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Role of Protein Degradation in the Survival of Carbon-Starved *Escherichia coli* and *Salmonella typhimurium*

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When an *Escherichia coli* K-12 culture was starved for glucose, 50% of the cells lost viability in about 6 days. When a K-12 mutant lacking five distinct peptidase activities, CM89, was starved in the same manner, viability was lost much more rapidly; 50% of the cells lost viability in about 2 days, whereas a parent strain lacking only one peptidase activity lost 50% viability in about 4 days. Compared with the wild-type strain and with its parent strain CM17, CM89 was defective in both protein degradation and protein synthesis during carbon starvation. Similar results were obtained with glucose-starved *Salmonella typhimurium* LT2 and LT2-derived mutants lacking various peptidase activities. An *S. typhimurium* mutant lacking four peptidases, TN852, which was deficient in both protein degradation and synthesis during carbon starvation (Yen et al., J. Mol. Biol. **143**:21–33, 1980), was roughly one-third as stable as the isogenic wild type. Isogenic *S. typhimurium* strains that lacked various combinations of three of four peptidases and that displayed protein degradation and synthesis rates intermediate between those of LT2 and TN852 (Yen et al., J. Mol. Biol. **143**:21–33, 1980) displayed corresponding stabilities during carbon starvation. These results point to a role for protein degradation in the survival of bacteria during starvation for carbon.

We have been interested in characterizing the survival strategies employed by bacterial populations subjected to starvation (6, 17). Bacteria frequently undergo periods of starvation in their natural environment, making such studies important in the understanding of normal bacterial life cycles. Further, in recent years the value of separating the growth and production phases of bacteria used in industrial fermentation processes has gained increasing recognition. Obviously, such a separation allows a more efficient conversion of raw material to product, reducing the amount of metabolic energy expended for growth-related processes. The use of nondividing cells also obviates the problem of reversion in genetically engineered organisms. Finally, in processes utilizing immobilized cells, considerations of containment volume become important, and the need for stable nongrowing cells becomes paramount (3a; D. S. Inloes, Ph.D thesis, Stanford University, 1982).

In this paper, we examine the role of protein degradation in the survival of carbon-starved bacteria. It is well established that growing cells of *Escherichia coli* K-12 degrade their bulk protein at an average rate of about 1 to 2%/h and that, when the cells are subjected to starvation for carbon, nitrogen, or inorganic nutrients, this rate increases to 4 to 5%/h (3). The mechanism underlying this phenomenon has not been elucidated, although an increased rate of protein degradation has been shown to correlate with various manifestations of the stringent response in these bacteria, in particular a rise in intracellular guanosine tetraphosphate (3, 10).

Peptidase-deficient mutants of *E. coli* K-12 and *Salmonella typhimurium* LT2 are available that are defective in protein degradation during starvation (8, 9). Growth and protein metabolism in the *S. typhimurium* peptidase mutants have been characterized in some detail by Miller and coworkers (15, 16), but the starvation survival of the *E. coli* and *S. typhimurium* mutants has not been investigated. To ascertain whether protein degradation plays a role in the survival of bacteria when they are starved for carbon, we have compared the viability behavior of these mutants with that of their corresponding wild-type strains under carbon starvation. Our results indicate that the degradation of proteins to free amino acids in carbon-starved cells plays an important role in cell survival.

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MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The *E. coli* K-12 and *S. typhimurium* LT2 strains used in this study are listed in Table 1. Minimal media are listed in Table 2; they are referred to as medium I, medium II, etc., below. Minimal salts media were of two basic compositions; M9 medium was used for *E. coli* cultures, and 0 salts medium was used for *S. typhimurium* cultures. M9 medium contained the following (per liter): Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.246 g; and $CaCl_2 \cdot 2H_2O$, 14.7 mg. 0 salts medium contained the following (per liter): KH_2PO_4, 2g; K_2HPO_4, 7 g; $(NH_4)_2SO_4$, 1 g; and $MgSO_4 \cdot 7H_2O$, 0.4 g. Growth media were supplemented with 0.4% glucose as the carbon source. All chemicals were reagent grade. Nutrient broth was from Difco Laboratories; amino acid and thymine supplements were from Sigma Chemical Co.

