Riftia pachyptila: a highly integrated symbiosis

Hydrothermal vents *Riftia pachyptila* Symbiosis Sulfide Pogonophora

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ABSTRACT

The deep-sea hydrothermal vent tubeworm *Riftia pachyptila* (Vestimentifera) lacks a digestive tract entirely. Nutriments are presumably provided to the animal by symbiotic chemoautotrophic bacteria which are harbored in a specialized organ of the animal, the trophosome. These bacteria are proposed to fix carbon dioxide from the ambient seawater using the energy gained from oxidation of sulfide which is emitted by the hydrothermal vents. Carbon dioxide is transported in a freely dissolved form in the blood to the bacteria and, after fixation in the plume, into 4-carbon compounds, mainly succinate and malate. The bacteria then decarboxylate the 4-carbon compounds or fix the dissolved carbon dioxide with their Calvin-Benson cycle. The sulfide which is oxidized by the bacteria is transported by a specialized hemoglobin which is abundant in the worms' blood. The hemoglobin prevents premature oxidation of the sulfide and prevents poisoning of the animals' tissues.

The significance of the trophosome for the evolution of the pogonophoran and vestimentiferan body plan is discussed.

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RÉSUMÉ

Riftia pachyptila : une symbiose très forte

Le ver tubulaire *Riftia pachyptila* (vestimentifère) des évents hydrothermaux profonds est totalement dépourvu de tube digestif. L'animal est probablement alimenté en sels nutritifs par des bactéries chimio-autotrophes symbiotiques localisées dans un organe spécialisé de l'animal, le trophosome. Ces bactéries sont censées fixer le dioxyde de carbone de l'environnement marin en utilisant l'énergie fournie par l'oxydation des sulfures émis par les évents hydrothermaux. Le dioxyde de carbone soluble dans le sang est apporté aux bactéries après s'être fixé dans la branchie sous forme de composés du carbone-4, principalement le succinate et le malate. Ensuite les bactéries séparent le carboxylate des composés du carbone-4, ou fixent le dioxyde de carbone dissous au cours de leur cycle de Calvin-Benson. Le sulfure oxydé par les bactéries est transporté par une hémoglobine spécialisée abondante dans le sang du ver. L'hémoglobine évite l'oxydation prématurée du sulfure et l'empoisonnement des tissus de l'animal.

Le rôle du trophosome dans l'évolution du pogonophore et l'organisation du corps des vestimentifères est discuté.

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INTRODUCTION

The deep-sea hydrothermal vents were discovered in 1977 by geologists investigating sea floor spreading centers (Weiss *et al.*, 1977, Corliss *et al.*, 1979, Rise Project Group, 1980). They also found, to everyone's surprise, dense animal communities at the vent sites. These dense assemblages of animals were immediately around the areas where sea water, heated and chemically altered by contact with the hot magma deep in the earth's crust, is ejected again. Large molluscs, crabs, sea anemones, fishes and the giant tubeworms *Riftia pachyptila* were among the distinctive species which were previously unknown, but lived in abundance at the vent sites (Hessler, Smithey, 1983; Grassle, 1985).

