THE EFFECT OF IONIZING IRRADIATION ON *SALMONELLA* SPECIES IN EGG PRODUCTS OF LOW WATER ACTIVITY

by

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ABSTRACT

Salmonella give, Salmonella heidelberg, Salmonella worthington, Salmonella typhimurium, Salmonella enteritidis and Salmonella senftenberg 775 W were artificially introduced into powdered egg albumen, powdered egg yolk and powdered whole egg.

All known variables which could produce biased results were investigated in preliminary studies before the main research was started. The gamma irradiation dosage required to destroy these organisms in the powdered egg products was then determined.

It was observed that complete destruction of all Salmonella species in powdered whole egg, was obtained at a level of 0.62 Mrads. S. worthington proved to be the most irradiation resistant species when present in this product. A dosage of 0.62 Mrads was required to destroy it. S. senftenberg, the least resistant species, required a dosage of 0.40 Mrads to eliminate it.

In powdered egg yolk complete destruction was obtained at 0.66 Mrads. S. worthington was the most resistant organism requiring 0.66 Mrads for complete destruction, while 0.37 Mrads was required to destroy S. enteritidis, the least resistant species.

Powdered egg albumen provided the greatest degree of protection of the three powdered products considered. S. heidelberg, the most resistant species in powdered egg yolk, required a dosage of 0.56 Mrads to destroy it. Elimination of the least resistant species, S. typhimurium, required 0.40 Mrads.

There was a definite variation in sensitivity to gamma irradiation between products and between species studied. However a dosage of 0.7 Mrads would be suitable level for complete destruction of all species in the powdered egg products investigated.

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INTRODUCTION

At the present time, the thermal pasteurization methods used to destroy Salmonellae in egg products prior to drying for the production of powdered egg products, have proved to be only partially successful. Thermal pasteurization occurs before the drying process, making the powdered product highly susceptible to contamination, during and after drying and during packaging. As a result, in certain cases, powdered egg products were found to be contaminated with Salmonella organisms. It is conceivable that most of this may be due to post-pasteurization contamination. In some instances, it has been reportedthat the thermal pasteurization temperature, 142°F for 3½ minutes, was inadequate to destroy the Salmonellae when the product was grossly contaminated. The occurrence of improper pasteurization is rare however, since a considerable safety factor is included in the temperatures used.

The presence of Salmonellae in the finished product normally necessitates re-processing of the powdered eggs. However, this is a costly approach and usually results in a poor quality product. In most cases, the powder is destroyed, resulting in a considerable loss of revenue to the manufacturer.

These problems could be avoided to a large extent, if gamma irradiation could be used to destroy the micro-organisms. In addition, it would not be necessary to subject the powdered egg products to gamma irradiation until after drying and packaging had occurred, since this method of food preservation is not limited to the form of the product. Re-pasteurization may also be possible using this method without adversely affecting the physio-chemical characteristics of the product.

The present study was initiated to determine whether an acceptable level of ionizing irradiation could be used to destroy Salmonellae in egg products of low water activity.

REVIEW OF LITERATURE

A. Historical Summary

I. The Presence of Salmonellae in Food

According to reasonably accurate historical records, the first known Salmonella (91) outbreak occurred in the small village of Frankenhauser, Germany, in 1888 (32). At that time the organism that was to take its generic name from the late Dr. Salmon was identified as Bacillus enteritidis (Salmonella enteritidis). This organism produced severe gastroenteritidis symptoms in 57 people and led to the death of one person who had consumed a large amount of the infected meat.

A second incidence of a Salmonellosis outbreak occurred in a village in Belgium, in 1895 (22). The importance of this outbreak was that, although the infected sausage meat looked and smelled alright, it resulted in one death and produced illness in a number of other individuals. Once again Salmonella enteritidis was isolated as the causative agent. This second outbreak suggested two important conclusions. First, the severity of the symptoms was not proportional to the meat consumed. Second, a time lapse of 12 to 24 hours occurred before the onset of the first symptoms.

II. Irradiation as a Means of Pasteurizing and Sterilizing Foods.

The value that irradiation has in producing biological changes in food was discovered 70 years ago. In 1898, Pacionotti and Porcelli first observed the effect of irradiation on microbes (112). In 1904, Prescottin discovered that radium radiation produced profound effects on pathogenic organisms. (112)

A patent was issued in 1930 to O. Wust, a Frenchman, to use ionizing irradiation as a means of preservation. Also, Proctor, (112) Van de Graaf and Fram of the Massachusetts Institute of Technology carried out the first controlled experiments on foods under contract for the U. S. Quartermasters Corps in 1943. (112)

It was not until 1950, however, that the United States Atomic Energy Commission commenced support, on a limited scale, of research investigating the potentials of gamma emitting isotopes. After two years of preliminary research, the Quartermaster General requested, in May 1953, authorization to initiate a five-year research program. By 1956, this program was supporting 80 institutions both in the academic field and food industry. (111). This program continued to the end of the five-year contract and was evaluated and realigned in 1960. After 1960, a considerable expansion of facilities was

carried out. By this time, research was also well established in Canada and the United Kingdom.

Three dates from the next four years became extremely important: February 8, 1963, August, 1963, and June 30, 1964. It was on these dates that the first three foods preserved by ionizing irradiation, bacon, wheat and wheat products, and potatoes were cleared by the U. S. Food and Drug Administration for human consumption. Since this time, however, the Food and Drug Association has become increasingly more cautious about the release of irradiated foods for human consumption. This was evident in the recent decision to prevent the marketing of irradiated canned ham (52).

B. Incidence and Types of Salmonellae in Foods

I. Incidence

Last year, 19,723 Salmonella isolations were reported from human sources. This represented a 1.6% decrease from the 20,040 isolations report in the U.S. in 1966. A total of 8,794 isolations were also reported from non-human sources, an increase of 14.1% over 1966 (114). In the case of non-human isolations, the belief is that this increase can be attributed to increased surveillance of non-human vectors rather than an actual increase in isolations.

During 1967, Canada reported 2,673 isolations, a

slightly higher per-capita level than was encountered in the U. S. This represents an increase of 4.8% from the 2,551 isolates of 1966 (115). There was a 31% increase in non-human isolates.

In the United States the serotype frequency increased from 153 in 1966 to 155 in 1967, while in Canada the number of serotypes increased to 68 from a previous year's high of 58. These figures represented only a small percentage of the more than 1,200 known Salmonella serotypes.

Vehicles for 29 major Salmonella outbreaks in the United States included seven caused by contaminated eggs, five by contaminated turkey, two by beef, two by pork, one by raw milk, and one by potato salad. The food for four other large scale outbreaks was not identified. These 29 outbreaks represented a total of 5,761 isolates and 41 different serotypes (Table 1) (114). These outbreaks, revealed the importance of animal reservoirs in the transmission of salmonellosis.

The incidence of salmonellosis increased markedly between 1942 and 1963 in the United States. This was attributed directly to a number of factors including availability of laboratory facilities, better techniques of isolation and identification, and improved systems of reporting salmonellosis. From 1964 onward, the incidence of salmonellosis has been essentially constant (Figures 1 and 2) (114).

TABLE 1

Outbreaks Reported in the Salmonella Surveillance Reports

1967

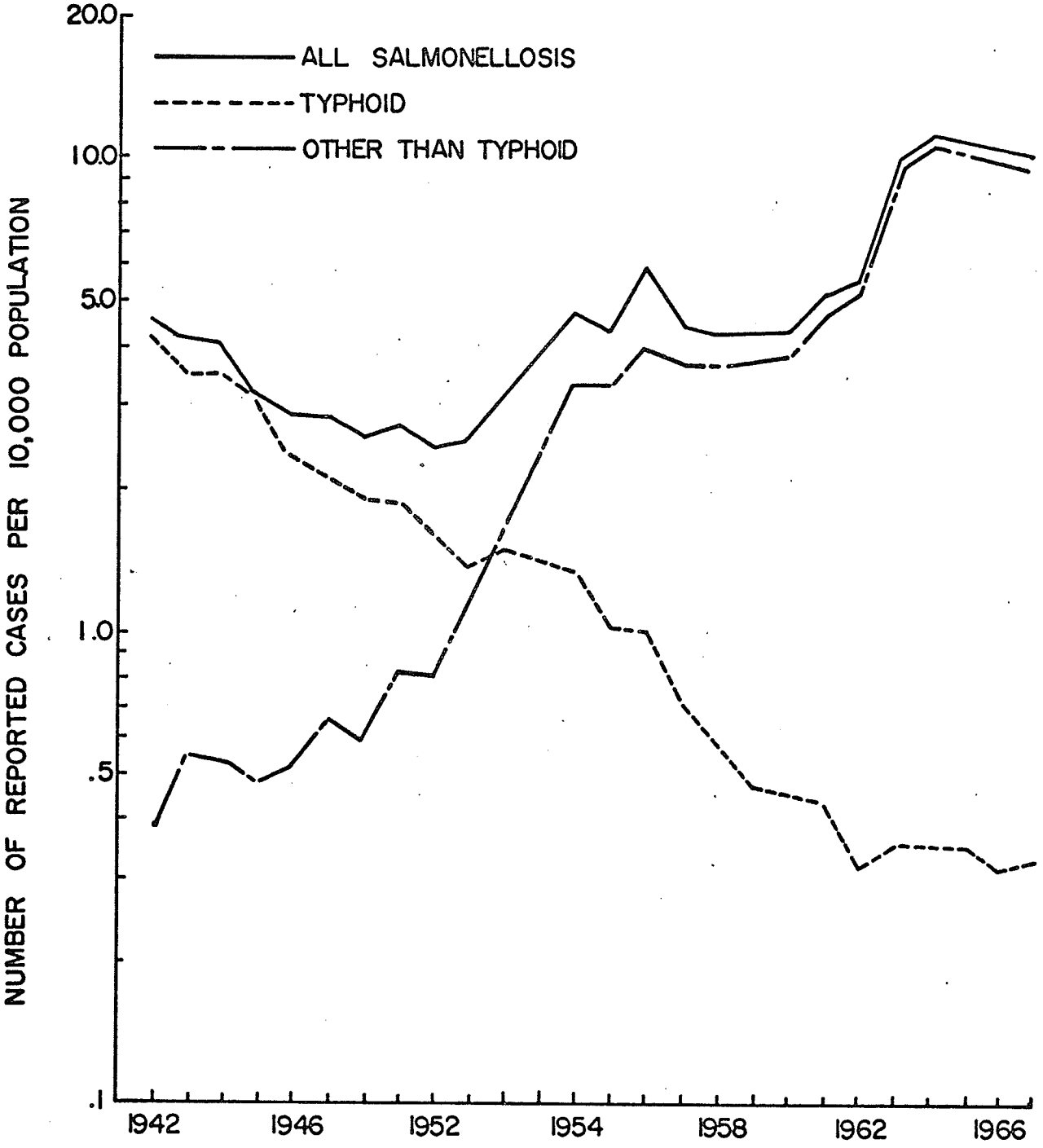
Vehicle of Infection	No. Persons Ill	Location	Serotype
Eggs	21	Hospital	S. infantis
	13	Hospital	S. typhi-murium
	1,800	Banquets	S. typhi-murium & S. braenderup
	250	School cafeteria	S. montevideo
	7	Home	S. enteritidis
	5	Home	S. pullorum
	5	Home	S. typhi-murium var. copenhagen
Turkey	185	Dormitory	S. manhattan & S. heidelberg
	1,900	Banquet	S. typhi-murium S. manhattan & S. newport
	172	Banquet	S. typhi-murium
	7	Home	S. heidelberg
	31	Nursing Home	S. enteritidis
Pork	90	Restaurant	S. chester
	10	Restaurant	S. typhi-murium
Beef Jerky	100	Product	S. thompson
Beef	300	School	S. thompson
Potato Salad	210	Banquet	S. typhi-murium var. copenhagen
Raw Milk	40	Home	S. typhi-murium
Food-borne, vehicle unidentified	41	Restaurant	S. typhi-murium
	14	Home	S. typhi
	42	Banquet	S. montevideo
	31	Dormitory	S. typhi
	12	Home	S. typhi
	319	Restaurant	S. newport
	3	Home	S. typhi
Contact-spread	104	Hospital	S. typhi-murium
	8	Hospital	S. Javiana
	9	Hospital	S. typhi-murium
	32	Hospital	S. heidelberg

TOTALS: Outbreaks - 29 Cases - 5,761 Serotypes - 14

FIGURE 1

REPORTED INCIDENCE OF HUMAN SALMONELLOSIS

UNITED STATES, 1942 - 1967

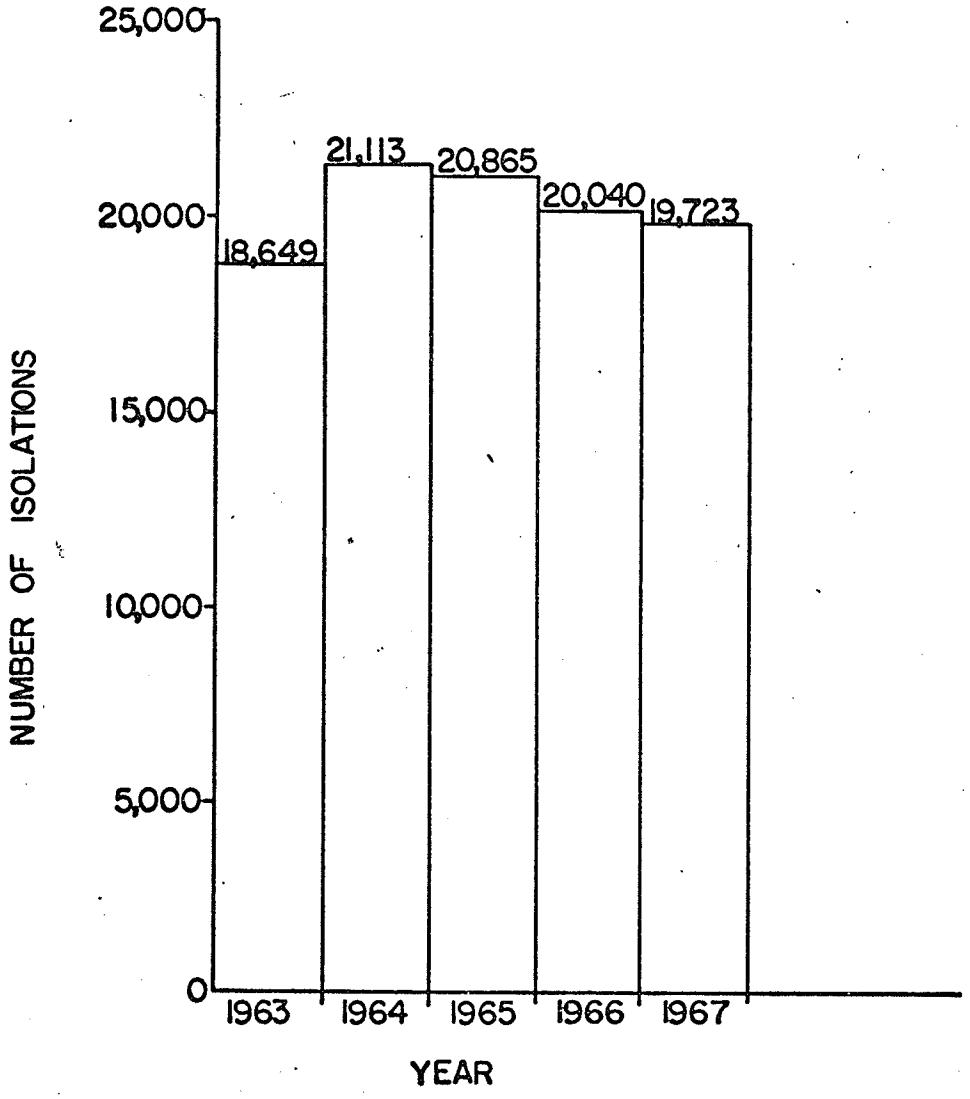


SOURCE: 1942 – 1962 MMWR, ANNUAL SUPPLEMENTS, 1951, 1954 AND 1964
1963 – 1967 SALMONELLA SURVEILLANCE REPORTS

FIGURE 2

REPORTED HUMAN ISOLATIONS OF SALMONELLA

UNITED STATES, 1963 - 1967


25,000
20,000
15,000
10,000
5,000
0
NUMBER OF ISOLATIONS
18,649
21,113
20,865
20,040
19,723
1963
1964
1965
1966
1967
YEAR

Incidence of deaths due to salmonellosis are difficult to assess, because many deaths that could be attributed to salmonellosis are compounded by the fact that the patients were also severely ill with other diseases. Of the 29 outbreaks representing 5,761 isolations, 13 deaths occurred. This represented a case-to-death ratio of 0.22%. Most of the deaths were among the very young, the elderly or the severely ill.

