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Barophilic Bacteria Associated with Digestive Tracts of Abyssal Holothurians

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Abyssal holothurians and sediment samples were collected at depths of 4,430 to 4,850 m in the Demerara abyssal plain. Bacterial concentrations in progressive sections of the holothurian digestive tract, as well as in surrounding surface sediments, were determined by epifluorescence microscopy. Total bacterial counts in sediments recently ingested by the animals were 1.5- to 3-fold higher than in surrounding sediments at the deepest station. Lowest counts were observed consistently in the foregut, where the digestive processes of the holothurian are believed to occur. In most animals, counts increased 3- to 10-fold in the hindgut. Microbial activity at 3°C and in situ and atmospheric pressure were determined for gut and sediment samples by measuring the utilization of [^{14}C]glutamic acid, the doubling time of the mixed-population of culturable bacteria, and the percentage of the total bacterial count responsive to yeast extract in the presence of nalidixic acid, using epifluorescence microscopy. A barophilic microbial population, showing elevated activity under deep-sea pressure, was detected by all three methods in sediments removed from the hindgut. Transmission electron micrographs revealed intact bacteria directly associated with the intestinal lining only in the hindgut. The bacteria are believed to be carried as an actively metabolizing, commensal gut flora that transforms organic matter present in abyssal sediments ingested by the holothurian. Using data obtained in this study, it was calculated that sediment containing organic matter altered by microbial activity cleared the holothurian gut every 16 h, suggesting that abyssal holothurians and their associated gut flora are important participants in nutrient cycles of the abyssal benthic ocean.

Most bacteria present in the nutrient-poor environment of the cold, abyssal ocean have been characterized as being slowly metabolizing or inactive (3, 15, 24, 34, 39). Rapid microbial activity, under in situ conditions of elevated hydrostatic pressure and low temperature, is reported to occur only in those specific microenvironments where nutrients are concentrated above background concentrations of the water column or sediment. For example, barophilic bacteria, i.e., those bacteria achieving optimal growth under elevated pressure, have been suggested to be components of the gut flora carried by abyssal scavenging amphipods (4, 11, 31, 37).

Deep-sea amphipods are highly visible members of the abyssal benthos, readily attracted to bait (12) and, thus, easily trapped for in situ experiments designed to clarify feeding patterns and behavior (16, 36). By contrast, little information is available on the in situ activities of large, deposit-feeding animals, which generally are collected only in trawling nets. A dominant member of many deep benthic communities is the deposit-feeding holothurian (10, 19, 23; Deming, ALVIN Dive 1205). These animals are believed to mix and till the deep-sea mud on an enormous scale (10), deriving nutrients from the small fraction (<10%) of particulate organic carbon, produced photosynthetically in surface waters, which ultimately sinks to the ocean floor (2, 22). The role of deposit-feeding holothurians in the turnover of organic matter reaching the deep sea may be more predictable, if not more important, than the role of opportunistic scavengers that feed intermittently.

In an earlier study (4), we collected a holothurian specimen (*Psychropotes* sp.) at a depth of 4,575 m in the Cape Basin of the South Atlantic Ocean. The culturable, mixed population of bacteria, scraped from the intestinal lining of the cloacae, demonstrated a relatively rapid doubling time of 11 h under in situ conditions of temperature and pressure, compared with 4 h at 3°C and 1 atm (ca. 1.013 × 10^2 kPa). Subse-

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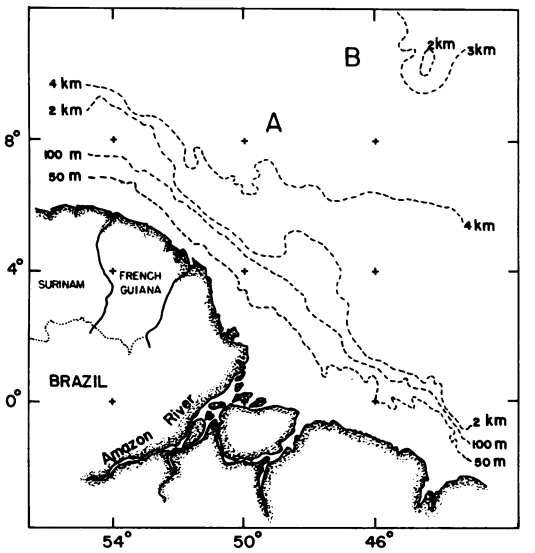