The existence of the most conspicuous member of this community, the vestimentiferan tubeworm, Riftia pachyptila came as a complete surprise since the pogonophorans, which were initially believed to be of the same phylum and the few other vestimentiferans known at this time were much smaller (Jones, 1981 a; b). All vestimentiferans including Riftia pachyptila, however, have very similar body plans, whose most striking characteristic is the complete absence of a digestive system. This body plan has intrigued biologists since the discovery of the pogonophorans and there have been many attempts to explain the nutrition of this group (Southward, Southward, 1981). Suggestions were made that the animals thrive entirely by the uptake of dissolved organic matter, by phagocytosis of small particulate material or by the uptake of dissolved organics released from the external digestion of particulate matter in the spaces between tube and body. The trophosome was considered an organ to provide the gonads, which are located within the trophosome, with nutrients (Southward, Southward, 1981). None of these explanations for the unusual morphology was fully satisfying. The body plan was clearly modified for some reason and the animals had to obtain nutrition from somewhere, but no single explanation for these problems was generally accepted. The discovery of the giant tubeworms made the usual explanations for the body plan even more unlikely because, due to their size, these animals had to obtain so much more nutrition through a relatively much smaller surface than the tiny pogonophorans. Soon after the second biological expedition to the hydrothermal vents in late 1979, several workers found the probable explanation for how the animals obtain energy and material for their growth and metabolism (Cavanaugh et al., 1981; Felbeck, 1981; Southward et al., 1981; Southward, 1982). This explanation, a symbiosis of the worms with endosymbiotic chemolithoautotrophic bacteria. also provides the basis for understanding the pogonophoran body plan. The bacteria live in animal cells in a specialized internal organ, the trophosome, and are proposed to provide the hosts with reduced organic matter which they synthesize from inorganic substances provided by the worms' circulatory system. This manuscript will describe this symbiosis and will discuss the physiological and biochemical characteristics of the symbiotic system consisting of the hydrothermal vent tubeworm Riftia pachyptila and its endosymbionts.

HABITAT

Deep-sea hydrothermal vents have been found along seafloor spreading centers, *i.e.* where two plates of the earth's crust move apart, and other tectonically active areas. At the sites of venting, water is seeping into the seafloor and is heated by very hot rock deep below the surface of the ocean floor. The geology of these sites has been described extensively elsewhere (Francheteau et al., 1979; MacDonald, Luyendyk, 1981; Edmond et al., 1982). The distinctive animal communities are associated mainly with areas where warm water is being released. These vent communities are characterized by the presence of the giant vestimentiferan tubeworm Riftia pachyptila, the large clam Calvptogena magnifica (Boss, Turner, 1980), the mussel Bathymodiolus thermophilus (Kenk, Wilson, 1985) or their respective close relatives. These animals live in the area where the venting waters are actively mixing with the surrounding ambient waters (Hessler, Smithey, 1983; Grassle, 1985). The dynamic nature of this environment as well as the variation in flow and mixing patterns in relation to animal size has made the description of the water chemistry around the animals quite difficult (Edmond et al., 1979; Welhan, Craig, 1979). In addition, since the vent water is very reactive due to the mixture of reduced (from the vents) and oxidized (from the ambient seawater) compounds the time from sampling at the site to the surface where samples were analyzed was too long. Reactions could happen which alter e.g. the concentrations of oxygen and sulfide in a given sample. In addition to this problem, the sampling methods were not precise enough to deal with the distribution of chemical parameters around the animals. To be able to monitor on a small scale, however, was crucial since the mixing patterns around the animals change constantly and are different at the top of the tubeworms than at the posterior end which is attached to the rocks. The "scanner", a device to measure in situ temperature and the concentrations of sulfide, oxygen, and silicate was developed by Johnson et al., to carry out these analyses around the animals (Johnson et al., 1986 a; b). For the analyses a continuous stream of water is sampled with a small intake tube positioned by the arm of a research submarine. The waterstream is then immediately chemically modified in situ according to standard techniques of seawater analysis and the concentrations of the different components measured photometrically. The results are stored in a computer memory and also displayed on a terminal inside of the submarine. This "real time" display of the concentrations only 4 minutes after intake of the sample together with the recorded position of the intake tube for the "scanner" provides information about the vent water flow around the animals. In series of measurements at the Galapagos Rift it was found that Riftia pachyptila lives close to vent openings where water with high sulfide and low oxygen concentrations is emitted. The posterior end of the tubeworms is attached to the rocks and bathed in relatively warm vent water while the plumes are exposed to a mixture of vent and ambient seawater characterized by high sulfide ($\approx 250 \ \mu M$) and warm temperatures. The clams are positioned with their feet extending down into cracks of the rocks which emit water slightly warmer than

ambient (Arp *et al.*, 1984). The mussels are found in large clumps where venting is quite active as well as in less strongly venting areas, but at "Rosegarden" (Galapagos) always associated with measurable concentrations of hydrogen sulfide (Johnson, pers. comm.).

