorporated into dried and frozen egg products, a high incidence of Salmonellae in these products could be expected.

Thatcher and Montford in 1962 (47), surveyed the bacteriological quality of commercial frozen egg products. They detected the presence of Salmonellae in 27 of 114 samples of frozen whole eggs. However, they did not find any Salmonellae in 13 samples of frozen yolk and 9 samples of frozen albumen. The serotypes identified were Salmonella gallinarum, Salmonella oranienberg, Salmonella thompson, Salmonella manhatten and Salmonella barielly.

Thatcher in 1963 (46), stated in a report that the presence of Salmonellae in frozen egg products was a health hazard and recommended that appropriate processing techniques be used to destroy these organisms. He further recommended that laboratory methods be adopted to verify that the destruction of the organisms had been accomplished and that no recontamination had occurred.

Although more stringent government controls and more knowledge of Salmonellae contamination are now in existance, numerous outbreaks of Salmonellosis have been reported in the United States and Canada in the past few years. In 1967, the Salmonella Suviellence (1) reported 553 cases of Salmonellae isolated from eggs and egg products, however, this figure was dropped to 312 in 1968 (2). For the first

three months of 1969, 82 isolations have been reported (2).

Sterilization of Egg Shells Prior to Shelling

Rizk et al (34) reported in 1966, that the contents of the egg as laid by a normal, healthy hen, were sterile. However, occasionally, the ovaries may become infected with Salmonellae, which could possibly be transferred to the egg contents. Consequently, according to Rizk et al, incidence of Salmonellae on or in shell eggs was dependant on several factors, i.e. the handling, the storage, and the physical structure of the shell.

Lancaster and Crabb (23) in 1953, demonstrated that although bacteria could penetrate the shell of eggs, only rarely did these organisms pass through the shell membrane and into the white of the eggs.

Elliot in 1954 (15), inoculated Pseudomonas into the air cell of eggs and showed that it took 4-8 days before bacteria could be isolated from the egg contents. Walden et al (49) in 1956, using Pseudomonas labelled with P^{35}, showed that the egg membrane could resist the penetration of bacteria for 20 hours.

Lifshitz et al (26) in 1963, also using Pseudomonas flourescens, showed that the inner shell membrane of an egg was the most effective bacterial barrier, the shell was ranked second and the outer membrane was the least important.

The above information indicated that the contents of

fresh eggs, less than 24 hours old, taken from healthy hens, should be sterile. The problem of bacterial contamination, therefore, can be prevented by retaining the sterile condition after the shell has been removed. In order to achieve this, the shell and surrounding atmosphere of the egg must be free of bacteria.

Garibaldi and Stokes (18) in 1957, sterilized the shells of eggs by immersing the eggs in 70% alcohol for several minutes. The eggs were then withdrawn, drained and flamed to remove the last traces of alcohol.

In another study reported by Winter and Stewart (51) in 1946, the eggs were sterilized by soaking the specimens in 1 % NaOH for 2-5 minutes. They were then drained, dipped into 95% alcohol and flamed to remove any residual alcohol.

To obtain sterile eggs, Lifshitz et al (26) 1963, washed the eggs with a sanitizing detergent, then dried and stored the eggs at 2° C. Just prior to being processed, the eggs were immersed in a 1 % solution of mercuric chloride for 1 minute and in 70 % alcohol for 2 minutes.

Rizk et al (38) in 1966, employed a different procedure for sterilizing egg shells. The eggs were immersed in a 1:1000 Roccal solution for 5 minutes which was followed by dipping into 75 % ethanol for 2 minutes. The blunt end of the shell was flamed and, after fracturing the shell with sterile forceps, the contents of the eggs were poured into

a sterile jar.

Effect of Low Temperatures and Freezing Conditions on the Survival of Salmonellae

Prucha and Brannon (37) in 1926 inoculated samples of ice cream with approximately 2.5 x 10^6 cells of Bacterium typhosum per cubic centimeter of product. The samples were frozen immediately and then placed in a hardening room which had a temperature of -4° F. Bacterological examinations were performed on samples taken from the unfrozen mix, the freshly frozen ice cream and then at varying during a period of 28 months of storage. They found that after five days of storage, the bacterial population was reduced by about 90% and after twenty days of storage more than 95% reduction was noted. After two years and four months of storage only about one cell in ten thousand survived.

Orr and Moore (35) in 1953, investigated Salmonella gallinarum, the causative agent of fowl typhoid. Three separate experiments using water as the medium were carried out at different temperatures. In this study one sample was kept at room temperature, the second sample was frozen and thawed daily and the third sample was frozen at -20° C and maintained at that temperature until it was used. They reported that those organisms in the water which was frozen and thawed daily, survived for 43 days while those that were frozen and maintained frozen revealed the presence of

Salmonella gallinarum irregularly for 93 days.

Woodbun and Strong (52) in 1959, examined the survival of Salmonella typhimurium in various foods including egg whites. The suspended cells were frozen at -11, -21 or -30° C and retained at those temperatures for 24 hours, 1 week, 4 weeks and 10 weeks. The survival of Salmonella typhimurium at -21° C was reduced from 16,000/ml at zero time to 8,800/ml after 24 hours, and 7,500/ml after 1 week; 5,800/ml after 4 weeks and 4,100/ml after 10 weeks of storage, respectively.

The effect of temperature on the viability of Salmonella and Staphlococcus in pre-cooked, ready to serve foods was studied by Angelotti et al (4) in 1961. A mixed culture consisting of Salmonella senftenberg 775W, Salmonella enteritidis and Salmonella manhatten stored in custard and ham salad at temperatures ranging from 40-50° F, resulted in a gradual decline in the numbers of organisms at all temperatures. However, they noted that growth occurred in chicken à la king at temperatures of 44° F and above.

Georgala and Hurst (19) in 1963, investigated the survival of food poisoning bacteria in frozen foods. They reported that Salmonellae in liquid egg stored at -17.8° C survived at least 10 months. They also observed that there was better survival of Salmonellae at -17.8° C than at -1° C.

In 1965, Foley and Sheuring (17), studied the lethal

rates of Escherichia coli, Pseudomenas flourescens, Staphlococcus aureus, Saccharomyces lactis and spores of Bacillus subtilis in soft ice cream during 30 minutes of freezing. They reported that destruction rates for Escherchia coli, Pseudomonas flourescens and Saccharomyces lactis were nonlinear, but rather similar. On the other hand Staphlococcus aureus exhibited considerable resistance to freezing, whereas spores of Bacillus subtilis were unaffected. They further reported that the mean lethal rates of the organisms and shape of the destruction rate curves were not influenced by initial cell concentration. Finally, the shape of the destruction rate curve was not influenced by the freezing rate.

There are several theories advanced to explain the cause of death of living cells in frozen states. The most acceptable theory is summarized in the work of Mazur (29) 1965. He concluded that most of the freezing injury to yeast and red blood cells was due to the combined effects of exposure to concentrated solutes and the formation of large intracellular ice crystals. This explanation also appeared to hold true for a number of species of micro-organisms.

Some investigators who were studying the lethal effects of radiation on microbial cells suggested that the temperature of irradiation may have a significant influence on the radiosensitivity of the cells.

Matsuyama et al (28) reported in 1963, the effect of freezing on the radiation sensitivity of bacterial spores. Gamma rays from Cobalt 60 were used to irradiate spores of Bacillus pumilis and Clostridium welchii at 10-13° C and at -79° C. In general, they found no difference in the sensitivity of the two types of spores studied when irradiated at room temperature and at -79° C.

In a second study in 1963, Matsuyama et al (27) examined the effect of freezing on the radiation sensitivity of vegetative bacteria. Five strains of Pseudomonas, Alcaligenes, Streptococcus faeceium and Escherchia coli were irradiated with gamma rays. Cells were suspended in heart infusion broth or in a phosphate buffer at temperatures of 10-13° C or -79° C. Generally, the shapes of the survival curves obtained from cells irradiated in a frozen state differed from those obtained at room temperature. The authors concluded that there was a reduction in radiosensitivity of the cells in the frozen state.

Licciardello (25), 1963, investigated the possible effect of temperature on the radiosensitivity of Salmonella typhimurium suspended in whole egg magma and liquid yolk. Irradiation was performed by gamma rays at temperatures varying from 32-130° F. Radiosensitivity appeared to increase in a nonlinear fashion as the irradiation temperature increased over a range of 32-130° F. The greatest lethal

effect occurred when irradiation was carried out at temperatures above 120° F.

Effect of Irradiation on Salmonellae

The possibility of eliminating Salmonellae from food products, particularly egg products, by means of irradiation with cathode rays or gamma rays has been investigated for some years (12, 24, 16, 22, 36, 20, 33, 9, 10).

Procter et al (36) in 1953 attempted to apply the use of cathode rays to sterilization of whole egg magma. They found that Salmonella organisms in heavily inoculated (10^6-10^7 cells/ml) whole egg magma were completely destroyed by cathode ray irradiation. The dosage of irradiation necessary for complete destruction of the organisms varied according to the species. They reported that Salmonella paratyphi and Salmonella typhimurium required a substantially higher irradiation dose than did Salmonella senftenberg.

In 1955, Gunter and Kohn (20), carried out a comparative study on the effect of x-ray irradiation on the survival of bacteria and yeast. Washed cell suspensions of 10^4 - 10^5 cells/ml, prepared from 16 to 17 hour plate cultures were irradiated in potassium phosphate buffer supplimented with magnesium sulfate. Irradiation was carried out at 24-27° C. They found that the least resistant organism was Pseudomonas flourescens, while the most resistant was Azotobactes agile. Saccharomyces cereviseae (haploid) and Rhodopseudomonas

spheroides displayed similar LD_{50} values.

Nickerson et al (33) in 1956, investigated the use of high voltage cathode rays to destroy Salmonellae in liquid and frozen egg white and in egg white solids. A level of 5×10^5 to 5×10^6 cells per gram of product of Salmonella typhimurium and Salmonella senftenberg were incorporated into the egg products. It was found that Salmonella was more resistant to irradiation in dried egg white products than in liquid or frozen egg white. In general, Salmonella typhimurium was found to be more resistant than Salmonella senftenberg.

Broglé et al (9) in 1957, determined 10^7 reduction values of Salmonella typhimurium and Salmonella senftenberg in whole egg solids, egg yolk solids and frozen egg yolk. They found that there was very little difference in the resistance of Salmonellae to cathode rays in all products, although it was observed that, in general, Salmonellae were slightly more resistant in egg yolk solids than in frozen egg yolk. Atmospheres of air, oxygen and nitrogen had very little or no effect on the resistance of these organisms to irradiation.

In 1959, Brookes et al (10) irradiated frozen whole egg contaminated with Salmonella at a temperature of -10° C. They reported that doses ranging from 0.3 to 0.5 Mrads, effectively eliminated viable Salmonella cells in the product.

Furthermore, they reported that organoleptic changes produced as a result of irradiation at these levels had little or no effect on the taste and odour of the baked goods prepared from the irradiated products.

Erdman et al (16), in 1960, carried out a study on the irradiation of micro-organisms in relation to food preservation. They investigated the comparative resistance of a number of bacteria cultures to gamma irradiation and the influence of different suspending media upon specific resistance of the micro-organisms. Their findings revealed that the nature of the suspending medium could influence greatly the relative radiation sensitivity of a specific culture. They also observed that food pathogens such as Salmonellae and Staphlococci were substantially more resistant to irradiation than the coliform organisms.

Ingram et al (22), in 1961, carried out a study on the application of gamma irradiation for the purpose of elimination of Salmonellae in frozen whole egg. They inoculated Salmonella gallinarum, Salmonella senftenberg and Salmonella typhimurium into liquid egg and into frozen egg. They observed that Salmonella gallinarum and Salmonella typhimurium were more resistant to irradiation in the frozen whole egg than in the liquid product, while Salmonella senftenberg exhibited equal resistance in both products. Among the three species, Salmonella typhimurium appeared to

be the most resistant while Salmonella gallinarum was the least resistant.

Ley et al (24), in 1963, investigated the use of gamma irradiation as a means of eliminating Salmonellae from various foods. 1000 gallons of liquid egg was inoculated with Salmonella gallinarum to a concentration of 5×10^5/ml samples were removed, frozen at -15° C for 2 days and exposed to a dose of 0.5 Mrad. They reported that no viable cells were found in 50 ml samples of the irradiated product.

Comer et al (12), in 1963, examined the effects of gamma irradiation on 18 Salmonella species in frozen whole egg. According to them, levels of irradiation necessary for a 10^7 reduction of each species of Salmonella, ranged from 0.36 Mrad to 0.54 Mrad. Furthermore, they found variation in sensitivity within all species of Salmonella studied, and suggested that due to the variation of micro-organisms within the species, no one species could be considered as more resistant than the others. The authors felt that a level of 0.54 Mrad of gamma irradiation would reduce the potential hazards of Salmonella to a safe level in frozen egg.

Recovery Techniques

In order to obtain an accurate estimation of the surviving fraction of Salmonellae following irradiation, an acceptable recovery technique was desirable.

Possibly, one of the most accurate means of enumerating

a bacterial population is by the most probable number technique as outlined by Hoskins (21) in 1934. This technique was established for the computation of the most probable number (M.P.N.) of coli-aerogenes organisms during the bacteriological analysis of water.

Ayres (5) in 1949, used a modification of the MPN technique to recover Salmonella organisms from dried egg products. Results were obtained by direct transfer of the reconstituted egg products and by the use of MPN data from selenite-F enrichment followed by streaking on SS agar. Three tubes of selenite-F broth were inoculated at each dilution level rather than the five tubes which were normally used in the MPN determinations of the coli-aerogenes group by Hoskins. Ayres discovered that, when the level of contamination was low, no valid counts were obtained by direct plating, whereas positive findings were obtained by using the MPN technique. Ayres concluded that the MPN method, whereby any amount of product could be sampled, was clearly superior to the plating technique.

Banwart and Ayres (7), in 1953, investigated the effect of various enrichment broths and selective agars for the recovery of several species of Salmonella. They found that selenite-F, tetrathionate or Ruy's medium did not support the growth of 8 species of Salmonella as well as did nutrient broth. Although, tetrathionate broth supported the

growth of most Salmonella species studied, it was definitely inhibitory to Salmonella paratyphi. Selenite-F appeared to be one of the better broths; however, during the initial incubation period, inhibition and actual destruction of Salmonella anatis was observed. They also discovered that when whole egg was added to these broths the inhibition effect was reduced. It was noted that brilliant green agar resulted in a more luxuriant growth of all species examined than did Salmonella-Shigella agar and DCLS agar.

Wells et al (50) in 1957, performed a comparative study of various selective media for the recovery of Salmonellae from egg white solids. The procedure followed for determining the number of Salmonella in the product was basically the M.P.N. technique as adopted by Ayres in 1953. Samples of 10, 1, 0.1, 0.01 and 0.001 gram were introduced in triplicate into four enrichment broths being studied. The broths employed were selenite-F, selenite brilliant green, commercial selenite brilliant green sulfapyridine (SBS) and laboratory prepared SBS, while the selective agars used were bismuth sulfite, brilliant green and brilliant green sulfa. After the inoculated tubes were incubated in each of the broths for 20-24 hours at 37° C, the contents were streaked onto the selective agar plates, which were also incubated for 20-24 hours at 37° C. The results of this study revealed little differences between bismuth sulfite, brilliant green

and brilliant green sulfa agars. The main exception was the low MPN value obtained when brilliant green agar was streaked from selenite brilliant green enrichment broth. The authors suggested that the larger number of Salmonella recovered from egg white solids after enrichment in SBS broth compared to selenite-F broth was due to its ability to support the growth of a greater variety of Salmonella types.

Taylor et al (45), 1957, stated that selenite-F broth was the medium of choice over tetrathionate broth, but mentioned that this did not preclude the possibility that there were changes in formulae which would enhance the ability of selenite-F to detect Salmonellae. The MPN determinations for Salmonellae were obtained using cystine-selenite broth, mannitol-selenite broth, selenite brilliant green sulfapyridine broth and dulcitol-selenite broth. The MPN determinations for coliform organisms involved the comparison of lactose broth with brilliant green lactose bile broth. Verification of positives in selenite broth was made on brilliant green agar.

Six different dilution levels consisting of three tubes at each level of mannitol selenite versus selenite-F revealed no statistically significant differences for the isolations of Salmonellae between these two media. Similar results were obtained when selenite-dulcitol was compared to selenite-F. Contrary to the results obtained by Wells et

al (50), it was found that selenite brilliant green sulfapyridine enrichment broth did not produce more isolations of Salmonella and in some instances was markedly inferior to selenite-F broth. The authors also found that coliforms were greatly inhibited by brilliant green, SS and bismuth sulfite agars as compared to TGY plates. They concluded that cystine-selenite-F enrichment broth and the subsequent streaking on brilliant green agar was the method of choice for detection and enumeration of Salmonellae in foods.

Silliker and Taylor (41), 1957, observed in previous investigations that quite frequently Salmonella organisms were not isolated from enrichment broth aliquots representing the largest inocula, but were detected with ease in those which had received much smaller inocula. The levels at which these "skips" occured were usually at the 10, 1.0 and 0.1 gram replicates.

The effect of an unfavourable imbalance of coliforms to Salmonella was determined by establishing varying ratios of coliforms to Salmonella and inoculating enrichment broths. The ability of added food to alter the performance of enrichment broths was tested with a variety of dried powdered foods. It was found that neither overwhelming numbers of coliform organisms nor an unfavourable imbalance of the ratio of coliforms to Salmonella materially affected the selectivity of selenite or tetrathionate enrichment broths

for Salmonella. The performance of these was, however, greatly diminished by the addition of many kinds of food materials. Gelatin and albumen caused the most reduction while egg yolk caused the least.

Taylor and Silliker (44), in 1961, demonstrated that an 18-24 hour pre-enrichment in lactose broth before inoculation of enrichment media was vastly superior to reconstitution in water for enrichment broths. The experiment was carried out with dried egg albumen, naturally contaminated with Salmonellae.

Taylor (43) in 1961, also demonstrated the advantage of pre-enriching with lactose broth before inoculation into the selective broth.

North (34) in 1961, investigated the possibility of using lactose broth pre-enrichment as a means of eliminating skips in the MPN analysis. The work was carried out using commercially produced dried egg white. North noticed a marked increase in the MPN recovery by the 24 hour lactose pre-enrichment method over direct inoculation in any of the enrichment broths. The inhibition of a high concentration of egg albumen at the lower dilutions was not observed when lactose pre-enrichment was employed.

