and 75 percent H_2O) and were not influenced significantly by immersion of the phantom in the H_2O simulating the chest wall. For the three peaks in Fig. 1, C and D, from H_2O/D_2O mixtures of 50, 25, and 75 percent H_2O, the average absolute deviation from the predetermined H_2O concentrations was 2.7 percent. When one of the tubes in Fig. 1A was filled with whole human blood, the NMR signal intensity was 82 percent of the signal from pure H_2O, indicating that 82 percent of the blood was H_2O. This result compares well with published confidence limits for H_2O in whole human blood of 83 to 86.5 percent (*9*).

Conventional NMR measurement of the total water content of an excised drying lung fragment was validated by weighing the fragment after each NMR measurement of the free induction decay (FID) following an excitation pulse (Fig. 2A). The extrapolated sample weight at zero NMR signal (dry weight) was determined from a least-squares regression to be 0.234 g, which was 98 percent of the gravimetric dry weight (0.248 g).

Regional lung edema, simulated by saline instillation, was detected by NMR in both the right lung of an isolated lung preparation (Fig. 2B) and the right lower lobe of an in situ lung (Fig. 2C). Good agreement was observed between the ratio of gravimetric water contents [(*A*)/(*B*)] and the ratio of NMR signal intensities [(*C*)/(*D*)] of the normal and saline-filled lungs (both the isolated right lung and the in situ right lower lobe) (Table 1).

These results provide evidence of the power of NMR imaging as a technique for performing quantitative studies of lung water. It is a noninvasive, perfusion- and ventilation-independent, technique that shows promise of becoming the standard method for in vivo measurement of the absolute amount of lung water and its distribution.

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10. Supported by grant 2 R01 HL 23746-03 from the National Institutes of Health.

22 September 1981; revised 26 January 1982

Deep-Sea Bacteria: Isolation in the Absence of Decompression

Abstract. *Sampling and pure culture isolation of deep-sea bacteria without loss of in situ pressure is required in order to determine the viability of decompression-sensitive strains. This was achieved by using a pressure-retaining sterilizable seawater sampling system in connection with a prepressurized hyperbaric isolation chamber. Rates of growth and substrate uptake of the majority of isolates showed highly barotolerant characteristics, while the remainder (4 out of 15) exhibited barophilic characteristics.*

During the last decade, deep-sea microbiology advanced in three areas, namely, the development of pressure-retaining sampling equipment (*1*, *2*), in situ studies with the research submersible *Alvin* (*3*), and the isolation of specifically pressure-adapted, barophilic bacteria (*4*). This work has led to a general understanding of the fact that the microbial decomposition and remineralization of natural and man-made materials on 60 percent of the globe's surface, covered by seawater of a depth of 1000 m or more, are strongly influenced by conditions characteristic of the deep sea. These are primarily the generally low concentrations of metabolizable organic substrates, uniformly low temperatures of 2° to 3°C, and hydrostatic pressure which increases approximately 1 atm for every 10 m of depth (*5*).

Microbial adaptations to life at extremely low nutrient levels (*6*) and low temperatures (*7*) have been well studied. Cold-adapted, psychrophilic bacteria, growing optimally at about 8° to 15°C, do not survive temperatures in the range of 20° to 25°C. In response to pressure,

