Heterotrophic Activity of Deep-Sea Sediment Bacteria

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Sediment samples, containing mixed microbial populations that were decompressed during retrieval from 7,750 and 8,130 m in the Puerto Rican Trench, were recompressed and incubated at the approximate in situ temperature (3 C) and pressure (775 or 815 atm) in the presence of 14C-labeled amino acids. Heterotrophic activity (total uptake, CO₂ respiration, and cellular assimilation) and cellular-associated "pool" concentrations were measured. Compared with atmospheric controls held at 3 C, the total uptake at elevated pressure at 3 C was reduced, on an average, 55 times, CO₂ respiration was reduced 45 times, and cellular assimilation was reduced 69 times. Rate of total uptake at elevated pressure was found to range from 4.0 × 10⁻¹⁰ μg/cell per h for leucine to 2.61 × 10⁻⁰ μg/cell per h for an amino acid mixture. Also, the percentage of total uptake at elevated pressures, respirized as CO₂, increased at the expense of cellular assimilation (ca. 22% increase). Two cellular-associated amino acid pools were detected, a large, loosely bound, outer pool and a small, tightly bound internal pool. The loosely bound outer pool was removed by a change in the pH of the incubation medium. Even though heterotrophic uptake and the outer, cellular-associated pool were markedly reduced at an elevated pressure, the percentage of total uptake calculated for the unincorporated, tightly bound, intracellular pool was 2 to 19 times that obtained for cultures held at 1 atm. The results were interpreted as indicating that bacterial metabolism and biosynthesis in the deep sea are markedly reduced, with a greater proportion of metabolic activity devoted to cellular maintenance.

Numerous studies of bacterial heterotrophic activity, in both pure and natural populations, have been reported (1, 5, 8–12, 14, 16, 22, 25, 26, 32, 34, 37–42). The majority of these investigations have employed shallow or surface-water inocula from the aquatic environment. In the sea, where phytoplankton are the primary producers in the food chain, bacteria are generally considered to be the decomposers of both dissolved and particulate matter (29). Bacteria utilize many complex molecules present in concentrations too dilute to be utilized by phytoplankton. In turn, decomposition carried out by bacteria results in the release of nutrients necessary for primary production. Approximately 1.6 × 10¹⁰ kg of carbon per year is fixed by phytoplankton in the ocean (29), an amount that, in general, is presumed to be balanced by decomposition. That is, it has been estimated that 90% of the carbon dioxide produced from organic matter is the result of bacterial respiration (29). Thus, the importance of bacteria in the various nutrient and energy cycles should not be underestimated.

The average depth of the world's oceans is 3,800 m (44) and the majority of the water mass, with its resident biological life, is a cold, dark region, subjected to elevated levels of hydrostatic pressure (ca. 380 atm at 3,800 m). Low temperature, combined with increased pressure, is known to be inhibitory for a variety of cellular activities associated with growth, maintenance, and reproduction, not only of all terrestrial bacterial species tested, but also of many marine bacteria (2, 3, 15, 18–21, 23, 26, 27, 30–32, 33, 35, 43–46).

Bacterial life below the euphotic zone has received little attention, with those investigators studying deep-sea microorganisms reporting low levels of activity (13, 14, 21, 44, 45). Generally, the low activity levels are a consequence of the environmental conditions since raising the temperature and lowering the pressure from that in situ results in "normal" rates of growth and metabolism (13, 14, 27, 32, 35, 44). Evaluations of deep-sea bacterial activities, in general, are based on static measurements, whereby initial and final values are measured (13, 14, 35, 44). These data give no indication of the rates associated with various cellular processes. Knowledge of rates (total uptake, respiration, incorporation, increase in biomass, etc.) is necessary if bacterial activities in the deep sea are to be properly assessed. Equally important,
the response of autochthonous populations to added nutrients should be investigated since waste disposal in the world’s oceans is on the increase. The ability of naturally occurring deep-sea bacteria to mineralize allochthonous substances may be limited, thereby necessitating a reevaluation of current ocean dumping practices. The goals of the study reported here were to investigate selected activities of mixed bacterial sediment populations, to determine limitations and mechanisms for growth and survival of microorganisms under simulated in situ conditions, and to assess the potential contribution of these microorganisms to nutrient dynamics of the sea.

