DEEP-SEA BACTERIA

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SUMMARY[1]

The presence of living bacteria in some of the deepest parts of the ocean was demonstrated on the Galathea Expedition. Sediment samples taken from the bottom of the Philippine Trench at depths exceeding 10 000 meters were found to contain from 10^4 to 10^6 living bacteria per ml. Large bacterial populations were also detected in sediment samples from the floor of the Kermadec-Tonga Trench (6790 to 9820 meters), the Sunda Deep (7020 meters) in the Java Trough, and the Weber Deep (7250 meters) in the Banda Sea. Several hundred microbial analyses were made on nine sediment samples taken from depths exceeding 10 000 meters.

The occurrence of bacteria in deep-sea sediment samples was demonstrated by direct microscopic examinations shortly after the samples were hauled aboard the Galathea. Growth or reproduction of the bacteria in nutrient sea water media proved that the bacteria were alive. Their indigenity was indicated by three lines of evidence: (1) Every possible precaution was exercized to prevent the contamination of the deep-sea samples, (2) More bacteria were found in the sediment samples than in the overlying water, and (3) The deep-sea bacteria were unique in their ability to grow preferentially or exclusively at *in situ* hydrostatic pressures (700 to 1 000 atmospheres) and low temperatures (3 to 5°C).

Apparatus is described which was designed to maintain sediment samples and bacterial cultures at any desired pressure in the ship's refrigerator. This apparatus made it possible to send by Air Express deep-sea sediment samples held at 1 000 atm to our laboratory at Scripps Institution for further observation.

The deep-sea bacteria appear to be even more heat-sensitive than pressure-sensitive. Many were killed by holding them for ten minutes at 30°C. Less than 20 per cent of the deep-sea bacteria survived for ten minutes at 40°C.

The unique pressure, temperature, and nutrient requirements of the deep-sea bacteria presents an enigma in bacterial taxonomy, because the bacteria cannot be characterized by conventional or standard method cultural procedures. Morphologically the deep-sea bacteria resemble common soil and water forms, but physiologically and culturally they show differences which suggest that most deep-sea bacteria are new and undescribed species or genera.

Though predominantly aerobic, some of the deep-sea bacteria develop anaerobically. Among the physiological types that were present and active at *in situ* pressures and temperatures were starch hydrolyzers, nitrate reducers, sulfate reducers, ammonifiers, and nitrifiers. The types of bacteria present as well as the organic and sulfate content of profile series of mud cores from the Kermadec-Konga Trench suggests that the bacteria have been active *in situ* in altering organic compounds and in reducing sulfate.

Besides affecting the non-conservative chemical composition of sea water and sediment, the deep-sea bacteria are believed to contribute to the nutrition of benthic fauna. The "standing crop" of organic carbon in the cells of living bacteria is estimated at from 0.2 to 2.0 mg per liter of sediment at the mud-water interface. There may be from 10 to 100 "crops" or bacterial generations per year. The bacteria are believed to obtain their carbon and energy requirements from organic detritus settling to the sea floor and to a much larger extent from dissolved or colloidal organic compounds carried by the movements of water masses.

1. Contribution from the Scripps Institution of Oceanography, University of California, La Jolla, California, U.S.A., New Series No. 926. These investigations were supported in part by grants from the Office of Naval Research, U.S.A. Department of Navy, and the Rockefeller Foundation.

INTRODUCTION

Observations on the Galathea Expedition in 1951 demonstrated for the first time the occurrence of living bacteria in some of the deepest parts of the ocean. Prior to this time, 5942 meters was the greatest depth at which bacteria had been found. Most microbiologists questioned whether bacteria could exist in oceanic abysses, although an abundant microflora has been shown to occur in near-shore sediments (ZoBELL, 1946).

On the Talisman Expedition, CERTES (1884a) found a few bacteria in bottom deposits taken from a depth of 5100 meters. The indigenous nature of these bacteria was indicated by their ability to grow in nutrient medium at hydrostatic pressures that were approximately isobaric with the depth from which they were taken (CERTES, 1884b). While on the Humboldt Plankton Expedition to the West Indies, FISCHER (1894) found a few bacterial colonies on nutrient agar plates inoculated with deep-sea sediment samples, one of which came from a depth of 5280 meters, but there was nothing to indicate whether these bacteria were indigenous or adventitious species.

In 1948, we obtained from a 5800-meter deep off Bermuda a mud sample in which numerous barophilic bacteria were found. The term "barophilic" was coined by ZoBELL and JOHNSON (1949) to characterize microbes which grow preferentially or exclusively at high hydrostatic pressures. During the 1950 Mid-Pacific Expedition to the Marshall Islands, large bacterial populations were demonstrated in pelagic sediments, including 14 samples taken from depths exceeding 4000 meters; one of these samples containing bacteria came from a depth of 5942 meters (MORITA and ZoBELL, 1955).

Cultural methods, supplemented by direct microscopic observations, were employed to investigate the numbers and kinds of bacteria in marine materials collected by the Galathea. Immediately after being hauled aboard the ship, deep-sea sediment and water samples were transferred aseptically to steel vessels for compression to hydrostatic pressures approximately isobaric with the depth from which taken, and placed in a refrigerator at 3-5°C. This operation ordinarily required from 5 to 15 minutes. Portions of each sample were removed for detailed examination as time and facilities permitted.

Temperature Tolerance of Deep-Sea Bacteria.

Haste in handling the deep-sea samples proved to be of utmost importance owing to the temperature sensitivity of the bacteria. About 25 per cent of the marine bacteria collected off the coast of California by ZoBELL and CONN (1940) were killed in 10 minutes when warmed to 30°C and only 20 per cent survived for 10 minutes at 40°C. Deep-sea bacteria have proved to be even more sensitive to heat than those from shoal waters.

The temperature of most of the deep-sea samples ranged from 5° to 10°C when hauled aboard the Galathea, having warmed up from about 3°C while being brought to the surface. The air temperature in the tropics, like that of the surface water, ranged from 28° to 30°C. Room temperature in the Galathea laboratory was usually a few degrees higher, and the temperature in the stateroom used as a microbiological laboratory often exceeded 40°C. Consequently we were working near or beyond the threshold of temperature tolerance of many marine microbes.

In spite of haste in handling materials and precautionary measures designed to keep them cool, many of the more sensitive organisms were probably killed by the heat. Lacking a cold room in which to make the necessary transfers involving minute amounts of inocula (often only 0.01 ml), part of the material got warmed up to near laboratory temperature during the measuring, transfer, dilution, and inoculation of the bacteria therein. Fortunately, a good many of the deep-sea bacteria did survive the extreme change in climate to which they were subjected during sampling and analytical operations, but we do not know how many may have perished. Data summarized in Table I illustrate the loss of viability of bacteria in deep-sea samples intentionally subjected to different temperatures for 10 minutes prior to plating on nutrient agar.

Only a small percentage (2.3 to 4.7) of the bacteria survived heating to 50°C for 10 minutes and less than 20 per cent survived for 10 minutes at 40°C. Most of those that survived proved to be spore formers. From the data in Table I, it might appear that most of the deep-sea bacteria tolerated a temperature of 30°C for 10 minutes, but this may not be true because, even at "room" temperature, the samples were subjected for several seconds to temperatures between 30° and 40°C or higher. Perhaps all of

Table I. Relative numbers of bacteria in sediment samples from Philippine Trench which developed on nutrient agar after small portion of sample was held for 10 minutes at stated temperature. The results are expressed as percentages of the number of colonies which developed from portions of the same samples subjected to "room" temperature during rapid routine manipulations.

Station Number	Water depth	Rapid room	Held 10 minutes at		
			30° C	40° C	50° C
	meters	per cent	per cent	per cent	per cent
413	10 060	100	91.6	18.0	4.7
418	10 190	100	82.5	7.4	2.3
419	10 210	100	93.7	11.8	3.0
420	10 160	100	78.4	6.5	2.8

the more heat-sensitive microbes were lost. This should stress the importance of providing properly air-conditioned rooms for microbiological manipulations on subsequent deep-sea expeditions. Maintaining the organisms at *in situ* temperatures and pressures during their transposition from the deep-sea floor to the surface may also be a useful innovation.

