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Mechanism by Which Gamma Irradiation Increases the Sensitivity of *Salmonella typhimurium* ATCC 14028 to Heat

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Effects of irradiation and heating on survival of *Salmonella typhimurium* ATCC 14028 were examined by measuring DNA damage and the integrity of the cytoplasmic membrane. *S. typhimurium* cells fell into two distinct groups following heating: (i) heat-sensitive cells, which were rapidly inactivated at 65°C and (ii) heat-resistant cells, which were only slowly inactivated at 65°C. Radiation sensitivity of *S. typhimurium* was greater in the presence of air than in the presence of N_2 gas (radiation doses required to inactivate 90% of the cells, 0.394 ± 0.029 in air and 0.561 ± 0.035 in N_2). Recovery of the covalently closed circular form of plasmid pBR322 from *S. typhimurium* transformants (Amp^r Tet^r) was decreased by irradiation but not by heating. Heating prior to irradiation significantly decreased the recovery of plasmid DNA without affecting survival of *S. typhimurium*. Transformability of the recovered plasmid pBR322 was affected by neither irradiation nor heating, and mutation of antibiotic resistance genes was not detected in *S. typhimurium*. Heating, but not irradiation, caused destabilization of the cytoplasmic membrane, allowing penetration of hydrophobic dye. These results suggest that lethality of heating followed by irradiation for *S. typhimurium* was additive, reflecting irradiation-induced DNA damage and heat-induced membrane destabilization. When irradiation preceded heating in the absence of air, more cells were inactivated than was expected, because of heat-inactivating radiation-damaged DNA.

Recent outbreaks of food poisoning in the United States have increased demand for a safer supply of meat and poultry products without sacrifice of wholesomeness and nutritional value. Thirty-five percent of the chicken carcasses processed in the United States are suspected of being contaminated with *Salmonella* spp. (5). The use of ionizing irradiation has been suggested as a method for eliminating or reducing contamination of foods by pathogens, such as *Salmonella* and *Campylobacter* spp. and *Escherichia coli* O157:H7 from meat and poultry products. Its use has been approved by the Food and Drug Administration and U.S. Department of Agriculture for raw poultry products (19).

Both direct and indirect reactions between ionizing radiation and cellular components occur in direct proportion to the amount of energy that is absorbed. Since 50 to 70% of the bacterial cell mass is water, it absorbs much of the radiation. As a result, hydroxyl radicals and hydrated electrons, which are important in irradiation-induced cell inactivation, are produced. Irradiation damage of DNA is considered a major cause of cell inactivation, while those of protein, lipid, and RNA contribute less (27). Reaction of DNA with hydroxyl radicals may result in single- and/or double-strand breaks, protein-DNA cross-linkage, and base alterations leading to cellular inactivation (27). Survival of bacterial cells following irradiation depends upon intrinsic factors, such as the physiological condition of individual cells and their potential for repair (27). However, the mechanisms of bacterial inactivation by ionizing irradiation are not completely understood.

The process of thermal control of food-borne pathogens in various food systems is well established. Typically, the bacterial population decreases exponentially, while a portion is heat resistant. Gould (8) and Hurst (10) summarized some of the mechanisms by which heat may cause cellular inactivation: (i) damage of DNA, (ii) inhibition of protein synthesis, (iii) damage of cell membrane, and (iv) inactivation of critical metabolic enzymes. Because multiple changes may occur during the inactivation of bacteria by heat, thermal inactivation is not linear (8, 10). Some or all of these mechanisms may also be applicable during inactivation of bacterial cells by irradiation.

Simultaneous applications of ionizing irradiation and heating to control food-borne pathogens have been proposed to maximize food safety, while preserving food quality by reducing the detrimental effects of heating and irradiation on the food (18, 19). However, current regulations require irradiation of poultry at 40°F (ca. ≤4°C), and the temperature must not exceed 55°F (ca. 13°C) during processing. An enhanced (synergistic) heat sensitivity of irradiated *Salmonella typhimurium* has been reported by Thayer et al. (25), suggesting that, if a few *Salmonella* cells should survive the irradiation treatment, they would be very unlikely to survive cooking, assuming that the irradiated product has been properly refrigerated, thus preventing recovery and multiplication of the cells.

The objective of this study was to identify mechanisms by which irradiation and heating act synergistically to produce greater inactivation of *S. typhimurium* than would be predicted from the effects of heating and irradiation alone. The relative amounts of DNA and cellular membrane damage were determined.