MATERIALS AND METHODS

Cultures employed. Sediment samples were collected at two stations in the Puerto Rican Trench, at depths of 8,130 m (station 3; 19°36’N, 68°20’W) and 7,750 m (station 4; 19°28’N, 66°30’W), during R/V EASTWARD Cruise E-1G-74, March 1974. Sediment samples were collected with a gravity corer. The sediment samples underwent compression during retrieval since there is no sampler in existence that permits retrieval of aseptically collected sediment samples, and also maintains in situ pressure. The sediment collected when using the corer was light brown and fine grained in appearance. Sediment samples, containing mixed cultures of microorganisms, were aseptically removed from the uppermost 2 cm (water-sediment interface) of the cores immediately after retrieval. The sediment samples were diluted 1:10 (vol/vol) with cold (3 C) artificial sea-water containing (grams per liter): NaCl, 23.8; MgCl2.6H2O, 12.2; MgSO4.7H2O, 5.8; Na2SO4, 3.6; CaCl2, 2.4; KCl, 0.75; NaHCO3, 0.3; KBr, 0.15; andSrCl2.6H2O, 0.05. The core sediment temperature, measured immediately prior to dilution, was ca. 8 C.

14C-labeled amino acids. The sole source of added nutrient was in the form of uniformly labeled amino acids ([U-14C]-labeled l-lysine mixture; l-[U-14C]glutamic acid; l-[U-14C]alanine; and l-[U-14C]leucine obtained from New England Nuclear Corp, Boston, Mass.) Each of the four labeled substrates was made up to the same concentration (56 µg/ml; 100 µCi/ml) with the respective unlabeled d-lysine mixture (Nutritional Biochemicals Corp., Cleveland, Ohio) and added to the diluted sediment to yield a final concentration of 0.0224 µCi/ml (0.04 µCi/ml).

Experimental procedure. All work was performed aboard ship immediately after core retrieval either in the cold or with prechilled materials. After the addition of labeled substrate, 5-ml portions of the diluted sediment were drawn into sterile, plastic, disposable syringes (four syringes of each substrate) and placed into water-filled pressure reactors (High Pressure Equipment Co., Erie, Pa.) previously equilibrated at 3 C. Hydrostatic pressure, equivalent to that in situ (station 3, 815 atm; station 4, 775 atm), was applied to the reactors, which were then incubated at 3 C for indicated time intervals. Samples held at atmospheric pressure were treated identically but without pressurization and were incubated in closed, water-filled reactors at 3 C. Two sets of controls were included. Data from one control set were collected to correct for activity occurring prior to application of pressure. Measurements were terminated for this control when pressurized cultures reached the incubation pressure. A second control, in which the mixed culture was poisoned by the addition of 100 µg of HgCl2 per ml immediately prior to the addition of labeled substrate, was used to determine the extent of abiotic transformation and/or substrate loss. The control samples were treated similarly, with respect to the respective pressure or atmospheric control samples, for each incubation period. All pressure and control samples were run in duplicate. Incubation was at 3 C.

Determination of uptake and utilization. The assay procedure employed, in general, was the method of Hobbie and Crawford (11) as modified by Paul and Morita (26). Individual pressure reactors were decompressed at monthly sampling intervals. The pressurized samples, or atmospheric samples, were treated in one of two ways. To measure cellular respiration, i.e., 14CO2 production, the contents of duplicate syringes were injected through a rubber serum cap fitted on a 30-ml serum bottle containing 0.2 ml of 6 N H2SO4. A plastic bucket (Kontes Glass Co., no. K-882320-0000, Vineland, N. J.) containing a piece (2 by 6 cm) of fluted Whatman no. 1 filter paper was suspended from the serum cap over the liquid. At least 1 h after acidification of the sample, 0.3 ml of β-phenylethylamine was injected through the rubber cap onto the filter paper. The bottles were then slowly shaken for 1.5 h to allow the phenylethylamine-saturated filter paper sufficient time to absorb the evolved 14CO2. The filter paper was removed and placed in a liquid scintillation vial containing 10 ml of counting fluid (3a70; Research Products International, Elk Grove, Ill.) for subsequent determination of radioactivity.

The contents of each serum bottle were filtered (Millipore Corp., Bedford, Mass., 0.45-µm pore size), and the bottle was rinsed with 5 ml of cold artificial seawater. This procedure was repeated three times, with each rinse used to wash the filtered cells. The filters were dried at 70 C for 30 min and placed in a liquid scintillation vial containing 10 ml of Omnifluor solution (New England Nuclear Corp., Boston, Mass., 4 g of Omnifluor per liter of toluene). The filters were used to determine the extent of substrate incorporation into cellular material and entry into any intracellular pool. The filtrate was assayed for radioactivity by adding a 0.25 ml-volume to 10 ml of 3a70 counting fluid in a liquid scintillation vial.

