Survival, Deoxyribonucleic Acid Breakdown, and Synthesis in *Salmonella typhimurium* as Compared with *Escherichia coli* B Strains

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*Salmonella typhimurium* LT-2 was compared with radioresistant (B/r) and radiosensitive (B−) strains of *Escherichia coli* in respect to the survival, deoxyribonucleic acid (DNA) breakdown, and DNA synthesis after X irradiation. It is shown that *S. typhimurium* LT-2 is about four times more sensitive than *E. coli* B/r but less sensitive than B−. The DNA breakdown is in *S. typhimurium* LT-2 lower than the postirradiation breakdown of DNA in both *E. coli* strains and DNA synthesis proceeds in this bacterium in spite of a much lower survival, as in the radioresistant *E. coli* B/r.

The most extensively studied biochemical processes after exposure of microorganisms to ionizing radiations were deoxyribonucleic acid (DNA) degradation (1, 9–11, 15–18) and DNA synthesis (15–17). These processes seem to be especially sensitive to radiation and could therefore have some connection with the radiation-induced cell death. Attempts were made to correlate the radiosensitivity of *Escherichia coli* strains with the postirradiation degradation of the bacterial DNA, estimated in conditions where DNA synthesis was prevented (20), and to correlate the radiosensitivity of a bacterial strain with the extent of the inhibition of DNA synthesis after different exposures to ionizing radiation (15). It was shown that the radiosensitivity of *E. coli* strains can be correlated with the extent of DNA degradation after irradiation (20), and that the inhibition of DNA synthesis after the same irradiation exposure is greater in a sensitive strain of *E. coli* than in a resistant one (15).

This study was undertaken to see whether these criteria are of any general significance and are true at least for genetically related bacterial species. For this purpose, the survival, DNA synthesis, and DNA degradation in wild-type *Salmonella typhimurium* LT-2 were compared with the same parameters in *E. coli* B/r and *E. coli* B−, after X irradiation.

**MATERIALS AND METHODS**

Chemicals. *1H*-methyl-thymidine (*C*H3-thymidine, 5 c/mmole) was obtained from the Radiochemical Centre, Amersham, England. Casamino Acids, Agar, and Nutrient Broth were purchased from Difco. All other chemicals used were analytical grade products of The British Drug Houses Ltd., England.

**Bacterial strains.** *E. coli* B/r from the laboratory of Tikvah Alper, London, and *E. coli* B−, from the laboratory of Ruth Hill, New York, were provided by Erika Kos, Zagreb, Yugoslavia. *S. typhimurium* LT-2 was obtained from Joseph S. Gots, Philadelphia.

**Media and conditions of cultivation.** The bacteria were grown in minimal salts medium (MA) described elsewhere (13) enriched with 0.2% Casamino Acids with aeration at 37 °C. The bacterial growth was followed by measuring the optical density at 650 nm (OD650) in a Beckman model D spectrophotometer. Two OD units corresponded to 10^9 cells per ml in *E. coli* strains and to approximately 1.5 × 10^9 cells per ml in *S. typhimurium*.

For labeling bacterial DNA with *1H*-thymidine the growth medium described above was supplemented with 200 μg of deoxyadenosine per ml (7) and 1 nmole of *1H*-thymidine per ml (radioactive medium).

**Preparation of bacteria for irradiation.** To prepare radioactive bacteria, bacteria from an overnight culture were diluted 100 times into radioactive medium and aerated for 4 to 6 hr. During this time, about 60% of the radioactive thymidine was taken up by the cells. The bacteria were then harvested by centrifugation, washed twice in chilled MA without glucose, resuspended in a double volume of nonradioactive medium containing twice as much glucose and Casamino Acids as normal, and aerated for another 60 min. After that time, the cells were centrifuged again, washed as above, suspended in cold MA without glucose at OD650 = 2.0, and stored in ice until use. Nonradioactive bacteria were prepared in the same way, except that deoxyadenosine and radioactive thymidine were...
omitted from the medium. After this treatment, all three bacterial strains are in the late-exponential phase of growth.

Irradiation of bacteria. The bacterial suspension (2.5 ml), prepared as described above, was placed in a chilled petri dish (φ 14 mm) and irradiated at ice-bath temperature with a Siemens therapeutic roentgen machine (200 kv, 16 ma, without filter, dose rate of 1.1 kr per min). All irradiations were performed in the presence of oxygen.

Survival curves. Irradiated and nonirradiated bacteria were appropriately diluted in saline and 0.1 ml of the suspension was plated on nutrient agar plates containing 1.5 g of Agar per 100 ml of Nutrient Broth, for determination of colony-forming units.