MATERIALS AND METHODS

Bacterial culture and media. *S. typhimurium* ATCC 14028 was obtained from the American Type Culture Collection and maintained on tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich.). *S. typhimurium* cultures in tryptic soy broth (TSB; Difco) were incubated at 35°C and agitated at 150 rpm. CFU were estimated by standard pour plate procedures on TSA following serial dilution with Butterfield's phosphate (0.25 M KH_2PO_4 adjusted to pH 7.2 with NaOH) (9). Following incubation at 35°C for 24 h, the CFU were counted at a dilution giving 30 to 300 colonies per plate with a Biotran II automated colony counter (New Brunswick Scientific, New Brunswick, N.J.). *S. typhimurium* transformants containing plasmid pBR322 were cultured on TSA or in TSB supplemented with

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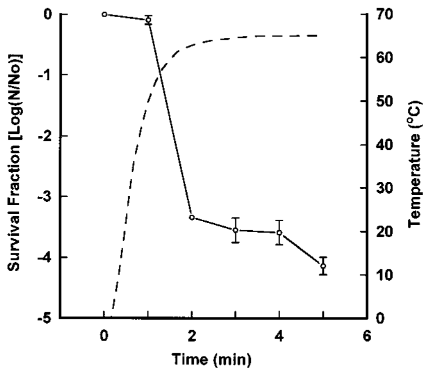

FIG. 1. Inactivation of *S. typhimurium* ATCC 14028 following heat treatments at 65°C for 0 to 5 min (○) and temperature profile of cell suspension during heating (– – –). The survival data are plotted as the $\log_{10}$ of the ratio of the number of surviving CFU per milliliter divided by the initial number of CFU per milliliter at time zero. The temperature profile represents the temperature of the cell suspension plotted against time. The come-up time (time required for the temperature of the cell suspension to reach that of the water bath) can be determined from this curve to be approximately 2 min.

ampicillin (100 μg/ml; Sigma Chemical Co., St. Louis, Mo.) and tetracycline (15 μg/ml; Sigma).

Irradiation. Stationary-phase (18 h) *S. typhimurium* cells were harvested by centrifugation and suspended in an equal volume of Butterfield's phosphate (i.e., 9.0×10^9 cells per ml). Cell suspensions (4 ml) in screw-cap test tubes were irradiated in a uniform portion of the radiation field in a self-contained ^{137}Cs gamma radiation source with a dose rate of 0.114 kGy/min. The temperature during irradiation was maintained at 0 ± 0.5°C by injection of the gas phase from liquid nitrogen. The injection of gas was controlled by a thermocouple within the chamber, and sample temperature was monitored continuously with calibrated thermocouples. The irradiation dose rate was established by using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Anaerobic conditions were established by bubbling cell suspensions (30 ml) on ice for 15 min with ultrapure grade nitrogen gas (Airco Gas Co., Murray Hill, N.J.). N_2 gas-saturated cell suspensions were dispensed into screw-cap test tubes which were flushed before capping.

Heat treatment. Cell suspensions (4 ml) of *S. typhimurium* in screw-cap test tubes were immersed into an agitated water bath (RTE-220; NESLAB Instrument Inc., Newington, N.H.) at 65°C. Temperatures of cell suspensions were monitored by TELE-Thermometer (42SC; Yellow Springs Instrument Inc., Yellow Springs, Ohio) and reached the set temperature within 2 min (come-up time). To determine the relationship between irradiation and heating, cell suspensions were heated at 65 ± 0.1°C for 2 min either before or after irradiation. After heating, cell suspensions were rapidly cooled to 0°C in ice water.