For the determination of total material associated with the cells, the acidification step was omitted prior to filtering the duplicate samples. Acidification of the sample has been shown to reduce significantly the substrate retention of cells (4, 7, 8, 36). The difference in radioactivity between nonacidified and acidified samples was used as an estimation of a loosely bound amino acid pool (8), designated amino acid pool 1 (AAP-1) (7).
Total uptake of substrate, as cited in this report, is defined as the sum of nonacidified, filtered cells and the CO₂ respired.

Incorporation of labeled substrate into cold trichloroacetic acid-insoluble fraction was determined by acid extraction (4). The acid-soluble fraction (4) was used to estimate the small, tightly bound internal amino acid pool (AAP-2) (7). Portions (0.2 ml) of both the acid-insoluble and acid-soluble fractions were added to 10 ml of 3a70 counting fluid in a liquid scintillation vial for radioactivity determinations. Counting of the radioactively labeled fractions was performed in an Intertechnique model SL-40 liquid scintillation spectrometer (Teledyne Intertechnique, Westwood, N.J.). Quench corrections were made by the channel ratio method for filters and by the external standard ratio method for liquid samples. The recovery efficiency of the overall procedure averaged 93.4%.

Viable cells present as colony-forming units in the original sediment samples, pressurized cultures, and atmospheric control samples were determined by plating duplicate samples from each ¹⁴C-labeled substrate syringe on cold MSWYE agar (28). Incubation of the inoculated plates was for 4 weeks at 3°C.

RESULTS AND DISCUSSION

The mixed populations present in the original sediment samples, as enumerated on MSWYE agar, contained ca. 3.0 × 10⁵ and 1.8 × 10⁵ colony-forming units per ml (wet weight) of sediment at stations 3 and 4, respectively. During the course of incubation under pressure at 3°C, the total colony-forming units of the diluted sediment populations increased, the extent of increase being dependent upon the substrate added (Fig. 1a and b). As was expected, samples eliciting the greatest growth response to added

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**Fig. 1.** Growth of the mixed sediment culture at elevated pressure and 3°C. Amino acids were added to diluted sediment to yield a final concentration of 0.0224 μg/ml. (a) Station 3, incubation at 815 atm; (b) station 4, incubation at 775 atm.
nutrient (80% increase) were those receiving the 14C-labeled amino acid mixture. Samples to which individual amino acids, i.e., alanine, glutamic acid, and leucine, were added yielded smaller population increases (5 to 35%).

No substantial increase or decrease was noted for those experiments carried out at 1 atm at 3 C over a 6-h sampling period, i.e., colony-forming units per ml of diluted sediment remained at ca. 3.0 x 10^4 cells and ca. 1.8 x 10^4 cells for stations 3 and 4, respectively. Total uptake at 1 atm and 3 C (Fig. 2a and b) of each substrate was linear with time at the concentration employed (0.0224 μg/ml of diluted sediment). The three amino acids were taken up at different rates, indicating different substrate affinities of the binding proteins, the transport proteins, or of both. The total uptake of the individual amino acids at 1 atm and 3 C, relative to the amino acid mixture, varied from 31%

![Graphs showing uptake of amino acids][1]

**Fig. 2.** Total uptake of 14C-labeled amino acids at 3 C. Final concentration of added 14C-labeled amino acids was 0.0224 μg/ml. (a) Station 3, 1 atm; (b) station 4, 1 atm; (c) station 3, 815 atm; (d) station 4, 775 atm.
(leucine; station 3) to 83% (glutamic acid; station 4). One possible interpretation of these results is that there is competition among the components of the amino acid mixture for transport sites (5), each of which may not be specific for a particular amino acid but, rather, possess binding and/or transport proteins with variable amino acid affinities.

Total uptake studies at elevated pressure revealed rather interesting results (Fig. 2c and d). The rates of uptake were observed to be linear from the point of pressure application, indicating an immediate inhibition on some cellular processes. Furthermore, the four labeled substrates employed could be separated into two distinct groups, with respect to total uptake. Bacteria under elevated pressure appear to preferentially take up glutamic acid and individual amino acids in the mixture when compared with alanine and leucine. Also, the cells did not appear to favor any given substrate on the basis of carbon number since leucine contains six carbon atoms and glutamic acid contains five.

Total uptake at the two stations (ca. 110 miles [ca. 176.9 km] apart) was comparable (Table 1), indicating similar responses of the microorganisms in the deep-sea trench sediment samples to incubation under pressure. The actual rate of uptake per cell per hour (Table 1) was markedly lower under pressure than at 1 atm (32 to 90 times less; see Table 3). Alanine and leucine transport were observed to be most susceptible to inhibition by pressure.