Montford and Thatcher (30) in 1961, compared four methods of isolating Salmonella from egg products, using six enrichment broths and five selective media. They found that

selenite-F enrichment broth produced the best MPN recoveries of Salmonellae from frozen whole egg. Large numbers of coliforms present in the product, apparently, did not appear to affect the recovery of low numbers of Salmonellae in the samples (0.44/gram). These authors recommended the pre-enrichment of the egg samples in a mixture of buffer and lactose broth for 5 hours at 37° C. They found that the 24 hour pre-enrichment in lactose broth produced a pronounced overgrowth of coliforms when frozen whole egg was investigated.

Silliker et al (40) in 1964, stated that, although the lactose pre-enrichment promoted Salmonella recovery from samples containing small numbers of dormant cells, the efficiency of the method was adversely affected by unfavourable coliform-Salmonella ratios. Their work showed that early subculture from lactose broth was important. When lactose broth sub-cultures were incubated beyond 8 hours, coliform overgrowth was likely to occur.

The report of a subcommittee on sampling and methodology for the recovery of Salmonellae in eggs (3) in 1966, recommended a 1:10 ratio of inoculum to lactose broth. This should then be followed by a six hour pre-enrichment in the lactose broth at 35° C and subsequent transfer into selenite-cystine broth. After 24 hours at 35° C in the selenite-cystine broth, the cultures were to be streaked on brilliant green agar plates and inoculated for a further 24 hours at 35° C.

SCOPE OF INVESTIGATION

The scope of this study was to determine the effect of gamma irradiation on selected species of Salmonellae in frozen egg products. Specifically the following areas were investigated.

1. The survival of Salmonella give, Salmonella worthington, Salmonella enteritidis, Salmonella typhimurium, Salmonella heidelberg, and Salmonella senftenberg in sterile frozen whole egg, sterile frozen egg yolk and sterile frozen egg albumen following irradiation treatments.
2. The survival of Salmonella give, Salmonella worthington, Salmonella enteritidis, Salmonella typhimurium, Salmonella heidelberg and Salmonella senftenberg, following irradiation treatments in frozen whole egg which also contained a high level (10^6 to 10^8 cells/gram) of natural flora.
3. The survival of the natural flora in frozen whole egg following irradiation treatments.

MATERIALS AND METHODS

Phase one of this investigation involved the destruction of selected species of Salmonella in sterile frozen egg products by gamma irradiation. The procedure used to obtain sterile egg products was as follows:

Fresh eggs were washed in warm soapy water, rinsed, and immediately placed in a sanitizing solution containing 200 ppm available chlorine for 15 minutes. The eggs were obtained weekly from the University of Manitoba Poultry Department. The sanitized eggs were then placed in a sterile isolation chamber. The isolation chamber used was manufactured by Lab Con Co. Ltd. of Kansas City and was equipped with an ultraviolet light for sterilization purposes. The interior of the chamber and contents were exposed to U.V. light for at least two hours before use.

In the isolation chamber, the sanitized eggs were immersed in a 70% ethanol solution and flamed to remove any residual ethanol. The eggs were then cracked and the albumen, yolk and whole egg placed in separate one-pint sterile mason jars. If not used immediately, these jars were stored at 6° C until used.

In order to establish the effectiveness of this technique to produce samples free of micro-organisms, 4 ml

aliquots were plated out on Tryptone-glucose-yeast-extract (TGY) agar and incubated for 2 days at 37° C. A further and more demanding test was also performed by adding 11 ml of egg product to 99 ml of sterile nutrient broth and incubating this for 1 week at 37° C. At the end of this period, loop-fulls of the mixture were streaked on TGY plates and incubated at 37° C for 2 days. The samples were considered bacteria free if no growth was observed on the TGY agar plates.

The second phase of this investigation was similar to phase one, with the exception that no attempt was made to exclude the natural microbial flora in the egg products. In fact a high level of natural flora was encouraged for the purposes of this investigation. The eggs used were unwashed cracks or other eggs of inferior quality. They were obtained from a local egg grading plant and broken at the Food Science Department.

The egg contents when cracked were mixed in a Waring blender and the resulting melange placed in sterile one pint mason jars. These jars were then stored at 6° C until used. Fifty gram samples were taken from these jars and examined for the presence of Salmonellae. The procedure followed for this analysis was the official procedure as outlined by the Food and Drug Directorate (30).

Prior to inoculation with the Salmonella species the egg melange was placed in a 35° C incubator for 12 hours to

obtain a natural flora in the order of 10^6 to 10^8 cells/gram.

The population of the flora was enumerated by the Standard Plate Count method (53) and by the Crystal Violet technique (53), which determined the gram negative flora present in the sample. The Standard Plate Count plates were incubated at 35° C for 48 hours while the Crystal Violet Agar plates were incubated at 32° C for the same period of time.

Preparation and Inoculation of Cultures

The preparation of the Salmonellae cultures to be used to inoculate the different egg products varied slightly throughout the study due to the different cultural peculiarities of each species studied. All species were preserved on nutrient agar slants and transferred to nutrient broth containing 1% peptone, as they were used. The tubes of nutrient broth plus 1% peptone containing the Salmonellae cultures were incubated for 18 hours at 37° C.

In the case of Salmonella give and Salmonella heidelberg an 18 hour old culture was inoculated into each of three erylenmeyer flasks containing 650 ml of nutrient broth plus 1% peptone. These flasks were then incubated at 37° C for 36 hours. The same procedure was followed for Salmonella enteritidis except for the fact that the flasks were placed on a rotary shaker during incubation at 37° C for 36 hours.

Salmonella typhimurium, Salmonella senftenberg and Salmonella worthington were cultured in an aerated four litre flask for 36 hours at 37° C after being inoculated with 18 hour cultures. The air used for aeration was filtered to render it free from micro-organisms. All six species studied in frozen whole egg containing a natural flora were cultured using the aeration technique.

At the end of the 36 hour incubation period the contents of the flasks were poured into sterile stainless steel centrifuge tubes and centrifuged in a Sowall high speed centrifuge at 4000 RPM for 30 minutes. The model used was the Sowall Superspeed RC2-B refrigerated centrifuge equipped with a 2 litre anodized aluminum angle head. After centrifugation, the supernatant was poured off and discarded. The remaining cells were suspended in 10 ml of 1% peptone water and served as the inoculum. This 10 ml inoculum was then added to 400 grams of egg product (in sterile 1 pint mason jar) and thoroughly mixed at a low speed on a Sowall Omni mixer. After mixing, 15 gram aliquots were asceptically transferred into sterile test tubes (18x150 mm). One batch (400 grams) of egg product yielded from 16 to 24 such tubes. These inoculated tubes of egg product were then frozen at -15° C varying from 1-3 days prior to irradiation.

Test Organisms

The Salmonella organisms used in this study were

Salmonella give ATCC 9268, Salmonella heidelberg ATCC 8326, Salmonella enteritidis ATCC 13076, Salmonella typhimirium, ATCC 1311, Salmonella senftenberg ATCC 8400, and Salmonella worthington ATCC 9607; all obtained from the American Type Culture Collection.

Irradiation Unit

The irradiation unit used throughout this study was a Gammacell 220, supplied by the Atomic Energy Commission of Canada. The irradiation source was Cobalt 60 with a maximum dose rate of 2.0×10^6 roetgens/hour at the centre of the sample chamber. The irradiation chamber was 6.0 inches in diameter by 8.1 inches in height, with a volume of 220 cubic inches. The cobalt 60 had a half life of 5.3 years with a decrease in radioactivity of about 1% per month. The unit had an energy source of 1.17 and 1.33 MeV and the ability to penetrate 40 cm if the material was irradiated from both sides. Due to the decaying factors of the source, the exposure times for specific dose levels were calculated monthly by using the appropriate decay factors.

Irradiation Procedure

Irradiation of each product was performed while the product was in the frozen state (-15° C), although the temperature rose to -1.1° C in egg albumen, egg yolk and whole egg after exposure to 0.7 Mrads of irradiation. This

was achieved by placing the 18 x 150 mm test tubes of frozen egg product into an insulated container with appropriate holes distributed in a symmetrical arrangement. Each styrofoam container could accomodate from seven to fourteen test tubes. This insulated container was then placed in the irradiation chamber of the gammacell 220 and the contents subjected to the following levels of irradiation; 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 Mrads.

Temperature studies were carried out in the irradiation chamber to determine if the temperature varied and to what extent it varied during irradiation. A thermocouple (copper-constantan)was placed in a test tube of the egg product to be irradiated. After freezing, this tube was taken to the gammacell in an insulated chamber and placed in the styrofoam container in the irradiation chamber. The thermocouple was attached to a Model #2736 Honeywell Potentiometer. The cell chamber was lowered into position and temperature readings taken at one minute intervals throughout the 0.7 Mrad exposure period (approximately 35 minutes).

Enumeration of Salmonellae after Irradiation

The most probable number enumeration technique was chosen for its simplicity of use, its acceptable degree of accuracy and for its ability to selectively enumerate Salmonellae amongst a mixed flora. This aspect was particu-

larly important as the second phase of this study involved the enumeration of Salmonellae organisms from egg products which contained a large natural flora.

It was assumed that at the lower levels of irradiation (0.1-0.2 Mrads) the possibility of injured cells surviving could occur. For this reason and the fact that the possibility of some cells being dormant as a result of the freezing temperatures, a pre-enrichment of 6 hours in a non-selective (lactose) broth was adopted. This procedure might permit any injured or dormant cells to recover and therefore be detected by the MPN technique.

The frozen irradiated egg samples were thawed in running water at 10° C. Eleven grams of the thawed product were aseptically pipetted into 99 ml of sterile lactose broth. This was shaken vigorously and incubated at 37° C for 6 hours.

From the lactose broth (1:10 dilution) serial dilutions were performed in sterile buffered water up to a dilution of 10^{-11}/gram. Three tubes of Selenite-F broth were inoculated for each of six dilution levels and incubated at 37° C for 20-24 hours.

Following the incubation period and after mixing each tube thoroughly on a Vortex mixer the contents of each selenite-F tube was streaked on BGS plates and incubated for a further 20-24 hours at 37° C. From the growth obtained on

the BGS plates the number of positive selenite-F tubes at each level was determined and the MPN/gram of product was calculated from "Hoskins, J. K., MPN tables." (21)

During the phase of the work on Salmonellae in egg products containing a natural flora, several trials were conducted to determine the presence of any natural flora surviving after treatments of 0.6 and 0.7 Mrads, respectively. This was accomplished by plating the irradiated samples on TGY agar and incubating at 35° C for 48 hours.

Freezing of Egg Products

As the egg product was irradiated in the frozen state, a study was conducted to determine the extent of Salmonellae surviving after the freezing process. The desired level of Salmonellae in the egg products before irradiation was 10^9/gram.

This investigation was carried out by enumerating the number of viable Salmonellae cells in the mixed unfrozen product and by following the same procedure after the product had been frozen at -15° C for 1-3 days. The lactose pre-enrichment and the MPN technique were used to enumerate the number of Salmonellae surviving the freezing process.

RESULTS

1. PRELIMINARY STUDIES

A. Sterile Egg Products

In order to ascertain the effects of gamma irradiation on selected species of Salmonella in frozen egg products with few or no micro-organisms, it was necessary to establish a reliable method to supply these products. It was found that the method as outlined in the procedure section gave products showing little or no contamination. The data presented in tables 1, 2, and 3 demonstrated that the method adopted to supply low bacteria egg products was effective. A certain number of the samples presented in Table 2 indicated the presence of bacteria in the product. This could possibly be due to contamination during handling and/or the presence of bacteria within the shelled eggs. In order to avoid the possibility of contamination, extra precautions were subsequently taken during the preparation of egg samples. As can be seen in Table 3, the method of preparing egg products was adequate in that no growth was evident in any of fifteen samples. As a rule, all egg products that were used in later studies were checked for the presence of bacteria before use.

TABLE 1

THE BACTERIAL CONTENT OF FRESHLY CRACKED EGG PRODUCTS PLATED ON TGY AND INCUBATED AT 37° C FOR 48 HOURS

Product	Samples	Av. Colonies/4 ml
Whole egg	8	0
Egg yolk	8	0
Egg albumen	8	0

TABLE 2

THE BACTERIAL CONTENT OF CRACKED EGG PRODUCTS INOCULATED INTO NUTRIENT BROTH AND INCUBATED FOR 7 DAYS

Product	Samples	No. positive TGY plates	% positive TGY plates
Trial #1			
Whole egg	20	2	10%
Egg yolk	20	6	30%
Egg albumen	20	0	0%
Trial #2			
Whole egg	20	1	5%
Egg yolk	20	2	10%
Egg albumen	20	0	0%

TABLE 3

THE BACTERIAL CONTENT OF FRESHLY CRACKED EGG PRODUCTS
A COMPARISON OF PLATING ON TGY TO INCUBATION IN NUTRIENT BROTH
FOR 7 DAYS AT 37^0 C

Product	Samples	No. of Positive on TGY	% Positive on TGY	No. of Positive on Nutrient Broth	% Positive on Nutrient Broth
Trial #3					
Whole egg	15	0	0	0	0
Egg yolk	15	0	0	0	0
Egg albumen	15	0	0	0	0

B. Reproducibility of the MPN Technique

The first attempts at using the MPN technique as outlined in the procedure section, were carried out using an 11 ml sample of egg product plus Salmonellae in 99 ml of sterile phosphate buffer (1:10 dilution). As is shown in Table 4, 10 samples of egg product were taken from one lot of egg products inoculated with Salmonella give. The MPN determinations performed on these samples revealed relatively good reproducibility with a few exceptions (Table 4). However, it was felt that MPN/gram determinations should be more consistent than the results presented in Table 4. For this reason 11 gram samples were used instead of the 11 ml samples. An immediate improvement in the reproducibility of the method was noted as indicated by the data presented in Table 5. Because of this all future MPN determinations involved the utilization of 11 gram samples rather than 11 ml samples in the MPN procedure.

C. Lactose Pre-enrichment of Irradiated Samples

The use of a 6 hour pre-enrichment period in lactose broth was used to determine if larger numbers of viable cells could be recovered from the egg product following irradiation. By performing the MPN determinations on the product before and after pre-enrichment treatments (Table 6) it was found that pre-enrichment resulted in MPN values

TABLE 4

DATA SHOWING THE REPRODUCIBILITY OF THE MPN TECHNIQUE WHEN USING AN 11 ml INOCULUM OF Salmonella give MPN/ml

Sample	Egg Yolk	Whole Egg	Egg Albumen
(1)	2.3×10^6	4.3×10^6	4.3×10^6
(2)	7.5×10^6	4.3×10^6	2.4×10^7
(3)	2.3×10^6	2.3×10^6	2.4×10^7
(4)	6.2×10^6	1.5×10^6	9.3×10^6
(5)	1.5×10^6	2.3×10^6	4.3×10^6
(6)	4.3×10^6	7.5×10^6	9.3×10^6
(7)	9.3×10^6	2.3×10^6	3.9×10^6
(8)	2.3×10^6	9.3×10^5	2.3×10^6
(9)	2.3×10^6	2.4×10^7	7.5×10^6
(10)	2.3×10^6	2.8×10^5	7.5×10^6
Average	4.0×10^6	5.0×10^6	9.6×10^6

TABLE 5

DATA SHOWING THE REPRODUCIBILITY OF THE MPN TECHNIQUE WHEN USING AN 11 gram INOCULUM OF *Salmonella give* MPN/gram

Sample	Egg Yolk	Whole Egg	Egg Albumen
(1)	2.3×10^8	4.3×10^8	1.5×10^8
(2)	2.3×10^8	4.3×10^8	1.5×10^8
(3)	7.5×10^8	3.9×10^8	4.3×10^8
(4)	7.5×10^8	4.3×10^8	3.9×10^8
(5)	1.5×10^9	4.3×10^8	4.3×10^8
Average	6.9×10^8	4.2×10^8	3.1×10^8

TABLE 6

Salmonellae ORGANISMS PER GRAM OF VARIOUS EGG PRODUCTS AS DETERMINED BY THE MPN TECHNIQUE. A COMPARISON OF PRE-ENRICHMENT IN LACTOSE TO NO PRE-ENRICHMENT

Egg Albumen		Whole Egg	
No Pre-Enrichment	Pre-Enrichment	No Pre-Enrichment	Pre-Enrichment
(1) 1.2×10^8	4.3×10^9	(1) 9.3×10^8	7.5×10^9
(2) 4.3×10^7	9.3×10^9	(2) 4.3×10^8	4.3×10^9
(3) 4.3×10^8	4.3×10^9	(3) 4.3×10^8	4.3 xx 10^9
(4) 9.3×10^7	9.3×10^9	(4) 2.3×10^8	4.3×10^9
(5) 2.4×10^8	2.4×10^9	(5) 9.3×10^8	9.3×10^9
Average 2.05×10^8	5.92×10^9	Average 5.9×10^8	5.94×10^9

Egg Yolk	
No Pre-Enrichment	Pre-Enrichment
(1) 1.5×10^9	9.3×10^9
(2) 2.4×10^9	4.3×10^9
(3) 4.3×10^8	2.3×10^9
(4) 9.3×10^9	4.3×10^{10}
(5) 9.3×10^8	L.A.
Average 2.9×10^9	1.2×10^{10}

TABLE 7

Salmonellae ORGANISMS PER GRAM OF VARIOUS EGG PRODUCTS AS DETERMINED BY THE MPN TECHNIQUE. A COMPARISON OF FROZEN TO NON-FROZEN PRODUCTS. ALL SAMPLES ARE PRE-ENRICHED WITH LACTOSE BROTH

	EGG ALBUMEN			WHOLE EGG	
	Non-Frozen	Frozen at -16°C		Non-Frozen	Frozen at -15°C
(1)	1.5×10^{10}	9.3×10^{9}	(1)	7.5×10^{9}	7.5×10^{9}
(2)	9.3×10^{9}	4.3×10^{9}	(2)	7.5×10^{9}	L. A.
(3)	9.3×10^{9}	4.3×10^{9}	(3)	4.3×10^{9}	2.3×10^{9}
(4)	4.3×10^{9}	2.3×10^{9}	(4)	2.4×10^{10}	4.3×10^{10}
(5)	2.4×10^{10}	4.3×10^{9}	(5)	4.3×10^{9}	4.3×10^{10}
Average	1.2×10^{10}	4.9×10^{9}	Average	9.5×10^{9}	1.14×10^{10}

	EGG YOLK	
	Non-Frozen	Frozen at -15°C
(1)	9.3×10^{9}	4.3×10^{9}
(2)	3.9×10^{9}	2.1×10^{9}
(3)	4.3×10^{9}	4.3×10^{9}
(4)	1.5×10^{10}	2.3×10^{9}
(5)	2.4×10^{10}	L. A.
Average	1.1×10^{10}	3.5×10^{9}

approximately one log cycle greater than those samples which did not receive a pre-enrichment treatment. In view of this development, all samples were subsequently pre-enriched in lactose broth for 6 hours prior to determination of MPN values.

