showed distant linkage (39 map units) between *Fv-1* and *b*, the locus for brown (the *b* allele) or black (*B*) coat color. In an initial attempt to relate *Fv-1* to other linkage group VIII markers, we obtained evidence that *Fv-1* might be close to *Gpd-1*, the locus for isozymes of glucose-6-phosphate dehydrogenase (*6*), which is about 32 map units from *b* (*7*). This was suggested by the finding that an *Fv-1* congenic strain, SIM.R (*8*), was also congenic for *Gpd-1* (*5*).

We have examined the linkage of *Fv-1* with *Gpd-1* by a three-point cross involving segregation of *Fv-1*, *Gpd-1*, and *b*. (C57BL/6J × DBA/2J)F_1 mice, mating genotype *Fv-1*^{b}, *Gpd-1*^{a}, *B* × *Fv-1*^{n}, *Gpd-1*^{b}, *b*, were backcrossed to DBA/2. The progeny were typed for *Fv-1* by the spleen focus assay of Axelrad and Steeves (*9*); they were inoculated intravenously into the orbital sinus with the F-S virus (*10*), an N-tropic line (*2*) of Friend leukemia virus, and the spleens were examined for foci 9 days later.

Gpd-1 type was determined by starch-gel electrophoresis of kidney extracts (*7*). The results (Table 1) show that *Fv-1* and *Gpd-1* are very closely linked, there being only one *Fv-1* to *Gpd-1* recombinant among the 107 mice tested. This one recombinant might suggest that the gene order is *b-Fv-1-Gpd-1*, but in view of the long distance from *b* to the *Fv-1-Gpd-1* region, this could have been a double recombinant.

In view of its close linkage to *Fv-1*, *Gpd-1* should be a useful marker in further delineating the effect of the *Fv-1* locus, and of murine leukemia virus, on leukemogenesis and other types of tumorigenesis in the mouse.

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Deep-Sea Microorganisms: In situ Response to Nutrient Enrichment

Abstract. *After inoculation of sterile organic materials on the deep-sea floor and in situ incubation for 1 year, relatively minute rates of microbial transformation were recorded. This extremely slow conversion rate, as well as the type and quantity of organic matter normally reaching the ocean floor, appear to characterize microbial life in the deep sea.*

We have reported (*1*) that microbial conversion of a number of organic substrates was considerably retarded when laboratory cultures and mixed populations of surface-born marine bacteria were incubated in the deep sea. The assumption was made that the indigenous microbial flora of the deep water or sediment may respond differently.

To check this possibility, a housing for sterilized sample bottles was devised that permitted inoculation directly on the deep-sea floor. A rack holding 20 120-ml bottles was enclosed in a pressure-tight aluminum cylinder (Fig. 1). As in earlier experiments, the bottles contained the media in concentrated form in quantities of 1 to 10 ml and were equipped with punctured serum caps for self-inoculation (*1*). After closing, the cylinder was evacuated (i) to lower the concentration of gases that dissolve in the samples under elevated hydrostatic pressure and (ii) to give the lid a tight fit during the submersion operation.

This sample-housing vessel was attached to the research submarine *Alvin*. Upon reaching the sea floor, a valve was operated by the mechanical arm to fill the cylinder and the sample bottles with water from the top sediment, including suspended sediment particles. When the pressure had equalized after about 2 minutes, the sample housing opened, and the rack with the sample bottles was removed and placed on the sea floor by the mechanical arm of the submarine. The empty cylinder was returned to the surface. The place of incubation was the permanent bottom station at 39°46′N, 70°41′W, 1830 m depth; the temperature was 4°C. The site was marked by sonar reflectors and revisited by *Alvin* several times during the summer seasons in 1971 and 1972 for placing and retrieval of instruments and samples.

After being filled at the site of incubation, the bottles contained 0.1 percent starch, 0.033 percent agar, or 0.1 percent gelatin. In addition, a duplicate set contained 0.01 percent KH_2PO_4 plus K_2HPO_4 and 0.025 percent NH_4Cl. After retrieval and poisoning with 1.0 ml of 0.2 percent $HgCl_2$, starch and agar concentrations were determined by the anthrone method, and gelatin by the protein determination technique of Lowry *et al.* (*2*). Sterile samples of some solid materials (bond paper, paper towels, balsa wood, beech wood, and thalli of the marine algae *Ulva*) were also included in separate bottles and their decomposition determined by weight loss of dry material (samples were dried at 103°C to constant weight after washing for the removal of salt). The bottles were deposited on 22 June 1971 and retrieved on 12 June 1972 (51 weeks of in situ incubation). Sterile controls were kept in the laboratory for the same time period and at the approximate deep-sea temperature of 3° to 4°C.