Cellular respiration, a measure of substrate mineralization by microorganisms, was estimated by measuring evolution of $^{14}$CO$_2$ (11). Respiration was linear with time at 1 atm (Fig. 3a and b), and the sediment cultures responded to the four substrates similarly, but the rates of mineralization differed for each of the substrates. The percentage of $^{14}$CO$_2$ respired ($^{14}$CO$_2$ respired/total uptake, see Table 1) was high for glutamic acid (61%), moderate for alanine (41%) and the amino acid mixture (45%), and low for leucine (30%). When the sediment cultures were incubated at a temperature and pressure simulating in situ conditions, the percentage of $^{14}$CO$_2$ respired increased relative to 1 atm (Table 1), although the actual rate of substrate conversion to $^{14}$CO$_2$ decreased (Fig. 3c and d). Also, the ratio of respiration rate at 1 atm to the respiration rate at elevated pressure was lower than that calculated for total uptake (see Table 3), suggesting that respiratory enzymes may be more resistant to, or tolerant of, increased pressure (26).

Several investigators have reported cellular

| Station | 3° |
|---------|----|
| 1 | Amino acid mixture | 6.58 x 10^{-9} | 3.18 x 10^{-8} | 48.3 | 3.40 x 10^{-9} | 51.7 | 113 |
| Glutamic acid | 5.67 x 10^{-9} | 3.44 x 10^{-8} | 60.7 | 2.23 x 10^{-9} | 39.3 | 132 |
| Alanine | 3.68 x 10^{-9} | 1.80 x 10^{-8} | 43.3 | 2.08 x 10^{-9} | 56.7 | 203 |
| Leucine | 1.69 x 10^{-9} | 0.51 x 10^{-8} | 30.0 | 1.18 x 10^{-9} | 70.0 | 442 |
| 815 | Amino acid mixture | 1.71 x 10^{-10} | 0.98 x 10^{-10} | 57.6 | 0.73 x 10^{-10} | 42.4 | 4,366 |
| Glutamic acid | 1.77 x 10^{-10} | 1.44 x 10^{-10} | 81.5 | 0.33 x 10^{-10} | 18.5 | 4,218 |
| Alanine | 0.52 x 10^{-10} | 0.30 x 10^{-10} | 57.7 | 0.22 x 10^{-10} | 42.3 | 14,359 |
| Leucine | 0.40 x 10^{-10} | 0.13 x 10^{-10} | 32.5 | 0.27 x 10^{-10} | 67.5 | 18,567 |

| Station | 4° |
|---------|----|
| 1 | Amino acid mixture | 10.3 x 10^{-9} | 4.29 x 10^{-8} | 41.8 | 6.01 x 10^{-9} | 58.2 | 121 |
| Glutamic acid | 8.94 x 10^{-9} | 5.47 x 10^{-8} | 61.2 | 3.67 x 10^{-8} | 38.8 | 139 |
| Alanine | 7.07 x 10^{-9} | 2.80 x 10^{-8} | 39.1 | 4.27 x 10^{-8} | 60.4 | 176 |
| Leucine | 3.53 x 10^{-9} | 1.02 x 10^{-8} | 28.9 | 2.51 x 10^{-8} | 71.1 | 333 |
| 775 | Amino acid mixture | 2.61 x 10^{-10} | 1.17 x 10^{-10} | 44.8 | 1.44 x 10^{-10} | 55.2 | 4,768 |
| Glutamic acid | 2.11 x 10^{-10} | 1.59 x 10^{-10} | 75.3 | 0.52 x 10^{-10} | 24.7 | 5,899 |
| Alanine | 0.78 x 10^{-10} | 0.39 x 10^{-10} | 50.0 | 0.39 x 10^{-10} | 50.0 | 15,964 |
| Leucine | 0.44 x 10^{-10} | 0.18 x 10^{-10} | 29.3 | 0.31 x 10^{-10} | 70.7 | 28,283 |