In Canada, deaths due to salmonellosis are not listed separately. In 1966 ten deaths were attributed to paratyphoid and other Salmonella infections, an incidence of 0.1 cases per 100,000 population (115).

II Types

In Canada, isolations of Salmonellae from human sources, during 1967 in the months of June, July and August, indicated that S. typhimurium and S. typhimurium var. copenhagen were the most commonly identified serotypes. These two serotypes represented 29.6% of all isolations. The second most common serotype was S. Newport with 16.2%. Other common serotypes were S. saint-paul (11.1%), S. infantis (6.7%) and S. enteritidis. The incidence of S. heidelberg decreased from 11.8% in 1966 to 4.7% in 1967. S. thompson also continued to decline from 5.8% to 4% in the respective years. These eight most frequent types accounted for 79.3% of all isolations (85).

In the case of non-human isolations, S. saint-paul replaced S. typhimurium as the most commonly reported isolate in 1967 with 15.3%. S. infantis accounted for 14.9% and S. typhimurium together with S. typhimurium var. copenhagen represented 14.6% of the isolations (85).

Again in 1967, twenty-three different serotypes were isolated for the first time in Canada. Ten of these (S. albony, S. albuquerque, S. bulawayo, S. coleypark, S. flint, S. haifa, S. haouaria, S. norwich, S. wassenaar and S. waycross) were isolated from human sources. Thirteen were isolated from non-human sources: S. chameleon, S. chicago, S. chingola, S. christiansborg S. dessau, S. good, S. matopeni, S. muenster, S. onderstepoort, S. saphra, S. stendal, S. westhampton and one not yet named (85).

The ten most common serotypes from human sources in the United States in 1967 were: S. typhimurium, S. heidelberg, S. enteritidis, S. newport, S. infantis, S. saint-paul, S. blockley, S. thompson, S. oranienberg, S. montevideo. These serotypes were responsible for 71% of all isolates reported. S. typhimurium and S. typhimurium var. copenhagen were encountered most often making up 29.4% of all isolations. S. heidelberg, the second most commonly encountered serotype, represented 8.4% of all isolations (114).

C. Commercial Significance of Salmonellae in Food

The presence of Salmonellae in food is of great importance, when consideration is given to food poisoning and food-borne infections throughout the world (17, 20). Considerable effort has been initiated to combat salmonellosis in some of the more advanced countries. In the remainder of the world, however, a far greater effort is required, since in many of these areas, outbreaks are becoming more common (20, 53).

I. Meat

Throughout many areas of the world infected meat has often been implicated in the epidemology of Salmonellae (5, 24, 42, 43, 64, 90). In Ireland, S. dublin was isolated in the feces of 24.5% of apparently healthy slaughter cattle (73). According to Lntje (61) adult slaughter cattle have also often been contaminated with S. typhimurium. A number of other studies (15, 21, 93) have also shown cattle to be contaminated with a variety of other Salmonella serotypes.

Other ruminants have also been implicated in the commercial transmission of Salmonellosis. Sheep have been especially singled out for criticism (42, 76).

In an examination of apparently normal hogs, Hormaeche and Salsmendi (49) identified a large number of different bacterial types including Salmonellae. As a result of this study, investigators have stressed

the importance of the stock yard, the holding lot and the abattoir in the spread of Salmonellae in hogs (29).

Perelli-Minetti et al (79) maintained that, if such high contamination levels were encountered in the animal before slaughter it is not surprising that Salmonella contamination often appears in foods prepared from meat. Salmonellae was recovered from 3% of 24,442 meat samples examined by Holtz (48). Other studies have shown a similarity high rate of contamination in both the United States and Europe (23, 31, 116).

Frozen meat has also been implicated in the spread of Salmonellae (47). It has been reported that 5 to 90% of all beef, veal, and mutton shipped from several countries was contaminated with Salmonellae (108). Frozen meats, intended for pet food were consistently contaminated with Salmonellae (28, 36, 46). The degree of contamination varied widely from 0 to 100% and was attributed to several factors including seasonal variations and the effect of the individual killing and packing establishments.

II. Poultry

Domestic fowl, it is believed, is the greatest reservoir of all Salmonella serotypes. Studies by Hinshaw et al, (44) have shown that 41.4% of turkeys slaughtered in 1948 were contaminated with Salmonellae.

Similarly, dressed poultry also has been implicated as a reservoir of Salmonellae (11, 30) Henshaw and McNeil (44) stated that "56 of the 60 Salmonella types and varieties isolated from birds have also been the cause of illness in man." U.S. Public Health Service reports showed that during the first nine months of 1967, 20 outbreaks of salmonellosis were reported. Of this number nine were traced directly to turkeys, (65). Galton et al (30) in his study of 129 chickens and turkeys found positive Salmonellae cultures in all of the 872 samples that he took.

III. Eggs and Egg Products

Egg and egg products have been implicated in a number of studies as a vehicle for the spread of Salmonellosis (67, 68, 73, 117). Two possible methods of contamination of eggs have been suggested; Ovarian infection or penetration of the egg shell by the organisms. Stokes et al (100) in turn observed that if penetration of the shell did occur, the temperature had to be in excess of 10°C. In support of this statement Rizk et al (86) in studies with inoculated hen eggs concluded that the higher the temperature, the higher the number of Salmonellae on the outer surface of the shell, and consequently the greater the number of Salmonellae penetrating the shell. On the other hand

at 2°C there was a decrease of Salmonellae on the surface of the shell and, therefore, fewer Salmonellae penetrating it.

In the case of processed egg products it has been reported (97) that Salmonellae gain entrance to the products by way of contaminated egg shells. Therefore, according to Solowey et al (97) only carefully cleaned eggs should be used in processing plants. Furthermore Edwards (20) suggested that as long as cracked and soiled eggs are used in the manufacture of dried and frozen egg products a higher incidence of Salmonellae can be expected to be present in these products.

Two outbreaks that were directly traced to eggs and egg products occurred in 1966 (75). These outbreaks involved the use of imitation cream to fill cakes in bakeries. It was discovered that the cream had been prepared from frozen egg white imported from China which was grossly contaminated with Salmonellae paratyphi. Further investigation showed that the hands of the baking staff were also contaminated.

D. The Importance of Powdered Egg Product in the Transmission of Salmonellosis

The presence of Salmonellae in powdered egg products has been reported by several investigators (96, 107, 109). It has also been observed that in most cases

unless gross contamination had occurred before drying the numbers of Salmonellae encountered are not usually large (34). Normally, it has been found that the danger of salmonellosis arises only after reconstitution of the powdered product, due to the multiplication of the small numbers of Salmonellae present if the product was contaminated. One outstanding example of this involved a four and a half year old child who became severely ill after consuming one teaspoonful of reconstituted egg yolk powder. It was found that the product was extensively contaminated with S. montevideo. The presence of such an organism is surprising since this product was required to pass what were thought to be reliable control tests in the bacteriological control laboratory of the Company. Spray-drying of such liquid egg cannot, then, be relied on to effectively eliminate the organism (4).

In England, 9.9% of over 7000 samples of spray-dried egg powder imported from the United States were contaminated with Salmonella organisms (91). Subsequent use of such powder by confectioners and bakers resulted in a number of Salmonella outbreaks. This was attributed to an absence of high-enough thermal temperatures during processing. Also strains isolated from such outbreaks were new to Great Britain (91). The United States Army conducted studies on 32 lots of powdered whole egg, (92),

of these, 3.18% were positive for Salmonellae. Five serotypes were isolated: S. oranienberg, S. tennessee, S. oregon, S. montevideo and S. pullorum. Excluding S. pullorum, of 901 samples of high quality powder, 0.67% were positive for Salmonellae. Also, of 797 lots processed with the aid of both a preheater and multi-stage units, Salmonella organisms were isolated from 13 or 1.63% of the samples. Without the preheater, of 104 lots there were 18.27% positive isolations. A number of other reports of salmonellosis outbreaks related to powdered egg products have also been noted (109).

I. The Occurrence of Salmonellae in Powdered Egg Products

Many workers have isolated numerous Salmonella serotypes from powdered egg products (3, 7, 45). Schneider (92) isolated Salmonellae in over 3% of 901 egg powder samples, while in another study, 28% of 400 samples of powdered whole egg were observed to be contaminated with Salmonellae (33). A third study found 5% of 507 samples of powdered whole egg positive for Salmonellae (66). Gibbons and Moore (34) isolated nine different serotypes from powdered whole egg. These included S. bareilly, S. pullorum, S. oranienburg,

S. typhimurium, S. thompson, S. minnesota, S. newport, S. manhatten, and S. potsdam. The number of Salmonella organisms found, however, was small, the highest recorded count being 54 organisms per gram of product.

II. The Effect of the Drying Process and Storage of Egg Powder on the Bacterial Population Present

Studies using whole egg have shown that it was possible to reduce the direct microscopic counts in both experimental and commercial liquid egg batters by 63.7 and 88.1% respectively as a result of drying (6). Microscopic counts in excess of 10 million organisms per gram were not encountered unless the liquid egg was extensively abused prior to dehydration (6). Also, if the powder was produced from highly contaminated eggs, rancidity develops sooner in powdered egg yolks and powdered whole egg due to the presence of the large number of organisms. This will result in a low quality product (18). During processing it has been demonstrated that pH has a definite effect on the Salmonella organism's resistance to pasteurizing temperatures (13). The lower the pH, the less resistance are the organisms to the pasteurizing temperature before drying is carried out.

It has been shown that storage temperature variations do not produce an increase in bacterial populations, in

dehydrated food (37, 40, 62). Moreover, as storage time and temperature are increased, a marked decrease in the bacterial count occurs. De Bord (18) supports this finding. In addition he found that the higher the contamination in the eggs prior to drying, the higher the count in the dried product. But the numbers in the dried product did not increase because there was not enough available water in the dehydrated product, to support bacterial growth. Gibbons and Fulton (35) arrived at a similar conclusion.

Bunwart and Ayres (13) achieved considerable success in destroying S. oranienburg, S. senftenberg and S. pullorum, by holding powdered egg albumen at elevated temperatures of 50, 60 or 70°C. Furthermore, there was no impairment of functional properties of the products held at these temperatures.

Other investigations, (18, 27, 35, 101) have shown that the kinds of and numbers of bacteria found in dried egg powder are largely determined by the following factors: (1) the number and the kind of the bacteria found in the liquid whole egg, (2) the temperature to which the bacteria are subjected, while being processed and then stored, (3) lysozyme activity, (4) whether or not the eggs are pasteurized before they are broken out, (5) eveness and method of drying and finally (6) the amount of moisture in the finished product.

E. The Effect of Irradiation on Salmonellae in Foods

I. The General Effects of Irradiation on Bacteria

Early investigators using irradiation as a means to destroy bacteria often employed X-rays as the irradiation source (39). The methods of application employed were crude by today's standards. The percent survival of bacteria in the samples was determined by microcolony growth. The survival percentage was normally determined by means of an exponential function of the dosage employed.

Fran et al (25) used X-rays in his studies with E. coli, A. aerogenes, S. aureus, S. morcesans, P. aeruginosa, P. fluorescens. They found that the rate of destruction followed a first-order reaction and that S. aureus was found to be most radiation resistant among the species examined. They concluded that the percentage of organisms killed was the same regardless of initial concentration.

During the 1950's many of the irradiation investigations were carried out using cathode rays (10, 63, 82). Proctor et al (82), in three experiments with spices, found that 1.33×10^6 reps was sufficient to destroy all or most of the micro-organisms present in the spices. Similar success was attained when eggs were sterilized using 500,000 reps transmitted by cathode rays (63). The eggs were previously inoculated with

10,000 cells of E. coli, P. fluorescens, and S. aureus. However, one problem was a reduction of egg quality. Grade A eggs were immediately reduced to Grade B or Grade C by sterilization with cathode rays.

Brogle et al (10) using cathode rays found that there was little difference in the resistance of Salmonellae to irradiation. He reported that the atmospheres of air, oxygen and nitrogen had no effect on the organism's resistance.

In recent investigations, the destruction of micro-organisms by ionizing irradiation has been achieved by employing gamma rays as the source of radiation (80, 113). Considerable success has been achieved using gamma rays in extending the shelf life of fish products (78, 80, 113). Ronsivalli and Slaom (87) observed that properly stored fishery products could be held for at least one month after gamma irradiation treatment and still be acceptable for consumption. In the same study it was found that 0.25 Mrads of irradiation was capable of destroying 99% of the microflora in haddock fillets.

In other areas, it has been observed that low dose cobalt 60 irradiation of tray-packed, cut-up fryer chickens, suppressed surface bacterial growth during refrigerated storage (69). Other studies have shown that it is possible to use ionizing irradiation as a means of bacterial destruction in many types of foods (8, 14, 41, 59).

II. The Effect of Irradiation on Salmonellae in Egg and Egg Products

Mossel (71), in studies with S. typhimurium and S. senftenberg recorded a six-decimal reduction of both organisms when subjected to 2×10^5 rads of ionizing irradiation and a nine-decimal reduction at slightly more than 5×10^5 rads. Further, he observed that the odour and functional properties of the whole eggs were impaired at dose levels above 10^4 rads. Brogle et al (10), using liquid egg albumen inoculated with S. typhimurium and S. senftenberg, obtained a 10^7 reduction in the number of each organism with dosages of 0.3 Mrads and 0.23 Mrads respectively. In another study, Proctor et al (81) found that the levels of irradiation required to destroy different types of Salmonellae in whole egg melange varied considerably with the particular serotype. Off flavours were also noted when scrambled eggs were prepared from this liquid egg melange but disappeared to a large extent when the egg melange was dried prior to cooking.

Comer et al (16) conducted extensive studies with 18 serotypes of Salmonellae in frozen whole egg. A wide range of irradiation levels were required to produce a 10^7 fold reduction in the different serotypes. An irradiation level of 0.35 Mrads was required to reduce S. senftenberg by a 10^7. While the most irradiation

resistant organism, S. give, required a dosage of 0.54 Mrads plus or minus 0.02 Mrads. Variation in sensitivity to irradiation was also detected in 3 culturs of S. pullorum which were identical with the exception of their origin. It was concluded that this variation in sensitivity within all species of Salmonellae makes it impossible to state that any one species is most resistant.

It has been observed (9) that dried egg albumen produced greater protection for Salmonellae than either liquid or frozen egg albumen. Studies (10) using S. typhimurium and S. senftenberg inoculated into dried egg albumen indicated that 700,000 rep and 1,000,000 rep, respectively, were required to produce a 10^7 fold reduction as opposed to 300,000 and 230,000 respectively with liquid egg albumen and 260,000 and 150,000, respectively with frozen egg albumen. As previously mentioned Proctor et al (81) had considerable success in eliminating Salmonellae by irradiating whole egg melange prior to drying. Brogle et al (10), also working with S. typhimurium and S. senftenberg, found that a 10^7 fold reduction could be achieved in egg yolk solids by subjecting the samples to 650,000 rep and in whole egg solid at 450,000 rep. No attempt was made to determine the difference in resistance of the two organisms.

F. Media Used to Isolate and Identify Salmonellae

I. Pre-enrichment Media

A great variety of pre-enrichment media have been used by various investigators to isolate Salmonellae from food products. The importance of pre-enrichment in isolating Salmonellae from large numbers of gram negative rods in dried foods has been reported by Taylor and Silliker (103), and Taylor et al (105). Jameson (51) working with dried eggs observed that considerably higher counts could be obtained when nutrient broth was used as a pre-enrichment. Byrne et al (12) reported that higher levels of Salmonellae could be obtained from inactivated dry yeast if the yeast was first incubated for 24 hours at 30°C prior to transfer into a selective enrichment media.

II. Enrichment Media

Numerous studies have been conducted to determine the most effective enrichment media to recover Salmonellae from egg products. Silliker et al (95) found that selenite containing 10% sterile feces produced a better recovery of small number of dormant Salmonellae from egg products, than if the eggs were first pre-enriched in lactose broth and inoculated into selenite broth. Whereas, Taylor et al (103) in their investigations of

Salmonellae in dried egg albumen recovered a higher number of organisms when the enrichment media was selenite cystine rather than selenite brilliant green sulfapyridine. Byrne et al (12) supported these findings, and reported that selenite F containing cystine was superior to both selenite F broth and also tetrathionate broth.

III. Selective Plating Media

Many different types of selective plating media have been used in a number of studies to identify Salmonellae. The most commonly used types included the following: brilliant green agar (56), salmonella shigella agar (58) bismuth sulfite agar (118, 119) and brilliant green sulfa agar (31).

