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ROOTS AS A SITE OF HYDROGEN SULFIDE UPTAKE IN THE HYDROCARBON SEEP VESTIMENTIFERAN *LAMELLIBRACHIA* SP.

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Summary

Vestimentiferan tubeworms have no mouth or gut, and the majority of their nutritional requirements are provided by endosymbiotic bacteria that utilize hydrogen sulfide oxidation to fix CO_2 into organic molecules. It has been assumed that all vestimentiferans obtain the sulfide, O_2 and CO_2 needed by the bacteria across the plume (gill) surface, but some live in locations where very little sulfide is available in the sea water surrounding the plume. We propose that at least some of these vestimentiferans can grow a posterior extension of their body and tube down into the sea-floor sediment, and that they can use this extension, which we call the 'root', to take up sulfide directly from the interstitial water. In this study of the vestimentiferan *Lamellibrachia* sp., found at hydrocarbon seeps in the Gulf of Mexico at depths of approximately 700 m, we measured seawater and interstitial sulfide concentrations in the hydrocarbon seep habitat, determined the structural characteristics of the root tube using transmission electron microscopy, characterized the biochemical composition of the tube wall, and measured the sulfide permeability of the root tube. We found that, while the sulfide concentration is less than 1 μmol l^{-1} in the sea water surrounding the gills, it can be over 1.5 mmol l^{-1} at a depth of 10–25 cm in sediment beneath tubeworm bushes. The root tube is composed primarily of giant β-chitin crystallites (12–30 % of total mass) embedded in a protein matrix (50 % of total mass). Root tubes have a mean diameter of 1.4 mm, a mean wall thickness of 70 μm and can be over 20 cm long. The tubeworm itself typically extends its body to the distal tip of the root tube. The root tube wall was quite permeable to sulfide, having a permeability coefficient at 20 °C of 0.41×10^{-3} cm s^{-1}, with root tube being 2.5 times more permeable to sulfide than trunk tube of the same diameter. The characteristics of the root suggest that it reaches down to the higher sulfide levels present in the deeper sediment and that it functions to increase the surface area available for sulfide uptake in a manner analogous to a respiratory organ.

Key words: *Lamellibrachia* sp., hydrogen sulphide, sulphide, hydrocarbon seep, vestimentiferan, tubeworm, root tube, chitin.

Introduction

Vestimentiferan tubeworms are one of the dominant faunal types at many hydrothermal vents and hydrocarbon seeps in the deep sea (Fisher et al., 1997; Gage and Tyler, 1991). Vestimentiferans lack a mouth or gut and possess a large, well-vascularized trophosome sac housing a dense colony of sulfide-oxidizing bacteria (Jones, 1981, 1985). Sulfide, which is obtained from the surrounding environment, is delivered by the tubeworm's circulating hemoglobin to the trophosome, where the bacteria fix CO_2 into organic molecules using sulfide as the energy source (Arp et al., 1987; Childress and Fisher, 1992; Fisher et al., 1988). In this symbiotic relationship, the bacteria provide the host tubeworm with the majority of its nutritional needs (Brooks et al., 1987; Fisher, 1990). Vestimentiferans live in a tube composed of giant β-chitin crystallites embedded in a protein matrix (Gaill et al., 1992a). The tube, which grows as the tubeworm elongates, provides external structural support for the tubeworm and protection from predation and from environmental challenges. The chitin of the vestimentiferan tube is secreted from specialized glands located in the body wall, along the entire length of the trunk and in the vestimentum and opisthosome (Gaill et al., 1997; Shillito et al., 1995b).

To maintain their metabolism and to support symbiotic bacterial chemosynthesis, vestimentiferans must extract O_2, CO_2 and sulfide from their surroundings and supply these molecules to their bacterial symbionts. At hydrothermal vent sites, vestimentiferans such as *Riftia pachyptila* are bathed in rapidly venting hydrothermal fluid that is continuously mixing with the surrounding sea water. In this animal, a set of highly vascularized gills called the respiratory plume or branchia, located at the anterior of the tubeworm, is the primary site of sulfide, O_2 and CO_2 uptake (Arp et al., 1985; Goffredi et al.,

1997a,b). Once these molecules cross the plume epithelium, they are transported in a closed circulatory system to the trophosome. While CO_2 is carried in the vascular blood in solution (Goffredi et al., 1997b), sulfide and O_2 are both transported in the blood by an extracellular hemoglobin of high molecular mass that binds sulfide and O_2 at separate sites (Arp et al., 1987; Zal et al., 1997).

Differences in the mechanism of sulfide production at hydrocarbon seeps compared with hydrothermal vents result in a difference in the availability of sulfide between the sites. At hydrothermal vents, sulfide is produced by a series of chemical reactions resulting from the passage of superheated sea water through the basalt that comprises the oceanic crust beneath the sea floor (Edmond and Von Damm, 1983; Hekinian, 1985). This hot, sulfide-containing water then issues rapidly from vents in the sea floor and rises in the water column as result of the lower density of the high-temperature fluid. In contrast, at hydrocarbon seeps, sulfide is primarily produced biogenically in the anoxic sediment just below the sea floor as a result of bacterial reduction of sulfate, which utilizes methane or other hydrocarbons as the energy source (Carney, 1994). The sulfide at hydrocarbon seeps is then brought up to the sea floor surface by convection of the interstitial water, probably induced by the upward migration of methane and other hydrocarbons. Irrespective of the source, as sulfide-containing water mixes with oxygenated sea water, the sulfide and O_2 immediately begin to react, forming thiosulfate and other thiols. Because the interstitial fluid is not buoyant and is not flowing rapidly into the water column, it is likely that very little sulfide at the hydrocarbon seeps persists long enough to diffuse up to the level of adult vestimentiferan plumes. Thus, at hydrocarbon seeps, the highest concentrations of sulfide are likely to be in the anoxic, interstitial water of the sediment beneath the tubeworm colonies. This was noted by MacDonald et al. (1989), who did not detect sulfide at levels above 3 $\mu mol\,l^{-1}$ in most of the large-volume bottom-water samples they analyzed at one hydrocarbon seep area; from this, they concluded that sulfide release was episodic, that the tubeworms were able to utilize trace concentrations of sulfide or that the tubeworms were somehow obtaining sulfide from buried portions of their tubes.

In the present study, we propose that *Lamellibrachia* sp. take up sulfide directly from the anoxic, interstitial water using a long, narrow thin-walled extension of the tube that reaches posteriorly down into the sediment. We name this extension the ‘root’ because of the functional and morphological analogy it may have with plant roots. This extension has not been recognized in previous anatomical descriptions of *Lamellibrachia* sp. or other vestimentiferans (Desbruyères and Segonzac, 1997), although we have previously presented the concept (MacDonald and Fisher, 1996). We now distinguish between the ‘trunk tube’, which is the familiar portion of the tube anterior to the point of substratum attachment, and the ‘root tube’, which is the portion of the tube posterior to the point of substratum attachment. To begin to test the hypothesis that *Lamellibrachia* sp. is capable of using the root for sulfide uptake, sulfide concentrations in the sea water surrounding the tubeworms and in the interstitial water of the sediment surrounding the roots were measured, the composition and ultrastructure of the root and trunk tubes were compared, and the sulfide permeability of *Lamellibrachia* sp. root and trunk tube were determined *in vitro*.

Materials and methods

Animal collection and maintenance

Lamellibrachia sp. were collected by submersible from three previously described hydrocarbon seep sites on the Louisiana Slope in the Gulf of Mexico: Bush Hill, GC 234 and GC 272 (MacDonald et al., 1989; Nix et al., 1995). The animals were brought to the surface in a temperature-insulated container and then immediately placed in chilled, aerated sea water on board the ship. At the end of the cruise, seven tubeworms, each approximately 0.3–0.5 m in length, were brought back to the laboratory. These were maintained at 9 °C in filtered, aerated sea water. Boluses of sodium sulfide were added approximately three times weekly to achieve a final concentration of 25 $\mu mol\,l^{-1}$. No additional maintenance was required, other than periodic exchanges of the sea water, and there was no mortality over the 4 month period between collection and dissection.

