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10 June 1980; revised 8 December 1980

Prokaryotic Cells in the Hydrothermal Vent Tube Worm *Riftia pachyptila* Jones: Possible Chemoautotrophic Symbionts

Abstract. *The existence of a symbiotic association between vestimentiferan tube worms from deep-sea hydrothermal vents and chemoautotrophic sulfur-oxidizing prokaryotes, based on histological and enzymatic evidence, is suggested.*

A major recent biological discovery is that of the dense benthic animal populations clustered tightly around the newly explored deep sea hydrothermal vents at the Galápagos Rift and East Pacific Rise ocean spreading centers (*1, 2*). The primary or sole base of the food chain in these communities appears to be the chemoautotrophic production of bacterial biomass with hydrogen sulfide emitted from the vents as the geothermic source of energy (*3*).

The most conspicuous animal of these vent communities is the large red-plumed vestimentiferan tube worm, *Riftia pachyptila* Jones (*4*), of the phylum Pogonophora (*5*). Observations from the submersible *Alvin* indicate that the tubes of *Riftia* are attached to rocks situated directly in the flow of sulfide-rich seawater from the vent (H_2S, up to 160 μM) (*2*). *Riftia* is superficially similar but much larger (up to 1.5 m in length and a diameter of 38 mm) than other related benthic pogonophorans (*4*). The most striking feature of this phylum is the lack of mouth and gut. *Riftia*'s large size is astonishing because speculation on the mode of nutrition of the Pogonophora has centered on the uptake of dissolved organic material via the epidermis (*6*). However, uptake rates of dissolved amino acids at ambient concentrations by the pogonophoran *Siboglinum fiordicum* were shown to be insufficient to account for the animal's metabolic requirements (*7*). Thus, the mechanism of nutrition of this group remains unclear.

Specimens of *Riftia* were collected at a number of geothermal vents in the Galápagos Rift and East Pacific Rise. The body of the animal consists of four regions (Fig. 1A). The cavity of the trunk, the third and most extensive region (at least 50 percent of the total length in postjuvenile specimens), is occupied by the gonads and the trophosome. The latter, of irregular and variable development along its length, is compact, of many lobules, and is well-supplied with vascular elements. Prior to this study the function of the trophosome in vestimentiferans was unknown. In *Lamellibrachia luymesi* it was suggested to serve as a source of nutrition for developing sperm or as a detoxifying organ (*8, 9*). In 21 of 31 specimens of *Riftia* examined, crystals of elemental sulfur up to 100 μm in size were found within the trophosomal tissue (*10*). This observation suggested a capacity for using the chemoautotrophic oxidation of sulfide as an internal source of nutrition. We report here a number of subsequent observations demonstrating the presence of prokaryotic cells within the trophosomal tissue of *Riftia pachyptila* Jones.

In stained paraffin sections the trophosomal tissue is granular (Fig. 1A) (*11*). The granules are usually aggregated in lobelike accumulations. There are few nuclei present, and the majority of these are associated with blood vessels and the squamouslike covering of the lobular surfaces. Juveniles as small as 1.44 mm long and 0.33 mm in diameter have a trophosome identical in appearance to that of post-juveniles (Fig. 1A). A trophosomal tissue homogenate stained with 4′,6-diamidino-2-phenylindole (a specific and sensitive DNA stain) and examined with epi-fluorescence microscopy (*12*) revealed that the morphologically distinct granules (3 to 5 μm in diameter) uniformly produced a brilliant blue fluorescence. This is interpreted as their being either prokaryotic cells or eukaryotic organelles (such as mitochondria). Direct counts of this trophosome homogenate indicated that there were 3.7×10^9 cells measuring 3.0 μm or more per gram of tissue (wet weight) (*13*).

The trophosomal tissue available for examination by electron microscopy was collected at the Rose Garden geothermal vent (*14*) and had been fixed in 5 percent Formalin (in seawater) as a general preservative. Consequently, the fixation for transmission electron microscopy is not of the highest quality but is sufficient to resolve important structural features. Scanning electron microscopy revealed that the characteristic lobes of the trophosomal tissue consist of densely packed spherical bodies (Fig. 1B). Transmission electron microscopy (TEM) of the same tissue (*15*) indicated that these bodies are prokaryotic cells varying in size between 3 and 5 μm (mean = 4.20 ± 0.64 μm; n = 16) and having a cell wall resembling that of gram-negative bacteria (Fig. 1C). To date we have been unable to determine if the prokaryotic cells are located within or outside the trophosomal cells.