Plasmid pBR322 transformation. Plasmid pBR322 (Amp^r Tet^r) was purchased from Life Technologies Inc. (Grand Island, N.Y.) and introduced into *S. typhimurium* cells by electrotransformation. *S. typhimurium* electroporation-induced intact cell transformation competence was achieved as described below. One milliliter of an overnight culture of *S. typhimurium* was inoculated into 100 ml of TSB and incubated at 35°C until the optical density of the culture reached 1.0 at 600 nm. Cells were harvested and washed with an equal volume of 10% polyethylene glycol solution (molecular weight, 8,000) (Amresco Inc., Solon, Ohio) and suspended in 1/20 volume of 10% polyethylene glycol solution. Aliquots of the electroporation-competent cell suspensions were stored at −50°C for future use. For electroporation, 50 μl of the cell suspension was mixed with 1 μl of the DNA solution (i.e., 0.1 μg of plasmid DNA per μl). Twenty microliters of the cell-DNA mixture was electroporated by using Electroporator (Life Technologies) set at 2.4 kV and 3.3 kΩ with a microelectroporation cuvette (Life Technologies). Following electroporation, 10 μl of the cell mixture was transferred into 1 ml of TSB and incubated for 3 h before being plated on TSA supplemented with ampicillin and tetracycline. Ampicillin- and tetracycline-resistant transformants of *S. typhimurium* (Amp^r Tet^r) were selected after incubation at 35°C for 24 h. The presence of plasmid pBR322 was confirmed by isolation of plasmid DNA from *S. typhimurium* transformants.

Analysis of biological activity of plasmid pBR322. (i) Isolation of the covalently ccc form of plasmid pBR322. The covalently closed circular (ccc) form of plasmid DNA was isolated from *S. typhimurium* transformants following irradiation to a dose of 0.8 kGy at 0°C and/or heating at 65°C for 2 min by an alkaline lysis method described by Maniatis et al. (15). Following ethanol precipitation, the recovered plasmid DNA was suspended in deionized water. The concentration of the ccc form of plasmid pBR322 was measured by image analysis of the stained agarose gel rather than by spectroscopic measurement of the total concentration of plasmid DNA, because *S. typhimurium* has an indigenous 50-kDa plasmid. Plasmid DNA solutions were digested with a single-cut restriction endonuclease, *Eco*RI (Life Technologies), at 37°C for 2 h and were subjected to agarose gel electrophoresis using Tris-acetate buffer as a running buffer at 80 V for 2 h (15). The ethidium bromide-stained agarose gel was photographed with Polaroid film type 667 (Polaroid Co., Cambridge, Mass.), and the concentration of the recovered ccc form of plasmid pBR322 was estimated by image analysis of photographs using Image-Pro Plus software (Media Cybernetics Inc., Silver Spring, Md.) by comparison with a known concentration of plasmid pBR322.

(ii) Transformability of recovered plasmid pBR322. The transformability of the recovered plasmid pBR322 was assayed by reintroduction into wild-type *S. typhimurium* by electroporation, as described previously. The mutation spectrum of the transformed plasmid pBR322 was measured by the phenotypic expression of ampicillin and tetracycline resistance genes on TSA (Amp), TSA (Tet), and TSA (Amp, Tet) selection plates.

Measurement of membrane damage. The relative integrity of the cytoplasmic membrane of *S. typhimurium* following treatment by irradiation and/or heating was estimated with the LIVE/DEAD *Bac*LIGHT viability kit (Molecular Probes Inc., Eugene, Oreg.). The kit contains fluorescent stains which differ in their spectral characteristics and their abilities to penetrate the cell membrane. Cell suspensions were stained as suggested by the manufacturer following irradiation and/or heating. After staining, the fluorescence emission spectrum of each cell suspension (excitation at 470 nm and emission at 480 to 700 nm) was measured by using a Luminescence spectrometer (LS-5B; Perkin-Elmer Corp., Norwalk, Conn.). Bacteria with intact membranes fluoresce green (emission spectrum, 510 to 540 nm), while bacteria with damaged membranes fluoresce red (emission spectrum, 620 to 650 nm). The ratio of green to red fluorescence (F_{green}/F_{red}) was used as an estimate of the percentage of cells with membrane structural damage. As a control, *S. typhimurium* cells with damaged membranes were prepared by incubating cell suspensions in 70% of isopropyl alcohol at room temperature for 2 h. Untreated cells served as a 100% control.

Statistical analysis. The responses of *S. typhimurium* to irradiation and/or heating were expressed as the logarithm_{10} of the surviving fraction (number of CFU per milliliter [N] divided by the initial number of CFU per milliliter [N_0]). Radiation doses required to inactivate 90% of the cells (D_{10}), in kilograys, were determined by least-squares analysis and are the negative reciprocals of the slopes of the individual regressions of the logarithms (N/N_0) plotted against