Effect of Hydrostatic Pressure on Marine Microbes.

Although bacteria appear to require for growth or reproduction a hydrostatic pressure which is near that of their native environment, it seems likely that few if any are killed by the decompression resulting from their transposition from the deep-sea floor to the surface. Such decompression may actually be advantageous to the organisms, because the resultant adiabatic cooling (about 1.6°C from a depth of 10 000 meters to the surface) helps counteract warming during transposition through warmer surface water. Of course, microbes may be killed mechanically by the sudden release of pressure in a system supersaturated with gas, but in spite of the pressure, there is no more gas dissolved in deep-sea water than in surface water.

Prolonged storage of deep-sea sediment samples at refrigeration temperatures has resulted in the gradual death of the bacteria at atmospheric pressure. Very few living bacteria could be detected in sediment samples from the Philippine Trench (depth 10 000 meters) following six weeks' storage at 3° to 5°C at atmospheric pressure, although large numbers of barophilic bacteria have persisted for 30 months in similar samples maintained in the refrigerator at 1000 atm. Whether this die-off is directly attributable to reduced pressure or is an indirect effect on the metabolism and nutrition of the bacteria under the experimental conditions can be determined only by further investigations.

Working with freshly collected deep-sea material (less than an hour after it was hauled aboard the Galathea), we found many more bacteria developing in nutrient media incubated at *in situ* pressures than at atmospheric pressure. The barophilic property of deep-sea bacteria is illustrated by representative results with samples taken from the Philippine Trench (Table II).

Table II. Most probable numbers (MPN) of bacteria per gram wet weight demonstrated in sediment samples from the Philippine Trench by the minimum dilution method in nutrient medium incubated in refrigerator at different pressures.

Station Number	Location of station		Water depth	Incubation pressure	
	Latitude	Longitude		1 atm.	1000 atm.
	North	East	meters	MPN	MPN
413	10° 20′	126° 36′	10 060	2 300	760 000
418	10° 13′	126° 43′	10 190	930	3 500 000
419	10° 19′	126° 39′	10 210	680	210 000
420	10° 24′	126° 40′	10 160	8 400	920 000
421	10° 29′	126° 05′	1 000	540 000	0
422	10° 49′	126° 01′	1 960	2 300 000	0
424	10° 28′	126° 39′	10 120	5 900	2 800 000

The most probable numbers of bacteria were estimated by "Standard Methods for the Examination of Water and Sewage" (American Public Health Assoc., 1936), as amplified by HALVORSEN and ZIEGLER (1933) and by PRESCOTT *et al.* (1947). Methods of inoculating the nutrient medium and of applying pressure are outlined below.

From the data in Table II, it will be observed that from 10 to 100 times as many bacteria from a depth of about 10 000 meters grew in nutrient medium incubated at 1000 atm as the number which grew at 1 atm. We now know that the effect of hydrostatic pressure on bacteria is partly a function of the composition of the medium, suggesting that with proper nutrients there may be as many bacteria from the deep sea that would grow at 1 atm as at 1000 atm, but we have not yet learned what these proper nutrients might be.

Although data like those summarized in Table II indicate the existence of strict barophiles (high pressure dependent bacteria), as this progress report is

written we must admit that the pressure picture appears to be somewhat more complicated. Be this as it may, finding more bacteria in deep-sea sediments growing at *in situ* pressures (1000 atm) than at 1 atm is regarded as conclusive evidence that most, if not all, of the bacteria were indigenous to the deep and that they are physiologically active at high pressures. This conclusion is supported by finding in nearby shallow sediments from the sides of the Philippine Trench large numbers of bacteria which grew at 1 atm and none which grew at 1000 atm. This latter observation agrees with the findings of ZoBELL and JOHNSON (1949) and ZoBELL and OPPENHEIMER (1950), who reported that surface-dwelling microbes fail to grow at deep-sea pressures and many are slowly killed in nutrient media when held at pressures ranging from 400 to 600 atm. According to OPPENHEIMER and ZoBELL (1952), pressures ranging from 200 to 600 atm inhibited the normal growth of nearly all of 63 different species of marine bacteria, mostly from shoal water habitats. Five of these were killed in four days by compression to 200 atm, 11 were killed at 400 atm, and 23 were killed in four days at 600 atm.

Nutrient Media and Enumeration Procedures.

The nutrient medium employed routinely for demonstrating the most probable number of bacteria by the minimum dilution method had the following composition:

Peptone (Difco)	5.0 gm
Ferric phosphate	0.1 gm
Potassium nitrate	1.0 gm
Soluble starch	2.0 gm
Yeast extract (Difco)	1.0 gm
Sea Water	1000.0 ml

Following autoclave sterilization its pH was 7.7. It was dispensed in 9.0-ml quantities in 15-ml screw-cap bottles.

Into the first bottle of sterile medium in each series was introduced aseptically 1.0 gm of the sample. After vigorously shaking to distribute the sediment (or water) sample evenly throughout the medium, a 1.0-ml aliquot was transferred, with a semi-automatic pipette, to the second bottle of sterile medium. This gave a 1:10 dilution or provided a 0.1-gm inoculum. After similarly shaking this second bottle, 1.0 ml was removed to inoculate the third bottle of sterile medium to give a 1:100 dilution or a 0.01-gm inoculum. The dilution procedure was repeated seriatim by powers of 10 until the original sample was diluted to 1:1 000 000.

Assuming a random distribution of the bacteria in the sediment sample and throughout the dilution series, it follows that bacterial growth in the 1:10 000 and all lower dilutions but no growth in the 1:100 000 dilution, for example, would indicate the presence of at least 10 000 viable bacteria per gram of sample but not 100 000 per gram. The most probable number (MPN) of bacteria in certain samples was determined with greater accuracy by preparing quintuplets of each series. For example, finding growth in all 5 tubes of medium inoculated with the 1:10 series, 3 positive in the 1:100 series, and none of the 5 positive in the 1:1000 series would indicate a bacterial population (MPN) of 79 per gram.

For incubation at atmospheric pressure, the diluted material in the nutrient medium was left in the screw-cap bottles. These bottles provided about 5 ml of air space for aerobes. To estimate the abundance of anaerobic bacteria in the sediment samples, the inoculated bottles were filled to capacity with O_2-free medium and the caps screwed tightly in place to exclude atmospheric oxygen. Following incubation at the desired temperature and pressure, the medium was examined for evidence of bacterial growth as manifested by increased turbidity of the medium or by finding large numbers of bacteria by direct microscopic examinations.

Ammonia liberation from peptone by ammonifiers:

$$R\text{-}NH_2 + HOH \rightarrow R\text{-}OH + NH_3$$

was detected by nesslerizing 0.2 ml of medium in a spot plate.

The presence of nitrate-reducing bacteria was indicated by the appearance of nitrite or by the disappearance of nitrate in the inoculated nutrient medium. Iodine was added to aliquots placed in a spot plate to test for the terminal presence of starch; its absence indicated the activity of starch-hydrolyzing bacteria. Sulfate-reducing bacteria were demonstrated in medium M-10-E:

Calcium lactate	3.5 gm
Magnesium sulfate, hydrated	0.2 gm
Potassium phosphate, dibasic	0.2 gm
Sodium sulfite	0.1 gm
Ferrous ammonium sulfate	0.1 gm
Ascorbic acid	0.1 gm
Peptone (Difco)	1.0 gm
Yeast extract (Difco)	1.0 gm
Bacto-agar (Difco)	3.0 gm
Sea water	1000.0 ml

It was adjusted to pH 7.5 following autoclave sterilization. The medium was dispensed in 15-ml screw-cap bottles, which were filled to capacity to displace all air, since the sulfate reducers are strict anaerobes. Following inoculation by serial dilutions and incubation, the presence of sulfate reducers was indicated by the blackening of the medium, resulting from the formation of ferrous sulfide:

$$SO_4^{--} + 10H \rightarrow H_2S + 4H_2O$$
$$H_2S + FeSO_4 \rightarrow FeS + H_2SO_4$$

Sea water enriched with 0.1 per cent ammonium phosphate (dibasic) and buffered with 0.2 per cent calcium carbonate was used to detect the presence of nitrifying bacteria. Following inoculation and incubation at the desired temperature and pressure, the enriched sea water was examined for the appearance of nitrite:

$$NH_4^+ + 2O_2 \rightarrow NO_2^- + 2H_2O$$

Nitrifiers which oxidize ammonium to nitrite were demonstrated in deep-sea sediment samples only when the inoculated medium was incubated at high pressures.