G. The Effect of Lyophilization on Bacteria

In early studies with lyophilized bacterial cultures, it was observed that the percentage of organisms surviving, varied widely with the strain used (84). A well defined relationship was also observed between the concentration of the organisms in the suspension before drying, and the percentage of survivors recovered after reconstituting the dried product. In general, the more dilute the suspension, the lower the percentage of organisms surviving. The higher

survival rate was attributed to the amount of material present in the medium thus providing the organism considerable protection. Several other studies have also examined extensively the survival rates of many bacterial genera by lyophilization (83, 98). Fry and Greaves (26) conducted a detailed examination of lyophilization using paracolon bacillus "D 2014". They observed that suspending fluids of nutrient broth or nutrient gelatin gave a moderate survival rate. Various other protein solutions, although they produced good survival immediately after drying exhibited a large reduction in survival as the storage time was lengthened. The addition of glucose and lactose in concentrations of 5 to 10% produced a greatly increased survival rate both, immediately after drying and after storage. Also very young cultures were far more sensitive to drying than older cultures. However the bacteria surviving the initial drying were not more resistant to subsequent drying. Finally as the cell concentration was increased the level of survival increased.

Hutton et al (50) demonstrated that the temperature of the frozen samples of the bacterial culture was extremely critical and was in turn associated with the salt content of the sample. A correlation was shown between the degree of dryness and the viable recovery after drying. Storage at room temperature was shown to influence the dryness of the lyophilized samples.

H. The Effect of Temperature on Salmonellae Being Irradiated

Considerable data has been reported on the effectiveness of ionizing energy followed by subjection of bacteria to thermal energy, (54, 55, 70).

Some studies have been conducted on the effect of temperature during irradiation in the destruction of micro-organisms. Most of these studies were conducted over a wide temperature range. Licardello (60) did show that radiosensitivity of S. typhimurium increased as a function of irradiation temperature from 32 to 130°F. Also simultaneous application of temperature and irradiation produced a significantly greater destructive effect, than when each was applied individually.

Other work with bacteriophage showed that radio-sensitivity of the phage was markedly increased at irradiation temperatures above 113°F (2). A similar conclusion was reached in work with Saccharomyces cerevisiae (120). Even when enzymes were used a similar temperature level of 113°F was achieved (94).

SCOPE OF INVESTIGATION

This investigation was initiated primarily to determine the effect of gamma irradiation on selected species of *Salmonella* when incorporated into egg powders. Specifically, the following areas were investigated:-

1. The development of a method to incorporate *Salmonella* species into various powdered egg products.
2. The determination of the level of gamma irradiation required to produce a sterile powdered egg product.
3. To determine the relationship between the age of the *Salmonella* cultures and the irradiation resistance of the organisms when incorporated into powdered egg products.
4. The determination of the dosage of gamma irradiation required to achieve a 10^8 reduction in the number of *Salmonella* organisms in powdered egg products.

MATERIALS AND METHODS

A. Source of the Powdered Egg Products

Powdered egg yolk, powdered egg albumen and powdered whole egg for this investigation, were obtained in two-pound quantities from The Borden Company, Limited, Winnipeg, Manitoba. The egg powders were packed in clean plastic bags and transported from the plant to the laboratory. This supplier was chosen because its egg products were of high quality.

B. Media and Substrates Used

Nutrient broth containing 1% peptone was used. The nutrient broth is a good basic growth media. However, the peptone enhances its growth properties, producing more rapid growth of the Salmonella species (16, 72).

Standard plate count agar (tryptone glucose yeast agar) was used as the solid growth media in the Roux bottles and as the final plating media. This media is considered to be the best type for the growth of a wide range of different types of organisms, since it has an ideal supply of growth nutrients. This media could be used in this study, because no isolation of the Salmonella species from other types of organisms was necessary.

Triple sugar iron agar was used as a confirmation media in a small number of the final counts. Its use was necessary because the possibility existed that some low

counts on the tryptone glucose yeast agar may have contained non-salmonella organisms due to contamination during plating. This agar is recommended by the Canadian Food and Drug Directorate for the identification of Salmonellae. The identification here is based on biochemical testing. A number of researchers have been responsible for its development and use (57, 88, 102).

C. Salmonella Cultures Used

Six Salmonella serotypes were used in this investigation: S. give, S. heidelberg, S. enteritidis, S. worthington, S. typhimurium and S. senftenberg (775W). All of the serotypes were obtained from the American Type Culture Collection, Washington, D. C. These serotypes were selected for their varying degrees of resistance to irradiation as reported by Comer et al (16) in their studies with frozen whole egg melange.

Lyophilized cultures obtained from the American Type Culture Collection were inoculated aseptically into 10 mls. of nutrient broth containing 1% w/w of peptone. The inoculated broth was incubated at 37°C for 24 hours. After incubation, one loopful of broth was spread over the surface of a tryptone glucose yeast agar slant. The slant was incubated at 37°C for 24 hours. At the end of incubation the slant was placed under refrigeration at less than 50°F until ready for use. The cultures were transferred onto new tryptone

glucose yeast agar slants every 30 days.

D. Irradiation Apparatus

The apparatus used in this study was a Gammacell model 220 containing Cobalt 60 as the irradiation source. The unit is a self-contained irradiation facility, produced by the Commercial Products Division of Atomic Energy of Canada Ltd. The irradiation chamber is 6" in diameter and 8 1/8" in height. Cobalt 60 has a half-life of 5.3 years with an energy level of 1.17 and 1.33 Mev and a penetration ability of 40 cm. if the material is irradiated from both sides (El - Bisi 1968). The original dose-rate of the unit when installed was 1.6 Mrads. per hour and decreased about 1% per month. As a result, exposure time of the samples was programmed to correct for decrease in radio-activity.

E. Lyophilization Apparatus

In order that the test cultures of Salmonella organisms would more closely duplicate those types that are encountered in powdered egg products, lyophilized Salmonella cultures were used in this study.

A "Virtis" Freeze Dryer, model 10-146 MPRA, was used in the lyophilization of all Salmonella cultures used in this study. The batch chamber was used for lyophilization in place of the shell freezing attachment because of the

difficulty encountered in maintaining an uncontaminated culture with the shell freezing flasks. It was not necessary to seal the cultures with nitrogen as they were used almost immediately after lyophilization had been concluded.

F. Preparation of Inoculum

One loopful of the specific Salmonella species being investigated was removed from a tryptone glucose yeast agar slant and inoculated into 10 mls. of nutrient broth containing one percent w/w of peptone. The inoculated broth was incubated for 18 hours at 37°C. After incubation, the 10 mls. of broth was spread over the surface of ca. 300 mls. of tryptone glucose yeast agar in a Roux bottle. Excess broth was removed and the Roux bottles were incubated at 37°C for at least 36 hours. At the end of the incubation period, surface growth was washed off with 20 mls. of 1% w/w peptone water in two washings of 10 mls. each. The cell suspension was centrifuged at 20 gs in a "Sorvall", model GLC-1 centrifuge for twenty minutes. The supernatant peptone solution was decanted off; and the cells washed once with 10 mls. of one percent peptone water. The cells were re-suspended in 10 mls. of peptone water into which was mixed 2.5 grams of powdered whole egg. This mixture was transferred to a 500 ml. erlenmeyer flask. The flask was immersed into liquid nitrogen (195°C) and the contents frozen in a thin layer over the inner surface of the flask.

Lyophilization of the flask contents was then initiated in the "Virtis" freeze dryer. The time required for lyophilization varied between 9.5 and 11 hours.

G. Combination of Inoculum with the Powdered Egg Product

Using a long handled spatula, the lyophilized culture in the flask was dispersed aseptically into a fine homogenous powder, and mixed into:

(a) 10 gram samples of sterile powdered egg albumen containing 10% w/w of calcium phosphate, an anti-caking agent,

(b) 10 gram samples of sterile powdered whole egg containing 30% w/w of calcium phosphate,

(c) 10 gram samples of sterile powdered egg yolk containing 30% w/w of calcium phosphate.

The resulting mixture of samples each contained between 1×10^8 and 1×10^9 Salmonella organisms per gram of sample. This combination was then mixed in the "Fisher Kendall" mixer at a constant speed of 57 rpm for 20 minutes.

H. Irradiation Procedure

Eight one-gram samples were removed from the mixed inoculated powdered egg products and placed aseptically into sterile test tubes. Next, the samples were placed in the Gammacell 220 and irradiated at levels of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 Mrads respectively. The

irradiation was done in an air atmosphere and at a temperature of 35 - 50°C.

I. Enumeration

The numbers of Salmonella organisms surviving after irradiation was determined by the pour plate method. This method consisted of plating suitably prepared dilutions of the irradiated powdered egg products on tryptone glucose yeast agar and incubated at 37°C for 48 hours, before counting. This method was selected because it made possible reproduction of consistent results.

J. Distribution Studies

The purpose of these studies was to ascertain whether or not proper mixing of the organisms could be achieved by the method selected. This was of considerable importance since proper distrubution of the Salmonella organisms in the powdered egg products was essential to obtain reproductible results.

I. Determination of the Natural Microflora in Powdered Egg Products

Ten grams of powdered egg product was combined with 10% w/w of calcium phosphate in the case of powdered egg albumen; and with 30% by weight of calcium phosphate

in the case of powdered whole egg and powdered egg yolk. The calcium phosphate acted, in this case, as an anti-caking agent. This material was then mixed for 10 minutes in the "Fisher Kendall" mixer, rotating at a constant speed of 57 rpm. Eight one gram samples were removed and after proper dilutions had been prepared in phosphate buffer, pH 7.2, the dilutions were plated on tryptone glucose yeast agar and inoculated at 37°C for 48 hours. The counts at the end of this time were recorded.

II. The Inoculation of Liquid Cultures Into Powdered Egg Products

Ten grams of a powdered egg product was combined with calcium phosphate for the same purpose and in amounts identical to those in (I). This material was mixed in the "Fisher Kendall" mixer for 10 minutes. The container of Powder was then placed in a Labconco isolation chamber, model 8611 which had been sterilized previously by ultra-violet light for 30 minutes. The container with the powdered egg product was wiped with 95% ethanol and opened aseptically. The contents of the jar were spread evenly over a 1.5 square foot piece of sterile paper. Six mls. of a 30 hour liquid Salmonella culture, suspended in a phosphate buffer, was sprayed on the mixed powder in a manner which produced even distribution of the culture

throughout the powder. The inoculated powder was then placed aseptically back into the original container and from this seven one-gram portions were removed, and suitable dilutions were prepared in phosphate-buffered water, pH 7.2. The dilutions were then plated on tryptone glucose yeast agar using the pour plate method and incubated at 37°C for 48 hours. The counts were recorded at the end of this time.

III. The Inoculation of Lyophilized Cultures into Powdered Egg Products

Each test culture was suspended in six mls. of 10% reconstituted whole egg powder and the mixture was lyophilized in the "Virtis" freeze dryer. Lyophilization time varied between 9.5 and 11 hours depending on the particular Salmonella organism and the presence of other samples in the freeze dryer. The lyophilized culture was added to 10 grams of egg powder plus 10% by weight of calcium phosphate. This combination was mixed in the "Fisher Kendall" mixer for 10 minutes. The inoculated powder was then divided into seven one-gram portions and suitable dilutions were prepared in phosphate buffer pH 7.2. The dilutions were plated on tryptone glucose yeast agar and inoculated at 37°C for 48 hours. The counts were recorded at the end of this time.

K. Determination of Sterility of Powdered Egg Products

The object of this section of the preliminary investigation was to determine at what level of irradiation sterile egg powders could be obtained. The method used was that as set down in the British Pharmacopoeia for the determination of sterility in powdered samples.

Fifteen-gram samples of powdered whole egg, powdered egg yolk, and powdered egg albumen were mixed with the suitable levels of calcium phosphate in the "Fisher Kendall" mixer for 15 minutes after which the samples were irradiated at 0, 0.5, 1.0, 1.5 and 3.0 Mrads respectively. One-gram samples were removed and added to 10 ml . of nutrient broth. The inoculated broth was incubated for one week at 37°C. Evidence of growth was checked at the end of the incubation period by plating the 10 mls. of inoculated nutrient broth in one ml. aliquots on tryptone glucose yeast agar and incubated at 37°C for 48 hours. The plates were examined for growth at the end of this time period.

L. A Method for Estimating the Number of Lyophilized Salmonellae Being Incorporated into the Egg Powders

The method used was similar to that used by Gunter et al (39) and Dyker et al (19). Changes in the methods were necessary to suit the specific purposes of this investigation.

One loopful each of Salmonella typhimurium and Salmonella pullorum was removed from a tryptone glucose yeast agar slant and inoculated into 10 mls. of nutrient broth containing 1% of peptone. The inoculated broth was incubated for 18 hours at 37°C. After incubation, the 10 mls. of broth was spread over the surface of ca. 300 mls. of tryptone glucose yeast agar in a Roux bottle. Excess broth was removed and the Roux bottle was incubated at 37°C for at least 36 hours. At the end of the incubation period, surface growth was washed off with 20 mls. of 0.85% saline in two washings of 10 mls. each. The cell suspension was centrifuged at ca 20 gs in a "Sorvall" centrifuge, model GLC-1, for 15 minutes. The supernatant saline solution was decanted off and the cells washed twice more with 0.85% saline. The cells were then re-suspended in 20 mls. of pH 7.2, phosphate buffered solution.

Appropriate cell suspensions were prepared with phosphate buffer, pH 7.2, so that a reading of 45% plus or minus one % transmittance at 575 mu was recorded on a spectronic 20 spectrophotometer. Then, 20 mls. of a dilution identical to that which gave 45% plus or minus 1% transmittance at 575 mu was prepared. From this dilution, a one ml. aliquot was removed, plated on tryptone glucose yeast agar and incubated at 37°C for 48 hours. The remaining 19 mls. of solution were combined with two grams of whole egg

powder and lyophilized in the "Virtis" Freeze dryer for 10 hours. This sample was reconstituted with 19 mls. of phosphate - buffered water pH 7.2. Then a one ml. aliquot of the reconstituted sample was removed, plated on tryptone glucose yeast agar and incubated at 37°C for 48 hours. Counts were recorded at the end of the incubation period.

Counts before and after lyophilization were prepared and the percent survival was calculated.

M. The Relationships Between the Age of Cultures and the Irradiation Resistance of the Organism

This study was initiated to determine if the age of the culture makes any difference in the irradiation resistance of the Salmonella cultures. If it does, then the age of the culture with greatest irradiation resistance would be chosen for use in this study.

Three cultures of Salmonella give, grown at 37°C for 18, 24 and 36 hours, respectively, were harvested by the method previously described in (c) except that the two washings with 0.85% saline were omitted. Dilutions were then prepared which gave a 45% plus or minus 1% transmittance at 575 mu on the Spectronic 20 Spectrophotometer. From these dilutions it was possible to prepare samples of whole egg powder containing approximately 1×10^9 lyophilized Salmonella give cells

per gram of powder. One-gram samples were removed and subjected to irradiation levels of 0, 0.06, 0.3, and 0.5 Mrads in the first set of trials and 0, 0.03, 0.06, 0.1, 0.2, 0.3, 0.5, and 0.6 Mrads in the second set of trials. The number of Salmonella give organisms surviving at each level in both sets of trials were determined by preparing suitable dilutions in phosphate buffered water, pH 7.2, plating these dilutions on tryptone glucose yeast agar, incubating at 37°C for 48 hours and recording the counts at the end of this time.

RESULTS

A. Preliminary Investigations

I. Distribution Studies of the Natural Microflora in Powdered Egg Products

The data presented in Table 2 indicated that the natural microflora of different samples of powdered egg yolk, whole egg and egg albumen, were rather uniformly distributed by the mixing technique employed. Some variation in counts were encountered but a statistical analysis by the ANOVA Log transformation technique indicated that the variation noted within each sample and between samples was not significant. The difference in counts could be attributed to chance rather than a real difference. In general, the average total count in egg yolk varied between 5300 and 7300 colonies per gram. For whole egg, the total count varied between 5300 and 6800 colonies per gram, whereas the counts in egg albumen varied between 7600 and 69000 colonies per gram.

