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THE EFFECT OF REPEATED TREATMENT WITH
GAMMA RAYS ON THE RADIO-RESISTANCE,
VIRULENCE, AND CULTURE CHARACTERISTICS
OF CERTAIN PATHOGENIC BACTERIA

Final Report

October 1968

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EFFECT OF REPEATED TREATMENT WITH GAMMA RAYS ON THE
RADIO-RESISTANCE, VIRULENCE AND CULTURE CHARACTERISTICS
OF CERTAIN PATHOGENIC BACTERIA

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EXPERIMENTAL METHODS

Preparation of Stock Cell Suspension

The Salmonella serotypes used in this investigation were S. typhimurium, S. newport ATCC 6962, S. thompson ATCC 8391, and S. heidelberg ATCC 8326.

One ml of a 15-hour broth culture was spread over the surface of trypticase soy agar enriched with 5 g yeast extract per liter (TSYE agar) and allowed to grow for 4 days at 37°C. The cells were harvested, washed with phosphate buffer pH 7, resuspended in phosphate buffer and held at 2.2-4.4°C until used. These cells were used in subsequent experiments as the initial inoculum to initiate growth. The amount added was such as to give an initial concentration of 100 cells per ml.

Effect of Growth Stage on Radio-Resistance

Culture tubes (16 x 50 mm) containing 10 ml trypticase soy broth enriched with yeast extract (5 g per liter) were inoculated with the stock cell suspension and then incubated at 37°C for such various periods as 8, 14, 24, 32 and 48 hours. At the end of each period growth was stopped by placing the tube in ice water. One ml of the culture was plated out to obtain the original concentration. The remaining broth was oxygenated for 10 minutes, irradiated at various doses in ice water, then plated on pre-poured plates of TSYE agar by spreading 0.1 ml aliquots of the appropriate dilution over the surface of the agar. Duplicate plates were incubated at 20, 30 and 37°C. Plates held at 20°C were counted after 5 to 7 days, and plates held at 30 or 37°C were counted after 2 to 3 days.

Effect of Aerobic or Anaerobic Growth on Radio-Resistance

Deep culture tubes containing 10 ml broth and 500 cc erlenmeyer flasks containing 100 ml broth were inoculated with cells from the stock suspension. The flasks were then agitated on mechanical shakers for 8 to 14 hours at 37^o C to permit aerobic growth; the deep culture tubes were not agitated during the same time period in order to encourage anaerobic growth.

A 10 ml aliquot of broth culture from the shake flask was transferred to a culture tube and both cultures (aerobic and anaerobic) were oxygenated prior to irradiation in ice water.

Effect of Temperature During Irradiation

The results of this experiment represent a preliminary investigation to establish optimum irradiation conditions for destroying Salmonella in poultry. Although the objective of this particular experiment differs from that of the current investigation, the results are included in this report because they are applicable.

White meat, dark meat and skin of chicken was removed from the bone using aseptic technique and then irradiated with 100,000 rad to destroy most of the natural bacterial flora. The irradiated chicken was held for 24 hours at 2.2-4.4^o C and then comminuted in a Hobart laboratory-model silent cutter while slowly adding an aliquot of a stock suspension of S. typhimurium. The components of the silent cutter had been refrigerated to maintain a low temperature during comminution. The comminuted chicken was filled into 2-ounce screw-cap polyethylene bottles. Six bottles (0^o F) and then irradiated at -17.6^o C in a 30% alcohol-water solution. These were stored at -17.6^o C. Six bottles were chilled to 0^o C and then irradiated in ice water, and six other

bottles were equilibrated to 43.3°C (110°F) and then irradiated at 43.3°C in a water bath. These last two sets of samples were then quick-frozen at -40°C to a center temperature of -17.6°C and stored at -17.6°C .

Bacterial counts were made after 6 days storage.

Determination of Variation in Radio-Resistance

A number of isolated colonies were selected from a spread plate and inoculated into separate tubes of broth. These were incubated for 15 hours at 37°C , oxygenated and irradiated at $0-0.55^{\circ}\text{C}$ ($32-33^{\circ}\text{F}$).

S. newport was irradiated at 35 and 70 K rad, and S. typhimurium was irradiated at 75 and 150 K rad. The decimal reduction dose was determined for each culture from the radiation-survival curve.

Colonies from cells which had survived the high irradiation dose were subcultured in broth for 15 hours at 37°C , oxygenated and irradiated as before. The decimal reduction dose was determined for each of these cultures.

Radio-Resistance of a Broth Culture Grown at a Low Flux of Gamma Rays

A broth culture of S. typhimurium or S. newport was incubated for 2 to 3 hours at 37°C and then transferred to a thermos flask containing water at 38°C . The thermos flask was lowered into a gamma radiation field to a position where the dose rate was low. These dose rates were as follows: run 1 = 60 rad/min; run 2 = 90 rad/min; run 3 = 160 rad/min; run 4 = 280 rad/min. The cultures were exposed to this low intensity field for about 4 to 5 hours at an average temperature of 36°C . A one ml portion of the irradiated culture was subcultured in 10 ml broth overnight and the radio-resistance of the progeny culture was determined.

Effect of Repeated Irradiation on Radio-Resistance of Subcultures.

A tube containing 10 ml broth was inoculated with a culture of Salmonella and then incubated overnight (15-16 hours) at 37°C. One ml of the culture was added to 10 ml of chilled fresh broth, and this was oxygenated by bubbling oxygen through it for 20 minutes in ice water. A portion of the oxygenated broth culture was plated out to determine the initial cell concentration, and the remainder was apportioned equally into two small tubes (12 x 100 mm). These two tubes were then irradiated with two different doses, one that was expected to reduce the concentration of cells from about 10^8 to 10^2 survivors per ml and an intermediate dose. After irradiation an aliquot was taken from each tube for counting survivors, and the remainder of the irradiated culture was incubated for approximately 5 hours at 37°C. One ml portions were subcultured in 10 ml broth overnight (15-16 hours) at 37°C, and then one ml of these cultures was added separately to two tubes containing 10 ml chilled fresh broth. The process of oxygenation, division into two portions, irradiation at the same two previous doses, etc. was repeated. In this manner the survivors of the low irradiation dose were used for perpetuating the culture which was cycled at the low dose, and survivors of the high dose were used for perpetuating the culture cycled at the high dose. This procedure was repeated through 20 irradiation-subculture cycles for cultures of S. typhimurium and S. newport and through 15 irradiation-subculture cycles for cultures of S. thompson and S. heidelberg with two different recycling irradiation doses for each serotype and also two different cultures of each serotype started from different isolates.

Double Irradiation Plating Method for Detecting Resistant Mutants.

Approximately one million cells from a 16 hour broth culture were spread over the surface of TSYE agar plates with a bent glass rod. The plates were then irradiated with a dose that would destroy about 90 per cent of the cells. Following the irradiation the plates were incubated for 3 hours at 37°C and then irradiated with a second dose which would hopefully permit not more than 10 colonies to grow out on the plate after a 48 hour incubation. The first and second irradiation doses were determined by trial and error for two different Salmonella serotypes, and these were as follows:

<u>Serotype</u>	<u>First irradiation dose (Krad)</u>	<u>Second irradiation dose (Krad)</u>
<u>S. newport</u>	15	60
<u>S. typhimurium</u>	25	80

The colonies surviving the double irradiation were tested for radio-resistance using a technique suggested by Greenberg and Woody-Karrer (1963). A standardized loopful of a 16 hour broth culture was streaked linearly across an agar plate. Fourteen different cultures were streaked on a single plate. With S. newport the plate was first irradiated with 15 Krad, incubated at 37°C for 3 hours, then irradiated with 45 Krad and incubated 48 hours. A confluent streak indicated a radio-resistant culture whereas a streak of sparse isolated colonies was indicative of a sensitive culture. The radio-resistance of resistant cultures screened by this method was further verified by determining radiation survival curves. When cultures of S. newport were being tested, a loopful of S. typhimurium was streaked on the same plate since it was

representative of a resistant culture with respect to S. newport and could be used for comparison.

Colonies of S. typhimurium surviving the double irradiation plating technique were screened for radio-resistance in a similar fashion except that the streaked plated were irradiated with 25 Krad, incubated 3 hours at 37°C, irradiated again with 75 Krad and then incubated for 48 hours before examining for confluent or sparse growth.

Comparison of the Virulence of Radio-Resistant Cultures with that of the Non-Resistant Parent Culture.

Radio-resistant cultures of S. typhimurium or S. newport were propagated from isolated colonies recovered from the cultures which had undergone 20 irradiation-subculturing cycles. For virulence testing in mice it was desired to have a cell suspension with a high concentration of viable cells so that the mice could be titrated over a broad range, but also to have a minimal amount of dead cells. It was believed that the dead or non-viable cells might exert a toxic effect, but their numbers could not be quantitated. Therefore, the cultures were grown under aerobic (shakeflask) conditions for 8 hours at 37°C at which time the cells would be in the logarithmic phase of growth. The cultures were centrifuged and then resuspended in chilled, phosphate-buffered peptone water (1 g peptone/liter, pH 7). The volume of peptone water was adjusted so that the final cell concentration would be of the order of 10^{10} per ml. Decimal dilutions were made of this primary cell suspension using chilled peptone water, and these serial dilutions were used for injecting either mice or embryonated eggs. Swiss, white, male mice weighing approximately 18-25 g served as the species of test animal. One-half ml portions of the serially-diluted cell suspensions were

injected intraperitoneally into groups of 10 mice. The mice were observed for deaths over a 3 day period. In some cases 8 day old embryo-nated eggs were used as the test species. The eggs were previously candled to eliminate those with dead embryos. One-half ml portions of the serially-diluted cell suspensions were inoculated into the yolk sac in groups of 6 eggs, and the eggs were observed during a 3 day incubation period at 37°C for mortality of the embryo by candling. A control group of 6 eggs was inoculated with 0.5 ml peptone water and observed during the same period. No embryos in this group died. A plate count was made either on the primary dilution or on several of the dilutions so as to determine the number of viable cells being injected into the test host. With S. thompson or S. heidelberg one ml from the original broth culture which had been subjected to 14 cycles of irradiation and sub-culturing was used for propagating the test culture rather than a colony isolated from the original resistant culture.

Biochemical Characteristics of Radio-Resistant and Sensitive Cultures.

Radio-resistant and sensitive cultures of S. typhimurium, S. newport and S. thompson were streaked on TSYE agar plates. The resistant cultures of S. typhimurium and S. newport were obtained after 20 cycles of irradiation and subculturing, and the resistant culture of S. thompson was obtained after 15 irradiation cycles. Fifty isolated colonies from a culture of each radio-resistant strain and of each sensitive strain were inoculated into each of the following media to test for the biochemical reaction characteristic for Salmonella.

Triple sugar iron agar-----to observe for typical reaction (alkaline slant, acid butt) and production of hydrogen sulfide.

Lysine iron agar----to observe for presence of lysine decarboxylase and hydrogen sulfide.

Phenylalanine agar----to observe for the absence of phenylalanine deaminase.

Simmons citrate agar----to observe for the ability to utilize citrate as carbon source.

Malonate broth----to observe for the inability to utilize malonate.

Urea broth----to observe for the absence of urease.

Tryptone broth----to test for the failure to produce indol.

K C N broth----to observe for failure to grow in the presence of K C N.

All inoculated media was incubated at 37°C and observed after 24 hours. Negative tubes were incubated up to 72 hours. Strains of Proteus, Aerobacter and E. coli were inoculated into the various media to serve as positive controls.

All media were obtained from Difco. The above tests are described by Edwards and Ewing (1964).

Serological reaction

A broth culture of the organism was surface streaked on TSYE agar plates to obtain isolated colonies. Random colonies were inoculated into triple sugar iron agar, and these cultures were used for agglutination tests. Tests for O (somatic) antigens were made by the slide method using Difco Salmonella O Antiserum Poly A-I or a group-specific Difco Salmonella O Antiserum. Tests for H (flagellar) antigens were made by the tube test method with Difco Salmonella Poly H Antiserum. These tests are described in the Difco Supplementary Literature.

Antibiotic Sensitivity

Random colonies from agar plates of radio-resistant or sensitive cells were inoculated into tubes of TSYE broth and incubated overnight.

Approximately five million cells were spread on the surface of pre-poured TSYE agar plates (20 ml agar per 100 mm plate). Sensitivity discs impregnated with the following antibiotics were placed on each plate: Chloramphenicol 5 mcg (BBL), tetracycline 5 mcg (BBL), ampicillin 2 mcg (BBL), ampicillin 5 mcg (BBL), ampicillin 10 mcg (BBL) and penicillin 5 units (Difco). The plates were incubated overnight at 37°C, and the zone of inhibition was then measured with a millimeter rule using the magnifying lens of a Quebec colony counter.

Measurement of Growth Rates

Anaerobic growth: Approximately 100 cells from a 15 hour TSYE broth culture of the radio-resistant or radio-sensitive strain were inoculated into 30 ml TSYE broth in large culture tubes (20 x 175 mm). The tubes were incubated in a water bath at some appropriate temperature, and a 1 ml aliquot was periodically withdrawn. Plate counts were made on TSYE agar with the plates being incubated at 37°C.

Aerobic growth: Approximately 100 cells from a 15 hour TSYE broth culture of the radio-resistant or radio-sensitive strain were inoculated into 100 ml TSYE broth pre-tempered to the desired temperature and contained in a 500 ml Erlenmeyer flask. The flasks were shaken on a mechanical shaker within a walk-in incubator. Aliquots were extracted periodically and analyzed for total viable count.

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RESULTS

In the early stages of this investigation the primary interest was to determine whether or not the survivors of an irradiated broth culture became more resistant to subsequent irradiation. The procedure for determining radio-resistance of the Salmonella serotypes was to inoculate a broth tube from a slant culture maintained at 2.2 to 4.4°C and incubate at 37°C. Decimal reduction doses (D_{10} dose) were estimated from the linear portion of the survival curves which usually exhibited an initial "shoulder" and a "tail". The average D_{10} dose obtained for different isolates of S. typhimurium was 60,000 rad and for S. newport it was 38,000 rad.

Twenty different isolates of S. newport from cells which had survived an irradiation dose of 200,000 rad were sub-cultured in broth for 24 hours and then irradiated a second time. None of these re-irradiated cultures was found to have a D_{10} dose exceeding 47,000 rad (the average D_{10} dose of the control cultures plus 3 standard deviations), thus indicating that their radio-resistance had not changed as a result of having survived a previous irradiation.

Throughout the course of this preliminary work it was noted with both S. typhimurium and S. newport that there was some variability in radio-resistance among control cultures which occasionally gave rise to a pseudo-resistant mutant. Therefore, in order to minimize this variability so as to eliminate these pseudo mutants, it was considered important to study some factors which might influence radio-sensitivity in order to establish a standard protocol.

The first consideration was the possibility of a change occurring in the stock slant culture during storage at refrigerator temperature. However, it was found that there was no significant change in radio-resistance up to at least 5 weeks storage.

It was felt that perhaps the initial quantity of inoculum used for inoculating broth tubes from a slant culture might vary sufficiently to affect the final concentration of cells reached after a 24-hour incubation. Therefore, this procedure was changed to inoculating the broth tubes with a constant known number of cells from a stock cell suspension maintained at refrigerator temperatures.

Effect of Oxygenation on Radio-Resistance.

Another change that was instituted was to bubble oxygen through each broth culture for 15 minutes before irradiating to eliminate any variation in dissolved oxygen content, since it has been well established that dissolved oxygen can markedly affect the radio-sensitivity of vegetative bacteria. As a result the D_{10} dose for S. typhimurium decreased from approximately 60 K rad (anoxic) to approximately 19 K rad (oxygenated); for S. newport the change was from approximately 38 K rad (anoxic) to 12 K rad (oxygenated). This represents a ratio of approximately three for D_{10} anoxic to D_{10} oxygenated. Ley et al (1963) similarly reported a value of approximately three for D_{10} anoxic/ D_{10} aerated for several Salmonella serotypes irradiated in phosphate buffer.

Effect of Growth Stage on Radio-Resistance

It has been stated in the literature that, in general, vegetative bacteria are most resistant to ionizing radiation during the lag period; they are least resistant during the period of logarithmic growth; and their radiation resistance returns when they have progressed into the stationary phase (Stapleton, 1955). A study was carried out to determine the degree of variability that might exist as a result of irradiating Salmonella at different stages of their growth cycle. Growth curves were determined for S. typhimurium and S. newport at 37°C to establish

the approximate times when the cells were in a particular phase of growth. There has been some evidence presented in the literature demonstrating that for some organisms an increase in recovery following irradiation results of the irradiated cells are incubated at a lower temperature than the optimum growth temperature (Stapleton et al, 1953). An explanation of this phenomenon may be that if an irradiated cell, which may have damaged DNA, is incubated at optimum growth temperature, the cell attempts to undergo division, and replication of the damaged DNA may lead to a lethal mutation. On the other hand, if the irradiated cell is incubated at a sub-optimal temperature so that its metabolism is slowed down, then certain repair mechanisms may have a chance to operate and repair the irradiation damage before the cell divides. The results of a combined study on the effect of growth stage and incubation temperature of the plates following irradiation are presented in Tables 1 and 2 for the Salmonella serotypes, typhimurium and newport. The 8-hour culture represents the logarithmic growth phase, the 14-hour culture represents the transitory stage from logarithmic to stationary phase, and the 24-, 32- and 48-hour cultures represent different periods of the stationary phase.

With regard to the incubation temperature of plates for maximum recovery of survivors following low dose irradiation treatments, there did not appear to be a significant difference between 20, 30 and 37°C. It seems, however, that at the higher irradiation doses, there was a slightly greater recovery of S. typhimurium at 37°C compared to 20°C, whereas with S. newport the reverse seemed to be the case.

For each run the D_{10} dose was estimated from the slope of the linear portion of the survival curve and these values are given as the last

entry in each row for a given set of data in the Tables 1 & 2. These D_{10} values have been plotted in Fig. 1 as a function of age of culture. It can be seen that cultures of S. typhimurium were least resistant to radiation when in the log phase (8-hour culture), more resistant when in the early stationary phase (14- to 24-hour culture) and most resistant in the late stationary phase, particularly at 48 hours. S. newport showed similar behavior except that there was no difference in radio-resistance among the 8-, 14- and 24-hours cultures. This general pattern of radio-resistance as a function of culture age is well documented, but what is of interest here is the fact that in the case of Salmonella, the decimal reduction dose can vary by a factor of two to three due to the age of the culture. Since these results indicated no difference in radio-resistance among 14- and 24-hour cultures for the 2 serotypes, a 15-hour growth period prior to irradiation was adopted as standard procedure.

Effect of Incubation Temperature of Plates after Irradiation

The previous observation that at the high irradiation dose a slightly greater recovery of survivors was obtained at 37°C for S. typhimurium and at 20°C for S. newport aroused an interest as to the effect of incubation temperature on recovery of other irradiated Salmonella serotypes. Consequently, 15-hour broth cultures of S. heidelberg and S. thompson were irradiated with various doses and petri plates were incubated at either 20, 30 or 37°C. The results of these experiments (Tables 3 and 4) are similar to those obtained with S. typhimurium, i.e., no drastic effect of incubation temperature on the D_{10} dose but a slightly greater recovery rate at 37°C as compared to 20°C for the survivors of the high dose irradiation.

Effect of Time Lag between Irradiation and Plating

The experimental procedure had been to irradiate the broth cultures in ice water and to maintain the irradiated cultures in ice water until plated out which usually occurred within two hours following irradiation. The thought arose the perhaps repair of irradiation damage was completed during the time lapse between irradiation and plating and for this reason, the harmful effect to be expected from incubating radiation survivors at the optimum growth temperature (37°C) was not observed. Roberts and Aldous (1949) had shown that there was a tremendous difference in the recovery of u.v.-irradiated E. coli depending upon the time after irradiation at which the plating was done. Therefore, 2 tubes of a broth culture of S. typhimurium were irradiated in ice water at 2 doses, one at 100 K rad, the other at 140 K rad.