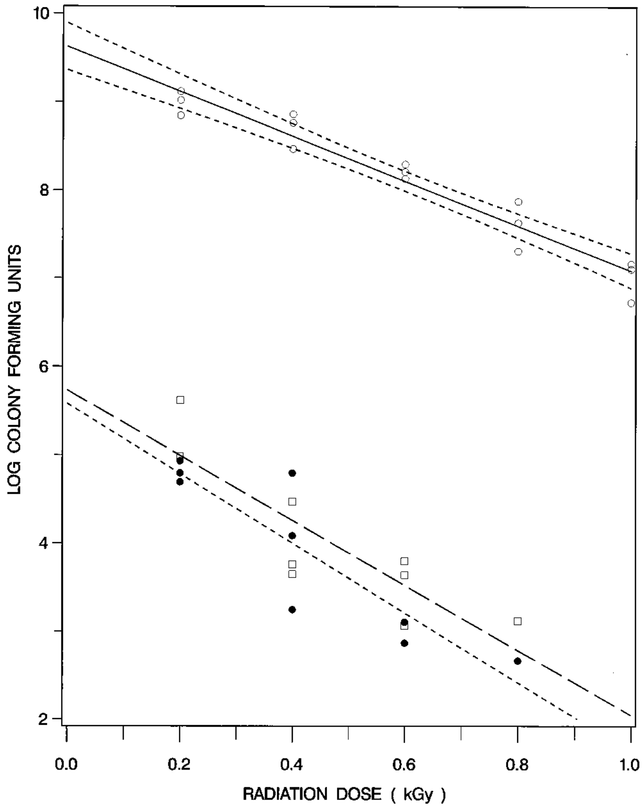

FIG. 2. Responses of *S. typhimurium* ATCC 14028 to irradiation in the presence of air (○) (dashed lines, 95% confidence intervals for the regression), heat treatment at 65°C for 2 min followed by irradiation (□, ——), and irradiation followed by heat treatment at 65°C for 2 min (●, – –).

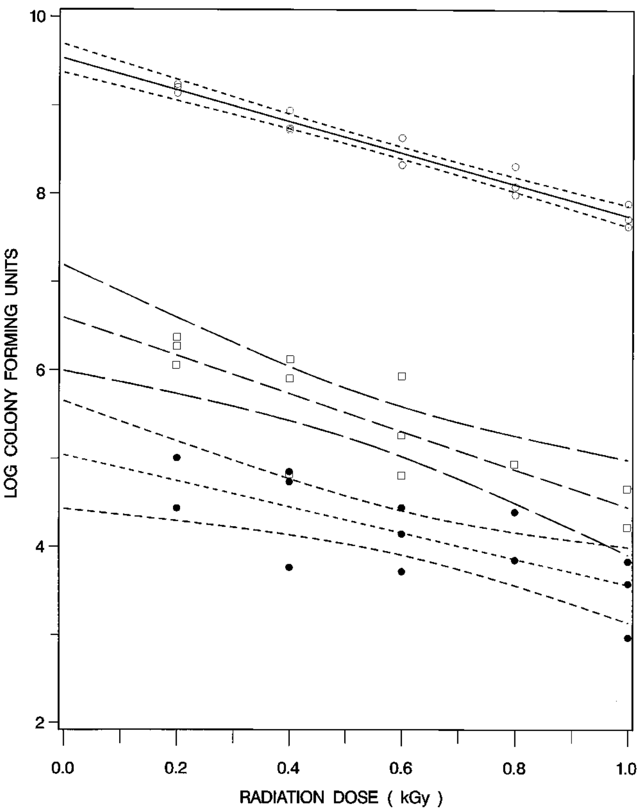

FIG. 3. Responses of *S. typhimurium* ATCC 14028 to irradiation in the presence of N_2 (○), heat treatment at 65°C for 2 min followed by irradiation (□, — —), and irradiation followed by heat treatment at 65°C for 2 min (●, – – –); 95% confidence intervals for the regressions are indicated.

radiation doses. The N_0 values were not themselves used in the computation of the regression to eliminate possible shoulder effects. Radiation doses of 0.2, 0.4, 0.6, 0.8, and 1.0 kGy, within the linear portion of the inactivation curve, were included in the calculation of the slope. Statistical calculations were performed with the general linear-models procedure of the SAS statistical package (6, 20). The regressions were tested for differences by analysis of covariance.