Inoculated media or samples to be held at high pressures were transferred to small glass vials, size 10×50 mm. After filling to capacity (5 ml) each vial was closed with a tapered No. 000 Neoprene stopper. When subjected to pressure in a steel vessel filled with hydraulic fluid, the stopper functioned as a piston, compressing the contents of the vial to approximately the same pressure as was built up in the steel vessel. Numerous tests have established that the compressed Neoprene piston stoppers provide an effective seal against contamination of any kind, the higher the pressure the better the seal.

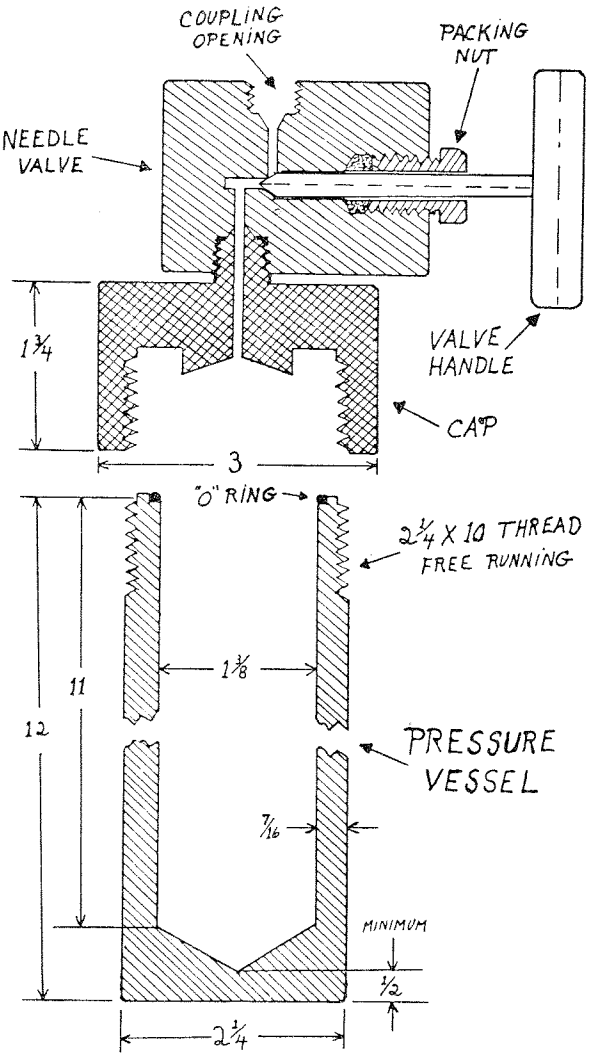

Fig. 1. Cross-section of stainless steel pressure vessel with its connecting cap and needle valve used for maintaining deep-sea sediment samples and bacterial cultures at high hydrostatic pressures. Dimensions are given in inches.

High Pressure Apparatus.

The pressure cylinders (Figure 1) were constructed of 18-8 type 303 stainless steel. Such a vessel having an inside diameter of 1-3/8 inches and an inside length of 11 inches holds 25 10×50-mm stoppered culture tubes and weighs only 6 kg. The vessels were fabricated by drilling a radially central hole, with tapered bottom, in 12-inch lengths of 2-1/4-inch steel bar stock. The caps were prepared by machining 3-inch lengths of 3-inch steel bar stock as illustrated. Machining also provided for a male-threaded top extension for the permanent attachment of a needle valve and a perforated cone-shaped ceiling for exhausting air from the vessel. When fitted with an "O" ring and the vessel is filled level full with hydraulic fluid, the latter is displaced through the cap and attached needle valve when the cap is screwed on the cylinder.

In practice the pressure vessel, equilibrated to the exact temperature at which it is to be incubated, is loaded with piston-stoppered culture tubes and filled level full with water at the same temperature. Hastily the cap is screwed on, an operation that can be performed by hand in a few seconds without the use of a wrench, and connected to the hydraulic pump (Figure 2). A few strokes with the handle of the pump produce a pressure up to 1000 atm in 20 to 30 seconds. After closing the needle valve, the pressurized vessel is disengaged from the pump for storage in the refrigerator or other thermostat. Con-

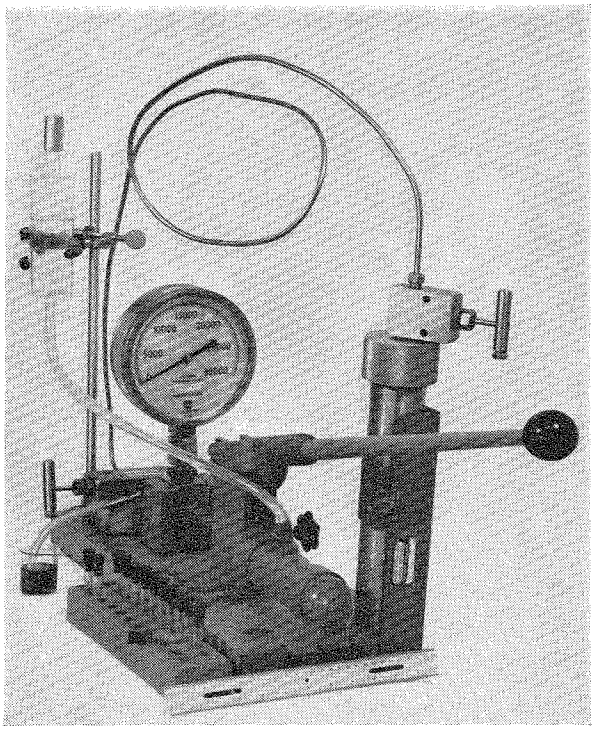

Fig. 2. High pressure vessel connected by semi-flexible steel tubing to hydraulic pump and Bourdon gauge. A rack of small culture tubes with piston stoppers is shown in foreground. ($^1/_{10}$ natural size).

stant pressure is maintained almost indefinitely provided the temperature is kept constant. The pressure changes between 6 and 7 atm per 1 °C change in temperature. Adiabatic heating amounts to about 2 °C during compression to 1000 atm and it requires about 30 minutes for temperature equilibrium to be established by heat conduction in the thermostat, the half-life of the adiabatic temperature increase being between 5 and 10 minutes.

The portable pump pictured in Figure 2 was prepared from a hydraulic truck jack having a capacity of 30 tons or about 4000 atm. The pump is mounted on a block of wood along with the pressure gauge, high-pressure valves, hydraulic fluid reservoir, and semi-flexible steel tubing for connecting the pump to pressure vessel. The clamp on the corner of the assembly is for holding the pressure vessel in an upright position during pressurization. Immediately after applying pressure the needle valve on the pressure vessel is closed and the vessel is returned to the thermostat after disconnecting the top coupling.

The pumps were obtained from the Blackhawk Mfg. Co., Milwaukee, Wisconsin. Needles valves, steel tubing, couplings, and pressure gauges were supplied by the American Instrument Co., Silver Springs, Maryland. "O" rings were provided by The Parker Appliance Co., Cleveland, Ohio. The pressure vessels were prepared by the Special Developments Division, Scripps Institution of Oceanography, La Jolla, under the supervision of James M. Snodgrass. The hydraulic fluid employed in the pump was a 1:1 mixture of water and glycerol.

