mer and of epithelium in the latter. There is a thin epithelial covering over the bacteria which comprise the mass of the trophosome and over the gonads and gonoducts as well; there is no covering layer over the peripheral feather muscles. The paired cavities of each opisthosomal segment are separated by septa, which bear muscle fibers on both the anterior and posterior faces, as well as long glandular structures situated in blood vessels or sinuses on the anterior septal face; the internal layer of the body wall of each segment is made up of bundles of normal longitudinal muscles in a connective tissue matrix; within each segment, between septa, there are thin circular bands of muscles which form partial, incomplete septa. In addition, there are transverse segmental blood vessels running just internal to the body wall. All of these morphological features appear to lack a covering of epithelial cells.

Considering the most widely accepted definition of a coelom as provided by Hyman (*7*), only the perivascular cavity of the obturacular vessels would appear to qualify as a coelomic cavity; the status of the other cavities is equivocal because they lack to a greater or lesser extent a complete peritoneal lining. Fransen (*8*) has found a similar perplexing series of linings in her examination of the coelomic cavities of a number of species of polychaetes and archiannelids. Previously Southward commented, in passing, on the equivocal nature of the coelomic lining of pogonophorans (*9*). In light of the present findings, use of the term "coelom" by previous workers in reference to the differing cavities of pogonophorans (in the strict sense) and vestimentiferans (*10*), as well as for other taxonomic groups, now requires a reexamination.

In the case of a marine animal as massive as *Riftia* (the largest specimen is 1.5 m long, with an average diameter of about 35 to 40 mm) the problem of nutrition is of more than passing interest because the worms lack a mouth and gut. The most obvious method of food uptake would appear to involve a direct pathway across the body wall, and the obturacular plume, in life extending from the opening of a thick-walled tube, would seem to be the obvious site for such food transport. With the large numbers of tentacles, each with more than 200 pairs of intraepidermal vascular loops and the large additional surface of up to 200 pairs of vascularized pinnules on each tentacle, the plume is an exceptional organ for the uptake of organic molecules (*11*). The trophosomal bacteria may also play a role in the nutrition of *Riftia*. The similarity of ratios of ^{13}C to ^{12}C in the trophosome (bacteria) and in the vestimental musculature found by Rau (*6*) suggests that *Riftia* may utilize the bacteria or their metabolites (or both) as a source of organic carbon.

MEREDITH L. JONES
Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560

References and Notes

1. J. B. Corliss *et al.*, *Science* **203**, 1073 (1979).
2. RISE Project Group: F. N. Spiess *et al.*, *ibid.* **207**, 1421 (1980).
3. M. L. Jones, *Proc. Biol. Soc. Wash.* **93**, 1295 (1980).
4. It is my opinion that the vestimentiferans, as well as the remainder of the Pogonophora, stand in close relationship to the Annelida; I consider the nerve cord to be ventral and the major longitudinal blood vessel, provided with a thick muscular layer, to be dorsal. For further discussions concerning the orientation of these taxa, see J. van der Land and A. Nørrevang, *Z. Zool. Syst. Evolutionsforsch.*, Sonderheft **1**, 86 (1975).
5. For observations on the functional aspects of oxygenation characteristics of extracellular hemoglobin of *Riftia*, see A. J. Arp and J. J. Childress, *Science* **213**, 342 (1981). For data on the kinetic constants for combination and dissociation rates of the hemoglobin of *R. pachyptila*, see J. B. Wittenberg, R. J. Morris, Q. H. Gibson, M. L. Jones, *ibid.*, p. 344.
6. For an account of enzymes possibly attributable to these bacteria, see H. Felbeck, *ibid.*, p. 336. For data on $^{13}C/^{12}C$ pertaining to them, see G. H. Rau, *ibid.*, p. 338. For a description of and observations on these organisms, see C. M. Cavanaugh, S. L. Gardiner, M. L. Jones, H. W. Jannasch, J. B. Waterbury, *ibid.*, p. 340.
7. L. H. Hyman, *Invertebrates*, vol. 2, *Platyhelminthes and Rhynchocoela, the Acoelomate Bilateria* (McGraw-Hill, New York, 1951).
8. M. Fransen, *Zoomorphologie* **95**, 235 (1980).
9. E. C. Southward, *Symp. Zool. Soc. London* **36**, 235 (1975).
10. M. Webb, *Bull. Mar. Sci.* **19**, 18 (1969); J. van der Land and A. Nørrevang, *K. Dan. Videnskr. Selsk. Biol. Skr.* **21**, (No. 3), 1 (1977); E. C. Southward, *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **10**, 264 (1980).
11. A. J. Southward, E. C. Southward, T. Brattegard, T. Bakke, *J. Mar. Biol. Assoc. U.K.* **59**, 133 (1979).
12. I thank J. B. Corliss, Oregon State University, for placing the first specimens of *Riftia* at my disposal; J. F. Grassle, Woods Hole Oceanographic Institution, for further specimens; R. Rieger, M. Fransen, and S. Gardiner, University of North Carolina, and K. Fauchald, National Museum of Natural History, for stimulating discussions, and the latter two for reviewing this manuscript; L. Cullen, of J. Harshbarger's Registry of Tumors of Lower Animals, National Museum of Natural History, for assistance in histological procedures; and E. Jarosewich and P. Dunn, National Museum of Natural History, for determination of sulfur crystals. Travel support, which allowed me the opportunity of making personal observations of living *Riftia*, was from the Research Fund of the Secretary, Smithsonian Institution. *Alvin* dive time was made available through D. Cohen, National Marine Fisheries Service, National Oceanic and Atmospheric Administration. This is contribution No. 19 of the Galápagos Rift Biology Expedition, supported by the National Science Foundation.