RESULTS

Heat sensitivity of *S. typhimurium*. The greatest decrease in viable cells occurred during the come-up period with only a small additional decrease in viable cell number when heating times were extended to 5 min at 65°C (Fig. 1). The inactivation during the come-up period was responsible for an initial 99.9% reduction in viability. The results indicate that *S. typhimurium* can be divided into two physiologically distinct groups following heating at 65°C (Fig. 1): (i) a heat-sensitive subpopulation, killed during the come-up period by heating at 65°C and (ii) a heat-resistant subpopulation, which is only slowly inactivated at 65°C. Cells surviving heat treatments did not exhibit elevated heat resistance when subcultured, indicating that they were not genetically distinct. The nonuniform response of individual cells to heating probably originates from differences in intrinsic factors of individual cells as suggested by Gould (8).

Irradiation sensitivity of *S. typhimurium*. Irradiation sensitivity of *S. typhimurium* was greater when the cell suspensions were saturated with air than with N_2 gas (Fig. 2 and 3 and Table 1). Previous experimental data (13) indicated that irradiation-induced inactivation of *S. typhimurium* at a radiation dose of 0.2 to 1.0 kGy was due to (i) oxygen-dependent cell surface damage as a result of the interaction of extracellular hydroxyl radicals and oxygen and (ii) oxygen-independent intracellular damage, primarily to DNA. Similar results were obtained with oxygen-free nitrogen, nitrous oxide, and argon, indicating the importance of the lack of oxygen, not the gas (13). In this study, *S. typhimurium* transformants containing plasmid pBR322 (Amp^r Tet^r) had similar oxygen-dependent and oxygen-independent sensitivities to gamma irradiation.

Combined effects of irradiation and heating in *S. typhimurium* inactivation. If the effects of irradiation and heating were simply additive, then survival of *S. typhimurium* should be the same regardless of the order in which the treatments were given. Use of both irradiation and heating treatments significantly decreased cell survival of *S. typhimurium* regardless of treatment order (Fig. 2 and 3). Irradiation preceding rather than following heating was consistently more lethal; however, the regressions were significantly ($P < 0.0001$) separated (Fig. 2 and 3) only when irradiation took place in the absence of O_2. The results indicate that irradiation acts synergistically with heating during inactivation of *S. typhimurium* in the absence of O_2 (Fig. 3). However, the order of irradiation or heating did not significantly alter the D_{10} values in the presence of O_2 (Table 1). This indicates that the increased sensitivity of irradiated salmonella cells is not completely dose dependent.

Plasmid DNA recovery from *S. typhimurium* transformants (Amp^r Tet^r) following irradiation and heating. Development of an efficient method for transformation of plasmid DNA into *S. typhimurium* by electroporation allowed the relative amount of DNA damage in vivo to be estimated. Neither the radiation nor the heat sensitivities of *S. typhimurium* were changed by transformation with plasmid pBR322 (Amp^r Tet^r). Heating at 65°C for 2 min had no effect on the recovery of the ccc form of plasmid pBR322, while irradiation at 0.8 kGy decreased its recovery (Fig. 4 and Table 2). This suggests that irradiation could cause alkaline labile DNA damage, such as strand breaks and base alterations in plasmid pBR322, which would be denatured during alkaline lysis processing (27). When cell suspensions of *S. typhimurium* transformants were heated before irradiation, recovery of the ccc form of plasmid pBR322 was greatly reduced, but its recovery was not significantly affected by heating after irradiation (Table 2). Heating could induce dissociation of DNA-binding proteins, and subsequent irradiation could increase DNA damage because of increased exposure of plasmid DNA to hydroxyl radicals during irradiation.

Reintroduction of recovered plasmid DNA into *S. typhimurium*. The biological activity of the recovered ccc form of plasmid pBR322 following irradiation and/or heating was assessed by measuring transformability into *S. typhimurium*. If

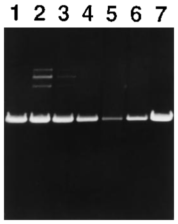

FIG. 4. Agarose gel electrophoresis analysis of restriction enzyme *Eco*RI-digested plasmid pBR322 isolated from *S. typhimurium* transformants following a radiation dose of 0.8 kGy and heating at 65°C for 2 min. Lanes 1 and 7, 0.5 and 1 μg of control plasmid pBR322, respectively; lane 2, plasmid pBR322 from untreated cells; lane 3, plasmid pBR322 from heat-treated cells; lane 4, plasmid pBR322 from irradiated cells; lane 5, plasmid pBR322 from cells irradiated following the heat treatment; lane 6, plasmid pBR322 from cells irradiated and then heat treated. Three extra bands were generated from the digestion of a 50-kDa indigenous plasmid which has at least three *Eco*RI-cut sites.