The pressure had to be released from the vessels before the latter could be opened for the examination of cultures or stored samples. For depressurization the vessel was reconnected to the pump and gauge assembly. Then pressure was applied by operating the pump until the pressure registered on the Bourdon gauge corresponded with the pressure initially applied to the vessel. If the pressure indicator did not move upon opening the needle valve, it showed that the pressure in the vessel was the same as that initially applied. Should the terminal pressure in the vessel be either more or less than that initially applied, the gauge would register an increase or decrease as the case might be. This procedure was always used to check the terminal pressures in the vessels. Interestingly, in thousands of cases the terminal pressure was always found to be the same as that initially applied, provided the temperature had been kept constant.

Air Express Shipment of Pressurized Samples.

Most of the microbiological analyses were made on the Galathea. However, because of their great significance, several sub-samples of sediment from the Philippine Trench were shipped by air express from Cebu to California, where they were examined in our laboratories at the Scripps Institution of Oceanography. This unique operation was made possible only through the cooperation of personnel from several organizations, including the officers and men on the Galathea, the East Asiatic Company, Royal Danish Consulate Service, Philippine Airlines, and the University of California.

Indispensable services were rendered by Mr. KJELD DANOE, who had our high pressure (1000 atm) vessels, containing sediment samples recently taken from the bottom of the Philippine Trench, insulated and packed in dry ice at Cebu for sending by air express to Manila. There this unique package was received two hours later by GUSTAV HALBERG and K. RAASCHOU NIELSEN, who made arrangements for its immediate transfer to a refrigerator on a Philippine Airlines plane. Thirty hours later the P.A.L. hostess placed the refrigerated pressurized

vessel in the hands of a colleague, who met the plane in San Francisco, thanks to Royal Danish Navy radiograms that had forwarded instructions from the Galathea. Neither the temperature nor the pressure of the vessels changed perceptibly from the time they left the Galathea until they were in the laboratory at La Jolla. Examination by direct microscopic and cultural procedures confirmed the presence of numerous living bacteria in sediment samples from depths exceeding 10 000 meters.

Direct Microscopic Observations.

As soon as time permitted on the Galathea, small sub-samples of deep-sea sediment were examined with a phase contrast microscope at a magnification of 970 diameters. By means of a sterile platinum loop designed to deliver approximately 0.01 ml, portions were spread evenly over an area of 1.0 cm^2 on a clean glass slide and protected with a cover glass. Each bacterium observed in the area (0.0002 cm^2) covered by one field of the microscope represents 500 bacteria per 0.01 ml or 50 000 per ml of the original sample. With samples containing fewer than 50 000 per ml, it was necessary to scan several fields in order to find any bacteria. It so happened, however, that a good many of the sediment samples had bacterial populations exceeding 10^5 per ml. Thus, in spite of the obscuring effects of sediment particles with which the bacteria were associated, several bacteria were observed in some fields.

The first significant microscopic observation was made on a sample of silty clay dredged from a depth of 8870 meters in the Philippine Trench on 13 July 1951 at Station No. 412. Twelve sub-samples were prepared for microscopic examination; 50 or more fields of each being scanned. In nearly every field were found definite discrete bacterial cells, including ten different morphological types. Most common were non-sporulating rods with rounded ends, size about 0.5 microns in width by from 2 to 3 microns in length. Fairly abundant were capsulated ovoid rods having an average width of 0.8 microns and a length of 2 microns. Slender rods, 0.6 microns in width and up to 6 microns in length were found attached to sediment particles. Also recognized were several small vibrio, two different types of sporulating bacilli, two kinds of spirilla, and a few diplococci. Motility was not observed in wet preparations. Ostensibly the deep-sea bacteria lose their powers of locomotion due to the change in climate (lower pressure and higher temperature), because it was established by subsequent observations that the vibrio and some of the rods are flagellated.

These microscopic observations on the sediment sample from Galathea Station No. 412 were considered as presumptive evidence for the occurrence of bacteria from a depth of 8870 meters. However, conclusive evidence that they were indigenous species in this, and, eventually, in sediment samples from even greater depths had to await cultural tests, the first of which were completed 24 July 1951. Also observed in this sediment sample were a few empty diatom tests and some fish scales with attached bacteria. There was no evidence of cellulose fibers or of any kind of particulate organic matter besides bacterial cells. The absence of plankton organisms helped to rule out the possibility of the bacteria's having been picked up inadvertently while the dredge was passing through the photosynthetic zone, where the bacterial population was of the order of 10^3/ml.

The microscope also revealed the presence of bacteria in the topmost strata of a clay core collected 15 July 1951 at Station No. 413 from a depth of 10 060 meters. The core was 75 cm long. Detecting no bacteria in the lower strata of the core either by cultural or by direct microscopic examination is highly significant; negative results proved that sediment samples could be collected and analyzed on the Galathea without contamination. The topmost 0.1 cm layer of sediment was estimated to contain at least 3×10^7 bacteria per ml. Cultural methods showed the presence of 7.6×10^5 bacteria which grew at 1000 atm and only 2.3×10^3 which grew at 1 atm.

Predominating in the clay sample from Station No. 413 were rod-shaped bacteria with only a few helicoidal-shaped (vibrio and spirilla) cells, and still fewer spherical-shaped (cocci) bacteria. A good many of the bacteria occured in pairs or short chains, suggesting that the bacteria were reproducing. No protozoans, or other form of microscopic life other than bacteria, were observed.

It was extremely difficult to distinguish bacteria from sediment particles in clay samples dredged from depths of 10 190 and 10 210 meters at Stations No. 418 and 419 respectively. The direct microscopic examination of material dissected from the interior of balls of clay revealed the presence of fewer than 10^5 bacteria per ml, but exterior material was primarily from the mud-water interface. None of the bacteria observed in wet preparations exhibited any motility. Most of the bacteria were small, 1 to 2 microns long by 0.5 to 0.8 microns in diameter.

That the majority of them came from the deep-sea floor was indicated by cultural tests; the MPN at 1000 atm being 10^5 to 10^6 per ml as compared with MPN of only 10^2 per ml at 1 atm (see Table II).

The microscopic examination of greyish-green clay from a depth of 10 120 meters at Station No. 424 was more rewarding. Besides showing the presence of numerous bacilli (order of 10^7 per ml) there were many large (1.6×8 microns) spirilla unlike any bacteria previously described. None of the latter appeared among the many bacteria that grew in nutrient media incubated at 1000 atm (MPN 2.8×10^6 per ml). The microscope also showed many bodies believed to be fungous spores. None of these germinated in nutrient medium incubated at 1000 atm, but in similar medium incubated at 1 atm bacteria were overgrown by fungi. This observation suggests the occurrence of viable fungous spores on the deep-sea floor. Failure to find fungous mycelial material in any of the deep-sea samples indicates that fungi are not growing on the deep-sea floor. Four different kinds of diatom tests were observed with no evidence of protoplasm.

A good many of the minimum dilution cultures prepared for MPN determinations were examined microscopically to confirm bacterial growth. Most of the deep-sea bacteria were morphologically similar to those from soil and shallow water environments with which we are more familiar, showing even less variety among the forms found. Short rods predominated. About 5 per cent were vibrio. Cocci were encountered only occasionally. Differential staining procedures proved that the majority of the deep-sea bacteria are Gram-negative. Capsulated forms were common. Endospores were observed in about 20 per cent of the bacilli.

Physiological Characteristics of Bacteria From Philippine Trench.

The deep-sea bacteria exhibited considerable physiological versatility in differential media at 1000 atm (Table III). Apparently there were almost as many anaerobes as aerobes and a good many reduced nitrate. Some seemed to be able grow in the absence of free oxygen by utilizing nitrate as the hydrogen acceptor. The sulfate reducers were the only obligate anaerobes demonstrated.