11 September 1980; revised 19 February 1981

Chemoautotrophic Potential of the Hydrothermal Vent Tube Worm, *Riftia pachyptila* Jones (Vestimentifera)

Abstract. *Trophosome tissue of the hydrothermal vent tube worm,* Riftia pachyptila *(Vestimentifera), contains high activities of several enzymes associated with chemoautotrophic existence. Enzymes catalyzing synthesis of adenosine triphosphate using energy contained in sulfur compounds such as hydrogen sulfide, and two diagnostic enzymes of the Calvin-Benson cycle of carbon dioxide fixation, ribulosebisphosphate carboxylase and ribulose 5-phosphate kinase, are present at high levels in trophosome, but are absent in muscle. These data are consistent with an autotrophic mode of nutrition for this worm, which lives in hydrogen sulfide–rich waters and lacks a mouth and digestive system.*

The sparse food supply of the deep sea has been thought to preclude a high biomass in this environment (*1*). Biomass decreases rapidly with increasing distance from the surface zone of primary productivity, and the metabolic rates of deep-living organisms are often vastly lower than those of shallow-living species (*1, 2*). The discovery of dense animal communities at hydrothermal vents near the Galápagos Islands (*3, 4*) and off the coast of Mexico (*5*) at depths of approximately 2500 m has led to a major reevaluation of our concepts of trophic interactions and biomass densities in the deep sea. The ultimate source of nutrition for the vent community animals, which include clams, mussels, crabs, anemones, fishes, and, particularly, large tube worms of the class Vestimentifera, is still in question (*3–5*). According to the main theory (*6*) the vent communities have chemosynthetic bacteria as the ultimate food source; food reaching these deep vent communities from surface productivity is quantitatively unimportant. Thus, the vent communities would differ from all known food chains in not depending on photosynthetic carbon fixation. The energy and reducing power needed by the vent bacteria for carbon fixation are thought to be generated by the oxidation of sulfur compounds, especially hydrogen sulfide (H_2S), which is dissolved in high concentrations (millimolar) in the hot effluent waters of the vents (*4, 7*).

Whereas a food chain based on chemoautotrophic bacteria appears probable in the case of filter-feeding animals

0036-8075/81/0717-0336$00.50/0

like clams and mussels, it is less apparent that the large vestimentiferan worms [*Riftia pachyptila* (*8*)] can be nourished adequately by this method. Like their close relatives the Pogonophora, the vestimentiferans lack a mouth, gut, and anus (*9*). Although the Pogonophora have been known for 66 years (*10*), it is not yet clear how they take up nourishment from seawater. The most recent theory is that the Pogonophora live largely, if not entirely, on dissolved organic matter (such as amino acids and carboxylic acids) (*11*). However, studies with radioactively labeled organic molecules have shown that uptake rates are inadequate to account for the metabolic rates of the Pogonophora (*11*). This disparity between the amounts of dissolved organic matter accumulation and the metabolic rates may be even greater in the larger vestimentiferan worms because the ratio of surface to body mass is vastly smaller than in the case of the pogonophorans studied, which measure only millimeters to centimeters in length compared to as much as 3 m for *R. pachyptila* (*8*).