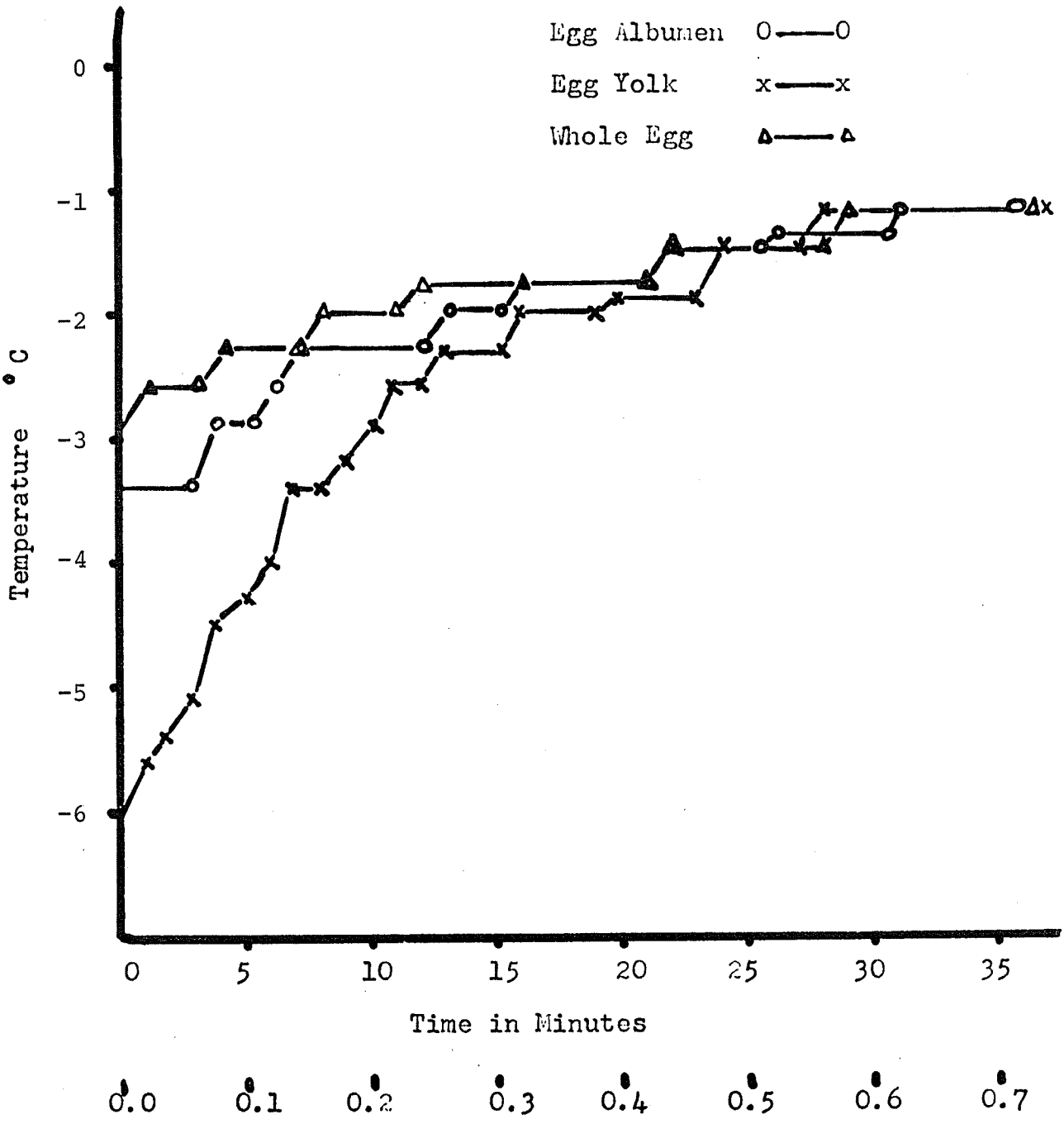
D. Effect of Freezing on the Survival of Salmonellae in Egg Products

As all egg products inoculated with Salmonella organisms were to be irradiated in the frozen state, a study was conducted to determine the number of Salmonellae surviving after the freezing process. The data presented in Table 7 indicated that a very small decrease in the bacterial population occurred after freezing at -15° C for 1-3 days. Salmonella give in egg albumen and egg yolk decreased by less than one log cycle while no significant change occurred for Salmonella give, frozen in whole egg melange. Subsequent results revealed that freezing of egg products containing the other 5 Salmonella test organisms did not appreciately reduce the population, because the number of organisms were in the range of 10^9 - 10^{10}/gram of product (Table 8).

E. Temperature Changes in Frozen Egg Products During Irradiation

A study of the temperature changes in the egg product during irradiation is presented in Figure 1. It was found that the initial temperatures of -6.1° C in egg yolk, -3.3° C

Fig. 1 Temperature Changes in Frozen Egg Yolk, Frozen Egg Albumen and Frozen Whole Egg During Irradiation



(Appendix Tables XXVIII to XXX)

TABLE 8

THE NUMBER OF *Salmonellae* SURVIVING FREEZING AT -15° C FOR 1-3 DAYS

MPN/gram

Organism	Product		
	Egg Yolk	Egg Albumen	Whole Egg
S. give	4.6×10^{9}	1.02×10^{10}	1.3×10^{10}
S. enteritidis	1.2×10^{10}	4.3×10^{9}	8.1×10^{9}
S. heidelberg	1.1×10^{10}	9.7×10^{9}	5.9×10^{9}
S. worthington	1.1×10^{10}	2.0×10^{10}	1.5×10^{10}
S. Typhimurium	2.6×10^{9}	3.5×10^{9}	4.9×10^{9}
S. senftenberg	2.2×10^{10}	1.5×10^{11}	1.8×10^{10}

Average of Five Determinations

(Appendix Tables I to XVIII)

in egg albumen and -2.8° C in whole egg rose to -1.1° C after an exposure of 0.7 Mrads. After 0.7 Mrads of irradiation the three egg products were still in the frozen state, indicating that the samples did not thaw.

2. IRRADIATION OF *SALMONELLAE* IN STERILE EGG PRODUCTS

A. Frozen Egg Albumen

The effect of gamma irradiation on the six species of *Salmonella* in frozen egg albumen appeared to vary with the species. *Salmonella give*, *Salmonella heidelberg*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella senftenberg* were completely destroyed by a dosage of 0.7 Mrads while *Salmonella worthington* displayed only slight growth (i.e. less than 1 colony per gram of product) at the same level of irradiation treatment. Furthermore, it was noted that *Salmonella enteritidis* and *Salmonella typhimurium* were completely destroyed by a dosage of 0.5 Mrads. (Table 9). A slight lag in the destruction of each organism except *Salmonella enteritidis* in frozen egg albumen was observed. *Salmonella enteritidis* was destroyed at the same rate at all levels of irradiation. With the exception of the slight lags in the destruction rates, straight line survival curves were obtained for all six species of *Salmonella* when plotted on semi-log paper (Figures 2 and 3).

According to the 10^8 reduction values per gram of product obtained from the survival curves (figures 2 and 3),

TABLE 9

THE EFFECT OF GAMMA IRRADIATION ON *Salmonellae*

Organisms in Frozen Egg Albumen

Organism	Irradiation Level MPN/gram							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
S. give	1.02×10^{10}	1.02×10^{9}	2.8×10^{8}	3.9×10^{6}	9.5×10^{4}	1.0×10^{2}	5.8	0
S. heidelberg	9.7×10^{9}	1.3×10^{9}	1.2×10^{8}	9.5×10^{4}	1.2×10^{3}	5.4×10^{1}	1.3	0
S. enteritidis	4.3×10^{9}	5.6×10^{6}	1.1×10^{5}	1.2×10^{2}	0.7	0	0	0
S. typhimurium	3.5×10^{9}	2.2×10^{8}	6.8×10^{6}	1.3×10^{4}	7.8	0	0	0
S. worthington	2.0×10^{10}	9.6×10^{8}	1.2×10^{8}	1.2×10^{6}	1.7×10^{3}	7.3×10^{1}	1.2	0.6
S. senftenberg	1.5×10^{10}	5.6×10^{8}	7.3×10^{7}	2.9×10^{6}	4.1×10^{4}	1.9×10^{3}	2.8×10^{1}	0

Average of Five Determinations

(Appendix Tables I, IV, VII, X, XIII, XVI)

Fig. 2 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Egg Albumen

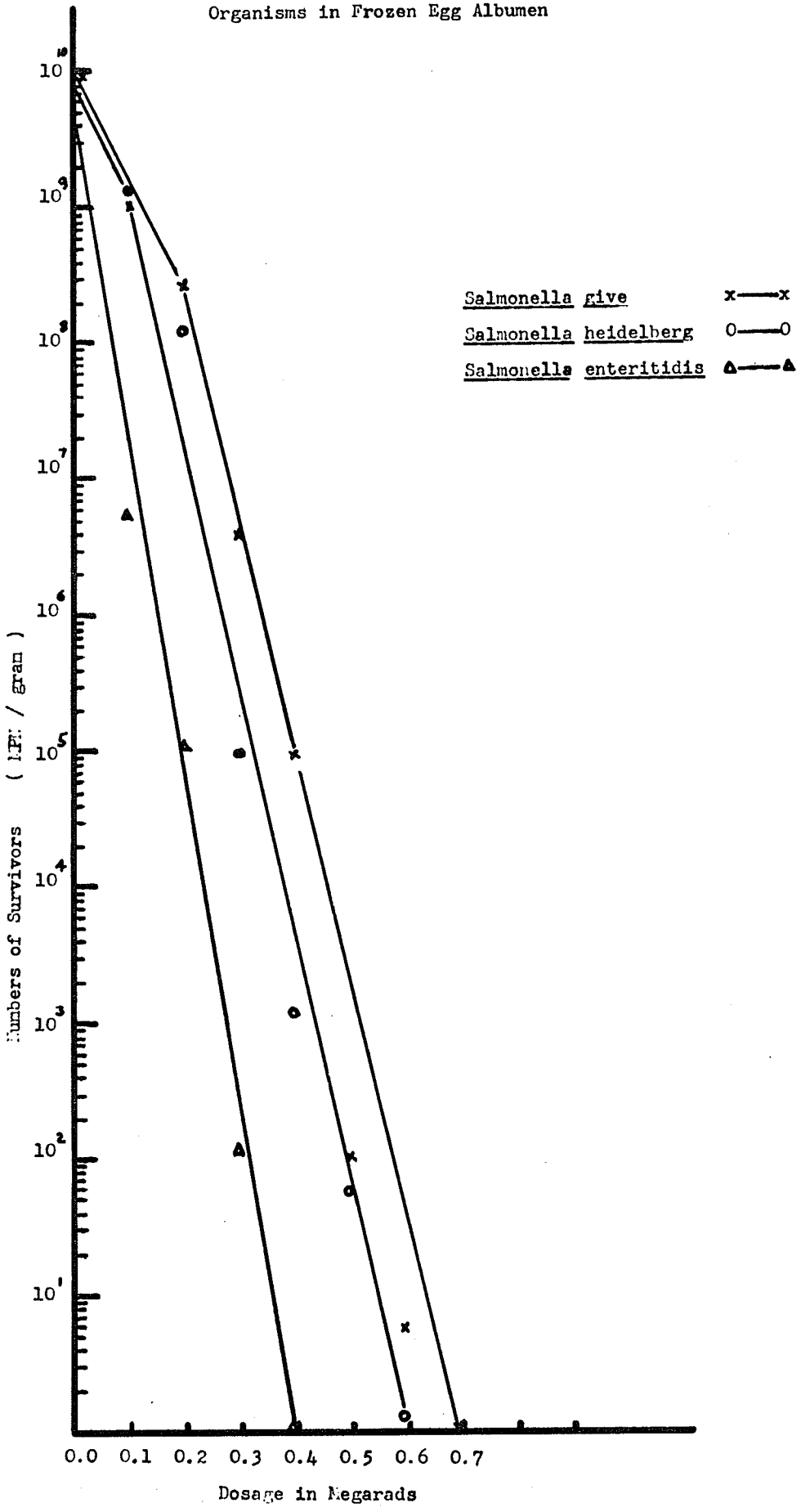
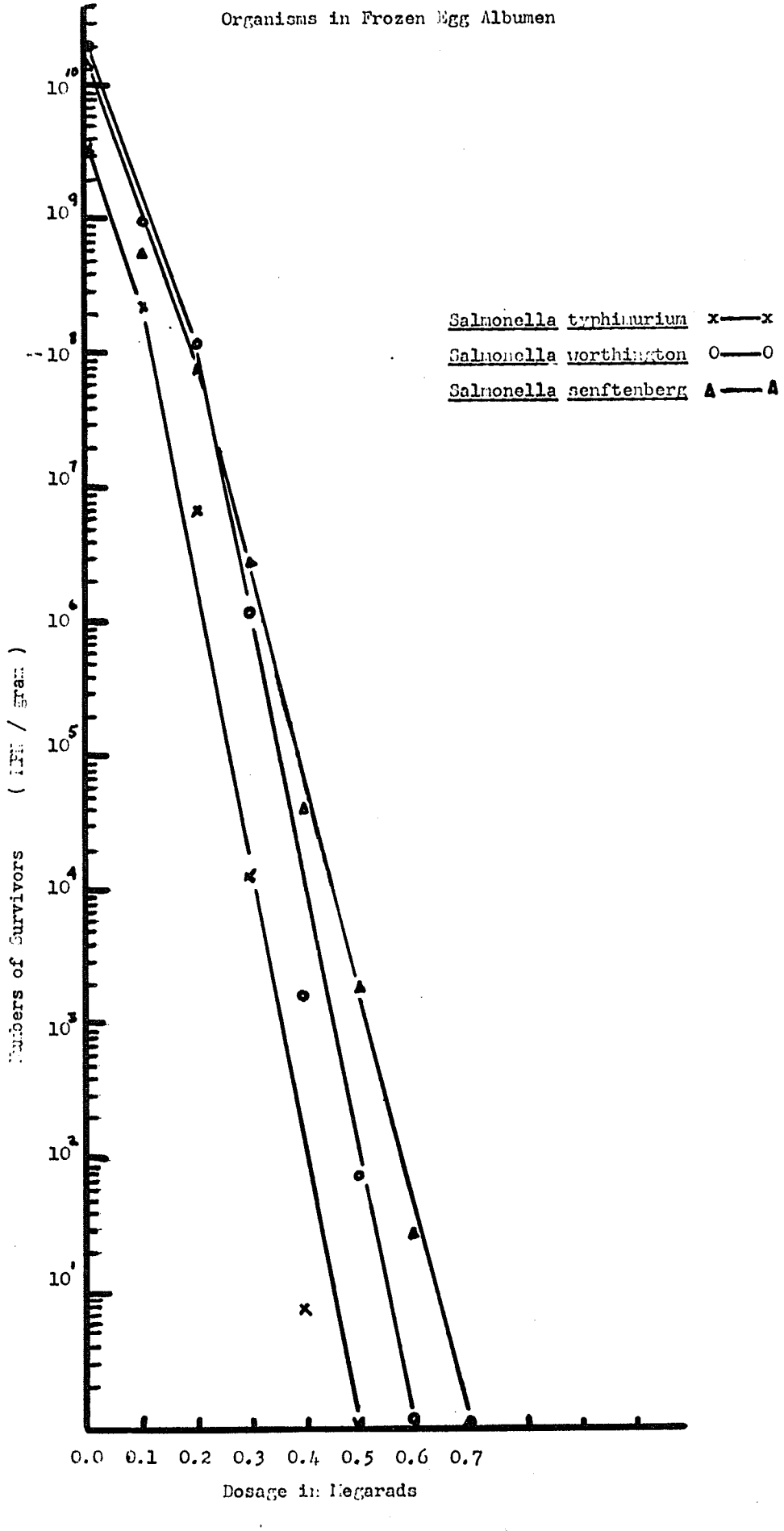


Fig. 3 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Egg Albumen



the species displaying the greatest radio-resistance to gamma irradiation in frozen egg albumen was Salmonella give, followed by Salmonella senftenberg, Salmonella heidelberg, Salmonella worthington, Salmonella typhimurium and Salmonella enteritidis respectively (Table 12). This sequence of radio-resistance of each test organism was similar to that as expressed by the D values of each test organism (Table 13) except for the fact that Salmonella senftenberg has a higher D value than Salmonella give due to the difference in the slopes of the survival curves.