Cells were grown in Erlenmeyer flasks at 37°C with shaking at 200 rpm. Growth was monitored by the increase in absorbance at 660 nm; 1 optical density unit was equivalent to $\simeq 10^9$ cells per ml.

Starvation for carbon. Cells were grown in the appropriate medium to a density of 2×10^8 to 3×10^8 cells per ml (midlog phase). They were harvested aseptically by centrifugation at 10,000 × *g* for 10 min at 5°C, washed twice by suspension in the growth medium minus glucose, and suspended to the original density in growth medium minus glucose. During starvation, cultures were shaken in Erlenmeyer flasks at 200 rpm at 37°C.

Viability determinations. The viability of starving cultures was determined by serial dilution spreading of cells. At each

time point, duplicate plates were counted. To make agar medium, minimal salts media were supplemented with 1.5% Difco Bacto-Agar and with 0.02% glucose as the carbon source; this low glucose concentration was chosen to minimize the possibility of substrate-accelerated death (1). Nutrient broth agar was made with Difco nutrient agar.

E. coli strains were spread on agar medium I, medium II, and nutrient broth. A comparison of the results obtained with these three media showed the following: (i) Wild-type *E. coli* K-12 did not recover well on nutrient broth; as much as a 10-fold lower recovery occurred on nutrient broth than on medium I or II, which yielded equally high recoveries. (ii) CM17 and CM89 recovered equally well on nutrient broth and medium II. (iii) CM17 also recovered equally well on medium I, but CM89 showed nearly a 10-fold lower recovery on this medium. These differences illustrate the need for testing a variety of recovery media when measuring the viability of starving cells and led us to use medium II plus 0.02% glucose for recovery of all *E. coli* strains during carbon starvation.

S. typhimurium strains were spread on agar medium III, medium IV, and nutrient broth. In contrast to the difficulties encountered with recovery of carbon-starved *E. coli* strains on various media, the *S. typhimurium* strains recovered equally well on all three media. The recovery data presented in this paper were obtained by spreading on medium III agar plus 0.02% glucose.

Determination of protein degradation. Medium I plus 0.4% glucose was inoculated with *E. coli* cells from an overnight culture, to an optical density at 660 nm of 0.075. The culture was grown for one generation, at which time 1 μCi of L-[4,5-^{3}H]leucine (59.2 Ci/mmol; New England Nuclear Corp.) per ml was added. The culture was grown for another generation in the presence of the radioactive label and then harvested, washed twice, and suspended in medium I minus glucose, plus 100 μg of nonradioactive leucine per ml. The culture was split into two halves at this point; 0.4% glucose was added to one half, and this half was used to determine protein degradation in growing cells. The other half was used to determine protein degradation in carbon-starved cells.

At zero time for starvation and at desired intervals thereafter, duplicate trichloroacetic acid (TCA) precipitates were formed by adding 0.5 ml of culture to 50 μl of 50% TCA and allowing the mixture to incubate at room temperature for 1 h. The mixture was then centrifuged in a Fisher model 235A microcentrifuge for 5 min. A 0.25-ml sample of the supernatant was counted in 5 ml of scintillant (3:1 [vol/vol] xylene–Triton X-114, 0.4% 2,5-diphenyloxazole). At zero time only, total TCA-precipitable counts were determined as follows. The TCA precipitates were washed with 100 μl of 1% bovine serum albumin plus 1 ml of cold 5% TCA and then with 1 ml of ice-cold 1:1 (vol/vol) absolute ethanol-anhydrous ethyl ether. They were then dried and dissolved in 50 μl of 1 N NaOH. After the addition of 0.4 ml of water and 50 μl of 1 N HCl, 0.25 ml of the dissolved precipitate was counted in 5 ml of scintillant. The rate of protein degradation was expressed as the percentage of the zero time TCA-precipitable counts released as TCA-soluble material per hour.