The deep-sea nitrifiers differ in their activity at high pressure from surface-dwelling forms as do the nitrate reducers. Nitrate reduction by several shoal water species, which have been tested by ZoBELL and BUDGE (1954), is retarded by pressures of 400 to 600 atm and at 1000 atm their nitratase system is slowly inactivated.

Table III. Minimum numbers of different physiological types of bacteria detected per ml of sediment from the Philippine Trench.

Station number	418	419	420	424
Water depth in meters	10 190	10 210	10 160	10 120
Total aerobic bacteria	10^6	10^5	10^5	10^6
Total anaerobic bacteria	10^5	10^5	10^5	10^5
Starch hydrolyzers	10^3	10^2	10^2	10^3
Nitrate reducers	10^5	10^4	10^5	10^5
Sulfate reducers	10^2	10	10^2	0
Ammonifiers	10^5	10^4	10^5	10^5
Nitrifiers	10	0	0	10

Bacteria in Indian Ocean Deeps.

Two sediment samples, suitable for bacteriological analyses, were obtained from depths exceeding 7000 meters in the Indian Ocean. Large numbers of viable bacteria were found in both samples, but surprisingly not nearly as many as in sediment samples taken from the Philippine Trench at depths exceeding 10 000 meters. Moreover, almost as many bacteria grew in nutrient medium at 1 atm as at 700 atm, and only a few from the Sunda and Weber Deeps grew at 1000 atm (Table IV).

Finding in these deeps starch hydrolyzers, nitrate reducers, sulfate reducers, ammonifiers, and nitrifiers which were active in differential media at 700 atm indicates that they are indigenous species which are probably active *in situ*. The activity of the deep-sea bacteria *in situ* is probably limited by the nutrient content of sea water and sediment and not by the high hydrostatic pressure or low temperature.

Bacteria in Kermadec-Tonga Trench.

Barophilic bacteria were found in 4 different sediment samples from the Kermadec-Tonga Trench (Table V).

Unlike the microflora found predominating in the Philippine Trench, nearly as many and in one sediment sample from the Kermadec-Tonga Trench (Station No. 686) more bacteria developed in nutrient media incubated at 1 atm than at high *(in situ)* pressures when isolates were examined. The inability of these bacteria recovered from the deep-sea floor to reproduce at *in situ* pressures suggests that

Table IV. Most probable numbers (MPN) of bacteria per gram wet weight demonstrated in sediment samples from Indian Ocean by minimum dilution method in nutrient media incubated in refrigerator at different pressures.

Station number	463 (Sunda Deep)	492 (Weber Deep)
Station location	10°16′ S × 109°51′ E	5°31′ S × 131°01′ E
Water depth	7020 meters	7250 meters
MPN at 1 atm	690 000	810 000
MPN at 700 atm	1 050 000	2 300 000
MPN at 1000 atm	4 800	17 000
Starch hydrolyzers at 700 atm	1 000	1 000
Nitrate reducers at 700 atm	10 000	10 000
Sulfate reducers at 700 atm	100	10
Ammonifiers at 700 atm	100 000	10 000
Nitrifiers at 700 atm	0	10

Table V. Minimum numbers of bacteria per gram of sediment from the Kermadec-Tonga Trench as determined by minimum dilution method in differential media incubated in refrigerator at different pressures.

Station number	649	650	658	686
Latitude	35°15′S	32°20′S	35°51′S	28°30′S
Longitude	178°40′W	176°54′W	178°31′W	176°53′W
Water depth (meters)	8300	6620	6720	9820
Total aerobes at 1 atm	10^5	10^6	10^4	10^6
Total aerobes at near *in situ* pressure*	10^6	10^6	10^6	10^5
Total anaerobes at 1 atm	10^4	10^6	10^5	
Starch hydrolyzers at 1 atm	0	10		
Nitrate reducers at 1 atm	0	0		
Sulfate reducers at 1 atm	0	0		

* Since the pressure-depth gradient in the sea approximates 0.1 atm/M, nutrient media inoculated with these four sediment samples were incubated at about 850, 680, 790. and 1000 atm respectively.

Table VI. Minimum numbers of bacteria per gram wet weight of sediment from different core depth detected in nutrient media incubated in refrigerator at 1 atm.

Station number	677		686	
Location of station	28°30′S × 175°53′W		20°53′S × 173°31′W	
Water depth	9190 meters		9820 meters	
Core depth	Aerobes	Anaerobes	Aerobes	Anaerobes
0-5 cm	1 000 000	1 000	1 000 000	1 000 000
5-10 cm	1 000 000	100 000	1 000 000	1 000 000
10-15 cm	100 000	10 000	1 000 000	100 000
15-20 cm	1 000 000	100 000	1 000 000	100 000
20-25 cm	100 000	10 000	1 000 000	10 000
25-30 cm	10 000	1 000	1 000 000	1 000
45-50 cm			10 000	10 000
85-90 cm			10 000	1 000

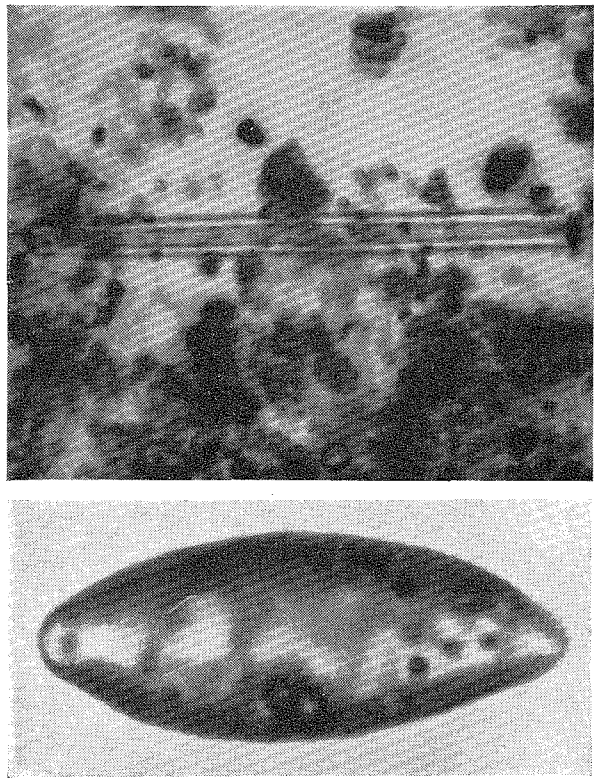

Fig. 3. Photomicrographs of two apparently intact diatoms found in deep-sea sediment following two years' storage in refrigerator at a pressure of 1000 atm.

they are not native to the deep-sea floor. The implication is that they have sunk to the sea floor where they may remain dormant for long periods of time. Such forms that tolerate high pressure without being able to reproduce are termed *baroduric*.

What appeared to be baroduric foraminifera and diatoms were detected in some of the Kermadec-Tonga Trench sediment samples examined by phase contrast microscope. This was confirmed by E. J. F. Wood (1956) working in our laboratory at La Jolla two years after the samples were taken. Since their collection the sediment samples had been stored in piston-stoppered tubes under conditions which were approximately isothermic and isobaric with the environment from which originally taken. Every one in the Scripps Institution laboratory who examined these preparations agreed that certain *Globigerina* contained protoplasm as did the *Coscinodiscus* and *Navicula* specimens. Extensive efforts to cultivate these foraminifera and diatoms have been unsuccessful. They are believed to be transient or adventitious forms which, like some of the baroduric bacteria, have sunk to the deep-sea floor. Regardless of their origin or significance, it is noteworthy that they have been preserved in refrigerated pressure vessels for many months. Two of the diatoms are shown in Figure 3.

The prolonged survival of baroduric bacteria buried at considerable depth was indicated by finding them in adundance to the bottom of cores collected from the Kermadec-Tonga Trench (Table VI). The vertical distribution of aerobes as well as anaerobes appeared to be sporadic, the general trend being decreasing abundance with core depth. Finding numerous aerobes buried at depths where there is probably no free oxygen constitutes further evidence of their dormancy. Some activity of bacteria is indicated by changes with core depth in the non-conservative chemical content of the sediment.