B. Frozen Egg Yolk

Some differences in results were obtained when the same six species of Salmonellae were irradiated in sterile frozen egg yolk. In general, the organisms appeared to exhibit slightly more resistance to gamma irradiation when they were suspended in frozen egg yolk than when the same organisms were suspended in frozen egg albumen. Salmonella give and Salmonella enteritidis were the only two organisms which were completely eliminated after the 0.7 Mrads of irradiation (Table 10). In fact, Salmonella enteritidis was completely destroyed with a dosage of only 0.5 Mrads while all other species displayed some survival at the 0.7 Mrad level. Contrary to that observed in frozen egg albumen Salmonella give and Salmonella heidelberg were the only

TABLE 10

THE EFFECT OF GAMMA IRRADIATION ON *Salmonellae* ORGANISMS IN FROZEN EGG YOLK

Organism	Irradiation Level MPN/gram							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
S. give	4.6×10^{9}	1.7×10^{9}	1.03×10^{8}	7.1×10^{5}	1.5×10^{3}	1.3	1.8	0
S. heidelberg	1.1×10^{10}	5.0×10^{9}	2.5×10^{8}	1.1×10^{6}	3.4×10^{3}	2.5×10^{2}	9.7×10^{1}	4.0
S. enteritidis	1.2×10^{10}	3.7×10^{7}	1.4×10^{5}	2.7×10^{3}	1.8×10^{1}	0	0	0
S. typhimurium	2.6×10^{9}	1.1×10^{8}	3.0×10^{6}	4.8×10^{5}	2.5×10^{2}	2.9×10^{1}	1.0×10^{1}	4.6
S. worthington	1.1×10^{10}	7.8×10^{8}	2.1×10^{7}	3.0×10^{5}	9.7×10^{2}	5.3	2.0×10^{1}	0.7
S. senftenberg	2.2×10^{10}	8.5×10^{8}	1.2×10^{8}	2.4×10^{6}	1.9×10^{5}	3.6×10^{2}	6.6×10^{1}	1.9×10^{1}

Average of Five Determinations

(Appendix Tables III, VI, IX, XII, XV, XVIII)

Fig. 4 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Egg Yolk

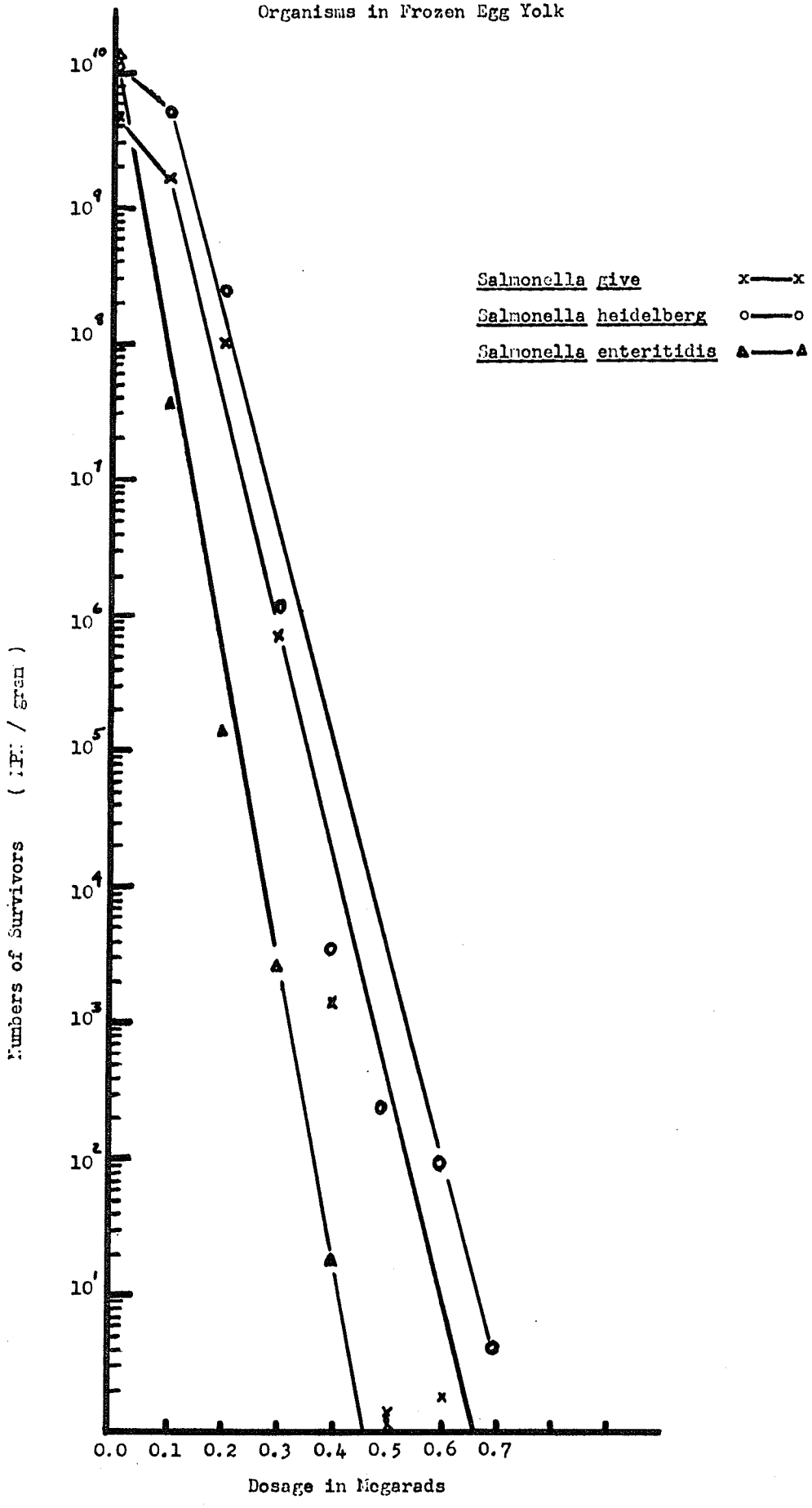
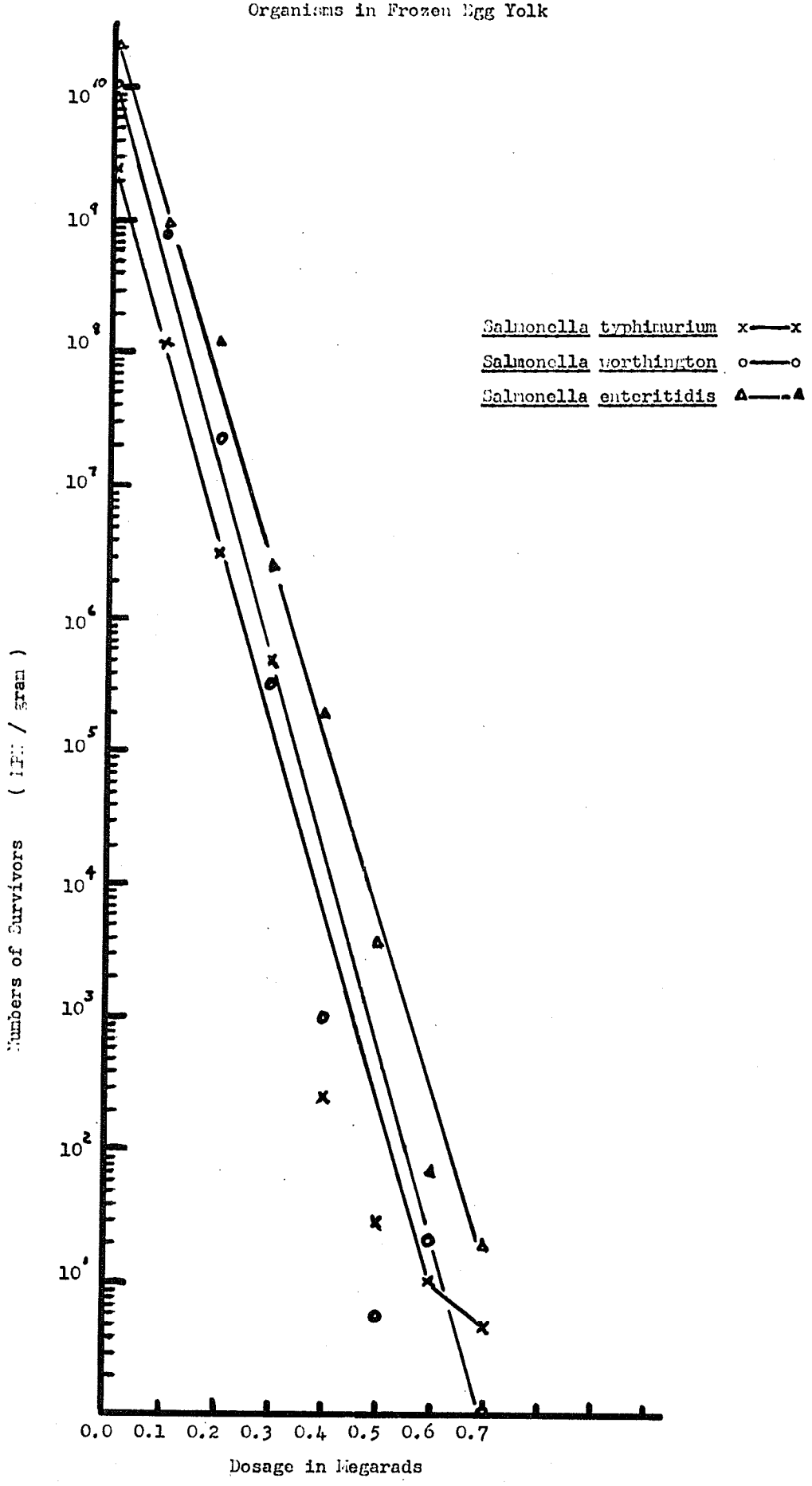


Fig. 5 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Egg Yolk



species which displayed a lag in their survival curves (Figures 4 and 5). With the exception of these two lag phases, all other survival curves appeared as straight lines when plotted on semi-log paper indicating that the destruction rates were equal at all levels of irradiation. On the other hand Salmonella typhimurium exhibited a curious "tailing" effect between the 0.6 and 0.7 Mrad levels (Figure 5).

The order of resistance of the test organisms was much different in frozen egg yolk than in frozen egg albumen. The 10^8 reduction values shown in Table 12 showed that the most resistant species was Salmonella senftenberg followed by Salmonella heidelberg, Salmonella typhimurium, Salmonella give, Salmonella worthington and Salmonella enteritidis. It was also observed that the 10^8 reduction values were all slightly higher for each species in frozen egg yolk.

The extrapolated D values for the six organisms were also greater than those obtained in frozen egg albumen. Salmonella senftenberg had the highest D value (0.075 Mrad) while Salmonella heidelberg, Salmonella typhimurium and Salmonella worthington all had D values of 0.070 Mrad (Table 13). Salmonella give had a D value of 0.065 Mrad and Salmonella enteritidis had the lowest at 0.045 Mrad in frozen egg yolk.

C. Frozen Whole Egg

The results obtained when the Salmonella organisms were irradiated in frozen whole egg (Table 11) differed from the results obtained when irradiation was performed in the other two egg products. Salmonella give and Salmonella heidelberg were completely eliminated after exposure to 0.7 Mrads of irradiation, and Salmonella enteritidis after 0.6 Mrads and Salmonella typhimurium after 0.5 Mrads. However, both Salmonella worthington and Salmonella senftenberg were recovered after a dosage of 0.7 Mrads. With the exception of Salmonella enteritidis and Salmonella senftenberg, lag phases were observed in the survival curves for the other four organisms when irradiated in frozen whole egg. As was noted in frozen egg albumen and frozen egg yolk the survival curves of all six organisms appeared as straight lines when plotted on semi-log paper, again indicating that the destruction rates were constant at all levels of irradiation.

The resistance to irradiation was similar to that seen when the test organisms were irradiated in frozen egg albumen. The 10^8 reduction values were, however, slightly higher in all species except Salmonella give which was lower and Salmonella typhimurium which was the same as that observed in frozen egg albumen. On the other hand the 10^8 reduction values were, in general, slightly lower in frozen whole egg than in frozen egg yolk. Exceptions were

TABLE 11

THE EFFECT OF GAMMA IRRADIATION ON *Salmonellae* ORGANISMS IN FROZEN WHOLE EGG

Organism	Irradiation Level MPN/gram							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
S. give	1.3×10^{10}	9.2×10^{9}	3.4×10^{8}	4.1×10^{6}	2.2×10^{4}	1.5×10^{3}	3.4×10^{1}	0
S. heidelberg	5.9×10^{9}	9.2×10^{8}	5.5×10^{7}	1.7×10^{5}	9.2×10^{3}	9.9×10^{1}	1.0	0
S. enteritidis	8.1×10^{9}	5.6×10^{7}	6.4×10^{5}	8.9×10^{2}	2.1×10^{1}	1.0	0	0
S. typhimurium	4.9×10^{9}	2.0×10^{8}	6.2×10^{7}	1.0×10^{5}	2.7×10^{1}	0	0	0
S. worthington	1.5×10^{10}	1.5×10^{9}	3.4×10^{7}	2.6×10^{6}	2.5×10^{3}	2.1×10^{2}	5.4	7.7
S. senftenberg	1.8×10^{10}	9.2×10^{8}	2.0×10^{8}	3.5×10^{6}	1.1×10^{4}	6.3×10^{2}	9.1×10^{1}	1.0×10^{1}

Average of Five Determinations

(Appendix Tables II, V, VIII, XI, XIV, XVII)

Fig. 6 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Whole Egg

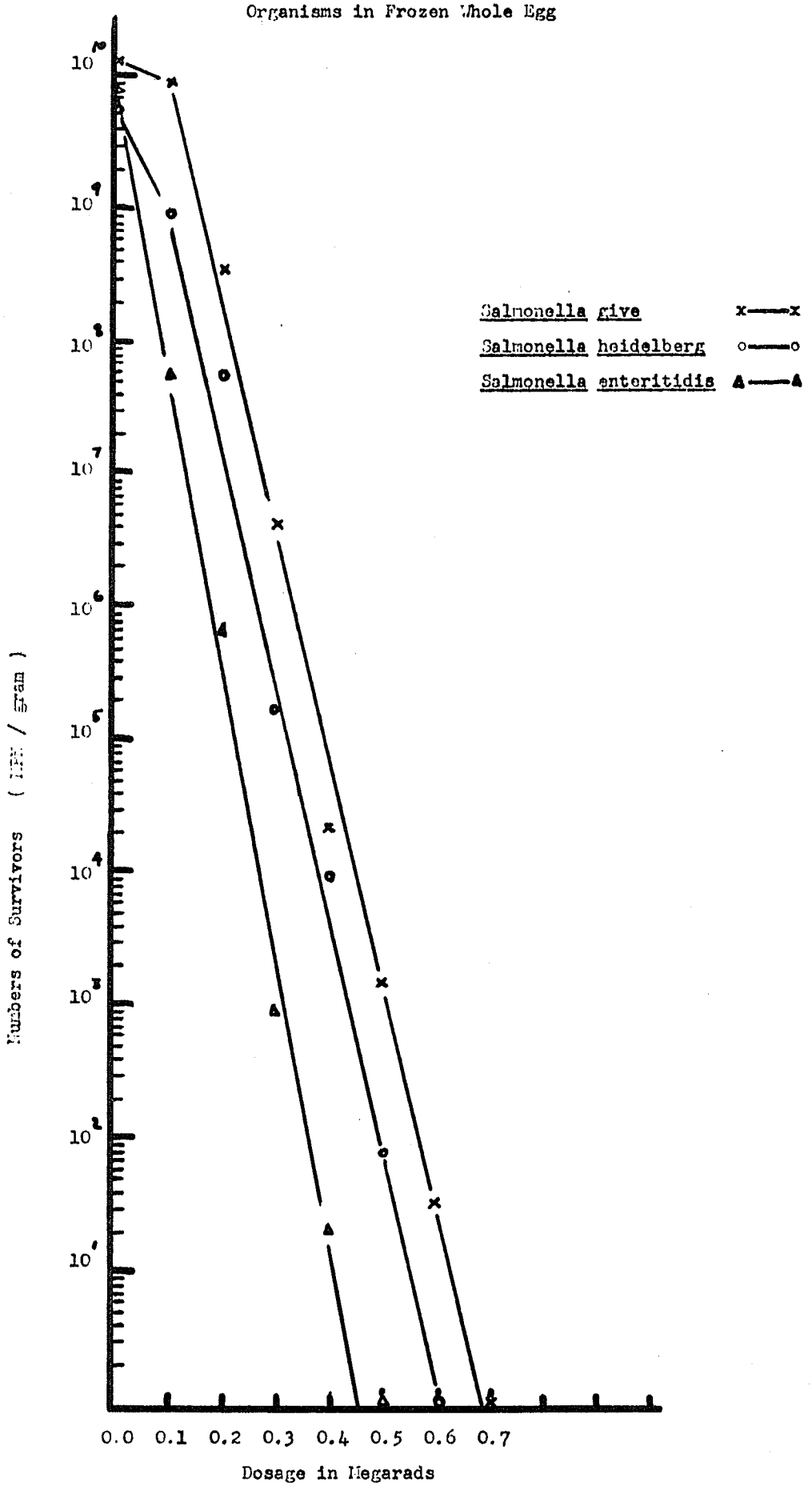


Fig. 7 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Whole Egg

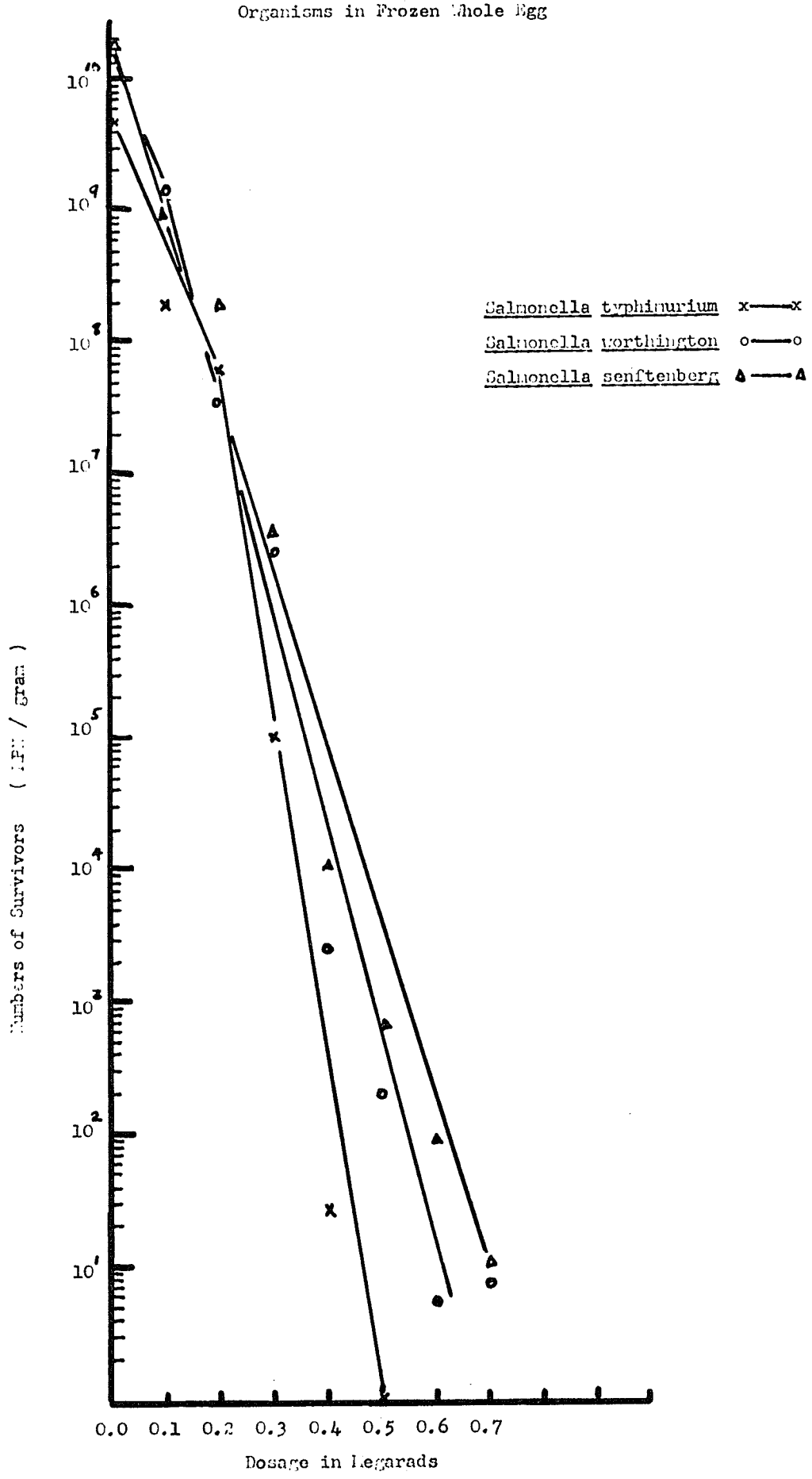


TABLE 12

GAMMA IRRADIATION DOSAGES REQUIRED TO OBTAIN $A10^8$ REDUCTION OF *Salmonellae* ORGANISMS PER GRAM OF FROZEN EGG YOLK, FROZEN EGG ALBUMEN AND FROZEN WHOLE EGG