* Expressed in micrograms.
* Expressed as percentage of total uptake.
* Calculated from: hours of incubation + fraction of available substrate taken up during incubation period.
* At 8,130 m, 3 C; 5.0 x 10^10 cells/ml.
* At 7,725 m, 3 C; 1.8 x 10^10 cells/ml.
assimilation of external substrates, i.e., substrate incorporated into cell material, or associated with the cell, or both, either extra- or intracellularly, using various methods (7, 8, 11, 37). Hobbie and Crawford (11), in correcting total uptake for CO₂ respiration, used an acidification technique to lower pH to <2, which terminates the incubation and results in the evolution of respired CO₂ that can subsequently be collected. This procedure, as any physical or chemical treatment altering the natural environment, removes a major portion of the amino acid pool (7, 8), resulting in an underestimation of cellular assimilation of external substrate. In the study reported here, a comparison of procedures was employed. The cellular fraction was measured with nonacidified cells that had been filtered and washed with unlabeled media immediately after incubation. Cellular assimilation of exogenous amino acids at both 1 atm and
at elevated pressure was, by this method, linear with time (Fig. 4a–d). The assimilation of given substrates reflected the increase in cell numbers shown in Fig. 1a and b. Cellular assimilation at the pressure simulating the in situ pressure was markedly reduced at elevated pressure (Tables 1 and 2), and the increased value obtained when calculating the ratio of the rate of 1 atm to the rate at elevated pressure for each substrate is interpreted to indicate that cellular assimilation is inhibited by pressure to a greater extent than is total uptake or mineralization (Table 3).

Several investigators have reported that various steps in protein synthesis are inhibited by pressure (2, 26, 27, 30, 31, 33, 35). It has also been demonstrated, as shown in this study, that uptake of amino acids by pure cultures of bacteria is markedly reduced when whole cells are subjected to increased hydrostatic pressure.
TABLE 2. Fate of 14C-labeled amino acids taken up and associated with the cellular fraction at 3 C and atmospheric and in situ pressures*  

| Atm | Substrate               | Cellular fractiona (acidified) | Incorporationb (TCA insoluble) | Total pool fractionc | Total pool/cellular fractiond | AAP-1e | AAP-2f | AAP-1/ AAP-2 |
|-----|-------------------------|-------------------------------|-------------------------------|----------------------|-------------------------------|--------|--------|-------------|
| 815 | Amino acid mixture      | 2.68 × 10⁻⁴                  | 2.61 × 10⁻⁴                  | 7.9 × 10⁻¹⁹         | 0.23                          | 7.2 × 10⁻¹⁹ | 7.25 × 10⁻¹¹ | 9.93        |
|     | Glutamic acid           | 6.11 × 10⁻¹⁹                | 5.6 × 10⁻¹⁹                  | 1.67 × 10⁻⁹         | 0.75                          | 1.62 × 10⁻⁹ | 5.1 × 10⁻¹¹  | 31.76       |
|     | Alanine                 | 2.82 × 10⁻¹⁰                | 1.9 × 10⁻¹⁰                  | 1.89 × 10⁻⁹         | 0.91                          | 1.8 × 10⁻⁹  | 9.22 × 10⁻¹¹ | 19.52       |
|     | Leucine                 | 1.54 × 10⁻¹⁰                | 1.3 × 10⁻¹⁰                  | 1.06 × 10⁻⁹         | 0.89                          | 1.03 × 10⁻⁹ | 2.38 × 10⁻¹¹ | 45.27       |
| 775 | Amino acid mixture      | 6.02 × 10⁻¹⁰                | 5.4 × 10⁻¹¹                  | 1.9 × 10⁻¹⁰         | 0.26                          | 1.28 × 10⁻¹⁰| 6.18 × 10⁻¹² | 2.07        |
|     | Glutamic acid           | 1.75 × 10⁻¹⁰                | 0.6 × 10⁻¹¹                  | 2.7 × 10⁻¹⁰         | 0.82                          | 1.55 × 10⁻¹⁰| 1.15 × 10⁻¹² | 1.35        |
|     | Alanine                 | 0.46 × 10⁻¹¹                | 0.3 × 10⁻¹¹                  | 1.9 × 10⁻¹⁰         | 0.86                          | 1.74 × 10⁻¹⁰| 1.6 × 10⁻¹²  | 10.88       |
|     | Leucine                 | 0.59 × 10⁻¹¹                | 0.4 × 10⁻¹¹                  | 2.3 × 10⁻¹⁰         | 0.85                          | 2.11 × 10⁻¹⁰| 1.88 × 10⁻¹² | 11.22       |

* Acid-terminated mixed cultures were used as a measure of incorporation into peptides and into AAP-2. AAP-2 was assayed by extracting the cells with cold 5% trichloroacetic acid and determining the radioactivity associated with the acid-soluble fractions. Incorporation into peptide was assayed by cold acid extraction and determination of the radioactivity associated with the acid-insoluble fraction. AAP-1 was the difference between nonacidified and acidified mixed cultures.

Total colony-forming units were 3.0 × 10⁹ and 1.8 × 10⁹ per ml at stations 3 and 4, respectively.