The presence of lipopolysaccharide (LPS), a compound characteristic of the outer cell wall of gram-negative bacteria, was confirmed in frozen trophosomal tissue by the *Limulus* amoebocyte lysate test (*16*). The result of the assay was strongly positive (0.8 μg of LPS per milligram of wet tissue) indicating the presence of a large population of gram-negative prokaryotic cells far in excess of what could be attributed to bacterial contamination of the frozen sample.

Felbeck (*17*) found in the trophosomal tissue high activities of enzymes used in generating adenosine triphosphate (ATP) from the oxidation of reduced sulfur compounds, that is, thiosulfate sulfurtransferase (rhodanese), APS reductase, and ATP sulfurylase. In addition, high activities of RuBP carboxylase and ribulose 5-phosphate kinase (enzymes of the Calvin-Benson cycle of CO_2 fixation) have been measured in the trophosome in activities comparable to those of spinach leaves (*17*). The prokaryotic cells make up a major portion of the trophosome in *Riftia*. This suggests strongly that they are responsible for these enzymatic activities and are symbiotic chemoautotrophic bacteria that are capable of generating ATP by way of sulfide oxidation and reducing CO_2 to organic matter. Preliminary studies by Rau (*18*) on $^{13}C/^{12}C$ ratios in *Riftia* lend support to

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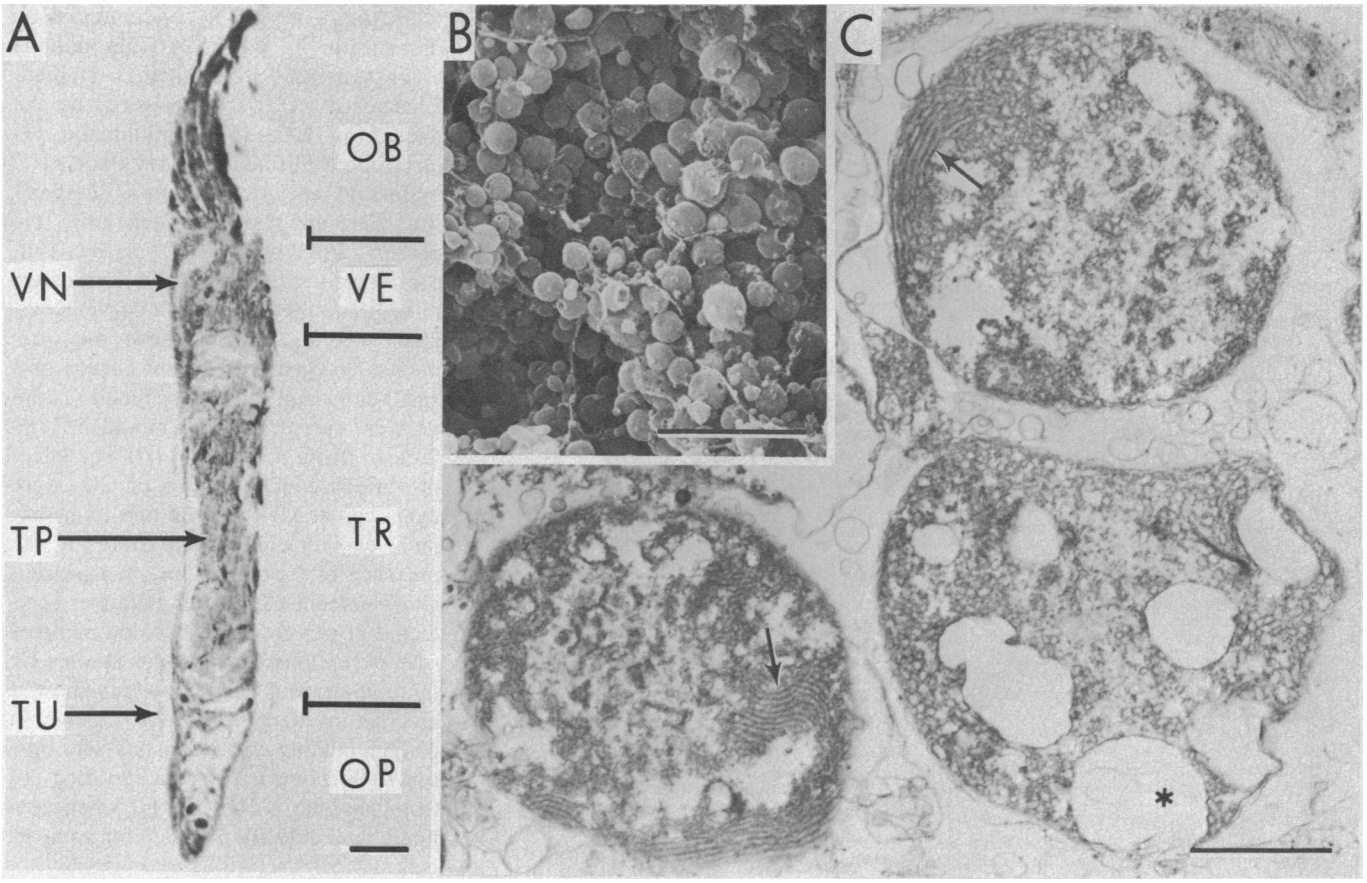