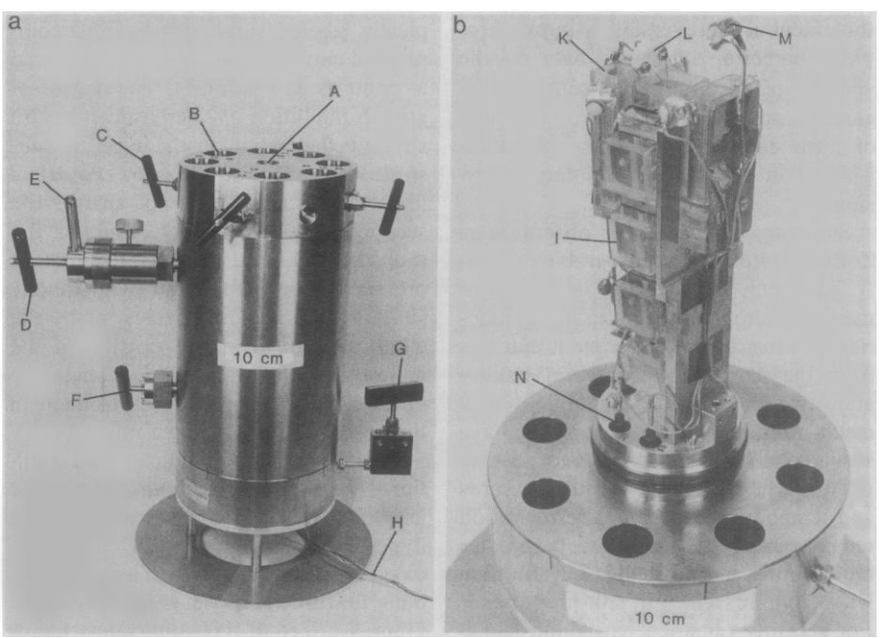

Fig. 1. (a) Isolation chamber assembled. *A*, viewing window; *B*, reset end cap bolts; *C*, valves for sample transfer into four vials; *D*, handle for horizontal movement of streaking loop; *E*, lever for rotary movement of streaking loop; *F*, handle for movement of agar plates; *G*, valve for pressurization; and *H*, wires for illumination and loop sterilization. (b) Bottom end cap with inner parts of isolation chamber (top removed); *I*, agar plates (nine); *K*, vials for liquid medium (four); *L*, ceramic loop holder with streaking loop; *M*, halogen lamps (five); and *N*, wire penetrators.

0036-8075/82/0618-1315$01.00/0

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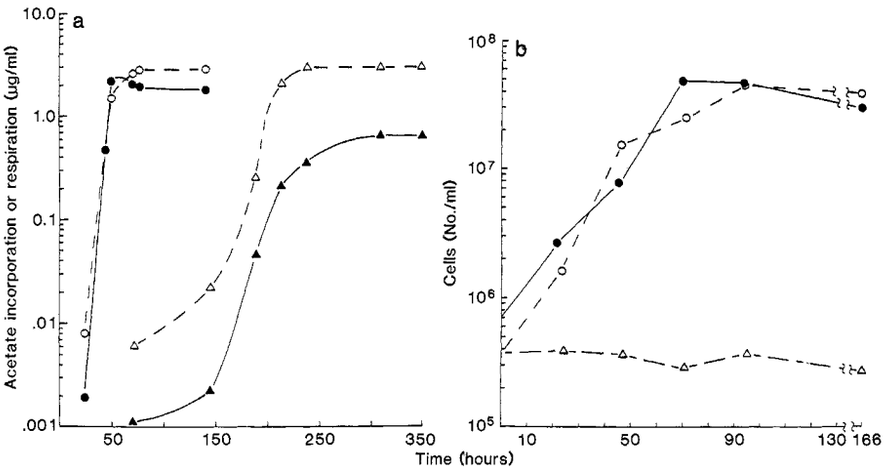

Fig. 2. (a) Incorporation (closed symbols) and respiration (open symbols) of labeled carbon from ^{14}C-labeled acetate by a deep-sea bacterium (strain C-5, isolated in the absence of decompression) at in situ pressure of 400 atm (triangles) and at 1 atm (circles), and (for both) in situ temperature of 3°C. The initial concentration of sodium acetate was 5.16 μg/ml in a mineral seawater medium (KH_2PO_4, 4 μg/ml; $(NH_4)_2SO_4$, 40 μg/ml). (b) Growth of a psychrophilic deep-sea bacterium (strain B-3, isolated in the absence of decompression) in a peptone-yeast extract medium (Difco 2216) measured by microscopic epifluorescent counting at in situ temperature of 3°C and at three pressures (○, 1 atm; ●, 400 atm; and △, 500 atm).

organisms are either barotolerant (that is, tolerating elevated pressures up to given limits) or barophilic (that is, growing optimally at pressures above 1 atm). Obligately barophilic organisms grow at elevated pressures only. Since the barophilic bacteria thus far studied have survived recovery from the deep sea (*4*, *8*), it is generally believed that decompression has no lethal or irreversible damaging effect. However, in contrast to studies on thermal growth inactivation and the extent of its reversibility in psychrophilic bacteria, analogous data on the viability of decompression-sensitive organisms are not available for the lack of pure cultures of deep-sea bacteria isolated in the absence of decompression.