* Expressed as micrograms per cell per hour. TCA, Trichloroacetic acid.

* Cellular fraction from nonacidified cultures.

* At 8,130 m, 3 C.

** At 7,750 m, 3 C.

TABLE 3. Ratio of rate 1 atm/rate in situ pressure for various cellular activities and fractions  

| Atm | Substrate               | Total uptake | CO₂ respired | Cellular fractiona | Incorporation | AAP-1 | AAP-2 |
|-----|-------------------------|--------------|--------------|-------------------|---------------|-------|-------|
| 815 | Amino acid mixture      | 38.5         | 32.4         | 46.7              | 48.3          | 56.3  | 11.7  |
|     | Glutamic acid           | 32.0         | 23.9         | 67.6              | 93.3          | 104.5 | 4.4   |
|     | Alanine                 | 70.7         | 53.3         | 94.5              | 63.3          | 103.4 | 57.6  |
|     | Leucine                 | 42.3         | 39.2         | 43.7              | 32.5          | 46.6  | 12.6  |
|     | Avg                     | 45.9         | 37.2         | 63.1              | 59.4          | 78.3  | 21.6  |
| 775 | Amino acid mixture      | 39.5         | 36.7         | 41.7              | 40.6          | 58.8  | 17.6  |
|     | Glutamic acid           | 42.4         | 34.4         | 66.7              | 64.5          | 81.5  | 10.0  |
|     | Alanine                 | 90.6         | 71.8         | 109.5             | 107.5         | 116.5 | 61.7  |
|     | Leucine                 | 80.2         | 78.5         | 81.0              | 70.0          | 87.9  | 21.3  |
|     | Avg                     | 63.2         | 55.3         | 74.7              | 70.7          | 86.2  | 27.7  |

* Calculated from nonacidified culture data.

* At 8,130 m, 3 C.

* At 7,750 m, 3 C.
and low temperature (26). Paul and Morita (26) suggested that the observed decrease in amino acid transport may be a primary reason for the inability of cells to grow in the deep-ocean environment.

Binding proteins, involved in exogenous substrate capture, have been demonstrated to be present on the cell surface of bacteria (24). A free intracellular acid-soluble pool of amino acids exists in many gram-positive and gram-negative bacteria (4, 6, 8, 17, 36). Only recently, it was reported that AAP-1 and AAP-2 are present in a marine bacterium, *Vibrio marinus* MP-1 (7). The properties of AAP-1 are such that it is rapidly and easily removed by physical or chemical alterations in the external environment (lowered pH, osmotic shock), with no apparent damage to the viability of the cells. Our data suggest that AAP-1 is loosely bound to the outer surface of the cell.

AAP-2 is very resistant to removal, short of cell lysis, and appears to be located intracellularly. When AAP-1 is removed by acidification of cells previously incubated in the presence of $^{14}$C-labeled amino acids, the amount of radioactivity remaining corresponds very closely to the radioactivity present in the acid-precipitable fraction (incorporated). Cells incubated in the presence of $^{14}$C-labeled amino acids and not acidified prior to filtering provide a measure of the incorporated and cell-associated free (AAP-1) and bound (AAP-2) amino acids (8). When the radioactivity present in acidified cells is subtracted from the radioactivity determined for nonacidified cells, the easily removed cellular-associated pool, AAP-1, can be calculated. This pool was found to comprise 23 to 91% of the total radioactivity associated with the nonacidified cells (Table 2). Radioactivity remaining after incorporation into acid-precipitable material was subtracted from that for acidified cells, and the result is interpreted to be a measure of AAP-2. This pool, smaller than AAP-1, contains 2 to 34% of the labeled material assimilated by the nonacidified cells (Table 2). The ratio of AAP-1 to AAP-2 was 6.5 to 47.4 at 1 atm and 1.3 to 11.5 at elevated pressure (Table 2). The observed decrease in the ratio after application of pressure suggested that in relation to each other, AAP-1 decreased more or AAP-2 increased more. Since incorporation was the same at a given pressure for the substrates examined, a comparison of the change in ratio for each pool, relative to incorporation before and after pressure application, delineates the more sensitive process. The results indicate that the formation, or maintenance, of AAP-1 at elevated pressures was more susceptible to inhibition (Table 2). However, the results also suggest that, relative to incorporation, a greater fraction of the material taken up under pressure was intracellular and available for incorporation. Protein biosynthesis may, therefore, be a more immediate limiting factor for cell growth at in situ temperature and pressure.