TABLE 1. D_{10} values for *S. typhimurium* ATCC 14028 following irradiation and/or heating in the presence or absence of oxygen[a]

Sample	D_{10} (kGy)		
	Rad	Heat + rad	Rad + heat
Air	0.394 ± 0.029	0.259 ± 0.047	0.247 ± 0.032
N_2	0.561 ± 0.035	0.466 ± 0.097	0.611 ± 0.143

[a] The reported results are the means ± standard deviations from four independent experiments. Cell suspensions were heated at 65°C for 2 min either before or after irradiation (Rad).

the recovered plasmid pBR322 contains alkaline-insensitive DNA damage, such as base alteration, the transformed plasmid DNA would have to be repaired before antibiotic resistance genes are expressed. The repair process might increase mutation frequency in *S. typhimurium*. Transformation efficiencies (ca. 1.6×10^9 to 6.2×10^9 transformants per μg of plasmid DNA) did not differ when the transformants were selected on TSA (Amp), TSA (Tet), and TSA (Amp, Tet) and were directly dependent upon the amount of plasmid DNA recovered following heating and/or irradiation. These results indicated that base alteration in vivo by heating at 65°C for 2 min and/or irradiation at a dose of 0.8 kGy was not a major cause of cell inactivation and/or mutation of *S. typhimurium*. Alternatively, base alterations were efficiently repaired in *S. typhimurium*.

Membrane damage of *S. typhimurium* following irradiation and/or heating. In a manner similar to that of isopropyl alcohol, heating, but not irradiation, induced destabilization of the cellular membrane which allowed penetration by the red hydrophobic fluorescent dye. The F_{green}/F_{red} ratio following irradiation and heating indicated that irradiation did not cause further destabilization of the cytoplasmic membrane regardless of the order of treatment (Table 2). This indicates that the cytoplasmic membrane was a primary target for heat inactivation of *S. typhimurium* as suggested previously (8, 10, 12).

DISCUSSION

The results suggest that gamma irradiation and heating act synergistically when cells are irradiated first and then heated or additively when cells are heated first and then irradiated. Our results are in agreement with those of Thayer et al. (25).

Synergistic and additive effects on bacterial inactivation following separate irradiation and heating treatments have been reported (2, 11, 18, 21, 25, 29). Heat can damage protein, lipids, and nucleic acids and destabilize membranes (8, 10). However, heat induces primarily blebbing and vesiculation of the outer membrane, leading to bacterial cell inactivation, and consequently increases its permeability to hydrophobic compounds (22, 26). Heating-induced membrane destabilization sequentially initiates indirect DNA damage as a consequence of increased nuclease activity in bacteria and is often the critical injury, resulting in death of the cell (7, 8, 11, 23). Irradiation induces primarily DNA damage, such as strand breaks and base alterations, and causes much less damage to protein, lipid, and RNA (27). It is possible that heat and radiation act on different sites but also share some targets such as DNA to cause additive or synergistic effects on cells (2, 29). Alternatively, heated cells could be more sensitive to irradiation either because the heat-labile recovery capacity of bacterial cells is affected (2) or because recovery from heating-induced damage by irradiation is abolished, preventing protein synthesis as a consequence of DNA damage in vegetative bacterial cells (17). The results suggest that irradiation-enhanced heat sensitivity of *S. typhimurium* originated from oxygen-independent intracellular damage, such as DNA damage, at radiation doses of 0.2 to 1.0 kGy as suggested by previous experiments (13). However, the irradiation-enhanced heat sensitivity was not dose dependent.

Radiation-induced DNA damage in vitro and its repair in vivo has been measured by analysis of mutational spectra following transformation of irradiated DNA (14, 16, 24, 28). To evaluate involvement of DNA damage in vivo in irradiation-induced cell inactivation, the *E. coli* plasmid pBR322 was transformed into *S. typhimurium*. Irradiation of plasmid pBR322 in vivo in *S. typhimurium* produced far less damage than its irradiation in vitro in a nonprotective solution. This was expected because of protection by conformational restraints, scavenging effects of small molecules, and DNA-binding proteins in biological systems (3, 27). Although the recovery of the ccc form of plasmid pBR322 was affected by irradiation, but not by heating, the rate of recovery of the plasmid was not correlated with cell survival, suggesting that primary interactions between DNA and radiation are limited. Heating could induce dissociation of DNA-binding proteins from DNA, making it more sensitive to irradiation as a result of the increased exposure of the DNA to hydroxyl radical attacks in *S. typhimurium*, as suggested by Dikomey and Franzke (3). This was observed in our study (Fig. 4). However, the increased irradiation-induced DNA damage following heating is not directly related to cell survival of *S. typhimurium* (Fig. 4).