Summing up his earlier observations ZoBell (*9*) stated: "Considerable difficulty has been experienced in trying to maintain barophilic bacteria in the laboratory. Most of the enrichment cultures from the deep sea lose their viability after two or three transplants to new media, although some barophiles have survived in sediment samples for several months when stored at in situ pressures and temperatures. Even at refrigeration temperatures, the deep-sea barophiles die off much more rapidly at 1 atm than when compressed to in situ pressures." For recently obtained barophilic bacteria, which survived decompression, growth at 1 atm was either reduced by 90 percent or, in an obligately barophilic isolate, completely inhibited (*4*). Probable physiological and molecular bases for the loss of barotolerance and barophilism upon decompression have been related to effects on protein synthesis, replication and membrane transport (*10*).

In work with deep-sea bacteria two questions remain: does decompression of deep-sea water or sediment samples have a selective effect on the natural population of microorganisms, and does it prevent the isolation of some highly pressure adapted organisms? An attempt to answer the former has been made by measuring transformation rates of isotopically labeled substrates by undecompressed natural populations as compared to controls at 1 atm (*11*). The degree of growth inhibition by increased pressure strongly depended on the type of substrate used. This result is of ecological importance, but it does not answer the second question, namely, whether the rate changes observed as an effect of pressure are brought about by metabolic shifts in one or more species, or by the elimination of an entire portion of the natural population. Again, conclusive proof requires pure cultures isolated in the absence of decompression.

Data of Taylor (*12*) indicate that traditional agar plating techniques may be used for the isolation of bacteria in a hyperbaric chamber pressurized with an oxygen-helium mixture. The chamber built for this purpose (Fig. 1) can be prepressurized to 1000 atm (safety factor of 4). It has been used so far at pressures between 350 and 600 atm with a final pO_2 of 0.2 atm (*12*). Deep-sea samples are obtained by an existing pressure-retaining sampler (*2*) that, during in situ filling concentrates 3 liters of seawater over a Nuclepore filter (pore size, 0.2 μm) to a final volume of 13 ml. During retrieval a temperature increase of up to 6°C over ambient, dependent on depth of thermocline and surface temperature, may occur for a period of 5 to 10 minutes. Transfer units (*2*) are used to introduce the undecompressed sample into one of four 8-ml vials housed inside the prepressurized isolation chamber. A rotating metal belt for the manipulation of nine agar-filled plates, the sterilizable platimum loop for streaking, and five halogen lamps for illumination, are all operated from the outside. The loop is sterilized electrically and dipped into the sample-containing vial, and the inoculum is streaked on one or more of the agar plates positioned under the viewing window. Transfer of cultures from one plate to another is done in the same manner. When a pure culture is attained, the isolated colony is transferred into one of the three remaining vials containing sterile nutrient media. After growth, the liquid culture is removed by a transfer unit and introduced into a prepressurized 1-liter growth chamber (*2*). Thus original deep-sea conditions of pressure (continuously monitored) and temperature (3° ± 0.5°C) are maintained throughout the entire transfer, purification, and cultivation procedures. As controls, samples of liquid pure cultures are decompressed and inoculated into fresh media maintained at 1 atm and 3°C.

Figure 2 shows data on the metabolic activity of two representative bacterial isolates from water samples collected on the Bermuda Rise (33°45'N, 57°40'W) from a depth of 4000 m. The metabolism of ^{14}C-labeled acetate (strain C-5) at a relatively low concentration in seawater media is clearly affected by the in situ pressure of 400 atm relative to the 1-atm control (Fig. 2a). This is reflected predominantly by a substantial lag phase and by a greater proportion of the substrate being respired to CO_2 than incorporated into cell material. The isolation and growth of another psychrophilic isolate (strain B-3) was conducted in a rich peptone yeast extract medium (Fig. 2b). Growth as determined by direct cell counts was not affected at the pressure of its sampling depth as compared to the 1-atm control, but was completely inhibited at 500 atm.

Colonies of barotolerant and barophilic bacteria cannot be distinguished in the isolation chamber. Therefore, the selection of colonies for transfer into liquid culture is arbitrary. Both of the isolates presented in Fig. 2 grew as well or better at 1 atm than at elevated pressure and must be defined as barotoler-

ant. They are representative of 11 out of 15 isolates thus far obtained. The other four isolates showed barophilic growth characteristics in a pressure range of 300 to 500 atm.