II. Incorporation of the Test Organisms Into Egg Powders by Atomization

Table 3 presents the results of inoculating *Salmonella pullorum* and *Salmonella typhimurium* into dried egg powders by the atomization technique described previously. A total of three trials were conducted on each species. The results revealed that it was possible to incorporate the

TABLE 2

Distribution of the Natural

Microflora in Powdered Egg Products

Trial	Egg Yolk	Whole Egg	Egg Albumen
	counts/gram		
1	7281	5300	69,325
2	5329	6369	7,600
3	5944	6880	
4	5461	6013	
5	6063	5413	

(See appendix tables 1, 2 and 3)

TABLE 3

Distribution of *Salmonella typhimurium* and *Salmonella pullorum* Liquid Cultures After Their Addition by Atomization to Powdered Egg Products

Trial	Egg Yolk Counts/gram	Whole Egg	Egg Albumen
	S. pullorum		
1	3.13×10^8	2.60×10^6	3.52×10^7
2	1.28×10^9	9.42×10^7	3.72×10^8
3	1.91×10^7	2.44×10^7	8.64×10^7
	S. typhimurium		
1	4.86×10^8	1.15×10^8	6.45×10^7
2	7.05×10^8	6.46×10^7	1.26×10^8
3	2.03×10^7		6.12×10^7

(See appendix tables 4, 5 and 6)

TABLE 4

Distribution of *Salmonella typhimurium* and *Salmonella pullorum* Lyophilized Cultures in Powdered Egg Products

Trial	Egg Yolk Counts/gram	Whole Egg	Egg Albumen
	S. pullorum		
1	1.48×10^7	1.77×10^5	1.23×10^6
2	4.55×10^6	2.85×10^6	1.80×10^7
3	4.19×10^6		5.33×10^6
	S. typhimurium		
1	1.75×10^6	6.48×10^4	8.77×10^6
2	8.71×10^5	5.74×10^6	5.90×10^7
3		3.32×10^6	8.72×10^6

(See appendix tables 7, 8 and 9)

liquid Salmonella cultures into powdered egg products, by this method and to obtain a certain degree of uniformity. There was no statistical difference in the Salmonella counts when different samples were removed and plated.

Although atomization appeared to offer a method by which cultures might be incorporated in egg powders, difficulties were encountered. First it was not possible to accurately control the number of organisms being added. Secondly the method was very awkward and cumbersome to work with. In addition the moisture content of the different powders were difficult to control, consequently, this method was discontinued.

III. Addition of Lyophilized Cultures of Salmonellae Into Powdered Egg Products

In order to test the feasibility of incorporating lyophilized cultures into powdered egg products, S. pullorum and S. typhimurium were selected as test organisms. The data presented in Table 4 indicated that the technique employed appeared to be acceptable. Rather uniform distribution of the two test cultures in the different products were obtained. Although variation in counts were encountered within replicates in the different trials for all three products, statistical analysis of the data revealed that these variations were not significant.

Since this procedure of incorporating Salmonella test organisms into powdered egg products was judged to be more convenient and effective than the atomization method, the technique was adopted for all subsequent experiments.

The data presented in Tables 5 and 6, indicated that there were differences in the number of survivals following lyophilization between S. pullorum and S. typhimurium. The average number of S. pullorum surviving lyophilization in whole egg powder was 6.47% and the average number for S. typhimurium was 14.21%. Furthermore, larger variations in the percentage survival after lyophilization were encountered with S. typhimurium than with S. pullorum.

Such variation within serotypes and differences between serotypes in sensitivity to lyophilization produced some difficulty in obtaining suitably high numbers of viable cells in lyophilized cultures of S. enteritidis and S. worthington in subsequent studies. A one log reduction was encountered when these cultures were lyophilized for the same period of time as the other serotypes. However, this problem was corrected by reducing lyophilization time from 11 hours to 9½ hours.

TABLE 5

Survival of *Salmonella pullorum*

After Lyophilization in Whole Egg Powder

Trial	*S. pullorum* counts/gram (Millions) Before Lyophilization	After Lyophilization	% Survival
1	625	32	5.04
2	715	33	4.64
3	660	45	6.82
4	610	50	8.11
5	685	55	8.03
6	700	43	6.14
Average	686	43	6.47

TABLE 6

Survival of *Salmonella typhimurium*
Following Lyophilization in
Whole Egg Powder

Trial	*S. typhimurium* counts/gram (Millions) Before Lyophilization	After Lyophilization	% Survival
1	910	55	6.04
2	450	104	23.11
3	490	60	12.24
4	430	57	13.14
5	340	41	11.91
6	960	70	7.24
7	915	129	14.04
8	905	130	14.31
9	835	129	15.45
Average	693	86	14.21

IV. A Comparison of Age of the Culture and the Radiation Resistance of the Organism

Since the main objective of this investigation was to destroy Salmonella organisms in powdered egg products by gamma irradiation, it was deemed necessary to evaluate the effect of age of the cultures and their sensitivity to irradiation. Cultures of S. give were grown at 37°C for 18, 24 and 36 hours, respectively. These cultures were then lyophilized and incorporated into powdered whole egg before irradiation.

The data presented in Table 7 showed that the 36 hour Salmonella give cultures were more resistant to gamma irradiation than the 18 or 24 hour cultures. All viable cells of the 18 and 24 hour cultures were destroyed at an irradiation dosage of 0.6 Mrads. However, a small number of viable cells of a 36 hour culture of S. give were still present after exposure to 0.6 Mrads. The results indicated that the 36 hour cultures exhibited greater resistance to lyophilization. As a result, because of this preliminary study, all subsequent investigations in this project utilized cultures grown at 37°C for at least 36 hours.

V. Radiation Dosages Required to Obtain Sterile Powdered Egg Products

In order to determine the irradiation dosages required to obtain sterile egg products, samples of powdered whole egg

TABLE 7

Resistance of Lyophilized Cultures of Salmonella give grown at 37°C for 18, 24 and 36 Hours to Gamma Irradiation

Age of Culture at 37°C (Ave. 3 Trials) Hours	Dosage in Megarads							
	0	0.03	0.06	0.1	0.2	0.3	0.5	0.6
18	6.50×10^8	1.50×10^8	8.60×10^7	1.30×10^7	1.10×10^7		0.00	0.00
	9.97×10^9		7.50×10^7			3.90×10^3	10.00	
24	7.80×10^8	1.90×10^8	3.90×10^7	2.00×10^7	3.60×10^7		0.00	0.00
	8.80×10^9		5.40×10^7			4.00	0.00	
36	2.70×10^9	1.80×10^9	9.00×10^8	3.10×10^8	1.10×10^8		2.90×10^2	1.00
	1.42×10^9		9.20×10^7			3.80×10^3	8.00	

The Lyophilized Cultures were Mixed into Powdered Whole Egg before Exposure to Irradiation Treatments

and powdered egg albumen were exposed to 0.5, 1.0 and 1.5 Mrads of radiation. The results presented in Tables 8 and 9 revealed that there were no survivals following 1.5 Mrads of irradiation in both products. In addition, the results revealed that 1.0 Mrads of radiation was not adequate to produce sterile products. However, on subsequent trials, (Table 10) samples of powdered whole eggs and egg albumen which were irradiated at 1.5 Mrads, showed that some organisms of the natural microflora of these products survived the irradiation treatment. These results were not consistent with those obtained previously. As a result, subsequent samples of powdered egg products were subjected to 3.0 Mrads of radiation to achieve complete sterility. The data presented in Table 11 indicated that a radiation dosage of 3.0 Mrads was sufficient to render the samples sterile. Furthermore, the sterility of the samples were determined on the basis of 10 grams of product. This procedure has given a certain degree of confidence that the samples were indeed sterile after exposure to 3.0 Mrads of radiation.

TABLE 8

The Effect of Irradiation on the Natural Flora of Powdered Whole Egg

Trial	Irradiation Level (Megarads) 0.	0.5	1.0	1.5
		Counts/Gram		
1	12,650	200	150	0
2	17,500	250	150	0
3	6,800	0	50	0
4	4,350	100	0	0

TABLE 9

The Effect of Irradiation on the Natural Flora of Powdered Egg Albumen

Trial	Irradiation Level (Megarads)			
	0.	0.5	1.0	1.5
	Counts/Gram			
1	5,500	200	100	0
2	11,300	50	0	0
3	8,700	100	0	0
4	4,850	250	150	0

TABLE 10

The Reduction in the Number of Viable Cells in Powdered Whole Egg and Powdered Egg Albumen After Exposure to 1.5 Megarads of Gamma Irradiation

Trial	Powdered Whole Egg		Powdered Egg Albumen	
	Control	Irradiated	Control	Irradiated
	Counts/Gram			
1	7,800	40	13,400	0
2	9,700	20	8,700	3
3	4,000	0	3,650	0
4	11,650	0	9,700	0

TABLE 11

The Reduction in the Number of Viable Cells

in Various Powdered Egg Products

After Exposure to 3.0 Mrads of Gamma Irradiation

Trial	Whole Egg Powder		Egg Albumen Powder		Egg Yolk Powder	
	Untreated counts/gm	Irradiated counts/10gms	Untreated counts/gm	Irradiated counts/10gms	Untreated counts/gm	Irradiated counts/10gms
1	4000	0	280	0	3140	0
2	5850	0	270	0	3320	0
3	5250	0	250	0	3800	0

B. Gamma Irradiation of Selected Salmonella Species in Powdered Whole Egg

The effect of gamma irradiation on the destruction of S. enteritidis, S. typhimurium, S. worthington, S. senftenberg S. heidelberg and S. give in powdered whole egg is presented in figures 1 and 2 (Appendix Tables 10 to 15). The results indicated that there were variations in the sensitivity of each test organism to gamma irradiation. The pattern of destruction of the six organisms was characterized by a very rapid initial destruction of Salmonella after exposure to very low dosages of gamma irradiation and then by a less rapid but constant destruction rate as the dosage level increased. The rate of initial destruction at low dosages of gamma radiation and the death rate at high dosages were both noted to be exponential in nature. After exposure to 0.05 Mrads of radiation it was noted that there was a reduction of 2 log cycles for S. worthington and a reduction of 4 log cycles from the initial count for S. typhimurium in powdered whole eggs. As the dosages were increased up to a level of 0.6 Mrads the rate of destruction for these two organisms appeared to be the same, however, a small number of S. worthington survived after exposure to 0.6 Mrads of radiation. There were no S. typhimurium surviving after 0.6 Mrads of radiation.

The destruction rate of S. enteritidis, S. heidelberg and S. give appeared to be exponential after 0.1 Mrads. A reduction by 3 log cycles from the initial count was noted for S. give, S. heidelberg and a reduction by 4 log cycles was observed for S. enteritidis. After exposure to dosages up to 0.6 Mrads, no viable cells of S. give or S. heidelberg were found, however, an average of less than 10 organisms per gram of S. enteritidis survived 0.6 Mrads of radiation.

An apparent similar rate of destruction by gamma irradiation in powdered whole egg was noted for S. typhimurium, S. give, S. heidelberg, and S. worthington. The rate of destruction was apparently slower for S. enteritidis when compared to others, indicating that it was slightly more resistant.

C. Gamma Irradiation of Selected Species of Salmonellae in Powdered Egg Albumen

The same six test organisms utilized in the powdered whole egg study were used here (figures 3 and 4) (appendix tables 16 to 21). Similar trends in the destruction of the test organisms in powdered egg albumen were noted. However different rates of destruction were noted for the different test organisms. The death of the test organisms were characterized by a rapid initial reduction in the

population of the test organisms after exposure to low doses of radiation, and followed by a less rapid destruction as the dosages were increased to a level of 0.6 Mrads.

Although the patterns of destruction by gamma irradiation of the Salmonella test organisms in powdered egg albumen were rather similar to that seen in powdered, whole eggs, some marked variations were apparent. This variation in destruction by radiation was observed for S. senftenberg S. worthington and S. give. The destruction of S. senftenberg in powdered egg albumen appeared to be the only organism among the six test organisms which was exponential at all levels of radiation. For the others, a very large number of organisms were destroyed after exposure to low dosages of radiation and this was then followed by a less rapid but constant rate of destruction as the irradiation levels were increased to 0.6 Mrads. With the exception of S. worthington and S. give no survivals were noted in powdered egg albumen after 0.6 Mrads or irradiation. As was noted previously, variations in the rate of destruction of the six Salmonella test organisms in powdered egg albumen were noted.

D. Gamma Irradiation of Selected Species of Salmonellae in Powdered Egg Yolk

The destruction of Salmonellae by gamma irradiation in powdered egg yolk is shown in figures 5 and 6 (appendix tables 22 to 27). The levels of inoculant used in this

phase varied between 10^8 and 10^9 organisms per gram of product. A very rapid reduction in the number of S. enteritidis, S. give and S. typhimurium were observed following 0.1 Mrads of radiation. The rapid destruction at low dose radiation was not observed for S. heidelberg S. senftenberg and S. worthington, in powdered egg yolk. Furthermore, the destruction of S. heidelberg, S. senftenberg were quite similar at all irradiation levels. The reduction, in the number of S. enteritidis, S. give and S. typhimurium as the dosages of radiation were increased, followed the same trend seen previously in powdered whole eggs, and powdered egg albumen. This trend was not as pronounced for the destruction of S. heidelberg, S. senftenberg and S. worthington in powdered egg albumen. With the exception of S. enteritidis and S. heidelberg , there were no viable organisms present in the product following 0.60 Mrads of irradiation. Both S. enteritidis and S. heidelberg had an average of 8 and 14 viable organisms per gram of product after being exposed to 0.6 Mrads of gamma radiation.

E. The Rates of Destruction of Selected Species of Salmonellae in Egg Products by Gamma Irradiation

The comparative rates of destruction of Salmonella organisms in powdered egg products by gamma irradiation are presented in Table 12. The rates of destruction at the various radiation levels are expressed by the

FIGURE 1

REDUCTION IN NUMBERS
OF VIABLE CELLS OF *SALMONELLAE*
SPECIES IN POWDERED WHOLE EGG
BY GAMMA IRRADIATION

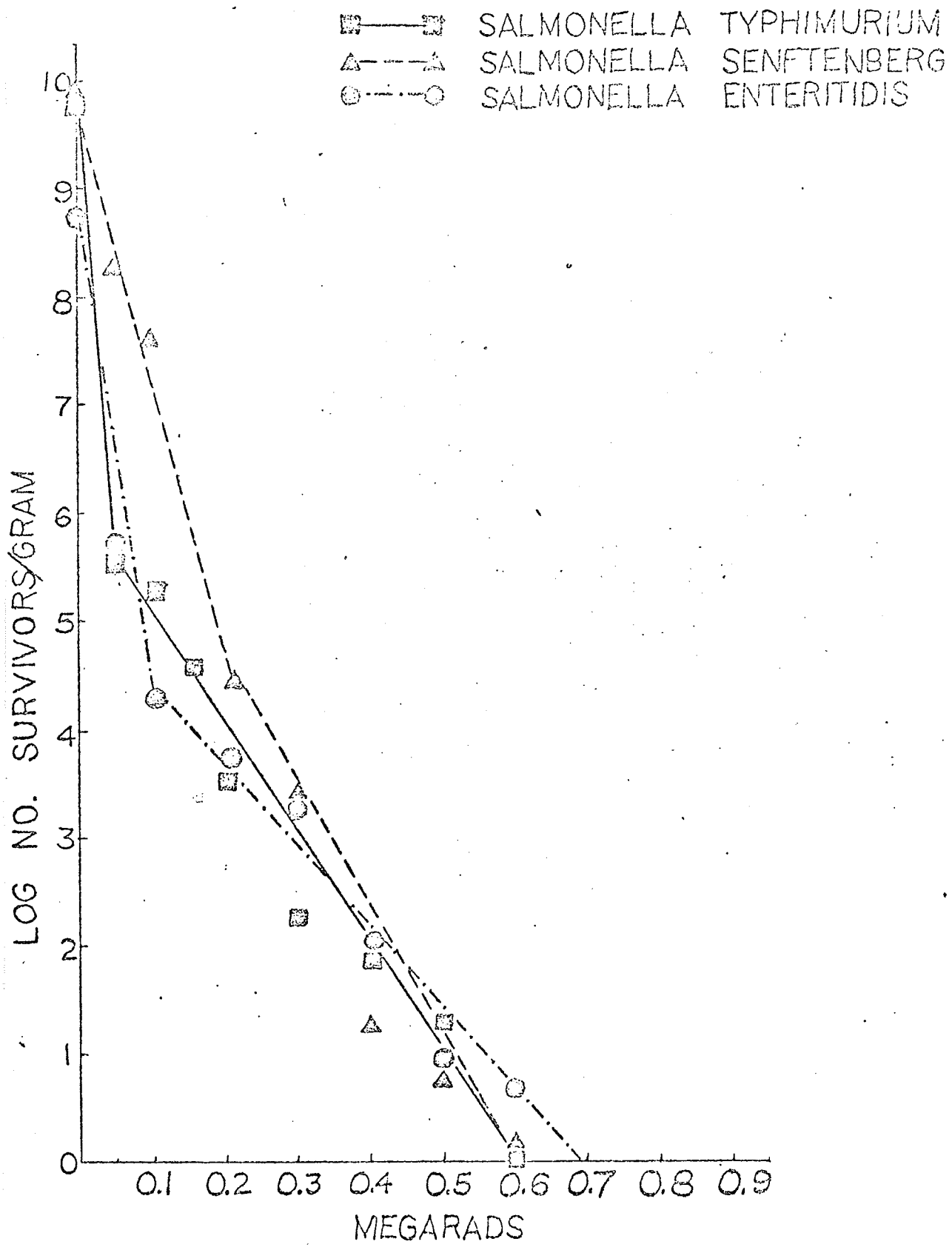

SALMONELLA TYPHIMURIUM
SALMONELLA SENFTENBERG
SALMONELLA ENTERITIDIS
LOG NO. SURVIVORS/GRAM
0
1
2
3
4
5
6
7
8
9
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
MEGARADS