FIG. 1. Stations A and B in the Demerara abyssal plain.

quently, we joined a French expedition aboard the N/O Jean Charcot in the Demerara abyssal plain of the North Atlantic Ocean and collected an additional seven holothurians at depths of 4,430 to 4,850 m. Because these animals were not as large as the *Psychropotes* specimen reported previously, it proved difficult to remove enough material from the gut lining to repeat the original growth studies. Instead, portions of the gut wall were fixed and examined by electron microscopy. Bacterial numbers and selected microbial activities, under deep-sea and atmospheric pressure, were measured in sediment samples removed from the holothurian digestive tract and from the undisturbed surface of box-cores. Our objectives were to determine what role bacteria play in the nutrition of abyssal holothurians and whether the digestive tract of an abyssal, deposit-feeding animal provides an appropriate microenvironment for the proliferation of barophilic bacteria in the deep sea.

MATERIALS AND METHODS

Collection and preparation of gut samples. Holothurians were collected at two stations in the Demerara abyssal plain (Fig. 1) during the Demeraby mission of September and October, 1980, organized by Myriam Sibuet of the Centre Océanologique de Bretagne, Brest, France. Animals were retrieved by beam trawl at station A (8°09'N, 49°02'W) at a depth of 4,430 m and at station B (10°24'N, 46°45'W) at a depth of 4,850 m. Only intact animals, packed in cold mud and thus thermally insulated during gear retrieval, were selected for study. These included members of the genera *Pseudostichopus*, *Diema*, and *Psychropotes*.

Each animal was dissected aseptically, using alcohol-sterilized dissecting tools. Because no histological studies differentiating functional segments of the digestive tract of an abyssal holothurian have been reported, the entire digestive tract was divided simply and arbitrarily into five sections (A through E) of roughly equivalent length, by the dissection scheme of Khripounoff and Sibuet (17; Fig. 2). Ingested sediment was removed from each gut section with a sterile spatula, weighed, and diluted 1:5 (wet wt/vol) with prefiltered (<0.2 μm), autoclaved, artificial seawater (ASW). When available, natural seawater collected from the same depth and station and similarly sterilized, was used as diluent. The animals were dissected on ice, and all subsequent procedures were completed in the shipboard coldroom at 3°C.

Enumeration of bacterial populations. Portions of each gut suspension were filtered onto a precombusted, preweighed 5.5 GF/C Whatman glass fiber filter, dried at 60°C for 18 h, and weighed again so that subsequent parameters could be normalized to grams (dry weight) of sediment. A sample of 10 to 20 ml of each suspension was fixed in a final concentration of 2% formaldehyde and stored at 3°C until return to the university laboratory at the end of 5 weeks, when the cruise terminated. Samples were diluted further ($>10^{-3}$) in ASW, stained with a final concentration of 0.01% acridine orange, and examined by epifluorescence microscopy, according to Hobbie et al. (14). Each sample was examined in duplicate or triplicate, and at least 200 bacteria (all of the green-, yellow-, and orange-fluorescing cells of distinct bacterial size and shape) were counted for each slide preparation. No attempt was made to correct final counts for bacteria present on hidden surfaces of sediment particles, since Rublee and Dornseif (29), in a recent review of the use

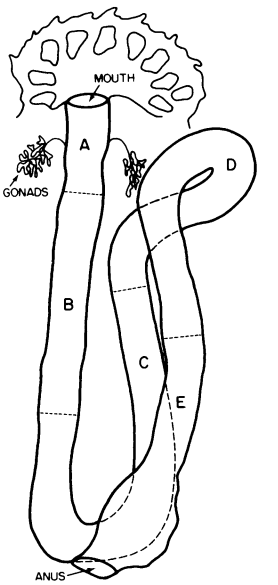

FIG. 2. Digestive tract of an abyssal holothurian and the dissection scheme used to sample gut contents, adapted from Khripounoff and Sibuet (17).