Immediately after irradiation a bacterial count was made on both tubes and then again after 1, 2 and 3 hours during which time the tubes were kept in ice water. The result of this experiment, which is presented in Table 5, indicated that the number of irradiation survivors remained constant during the 3-hour period at 0°C following the irradiation.

It then became of interest to determine the fate of irradiated cells held at refrigerator temperatures over a long time period since in this study we have only been determining radiation survival immediately following irradiation. Consequently, broth cultures of S. typhimurium and S. newport were irradiated with various doses, plated out immediately following irradiation and then again after different storage periods up to 15 days at 2.2 to 4.4°C . The results are presented in Table 6. During the 15-day storage period the concentration of the non-irradiated

cells remained constant. The cultures treated with a low irradiation dose had decreased slightly by the end of the storage period, but the cultures which had received the highest dose had decreased by a factor of about 10 at the end of the storage period. It does not appear that any significant repair of irradiation damage is taking place during a holding period at 2.2 to 4.4°C. On the contrary, the irradiated cells appear to be slowly dying off.

Roberts and Aldous (1949) noted that the recovery of U.V.-irradiated E. coli was influenced by the moisture content of the agar plates. Therefore, an experiment was carried out with S. typhimurium and S. newport to determine whether the age of the pre-poured plates which were being used had any effect on the quantitative recovery of irradiation survivors. No difference in recovery was observed when irradiated cells were plated on agar plates which had been previously held 1, 2, 3, or 4 days at room temperature.

Effect of Broth Composition

Another possible source of variation in radio-resistance among individual broth cultures might be due to differences in the final composition of the broth itself. Since the cultures were being irradiated in the original growth medium, there may be differences in the final concentration of metabolic end products which may either protect or sensitize the culture. Therefore, the radio-resistance of S. typhimurium and S. newport was determined in the original growth medium and in the original growth medium diluted 1 to 10 with fresh broth (pH adjusted to that of final growth). The radiation inactivation in these two different substrates was about the same (Figures 2 and 3) and this would rule out any variability in radio-resistance arising from the particular substrate used in this study.

Effect of Aerobic or Anaerobic Growth

Hollaender and his associates (1951) have shown that for E. coli differences in radio-resistance resulted depending on whether the organism was grown aerobically or anaerobically and irradiated aerobically or anaerobically. In the present investigation the organism has been grown overnight in culture tubes, which essentially is an anaerobic fermentation although some aerobic growth may occur close to the surface, and the culture was oxygenated prior to irradiation. Some experiments were conducted to compare the radio-resistance of cultures grown aerobically and cultures grown anaerobically. Irradiation inactivation curves for cells grown under these conditions are presented in Figures 4, 5, 6 and 7. In the first experiment S. typhimurium and S. newport were grown aerobically (shake flask) or anaerobically (culture tube) for 14 hours. The radiation inactivation was much greater for the cultures grown anaerobically than for those grown aerobically. However, the cells grown aerobically had a much higher initial count and could have been in a different stage of growth compared to the anaerobic cultures. Therefore, the experiment was repeated but this time the cells were only allowed to grow for 8 hours to ensure that both cultures (aerobic and anaerobic) would be in the same stage of growth (logarithmic) during irradiation. The result of this experiment was inconclusive. With S. newport and S. thompson the radio-resistance of the aerobic and anaerobic cultures was the same, but with S. typhimurium the anaerobic culture was more radio-resistant than the aerobic culture and with S. heidelberg the aerobic culture was the more radio-resistant. This discrepancy was probably due to some environmental factor which

could not be explained. No further experiments were conducted along this line.

Effect of Growth Temperature

The temperature at which the cultures were grown prior to irradiation was found to influence radio-resistance. Cultures of S. newport or S. heidelberg were more radio-resistant when grown at 37°C than at 30 or 20°C (Figures 8 and 9). Bruce and Maaløe (1956) also found S. typhimurium to be more radio-resistant when grown at 37°C as compared to 25°C. There did not appear to be any difference in radio-resistance among cultures grown at 20 to 30°C. Since the optimum growth temperature for Salmonella is about 37°C, cells grown at this temperature may have synthesized a greater amount of protein or DNA than cells grown at sub-optimal temperature and this might be a possible explanation for the higher radio-resistance.

Effect of Temperature during Irradiation

That there is an effect of temperature during irradiation on radio-resistance of microorganisms is known, and Figure 10 shows the advantage to be gained in inactivating S. typhimurium in chicken by irradiation at a moderately high temperature (43.3°C) compared to lower temperatures (0 or -17.6°C). For example, the per cent survival after an irradiation dose of 200,000 rad was 0.01, 0.38 and 3.8 for irradiation temperatures of 43.3°C (110°F), 0°C (32°F) and -17.6°C (0°F), respectively. There was no significant reduction in viable cells at 43.3°C for the length of time (36 minutes) required to administer the maximum irradiation dose.

Based upon the previous findings, a standard procedure was adopted for determining radio-resistance of salmonella cultures. This was to inoculate and incubate tubes of TSYE broth for 15-16 hours at 37°C, arrest growth by placing the culture tubes in ice water, and oxygenate for 15 minutes and then irradiate in ice water. Maintain culture tubes in ice water following irradiation, but plate out survivors within 2 hours.

Effect of Incubation Temperature on Survival Following a High Irradiation Dose

To resolve the question of optimum incubation temperature for irradiated cultures, 41 random isolates of S. newport and 19 random isolates of S. typhimurium were sub-cultured in broth and irradiated with a dose resulting in 10^{-5} to 10^{-7} per cent survival. Portions of 0.5 ml were plated on 10 replicate plates and a set of 5 plates was incubated at 20°C and another set at 37°C. The results are tabulated in Tables 7 and 8. With S. typhimurium it is readily apparent from the high frequency of values of survival ratio 20/37°C of less than 1 that an incubation temperature of 37°C yielded a greater number of survivors. In the case of S. newport the results were not as one-sided and thus had to be subjected to a statistical analysis. The average ratio of per cent survival at 20°C to per cent survival at 37°C was found to be 0.98 and when this value was compared to 1.0 (ratio when % survival at 20°C = % survival at 37°C) by the t test, the difference was not significant at the 95 per cent level. This indicates that for S. newport an incubation temperature of 20°C is as good as 37°C for quantitative recovery of irradiation survivors.

Radio-Resistance of Cultures Grown in a Low-Intensity Radiation Field

Thus far only a fraction of the survivors in the irradiated broth culture had been tested for radio-resistance. In this manner there is

a possibility of missing a resistant mutant in the random selection of isolates to be tested. Therefore, an experiment was initiated whereby the culture was allowed to grow while being exposed to a low dose rate of gamma rays. It was considered that any resistant mutants that were present initially in the culture or that might be induced during the irradiation would continue to grow and multiply at the low dose rate, whereas the sensitive cells would be inactivated, thus enriching the culture with resistant mutants. After exposure to the low intensity radiation, the survivors were subcultured and the radio-resistance of the progeny was determined. These results are shown in Table 9 for S. typhimurium and S. newport. It is not considered that the condition of growing the cells in a low intensity radiation field effected an increased radio-resistance in the cells. In run 1 the concentration of S. newport just prior to being placed in the radiation field was 2.9×10^8 per ml and after 5 hours exposure at 37°C to a dose rate of about 60 rad per minute (total absorbed dose = 18,000 rad), the final concentration of viable cells was 3.2×10^8 per ml. Thus, the growth rate was balanced by the death rate. With S. typhimurium the concentration of viable cells before and immediately after the radiation exposure was 4.7×10^6 per ml and 1.8×10^8 per ml, respectively. In this case the growth rate exceeded the death rate which would be expected in this particular case since typhimurium is more radio-resistant than newport. Nevertheless, in either case this would indicate that the intensity of the radiation should have been sufficiently mild for favoring survival of resistant mutants had there been any. In the other 3 runs, in which the cultures were exposed

to higher radiation intensities, a quantitative count before and after exposure was not made. However, observation of the culture under phase microscope revealed less viable cells after irradiation than before, indicating that the death rate exceeded the growth rate. With both S. typhimurium and S. newport many filamentous cells were observed after the radiation exposure and this phenomenon has been previously reported for other irradiated bacterial species, particularly E. coli.

Effect of Repeated Irradiation on Radio-Resistance of Subcultures

Some investigators (Gaden and Henley, 1953; Erdman et al, 1961) have been able to increase the radio-resistance of some bacterial species by repeated irradiation and subculture of the survivors. However, Erdman et al (1961) did not succeed in increasing the radio-resistance of Salmonella gallinarum with 14 successive irradiation cycles. In this present study an experiment of this type was initiated with cultures of S. typhimurium, S. newport, S. thompson, and S. heidelberg. Two cultures of each serotype were recycled (irradiated and subcultured) with two different doses, a low dose expected to result in about a 2 log cycle reduction in count and a higher dose expected to give about a 5 log cycle reduction. The rationale for studying the effects of two different cycling doses was that although one might have expected a priori that a higher dose might be more favorable in selecting the more resistant cells, the possibility exists that a massive dose may inactivate resistant as well as sensitive cells, particularly if the difference in radio-resistance is not too great, and the survivors that would be obtained under this condition might only be chance survivors of sensitive cells.

The results of these cyclic irradiation experiments are presented in Tables 10 through 25. The tables show the concentration of cells

prior to irradiation and after irradiation at the two different doses, and the same data converted to per cent survival. To determine whether or not a change in radio-resistance was occurring with each radiation cycle, one might compare either per cent survival at a given dose of the D_{10} dose. In the tables an estimate of the D_{10} dose is given; however, it should be pointed out that the survival curves usually had an initial lag or shoulder; and, therefore, the value of the D_{10} dose was estimated from survival points at two irradiation doses only and did not include the zero dose point. Therefore, a comparison of radio-resistance cannot be made on the basis of D_{10} value alone since in some instances an increase in radio-resistance might have occurred; but this was only manifested by an extension of the shoulder of the survival curve while the slope of the secondary linear portion of the survival curve did not change.

A study of the data presented in Tables 10 through 25 shows that the various cultures did become more radio-resistant upon repeated irradiation and sub-culturing. The increase in resistance appeared to occur in steps rather than at a progressively increasing rate. This phenomenon is indicated in Fig. 11 with S. typhimurium whereby the percentage of cells surviving a dose of 110 Krad has been plotted as a function of the radiation cycle. The graphs have been drawn by visual approximation and thus should only be considered to indicate the trend in which radio-resistance is changing with repeated irradiation and sub-culturing. Nevertheless, these trend lines do suggest that the cultures had not attained maximum radio-resistance after 20 radiation cycles and also that the first change in radio-resistance occurred earlier in the cultures subjected to the 60 Krad cycling dose compared

to cultures cycled at 110 Krad. If for reasons of comparison we arbitrarily designate a culture as having become radio-resistant when the per cent survival at the highest dose used (in this study) increases for the first time by a factor of 100 (2 log cycles), the following statements can be made: Cultures of S. typhimurium cycled at 60 Krad became radio-resistant after the fifth or sixth cycle, whereas with a cycling dose of 110 Krad the change in radio-resistance was not evident until the tenth or eleventh cycle. With a cycling dose of 30 Krad cultures of S. newport became radio-resistant after the fifth or sixth radiation cycle and with a cycling dose of 60 Krad the cultures were considered radio-resistant after the ninth radiation cycle. Cultures of S. heidelberg were regarded as being radio-resistant after six radiation cycles at 70 Krad or eight to ten radiation cycles at 120 Krad. In the case of S. thompson cultures cycled either at 70 Krad or 120 Krad became radio-resistant after twelve or thirteen cycles.

To determine whether or not the mere sub culturing of a broth culture affected the radio-resistance, one of the S. thompson cultures (Isolate 1, Table 18) was subjected to 14 sub-culture treatments with no irradiation. The radio-resistance of the culture was checked at various transfers. The following results were obtained:

Transfer	Cells/ml after a dose of:			% Survival after a dose of			D ₁₀ Krad
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	50 x 10 ⁶	48 x 10 ⁴	104 x 10 ¹	100	0.95	0.0021	19
10	114 x 10 ⁶	12 x 10 ⁴	19 x 10 ¹	100	0.18	0.0002	18
14	108 x 10 ⁶	24 x 10 ⁴	54 x 10 ¹	100	0.22	0.0005	19

Since there was no increase in radio-resistance of the culture after 14 transfers, it is concluded that the act of subculturing did not contribute to the increased radio-resistance which resulted from repeated irradiation and sub-culturing.

Stability of Radio-Resistance Characteristic of Resistant Cultures.

Two random isolated colonies were selected from the agar plates which contained the survivors of the twentieth irradiation-subculture cycle for S. typhimurium and S. newport. Since originally two different broth cultures (Isolate 1 and Isolate 2) of each serotype had been treated with two different cycling doses, this meant a total of eight colonies each of S. typhimurium and S. newport had been selected. These individual colonies were sub-cultured in broth, and the radio-resistance of the individual broth cultures was determined. The individual isolates were found to be as radio-resistant as the resistant culture from which they were derived. Cultures from these isolates were then put through a series of transfers until each culture had been transferred 24 times in broth. At various intervals of transfer the radio-resistance of the cultures was determined. The results appear in Tables 26 through 29. Although some fluctuation in radio-resistance was observed during the 24 transfers, this can probably be attributed to experimental error; and it is concluded that the cultures retained their radio-resistance throughout the 24 transfers. Thus, the increased radio-resistance of these cultures is an acquired characteristic and not a transient one. The criterion for radio-resistance was regarded as the value of per cent survival following the highest irradiation dose used in obtaining survival data. In this particular study this was 110 Krad for the culture of S. typhimurium and 60 Krad for the culture of S. newport.

Effect of Stage of Growth Cycle on Radio-Resistance of Resistant Cultures.

The radio-resistance of the resistant cultures had been determined consistently on overnight (15 hours) broth cultures. To determine whether the increased resistance was characteristic of the culture at other stages of growth, broth cultures of the resistant strain and the non-resistant parent strain were irradiated while in the logarithmic phase of growth (7 hours), the early stationary phase (15 hours), and a later period of stationary phase (24 hours). Survival curves of S. typhimurium and S. newport are presented in Figures 12 and 13. It is apparent that the increased resistance of the resistant cultures was inherent at the various stages of growth. The 24 hour cultures (Stationary phase) were more radio-resistant than the 7 hour cultures (log phase), as would be expected; but it should be noted that the increased radio-resistance was manifested more so in an extension of the shoulder of the survival curve than in a change of slope of the linear portion of the survival curve. It should also be noted that the radio-resistant cultures while in the logarithmic growth phase were still more resistant than the non-resistant cultures in the stationary phase.

Double Irradiation Plating Method for Detecting Resistant Mutants

Witkin (1946) devised a double irradiation technique for selecting radio-resistant mutants from a population of E. coli cells. The method consisted of irradiating cells, which had been streaked on an agar plate, with a 90 per cent reduction dose; incubating the survivors, which theoretically should be enriched with resistant cells, for 3 hours to allow microcolony formation; then giving a second higher dose which conceivably would inactivate all the cells in a microcolony made up of sensitive cells, but which would permit some cells to survive in a microcolony composed of resistant cells and ultimately form a colony of

resistant cells. Colonies isolated in this fashion were then tested for radio-resistance by irradiating a broth culture and then streaking a loopful of the irradiated culture on an agar plate. After a brief incubation period the streaks were observed microscopically (high-dry power). The presence of normally dividing cells in microcolonies was indicative of radio-resistance, whereas the presence of elongated filamentous cells was taken to indicate that the cells were sensitive to radiation. An attempt was made to apply this same technique for testing radio-resistance of some Salmonella isolates obtained by the double irradiation plating method; however, this technique was soon abandoned because no clear cut association was observed between radio-resistance and normal cell division following irradiation. Curry and Greenberg (1962) also reported that some radio-resistant strains of E. coli became filamentous after irradiation. Probably the most reliable method for assaying radio-resistance is to obtain radiation survival data at several doses, however, it was felt that this method would be too tedious for application in testing a large number of double irradiation survivors. Therefore, a rapid qualitative technique was adopted in which a loopful of broth culture of the isolate being tested is streaked linearly across an agar plate and then subjected to two intermittent irradiations. A confluent streak after incubation denotes a radio-resistant culture and a streak with sparse isolated colonies indicates a sensitive culture (see section on Methods).

Five hundred and two isolates of S. newport obtained by the double irradiation plating method were screened for radio-resistance by the loop streak method. Seven of these isolates were found to be radio-resistant. Broth cultures of these seven isolates were then irradiated at several dose levels, and the survival data is presented in Table 30.

A control culture is included for comparison. From the radiation survival data it can be seen that all seven isolates were truly radio-resistant.

These seven radio-resistant isolates were found among 502 isolates which had survived the double irradiation plate technique. The 502 isolates had been collected from a total of 65 plates. The total number of cells deposited on these 65 plates prior to the double irradiation treatment was quantitatively determined to be 62.3 million. Thus, for an estimate of the frequency of radio-resistant cells of S. newport in a population, a value of seven resistant per 62.3 million cells, or one resistant per 8.9 million cells was obtained.

To determine the effect of irradiation on the induction of mutation to radio-resistance, the following experiment was conducted: A broth culture of S. newport was irradiated with a dose of 75 Krad so as to reduce the population of cells from an initial concentration of about 1×10^8 per ml to a final concentration of about 10 per ml. One ml of the irradiated culture was sub-cultured in fresh broth overnight, and the progeny of the irradiation survivors were streaked on plates which were then subjected to the double irradiation plating technique. Two hundred and sixty isolates were recovered after the double irradiation on a total of 64 plates. Of the 260 isolates only four proved to be radio-resistant. Thus, in this particular case the frequency of radio-resistant cells was estimated to be four per 64 million cells or one resistant cell per 16 million cells. Since in a population of Salmonella derived from irradiated parents the frequency of radio-resistant mutants was not greater than the case in which the population was derived from non-irradiated parents, it does not appear that the radio-resistant mutants are arising so much as a result of an induction

process but more so as the result of a selection process by the irradiation treatment.

Luria and Delbruck (1943) devised a method known as the fluctuation test for specifically determining whether a mutation is spontaneous or induced. An attempt was made to apply this technique to determine the origin of mutation to radio-resistance of S. newport. Briefly, the method consists of comparing the number (variance) of mutants obtained from a number of independent cultures with the number (variance) of mutants obtained from the same number of samples taken from a single culture. The results of two trials are presented in Table 31. In general, this experiment was not as successful as it was hoped to have been. The reason for this was that the double-irradiation did not exclusively permit survival of resistant cells. Had this been the case, then the analysis of the experiment would have simply been to determine the variance for the number of cells after double-irradiation for the situation when the samples were taken from independent cultures and also for the case when the samples originated from a single culture and then to compare the two variances. However, since some of the survivors of the double-irradiation could have been sensitive cells, then each survivor had to be screened for radio-resistance by the loop-streak method. As a result, in the first experiment a total of three resistant cells, each from a different tube, was found among samples taken from independent cultures; but no resistant cells were found among samples taken from a single culture. These results suggested that the mutation to resistance was spontaneous since resistant cells were uniformly absent among tubes from the single culture and randomly present in a few tubes of the independent cultures. The experiment was repeated, and this

time there were no resistant cells among 15 independent samples, but, three resistant cells in 15 samples from the same culture. This result is not in accord with the previous result. The few resistant cells among the samples from the single culture can be explained on the basis that in the single culture a mutation to resistance had occurred just a few divisions before the samples were taken thus yielding a low probability of finding a resistant cell in every sample. However, the presence of no resistant cells among any of the samples from the independent cultures cannot be explained except as was previously stated, the particular conditions employed in the double-irradiation method may not have been optimum for quantitative recovery of resistant cells.

