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Starvation-Survival Processes of a Marine Vibrio†

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Levels of DNA, RNA, protein, ATP, glutathione, and radioactivity associated with [^{35}S]methionine-labeled cellular protein were estimated at various times during the starvation-survival process of a marine psychrophilic heterotrophic *Vibrio* sp., Ant-300. Values for the macromolecules were analyzed in terms of total, viable, and respiring cells. Electron micrographs (thin sections) were made on log-phase and 5.5-week-starved cells. On a per-cell basis, the levels of protein and DNA rapidly decreased until a constant level was attained. A second method in which radioactive sulfur was used for monitoring protein demonstrated that the cellular protein level decreased for approximately 2.5 weeks and then remained constant. An initial decrease in the RNA level with starvation was noted, but with time the RNA (orcinol-positive material) level increased to 2.5 times the minimum level. After 6 weeks of starvation, 45 to 60% of the cells remained capable of respiration, as determined by iodonitrotetrazolium violet-formazan granule production. Potential respiration and endogenous respiration levels fell, with an intervening 1-week peak, until at 2 weeks no endogenous respiration could be measured; respiratory potential remained high. The cell glutathione level fell during starvation, but when the cells were starved in the presence of the appropriate amino acids, glutathione was resynthesized to its original level, beginning after 1 week of starvation. The cells used much of their stored products and became ultramicrocells during the 6-week starvation-survival process. Ant-300 underwent many physiological changes in the first week of starvation that relate to the utilization or production of ATP. After that period, a stable pattern for long-term starvation was demonstrated.

Any heterotrophic marine bacterium faces the pressures of species survival in an environment where there is a severe lack of nutrients. The dissolved organic carbon content of open ocean water is often <0.5 mg of carbon per liter (20), and much of the deep-water carbon is refractory to microbial degradation (1). Hence, the amount of organic matter available to bacteria in the deep sea is extremely limited. When the long residence time of water masses is also considered, bacterial cells may have to withstand nutrient deprivation for extremely long periods of time (21).

Novitsky and Morita (24–26) demonstrated that Ant-300 (a marine psychrophilic heterotrophic vibrio) has the ability to survive for long periods of time (1 year) in the absence of an organic energy source. One culture was held under starvation conditions for nearly 2.5 years (R. Y. Morita, unpublished data). However, these studies did not address the specific physiological changes that occur in heterotrophic marine bacteria during the process of starvation-survival. This study investigates some of those changes, and the data aid in the understanding of how these changes occur in bacteria during starvation-survival in their natural environments.

MATERIALS AND METHODS

Organism and media. A marine psychrophilic heterotrophic bacterium (Ant-300), tentatively identified as a *Vibrio* sp. (2), was used for this study. It was isolated in 1972 from the antarctic convergence (300 m) during cruise 46 of the R. V. Eltanin.

Ant-300 cells were grown either in liquid or on solid (1.2% agar added) Lib-X medium (2). Cells were harvested from cultures in the log phase (optical density at 600 nm, 0.6 to 0.9) by centrifugation (4,080 × *g*) and washed twice in mineral salts solution (SM) (24). The washed cells were suspended in SM, buffered with 0.01 M Tris (pH 7.8), and incubated for various periods at 5°C. Starved cell suspensions having an initial cell density of 10^7 to 10^8/ml were shaken at 100 rpm in a Psychrotherm incubator shaker (New Brunswick Scientific Co.). All media, dilution blanks,

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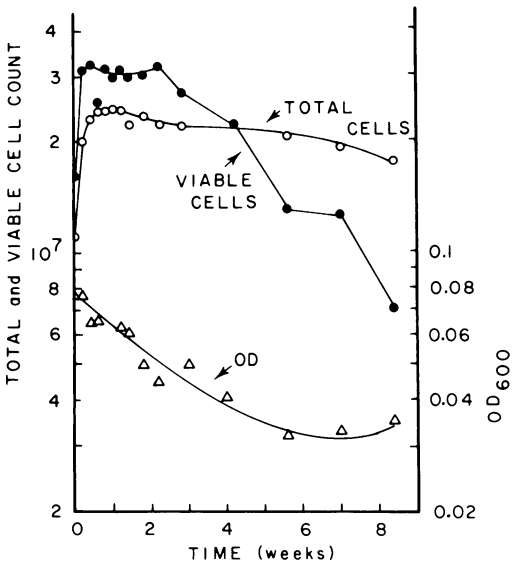

FIG. 1. Total cells counts, viable cells counts, and optical density (OD) with starvation time. During the initial fragmentation period (12 to 36 h), total cell counts and viable cell counts increased dramatically. OD_{600}, Optical density at 600 nm.

