are yellowish like Venus, there are many unanswered questions about the substance (Are the low polymers liquid? Is their vapor pressure low enough for the gas to go undetected? What is the refractive index? Are the low polymers stable at the expected temperatures and pressures? Are sufficient production rates plausible?). Carbon suboxide is hence a very uncertain material, but laboratory studies of it would be useful.

It is conceivable that water of hydration could reduce the refractive index of some minerals to $n_r \sim 1.45$ (*20*) or that dissolved salts may raise the refractive index of water sufficiently. However, such possibilities are very speculative without independent evidence for a particular material.

In summary, the comparison of polarization observations with theoretical calculations has accurately yielded the optical properties of the visible clouds of Venus. These properties rule out most of the materials that have been suggested for these clouds, including water and ice. An aqueous solution of hydrochloric acid (HCl–H_2O) and carbon suboxide (C_3O_2) are not absolutely excluded, but the likelihood for either is not high. A new look at the question of the Venus cloud composition seems in order.

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7. The imaginary part of the refractive index must be very small for the particles in the upper cloud layer on Venus, and the single particle albedo, ω_0, must be close to unity. Since the spherical albedo of Venus is less than 100 percent at each wavelength of interest, we may choose either (i) a finite optical thickness with a partially absorbing ground, (ii) some interparticle gaseous absorption, (iii) a small absorption within the particle, or some combination of (i), (ii), and (iii). However, we found the differences in the resulting polarization under these assumptions to be negligible, and the calculations for the figures in this report were made on the basis of assumption (iii).
8. The effect of the particle size distribution may be described by a small number of parameters. For the amount of detail that may be extracted from the presently available observations of Venus, two parameters are sufficient: the mean scattering radius, $\bar{r}$, which is the mean radius with the particle scattering cross section included as a weight factor, and the dispersion of particle sizes, $\bar{r}^2$, which is the second moment about the mean, again with the cross section included as a weight factor. Results shown in Figs. 2–4 were obtained with the gamma function size distribution

$$n(r) \propto r^{-p_2} \exp(-rp_2/p_1)$$

with $p_2 = 6$, which corresponds to a moderate dispersion; p_1, the mode radius, was allowed to vary to obtain different values for the mean radius.
9. The phase variation of line strengths [J. W. Chamberlain and G. R. Smith, *Astrophys. J.* **160**, 755 (1970); L. D. Gray Young, R. A. Schorn, E. S. Barker, M. MacFarlane, *Icarus* **11**, 390 (1969)] and several estimates of a long mean free path in the cloud layer [R. Goody, *Planet. Space Sci.* **15**, 1817 (1967); M. J. S. Belton, D. M. Hunten, R. M. Goody, in *The Atmospheres of Venus and Mars*, J. C. Brandt and M. B. McElroy, Eds. (Gordon & Breach, New York, 1968); J. E. Hansen, *Astrophys. J.* **158**, 337 (1969)] indicate that the assumption of uniform mixing is probably better than the often used hypothesis of a dense "reflecting layer" cloud with a gas above. The mixed model may be less valid for polarizations than for intensities, but the particle size, shape, and refractive index which we derive are independent of this assumption and the derived pressure does not depend greatly on it either.
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11. Despite the small magnitude of the variation in n_r with λ, the variation is significant. Any model (with a given size distribution and refractive index, including a possible dispersion in n_r) must have a larger refractive index in the ultraviolet than in the infrared region in order to obtain agreement in both wavelength regions.
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25 September 1970; revised 7 January 1971 ■

Microbial Degradation of Organic Matter in the Deep Sea

Abstract. *Food materials from the sunken and recovered research submarine* Alvin *were found to be in a strikingly well-preserved state after exposure for more than 10 months to deep-sea conditions. Subsequent experiments substantiated this observation and indicated that rates of microbial degradation were 10 to 100 times slower in the deep sea than in controls under comparable temperatures.*

On 16 October 1968, the research submersible *Alvin* of the Woods Hole Oceanographic Institution sank in about 1540 m of water, 135 miles southeast of Woods Hole, Massachusetts. The accident occurred when, because of a broken cable, the vessel dropped into the sea with an open hatch and sank after the crew of three escaped safely. A photograph taken on 13 June 1969 by U.S.N.S. *Mizar* prior to the retrieval operations showed the position of the vessel on the sea floor, the hatch still being open (Fig. 1). On 1 September 1969, *Alvin* was brought to the surface (*1*). Among the items recovered was the crew's lunch consisting of two thermos bottles filled with bouillon and a plastic box containing sandwiches and apples. From general appearance, taste, smell, consistency, and preliminary bacteriological and biochemical assays, these food materials were strikingly well-preserved. When kept under refrigeration at 3°C, the starchy and proteinaceous materials spoiled in a few weeks.