By the same inoculation and incubation procedure, isotopically labeled mannitol, sodium acetate, sodium glutamate, and casamino acids (in concentrations of 30, 10, 5, and 2 μg/ml were deposited at the same site from 12 June to 25 September 1972 (14 weeks). In a duplicate set, the bottles were filled during *Alvin*'s descent at a depth of 200 m, also to be incubated at 1830 m. Upon retrieval and poisoning the degree of substrate conversion into cell material and CO_2 was measured by liquid scintillation spectrophotometry. Quenching corrections were made by the channel ratio method. The total recovery of labeled material averaged 97 percent.

Table 1 shows the percentage of substrate converted in 1 year on the deep-sea floor. The controls were parallel samples incubated at 4°C for 1 month in the laboratory. One set was inoculated with water from 1830-m depth, the other with surface water (Eel Pond, Woods Hole). The data for in situ in-

cubation relative to controls are corrected for the difference in the times of incubation and indicate that the deep-water population converted the substrates 17.5 to 125 times faster when incubated in the laboratory (at deep-sea temperature) rather than in situ. In this response, this population hardly differs from the microbial population of surface water.

The changes of dry weight of the paper, wood, and algal (*Ulva*) samples were statistically not significant (± 1 percent) and indicated no measurable degradation during the 1-year incubation. Parallel samples, incubated for 14 weeks at 1830 m, were sent for examination for evidence of bacterial and fungal decay to J. Kohlmeyer (Institute of Marine Sciences, University of North Carolina at Morehead City). None was found. Wood samples incubated for 1 year in open containers, not protected from animal attack as in the bottles, were attacked by boring mollusks (*3*) in the absence of visible microbial degradation.

In Table 2, conversion of ^{14}C-labeled substrates is given as percentage relative to control samples incubated in the laboratory at 4°C. Data for the 5300-m incubation were obtained in an earlier experiment; the same controls were used for these data and for the samples taken at 1830 and 200 m and incubated at 1830 m in the present experiment. Conversion data for the latter samples

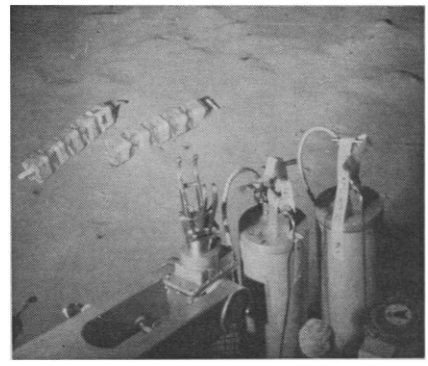

Fig. 1. Two racks of 20 sample bottles (120 ml each) at the permanent bottom station at a depth of 1830 m. In the foreground are *Alvin*'s mechanical arm and two closed pressure-tight sample housings.

are essentially similar to those for samples inoculated and incubated at 1830 m, the differences probably being related to the size of the inoculated population.

In all experiments, the data indicate that the in situ microbial response to enrichment of deep-sea water and sediment with various organic substrates was between one to three orders of magnitude lower than in the controls. This was most recently supported by measurements of oxygen uptake in deep-sea sediments at the same site (*4*). Further, the response of microbial deep-sea populations was similar to that of surface-water populations incubated in the deep sea or in the laboratory at normal pressure and comparable temperature.

Two technical points have to be discussed. During the filling of the sample bottles, part of the inoculum underwent decompression, which must be assumed (until evidence to the contrary is available) to affect the viability of adapted deep-sea microorganisms. ZoBell (*5*) discussed this problem, giving experimental proof that successive compression and decompression of strains of marine bacterial isolates had little or no effect on their viability. Before the present study, however, no work had been reported on deep-sea bacteria that did not undergo previous decompression. In our experiments, this possible effect is minimized because decompression lasted only seconds and did not affect the entire inoculum. In the absence of studies on pure cultures, no conclusive statement on the barophobic or barotolerant behavior of these populations can be made.