It is obvious that bacterial processes in the deep sea are differentially inhibited (Tables 2 and 3). AAP-2 (intracellular pool) appears to be the least susceptible to pressure, indicating that an adequate pool of amino acids is available for levels of cellular metabolism and macromolecular biosynthesis required for maintenance or survival. Actual transport of amino acids into the cells was ruled out as the immediate limiting factor for cellular growth. Cellular respiration-respiratory enzymes apparently function adequately for the level of metabolism required for survival under deep-sea conditions, with a greater fraction of the material taken up being devoted to respiration under pressure, perhaps in response to the environmental stress imposed upon the cell. Morita and Buck (22) have shown that as the incubation temperature is lowered at 1 atm, the percentage of total uptake respired as CO$_2$ increases. Elevated pressure places an additional burden upon the cell by further limiting total uptake while increasing the percentage of CO$_2$ respiration. The cell requires more energy to overcome the environmental stress, but, in every instance observed, sufficient uptake to channel a portion of the energy available to the cell for growth and repair was available.

Incorporation into acid-insoluble material was found to be greatly inhibited by pressure, resulting in slowed bacterial growth and reproduction under conditions. If the comparison of the various rates of 1 atm to rates at elevated pressure is a suitable criterion, then the formation or integrity of the extracellular pool is more inhibited or disrupted by pressure than the internal pool and may cause the slower cell growth that was observed.

Binding proteins, present on or in the cell surface (24), bind and concentrate exogenous substrate for subsequent transport to the cell interior. These proteins are highly efficient since the most concentrated constituent in the amino acid mixture (glutamic acid) was $10^{-8}$ M. Whether these proteins also are the actual vehicle for the transport of material into the cell cannot be resolved in the present study. Presumably, different affinities of the cell for various amino acids is manifested in binding proteins, in transport proteins, or in both. Pres-
sure and low temperature may differentially lower these affinities by inhibiting protein conformational changes (allosteric or active site) that result in molecular volume increases, and, hence, result in inhibition by pressure (15). A minimum concentration of amino acids may be required for transport to occur and may not always be present in the natural environment. Furthermore, reduced substrate affinity under pressure may not be evenly distributed among the various amino acids, resulting in a limiting condition for growth, reproduction, or both. Thus, an increase in selectivity for a particular amino acid, such as glutamic acid (Fig. 2a–d), can occur. The cell will, thereby, be assured of a sufficient and readily utilizable supply of energy under environmental stress.

A comparison of AAP-1 with AAP-2 (Table 2) showed that, under pressure, AAP-2 increased relative to AAP-1 and to incorporation. Previous studies have shown that, when incorporation or utilization of a substrate is reduced by elevated pressure to 3 to 5% that of an atmospheric control, the amount of substrate present in the pressurized cultures intracellularly is two to eight times that found in the control cultures (30, 32). The site of pressure inhibition of cellular growth appears to reside in the transfer ribonucleic acid-ribosome-messenger complex (2, 27, 31). Thus, the internal amino acid concentration appears to be sufficient at elevated pressure, even though the content of AAP-1 is somewhat reduced (10 to 30%, Table 3) and the limiting factor for cellular growth is the translational mechanism.

The data accumulated to date support the hypothesis that AAP-1 serves as a storehouse, accumulating nutrient primarily for cellular maintenance and, thereafter, for growth and reproduction. The present indications are that bacteria in the deep-ocean sediment, under conditions of elevated pressure and low temperature, function very slowly with respect to terrestrial or surface-water conditions. The reduction in metabolism and biosynthesis observed in the sediment cultures held under pressure and reported here was reversible if the cultures were brought to ambient temperatures and pressures. Microorganisms adapt to their environment, pooling resources, albeit very slowly, when subjected to elevated pressures. The microorganisms, thus, may remain viable under those conditions that are routinely observed to exist in the deep sea.

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LITERATURE CITED

1. Andrews, P., and P. J. LeB. Williams. 1971. Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of the oxidation rates and concentrations of glucose and amino acids in seawater. J. Mar. Biol. Assoc. 51:111–125.

2. Arnold, R. M., and L. J. Albright. 1971. Hydrostatic pressure effects on the translation stages of protein synthesis in a cell-free system from Escherichia coli. Biochim. Biophys. Acta 265:347–354.

3. Baross, J. A., F. J. Hanus, and R. Y. Morita. 1974. 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.

4. Britten, R. J., and F. T. McClure. 1962. The amino acid pool in Escherichia coli. Bacteriol. Rev. 26:392–395.

5. Burnison, B. K., and R. Y. Morita. 1973. Competitive inhibition for amino acid uptake by the indigenous microflora of Upper Klamath Lake. Appl. Microbiol. 25:103–106.