Possible implications of this unexpected finding led us to make some additional observations. The environmental conditions at a depth of 1500

m are assumed to be fairly constant at about 3° to 4°C and 150 atm of pressure. There was no evidence of reducing conditions nor was there a noticeable lack of dissolved oxygen either in the pressure hull of the vessel or in the box containing the food materials. In addition, there was no evidence for the presence or the possible leakage of a soluble material that could have acted as a preservative. The plastic lids of the stainless steel thermos bottles were crushed by pressure, and some seawater must have penetrated and mixed with the contents.

Besides being soaked with seawater, the six sandwiches wrapped in waxed paper (Fig. 2a) appeared fresh by taste and smell. When pieces of the bread were streaked on seawater agar, bacteria and molds grew profusely. Placed in tubes with sterile seawater and kept at 3°C, the bread decayed with slight gas production (floating to the surface) within 6 weeks. The slices of meat (bologna) were grayish on the outside but still pink in the center. Submerged in sterile seawater, the meat spoiled with a putrefactive smell within 4 weeks at 3°C and within 5 days at 30°C.

The two apples found in the lunch box had a pickled appearance (Fig. 2b) but showed no sign of obvious decay. The *p*H of the tissue was the same *p*H (3.2), and the tyrosinase activity (*2*) was about half that of a fresh apple tested. The soup, originally prepared with hot (not boiling) water from canned meat extract, was perfectly palatable in hot and cold condition. Samples of this broth showed a maximum turbidity caused by bacterial growth in 22 days when incubated at 3°C, and in 5 days when incubated at 30°C. Sporeforming bacteria were observed while the majority of bacteria were represented by Gram-negative rods that grew well on seawater media.

In conclusion, the food materials recovered from *Alvin* after 10 months of exposure to deep-sea conditions exhibited a degree of preservation that, in the case of fruit, equaled that of careful storage and, in the case of starch and proteinaceous materials, appeared to surpass by far that of normal refrigeration.

The implications of this finding, if generally true, are of theoretical and practical interest. Viewing the ocean as the ultimate sink of inorganic as well as organic materials, we have virtually no knowledge of qualitative and quantitative microbial decomposition processes. While the absolute amounts of nonliving organic matter calculated for all oceans by far exceeds that of the landmasses, the actual concentrations in seawater are extremely low. In fact, in the larger part of the oceans the concentration of dissolved organic carbon is too low for a direct measurement of oxygen consumption or any other parameter as an indicator of degradation processes. The constancy of organic carbon concentrations with depth in the sea suggests little or no microbial activity (*3*). On the other hand, results of experimental work on the effect of low temperature and high hydrostatic pressure (*4*) do not exclude considerable microbial activities in the deep sea if suitable energy sources and nutrients are available.

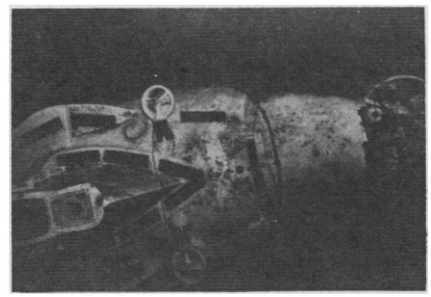

Fig. 1. *Alvin* resting at a depth of 1540 m with open hatch as photographed about 8 months after her sinking.

Research in this laboratory has been directed toward measuring in situ rates of growth and biochemical activities of marine bacteria as measured by chemostat systems fed with natural seawater (*5*). This approach has been limited to the richer surface waters. The *Alvin* accident stimulated a direct experimental study of microbial activities as affected by deep-sea conditions.

Various experimental approaches were readily conceivable in order to confirm the observed phenomenon on a more general basis. In cooperation with the Department of Physical Oceanography a program was designed in which specially designed sample racks were attached to deep-sea moorings about 10 m above the sea floor at depths of about 5000 m and recovered by an acoustic release mechanism after exposure for 2 to 5 months.