FIGURE 2

REDUCTION IN NUMBERS OF VIABLE CELLS OF *SALMONELLAE* SPECIES IN POWDERED WHOLE EGG BY GAMMA IRRADIATION

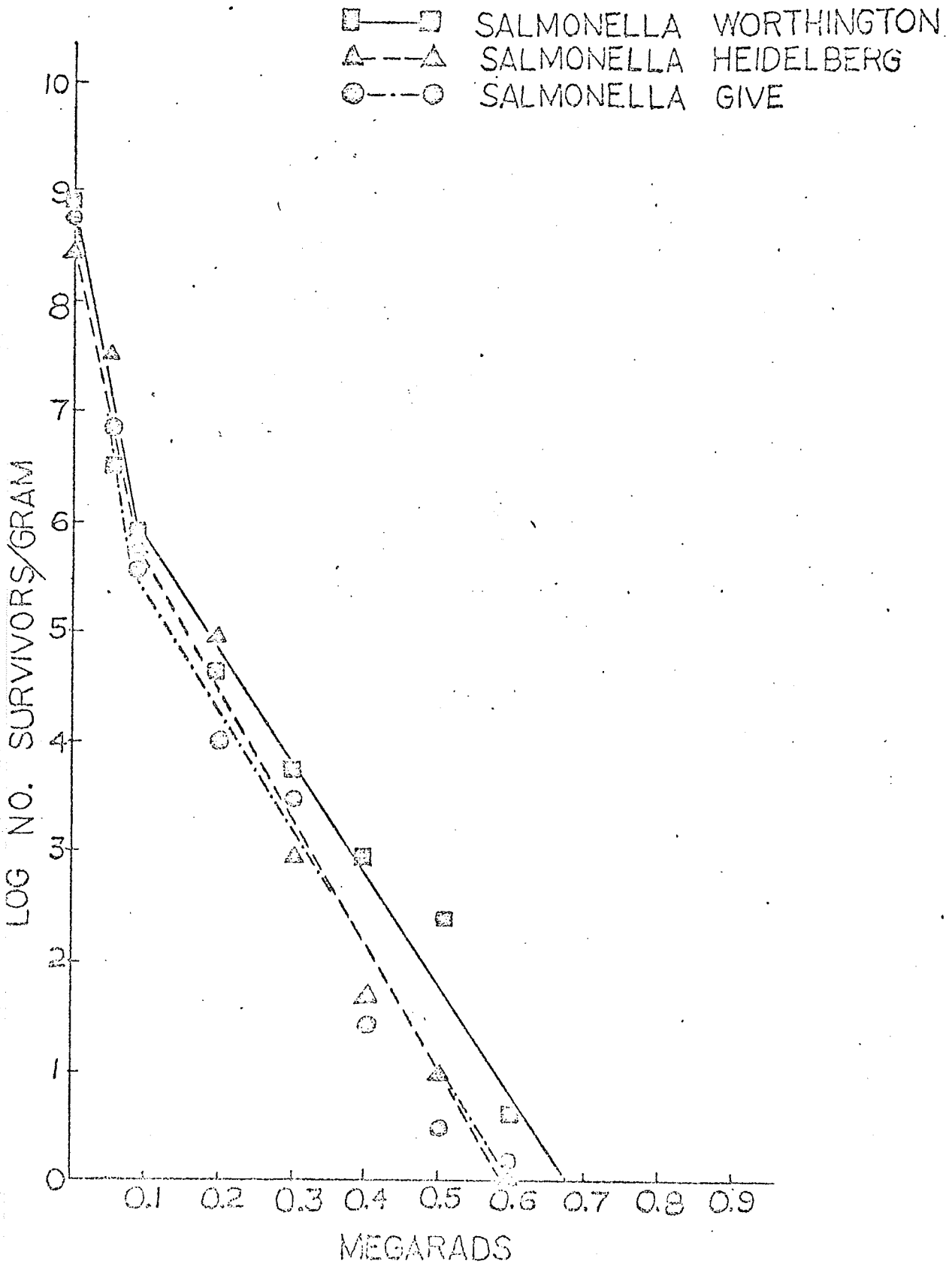

SALMONELLA WORTHINGTON
SALMONELLA HEIDELBERG
SALMONELLA GIVE
LOG NO. SURVIVORS/GRAM
0
1
2
3
4
5
6
7
8
9
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
MEGARADS

FIGURE 3

REDUCTION IN NUMBERS
OF VIABLE CELLS OF SALMONELLAE
SPECIES IN POWDERED EGG
ALBUMEN BY GAMMA IRRADIATION

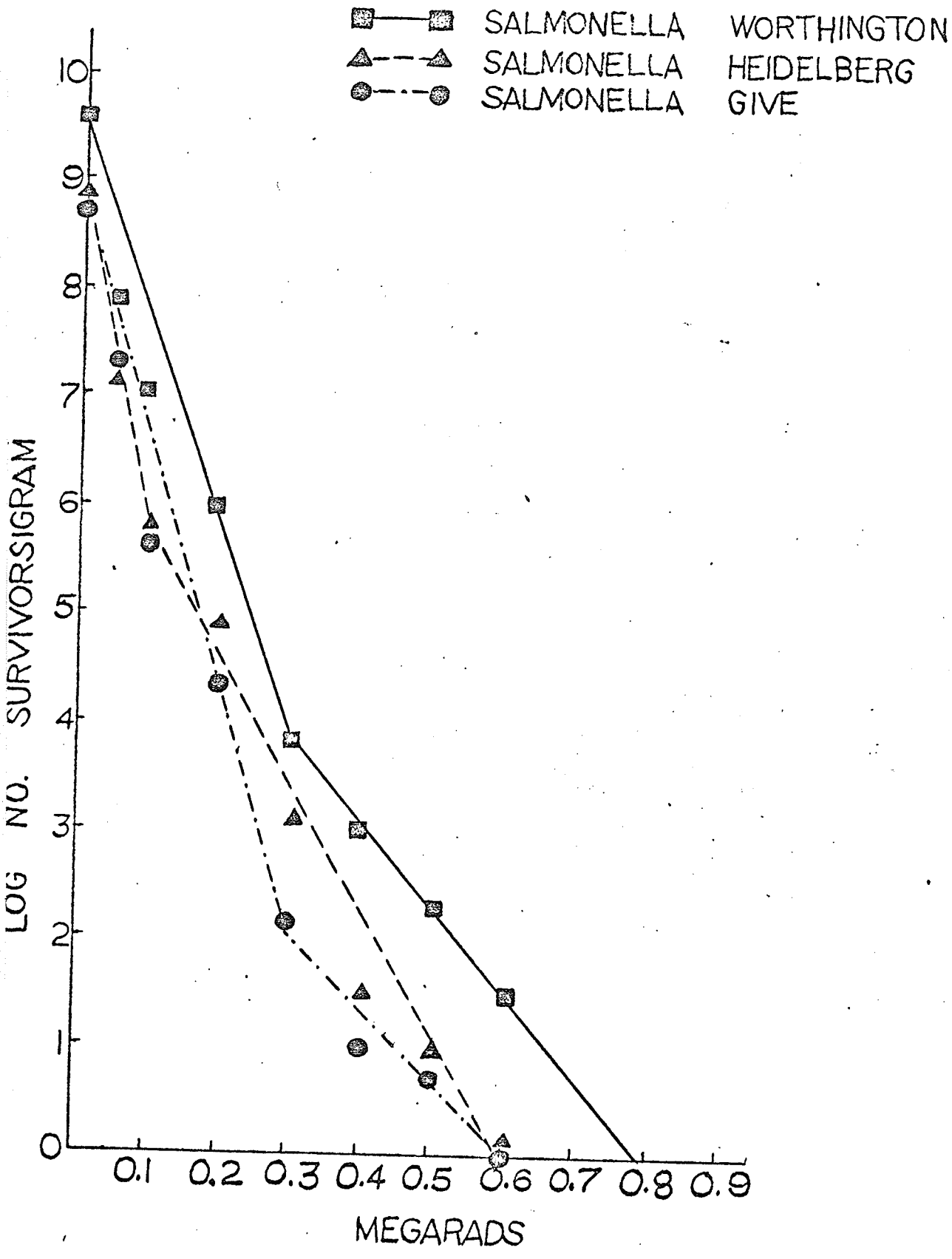

SALMONELLA WORTHINGTON
SALMONELLA HEIDELBERG
SALMONELLA GIVE
LOG NO. SURVIVORSIGRAM
0
1
2
3
4
5
6
7
8
9
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
MEGARADS

FIGURE 4

REDUCTION IN NUMBERS
OF VIABLE CELLS OF *SALMONELLAE*
SPECIES IN POWDERED EGG
ALBUMEN BY GAMMA IRRADIATION

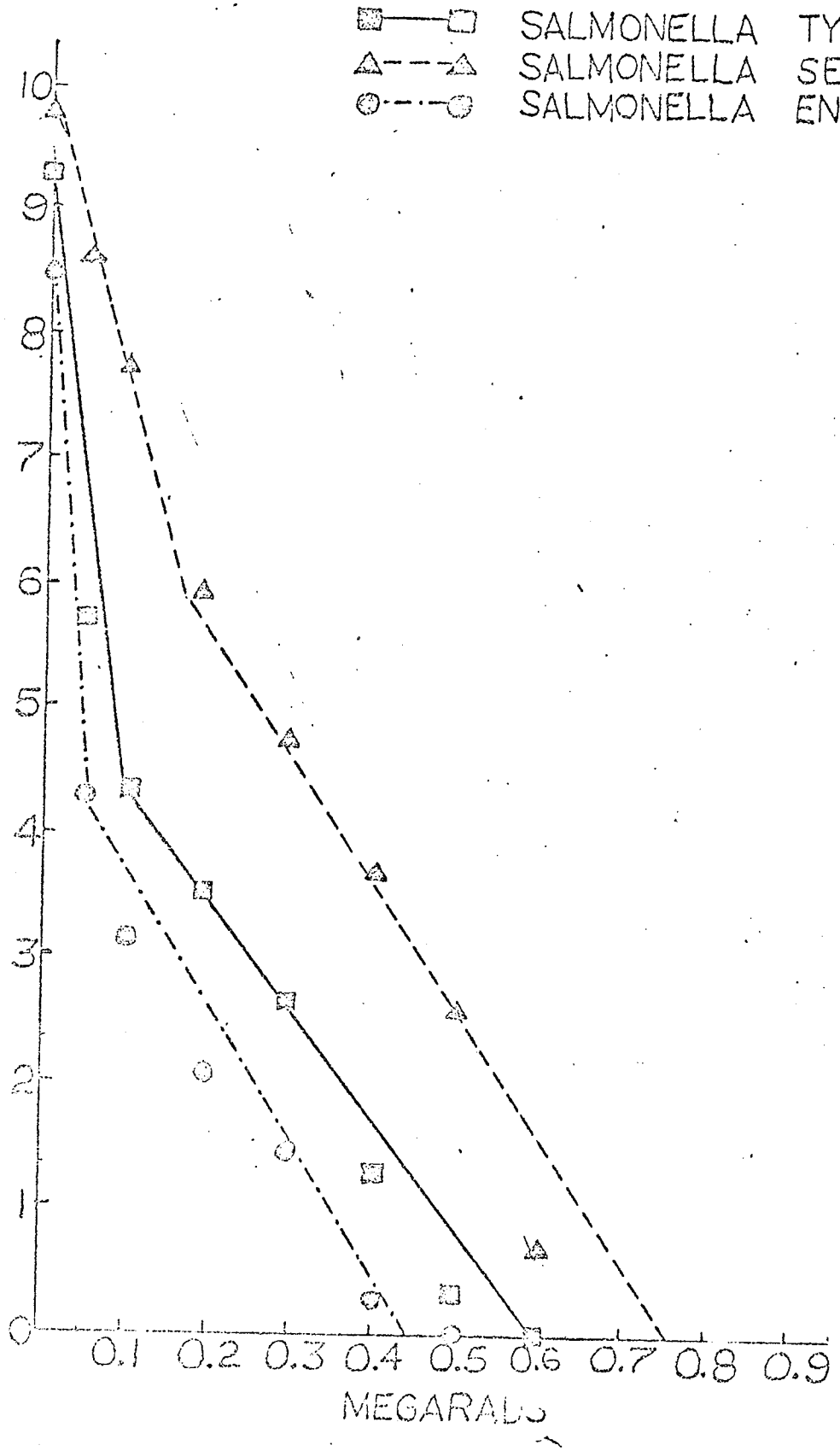

SALMONELLA TYPHIMURIUM
SALMONELLA SENFTENBERG
SALMONELLA ENTERITIDIS
10
9
8
7
6
5
4
3
2
1
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
MEGARADS