Fig. 1. Light and electron micrographs of *Riftia pachyptila* Jones. (A) Parasagittal section of juvenile (UNSM 59960) showing restricted distribution of trophosome in trunk region; ventral to left (*OB*, obturaculum; *OP*, opisthosome; *TP*, trophosome; *TR*, trunk; *TU*, tube; *VE*, vestimentum; *VN*, ventral nerve; scale bar, 100 μm). (B) Scanning electron microscopy of trophosome showing spherical prokaryotic cells (scale bar, 10 μm) [Photo by E. Seling, Harvard University]. (C) Transmission electron microscopy of prokaryotic cells in trophosome (scale bar, 1 μm). Intracytoplasmic membranes (arrows) and electron transparent areas (asterisk) are dispersed throughout the cytoplasm. The latter are similar in appearance in thin sections to those associated with sulfur inclusions in known sulfur-oxidizing bacteria [for example, *Thiovulum* (*28*)].

the possibility of an autotrophic nutrition.

The extended vascular system within the trophosome (*4*), the high oxygen affinity of the extracellular hemoglobin in *Riftia* (*19*), and the relative insensitivity of the blood oxygen-carrying capacity to changes in temperature and CO_2 concentration (*19*) suggest special adaptations in this worm to ensure a supply of O_2 and CO_2 to the trophosome bacteria. A transport system for H_2S has not been found in the blood of *Riftia* (*19*), and it is possible that sulfide is made available to the symbionts by diffusion across the body wall of the worm (into the coelomic fluid) or across tentacular surfaces into the bloodstream. The former process has been shown to occur in gastrotrichs and turbellarians that live in sulfide-rich systems (*20*). Whether the worm benefits from this microbial association and how the microbially produced organic substrate is used in the nutrition of *Riftia* remains to be assessed.

Preliminary TEM examination of eggs removed from the ovary of an adult female has failed to reveal the presence of prokaryotic cells, thereby leaving the mechanism of their transmission still to be resolved.

Structures resembling the *Riftia* symbionts have been described within the trophosome cells of the vestimentiferan *Lambellibrachia luymesi* (*9*), and the same type of stained granules can be seen in paraffin sections of *L. barhami* trunk tissue (USNM 55162). A "spongy tissue" similar to the trophosome of the Vestimentifera is described by Ivanov (*21*) in the perviate (*4*) Pogonophora. There is sufficient similarity between these described tissues and that of *Riftia* trophosome to merit examination of all pogonophorans for the presence of bacterial symbionts.

Our observations on the chemoautotrophic symbiosis in *Riftia* suggest that similar symbiotic relationships also may exist in other animals inhabiting sulfide-rich environments. Indeed, initial tests for RuBP carboxylase were positive, and prokaryotic symbionts were discovered by TEM examinations (*22*) in *Solemya velum*, an Atlantic coast bivalve known to have a small gut and to inhabit sulfide-rich sediments (*23*), as well as in *Calyptogena magnifica*, the new species of giant white clam found at the hydrothermal vents (*24*). Furthermore, the hitherto unknown mode of nutrition in gutless organisms, such as the West Coast species of *Solemya* (*25*), the archiannelid polychaete *Astomus taenoides* (*26*), and the nematode *Rhaptothyreum typicus* (*27*) suggests a widening of the survey for potential chemoautotrophic symbionts.