Chemical Content of Deep-Sea Cores.

Portions of two cores from the Kermadec-Tonga Trench were preserved in sealed bottles and brought back to the Scripps Institution laboratory for chemical analysis. Water content was determined by subtracting the constant dry weight (at 105°C) from the initial wet weight. The dried sediment was used for determining organic carbon, carbonate, Kjeldahl nitrogen, ammonia nitrogen, and sulfate.

Organic carbon was estimated by oxidizing at boiling point with a mixture of concentrated sulfuric acid, potassium iodate, and C.P. oxygen after first liberating carbonate carbon by treatment with phosphoric acid and flushing with a stream of pure nitrogen. The carbon dioxide resulting from both treatments was collected (separately) in standard barium hydroxide solution which was back-titrated with N/100 hydrochloric acid. The method of Bien (1952) for determining carbonate and organic carbon was used. This method employs a closed system which eliminates erroneous results from atmospheric CO_2.

Ammonia was driven off weighed sediment samples by sodium hydroxide treatment. Micro-Kjeldahl procedures were employed for determining organic nitrogen. Weighed samples were washed several times with distilled water accompanied by mechanical agitation to leach out sulfate, which was then determined gravimetrically as its barium salt. Results of the chemical analyses are summarized in Table VII.

The low carbonate content was expected, since carbonate in sediments usually decreases with depth in the ocean (Kuenen, 1950). This may be due to the increased solubility of calcium carbonate at the lower pH of sea water associated with the high hydrostatic pressure (Buch and Gripenberg, 1932).

Table VII. Certain non-conservative chemical constituents found in different strata of two mud cores from the Kermadec-Tonga Trench. Values are recored as milligrams per gram of dried (at 105°C) sediment.

Core from Galathea Station No. 677; water depth 9190 meters.

Core depth	Water content	Carbonate content	Organic carbon	Ammonia nitrogen	Kjeldahl nitrogen	Sulfate content
cm	per cent	mg/gm	mg/gm	mg/gm	mg/gm	mg/gm
0-5	46.2	0.7	0.5	0.8	0.5	1.9
5-10	43.8	0.0	0.2	0.2	0.3	1.5
10-15	40.1	0.2	0.1	0.1	0.3	1.2
15-20	40.0	0.6	0.2	0.1	0.3	1.6
20-25	41.8	0.2	0.2	0.1	0.4	1.2
25-28	38.4	0.4	0.1	0.1	0.3	0.9

Core from Galathea Station No. 686; water depth 9820 meters.

Core depth	Water content	Carbonate content	Organic carbon	Ammonia nitrogen	Kjeldahl nitrogen	Sulfate content
cm	per cent	mg/gm	mg/gm	mg/gm	mg/gm	mg/gm
0-10	53.7	0.2	0.3	0.1	0.2	2.0
15-20	51.7	0.5	0.3	–	0.2	1.6
25-30	50.7	0.5	0.2	0.2	0.2	1.3
35-40	52.5	0.3	0.3	0.1	0.3	1.1
45-50	53.0	–	0.1	0.0	0.1	–
55-60	50.8	0.2	0.2	0.1	0.3	0.2
65-70	53.4	0.4	0.2	0.0	0.4	0.4
75-80	52.0	0.2	0.2	–	0.2	0.2
85-90	47.7	–	0.2	0.1	0.2	–

The organic carbon as well as the organic nitrogen content of the deep-sea sediments was very low; in the deeper core strata the C/N ratio was 1:1. Total contents and the C/N ratios are considerably lower than in near-shore sediments, conditions which have been noted by WISEMAN and BENNETT (1940) and by ARRHENIUS (1952). Arrhenius postulates that the low C/N ratios in deep-sea clay deposits may be caused by some property, e.g., low calcium carbonate content, of the clay favoring conservation of nitrogen during the low rate of organic matter deposition, or it might be a reflection of differences in the way in which organic matter is decomposed in the deep sea. Most likely the relatively abundant ammonia-nitrogen content of the deep-sea deposits has resulted from the decomposition of proteinaceous materials. Bacteria (ammonifiers) which liberate ammonium from proteinaceous material were detected in virtually all deep-sea sediments tested for their presence.

There seems to be a general trend of decreasing organic content with core depth, which suggests its slow oxidation. In the absence of any obvious supply of free oxygen below the mud-water interface, the organic matter must be attacked by anaerobes, if by any kind of bacteria. The rate of attack appears to be very slow. Sulfate-reducing bacteria could account for the slow oxidation of organic matter in strictly anaerobic environments. Sulfate-reducing bacteria have been found in deep-sea sediments and, interestingly, there is a definite decrease in the sulfate content of the deep-sea cores.

Barophilic Sulfate-Reducing Bacteria.

Because of their importance as geochemical agents, special effort was devoted to the detection of sulfate-reducing bacteria in deep-sea sediment samples collected by the Galathea. Besides reducing sulfate to sulfide and oxidizing a great variety of organic compounds, certain sulfate reducers fix nitrogen (SISLER and ZOBELL, 1951), and utilize molecular hydrogen (ZOBELL, 1947; SISLER and ZOBELL, 1950). Sulfate-reducing bacteria, which commonly occur in near-shore sediments (ZOBELL and RITTENBERG, 1948), were also found by MORITA (1954) in several ocean basins. Populations exceeding 100 per gram of wet sediment were demonstrated in collections from a

depth of 5032 meters at Mid-Pacific Station No. 36 (MORITA and ZOBELL, 1955).

As shown in Table III above, barophilic sulfate-reducing bacteria were demonstrated in Philippine Trench sediment samples from depths exceeding 10 000 meters. Such bacteria which grew at 700 atm were also demonstrated in bottom deposits from the Sunda Deep and the Weber Deep (see Table IV). Sulfate reducers from the Weber Deep have been maintained in pressure vessels at 3-5°C and 700 atm for more than 4 years and are still under observation at the Scripps Institution. Upon decompressing and opening the pressure vessel for the examination of some of the original sediment sample, a strong odor of hydrogen sulfide emanated, indicating activity of the organisms during storage. Small quantities of the mud were used to inoculate medium M-10-E in piston-stoppered tubes for enrichment in the refrigerator at 700 atm. After 60 days' incubation there was no evidence of sulfate reduction, but after 10 months sulfide had been produced in all tubes of medium M-10-E inoculated with this stored mud from the Weber Deep (7250 meters). The uninoculated controls were negative. There was no evidence of sulfate reduction in similar medium after 17 months' incubation at 1 atm in medium inoculated either with some of the original mud sample or with enrichment cultures that developed at 700 atm.

The obligate barophilic sulfate-reducing bacteria from the Weber Deep have been transplanted four successive times and kept growing at 3-5°C and 700 atm. With the cooperation of Dr. JAMES W. BARTHOLOMEW, electron micrographs were made. They show a small ovoid-shaped microbe about 0.3 microns in width by 0.5 to 0.8 microns in length, which differs in both shape and size from other known sulfate reducers commonly classified as *Desulfovibrio* species. Though highly pleomorphic, known *Desulfovibrio* species are usually comma-shaped organisms 0.5 to 1.0 by 3 to 5 microns in size. The barophilic sulfate reducer from the Weber Deep is believed to be a new genus, but further studies must precede its naming. Like most barophilic bacteria, it grows very slowly (ZOBELL and MORITA, 1957).

An Enigma in Bacterial Taxonomy.

In their unique ability to grow at high hydrostatic pressures, a good many of the bacteria isolated from the deep sea differ from known or described species whose habitat is soil, sewage, near-shore sediments, and other surface or shallow-water environments. However, pressure tolerance is probably not a sufficiently distinctive characteristic to delineate species, particularly since the deep-sea bacteria may have become acclimatized to high pressure while sinking from the surface, or their pressure tolerance may be associated with the composition of the medium.