Breakdown of DNA. The postirradiation degradation of DNA was estimated by measuring the loss of radioactivity from the cells previously labeled, as described above, with radioactive thymidine. After irradiation, the suspensions of unirradiated and irradiated bacteria were diluted 10 times in MA without glucose to prevent reincorporation of the label into DNA (20) and incubated at 37 C. We considered our estimations of degradation to be correct, since experiments performed with B/r (the incorporation of ³H-thymidine is in this bacterium inhibited even after 5 kr of X-rays) showed that the breakdown of DNA is not dependent on the presence of the carbon source. Samples (1 ml) were withdrawn at the times indicated, pipetted into 1 ml of 10% trichloroacetic acid, and kept for 30 min at ice-bath temperature. After that time, 0.1 ml of 1% bovine serum albumin was added with stirring and the suspension was centrifuged for 30 min at 5,000 rev/min in the International refrigerated centrifuge. A 0.5-ml amount of the supernatant was counted after the addition of 10 ml of scintillation fluid (4 g of 2,5-diphenyloxazole, 0.2 g of p-bis-2-(5-phenyloxazolyl) benzene), 60 g of naphthalene, 20 ml of ethylene glycol, 100 ml of methanol, and dioxan to a final volume of 1,000 ml in a Mark I scintillation counter (Nuclear-Chicago Corp.).

One hundred percent radioactivity was determined as follows. A 0.1-ml amount of 10% HClO₄ was added to 0.1 ml of the labeled control suspension before dilution (1:10), and the mixture was heated for 60 min at 80 C. The hydrolysate was then diluted to 2 ml with 5% trichloroacetic and centrifuged at 5,000 rev/min in the cold. The radioactivity in 0.5 ml of the supernatant was measured as described above. This amount corresponds to 250 µlitters of the diluted (1:10) cell suspension, the amount counted in the samples, where the DNA degradation was followed after irradiation.

Incorporation of ³H-thymidine. Samples (50 µlitters) of the bacterial suspension were pipetted at ice-bath temperature into 0.3 ml of the radioactive medium. Fractions (25 µlitters) were removed from these suspensions at zero time (ice-bath temperature). At different time intervals after transferring the bacterial suspensions to 37 C, the fractions were pipetted onto Whatman 3MM filter paper discs, and the incorporation of radioactive thymidine into DNA was followed by measuring the radioactivity in the trichloroacetic acid-insoluble fraction by the paper disc method of Bollum (4). The radioactivity on the paper discs was counted in the same scintillation counter as described above, by using the toluene scintillation fluid.

DNA content. Irradiated and control bacteria were diluted 10 times with enriched MA and incubated at 37 C for different time intervals. DNA was estimated in appropriate samples by the method of Burton (5).

RESULTS

Survival curves for S. typhimurium LT-2, E. coli B/r, and E. coli B/r. The conditions of irradiation, especially the exposure rate, cited in the literature are very different and, accordingly, the survivals published by different authors for the same strain at the same X-ray exposure may vary considerably. Therefore, attempting to compare radiosensitivity of S. typhimurium with the radiosensitivities of E. coli B/r and E. coli B/r, we made survival curves for all three strains under identical conditions as described above.

The results of these experiments are presented in Fig. 1. It can be seen that the D₉₀ (13 kr) for colony-survival of S. typhimurium does not exceed 3 kr and is very close to the value reported for E. coli B/r (12) but is for a factor of 2 higher than the D₉₀ for E. coli B/r, as is evident from the graph. The D₉₀ for E. coli B/r, as determined by our conditions, is very similar to the value published by Boyce and Howard-Flanders (6) for this strain. One can therefore characterize S. typhimurium as a strain which is relatively sensitive to X irradiation.

X-ray-induced breakdown of DNA. DNA degradation after X irradiation was studied in two types of experiments. In the first, the exposure dependence of the amount of DNA degraded at 90 min after irradiation was determined to see whether this relationship can be correlated with the bacterial radiosensitivity. The results of these experiments are illustrated in Fig. 2. In the two E. coli strains studied, the more sensitive strain shows a higher breakdown of DNA than the less sensitive one at all exposures of X irradiation, an observation which is in agreement with the data reported by others (15, 20). Comparing, however, S. typhimurium and E. coli B/r, this regularity is not maintained; namely, the difference between these two bacterial species in the amount of DNA degraded at 90 min after irradiation depends on the radiation exposure. At doses below 5 kr, the DNA breakdown is higher in S. typhimurium than in E. coli B/r, whereas at exposures above 5 kr the DNA degradation in E. coli B/r exceeds that in S. typhimurium.

