sponding to the least contaminated samples) we obtain a range of ages from 139,000 to 160,000 years B.P. Although this range lies within the statistical error limits of the individual determinations, it may in fact be real because each sample dated has a separate stratigraphic location and stalagmites of such dimensions usually require a period of at least this duration to develop (*17*).

The maximum estimated tectonic submergence (*12*) of this site in 150,000 years is about 3 m; therefore, sea level was at least 42 m below its present level during this period (Fig. 1). It is likely that a lowering of more than 42 m took place because there has been sufficient time for cave passages to develop and integrate and large speleothems to form. It is tempting to suggest that rising sea level at the end of the Illinoian glaciation may have terminated growth of these stalagmites, but unfortunately the younger (outer) layers of the stalagmites, which might demonstrate this effect, have been totally replaced by marine deposits. Neumann and Moore (*18*) have observed a high sea stand on Andros Island at +6 m above sea level, which correlates with the maximum sea level of the last interglacial (isotopic stage 5e, see Fig. 1). Correcting for tectonic submergence, sea level on the Bahamas platform must have risen at the end of the Illinoian at no less than 3.2 m per 1000 years. Further, deeper dives into these blue holes may allow us to establish a time scale for a large part of glacial sea-level lowering.

M. GASCOYNE
Department of Geology, McMaster University, Hamilton, Ontario L8S 4M1 Canada
G. J. BENJAMIN
247 Richmond Street, East, Toronto, Ontario M5A 1P2
H. P. SCHWARCZ
D. C. FORD
Department of Geology, McMaster University

References and Notes

1. H. H. Veeh, *J. Geophys. Res.* **71**, 3379 (1966).
2. W. S. Broecker and D. L. Thurber, *Science* **149**, 58 (1965).
3. T.-L. Ku, M. A. Kimmel, W. H. Easton, T. J. O'Neil, *ibid.* **183**, 959 (1974).
4. K. J. Mesolella, R. K. Matthews, W. S. Broecker, D. L. Thurber, *J. Geol.* **77**, 250 (1969); A. L. Bloom , W. S. Broecker, J. M. A. Chappell, R. K. Matthews, K. J. Mesolella, *Quat. Res. (N.Y.)* **4**, 185 (1974).
5. B. Heezen, M. Tharp, M. Ewing, *Geol. Soc. Am. Spec. Pap. 65* (1959).
6. W. L. Donn, W. R. Farrand, M. Ewing, *J. Geol.* **70**, 206 (1962).
7. J. Chappell, *Geol. Soc. Am. Bull.* **85**, 553 (1974).
8. N. J. Shackleton and N. D. Opdyke, *Quat. Res. (N.Y.)* **3**, 39 (1973).
9. R. S. Harmon, H. P. Schwarcz, D. C. Ford, *ibid.* **9**, 205 (1978).
10. R. F. Spalding and T. D. Mathews, *ibid.* **2**, 470 (1972).
11. G. J. Benjamin, *Natl. Geogr. Mag.* **138**, 347 (1970).
12. H. T. Mullins and G. W. Lynts, *Geol. Soc. Am. Bull.* **88**, 1447 (1977).
13. Speleothems are the familiar stalagmites and stalactites formed by the loss of carbon dioxide from groundwaters entering a cave, with the concurrent precipitation of calcium carbonate (usually as calcite). Speleothems consisting of clear detritus-free calcite can only have formed under vadose conditions from percolating freshwater.
14. J. McKenney, *Skin Diver* **21** (No. 3), 42 (1972).
15. D. Kobluk, personal communication.
16. M. Gascoyne, H. P. Schwarcz, D. C. Ford, *Br. Cave Res. Assoc. Trans.* **5** (No. 2), 91 (1978).
17. R. S. Harmon, P. Thompson, D. C. Ford, H. P. Schwarcz, *Bull. Natl. Speleol. Soc.* **37**, 21 (1975); the observed range of stalagmite growth rates is from 0.1 to 10 cm per 1000 years.
18. A. C. Neumann and W. S. Moore, *Quat. Res. (N.Y.)* **5**, 215 (1975).
19. Research supported by the National Research Council of Canada. Contribution 97 of the McMaster Isotope and Nuclear Studies Group.