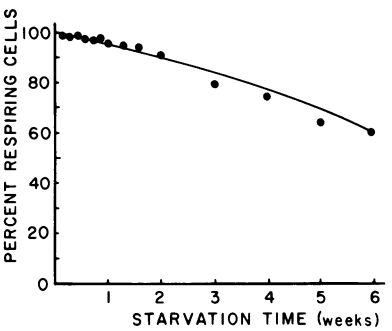

FIG. 2. Percentage of respiring cells during 6 weeks of starvation. A large percentage of Ant-300 cells retained their ability to respire during starvation.

and reagents were filter sterilized (0.22-μm filters; Millipore Corp.) or filtered and heat sterilized to minimize debris interference when total cell counts were determined. All materials, including pipettes and centrifuge tubes, were held at 5°C.

Radiolabeling of cells. A modified Lib-X broth with the organic contents reduced to 10% and L-glutamic acid eliminated was used for protein labeling. Cells were grown at 5°C to slight turbidity (ca. 10^6 to 10^7/ml). The culture was centrifuged at 3°C and 3,040 × *g* for 5 min, and the cells were suspended in fresh broth containing 53 μCi of [^{35}S]methionine per ml (specific activity, 1,001.8 Ci/mmol; New England Nuclear Corp.) and incubated at 5°C for 24 h. Cells were then prepared for starvation as described above.

At various times, triplicate 0.5-ml samples were filtered onto 0.22-μm Millipore filters, dried, and counted in vials containing 10 ml of Omnifluor in a Beckman LSC-100 scintillation counter. Filtrate samples were applied to filters and treated in the same way as cell samples.

Cell counts. Viable cell counts were made by diluting the cell suspensions in SM and spreading known volumes of the suspensions in duplicate onto Lib-X plates. Plates were incubated at 5°C for 1 week before the colonies were counted. Total cell counts were determined by epifluorescence microscopy with a Zeiss epifluorescence microscope and in accordance with the method of Zimmermann and Meyer-Reil (37) on Nucleopore filters (0.2-μm-pore size; Nucleopore Corp.) stained with Igralan black (35). A total of 10 fields were counted for each sample; replicate samples were used. Turbidities (optical densities) of cell suspensions were estimated at 600 nm with a Bausch and Lomb Spectronic 710 spectrophotometer.

To determine the fraction of respiring cells we used a modification of the iodonitrotetrazolium violet (INT; Calbiochem) method of Zimmermann et al. (36). Starved cells (5 ml) were harvested by centrifugation (Sorvall RC-2 centrifuge) at 3,000 × *g* for 10 min at 3°C. Cells were suspended in approximately 0.5 ml of SM, and to this suspension was added 0.1 ml of a 0.2% aqueous solution of INT; this suspension was incubated for 30 min. All solutions, including the incubation mixture, were kept on ice. The incubated cell suspension (0.1 ml) was observed with a Zeiss phase-contrast microscope. The total number of cells and the number of respiring cells (those with refractile granules of INT) were counted for each sample, and the percentage of respiring cells was calculated.

Endogenous respiration and potential respiration. At various times during the starvation process, 40-ml samples of a starving cell suspension (in duplicate) were centrifuged as described above. The cell pellet was suspended in 4.0 ml of SM and held on ice. The endogenous respiration mixture (in triplicate), which contained 2.3 ml of SM, 0.5 ml of 0.2% INT, and 0.2 ml of the cell suspension, was incubated on ice. After incubation, the reaction mixture was terminated with 1.0 ml of Zap (50% phosphoric acid–50% Formalin) (27). The amount of INT-formazan produced was estimated at 600 nm with a Beckman DB spectrophotometer and compared against a standard curve prepared with various amounts of INT-formazan (Sigma Chemical Co.) in 95% ethanol.

The reaction mixture for the determination of potential respiration was identical to the mixture for the determination of endogenous respiration except that 1.0 ml of substrate solution (28) replaced 1.0 ml of SM. The substrate solution was prepared by adding 7.6 g of sodium succinate, 1.8 ml (1:10 dilution) of Triton X-100, and distilled water to a final volume of 100 ml. To a 10-ml portion of this mixture 10 mg of NADH and 1 mg of NADPH were added just before use. These analyses were also run in triplicate.