Because of the high concentrations of reduced sulfur compounds (like H_2S) in the mixing zone of vent and ambient seawater, the biotope of the vestimentiferans, it appeared reasonable to ask whether these worms were able to use reduced sulfur compounds in ways similar to those of the chemoautotrophic bacteria in vent waters. Our interest was aroused especially by the observation (*12*) that the trophosome tissue of the worms, a tissue filling the greater share of the coelom (*8*), frequently was spotted with small particles of pure elemental sulfur. To find out whether the trophosome tissue of the vestimentiferans had enzyme systems capable of producing biologically useful energy as adenosine triphosphate (ATP) from sulfur compounds, several enzymes active in sulfur metabolism were assayed (Table 1). Tests were positive for rhodanese, APS-reductase, and ATP-sulfurylase (*13*). No activity was found for adenosine diphosphate–sulfurylase, but this negative result may be due to enzyme denaturation in the frozen tissue available to us. Tissues used were from two worms collected at the Galápagos vents and frozen immediately after capture (*14*). Extracts (*15*) prepared from trophosome and vestimental (*8*) muscle were used in standard tests for these enzymes (*13*). These extracts were used to demonstrate H_2S-stimulated oxygen consumption with the trophosome material, in an oxygen electrode system (*16*). This activity was heat-labile, indicating its enzymatic basis, but could not be quantified because of a lack of proportionality between added trophosome extract and the amount of H_2S-stimulated oxygen uptake.

Table 1. Activities of some enzymes involved in sulfur metabolism and carbon dioxide fixation via the Calvin-Benson cycle, measured in tissues of the hydrothermal vent tube worm *Riftia pachyptila*. Experimental techniques are described in (*13*, *15*). Activities are in international units (micromoles of substrate converted to product per minute) per gram (wet weight) of tissue.

Enzyme	Activities		
	Trophosome		Muscle
	Worm 1	Worm 2	
Rhodanese	4.0	7.6	0
APS-reductase	15.0	23.3	0
ATP-sulfurylase	17.0	74.0	0
RuBP carboxylase*	0.15	0.22	0
Ru5P kinase†	4.3	19.0	0

*Ribulose-1,5-bisphosphate carboxylase. †Ribulose 5-phosphate kinase.

In addition to these enzymes of sulfur metabolism, two diagnostic enzymes of the Calvin-Benson cycle, ribulose-1,5-bisphosphate carboxylase (E.C. 4.11.39) and ribulose 5-phosphate kinase (E.C. 2.7.1.19) were found in high activities—comparable to those in fresh spinach leaves (*17*)—in the trophosome (Table 1). Each of these two enzymes was demonstrated by means of two different analytical techniques (*18*). The Calvin-Benson cycle enzymes were not present in muscle.

The combination of all these enzymes in trophosome tissue leads to the conclusion that *R. pachyptila* is able to oxidize sulfide and make use of the ATP and reducing power generated by sulfur oxidation to reduce and fix CO_2.

Although ATP-sulfurylase especially is involved in sulfate reduction, this enzyme also works in the opposite direction generating ATP from APS and pyrophosphate (*19*). The high activities of these enzymes favor their participation in energy metabolism rather than in detoxification reactions (*20*, *21*). Generating energy by reducing sulfate is highly improbable as anaerobic conditions are needed for those reactions (*22*), and it has been shown that the worms use molecular oxygen at high rates (*23*) and the worms live in an aerobic environment (*3*–*5*).