TABLE 1. *E. coli* K-12 and *S. typhimurium* LT2 strains

Strain	Genotype	Source
E. coli		
K-12	Wild type	Stanford
CM17	*leu-9* Δ(*pro-lac*) *met thyA pepD*[a]	Miller (9)
CM89	*leu-9* Δ(*pro-lac*) *met thyA pepN102 pepA11 pepB1 pepQ10 pepD*[a]	Miller (9)
S. typhimurium		
LT2	Wild type	Miller (15)
TN843	*zfg-801*::Tn*10 pepN10 pepA1 pepB1 pepD3*	Miller (15)
TN852	*leu-485 zfg-801*::Tn*10 pepN10 pepA1 pepB1 pepD3*	Miller
TN858	*leu-485 zfg-801*::Tn*10 pepA1 pepD3 pepB1*	Miller
TN859	*leu-485 zfg-801*::Tn*10 pepN10 pepD3 pepB1*	Miller
TN860	*leu-485 zfg-801*::Tn*10 pepN10 pepA1 pepD3*	Miller
TN861	*leu-485 zfg-801*::Tn*10 pepN10 pepA1 pepB1*	Miller

[a] The *pepD* locus is included in the *pro-lac* deletion.

TABLE 2. Media

Medium	Description
I	M9 medium plus 0.3 mM methionine, 0.3 mM proline, 0.1 mM leucine, and 50 μg of thymine per ml
II	Medium I plus all 20 amino acids at 0.8 μg/ml
III	0 salts medium plus 0.3 mM leucine
IV	Medium III plus all 20 amino acids at 0.8 μg/ml

Determination of protein synthesis. Medium I plus 0.4% glucose was inoculated to an initial optical density at 660 nm of 0.075 with *E. coli* cells from an overnight culture. The culture was grown to an optical density at 660 nm of 0.3, and the cells were then harvested, washed twice, and suspended in medium I minus glucose. At zero time for starvation, 5 μCi of [^{3}H]leucine per ml was added to the culture. Then, at zero time and at desired intervals thereafter, duplicate TCA precipitates were formed and counted as described above. Protein synthesis was expressed as the increase in TCA-precipitable counts with time.

RESULTS

Protein degradation deficiency in carbon-starved *E. coli* peptidase mutants. The *E. coli* mutant CM89 lacks five peptidase activities (designated N, A, B, D, and Q) having distinct, but overlapping, peptide specificities (9). During a 6-h period (Fig. 1) growing *E. coli* K-12 cultures hydrolyzed bulk protein to TCA-soluble material at an average rate of about 1%/h; growing CM89 cultures hydrolyzed protein to TCA-soluble material at about the same rate, roughly 1.25%/h. This TCA-soluble, radioactive material probably consists largely of free leucine in the wild type, whereas TCA-soluble peptide products of protein degradation build up in the mutant, as was shown by Yen et al. for *S. typhimurium* LT2 and its peptidase-deficient mutants (16).

In wild-type *E. coli*, starvation for carbon caused a marked increase in protein degradation rate, whereas in CM89 this increase did not occur (Fig. 1). During the first 6 h of carbon starvation the wild type degraded an average of 3% of bulk protein to TCA-soluble material per h, whereas CM89 degraded only 1.5%/h to TCA-soluble material. In another experiment (results not shown), CM17, the *pepD*$^-$ parent strain of CM89, exhibited a rate of release of TCA-soluble material that was intermediate between that of wild-type K-12 and CM89, roughly 1.5 times the rate shown by CM89.

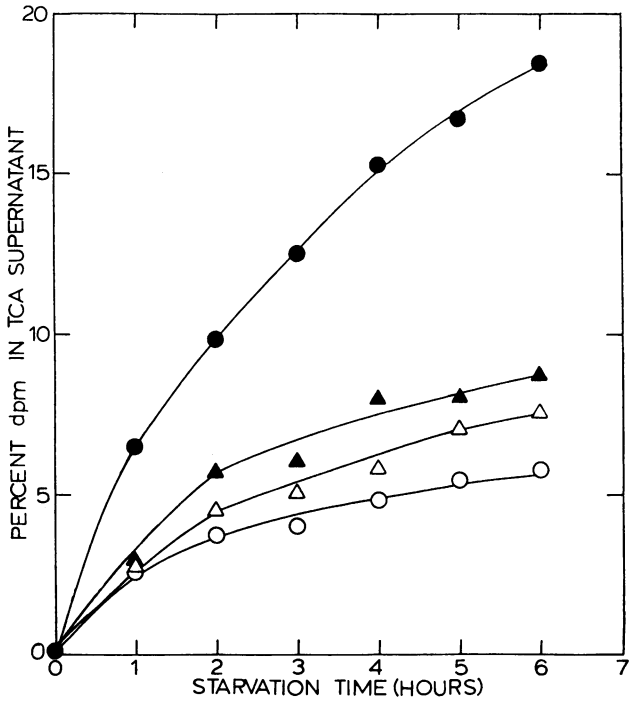

FIG. 1. Protein degradation in growing and carbon-starved *E. coli* K-12 and CM89. Protein degradation was measured in growing *E. coli* K-12 (○), carbon-starved *E. coli* K-12 (●), growing CM89 (△), and carbon-starved CM89 (▲), as described in the text.