Organism	Frozen Albumen	Frozen Yolk	Frozen Whole Egg
	MRADS		
S. give	0.580	0.560	0.560
S. heidelberg	0.495	0.600	0.510
S. enteritidis	0.330	0.360	0.370
S. typhimurium	0.430	0.570	0.430
S. worthington	0.490	0.550	0.540
S. senftenberg	0.570	0.610	0.610

Average of Five Trials

TABLE 13

MRADS OF GAMMA IRRADIATION REQUIRED TO ACHIEVE A 90% REDUCTION IN THE NUMBERS OF Salmonellae ORGANISMS IN FROZEN EGG PRODUCTS (D VALUES)

Organism	Frozen Albumen	Frozen Yolk	Frozen Whole Egg
	MRADS		
S. give	0.060	0.065	0.060
S. heidelberg	0.060	0.070	0.060
S. enteritidis	0.040	0.045	0.050
S. typhimurium	0.045	0.070	0.040
S. worthington	0.055	0.070	0.065
S. senftenberg	0.070	0.075	0.075

D Values Obtained From Survival Curves Presented in Figures 2 to 7

TABLE 14

MRADS OF GAMMA IRRADIATION REQUIRED TO ACHIEVE A 90% REDUCTION IN THE NUMBERS OF *Salmonellae* ORGANISMS IN FROZEN EGG PRODUCTS (D VALUES)

Organism	Frozen Albumen	Frozen Yolk	Frozen Whole Egg
	MRADS		
S. give	0.064	0.064	0.069
S. heidelberg	0.061	0.074	0.061
S. enteritidis	0.041	0.045	0.050
S. typhimurium	0.046	0.070	0.048
S. worthington	0.059	0.069	0.075
S. senftenberg	0.069	0.077	0.075

D Values calculated from data presented in Tables 8, 9, 10

Salmonella enteritidis which was slightly higher and Salmonella senftenberg and Salmonella give which were the same in both products (Table 12).

The D values as presented in Table 13 indicated a similar trend in the resistance of the organisms as that seen in the 10^8 reduction values. According to the D values Salmonella senftenberg was noticed to show the greatest resistance to gamma irradiation (0.075 Mrads). This was followed by Salmonella worthington at 0.065 Mrads, Salmonella give and Salmonella heidelberg at 0.060 Mrads, Salmonella enteritidis at 0.050 Mrads and Salmonella typhimurium at 0.040 Mrads, respectively. The resistance of each species in frozen whole egg as compared to its resistance in frozen egg yolk and frozen egg albumen is essentially the same as that observed in the latter two egg products.

In general, it appeared that Salmonellae was most resistant to irradiation in frozen egg yolk and least resistant in frozen egg albumen with frozen whole egg affording intermediate protection to the organism.

3. IRRADIATION OF SALMONELLAE IN FROZEN WHOLE EGG WITH THE PRESENCE OF A NATURAL FLORA

The results of irradiating Salmonella organisms in frozen whole egg in the presence of a natural flora are presented in Table 15. Gamma irradiation affected Salmonella organisms suspended in frozen whole egg quite differently

when a natural flora was present. Salmonella give appeared to be much more resistant as growth (58 cells/gram) was found after 0.7 Mrads of irradiation. No growth for Salmonella give was found at this level of irradiation in the sterile product (Tables 11 to 14). Salmonella heidelberg, on the other hand, displayed similar results as those observed in the sterile product (i.e. little or no growth at the 0.6 Mrad level). Salmonella enteritidis and Salmonella typhimurium were completely eliminated after exposure to a dosage of 0.5 Mrads. The same two organisms were also completely destroyed with 0.5 Mrads of irradiation in frozen sterile whole eggs. The results obtained when Salmonella worthington was irradiated with a natural flora present were much different than those obtained in sterile whole egg. A rapid initial decline was noted with a tailing effect persisting from the 0.4 to the 0.7 Mrad level. Approximately one cell per gram of product was obtained at each of these levels of irradiation (Table 15). Salmonella senftenberg appeared to be quite resistant in the presence of the natural flora, with some growth observed at the 0.7 Mrad level. This growth was also observed for Salmonella senftenberg when it was irradiated in sterile frozen whole egg.

Except for Salmonella enteritidis the survival curves of all species studied, when plotted on semi-log paper,

TABLE 15

THE EFFECT OF GAMMA IRRADIATION ON *Salmonellae* ORGANISMS IN FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT

NATURAL FLORA Cells/gram		Salmonella	IRRADIATION LEVEL (MRADS)							
Total Count	Gm (-)ve Count	Organism	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
			MPN Salmonella/gram							
1.6×10^8	1.8×10^6	S. *senftenberg*	5.6×10^9	1.2×10^9	1.0×10^8	2.9×10^6	4.9×10^4	3.2×10^3	6.6×10^2	1.6
1.4×10^8	8.2×10^7	S. *give*	2.7×10^{10}	2.1×10^9	3.9×10^8	2.9×10^7	4.5×10^5	1.5×10^3	3.2×10^2	5.8×10^1
2.2×10^8	1.8×10^8	s. *heidelberg*	2.3×10^{10}	2.1×10^9	2.2×10^8	3.7×10^5	5.1×10^3	6.8×10^1	0	0.7
3.0×10^8	2.6×10^8	s. *worthington*	3.8×10^9	8.3×10^7	4.1×10^5	1.0×10^3	0.45	0.37	0.37	1.7
1.1×10^9	1.3×10^9	s. *enteritidis*	6.0×10^9	2.7×10^7	1.6×10^5	3.2×10^3	7.1	0	0.6	0
5.8×10^8	5.0×10^8	s. *typhimurium*	6.9×10^8	5.6×10^7	7.2×10^5	2.0×10^3	1.4×10^1	0	0	0

Average of at least Five Trials

(See Appendix Tables XIX to XXIV

Fig. 8 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Whole Egg With a Natural Flora Present

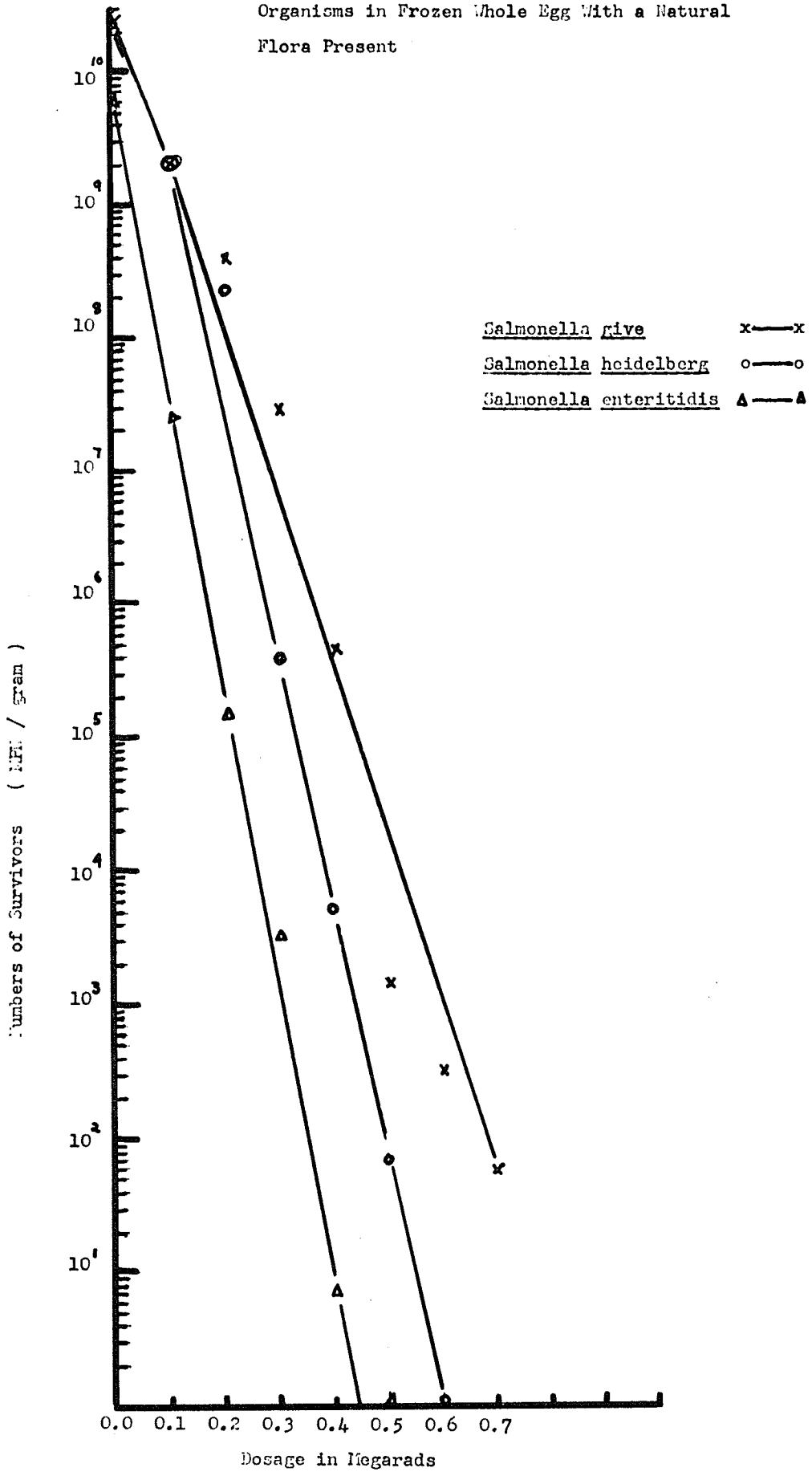


Fig. 9 The Effect of Gamma Irradiation on Salmonellae Organisms in Frozen Whole Egg With a Natural Flora Present

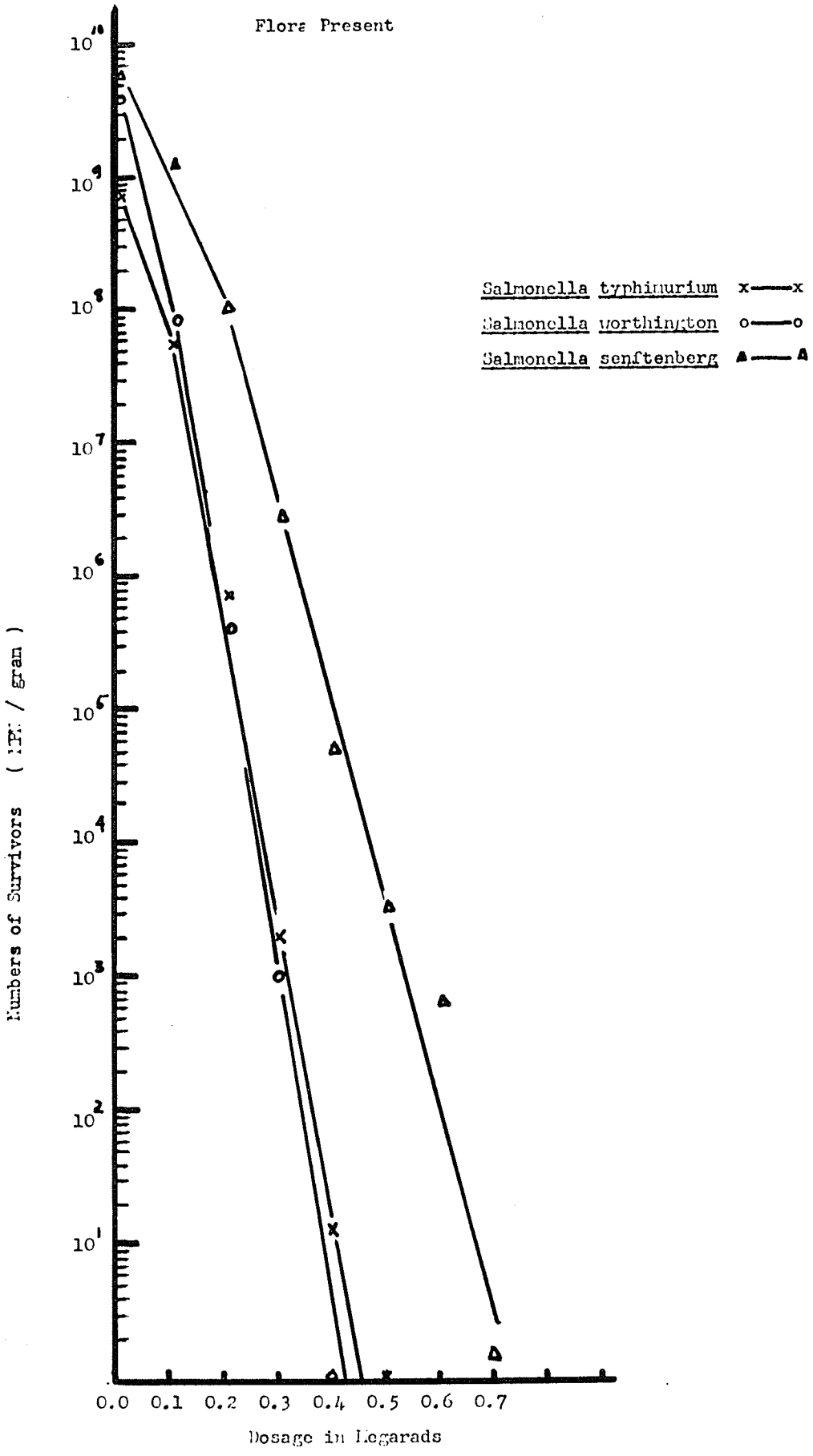


TABLE 16

A COMPARISON OF THE AMOUNT OF GAMMA IRRADIATION REQUIRED TO OBTAIN A 10^8 REDUCTION OF *Salmonellae* ORGANISMS PER GRAM OF STERILE FROZEN WHOLE EGG AND FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT

Organism	10^8 Reduction Values: Sterile Frozen Whole Egg		Frozen Whole Egg Plus Natural Flora	
S. give	0.560	MRAD	0.650	MRAD
S. heidelberg	0.510	MRAD	0.470	MRAD
S. enteritidis	0.370	MRAD	0.375	MRAD
S. typhimurium	0.430	MRAD	0.410	MRAD
S. worthington	0.540	MRAD	0.360	MRAD
S. senftenberg	0.610	MRAD	0.610	MRAD

Average of at Least Five Trials

TABLE 17

A COMPARISON OF THE AMOUNT OF GAMMA IRRADIATION REQUIRED TO OBTAIN A 90% REDUCTION IN THE NUMBERS OF *Salmonellae* ORGANISMS IN STERILE FROZEN WHOLE EGG AND FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT

(D Values)

Organism	MRADS	
	Sterile Frozen Whole Egg	Frozen Whole Egg Plus Natural Flora
S. give	0.060	0.080
S. heidelberg	0.060	0.055
S. enteritidis	0.050	0.045
S. typhimurium	0.040	0.045
S. worthington	0.065	0.040
S. senftenberg	0.075	0.065

D Values Obtained From Survival Curves Presented in Figures 2 to 9

TABLE 18

A COMPARISON OF THE AMOUNT OF GAMMA IRRADIATION REQUIRED TO OBTAIN A 90% REDUCTION IN THE NUMBERS OF *Salmonellae* ORGANISMS IN STERILE FROZEN WHOLE EGG AND FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT (D VALUES)

Organism	MRADS	
	Sterile Frozen Whole Egg	Frozen Whole Egg Plus Natural Flora
S. give	0.069	0.080
S. heidelberg	0.061	0.058
S. enteritidis	0.050	0.045
S. typhimurium	0.048	0.051
S. worthington	0.075	0.045
S. senftenberg	0.075	0.073

D Values Calculated From Data Presented in Tables 8, 9, 10 & 13

appeared as straight lines with slight initial lag phases (Figures 8 and 9). The survival curve of Salmonella enteritidis appeared as a straight line but did not display any initial lag phase.

According to the 10^8 reduction values obtained from the survival curves, Salmonella give displayed the greatest resistance. This was followed by Salmonella senftenberg, Salmonella heidelberg, Salmonella typhimurium, Salmonella enteritidis and Salmonella worthington (Table 16). This order of resistance was quite different than the order of resistance in the frozen sterile whole egg. Salmonella give and Salmonella enteritidis displayed more resistance when the natural flora was present, while Salmonella heidelberg, Salmonella typhimurium and Salmonella worthington were less resistant. Salmonella senftenberg had the same 10^8 reduction value in both products.

The order of resistance as determined by the D values in Table 17 was essentially the same as the trend observed in the 10^8 reduction values. Salmonella enteritidis and Salmonella typhimurium each had a D value equal to 0.045 Mrads while Salmonella give and Salmonella typhimurium were the only two species that showed an increase in D value. The remaining four species had lower D values when irradiated in the presence of a natural flora.

It was interesting to note that the natural flora was

TABLE 19

THE EFFECT OF GAMMA IRRADIATION

ON THE NATURAL FLORA IN FROZEN WHOLE EGG

Organism	MPN/gram at 0 MRAD	Cells/gram Natural Flora: Original Total Count	Count at 0.6 MRAD	Count at 0.7 MRAD
S. worthington	3.8×10^9	3.0×10^8	0	0
S. enteritidis	6.0×10^9	1.1×10^9	0	0
S. typhimurium	6.9×10^8	5.8×10^8	0	0

Average of at least Five Trials

(See Appendix Tables XXV, XVI, XVII)

completely eliminated by a dosage of 0.6 Mrads. Only three Salmonella species plus the natural flora were studied for this effect (Table 19).