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20 October 1980; revised 19 February 1981

Blood Function in the Hydrothermal Vent Vestimentiferan Tube Worm

Abstract. *Extracellular hemoglobin in the whole blood of* Riftia pachyptila *has a high oxygen affinity (P_{50} = 1.8 millimeters of mercury at 3°C), a moderate decrease in oxygen affinity at higher temperatures (P_{50} = 2.7 millimeters of mercury at 14°C), a small effect of carbon dioxide on oxygen affinity ($\Delta \log P_{50}/\Delta pH = -0.12$), and a high oxygen carrying capacity (up to 11 milliliters of oxygen per 100 milliliters of blood). These characteristics are compatible with the high oxygen demand of chemoautotrophic metabolism in the variable vent environment.*

Giant vestimentiferan worms (up to 3 m in length) are found at a depth of 2500 m around the deep-sea hydrothermal vents (*1*). These sessile animals, *Riftia pachyptila*, live where the vent water (up to 22°C, 350 μ*M* H_2S, anoxic) is actively mixing with the surrounding water (2°C, no H_2S, 110 μ*M* O_2) and they are therefore exposed to conditions continually changing between these extremes (*1–3*). It has been postulated that these worms, or their symbionts, oxidize H_2S in the presence of oxygen for their metabolic energy source (*4*).

The vent worm has no mouth or gut but does have a ventral heart and a well-developed circulatory system that supply blood to the trophosome, the proposed site of autotrophic metabolism (*2, 4*). The red color of the animal is due to an extracellular hemoglobin, which occurs in both the vascular blood and the coelomic fluid, and is similar in structure to that of annelid worms (*5*). We examined the oxygenation characteristics of the whole blood of *R. pachyptila* to gain a better understanding of respiratory function of animals in this unusual habitat. We present data showing that the concentrated extracellular hemoglobin is suited to the needs of an autotrophic organism in this variable environment.

Vestimentiferan worms were collected by the submersible *Alvin* during November and December of 1979 at the Galápagos Rift valley sites Garden of Eden and Rose Garden (*1, 6*). As soon as the specimens were brought to the surface, blood was sampled and introduced into a chamber that was regulated to the same temperature and CO_2 concentration as the subsample whose oxygen binding characteristics were being studied. Changes in *p*H were monitored by the use of an E and K combination *p*H electrode calibrated with *p*H 4 and *p*H 7 buffers at each experimental temperature. The effect of 0 percent (air), 0.4 percent, and 2 percent CO_2 on the *p*H of the oxygen saturated blood was determined, and the *p*H of each curve generated was taken from these measurements. Oxygen equilibrium curves of a small subsample of whole blood (2 to 4 μl) were measured with a modified Hem-O-Scan (from 3° to 30°C) (*7*). The effect of various concentrations of CO_2 were examined at each temperature by generating oxygen equilibrium curves in the presence of 0 percent (air), 0.4 percent and 2 percent CO_2. Most data were collected at sea from fresh blood samples (the exceptions being some studies on dilution and Bohr effects) which showed no sign of denaturation or change in oxygen binding characteristics with time (up to 14 hours). All data reported are from vascular blood, except when coelomic fluid data are included for comparison.

The whole blood of *R. pachyptila* shows a small Bohr effect (a shift of the oxygen equilibrium curve to the right) with increased CO_2 concentrations and decreased *p*H ($\Delta \log P_{50}/\Delta pH = -0.12 \pm 0.09$; 95 percent confidence interval, $n = 21$) (P_{50} is the oxygen partial pressure at which the hemoglobin is half saturated with oxygen). Oxygen equilibrium curves were generated without CO_2 gas and with 2 percent CO_2 gas repeatedly at 8°C; *p*H values were determined from subsamples of whole blood equilibrated with these same gas mixtures. In general, for annelids containing extracellular hemoglobins, worms that inhabit permanent, well-ventilated burrows or tubes tend to have large Bohr effects (for *Diopatia*, −0.86); but transient burrow dwellers tend to have small Bohr effects and sometimes reverse Bohr effects (*Marphysa*, −0.25; *Nephtys*, +0.09) (*8*). *Riftia pachyptila*, although inhabiting a permanent tube, is unusual in that the whole blood has a small Bohr effect. This unusual characteristic may be important for an autotroph that transports CO_2 simultaneously with oxygen to the tissues.

The vascular blood and the coelomic fluid both showed a high affinity for oxygen. The P_{50} values for vascular blood increased with temperature and ranged from 1.2 mmHg at 3°C to 7.2 mmHg at 25°C (Fig. 1), while coelomic

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