Protein synthesis in carbon-starved CM89. Carbon-starved CM89 and its *pepD*⁻ parent strain CM17 were defective in protein synthesis when compared with the wild-type K-12 (Fig. 2), with CM89 having virtually no capacity, and CM17 possessing an intermediate capacity. That this inability to synthesize protein is due to the unavailability of free amino acids in the starved mutant cells is shown by the experiment of Fig. 3. When amino acids were supplied to the peptidase-deficient mutant CM89 at levels determined to be too low to allow an increase in cell number, protein synthesis was spurred, even when the amino acids were added after 4 h of starvation. Immediately upon the addition of the amino acids, the protein synthesis rate exhibited a drastic increase, which continued for approximately 1 h. After 1 h, this rate decreased to match the rate observed before amino acid addition.

A control experiment was done, in which wild-type K-12 was subjected to the same treatment (data not shown). The wild type exhibited the same pattern of protein synthesis upon amino acid addition, i.e., a rapid increase in rate followed by a decrease, after 1 h, to the initial rate. At 1 h after amino acid addition, the absolute difference between the amount of label incorporated into protein by the wild-type control culture and by the wild-type culture to which amino acids were added was the same as that observed between control and amino acid-treated CM89 cultures (Fig. 3), whether the amino acids were added at 0 or 4 h of starvation. Thus, the drop in rate of protein synthesis by amino acid-treated CM89 observed after 1 h probably resulted from the exhaustion of added amino acids, and we may conclude that CM89 has no innate deficiency in protein synthesis when the necessary amino acids are available.

Viability of *E. coli* K-12, CM17, and CM89 during carbon starvation. Peptidase-deficient mutants are thus deficient in both degradation and synthesis of protein during carbon starvation and so afford a means of investigating whether protein turnover plays a role in the survival of starving bacteria. Accordingly, we compared the starvation survival of wild-type *E. coli* K-12, the peptidase-deficient mutant CM89, and its *pepD*⁻ parent CM17.

As mentioned above, the viability of carbon-starved *E. coli* K-12, CM17, and CM89 was monitored by serial dilution spreading on medium II agar plus 0.02% glucose. Carbon-starved CM89 lost viability more rapidly than did wild-type K-12 (Fig. 4); the wild type lost 50% viability in about 6 days, whereas CM89 lost 50% viability in about 2 days. CM17 exhibited an intermediate behavior, losing 50% viability in about 4 days. (Note that for an initial period of about 1.5 days, all cultures maintained 100% viability.)

These differences in stability were reproducible. When the linear death rates of cultures were measured after 48 h of starvation in four separate experiments, wild-type K-12 exhibited an average half-life of 4.8 ± 0.9 days. CM17 exhibited an average half-life of 2.4 ± 0.9 days, and CM89 had an average half-life of 1.1 ± 0.2 days.

The biphasic nature of the death curve for CM89 was also a reproducible phenomenon; the reason for this behavior is unknown. It may have resulted from selection during starvation for peptidase-containing revertants, which may have arisen during growth of the original culture. Samples taken after 5 days of starvation and replica plated on valine peptide-containing medium did contain cells sensitive to the peptides, an indication of peptidase activity (9). This biphasic behavior was not observed with CM17 or with the *S. typhimurium* mutants discussed below.