In an effort to improve the selectivity of the double-irradiation method several modifications were made, but first of all survival curves were determined for radio-resistant and non-resistant cultures of S. newport irradiated on the surface of TSYE agar plates. These survival curves are shown in Fig. 14A. In one experiment in which a modified double-irradiation procedure was employed the primary irradiation dose was increased to 20 Krad, the incubation period was still maintained at 37°C for three hours, and the secondary irradiation dose was decreased to 40 Krad. All the cells surviving this particular sequential treatment were radio-sensitive, yet from the survival curve it can be seen that had there been any resistant cells present, they should have survived the irradiation treatment which was relatively mild for the resistant cells compared to the sensitive cells. Whereas the primary irradiation dose of 20 Krad was expected to inactivate about 95 per cent of the sensitive cells, the same dose should only inactivate about 40 per cent of the resistant cells. The secondary irradiation dose of 40 Krad should only have destroyed about 70 to 75 per cent of the resistant cells. Thus, if any resistant cells had survived the first irradiation treatment, and had formed microcolonies

containing as few as 10 to 20 cells after the three hour incubation period, a sufficient number of resistant cells in each microcolony should have survived the second irradiation treatment (40 Krad) to ultimately produce a colony. In another experiment the cell inoculum per plate was increased to 1×10^8 to raise the probability of finding a resistant mutant, the primary irradiation dose was increased to 20 Krad, the incubation period still remained three hours at 37°C , and the secondary irradiation dose was increased to 80 Krad. Twenty three survivors were obtained on a total of three plates and all were radio-sensitive. Thus, it was considered that although it may have been possible to arrive at some prescribed set of conditions for the double-irradiation which would exclusively isolate the resistant cells in a population, time did not allow it. Therefore, the fluctuation test of Luria and Delbruck was discontinued, and it was not possible to determine the mutation rate to radio-resistance.

All experiments with Salmonella in this investigation were conducted with smooth colony forms. The double irradiation technique was applied to cultures derived from rough colonies of S. newport to compare the frequency of resistant cells with those found in cultures propagated from smooth colonies. The same conditions of double irradiation were employed for the rough cultures as for the smooth cultures, that is, a first dose of 15 Krad, a three hour incubation at 37°C , then a second dose of 60 Krad. A total of 9.2×10^6 cells was deposited on the surface of six agar plates. From these six agar plates 102 survivors were recovered after double irradiation. Five of these survivors were found to be radio-resistant by the loop streak test. This gives a frequency of one resistant cell per 1.8×10^6 cells for the rough culture compared to a frequency of one resistant cell per 8.9×10^6 cells for the smooth

culture.

The double irradiation technique was also applied to cultures of S. typhimurium to determine the frequency of resistant cells. Since this serotype was more radio-resistant than S. newport, the doses for the double irradiation were slightly higher. The primary dose was 25 Krad; the incubation period was three hours at 37°C; the secondary dose was 80 Krad. From a total of 50 plates containing a total of about 56 million cells, 240 survivors were recovered. Only three of the 240 survivors were proved to be resistant by the loop streak test (also confirmed by survival curves). A frequency of about one resistant cell per 19 million cells was thus obtained.

Comparison of the Virulence of Radio-Resistant Cultures with that of the Non-Resistant Parent Culture.

A comparison of the virulence of radio-resistant cultures of S. thompson, S. newport, S. typhimurium and S. heidelberg with their corresponding non-resistant parent culture can be made by referring to Tables 32 to 35. In these tables the number of deaths which occurred in a group of 10 mice, one two or three days after being injected with viable cells, is recorded as a function of the number of viable cells injected. The LD₅₀ (number of cells required to kill 50% of the mice) was estimated for both the radio-resistant cells and the non-resistant cells. The per cent mortality which had occurred by the end of the maximum observation period (usually 3 days) was graphed as a function of the log of the number of viable cells injected. The number of cells causing 50 per cent mortality was determined by graphical interpolation. Using this LD₅₀ value as the criteria of virulence, it can be concluded that the radio-resistant cells were not more virulent than the non-resistant cells since the differences between corresponding LD₅₀s were

usually not remarkable and could be ascribed to experimental error. However, in the experiment involving S. typhimurium as the test organism (Table 34) the LD₅₀ for the radio-resistant culture was approximately 10 times greater than the LD₅₀ for the non-resistant culture. A difference of this magnitude might be large enough to be of statistical significance. This would indicate that for S. typhimurium the resistant cells were less virulent than the non-resistant cells. The number of cells required to cause death was generally of the order of 10^7 to 10^8 . This is in accord with the results of Krishnapillae et al (1964) who reported that some strains of Salmonella must be injected in large numbers, about 10^8 cells, to produce toxic effects in mice of a similar size used in the present study.

The results using chick embryos as host seemed to confirm those obtained with mice, that is, that the radio-resistant cells were not more virulent than the non-sensitive cells (see Tables 36 and 37). The chick embryo was considerably more sensitive as a host than the mouse since the introduction of only a few cells was sufficient to eventually result in death. In the case of the chick embryo death was most likely due to an infection whereas with the mice death was more likely caused by an intoxication since a tremendously large number of cells were involved. Another criterion of virulence is the rapidity with which death occurs once the host has been infected. There did not appear to be any important difference in this respect between the radio-resistant cultures and the non-resistant cultures. The data obtained at the highest dilution injected (0.03 cells) of the non-resistant culture of S. newport were judged to be erratic since they were not consistent with the other data from the same experiment. These data were not considered in estimating the LD₅₀ for the non-resistant cells.

Passage of microorganisms through animals has been known to effect an increase in virulence. Therefore, one experiment was directed to ascertain whether the radio-resistant culture might become more virulent after passage through a live host as compared with the non-resistant culture. For this experiment radio-resistant and non-resistant cells of S. thompson were recovered from chick embryos which had previously died as the result of being infected with a few cells (about 3 to 5) of S. thompson, either the radio-resistant or non-resistant strain. One ml of yolk fluid was pipetted from a dead embryo, and suitable dilutions were streaked on brilliant green sulfadiazene agar. An eight hour broth culture was prepared from typical isolated colonies after being confirmed as Salmonella by serological agglutination and growth on triple sugar iron agar. The virulence of the radio-resistant and non-resistant cultures was then determined using mice as the test animal. The results which appear in Table 38 again indicate that the radio-resistant culture was not more virulent than the non-resistant parent culture. Prior to animal passage the mouse LD₅₀ for the non-resistant culture was about 1.5×10^8 cells, and after one passage through chick embryo the mouse LD₅₀ was about 3.5×10^7 cells. This decrease in LD₅₀ value after animal passage is indicative of an increase in virulence. Similarly with regard to the radio-resistant culture, the mouse LD₅₀ value before chick embryo passage was about 3.5×10^8 cells and after chick embryo passage was about 1.1×10^8 cells. This slight decrease in LD₅₀ value would also indicate that the radio-resistant culture became more virulent after one passage through the chick embryo. Since the variability associated with this type of experiment was not known, it cannot be stated with any degree of statistical certainty whether the increases in virulence

which occurred in the radio-resistant and non-resistant cultures after one animal passage were significant. However, since the extent of the change in mouse LD₅₀ for both cultures after chick embryo passage was not too different, it can be concluded that the radio-resistant culture was not more prone to increase in virulence after one animal passage as compared to the non-resistant culture.

Virulence in pathogenic bacteria has been associated with "smooth" colonies, the "rough" colonies usually giving rise to avirulent cells. Smooth colonies sometimes revert to the rough form, but the opposite reversion has not usually been observed. It was therefore of interest to determine whether or not conversion of rough to smooth could be induced by irradiation. Fourteen agar plates were each streaked with approximately one million cells from a "rough" broth culture of S. newport. These plates were then subjected to a double irradiation treatment (15 Krad primary dose, 3 hours incubation at 37°C, then a second dose of 60 Krad). Two hundred and thirty four cells survived this treatment, and the resulting colonies were all "rough".

Biochemical Characteristics of Radio-Resistant and Radio-Sensitive Cultures.

The biochemical reactions of radio-resistant and radio-sensitive cultures of several Salmonella serotypes on various media are presented in Table 39. The object of this study was to determine whether or not irradiation might produce mutants with abnormal biochemical characteristics in which case identification of these mutants as Salmonella by biochemical tests would be hindered. The particular medium or test employed are among those most commonly used in the identification of Salmonella.

The sensitive cultures of the four serotypes tested (newport, thompson, heidelberg, typhimurium) all gave typical reactions on the various media used with one exception.

A few radio-sensitive cultures of S. newport were able to grow in KCN medium, therefore, this particular test was discontinued since the test characteristic also varied among the control cultures. Some of the radio-resistant cultures of S. newport also exhibited growth in the KCN medium.

However, some radio-resistant cultures were found which did have one or two abnormal biochemical characteristics. Eleven of the resistant cultures of S. newport did not utilize citrate; among the resistant cultures of S. thompson five did not utilize citrate, two did not produce hydrogen sulfide nor utilized citrate. Radio-resistant cultures of S. heidelberg or S. typhimurium did not show a change in any of the biochemical reactions tested. The known controls (Proteus, Aerobacter, E. coli) all gave reactions on the various media which were typical for the species thus validating the test procedure. There are normally some Salmonella serotypes which do not utilize Simmon's citrate and some which do not produce hydrogen sulfide on triple sugar iron agar. Therefore, while the cyclic irradiation did not cause a basic change in the biochemical pattern, thus making genus identification still possible with biochemical tests, the irradiation did in some instances produce a minor change which based on biochemical properties would appear to have converted on Salmonella serotype to another. Unfortunately, the biochemical mutants that were found among the radio-resistant cultures were not typed serologically to determine whether conversion to a different serotype had occurred. Erdman et al (1961) found no change in biochemical reactions of a culture of S. gallinarum which had been subjected to 14 cyclic irradiations at 200,000 rep whereas a culture of E. coli failed to produce indol after 12 irradiation cycles.

The appearance and reactions of Salmonella on certain differential media is often employed as an aid in identification. Therefore, colony appearance of the parent radio-sensitive cultures and radio-resistant cultures of four Salmonella serotypes was compared on brilliant green agar, bismuth sulfite agar, Salmonella-Shigella agar and MacConkey agar. Results of this experiment appear in Table 40. There was no noticeable difference in appearance or reaction between the sensitive colonies and resistant colonies on any of the four diagnostic media. All the colonies of the four serotypes (S. newport, S. thompson, S. heidelberg and S. typhimurium) appeared similar on brilliant green agar, on Salmonella-Shigella agar and also on MacConkey agar. However, there were differences among serotypes and even within a serotype of the sensitive cultures on bismuth sulfite agar. Thus, on this particular medium there was more variation between serotypes than there was between radio-resistant and radio-sensitive cultures.

One experiment was carried out to compare the recovery of radio-sensitive and radio-resistant cultures of S. newport on various differential media since the previous experiment indicated that there was an apparent difference. The test media included tryptic soy yeast extract agar (a non-selective medium), brilliant green agar, bismuth sulfite agar, MacConkey agar, desoxycholate citrate agar and Salmonella-Shigella agar. Counts were made by surface streaking one-tenth ml portions of various dilutions on pre-poured plates and counting colonies after 48 hour incubation. The result of this experiment was as follows: Maximal recovery of the sensitive culture was obtained on TSYE agar, brilliant green agar, bismuth sulfite agar, and MacConkey agar; but only 25 to 50 per cent recovery was obtained on desoxycholate citrate

agar and Salmonella-Shigella agar. With the radio-resistant culture, recovery was maximal on TSYE agar and MacConkey agar, somewhat less on brilliant green agar or bismuth sulfite agar, but considerably less (by a factor of 10^4) on desoxycholate citrate agar of Salmonella-Shigella agar (See Table 41.).

Another similar experiment was conducted with radio-sensitive and radio-resistant cultures of S. typhimurium except in this case the cells were recovered from comminuted chicken meat; and, in addition to making plate counts on various solid selective media, the counts were also estimated by the most probable numbers (MPN) method using five tubes per dilution and confirmation on brilliant green agar. The results of this experiment, shown in Table 42, also indicated that recovery of the radio-resistant culture of S. typhimurium was poor on desoxycholate citrate agar. Counts obtained by the MPN method with either selenite-cystine broth or tetrathionate broth were comparable with direct plate counts on the solid selective media and neither of the two selective broths appeared inhibitory to the radio-resistant culture. Pre-enrichment in nutrient broth prior to inoculation into tetrathionate broth did not seem to be of any advantage in recovery of cells.

Ley et al (1963) also reported that recovery of irradiated salmonellae was poor on desoxycholate-citrate agar as compared to a nutrient agar or MacConkey agar. Desoxycholate citrate agar and Salmonella-Shigella both contain a surface active agent (bile salts or sodium desoxycholate) and this may indicate that the cell wall of the radio-resistant cultures is more sensitive to surface active agents. However, these two media also have sodium citrate in common and it is possible that this may be the inhibitory agent.

It then became of interest to compare the efficiency of various selective media on the quantitative recovery of salmonellae in an irradiated substrate. For this experiment three serotypes, S. thompson, S. newport, or S. typhimurium were irradiated in TSYE broth and then 0.1 ml portions of the appropriate dilution were surface-streaked on pre-poured plates of various selective media. Results appear in Table 43. In general, recovery was best on TSYE agar, poorest on Salmonella-Shigella agar, and good on Desoxycholate lactose agar, brilliant green agar, bismuth sulfite agar and MacConkey agar. During the course of preliminary recovery tests with bismuth sulfite agar, it was observed that variable results were obtained when refrigerated plates more than one to two days old were used. Therefore, in this particular series of tests the bismuth sulfite agar plates were used within 24 hours of pouring.

Table 44 presents the results of a similar recovery experiment with irradiated cells of S. heidelberg and S. anatum, except in these particular tests a substrate of phosphate buffer (pH 7) was used so as to preclude the addition of any nutrient material in the inoculum being plated. The results were similar to that of the previous study (Table 43), that is, maximum counts obtained on TSYE agar and lowest counts obtained on Salmonella-Shigella agar and also on desoxycholate citrate agar.

It will be recalled that in the previous study a good recovery was effected with desoxycholate lactose agar, however, this medium only contains one-tenth as much desoxycholate and citrate as does desoxycholate citrate agar. Also, note the poor recovery of S. anatum on brilliant green sulfadiazene agar. This same phenomenon was encountered in another study with S. oranienburg. It is not known whether

this was due to the culture, or possible addition of an excess amount of sulfadiazene, or a non-uniform distribution of the inhibitory agents in the dry medium.

The count on minimal media is an indication of the numbers of non-injured irradiation survivors. In the case of S. heidelberg the counts on brilliant green sulfadiazene agar, bismuth sulfite agar or MacConkey agar were at least equal to or greater than the count on minimal media. Thus, only a fraction of the radiation-injured cells were inhibited on these three selective media. However, with S. anatum, a higher recovery of irradiation survivors resulted on minimal media compared to the selective media.

Serological Reaction

One hundred colonies of S. typhimurium isolated from a radio-sensitive culture and 100 colonies from a radio-resistant culture were tested for agglutination with Salmonella O antiserum poly A-I and all gave a positive reaction although a few of the resistant cultures reacted weakly. Twenty-five colonies from each culture were then tested with the Salmonella O antiserum specific for group B, and in all instances a positive reaction was obtained. These same cultures, both sensitive and resistant, also were agglutinated with Salmonella H antiserum specific for group 1 (phase 1).

Seventy radio-resistant and 70 radio-sensitive cultures of S. newport were tested with Salmonella O antiserum poly A-I; and although a positive reaction resulted in every case, it appeared, in general, that the resistant cultures gave a weaker reaction. Several of the radio-sensitive cultures were reacted with Salmonella O antiserum group C₂

and all agglutinated strongly, but with the radio-resistant cultures there was either no agglutination or a very weak reaction.

With respect to flagellar antigens radio-sensitive cultures of S. newport were agglutinated with Salmonella H antiserum group e, h (phase 1), but the radio-resistant cultures only gave a very weak reaction. It should be mentioned at this point that in the early stage of the logarithmic growth phase broth cultures of the radio-resistant strain were motile, but as the culture approached the stationary phase of growth most of the cells had lost their motility and had sedimented to the bottom of the culture tube. Thus, the overnight cultures that were tested for flagellar antigens in this case only contained a small percentage of motile cells; and this may account for the weak agglutination.

Twenty sensitive and resistant cultures of S. heidelberg were tested for serological reaction with Salmonella antiserum. Whereas the radio-sensitive cultures all showed a typical agglutination reaction with the poly H antiserum, many of the radio-resistant cultures only gave a weak agglutination. In these instances if the tubes were incubated an additional 20 minutes at 50°C, an increased flocculation resulted. Both sensitive and resistant cultures were agglutinated with Salmonella O antiserum group B specific.

Antibiotic Sensitivity

The relative sensitivity of radio-resistant (radiation-cycled) and radio-sensitive (non-cycled) cultures of S. typhimurium, S. newport, S. heidelberg and S. thompson to various antibiotics, as measured by the sensitivity disc method, can be evaluated from the information presented in Tables 45, 46, 47, and 48. The data shown represent the diameter in millimeters of the zone of inhibition around each antibiotic

disc. The discs themselves were 6 mm in diameter, thus a value of 6 for zone of inhibition would indicate no inhibition at the particular concentration.

The average value of zone of inhibition for the total number of cultures tested and also the standard deviation are given at the bottom of each column. A statistical difference between radio-resistant and radio-sensitive cultures to a particular antibiotic was determined by the student t test, and the result is indicated at the bottom of each column as "significant" or "not significant" at the 95 per cent level.

Radio-resistant cells of *S. typhimurium* or *S. newport* were more resistant to chloramphenicol than were radio-sensitive cells. There was no difference in sensitivity between radio-resistant of sensitive cultures of *S. thompson* or *S. heidelberg*.

With regard to tetracycline the radio-resistant cells of *S. typhimurium* were more resistant; the radio-resistant cells of *S. heidelberg* were more sensitive; the radio-resistant and radio-sensitive cells of *S. newport* and *S. thompson* were equally sensitive.

There was no difference in sensitivity to 2 mcg ampicillin among radio-resistant or radio-sensitive cultures of *S. typhimurium*, *S. thompson*, or *S. heidelberg*; however, radio-resistant cultures of *S. newport* were more resistant than radio-sensitive cultures. At higher concentrations of the antibiotic, 5 mcg or 10 mcg, the radio-resistant cultures of *S. newport*, *S. thompson* and *S. heidelberg* were more sensitive to the antibiotic than their radio-sensitive counterparts.

Radio-resistant cells of *S. typhimurium* and *S. newport* were more resistant to penicillin than the radio-sensitive cells. With *S. heidelberg* both groups (radio-resistant and radio-sensitive) were resistant to the antibiotic

Of the antibiotics tested only chloramphenicol gave a sharply defined boundary at the zone of inhibition. With tetracycline or penicillin the boundary was usually hazy or diffuse, and with ampicillin there were usually many small colonies at the periphery of the inhibition zone for both radio-resistant and radio-sensitive cultures indicating a heterogeneity in resistance among the population.

Although a statistical difference in antibiotic sensitivity may have been shown to exist between radio-resistant and radio-sensitive cultures, the numerical differences were not large enough to be of practical importance. Therefore, it does not appear that irradiation would generally produce mutant strains of Salmonella that would be resistant to antibiotics ordinarily effective against this species. Nevertheless, if the statistical difference does indicate a trend toward increased resistance or sensitivity, and if the mode of action of the antibiotic is known, one might speculate upon the nature of the change causing radio-resistance. In this regard the increased resistance of radio-resistant cells of S. typhimurium or S. newport to penicillin is of particular interest. Witkin (1946) also reported the radio-resistant mutant B/r of E. coli to be more resistant to penicillin than the parent strain. If this is a common property of radio-resistant cells, then the nature of the change to increased radio-resistance may be related in some manner to the cell wall since penicillin is known to affect cell wall synthesis. A further study on sensitivity was carried out except this time the antibiotic was present in a broth culture. Two different cultures of radio-resistant and radio-sensitive serotypes were grown in TSYE broth overnight. Approximately 200 cells from each culture

were inoculated into tubes containing 2 ml TSYE broth and potassium penicillin G with concentrations ranging from 0 to 40 units per ml. The tubes were incubated at 37^o C and observed for growth after 24 and 48 hours. The result of this experiment is given in Table 49. The degree of growth or turbidity is indicated by the number of plus signs. This experiment again confirmed the increased resistance to penicillin of the radio-resistant S. newport cultures, but with the other three serotypes there appeared to be scarcely any difference in sensitivity to penicillin among radio-resistant and radio-sensitive cultures.