Ordinarily, the taxonomic position of bacteria is based upon their physiological, cultural, and morphological characteristics as determined at 1 atm. Since some of these characteristics are altered and others are indeterminate at high pressure, we are confronted by an enigma in bacterial taxonomy. At least superficially a good many of the deep-sea bacteria appear to be new species or even new genera, but this can be established only by much more study.

The only deep-sea microbe to which we have applied a generic name is a small (0.4 micron) Gram-negative sphere, usually occurring singly but occasionally in pairs. It has exhibited no evidence of motility and does not form endospores. It liquefies gelatin and liberates ammonia from peptone in sea water medium in the refrigerator at 1000 atm. It oxidizes glucose with the formation of acid; sucrose, lactose, and starch are not attacked. In the refrigerator (3-5°C) it survives at 1 atm, but is killed in a few minutes when warmed to 25°C. It was isolated from two different mud samples from the Philippine Trench. Tentatively it has been named *Bathycoccus galathea*. The Greek suffix *bathy-* denotes great depth and *coccus* indicates that the cells are spherical.

Oceanographic Significance of Deep-Sea Bacteria.

What is the ecological and oceanographic significance of the bacterial populations found in oceanic deeps? Are they primarily passive inhabitants on the deep-sea floor or is their rate of metabolism and reproduction rapid enough for them to affect substantially chemical or biological conditions? Our observations prove only the presence of viable bacteria in some of the deepest parts of the ocean and show that these deep-sea bacteria are physiologically active in the laboratory under experimental conditions designed to duplicate deep-sea conditions, including high hydrostatic pressure and low temperature. Prerequisite to assessing the oceanographic significance of these deep-sea bacterial populations is much more information on the circulation of water masses, the amounts of organic matter reaching the deep-sea floor, the non-conservative chemical composition of the water and bottom deposits, abundance and

growth rates of other organisms inhabiting the deeps, and more information on the characteristics of the bacteria.

An estimate of an average bacterial population of 10^6 per ml in the topmost cm of deep-sea sediment is believed to be conservatively low. Approximately this many were demonstrated in nutrient medium by the minimum dilution method, which does not detect all living microbes, since no one medium can provide for the nutrient requirements and essential environmental conditions for the growth of all bacteria. Moreover, groups of bacteria occurring in agglomerates or attached to solid particles register only as single individuals in the minimum dilution method. Extensive experience by bacteriologists who have been analyzing soil, sewage, milk, and similar materials for several decades shows that conventional cultural methods usually detect only about 10 per cent of the viable bacterial population. Actually bacterial populations of the order of 10^7 per ml of deep-sea sediment were indicated by the direct microscopic method, but the microscope fails to distinguish living from dead bacteria.

Taking 10^6 per ml as the order of magnitude of the bacterial population and considering these bacteria to contain an average of 2×10^{-13} gm of organic carbon per cell, it follows that 1.0 ml of such sediment would contain 2×10^{-7} gm of bacterial carbon, or 0.2 mg per liter. In a layer 1 cm thick there would be 2 mg of bacterial carbon per square meter.

Such a standing crop of bacteria may contribute substantially to the nutrition of benthic animals. Protozoans, worms, sponges, filter feeders, and mud eaters are among the animals known to ingest and digest bacteria (ZoBell and Feltham, 1938). Certain animals can survive and thrive on an exclusive diet of bacteria (ZoBell and Landon, 1937), and some can reduce the bacterial population of sea water to 10^2 to 10^3/ml. Bacteria consist largely of easily digestible proteins and lipids. Many are rich in vitamins and other accessory growth factors which may be beneficial to benthic fauna far removed from products of photosynthetic activity.

Kriss (1954) has stressed the importance of bacteria as food for marine animals and in the organic cycles in the sea. According to Kriss and Rukina (1952), the microbial biomass in marine sediments and overlying water column amounts to from 0.2 to 1.4 gm per square meter.

The extent to which bacteria contribute to the nutrition of deep-sea animals cannot be estimated from the standing crop or population of either, but must be based upon reproduction or growth rates. The population is only an expression of a dynamic balance between the rate of reproduction and the rate of death. Laboratory observations indicate that deep-sea bacteria reproduce (by transverse fission) once every 2 to 20 hours in nutrient medium incubated at 1000 atm and 3°C; somewhat slower in deep-sea sediment or sea water to which no nutrients have been added. They may be dormant *in situ,* as indeed many appear to be in the lower strata of cores from the Kermadec-Tonga Trench (Table VI), but at the mud-water interface they are probably reproducing. The low content of organic matter in deep-sea sediments and immediately overlying water must be attributable to bacterial activity, and there is pretty good evidence for the activity of sulfate-reducing bacteria. Should the rate of reproduction *in situ* be comparable to that found in the laboratory under conditions designed to simulate the *in situ* environment, we may think with confidence of an *in situ* reproduction rate which could provide a hundred or more bacterial "crops" per year, but other dynamic factors must be evaluated before concluding that this is a reasonable estimate.

The maximum amount of bacterial cell substance producible per unit of time is limited by the amount of available energy source. This is believed to consist largely of dissolved or colloidal organic matter reaching the bacteria. Chemosynthetic autotrophs may fix some carbon on the deep-sea floor, but such bacteria appear to play a very minor role. Of course, bacteria are highly efficient in recycling the organic waste products of animals, but there must be an influx of energy to keep the processes going. This energy, originating as fixed carbon in the photosynthetic zone, must reach the deep-sea floor primarily by settling or by the movements of water masses.

Ordinarily an average of one-third of the organic carbon assimilated by heterotrophic bacteria in dilute media is converted into bacterial cell substance, the other two-thirds being oxidized to CO_2. With similar efficiency a bacterial population of 10^6/ml representing a standing crop of 0.2 mg bacterial carbon per liter would require 0.6 mg organic carbon per liter and about the same amount of O_2. The complete oxidation of organic matter requires somewhat more than its own weight in O_2; 1.0 mg of various carbohydrates requiring 1.07 to 1.18 mg O_2, common fatty acids 1.06 to 2.97 mg O_2, triglycerides 2.40 to 2.92 mg O_2, amino acids and proteins 0.64 to 2.58 mg O_2/mg.

Replacing a bacterial population of 10^6/ml 100

times per year would require 60 mg of organic carbon per liter and somewhat more than this amount of O_2. This is much more than occurs in sea water at any one time, but throughout the year organic matter on the sea floor may be replenished by the movements of water masses and to a lesser extent by sedimentation; O_2 by diffusion and water movements. Apparently bacterial populations of the order of 10^6/ml occur only in the immediate vicinity of the mud-water interface, falling off rapidly with distance off the bottom. Only a few samples of sea water from depths exceeding 7000 meters and a meter or more off the bottom have been examined for the presence of bacteria, but all of these, like hundreds of water samples collected from depths exceeding 1000 meters, have bacterial populations of less than 10/ml. If this is representative of conditions throughout deep water masses, organic matter and O_2 from a water column of considerable thickness may be supplying the active biotic zone at the mud-water interface.

Besides oxidizing most kinds of organic matter with the consumption of O_2 and contributing to the nutrition of benthic fauna, bacteria may affect the non-conservative chemical composition of the deep-sea floor. The decomposition of organic matter probably results in the formation of complex marine humus and the liberation of phosphate, sulfate or sulfide, and nitrogenous compounds. Ammonifiers liberate ammonia from certain nitrogenous substances and nitrifiers may oxidize the ammonia to nitrite or nitrate. In the absence of free oxygen, sulfate reducers may produce hydrogen sulfide. If the latter diffuses into oxygenated waters, it may be oxidized with the formation of sulfate or sulfur. Though not detected on the Galathea Expedition, other bottom-dwelling bacteria may fix nitrogen or oxidize hydrogen or methane, which often result from the fermentation of organic compounds.

Bacteria in Water.