The dose-independent irradiation-enhanced heat sensitivity of *S. typhimurium* may be explained by the degree of association between DNA and the cytoplasmic membrane as has been suggested by studies of *E. coli* (30). When the chromosomal DNA is attached to the cytoplasmic membrane at multiple sites (4), both single and double DNA strand breaks which are closely located are easily repaired. However, the denaturation or dissociation of broken DNA strands from the cellular membrane by heating following irradiation makes them unrepairable. Alternatively, (i) heating may alter DNA polymerase and topoisomerase I as in yeast cells (1), or (ii) irradiation may induce functional changes in the cytoplasmic membrane in addition to the heating-induced destabilization, such as oxidation of sulfydryl groups of the membrane-binding bound proteins, resulting in cell death in addition to direct DNA damage (30).

Ionizing irradiation can produce a variety of damaging effects in base and sugar moieties of the plasmid or viral DNA, in vitro, and the transformed plasmid or viral DNA can induce mutations in vivo as a consequence of repair processes and

TABLE 2. Comparative responses of *S. typhimurium* ATCC 14028 to irradiation and/or heat[a]

Treatment	Cell survival (avg no. of CFU)	Recovery of plasmid DNA (%)[b]	F_{green}/F_{red}
Control	8.1×10^9	100 ± 0.0	14.44 (1.07)[c]
Heat	1.6×10^6	96 ± 4.0	4.24
Rad	4.6×10^7	79 ± 4.0	12.00
Heat + rad	1.3×10^3	49 ± 11.0	4.09
Rad + heat	4.6×10^2	67 ± 7.0	3.83

[a] The results are from at least two independent experiments. The radiation (Rad) dose was 0.8 kGy at 0°C, and the heat treatment was 65 ± 0.1°C for 2 min.

[b] The data are means ± standard deviations.

[c] *S. typhimurium* cell membranes were damaged by treatment with 70% isopropyl alcohol.

eventually lead to mutation (24, 28) and cell inactivation (27). Heating- and/or irradiation-induced base alterations are presumably distributed randomly; thus, it would be expected that the transformation efficiency should be lowest during double selection on TSA (Amp, Tet). Instead, transformation frequencies of recovered plasmid pBR322 were similar regardless of selection pressure. This suggests that radiation damage of plasmid pBR322, in vivo, was not directly related to cell lethality or mutation of *S. typhimurium*.

Although the relationship between heating and irradiation effects on cell lethality and the DNA repair system of *S. typhimurium* requires further study, the irradiated meat and poultry products will be much safer for consumers than expected because of irradiation-enhanced heat sensitivity of *S. typhimurium*.