Finally, it should be pointed out that the pertinent data presented in this study were statistically analysed, (appendix tables XXXI through XXXVIII). The results showed statistically significant differences between the sensitivity of the six test organisms to gamma irradiation, as well as, the rate of destruction of each test organism in the three different egg products.

DISCUSSION

Some work has already been done on the subject of gamma irradiation of egg products. Three investigations in particular involved work similar to that performed in this study; Comer et al in 1963, Ley et al in 1963 and Ingram et al in 1961.

As outlined in the scope of investigation section of this thesis an attempt was made to investigate some aspects of the effects of gamma irradiation on selected species of Salmonellae in frozen egg products. Specifically, a high initial level of Salmonella contamination (10^9-10^{10} cells/gram) in the product was achieved prior to irradiation and the effects of a large natural flora (10^6 to 10^8 cells/gram) on the resistance of the Salmonella organisms to gamma irradiation was examined. Throughout this investigation the initial level of Salmonellae inoculated into the products ranged between 10^8 and 10^9 cells per gram. An attempt was made in this study to achieve a level of 10^9 to 10^{10} cells per gram for each of the six species studied. It was felt that this higher initial level might have some effect on the numbers of Salmonellae surviving irradiation and ultimately on the radioresistance of each species studied. This higher level of inoculum was attained for every species

except Salmonella typhimurium which did not grow as well as the others. A level of 10^8 to 10^9 cells per gram was obtained with this organism. Unlike the other studies, the effect of gamma irradiation on Salmonella species in frozen egg and frozen egg albumen was studied in addition to frozen whole egg. Because of the higher initial level of contamination it was possible to obtain 10^8 reduction values instead of 10^7 reduction values.

In the investigations of Ley et al and Ingram et al some work was done with a very low level natural flora present. A portion of this study was concerned with the possible effect of a high level of natural flora on the 10^8 reduction values and D values of each Salmonella species in frozen whole egg.

The preliminary work performed in this investigation established the acceptability of the method used to obtain bacteria free egg samples, the validity and reproducibility of the MPN technique, the positive effect of lactose pre-enrichment on the recovery of Salmonella organisms and the effects of freezing on the total Salmonella population. It was established by the preliminary data that the method of mixing the Salmonella organisms into the egg product produced a uniform population in the product.

The temperature studies carried out on the egg products during irradiation showed that although the temperature

did rise, thawing did not occur.

It was observed that when Salmonellae were irradiated in frozen egg albumen Salmonella give was the most resistant species with a 10^8 reduction value of 0.580 Mrads and Salmonella senftenberg the second most resistant with a 10^8 reduction value of 0.570 Mrads. This order of resistance was reversed, however, when the D values were compared. Salmonella senftenberg had a D value of 0.070 Mrads while Salmonella give had one equal to 0.060 Mrads. This apparent reversal in resistance may be explained by the fact that Salmonella give displayed a much more prominent lag than did Salmonella senftenberg. The D value is in fact equal to the slope of the survival curve and therefore is greatly affected by any lag period.

The order of resistance as determined by the 10^8 reduction values for the remaining four species was Salmonella heidelberg (0.495 Mrads), Salmonella worthington (0.490 Mrads), Salmonella typhimurium (0.430 Mrads) and Salmonella enteritidis (0.330 Mrads). This order was identical when the D value for each species was compared. This was likely due to the fact that Salmonella heidelberg, Salmonella worthington and Salmonella typhimurium had only slight lags while none at all was displayed by Salmonella enteritidis.

The investigation of Comer et al established the

following order of resistance based on 10^7 reduction values; Salmonella give, Salmonella heidelberg, Salmonella typhimurium, Salmonella enteritidis and Salmonella senftenberg. Although these values were obtained in frozen whole egg it is interesting to note that Salmonella senftenberg was the least resistant of the species listed whereas it was the second most resistant species in frozen egg albumen in our studies. This would suggest that there are differences in resistance within serotypes as well as between species.

Ley et al and Ingram et al in their studies in frozen whole egg established Salmonella typhimurium as being more resistant than Salmonella senftenberg although Ley et al used D values and Ingram et al used 10^7 reduction values for comparison. Nickerson et al, using high voltage cathode rays found that greater dosages were required to achieve a 10^7 reduction of Salmonella typhimurium than Salmonella senftenberg in frozen egg albumen. Possibly, the fact that Ley et al and Nickerson et al used Salmonella senftenberg ATCC #775W as compared to the Salmonella senftenberg ATCC #8400 used in this study would explain why Salmonella senftenberg was found to be more resistant than Salmonella typhimurium. This would tend to support the concept of differing resistances among serotypes of the same species. Comer et al felt that sensitivity to ionizing radiation was not a fixed species characteristic. They believed that the

chief difference between species of Salmonella lay in the serological characteristics of the cells.

As mentioned earlier, a lag was noted in all survival curves except for the one representing Salmonella enteritidis. Other than for this lag, the survival curves represented a geometric death rate consistent with the concepts advanced by the target theory. When plotted on semi-log paper the survival curves appeared as straight lines.

The "target theory" as outlined by Cassarett in 1968 (11), states that the number of viable organisms decreases in a geometric progression when exposed to irradiation. If the surviving fraction is plotted on a log scale a straight line survival curve is obtained.

The order of resistance was changed considerably when the Salmonella organisms were irradiated in frozen egg yolk. The most resistant species was Salmonella senftenberg with a 10^8 reduction value of 0.610 Mrads, followed by Salmonella heidelberg at 0.600 Mrads. Salmonella typhimurium, Salmonella give and Salmonella worthington displayed similar 10^8 reduction values of 0.570, 0.560 and 0.550 Mrads respectively. As in frozen egg albumen Salmonella enteritidis exhibited the least resistance with a 10^8 reduction value of 0.360 Mrads. The order of resistance remained essentially the same when the D values for each organism were compared. The only exception was Salmonella give which had a lower D

value than Salmonella worthington. This was possibly due to the fact that while Salmonella give had a very marked lag, Salmonella worthington had none at all.

Except for Salmonella give a slight increase was noted in the resistance of each species to gamma irradiation. This was established by examining both the 10^8 reduction values and the D values (Tables 12 and 13). However, the D value of Salmonella give was greater in frozen egg yolk than in frozen egg albumen. These observations would suggest that frozen egg yolk offered considerably more protection from irradiation than did frozen egg albumen. This observation was also made by Nickerson et al in 1956 and Brogel et al in 1957. In both studies Salmonella senftenberg and Salmonella typhimurium were subjected to successive dosages of high voltage cathode rays. Nickerson et al studied the effects produced when these organisms were suspended in frozen egg albumen whereas Brogel et al investigated these organisms when they were suspended in frozen egg yolk. The results showed that a very marked increase in resistance was experienced by each species when suspended in frozen egg yolk.

An effect that was observed in frozen egg yolk was the "tailing" effect of Salmonella typhimurium between the 0.6 and 0.7 Mrad levels. This tailing effect was also reported by Dyer et al (13) in 1965, who investigated the

radiation survival of food pathogens in solid crab meat. Growth was found in three of the five trials at the 0.6 Mrad level and in one of the trials at the 0.7 Mrad level. The one trial that showed growth at the 0.7 Mrad level remained essentially constant from the 0.5 Mrad level onwards; with counts of 23, 43 and 23 /gram respectively. (Appendix XII). The reason for this "tailing" effect could possibly be that the yolk was capable of affording some protection to this species at these levels or that the inoculum was not properly mixed throughout the yolk before freezing. Possibly clumps of Salmonellae are more resistant to irradiation than are individual cells.

Salmonella give and Salmonella heidelberg were the only species which displayed a lag in their survival curves in frozen egg yolk. The same explanation as used to explain the lag in frozen egg albumen can be used to explain the lag in these two species in frozen egg yolk. Comer et al suggested that this lag was a result of a protective effect exerted by substances in the water soluble fraction of food or complex media. The remaining four species displayed no initial lag period and all survival curves appeared as straight lines when plotted on semi-log paper. Thus, it would appear that the concepts of the target theory also apply for Salmonella organisms irradiated in frozen egg yolk.

The resistance of Salmonella organisms was altered

again when they were suspended in frozen whole egg. Salmonella give and Salmonella senftenberg remained unchanged as compared to frozen egg yolk but differed slightly from frozen egg albumen. Salmonella heidelberg, Salmonella typhimurium and Salmonella worthington were less resistant in frozen whole egg than in frozen egg yolk, but except for Salmonella typhimurium which was the same, were more resistant than in frozen egg albumen. The order of resistance as determined by the 10^8 reduction values was therefore altered. The D values when examined gave a similar order of resistance with the differences being attributable to the initial lag observed in all species except Salmonella senftenberg and Salmonella enteritidis. For example, Salmonella give had a much higher 10^8 reduction value than did Salmonella heidelberg while both species displayed the same D value. The survival curves (Figure 6) show that Salmonella give had a very pronounced initial lag while Salmonella heidelberg displayed only a slight lag. A similar observation can be made with Salmonella enteritidis and Salmonella typhimurium. In this case Salmonella enteritidis displayed no lag while Salmonella typhimurium had a very noticeable one.

The D values obtained in this study differed considerably from those found by Ley et al in 1963. According to their investigation the D values for Salmonella typhimurium and Salmonella senftenberg were 0.068 and 0.047

Mrads respectively. The values found in this study were 0.075 Mrads for Salmonella senftenberg and 0.040 Mrads for Salmonella typhimurium. These descrepancies can possibly be explained by differences among serotypes and the varied effects of different batches of whole egg used in the two studies. Comer et al suggested that variation in the melange was possibly responsible for the apparent variation in radiation sensitivity among the species. On the basis of 10^7 reduction values obtained, Ingram et al found Salmonella typhimurium to be more resistant than Salmonella senftenberg in frozen whole egg. Comer et al established the following order of resistance in frozen whole egg by using 10^7 reduction values; Salmonella give, Salmonella heidelberg, Salmonella typhimurium, Salmonella enteritidis and Salmonella senftenberg.

It must be kept in mind that the recovery techniques used by these three research groups differed from the technique used in this study. Ley et al used serial dilutions and surface counts on oxoid nutrient agar; Ingram et al followed the same procedure using nutrient agar, while Comer et al used nutrient agar plus 1% peptone to obtain plate counts. In none of these studies was a lactose pre-enrichment used; a fact which may help explain the higher recovery rates obtained in this study. Also, the MPN recovery technique has been established by Ayres (1949) to

be clearly superior to the plate count technique for the recovery of Salmonella organisms; a belief that was upheld by Taylor et al in 1957.

The survival curves obtained for each species followed a straight line when plotted on semi-log paper. Salmonella give, Salmonella heidelberg, Salmonella typhimurium and Salmonella worthington displayed an initial lag while Salmonella senftenberg and Salmonella enteritidis did not. Comer et al, on the other hand, found a lag in all of these species. Ley et al did not show a lag in Salmonella typhimurium in frozen whole egg, but neither did the survival curve for this organism reach the y-axis.

It would appear that the results obtained by irradiating Salmonella organisms in frozen whole egg also can be explained on the basis of the target theory.

As mentioned earlier in the discussion, an attempt was made in this study to examine the possible effects of a high level of natural flora on the amount of irradiation required to achieve a 10^8 reduction in the numbers of Salmonella organisms present in frozen whole egg. It was postulated that if more cells were present there would be more targets and thus more irradiation required to achieve the same reduction in numbers.

Salmonella give and Salmonella enteritidis were the only species that displayed higher 10^8 reduction values when

a natural flora was present. Salmonella senftenberg remained unchanged while Salmonella heidelberg, Salmonella typhimurium and Salmonella worthington displayed lower 10^8 reduction values. Salmonella worthington in fact showed a very marked decrease; from 0.540 Mrads in the sterile product to 0.360 Mrads with a natural flora present.

The data presented in Table 15 shows that the natural flora consisted mainly of gram negative organisms. However, in the case of Salmonella give and Salmonella senftenberg larger numbers of gram positive organisms were present. Possibly, gram positive organisms display a greater resistance than do gram negative organisms to gamma irradiation, thus necessitating higher dosages for inactivation. Erdman et al (16) in 1961, showed that the gram positive organisms, Streptococcus faecalis and Staphlococcus aureus were more resistant to gamma irradiation than were the gram negative Salmonella pullown and Escherchici coli organisms. Desrosier and Rosenstock (12a) in 1960 stated that the species type was a most critical factor to be considered when a bacterial population was subjected to irradiation. They felt that, in general, vegetative cells required a substantially lower dosage than did spores to accomplish a given degree of kill. They also stated that cocci and non-spore forming rods were generally more radiation sensitive. Population reductions of 90 per cent with Escherichia coli were obtained with as

little as 20,000 rads. Ley et al also investigated a low level of natural flora present but no attempt was made to see if this had any effect on the amount of irradiation required.

Furthermore, it would appear that the natural egg flora used in this investigation did not contain any appreciable number of spore forming organisms, as spores have been shown to survive a dosage of 0.7 Mrads of irradiation. No attempt was made to determine the presence of spores in the natural flora. However, since the natural flora consisted of a culture which was grown in an enriched medium prior to irradiation, in order to obtain a large population, all organisms in the inoculum may be in the vegetative state including spore formers. This may explain the absence of any survivors after 0.7 Mrads of irradiation.

The decrease in 10^8 reduction values of three of the species studied is difficult to explain. Perhaps the gram negative organisms present, produced some antagonistic effect on the Salmonellae and thus enhanced the effect of gamma irradiation. There is the possibility that natural inhibitors present in the sterile whole egg were tied up by the natural flora in the lower quality eggs. It could also be that the natural flora utilized some nutritional factor that was used by the Salmonellae during recovery in the sterile whole egg.

An examination of the D values revealed a somewhat

different picture with regards to radio-resistance. According to these values only Salmonella give and Salmonella typhimurium appear to be more resistant when a natural flora is present. All other species showed a decrease in resistance. This, possibly, can be explained by the fact that all species, with the exception of Salmonella enteritidis, displayed an initial lag on their survival curves.

The survival curves, except for the initial lags, appeared as straight lines on semi-log paper. As this represents a geometric decrease in numbers it would appear that the target theory can be used to explain the results in this aspect of the work as well.

Irradiation levels of 0.6 and 0.7 Mrads completely eliminated the natural flora when Salmonella worthington, Salmonella enteritidis and Salmonella typhimurium were present (Table 19). Each of these Salmonella organisms were essentially eliminated at the 0.5 Mrad level; thus any colonies appearing on the plates at the 0.6 and 0.7 Mrad levels would have to be the natural flora. As none was observed, it would seem that a dosage of 0.5 to 0.6 Mrads will eliminate the natural flora as well as these particular Salmonellae, yielding an essentially sterile product.

For the purposes of this investigation, 10^8 reduction values were used as a measure of an organisms' resistance to irradiation. It was felt that this eight log reduction would possibly give a truer picture of radio-resistance than

the seven log reduction and thus produce more accurate D values. The D values were obtained not primarily to gauge resistance but to be used as a means of calculating the desired dosages required to produce a given inactivation. For example, if a product contained 1000 Salmonellae per gram and the desired level was on Salmonellae per 1000 grams, the reduction needed would be equivalent to six log cycles. To determine the dosage required, the D value for the Salmonella species present would be multiplied by a factor of six (6D). This reasoning was also followed by Ley et al in 1963.

SUMMARY

This study involving the irradiation of selected species of Salmonella in frozen egg products in the presence and absence of a natural flora indicated the following;

1. Of the six species of Salmonellae investigated in sterile frozen egg albumen, the 10^8 reduction values revealed Salmonella give to be the most radio-resistant followed by Salmonella senftenberg, Salmonella heidelberg, Salmonella worthington, Salmonella typhimurium and Salmonella enteritidis.
2. Of the six species of Salmonellae investigated in sterile frozen egg yolk, the 10^8 reduction values revealed Salmonella senftenberg to be the most radio-resistant followed by Salmonella heidelberg, Salmonella typhimurium, Salmonella give, Salmonella worthington and Salmonella enteritidis.
3. Of the six species of Salmonellae investigated in sterile frozen whole egg, the 10^8 reduction values revealed Salmonella senftenberg to be the most resistant followed by Salmonella give, Salmonella worthington, Salmonella heidelberg, Salmonella

typhimurium and Salmonella enteritidis.

4. Of the three egg products studied, it appeared that, the least protection was offered to the Salmonellae by frozen egg albumen and the most by frozen egg yolk with frozen whole egg offering intermediate protection.
5. The presence of a high level of natural flora in frozen whole egg altered the resistance of each Salmonella organism. Due to the presence of a natural flora in the frozen whole egg the most radio-resistant species appeared to be Salmonella give followed by Salmonella senftenberg, Salmonella heidelberg, Salmonella typhimurium, Salmonella enteritidis and Salmonella worthington.
6. The target theory of irradiation was upheld by the data obtained for each product and no selectivity was noted under irradiation.