Although attention was concentrated on the microbial content of deep-sea sediments, several samples of sea water were examined for the presence of bacteria. Aseptic samples were collected in sterile evacuated bottles hermetically sealed with glass capillary tubes which could be sheared off at any desired depth to provide for the aspiration of water (ZoBell, 1941). Near-surface samples were collected in glass bottles tossed forward, with a hand line, off the bow of the Galathea in order to avoid contamination from the ship. Water samples from depths of 7000 to 8000 meters were collected in 300 ml collapsible rubber bulbs attached in properly spaced series to the hydrographic wire (Figure 4). At greater depths the elasticity of the rubber was adversely affected by the high pressure as has been described by ZoBell (1954).

From 12 July to 15 August 1951 a total of 44 surface water samples collected over the Philippine Trench were examined for bacteria by the minimum dilution method. In every one of these samples were detected from several hundred to a few thousand bacteria per ml, which grew in nutrient medium incubated at air temperature (ca. 30°C). There was no growth in similarly inoculated medium incubated at 1000 atm, and very little growth at 3-5°C, unlike the bacteria found in Philippine Trench sediments.

In organic-rich waters from Philippine Island bays and harbors visited by the Galathea were found

Table VIII. Minimum numbers of bacteria per ml of surface sea water collected on Galathea at different locations. The bacteria were demonstrated in nutrient medium incubated at air temperature and atmospheric pressure.

Total samples	General location of stations from which samples were collected	Date of collection	Bacteria per ml
2	Manila Bay	9-10 July	10^4 to 10^5
11	Over Philippine Trench	12-15 July	10^2 to 10^3
3	Tubajon Bay off Dinagat Island	17-19 July	10^3 to 10^4
12	Over Philippine Trench	21-24 July	10^2 to 10^3
2	Cebu Harbor	25 July	10^4 to 10^6
9	Over Philippine Trench	26-29 July	10^2 to 10^3
1	Kanlanuk Bay off Bucas Grande	30 July	10^4
2	Candos Bay off Lapinigan Island	31 July	10^4
8	Over Philippine Trench	2-8 August	10^2 to 10^3
6	Cebu Harbor	10-12 August	10^4 to 10^5
4	Over Philippine Trench	14-15 August	10^3

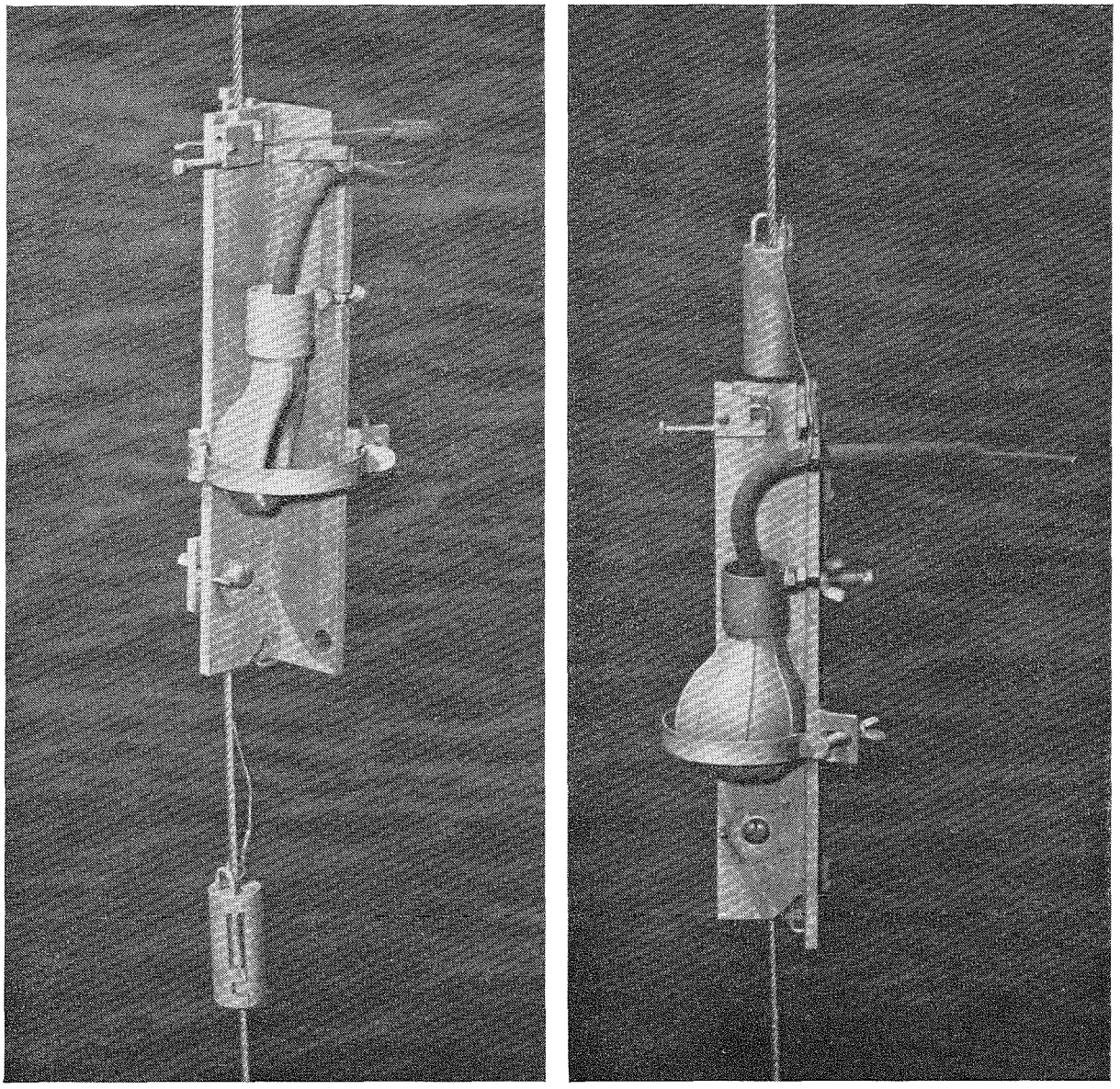

Fig. 4. Bacteriological sampler attached to hydrographic wire; at left the sterile rubber bulb is shown collapsed ready to be lowered into the water. Upon reaching the desired depth a messenger is dropped which shears off the capillary glass tubing thus permitting the thick-walled rubber tubing to flip out to the position shown at the right so water is aspirated from an area considerable distance from the apparatus in order to minimize self-contamination. Simultaneously the messenger suspended from the apparatus (left) is released, permitting it to drop for activating similar or other hydrographic apparatus at greater depth.

from 10 to 1000 times as many bacteria as in near-surface water collected over the Philippine Trench (Table VIII).

Throughout the photosynthetic zone over the Philippine Trench to a depth of 100 to 200 meters, the bacterial population ranged from 10^2 to 10^3 per ml. Not enough samples were analyzed to determine whether the vertical abundance of bacteria corresponded with vertical distribution of phytoplankton, as has been found to be the case in certain areas (ZoBELL, 1946). At depths exceeding 1000 meters fewer than 10 bacteria per ml were found. In northern Pacific Ocean deeps Kriss (1952) found few bacteria in water samples, but in bottom sediments he found from 10^6 to 10^8 bacteria per gram.

Acknowledgments.

The authors appreciate the financial support of the University of California Research Committee (Grant No. 1250), the U.S.A. Office of Naval Research (contract N6onr-27518), and The Rockefeller Foun-

dation. It is also a pleasure to acknowledge the invaluable assistance of Dr. ANTON F. BBUUN together with all the officers, crew members, and scientists on the Galathea, who contributed in many ways to the success of the bacteriological investigations. Special thanks are due to Cmdr. TAGE FEDDERSEN, Lt. L. FERDINAND, Capt. SVEN GREVE, Cmdr. SIGURD BARFOED, TORBEN WOLFF, POUL JACOBSEN, ALF KIELERICH, and P. ANDREASEN for technical help. A special debt of gratitude is due the Royal Danish Navy and the people of Denmark who subsidized the Expedition.

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