Water sampling

To characterize the sulfide concentrations of the hydrocarbon seep habitat around *Lamellibrachia* sp., we used the hydraulic arm of the submersible to position water-sampling devices at several different locations within tubeworm colonies. Small (approximately 1 ml) water samples were taken using devices similar to those described by Nix et al. (1995). Water samples from the region adjacent to the tubeworm plumes and from the region among the tubes, half-way between the plumes and the sediment surface, were taken using a wand sampler with a 10 µm Nalgene filter at the end. Interstitial water samples from a sediment depth of 10 cm were taken using the sampler described by Nix et al. (1995), which resembles a large thumbtack with the sample drawn from a point 10 cm below the surface plate through a fritted stainless-steel filter. This was inserted near the base of a tubeworm colony until the surface plate was resting against the sediment surface. Samples taken from deeper than 10 cm beneath tubeworm colonies were obtained using a long tapered probe fitted with a similar inlet filter and marked with graduations on the side, which allowed the depth of penetration to be recorded. This ‘deep-probe’ sampler was inserted until it touched the buried rock to which the tubeworm colony was attached and therefore sampled water in the vicinity of the buried tubeworm roots. All samples were drawn through approximately 7 m of 0.01 inch (0.025 mm) inner diameter polyetheretherketone (PEEK) tubing into the submersible and into a syringe primed with a basic zinc acetate fixative solution to inhibit sulfide oxidation (Nix et al., 1995). These syringes were then kept on ice until analysis (always less than 8 h). The samples were

analyzed on board the ship using gas chromatography for total sulfide concentration (the sum of S^{2-}, HS^- and H_2S), as described previously (Childress et al., 1984).

Structural and biochemical analyses

All tube samples used for ultrastructural and biochemical studies were air-dried after collection. Samples for structural studies were fixed with buffered 3 % glutaraldehyde, post-fixed in 1 % OsO_4 and embedded in Araldite resin, as previously described (Gaill and Hunt, 1986). Thin sections were obtained using a Reichert-Jung ultramicrotome and stained with uranyl acetate and lead citrate. Observations were carried out using a Zeiss 912 transmission electron microscope operating at 100 kV.

For biochemical analysis, pieces of air-dried tubes were carefully rinsed several times in distilled water and then dried *in vacuo* over NaOH and weighed. Samples were then placed in 0.5 mol l^{-1} HCl for 4 h at 20 °C, washed in distilled water and dried. Finally, samples were placed in 0.5 mol l^{-1} NaOH for two 3 h treatments at 100 °C. Proteins were assayed in NaOH extracts using standard techniques (Lowry et al., 1951), with modifications as described previously (Schacterle and Pollack, 1973). Chitin was assayed enzymatically in residues after HCl and NaOH extractions (Gaill et al., 1997; Jeuniaux, 1963). For the determination of the amino acid composition of tube fragments, approximately 5 nmol of tube protein was first hydrolyzed in 6 mol l^{-1} HCl at 107 °C for 24 h in evacuated, sealed tubes. This was followed by amino acid analysis on a Dionex DC 300 amino acid analyzer fitted with a high-performance liquid chromatography (HPLC) polystyrene sulfonic ion-exchange column (0.4 cm×25 cm) and a Waters model 440 dual-wavelength absorbance detector. The small size of some samples precluded complete amino acid analysis.

Sulfide permeability

The permeability of tube sections was measured using either a flow-through method or a recirculating method. Both trunk and root tube sections used in the recirculating method were obtained from freshly collected animals. Trunk tube sections used in the flow-through method were from animals that had been maintained in the laboratory for several months. Root tube sections used in this system were from tube sections that had been attached to rocks that were brought up by the submersible. These had been immediately frozen on the ship and kept at −20 °C until used for permeability measurements 4 months later.

After a tubeworm had been removed from its tube, the tube was cut into sections ranging in length from 2 to 20 cm. These sections were selected such that each appeared reasonably homogeneous, having a similar stiffness, wall thickness and opacity throughout its length. The tubes were then maintained in cold sea water until used for permeability measurements. Immediately prior to use, the section of tube was rinsed with sea water and sealed at each end to borosilicate glass tubing using cyanoacrylate cement (Loctite 447 surface-insensitive adhesive). Each piece of glass tubing had previously been modified over a flame such that it formed a tight seal with the end of the tube. This apparatus was then placed inside an acrylic chamber, such that the two sections of glass tubing extended upwards out of the lid of the chamber (Fig. 1). To minimize movement, each piece of glass tubing was secured to the lid using rubber gaskets and compression fittings. The diffusion chamber was then filled with 900 ml of deoxygenated buffered artificial sea water (BASW) consisting of artificial sea water (Instant Ocean, Aquarium Systems Inc.) containing 50 mmol l^{-1} Tris base (Sigma). Deoxygenation was achieved by thoroughly stripping the O_2 by vigorously bubbling N_2 gas. Throughout the diffusion experiment, the chamber BASW was kept well stirred using a magnetic stir bar, and a gentle stream of compressed N_2 gas was flushed through the chamber head-space to prevent any O_2 from entering the solution.

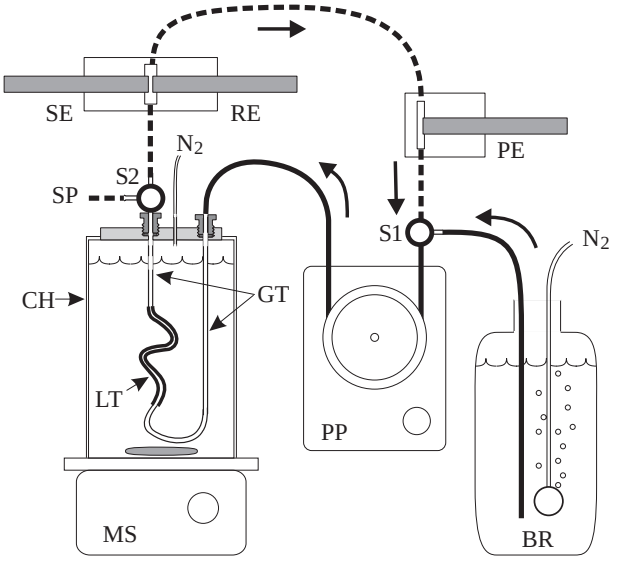

Fig. 1. Diagram of the permeability measurement system. BR, buffer reservoir; CH, chamber; GT, glass tubing; LT, *Lamellibrachia* sp. tube; MS, magnetic stirrer; PE, pH electrode; PP, peristaltic pump; RE, reference electrode; S1 and S2, three-way stopcocks; SE, sulfide electrode; SP, sampling port. For use as a flow-through system, buffer was pumped from the reservoir through the *Lamellibrachia* sp. tube and out through the sampling port. For use as a recirculating system, the reservoir and sampling port were not used, and the stopcocks were adjusted so that the buffer recirculated continuously through the electrode chambers (as indicated by the broken lines) and the *Lamellibrachia* sp. tube.

In the flow-through method, a peristaltic pump was used to pump deoxygenated BASW from a reservoir through the tube lumen at 0.4 ml min^{-1}. The tube BASW reservoir was kept deoxygenated by continuous bubbling with compressed N_2. The absence of leaks in the system was then tested under a back pressure of 3 kPa. At the initiation of each recording period, sodium sulfide was added to the diffusion chamber solution to a final concentration of 10 mmol l^{-1}. To check for a significant difference in permeability between H_2S and HS^-, both chamber and tube BASW solutions were adjusted to either

pH 7.0 or 8.0 (Julian and Arp, 1992), with the order alternating between experiments.