When examined under phase microscope radio-sensitive and radio-resistant cells of the various Salmonella serotypes appeared as follows: S. newport--radio-sensitive cultures consisted of slender rods whereas radio-resistant cultures consisted of plump rods many oval in shape; S. typhimurium--radio-sensitive cultures made up predominantly of slender rods although a few oval cells were present, but in radio-resistant cultures the cells were plumper and mostly oval. S. thompson and S. heidelberg showed a similar pattern. The radio-sensitive cultures consisted of slender rods whereas the cells of radio-resistant cultures were somewhat plumper although with these two serotypes the difference was not as pronounced as it was in the case of S. newport. Photographs of radio-sensitive and radio-resistant cells of S. newport taken through an electron microscope are presented in Fig. 14B. The coccoid form of the radio-resistant cells is readily apparent as is also the lack of flagella. In general, bacterial species with coccoid vegetative cells have usually been found to be more radio-resistant than rod-shaped bacteria. It may be that the increased radio-resistance of these particular Salmonella serotypes is related to this coccoid shape. Whether or not this phenomenon is related to an increased surface to volume ratio afforded by the

coccoid form or an increase in the structural integrity of the cell wall for the coccoid form is not known. It was reasoned that if a change, such as a thickening, had occurred in the cell wall of the radio-resistant cells, it might be manifested in a change in gram staining characteristics. However, all radio-resistant cultures of the four serotypes were still found to be gram negative.

Growth Rate of Radio-resistant and Radio-sensitive Cultures.

In devising a new process for destroying microorganisms, one has to be concerned, in addition to the induction of resistant mutants, that the process does not produce mutants which can grow at a faster rate. With this thought in mind growth rates of the radiation-cycled cultures were compared with those of the parent cultures. The growth curves obtained for radio-resistant or radio-sensitive cultures of S. typhimurium, S. newport, S. thompson and S. heidelberg, under aerobic or anaerobic conditions at various temperatures, are shown in Figures 15 through 39. The straight lines drawn to depict the linear portion of the growth curves are regression lines. There was no apparent difference between growth rates of radio-resistant and radio-sensitive cultures except for S. newport. At low temperatures, 10°C or 20°C, radio-resistant cultures of S. newport grew at a slower rate than the non-resistant cultures. In Fig. 40 growth rates (regression slope) have been plotted as a function of growth temperature (anaerobic) for cultures of S. typhimurium and S. thompson to illustrate the similarity of growth rates between radio-resistant and non-resistant cultures. Witkin (1946) reported there to be no difference in growth rates of E. coli B and the radio-resistant mutant B/r. However, the lag period was shorter for the radio-resistant mutant B/r, and the final titer of the resistant

culture was usually slightly less than that of sensitive cultures. Erdman et al (1961) similarly found that radio-resistant cultures of S. faecalis did not achieve as high a final concentration as sensitive cultures. This latter observation, the radio-resistant cultures reaching a slightly lower final concentration, was also confirmed in this present study. In this regard the biggest difference occurred with S. newport. However, there did not appear to be any apparent difference in lag time for the radio-resistant or radio-sensitive cultures. The lag times (defined by Witkin as the time required for the initial inoculum to double in numbers) were determined by graphical interpolation of the growth curves and are recorded in Table 50. Although these experimentally determined lag times are at best only approximations of the true lag times, still one can see from Table 50 that there is no particular trend for the resistant cultures to have a shorter or longer lag period than sensitive cultures.

SUMMARY AND CONCLUSIONS

Several Salmonella serotypes were subjected to consecutive irradiation and sub-culturing treatments to determine whether mutants with increased radiation resistance, increased virulence or altered biochemical characteristics might be induced or selected which could present a threat to a potential irradiation process designed to destroy Salmonella in a food.

This cyclic irradiation treatment did indeed produce cultures with increased radio-resistance. The number of radiation cycles required to effect a significant change in radio-resistance depended upon the irradiation dose. With a $2D_{10}$ dose a notable increase in radio-resistance was apparent after about 6 irradiation cycles whereas with a $5D_{10}$ dose about 10 cyclic irradiations were necessary. These radio-resistant mutants were resistant at all stages of their growth cycle and maintained their resistance after 24 transfers in broth. Considering the manipulation required to obtain these resistant mutants and also the number of radiation cycles needed, it is highly improbable that these specialized conditions could be duplicated in a food plant employing an irradiation process.

The virulence of radio-resistant cultures (radiation-cycled) was compared with that of the parent cultures using mice or embryonated eggs as hosts. The radio-resistant cultures were not more virulent than the parent cultures and with one radio-resistant serotype a decrease in virulence had occurred. The irradiation treatment also did not appear to transform rough colony types (avirulent) to smooth (virulent).

Cyclic irradiation did not cause a gross change in the biochemical pattern although one or two biochemical reactions of two of the serotypes

were altered. This is not surprising since ionizing and non-ionizing radiations have been used for years to produce bacterial mutants with some particular metabolic trait. In the present study the biochemical mutations that did occur were not of such an order as to make identification of Salmonella on irradiated foods by biochemical reactions difficult.

With two Salmonella serotypes cyclic irradiation did not cause any perceptible alteration in serological properties although a few isolates gave a weak agglutination reaction with poly Salmonella O or H antisera. However, cyclic irradiation did appear to modify the antigenic properties of S. newport. Isolates from a 20-cycled culture either reacted weakly or not at all with specific somatic antiserum and reacted very weakly with flagella antiserum. These cultures exhibited a marked reduction in motility.

Cyclic irradiation produced a morphological change in cell shape from slender rods to plump oval cells. This phenomenon was most pronounced with S. newport. In spite of the changes that may have occurred in biochemical properties, serology or morphology of the cultures as a result of cyclic irradiation, it is believed that these cultures would still be typed as Salmonella if one resorted to a protocol which included comprehensive biochemical and serological testing.

There was no difference between growth rates of radiation-cycled cultures and the parent cultures under aerobic or anaerobic conditions over a temperature range of 10 to 45°C. One of the radiation-cycled cultures (S. newport) grew at a slower rate at the lower temperatures than the parent culture. Radio-resistant cultures usually attained a lower titer than the non-resistant cultures.

Radiation-cycled cultures of Salmonella showed mixed reactions to antibiotic sensitivity. In some instances a statistically significant increase or decrease to a particular antibiotic was shown; in other instances there was no significant difference. Any difference that did exist appeared small in magnitude from a practical standpoint, and thus it did not appear that the irradiation treatment would produce strains of Salmonella that could not be controlled by the antibiotics of choice.

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Table 1. Effect of growth stage and incubation temperature after irradiation on radiation inactivation of S. typhimurium.

8-hour culture - per cent survival at incubation temperature of:						
Dose (K rad)	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
20	63.0	20.4	68.7	15.6	57.0	10.5
40	32.8	2.5	57.0	3.5	37.0	2.3
60	2.0	0.16	2.9	0.24	2.3	0.16
80	0.18	8×10^{-3}	0.15	1.3×10^{-2}	0.20	9×10^{-3}
100	2.0×10^{-3}	4.1×10^{-4}	4.6×10^{-3}	6.0×10^{-4}	1.2×10^{-2}	1.1×10^{-3}

D_{10} dose
(K rad)

16 15 15 16 17 18

14-hour culture - per cent survival at incubation temperature of:						
Dose (K rad)	23°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	17.5	23.1	19.1	27.7	19.3	25.0
60	1.4	1.4	1.6	2.0	1.7	1.8
90	2.8×10^{-2}	2.1×10^{-2}	4.9×10^{-2}	5.0×10^{-2}	6×10^{-2}	7.2×10^{-2}
120	2.2×10^{-4}	1.8×10^{-4}	9.8×10^{-4}	5.5×10^{-4}	1.8×10^{-3}	1×10^{-3}
150	3.5×10^{-5}	6.1×10^{-5}	1.1×10^{-4}	2.9×10^{-5}	3.6×10^{-4}	6.8×10^{-5}

D_{10} dose
(K rad)

19 18 21 19 24 21

Table 1 continued

48-hour culture - per cent survival at incubation temperature of:						
Dose (K rad)	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	11.4	17.9	16.0	16.2	17.5	16.5
60	1.9	1.8	3.4	1.6	3.1	1.6
90	0.20	0.35	0.34	0.56	0.37	0.52
120	4.1×10^{-2}	8.5×10^{-2}	8.0×10^{-2}	0.15	9.7×10^{-2}	0.18
150	1.3×10^{-3}	2.0×10^{-2}	2.7×10^{-2}	6.0×10^{-2}	2.9×10^{-2}	7.0×10^{-2}
D ₁₀ dose (K rad)	37	39	40	44	41	47

Table 1 continued

24-hour culture - per cent survival at incubation temperature of:									
Dose (K rad)	20°C			30°C			37°C		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
0	100	100	100	100	100	100	100	100	100
30	13.9	10.8	11.9	13.9	11.7	10.8	16.7	12.4	12.2
60	1.4	0.27	0.23	1.1	0.39	0.37	1.4	0.42	0.47
90	2.1×10^{-2}	4.9×10^{-3}	1.7×10^{-3}	2.8×10^{-2}	1.1×10^{-2}	5.6×10^{-3}	3.0×10^{-2}	1.6×10^{-2}	6.9×10^{-3}
120	1.4×10^{-4}	9.5×10^{-5}	3.3×10^{-4}	3.5×10^{-4}	1.7×10^{-4}	1×10^{-4}	7.3×10^{-4}	2.4×10^{-4}	3.3×10^{-4}
150	2.6×10^{-5}	5.8×10^{-5}	9.7×10^{-7}	6.4×10^{-5}	6.7×10^{-5}	9.4×10^{-6}	8.3×10^{-5}	2.4×10^{-4}	3.5×10^{-5}

D ₁₀ dose (K rad)	19	18	17	20	20	19	20	20	20
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32-hour culture - per cent survival at incubation temperature of:									
Dose (K rad)	20°C			30°C			37°C		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
0	100	100	100	100	100	100	100	100	100
30	11.0	10.0	8.3	15.0	10.0	12.8	10.5	10.0	11.2
60	0.57	0.52	0.43	1.0	0.68	0.72	0.66	0.55	0.62
90	1.0×10^{-2}	6.1×10^{-2}	3.6×10^{-3}	2.8×10^{-2}	1×10^{-1}	1.2×10^{-2}	2.3×10^{-2}	1.2×10^{-1}	1.4×10^{-2}
120	2.6×10^{-3}	2.9×10^{-4}	1.4×10^{-4}	1.0×10^{-2}	4.5×10^{-4}	3.7×10^{-4}	9.3×10^{-3}	5.9×10^{-4}	8.5×10^{-4}
150	1.5×10^{-3}	5.7×10^{-5}	4.3×10^{-5}	4.2×10^{-3}	1.8×10^{-4}	2.0×10^{-4}	4.6×10^{-3}	2.5×10^{-4}	3.8×10^{-4}

D ₁₀ dose (K rad)	21	23	20	23	25	20	23	25	22
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Table 2. Effect of growth stage and incubation temperature after irradiation on radiation inactivation of S. newport.

8-hour culture - per cent survival at incubation temperature of:						
Dose (K rad)	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
20	12.2	7.1	12.7	5.9	12.0	5.2
40	0.36	0.25	0.45	0.12	0.42	0.10
60	2.2×10^{-2}	9.0×10^{-3}	1.7×10^{-2}	1.1×10^{-2}	1.2×10^{-2}	2.0×10^{-3}
80	3.9×10^{-4}	3.2×10^{-4}	2.6×10^{-4}	1.7×10^{-4}	3.4×10^{-4}	1.0×10^{-4}
100	4.2×10^{-5}	2.5×10^{-5}	5.4×10^{-5}	1.0×10^{-5}	5.5×10^{-5}	1.0×10^{-5}
D ₁₀ dose (K rad)	14	14	14	14	14	13

14-hour culture - per cent survival at incubation temperature of:						
Dose (K rad)	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	1.88	2.70	1.96	2.90	1.63	2.72
60	1.2×10^{-2}	2.1×10^{-2}	8.0×10^{-3}	1.6×10^{-2}	6.0×10^{-3}	1.6×10^{-2}
90	2.1×10^{-4}	6.0×10^{-5}	1.1×10^{-4}	5.0×10^{-5}	1.4×10^{-4}	7.0×10^{-5}
120	5.1×10^{-5}	5.0×10^{-6}	3.2×10^{-5}	2.0×10^{-6}	1.9×10^{-5}	1.0×10^{-6}
150	6.0×10^{-6}	6.0×10^{-7}	1.6×10^{-5}	no survivors	2.4×10^{-5}	no survivors
D ₁₀ dose (K rad)	14	14	13	13.5	13	13.5

Table 2 continued

24-hour culture - per cent survival at incubation temperature of:								
Dose	20°C		30°C		37°C			
(K rad)	run 1	run 2	run 1	run 2	run 1	run 2		
0	100	100	100	100	100	100		
30	1.15	0.69	1.60	0.49	0.90	0.46		
60	1.1×10^{-3}	1.7×10^{-3}	7.9×10^{-3}	8.0×10^{-4}	3.3×10^{-3}	1.1×10^{-3}		
90	1.0×10^{-4}	9.0×10^{-6}	7.0×10^{-5}	8.0×10^{-6}	8.0×10^{-5}	9.0×10^{-6}		
120	5.0×10^{-6}	2.3×10^{-7}	3.0×10^{-6}	4.1×10^{-7}	---	4.6×10^{-7}		
150	2.0×10^{-6}	9.0×10^{-8}	1.8×10^{-6}	8.0×10^{-8}	1.3×10^{-6}	1.8×10^{-7}		
D ₁₀ dose								
(K rad)	14	12	14	12	14	12		
32-hour culture - per cent survival at incubation temperature of:								
Dose	20°C			30°C			37°C	
(K rad)	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 3
0	100	100	100	100	100	100	100	100
30	1.9	2.8	1.8	1.5	1.5	0.7	1.5	1.4
60	7.7×10^{-2}	1.9×10^{-2}	1.2×10^{-2}	4.9×10^{-2}	4.0×10^{-3}	5.7×10^{-3}	4.2×10^{-2}	3.6×10^{-3}
90	1.2×10^{-2}	2.3×10^{-4}	2.1×10^{-3}	5.0×10^{-3}	6.7×10^{-5}	5.7×10^{-4}	4.0×10^{-3}	4.3×10^{-4}
120	2.4×10^{-3}	6.0×10^{-5}	4.5×10^{-4}	6.0×10^{-4}	6.5×10^{-6}	1×10^{-4}	6.0×10^{-4}	1.0×10^{-4}
150	4.7×10^{-4}	2.5×10^{-5}	2.1×10^{-4}	1.0×10^{-4}	5.2×10^{-6}	3.8×10^{-5}	1.0×10^{-4}	2.5×10^{-5}
D ₁₀ dose								
(K rad)	22	15	17	20	13	15	20	15

Table 2 continued

Dose (K rad)	48-hour culture - per cent survival at incubation temperature of:					
	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	6.1	26.2	4.5	30.0	4.3	23.0
60	0.114	6.0	5.3×10^{-2}	6.8	3.3×10^{-2}	5.0
90	6.3×10^{-3}	1.02	2.3×10^{-3}	1.04	1.6×10^{-3}	0.64
120	2.3×10^{-4}	0.21	6.0×10^{-5}	0.16	3.0×10^{-5}	0.10
150	5.3×10^{-4}	2.8×10^{-2}	1.1×10^{-4}	4.3×10^{-2}	7.0×10^{-5}	2.3×10^{-2}
 D ₁₀ dose (K rad)	 22	 43	 19	 41	 19	 40

Table 3. Effect of incubation temperature after irradiation on radiation-inactivation of S. heidelberg (15-hour culture).

per cent survival at incubation temperature of:						
Dose (K rad)	20°C		30°C		37°	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	6.5	12.9	6.8	13.9	5.7	11.3
60	2.2×10^{-2}	1.3×10^{-1}	6.4×10^{-2}	1.5×10^{-1}	5.9×10^{-2}	9.5×10^{-2}
90	2.7×10^{-4}	1.5×10^{-4}	5.7×10^{-4}	5.4×10^{-4}	5.3×10^{-4}	4.4×10^{-4}
120	4.7×10^{-6}	1.0×10^{-6}	1.3×10^{-5}	6.3×10^{-6}	1.5×10^{-5}	7.1×10^{-6}
150	5.9×10^{-8}	7.0×10^{-8}	4.5×10^{-7}	2.6×10^{-7}	2.2×10^{-7}	3.5×10^{-7}
D ₁₀ dose (K rad)	15	14	15	14	15	14

Table 4. Effect of incubation temperature after irradiation on radiation-inactivation of S. thompson (15-hour culture).

Dose (K rad)	per cent survival at incubation temperature of:					
	20°C		30°C		37°C	
	run 1	run 2	run 1	run 2	run 1	run 2
0	100	100	100	100	100	100
30	10.7	22.6	13.7	28.6	14.5	20.5
60	0.13	0.88	0.11	0.98	0.10	0.59
90	1.1×10^{-4}	6.5×10^{-4}	1.2×10^{-4}	7.0×10^{-4}	1.5×10^{-4}	5.1×10^{-4}
120	no growth	1.0×10^{-7}	1.1×10^{-6}	1.4×10^{-7}	1.7×10^{-6}	9.4×10^{-7}
 D ₁₀ dose (K rad)	 13	 15	 13	 15	 13	 15

Table 5. Survival of irradiated cells of S. typhimurium held for different times at 0°C immediately after irradiation.

Holding time after irradiation (hours)	number of cells per ml surviving a dose of:		
	0	100 K rad	140 K rad
0	3.6×10^8	1.1×10^4	1.2×10^3
1	---	1.2×10^4	8.0×10^2
2	---	2.4×10^4	1.3×10^3
3	---	1.0×10^4	1.4×10^3

Table 6. Survival of irradiated cells of S. typhimurium
(15-hour culture) during storage at 2.2 to 4.4°C.

Days storage after irradiation	number of cells per ml surviving an irradiation dose of:			
	0	40 k rad	80 K rad	120 K rad
0	1.6×10^9	2.1×10^7	10.0×10^3	2.8×10^1
1	1.2×10^9	1.6×10^7	9.3×10^3	9.3×10^1
2	1.4×10^9	1.3×10^7	5.0×10^3	10.0×10^1
3	1.2×10^9	1.4×10^7	5.7×10^3	2.3×10^1
6	1.3×10^9	9.6×10^6	2.3×10^3	1.7×10^1
7	2.5×10^9	8.3×10^6	2.1×10^3	4.0×10^0
8	1.6×10^9	8.1×10^6	2.2×10^3	8.0×10^0
9	1.5×10^9	9.0×10^6	2.0×10^3	4.0×10^0
15	1.3×10^9	4.0×10^6	5.5×10^2	2.0×10^0

Survival of irradiated cells of S. newport
(15-hour culture) during storage at 2.2 to 4.4°C.

Days storage after irradiation	number of cells per ml surviving an irradiation dose of:		
	0	40 K rad	80 K rad
0	1.7×10^9	3.0×10^5	10×10^1
1	1.7×10^9	2.3×10^5	3.7×10^1
2	1.3×10^9	1.6×10^5	2.4×10^1
3	1.8×10^9	3.2×10^5	8.6×10^1
6	1.4×10^9	1.8×10^5	5.1×10^1
7	1.4×10^9	1.2×10^5	3.7×10^1
8	1.5×10^9	1.3×10^5	3.0×10^1
9	1.8×10^9	1.1×10^5	1.8×10^1
15	1.4×10^9	1.6×10^5	9.0×10^0

Table 7. Effect of incubation temperature (20 or 37°C)
on radiation survival of S. typhimurium.