In the second type of experiment, we were interested in the rate of DNA degradation in the
three strains under investigation at different times after exposure to 20 kr of X rays. It is to be expected that, in the strain with an efficient repair mechanism, the degradation of the DNA should slow down or stop as soon as most (or all) breaks are repaired. If this is true, the decrease in the rate of DNA breakdown with time for *S. typhimurium* and *E. coli* B–1 should be similar, whereas in *E. coli* B/r the degradation should slow down more rapidly. It is evident from Fig. 3 that, in all three strains, the DNA breaks down at the highest rate during the first 30 min after the irradiation; the extent of the DNA degraded during this time interval decreases in the following order: *S. typhimurium*, *E. coli* B/r, *E. coli* B–1.

However, if we compare the decrease in the rate of DNA degradation with time (amount of DNA degraded between zero time and 30 min compared with the amount of DNA degraded between 30 and 60 min), the rate of DNA degradation decreases during the second 30 min after irradiation in *E. coli* B/r by approximately 36%, in *S. typhimurium* by 18%, and in *E. coli* B–1 by 19% of the initial rate of degradation. The results indicate that, in the radioresistant strain, the rate of DNA breakdown slows down more rapidly than in the sensitive strains, even when the degradation of DNA is much lower in the more sensitive strain, as in *E. coli* B/r compared to *S. typhimurium*.

**Incorporation of *H*-thymidine and DNA content.**

It is known that ionizing radiation inhibits net DNA synthesis in radioresistant bacteria such as *Micrococcus radiodurans* (8) or *E. coli* B/r (14) during the period of DNA repair. On the other hand, it was shown that even low exposures to gamma-irradiation permanently stop DNA synthesis followed by the incorporation of radioactive thymidine into bacterial DNA (15) in a sensitive strain such as *E. coli* B–1. One can conclude, on the basis of these data, that the ability to incorporate radioactive thymidine into DNA after ionizing radiation is related to the sensitivity of the bacterial strain. Thus, it was of interest to see whether *S. typhimurium* behaves in respect to

![Fig. 1. Survival curves of *S. typhimurium* LT-2, *E. coli* B/r, and *E. coli* B–1 after X irradiation in the late-exponential phase of growth.](http://jb.asm.org/)

![Fig. 2. Loss of radioactivity from *S. typhimurium* LT-2, *E. coli* B/r, and B–1 after different exposures to X rays. *Bacteria* labeled with *H*-thymidine were exposed to different doses of X irradiation and incubated for 90 min in nonradioactive medium lacking glucose. The differences between the acid-soluble radioactivities from control and irradiated cultures are plotted against the corresponding radiation exposure.](http://jb.asm.org/)
net DNA increase and to the incorporation of radioactive thymidine as the radiosensitive *E. coli* B8 or as the radioresistant *E. coli* B/r.

Surprisingly, the ability to incorporate radioactive thymidine into DNA after X irradiation is nearly the same in *S. typhimurium* and in *E. coli* B/r, whereas in *E. coli* B8 a 5-kr dose completely abolishes any incorporation of the label into the trichloroacetic acid-insoluble fraction (Fig. 4).

The DNA content of the bacterial strains, after identical X-ray exposures and then incorporation of radioactive thymidine, is presented in Table 1. The DNA content is the result of DNA synthesis and DNA degradation. In *E. coli* B8, after the applied exposures to X rays, only DNA degradation takes place and, therefore, the figures presented for this strain in Table 1 reflect grossly what is evident from Fig. 2. Comparing *S. typhimurium* with *E. coli* B/r after the exposures listed in Table 1, one can observe the following. After the exposure to 5 kr of X irradiation, the DNA content is higher in *E. coli* B/r than in *S. typhimurium*. This is in agreement with the observations that, after this exposure, there is less DNA degraded and more thymidine incorporated in *E. coli* B/r than in *S. typhimurium*. After an exposure of 20 and 40 kr, the DNA

### Table 1. DNA content of the bacterial suspension 90 min after exposure to 5-, 20-, and 40-kr doses of X rays, expressed as per cent of the non-irradiated control at zero time

<table>
<thead>
<tr>
<th>Strain</th>
<th>Doses of X irradiation (kr)</th>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>546</td>
<td>440</td>
<td>210</td>
<td>113</td>
</tr>
<tr>
<td><em>Escherichia coli</em> B/r</td>
<td>535</td>
<td>520</td>
<td>157</td>
<td>75</td>
</tr>
<tr>
<td><em>E. coli</em> B8</td>
<td>520</td>
<td>49</td>
<td>19</td>
<td>61</td>
</tr>
</tbody>
</table>