21 December 1978; revised 27 February 1979

Isolation of a Deep-Sea Barophilic Bacterium and Some of Its Growth Characteristics

Abstract. *A bacterium, a spirillum, has been isolated from a deep-sea sample and has been found to grow optimally at about 500 bars and 2° to 4°C. These conditions are similar to those prevailing at the 5700-meter depth from which the sample was collected. The organism grows at these pressures and temperatures with a generation time of between 4 and 13 hours; at atmospheric pressure and 2° to 4°C, the generation time is about 3 to 4 days.*

Barophilic bacteria are those that "grow preferentially or exclusively at high hydrostatic pressures" [(*1*), p. 771]. ZoBell and Morita (*2*) have described some characteristics of an obligately barophilic bacterium that functioned slowly at 700 bars, and they have found other probably barophilic bacteria associated with deep-sea animals. Recent efforts to isolate barophilic bacteria, let alone obligately barophilic ones, have been less successful. Schwarz *et al.* (*3*) and Jannasch and his colleagues (*4*, *5*) have found only barotolerant bacteria. We report here the isolation and some growth characteristics of a barophilic deep-sea bacterium.

Amphipods (crustaceans) that had been retrieved alive (*6*) were maintained at deep-sea temperatures and pressures; after they died, deep-sea conditions of temperature (2° to 4°C) and pressure (580 bars) were maintained in the trap for 5 months. During that time autolytic and microbial processes led to the disintegration of the amphipod tissues. We then decompressed and opened the trap and found that it contained clear seawater overlaying a turbid suspension; an examination of this suspension with phase microscopy revealed the presence of bacteria. This suspension was used as the inoculum for the cultivation of bacteria. The deep-sea bacteria in this inoculum could have originated from the exterior of the dead animals (*7*), from the seawater, or from the gut of the amphipods (*8*).

We grew colonies of bacteria at high pressure in silica gel (*9*) containing nutrient medium. The silica-gel medium was inoculated at 2°C and atmospheric pressure, and it gelled within 10 minutes. Test tubes containing the gelled medium were sealed, placed in pressure vessels, and incubated at 570 atm and 2° to 4°C. After 3 weeks, the vessels were decompressed; four colonies were randomly selected and were used to inoculate more pour tubes. We examined these tubes after 5 days and found that they contained colonies. Such a rapid appearance of colonies suggested that the bacteria had a rapid doubling rate (*10*). One of these colonies was found to contain axenically a spirillum-like organism that did not grow into colonies in pour tubes incubated at atmospheric pressure for several weeks—the organism was apparently barophilic. The morphology of the cells is shown in Fig. 1. Cultures established from this colony served as the inocula for the experiments described below.

The data in Fig. 2 show the amount of growth (increase in cell numbers) observed in separate cultures that were begun with parts of the same starting culture and that were incubated at different pressures for the same amount of time (7 days). The curve shows that growth occurs at pressures between atmospheric pressure and somewhat above 825 bars. We calculate a maximum doubling time of 86 hours at atmospheric pressure. The optimum pressure for growth, about 500 bars, was not accurately determined by this experiment, probably because the incubation was too long (7 days). The growing cells were thus able to reach the concentration of 2×10^8 to 3×10^8 cells per milliliter, which is the maximum cell yield possible under these growth conditions. This pressure dependence has

0036-8075/79/0824-0808$00.50/0

been observed in four additional experiments which include growth in a mixed culture and which were done with the same protocol as in the first experiment but in the silica-gel medium. We determined the cell concentrations by microscopic counts. The optimum growth pressure in these experiments was between 425 and 500 bars at 2° to 4°C. The distribution of cell size was narrow in those cultures at 282, 480, and 518 bars but was broader in those cultures at 104, 656, and 725 bars.

We have observed doubling times of between 4 and 13 hours at a pressure of 580 bars, equivalent to that at the depth where the bacterium originated, in more than five experiments at 2° to 4°C. This generation time for the spirillum in pure culture is coincidentally about the same as the one deduced by Seki *et al.* (*11*) for his in situ experiments. A doubling time more rapid than this was observed in a single experiment with bacteria in mixed culture at 700 atm by Schwarz *et al.* (*12*). Isolates from this mixed culture, however, have been organisms with slower doubling times of about 24 hours at deep-sea conditions, and none have shown barophilic growth (*12*).

There is evidence (*13*) that the barophilic or barotolerant nature of some organisms can be lost during laboratory maintenance at atmospheric pressure. The bacterium CNPT-3 has not lost any of its barophilic characteristics after at least ten transfers but is maintained at 580 bars between transfers.