Initial experiments had demonstrated that the rate of sulfide influx reached a steady state within 30 min for tubes with thin walls (<0.3 mm) and within 60 min for tubes with thicker walls (0.3–0.8 mm). Accordingly, each experiment was run for at least 60 min, at which time the tube solution exiting the chamber and the diffusion chamber solution were each assayed in triplicate for sulfide concentration using a spectrophotometric assay (Cline, 1969). The chamber solution was first diluted 20-fold in deoxygenated BASW to avoid saturating the assay. In each case, the permeability measurement was then repeated at the other pH. At the end of each experiment, the length, outer diameter and wall thickness of each tube were measured using digital calipers (0.01 mm resolution). When measuring the thickness of root wall, the tube was gently compressed and the thickness of two walls, sandwiched together, was measured and divided by two.

The rate of sulfide flux, J (mol s^{-1}), across the tube in this system can be represented by $J=\dot{Q}C$, where $\dot{Q}$ is the rate of fluid flow ($cm^3\,s^{-1}$) through the tube and C is the sulfide concentration (mol cm^{-3}) of the fluid as it exits the tube. At steady state, the permeability coefficient p ($cm\,s^{-1}$) was determined from the flux rate as:

$$p = J/(S\overline{\Delta C}), \quad (1)$$

where S is the tube surface area (cm^2) and $\overline{\Delta C}$ is the mean concentration difference (mol cm^{-3}) across the tube wall. This ideal condition assumes that the rate of change in the sulfide concentration gradient was uniform across the length of the tube, which may not have been true. However, the error caused by any non-uniformity was minimal because, in all experiments, the ratio between the outer (chamber) sulfide concentration and the effluent inner (tube) sulfide concentration was never less than 20. The diffusion coefficient D ($cm^2\,s^{-1}$) was calculated from the permeability coefficient as:

$$D = ph/\alpha, \quad (2)$$

where h is the wall thickness (cm) and α is the distribution coefficient, which is unitless and can vary from 0 to 1. Since α is not known for vestimentiferan tube wall, it was assumed to be equal to 1, which yields a minimum estimate of D.

In the recirculating method, the tube solution was continuously recirculated in a loop using a peristaltic pump (Fig. 1, broken lines). The total volume of fluid in this loop, excluding the inner volume of the tube, was 4.6 ml. In-line with this loop were three electrodes: a solid-state silver sulfide electrode, a double-junction calomel reference electrode and an internal reference pH electrode. The silver sulfide electrode and the calomel reference electrode were placed in apposition to each other, with less than 2 mm separating their tips. To reduce contamination, the outer chamber of the reference electrode was flushed with fresh filling solution and the silver sulfide electrode was rinsed briefly in 2 mol l^{-1} NaOH after each experiment.

At the initiation of each experiment, sodium sulfide was added to the diffusion chamber solution to a final concentration of 1 mmol l^{-1}, and the pH was adjusted to 7.5. The tube was then flushed with a sample of the chamber solution, and this was allowed to remain in the tube for 10–15 min to allow equilibration of sulfide through the tube wall to minimize the time lag in sulfide influx that would otherwise occur. The tube was then completely flushed with sulfide-free, deoxygenated BASW (composition as described above, except with Hepes buffer instead of Tris), and the peristaltic pump was started at a flow rate of 2 ml min^{-1}. The rate of sulfide accumulation in the tube solution was then monitored and recorded at 2 Hz for 20–40 min. At the end of the experiment, the tube was removed and its dimensions were measured as described above.

The silver sulfide electrode was calibrated after each experiment with known concentrations of sodium sulfide in BASW. Because the offset was found to drift between experiments, the calibration was also used to adjust mathematically the baseline of the recordings *post hoc*. Although we were using the electrodes well below the manufacturer's suggested minimum concentration range, the slopes of the calibration curves under the controlled conditions of our experiments were uniformly very close to the ideal (–28 mV per decade change in sulfide concentration) over the range of 15 μmol l^{-1} to 5 mmol l^{-1} total sulfide (slope of linear regression for eight calibrations, –27.8 mV per decade; r^2=0.94; 95 % confidence limits for slope, –26.3 to –29.4 mV per decade). The use of similar electrodes at total sulfide concentrations as low as 0.1 μmol l^{-1} has been described previously (Vismann, 1996).

In this system, the sulfide concentration in the tube solution was initially zero and then increased gradually throughout the duration of the experiment, approaching the chamber (outer) concentration C_o. Accordingly, the permeability coefficient p was calculated as:

$$p = -v[\ln(1 - C_i/C_o)]/(St), \quad (3)$$

where v is the volume (cm^3) of the tube solution, C_i is the concentration (mol cm^{-3}) of the tube solution and t is the elapsed time (Sten-Knudsen, 1978).

To control for effects of tube diameter on the apparent permeability coefficient of sulfide, a control experiment was conducted with sections of synthetic cellulose ester dialysis tubing of two different diameters. Other than diameter, the different samples of dialysis tubing were identical in all other characteristics (10^4 M_r cut-off, Spectrum Laboratories Inc.). Membranes 2.5 and 6.4 mm in diameter were mounted on glass tubing in the diffusion chamber, and the sulfide permeability was then determined using the flow-through method as described above, except that the chamber solution contained 1 mmol l^{-1} sulfide and the experiment was run only at pH 7.5.

Statistical analyses

Except where indicated otherwise, data are presented as mean ± standard deviation (S.D.). Paired *t*-tests, least-squares linear regression and statistical comparisons between two regressions were calculated using commercial statistical

software (Glantz, 1997). Non-linear regression and chart preparation were performed using graphics software (DeltaGraph v. 4.02, SPSS Inc.).

Results

Sulfide levels in seep tubeworm habitats

The sulfide concentration is very low (undetectable) in the sea water surrounding the plumes and tubes of *Lamellibrachia* sp. in most aggregations (Table 1). Eight of nine samples from the region adjacent to *Lamellibrachia* sp. plumes (89 %) yielded no detectable sulfide. Closer to the sediment, at a point among the tubes half-way between the plumes and the sediment surface, sulfide was undetectable in 15 of 17 samples (88 %). Even at a depth of 10 cm into the sediment, sulfide was undetectable in five of 12 samples (42 %).

However, sulfide concentrations reached substantial levels in the deeper interstitial samples. At a sediment depth of 10 cm adjacent to the bases of tubeworm bushes, the mean sulfide concentration was 230 μmol l^{-1} (N=12) and reached as high as 1200 μmol l^{-1}. Samples taken from a depth of 10–25 cm beneath young tubeworm bushes had a mean sulfide concentration of 1800 μmol l^{-1} (N=4), with a range of 1400–2700 μmol l^{-1} (sulfide was detected in all samples). At a depth of 35–70 cm below the bases of older tubeworm bushes, the mean sulfide concentration was lower (170 μmol l^{-1}, N=14), and sulfide was detected in 13 of 14 samples.

Root morphology

Intact tubeworm roots have proved to be very difficult to obtain for study since they are extremely delicate and are anchored into the substratum. In medium-sized and large animals, the point of anchorage is typically buried in sediment. As a result, the roots usually break off from the trunk tubes when tubeworm bushes are pulled up from the substratum. Nonetheless, we have occasionally collected short sections of roots, especially when the worms were anchored to rocks that were retrieved by submersible.

The root of an individual *Lamellibrachia* sp. is a single, small-diameter, transparent or translucent tube that is a direct extension of the basal portion of the trunk tube. It is unclear how long the root of an adult tubeworm is, but we have retrieved roots longer than 30 cm. A single bush (or 'aggregation') of tubeworms can contain thousands of individuals, and the roots of these tubeworms become tangled together. The roots of several individual tubeworms can frequently be found grouped together into 'ropes' that travel down the empty tubes of dead tubeworms or run through holes in rocks (it is unclear whether the roots have grown through pre-existing holes in the rock or have somehow dissolved away the rock). We generally find that the tubeworm's body extends well into its root. Anatomically, this portion of the tubeworm body appears similar to, although much narrower than, the body in the trunk. For example, the body in the root is typically well vascularized and contains both coelomic fluid and trophosome.