ANATOMY

The major tissues of Riftia pachyptila are the plume, the vestimentum, the trophosome, the vascular system, the fluid filled coelom, the body wall and the opisthosome (Figure: Jones, 1981, a; b). Vascular blood, coelomic fluid, and trophosome account for about 4, 26, and 15 % of the total body mass, respectively, the rest is mainly accounted for by the vestimentum and the plume (Childress et al., 1984). Higher estimates of trophosome mass (40-60 % of the total bodymass) in the literature (Wittenberg, 1985; Jannasch, 1985) are apparently based on estimates rather than actual measures of this tissue. The worm is free to move up and down in the tube and often extends the large obturacular plume or withdraws it from contact with the surrounding water. This plume is the only part of the worm which comes into free contact with the venting waters, the remaining body surface being always within the thick-walled tube. This plume has a large surface area due to lamellae which are finely divided into tentacles which bear smaller extensions, pinnules. This organ clearly appears to be the major source of exchange of materials between the environment and the animal. The plume is highly vascularized with afferent and efferent vessels reaching all the way to the pinnules. It is supplied from the dorsal vessel which collects blood from the trophosome, the blood is transported back to the trophosome through the major ventral vessel. In the trophosome the ventral and dorsal vessels are apparently



Figure

Metabolic scheme of Riftia pachyptila. The transport of metabolically important compounds is shown in relation to the major organs of the animals. $4 \cdot C$: carbon compound; $0 \cdot C$: organic carbon; bact: bacteria.

Schéma du métabolisme de *Riftia pachyptila*. Principaux organes des animaux et transport des composés importants dans le métabolisme : 4-C : composé du carbone-4 ; 0-C : carbone organique : bact : bactéries.

connected via an extensive net of vessels and sinuses. The blood is presumably pumped by a heart like structure in the anterior portion of the dorsal vessel as well as auxilliary hearts within the tentacles. The vascular blood undoubtedly accounts for substantially more than the 4 % of the body weight estimated by attempting to drain the vascular system. This blood is hemoglobin-rich (3 to 5 mM heme) and its difference in concentration from the coelomic fluid (1 to 3 mM heme) indicates clearly that it is not confluent although some dissolved materials are readily exchanged between these compartments (Childress et al., 1984). Posterior to the plume is the muscular tissue, the vestimentum. This muscle is used by the animal to position itself in the tube and to grip the tube. Posterior to the vestimentum is the large sac formed by the body wall and terminated by the setate opisthosome. Within the sac are two of the major tissues of this worm, one, the coelomic fluid, bathes the other, the trophosome. Since the coelomic fluid accounts for about 26 % of the worm's mass and is rich in hemoglobin it represents a major possible store for oxygen and metabolites within the animal. Although this compartment has no obvious means of circulation it is clearly able to exchange material with the vascular blood rapidly (Childress et al., 1984) and, therefore, probably plays a significant physiological role beyond the obvious hydraulic one. The trophosome is the most distinctive tissue in the worm. Here the symbiotic bacteria are found inside of animal cells (Cavanaugh et al., 1981; Bosch, Grasse, 1984 a; b; DeBurgh, 1986). This tissue with the coelomic fluid and the gonads fills the posterior part of these worms. It is highly vascularized. The trophosome is formed of lobes which are enclosed by a layer of cells without bacteria. These lobes are penetrated by a large number of small blood vesicles forming an extremely large contact area between the blood system and trophosome cells. The inside of the lobes is formed by cells containing large numbers of gram negative bacteria. Usually one or a few of the bacteria are enclosed by an animal membrane. The bacteria frequently contain glycogen-like inclusions and structures resembling carboxysomes. In Riftia pachyptila the bacteria are primarily one species as indicated by the sequences of 5s RNA (Stahl et al., 1984) and analyses of DNA base composition (Nelson et al., 1984). Contrary to these observations a variety of bacterial strains have been isolated and cultured from trophosome tissue (Jannasch, 1985; Jannasch, Mottl, 1985). These, however, could be components of the trophosome's bacteria minor complement. Anatomically only one kind of bacteria has been identified in the trophosome of *Riftia pachyptila* but several have been seen in the trophosome of other species of vestimentifera, although also here one species usually was predominant.