The high activities of sulfur metabolism and Calvin-Benson cycle enzymes in trophosome tissue, coupled with the observations (*11*) that closely related pogonophoran worms are unable to obtain adequate nutrition from the uptake of dissolved organic material, leads us to ask whether the vestimentiferan worms and, by extension, pogonophorans in general, which may inhabit sulfide-rich muds (*24*), can appropriately be considered as "autotrophic animals." Analyzed materials from the same specimens of *R. pachyptila* used for the enzyme studies (*25*) support the concept that this species is largely, if not fully, autotrophic. These data indicate that different mechanisms of CO_2 fixation at the base of the food chain (or chains) of the vent exist (*25*). That is, bacterially derived carbon, as found in the filter-feeding animals, is fixed by a different biochemical process from that involved in primary CO_2 fixation by the vestimentiferans. The observed ^{13}C depletion value of both vestimental muscle and trophosome tissue is only −10.8 per mil, however. This difference in ^{13}C depletion between the vestimentiferan worms and other vent community animals studied, and the identity of the $^{13}C/^{12}C$ ratio in trophosome and muscle tissues, suggests that the worms are fixing adequate amounts of CO_2 in their trophosome-based chemoautotrophic processes to nourish the rest of the worm.

The ^{13}C depletion characteristic of the tissues of *R. pachyptila* is known only in C_4 plants, which fix CO_2 primarily into phosphoenolpyruvate (PEP) with the enzyme PEP carboxylase. Activity of PEP carboxylase could not be detected in trophosome or muscle, a negative result possibly due to enzyme denaturation during freezing-thawing of the tissues. However, a suggestion that the C_4 plant type of CO_2 fixation mechanism may be involved in *R. pachyptila* metabolism is the observation that, of all enzymes of intermediary metabolism tested so far in this species, malate dehydrogenase has the highest activities (*26*). Such activity would facilitate rapid interconversion of oxaloacetate and malate, an important step in the C_4 plant type of carbon fixation process.

In view of the fact that the sulfur metabolism and carbon fixation reactions discovered in the trophosome tissue of *R. pachyptila* either are not found in animal cells (the Calvin-Benson cycle reactions), or are present only at extremely low levels for detoxification purposes (certain of the sulfur metabolism enzymes), it is appropriate to inquire whether these enzymes are actually present in the animal's cells; that is, whether they are coded by *R. pachyptila* DNA, or contained within symbiotic bacteria located in the trophosome. Descriptions based on light microscopy of the structure of trophosome cells of the vestimentiferan *Lamellibrachia barhami* (*8*, *22*)

refer to granules within the trophosome cells having the dimensions of bacteria.

From the standpoint of the trophic structure of the vent community, the precise localization of the sulfur metabolism and Calvin-Benson cycle enzymes within the trophosome is of less importance than the fact that *R. pachyptila* may represent the first example of an autotrophic animal situated at the base of a food chain.

HORST FELBECK

Scripps Institution of Oceanography, University of California, San Diego La Jolla 92093