Tube structure

The root tube does not appear to change in diameter or wall thickness with distance from the trunk tube. In the longest sections that were analyzed, both the diameter and the wall thickness were relatively unchanged along the entire length. Unlike the root, and unlike the tube of *R. pachyptila*, the trunk tube of *Lamellibrachia* sp. is roughly conical, increasing in diameter anteriorly from the point of substratum attachment. We took three *Lamellibrachia* sp. trunk tubes from the same bush, each similar in size and complete except for the root, and measured the outer diameter at 2 cm intervals along the length of the tubes. The change in tube diameter was found to be approximately linear with respect to length, with little variability between the three individuals (Fig. 2).

The outer diameter of the tube samples used for permeability measurements ranged from 0.95 mm for roots (mean = 1.4 mm) to 6.3 mm near the anterior opening of the trunk tube. Wall thickness ranged from 0.03 to 0.12 mm for root tubes (mean = 0.07 mm), and from 0.06 to 0.75 mm for trunk tubes. Trunk tube wall thickness was linearly correlated with diameter (Fig. 3, r^2=0.89, P<0.001, N=28). Thus, the most anterior trunk

Table 1. *Sulfide levels in the habitat of the seep tubeworm*

Location	Number of samples	Range (μmol l^{-1})	Mean concentration (μmol l^{-1})	Samples with undetectable sulfide	
				Number	Percentage
Sea water; near plumes[a]	9	ND to 15	2	8	89
Sea water; among trunk tubes[b]	17	ND to 8	1	15	88
Interstitial; 10 cm depth[c]	12	ND to 1200	230	5	42
Interstitial; base of young bushes[d]	4	1400–2700	1800	0	0
Interstitial; base of mature bushes[e]	14	ND to 1100	170	1	7

Not detectable (ND) indicates that the sulfide concentration was below the detection limit of the gas chromatograph (approximately 2 μmol l^{-1}).

[a]Samples taken from sea water surrounding tubeworm plumes; [b]samples taken from among the tubeworm trunk tubes, approximately half-way between the sediment surface and the plumes; [c]interstitial samples taken using a 'thumbtack' sampler at a depth of 10 cm below a tubeworm colony; [d]interstitial samples taken using a deep-probe sampler at an interstitial depth of 10–25 cm at the buried bases of young tubeworm bushes; [e]interstitial samples taken using a deep-probe sampler at an interstitial depth of 35–70 cm at the buried bases of mature bushes.

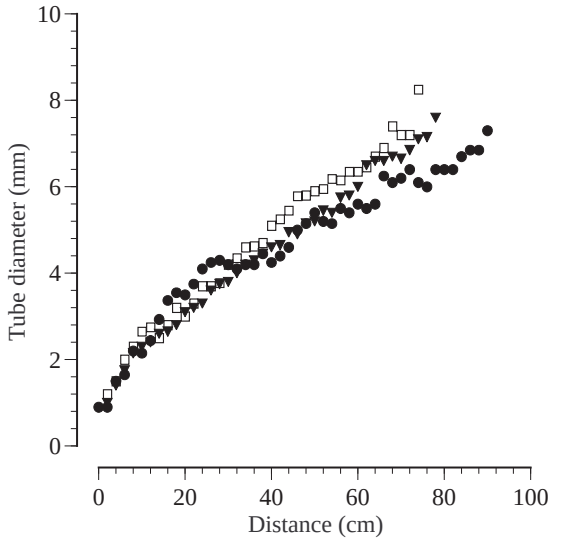

Fig. 2. Increase in *Lamellibrachia* sp. trunk tube outer diameter with length from the point of substratum attachment. The diameter was measured at 2 cm intervals, with the three different symbols (circles, squares and triangles) representing individual measurements from three different tubes. The tubes were from animals of similar size and from the same bush.

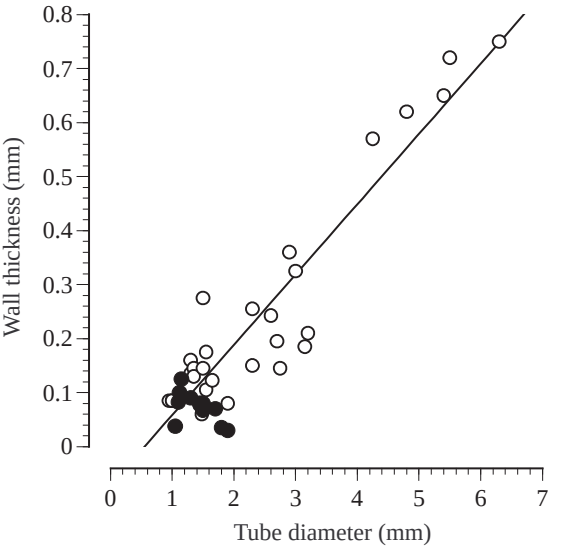

Fig. 3. Relationship between wall thickness and tube outer diameter in representative *Lamellibrachia* sp. tube sections. Filled symbols represent root tubes and open symbols represent trunk tubes, with each data point representing a single measurement. The regression line (y=0.13x–0.072) is drawn through all trunk tube data (r^2=0.89, P<0.001, N=28).

tube sections are both larger in diameter and thicker-walled than those at the posterior. In contrast, the wall thickness of root tubes tended to be negatively correlated with diameter, although the correlation was weak (r^2=0.33, P=0.053, N=12). Near the point of transition from trunk tube to root tube, which is presumably at the point of substratum attachment, there is some overlap in dimension between the trunk tube and the root tube, as both have similar diameters and wall thicknesses. Despite this, the roots are easy to recognize because the walls are translucent and flexible. This is in contrast to the trunk tube, which is opaque and comparatively inflexible (even that part closest to the root).

Lamellibrachia sp. tube wall, like that of *R. pachyptila*, is made of layers of chitin crystallites embedded in an amorphous protein matrix (Shillito et al., 1995a), forming a plywood-like structure (Gaill et al., 1992a). The orientation of the crystallites is parallel within one layer, but varies from one layer to the next without any significant periodicity. This differs from the organization of chitin in Crustacea, in which the layers are organized in a regular twisted system with long-range periodicity (Bouligand, 1971; Gaill et al., 1992a). When viewed using an electron microscope, the wall appears as electron-lucent fibrils embedded in a dark matrix. Fig. 4 illustrates the variation in the tube wall structure along the tube length from the anterior-most trunk tube wall (Fig. 4A) to the root tube wall (Fig. 4D). There are a number of structural differences between the trunk tube wall and the root tube wall. First, the lamellated structure of the trunk tube wall is not as apparent in the root tube. Second, the electron-lucent fibril regions, which have been shown to be chitin crystallites in other vestimentiferans (Gaill et al., 1992a), are not present in the distal root tube wall. Finally, the staining intensity of the matrix, which is correlated with the protein content (Shillito et al., 1995a), is decreased in the root tube wall.

Trunk and root tube also differ in their association with microorganisms and in the presence of mineral particles on the outer surface. In trunk tube, both the outer surface and the wall matrix were occasionally colonized by bacterial populations (not shown). Such associations were not seen in the root tube. In addition, the root tube was often surrounded by mineral particles on the outer surface (not shown).

Both root tube wall and trunk tube wall are principally composed of protein and chitin (Fig. 5). On the basis of a small data set (N=8), the chitin content appears to decrease from the trunk tube to the root tube (P=0.040 by t-test; linear regression r^2=0.55, P=0.036). In contrast, the protein content is relatively constant at approximately 50 % throughout the whole tube length, with no apparent dependence on tube type or diameter (P=0.80 by t-test; linear regression r^2=0.01, P=0.84). In the root tube, 10–45 % of the tube wall is not accounted for by chitin or protein. Mineral particles are probably the primary source of this discrepancy.