The anatomy and the ultrastructure of the trophosome indicate that this organ is designed to provide a means to exchange substances between the bacteria-containing cells and the blood and, thus, the rest of the animal. An organ like the trophosome of *Riftia* is characteristic for pogonophoran and vestimentiferan tubeworms. Trophosomes containing bacteria have been found in a variety of pogonophorans where they have been proposed to assume the same or similar functions (Southward, 1982; Southward *et al.*, 1981). We can now summarize

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the body plan of *Riftia pachyptila* (and other Vestimentifera and Pogonophora). These animals are characterized by a large exchange surface which brings blood in close contact with the environment. This exchange surface is connected by a closed circulatory system with a tissue inside the animals' body containing symbionts. The muscular tissues, vestimentum and body wall appear to be related to positioning the animal in its tube and producing the tube. In the absence of a mouth and a gut this system is clearly set up to facilitate the exchange of materials between the trophosome and the external sea water.

PHYSIOLOGY AND METABOLIC PATHWAYS

Clearly, some respiratory gases as well as reduced material for energy production has to be collected from the outside and transported to tissues able to acquire the necessary organic matter for the worm, e. g. the trophosome. Undoubtedly dissolved reduced organic matter is taken up via the plume and utilized for this purpose but due to the large size of the animals and apparent low levels of DOC in the vent waters (Smith, 1985) this route appears unlikely to satisfy the animals. Also, if this were the primary nutritional mode the presence of bacteria would not be necessary. The obvious conclusion is that the bacteria must be metabolizing something else than reduced organics and synthesize their own organic material which they could in part transfer to the host. To examine this hypothesis we will describe the metabolism and enzymology of the animal and the symbiotic bacteria and properties of the blood which is the vital link connecting the trophosome to the waters around the animal.

The first indicators that something unusual was used to provide the worms nutritional requirements were data on the isotopic composition of carbon, nitrogen, and sulfur of the bacteria and the tubeworms (Rau, Hedges, 1979; Rau, 1981 a, b; Williams et al., 1981; Fry et al., 1983). The importance of these ratios can best be explained by the example of the C^{13}/C^{12} ratios. One can assume that all animals depending on the same source of reduced carbon at the vents, initially believed to be the free-living sulfur bacteria in the warm waters, should have a similar C¹²/C¹³ ratio (see Rau, 1985 for review). This, however, was not the case, Riftia pachyptila had a C13/C12 ratio that most closely resembled that of plants using the C4 cycle for carbon fixation. It was proposed therefore, that the tubeworms were not included in the normal food chain at the vents but that they had to have a different way to obtain their carbon (Rau, 1981 b). Since the C^{13}/C^{12} ratio of the animal tissue (without bacteria) was the same as that of the trophosome it also could be deduced that the worms have the same carbon source as the symbiotic bacteria within the trophosome. Similar conclusions could be drawn from results obtained for nitrogen and sulfur isotopes, all data indicate a nonphotosynthetic origin of the organic compounds of the tubeworms' bodymass.