Jannasch and Wirsen (*4*) have argued that, since psychrophiles are sensitive to temperature increases, barophiles might be sensitive to decompressions. The barophilic spirillum that we have isolated is apparently not inactivated by decompression, although its apparent growth is considerably slowed (Fig. 2).

Some of our knowledge of how deep-sea microbes function is derived from studies of microbes that are isolated from deep-sea samples but that do not grow best under high pressures and low temperatures (*14*). The determination of which of these microbes are truly deep-sea inhabitants is a difficult question to answer because deep-sea temperatures and pressures are not necessarily lethally hostile to intruders from shallow waters (*15*) who could metabolize, albeit slowly, in the deep sea. It is also possible, as has been pointed out by Jannasch and Wirsen (*4*), that slow growth may be advantageous in the deep sea. Slow growth may be characteristic of some microbial communities (*4*, *5*), rapid growth of others (*3*). It remains to be shown whether

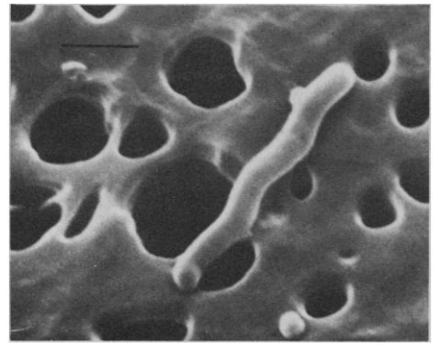

Fig. 1. The spirillum bacterium CNPT-3 as seen with the scanning electron microscope. The scale is 1 μm.

the isolate CNPT-3 will exhibit rapid growth when reintroduced into the deep sea or when placed in environments that simulate the deep sea not only in temperature and pressure but also in nutrient supply, sediment type, trace-element concentrations, microbial community structure, and many other variables.

Most of the work on the mechanisms of pressure effects on microbial cells has been carried out with relatively well studied microbes such as *Escherichia coli*, *Streptococcus faecalis*, and *Tetrahymena pyriformis* (*16*, *17*). Protein synthesis, energy metabolism, cell division, and other processes have been found to be pressure-sensitive, and each has been often investigated as the determinant of cellular pressure sensitivity.

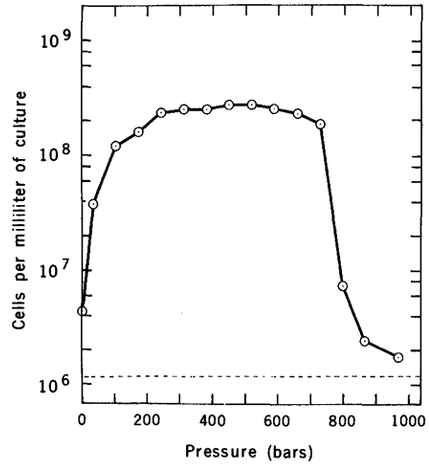

Fig. 2. The amount of growth at each of 15 different pressures in cultures begun with the same inoculum size (indicated by the dashed line). The incubations were for 7 days at the indicated pressures and at 2° to 4°C. The medium was a standard 2216 marine broth (Difco). The concentration of cells was determined with a Coulter counter (30-μm aperture tube and 100-μl manometer) (Coulter model B). We checked all samples for the presence of a monoculture of the spirillum, using phase microscopy.

All views may prove to be correct since barophilic cells may be shown to be like thermophilic cells, which show extensive (*18*) although subtle (*19*) molecular adaptations. Further work with the spirillum CNPT-3 should help to define essential changes in molecular architecture.

We feel that it is a significant breakthrough to be able to cultivate a rapidly growing barophilic deep-sea bacterium. The physiology, molecular biology, and radiation biology of this cell can now be studied. New products for bioengineering may emerge; for example, enzymes with unusual specificities may be found and certain barophilic cells may prove to be ideal vehicles for recombinant DNA-directed synthesis. The latter may be feasible if it can be established that certain barophilic species die at atmospheric pressure. They would then be evolutionally designed to be a contained system.