Lamellibrachia sp. tube is characterized by a high content of glycine, cysteine, asparagine, arginine and tyrosine (Table 2) that is consistent throughout the trunk tube and root tube (not shown), indicating that the protein fraction of the tube does not differ along its length. The amino acid content of *Lamellibrachia* sp. tube is similar to that of trunk tubes analyzed from the vestimentiferans *R. pachyptila*, *Tevnia jerichonana* and *Ridgeia piscesae* (Gaill and Hunt, 1986, 1991).

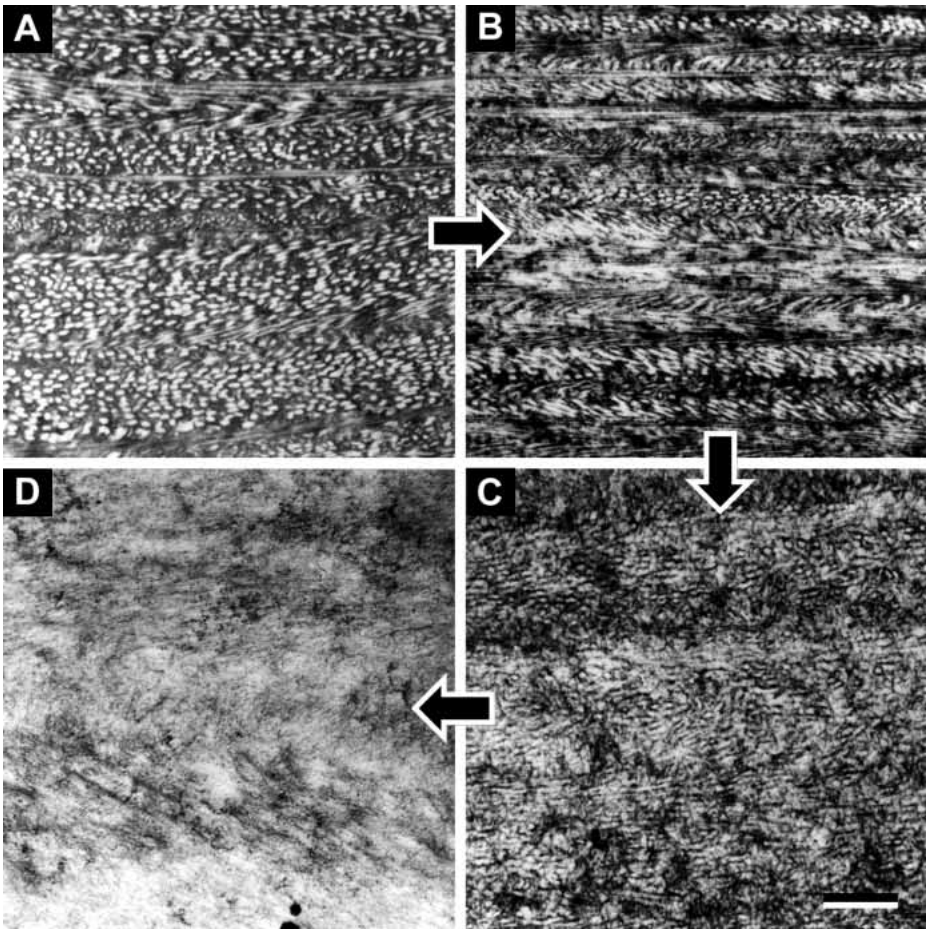

Fig. 4. Transmission electron microscope images of cross sections of *Lamellibrachia* sp. tube wall. Sequential images are from samples at four positions along the tube length from the anterior-most trunk tube wall (A) to the root tube wall (D). Scale bar, 10 μm.

Sulfide permeability

Sulfide influx was measured in 18 tube sections from five individuals at pH 7.0 and 8.0 using the flow-through method, and in 22 tube sections from six individuals at pH 7.5 using the recirculating method. In theory, each ionization species of sulfide has a specific permeability coefficient p_{H_2S} and p_{HS^-} (the contribution of S^{2-} is assumed to be negligible at the pH range used in these experiments). Thus, for a given section of tube, if the permeability to H_2S is substantially different from that to HS^-, then the measured total sulfide permeability would be dependent on the test pH (Julian and Arp, 1992). This was tested by measuring the total sulfide permeability at pH 7.0 and pH 8.0 for individual tube sections in the flow-through system. There was no significant effect of pH on the rate of sulfide influx (P=0.65 by paired t-test), indicating that H_2S and HS^- are equally permeable through the tube wall. For the remainder of the analyses, the permeability coefficients at the two pH conditions for each tube section measured in the flow-through system were averaged.

Regardless of measurement technique, sulfide permeability was strongly dependent on wall thickness, with the thinnest tubes having a substantially higher permeability (Fig. 6). Because the range of wall thickness for the tube sections used in the flow-through method (0.030–0.75 mm) was greater than that of tube sections used in the recirculating method

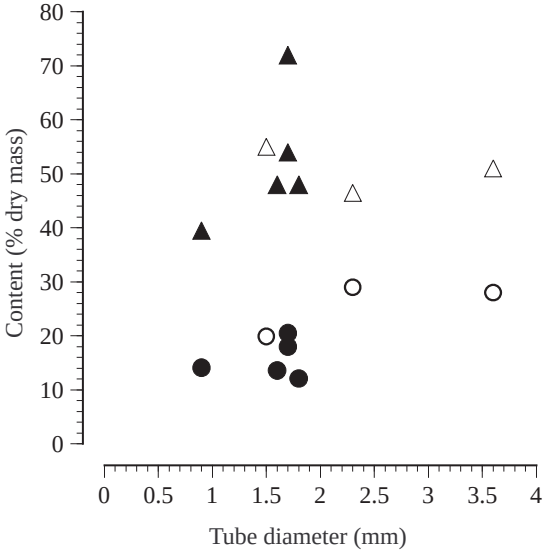

Fig. 5. Protein and chitin content of the tube wall as a function of tube outer diameter. Protein (triangles) and chitin (circles) are expressed as a percentage of tube total dry mass, with filled symbols representing the root tube and open symbols representing the trunk tube. Each data point represents a single protein or chitin measurement from one of eight tube sections (five root sections and three trunk sections).

Table 2. *Comparison of trunk tube amino acid composition between* Lamellibrachia *sp. and other vestimentiferans*

Amino acid	*Lamellibrachia* sp.	*Riftia pachyptila*[a]	*Tevnia jerichonana*[b]	*Ridgeia piscesae*[b]
Asp	10.8±0.84	11.1	12.6	11.7
Thr	4.1±0.94	4.9	3.1	4.1
Ser	7.9±0.86	7.5	3.7	4.1
Glu	6.0±0.52	7.3	8.6	9.1
Pro	5.1±0.42	5.8	4.8	5.0
Gly	11±0.76	14.4	13.9	12.7
Ala	4.0±1.3	7.8	4.9	5.5
Cys	11±0.81	9.8	5.4	3.6
Val	5.4±0.50	7.2	7.1	6.8
Met	+	0.2	0	0
Ile	2.1±0.42	3.2	2.3	3.0
Leu	3.2±0.28	3.9	4.0	4.4
Tyr	9.5±1.2	5.3	9.0	8.5
Phe	3.9±0.61	0	4.4	4.4
Lys	4.6±0.63	6.2	Trace	4.1
His	1.7±0.23	2.4	4.6	4.1
Arg	9.5±0.76	5.9	11.5	8.7

Amino acid contents are expressed as the number of residues per 100 total residues (mean ± S.D.).

The number of samples was seven for each analysis of *Lamellibrachia* sp. tube except for Ile (N=6) and Tyr (N=5).

Met was present in all *Lamellibrachia* sp. samples (+), but was not quantified.

[a]From Gaill and Hunt (1986); [b]from Gaill and Hunt (1991).