Isolate	per cent survival after 150 K rad		ratio 20°C/37°C
	20°C	37°C	
1	$< 1.3 \times 10^{-7}$	9.2×10^{-7}	< 0.14
2	8.1×10^{-6}	2.8×10^{-6}	2.9
3	$< 1.4 \times 10^{-7}$	$4.9 \times 10 \times 10^{-7}$	< 0.3
4	3.2×10^{-7}	4.2×10^{-7}	0.76
5	$< 1.2 \times 10^{-7}$	2.0×10^{-7}	< 0.60
6	$< 1.0 \times 10^{-7}$	1.6×10^{-7}	< 0.62
7	$< 1.2 \times 10^{-7}$	9.2×10^{-7}	< 0.13
8	2.7×10^{-7}	3.8×10^{-7}	0.71
9	10.6×10^{-7}	7.5×10^{-7}	1.4
10	0.6×10^{-6}	9.5×10^{-6}	0.06
11	$< 0.9 \times 10^{-7}$	5.8×10^{-7}	< 0.15
12	$< 1.4 \times 10^{-7}$	2.2×10^{-7}	< 0.6
13	0.3×10^{-6}	1.8×10^{-6}	0.17
14	$< 0.6 \times 10^{-7}$	2.2×10^{-7}	< 0.27
15	$< 0.7 \times 10^{-7}$	0.9×10^{-7}	< 0.77
16	$< 1.8 \times 10^{-7}$	4.2×10^{-7}	< 0.43
17	1.3×10^{-7}	2.4×10^{-7}	0.54
18	$< 0.6 \times 10^{-7}$	1.8×10^{-7}	< 0.33
19	$< 0.6 \times 10^{-7}$	1.8×10^{-7}	< 0.33

Table 8. Effect of incubation temperature (20 or 37°C) on radiation survival of S. newport.

Isolate	per cent survival after 70 K rad		ratio 20°C/37°C
	20°C	37°C	
1	6.7×10^{-6}	13×10^{-6}	0.52
2	9.0×10^{-6}	14×10^{-6}	0.64
3	7.0×10^{-5}	8.9×10^{-5}	0.79
4	7.2×10^{-5}	8.8×10^{-5}	0.82
5	0.0×10^{-5}	7.7×10^{-5}	1.03
6	5.1×10^{-6}	16×10^{-6}	0.32
7	2.2×10^{-6}	2.6×10^{-6}	0.85
8	2.8×10^{-5}	2.6×10^{-5}	1.08
9	1.5×10^{-5}	2.6×10^{-5}	0.58
10	6.9×10^{-5}	3.5×10^{-5}	1.97
11	5.3×10^{-5}	6.5×10^{-5}	0.82
12	1.2×10^{-4}	1.3×10^{-4}	0.93
13	2.9×10^{-5}	1.0×10^{-5}	2.90
14	1.2×10^{-5}	1.8×10^{-5}	0.67
15	3.1×10^{-5}	3.7×10^{-5}	0.84
16	2.7×10^{-5}	4.2×10^{-5}	0.64
17	1.2×10^{-4}	1.1×10^{-4}	1.10
18	9.4×10^{-5}	10×10^{-5}	0.94
19	5.9×10^{-4}	8.7×10^{-4}	0.68
20	1.3×10^{-4}	1.3×10^{-4}	1.0
21	1.1×10^{-4}	1.7×10^{-4}	0.65
22	6.1×10^{-5}	30×10^{-5}	0.20
23	6.6×10^{-5}	9.0×10^{-5}	0.73
24	10×10^{-5}	9.3×10^{-5}	1.10
25	7.5×10^{-5}	13×10^{-5}	0.58

Table 8 continued

Isolate	per cent survival after 70 K rad		ratio
	20°C	37°C	20°C/37°C
26	1.2×10^{-5}	2.9×10^{-5}	0.41
27	3.3×10^{-5}	5.3×10^{-5}	0.62
28	1.8×10^{-4}	2.1×10^{-4}	0.86
29	1.8×10^{-4}	1.2×10^{-4}	1.50
30	4.2×10^{-5}	2.2×10^{-5}	1.90
31	1.9×10^{-4}	2.7×10^{-4}	0.70
32	1.2×10^{-4}	1.2×10^{-4}	1.00
33	6.1×10^{-5}	5.8×10^{-5}	1.10
34	2.5×10^{-5}	2.3×10^{-5}	1.10
35	8.7×10^{-6}	6.2×10^{-6}	1.40
36	1.7×10^{-5}	1.7×10^{-5}	1.00
37	4.3×10^{-5}	2.5×10^{-5}	1.72
38	9.6×10^{-6}	11×10^{-6}	0.87
39	3.6×10^{-6}	2.4×10^{-6}	1.50
40	4.8×10^{-5}	3.1×10^{-5}	1.55
			average = 0.98

Table 9. Radiation survival of salmonella broth cultures derived from cultures which had been grown while exposed to a low flux of gamma radiation.

Run number	growth condition of parent culture	<u>S. newport</u> per cent survival of subculture at:	
		45 K rad	90 K rad
1	2 hrs pre-incubation at 37°C, then 5 hrs at 60 rad per min	3.3×10^{-2}	1.2×10^{-5}
2	2 hrs pre-incubation, at 37°C, then 4.5 hrs at 90 rad per min	6.2 5.2	1.2×10^{-1} 7.0×10^{-2}
3	2 hrs pre-incubation at 37°C, then 5 hrs at 160 rad per min	1.4×10^{-2}	5.0×10^{-5}
4	2 hrs incubation at 37°C, then 4 hrs at 280 rad per min	2.1×10^{-2} 1.2×10^{-2}	8.0×10^{-8} 6.8×10^{-7}

Table 9 continued

Radiation survival of salmonella broth cultures
derived from cultures which had been grown while
exposed to a low flux of gamma radiation.

Run number	growth condition of parent culture	<u>S. typhimurium</u>	
		per cent survival of subculture at:	
		70 K rad	140 K rad
1	2 hrs pre-incubation at 37°C, then 5 hrs at 60 rad per min	4.0×10^{-3}	1.0×10^{-6}
2	2 hrs pre-incubation at 37°C, then 4.5 hrs at 90 rad per min	6.0×10^{-3} 7.7×10^{-3}	3.0×10^{-5} 1.8×10^{-5}
3	2 hrs pre-incubation at 37°C, then 5 hrs at 160 rad per min	4.4×10^{-3}	1.1×10^{-7}
4	2 hrs incubation at 37°C, then 4 hrs at 280 rad per min	9.8×10^{-2} 4.5×10^{-2}	6.8×10^{-7} 5.9×10^{-6}

Table 10. Effect of Cyclic Irradiation at 60 Krad and Sub-culture
of Survivors on Radio-Resistance of S. typhimurium
(Isolate 1).

Radiation Cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
1	140 x 10 ⁶	20 x 10 ⁴	10 x 10 ¹	100	0.14	0.00007	15
2	50 x 10 ⁶	68 x 10 ⁴	36 x 10 ¹	100	1.4	0.0007	15
3	62 x 10 ⁶	39 x 10 ⁴	39 x 10 ¹	100	0.63	0.0006	16
4	41 x 10 ⁶	26 x 10 ⁴	60 x 10 ¹	100	0.63	0.0015	19
5	64 x 10 ⁶	14 x 10 ⁵	48 x 10 ²	100	2.1	0.0075	21
6	65 x 10 ⁶	20 x 10 ⁵	22 x 10 ³	100	3.1	0.033	25
7	45 x 10 ⁶	34 x 10 ⁵	33 x 10 ³	100	7.6	0.073	25
8	47 x 10 ⁶	25 x 10 ⁵	32 x 10 ³	100	5.3	0.068	26
9	64 x 10 ⁶	37 x 10 ⁵	15 x 10 ³	100	5.8	0.023	21
10	40 x 10 ⁶	54 x 10 ⁵	23 x 10 ³	100	13.5	0.058	21
11	47 x 10 ⁶	41 x 10 ⁵	24 x 10 ³	100	8.7	0.051	22
12	42 x 10 ⁶	32 x 10 ⁵	20 x 10 ³	100	7.6	0.048	23
13	55 x 10 ⁶	36 x 10 ⁵	7 x 10 ³	100	6.5	0.013	19
14	53 x 10 ⁶	45 x 10 ⁵	26 x 10 ³	100	8.5	0.058	23
15	57 x 10 ⁶	38 x 10 ⁵	32 x 10 ³	100	6.7	0.056	24
16	56 x 10 ⁶	51 x 10 ⁵	36 x 10 ³	100	9.1	0.064	23
17	44 x 10 ⁶	53 x 10 ⁵	75 x 10 ³	100	12.0	0.17	27
18	24 x 10 ⁶	33 x 10 ⁵	51 x 10 ³	100	13.7	0.21	28
19	41 x 10 ⁶	10 x 10 ⁵	84 x 10 ³	100	25.2	0.20	27
20	36 x 10 ⁶	62 x 10 ⁵	79 x 10 ³	100	17.2	0.22	26

Table 11. Effect of Cyclic Irradiation at 110 Krad and Sub-culture of Survivors on Radio-Resistance of S. typhimurium (Isolate 1).

Radiation Cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
1	140 x 10 ⁶	20 x 10 ⁴	10 x 10 ¹	100	0.14	0.00007	15
2	49 x 10 ⁶	60 x 10 ⁴	78 x 10 ¹	100	1.20	0.0016	17
3	47 x 10 ⁶	32 x 10 ⁴	59 x 10 ¹	100	0.68	0.0013	18
4	28 x 10 ⁶	30 x 10 ⁴	57 x 10 ¹	100	1.1	0.0020	18
5	40 x 10 ⁶	88 x 10 ⁴	160 x 10 ¹	100	2.2	0.0040	19
6	36 x 10 ⁶	52 x 10 ⁴	62 x 10 ¹	100	1.5	0.0017	17
7	44 x 10 ⁶	47 x 10 ⁴	76 x 10 ¹	100	1.1	0.0017	18
8	42 x 10 ⁶	12 x 10 ⁵	21 x 10 ²	100	2.9	0.0050	18
9	42 x 10 ⁶	10 x 10 ⁵	13 x 10 ²	100	2.4	0.0032	17
10	64 x 10 ⁶	18 x 10 ⁵	51 x 10 ²	100	2.8	0.008	20
11	51 x 10 ⁶	40 x 10 ⁵	39 x 10 ³	100	7.9	0.077	25
12	29 x 10 ⁶	35 x 10 ⁵	26 x 10 ³	100	12.0	0.090	23
13	40 x 10 ⁶	66 x 10 ⁵	44 x 10 ³	100	16.5	0.11	23
14	23 x 10 ⁶	65 x 10 ⁵	57 x 10 ³	100	28.0	0.25	25
15	43 x 10 ⁶	10 x 10 ⁶	18 x 10 ⁴	100	23.0	0.41	29
16	60 x 10 ⁶	12 x 10 ⁶	17 x 10 ⁴	100	21.0	0.28	27
17	55 x 10 ⁶	12 x 10 ⁶	24 x 10 ⁴	100	21.2	0.43	29
18	44 x 10 ⁶	9 x 10 ⁶	24 x 10 ⁴	100	20.9	0.55	31
19	45 x 10 ⁶	8 x 10 ⁶	19 x 10 ⁴	100	17.8	0.41	30
20	34 x 10 ⁶	10 x 10 ⁶	25 x 10 ⁴	100	28.8	0.74	31

Table 12. Effect of Cyclic Irradiation at 60 Krad and Sub-culture of Survivors on Radio-Resistance of S. typhimurium (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
1	94 x 10 ⁶	84 x 10 ⁴	82 x 10 ¹	100	0.89	0.0009	17
2	53 x 10 ⁶	35 x 10 ⁴	55 x 10 ¹	100	0.66	0.0010	18
3	59 x 10 ⁶	44 x 10 ⁴	42 x 10 ¹	100	0.75	0.0007	17
4	47 x 10 ⁶	35 x 10 ⁴	49 x 10 ¹	100	0.74	0.0010	17
5	95 x 10 ⁶	14 x 10 ⁵	61 x 10 ²	100	1.5	0.0064	21
6	53 x 10 ⁶	45 x 10 ⁵	28 x 10 ³	100	8.5	0.052	23
7	58 x 10 ⁶	32 x 10 ⁵	30 x 10 ³	100	5.5	0.052	24
8	46 x 10 ⁶	47 x 10 ⁵	22 x 10 ³	100	10.2	0.048	21
9	66 x 10 ⁶	30 x 10 ⁵	13 x 10 ³	100	4.6	0.019	21
10	46 x 10 ⁶	53 x 10 ⁵	20 x 10 ³	100	11.5	0.044	21
11	45 x 10 ⁶	27 x 10 ⁵	26 x 10 ³	100	6.0	0.058	25
12	49 x 10 ⁶	45 x 10 ⁵	20 x 10 ³	100	9.2	0.040	21
13	64 x 10 ⁶	49 x 10 ⁵	10 x 10 ³	100	7.7	0.015	19
14	57 x 10 ⁶	57 x 10 ⁵	24 x 10 ³	100	10.0	0.042	21
15	66 x 10 ⁶	51 x 10 ⁵	34 x 10 ³	100	7.7	0.052	24
16	48 x 10 ⁶	57 x 10 ⁵	32 x 10 ³	100	12.0	0.067	23
17	43 x 10 ⁶	53 x 10 ⁵	74 x 10 ³	100	12.3	0.11	27
18	17 x 10 ⁶	56 x 10 ⁵	112 x 10 ³	100	33.0	0.66	30
19	6 x 10 ⁶	10 x 10 ⁵	44 x 10 ³	100	16.7	0.74	36
20	24 x 10 ⁶	50 x 10 ⁵	55 x 10 ³	100	20.8	0.23	26

Table 13. Effect of Cyclic Irradiation at 110 Krad and Sub-culture
on Radio-Resistance of S. typhimurium (Isolate 2).

Radiation Cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
1	94 x 10 ⁶	84 x 10 ⁴	82 x 10 ¹	100	0.89	0.0009	17
2	37 x 10 ⁶	42 x 10 ⁴	63 x 10 ¹	100	1.1	0.0017	18
3	28 x 10 ⁶	31 x 10 ⁴	49 x 10 ¹	100	1.1	0.0018	18
4	32 x 10 ⁶	20 x 10 ⁴	61 x 10 ¹	100	0.62	0.0019	20
5	40 x 10 ⁶	86 x 10 ⁴	80 x 10 ¹	100	2.3	0.0020	16
6	46 x 10 ⁶	66 x 10 ⁴	110 x 10 ¹	100	1.4	0.0024	18
7	80 x 10 ⁶	62 x 10 ⁴	58 x 10 ¹	100	0.78	0.0007	17
8	42 x 10 ⁶	105 x 10 ⁴	168 x 10 ¹	100	2.5	0.0040	18
9	47 x 10 ⁶	58 x 10 ⁴	82 x 10 ¹	100	1.2	0.0017	18
10	50 x 10 ⁶	80 x 10 ⁴	37 x 10 ²	100	1.6	0.0074	22
11	27 x 10 ⁶	59 x 10 ⁵	61 x 10 ³	100	21.8	0.23	25
12	21 x 10 ⁶	57 x 10 ⁵	38 x 10 ³	100	27.0	0.18	23
13	24 x 10 ⁶	90 x 10 ⁵	64 x 10 ³	100	37.5	0.27	23
14	37 x 10 ⁶	64 x 10 ⁵	85 x 10 ³	100	17.0	0.23	27
15	46 x 10 ⁶	16 x 10 ⁶	19 x 10 ⁴	100	35.0	0.41	26
16	42 x 10 ⁶	11 x 10 ⁶	17 x 10 ⁴	100	25.0	0.41	28
17	29 x 10 ⁶	18 x 10 ⁶	25 x 10 ⁴	100	63.0	0.87	27
18	32 x 10 ⁶	11 x 10 ⁶	36 x 10 ⁴	100	33.0	1.12	32
19	36 x 10 ⁶	12 x 10 ⁶	53 x 10 ⁴	100	34.0	1.47	37
20	31 x 10 ⁶	9 x 10 ⁶	23 x 10 ⁴	100	30.0	0.74	31

Table 14. Effect of Cyclic Irradiation at 30 Krad and Sub-culture of Survivors on Radio-Resistance of S. newport (Isolate 1).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
1	64 x 10 ⁶	9 x 10 ⁵	84 x 10 ¹	100	1.4	0.0013	10
2	58 x 10 ⁶	14 x 10 ⁵	11 x 10 ²	100	2.4	0.0019	10
3	95 x 10 ⁶	14 x 10 ⁵	18 x 10 ²	100	1.5	0.0019	10
4	97 x 10 ⁶	23 x 10 ⁵	38 x 10 ³	100	2.4	0.039	17
5	100 x 10 ⁶	80 x 10 ⁵	23 x 10 ⁴	100	8.0	0.23	20
6	100 x 10 ⁶	176 x 10 ⁵	67 x 10 ⁴	100	18.0	0.67	20
7	49 x 10 ⁶	130 x 10 ⁵	63 x 10 ⁴	100	26.0	1.3	23
8	90 x 10 ⁶	113 x 10 ⁵	89 x 10 ⁴	100	13.0	1.0	26
9	90 x 10 ⁶	145 x 10 ⁵	140 x 10 ⁴	100	16.0	1.5	29
10	48 x 10 ⁶	175 x 10 ⁵	130 x 10 ⁴	100	18.2	2.6	35
11	80 x 10 ⁶	200 x 10 ⁵	150 x 10 ⁴	100	25.0	1.9	27
12	76 x 10 ⁶	120 x 10 ⁵	92 x 10 ⁴	100	15.8	1.2	27
13	58 x 10 ⁶	130 x 10 ⁵	54 x 10 ⁴	100	22.0	0.93	22
14	54 x 10 ⁶	210 x 10 ⁵	250 x 10 ⁴	100	39.0	4.7	33
15	64 x 10 ⁶	128 x 10 ⁵	97 x 10 ⁴	100	20.0	1.5	27
16	65 x 10 ⁶	115 x 10 ⁵	64 x 10 ⁴	100	18.0	1.0	24
17	88 x 10 ⁶	68 x 10 ⁵	206 x 10 ⁴	100	7.7	0.23	20
18	47 x 10 ⁶	100 x 10 ⁵	75 x 10 ⁴	100	21.0	1.6	26
19	44 x 10 ⁶	82 x 10 ⁵	92 x 10 ⁴	100	18.6	2.1	30
20	66 x 10 ⁶	98 x 10 ⁵	150 x 10 ⁴	100	14.8	2.3	34