FIGURE 5

REDUCTION IN NUMBERS
OF VIABLE CELLS OF SALMONELLAE
SPECIES IN POWDERED EGG YOLK
BY GAMMA IRRADIATION

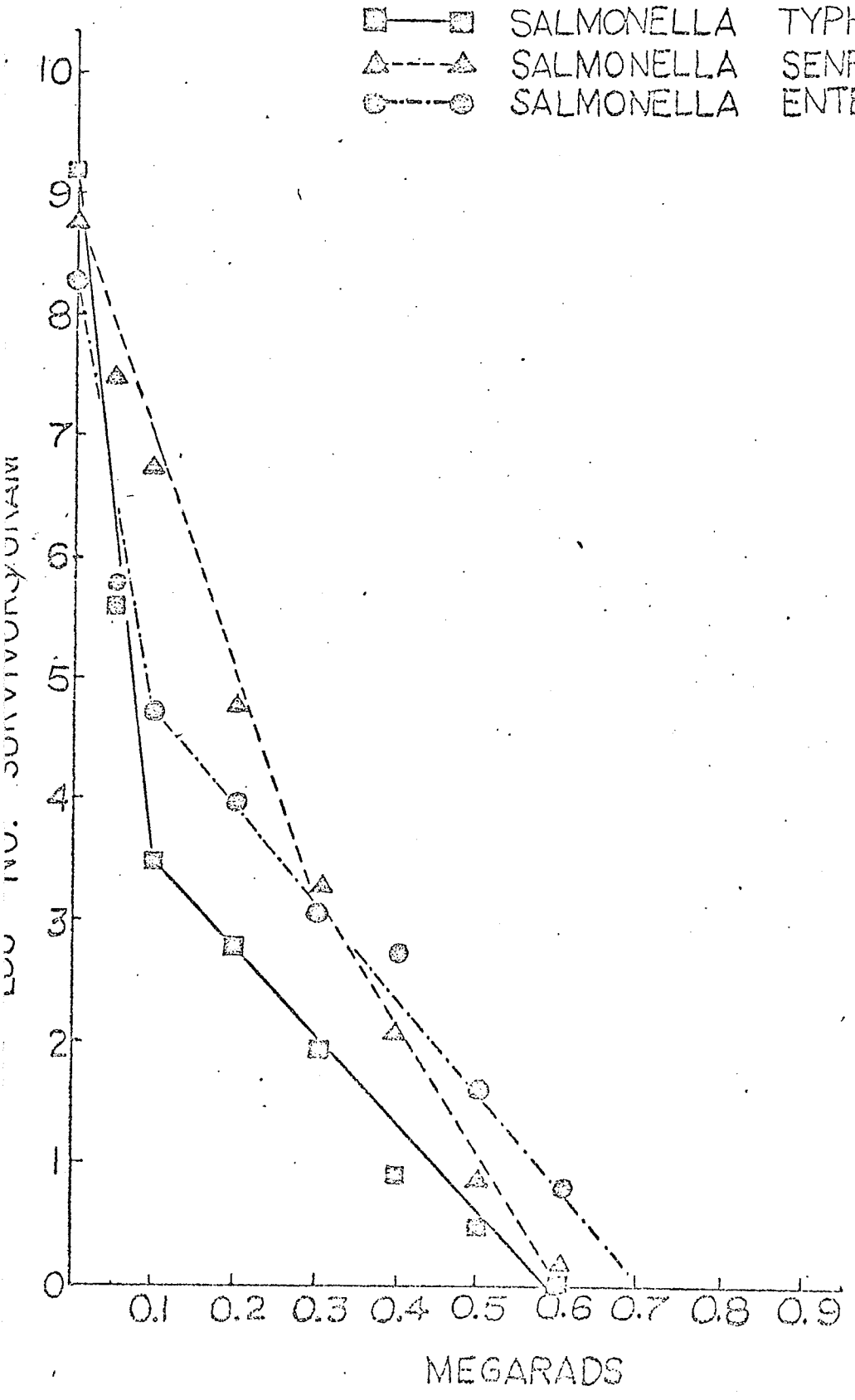
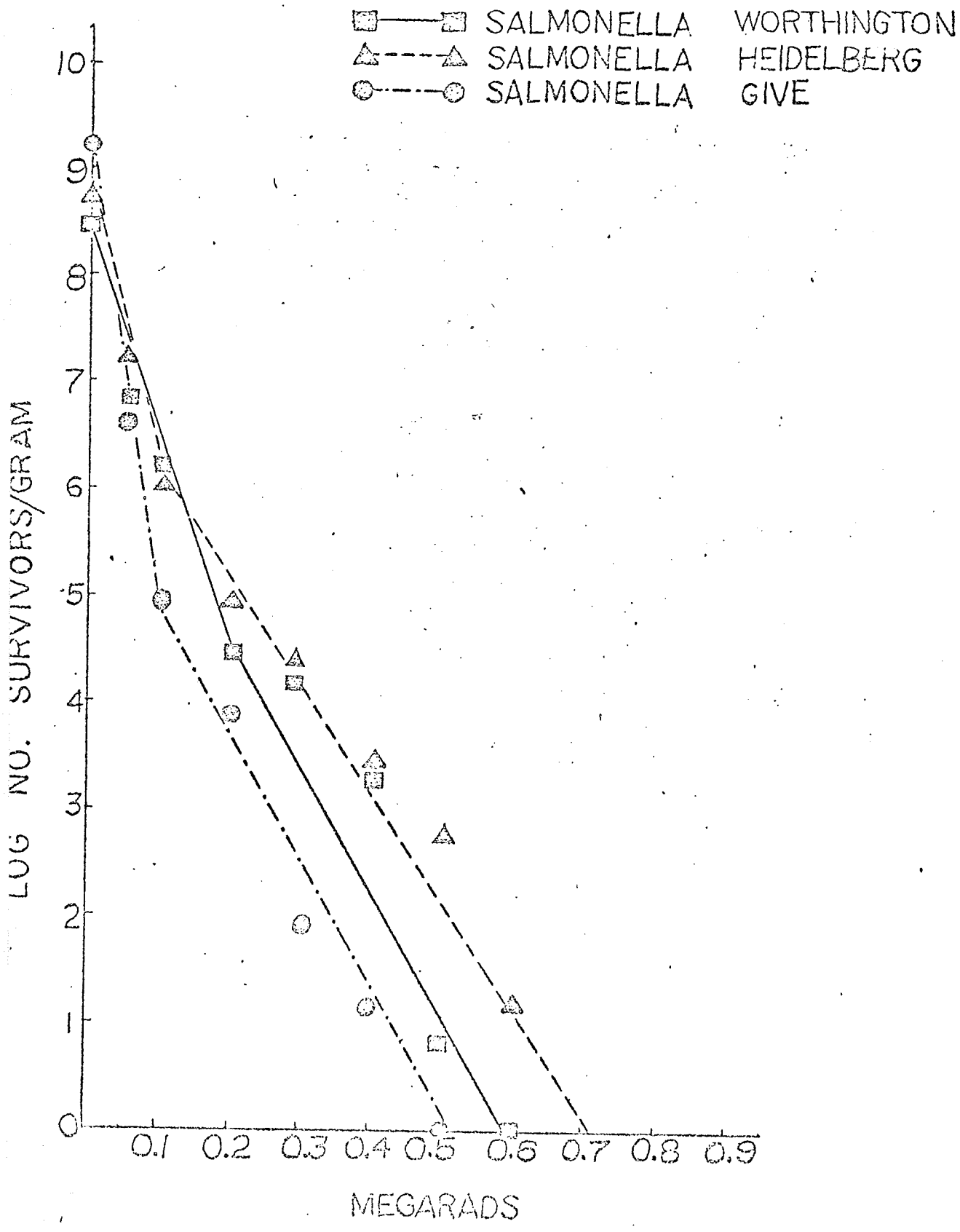

SALMONELLA TYPHIMURIUM
SALMONELLA SENFTENBERG
SALMONELLA ENTERITIDIS
LOG NO. SURVIVORS/UNIT
10
9
8
7
6
5
4
3
2
1
0
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9
MEGARADS

FIGURE 6

REDUCTION IN NUMBERS
OF VIABLE CELLS OF *SALMONELLAE*
SPECIES IN POWDERED EGG YOLK
BY GAMMA IRRADIATION


SALMONELLA WORTHINGTON
SALMONELLA HEIDELBERG
SALMONELLA GIVE
LOG NO. SURVIVORS/GRAM
0
1
2
3
4
5
6
7
8
9
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
MEGARADS

respective D_M values. The destruction of Salmonellae in powdered egg products was not exponential at the different radiation levels. In fact, the destruction by gamma irradiation in the different powdered egg products for each test organism, appears to have two distinctive rates of destruction. The value under D_M1 is the rate of destruction at low irradiation levels while D_M2 describes the other destruction rate as the radiation dosages were increased. The two different D_M values for each test organism presented in Table 12 are estimated D_M values derived from the graphs presented in figures 3 through 8 (appendix tables 10 through 26). These D_M values represent the radiation dosages required to reduce the number of organism by 90% or 1 log cycle.

The result obtained from this study showed that all six test organisms in powdered whole egg had D_M1 values of 0.02 Mrads. The D_M2 values for the same organisms in the whole egg varied between 0.08 and 0.14 Mrads with S. enteritidis requiring 0.14 Mrads to reduce the counts by 90%.

The destruction rates of Salmonellae in powdered egg yolk varied between 0.02 to 0.05 Mrads for the D_M1 values and for the D_M2 values, they varied between 0.08 and 0.13 Mrads. S. typhimurium and S. enteritidis each had D_M2 values of 0.13 Mrads.

Greater apparent variation in D_M1 and D_M2 values were

TABLE 12

Rates of Destruction of Selected Species of *Salmonellae* in Various Powdered Egg Products by Gamma Irradiation

	Whole Egg		Egg Yolk		Egg Albumen	
	Mrads					
	D_M1	D_M2	D_M1	D_M2	D_M1	D_M2
S. give	0.02	0.08	0.02	0.09	0.08	0.14
S. heidelberg	0.02	0.08	0.04	0.11	0.03	0.09
S. typhimurium	0.02	0.09	0.02	0.13	0.02	0.04
S. senftenberg	0.02	0.08	0.05	0.08	0.06	0.08
S. worthington	0.02	0.10	0.04	0.08	0.05	0.12
S. enteritidis	0.02	0.14	0.02	0.13	0.01	0.09

observed for the destruction of Salmonella organisms in powdered egg albumen. The D_M1 values for the six test organisms in this product, ranged from 0.01 to 0.06 Mrads with S. senftenberg having the greatest D_M1 value. This large variation in D_M1 values indicated that greater variation in resistance were observed for the Salmonella test organisms when present in powdered egg albumen.

Similarly, a wider variation in D_M2 values were observed for the same organisms in powdered egg albumen. The D_M2 value varied between a low of 0.04 for S. typhimurium to a high of 0.14 Mrads for S. give. Surprisingly, S. enteritidis did not possess high D_M1 and D_M2 values in powdered egg albumen whereas, the D_M2 values for this organism was considerably larger in powdered egg yolk and whole egg. In addition, S. typhimurium had relatively low D_M1 and D_M2 values, they were 0.02 and 0.04 respectively, indicating of course, that this organism was easily destroyed by relatively low dosages of gamma irradiation.

F. Radiation Dosages Required to Achieve a 10^8 Reduction in the Number of Salmonella Organisms in Powdered Egg Products

The dosages which were necessary to obtain a 10^8 reduction in the number of each test organism in all three

powdered egg products are presented in Table 13. The date in Table 13 was derived directly from Figures 3 through 8.

In powdered whole eggs, relatively low dosages, i.e. 0.40 and 0.43 Mrads were required to achieve a 10^8 reduction for S. senftenberg and S. typhimurium. A radiation level 0.62 Mrads was required for S. worthington and 0.59, 0.51 and 0.50 Mrads, respectively for S. enteritidis, S. heidelberg and S. give.

The dosages required to reduce the number of Salmonellae by 10^8 organisms in powdered egg yolk varied between a high of 0.66 Mrads for S. heidelberg and a low of 0.39 Mrads for S. typhimurium. S. senftenberg and S. enteritidis each required a dosage of 0.52 Mrads and S. worthington, a dosage of 0.54 Mrads to achieve the same level of destruction of the organisms.

With respect to powdered egg albumen, the radiation level required to obtain the same magnitude of reduction in the number of organisms in one gram of product varied between 0.37 for S. enteritidis and 0.56 Mrads for S. worthington. S. Seftenberg and S. heidelberg each required a dosage of 0.53 and 0.51 Mrads, respectively. Whereas, 0.48 Mrads was necessary for S. give and 0.40 Mrads for S. typhimurium.

In general, the data indicated that the destruction of the same test organism in each of the three different

TABLE 13

Radiation Dosages Necessary to Obtain a 10^8 Reduction of *Salmonella* Organisms in Powdered Egg Products

	Powdered whole egg	Powdered egg yolk	Powdered egg albumen	All these products Ave. dosage
	..... Dosage (Mrads)			
S. give	0.50	0.40	0.48	0.460
S. heidelberg	0.51	0.66	0.51	0.560
S. typhimurium	0.43	0.39	0.40	0.405
S. senftenberg	0.40	0.52	0.53	0.483
S. worthington	0.62	0.54	0.56	0.573
S. enteritidis	0.59	0.52	0.37	0.493

products were different. Some of these differences were rather pronounced as in the case of S. enteritidis. Whereas, for S. typhimurium, there were only very small differences in the radiation dosage required to reduce the number of organisms in all three products.

Finally, the dosages for 10^8 reduction for each test organisms in all three products were averaged, and are presented in Table 13. The average radiation dosage was derived in order to determine the relative radio-resistance of each test organism.

The organisms were ranked according to the highest average dosage required to obtain a 10^8 reduction in all three products revealing that S. worthington was more resistant than S. heidelberg and followed by S. enteritidis, S. senftenberg, S. give and S. typhimurium, respectively. In general, S. worthington and S. heidelberg were rather similar in their resistance to gamma irradiation both required considerably larger dosages to obtain 10^8 reduction. The next group of organisms which required relatively similar dosages were S. enteritidis, S. senftenberg and S. give. Finally S. typhimurium appears to be the least resistant of all six species studied.

It should be pointed out that the average temperature at which the various products were irradiated was 100 ^{o}F ± 5 ^{o}F. Attempts to control the temperature of the product during irradiation was not successful with

TABLE 14

Megarad-Temperature Relationship
Within the Gammacell 220 Irradiation Chamber
During the Irradiation of the Powdered Egg Products

Irradiation Level (Megarads)	Temperature (°F)
0	85.0
0.05	95.0
0.1	97.5
0.2	101.0
0.3	101.5
0.4	102.5
0.5	102.5
0.6	102.5
0.7	104.0

conventional methods. Part of the reason for this could be due to the design of the gamma cell which was used in this investigation. Furthermore, all samples were irradiated in air atmosphere.

DISCUSSION

In recent years, food borne infection caused by salmonellosis resulting from consumption of contaminated foods have been reported by numerous investigators (1, 4, 7, 28, 44). Egg and egg products have been implicated in many such outbreaks as the vehicle of transmission. The contamination of powdered egg products may arise from one of many causes, such as:-

1. The organisms are not completely destroyed by the pre-heating treatment and/or the drying processes.
2. Incorporation of the organisms into the product from contaminated air used in the drying process.
3. The powdered product being contaminated by unsanitary equipment.
4. Post-drying contamination.

This study was initiated to examine the possibility of utilizing low dosages of gamma radiation to destroy selected species of Salmonella organisms in powdered egg and egg products, as well as, to determine the dosages required to obtain a decimal reduction of eight log cycles.

The results obtained from this study, revealed that low dose gamma irradiation may be employed as a preservation method, and in particular, to destroy a large number of Salmonella organisms contained in one gram of product may be achieved by subjecting the products to a dosage of 0.6 Mrads. Although, it was found that on

the average 0.66 Mrads was required to destroy 10^8 S. heidelberg organisms in powdered egg yolk. These findings confirmed the results obtained by Comer et al (16), and Nickerson et al (77), that gamma irradiation was effective in destroying Salmonella organisms in frozen and powdered egg albumen. However the dosages reported by these investigators were considerably less than those reported in this study. These investigators (16, 77) reported that 0.42 Mrads was required to destroy approximately 10^7 S. typhimurium and 0.36 Mrads was required for S. senftenberg in frozen whole egg. These differences may be explained by the fact that experimental conditions were not similar as well as by the fact that the level of inoculum was larger in the present study. Furthermore, the composition of the products used in the present study were powdered samples.

This study also revealed that the age of the culture was a factor in determining the radio-sensitivity of the organism. In an attempt to utilize the most resistant cultures for the irradiation studies it was found that cultures grown at 37°C for at least 36 hours were more resistant than those grown either at 18 or 24 hours. The resistance to gamma irradiation of Salmonellae as well as other micro-organisms with increased age has been reported by Proctor et al (81).

This study also revealed that radio-sensitivity differences were found among the six test organisms, and that these differences were found to be statistically significant. The results suggest that sensitivity to ionizing irradiation was not a fixed species characteristic. Such differences in radio-sensitivity have been noted in several other studies (16, 77, 94). These findings indicated that the differences not only occured between species of Salmonellae but also may occur within the same species (16).

In the present study, it was found that when all three products were taken into consideration, the average dosage which was required to obtain a 10^8 reduction in the number of Salmonellae

showed that S. worthington was the most resistant species, followed in order of decreasing radio-sensitivity, by S. heidelberg, S. enteritidis, S. senftenberg, S. give and the least resistant species, S. typhimurium. Furthermore, it was observed that the order of sensitivity changed for these organisms when each product was considered alone. In powdered whole egg, the most resistant species was S. worthington (0.62 Mrads) and the least resistant ones S. senftenberg (0.40 Mrads) and S. typhimurium, (0.43 Mrads). In powdered egg yolk, the most resistant organism was S. heidelberg (0.66 Mrads) and again the least resistant one S. typhimurium. Whereas, in powdered egg

albumen, S. worthington was again found to be the most resistant species and the least resistant species S. typhimurium. The data indicated that with the possible exception of S. typhimurium, which was the least resistant serotype, S. worthington and S. heidelberg were the more resistant serotypes. S. give may be ranked as the medium resistant species, whereas the other two test organisms, i.e. S. senftenberg and S. enteritidis exhibited varied radio-sensitivity to low dose gamma irradiation. These results suggest that the composition of the product greatly affected the resistance of the organisms. The different products apparently imparted selective protective action to gamma irradiation for some organisms but not to others.

The radio-resistance of the six serotypes differed from the results reported by Comer et al (16). In their study with frozen whole egg they reported in order of decreasing resistance to gamma radiation S. give, S. heidelberg, S. typhimurium, S. enteritidis, and S. senftenberg. This difference in radio-resistance between the results of this study and those reported by others could possibly be explained by the fact that these investigators used frozen egg product and a different mode of preparing the inoculum. In the present study the inoculum was added in the form of a lyophilizate. It is conceivable that the sensitivity of the organisms

were affected as a result of lyophilization.

With respect to the Decimal Reduction rates, the test organisms appeared to be destroyed at two distinctive rates in all three products. When the organisms were exposed to very low levels of gamma irradiation, there was a very large initial reduction in the number of organisms, this was next followed by another rate of reduction when the dosage was increased up to a level of 0.60 Mrads. With the exception of S. typhimurium, the least resistant species of the test organisms used, all species exhibited two distinctive rates of reduction. The results suggest that within the population of the lyophilized test organisms, a large fraction of the organisms were extremely sensitive to gamma irradiation. This may be attributed to the sensitization effect of the lyophilization treatment and/or perhaps that within the culture, a large fraction of the organisms were more susceptible to gamma irradiation. This phenomena has been observed by other investigators (72). Furthermore, the present study dealt with very large numbers of test organisms, levels of 10^8 - 10^9 were utilized, and it may be expected that a fraction of the culture was less resistant to gamma irradiation. On the other hand several cultures which included S. worthington, S. senftenberg in egg albumen; S. enteritidis, S. heidelberg in egg yolk and S. enteritidis, S. worthington in whole

egg were not completely destroyed after exposure to 0.60 Mrads of irradiation. This may be explained on the basis of natural resistance of the organisms (8, 19, 59). In addition, when the initial population of the samples were large, and the rate of destruction was constant, it may be expected that not all of the organisms will be destroyed with 0.60 Mrads and that in order to obtain no survivors, larger dosages must be employed.