The barophilic isolates described earlier (*4, 13*) were obtained from decomposing deep-sea amphipods and from invertebrate intestines, that is, from nutrient-rich niches. While we have also recently been able to isolate baro- and psychrophilic strains from these niches as well as from decompressed deep-sea water, most organisms in these samples appeared to be of the same type as those thus far obtained with the isolation chamber, namely, psychrophilic and highly barotolerant.

An explanation for the distribution of more or less temperature- and pressure-adapted bacteria in the deep sea might be found in accumulating data on the considerable particle flux from surface waters to the deep sea and observations of bacterial attachments to these particles, such as organic detritus and fecal pellets (*14*). Most of the deep-sea bacteria in the sediments and water column may thus represent relatively recent arrivals. Their metabolic rates are affected by the decreasing temperature and increasing pressure but, as comparative data from undecompressed and decompressed natural deep-sea populations show (*11*), at least some bacteria recover immediately and exhibit maximum activity at 1 atm. While these surface-originated bacteria are favored by decompression, some barophilic organisms may be irreversibly affected by a decrease of pressure as many psychrophilic bacteria are affected by an increase of temperature. This approach of sampling and isolating microorganisms in the absence of decompression may provide answers to questions concerning the viability of decompression-sensitive deep-sea bacteria and will furnish undecompressed pure cultures for studies of pressure effects on the physiological and molecular level.

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21 January 1982

Lyme Disease—A Tick-Borne Spirochetosis?

Abstract. *A treponema-like spirochete was detected in and isolated from adult* Ixodes dammini, *the incriminated tick vector of Lyme disease. Causally related to the spirochetes may be long-lasting cutaneous lesions that appeared on New Zealand White rabbits 10 to 12 weeks after infected ticks fed on them. Samples of serum from patients with Lyme disease were shown by indirect immunofluorescence to contain antibodies to this agent. It is suggested that the newly discovered spirochete is involved in the etiology of Lyme disease.*

Lyme disease is an epidemic inflammatory disorder that usually begins with a skin lesion called erythema chronicum migrans (ECM). Weeks to months later the lesion may be followed by neurologic or cardiac abnormalities, migratory polyarthritis, intermittent attacks of oligoarticular arthritis, or chronic arthritis in the knees (*1*).

Although in the United States cases of ECM were first reported from Wisconsin (*2*) and southeastern Connecticut (*3*), Lyme disease as a new form of inflammatory arthritis was first recognized in 1975 in Lyme, Connecticut (*4*). It has since been reported from other northeastern, midwestern, and western states (*5*).

Epidemiologic evidence suggests that Lyme disease is caused by an infectious agent transmitted by ticks of the genus *Ixodes*. In the Northeast and Midwest *Ixodes dammini* and, in the West, *I. pacificus* have been incriminated as potential vectors (*6, 7*). Until recently, all attempts to isolate the causative agent either from ticks or from patients were unsuccessful.

Recently we isolated from *I. dammini* a spirochete that binds immunoglobulins of patients convalescing from Lyme disease. We also recorded the development of lesions resembling ECM in New Zealand White rabbits on which ticks harboring this spirochete had fed.

Adult *I. dammini* were collected in late September and early October 1981 by flagging lower vegetation on Shelter Island, New York—a known endemic focus of Lyme disease (*8*). Of 126 such ticks that were dissected, 77 (61 percent; 65 males and 12 females) contained spirochetes. The spirochetes were distributed mainly in the midgut but were occasionally also seen in the hindgut and rectal ampule. No other tissues, including the salivary glands, contained spirochetes. The organisms stained moderately well with Giemsa (Fig. 1); in wet preparations examined by dark-field mi-

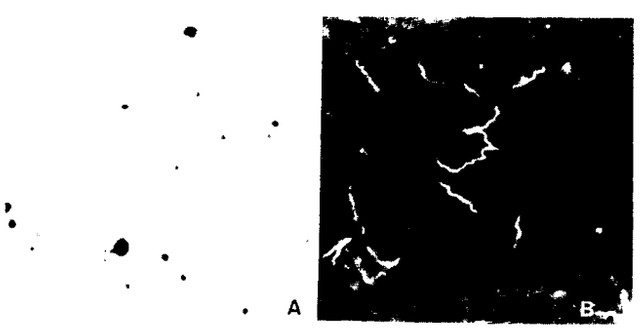

Fig. 1. *Ixodes dammini* spirochetes in midgut tissues of its tick vector. (A) Giemsa staining (×1200). (B) Serum of patient J.G. examined by indirect immunofluorescence (×570).

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