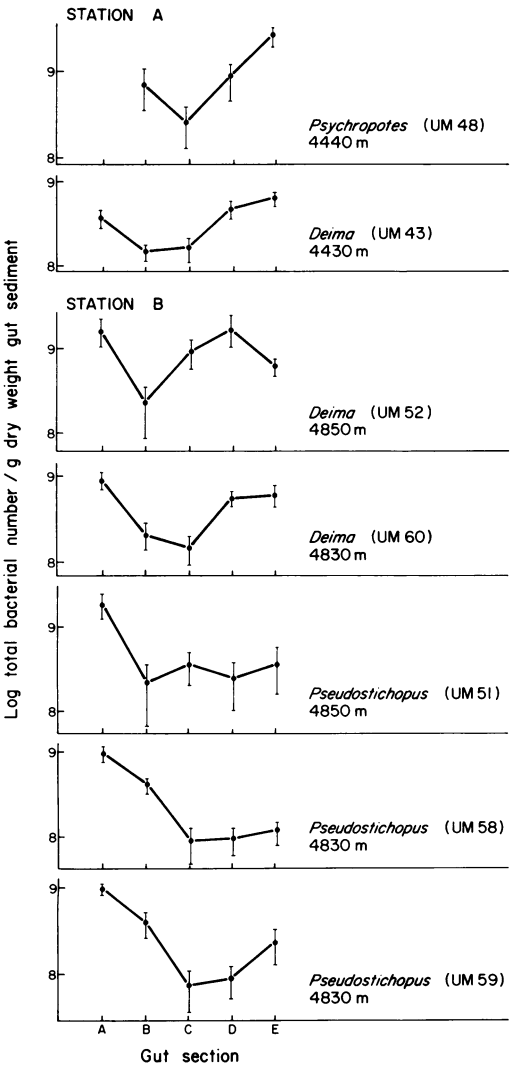

FIG. 3. Bacterial profiles, determined by epifluorescence microscopy, of the digestive tract of holothurians collected at stations A and B. Gut sections A through E represent progressive sections through the digestive tract from mouth (section A) to anus (section E). Error bars indicate ±1 standard deviation of the mean bacterial count.

of acridine orange and epifluorescence microscopy to examine sediment bacteria, concluded that particle-hidden bacteria in similarly diluted sediment samples account for only about 10% of the total bacterial number.

Microbial activity. Three methods were used to determine relative microbial activity, under in situ and atmospheric pressure at 3°C, for gut sections B through E of holothurian UM48 (*Psychropotes bug-lossa*). Sample suspensions used in the following procedures were prepared by diluting the original material present in each gut section 1:20 (wet wt/vol) in ASW.

The microbial utilization (uptake and incorporation plus respiration) of ^{14}C-labeled glutamic acid was determined by the method of Hobbie and Crawford (13), as modified by Paul and Morita (25). Complete procedural details for measuring ^{14}C-substrate utilization in sediment suspensions incubated at 3°C and in situ or at atmospheric pressure have been described in earlier publications (30, 33). [^{14}C]glutamic acid (1.97 μCi/μg; Commissariat à l'Energie Atomique, France) was added at a final concentration of 0.92 and 0.88 μg/g (dry weight) of sediment in UM48 gut sections B and E, respectively. Total substrate utilization was measured in duplicate subsamples at approximately daily intervals for a total incubation period of 5 to 7 days. All counts per minute values were corrected for abiotic $^{14}CO_2$ evolution or the retention of nonincorporated labeled substrate by the filter and filtered sediments, based on values obtained from control samples acidified with 0.3 ml of 4 N H_2SO_4 immediately after the addition of label. Counting efficiency and quench corrections were determined by the use of internal standards. Overall efficiency averaged 44%. Glutamic acid utilization rates were calculated by linear regression analysis of uptake curves (see Fig. 4).

Experiments to determine the growth rates of the mixed population of culturable bacteria present in gut sections UM48 B and E were conducted simultaneously with ^{14}C-uptake experiments, again using incubation conditions of 3°C and both in situ and atmospheric pressure. Procedural details have been described elsewhere (4, 33). Unlabeled glutamic acid was added to each sample at a final concentration of 5 μg/liter, making sample treatment for growth experiments equivalent to that for [^{14}C]glutamic acid utilization experiments. At approximately daily intervals during a 7-day incubation period, duplicate subsamples were diluted serially in AWS and plated, in duplicate, on 2216 marine agar (Difco Laboratories, Detroit, Mich.). All manipulations were conducted in the shipboard coldroom. Plates were incubated at 3°C for 6 weeks before enumerating total colony-forming units. A mean population generation time was calculated from the logarithmic portion of each growth curve, using linear regression analysis and the following equation: $[t - t_0]/G = [3.3 \log (n_t/n_0)]$ where G is the generation time in hours, $t - t_0$ is the time interval in hours during which logarithmic growth occurred, and n_t/n_0 is the increase in colony-forming units per gram (dry weight) of sediment during that time interval.