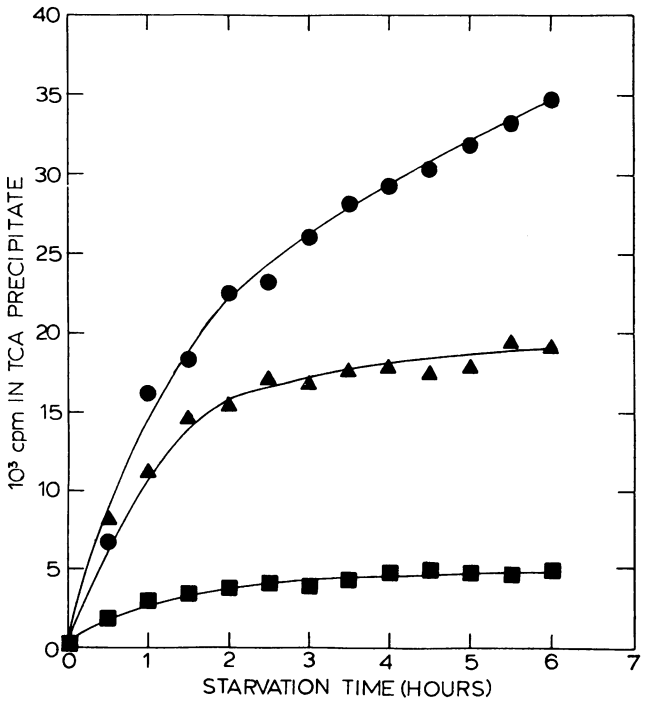

FIG. 2. Protein synthesis in carbon-starved *E. coli* K-12, CM17, and CM89. Protein synthesis was measured in carbon-starved *E. coli* K-12 (●), CM17 (▲), and CM89 (■) as described in the text.

Viability of *S. typhimurium* LT2 and its peptidase-deficient mutants during carbon starvation. Interpretation of the above data obtained with *E. coli* peptidase mutants was hampered by the fact that CM89 was the original mutagenized strain in which the peptidase mutations were isolated and may have contained other, unknown mutations. CM89 may not even be isogenic with CM17, since it was derived from CM17 by 2-aminopurine mutagenesis (9). To confirm our conclusions, it was necessary to duplicate our results in a system affording a clean genetic background. For this reason, we determined the survival ability during carbon starvation of *S. typhimurium* LT2 and a number of isogenic peptidase-deficient strains derived from LT2 by P1 transduction.

Yen et al. (15) have isolated and characterized *S. typhimurium* TN852, which lacks peptidases N, A, B, and D, and a series of so-called 1+ strains, in which individual peptidase activities have been restored to a TN852 background by transduction. When these strains were starved for carbon, wild-type LT2 showed the greatest stability (Fig. 5). The N^+ and A^+ strains, TN858 and TN859, showed the next highest stabilities, being roughly equal to one another. TN861 (D^+) and TN860 (B^+) were about equally stable, but were less stable than the N^+ or A^+ strains. The strain lacking all four peptidases, TN852, was the least stable. In a separate experiment (data not shown), the viability behavior of TN843, a strain isogenic with TN852 but lacking the leucine auxotrophy, was compared with that of wild-type LT2.

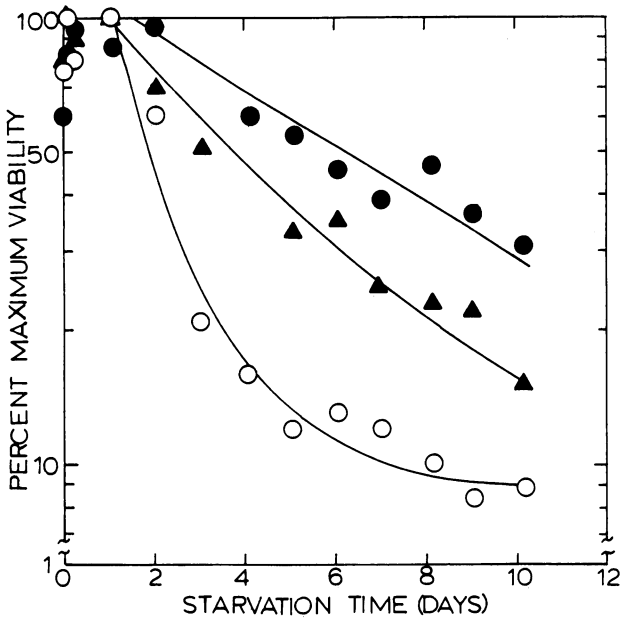

FIG. 4. Viability of carbon-starved *E. coli* K-12, CM17, and CM89. *E. coli* K12 (●), CM17 (▲), and CM89 (○) were grown for two generations in medium I plus 0.4% glucose, harvested, washed, and starved for carbon as described in the text. Viability was determined for each culture by serial dilution spreading on medium II agar plus 0.02% glucose.