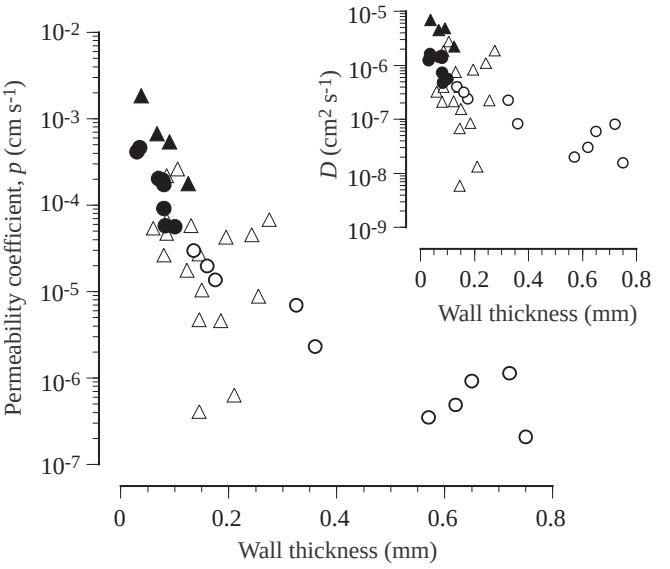

Fig. 6. Sulfide permeability of *Lamellibrachia* sp. tubes. Filled symbols represent root tube sections, and open symbols represent trunk tube sections. Circles represent flow-through measurements, and triangles represent recirculating measurements. Each data point for the recirculating experiments represents the mean of two permeability measurements (one at pH 7.0 and the other at pH 8.0). Each data point for the flow-through experiments represents a single permeability measurement (at pH 7.5). The diffusion coefficient (D, inset) was calculated by multiplying the permeability, p, by the wall thickness.

(0.037–0.27 mm), the flow-through measurements yielded a broader range of permeability coefficients. Linear regression of log$p$ against wall thickness yielded reasonable fits for the flow-through measurements (r^2=0.63, P<0.001) and recirculating measurements (r^2=0.26, P<0.016), which were not significantly different (P=0.36 for the slopes and P=1.0 for the regressions). Combining the data from the flow-through and recirculating measurements, the sulfide permeability coefficient ranged from 0.2×10^{-6} to 1.8×10^{-3} cm s^{-1} for the different portions of the tubes. The mean permeability coefficient of root tubes ($0.41\times10^{-3}\pm0.49\times10^{-3}$ cm s^{-1}) was 10 times higher than that of trunk tubes ($0.037\times10^{-3}\pm0.062\times10^{-3}$ cm s^{-1}) (P<0.001).

If the composition of the tube wall were homogeneous and invariant, then D would be constant, irrespective of the wall thickness or location. However, multiplying the permeability coefficient by the wall thickness for each tube (equation 2, assuming α=1) still produced a strong dependence of permeability on wall thickness (Fig. 6, inset), with the diffusion coefficient ranging from 0.016×10^{-6} to 1.6×10^{-6} cm^2 s^{-1}. The mean diffusion coefficient of root tubes ($2.3\times10^{-6}\pm2.0\times10^{-6}$ cm^2 s^{-1}) was significantly higher than that of trunk tubes ($0.48\times10^{-6}\pm0.67\times10^{-6}$ cm^2 s^{-1}) (P<0.001), suggesting that thinner-walled tubes are structurally different from thicker-walled tubes, such that the sulfide distribution coefficient or the kinetics of sulfide diffusion are affected.

Flow velocity as a source of experimental artifact

For a given flow rate, the mean flow velocity through a tube is inversely proportional to the square of the tube diameter. In the permeability experiments, the flow rate was constant at 0.4 ml min^{-1} for the flow-through method and 2.0 ml min^{-1} for the recirculating method, irrespective of tube diameter. Thus, an alternative explanation for the apparent dependence of permeability on wall thickness is that there was an experimental artifact in measurement resulting from the lower flow velocity in larger-diameter tubes (which typically had the thickest walls). This would occur, for example, if sulfide flux across the tube was strongly flow-limited, instead of diffusion-limited, at low flow velocities.

This was tested by replacing the *Lamellibrachia* sp. tube in the flow-through permeability apparatus with a tube of cellulose ester dialysis membrane with a diameter of 2.5 or 6.4 mm. Dialysis tubes of different diameters were otherwise identical. If there were no effect of tube diameter (and thus fluid flow velocity) on sulfide flux, then the permeability coefficient for each of the two dialysis tubes would have been identical. Instead, the measured permeability coefficient of the smaller-diameter tube was 2.9 times larger than that of the larger-diameter tube, indicating that at least some of the lower sulfide permeability observed in more anterior tubes sections is due to tube diameter flow velocity effects. However, under the same experimental conditions, the sulfide diffusion coefficient of *Lamellibrachia* sp. tubes was 160 times higher in tubes

1.9–2.7 mm in diameter than in tubes 5.4–6.5 mm in diameter. Thus, the flow velocity effect is not sufficient to explain the higher permeability of more-posterior tube sections.

Additional evidence suggests that root tubes are more permeable to sulfide than are trunk tubes. The smallest trunk tubes had cross-sectional areas that were in the same range as those of root tubes. When the permeability coefficient in these tubes was measured under the same experimental conditions, both the root and trunk tubes would have had similar, if not identical, boundary layer velocity and thickness. The range of cross-sectional areas for the roots in the permeability studies was 0.6–2.6 mm^2 (mean 1.34±0.67 mm^2, N=12). There were 15 sections from the trunk tube with similar cross-sectional areas (range 0.5–2.5 mm^2, mean 1.15±0.62 mm^2). Although the mean cross-sectional areas of these two groups were not significantly different (P=0.49 by t-test), the mean diffusion coefficient of the root group ($2.12\times10^{-6}\pm1.67\times10^{-6}$ $cm^2\,s^{-1}$) was 2.5 times higher than that of the non-root group ($0.83\times10^{-6}\pm0.78\times10^{-6}$ $cm^2\,s^{-1}$) (P=0.025). Furthermore, this difference was found in the diffusion coefficient, and not just the permeability coefficient, indicating that the wall of root tubes is intrinsically more permeable to sulfide than the wall of trunk tubes.

Although the recirculating method (which uses silver sulfide electrodes) and the flow-through method (which uses a spectrophotometric assay) were substantially different, the results demonstrating a dependence of sulfide permeability on wall thickness were remarkably similar. If the two methods are compared by performing linear regressions through the permeability coefficient and diffusion coefficient data in Fig. 6, the slopes are found not to be statistically different. This is true irrespective of whether the regressions are through all the tubes analyzed with each method (P=0.78 for p and P=0.89 for D) or just the root tubes (P=0.84 and P=0.99, respectively) and trunk tubes (P=0.95 and P=0.91, respectively), indicating that the dependence of permeability on wall thickness is consistent, regardless of the measurement technique. However, the recirculating method produced somewhat higher coefficients for root tubes (but not trunk tubes), as indicated by a statistical comparison of the intercepts for regressions through the root tube data (P<0.001 for both p and D), suggesting a systematic bias in one of the techniques.

A potential explanation for this bias in the permeability results was that each of the roots used in the flow-through method had been frozen. For example, it might have been possible for a cycle of freeze-thawing to cause some disruption of the root tube wall structure, decreasing the permeability. However, this is somewhat counterintuitive, since this disruption might be expected to increase the permeability. It is more likely that the lower permeability and diffusion coefficients measured using the flow-through method were due to increased boundary layer effects, as described above.

Discussion

Large colonies of *Lamellibrachia* sp. are found at hydrocarbon seep sites on the floor of the Gulf of Mexico. *Lamellibrachia* sp., like the better-known giant tubeworm *R. pachyptila* found at hydrothermal vents, must provide sulfide to its symbiotic bacteria in order to survive. However, unlike the situation at hydrothermal vents, the sulfide concentration in the sea water around the respiratory plumes of *Lamellibrachia* sp. is very low. In fact, sulfide was undetectable midway between the plume and the sediments. Since the sediment is the source of the sulfide, and sulfide leaving the sediment must diffuse up to the plume, this is further evidence for an absence of sulfide around the plumes of adult *Lamellibrachia* sp. However, sulfide reached very high levels in the interstitial waters of the sediments around the buried bases of the trunk tubes. Because *Lamellibrachia* sp. extend long, thin tubes into the sediment, we hypothesize that these tubes, which we call roots, give *Lamellibrachia* sp. the ability to withdraw sulfide directly from deep interstitial waters, which is probably the only reliable source of sulfide for large, old colonies.