The sample racks held about 50 bottles of 120-ml volume each and 20 plastic syringes containing liquid media of various types and concentrations. In experiments with ^{14}C-labeled substrates, the bottles were filled with seawater from 200 m collected at the site of launching. The substrates were added just before the samples were submersed. The serum stoppers used for sealing permitted pressure equalization. Parallel controls were kept under refrigeration at 3°C in the laboratory at 1 atm in the dark. In other experiments, bottles were inoculated with mixed microbial populations of heavily contaminated surface water (Eel Pond, Woods Hole). Some bottles were equipped with a simple device that provided for self-inoculation by hydrostatic pressure at depths from 150 m down to the sea floor. Other samples were inoculated with pure cultures of specific isolates and submerged in plastic syringes of 50-ml volume containing air in addition to the liquid media. The mechanical behavior of the bottles and syringes at increased hydrostatic pressure was tested in special pressure chambers equipped with viewing ports (*6*).

For the data presented in this account, the following brief indication of analytical methods may suffice. ^{14}C-Labeled substrates and metabolic products were counted in a Packard (Tri-Carb, model 3380) scintillation spectrometer in 10 ml of Bray's solution. The efficiency of all counts was corrected for quenching from a prepared external standard ratio curve. Ammonia released from nitrogenous substrates was determined by micro-

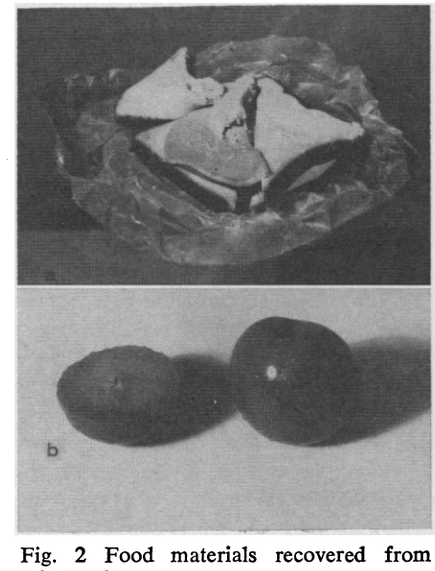

Fig. 2 Food materials recovered from *Alvin* after exposure to seawater at a depth of 1540 m for 10 months.

Table 1. Microbial degradation of four substrates exposed for 8 weeks at a depth of 5300 m (location 33°58′N, 70°W) as compared to controls kept at 3°C for 6 weeks. Percent values are corrected for the unequal exposure time. The microgram values are calculated from counts of ^{14}C radioactivity and are given for total volume of sample (120 ml). Cold Difco casamino acids were added to a mixture of 14 uniformly labeled ^{14}C-amino acids. The inoculum consisted of about 120 ml of seawater sampled separately at a depth of 200 m at the site of launching.

Substrate added (μg)	Substrate in particulate fraction (μg)		Sample control (%)
	Control	Sample	
	Acetate		
3600	88.9	3.58	3.0
1200	146.1	2.08	1.07
600	138.7	0.29	0.15
240	16.2	0.073	0.34
	Mannitol		
3600	166.6	3.45	1.55
1200	46.0	1.06	1.7
600	41.1	0.60	1.1
240	40.1	0.13	0.24
	Sodium glutamate		
3600	252.5	6.50	1.9
1200	130.6	1.66	0.95
600	59.6	2.20	2.77
240	43.8	0.50	0.86
	Casamino acids		
3600	406.7	48.80	9.0
1200	336.0	14.40	3.2
600	123.6	17.10	10.4
240	49.9	8.60	12.9

Kjeldahl distillation. Residual carbohydrates and sugars were determined by the phenol-sulfuric acid method (*7*). For the determination of bacterial growth, colonies were counted on seawater agar containing the particular substrate studied.

Table 1 represents data of an experiment with ^{14}C-labeled substrates in concentrations of 2 to 30 μg/ml. The total recovery of added ^{14}C activity in the three fractions—residual substrate, CO_2, and particulate carbon—ranged from 95 to 99 percent. The ratio of the amount of labeled CO_2 to the amount of particulate carbon in the laboratory controls ranged from 1.5 to 3.4. In the deep-sea samples, however, the amount of labeled CO_2 was too small for significant measurements and very low relative to the amount of labeled carbon in the particulate fraction. For this reason, only the data for the conversion of substrate into particulate carbon are given in Table 1. It might be assumed that dissolved products other than CO_2 were formed by fermentative interconversions. However, there was no indication of anaerobic or reducing conditions in any of the samples.