![Fig. 3. Percentage of acid-insoluble radioactivity in labeled bacteria plotted against time at 37 C after exposure to 20 kr X rays. One hundred per cent represents approximately 7,000 counts per min per 250-μl sample and is the average of three unirradiated samples at zero time.](image)

![Fig. 4. Incorporation of 3H-thymidine into bacteria. Part A, relationship between counts per minute per 25 μl of LT-2 cell suspension and minutes of incubation at 37 C in the presence of 3H-thymidine after 0 kr (O), 5 kr (X), 20 kr (Δ), and 40 kr (□) X rays. Parts B and C as for part A, except with *E. coli* B/r and *E. coli* B8, respectively.](image)
content is, however, greater in *S. typhimurium* than in *E. coli* B/r; this is understandable since the degradation after these exposures is lower and incorporation of thymidine the same or higher in *S. typhimurium* than in *E. coli* B/r.

**DISCUSSION**

The correlation between the radiosensitivity and DNA breakdown observed in various *E. coli* strains with different radiosensitivities (20) can be extended to *S. typhimurium* only under restricted conditions, namely, at exposures below 5 kr of X-rays. It is believed that single-strand breaks serve as starting points for DNA degradation after ionizing radiation (15, 20) in bacteria. There are data available that irradiated DNA is in vitro a better substrate for degradation by a crude extract of *E. coli* B than a native DNA preparation (19). One can anticipate therefore that DNA degradation in vivo depends on the activity of at least two types of enzymes. One is involved in the degradation of DNA and the other in rejoining of broken strands (14). The dose at which the maximal degradation takes place and the maximal amount of the DNA degraded will depend on the relative amounts of the enzymes which contribute to the postirradiation degradation and rejoining. The low level of DNA degraded and a relatively high degradation at low exposures in *S. typhimurium* is consistent with the explanation that *S. typhimurium* contains, in comparison with *E. coli* B/r, smaller amounts of both types of the corresponding enzymes, namely, less of the degradative enzyme(s) and less of the rejoining enzyme. On the other hand, the higher degradation of DNA in *E. coli* B strains in comparison with *S. typhimurium*, is adequately explained by the absence or by a low level of the rejoining enzyme and by a higher level of the degradative enzyme(s). It is, however, also possible that B strains lack in addition a mechanism regulating DNA breakdown induced by radiation.

Analogous to the findings of other authors (10, 15, 20), all three strains show maximal degradation of DNA at a certain exposure to ionizing radiation. The exposure at which this maximal degradation takes place in *S. typhimurium* is lower than in both *E. coli* B strains studied; by increasing the exposure of radiation, the amount of DNA degraded decreases only slightly in *S. typhimurium*, whereas in *E. coli* strains a very expressed maximum is observed. It is clear that no correlation can be made between maximal degradation and radiosensitivity when bacteria of different species are compared. This is probably due to differences in the level of enzymes(s) responsible for the DNA degradation after irradiation in different bacterial species, although the role of mechanisms controlling the DNA breakdown should also be considered.

The results of the experiments on the incorporation of radioactive thymidine into DNA of X-irradiated bacteria show that DNA replication proceeds at nearly the same rate in *S. typhimurium* and *E. coli* B/r after the same radiation exposure and yet the survival of *S. typhimurium* is lower by a factor of 1,000 compared to *E. coli* B/r. The following questions thus arise. What kind of DNA replication takes place in *S. typhimurium* after irradiation in comparison with *E. coli* B/r and what does the DNA synthesized in *S. typhimurium* under these conditions look like? Billen showed (2, 3) that X irradiation induces new replication points in DNA, which leads to an aberrant DNA synthesis. It is possible that, in *S. typhimurium*, this kind of replication in the absence of an efficient repair by rejoining makes some kind of dysfunctional DNA. A final answer on these questions can be given only after a thorough analysis of the DNA synthesized after irradiation in *S. typhimurium*.

The biochemical changes which occur after X irradiation in *S. typhimurium*, as compared with those in *E. coli* B strains, can be most adequately explained by a low level of the exonuclease enzyme(s) involved in the DNA breakdown and by a low level of the repair enzyme(s). Data which confirm our assumptions will be published later. We also demonstrated that X-ray damage alone has much less influence on the extent of DNA replication in vivo than can be expected from data on the sensitive strains of *E. coli* B.

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**LITERATURE CITED**