Table 15. Effect of Cyclic Irradiation at 60 Krad and Sub-culture of Survivors on Radio-Resistance of S. newport (Isolate 1).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
1	64 x 10 ⁶	9 x 10 ⁵	84 x 10 ¹	100	1.4	0.0013	10
2	56 x 10 ⁶	13 x 10 ⁵	14 x 10 ²	100	2.3	0.0025	10
3	97 x 10 ⁶	9 x 10 ⁵	8 x 10 ²	100	0.9	0.0008	10
4	93 x 10 ⁶	10 x 10 ⁵	15 x 10 ²	100	1.0	0.0016	11
5	74 x 10 ⁶	27 x 10 ⁵	48 x 10 ²	100	3.7	0.0065	11
6	114 x 10 ⁶	56 x 10 ⁵	24 x 10 ³	100	4.9	0.021	13
7	82 x 10 ⁸	86 x 10 ⁵	22 x 10 ³	100	10.5	0.027	12
8	115 x 10 ⁶	114 x 10 ⁵	39 x 10 ³	100	10.0	0.034	12
9	82 x 10 ⁶	120 x 10 ⁵	26 x 10 ⁴	100	14.6	0.31	18
10	83 x 10 ⁶	110 x 10 ⁵	33 x 10 ⁴	100	13.2	0.40	20
11	65 x 10 ⁶	127 x 10 ⁵	35 x 10 ⁴	100	19.6	0.54	19
12	46 x 10 ⁶	71 x 10 ⁵	20 x 10 ⁴	100	15.4	0.44	19
13	44 x 10 ⁶	100 x 10 ⁵	16 x 10 ⁴	100	23.0	0.37	17
14	50 x 10 ⁶	233 x 10 ⁵	170 x 10 ⁴	100	47.0	3.4	28
15	100 x 10 ⁶	100 x 10 ⁵	16 x 10 ⁴	100	10.0	0.16	17
16	60 x 10 ⁶	92 x 10 ⁵	6 x 10 ⁴	100	15.0	0.10	14
17	66 x 10 ⁶	101 x 10 ⁵	33 x 10 ⁴	100	15.3	0.50	20
18	64 x 10 ⁶	134 x 10 ⁵	105 x 10 ⁴	100	24.0	1.6	26
19	50 x 10 ⁶	118 x 10 ⁵	84 x 10 ⁴	100	23.6	1.7	26
20	65 x 10 ⁶	198 x 10 ⁵	228 x 10 ⁴	100	19.8	3.5	32

Table 16. Effect of Cyclic Irradiation at 30 Krad and Sub-culture of Survivors on Radio-Resistance of *S. newport* (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
1	54 x 10 ⁶	47 x 10 ⁴	87 x 10 ¹	100	0.87	0.0016	11
2	52 x 10 ⁶	15 x 10 ⁵	130 x 10 ¹	100	2.9	0.0025	10
3	90 x 10 ⁶	11 x 10 ⁵	120 x 10 ¹	100	1.2	0.0013	10
4	100 x 10 ⁶	13 x 10 ⁵	61 x 10 ²	100	1.3	0.0061	13
5	104 x 10 ⁶	38 x 10 ⁵	57 x 10 ³	100	3.6	0.055	16
6	113 x 10 ⁶	123 x 10 ⁵	27 x 10 ⁴	100	10.9	0.24	18
7	96 x 10 ⁶	72 x 10 ⁵	21 x 10 ⁴	100	7.5	0.22	19
8	94 x 10 ⁶	134 x 10 ⁵	44 x 10 ⁴	100	14.2	0.47	20
9	94 x 10 ⁶	155 x 10 ⁵	113 x 10 ⁴	100	16.5	1.2	27
10	19 x 10 ⁶	170 x 10 ⁵	14 x 10 ⁴	100	9.0	0.72	28
11	62 x 10 ⁶	81 x 10 ⁵	41 x 10 ⁴	100	13.1	0.66	21
12	68 x 10 ⁶	76 x 10 ⁵	40 x 10 ⁴	100	11.2	0.59	23
13	66 x 10 ⁶	108 x 10 ⁵	38 x 10 ⁴	100	16.0	0.58	21
14	58 x 10 ⁶	173 x 10 ⁵	90 x 10 ⁴	100	30.0	1.6	23
15	71 x 10 ⁶	124 x 10 ⁵	52 x 10 ⁴	100	17.5	0.73	22
16	107 x 10 ⁶	86 x 10 ⁵	40 x 10 ⁴	100	8.0	0.37	23
17	56 x 10 ⁶	118 x 10 ⁵	36 x 10 ⁴	100	21.0	0.64	20
18	62 x 10 ⁶	118 x 10 ⁵	106 x 10 ⁴	100	19.1	1.7	29
19	62 x 10 ⁶	170 x 10 ⁵	80 x 10 ⁴	100	27.4	1.3	23
20	56 x 10 ⁶	93 x 10 ⁵	146 x 10 ⁴	100	16.6	2.6	37

Table 17. Effect of Cyclic Irradiation at 60 Krad and Sub-culture of Survivors on Radio-Resistance of S. newport (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
1	54 x 10 ⁶	47 x 10 ⁴	87 x 10 ¹	100	0.87	0.0016	11
2	48 x 10 ⁶	12 x 10 ⁵	96 x 10 ¹	100	2.5	0.0020	10
3	62 x 10 ⁶	10 x 10 ⁵	80 x 10 ¹	100	1.6	0.0013	10
4	82 x 10 ⁶	12 x 10 ⁵	12 x 10 ²	100	1.5	0.0015	10
5	78 x 10 ⁶	15 x 10 ⁵	27 x 10 ²	100	1.9	0.0035	11
6	88 x 10 ⁶	35 x 10 ⁴	17 x 10 ²	100	0.40	0.0019	13
7	66 x 10 ⁶	22 x 10 ⁴	29 x 10 ²	100	0.33	0.0044	16
8	61 x 10 ⁶	32 x 10 ⁴	51 x 10 ³	100	0.52	0.083	17
9	59 x 10 ⁶	36 x 10 ⁵	28 x 10 ⁴	100	6.1	0.48	26
10	61 x 10 ⁶	92 x 10 ⁵	65 x 10 ⁴	100	15.1	1.1	26
11	73 x 10 ⁶	55 x 10 ⁵	68 x 10 ⁴	100	7.6	0.93	27
12	44 x 10 ⁶	34 x 10 ⁵	50 x 10 ⁴	100	7.7	1.1	29
13	68 x 10 ⁶	14 x 10 ⁵	8 x 10 ⁴	100	2.1	0.12	20
14	136 x 10 ⁶	138 x 10 ⁵	54 x 10 ⁴	100	10.0	0.40	22
15	50 x 10 ⁶	34 x 10 ⁵	11 x 10 ⁴	100	6.8	0.23	21
16	64 x 10 ⁶	56 x 10 ⁵	6 x 10 ⁴	100	8.8	0.09	15
17	68 x 10 ⁶	117 x 10 ⁵	68 x 10 ⁴	100	17.2	1.0	24
18	62 x 10 ⁶	134 x 10 ⁵	118 x 10 ⁴	100	21.6	1.9	29
19	68 x 10 ⁶	118 x 10 ⁵	116 x 10 ⁴	100	17.4	1.7	30
20	64 x 10 ⁶	104 x 10 ⁵	109 x 10 ⁴	100	16.2	3.0	40

Table 18. Effect of Cyclic Irradiation at 70 Krad and Sub-culture of Survivors on Radio-Resistance of S. thompson (Isolate 1).

Radiation Cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	50 x 10 ⁶	48 x 10 ⁴	10 x 10 ²	100	0.95	0.0021	19
2	57 x 10 ⁶	10 x 10 ⁴	20 x 10 ²	100	0.18	0.0035	29
3	37 x 10 ⁶	36 x 10 ⁴	13 x 10 ²	100	0.97	0.0035	21
4	77 x 10 ⁶	15 x 10 ⁴	16 x 10 ¹	100	0.20	0.0002	17
5	52 x 10 ⁶	45 x 10 ⁴	12 x 10 ²	100	0.87	0.0022	19
6	72 x 10 ⁶	90 x 10 ⁴	58 x 10 ²	100	1.3	0.0081	21
7	56 x 10 ⁶	96 x 10 ⁴	51 x 10 ²	100	1.7	0.0091	22
8	51 x 10 ⁶	134 x 10 ⁴	238 x 10 ²	100	2.6	0.0470	29
9	96 x 10 ⁶	173 x 10 ⁴	96 x 10 ²	100	1.8	0.0100	23
10	74 x 10 ⁶	90 x 10 ⁴	35 x 10 ²	100	1.2	0.0047	21
11	55 x 10 ⁶	18 x 10 ⁵	92 x 10 ²	100	3.3	0.017	21
12	38 x 10 ⁶	21 x 10 ⁵	13 x 10 ³	100	5.4	0.033	23
13	64 x 10 ⁶	54 x 10 ⁵	52 x 10 ³	100	8.4	0.081	24
14	69 x 10 ⁶	40 x 10 ⁵	51 x 10 ³	100	5.8	0.074	25
15	66 x 10 ⁶	36 x 10 ⁵	44 x 10 ³	100	5.5	0.067	26

Table 19. Effect of Cyclic Irradiation at 120 Krad and Sub-culture of Survivors on Radio-Resistance of S. thompson (Isolate 1).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	50 x 10 ⁶	48 x 10 ⁴	10 x 10 ²	100	0.95	0.0021	19
2	75 x 10 ⁶	8 x 10 ⁴	14 x 10 ²	100	0.11	0.0019	29
3	68 x 10 ⁶	22 x 10 ⁴	7 x 10 ²	100	0.32	0.0011	20
4	58 x 10 ⁶	46 x 10 ⁴	12 x 10 ²	100	0.79	0.0020	19
5	48 x 10 ⁶	37 x 10 ⁴	6 x 10 ²	100	0.77	0.0013	18
6	47 x 10 ⁶	86 x 10 ⁴	26 x 10 ²	100	1.8	0.0055	20
7	50 x 10 ⁶	118 x 10 ⁴	60 x 10 ²	100	2.4	0.012	22
8	40 x 10 ⁶	89 x 10 ⁴	72 x 10 ²	100	2.2	0.018	24
9	50 x 10 ⁶	174 x 10 ⁴	270 x 10 ²	100	3.5	0.054	27
10	72 x 10 ⁶	114 x 10 ⁴	22 x 10 ²	100	1.6	0.003	19
11	48 x 10 ⁶	30 x 10 ⁵	160 x 10 ²	100	6.3	0.033	22
12	44 x 10 ⁶	24 x 10 ⁵	120 x 10 ²	100	5.5	0.027	22
13	52 x 10 ⁶	56 x 10 ⁵	91 x 10 ³	100	11.1	0.18	28
14	52 x 10 ⁶	50 x 10 ⁵	93 x 10 ³	100	9.6	0.18	29
15	47 x 10 ⁶	44 x 10 ⁵	108 x 10 ³	100	9.4	0.23	31

Table 20. Effect of Cyclic Irradiation at 70 Krad and Sub-culture of Survivors on Radio-Resistance of S. thompson (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	77 x 10 ⁶	57 x 10 ⁴	9 x 10 ²	100	0.74	0.0012	18
2	49 x 10 ⁶	51 x 10 ⁴	42 x 10 ²	100	1.0	0.0086	24
3	58 x 10 ⁶	46 x 10 ⁴	12 x 10 ²	100	0.79	0.0021	20
4	75 x 10 ⁶	31 x 10 ⁴	3 x 10 ²	100	0.41	0.0004	17
5	54 x 10 ⁶	54 x 10 ⁴	13 x 10 ²	100	1.0	0.0025	19
6	52 x 10 ⁶	92 x 10 ⁴	38 x 10 ²	100	1.8	0.0073	21
7	56 x 10 ⁶	98 x 10 ⁴	61 x 10 ²	100	1.8	0.011	23
8	37 x 10 ⁶	90 x 10 ⁴	120 x 10 ²	100	2.4	0.032	27
9	64 x 10 ⁶	33 x 10 ⁵	20 x 10 ³	100	5.2	0.032	23
10	52 x 10 ⁶	46 x 10 ⁵	36 x 10 ³	100	8.8	0.069	24
11	100 x 10 ⁶	42 x 10 ⁵	42 x 10 ³	100	4.2	0.042	25
12	50 x 10 ⁶	80 x 10 ⁵	82 x 10 ³	100	16.0	0.16	25
13	76 x 10 ⁶	71 x 10 ⁵	116 x 10 ³	100	9.3	0.15	28
14	77 x 10 ⁶	76 x 10 ⁵	140 x 10 ³	100	9.9	0.18	29
15	79 x 10 ⁶	48 x 10 ⁵	106 x 10 ³	100	6.1	0.13	30

Table 21. Effect of Cyclic Irradiation at 120 Krad and Sub-culture of Survivors on Radio-Resistance of S. thompson (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	77 x 10 ⁶	57 x 10 ⁴	90 x 10 ¹	100	0.74	0.0012	18
2	64 x 10 ⁶	4 x 10 ⁴	83 x 10 ¹	100	0.063	0.0013	30
3	20 x 10 ⁶	34 x 10 ⁴	74 x 10 ¹	100	1.7	0.0037	19
4	70 x 10 ⁶	16 x 10 ⁴	24 x 10 ¹	100	0.23	0.0003	18
5	30 x 10 ⁶	36 x 10 ⁴	70 x 10 ¹	100	1.2	0.0023	18
6	76 x 10 ⁶	86 x 10 ⁴	22 x 10 ²	100	1.1	0.0029	19
7	84 x 10 ⁶	164 x 10 ⁴	11 x 10 ²	100	2.0	0.0005	21
8	42 x 10 ⁶	120 x 10 ⁴	98 x 10 ²	100	2.9	0.023	24
9	75 x 10 ⁶	190 x 10 ⁴	214 x 10 ²	100	2.6	0.028	26
10	54 x 10 ⁶	66 x 10 ⁴	68 x 10 ²	100	1.2	0.013	25
11	38 x 10 ⁶	130 x 10 ⁴	68 x 10 ²	100	3.4	0.018	22
12	32 x 10 ⁶	34 x 10 ⁵	116 x 10 ²	100	11.0	0.036	20
13	68 x 10 ⁶	32 x 10 ⁵	74 x 10 ³	100	4.7	0.11	31
14	56 x 10 ⁶	26 x 10 ⁵	94 x 10 ³	100	4.7	0.17	35
15	82 x 10 ⁶	35 x 10 ⁵	177 x 10 ³	100	4.3	0.22	38

Table 22. Effect of Cyclic Irradiation at 70 Krad and Sub-culture of Survivors on Radio-Resistance of S. heidelberg (Isolate 1).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	120 x 10 ⁶	25 x 10 ³	2 x 10 ¹	100	0.025	0.00002	16
2	100 x 10 ⁶	31 x 10 ³	4 x 10 ¹	100	0.031	0.00004	17
3	100 x 10 ⁶	80 x 10 ³	24 x 10 ¹	100	0.080	0.0002	20
4	98 x 10 ⁶	55 x 10 ⁴	170 x 10 ¹	100	0.56	0.0017	20
5	120 x 10 ⁶	18 x 10 ⁴	46 x 10 ¹	100	0.15	0.0004	19
6	94 x 10 ⁶	76 x 10 ⁴	78 x 10 ²	100	0.81	0.0083	25
7	91 x 10 ⁶	12 x 10 ⁵	59 x 10 ²	100	1.3	0.0065	22
8	57 x 10 ⁶	14 x 10 ⁵	75 x 10 ²	100	2.5	0.013	22
9	78 x 10 ⁶	12 x 10 ⁵	140 x 10 ²	100	1.5	0.018	26
10	63 x 10 ⁶	8 x 10 ⁵	120 x 10 ²	100	1.2	0.019	28
11	30 x 10 ⁶	7 x 10 ⁵	240 x 10 ²	100	2.3	0.008	21
12	59 x 10 ⁶	14 x 10 ⁵	80 x 10 ²	100	2.4	0.014	22
13	74 x 10 ⁶	19 x 10 ⁵	92 x 10 ²	100	2.6	0.012	22
14	60 x 10 ⁶	21 x 10 ⁵	100 x 10 ²	100	3.5	0.017	22
15	58 x 10 ⁶	17 x 10 ⁵	82 x 10 ²	100	3.0	0.014	22

Table 23. Effect of Cyclic Irradiation at 120 Krad and Sub-culture of Survivors on Radio-Resistance of S. heidelberg (Isolate 1).

Radiation Cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	120 x 10 ⁶	25 x 10 ³	20 x 10 ⁰	100	0.025	0.00002	16
2	99 x 10 ⁶	32 x 10 ³	62 x 10 ⁰	100	0.032	0.00006	18
3	100 x 10 ⁶	34 x 10 ³	54 x 10 ⁰	100	0.034	0.00005	18
4	130 x 10 ⁶	32 x 10 ³	90 x 10 ⁰	100	0.025	0.00007	20
5	150 x 10 ⁶	37 x 10 ³	44 x 10 ⁰	100	0.025	0.00003	17
6	120 x 10 ⁶	67 x 10 ³	84 x 10 ⁰	100	0.056	0.00007	17
7	86 x 10 ⁶	64 x 10 ³	11 x 10 ¹	100	0.074	0.0001	18
8	70 x 10 ⁶	51 x 10 ³	12 x 10 ¹	100	0.073	0.0002	19
9	150 x 10 ⁶	11 x 10 ⁴	70 x 10 ¹	100	0.073	0.0005	23
10	120 x 10 ⁶	65 x 10 ⁴	83 x 10 ²	100	0.54	0.0069	27
11	110 x 10 ⁶	12 x 10 ⁵	81 x 10 ²	100	1.1	0.0074	23
12	110 x 10 ⁶	14 x 10 ⁵	99 x 10 ²	100	1.3	0.0090	23
13	110 x 10 ⁶	18 x 10 ⁵	15 x 10 ³	100	1.6	0.014	24
14	97 x 10 ⁶	21 x 10 ⁵	21 x 10 ³	100	2.2	0.022	25
15	88 x 10 ⁶	32 x 10 ⁵	35 x 10 ³	100	3.6	0.040	26

Table 24. Effect of Cyclic Irradiation at 70 Krad and Sub-culture of Survivors on Radio-Resistance of S. heidelberg (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	130 x 10 ⁶	31 x 10 ³	20 x 10 ⁰	100	0.024	0.00002	16
2	110 x 10 ⁶	29 x 10 ³	70 x 10 ⁰	100	0.026	0.00006	19
3	130 x 10 ⁶	11 x 10 ⁴	39 x 10 ¹	100	0.084	0.0003	20
4	130 x 10 ⁶	78 x 10 ⁴	160 x 10 ¹	100	0.58	0.0012	19
5	89 x 10 ⁶	23 x 10 ⁴	75 x 10 ¹	100	0.26	0.0008	20
6	110 x 10 ⁶	87 x 10 ⁴	12 x 10 ²	100	0.80	0.0011	18
7	76 x 10 ⁶	50 x 10 ⁴	20 x 10 ²	100	0.66	0.0026	21
8	63 x 10 ⁶	40 x 10 ⁴	12 x 10 ²	100	0.63	0.0019	20
9	120 x 10 ⁶	47 x 10 ⁴	33 x 10 ²	100	0.39	0.0029	23
10	100 x 10 ⁶	87 x 10 ⁴	14 x 10 ³	100	0.87	0.014	28
11	82 x 10 ⁶	28 x 10 ⁵	47 x 10 ³	100	3.4	0.057	28
12	73 x 10 ⁶	30 x 10 ⁵	66 x 10 ³	100	4.1	0.090	30
13	130 x 10 ⁶	42 x 10 ⁵	89 x 10 ³	100	4.0	0.068	30
14	72 x 10 ⁶	35 x 10 ⁵	73 x 10 ³	100	4.9	0.100	30
15	40 x 10 ⁶	5 x 10 ⁵	9 x 10 ³	100	1.1	0.023	30

Table 25. Effect of Cyclic Irradiation at 120 Krad and Sub-culture of Survivors on Radio-Resistance of S. heidelberg (Isolate 2).