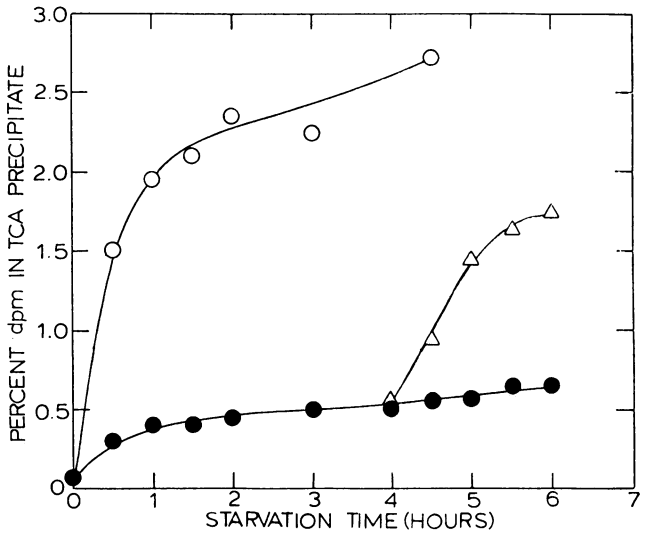

FIG. 3. Stimulation of protein synthesis in carbon-starved CM89 by the addition of amino acids. *E. coli* CM89 was grown for two generations in medium I plus 0.4% glucose. The cells were then harvested, washed, and suspended in medium I minus glucose as described in the text. At zero time for starvation, 5 μCi of [^{3}H]leucine per ml was added to the culture, and the culture was split into two parts. To one part, the control culture (●), no additions were made. To the other (○), a mixture of all 20 amino acids at 0.8 μg/ml of culture was added. At 4 h of starvation, a mixture of all 20 amino acids at 0.8 μg/ml of culture was added to another sample of the control culture (△). (Since excess leucine was present in the starvation medium, omitting leucine in the amino acid mixture did not have any effect on the results.) Protein synthesis, expressed as the percentage of the added disintegrations per minute incorporated into TCA-precipitable material, was monitored in all cultures as described in the text.

Growth, starvation, and recovery were done in medium III lacking leucine. TN843 exhibited the same behavior relative to the wild type as did TN852. Thus, the difference in stability of LT2 and the *S. typhimurium* 1+ strains is not dependent upon the amino acid auxotrophy of these peptidase-deficient strains.

Again, the differences in stability seen in Fig. 5 were reproducible. When the linear death rates of cultures were measured after 48 h of starvation in six experiments, the wild type had an average half-life of 2.8 ± 0.5 days, whereas TN852 had an average half-life of 0.9 ± 0.5 days. Only two experiments were performed with the various 1+ strains, but in both cases their stability was intermediate between that of the wild type and TN852.

The stabilities of the peptidase-deficient *S. typhimurium* strains during carbon starvation correlated well with their ability to degrade protein, release free amino acids via protein degradation, and synthesize new protein under these conditions (15). We found that all *S. typhimurium* strains exhibited roughly the same doubling time for growth (ca. 1 h) as did the wild type (data not shown), although lag times after dilution of the overnight cultures into new growth medium were generally longer for the peptidase-deficient mutants than for the wild type, agreeing with the findings of Yen et al. (15).

Possible peptide toxicity in starving peptidase-deficient mutants. Yen et al. (15) showed that lack of four peptidases, N, A, B, and D, resulted in a buildup of small, TCA-soluble peptides in peptidase-deficient *S. typhimurium* mutants during carbon starvation. The possibility exists that these small peptides may be toxic to starving cells, accounting for the more rapid loss of viability by carbon-starved peptidase-deficient mutants (Fig. 4 and 5). It is known that some small

peptides inhibit the growth of organisms that are unable to hydrolyze them (7).

To determine whether peptides played a role in the decreased resistance of the mutants to starvation, an experiment was done in which *S. typhimurium* TN843 cells starved for carbon were harvested and suspended in fresh starvation medium at various times to remove peptides excreted into the medium. Since the major portion of the TCA-soluble material generated by protein degradation during starvation could be assumed to be small peptides (15), labeling of cellular protein during growth allowed us to trace the fate of these peptides in the starved culture.