6. Clark, V. L., D. E. Peterson, and R. W. Bernlohr. 1972. Changes in free amino acid production and intracellular amino acid pools of Bacillus licheniformis as a function of culture age and growth media. J. Bacteriol. 112:715–725.

7. Griffiths, R. P., J. A. Baross, F. J. Hanus, and R. Y. Morita. 1974. Some physical and chemical parameters affecting the formation and retention of glutamate pools in a marine psychrophilic organism. Z. Allg. Mikrobiol. 14:359–369.

8. Griffiths, R. P., F. J. Hanus, and R. Y. Morita. 1974. The effects of various water sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. Can. J. Microbiol. 20:1281–1296.

9. Hamilton, R. D., K. M. Morgan, and J. D. H. Strickland. 1966. The glucose uptake kinetics of some marine bacteria. Can. J. Microbiol. 12:995–1003.

10. Harrison, M. J., R. T. Wright, and R. Y. Morita. 1971. Method for measuring mineralization in lake sediments. Appl. Microbiol. 21:698–702.

11. Hobbie, J. E., and C. C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14:528–532.

12. Hobbie, J. E., and R. T. Wright. 1965. Bioassay with bacterial uptake kinetics: glucose in fresh water. Limnol. Oceanogr. 10:471–474.

13. Jannasch, H. W., K. Eimhjellen, C. O. Wirsen, and A. Farmanfarmaian. 1971. Microbial degradation of organic matter in the deep sea. Science 171:672–675.

14. Jannasch, H. W., and C. O. Wirsen. 1973. Deep-sea microorganisms: in situ response to nutrient enrichment. Science 180:641–643.

15. Johnson, F. H., F. Eyring, and M. J. Polissar. 1964. The kinetic basis of molecular biology, p. 258–368. John Wiley and Sons, New York.

16. Kadota, H., Y. Hata, and H. Miyoshi. 1966. A new method for estimating the mineralization activity in lake water and sediment. Mem. Res. Inst. Food Sci., Kyoto Univ. 27:28–30.

17. Kay, W. W., and A. P. Gronlund. 1969. Amino acid pool
formation in Pseudomonas aeruginosa. J. Bacteriol. 159:2682-2687.
33. Shen, J. C., and L. R. Berger. 1974. Measurement of active transport by bacteria at increased hydrostatic pressure, p. 173-179. In R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
34. Sieburth, J. McN. 1974. Distribution and activity of oceanic bacteria. Deep-Sea Res. 18:1111-1121.
35. Swartz, M. E., and J. R. Schwartz, and J. V. Landau. 1974. Comparative effects of pressure on protein and RNA synthesis in bacteria isolated from marine sediments, p. 145-159. In R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
36. Tempest, D. W., J. L. Meers, and C. M. Brown. 1970. Influence of environment on the content and composition of microbial free amino acid pools. J. Gen. Microbiol. 64:171-185.
37. Thompson, B. M., and R. D. Hamilton. 1973. Heterotrophic utilization of sucrose in an artificially enriched lake. J. Fish. Res. Board Can. 30:1547-1552.
38. Vaccaro, Ralph F. 1969. The response of the natural microbial populations in seawater to organic enrichment. Limnol. Oceanogr. 14:726-735.
39. Wiebe, W. J., and C. W. Hendricks. 1974. Distribution of heterotrophic bacteria in a transect of the Antarctic Ocean, p. 524-536. In R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
40. Wirsen, C. O., and H. W. Jannasch. 1974. Microbial transformations of some 14C-labeled substrates in coastal water and sediment. Microbial Ecol. 1:25-37.
41. Wright, R. T. 1974. Mineralization of organic solutes by heterotrophic bacteria, p. 549-565. In R. Colwell and R. Y. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
42. Wright, R. T., and J. E. Hobbie. 165. The uptake of organic solutes by planktonic bacteria and algae. Trans. Limnol. Oceanogr. Mar. Tech. Soc. 1:116-127.
43. Yayanos, A. A., and E. C. Pollard. 1969. A study of the effects of hydrostatic pressure on macromolecular synthesis in Escherichia coli. Biophys. J. 9:1464-1462.
44. ZoBell, C. E. 1968. Bacterial life in the deep sea. Bull. Mar. Sci. Jpn. 12:77-96.
45. ZoBell, C. E., and R. Y. Morita. 1957. Barophilic bacteria in some deep sea sediments. J. Bacteriol. 73:563-568.
46. ZoBell, C. E., and C. H. Oppenheimer. 1950. Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. J. Bacteriol. 60:771-781.