This strategy for acquiring sulfide is roughly analogous to that proposed for some symbiont-containing bivalves. For example, it has been proposed that the hydrothermal vent clam *Calyptogena magnifica* stretches its well-vascularized foot down through fissures to reach higher sulfide concentrations (Arp et al., 1984). Similarly, in the family of burrowing clams Lucinidae, which are found in a wide range of sulfidic marine habitats, at least some species appear to extend their feet deep into the sediment to reach highly sulfidic mud (for a review, see Anderson, 1995). However, unlike the muscular, flexible foot of a clam, the root of *Lamellibrachia* sp. is almost certainly unable to change its position or location once formed, except by extending further into the sediment.

Tube structure

Vestimentiferan tubes are composed of parallel bundles of chitin microfibrils embedded in a protein matrix (Gaill et al., 1992b; Shillito et al., 1995a). Previous studies have demonstrated that the electron-lucent fibrils are chitin crystallites and that the proteins are concentrated in the surrounding matrix (Shillito et al., 1995a). The chitin and protein contents of the *Lamellibrachia* sp. trunk tubes used in this study are similar to those reported previously (28 % and 75 % of dry mass, respectively; Shillito et al., 1995a). Thus, the 10–20 % chitin content (by dry mass) found in *Lamellibrachia* sp. root tubes represents a low chitin content by comparison. Shillito et al. (1995a) found that chitin and protein accounted for all the dry mass of the *Lamellibrachia* sp. trunk tubes. Finally, in both the trunk and root tube, the outer portion of the wall differed structurally from the inner portion. This difference is probably indicative of ‘weathering’ effects on the outer tube surface and continuous repair of the inner tube surface.

Permeability

Under experimental conditions, the roots were over 700 times more permeable to sulfide than the most anterior portion

of the trunk tube. Since the trunk tube wall is much thicker than the root tube wall, most of this higher permeability was due to a shorter diffusion distance across the root wall. However, at least some of this difference in permeability is clearly due to an intrinsically higher sulfide permeability of the root tube wall, as shown by the higher sulfide diffusion coefficient, which is, ideally, independent of wall thickness. When trunk tubes and roots of similar diameter were compared, the material of the root tube wall was found to be 2.5 times more permeable to sulfide than that of the trunk tube wall. This strongly suggests that the roots are structurally specialized for sulfide uptake. Unfortunately, it is difficult to compare diffusion coefficients between tubes of widely different diameter because of experimental complications resulting from differences in flow velocity through tubes of different inner diameter, and permeability measurements in large-diameter trunk tubes are probably an underestimate. Nonetheless, the control experiments utilizing dialysis tubing of different diameters demonstrated that the effects of flow velocity are small compared with the observed differences in diffusion coefficients.

The diffusion coefficient of sulfide in root tube wall ($2.3\times10^{-6}\pm2.0\times10^{-6}\,cm^2\,s^{-1}$, at 20 °C) was approximately 14 % of the H_2S and HS^- diffusion coefficient in sea water at the same temperature ($17\times10^{-6}\,cm^2\,s^{-1}$; Tamimi et al., 1994). Since the distribution coefficient was assumed to be 1, which is a maximum estimate, the actual diffusion coefficient may be somewhat higher. Nonetheless, the sulfide diffusion coefficient in root tube wall is similar to the H_2S diffusion coefficient of $1.5\times10^{6}\,cm^2\,s^{-1}$ measured in the body wall of *Urechis caupo* (Arp et al., 1995; Julian and Arp, 1992). However, whereas H_2S and HS^- were both equally permeable in *Lamellibrachia* sp. tube, the permeability of HS^- was one-third that of H_2S in *U. caupo* body wall. It was proposed that the higher permeability of H_2S in *U. caupo* body wall was due to the HS^- diffusion barrier provided by cell membranes. *Lamellibrachia* sp. tube wall, in contrast, has no lipid membranes, and all diffusion is through its network of protein and chitin fibers. Thus, any differences in permeability to H_2S and HS^- in tube wall would have to be due to such factors as solute molecular mass, molecular radius and interactions with charges on the network fibers. In *Lamellibrachia* sp., variations in the characteristics of chitin and protein in the different regions of the tube wall are correlated with differences in sulfide permeability between the root and trunk tube. On the basis of theoretical models developed for permeability in the fiber matrix of capillaries (Curry, 1984; Ogston et al., 1973), the increased diffusion coefficient of root tube compared with trunk tube could only be achieved either by decreasing the effective fiber radius or by decreasing the fraction of the matrix volume occupied by fibers. Both appear to be true in the *Lamellibrachia* sp. tube, since the fiber radius and density are both lower in the root tube wall than in the trunk tube. In fact, the chitinous fibrils become thinner and can even be undetectable in the root tube wall. The disorganization of the fibrillar system results in a decrease in the relative volume occupied by the fibers, which is consistent with the decrease in chitin content observed in the roots.

What is the role of the root?

One possible function of the roots would be to anchor the tubeworms to the substratum. However, this is probably unnecessary since the animals are normally attached to rocks, the roots forming after the animals have been partially buried by sediment, and water currents at this depth in the Gulf of Mexico are unlikely to exceed a few centimeters per minute. We propose instead that the roots are important for taking up sulfide from interstitial waters.

The smallest, and presumably youngest, *Lamellibrachia* sp. aggregations we find in the Gulf of Mexico are attached to exposed authegenic carbonate rocks. These carbonates form in places where dissolved inorganic carbon concentrations are supersaturated as a result of the oxidation of methane that seeps up through the underlying sediments. At these same locations, the water seeping from the sediments would probably also bring sulfide to the surface. In the early years of their lives, when their gills are positioned very close to the sediment–water interface, the tubeworms may be able to obtain this sulfide across their gills. Thus, the locations where the substratum is suitable for *Lamellibrachia* sp. colonization may also be those where there is sufficient sulfide at the sediment–water interface to support the young animals. As time progresses, however, continued precipitation of carbonates and partial burial of the tubeworms with sediment both obstruct the upward diffusion of sulfide. Furthermore, continued growth of the tubeworms, which can extend their anterior end as much as 1.5 m above the sediment, moves the gills even farther from the sulfide source. Therefore, the role of the roots as sulfide uptake organs becomes more important to the tubeworms as they grow.

As structures for enhancing sulfide uptake, roots could function by two different mechanisms. First, the roots could provide a low-resistance pathway for sulfide diffusion from the deeper sediment. In this mechanism, which is roughly analogous to the aerenchyma of some wetland plant stems (Armstrong, 1978), the optimal arrangement would be a very straight root (i.e. low tortuosity) and an empty root lumen (i.e. high porosity). Alternatively, the roots could increase the surface area available for uptake, with convection of sulfide in the roots being provided by the animal's vascular system. In this mechanism, which would be analogous to a respiratory surface, the optimal arrangement would be a tortuous root with the animal's body extending far down into the root lumen.

For the roots to function as a low-resistance pathway, the longitudinal resistance of the roots would have to be less than that of the surrounding substratum. The diffusion of water molecules through a variety of substrata of broadly different porosity (0.41–0.97, with 0 as a complete barrier and 1 as pure water) is rather similar, being 0.55–0.67 of that in pure water (Sweerts et al., 1991). Although the porosity of sediment surrounding the roots is not specifically known, the effect of porosity on sulfide diffusion in the same sediment would

probably be nearly identical. However, this assumes that the roots are relatively straight (tortuosity less than approximately 1.5) and have a high porosity. Neither of these is likely to be true since the tubeworm's body wall extends into the root portion of the tube and the roots are typically very tortuous. Furthermore, the body wall in the root is vascularized and contains coelomic fluid, which suggests that convective transport could occur in the roots.