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REFERENCES

1. **Borehan, D. R., A. Trivedi, P. Weinberger, and R. E. Mitchel.** 1990. The involvement of topoisomerases and DNA polymerase I in the mechanism of induced thermal and radiation resistance in yeast. Radiat. Res. **123:**203–212.
2. **Bridges, B. A., M. J. Ashwood-Smith, and R. J. Munson.** 1969. Correlation of bacterial sensitivities to ionizing radiation and mild heating. J. Gen. Microbiol. **58:**115–124.
3. **Dikomey, E., and J. Franzke.** 1992. Effects of heat on induction and repair of DNA strand breaks in X-irradiated CHO cells. Int. J. Radiat. Biol. **61:**221–233.
4. **Driedger, A. A.** 1970. Are there multiple attachments between bacterial DNA and the cell membrane? Can. J. Microbiol. **16:**881–882.
5. **Dubbert, W. H.** 1988. Assessment of *Salmonella* contamination in poultry—past, present, and future. Poultry Sci. **67:**944–949.
6. **Freund, R. J., R. C. Littell, and P. C. Spector.** 1986. SAS system for linear models. SAS Institute, Inc., Cary, N.C.
7. **Gomez, R. F.** 1977. Nucleic acid damage in thermal inactivation of vegetative microorganisms. Adv. Biochem. Eng. **5:**50–67.
8. **Gould, G. W.** 1989. Heat-induced injury and inactivation, p. 11–42. *In* G. W. Gould (ed.), Mechanisms of action of food preservation procedures. Elsevier Applied Science, New York.
9. **Horwitz, W.** 1980. Official methods of analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists. Washington, D.C.
10. **Hurst, A.** 1977. Bacterial injury: a review. Can. J. Microbiol. **23:**935–944.
11. **Kadota, H., A. Uchida, Y. Sako, and K. Harada.** 1978. Heat-induced DNA injury in spores and vegetative cells of *Bacillus subtilis*, p. 27–30. *In* G. Chambliss and J. C. Vary (ed.), Spores VII. American Society for Microbiology, Washington, D.C.
12. **Katsui, N., T. Tsuchido, R. Hiramatsu, S. Fujikawa, M. Takano, and I. Shibasaki.** 1982. Heat-induced blebbing and vesiculation of the outer membrane of *Escherichia coli*. J. Bacteriol. **151:**1523–1531.
13. **Kim, A., and D. Thayer.** 1994. Radiation-induced cell lethality of *Salmonella typhimurium* ATCC 14028: cooperative effect of hydroxyl radical and oxygen. Radiat. Res. **144:**36–42.
14. **Kow, Y. W., G. Faundez, R. J. Melamede, and S. S. Wallace.** 1991. Processing of model single-strand breaks in 0X-174 RF transfecting DNA by *Escherichia coli*. Radiat. Res. **126:**357–366.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Milligan, J. R., J. A. Aguilera, and J. F. Ward.** 1993. Variation of single-strand break yield with scavenger concentration for plasmid DNA irradiated in aqueous solution. Radiat. Res. **133:**151–157.
17. **Mukherjee, P., and S. B. Bhattacharjee.** 1970. Recovery of bacteria from damages induced by heat. J. Gen. Microbiol. **60:**233–238.
18. **Pallas, J. E., III, and M. K. Hamdy.** 1976. Effects of thermoradiation on bacteria. Appl. Environ. Microbiol. **32:**250–256.
19. **Radomyski, T., E. A. Murano, D. G. Olson, and P. S. Murano.** 1994. Elimination of pathogens of significance in food by low-dose irradiation: a review. J. Food Prot. **57:**73–86.
20. **SAS Institute, Inc.** 1987. SAS-STAT guide for personal computers, version 6. SAS Institute, Inc., Cary, N.C.
21. **Schaffner, D. F., M. K. Hamdy, R. T. Toledo, and M. L. Tift.** 1989. *Salmonella* inactivation in liquid whole egg by thermoradiation. J. Food Sci. **54:**902–905.
22. **Scheie, P., and S. Ehrenspeck.** 1973. Large surface blebs on *Escherichia coli* heated to inactivating temperature. J. Bacteriol. **114:**814–818.
23. **Sedgwick, S. G., and B. A. Bridges.** 1972. Evidence for indirect production of DNA strand scissions during mild heating of *Escherichia coli*. J. Gen. Microbiol. **71:**191–193.
24. **Sikpi, M. O., M. L. Freedman, S. M. Dry, and A. G. Lurie.** 1992. Mutation spectrum in γ-irradiated shuttle vector replicated in ataxia-telangiectasia lymphoblast. Radiat. Res. **130:**331–339.
25. **Thayer, D. W., S. Songprasertchai, and G. Boyd.** 1991. Effects of heat and ionizing radiation on *Salmonella typhimurium* in mechanically deboned chicken meat. J. Food Prot. **54:**718–724.
26. **Tsuchido, T., and M. Takano.** 1988. Sensitization by heat treatment of *Escherichia coli* K-12 cells to hydrophobic antibacterial compounds. Antimicrob. Agents Chemother. **32:**1680–1683.
27. **von Sonntag, C.** 1987. The chemical basis of radiation biology. Taylor & Francis, London.
28. **Waters, L. C., M. O. Sikpi, J. Preston, S. Mitra, and A. Jaberaboansari.** 1991. Mutation induced by ionizing radiation in a plasmid replicated in human cells. Radiat. Res. **127:**190–201.
29. **Yatvin, M. B., J. J. Gipp, D. R. Klessig, and W. H. Dennis.** 1986. Hyperthermic sensitivity and growth stage in *Escherichia coli*. Radiat. Res. **106:**78–88.
30. **Yatvin, M. B., and M. A. Grummer.** 1987. Membrane structure and radiation and hyperthermic damage. Radiat. Phys. Chem. **30:**351–364.