The percentage of the total bacterial number responsive to nutrient enrichment was determined by the method of Kogure et al. (18). Yeast extract and nalidixic acid were added to the sample to give a final concentration of 0.025 and 0.002%, respectively. Two-milliliter samples were loaded into sterile 3-ml syringes (Becton, Dickinson & Co., Rutherford, N.J.). Each syringe contained a sterile glass bead for mixing and was fitted with a sterile 20-gauge needle inserted into a rubber stopper after sample loading. Syringes were incubated at 3°C and 440 atm, with a duplicate set held at 3°C and 1 atm. After an incubation period of 3 days, all samples were fixed in a final concentration of 2% formaldehyde and stored at 3°C. At the university laboratory, samples were stained with acridine orange and examined by epifluorescence microscopy, as described above. The number of yeast extract-responsive bacteria, i.e., abnormally enlarged, orange-fluo-

TABLE 1. Bacterial concentrations, determined by epifluorescence microscopy, in boxcore surface sediments and sediments recently ingested by holothurians (gut samples from Fig. 2A)

Station	Boxcore deployment depth (m)	Bacteria (10^8 ± SD) per g (dry wt) of sediment		Trawling depth (m)	Holothurian specimens collected	Bacteria (10^8 ± SD) per g (dry wt) of sediment	
		Individual boxcore	Mean (±SEM) for each station			Individual animal	Mean (±SEM) for animals from the same trawl
A	4,440	10.7 (±1.58)	9.82 (±0.44)	4,438	UM43	3.57 (±0.85)	
	4,430	10.0 (±1.99)					
	4,434	9.97 (±2.63)					
	4,440	8.60 (±2.20)					
B	4,850	4.40 (±1.07)	6.20 (±1.39)	4,830	UM58	9.38 (±1.89)	9.10 (±0.37)
	4,850	7.24 (±1.92)			UM59	9.55 (±1.27)	
	4,850	7.35 (±2.58)			UM60	8.37 (±1.88)	
	4,850	5.81 (±1.81)		4,850	UM51	18.5 (±6.57)	17.6 (±0.90)
					UM52	16.7 (±6.20)	

rescing bacteria, as well as the total number of bacteria (including yeast extract-responsive bacteria), was determined for each slide preparation. Total numbers of bacteria were not significantly different from counts obtained for separate, untreated samples fixed in formaldehyde immediately after dissection.

Electron microscopy. Small portions of the intestinal wall from sections B and E of holothurian UM48 were rinsed in ASW and fixed in a final concentration of 2% glutaraldehyde for subsequent examination by electron microscopy. Tissue samples were postfixed in 1% OsO_4 for 1.5 h, dehydrated in an ethanol series, and embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate, examined in a Zeiss EM9S-2 transmission electron microscope, and photographed with DuPont COS7 film.

Microbial analysis of boxcore sediments. In addition to holothurian specimens, abyssal surface sediments were collected at stations A and B, using a 0.25-m^2 boxcore. Immediately upon retrieval, overlying seawater was removed carefully by siphon. Undisturbed surface sediment was sampled aseptically to a depth of 1 cm and removed to the coldroom for further manipulations. Total numbers of bacteria per gram (dry weight) of sediment were determined for sediment samples from four separate boxcores at each station, as described above for gut samples. A ninth sediment sample, collected at the same location and depth from which holothurian UM48 was retrieved, was analyzed in the same manner as gut sections UM48 B through E to determine: (i) the microbial utilization of glutamic acid, using a final added concentration of 0.14 μg of [^{14}C]glutamic acid per g (dry weight) of sediment; (ii) the mean generation time for the mixed population of bacteria culturable on marine agar at 3°C; and (iii) the percentage of the total bacterial number responsive to

TABLE 2. Microbial activity in sediment samples removed from the upper centimeter of a boxcore and from gut sections of holothurian UM48[a]