Radiation cycle	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	70 Krad	120 Krad	0 Krad	70 Krad	120 Krad	
1	130 x 10 ⁶	31 x 10 ³	20 x 10 ⁰	100	0.024	0.00002	16
2	95 x 10 ⁶	45 x 10 ³	12 x 10 ¹	100	0.047	0.0001	19
3	150 x 10 ⁶	39 x 10 ³	15 x 10 ¹	100	0.026	0.0001	21
4	110 x 10 ⁶	42 x 10 ³	20 x 10 ¹	100	0.038	0.0002	21
5	190 x 10 ⁶	68 x 10 ³	14 x 10 ¹	100	0.036	0.00007	19
6	160 x 10 ⁶	13 x 10 ⁴	32 x 10 ¹	100	0.081	0.0002	19
7	110 x 10 ⁶	16 x 10 ⁴	47 x 10 ¹	100	0.15	0.0004	20
8	71 x 10 ⁶	38 x 10 ⁴	110 x 10 ¹	100	0.54	0.0015	20
9	130 x 10 ⁶	21 x 10 ⁴	73 x 10 ¹	100	0.16	0.0006	20
10	110 x 10 ⁶	54 x 10 ⁴	200 x 10 ¹	100	0.49	0.018	21
11	87 x 10 ⁶	74 x 10 ⁴	49 x 10 ¹	100	0.85	0.0006	16
12	64 x 10 ⁶	79 x 10 ⁴	63 x 10 ¹	100	1.2	0.001	16
13	74 x 10 ⁶	11 x 10 ⁵	26 x 10 ²	100	1.5	0.004	19
14	110 x 10 ⁶	22 x 10 ⁵	10 x 10 ³	100	2.0	0.10	21
15	91 x 10 ⁶	20 x 10 ⁵	14 x 10 ³	100	2.2	0.15	23

Table 26. Radio-Resistance of Resistant Cultures of
S. typhimurium upon Successive Transfers in Broth.

Transfer	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
	(Culture started from Isolate 1 after 20 irradiation cycles at 60 Krad.)						
1	61 x 10 ⁶	71 x 10 ⁵	10 x 10 ³	100	11.6	0.016	18
6	36 x 10 ⁶	35 x 10 ⁵	50 x 10 ³	100	9.8	0.14	24
16	35 x 10 ⁶	53 x 10 ⁵	28 x 10 ³	100	15.1	0.080	22
24	30 x 10 ⁶	44 x 10 ⁵	19 x 10 ³	100	14.7	0.063	21
	(Culture started from Isolate 1 after 20 irradiation cycles at 110 Krad.)						
1	49 x 10 ⁶	51 x 10 ⁵	8 x 10 ³	100	10.4	0.017	18
6	52 x 10 ⁶	63 x 10 ⁵	47 x 10 ³	100	12.1	0.091	23
16	42 x 10 ⁶	60 x 10 ⁵	26 x 10 ³	100	14.3	0.062	21
24	50 x 10 ⁶	38 x 10 ⁵	27 x 10 ³	100	7.6	0.054	23
	(Culture started from Isolate 1 after 20 irradiation cycles at 110 Krad.)						
1	40 x 10 ⁶	140 x 10 ⁵	35 x 10 ⁴	100	35.0	0.88	31
6	43 x 10 ⁶	135 x 10 ⁵	60 x 10 ⁴	100	31.4	1.4	37
16	36 x 10 ⁶	108 x 10 ⁵	37 x 10 ⁴	100	30.0	1.0	34
24	30 x 10 ⁶	29 x 10 ⁵	8 x 10 ⁴	100	9.7	0.28	32
	(Culture started from Isolate 1 after 20 irradiation cycles at 110 Krad.)						
1	44 x 10 ⁶	154 x 10 ⁵	36 x 10 ⁴	100	35.0	0.82	30
6	40 x 10 ⁶	149 x 10 ⁵	45 x 10 ⁴	100	37.3	1.1	32
16	41 x 10 ⁶	92 x 10 ⁵	31 x 10 ⁴	100	22.4	0.75	34
24	38 x 10 ⁶	44 x 10 ⁵	10 x 10 ⁴	100	11.6	0.25	30

Table 27. Radio-Resistance of Resistant Cultures of
S. typhimurium upon Successive Transfers in Broth.

Transfer	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	60 Krad	110 Krad	0 Krad	60 Krad	110 Krad	
	(Culture started from Isolate 2 after 20 irradiation cycles at 60 Krad.)						
1	57 x 10 ⁶	44 x 10 ⁵	23 x 10 ³	100	7.7	0.041	22
6	67 x 10 ⁶	90 x 10 ⁵	25 x 10 ³	100	13.4	0.036	20
16	94 x 10 ⁶	17 x 10 ⁵	14 x 10 ³	100	1.8	0.014	24
24	84 x 10 ⁶	17 x 10 ⁵	5 x 10 ³	100	2.0	0.005	19
1	65 x 10 ⁶	24 x 10 ⁵	81 x 10 ²	100	3.7	0.012	20
6	82 x 10 ⁶	22 x 10 ⁵	70 x 10 ²	100	2.8	0.009	20
16	67 x 10 ⁶	7 x 10 ⁵	25 x 10 ²	100	0.97	0.004	21
24	98 x 10 ⁶	10 x 10 ⁵	53 x 10 ²	100	0.98	0.005	22
	(Culture started from Isolate 2 after 20 irradiation cycles at 110 Krad.)						
1	42 x 10 ⁶	92 x 10 ⁵	54 x 10 ³	100	21.9	0.13	22
6	51 x 10 ⁶	95 x 10 ⁵	74 x 10 ³	100	18.6	0.15	23
16	61 x 10 ⁶	29 x 10 ⁵	15 x 10 ³	100	4.8	0.025	22
24	74 x 10 ⁶	25 x 10 ⁵	8 x 10 ³	100	3.4	0.010	20
1	62 x 10 ⁶	195 x 10 ⁵	360 x 10 ³	100	31.4	0.58	28
6	64 x 10 ⁶	128 x 10 ⁵	410 x 10 ³	100	20.0	0.64	33
16	78 x 10 ⁶	59 x 10 ⁵	75 x 10 ³	100	7.6	0.096	25
24	103 x 10 ⁶	65 x 10 ⁵	97 x 10 ³	100	6.3	0.094	27

Table 28. Radio-Resistance of Resistant Cultures of S. newport upon Successive Transfers in Broth.

Transfer	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
	(Culture started from Isolate 1 after 20 irradiation cycles at 30 Krad.)						
1	47 x 10 ⁶	20 x 10 ⁶	85 x 10 ⁴	100	42.5	1.0	22
6	51 x 10 ⁶	7 x 10 ⁶	22 x 10 ⁴	100	12.9	0.42	20
16	86 x 10 ⁶	13 x 10 ⁶	34 x 10 ⁴	100	15.1	0.40	19
24	38 x 10 ⁶	18 x 10 ⁶	260 x 10 ⁴	100	47.5	6.8	36
1	62 x 10 ⁶	18 x 10 ⁶	73 x 10 ⁴	100	28.8	1.2	22
6	41 x 10 ⁶	6 x 10 ⁶	34 x 10 ⁴	100	13.9	0.83	25
16	30 x 10 ⁶	1 x 10 ⁶	6 x 10 ⁴	100	4.3	0.19	22
24	60 x 10 ⁶	19 x 10 ⁶	200 x 10 ⁴	100	30.8	3.4	31
	(Culture started from Isolate 1 after 20 irradiation cycles at 60 Krad.)						
1	67 x 10 ⁶	11 x 10 ⁶	36 x 10 ⁴	100	15.7	0.54	21
6	39 x 10 ⁶	7 x 10 ⁶	13 x 10 ⁴	100	17.9	0.33	17
16	51 x 10 ⁶	11 x 10 ⁶	11 x 10 ⁴	100	21.8	0.21	15
24	58 x 10 ⁶	22 x 10 ⁶	182 x 10 ⁴	100	36.5	3.1	28
1	56 x 10 ⁶	14 x 10 ⁶	226 x 10 ⁴	100	24.4	4.0	37
6	55 x 10 ⁶	20 x 10 ⁶	187 x 10 ⁴	100	35.6	3.4	30
16	56 x 10 ⁶	17 x 10 ⁶	178 x 10 ⁴	100	29.8	3.2	31
24	67 x 10 ⁶	27 x 10 ⁶	394 x 10 ⁴	100	40.0	5.9	34

Table 29. Radio-Resistance of Resistant Cultures of S. newport
upon Successive Transfers in Broth.

Transfer	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ (Krad)
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
	(Culture started from Isolate 2 after 20 irradiation cycles at 30 Krad.)						
1	51 x 10 ⁶	21 x 10 ⁶	31 x 10 ⁵	100	41.3	6.0	36
6	45 x 10 ⁶	20 x 10 ⁶	29 x 10 ⁵	100	43.7	6.4	36
16	43 x 10 ⁶	23 x 10 ⁶	19 x 10 ⁵	100	52.0	4.4	28
24	38 x 10 ⁶	24 x 10 ⁶	44 x 10 ⁵	100	63.0	11.4	40
1	25 x 10 ⁶	15 x 10 ⁶	16 x 10 ⁵	100	60.0	6.4	31
6	19 x 10 ⁶	24 x 10 ⁶	22 x 10 ⁵	100		11.6	29
16	64 x 10 ⁶	27 x 10 ⁶	21 x 10 ⁵	100	42.6	3.3	28
24	95 x 10 ⁶	28 x 10 ⁶	32 x 10 ⁵	100	29.7	3.4	32
	(Culture started from Isolate 2 after 20 irradiation cycles at 60 Krad.)						
1	96 x 10 ⁶	19 x 10 ⁶	15 x 10 ⁵	100	20.1	1.5	27
6	25 x 10 ⁶	26 x 10 ⁶	28 x 10 ⁵	100		11.2	31
16	76 x 10 ⁶	16 x 10 ⁶	15 x 10 ⁵	100	20.6	2.0	29
24	86 x 10 ⁶	23 x 10 ⁶	36 x 10 ⁵	100	27.0	4.2	37
1	26 x 10 ⁶	5 x 10 ⁶	19 x 10 ⁵	100	19.6	7.4	
6	20 x 10 ⁶	6 x 10 ⁶	2 x 10 ⁵	100	30.0	1.0	21
16	86 x 10 ⁶	13 x 10 ⁶	10 x 10 ⁵	100	15.3	1.2	27
24	86 x 10 ⁶	25 x 10 ⁶	26 x 10 ⁵	100	28.6	3.1	31

Table 30. Radiation Survival Data for 7 Radio-Resistant Isolates of S. Newport Obtained by the Double-Irradiation Plate Method.

Sample	Cells/ml after a dose of:			% Survival after a dose of:			D ₁₀ Krad
	0 Krad	30 Krad	60 Krad	0 Krad	30 Krad	60 Krad	
Control	127 x 10 ⁷	15 x 10 ⁶	6 x 10 ³	100	1.2	0.00047	9
Resistant 1	270 x 10 ⁷	106 x 10 ⁶	205 x 10 ⁴	100	3.9	0.076	17
Resistant 2	131 x 10 ⁷	30 x 10 ⁶	23 x 10 ⁴	100	2.3	0.018	14
Resistant 3	117 x 10 ⁷	38 x 10 ⁶	6 x 10 ⁴	100	3.2	0.005	11
Resistant 4	108 x 10 ⁷	54 x 10 ⁶	40 x 10 ⁴	100	5.0	0.037	14
Resistant 5	108 x 10 ⁷	20 x 10 ⁶	11 x 10 ⁴	100	1.8	0.010	13
Resistant 6	106 x 10 ⁷	105 x 10 ⁶	174 x 10 ⁴	100	10.0	0.16	17
Resistant 7	112 x 10 ⁷	98 x 10 ⁶	116 x 10 ⁴	100	8.7	0.10	16

Table 31. Number of Radio-Resistant Cells of S. newport in samples from Independent Cultures and in Samples from Single Cultures.

Samples from independent cultures				Samples from single cultures			
Sample Number	Initial number of cells	Number of cells after double-irradiation*	Number of resistant cells	Sample Number	Initial Number of cells	Number of cells after double-irradiation*	Number of resistant cells
Experiment 1				Experiment 1			
1	92×10^4	12	0	1	85×10^4	24	0
2	100×10^4	10	0	2	"	4	0
3	93×10^4	18	0	3	"	7	0
4	113×10^4	11	0	4	"	10	0
5	92×10^4	7	1	5	"	3	0
6	98×10^4	11	1	6	"	8	0
7	94×10^4	15	0	7	"	8	0
8	92×10^4	6	0	8	"	3	0
9	84×10^4	4	0	9	"	4	0
10	92×10^4	4	1	10	85×10^4	4	0
Experiment 2				Experiment 2			
1	150×10^4	1	0	1	72×10^4	0	0
2	135×10^4	0	0	2	"	5	0
3	107×10^4	5	0	3	"	0	0
4	120×10^4	3	0	4	"	2	0
5	98×10^4	1	0	5	"	2	1
6	111×10^4	4	0	6	"	2	0
7	125×10^4	1	0	7	"	2	0
8	106×10^4	2	0	8	"	2	0
9	92×10^4	3	0	9	"	4	1
10	93×10^4	3	0	10	"	4	1
11	114×10^4	1	0	11	"	1	0
12	106×10^4	2	0	12	"	1	0
13	150×10^4	6	0	13	"	9	0
14	89×10^4	3	0	14	"	3	0
15	102×10^4	2	0	15	72×10^4	4	0

* double-irradiation = irradiate with 15 Krad, incubate 3 hours, then irradiate with 60 Krad

Table 32. Mortality of Mice After Intraperitoneal Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. thompson.

Non-resistant culture					Radio-resistant culture				
Number of cells injected	Number of deaths/group of 10 mice				Number of cells injected	Number of deaths/group of 10 mice			
	after 1 day	after 2 days	after 3 days	after 5 days		after 1 day	after 2 days	after 3 days	after 5 days
5×10^8	3/5	5/5	5/5	5/5	3.5×10^8	0/5	2/5	2/5	2/5
5×10^7	0/10	0/10	0/10	0/10	3.5×10^7	0/10	0/10	0/10	0/10
5×10^6	0/10	0/10	0/10	0/10	3.5×10^6	0/10	0/10	0/10	0/10
5×10^5	0/10	0/10	0/10	0/10	3.5×10^5	0/10	0/10	0/10	0/10
5×10^4	0/10	0/10	0/10	0/10	3.5×10^4	0/10	0/10	0/10	0/10
5×10^3	0/10	0/10	0/10	0/10	3.5×10^3	0/10	0/10	0/10	0/10
5×10^2	0/10	0/10	0/10	0/10	3.5×10^2	0/10	0/10	0/10	0/10
$LD_{50} \approx 1.5 \times 10^8$ cells					$LD_{50} \approx 3.5 \times 10^8$ cells				

Table 33. Mortality of Mice After Intraperitoneal Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. newport.

Number of cells injected	Non-resistant culture			Number of cells injected	Radio-resistant culture		
	Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days		Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days
2.5×10^9	10/10	10/10	10/10	1.5×10^9	8/10	9/10	9/10
2.5×10^8	10/10	10/10	10/10	1.5×10^8	8/10	8/10	8/10
2.5×10^7	0/10	0/10	0/10	1.5×10^7	0/10	0/10	0/10
2.3×10^6	0/10	0/10	0/10	1.2×10^6	0/10	0/10	0/10
2.3×10^5	0/10	0/10	0/10	1.2×10^5	0/10	0/10	0/10
2.1×10^4	0/10	0/10	0/10	1.5×10^4	0/10	0/10	0/10
$LD_{50} \approx 8 \times 10^7$ cells				$LD_{50} \approx 6 \times 10^7$ cells			

Table 34. Mortality of Mice After Intraperitoneal Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. typhimurium.

Non-resistant culture				Radio-resistant culture			
Number of cells injected	Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days	Number of cells injected	Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days
1.4×10^9	9/10	10/10	10/10	0.8×10^9	10/10	10/10	10/10
1.4×10^8	10/10	10/10	10/10	0.8×10^8	8/10	10/10	10/10
1.4×10^7	4/10	10/10	10/10	0.8×10^7	0/10	1/10	1/10
2.3×10^6	1/10	2/10	4/10	1.3×10^6	0/10	0/10	0/10
2.3×10^5	0/10	0/10	0/10	1.3×10^5	0/10	0/10	0/10
3.2×10^4	0/10	0/10	0/10	1.6×10^4	0/10	0/10	0/10
$LD_{50} \approx 3 \times 10^6$ cells				$LD_{50} \approx 2 \times 10^7$ cells			

Table 35. Mortality of Mice After Intraperitoneal Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. heidelberg.

Non-resistant culture				Radio-resistant culture			
Number of cells injected	Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days	Number of cells injected	Number of deaths/group of 10 mice after 1 day	after 2 days	after 3 days
3.6×10^9	10/10	10/10	10/10	2.8×10^9	8/10	9/10	9/10
3.0×10^8	10/10	10/10	10/10	2.5×10^8	10/10	10/10	10/10
3.0×10^7	0/10	1/10	2/10	2.5×10^7	4/10	5/10	5/10
3.8×10^6	0/10	0/10	0/10	3.6×10^6	0/10	1/10	1/10
3.8×10^5	0/10	0/10	0/10	3.6×10^5	0/10	0/10	0/10
4.8×10^4	0/10	0/10	0/10	2.6×10^4	0/10	0/10	0/10
$LD_{50} \approx 7 \times 10^7$ cells				$LD_{50} \approx 2.5 \times 10^7$ cells			

Table 36. Mortality of Chick Embryos After Yolk-Sac Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. thompson.

Non-resistant culture				Radio-resistant culture			
Number of cells injected	Number of deaths/group after 1 day	after 2 days	after 3 days	Number of cells injected	Number of deaths/group after 1 day	after 2 days	after 3 days
5000	6/6	6/6	6/6	3500	5/6	6/6	6/6
500	6/6	6/6	6/6	350	2/6	6/6	6/6
50	6/6	6/6	6/6	35	1/6	6/6	6/6
5	1/6	6/6	6/6	3.5	0/6	3/6	3/6
LD ₅₀ $\approx$ < 5 cells				LD ₅₀ $\approx$ 3.5 cells			

Table 37. Mortality of Chick Embryos After Yolk-Sac Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. newport.

Non-resistant culture				Radio-resistant culture			
Number of cells injected	Number of deaths/group after 1 day	after 2 days	after 3 days	Number of cells injected	Number of deaths/group after 1 day	after 2 days	after 3 days
1700	6/6	6/6	6/6	1200	6/6	6/6	6/6
230	6/6	6/6	6/6	180	6/6	6/6	6/6
32	6/6	6/6	6/6	12	5/6	6/6	6/6
3.2	5/6	5/6	5/6	1.5	1/6	1/6	4/6
0.3	1/6	1/6	1/6	0.15	1/6	1/6	1/6
0.03	3/6	3/6	4/6	0.015	0/6	0/6	0/6
LD ₅₀ $\approx$ 0.9 cells				LD ₅₀ $\approx$ 0.7 cells			

Table 38. Mortality of Mice After Intraperitoneal Injection with Various Numbers of Radio-Resistant or Non-Resistant Viable Cells of S. thompsoni Which had been Passed once Through Chick Embryo.

Non-resistant culture					Radio-resistant culture				
Number of cells injected	Number of deaths/group of 10 mice after 18 hrs	after 1 day	after 2 days	after 3 days	Number of cells injected	Number of deaths/group of 10 mice after 18 hrs	after 1 day	after 2 days	after 3 days
1.5×10^9	5/10	10/10	10/10	10/10	1.1×10^9	4/10	6/10	9/10	9/10
1.1×10^8	0/10	5/10	10/10	10/10	1.1×10^8	0/10	0/10	4/10	5/10
1.1×10^7	0/10	0/10	0/10	0/10	1.1×10^7	0/10	0/10	0/10	0/10
3.2×10^6	0/10	0/10	0/10	0/10	2.3×10^6	0/10	0/10	0/10	0/10
3.2×10^5	0/10	0/10	0/10	0/10	2.3×10^5	0/10	0/10	0/10	0/10
4.1×10^4	0/10	0/10	0/10	0/10	2.7×10^4	0/10	0/10	0/10	0/10
$LD_{50} \approx 3.5 \times 10^7$ cells					$LD_{50} \approx 1.1 \times 10^8$ cells				

Table 39. Biochemical Reactions of Radio-Resistant and Sensitive Cultures of Different Salmonella Serotypes on Various Media

Serotype	Number of cultures tested	Reaction on triple sugar iron agar.		Reaction on lysine iron agar.*		Citrate utilization	Malonate utilization	Phenyl-alanine deaminase	Urease	Growth in KCN medium	Indol production
		(Slant/Butt)	H ₂ S	(Slant/Butt)	H ₂ S						
S. newport (sensitive)	43	ALK/ACID	+	ALK/ALK	+	+	-	-	-	-	-
	4	ALK/ACID	+	ALK/ALK	+	+	-	-	-	+	-
	3	ALK/ACID	+	ALK/ALK	+	+	-	-	-	weak	-
S. newport (radio-resistant)	33	ALK/ACID	+	ALK/ALK	+	+	-	-	-	-	-
	8	ALK/ACID	+	ALK/ALK	+	-	-	-	-	-	-
	2	ALK/ACID	+	ALK/ALK	+	-	-	-	-	+	-
	1	ALK/ACID	+	ALK/ALK	+	-	-	-	-	weak	-
	6	ALK/ACID	+	ALK/ALK	+	+	-	-	-	+	-
S. thompson (sensitive)	50	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
S. thompson (radio-resistant)	41	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
	5	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
	2	ALK/ACID	-	ALK/ALK	-	-	-	-	-		-
	2	ALK/ACID	-	ALK/ALK	-	+	-	-	-		-

Table 39. Continued.