Initially it was only known that the bacteria are gram negative (Cavanaugh *et al.*, 1981) and contain enzymes indicating that they are chemoautotrophic (Felbeck, 1981). Ribulose -1,5- bisphosphate carboxylase and ribulose -5phosphate kinase, characteristic enzymes of the Calvin-

Benson cycle of carbon dioxide fixation were found as well as enzymes strongly pointing towards a role of sulfur metabolism. In addition, nitrate reductase could be detected which would enable the bacteria to obtain nitrogen from the environment (Felbeck et al., 1981). These sets of enzymes would allow the bacteria to survive and grow without addition of organic carbon but instead to obtain their carbon supply by fixing carbon dioxide. The worms, however, do not have the enzymes for a net carbon fixation and therefore have to rely on a supply of reduced organic substances from other sources. The identical C^{13}/C^{12} ratio of the animal and the bacteria-containing tissue can only be explained if the bacteria supply the host animal with reduced carbon. The energy necessary for this fixation by the bacteria can only be obtained by oxidizing a reduced substance since photosynthesis can obviously be excluded in the deep sea. Since enzymes characteristic for sulfide oxidation and mechanisms to concentrate and transport sulfide have been found in the trophosome tissue this energy is most likely gained by the oxidation of sulfide which is abundant in the surrounding seawater and originates in the vent fluids. The sulfide is clearly available to the bacteria since sulfide concentrations of up to several mM have been measured in blood and coelomic fluids of Riftia pachyptila (Arp, Childress, 1983; Childress et al., 1984). This presence of sulfide in the blood is also unusual in that it is a very potent inhibitor of cytochrome-c-oxidase. Because of that, animals without symbiotic bacteria, e. g. the tubeworms, exclude sulfide from their bodyfluids to a large extent oxidizing it mostly at the body surface and elsewhere. In contrast, Riftia pachyptila concentrates sulfide from the environment into the blood. Elemental sulfur, a metabolite of sulfide, can also be found in high concentrations (up to 20% of the dry weight in the posterior trophosome) in the trophosome of freshly captured animals. Since elemental sulfur is highly hydrophobic it cannot be collected on the outside of the animals and transported to the trophosome where it is stored.

Fisher and Childress (1984) have demonstrated that bacterial preparations of trophosome from Riftia pachyptila oxidize sulfide. They also show a rate of oxygen consumption for the bacteria which is an order of magnitude higher than that of the whole animal. Their trophosome preparations also oxidized methane which, however, as well as hydrogen is unlikely to be a major energy source for the bacteria. The concentrations of hydrogen and methane in the vent waters are very low (less than 1 mm) (Welhan, Craig, 1979), Riftia pachyptila lacks any apparent mechanism to concentrate them from the environment (Arp et al., 1985), and intact Riftia pachyptila do not consume methane (Childress et al., 1984). In fact, no evidence has been presented that indicates the presence of methanotrophic symbioses in any vestimentiferans, although the suggestion of methanotrophy has been made (Kulm et al., 1986; Paull et al., 1985). As discussed by Arp et al. (1986) and Childress et al. (1984), such symbioses are extremely unlikely because of the problem of transport of sufficient methane through the blood to the remotely located symbionts. In contrast a methanotrophic symbiosis has now been demonstrated in a hydrocarbon seep mussel which has the bacteria located at the surface of the gills where a methane

transport mechanism is unnecessary (Childress et al., 1986).

It presently appears that the trophosome bacteria in *Riftia* pachyptila oxidize sulfide with oxygen and fix carbon dioxide into organic matter. Some of this organic matter is then transferred to the animal to satisfy its nutritional needs (Felbeck, 1985; Felbeck *et al.*, 1985). Because of the similar C^{13}/C^{12} ratio in trophosome and the rest of the body, either the animal must derive most of its organic carbon from the bacteria or carbon must be shifted between bacteria and animal extensively. This scheme for the role of the bacteria places great emphasis on the blood and circulatory properties of the central and most important tissue, the trophosome containing the symbiotic bacteria.