Estimation of macromolecules. Samples (40 ml; in duplicate) were taken at various times during the starvation-survival process and harvested by centrifugation as described above. Fractionation of protein from nucleic acids was accomplished by treatment with trichloroacetic acid, perchloric acid, and NaOH as described by Gallant and Suskind (8). The precipitated material was dissolved in 1.0 ml of 1.0 N NaOH and assayed for protein by the Folin-phenol method (17). The DNA and RNA concentrations were estimated spectrophotometrically by the diphenylamine (5)

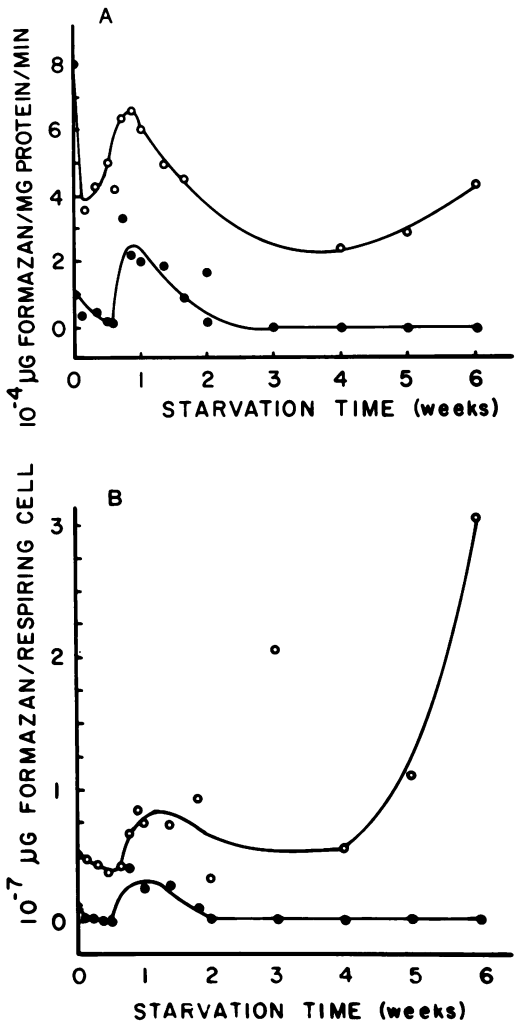

FIG. 3. INT-formazan formed by Ant-300 per milligram of protein (A) and per respiring cell (B) during starvation. Symbols: ●, endogenous respiration; ○, potential respiration.

and orcinol (19) methods. Corrections for any color development caused by the presence of DNA in the RNA fraction were made.

Estimation of ATP. The amount of ATP in starved cells was estimated by the method of Holm-Hansen and Booth (11) with boiling Tris buffer (pH 7.75) to extract the ATP from triplicate 2-ml cell suspensions concentrated onto 0.22-µm Millipore filters. The extracted ATP was placed in a vial, the volume was brought to 10 ml, and the mixture was frozen (−20°C) until assayed.

The assay mixture, which contained 10 µl of samples, 20 µl of buffer (20 mM Trizma base, pH 7.75 [Sigma]), 15 mM $MgCl_2$, and 2 mM sodium EDTA (ethylene dinitrolotetraacetic acid), was placed in an ATP photometer assay vial. A second vial identical to the first but containing an internal spike of 10 µl of ATP (10^{-8} or 10^{-9} M) in Tris-sodium EDTA-Mg buffer instead of 10 µl of buffer was also prepared for each sample. The reaction was initiated by injecting 50 µl of partially purified luciferin-luciferase (Du Pont Co.) into an ATP photometer assay vial, and the light emitted was measured with a Picolite photometer (Packard Instrument Co.).

Calculations were made by comparing maximum peak heights in 5 s of spiked and unspiked samples.

Estimation of glutathione. Samples (40 ml; in duplicate) of a starving cell suspension were centrifuged as previously described, and the cell pellet was suspended in 0.6 ml of distilled water. A portion (0.5 ml) of the suspended cells was treated with 0.1 ml of 1 N NaOH for protein analysis. The remaining sample was then prepared for high-pressure liquid chromatography analysis by the method of Reed et al. (30). Peak heights relative to standards were used to estimate the concentrations of glutathione in the cells.

Electron microscopy. Log-phase and 5.5-week-starved cells were harvested by centrifugation (at 3,200 × *g* for 10 min at 3°C; Sorvall RC-2B centrifuge), and the cells were suspended in fixative (0.2 M cacodylate buffer [pH 7.8], 0.3 M NaCl, 3% glutaraldehyde [Tousimis Research Corp.]). Cells were fixed for 2 h at 5°C and then centrifuged as described above. The cell pellet was mixed with approximately 1.0 ml of 2% Noble agar (Difco Laboratories) and allowed to solidify on a glass slide. Small cubes were cut with a razor blade, washed with four changes of cacodylate buffer, and post fixed in 1% OsO_4 in cacodylate buffer for 2 h on ice. The cubes were dehydrated in an acetone series (30, 50, and 70% [containing 1 g of uranyl acetate per 100 ml]) and finally with three changes in 100% acetone. Each acetone dehydration step lasted 15 min. Individual cubes were placed in capsules, covered with embedding resin (Spurrs; Polysciences, Inc.), and cured overnight at 70°C. Sections were cut with a Porter-Blum ultramicrotome and a diamond knife (F. A. Dehmer) and stained with lead citrate (31) before examination with a Philips EM 300 electron microscope.