A. ARISTIDES YAYANOS
ALLAN S. DIETZ
R. VAN BOXTEL
Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093

References and Notes

1. C. E. ZoBell and C. H. Oppenheimer, *J. Bacteriol.* **60**, 771 (1950).
2. C. E. ZoBell and R. Y. Morita, *ibid.* **73**, 563 (1957).
3. J. R. Schwarz, A. A. Yayanos, R. R. Colwell, *Appl. Environ. Microbiol.* **31**, 46 (1976).
4. H. W. Jannasch and C. O. Wirsen, *Sci. Am.* **236**, 42 (June 1977).
5. _____, C. D. Taylor, *Appl. Environ. Microbiol.* **32**, 360 (1976).
6. A. A. Yayanos, *Science* **200**, 1056 (1978); *Rev. Sci. Instrum.* **48**, 786 (1976).
7. P. J. Boyle and R. Mitchell, *Science* **200**, 1157 (1978).
8. R. R. Hessler, C. L. Ingram, A. A. Yayanos, B. R. Burnett, *Deep-Sea Res.* **25**, 1029 (1978); A. A. Yayanos, *Biophys. J.* **16**, 180a (1976).
9. A. S. Dietz and A. A. Yayanos, *Appl. Environ. Microbiol.* **36**, 966 (1978). This silica-gel pour tube method has been the easiest way for us to obtain pure deep-sea cultures. Enrichments in liquid media have not been as productive as those in silica-gel pour tubes. The pour tube method, however, does not yet allow for the cultivation of all of the morphological types of bacteria present in an inoculum.
10. For exponential growth,
$$dN/dt = kN$$
where N is the number of cells at time t and k is the exponential growth constant. Therefore, the doubling (generation) time, g, is
$$g = \ln^2(\Delta t)/\ln(N/N_0)$$
where Δt is the time needed for the number of cells in a culture to increase from a value of N_0 to N. Consequently, if the colony originated from one cell and had 10^8 cells after 5 days, then g = 4.5 hours, indicative of a rapid doubling rate.
11. H. Seki, E. Wada, I. Koike, A. Hattori, *Mar. Biol.* **26**, 1 (1974).
12. J. R. Schwarz, personal communication.
13. D. H. Pope, W. P. Smith, M. A. Orgrinc, J. V. Landau, *Appl. Environ. Microbiol.* **31**, 1001 (1976).
14. E. U. Arcuri and H. L. Ehrlich, *ibid.* **33**, 282 (1977); see also the discussion in (*16*), pp. 227–228.
15. T. C. Malone, C. Garside, R. Anderson, O. A. Roels, *J. Phycol.* **9**, 482 (1973); J. W. Barthole-

mew and G. Paik, *J. Bacteriol.* **92**, 635 (1966). See also the data on the survival of five different bacteria under deep-sea conditions summarized in table 2 (p. 289) of (*17*).
16. R. E. Marquis, *Adv. Microb. Physiol.* **14**, 159 (1976).
17. R. Y. Morita, in *Survival of Vegetative Microbes*, T. R. G. Gray and J. R. Postgate, Eds. (Cambridge Univ. Press, Cambridge, England, 1976), pp. 279–298.
18. M. R. Heinrich, Ed., *Extreme Environments: Mechanisms of Microbial Adaptation* (Academic Press, New York, 1976).
19. R. Singleton, Jr., C. R. Middaugh, R. D. MacElroy, *Int. J. Pept. Protein Res.* **10**, 39 (1977); R. Singleton, Jr., and R. E. Amelunxen, *Bacteriol. Rev.* **37**, 320 (1973).
20. We are indebted to Prof. C. E. ZoBell for his loan of pressure vessels and for his comments on the manuscript. Supported by contract No. 07-7911 from Sandia Laboratories (a prime contractor of the U.S. Department of Energy) and by grant OCE 76-12017 from the National Science Foundation.

8 December 1978; revised 9 April 1979

Voltage-Dependent Calcium and Potassium Ion Conductances: A Contingency Mechanism for an Associative Learning Model

Abstract. *Persistent light-induced depolarization results from Ca^{2+} influx across a photoreceptor membrane. The marked dependence on potential of this Ca^{2+} influx and a Ca^{2+}-dependent K^+ efflux accounts for enhancement of the light-induced depolarization when light is paired with rotation. A positive feedback cycle between light-induced depolarization and synaptic depolarization due to stimulus pairing can explain long-lasting behavioral changes produced by associative training but not control paradigms. The sensitivity of this Ca^{2+} influx to intracellular levels of adenosine 3′,5′-monophosphate suggests biochemical steps for this model of associative learning.*

Learning, as it has been most generally defined, refers to a change of an animal's behavior as a result of training. Specific definitions of learning have been quite different, depending on the viewpoints and interests of individual investigators. Habituation, the decrement of a behavioral response with repetition, has been regarded by some as a simple form of learning. Others require in their definitions features of associative learning (*1*) such as pairing specificity or contingency, acquisition, retention, and stimulus specificity.