Table 1 shows that the amount of substrate converted into the particulate fraction in the deep-sea samples ranged from 0.15 to 12.9 when expressed as the percentage of the corresponding conversion in the laboratory controls (in the calculation of these percentage figures, the values of columns 2 and 3 have been corrected for the unequal exposure time). In other words, in these two extreme cases the substrate decomposed 666 to 8.2 times more slowly in the deep-sea samples than in the refrigerated laboratory controls. The corresponding average figures for the two carbohydrates are 88 times, and for glutamate and casamino acids 62 and 11 times respectively. With the exception of the casamino acids, these rates appear to decrease with increasing concentration of the particular substrate. In addition, on the basis of the turnover of organic carbon, in the deep-sea samples the carbohydrates decomposed two to four times more slowly than the nitrogenous substrates.

In another experiment at the same location, chemical analyses were used. For the sake of analytical accuracy, the substrate concentrations were chosen to be five to ten times higher (Table 2). The controls were checked after an incubation period of 6 weeks, at which time the degradation was clearly completed. Therefore, the ratios between the amount of substrate utilized in the controls (corrected for an incubation time of 19 weeks) to that metabolized in the deep-sea samples represent maximum values.

There was no perceptible quantitative difference in the rates of decomposition per bacterial cell when rich surface water or offshore seawater collected at 200 m was used as an inoculum. In pure culture experiments, we selected mesophilic and psychrophilic strains that had been isolated from various depths in the open ocean. Only an obligately psychrophilic bacterium produced a small but significant amount of ammonia in a peptone-yeast extract medium (Table 3). In no instance did any of the liquid media incubated in the deep sea give rise to turbid cell suspensions.

From this study it appears that the degree of preservation of the food materials recovered from *Alvin* is no chance observation, although our experiments were carried out at greater depths than those where the *Alvin* accident occurred.

The surprisingly large difference between rates of degradation in samples exposed to deep-sea conditions and those in controls appears to be real. The data support the notion of a general slow-down of life processes in the deep sea. No obvious explanation is readily conceivable except for some clues derived from an apparent temperature-pressure relation in microorganisms indicated by some of our data.

The experiment with pure cultures (Table 3) included, in addition to typical mesophilic bacteria, several psychrophilic strains that all grew readily at −1°C (not identical with the minimal growth temperature) in the laboratory. At deep-sea conditions, however, only the culture with the

Table 2. Microbial degradation of four substrates in 50 ml syringes (10 ml of liquid medium, 20 ml of air) exposed for 19 weeks at a depth of 5300 m (location: 33°58′N, 69°58′W) as compared to controls kept at 3°C for 6 weeks. Percent values are corrected for the unequal exposure time. The substrate concentrations are given as micrograms of starch, galactose, or ammonia nitrogen per milliliter, respectively. The inoculum was 5 ml of surface water from Eel Pond, Woods Hole.

Initial concentration (μg/ml)	Change in concentration (μg/ml)		Sample control (%)
	Control	Sample	
	Starch		
1850	1330	260	6.2
	1290	170	4.2
	Galactose		
1800	1680	220	4.15
	1580	280	5.6
	Peptone		
57	263	9	1.1
	258	20	2.4
	Albumin		
57	172	15	2.8
	173	17	3.1

Table 3. Microbial degradation of a complex nitrogenous medium (0.02 percent yeast extract, 0.2 percent peptone, and an initial ammonia nitrogen concentration of 39.0 μg/ml) in 50 ml syringes (10 ml of medium, 20 ml air) exposed for 18 weeks at a depth of 4300 m (location: 28°N, 70°W) as compared to controls kept at 3°C for 6 weeks. The ammonia nitrogen values have a standard deviation of ± 0.5 μg/ml. The inoculum was six strains of mesophilic and psychrophilic bacteria isolated in a preceding study (*9*).

Strain	Temperature range for growth (°C)	Change in concentration *	
		Control	Sample
44	17 to 36	3	0.1
36	8 to 36	1.5	−0.7
7	−1 to 36	130	−1.1
20	−1 to 27	128	+0.4
60	−1 to 23	87	−0.1
58	−1 to 17	15	+2.2

* Micrograms of ammonia nitrogen per milliliter.

lowest maximal growth temperature (strain 58) caused detectable biochemical changes of the substrate within the given exposure time. But even in this case, the rate is strongly reduced as compared to that in the laboratory controls.