RESULTS AND DISCUSSION

Total and viable cell counts as well as the turbidity of a culture undergoing starvation are shown in Fig. 1. During the initial starvation phase the viable cell numbers increased and then decreased, accompanied by a decrease in microbial biomass, as indicated by the turbidimetric readings. The total cell count stayed high and rather constant, demonstrating that many of the cells remained structurally intact but not viable. The total cell count was consistently lower than the viable count because of inaccuracies caused by the increased number of manipulations necessary to determine total cell numbers. This pattern of a rapid increase in viable cell numbers followed by a gradual decrease in viable cell numbers were noted by Novitsky and Morita (26) and Kjelleberg et al. (13) and was also demonstrated in 7 out of 16 open-ocean isolates (P. S. Amy and R. Y. Morita, unpublished data).

Before starvation, cells were rod shaped and had average dimensions of ca. 1.3 by 4.0 µm.

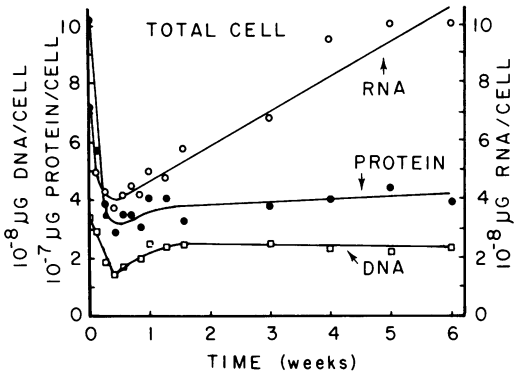

FIG. 4. Macromolecules per cell during 6 weeks of starvation in Ant-300.

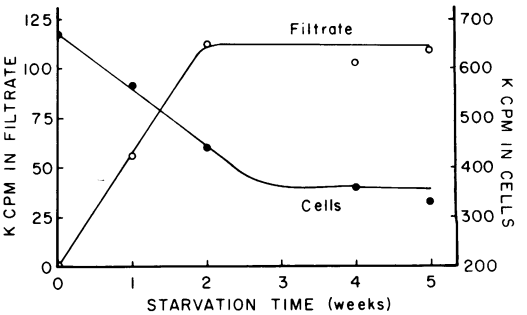

FIG. 5. ^{35}S-labeled material present in cells and cell filtrates during 5 weeks of starvation in Ant-300.

When cells were incubated with INT, up to 12 refractile granules were produced per cell. During starvation, the cells underwent division and became much more coccoid, and the number of refractile granules produced decreased to one per cell. The granules took up much of the cytoplasmic space of the cells. The failure to produce a granule detectable by light microscopy distinguished between respiring and nonrespiring cells. Typical experiments showed 45 to 60% of the cells capable of granule deposition after 6 weeks of starvation (Fig. 2). Using a marine pseudomonad, Kurath (M.S. thesis, Oregon State University, Corvallis, 1980) showed that in starving cultures, the number of viable cells became constant at 0.1% of the original population. However, compared with the viable cell count, 10 times more respiring cells were measured after 18 days of starvation. These data imply that many cells incapable of colony formation under the conditions used were able to respire sufficiently to deposit INT-formazan granules. In Ant-300 cultures, the number of respiring and viable cells was approximately the same throughout 5 weeks of starvation, and at 6 weeks the number differed by only 35%. Novitsky and Morita (24) demonstrated that log-phase cultures of Ant-300 had up to four nuclear bodies, whereas only one nuclear body was observed in starved cells.

The endogenous and potential respiration (with added substrate) rates dropped immediately upon starvation (Fig. 3A and B). A temporary increase that reached a peak in ca. 7 days was observed. Subsequent periods of starvation brought about lower rates of endogenous and potential respiration. After 2 weeks of starvation, the endogenous respiration rate was 6% of its original value and only 4% of its peak value. Using cells grown on [^{14}C]glucose, Novitsky and Morita (25) demonstrated that the endogenous respiration rate of cells starved 7 days or longer was 0.0071%. However, with the technique used in this investigation, the endogenous respiration rate could not be measured after 14 days. Other investigators have also reported rapid suppression of endogenous respiration during starvation-survival, and we assumed that our method was a way to prevent rapid self destruction (23). Nelson and Parkinson (23) demonstrated increased respiration with a *Bacillus* sp. and an *Arthrobacter* sp. but not with a *Pseudomonas* sp. The *Bacillus* sp. and *Arthrobacter* sp. underwent rapid cell death, whereas the *Pseudomonas* sp., which displayed a constant but decreased respiration level, survived very well. It may be that in the case of the two poorly surviving bacteria and of Ant-300 after 1 week of starvation, an effort to produce energy through respiration was necessary to maintain cell viability. In all three cases, cell death began or continued in spite of the increased respiration or perhaps because of it. After 1 week of starvation of Ant-300 and the *Pseudomonas* sp., low but constant respiratory activity occurred concomitantly with retention of viability.