It cannot be assumed that these phenomena (habituation, sensitization, conditioning, and so forth) depend on common neurophysiologic and biochemical mechanisms. It is quite reasonable that the training regimens and neural networks important for a nonassociative process such as habituation involve cellular bases different from those that underlie associative learning.

One approach to the study of cellular mechanisms responsible for learning is to use relatively unevolved species that have fairly uncomplicated neural systems. Changes within these neural systems are sought as they relate to behavioral changes produced by training procedures.

An outstanding disadvantage of these species for this approach has been their rather limited capacity for learned behavior. Yet, for any possible generalizability of mechanisms uncovered in "simple" neural systems, the learned behavior should closely resemble that of higher organisms. Learning behavior of gastropod molluscs (which have "simple" neural systems) cannot be identically compared to the sophisticated learning (such as conditioning) of which primates are capable. However, enough might be found in common for learned behavior of lower organisms and more evolved species to direct our attention to common biologic principles.

In previous studies, I have found that movement of the Pacific nudibranch *Hermissenda crassicornis* toward a light source is markedly reduced after repeated pairing of a light stimulus with rotation (*2*). Crow and Alkon showed that this behavioral change is associative (that is, randomized light and rotation do not produce the same effect), persists for at least several days after training, and increases with practice (*2*). Specificity of this training effect was suggested by the fact that trained animals did not show changes in responsiveness to food (*3*). Thus, this behavioral change of *Hermissenda* shares defining features of, and can serve as a model for, associative learning (*3*).

Because of the relative simplicity of the *Hermissenda* nervous system it has been possible to ascertain many of the invariant aspects of the three sensory pathways essential to the associative learning model: the visual, statocyst, and chemosensory pathways (*4*). These three sensory pathways interact with each other, but are selectively responsive to the sensory stimuli (light, rotation, and food) used for the training and control procedures (*4, 5*). Within these neural systems of *Hermissenda* specific changes occurred only in animals subjected to associative training paradigms and not to control paradigms (*5, 6*).

A possibly primary neural change (which could account for the other changes within the networks) occurred within the type B photoreceptor (*6*). This involved, in part, persistent depolarization and increased membrane resistance of this cell. I describe here how this cell, through its receptor and membrane properties, in addition to its synaptic relationships, provides for the associative nature or contingency of the associative learning model. The sensitivity of this cellular mechanism for contingency to intracellular adenosine 3′,5′-monophosphate (cyclic AMP) also suggests biochemical mechanisms for the behavioral changes produced by the associative training procedure.

There are three type B (and two type A) photoreceptors in each of two *Hermissenda* eyes. These photoreceptors have identifiable loci, geometries, and electrophysiologic characteristics (*4*). The type B photoreceptor, located in the anterodorsal portion of the eye, is ~ 35 μm in diameter. Its axon, ~ 1 μm in diameter, leaves the base of the eye, enters the optic nerve, passes ensheathed through the optic ganglion (for ~ 50 μm), enters the optic tract, and terminates in a spray of fine endings ~ 20 μm from its point of entry into the cerebropleural ganglion. Simultaneous intraaxonal and intrasoma recordings (*4*) and lesion experiments indicate that the generator potential arises at the rhabdome of the cell body and spreads passively down the axon. Impulses arise within the axon near the cerebropleural ganglion point of entry but do not actively invade the cell body. Synaptic interactions only occur at the terminal endings.

Thus, a lesion (cut nerve) proximal to the impulse-generating zone leaves a cell body that contains the phototransduction apparatus without impulses or synaptic interactions. Voltage and current clamp recordings can then be made with two electrodes in the cell body under favorable space-clamp conditions (*7*).

Type B photoreceptors in cut nerve preparations depolarize for 1 to 2 minutes (Fig. 1A) after a 30-second light step of moderate intensity (10^3 to 10^4 erg cm^{-2} sec^{-1}). This long-lasting depolarization (LLD) was always associated with a membrane resistance 1.5 to 3 times higher than the resting or dark value (*8*). Positive current injection, causing depolarization comparable to that produced by light, was not followed by an LLD or increased membrane resistance. With sufficient hyperpolarization during the light

0036-8075/79/0824-0810$00.50/0