The blood contains an abundant large molecular weight hemoglobin (Terwilliger et al., 1980; Terwilliger, Terwilliger, 1985) which is found in concentrations approaching 5 mM in some individual worms (Childress et al., 1984). This high concentration gives it about half of the oxygen carrying capacity of human blood and about the same as the highest found in annelids. The Hb has a high affinity for O₂ (P_{s0} between 2 and 3 mm Hg at 8°C) which undergoes a moderate decline in affinity as temperature increases (apparent H= -9.2 kcal/mol; Arp, Childress, 1981; Wittenberg et al., 1981). It also has a small Bohr effect (log $P_{50}/pH=-0.12$). In a general way these characteristics are similar to those of Lamellibrachia sp. hemoglobin and those of some annelids (Terwilliger, Terwilliger, 1985). This blood clearly has the ability to load O₂ in the low oxygen environment of the vents and the temperature effect on affinity might well facilitate unloading in the trophosome at the base of the animal. The small Bohr effect (Lamellibrachia has none; Terwilliger, Terwilliger, 1985) may be of importance for an animal wich transports both CO_2 and O_2 in the same direction instead of in the heterotrophic pattern. The high affinity may also serve to maintain a low PO₂ in the trophosome while at the same time providing a high oxygen concentration. This could be beneficial if the bacteria are sensitive to higher O₂ levels as has been shown for other sulfur oxidizing bacteria and as appears for the trophosome bacteria (Fisher, Childress, 1984).

Another important metabolite needed by the bacteria is the highly toxic sulfide. We have demonstrated that Riftia pachyptila held at low external sulfide concentrations (50 -200 nM) are able to concentrate this chemical up to concentrations of several mM in their vascular and coelomic blood (Childress, unpublished observations). This is particularly striking since the cytochrome-coxidase of *Riftia pachyptila* is inhibited by less than μM sulfide levels (Powell, Somero, 1983). These results are reconciled by the observations that the hemoglobin of Riftia pachyptila reversibly binds sulfide (0.7 - 2.5 mMol/heme depending on blood fraction) at a non-heme site (Arp, Childress, 1983; Childress et al., 1984; Arp et al., 1986) with a high enough affinity to protect the cytochrome-c-oxidase (Powell, Somero, 1983). This binding of sulfide also prevents the spontaneous reaction of O₂ and HS⁻ even when both are present at high concentrations (Fisher, Childress, 1984). The binding of sulfide does not appear to affect the Hb affinity for O₂. This sulfide-binding mechanism can, however, only

prevent toxicity in the face of a continuous supply of HSif there is removal of sulfide from the blood to prevent its becoming saturated. Fisher and Childress (1984) have shown that the bacteria can remove bound sulfide from the blood and thus serve as a sink in this mechanism to prevent poisoning. The extracellular hemoglobin of *Riftia pachyptila* thus not only serves to transport oxygen from the plume to the trophosome but also for the concentration and transport of sulfide. It also appears to protect the animal tissues from sulfide poisoning and inhibits the spontaneous oxidation of sulfide by free oxygen.

In addition to sulfide and oxygen the blood has to transport the carbon dioxide used by the bacteria to produce reduced organic material. This CO₂ may be transported in several ways, dissolved or bound to hemoglobin, or incorporated into low molecular weight organic acids. It has been demonstrated that CO₂ in the blood is transported both ways. After incubation of animals in radiolabelled CO₂ about half of the radioactivity in the blood is in acid labile form, *i. e.* CO_2 bound by hemoglobin or freely dissolved while the other half is in the form of succinate and malate (Felbeck, Somero, 1982; Felbeck, 1985; Felbeck et al., 1985). The acid labile portion of the transport is most likely freely dissolved in the plasma since the hemoglobin only shows a very small Bohr effect. The total concentration of CO₂ in Riftia pachyptila blood is generally quite high (about 4 mM at pH 7.5, at 8° C; Childress et al., 1984) and is thus a good potential source of CO₂ for the bacteria. For the carbon dioxide incorporated in organic acids it has been proposed that tissue pyruvate is carboxylated in the plume with carbon dioxide from the seawater. The C4 molecule synthesized is reduced to malate and succinate which can be decarboxylated again by the bacteria in the trophosome thus providing CO_2 to the bacteria.