Hypothetical model of sulfide uptake by a root

High surface area and the possibility of convective transport both support the idea that the roots are analogous to a respiratory surface. To begin to test this hypothesis, the experimental results for environmental sulfide concentrations, the dimensions of root and trunk tubes, and the sulfide permeability coefficients were incorporated into a mathematical model of sulfide uptake by the roots. In this model, a root was conceptually broken down into discrete segments, and the steady-state sulfide flux through each segment was calculated using equation 1. The root was assumed to reach down to a sediment depth of 40 cm, with the tortuosity being variable. The seawater sulfide concentration was assumed to be zero, and interstitial sediment sulfide concentration was assumed to increase exponentially from zero at the sediment surface to a maximum of 1500 μmol l^{-1} at 20 cm sediment depth, and then to decrease exponentially back towards zero at 40 cm sediment depth. Although the sulfide concentration data set from which these values are derived is not normally distributed and the concentrations are patchy, these are reasonable assumptions based on the observed ranges. Because H_2S and HS^- were found to be equally permeable through *Lamellibrachia* sp. tubes, and because the diffusion coefficients of H_2S and HS^- in water are approximately equivalent (Li and Gregory, 1974; Tamimi et al., 1994), it was not necessary to consider the dissociation constant of H_2S in the model (the concentration of S^{2-} was considered to be negligible at the pH range appropriate for the model). No estimates are yet available for vascular blood flow rate in *Lamellibrachia* sp., but it was assumed, for this model, that the vascular blood was capable of convecting all the sulfide entering the root from the surrounding sediment. The sulfide-binding characteristics of *Lamellibrachia* sp. blood have also not yet been reported, but if the sulfide-binding affinity and capacity of *Lamellibrachia* sp. hemoglobins are similar to those measured in other vestimentiferans, the free sulfide concentration in the blood probably never exceeds 1 μmol l^{-1} (Arp et al., 1987; Fisher et al., 1988; Somero et al., 1989).

With a peak sediment sulfide concentration of 1500 μmol l^{-1} for a straight root (tortuosity=1), with the body extending the full length of the root and with a free sulfide concentration in the vascular blood of less than 10 μmol l^{-1}, the hypothetical net rate of sulfide influx is approximately 7.2 μmol h^{-1}. If the peak sediment sulfide concentration is decreased, the net rate of sulfide influx decreases proportionally. Similarly, increasing the root tortuosity increases the net sulfide influx proportionally. If the body does not reach into the root, then no convective sulfide transport can take place, and all longitudinal sulfide transport in the root would be diffusive. This would result in a net sulfide influx of less than 1 % of that which would occur with full convective transport. It is important to note that, while the relative effects of some features, such as tube tortuosity, sediment sulfide concentration and blood sulfide concentration, are likely to be fairly well estimated by this model, it is unlikely to predict accurately the actual sulfide flux rates. This is because many processes were highly simplified, and several important variables, such as sulfide profiles in the interstitial water of the sediment and sulfide transport in the vascular system, were estimated.

We do not yet have measurements of metabolic sulfide uptake rate in *Lamellibrachia* sp., so it is difficult to determine whether the hypothetical rate of net sulfide influx calculated above is sufficient to supply *Lamellibrachia* sp. with its metabolic needs. However, Childress et al. (1991) measured the metabolic sulfide influx rate in *R. pachyptila* and found it to reach a maximum of approximately 4 μmol g^{-1} h^{-1} at 8.4 °C. A *Lamellibrachia* sp. similar to that in the hypothetical model described above would have a trunk tube length of 100 cm and a tissue mass of approximately 10 g (D. Bergquist and C. R. Fisher, unpublished observations). If we assume that the metabolic rate of *Lamellibrachia* sp. is at most one-tenth of that of *R. pachyptila* (Fisher et al., 1997), which is still probably a great overestimate, then the maximal metabolic sulfide uptake rate of the *Lamellibrachia* sp. in the hypothetical model would be 4 μmol h^{-1}. This is less than the hypothetical rate of net sulfide influx across the root (7.2 μmol h^{-1}), suggesting that sulfide uptake across the root alone could be sufficient for the animal's metabolic requirements.

That sulfide is permeable through tube wall implies that it could also 'leak' out of the trunk tube and into the surrounding sea water. However, this effect would probably be relatively small because the trunk tube wall is intrinsically less permeable to sulfide and much thicker than the root tube wall, and because the blood sulfide concentration is likely to be low as a result of sulfide binding. Although the conical shape of the trunk tube would cause the lateral surface area to increase disproportionately with trunk length, this is accompanied by thicker, less-permeable walls and a lower surface-to-volume ratio. Thus, the effect of doubling the trunk tube length from 0.5 to 1 m is a 570 % increase in body mass and a 300 % increase in lateral surface area, but an increase of only 80 % in total sulfide permeability. It should also be noted here that the respiratory plume could present an extremely low-resistance pathway for sulfide leakage. However, as discussed above for leakage through the trunk, sulfide binding by the blood would minimize the gradient. It may also be possible for sulfide-rich blood to be shunted away from the plume.

Enhancing sulfide production

If the bulk of the sulfide at the hydrocarbon seeps is produced biogenically through anaerobic sulfate reduction in relatively shallow sediments, as suggested by Carney (1994),

then the main limiting factor to sulfide production in these environments is probably sulfate (organic hydrocarbons including methane are abundant). This would become especially severe (for *Lamellibrachia* sp.) in areas with large and dense aggregations of the tubeworms. An interesting possibility is that *Lamellibrachia* sp. may enhance sulfide production in deeper sediments around the colonies by transporting the limiting reagent, sulfate, down to the deep organic rich and anoxic interstitial waters. For example, sulfate could be taken up from the sea water at the plume surface, either by diffusion or *via* a transporter, and then brought down to the roots in the vascular blood, from where it would diffuse out into the sediment. Once outside the tube, the sulfate would be reduced to sulfide by bacteria oxidizing organic molecules. The possibility would also exist for cycling of sulfate between sulfide-oxidizing bacteria in the trophosome and sulfate-reducing bacteria surrounding the root.

Fisher et al. (1997) have proposed that larval vestimentiferans at Gulf of Mexico hydrocarbon seeps settle gregariously on exposed rocks, ultimately forming dense colonies consisting of thousands of individuals. As the tubeworms grow upwards while secreting their protective tube, sediment accumulation gradually buries the attached posterior ends of the tubes by up to 1 m. *Lamellibrachia* sp., the most common vestimentiferan species at hydrocarbon seeps, grows very slowly, averaging $0.77\,cm\,year^{-1}$, and yet commonly reach lengths of over 2 m. On the basis of conservative calculations, Fisher et al. (1997) concluded that individuals in mature aggregations are a minimum of 100 years old and are probably much older. The longevity and low growth rates of the hydrocarbon seep vestimentiferans reflect life history strategies very different from those of their hydrothermal vent relatives. While sulfide is less abundant at hydrocarbon seeps, these environments are more stable and may provide sulfide for centuries, in contrast to hydrothermal vents, which only persist for years to decades (Fisher et al., 1997; Lutz and Kennish, 1993). Methane and hydrocarbon release is relatively stable and, with the addition of sulfate, bacterial methane oxidation/sulfate reduction may provide a steady flow of sulfide into the hydrocarbon seep communities over long periods. We propose that roots allow *Lamellibrachia* sp. (and perhaps other vestimentiferans) to tap sources of sulfide deep in the sediments, allowing them to flourish under conditions where insufficient sulfide leaves the sediment to reach their plumes. By no longer needing to be physically near the sulfide source, the plumes are now free to extend up into the water column, where O_2 is more abundant. Further experiments will be required to confirm that the roots are the primary site of sulfide uptake in adult *Lamellibrachia* sp., to clarify the mechanism by which the roots are formed and to explain what makes the root tube wall more permeable to sulfide than the trunk tube wall.

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