JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 1234-1236
Vol. 105, No. 3
Copyright © 1971 American Society for Microbiology
Printed in U.S.A.

Biosynthesis During Recovery of Heat-Injured *Salmonella typhimurium*

MERLE D. PIERSON,[1] RICHARD I. TOMLINS, AND Z. JOHN ORDAL
Departments of Food Science and Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received for publication 23 November 1970

Salmonella typhimurium 7136 incorporated label from glucose-U-^{14}C into nucleic acids, lipids, and pool material during recovery from heat injury. There was very little incorporation of label into protein during recovery.

Heat injury has been characterized by an alteration of the selective permeability mechanisms and the biosynthetic capabilities of the stressed cells (1, 2, 4, 5). When injured cells are placed in a suitable medium such as Trypticase Soy Broth (TSB), they will recover and grow (4, 5). Heat-injured staphylococci do not require either protein, cell wall, or cell wall mucopeptide synthesis for recovery; however, ribonucleic acid synthesis is required (5, 8).

Salmonella typhimurium 7136 (obtained from the National Center for Urban and Industrial Health, Cincinnati, Ohio) was grown in TSB. After incubation for 12 hr at 37 C on a rotary shaker, 200 ml of culture was centrifuged at 8,000 × *g* for 10 min. The pellet was washed once with 100 mM potassium phosphate buffer (*p*H 6.0), centrifuged, and resuspended in 10 ml of buffer. For injury, the cells were added to 190 ml of preheated 100 mM potassium phosphate buffer and held at 48 C for 30 min with constant agitation. At the termination of the heating period, the injured cells were harvested by centrifugation at 8,000 × *g* for 10 min at 0 to 2 C. The injured cells were resuspended in 10 ml of 100 mM potassium phosphate buffer (*p*H 6.0), added to 190 ml of TSB, and recovered at 37 C on a rotary shaker.

The injured population was estimated by pour plating samples on Trypticase Soy Agar (TSA) and surface plating on Levine's eosin methylene blue agar (Difco Laboratories, Inc., Detroit, Mich.) plus 2% salt (EMBS) (4). The TSA count represented the total number of viable cells present in the heating menstruum or recovery medium, and the EMBS count was an estimate of the number of uninjured cells present. The difference between the TSA and EMBS counts represented the injured population.

There was a rapid increase in the injured population of cells during the first 15 min of heating (Fig. 1A). After 30 min heating, over 95% of the viable cells would not grow on EMBS. For the following experiments recovery was done at a level of about 10^9 to $10^{9.5}$ cells per ml in TSB. The recovery curve for the $10^{9.5}$ cells per ml is given in Fig. 1B. Within 3 hr the injured population had recovered their tolerance to EMBS. During this period of time there was no cell growth as indicated by the TSA count; rather there was repair of the heat-induced lesions. After 3 hr there was some growth of cells as indicated by the increased TSA and EMBS counts.

Injured cells were resuspended to a final cell concentration of about 10^9 cells per ml in 200 ml of TSB containing 20 μCi of glucose-U-^{14}C (New England Nuclear Corp., Boston, Mass.). The cells were recovered in TSB at 37 C on a rotary shaker. At intervals during recovery a 20-ml sample was removed and centrifuged at 10,000 × *g* for 10 min at 0 to 2 C. The cells were washed once with cold 100 mM phosphate buffer (*p*H 7.2)

The washed cells were fractionated by the method of Roberts et al. (7). A 1-ml sample from each fraction was added to 15 ml of scintillation fluid composed of 0.7% 2,5 diphenyloxazole (PPO), 0.03% 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP), and 10% naphthalene in dioxane. A Tri-Carb liquid scintillation spectrometer (model 3320 Packard Instrument Co., Inc., Downers Grove, Ill.) was used to count all radioactive samples. Correction for quenching was made by the Bush channel ratio method (3). These data are presented in Fig. 2.

There was a rapid incorporation of label into the nucleic acid fraction during the first 2 hr of recovery (Fig. 2). One hour prior to the complete

[1] Present address: Department of Food Science and Technology, Virginia Polytechnic Institute, and University Blacksburg, Va. 24061.

return of tolerance to EMBS and growth, there was a plateau in the amount of ^{14}C incorporated into the nucleic acid fraction. After a 3-hr recovery the amount of ^{14}C incorporated into the nucleic acid fraction increased due to cell growth (Fig. 1B).

The in vivo degradation of ribosomal RNA (rRNA) during injury and its resynthesis during recovery has been reported for *Staphylococcus aureus* (8). The same type of phenomenon occurs in *Salmonella typhimurium* (R. I. Tomlins, Ph.D. Thesis, University of Illinois, 1971). There was a rapid incorporation of label into nucleic acids probably because of the utilization of glucose via the hexose monophosphate pathway to ribose and finally incorporation into RNA. There is no deoxyribonucleic acid synthesis, whereas there is RNA synthesis during recovery (9). The plateau in RNA synthesis could have represented a stage at which the cells resynthesized their full complement of rRNA. It has been reported that *S. typhimurium* has an rRNA content per unit cell mass that remains constant during steady state growth and is characteristic for a given broth medium (6). If RNA synthesis per se was the only thing required for recovery then a plateau probably would not have been seen. Rather, after recovery there would have simply been an increase in the amount of ^{14}C incorporated due to cell growth. It is possible that there are rate-limiting steps in the maturation of the newly synthesized rRNA. Finally, the increase in ^{14}C incorporation after the 3-hr recovery period could be accounted for by cell growth.