In pulse chase experiments using ¹⁴CO₂ the label in the blood is all in the form of organic material after 16h (Felbeck, unpublished results). Since the concentration of malate alone in the blood is high (10 mM has been measured) this mechanism could provide a large quantity of CO₂ to the trophosome. Not much is known so far about the tubeworms' sources of nitrogen. The finding of nitrate reductase in the trophosome indicates a reduction of nitrate to yield the ammonia necessary for many biosynthetic reactions. No experiments have been done so far to approach this question. Other possible nitrogen sources could be the uptake of dissolved amino acids from the seawater or the uptake and fixation of elemental nitrogen. The latter appears to be very unlikely since no trace of a nitrogenase could be identified in the trophosomal tissue (Wittenberg, pers. comm.). Uptake of amino acids has been shown in experiments; whether this mechanism could provide enough nitrogen for the animals metabolism is, however, unknown since the concentrations of free amino acids around the vents have not been measured thoroughly yet. Smith (1985) and Comita et al. (1983) found the concentrations of total dissolved organic material around the vents as low as those of ambient deep-sea water further away.

We can now consider the whole animal metabolism of *Riftia pachyptila*. The intact animals have rates of metabolism about as high as the most active annelids

previously studied in spite of the relative sluggishness of the vestimentiferans (Childress, Mickel, 1985). This is probably the result of the very high rates of metabolism of the trophosome bacteria (Fisher, Childress, 1984). These animals also have the ability to remove O₂ from seawater down to O₂ partial pressures below 10 mm Hg (Childress et al., 1984). This is one clear advantage of the high affinity hemoglobin. These animals are also able to survive anaerobic conditions for extended periods of time (> 36 h), a clear advantage in the variable vent environment. When intact Riftia pachyptila are maintained away from sulfide for a few days their balance of CO₂ production and O₂ consumption are typically heterotrophic (Childress et al., 1984). Preliminary experiments indicate that in the presence of sulfide CO₂ production declines indicating a shift to autotrophy in the presence of sulfide (Anderson, pers. comm.). It is also clear that the intact animals do not metabolize CH₄ or H₂, presumably because they lack a mechanism for concentrating and transporting them to the bacteria.

Whole animal experiments have also been carried out to determine the relation between the coelomic and vascular compartments (Childress et al., 1984). These experiments have shown that although relatively large differences can sometimes be observed between these compartments immediately after capture, after a period of 2 days the CO₂, H₂S concentrations and the pH in the two compartments generally show equilibrium distribution. This indicates that even though these compartments are not confluent, they exchange material rather freely. This was supported by a recent experiment in which radiolabeled CO₂ appeared in the coelomic blood within 15 min of being applied to the animal. The vascular system then seems to be the supply route from the plume to the trophosome and other animal tissues, while the abundant coelomic fluid can "buffer" the entire system against short term changes in the supply of metabolites.

To summarize the present view, it is proposed that sulfide is taken up and concentrated from the environmental seawater, bound to hemoglobin transported to the bacteria in the trophosome, and there released again to be used for energy production. The bacteria then use the energy and the reducing power to fix carbon dioxide in the Calvin Benson Cycle. The CO₂ for this cycle is transported to the bacteria either freely dissolved in the blood or in form of organic acids like malate or succinate. The organic acids are decarboxylated to release carbon dioxide in the trophosome. Using these mechanisms the bacteria are able to synthesize reduced organic material from inorganic substances provided by the host, *Riftia pachyptila*, via its circulatory system.