The scope of this investigation was rather limited as only six serotypes were considered. Furthermore, the test organisms were selected on their radio-resistance as reported by other investigators (10, 16, 77).

The results obtained from this study indicated that low dosage gamma irradiation may be employed as a means to destroy Salmonellae in egg products, but the relative resistance of the organisms were quite different from those reported by others (10, 16, 72). Perhaps this may be explained by the fact that the nature of the inoculum and the composition of the product used in this study could contribute to the difference in radio-resistance of each serotype.

Finally, this study did not investigate the effects of gamma irradiation on Salmonella species found naturally as a result of contamination in egg products. Furthermore, whether these contaminants will be more or less resistant to gamma irradiation remains to be ascertained. However,

it is known that the level of contamination would in no way approach the level employed in this study. It should be mentioned that the various egg products treated with 0.6 Mrads of gamma irradiation were used to prepare angel-food cakes. Organoleptic evaluation of the cakes found them to be acceptable by a panel of judges. The results of this investigation will be presented in a separate report.

CONCLUSIONS

Although considerable research still must be done in this area of study, the following conclusions may be stated:-

1. A 10^8 reduction for all of the organisms tested was obtained when the powdered egg products were subjected to a minimum of 0.7 Mrads of gamma irradiation.
2. There were differences in the amount of resistance that each Salmonella species possessed to gamma irradiation.
3. The Mrads required to destroy any one Salmonella species differed from one powdered egg product to another.
4. The most resistant Salmonella species in each of the three powdered egg products considered were:-
   (a) Salmonella worthington when present in powdered whole egg.
   (b) Salmonella heidelberg when present in powdered egg yolk.
   (c) Salmonella worthington when present in powdered egg albumen.

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APPENDIX

TABLE 1

Distribution of the Natural Microflora

in Powdered Egg Yolk

Sample No.	Trials Counts/Gram 1	2	3	4	5
1	6200	4200	5650	5050	5700
2	9550	5900	6350	6150	5050
3	4800	4000	6250	4800	6250
4	6300	4800	6000	5535	6500
5	9150	5500	6800	5700	6100
6	6650	----	5450	5550	5900
7	7400	7000	4750	5200	7050
8	8200	5900	6300	5700	5950

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	7	0.05268	0.00753	2.019
Trials	4	0.08162	0.02041	
Exp. Error	27	0.10070	0.00373	
Total	38	0.23500		

F Value ** Highly Significant

* Significant

TABLE 2

Distribution of the natural microflora in Powdered Whole Egg

Sample No.	1	2	3	4	5
1	4000	7400	6700	6700	3650
2	5900	6400	6650	6500	5700
3	5000	5700	6450	6800	4600
4	3200	6350	7050	4900	7100
5	6400	8000	6850	5350	6500
6	6700	7050	5950	5250	5150
7	5800	5700	7600	6550	5000
8	5400	4350	7550	7050	5600
Average	5300	6369	6850	6013	5413

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	7	0.02666	0.00381	0.545
Trials	4	0.08256	0.02064	
Exp. Error	28	0.19556	0.00698	
Total	39	0.30478		

F Value ** Highly Significant

* Significant

TABLE 3

Distribution of the Natural Microflora in Powdered Egg Albumen

Sample No.	Trials Counts/Gram 1	2
1	121,000	5150
2	39,600	6500
3	83,500	3550
4	83,000	4250
5	57,000	7350
6	55,500	10,250
7	51,500	13,200
8	63,500	10,550
Average	69,325	7600

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	7	0.09804	0.01401	0.280
Trials	1	3.81411	3.81411	
Exp. Error	7	0.35047	0.05007	
Total	15	4.26261		

F Value ** Highly Significant

* Significant

TABLE 4

Distribution of *Salmonella typhimurium* and *Salmonella pullorum* Liquid Cultures after Their Addition by Atomization to Powdered Whole Egg

Sample Number	*Salmonella pullorum* Trials Counts/Gram			*Salmonella typhimurium* Trials Counts/Gram		
	1	2	3	1	2	3
1	1.50×10^6	9.85×10^7	2.29×10^7	1.17×10^8	7.75×10^7	
2	3.10×10^6	9.05×10^7	2.45×10^7	1.30×10^8	7.65×10^7	
3	1.74×10^6	9.35×10^7	2.82×10^7	8.10×10^7	6.05×10^7	
4	1.95×10^6	8.90×10^7	2.38×10^7	1.10×10^8	Contaminated	Laboratory
5	4.05×10^6	1.00×10^8	2.55×10^7	1.29×10^8	5.60×10^7	Accident
6	2.22×10^6	9.05×10^7	1.90×10^7	1.25×10^8	7.20×10^7	
7	3.65×10^6	9.75×10^7	2.67×10^7	1.14×10^8	4.55×10^7	
A. M.	2.60×10^6	9.42×10^7	2.44×10^7	1.15×10^8	6.46×10^7	

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.07728	0.01288	1.394
Trials	2	8.99599	4.49799	
Exp. Error	12	0.11089	0.00924	
Total	20	9.18416		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.04411	0.00735	1.594
Trials	1	0.22958	0.22958	
Exp. Error	6	0.02767	0.00461	
Total	13	0.30136		

F Value ** Highly Significant

* Significant

TABLE 5

Distribution of *Salmonella typhimurium* and *Salmonella pullorum* Liquid Cultures After Their Addition by Atomization to Powdered Egg Yolk

Sample Number	*Salmonella pullorum* Trials Counts/Gram			*Salmonella typhimurium* Trials Counts/Gram		
	1	2	3	1	2	3
1	3.42×10^8	1.91×10^9	9.55×10^6	5.60×10^8	7.60×10^8	2.80×10^7
2	1.36×10^8	1.78×10^9	2.43×10^7	3.85×10^8	2.50×10^8	2.56×10^7
3	-------	1.25×10^9	1.22×10^7	7.50×10^8	5.95×10^8	2.26×10^7
4	2.13×10^8	-------	1.12×10^7	5.55×10^8	1.58×10^9	-------
5	6.05×10^8	1.12×10^9	2.26×10^7	8.55×10^8	2.55×10^8	4.20×10^6
6	2.70×10^8	1.26×10^9	3.50×10^7	1.32×10^8	6.15×10^8	1.27×10^7
7	3.14×10^8	3.35×10^8	-------	1.65×10^8	8.85×10^8	2.87×10^7
A. M.	3.13×10^8	1.28×10^9	1.91×10^7	4.86×10^8	7.05×10^8	2.03×10^7

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.44697	0.07449	0.569
Trials	2	9.67343	4.83671	
Exp. Error	9	1.17814	0.13090	
Total	17	11.29854		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	1.08242	0.18040	1.583
Trials	2	8.29782	4.14891	
Exp. Error	11	1.25336	0.11394	
Total	19	10.63360		

F Value ** Highly Significant

* Significant

TABLE 6

Distribution of Salmonella typhimurium and Salmonella pullorum Liquid Cultures After Their Addition by Atomization to Powdered Egg Albumen

Sample Number	Salmonella pullorum Trials Counts/Gram			Salmonella typhimurium Trials Counts/Gram		
	1	2	3	1	2	3
1	3.35×10^7	1.23×10^8	1.12×10^8	1.01×10^8	1.27×10^8	1.57×10^7
2	3.75×10^7	-------	9.75×10^7	9.70×10^7	1.06×10^8	2.87×10^8
3	2.48×10^7	5.15×10^8	8.70×10^7	4.40×10^7	-------	2.71×10^7
4	3.60×10^7	2.90×10^8	1.04×10^8	4.70×10^7	1.48×10^8	2.41×10^7
5	4.00×10^7	3.75×10^8	8.25×10^7	1.10×10^7	9.60×10^7	2.93×10^7
6	3.60×10^7	4.45×10^8	8.00×10^7	7.95×10^7	1.53×10^8	2.22×10^7
7	3.85×10^7	4.85×10^8	4.20×10^7	7.20×10^7	1.25×10^8	2.29×10^7
A. M.	3.52×10^7	3.72×10^8	8.64×10^7	6.45×10^7	1.26×10^8	6.12×10^7

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.06347	0.01058	0.251
Trials	2	3.10689	1.55344	
Exp. Error	11	0.46327	0.04212	
Total	19	3.63362		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.71245	0.11874	1.202
Trials	2	1.07348	0.53674	
Exp. Error	11	1.08638	0.09876	
Total	19	2.87231		

F Value ** Highly Significant

* Significant

TABLE 7

Distribution by Means of the Fisher Kendall Mixer of Salmonella typhimurium and Salmonella pullorum Lyophilized Cultures in Powdered Egg Albumen

Sample Number	Salmonella pullorum Trials Counts/Gram			Salmonella typhimurium Trials Counts/Gram		
	1	2	3	1	2	3
1	1.37×10^6	2.62×10^7	1.95×10^6	1.07×10^7	6.65×10^7	3.60×10^6
2	1.12×10^6	2.61×10^7	1.70×10^6	1.12×10^7	5.60×10^7	8.65×10^6
3	1.55×10^6	1.34×10^7	4.35×10^6	1.12×10^7	4.50×10^7	1.88×10^7
4	1.41×10^6	1.17×10^7	6.80×10^6	7.90×10^6	6.45×10^7	6.00×10^6
5	1.19×10^6	2.26×10^7	4.25×10^6	6.75×10^6	5.80×10^7	6.30×10^6
6	9.60×10^5	8.00×10^6	1.23×10^7	6.00×10^6	5.60×10^7	6.70×10^6
7	1.26×10^6	1.78×10^7	6.00×10^6	7.70×10^6	6.75×10^7	1.10×10^7
A. M.	1.23×10^6	1.80×10^7	5.33×10^6	8.77×10^6	5.90×10^7	8.72×10^6

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.04310	0.00718	0.113
Trials	2	4.40775	2.20387	
Exp. Error	12	0.76376	0.06365	
Total	20	5.21461		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.11256	0.01876	0.768
Trials	2	3.44530	1.72265	
Exp. Error	12	0.29319	0.02443	
Total	20	3.85105		

F Value ** Highly Significant

* Significant

TABLE 8

Distribution by Means of the Fisher Kendall Mixer of *Salmonella typhimurium* and *Salmonella pullorum* Lyophilized Cultures in Powdered Egg Yolk

Sample Number	*Salmonella pullorum* Trials Counts/Gram			*Salmonella typhimurium* Trials Counts/Gram		
	1	2	3	1	2	3
1	2.55×10^6	5.05×10^6	1.04×10^6	3.55×10^4	7.20×10^5	
2	9.35×10^6	3.45×10^6	1.85×10^6	3.10×10^6	1.51×10^5	
3	5.00×10^6	3.90×10^6	4.10×10^6	6.50×10^6	9.90×10^5	Laboratory
4	2.59×10^7	5.70×10^5	3.95×10^6	3.25×10^5	8.05×10^5	Accident
5	9.00×10^6	5.40×10^6	3.50×10^6	1.33×10^6	1.59×10^6	
6	3.08×10^7	4.30×10^6	6.35×10^6	3.45×10^5	7.90×10^5	
7	L.A.	4.05×10^6	8.55×10^6	3.10×10^5	1.05×10^6	
A. M.	1.48×10^7	4.55×10^6	4.19×10^6	1.75×10^6	8.71×10^5	

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.67910	0.11318	0.863
Trials	2	1.00011	0.50006	
Exp. Error	11	1.44256	0.3114	
Total	19	3.12177		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	1.73058	0.28843	0.748
Trials	1	0.02071	0.02071	
Exp. Error	6	2.31438	0.38573	
Total	13	4.06567		

F Value ** Highly Significant

* Significant

TABLE 9

Distribution by Means of the Fisher Kendall Mixer of *Salmonella typhimurium* and *Salmonella pullorum* Lyophilized Cultures in Powdered Whole Egg

Sample Number	*Salmonella pullorum* Trials Counts/Gram			*Salmonella typhimurium* Trials Counts/Gram		
	1	2	3	1	2	3
1	2.25×10^5	3.10×10^6		7.40×10^4	3.40×10^6	2.90×10^6
2	2.05×10^5	3.75×10^6		8.80×10^4	6.95×10^6	2.38×10^6
3	1.92×10^5	3.50×10^6	Laboratory	5.55×10^4	4.60×10^6	3.30×10^6
4	1.85×10^5	7.80×10^5	Accident	8.45×10^4	1.19×10^7	3.05×10^6
5	6.45×10^4	4.00×10^6		5.55×10^4	3.60×10^6	4.75×10^6
6	1.73×10^5	9.45×10^5		6.45×10^4	4.05×10^6	3.05×10^6
7	1.90×10^5	3.85×10^6		3.20×10^4	-------	3.80×10^6
A. M.	1.77×10^5	2.85×10^6		6.48×10^4	5.74×10^6	3.32×10^6

Salmonella pullorum

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.32086	0.05348	0.707
Trials	1	4.71389	4.71389	
Exp. Error	6	0.45377	0.07563	
Total	13	5.48851		

F Value ** Highly Significant

* Significant

Salmonella typhimurium

ANOVA

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatments	6	0.75287	0.12548	1.349
Trials	2	13.96772	6.98386	
Exp. Error	11	1.02333	0.09303	
Total	19	15.74392		

F Value ** Highly Significant

* Significant

TABLE 10

Reduction in Numbers of Viable Cells of *Salmonella heidelberg* in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	2.90×10^{8}	5.40×10^{6}	4.00×10^{5}	5.50×10^{3}	2.10×10^{2}	4.40×10^{1}	4.00	0.00
2	3.40×10^{8}	1.40×10^{7}	7.50×10^{5}	1.20×10^{4}	3.30×10^{3}	5.40×10^{1}	1.8×10^{1}	0.00
3	4.10×10^{8}	8.70×10^{6}	4.60×10^{5}	8.50×10^{3}	1.50×10^{2}	2.70×10^{1}	9.00	0.00
4	2.30×10^{8}	4.50×10^{6}	3.20×10^{5}	7.90×10^{3}	2.00×10^{2}	4.20×10^{1}	5.00	0.00
5	4.00×10^{8}	3.90×10^{7}	1.20×10^{6}	5.90×10^{5}	1.50×10^{3}	1.20×10^{2}	3.00×10	0.00
A.M.	3.34×10^{8}	1.43×10^{7}	6.26×10^{5}	1.25×10^{5}	1.07×10^{3}	5.74×10^{1}	1.32×10^{1}	0.00
LOG.OF AVE.NO.	8.5237	7.1553	5.7966	5.0969	3.0294	1.7589	0.1206	0.0000

TABLE 11

Reduction in Numbers of Viable Cells on *Salmonella give* in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	3.87×10^{8}	1.00×10^{7}	1.40×10^{6}	1.90×10^{4}	8.30×10^{3}	2.50×10^{1}	0.00	0.00
2	4.90×10^{8}	3.40×10^{7}	6.90×10^{5}	9.40×10^{3}	5.40×10^{2}	1.70×10^{1}	0.00	0.00
3	5.10×10^{8}	8.70×10^{5}	8.00×10^{4}	2.70×10^{3}	1.50×10^{2}	3.00×10^{1}	6.00	0.00
4	1.50×10^{9}	2.50×10^{6}	9.60×10^{4}	2.50×10^{3}	1.50×10^{2}	4.40×10^{1}	4.00	0.00
5	2.80×10^{8}	7.90×10^{5}	5.80×10^{4}	2.50×10^{4}	7.00×10^{3}	6.60×10^{1}	3.00	0.00
A.M.	6.33×10^{8}	9.63×10^{6}	4.65×10^{5}	1.17×10^{4}	3.23×10^{3}	3.64×10^{1}	3.00	0.00
LOG.OF AVE.NO.	8.8014	6.9836	5.6675	4.0682	3.5092	1.5611	0.4150	0.0000