In an unwashed control culture, 5% of the protein was converted to peptides during the first 6 h of starvation, as evidenced by the fact that 5% of the counts present in TCA-precipitable material at zero time became TCA soluble during this interval; this agreed with the results shown for CM89 in Fig. 1. Between 6.5 and 22.5 h of starvation, another 2.5% of the protein was converted to peptides. Washing the culture at 1.5, 3.0, 4.5, and 6.0 h always reduced the TCA-soluble counts to a value corresponding to less than 2% of the total protein, suggesting that the maximal level of peptides that could be retained by the cells corresponded to those generated by the breakdown of 2% of the original protein, the rest being excreted into the medium. Between 23 and 99 h of starvation, an average of only 0.13% of the TCA-insoluble counts were rendered TCA soluble per h in both the washed and unwashed cultures; at 99 h, the unwashed culture contained peptides generated from the breakdown of 20% of the protein in the original culture, whereas the washed culture contained about half this amount. Thus, the four washes employed in this experiment were sufficient to reduce the level of peptides in the culture at all times by at least 50%. Yet, this washing had no effect on culture viability; both the washed and unwashed cultures had lost about 85% viability by 100 h of starvation (data not shown).

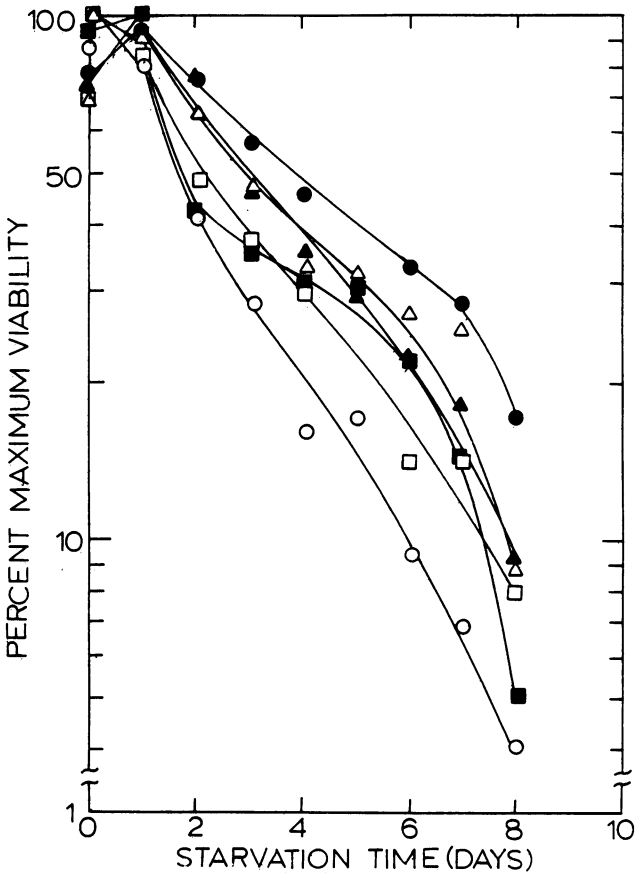

FIG. 5. Viability of carbon-starved *S. typhimurium* LT2 and its peptidase-deficient 1+ mutants. Cultures of *S. typhimurium* LT2 and the 1+ mutants were grown for two generations in medium III plus 0.4% glucose, harvested, washed, and starved for carbon as described in the text. Viability was determined by serial dilution spreading on medium III agar plus 0.02% glucose. Symbols: ●, LT2; ○, TN852 (*pepN⁻A⁻B⁻D⁻*); ▲, TN858 (*pepN⁺A⁻B⁻D⁻*); △, TN859 (*pepN⁻A⁺B⁻D⁻*); ■, TN860 (*pepN⁻A⁻B⁺D⁻*); □, TN861 (*pepN⁻A⁻B⁻D⁺*).

DISCUSSION

The results presented in this paper clearly demonstrate that peptidase activities are important to the survival of *E. coli* and *S. typhimurium* cells during carbon starvation. Yen et al. (15) have shown that these peptidases also play an important role in recovery of cells from nutritional shift-down. Thus, the peptidases are important to the survival of cells during nutrient limitation or deprivation, both conditions that normally result in enhanced rates of protein degradation.