EVOLUTION

It is evident that the trophosome plays the major role in providing nutrition to the vestimentiferan tubeworms. So far, in all vestimentiferans investigated, the trophosome contained symbiotic bacteria. Since all vestimentiferans lack a digestive tract, all of these bacteria probably play an important role for the worms' needs. Much work is also being carried out on some more easily accessible bivalves from shallow water and other hydrothermal vent bivalves

which have sulfide-oxidizing bacterial symbionts (Cavanaugh, 1983; 1985; Felbeck et al., 1981; Felbeck. 1983; Fiala-Medioni, 1984; Le Pennec, Hily, 1984; Fiala-Medioni, Metivier, 1985; Dando et al., 1985; 1986; Schweimanns, Felbeck, 1985; Vetter, 1985; Wittenberg, 1985). In those the bacteria are proposed to fulfill the same or at least a very similar role to that in the tubeworms. A major difference is the location of the bacteria in the animal, instead of in a trophosome-like structure they are housed inside the gill cells where the bacteria are only a very short distance away from their sources of energy and carbon. Some of these bivalves also (e.g. Solemva species and all Lucinidae) completely lack a digestive system or have a greatly reduced one (Reid. Bernard, 1980; Schweimanns, Felbeck, 1985). The question is obvious why the vestimentiferans and pogonophorans do not have symbionts in the organ located in close contact with the seawater, the plume.

One obvious advantage of the pogonophoran body plan is simply that the symbiotic tissue is protected inside the tube and not exposed to predation or the environmental risks as is the plume. However, we do not believe that this is the main selective advantage of the pogonophoran body plan. Rather, we believe that the primary selective values of this body plan are those relating to the value of organ level specializations within the bodies of animals. That is, organs specialized for particular functions can evolve sizes and characteristics relatively free from constraints which apply to organs, like molluscan gills containing symbionts, which must carry out several different functions. In addition, the organ level of organization allows the separate evolution of specialized exchange surfaces and transport systems to supply not only the needs of the specialized tissue but to provide an environment more tailored to its needs than if it were simply part of gills or some other superficial structure.

That is, we propose that the distinctive and distinguishing feature of the pogonophoran and vestimentiferan body plan which sets this body plan apart from others and provides the key to wherever it is found is the presence of an organ (the trophosome) specialized for sulfide-oxidizing, carbonfixing bacterial endosymbiosis located within the trunk of the animal. By limiting the symbiosis to this specialized organ, the animals have evolved the capacity to contain very large microbial biomasses, apparently obviating the need for the uptake of particulate matter and thereby the organs and tissues needed to process particulate matter. At the same time the development of this organ required the evolution of great abilities to exchange, concentrate and transport those molecules needed by the endosymbionts. As detailed earlier in this manuscript, these animals have evolved large exchange surfaces, specialized hemoglobins, high blood and coelomic fluid volumes and other characteristics which enable them to provide the trophosome with an abundant suply of O₂, HS and CO₂ while at the same time controlling the partial pressures of these substances and "buffering" the blood supply against major changes in supply caused by the animal's retraction into the tube.

The vent dwelling vestimentiferans like *Riftia pachyptila* are clearly the largest users of the pogonophoran body plan. We believe that this large size can best be understood in the context of the vent environment. In particular, this

environment has a vertically large transition zone where HS- and O_2 may both be found in the water, but the horizontal amount of space associated with this is quite limited. This is the opposite of other HS- rich environments where the transition zone has a very small vertical extent, but a large horizontal one. Clearly the vent habitat allows the evolution of large animals because of its vertical extent and selects for their evolution because of its very limited horizontal extent. In other HS- rich habitats the situation appears virtually reversed.

In summary, we propose that the pogonophoran body plan is distinguished by a unique organ of nutrition, the trophosome, which is specialized for HS- oxidizing, CO_2 fixing bacterial endosymbionts. The other characteristics of this body plan, such as the large gas exchange surface,

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unique hemoglobin, well developed vascular system, large coelomic fluid volume, and lack of a gut and associated structure have evolved in response to the needs, or lack of needs in some cases, of this peculiar organ.

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