There was very little incorporation of ^{14}C into protein during the first 3 hr of recovery. Sogin and Ordal (8) reported that during the recovery of heat-injured *S. aureus* there was a 90- to 100-min lag before there was a significant amount of protein synthesis. The protein that was synthesized by *S. typhimurium* during recovery was important since injured cells did not recover when 100 μg of chloramphenicol/ml was added to the recovery medium. This is unlike *S. aureus* where protein synthesis is not required for recovery (5, 8). The increase of ^{14}C incorporation into the protein fraction after the 3-hr recovery period could be accounted for by cell growth.

There was incorporation of ^{14}C into lipid during recovery. After 3 hr there was a further increase in the rate of lipid synthesis probably due to cell growth. The amount of incorporation of ^{14}C into pool material during recovery was somewhat greater than that into the lipid fraction.

Leakage of intracellular constituents (1), an increased salt sensitivity (5), and an increased sensitivity to food preservatives (R. I. Tomlins, M.S. Thesis, University of Illinois, 1969) after sublethal heat injury indicates an alteration in the selective permeability mechanism of heat-injured cells. Gabis et al. (Bacteriol. Proc., p. 3, 1970) reported that there is lipid synthesis in *E. coli* during recovery from freeze injury.

The lipid synthesized during recovery could have been used for the repair of the heat-damaged membrane. In this way the cells could re-

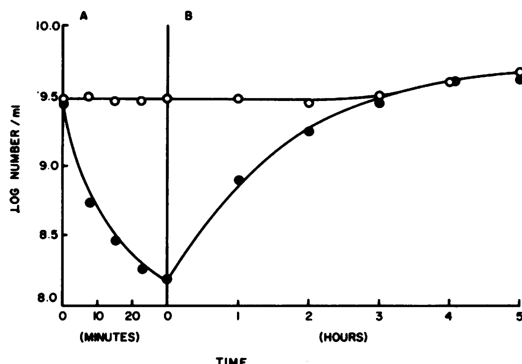

FIG. 1. *Survival and recovery curves for Salmonella typhimurium 7136. Cells were heated in 100 mM potassium phosphate buffer (pH 6.0) at 48 C. (A) At various time intervals samples were plated on TSA (○) and EMBS (●). Injured cells were placed in TSB and recovered at 37 C on a rotary shaker. (B) The degree of recovery was estimated by plating samples on TSA (○) and EMBS (●).*

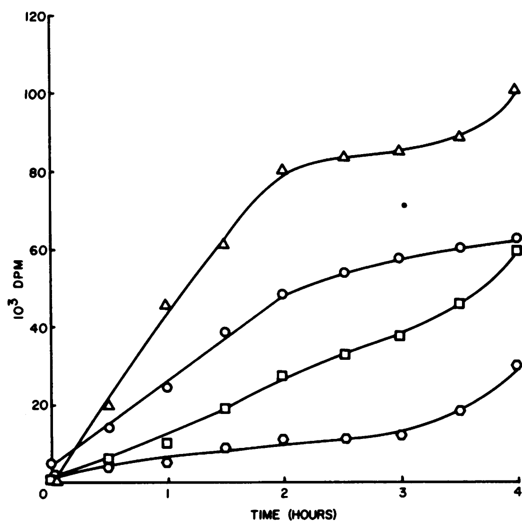

FIG. 2. *The incorporation of ^{14}C into heat-injured Salmonella typhimurium 7136 during recovery in TSB containing glucose-U-^{14}C. At various time intervals a sample of recovering cells was fractionated by the procedure of Roberts et al. (7) into pool material (○), lipids (□), nucleic acids (△), and proteins (⬭).*

synthesize and reconcentrate intracellular pools necessary for recovery and growth. The repair of the heat-damaged membrane must be rapid and probably occurs early during recovery so the cell can fully repair other heat-induced lesions. If this is the case, then lipid synthesis plays an important role in the repair process.

Results similar to those reported here were observed when *S. typhimurium* 7136 was grown in a citrate salts medium (CSM), heat-injured, and then recovered in CSM containing citrate-*2,4-^{14}C*.

This investigation was supported by Public Health Service grant 2-RO1-FD-00093 from the U.S. Food and Drug Administration.

LITERATURE CITED

1. Allwood, M. C., and A. D. Russell. 1967. The leakage of intracellular constituents from heated suspensions of *Staphylococcus aureus*. Experientia (Basel) **23**:878.
2. Bluhm, L., and Z. J. Ordal. 1969. Effect of sublethal heat on the metabolic activity of *Staphylococcus aureus*. J. Bacteriol. **97**:140-150.
3. Bush, E. T. 1963. General applicability of the channel ratio method of measuring liquid scintillation counting efficiencies. Anal. Chem. **35**:1024-1029.
4. Clark, C. W., and Z. J. Ordal. 1969. Thermal injury and recovery of *Salmonella typhimurium* and its effect on enumeration procedures. Appl. Microbiol. **18**:332-336.
5. Iandolo, J. J., and Z. J. Ordal. 1966. Repair of thermal injury of *Staphylococcus aureus*. J. Bacteriol. **91**:134-142.
6. Kjeldgaard, N. O. 1961. The kinetics of ribonucleic acid and protein formation in *Salmonella typhimurium* during the transition between different states of balanced growth. Biochim. Biophys. Acta. **49**:64-76.
7. Roberts, R. B., D. B. Cowie, P. H. Abelson, E. T. Bolton and R. J. Britten. 1957. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Wash. Publ. 607.
8. Sogin, S. J., and Z. J. Ordal. 1967. Regeneration of ribosomes and ribosomal ribonucleic acid during repair of thermal injury to *Staphylococcus aureus*. J. Bacteriol. **94**: 1082-1087.
9. Tomlins, R. I., and Z. J. Ordal. 1971. Requirements of *Salmonella typhimurium* 7136 for recovery from thermal injury. J. Bacteriol. **105**:512-518.