On the other hand, starved cells retained the ability to respire when challenged with substrate (Fig. 3A and B). The ability to respire with added substrate increased with cells starved longer than 4 weeks and became more evident when the data were plotted in terms of INT-formazan production per respiring (INT) cell in the system (Fig. 3B). The potential respiration activity increased since there was a decrease in the number of respiring cells with starvation time. Ant-300 was also shown to rapidly take up and utilize other substrates, such as amino acids, after various periods of starvation (Glick, M.S. thesis, Oregon State University, Corvallis, 1980). Other marine isolates have also shown this ability (P. S. Amy and R. Y. Morita, unpublished data).

During the initial starvation phase protein, RNA, and DNA showed a similar pattern of a rapid decrease in levels with starvation time

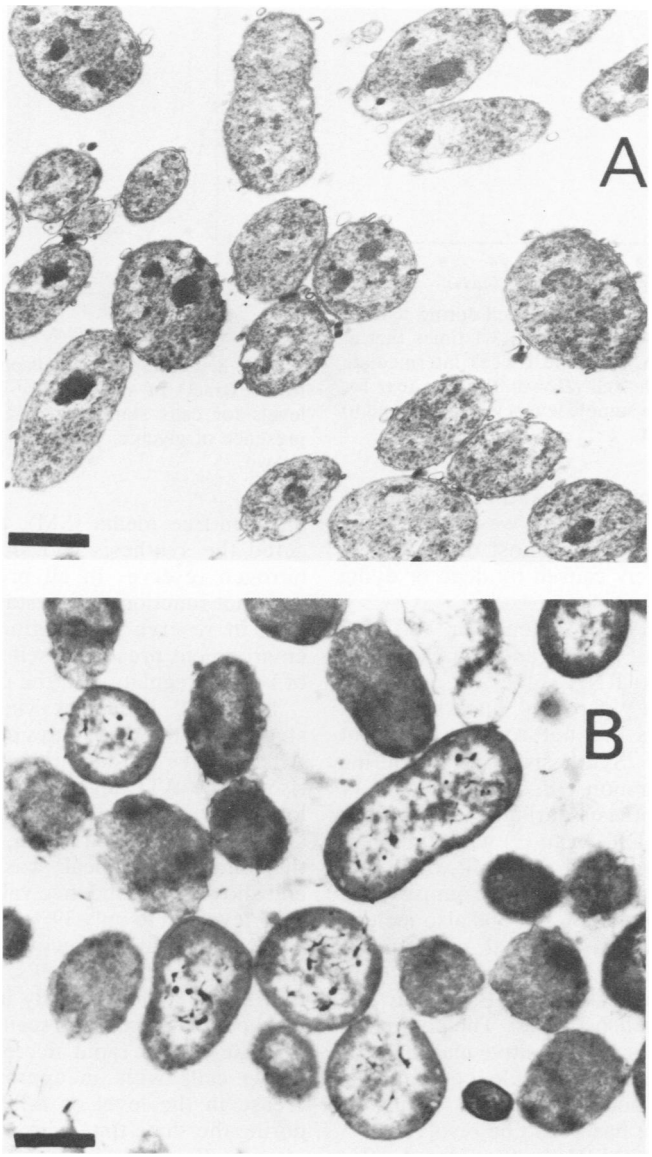

FIG. 6. Thin sections of log-phase (A) and 5.5-week-starved (B) cells examined by transmission electron microscopy. Bars = 1 μm.

(Fig. 4). The protein and DNA values increased slightly and stabilized at 2.9×10^{-8} and 2.5×10^{-7} μg per cell, respectively. According to Koch (14, 15), *Escherichia coli* is not capable of degrading certain classes of proteins; hence, the protein concentration in the culture suspension remained quite high. It is possible that there is a shift in the type of macromolecules within the starving viable cells; however, such a shift could not be detected by the methods used in this study.