Sample	Glutamic acid utilization rate (ng/g [dry wt] of sediment per day)[b] at atm:		Mean generation time for culturable bacteria (h)[c] at atm:		% Yeast extract-responsive bacteria[d] at atm:	
	1 (r)[e]	440 (r)[e]	1 (r)[e]	440 (r)[e]	1	440
Sediment	8.72 (0.997)	10.2 (0.980)	13 (0.90)	12 (0.99)	0.0	0.4
UM48 B	1.70 (0.877)	39.6 (0.999)	2.6 (0.93)	4.2 (0.87)	0.0	0.0
UM48 C	ND[f]	ND	ND	ND	0.9	0.9
UM48 D	ND	ND	ND	ND	0.0	19.7
UM48 E	67.4 (0.997)	147 (0.993)	7.9 (0.99)	2.4 (0.99)	0.4	21.1

[a] Both boxcore and holothurian were collected at station B (4,440 m). Sediment samples were incubated at 3°C and both 1 and 440 atm.

[b] The total microbial utilization of glutamic acid was calculated as uptake and incorporation (radioactivity associated with filtered, washed sediment) plus respiration (evolved $^{14}CO_2$). The percent respiration ranged from 80 to 94%.

[c] Calculated according to the equation given in the text.

[d] The percentage of the total bacterial number, determined by epifluorescence microscopy, that was responsive to yeast extract in the presence of nalidixic acid, i.e., abnormally enlarged and orange-fluorescing bacteria. The counting procedure used did not permit the detection of yeast extract-responsive bacteria if their numbers were <0.005% of the total bacterial count.

[e] Correlation coefficient, determined by linear regression analysis of substrate utilization (Fig. 4) and growth curves.

[f] ND, Not determined.

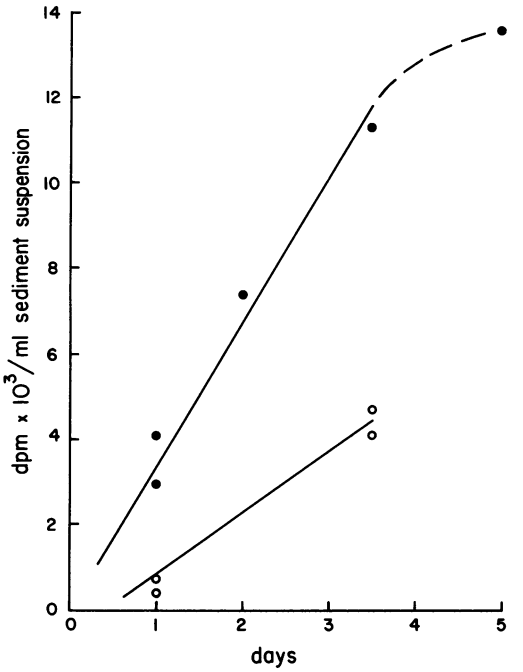

FIG. 4. Total microbial utilization (uptake and incorporation plus respiration) of [^{14}C]-glutamic acid in sediment removed from gut section E of holothurian UM48, after incubation at 3°C and 1 atm (○) and 440 atm (●). Duplicate subsamples were not examined at each time interval due to the limited amount of original sample available.

yeast extract in the presence of nalidixic acid. In all cases, duplicate subsamples were incubated at 3°C under pressures of 440 and 1 atm.

RESULTS

Bacterial counts. Bacterial profiles of the holothurian digestive tract, based on the total number of bacteria per gram (dry weight) of sediment in each gut section, are shown in Fig. 3. Error bars, indicating ± one standard deviation of the mean count, reflect the inaccuracy inherent in the microscopic enumeration of bacteria associated with sediment particles. The average coefficient of variation of the mean for samples examined in triplicate was 16%. A marked reduction in the concentration of bacteria, ranging from 59 to 92%, was observed in the "foregut" of each animal, i.e., between mouth section A and sections B and C. Although bacterial numbers stabilized in specimens UM51 and UM58, counts in the other animals increased 3- to 10-fold in "hindgut" sections C through E.

The total number of bacteria per gram (dry weight) of sediment observed in the upper centimeter of sediment boxcores collected at the same stations sampled by beam trawl for benthic animals is given in Table 1. Concentrations of bacteria in sediments recently ingested by holothurians, i.e., present in gut section A, are included for comparison. Total counts in boxcore sediments at station A were significantly different from those at station B ($P < 0.01$), with lower values observed at greater ocean depth. For sediments recently ingested by holothurians, bacterial concentrations were inversely proportional to the depth of collection and, at the deepest station which was studied more intensively, were 1.5- to 3-fold greater than numbers in the surrounding sediment.