References and Notes

1. K. Banse, *Prog. Oceanogr.* **2**, 53 (1964).
2. J. J. Childress, *Comp. Biochem. Physiol.* **50A**, 787 (1975); K. L. Smith and R. R. Hessler, *Science* **184**, 72 (1974); J. J. Torres, B. W. Belman, J. J. Childress, *Deep-Sea Res.* **26A**, 185 (1979).
3. P. Lonsdale, *Deep-Sea Res.* **24**, 857 (1977); Galápagos Biology Expedition Participants, *Oceanus* **22**, 2 (1979).
4. J. B. Corliss *et al.*, *Science* **203**, 648 (1979).
5. RISE Project Group: F. N. Spiess *et al.*, *ibid.* **207**, 1421 (1979).
6. H. W. Jannasch and C. O. Wirsen, *BioScience* **29**, 592 (1979); D. M. Karl, C. O. Wirsen, H. W. Jannasch, *Science* **207**, 1345 (1980).
7. J. M. Edmond *et al.*, *Earth Planet. Sci. Lett.* **46**, 1 (1979).
8. M. L. Jones, *Science* **213**, 333 (1981).
9. J. van der Land and A. Nørrevang, *Z. Zool. Syst. Evolutionsforsch.*, Sonderheft **1**, 86 (1975); *K. Dan. Videnskr. Selsk. Biol. Skr.* **21** (No. 3), 1 (1977). Very recently a mussel has been found also lacking a digestive tract [R. G. B. Reid and F. R. Bernard, *Science* **208**, 609 (1980)].
10. M. Caullery, *Bull. Soc. Zool. Fr.* **39**, 350 (1914).
11. A. J. Southward, E. C. Southward, T. Brattegard, T. Bakke, *J. Mar. Biol. Assoc. U.K.* **59**, 133 (1979).
12. M. L. Jones, personal communication.
13. Rhodanese (E.C. 2.8.1.1) was tested according to A. J. Smith and J. Lascelles [*J. Gen. Microbiol.* **42**, 357 (1966)], and APS-reductase (adenosine phosphosulfate-reductase; E.C. 1.8.99.2) was tested according to H. D. Peck, Jr., T. E. Deacan, and J. T. Davidson [*Biochim. Biophys. Acta* **96**, 429 (1965)]. The ATP-sulfurylase (E.C. 2.7.7.4) was tested in a continuous photometric test in which the ATP formed was used to phosphorylate glucose and the glucose 6-phosphate formed was oxidized with $NADP^+$ and glucose 6-phosphate dehydrogenase. The test mixture contained: triethanolamine-HCl buffer, 0.1 mole/liter, *p*H 7.3; magnesium acetate, 2.5 mmole/liter; glucose, 25 mmole/liter; $NADP^+$, 0.5 mmole/liter; sodium pyrophosphate, 25 mmole/liter; adenosine 5′-phosphosulfate (obtained from Sigma Chemical), 0.5 mmole/liter; Ap_5A [P^1,P^5-di(adenosine-5′)-pentaphosphate, an inhibitor of adenylate kinase] [G. E. Lienhard and I. I. Secemski, *J. Biol. Chem.* **248**, 1121 (1973)], 0.25 mmole/liter; hexokinase, 5 unit/ml; glucose-6-phosphate dehydrogenase 2.5 unit/ml in a final volume of 2 ml. The test could be started with pyrophosphate, APS, or extract. In addition, controls were made by dividing the continuous test into two steps, the first one being at the level of glucose-6-phosphate, which was tested subsequently in the neutralized perchloric acid extract according to G. Lang and G. Michal, in *Methoden der enzymatischen Analyse*, H. U. Bergmeyer, Ed. (Verlag Chemie, Weinheim, 1974), vol. 2, p. 1283.
14. Specimens were frozen immediately after recovery: worm 1, in March 1979 at −15°C and stored at −15°C; worm 2, in December 1979 at −78°C and stored at −80°C.
15. Homogenization was in triethanolamine-HCl buffer, 0.1 mole/liter, *p*H 7.3, 1:4 (weight to volume). Trophosome was homogenized gently in a glass-Teflon Potter Elvehjem homogenizer with large gap; muscle tissue in a glass-glass homogenizer with conical rough contact area. The supernatant of a homogenate centrifuged for 10 minutes at 27000*g* was used as extract. Grinding trophosome tissue extensively with a motor-driven homogenizer (like the one used for muscle) did not increase the yield of enzymes compared to gentle homogenization.
16. Yellow Springs Instrument Co. The test mixture contained: triethanolamine-HCl buffer, 0.1 mole/liter, *p*H 7.3; magnesium acetate, 2.5 mmole/liter; Na_2S, 10 mmole/liter. The reaction could be started with Na_2S or extract. After boiling the extract for 5 minutes in a water bath, the reaction velocity decreased significantly.
17. E. Latzko and M. Gibbs, *Plant Physiol.* **44**, 295 (1969).
18. E. Racker, *Arch. Biochem. Biophys.* **69**, 300 (1957); M. Wishnick and M. D. Lane, *Methods Enzymol.* **23**, 571 (1971).
19. The pyrophosphate used for this reaction could be provided by a reversed reaction of the inorganic pyrophosphatase [P. W. Robbins and F. Lipmann, *J. Biol. Chem.* **233**, 686 (1958)].
20. E. N. Powell, M. A. Crenshaw, R. M. Rieger, *J. Exp. Mar. Biol. Ecol.* **37**, 57 (1979); *Mar. Ecol. Prog. Ser.* **2**, 169 (1980).
21. H. D. Peck, Jr., in *The Enzymes*, P. Boyer, Ed. (Academic Press, New York, 1974), vol. 10, p. 651; J. Westley, *Adv. Enzymol.* **39**, 327 (1973); R. M. Lyric and I. Suzuki, *Can. J. Biochem.* **48**, 344 (1970).
22. P. D. Trudinger, *Adv. Microbiol.* **3**, 111 (1969).
23. J. J. Childress, personal communication.
24. Also in the Pogonophora a "spongy tissue" is found in the trunk comparable to the trophosome of the Vestimentifera [(*8*, *9*); A. V. Ivanov, *Pogonophora*, D. B. Carlisle, Transl. and Ed. (Consultants Bureau, New York, 1963)].
25. G. H. Rau and J. I. Hedges, *Science* **203**, 648 (1979); G. H. Rau, *ibid.* **213**, 338 (1981).
26. J. F. Siebenaller and G. N. Somero, personal communication.
27. This report is contribution No. 9 from the Galápagos Rift Biology Expedition. Research funds for the expedition were from NSF support grant OCE 78-10458 to Dr. Frederick Grassle (Woods Hole Oceanographic Institution); the biochemical studies were supported by NSF grant OCE 78-08853 to George N. Somero (Scripps Institution of Oceanography). Supported by Deutsche Forschungsgemeinschaft grant FE 183/1. I thank Dr. J. J. Childress for help in obtaining and shipping specimens and Dr. G. N. Somero for laboratory space and his comments on this manuscript.