Another point of discussion is the increased solubility of oxygen at elevated hydrostatic pressure and the possible inhibitory effect of high oxygen concentrations (*6*). However, when the valve of the evacuated housing vessel was opened on the sea floor at about 183 atm, the critical pressure forcing the remaining air in solution was not reached in the vessel until most sample bottles were filled. In addition, undissolved air was observed to escape when the lid of the housing vessel opened following pressure equilibration. Experimental tests in pressure chambers showed that no gas is trapped in upright bottles with punctured serum caps. Therefore, we assume that little of the oxygen remaining in the housing vessel had dissolved in the sampled water, and that the critical concentration could not have been reached (*6*). As an additional check, samples first flushed with nitrogen gas have been deposited for retrieval in the summer of 1973.

It is unlikely that, after a 1-year incubation, the low rate of microbial activity is explainable by too small an inoculum (about 120 ml of surface sediment slurry). Thus, it appears that enrichments of deep-sea sediment with various organic substrates in different amounts do not result in any substantial activity of the indigenous microflora within a 1-year in situ incubation. The assumption may be made that more than 1 year is required to complete possible adaptive processes that may ul-

Table 1. Percentage of substrate utilized after incubation with and without added nutrients (0.01 percent KH_2PO_4 plus K_2HPO_4 and 0.025 percent NH_4Cl). In situ samples (A) were inoculated with water at 1830 m and incubated for 12 months in situ at 1830 m. Controls were inoculated with water from 1830 m (B) or with surface water (C) and incubated for 1 month in the laboratory at 4°C. Columns A/B and A/C show in situ conversion as a percentage of control conversion, calculated on the basis of equal incubation periods.

Substrate	Added nutrients	In situ (A) (duplicates)		Controls		A/B (%)	A/C (%)
				B	C		
Starch, 1.0 mg/ml	Yes	7.7	3.9		28.0		1.7
	No	5.5	16.5	16.0	9.0	5.7	10.2
Agar, 0.33 mg/ml	Yes	1.3	1.7	13.0	26.5	0.9	0.5
	No	0.0	0.0	2.5	1.5		
Gelatin, 1.0 mg/ml	Yes	0.0	9.7	50.3	84.9	0.8	0.5
	No	0.0	1.8		48.5		0.2

Table 2. Microbial conversion of four ^{14}C-labeled substrates inoculated and incubated in the deep sea. Conversion in samples incubated in situ is expressed as percentage relative to control samples incubated in the laboratory at 4°C for an equal period. Data are given for incorporated substrate (particulate) and metabolized substrate (CO_2 plus particulate); I, incorporated; M, metabolized.

^{14}C-labeled substrate	Taken at 200 m; incubated at 5300 m		Taken at 200 m; incubated at 1830 m		Taken at 1830 m; incubated at 1830 m	
	I	M	I	M	I	M
Acetate	1.14	2.83	0.38	0.37	4.08	2.91
Mannitol	1.14	1.68	7.12	7.02	0.26	0.98
Glutamate	1.62	0.67	0.72	0.81	0.26	0.50
Casamino acids	8.87	7.71	11.74	30.86	1.70	3.08

timately result in faster metabolic rates.

The fact that not only surface-born bacteria (*1*) but also microorganisms collected at the deep-sea floor are exhibiting extremely slow metabolic rates when incubated in situ may be interpreted in two ways.

Life processes in general may be slower at deep-sea conditions than at surface pressures and temperatures for reasons other than the low nutrient supply. Studies on the deep-sea benthic fauna (*7*), have reported (i) extreme diversity of species, (ii) small brood size, (iii) preponderance of adult individuals in most species, and (iv) abundant cases of endemism. These characteristics suggest slow growth and long life of the individual animal and could be the result of a relative retardation of certain critical metabolic processes.