Protein is known to be metabolized during starvation (10, 12). [^{35}S]methionine-labeled Ant-300 cells were starved, and the radioactivity in the cells and cell filtrates was monitored during the course of the starvation period. There was a linear increase in the level of radiolabel in the cell filtrates for approximately 2 weeks (Fig. 5), and there was a concomitant loss of radioactivity in the cells. The net protein degradation appeared to stop after 2 to 3 weeks. This increase in the radioactivity in the cell filtrates suggests either that viable, dying, and/or dead cells degraded or leaked labeled cellular protein through normal metabolism or autolytic processes or that an extensive turnover of proteins took

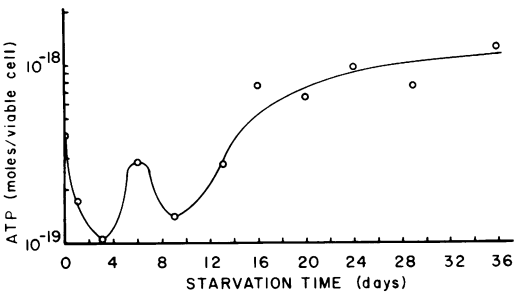

FIG. 7. ATP per viable Ant-300 cell during 36 days of starvation. The 36-day level was 3.1 times that at initiation of starvation. Note the 1-week intermediate rise in ATP per viable cell ($P = 0.01$). See text for explanation. Triplicate sample levels were averaged to obtain each time point.

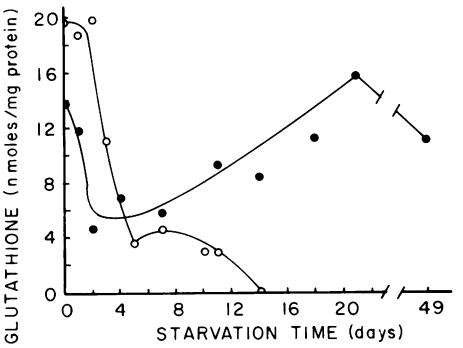

FIG. 8. Glutathione levels per nanogram of protein from extracts of starving Ant-300 cells. Glutathione levels for cells starved in SM only (○) and in the presence of glycine, glutamate, and cysteine (●) are shown.

place in starving Ant-300 cells. Since the majority of the original population was viable even after 3 weeks of starvation, most of these processes were not likely caused by dead or dying cells.

The change in RNA content per cell was different from that of protein and DNA (Fig. 4). After the first week, RNA values, as measured by the orcinol test, increased linearly and in typical experiments did not reach a constant value by 6 weeks. Synthesis of RNA during starvation is uncommon (18, 32). One case of increased RNA synthesis during starvation was reported by Borek and Ryan (3) with a methionine-requiring mutant of *E. coli* K-12 which was also incapable of the stringent response. The nutritional requirements of Ant-300 also include methionine (J. Baross, personal communication), and attempts to demonstrate the stringent response have to this date failed (P. S. Amy and R. Y. Morita, unpublished data). The possibility also exists that the orcinol-positive material was not RNA but another molecule containing a pentose, such as aldoheptoses and hexuronic acids (6). This issue has yet to be resolved.

The form in which RNA was stored after resynthesis is not known. The most abundant form of stable RNA was ribosomal. Thin sections of 5.5-week-starved cells (Fig. 6) did not shown an increase in ribosomal bodies. If ribosomes were degraded during the initial starvation period and only RNA was resynthesized new ribosomes would not be formed because of the lack of ribosomal proteins. Cells labeled with [2-^{14}C]uracil during growth showed no respiration of the ring structure during starvation but did show some leakage of non-macromolecular-labeled molecules into the starvation menstruum (P. S. Amy and R. Y. Morita, unpublished data). Postgate and Hunter (29) reported a similar breakdown of RNA with cellular leakage in starved bacteria. Since Ant-300 was starved in nitrogen-free media (SM), this may have triggered the synthesis and storage of RNA as a nitrogen reserve. In all probability this RNA does not function during starvation but either is held in reserve to function when the proper environment presents itself (10) or is the result of lack of regulation in the cell (7).

The ATP level per viable cell decreased steadily for the first 3 days of starvation (Fig. 7). After 3 days of starvation a small intermittent rise in the ATP level was seen. This was followed by a second decrease beginning after about 1 week of starvation. The significance of this intermediate peak was statistically tested and showed a confidence value of $P = 0.01$. The ATP level fell to only 39% of the initial level by 1 week, after which it recovered to within 92% of the initial level by 2 weeks. With longer starvation times, a slow, steady increase in the ATP level per viable cell was seen. This was primarily because of the rapid decrease in the level of viable cells with an approximately equal decrease in the level of ATP per ml of culture during the same time period. The ATP levels in starving *Pseudomonas* sp. and *Arthrobacter* sp. decreased to 75 and 71%, respectively, of initial levels within 5 days but remained at those levels for up to 10 days of starvation (23). Kurath (M.S. thesis, 1980) demonstrated that cells of a marine pseudomonad contained more ATP per viable cell after starvation for 1 week than they contained at the initiation of starvation. The patterns of ATP levels per viable cell in that pseudomonad and in Ant-300 were identical except that in the pseudomonad the level of ATP per viable cell continued to rise until, at its peak (25 days), it represented 1,058% of the initial level. Since it is known that 20% of the energy in a growing culture is used for substrate transport (34), the high levels of ATP in starving cells reinforce the concept that starved cells are phys-

iologically capable of metabolism if a substrate becomes available. This ATP is necessary for initial active transport but may not be readily renewable without further input of an energy-yielding substrate.