TABLE 12

Reduction in Numbers of Viable Cells of *Salmonella enteritidis* in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	4.40×10^{8}	4.30×10^{5}	1.20×10^{4}	2.50×10^{3}	2.00×10^{3}	1.80×10^{2}	2.10×10^{1}	9.00
2	7.50×10^{8}	4.00×10^{5}	4.20×10^{4}	5.70×10^{3}	3.50×10^{3}	9.66×10^{1}	8.00	6.00
3	6.20×10^{8}	-------	1.80×10^{4}	6.80×10^{3}	1.60×10^{3}	2.20×10^{1}	1.20×10^{1}	6.00
A.M.	6.03×10^{8}	4.15×10^{5}	2.40×10^{4}	5.00×10^{3}	2.37×10^{3}	9.93×10^{1}	1.37×10^{1}	7.00
LOG.OF AVE.NO.	8.7803	5.6180	4.3802	3.6990	3.3747	1.9969	1.1367	0.8494

TABLE 13

Reduction in Numbers of Viable Cells of Salmonella worthington in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	1.30×10^{8}	1.80×10^{6}	7.40×10^{5}	8.20×10^{3}	2.40×10^{2}	5.40×10^{1}	1.00×10^{1}	1.00
2	1.70×10^{9}	4.30×10^{6}	1.00×10^{6}	1.20×10^{5}	1.50×10^{4}	2.90×10^{3}	7.60×10^{2}	9.00
3	3.70×10^{8}	4.80×10^{6}	6.30×10^{5}	1.60×10^{4}	9.30×10^{2}	3.80×10^{1}	9.00	1.00
A.M.	7.33×10^{8}	3.63×10^{6}	7.90×10^{5}	4.80×10^{4}	5.37×10^{3}	9.97×10^{2}	3.00×10^{2}	4.00
LOG.OF AVE.NO.	8.8651	6.5599	5.8976	4.6812	3.7300	2.9987	2.4249	0.6021

TABLE 14

Reduction in Numbers of Viable Cells of *Salmonella typhimurium* in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	7.30×10^{9}	1.90×10^{5}	2.50×10^{4}	1.80×10^{3}	5.00×10^{1}	3.40×10^{1}	1.80×10^{1}	0.00
2	4.80×10^{9}	-------	5.00×10^{5}	6.90×10^{3}	6.10×10^{2}	2.00×10^{2}	4.80×10^{1}	0.00
3	3.70×10^{9}	5.30×10^{5}	7.50×10^{4}	1.40×10^{3}	8.10×10^{1}	2.10×10^{1}	0.00	0.00
A.M.	5.27×10^{9}	3.60×10^{5}	2.00×10^{5}	3.37×10^{3}	2.47×10^{2}	8.50×10^{1}	2.20×10^{1}	0.00
LOG.OF AVE.NO.	9.72181	5.5563	5.30103	3.52763	2.3927	1.92942	1.34242	0.0000

TABLE 15

Reduction in Numbers of Viable Cells of Salmonella senftenberg in Powdered Whole Egg by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	3.50×10^{9}	3.50×10^{7}	8.30×10^{6}	1.70×10^{4}	1.30×10^{3}	4.70×10^{1}	1.5×10^{1}	0.00
2	2.00×10^{10}	4.50×10^{8}	1.20×10^{8}	7.10×10^{4}	3.50×10^{3}	5.50×10^{1}	5.00	0.00
3	2.50×10^{9}	8.60×10^{7}	5.10×10^{6}	1.30×10^{4}	9.60×10^{2}	1.40×10^{1}	8.00	0.00
A.M.	8.60×10^{9}	1.90×10^{8}	4.45×10^{7}	3.37×10^{4}	1.92×10^{3}	3.87×10^{1}	9.00	0.00
LOG.OF AVE.NO.	9.9380	8.2788	7.6484	4.5276	3.2833	1.5877	0.9542	0.0000

TABLE 16

Reduction in Numbers of Viable Cells of Salmonella heidelberg in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	2.70×10^{8}	6.30×10^{6}	1.40×10^{4}	1.20×10^{3}	1.20×10^{2}	1.20×10^{1}	2.00	0.00
2	2.40×10^{8}	2.00×10^{7}	6.20×10^{4}	2.20×10^{3}	1.10×10^{2}	8.00	1.00	0.00
3	3.30×10^{8}	2.10×10^{6}	6.20×10^{4}	2.20×10^{3}	3.00×10^{2}	1.80×10^{1}	1.00×10^{1}	0.00
4	2.00×10^{9}	3.90×10^{7}	8.10×10^{5}	3.40×10^{5}	1.00×10^{3}	7.20×10^{1}	2.70×10^{1}	0.00
5	5.40×10^{8}	6.50×10^{6}	1.20×10^{6}	8.50×10^{4}	5.00×10^{3}	8.20×10^{1}	1.10×10^{1}	0.00
A.M.	6.76×10^{8}	1.50×10^{7}	4.30×10^{5}	8.63×10^{4}	1.31×10^{3}	3.84×10^{1}	1.02×10^{1}	0.00
LOG.OF AVE.NO.	8.8299	7.1761	5.6335	4.9360	3.1173	1.5843	1.0086	0.0000

TABLE 17

Reduction in Numbers of Viable Cells of Salmonella give in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	4.00×10^{8}	1.10×10^{7}	2.50×10^{5}	2.40×10^{4}	2.20×10^{2}	7.00	3.00×10^{1}	0.00
2	8.60×10^{8}	1.40×10^{7}	9.00×10^{5}	5.30×10^{4}	3.00×10^{2}	1.90×10^{1}	1.00	0.00
3	3.70×10^{8}	1.10×10^{7}	3.50×10^{5}	3.80×10^{4}	1.20×10^{2}	1.20×10^{1}	0.00	0.00
4	4.70×10^{8}	2.10×10^{7}	4.30×10^{5}	4.30×10^{3}	1.50×10^{2}	6.00	2.00	1.00
5	4.70×10^{8}	3.60×10^{7}	3.20×10^{5}	1.20×10^{3}	4.00×10^{1}	5.00	0.00	0.00
A.M.	5.14×10^{8}	1.86×10^{7}	4.50×10^{5}	2.41×10^{4}	1.66×10^{2}	1.00×10^{1}	7.00	0.00
LOG.OF AVE.NO.	8.7110	7.2695	5.6532	4.3820	2.2201	1.0000	0.8451	0.0000

TABLE 18

Reduction in Numbers of Viable Cells of Salmonella enteritidis in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	3.30×10^{8}	2.10×10^{4}	8.10×10^{2}	7.00×10^{1}	7.00	2.00	0.00	0.00
2	2.30×10^{8}	2.20×10^{4}	1.80×10^{3}	2.10×10^{2}	7.30×10^{1}	5.00	0.00	0.00
3	3.30×10^{8}	2.70×10^{4}	1.50×10^{3}	2.40×10^{1}	1.30×10^{1}	0.00	0.00	0.00
A.M.	2.97×10^{8}	2.33×10^{4}	1.37×10^{3}	1.01×10^{2}	3.10×10^{1}	2.00	0.00	0.00
LOG.OF AVE.NO.	8.4728	4.3674	3.1367	2.0043	1.4914	0.3010	0.0000	0.0000

TABLE 19

Reduction in Numbers of Viable Cells of Salmonella worthington in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	7.30×10^{8}	2.80×10^{6}	7.40×10^{5}	6.60×10^{4}	6.30×10^{2}	5.00×10^{1}	3.80×10^{1}	0.00
2	4.30×10^{9}	8.60×10^{7}	1.70×10^{7}	3.70×10^{5}	4.80×10^{3}	2.80×10^{3}	3.50×10^{2}	8.20×10^{1}
3	9.30×10^{9}	1.70×10^{8}	1.30×10^{7}	3.00×10^{6}	1.40×10^{4}	2.00×10^{3}	2.40×10^{2}	2.10×10^{1}
A.M.	4.78×10^{9}	8.63×10^{7}	1.03×10^{7}	1.15×10^{6}	6.48×10^{3}	1.62×10^{3}	2.09×10^{2}	3.43×10^{1}
LOG.OF AVE.NO.	9.6794	7.9360	7.0128	6.0607	3.8116	3.2095	2.3201	1.5353

TABLE 20

Reduction in Numbers of Viable Cells of Salmonella typhimurium in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	2.60×10^{9}	3.10×10^{5}	3.10×10^{4}	2.10×10^{3}	4.90×10^{2}	2.20×10^{1}	3.00	0.00
2	3.00×10^{9}	5.10×10^{5}	1.60×10^{4}	8.00×10^{3}	1.90×10^{2}	2.70×10^{1}	4.00	0.00
3	3.10×10^{8}	8.20×10^{5}	2.10×10^{4}	1.00×10^{3}	8.20×10^{2}	1.50×10^{1}	0.00	0.00
A.M.	1.97×10^{9}	5.47×10^{5}	2.27×10^{4}	3.70×10^{3}	5.00×10^{2}	2.13×10^{1}	2.00	0.00
LOG.OF AVE.NO.	9.2945	5.7380	4.3560	3.5682	2.6990	1.3284	0.3010	0.0000

TABLE 21

Reduction in Numbers of Viable Cells of Salmonella senftenberg in Powdered Egg Albumen by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	3.30×10^{9}	2.30×10^{7}	6.20×10^{6}	2.50×10^{5}	7.90×10^{4}	3.20×10^{3}	6.10×10^{2}	9.00
2	1.10×10^{10}	1.30×10^{9}	1.50×10^{8}	3.00×10^{6}	2.60×10^{4}	1.10×10^{4}	6.20×10^{2}	7.00
3	9.20×10^{9}	4.00×10^{7}	5.00×10^{6}	5.60×10^{5}	6.00×10^{4}	8.40×10^{2}	7.00×10^{1}	0.00
A.M.	7.83×10^{9}	4.54×10^{8}	5.37×10^{7}	1.27×10^{6}	5.50×10^{4}	5.01×10^{3}	4.33×10^{2}	5.00
LOG.OF AVE.NO.	9.8651	8.6571	7.7300	6.1038	4.7404	3.6998	2.6365	0.6990

TABLE 22

Reduction in Numbers of Viable Cells of Salmonella heidelberg in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
	COUNTS/GRAM							
1	2.30×10^{8}	2.30×10^{7}	8.70×10	4.30×10^{4}	8.40×10^{2}	6.30×10^{2}	1.50×10^{2}	2.70×10^{1}
2	4.30×10^{8}	4.70×10^{7}	2.40×10^{5}	3.50×10^{3}	3.70×10^{3}	5.10×10^{1}	6.00	1.00
3	4.10×10^{8}	3.80×10^{7}	8.10×10^{5}	4.50×10^{4}	5.10×10^{3}	5.10×10^{2}	5.90×10^{1}	9.00
4	5.30×10^{8}	2.30×10^{7}	4.30×10^{5}	3.10×10^{4}	3.80×10^{3}	6.30×10^{2}	1.90×10^{1}	4.00
5	3.40×10^{8}	8.60×10^{6}	-------	2.00×10^{3}	2.70×10^{2}	1.40×10^{1}	4.80×10^{1}	1.00
6	3.40×10^{8}	2.00×10^{6}	6.20×10^{5}	9.30×10^{4}	4.40×10^{4}	3.10×10^{3}	6.00×10^{1}	3.00
7	1.20×10^{9}	9.30×10^{6}	4.60×10^{6}	2.10×10^{5}	4.90×10^{4}	7.80×10^{3}	1.10×10^{2}	3.50×10^{1}
8	8.20×10^{8}	6.00×10^{6}	4.10×10^{6}	5.60×10^{5}	1.90×10^{5}	1.10×10^{4}	4.40×10^{3}	3.30×10^{1}
A.M.	6.63×10^{8}	1.96×10^{7}	1.46×10^{6}	1.23×10^{5}	3.71×10^{4}	2.97×10^{3}	6.07×10^{2}	1.80×10^{1}
LOG.OF AVE.NO.	8.8215	7.2923	6.1644	5.0899	4.5694	3.4728	2.7832	1.2430

TABLE 23

Reduction in Numbers of Viable Cells of Salmonella give in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	2.60×10^{4}	8.40×10^{6}	1.10×10^{5}	3.00×10^{4}	1.70×10^{2}	1.40×10^{1}	1.00	0.00
2	3.80×10^{9}	7.00×10^{6}	2.60×10^{5}	8.80×10^{3}	2.20×10^{2}	1.30×10^{1}	0.00	0.00
3	1.90×10^{9}	3.80×10^{6}	6.40×10^{4}	4.30×10^{3}	1.00×10^{2}	2.00×10^{1}	0.00	0.00
4	1.60×10^{8}	6.40×10^{5}	9.40×10^{3}	3.60×10^{2}	2.00×10^{1}	7.00×10	1.00	0.00
5	1.40×10^{8}	1.30×10^{6}	4.30×10^{4}	2.10×10^{2}	1.80×10^{1}	7.00	0.00	0.00
A.M.	1.78×10^{9}	4.23×10^{6}	9.73×10^{4}	8.73×10^{3}	1.06×10^{2}	1.26×10^{1}	0.00	0.00
LOT.OF AVE.NO.	9.2504	6.6263	4.9881	3.9410	2.0253	1.1004	0.00	0.00

TABLE 24

Reduction in Numbers of Viable Cells of *Salmonella enteritidis* in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	1.00×10^{8}	-------	4.20×10^{4}	6.90×10^{3}	2.80×10^{3}	1.90×10^{2}	3.50×10^{1}	9.00
2	1.90×10^{8}	6.30×10^{5}	1.40×10^{5}	3.40×10^{4}	-------	1.70×10^{3}	1.10×10^{2}	1.6×10^{1}
3	1.40×10^{8}	2.40×10^{5}	2.50×10^{4}	1.00×10^{3}	4.10×10^{2}	4.00×10^{1}	2.00	0.00
A.M.	1.43×10^{8}	4.35×10^{5}	6.90×10^{4}	1.40×10^{4}	1.61×10^{3}	6.43×10^{2}	4.90×10^{1}	8.00
LOG.OF AVE.NO.	8.1153	5.6386	4.8388	4.1461	3.2068	2.8082	1.6902	0.9031

TABLE 25

Reduction in Numbers of Viable Cells of Salmonella worthington in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	1.10×10^{8}	2.20×10^{6}	1.20×10^{5}	7.70×10^{3}	3.80×10^{2}	4.50×10^{1}	1.00×10^{1}	0.00
2	6.50×10^{8}	1.00×10^{7}	4.50×10^{6}	-------	-------	-------	2.00	0.00
3	1.40×10^{8}	8.30×10^{6}	4.20×10^{4}	2.30×10^{2}	4.80×10^{1}	2.80×10^{1}	8.00	0.00
A.M.	3.00×10^{8}	6.83×10^{6}	1.55×10^{6}	4.00×10^{3}	2.14×10^{2}	3.60×10^{1}	7.00	0.00
LOG.OF AVE.NO.	8.4771	6.8344	6.1903	4.5441	4.1614	3.3263	0.8451	0.0000

TABLE 26

Reduction in Numbers of Viable Cells of Salmonella typhimurium in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	4.40×10^{9}	9.40×10^{5}	4.00×10^{3}	8.80×10^{2}	8.30×10^{1}	1.00×10^{1}	0.00	0.00
2	1.90×10^{8}	3.00×10^{4}	2.80×10^{3}	4.80×10^{2}	1.10×10^{1}	6.00	2.00	0.00
3	1.60×10^{8}	4.70×10^{4}	3.70×10^{3}	4.80×10^{2}	1.70×10^{1}	4.00	4.00	0.00
A.M.	1.58×10^{9}	3.39×10^{5}	3.50×10^{3}	6.13×10^{2}	3.70×10^{1}	6.00	2.00	0.00
LOG.OF AVE.NO.	9.1987	5.5302	3.5441	2.7875	1.5682	0.7973	0.2718	0.0000

TABLE 27

Reduction in Numbers of Viable Cells of Salmonella senftenberg in Powdered Egg Yolk by Gamma Irradiation

Trial	IRRADIATION LEVEL (Megarads)							
	0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
				COUNTS/GRAM				
1	4.70×10^{8}	6.30×10^{7}	1.90×10^{6}	1.90×10^{4}	1.80×10^{3}	4.00×10^{1}	7.00	0.00
2	1.10×10^{8}	3.00×10^{7}	1.10×10^{7}	1.90×10^{5}	4.20×10^{3}	3.70×10^{2}	2.00	0.00
3	1.10×10^{9}	5.10×10^{6}	2.40×10^{6}	2.30×10^{4}	7.20×10^{2}	4.90×10^{1}	1.20×10^{1}	0.00
A.M.	5.60×10^{8}	3.27×10^{7}	5.10×10^{6}	7.73×10^{4}	2.24×10^{3}	1.53×10^{2}	7.00	0.00
LOG.OF AVE.NO.	8.7482	7.5145	6.7076	4.8882	3.3502	2.1847	0.8451	0.0000