Serotype	Number of cultures tested	Reaction on triple sugar iron agar. (Slant/Butt) H ₂ S		Reaction on lysine iron agar.* (Slant/Butt) H ₂ S		Citrate utilization	Malonate utilization	Phenylalanine deaminase	Urease	Growth in KCN medium	Indol production
S. heidelberg (sensitive)	50	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
S. heidelberg (radio-resistant)	50	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
S. typhimurium (sensitive)	50	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
S. typhimurium (radio-resistant)	50	ALK/ACID	+	ALK/ALK	+	+	-	-	-		-
Proteus		ALK/ALK	+	RED/ACID	+	-	-	+	+	+	-
Aerobacter		ACID/ACID	-	ALK/ALK	-	+	+	-	-	+	-
E. coli		ACID/ACID	-	ALK/ACID	-	-	-	-	-	-	+

* ALK/ALK denotes presence of lysine decarboxylase

Table 40. Colony Appearance of Radio-Resistant and Sensitive
Cultures of Four Salmonella Serotypes on Various
Differential Media.

Serotype	Brilliant-green agar	Bismuth sulfite agar	Salmonella-shigella agar	Mac Conkey agar
<i>S. newport</i> (sensitive)	Whitish, opaque raised colony surrounded by red zone.	Black, raised colony with flat top. Some with silvery sheen.	Raised, transparent colony. Same color as medium (yellowish).	Sl. raised tan colony with darker center.
<i>S. newport</i> (resistant)	Same as sensitive	Same as sensitive	Same as sensitive some with black center	Same as sensitive
<i>S. thompson</i> (sensitive)	Whitish, opaque raised colony surrounded by red zone.	Some raised black colonies. Some with silvery sheen. Some green flat (button) colonies.	Raised, transparent colony. Same color as medium (yellowish).	Sl. raised tan colony with darker center.
<i>S. thompson</i> (resistant)	Same as sensitive.	Same as sensitive.	Same as sensitive.	Same as sensitive.
<i>S. heidelberg</i> (sensitive)	Whitish, opaque raised colony surrounded by red zone.	Silvery-gray raised colony surrounded by black zone. Some with sheen.	Raised transparent colony. Same color as medium (yellowish). Some with black center.	Sl. raised tan colony with darker center.
<i>S. heidelberg</i> (resistant)	Same as sensitive.	Same as sensitive.	Same as sensitive.	Same as sensitive.
<i>S. typhimurium</i> (sensitive)	Whitish, opaque raised colony surrounded by red zone.	Green, flat colony.	Raised, transparent colony. Same color as medium (yellowish).	Sl. raised tan colony with darker center.
<i>S. typhimurium</i> (resistant)	Same as sensitive.	Same as sensitive.	Same as sensitive.	Same as sensitive.

Table 41. Recovery of Radio-sensitive and Radio-resistant cultures of S. newport on Various Differential Media.

Medium	Plate count per ml	
	sensitive culture	resistant culture
TSYE agar	128×10^5	80×10^5
Brilliant green agar	127×10^5	23×10^5
Bismuth sulfite agar	108×10^5	13×10^5
Mac Conkey agar	139×10^5	56×10^5
Desoxycholate citrate agar	30×10^5	60×10^1
Salmonella-Shigella agar	59×10^5	19×10^1

Table 42. Recovery of Radio-Sensitive and Radio-Resistant

Cultures of S. typhimurium on Various Differential Media.

Medium	Cells per gram	
	sensitive culture	resistant culture
(Direct plate count)		
Brilliant green sulfadiazene agar	23×10^4	32×10^4
Bismuth sulfite agar	75×10^4	76×10^4
MacConkey agar	87×10^4	81×10^4
Salmonella-Shigella agar	5×10^4	7×10^4
Desoxycholate citrate agar	73×10^4	5×10^4
(MPN method)		
Selenite-cystine broth	79×10^4	110×10^4
Tetrathionate broth	79×10^4	70×10^4
Nutrient broth → tetrathionate broth	130×10^4	46×10^4

Table 43. Efficiency of Various Selective Media on the
Quantitative Recovery of Salmonella after Irradiation
in TSYE Broth.

Irradiation dose (Krad)	Cells per ml on agar plates of:					
	TSYE	Brilliant green	Salmonella- Shigella	Bismuth sulfite	Desoxycholate lactose	MacConkey
			<u>S. thompson</u>			
0	60×10^7	67×10^7	$< 1 \times 10^6$	76×10^7	73×10^7	72×10^7
60	38×10^5	15×10^5	10×10^3	14×10^3	19×10^5	11×10^5
90	245×10^3	130×10^3	1×10^1	70×10^3	87×10^3	35×10^3
			<u>S. newport</u>			
0	170×10^7	12×10^7	10×10^7	150×10^7	137×10^7	146×10^7
30	103×10^5	132×10^5	2×10^5	257×10^5	148×10^5	140×10^5
60	40×10^2	72×10^2	5×10^1	180×10^2	38×10^2	32×10^2
			<u>S. typhimurium</u>			
0	195×10^7	95×10^7	24×10^7	200×10^7	230×10^7	175×10^7
60	49×10^4	5×10^4	12×10^3	28×10^4	19×10^4	31×10^4
90	275×10^1	24×10^1	8×10^0	40×10^1	50×10^1	14×10^1

Table 44. Efficiency of Various Selective Media on the Quantitative Recovery of S. heidelberg and S. anatum after Irradiation at 33°F (0.5°C) in Phosphate Buffer.

Radiation dose (Krad)	Recovery count per ml on agar plates of:						
	TSYE agar	Minimal (glucose-salts)	Brilliant green sulfadiazene	Bismuth sulfite agar	Salmonella-Shigella agar	Desoxycholate citrate agar	MacConkey agar
			<u>S. heidelberg</u>				
0	130×10^6	110×10^6	95×10^6	90×10^6	25×10^6	40×10^6	123×10^6
50	215×10^3	7×10^3	67×10^3	18×10^3	15×10^3	11×10^3	99×10^3
75	350×10^1	67×10^1	190×10^1	55×10^1	27×10^1	12×10^1	130×10^1
			<u>S. anatum</u>				
0	93×10^6	77×10^6	$< 1 \times 10^5$	82×10^6	9×10^6	9×10^6	64×10^6
50	190×10^2	60×10^2	2×10^1	36×10^2	23×10^1	7×10^1	26×10^2
75	no colonies at one-to-ten dilution						

Table 45. Antibiotic Sensitivity of Radio-Resistant and Radio-Sensitive Cultures of *S. typhimurium*.

Culture No.	(Radio-sensitive)				(Radio-resistant)			
	Diameter (mm) of zone of inhibition.				Diameter (mm) of zone of inhibition.			
	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Peni- cillin 5 units	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Peni- cillin 5 units
1	16	12	11		15	11	9	
2	16	12	9		16	11	9	
3	16	11	9		15	10	10	
4	16	12	8		15	9	8	
5	17	11	11		16	11	7	
6	16	11	8		16	11	8	
7	18	11	10	8	17	11	9	
8	15	10	11		15	10	8	
9	15	11	11	8	15	10	8	
10	17	11	8		15	11	7	
11	17	12	9	9	15	10	7	
12	16	11	7	8	16	11	7	
13	16	12	10		15	10	7	
14	16	11	7	8.5	16	10	7	
15	17	11	8		16	12	8	
16	16	11	7		15	10	9	
17	17	11	11		16	11	9	
18	18	12	9		17	12	8	
19	18	11	8		17	10	9	
20	16	12	11		16	11	8	
21	18	11	9		17	11	9	
22	18	10	8		15	11	7	
23	16	12	10		16	10	8	7
24	16	10	7	9	15	11	9	6
25	16	11	7		14	10	7	7
26	17	11	10		15	10	7	
27	17	11	7		15	11	8	8
28	16	12	7		15	10	9	7
29	18	11	7		15	11	10	9
Avg.	16.6	11.2	8.8	8.4	15.0	10.6	8.1	7.3
S.D.	0.91	0.62	1.49		0.97	0.68	0.96	
	Significant	Significant	Not Significant					

Table 46. Antibiotic Sensitivity of Radio-Resistant and Radio-Sensitive Cultures of S. newport.

Culture No.	(Radio-sensitive)						(Radio-resistant)					
	Diameter (mm) of zone of inhibition						Diameter (mm) of zone of inhibition					
	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg	Peni- cillin 5 units	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg	Peni- cillin 5 units
1	19	11	14	19	22	11	15	11	11	25	27	8
2	18	11	12	21	22	9	14	11	12	23	25	8
3	18	11	13	20	23	10	15	11	10	17	22	7
4	17	11	13	19	21	10	13	10	8	21	25	6
5	17	12	13	20	22	10	16	12	10	25	27	8
6	18	11	15	19	22	9	16	12	12	24	28	8
7	18	12	13	21	23	10	16	12	12	23	28	8
8	18	11	12	20	23	10	17	12	10	24	26	7
9	18	11	12	20	22	11	15	11	11	24	28	7
10	18	11	12	18	18	9	15	12	11	23	25	8
11	18	11	13	21	22	11	16	12	11	26	28	8
12	18	11	13	19	20	11	16	12	12	25	28	8
13	19	11	13	19	21	9	15	11	10	24	27	8
14	19	11	12	20	23	9	16	12	11	23	26	8
15	17	12	14		22		17	12	12		25	
16	19	12	12		20		15	11	12		25	
17	16	11	12		20		15	11	12		24	
18	18	12	14		22		16	12	10		30	
19	20	12	14		24		16	12	13		29	
20	20	13	13		22		18	13	12		27	
Avg.	18.2	11.4	12.9	19.7	21.7	9.9	15.6	11.6	11.1	23.4	26.5	7.7
S.D.	1.0	0.60	0.89	0.91	1.56	0.85	1.1	0.68	1.76	1.37	1.44	0.64
	Significant	Not Significant	Signi- ficant	Signifi- cant	Signifi- cant							

Table 47. Antibiotic Sensitivity of Radio-Resistant and Radio-Sensitive Cultures of S. heidelberg.

Culture No.	(Radio-sensitive)						(Radio-resistant)					
	Diameter (mm) of zone of inhibition.						Diameter (mm) of zone of inhibition.					
	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg	Peni- cillin 5 units	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg	Peni- cillin 5 units
1	18	10	11	17	21	6	17	12	10	20	23	6
2	18	10	11	18	20	6	19	13	14	21	24	6
3	16	10	10	17	20	6	16	12	10	19	22	6
4	17	10	10	18	20	6	19	12	11	20	23	6
5	18	10	13	18	21	6	16	12	10	20	23	6
6	18	10	11	17	21	6	17	12	10	20	22	6
7	17	9	9	18	22	6	17	12	9	18	22	6
8	16	10	12	17	21	6	17	12	11	19	21	6
9	16	9	9	17	20	6	14	11	11	19	23	6
10	16	10	10	18	20	6	18	12	10	20	24	6
11	18	10	10	20	24	6	18	12	10	20	24	6
12	15	10	12	19	22	6	16	12	10	20	22	6
13	17	10	10	19	22	6	17	12	12	20	22	6
14	20	11	14	21	23	6	16	12	9	19	22	6
15	16	10	11	18	21	6	16	12	9	20	23	6
16	18	10	10	18	22	6	17	13	10	19	22	6
17	18	10	10	19	21	6	18	13	10	20	24	6
18	17	10	10	20	22	6	15	12	10	20	22	6
19	18	10	10	18	20	6	17	12	10	20	24	6
20	17	11	11	19	21	6	17	12	10	20	23	6
Avg.	17.2	10.0	10.7	18.3	21.2	6	16.9	12.1	10.3	19.7	22.8	6
S.D.	1.13	0.45	1.18	1.12	1.08		1.23	0.46	1.04	0.66	0.91	
	Not Significant	Significant	Not Significant	Significant	Significant							

Table 48. Antibiotic Sensitivity of Radio-Resistant and
Radio-Sensitive Cultures of S. thompson.

Culture No.	(Radio-sensitive)					(Radio-resistant)				
	Diameter (mm) of zone of inhibition					Diameter (mm) of zone of inhibition				
	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg	Chlor- amphenicol 5 mcg	Tetra- cycline 5 mcg	Ampi- cillin 2 mcg	Ampi- cillin 5 mcg	Ampi- cillin 10 mcg
1	17	10	10	18	19	16	11	9	19	20
2	14	10	9	15	18	14	10	9	19	20
3	15	11	11	17	19	14	10	10	17	22
4	14	10	10	17	20	15	10	9	19	20
5	16	11	11	17	19	14	9	11	17	20
6	14	10	10	17	18	15	10	9	18	20
7	16	10	10	16	19	16	12	9	18	20
8	14	10	10	18	20	14	10	11	17	19
9	16	10	10	18	20	15	9	10	18	20
10	16	10	10	17	20	14	11	11	17	20
11	15	10	11	16	18	14	11	10	20	22
12	18	10	12	17	21	17	11	9	20	23
13	15	10	10	17	18	15	10	11	20	21
14	15	10	10	16	18	15	11	10	17	20
15	16	11	11	17	20	15	10	10	17	20
16	14	10	10	16	20	15	11	10	20	22
17	15	11	10		20	15	11	12		21
18	15	11	12		20	15	11	13		20
19	16	11	11		20	18	12	12		23
20	18	11	13		21	16	11	12		21
Avg.	15.5	10.4	10.1	16.8	19.4	15.1	10.6	10.4	18.3	20.7
S.D.	1.24	0.49	0.78	1.09	1.16	1.08	0.84	1.28	1.20	1.13
	Not Significant	Not Signifi- cant	Not Signifi- cant	Signifi- cant	Signifi- cant					

Table 49. Effect of Penicillin G on Growth of
Radio-Resistant or Radio-Sensitive Cultures
of Salmonella in Broth.

Serotype	Incubation period (hours)	Turbidity of culture at antibiotic concentration (units/ml) of:				
		0	5	10	20	40
newport sensitive(1)	24	+++	-	-	-	-
	48	+++	trace	-	-	-
newport sensitive(2)	24	+++	-	-	-	-
	48	+++	-	-	-	-
newport resistant(1)	24	+++	trace	-	-	-
	48	+++	++	-	-	-
newport resistant(2)	24	+++	trace	-	-	-
	48	+++	++	-	-	-
typhimurium sensitive(1)	24	+++	++	-	-	-
	48	+++	++	+	-	-
typhimurium sensitive(2)	24	+++	+	-	-	-
	48	+++	++	-	-	-
typhimurium resistant(1)	24	+++	+	-	-	-
	48	+++	+	++	-	-
typhimurium resistant(2)	24	+++	+	-	-	-
	48	+++	+	+	-	-
heidelberg sensitive(1)	24	+++	++	-	-	-
	48	+++	++	-	-	-
heidelberg sensitive(2)	24	+++	+	-	-	-
	48	+++	++	-	-	-
heidelberg resistant(1)	24	+++	++	-	-	-
	48	+++	++	-	-	-
heidelberg resistant(2)	24	+++	++	-	-	-
	48	+++	++	-	-	-
thompson sensitive(1)	24	+++	+	-	-	-
	48	+++	++	-	-	-
thompson sensitive(2)	24	+++	+	-	-	-
	48	+++	++	-	-	-
thompson resistant(1)	24	+++	+	-	-	-
	48	+++	++	-	-	-
thompson resistant(2)	24	+++	+	-	-	-
	48	+++	++	-	-	-

Table 50. Growth Characteristics under Aerobic or Anaerobic Conditions for Radio-Resistant or Radio-Sensitive Cultures of Various Salmonella Serotypes.

Serotype	Growth temp. °C	Generation time (min.)		Lag time (min.)	
		sensitive culture	resistant culture	sensitive culture	resistant culture
<u>S. typhimurium</u>		Anaerobic growth		Anaerobic growth	
	10	690	720	-	-
	20	91	86.5	180	210
	30	36.8	34.7	90	80
	34	26.0	26.3	85	95
	38	29.0	27.8	84	60
	43	24.0	24.5	35	40
	45	30.0	32.2	110	110
		Aerobic growth		Aerobic growth	
	20	85.8	79	190	200
	37	20.5	23	120	85
<u>S. heidelberg</u>		Anaerobic growth		Anaerobic growth	
	37	22.8	24.2	70	70
		Aerobic growth		Aerobic growth	
	20	79	79	350	380
	37	22.5	25.2	60	150
<u>S. newport</u>		Anaerobic growth		Anaerobic growth	
	10	590	780	-	-
	20	96.5	141	270	660
	37	22	25.4	85	75
		Aerobic growth		Aerobic growth	
	20	87	127.8	330	345
	37	19.3	21.6	140	150

Table 50 continued.

Serotype	Growth temp. °C	Generation time (min.)		Lag time (min.)	
		sensitive culture	resistant culture	sensitive culture	resistant culture
<u>S. thompson</u>		Anaerobic growth		Anaerobic growth	
	10	620	610	-	-
	20	98.5	103	375	375
	30	30	26.4	84	108
	34	24.3	24.3	60	60
	37	24.8	25.1	96	84
	43	26	26	96	60
	45	38	37.5	48	72
		Aerobic growth		Aerobic growth	
	20	81.5	83	252	260
	37	17.9	20.3	180	135

FIGURE 4

EFFECT OF GROWTH CONDITION (AEROBIC OR ANAEROBIC)
ON RADIATION INACTIVATION OF *S. typhimurium*.

FIGURE 6
EFFECT OF GROWTH CONDITION (AEROBIC OR ANAEROBIC)
ON RADIATION INACTIVATION OF *S. heidelberg*.

FIGURE 12

FIGURE 13

FIGURE 14B.
ELECTRON MICROSCOPE PHOTOGRAPHS OF RADIO-RESISTANT
AND RADIO-SENSITIVE CELLS OF S. newport.

RADIO-SENSITIVE
X5500

RADIO-RESISTANT
X5500

FIGURE 15.
ANAEROBIC GROWTH RATE AT 10°C OF RADIO-RESISTANT
AND RADIO-SENSITIVE CULTURES OF *S. typhimurium*.

FIGURE 35.
ANAEROBIC GROWTH RATE AT 45 C OF RADIO-RESISTANT
AND RADIO-SENSITIVE CULTURES OF *S. thompsoni*.

FIGURE 39.
AEROBIC GROWTH RATE AT 37° C AND 20° C OF RADIO-RESISTANT
AND RADIO-SENSITIVE CULTURES OF *S. Neideberg*.

Electron Micrograph of Radio-Resistant Strain of S. newport.

(5 hour broth culture)

Salmonella newport
radio-resistant X 5500

Electron Micrograph of Radio-Resistant Strain of S. newport.
(5 hour broth culture)

Salmonella newport
radio-resistant X 5500

Electron Micrograph of Radio-Sensitive Parent Strain of
S. newport (5 hour broth culture)

Salmonella newport
radio-sensitive X 5500

Electron Micrograph of Radio-Sensitive Parent Strain of
S. newport (5 hour broth culture)

Salmonella newport
radio-sensitive X 5500