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APPENDIX I

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella give* IN FROZEN EGG ALBUMEN

Trial \ MRADS	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^{9}	2.3×10^{10}	3.9×10^{8}	7.5×10^{6}	4.3×10^{5}	2.3×10^{2}	2.9×10^{1}	0
(2)	9.3×10^{9}	2.4×10^{10}	9.3×10^{8}	4.3×10^{6}	4.3×10^{4}	2.3×10^{2}	0	0
(3)	2.4×10^{10}	2.4×10^{9}	1.5×10^{7}	7.5×10^{6}	2.1×10^{3}	3.9×10^{1}	0	0
(4)	9.3×10^{9}	9.3×10^{8}	4.3×10^{7}	2.4×10^{5}	9.3×10^{1}	0	0	0
(5)	4.3×10^{9}	4.3×10^{8}	4.3×10^{7}	2.4×10^{4}	4.3×10^{1}	0	0	0
Average	1.02×10^{10}	1.02×10^{9}	2.8×10^{8}	3.9×10^{6}	9.5×10^{4}	1.0×10^{2}	5.8	0
Log of Av.	10.010	9.010	8.447	6.591	4.977	2.0	0.763	0

APPENDIX II

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella give* IN FROZEN WHOLE EGG

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^{9}	4.3×10^{9}	4.3×10^{8}	4.3×10^{6}	2.3×10^{4}	4.3×10^{3}	0	0
(2)	4.3×10^{9}	2.3×10^{9}	2.4×10^{8}	9.3×10^{6}	3.9×10^{4}	2.3×10^{3}	0	0
(3)	4.3×10^{10}	2.9×10^{10}	9.3×10^{8}	4.3×10^{6}	2.4×10^{4}	9.3×10^{2}	7.5×10^{1}	0
(4)	2.4×10^{9}	9.3×10^{9}	9.3×10^{7}	2.4×10^{6}	2.4×10^{4}	0	9.3×10^{1}	0
(5)	9.3×10^{9}	1.5×10^{9}	1.5×10^{7}	2.4×10^{4}	9.3×10^{1}	0	0	0
Average	1.3×10^{10}	9.2×10^{9}	3.4×10^{8}	4.1×10^{6}	2.2×10^{4}	1.5×10^{3}	3.4×10^{1}	0
Log of Av.	10.104	9.964	8.531	6.613	4.342	3.176	1.531	0

APPENDIX III

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella give* IN FROZEN EGG YOLK

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	7.5×10^{9}	7.5×10^{9}	4.3×10^{7}	2.3×10^{6}	2.1×10^{3}	0	0	0
(2)	2.4×10^{9}	2.1×10^{8}	1.5×10^{7}	4.3×10^{5}	4.3×10^{2}	0	0	0
(3)	1.5×10^{9}	4.3×10^{8}	4.3×10^{8}	7.5×10^{5}	4.3×10^{3}	3.6	9.1	0
(4)	2.4×10^{9}	1.5×10^{8}	2.4×10^{6}	2.4×10^{4}	2.4×10^{2}	0	0	0
(5)	9.3×10^{9}	3.9×10^{8}	2.4×10^{7}	2.4×10^{4}	4.3×10^{2}	3.0	0	0
Average	4.6×10^{9}	1.74×10^{9}	1.03×10^{8}	7.1×10^{5}	1.5×10^{3}	1.3	1.8	0
Log of Av.	9.662	9.240	8.013	5.851	3.176	0.114	0.260	0

APPENDIX IV

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella heidelberg* IN FROZEN EGG ALBUMEN

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	9.3×10^{9}	9.3×10^{8}	2.4×10^{8}	2.4×10^{4}	4.3×10^{2}	3.6	0	0
(2)	7.5×10^{9}	1.5×10^{9}	9.3×10^{7}	4.3×10^{5}	2.1×10^{3}	1.5×10^{1}	6.1	0
(3)	1.5×10^{10}	1.5×10^{9}	4.3×10^{6}	9.3×10^{3}	2.4×10^{3}	9.1	0	0
(4)	9.3×10^{9}	2.4×10^{9}	2.4×10^{8}	9.3×10^{3}	9.3×10^{2}	2.4×10^{2}	0	0
(5)	7.5×10^{9}	2.4×10^{8}	4.3×10^{6}	4.3×10^{3}	2.3×10^{1}	0	0	0
Average	9.7×10^{9}	1.3×10^{9}	1.2×10^{8}	9.5×10^{4}	1.2×10^{3}	5.4×10^{1}	1.3	0
Log of Av.	9.98	9.12	8.06	4.98	3.07	1.73	0.09	0

APPENDIX V

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella heidelberg* IN FROZEN WHOLE EGG

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	9.3×10^{9}	9.3×10^{8}	2.4×10^{8}	4.3×10^{5}	9.3×10^{3}	9.1	0	0
(2)	4.3×10^{9}	4.3×10^{8}	2.4×10^{7}	7.5×10^{4}	9.3×10^{2}	2.4×10^{2}	0	0
(3)	2.4×10^{9}	4.3×10^{8}	9.3×10^{5}	2.4×10^{5}	2.4×10^{3}	3.6	3.6	0
(4)	4.3×10^{9}	2.4×10^{9}	9.3×10^{6}	4.3×10^{4}	2.4×10^{4}	9.3×10^{1}	0	0
(5)	9.3×10^{9}	4.3×10^{8}	2.4×10^{6}	4.3×10^{4}	9.3×10^{3}	1.5×10^{2}	0	0
Average	5.9×10^{9}	9.2×10^{8}	5.5×10^{7}	1.7×10^{5}	9.2×10^{3}	9.9×10^{1}	1.0	0
Log of Av.	9.77	8.97	7.74	5.23	3.96	1.99	0	0

APPENDIX VI

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella heidelberg* IN FROZEN EGG YOLK

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	9.3×10^{9}	2.4×10^{9}	7.5×10^{8}	4.3×10^{5}	2.4×10^{3}	7.5×10^{1}	2.4×10^{2}	9.1
(2)	9.3×10^{9}	1.5×10^{9}	2.4×10^{8}	2.4×10^{6}	4.3×10^{3}	2.4×10^{2}	2.4×10^{2}	3.6
(3)	2.4×10^{10}	1.5×10^{9}	4.3×10^{6}	9.3×10^{4}	4.3×10^{2}	2.4×10^{2}	0	0
(4)	4.3×10^{9}	2.4×10^{9}	2.4×10^{7}	4.3×10^{4}	7.5×10^{2}	4.3×10^{2}	0	0
(5)	9.3×10^{9}	2.4×10^{9}	2.4×10^{8}	2.4×10^{6}	9.3×10^{3}	2.4×10^{2}	3.6	7.3
Average	1.1×10^{10}	5.0×10^{9}	2.5×10^{8}	1.1×10^{6}	3.4×10^{3}	2.5×10^{2}	9.7×10^{1}	4.0
Log of Av.	10.05	9.70	8.39	6.03	3.53	2.39	1.90	0.6

APPENDIX VII

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella enteritidis* IN FROZEN EGG ALBUMEN

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^9	2.9×10^6	4.3×10^4	2.3×10^1	0	0	0	0
(2)	1.5×10^9	9.3×10^6	9.3×10^3	4.3×10^2	3.6	0	0	0
(3)	2.4×10^9	9.3×10^6	9.3×10^3	2.3×10^1	0	0	0	0
(4)	4.3×10^9	2.4×10^6	4.3×10^4	9.3×10^1	0	0	0	0
(5)	9.3×10^9	4.3×10^6	4.3×10^5	2.3×10^1	0	0	0	0
Average	4.3×10^9	5.6×10^6	1.1×10^5	1.2×10^2	0.7	0	0	0
Log of Av.	9.63	6.75	5.04	2.08	0	0	0	0

APPENDIX VIII

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella enteritidis* IN FROZEN WHOLE EGG

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^{8}	4.3×10^{7}	9.3×10^{4}	4.3×10^{2}	9.1	3.6	0	0
(2)	3.9×10^{9}	4.3×10^{6}	3.9×10^{4}	7.5×10^{2}	4.3×10^{1}	0	0	0
(3)	2.4×10^{10}	9.3×10^{6}	2.4×10^{5}	4.3×10^{2}	7.3	0	0	0
(4)	4.3×10^{9}	2.4×10^{7}	2.4×10^{5}	2.4×10^{3}	4.3×10^{1}	0	0	0
(5)	7.5×10^{9}	7.5×10^{7}	4.3×10^{5}	4.3×10^{2}	3.6	0	0	0
Average	8.1×10^{9}	5.6×10^{7}	6.4×10^{5}	8.9×10^{2}	2.1×10^{1}	1.0	0	0
Log of Av.	9.91	7.75	5.81	2.95	1.32	0	0	0

APPENDIX IX

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella enteritidis* IN FROZEN EGG YOLK

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	7.5×10^{9}	4.3×10^{7}	9.3×10^{4}	4.3×10^{3}	3.6	0	0	0
(2)	2.1×10^{10}	9.3×10^{7}	9.3×10^{4}	4.3×10^{3}	7.5×10^{1}	0	0	0
(3)	9.3×10^{9}	2.4×10^{6}	9.3×10^{3}	1.5×10^{3}	0	0	0	0
(4)	9.3×10^{9}	3.9×10^{6}	9.3×10^{4}	9.3×10^{2}	0	0	0	0
(5)	1.5×10^{10}	4.3×10^{7}	4.3×10^{5}	2.4×10^{3}	9.1	0	0	0
Average	1.2×10^{10}	3.7×10^{7}	1.4×10^{5}	2.7×10^{3}	1.8×10^{1}	0	0	0
Log of Av.	10.08	7.57	5.15	3.43	1.24	0	0	0

APPENDIX X

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella typhimurium* IN FROZEN EGG ALBUMEN

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	9.3×10^{9}	4.3×10^{8}	4.3×10^{5}	9.3×10^{3}	3.6	0	0	0
(2)	9.3×10^{8}	9.3×10^{6}	2.4×10^{4}	2.3×10^{1}	0	0	0	0
(3)	9.3×10^{8}	4.3×10^{7}	2.4×10^{5}	4.3×10^{2}	9.1	0	0	0
(4)	4.3×10^{9}	4.3×10^{8}	2.4×10^{7}	4.6×10^{4}	2.3×10^{1}	0	0	0
(5)	2.4×10^{9}	2.4×10^{8}	9.3×10^{6}	9.3×10^{3}	3.6	0	0	0
Average	3.5×10^{9}	2.2×10^{8}	6.8×10^{6}	1.3×10^{4}	7.8	0	0	0
Log of Av.	9.55	8.35	6.83	4.11	0.89	0	0	0

APPENDIX XI

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella typhimurium* IN FROZEN WHOLE EGG

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	7.5×10^{9}	2.4×10^{8}	2.4×10^{8}	1.5×10^{5}	4.3×10^{1}	0	0	0
(2)	2.4×10^{8}	7.5×10^{7}	9.3×10^{6}	2.4×10^{5}	0	0	0	0
(3)	4.3×10^{8}	2.4×10^{7}	9.3×10^{6}	2.4×10^{3}	7.2	0	0	0
(4)	1.5×10^{9}	4.3×10^{8}	4.6×10^{7}	9.3×10^{4}	4.3×10^{1}	0	0	0
(5)	1.5×10^{10}	2.4×10^{8}	7.5×10^{6}	2.4×10^{4}	4.3×10^{1}	0	0	0
Average	4.9×10^{9}	2.0×10^{8}	6.2×10^{7}	1.0×10^{5}	2.7×10^{1}	0	0	0
Log of Av.	9.69	8.30	7.80	5.01	1.44	0	0	0

APPENDIX XII

THE EFFECT OF GAMMA IRRADIATION ON *Salmonella typhimurium* IN FROZEN EGG YOLK

Trial \ MRAD	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	2.4×10^{9}	4.3×10^{7}	2.4×10^{6}	2.4×10^{3}	2.3×10^{1}	7.5×10^{1}	0	0
(2)	4.3×10^{8}	1.2×10^{7}	1.5×10^{6}	1.5×10^{3}	2.4×10^{2}	2.3×10^{1}	4.3×10^{1}	2.3×10^{1}
(3)	3.9×10^{8}	4.3×10^{7}	2.4×10^{6}	4.3×10^{3}	3.9×10^{1}	3.0	3.6	0
(4)	2.4×10^{8}	7.5×10^{6}	4.3×10^{6}	9.3×10^{3}	2.3×10^{1}	3.6	0	0
(5)	9.3×10^{9}	4.3×10^{8}	4.3×10^{6}	2.4×10^{6}	9.3×10^{2}	4.3×10^{1}	3.6	0
Average	2.6×10^{9}	1.1×10^{8}	3.0×10^{6}	4.8×10^{5}	2.5×10^{2}	2.9×10^{1}	1×10^{1}	4.6
Log of Av.	9.41	8.03	6.47	5.68	2.40	1.47	1.0	0.66

APPENDIX XIII

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella worthington* IN FROZEN EGG ALBUMEN

Trial	Irradiation Level (MRAD) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^{9}	1.5×10^{9}	2.1×10^{8}	2.4×10^{6}	4.3×10^{2}	9.3×10^{1}	6.2	3.0
(2)	4.3×10^{10}	9.3×10^{8}	9.3×10^{7}	3.9×10^{5}	2.4×10^{3}	2.4×10^{2}	0	0
(3)	2.4×10^{10}	4.3×10^{8}	9.3×10^{7}	2.4×10^{5}	9.3×10^{2}	9.0	0	0
(4)	4.3×10^{9}	1.5×10^{9}	9.3×10^{7}	2.4×10^{6}	4.3×10^{3}	2.3×10^{1}	0	0
(5)	2.4×10^{10}	4.3×10^{8}	9.3×10^{7}	4.3×10^{5}	2.4×10^{2}	0	0	0
Average	2.0×10^{10}	9.6×10^{8}	1.2×10^{8}	1.2×10^{6}	1.7×10^{3}	7.3×10^{1}	1.2	0.6

APPENDIX XIV

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella worthington* IN FROZEN WHOLE EGG

Trial	Irradiation Level (MRAD) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	1.5×10^{10}	3.9×10^{8}	7.5×10^{6}	4.3×10^{5}	4.3×10^{2}	7.3	1.2×10^{1}	2.9×10^{1}
(2)	4.3×10^{9}	9.3×10^{8}	4.3×10^{7}	9.3×10^{5}	2.4×10^{2}	9.3×10^{1}	0	0
(3)	4.3×10^{9}	2.4×10^{9}	9.3×10^{6}	2.4×10^{4}	9.3×10^{3}	0	0	3.0
(4)	9.3×10^{9}	2.4×10^{9}	1.5×10^{7}	4.3×10^{6}	9.3×10^{2}	9.3×10^{2}	0	3.0
(5)	4.3×10^{10}	1.5×10^{9}	9.3×10^{7}	7.5×10^{6}	1.2×10^{3}	6.2	1.5×10^{1}	3.6
Average	1.5×10^{10}	1.5×10^{9}	3.4×10^{7}	2.6×10^{6}	2.5×10^{3}	2.1×10^{2}	5.4	7.7

APPENDIX XV

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella worthington* IN FROZEN EGG YOLK

Trial	Irradiation Level (MRAD) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	2.4×10^{10}	2.1×10^{9}	7.5×10^{7}	4.3×10^{5}	2.4×10^{1}	0	3.6	3.6
(2)	9.3×10^{9}	9.3×10^{8}	9.3×10^{6}	2.4×10^{5}	1.5×10^{1}	0	3.6	0
(3)	9.3×10^{9}	4.3×10^{8}	9.3×10^{6}	4.3×10^{4}	9.3×10^{1}	3.6	9.3×10^{1}	0
(4)	4.3×10^{9}	2.4×10^{8}	4.3×10^{6}	7.5×10^{5}	4.3×10^{2}	0	0	0
(5)	9.3×10^{9}	2.4×10^{8}	7.5×10^{6}	3.9×10^{4}	4.3×10^{3}	2.3×10^{1}	0	0
Average	1.1×10^{10}	7.8×10^{8}	2.1×10^{7}	3.0×10^{5}	9.7×10^{2}	5.3	2.0×10^{1}	0.7

APPENDIX XVI

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella senftenberg*

IN FROZEN EGG ALBUMEN

(Revised)

Trial	Irradiation Level (MRAD) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	9.3×10^{9}	7.5×10^{8}	4.6×10^{7}	1.1×10^{6}	3.9×10^{3}	2.3×10^{1}	9.3×10^{1}	0
(2)	9.3×10^{9}	2.4×10^{8}	1.1×10^{8}	2.1×10^{5}	4.3×10^{4}	9.3×10^{3}	3.9×10^{1}	0
(3)	9.3×10^{9}	4.3×10^{8}	4.3×10^{7}	9.3×10^{6}	9.3×10^{4}	4.3×10^{1}	0	0
(6)	2.1×10^{10}	4.3×10^{8}	7.5×10^{7}	1.5×10^{6}	4.3×10^{4}	9.3×10^{1}	9.1	0
(7)	2.4×10^{10}	9.3×10^{8}	9.3×10^{7}	2.4×10^{6}	2.4×10^{4}	2.3×10^{1}	0	0
Average	1.5×10^{10}	5.6×10^{8}	7.3×10^{7}	2.9×10^{6}	4.1×10^{4}	1.9×10^{3}	2.8×10^{1}	0

APPENDIX XVII

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella senftenberg* IN FROZEN WHOLE EGG

(Revised)

Trial	Irradiation Level (MRAD) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	4.3×10^{9}	1.5×10^{8}	4.3×10^{8}	4.3×10^{5}	2.4×10^{3}	4.3×10^{2}	L.A.	2.3×10^{1}
(2)	2.4×10^{10}	3.9×10^{8}	2.4×10^{8}	9.3×10^{6}	2.4×10^{4}	1.5×10^{3}	2.4×10^{2}	2.1×10^{1}
(3)	4.3×10^{10}	7.5×10^{8}	2.4×10^{8}	7.5×10^{6}	1.5×10^{4}	9.3×10^{2}	9.3×10^{1}	3.6
(9)	9.3×10^{9}	9.3×10^{8}	4.3×10^{7}	1.2×10^{5}	9.3×10^{3}	2.4×10^{2}	2.3×10^{1}	3.6
(10)	9.3×10^{9}	2.4×10^{9}	2.9×10^{7}	4.3×10^{4}	2.3×10^{3}	4.3×10^{1}	7.3	0
Average	1.8×10^{10}	9.2×10^{8}	2.0×10^{8}	3.5×10^{6}	1.1×10^{4}	6.3×10^{2}	9.1×10^{1}	1.02×10^{1}

APPENDIX XVIII

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella senftenberg* IN FROZEN EGG YOLK

(Revised)

Trial	Irradiation level (MRADS) MPN/gm							
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(2)	4.3×10^{10}	4.3×10^{8}	3.9×10^{7}	4.3×10^{5}	9.3×10^{3}	4.3×10^{2}	4.3×10^{1}	9.3×10^{1}
(3)	1.5×10^{9}	9.3×10^{8}	2.4×10^{8}	2.4×10^{6}	4.3×10^{3}	9.1	0	0
(4)	2.4×10^{9}	2.4×10^{8}	4.3×10^{7}	4.3×10^{5}	9.3×10^{5}	9.3×10^{2}	2.4×10^{2}	0
(6)	2.4×10^{10}	2.4×10^{9}	2.4×10^{8}	4.3×10^{6}	7.5×10^{3}	2.4×10^{2}	3.9×10^{1}	0
(7)	3.9×10^{10}	2.4×10^{8}	4.3×10^{7}	4.3×10^{6}	9.3×10^{3}	2.4×10^{2}	9.1	0
Average	2.2×10^{10}	8.5×10^{8}	1.2×10^{8}	2.4×10^{6}	1.9×10^{5}	3.6×10^{2}	6.6×10^{1}	1.9×10^{1}

APPENDIX XIX

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella senftenberg* IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