Microbial activity. The relative microbial activities at 3°C and both 440 and 1 atm in sediment samples removed from the upper centimeter of a boxcore and from gut sections of the digestive tract of holothurian UM48 are shown in Table 2. The microbial utilization of glutamic acid was linear during the first 3 days of incubation at both in situ and atmospheric pressure in all samples examined (Fig. 4), although little significant activity was detected in gut section B at 1 atm. Pressure enhanced the utilization rate of all of the samples, but especially in the holothurian gut samples. The most rapid rate at 3°C was observed in sediment removed from the hindgut of the holothurian and repressurized to 440 atm (Table 2, Fig. 4). Similarly, the most rapid generation time for the mixed population of culturable bacteria was observed in hindgut sediments, when incubated under in situ conditions. Logarithmic growth of bacteria in gut samples occurred during the first 3 days under both in situ and atmospheric pressure. Growth in boxcore sediment samples was detected only after a 3-day lag period under both incubation conditions. The initial number of colony-forming units per gram (dry weight) of sediment in boxcore, foregut (UM48B), and hindgut (UM48E) sediments was 1,060, 2,000, and 4,600, respectively.

Only a small fraction of the total bacterial count (0.0 to 0.9%) was responsive to yeast extract in either boxcore or sediment at 3°C and 1 atm. Under in situ pressure of 440 atm, however, a substantial increase (up to 21.1%, i.e., 3.78×10^8 yeast extract-responsive bacteria per g [dry weight] of sediment) was observed in the hindgut sediments of the holothurian. In gut section B, where total counts of bacteria were lowest in most specimens examined (Fig. 3), no yeast extract-responsive bacteria were observed at either in situ or atmospheric pressure. It should be noted, however, that the counting procedure used allowed the detection of yeast extract-responsive bacteria only if they represented ≥0.005% of the total bacterial count.

Electron microscopy. Thin sections of the intestinal lining of the foregut (section B) and hindgut (section E) of holothurian UM48 were

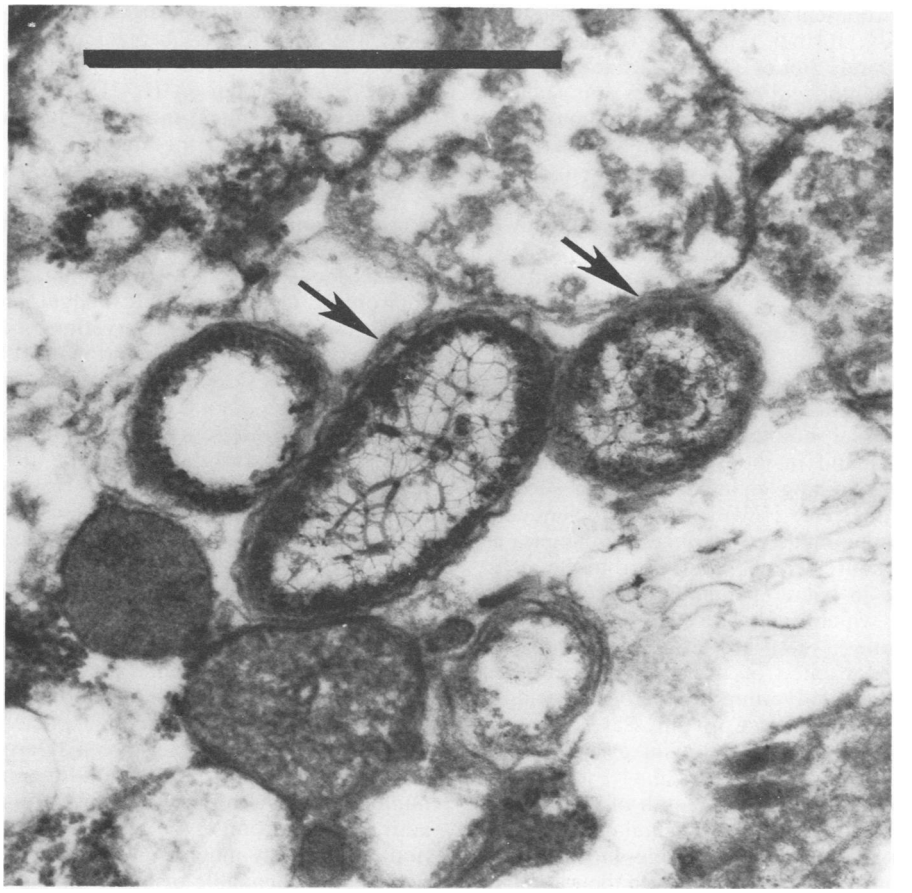

FIG. 5. Transmission electron micrograph of intact bacteria (arrows) directly associated with the digestive epithelial tissue of hindgut section E of holothurian UM48. Bar indicates 1.0 μm.