A single primary source of energy for the 60% of cells that continued to respire during a 6-week starvation period was not identified. Thin sections of growing and starved cells revealed radically different cellular internal structures. Large medium-dark pockets, peripheral white granules, and small, darkly stained granules were present in growing cells (Fig. 6A). These structures almost completely disappeared by 5.5 weeks of starvation. Only the central nuclear region surrounded by clearer cytoplasm remained in starved cells (Fig. 6B). The cells became coccobacilli, but the cell diameter at 5.5 weeks was approximately the same as that in growing cells. One type of granule showed an intense, nonstained white spot in the cell cytoplasm resembling poly-β-hydroxybutyrate, but repeated attempts to demonstrate poly-β-hydroxybutyrate with the technique of Law and Slepecky (16) failed. Sudan B black staining material was seen in growing cells under light microscopy, and it is therefore suspected that lipids may be stored in growing cells. Evidence for this was demonstrated by Stringer and Oliver (W. Stringer and J. Oliver, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, N107, p. 195), who showed a 35% reduction in lipid phosphate in Ant-300 during 3 weeks of starvation. Small, intense black granules disappeared slowly and could sometimes be seen in 5.5-week-starved cells. Polyphosphates, lipids, proteins, and carbohydrates are possible storage products in growing Ant-300 cells.

The presence of glutathione in eucaryotic cells has been related to the ability of cells to withstand oxidation because of the reduced sulfhydryl group of the cysteine moiety of glutathione (22). In this sense the tripeptide functions are very similar to the proposed action of vitamins E and C. In this study cells starved in SM alone were unable to resynthesize glutathione after the initial dramatic drop in the first 6 days of starvation; however, given the three amino acids which make up glutathione, the cells resynthesized this compound to the original level of 13 to 14 nmol per mg of protein (Fig. 8). These cells did not utilize the three amino acids for growth and cell division as one might expect. There appears to be an important function for glutathione in Ant-300, given that starving cells direct their energy to its resynthesis. It may be that oxidation of cellular components during starvation is a major cause of cell death, and glutathione may play an important role in maintaining a reduced intracellular state.

The coincidence of increased ATP content per viable cell, higher respiratory activities, the rapid change from the dramatic decrease in cellular macromolecules to a constant value (or even resynthesis in the case of RNA), and the resynthesis of glutathione when amino acids were present is apparently related to energy production and utilization. The reordering of the activities of cells at 1 week of starvation represents a line of demarcation between the initial response to starvation (cell fragmentation) and a long-term reaction to nutrient depletion. When 1- and 2-week-starved cells were challenged with 0.1 ml of Lib-X, there was a difference in the response time, as measured by the ability of cells to grow and divide. The 2-week-starved cells responded more slowly and divided several hours later than did the 1-week-starved cells (P. S. Amy and R. Y. Morita, unpublished data). It may be that there are short-term and long-term responses of Ant-300 to nutrient starvation which cause cells starved for longer periods of time to respond more slowly. Perhaps they are deeper into cell dormancy of the type described by Stevenson (33).

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LITERATURE CITED

1. **Barber, R. T.** 1968. Dissolved organic carbon from deep waters resists microbial oxidation. Nature (London) **220**:274–275.
2. **Baross, J. A., F. J. Hanus, and R. Y. Morita.** 1974. The effects of hydrostatic pressure on uracil uptake, ribonucleic acid synthesis, and growth of three obligately psychrophilic marine vibrios, *Vibrio alginolyticus* and *Escherichia coli*, p. 180–202. *In* R. R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
3. **Borek, E., and A. Ryan.** 1958. Studies on a mutant of *Escherichia coli* with unbalanced ribonucleic acid synthesis. II. The concomitance of ribonucleic acid synthesis with resumed protein synthesis. J. Bacteriol. **75**:72–76.
4. **Boylen, C. W., and J. C. Ensign.** 1970. Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical stage cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103**:578–587.
5. **Burton, K.** 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315–323.
6. **Dische, Z.** 1955. Color reactions of nucleic acid components. Nucleic Acids **1**:285–305.
7. **Gallant, J.** 1979. Stringent control in *Escherichia coli*. Annu. Rev. Genet. **13**:393–415.
8. **Gallant, J., and S. R. Suskind.** 1961. Relationship between thymineless death and ultraviolet inactivation in *Escherichia coli*. J. Bacteriol. **82**:187–194.
9. **Geesey, G. G., and R. Y. Morita.** 1979. Capture of arginine at low concentrations by a marine psychrophilic bacterium. Appl. Environ. Microbiol. **38**:1092–1097.
10. **Gronlund, A. F., and J. J. R. Campbell.** 1963. Nitrogenous substrates of endogenous respiration in *Pseudomonas aeruginosa*. J. Bacteriol. **86**:58–66.