	NATURAL FLORA		IRRADIATION LEVEL (MRADS)							
	Cells/gm		MPN Salmonella/gm							
Trial	Total Count	gm (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	1.97×10^8	2.41×10^6	9.3×10^8	1.5×10^8	4.3×10^6	3.9×10^4	2.3×10^3	3.9×10^1	0	0
(2)	1.97×10^8	2.41×10^6	1.5×10^9	9.3×10^8	2.4×10^6	1.5×10^5	9.3×10^2	0	7.3	0
(3)	2.47×10^8	1.04×10^5	4.3×10^9	2.4×10^8	9.3×10^7	1.5×10^6	9.3×10^3	2.4×10^2	0	0
(4)	2.47×10^8	1.04×10^5	2.4×10^9	7.5×10^8	2.4×10^8	7.5×10^6	4.3×10^4	L.A.	0	0
(5)	2.47×10^8	1.04×10^5	2.4×10^9	9.3×10^8	4.3×10^7	4.3×10^6	2.4×10^5	1.5×10^2	3.9×10^1	1.1×10^1
(6)	6.1×10^6	3.9×10^6	2.4×10^{10}	4.3×10^9	1.5×10^8	4.3×10^6	2.4×10^4	9.3×10^3	2.4×10^2	0
(7)	6.1×10^6	3.9×10^6	4.3×10^9	9.3×10^8	2.4×10^8	2.4×10^6	2.4×10^4	9.3×10^3	4.3×10^3	0
Ave.	1.63×10^8	1.84×10^6	5.6×10^9	1.2×10^9	1.03×10^8	2.9×10^6	4.9×10^4	3.2×10^3	6.6×10^2	1.6

APPENDIX XX

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella* give IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

	Cells/gm		IRRADIATION LEVEL (MRAD) MPN Salmonella/gm							
Trial	Total Count	gm (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	5.5×10^7	3.9×10^6	4.3×10^9	2.4×10^9	2.4×10^8	4.3×10^6	4.6×10^5	2.4×10^3	3.6	4.3×10^1
(2)	5.5×10^7	3.9×10^6	9.3×10^{10}	9.3×10^8	2.4×10^8	3.9×10^6	1.5×10^5	4.3×10^2	2.4×10^2	3.6
(3)	5.5×10^7	3.9×10^6	2.4×10^9	1.5×10^9	9.3×10^7	9.3×10^7	4.6×10^5	4.3×10^3	9.3×10^2	2.4×10^2
(4)	2.65×10^8	1.98×10^8	9.3×10^9	4.3×10^9	4.3×10^8	2.1×10^7	9.3×10^5	1.5×10^2	3.0	3.6
(5)	2.65×10^8	1.98×10^8	2.4×10^{10}	1.5×10^9	9.3×10^8	2.4×10^7	2.4×10^5	2.4×10^2	4.3×10^2	0
Average	1.4×10^8	8.2×10^7	2.7×10^{10}	2.1×10^9	3.9×10^8	2.9×10^7	4.5×10^5	1.5×10^3	3.2×10^2	5.8×10^1

APPENDIX XXI

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella heidelberg*

IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

	NATURAL FLORA Cells/gram		IRRADIATION LEVEL (MRADS) MPN Salmonella/gram							
Trials	Total Count	Gram (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	2.4×10^{8}	1.4×10^{9}	2.1×10^{10}	2.4×10^{9}	4.3×10^{7}	2.3×10^{5}	1.6×10^{4}	3.9×10^{1}	0	3.6
(2)	2.4×10^{8}	1.4×10^{9}	6.4×10^{10}	9.3×10^{8}	9.3×10^{7}	1.5×10^{5}	9.1×10^{2}	1.1×10^{2}	0	0
(3)	2.1×10^{8}	2.1×10^{8}	9.3×10^{9}	2.4×10^{9}	2.4×10^{7}	4.3×10^{5}	9.1×10^{2}	7.5×10^{1}	0	0
(4)	2.1×10^{8}	2.1×10^{8}	9.3×10^{9}	3.9×10^{9}	9.3×10^{8}	9.3×10^{5}	7.5×10^{3}	7.5×10^{1}	0	0
(5)	2.1×10^{8}	2.1×10^{8}	9.3×10^{9}	9.3×10^{8}	2.1×10^{7}	9.1×10^{4}	3.6×10^{2}	4.3×10^{1}	0	0
Average	2.2×10^{8}	1.8×10^{8}	2.3×10^{10}	2.1×10^{9}	2.2×10^{8}	3.7×10^{5}	5.1×10^{3}	6.8×10^{1}	0	0.7

APPENDIX XXII

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella worthington*

IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

	NATURAL FLORA		IRRADIATION LEVEL (MRAD)							
	Cells/gram		MPN Salmonella/gram							
Trials	Total Count	Gram (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	1.4×10^{7}	5.7×10^{6}	2.4×10^{9}	9.3×10^{7}	4.3×10^{5}	4.3×10^{3}	3.6	3.0	3.0	3.0
(2)	1.4×10^{7}	5.7×10^{6}	1.5×10^{9}	9.3×10^{7}	4.3×10^{5}	4.3×10^{2}	0	0	0	0
(3)	2.2×10^{7}	9.5×10^{6}	1.5×10^{9}	2.4×10^{8}	2.4×10^{5}	3.9×10^{2}	0	0	0	0
(4)	2.2×10^{7}	9.5×10^{6}	9.3×10^{9}	4.3×10^{7}	2.4×10^{4}	9.3×10^{1}	0	0	0	0
(5)	2.2×10^{7}	9.5×10^{6}	2.4×10^{9}	4.3×10^{7}	1.5×10^{6}	1.5×10^{3}	0	0	0	0
(6)	7.7×10^{8}	6.7×10^{8}	2.1×10^{9}	1.5×10^{7}	2.4×10^{4}	3.9×10^{1}	0	0	0	3.6
(7)	7.7×10^{8}	6.7×10^{8}	9.3×10^{9}	4.3×10^{7}	3.9×10^{5}	1.5×10^{3}	0	0	0	3.6
(8)	7.7×10^{8}	6.7×10^{8}	1.5×10^{9}	9.3×10^{7}	2.4×10^{5}	9.3×10^{1}	0	0	0	3.6
Average	3.0×10^{8}	2.6×10^{8}	3.8×10^{9}	8.3×10^{7}	4.1×10^{5}	1.0×10^{3}	0.5	0.4	0.4	1.7

APPENDIX XXIII

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella enteritidis* IN FROZEN WHOLE EGG WITH NATURAL FLORA PRESENT

Trials	NATURAL FLORA Cells/gram		IRRADIATION LEVEL (MRADS) MPN Salmonella/gram							
	Total Count	Gram (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	1.2×10^{9}	1.1×10^{9}	4.3×10^{9}	4.3×10^{7}	2.4×10^{5}	9.3×10^{3}	2.3×10^{1}	0	0	0
(2)	1.2×10^{9}	1.1×10^{9}	1.5×10^{10}	3.9×10^{7}	4.3×10^{4}	2.1×10^{3}	3.6	0	0	0
(3)	1.2×10^{9}	1.1×10^{9}	4.3×10^{9}	9.3×10^{6}	9.3×10^{4}	2.4×10^{3}	0	0	0	0
(4)	9.8×10^{8}	1.5×10^{9}	4.3×10^{9}	4.3×10^{7}	4.3×10^{5}	2.4×10^{3}	9.1	0	0	0
(5)	9.8×10^{8}	1.5×10^{9}	2.4×10^{9}	2.4×10^{6}	1.5×10^{3}	3.9×10^{1}	0	0	3.0	0
Average	1.1×10^{9}	1.3×10^{9}	6.0×10^{9}	2.7×10^{7}	1.6×10^{5}	3.2×10^{3}	7.1	0	0.6	0

APPENDIX XXIV

REDUCTION IN NUMBER OF VIABLE CELLS OF *Salmonella typhimurium* IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

	NATURAL FLORA Cells/gram		IRRADIATION LEVEL (MRADS) MPN Salmonella/gram							
Trials	Total Count	Gram (-)ve Count	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(1)	5.8×10^8	4.3×10^8	4.3×10^8	3.9×10^7	9.3×10^5	9.3×10^3	2.3×10^1	0	0	0
(2)	5.8×10^8	4.3×10^8	2.4×10^8	4.3×10^7	9.3×10^5	2.3×10^1	3.6	0	0	0
(3)	5.8×10^8	4.3×10^8	1.9×10^9	2.4×10^8	2.4×10^6	2.4×10^3	4.3×10^1	0	0	0
(4)	5.9×10^8	5.8×10^8	2.4×10^8	4.3×10^6	4.3×10^4	3.9×10^1	9.1	0	0	0
(5)	5.9×10^8	5.8×10^8	9.3×10^8	9.3×10^6	4.3×10^4	2.3×10^1	0	0	0	0
(6)	5.9×10^8	5.8×10^8	4.3×10^8	9.3×10^5	4.3×10^3	6.4×10^1	6.2	L.A.	0	0
Average	5.8×10^8	5.0×10^8	6.9×10^8	5.6×10^7	7.2×10^5	2.0×10^3	1.4×10^1	0	0	0

APPENDIX XXV

THE EFFECT OF GAMMA IRRADIATION ON THE NATURAL FLORA OF FROZEN WHOLE EGG WHEN *Salmonella worthington* is PRESENT

	S. worthington MPN/gram	Natural Flora Colonies/gram		
Trials	Count at 0 MRAD	Count at 0 MRAD	Count at 0.6 MRAD	Count at 0.7 MRAD
(1)	2.4×10^9	1.4×10^7	0	0
(2)	1.5×10^9	1.4×10^7	0	0
(3)	1.5×10^9	2.2×10^7	0	0
(4)	9.3×10^9	2.2×10^7	0	0
(5)	2.4×10^9	2.2×10^7	0	0
(6)	2.1×10^9	7.7×10^8	0	0
(7)	9.3×10^9	7.7×10^8	0	0
(8)	1.5×10^9	7.7×10^8	0	0
Average	3.8×10^9	3.0×10^8	0	0

APPENDIX XXVI

THE EFFECT OF GAMMA IRRADIATION ON THE NATURAL FLORA OF FROZEN WHOLE EGG WHEN *Salmonella enteritidis* IS PRESENT

	S. enteritidis MPN/gram	Natural Flora Colonies/gram		
Trials	Count at 0 MRAD	Count at 0 MRAD	Count at 0.6 MRAD	Count at 0.7 MRAD
(1)	4.3×10^{9}	1.2×10^{9}	0	0
(2)	1.5×10^{10}	1.2×10^{9}	0	0
(3)	4.3×10^{9}	1.2×10^{9}	0	0
(4)	4.3×10^{9}	9.8×10^{8}	0	0
(5)	2.4×10^{9}	9.8×10^{8}	0	0
Average	6.0×10^{9}	1.1×10^{9}	0	0

APPENDIX XXVII

THE EFFECT OF GAMMA IRRADIATION ON THE NATURAL FLORA OF FROZEN WHOLE EGG WHEN *Salmonella typhimurium* IS PRESENT

	S. typhimurium MPN/gram	Natural Flora Colonies/gram		
Trials	Count at 0 MRAD	Count at 0 MRAD	Count at 0.6 MRAD	Count at 0.7 MRAD
(1)	4.3×10^8	5.8×10^8	0	0
(2)	2.4×10^8	5.8×10^8	0	0
(3)	1.9×10^9	5.8×10^8	0	0
(4)	2.4×10^8	5.9×10^8	0	0
(5)	9.3×10^8	5.9×10^8	0	0
(6)	4.3×10^8	5.9×10^8	0	0
Average	6.9×10^8	5.8×10^8	0	0

APPENDIX XXVIII

TEMPERATURE CHANGES IN FROZEN EGG ALBUMEN DURING IRRADIATION

Time (Minutes)	MRADS	Temperature (° C)	Time (Minutes)	MRADS	Temperature (° C)
0		-3.3	21		-1.7
1		-3.3	22		-1.4
2		-3.3	23		-1.4
3		-3.3	24		-1.4
4		-2.8	25		-1.4
5.1	0.1	-2.8	25.5	0.5	-1.4
6		-2.5	26		-1.3
7		-2.2	27		-1.3
8		-2.2	28		-1.3
9		-2.2	29		-1.3
10.2	0.2	-2.2	30		-1.3
11		-2.2	30.6	0.6	-1.3
12		-2.2	31		-1.1
13		-1.9	32		-1.1
14		-1.9	33		-1.1
15.3	0.3	-1.9	34		-1.1
16		-1.7	35		-1.1
17		-1.7	35.7	0.7	-1.1
18		-1.7			
19		-1.7			
20.4	0.4	-1.7			

APPENDIX XXIX

TEMPERATURE CHANGES IN FROZEN EGG YOLK DURING IRRADIATION

Time (Minutes)	MRADS	Temperature (°C)	Time (Minutes)	MRADS	Temperature (°C)
0		-6.1	21		-1.7
1		-5.5	22		-1.7
2		-5.3	23		-1.7
3		-5.0	24		-1.4
4		-4.4	25		-1.4
5.1	0.1	-4.2	25.5	0.5	-1.4
6		-3.9	26		-1.4
7		-3.3	27		-1.4
8		-3.3	28		-1.1
9		-3.1	29		-1.1
10.2	0.2	-2.8	30		-1.1
11		-2.5	30.6	0.6	-1.1
12		-2.5	31		-1.1
13		-2.2	32		-1.1
14		-2.2	33		-1.1
15.3	0.3	-2.2	34		-1.1
16		-1.9	35		-1.1
17		-1.9	35.7	0.7	-1.1
18		-1.9			
19		-1.9			
20.4	0.4	-1.7			

APPENDIX XXX

TEMPERATURE CHANGES IN FROZEN WHOLE EGG DURING IRRADIATION

Time (Minutes)	MRADS	Temperature (° C)	Time Minutes	MRADS	Temperature (° C)
0		-2.8	21		-1.7
1		-2.5	22		-1.4
2		-2.5	23		-1.4
3		-2.5	24		-1.4
4		-2.2	25		-1.4
5.1	0.1	-2.2	25.5	0.5	-1.4
6		-2.2	26		-1.4
7		-2.2	27		-1.4
8		-1.9	28		-1.4
9		-1.9	29		-1.1
10.2	0.2	-1.9	30		-1.1
11		-1.9	30.6	0.6	-1.1
12		-1.7	31		-1.1
13		-1.7	32		-1.1
14		-1.7	33		-1.1
15.3	0.3	-1.7	34		-1.1
16		-1.7	35		-1.1
17		-1.7	35.7	0.7	-1.1
18		-1.7			
19		-1.7			
20.4	0.4	-1.7			

APPENDIX XXXI

THE EFFECTS OF GAMMA IRRADIATION ON SALMONELLAE ORGANISMS IN FROZEN EGG ALBUMEN

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replication	4	6.59	1.65	
Organisms	5	91.51	18.30	51.06**
Levels	7	2934.70	419.24	1169.79**
Interaction	35	52.16	1.49	4.16
Exp. Errors	188	67.38	0.36	
TOTALS	239	3152.34		

F Values ** Highly Significant

* Significant

(Data from Appendix Tables I, IV, VII, X, XIII, XVI)

APPENDIX XXXII

THE EFFECTS OF GAMMA IRRADIATION ON SALMONELLAE ORGANISMS IN FROZEN WHOLE EGG

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replication	4	1.98	0.494	
Organisms	5	110.70	22.14	51.20**
Levels	7	3014.49	430.78	996.15**
Interaction	35	56.98	1.63	3.76**
Exp. Errors	188	81.30	0.432	
TOTALS	239	3266.45		

F Values ** Highly Significant

* Significant

(Data from Appendix Tables II, V, VIII, XI, XIV, XVII)

APPENDIX XXXIII

THE EFFECTS OF GAMMA IRRADIATION ON SALMONELLAE ORGANISMS IN FROZEN EGG YOLK

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replications	4	9.30	2.33	
Organisms	5	141.09	28.22	83.03**
Levels	7	3166.36	452.34	1330.96**
Interaction	35	95.173	2.72	8.00**
Exp. Errors	188	63.89	0.340	
TOTAL	239	3475.83		

F Values ** Highly Significant Differences

* Significant Differences

No Significant Differences

(Data from Appendix Tables III, VI, IX, XII, XV, XVIII)

APPENDIX XXXIV

THE EFFECT OF GAMMA IRRADIATION ON SALMONELLAE ORGANISMS IN FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT

ANOV

Source of Variation	Degrees of Freedoms	Sums of Squares	Mean Squares	F Values
Organisms	5	62.14	12.43	19.07**
Levels	7	546.88	78.13	121.09**
Interactions	35	22.58	0.65	
TOTAL	47	631.61		

F Values ** Highly Significant

* Significant

(Data from Appendix Tables XIX, XX, XXI, XXII, XXIII, XXIV)

APPENDIX XXXV

GAMMA IRRADIATION DOSAGES REQUIRED TO OBTAIN A 10^8 REDUCTION OF *SALMONELLAE* ORGANISMS PER GRAM OF FROZEN EGG YOLK, FROZEN EGG ALBUMEN AND FROZEN WHOLE EGG

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replications	2	0.01081	0.00540	4.15*
Treatments	5	0.11183	0.02237	17.236**
Exp. Errors	10	0.01298	0.0013	
TOTAL	17	0.13561		

F Value ** Highly Significant

* Significant

(Data from Table 12)

APPENDIX XXXVI

MRADS OF GAMMA IRRADIATION REQUIRED TO ACHIEVE A 90% REDUCTION IN THE NUMBERS OF SALMONELLAE ORGANISMS IN FROZEN EGG PRODUCTS (D VALUES)

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replications	2	0.00037	0.00018	0.415
Treatments	5	0.00147	0.00030	7.203**
Exp. Errors	10	0.00041	0.00004	
TOTAL	17	0.00227		

F Value ** Highly Significant

* Significant

(Data from Table 13)

APPENDIX XXXVII

GAMMA IRRADIATION DOSAGES REQUIRED TO OBTAIN A 10^8 REDUCTION OF SALMONELLAE ORGANISMS IN FROZEN WHOLE EGG WITH A NATURAL FLORA PRESENT

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replication	5	0.09584	0.01917	
Treatment	1	0.00175	0.00175	0.445
Exp. Errors	5	0.01951	0.00390	
TOTAL	11	0.11711		

F Values ** Highly Significant

* Significant

(Data from Table 16)

APPENDIX XXXVIII

MRADS OF GAMMA IRRADIATION REQUIRED TO ACHIEVE A 90% REDUCTION IN THE NUMBER OF SALMONELLAE ORGANISMS IN FROZEN WHOLE EGG WITH THE NATURAL FLORA PRESENT

ANOV

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Values
Replications	5	0.00132	0.00026	
Treatments	1	0.00003	0.00003	0.294
Exp. Errors	5	0.00057	0.00011	
TOTAL	11	0.00192		

F Value ** Highly Significant Differences

* Significant Differences

No Significant Differences

(Data from Table 18)