examined extensively by transmission electron microscopy. Rod-shaped gram-negative bacteria, directly associated with the digestive epithelial tissue, were observed only in section E (Fig. 5). The condition of the digestive tissue did not permit a more refined definition of this association.

DISCUSSION

The nutrition of deep-sea holothurians has been investigated by Khripounoff and Sibuet (17), who examined the gut contents of 325 abyssal holothurians collected at depths of 2,100 to 4,600 m in the Bay of Biscay. They found that concentrations of organic carbon and nitrogen in sediments recently ingested by the animals were about four and six times greater, respectively, than concentrations in environmental sediments. Although they did not determine the bacterial content of gut or environmental samples, they hypothesized that selective feeding on organically enriched particulates provided not only a direct source of nutrients for the holothurian, to be degraded and assimilated by the animal's own digestive enzymes, but also concentrated substrates that would support bacterial proliferation in the gut. Metabolites released by an active microflora, as well as by lysed bacteria, would provide nutrients for the host (6). Since the amount of organic matter available in abyssal sediments is limited (2) and considered to be largely refractory (8), the stimulation of bacterial growth and the concomitant degradation of organic molecules not readily assimilated by the holothurian could be a critical element in the nutrition of abyssal holothurians. Furthermore, if rapid microbial activity in the deep sea is indeed limited to nutrient-enriched habitats, an active gut flora in the digestive tract of abyssal holothurians continuously "mining" the ocean floor could have a major impact on deep-sea nutrient cycles in general.

The relative concentrations of bacteria in gut

and environmental sediments, as determined in this study, further refine the results of Khripounoff and Sibuet (17). At station B, four environmental sediment samples and sediments present in the mouth section of five holothurians were available for comparison (Table 1). The increased number of bacteria present in sediment recently ingested by the animals suggests that the selective ingestion of organically enriched particulates includes a selection for particles colonized by bacteria. In experiments with ^{14}C-labeled food, Yingst (38) determined that a shallow water holothurian, *Parastichopus parvimensis*, is capable of utilizing bacteria directly as a food source with a feeding efficiency of 43% (±22% standard deviation). Similar experiments with abyssal holothurians would require the collection and maintenance of the animals in pressure-retaining chambers and, as such, are not yet feasible. However, the efficiency with which the animals utilize ingested bacteria as a nutrient source can be inferred from the marked decrease in bacterial numbers observed in the foregut, which averaged 80% (±14% standard deviation).

In the absence of direct experimental evidence, the relative importance of bacterial carbon as a food source for abyssal holothurians was estimated on the following assumptions: (i) organic carbon constitutes 0.44% of 1 g (dry weight) of sediment at a depth of 4,200 m (17), (ii) abyssal holothurians assimilate 15% of the total organic carbon available in surrounding sediments, an estimate based on the total organic carbon or ingested and egested sediments (17), (iii) as many as 1.45×10^9 bacteria per g (dry weight) of sediment are "cropped" in the foregut (Fig. 3), and (iv) a deep-sea bacterium consists of 1×10^{-14} g of carbon (35). Accordingly, the contribution of bacterial carbon to the overall amount of organic carbon consumed by an abyssal holothurian was determined to be about 3%. The conversion factor for cell carbon is a conservative estimate used by Williams et al. (35) for bacteria present in deep-sea water samples and, therefore, may be an under-estimate for bacteria in abyssal sediments, where nutrients are more concentrated than in the water column. Nevertheless, it appears that sediment bacteria, though utilized with great efficiency by an abyssal holothurian, provide only a minor source of the total caloric intake (1, 9, 38). Not obvious from calculations of this sort is the fact that the quality of organic matter ingested can be more important than the quantity, i.e., bacteria may provide the deposit-feeder with essential nutrients (nitrogen, vitamins, and trace elements) not otherwise available (5, 20, 24).