The slow metabolic rate may also lead to the argument that an active, adapted microflora does not exist in the deep-sea sediment. The high colony counts usually found when deep-sea sediment samples are streaked on nutrient agar (*5*) may originate solely from surviving and viable cells that reached the ocean floor with sedimenting detritus particles. Particulate organic matter readily available for microbial decomposition will hardly reach the deep ocean. It will largely be degraded during the slow sedimentation, estimated to take from several weeks to more than a year per 1000 m of depth (*8*). The particulate organic matter in deep waters was shown to be "refractory" (*9*), that is, no degradability could be demonstrated. The total amount of larger particulate material (for instance, animal carcasses) reaching the deep sea undegraded will probably be very small, although of considerable significance for the highly diverse but scanty fauna of benthic scavengers. Nothing is known about the quantity of nonrefractory organic matter reaching the deep-sea floor with the relatively fast-sinking fecal pellets of zooplankton (*8*).

Thus, the top sediment being virtually void of nonrefractory organic matter readily available for degradation, the activity of microorganisms in the deep sea may be largely confined to intestinal tracts of animals, where the enriched nutrient milieu will enable microorganisms to decompose refractory materials (chitin, cellulose, and so forth) in an endosymbiotic fashion. This notion is supported by the finding of an enlarged gut in deep-sea mollusks (*10*). According to this hypothesis, the role played by microorganisms in the turnover of organic matter in the deep-sea sediments appears to be fundamentally different from that in shallow-water sediments, or, for that matter, in soil. Experiments on incubations of solidified organic materials (agar, starch, gelatin) on the deep-sea floor in open containers (*11*) show that after 1 year of exposure, marks of animal feeding appeared to be almost the only sign of disintegration. No work on the intestinal flora of deep-sea invertebrates has yet been done. Complementing our earlier work (*1*), the data reported in this study confirm the conclusion that the deep sea must be considered extremely inefficient with respect to recycling of organic wastes.

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Polychlorobiphenyls in North Atlantic Ocean Water

Abstract. *Concentrations of polychlorobiphenyls (PCB's) have been measured at the surface and at various depths in the water of the North Atlantic Ocean between 26°N and 63°N. The concentrations average about 20 parts per trillion and amount to an estimated 2 × 10⁴ metric tons of PCB's in the upper 200 meters of water. The average concentrations of PCB's in the surface water of the Sargasso Sea are lower than those in the northern North Atlantic.*

Seawater is the most abundant solvent available for trapping hydrocarbons, such as the polychlorobiphenyls (PCB's), released into the environment. The volume of the oceans (10^{21} liters) is sufficient to dissolve all the PCB's that have been manufactured (*1*). Relatively few measurements have been made of PCB concentrations in open-ocean water to determine the extent to which it functions as a solvent trap (*2*). Most analyses have been confined to organisms (*3*), which comprise less than 1 part per million of the total volume.

During the summer of 1972 we analyzed the PCB concentrations of open-ocean water in the eastern and western North Atlantic between 26°N and 63°N. The stations and analyses are tabulated in Table 1 (*4*). For samples 1 to 9 and 13 we extracted 19 liters of seawater (*5*) with 2 liters of a hexane-ether mixture (94 : 6), concentrated the extract, and then analyzed it by electron-capture gas chromatography (ECGC). For the other samples we pumped 19 to 80 liters of water through a brass or glass column [16 by 2 cm (inside diameter)] packed with Amberlite XAD-2 resin (Rohm & Haas) to a height of 12 cm (*6*) at 250 ml/min. The PCB's were eluted from the column with 300 ml of boiling acetonitrile at full gravity flow. The acetonitrile was then diluted with 1 liter of water (distilled or seawater) and extracted with hexane. The concentrated hexane extract was analyzed by ECGC. No cleanup of the extract was required prior to ECGC. Unfiltered seawater samples collected and analyzed simultaneously with water filtered through a 0.3-μm glass fiber filter or a glass wool plug contained a maximum of 10 percent more PCB than the filtered seawater samples. All analyses were completed on shipboard within 4 hours of sampling (*7*).

Three observations can be made from the data:

1) Although the range is very broad, the concentrations of PCB's in the northern North Atlantic average 35 ng/kg [35 parts per trillion (ppt)] in surface waters, and 10 ppt at 200 m.

2) The PCB concentrations decrease with depth.

3) The surface waters of the Sargasso Sea (stations 25–41) have slightly lower surface concentrations of PCB's (27 ppt) than surface waters in other parts of the North Atlantic.

The widespread distribution of PCB's