In *E. coli* peptidase-deficient mutants starved for carbon, the rate of hydrolysis of TCA-insoluble polypeptides was much lower than that observed in the wild type (Fig. 1). These results are consistent with those of Yen et al. (15) for *S. typhimurium* LT2 and its peptidase mutants. These workers showed that loss of peptidases in *S. typhimurium* resulted in a lower rate of protein degradation during starvation and, further, that the products of protein degradation were small peptides, as opposed to the free amino acids generated by the wild type.

Is the decreased stability of peptidase-deficient mutants starved for carbon a result of the toxic effects of peptide buildup or of their inability to degrade protein? It is unlikely that toxicity of accumulated intracellular peptides accounted for the decreased stability of the mutants during starvation, since removal of peptides by washing the cells had no effect on their longevity. This is consistent with numerous other considerations. In all cases where a mode of action for a peptide effector is known, this mode involves inhibition of either amino acid biosynthesis (12, 13), protein synthesis (2), or peptidase activity (14). First, it is unlikely that inhibition of amino acid biosynthesis would be deleterious to carbon-starved cells, which are already severely limited in their ability to synthesize amino acids. We have shown (unpublished results) that peptidase deficiency has similar effects on the survival of nitrogen-starved cells, which are unable to carry out net amino acid biosynthesis. Second, we have shown (Fig. 3) that carbon-starved *E. coli* CM89 responds to amino acid addition at either 0 or 4 h of starvation with rapid synthesis of protein. Thus, the peptides resulting from protein breakdown in these cells do not inhibit protein synthesis when sufficient free amino acids are provided. The third possibility, peptide inhibition of peptidase activities, would not change the interpretation of our results. Overall, it appears most probable that the decreased stability of the peptidase mutants resulted not from any toxic effect of peptides, but rather from the inability of these mutants to degrade protein.

Why is protein degradation helpful in the survival of carbon-starved bacteria? One possibility is that the peptidases may carry out post-translational modification of key

survival-related proteins, which is necessary for enzyme function and is in turn necessary for the survival of the starved cells. However, the only known specificities of the peptidases missing in our strains (peptidases N, A, B, D, and Q) are for small peptides; none of these peptidase activities has so far been implicated in the post-translational modification of specific proteins.

Carbon-starved cells may need protein degradation to inactivate energy-wasting enzymes. It has been demonstrated that starving cells degrade their intracellular proteins at widely varied rates (3, 4), suggesting that it is advantageous to these cells to selectively degrade certain proteins. A number of enzyme activities involved in energy metabolism are known to be lost during starvation; this loss may be due to proteolysis, although only a very few examples of proteolytic inactivation have been documented (11). If the defect in proteolysis displayed by the peptidase-deficient mutants not only allows a buildup of large, TCA-insoluble polypeptides in the cells, but also allows the conservation of energy-wasting enzyme activities, this waste of energy may be detrimental to survival of the cells. Studies of the stability of protease-deficient mutants inhibited in the early steps of proteolysis would be informative in this regard.

Another possibility is that carbon-starved cells may need protein degradation as a source of free amino acids. Mandelstam (4, 5) suggested that starving cells need a pool of free amino acids to respond to their changed environment via the synthesis of new enzymes that are specific to the starvation condition. He observed that the rates of protein degradation and synthesis in starved cells are balanced, suggesting that protein degradation is the major source of the pool amino acids utilized for protein synthesis during starvation, and, conversely, that starving cells use amino acids derived from protein degradation mainly for new protein synthesis (4). Mandelstam's studies were done using amino acid- or nitrogen-starved cells, but protein degradation may have a similar role in carbon-starved cells, in which new amino acid synthesis is limited by the unavailability of carbon skeletons and protein degradation must serve as the major source of free amino acids. Indeed, our results and those of Yen et al. (15) show that carbon-starved, peptidase-deficient cells are defective in protein synthesis. After long periods of starvation, free amino acids might also be utilized as carbon or energy sources for the cell; a thorough investigation of this possibility is yet to be carried out (3).

The data presented in this paper do not allow us to choose among the possibilities discussed above. However, they strongly suggest a role for continued protein synthesis in the survival of starving cells. In a future communication, we will present evidence that the ability to synthesize protein is indeed important in the survival of carbon-starved bacteria.

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