The holothurian foregut has been described as the site of intracellular digestion and mucoid biosynthesis, whereas absorption and metabolite transport are believed to occur to a greater extent in the hindgut (7, 17, 21). In the abyssal holothurians examined in this study, an important component of the ingested bacterial population appears to survive the initial digestive processes of the foregut and to flourish in the hindgut (Fig. 3). Examination of the intestinal lining by transmission electron microscopy (Fig. 5), as well as measurements of microbial activity in environmental, foregut, and hindgut sediments (Table 2), supports this observation.

We recognize that some caution must be exercised in a direct comparison of glutamic acid utilization rates among different sample types, due to possible differences in naturally occurring concentrations of glutamic acid or in bacterial community size and structure. However, the results of ^{14}C-utilization experiments conducted in duplicate at both 440 and 1 atm can be compared directly for a given sample and clearly indicate a barophilic microbial response in the hindgut (Fig. 4). The results of growth studies and measurements of yeast extract-responsive bacteria by epifluorescence microscopy (Table 2) provide independent confirmation of the presence of a population of barophilic bacteria, even though each method is based on a different set of criteria. A population generation time, based on numbers of colony-forming units, reflects the activity of a portion of the total bacterial population, presumably much smaller and more selective than that capable of metabolizing glutamic acid. Certainly, the bacterial population culturable on marine agar at 3°C represented a negligible percentage ($<1 \times 10^{-5}$) of the total bacterial count determined by epifluorescence microscopy. The method of Kogure et al. (18) detects a significantly larger number of metabolically active bacteria than do culturing methods. However, the method involves sample enrichment with a final concentration of 25 mg of yeast extract per ml, providing a measure of potential or induced activity, rather than relatively undisturbed or in situ activity. The substitution of tracer levels of glutamic acid for enriching levels of yeast extract would have provided an experimental approach more compatible with the separate measurements of glutamic acid utilization and doubling time of culturable bacteria. However, Peele and Colwell (26) demonstrated that the use of nalidixic acid alone, or with the addition of a single substrate, as opposed to the complexity of nutrients in yeast extract, is ineffective in producing an optimal or even detectable number of abnormally enlarged, orange-fluorescing bacteria.

Microbial activity as measured in this study can be applied to the broader purpose of estimat-

ing the rate at which an abyssal holothurian feeds, if the following assumptions are valid: (i) sediment passes through a digestive tract of constant diameter without appreciable mixing, at a constant rate; and (ii) the increase in bacterial number in the hindgut is due to a constant growth rate. Using holothurian UM48 as an example and assuming bacterial numbers doubled every 3 hours as sediment moved through the distal portion of the gut (60% of the total gut volume), we calculated a sediment turnover time of 16 h for a total gut content of 70 g (wet weight) of sediment, i.e., about 105 g/day. This estimate is remarkably similar to that of Rowe (27), who used feces size and rate of production and the movement of a holothurian across the ocean floor, revealed by bottom photographs (10), to estimate that 100 g of sediment can pass through a single animal per day. These independent estimates of sediment turnover time indicate that abyssal holothurians and their associated gut flora have an important impact on the recycling of organic matter that has settled to the ocean floor and that relatively simple microbiological techniques can be used in understanding nutritional and behavioral characteristics of benthic animals otherwise difficult to observe and study in the inaccessible depths of the ocean.

The composite data from this study support the following hypothesis. Abyssal holothurians ingest sediments containing an abundance of bacteria which provide a minor, though probably stable and nutritious, food source. As organically enriched sediment particles are concentrated and passed through the holothurian, the digestive tract functions as a selective environment for barophilic bacteria, uniquely adapted for optimal growth and activity under deep-sea pressure. A portion of these bacteria are directly associated with the digestive tissue of the hindgut and may, in fact, be carried as a resident gut flora. Metabolites released by an active microflora provide additional nutrients for the host, an animal otherwise dependent on the limited and largely refractory sources of organic carbon available in abyssal sediments. More quantitative studies of the microbial transformation of sedimented organic carbon that passes through the digestive tracts of abyssal benthic animals should add new meaning to mass carbon budgets (28, 32) that imply significant biological utilization in the deep sea by balancing nonbiological parameters that can be measured directly, i.e., carbon flux to the deep sea and ultimate burial in abyssal sediments.

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