These data suggest that, superimposed on a quantitative reduction of the rate of biochemical activity, the increased hydrostatic pressure may exert an effect on the cells, raising the minimal growth temperature. When this increase exceeds the environmental temperature, the cells will become inactive. This effect would be similar to, but not necessarily biochemically linked to, the observed increase in temperature tolerance of bacteria (*4*) and of isolated enzymes (*8*) when exposed to similar increases of pressure.

We now propose the hypothesis that, in an environment of low temperature, an increasing pressure will eliminate growth and biochemical activity of bacterial types successively as their minimal growth temperatures are shifted toward, and ultimately surpass, the environmental temperature. Thus, psychrophilism of our isolates at normal pressure may be defined as an expression of adaptability to the combined effect of high pressure and low temperature. Or, in other words, psychrophilic bacteria would not necessarily react as psychrophiles in the deep sea. Laboratory experiments in this direction are under way.

Our hypothesis may be further supported by the fact that in marine sediments from depths of 1300 and 2600 m extremely obligate psychrophilic bacteria that exhibited maximal growth temperatures between 8° and 15°C have been isolated. These types are not found in shallower waters where obligate psychrophiles with maximal growth temperatures between 17° to 24°C are present (*9*). Strain 58 belongs to the latter group but appears to have the potential of being biochemically active at 2° to 3°C at a depth of 4300 m.

In seawater collected at a depth of 200 m (17.6°C), mesophilic bacteria were predominant while obligate psychrophilic bacteria were absent (*9*). This may explain the low absolute rates of degradation in these samples when exposed to deep-sea conditions.

One obvious implication of our findings concerns the use of the deep sea as a dumping site for organic wastes. The relatively low rates of microbial activity at deep-water conditions appear to render this way of waste disposal very inefficient compared to the degradation of organic wastes in land-disposal sites or in treatment plants. Accumulations of waste materials or intermediate decomposition products in the deep sea appear rather uncontrollable. Bruun and Wolff (*10*) mention the common recovery of water-logged wood materials from deep-sea dredgings even far from land.

Normally, few solid organic materials, produced on land or in the sea, can be expected to reach the deep sea without passing surface waters or shallow-water sediments where considerable degradation occurs. If this step during offshore disposal were eliminated, it seems possible to trap substantial amounts of nutrients in solid form in the deep sea, and thereby remove them from natural or technically enhanced recycling processes. The notion of fertilizing the sea with man-made wastes might not be applicable with regard to deep-sea dumping.

Although neither microbial population collected from surface or deep waters showed appreciable activities when exposed to deep-sea conditions, our data do not entirely disprove the possibility of long-term enrichments in deep-sea sediments. Whether or not adaptive processes occur, the rates of oxygen supply and microbial degradation activities will determine the extent to which anaerobic conditions will arise, with possible elimination of the benthic nonmicrobial fauna.

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11. We thank B.-A. Collins for technical assistance and G. H. Volkman and R. H. Heinmiller for performing the technical operations at sea. Supported by NSF grant BO 20956. Contribution No. 2573 of the Woods Hole Oceanographic Institution.

12 September 1970; revised 13 November 1970 ■

Fluorescence of the Purine and Pyrimidine Bases of the Nucleic Acids in Neutral Aqueous Solution at 300°K

Abstract. *Fluorescence of adenine, guanine, cytosine, and uracil at room temperature in neutral aqueous solution has been detected by means of a digital signal accumulation technique. Corrected emission and excitation spectra are presented and compared with low-temperature data. The quantum yields are, respectively,* 2.6×10^{-4}, 3.0×10^{-4}, 0.8×10^{-4}, *and* 0.5×10^{-4} *when the bases are excited at their low-energy absorption maxima.*

An understanding of the nature of the excited states of DNA is fundamental to an understanding of both electronic energy transfer processes and DNA photochemistry, and luminescence measurements are the most direct way of determining the properties of these states. Earlier work on neutral solutions has been carried out in low-temperature environments where quantum yields are more easily measurable ($\Phi \sim 10^{-1}$). A great amount of data has been accumulated, principally at 77°K in ethylene glycol–water (EG:H_2O) glasses, on the fluorescence, phosphorescence, and electron spin resonance properties of the purine and pyrimidine bases, the corresponding nucleotides, and various DNA's. This work has been reviewed recently (*1*). Extensive work, also at 77°K, has been carried out on homo- and heterodinucleotides and polynucleotides with the aim of understanding