11. **Holm-Hansen, O., and C. R. Booth.** 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. **11**:510–519.
12. **Jones, K. L., and M. E. Rhodes-Roberts.** 1981. The survival of marine bacteria under starvation conditions. J. Appl. Bacteriol. **50**:247–258.
13. **Kjelleberg, S., B. A. Humphrey, and K. C. Marshall.** 1982. Effect of interfaces on small, starved marine bacteria. Appl. Environ. Microbiol. **43**:1160–1165.
14. **Koch, A. L.** 1971. The adaptive responses of *Escherichia coli* to a feast or famine existence. Adv. Microb. Physiol. **6**:147–217.
15. **Koch, A. L.** 1979. Microbial growth in low concentration of nutrients, p. 261–279. *In* M. Shilo (ed.), Strategies of microbial life in extreme environments. Dahlem Konferenzen, Berlin.
16. **Law, J. H., and R. A. Slepecky.** 1961. Assay of poly-β-hydroxybutyric acid in connection with sporulation of *Bacillus megaterium*. J. Bacteriol. **82**:33–36.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
18. **Maaløe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis, p. 1–284. W. A. Benjamin, Inc., New York.
19. **Mejbaum, W. Z.** 1939. Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsaüre. Hoppe-Seyler's Z. Physiol. Chem. **258**:117–120.
20. **Menzel, D. W., and J. H. Ryther.** 1970. Distribution and cycling of organic matter in the oceans, p. 31–54. *In* D. W. Hood (ed.), Organic matter in natural water. Institute of Marine Science, College, Alaska.
21. **Morita, R. Y.** 1980. Low temperature, energy, survival, and time in microbial ecology, p. 323–324. *In* D. Schlessinger (ed.), Microbiology—1980. American Society for Microbiology, Washington, D.C.
22. **Nathan, C. F., B. A. Arrick, H. W. Murrays, N. M. DeSantis, and Z. A. Cohn.** 1981. Tumor cell anti-oxidant defenses: inhibition of the glutathione redox cycle enhances macrophage-mediated cytolysis. J. Exp. Med. **153**:766–782.
23. **Nelson, L. M., and D. Parkinson.** 1978. Effect of starvation on survival of three bacterial isolates from an arctic soil. Can. J. Microbiol. **24**:1460–1467.
24. **Novitsky, J. A., and R. Y. Morita.** 1976. Morphological characteristics of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. Appl. Environ. Microbiol. **32**:617–622.
25. **Novitsky, J. A., and R. Y. Morita.** 1977. Survival of a psychrophilic marine vibrio under long-term nutrient starvation. Appl. Environ. Microbiol. **33**:635–641.
26. **Novitsky, J. A., and R. Y. Morita.** 1978. Possible strategy for the survival of marine bacteria under starvation conditions. Mar. Biol. **48**:289–295.
27. **Owens, T. G., and F. D. King.** 1975. The measurement of respiratory electron-transport-system activity in marine zooplankton. Mar. Biol. **30**:27–36.
28. **Packard, T. T., M. L. Healy, and F. A. Richards.** 1971. Vertical distribution of the activity of the respiratory electron transport system in marine plankton. Limnol. Oceanogr. **16**:60–70.
29. **Postgate, J. R., and J. R. Hunter.** 1962. The survival of starved bacteria. J. Gen. Microbiol. **29**:233–263.
30. **Reed, D. J., J. R. Babdson, P. W. Beatty, A. E. Brodie, W. W. Ellis, and D. W. Potter.** 1980. HPLC analysis of nanomole levels of glutathione disulfide and related thiols and disulfides. Anal. Biochem. **106**:55–62.
31. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
32. **Schaechter, M.** 1961. Patterns of cellular control during unbalanced growth. Cole Spring Harbor Symp. Quant. Biol. **26**:53–62.
33. **Stevenson, L. H.** 1978. A case for bacterial dormancy in aquatic systems. Microb. Ecol. **4**:127–133.
34. **Stouthamer, H. A.** 1973. A theoretical study of the amount of ATP required for synthesis of microbial cell material. Antonie van Leeuwenhoek J. Microbiol. Serol. **39**:545–565.
35. **Watson, S. W., T. J. Novitsky, H. L. Quinby, and F. W. Valois.** 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. **33**:940–946.
36. **Zimmermann, R., R. Iturriaga, and J. Becker-Birck.** 1978. Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol. **36**:926–935.
37. **Zimmermann, R. R., and L. A. Meyer-Reil.** 1974. A new method for fluorescence staining of bacterial populations on membrane filters. Kiel. Meeresforsch. **30**:24–27.