27 May 1980; revised 11 November 1980

Hydrothermal Vent Clam and Tube Worm $^{13}C/^{12}C$: Further Evidence of Nonphotosynthetic Food Sources

Abstract. *The stable carbon isotope ratios in clam mantle tissues taken from both Galápagos and 21°N hydrothermal vent sites were similar to the unusually low ratios of carbon-13 to carbon-12 previously reported for a Galápagos hydrothermal vent mussel. In marked contrast to these bivalves, vestimentiferan worm tissues from a Galápagos vent had isotope ratios that were higher than those of open ocean biota. These observations suggest that more than one nonpelagic and nonphotosynthetic carbon fixation pathway is of nutritional importance to vent animals, and that at least one of these pathways is common to two geographically separated vent sites.*

After dense animal communities were discovered near Pacific Ocean hydrothermal vents (*1*), on-site microbial chemosynthesis was implicated as the primary source of reduced carbon for these organisms. The locally large standing crop and apparent rapid growth (*2*) of some of these animals require a food base that is much more abundant than is normally available to deep-sea benthos. These circumstances, however, do not preclude the possibility that the relatively sparse food resource sedimenting from the ocean's euphotic zone is being physically concentrated or entrained by advective currents near the vents (*3*). Still, high rates of microbial carbon fixation can occur in vent waters (*4*), and this chemosynthetic primary production could conceivably be an important source of energy and biomass for higher trophic levels. Support for the concept that vent animals do not rely on imported, photosynthetically derived food sources was found in the striking dissimilarity between the $^{13}C/^{12}C$ of a vent mytilid mussel and the $^{13}C/^{12}C$ of nonvent marine organisms (*5*). Further isotopic evidence for vent food web autochthony is presented in this report.

Portions of mantle tissue from whole, frozen vesicomyid clams, *Calyptogena magnifica* Boss and Turner, collected by the *Alvin* submarine from the Rose Garden site (Galápagos Rift) and from the 21°N site (East Pacific Rise), were obtained from Dr. George Somero, Scripps Institution of Oceanography. Also removed were samples of frozen vestimentum and trophosome of a vestimentiferan worm, *Riftia pachyptila* Jones, originally collected from Rose Garden. All of the above samples were dried for several days at 60°C and then ground or pulverized. A 50-mg portion of the dried, ground vestimentum was submerged in 8 percent HCl and the solution was then gently heated to dryness. Subsamples (5 to 10 mg) of all of the above tissues were then combusted and the resultant CO_2 was purified, collected, and isotopically analyzed by previously described methods (*6*). The $^{13}C/^{12}C$ of each sample is reported as $\delta^{13}C$, the relative per mil difference between the $^{13}C/^{12}C$ of the sample and the $^{13}C/^{12}C$ of the PDB carbonate standard (*7*).

The $\delta^{13}C$ of clam mantle tissues from both Galápagos and 21°N vents are quite similar to the previously reported $\delta^{13}C$ of a Galápagos vent mussel (Table 1). This earlier study (*5*) pointed out that such $\delta^{13}C$ values are lower than those of animals sampled from other marine envi-

0036-8075/81/0717-0338$00.50/0