for interaction with O_2, thus enabling the persistence of sulfide in the animal and the simultaneous transport of both sulfide and O_2 by the blood to internal symbionts. This further supports the proposed sulfide-based chemoautotrophy hypothesis (*4*).

The hemoglobin of *R. pachyptila* accounts for more than 90 percent of the total protein present in the blood (*11*) and is therefore a likely candidate for the sulfide-binding protein. Vertebrate methemoglobin (ferric hemoglobin produced by treatment with nitrite or ferricyanide) can form complexes with sulfide (*12*). The sulfide binding by *R. pachyptila* blood does not appear to involve the formation of methemoglobin, however, as substantial levels of methemoglobin have not been detected in *R. pachyptila* blood (fresh blood showed no spectral indication of methemoglobin or sulfhemoglobin formation when exposed to sulfide levels as high as 5.3 mmHg of H_2S gas) and determinations of methemoglobin in fresh blood were low (*13*). In addition, vertebrate methemoglobin does not occur naturally in high concentrations and is nonfunctional for O_2 transport, whereas *R. pachyptila* sulfide-binding protein is present in high concentrations and the blood has a high O_2 capacity simultaneously.

The binding of sulfide by vertebrate methemoglobin protects against sulfide poisoning both in vitro and in vivo (*14*). The binding protein in *R. pachyptila* has been shown to protect against sulfide poisoning in this species (*15*).

In conclusion, *R. pachyptila* blood contains a sulfide-binding protein that may, in analogy to oxygen-binding proteins, allow the animal to concentrate sulfide from its environment and transport sulfide to the point of utilization within the animal's body. The unloading of sulfide to the trophosome could occur in response to lower *p*H or lower sulfide concentrations. This protein may also have an important function in protecting the animal against sulfide toxicity.

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9. *Riftia pachyptila* blood with an initial sulfide concentration of 2.62 m*M* was titrated with 0.5*M* HCl to *p*H 5. Blood sulfide concentration dropped to 0.44 m*M* after 3 hours, 0.18 m*M* after 5.5 hours, and 0.03 m*M* after 11.5 hours.
10. Blood dialyzed against oxygenated 50 m*M* tris buffer (*p*H 7.76 at 5°C) that contained 0.74 m*M* sulfide accumulated and retained 3 m*M* sulfide for 5 days.
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Blood Components Prevent Sulfide Poisoning of Respiration of the Hydrothermal Vent Tube Worm *Riftia pachyptila*

Abstract. *Respiration of plume tissue of the hydrothermal vent tube worm* Riftia pachyptila *is insensitive to sulfide poisoning in contrast to tissues of animals that do not inhabit vents. Permeability barriers may not be responsible for this insensitivity since plume homogenates are also resistant to sulfide poisoning. Cytochrome c oxidase of plume, however, is strongly inhibited by sulfide at concentrations less than 10* μM. *Factors present in blood, but not in cytosol, prevent sulfide from inhibiting cytochrome c oxidase. Avoidance of sulfide poisoning of respiration in* Riftia pachyptila *thus appears to involve a blood-borne factor having a higher sulfide affinity than that of cytochrome c oxidase, with the result that appreciable amounts of free sulfide are prevented from accumulating in the blood and entering the intracellular compartment.*

The dense communities of organisms at the deep-sea hydrothermal vents are believed to be highly dependent on primary production that is driven by the oxidation of hydrogen sulfide (*1*). The sulfide that is produced by reduction of seawater sulfate in the hot basaltic rocks of the sea floor (*2*) is oxidized by chemolithotrophic bacteria that are either free-living or contained as symbionts within specialized tissues of certain vent animals (*3, 4*). The vent tube worm *Riftia pachyptila* Jones (*5*), which lacks a mouth and digestive system, harbors symbiotic bacteria at densities of approximately 10^9 cells per gram (fresh weight) in its trophosome tissue (*3*). Studies of enzyme systems (*4, 6, 7*) and stable carbon isotope ratios (*8*) of *R. pachyptila* indicate that reduced carbon compounds generated in the trophosome may supply a major share of the organism's needs for these materials.

While supplying an abundant source of energy for primary production in the vent ecosystem, hydrogen sulfide creates a potential problem for the vent organisms as a result of its extreme toxicity. With the exception of certain animals from sulfide-rich sediments (*9*), the animals that have been studied are killed by sulfide concentrations of a few micro-

Table 1. The effects of sulfide on oxygen consumption by whole tissues and plume homogenates. Percent inhibition values represent means ± 1 standard deviation, with the number of different tissue samples tested given in parentheses. Tissue oxygen consumption was measured at 20°C with an oxygen meter (Yellow Springs Instruments, model 53); surface seawater was used as the incubation medium. For measurements of sulfide effects, a small volume of a concentrated Na_2S solution was injected into the respiration chamber to yield a final sulfide concentration of 0.6 m*M*. The effect of sulfide on respiration was determined by first measuring the oxygen consumption of a fresh tissue sample (run A), and then repeating the measurement with the same sample and injecting sulfide after an initial rate was obtained (run B) (see Fig. 1). The degree of inhibition was calculated from the percent activity, given by (initial rate A/initial rate B)/(final rate A/final rate B) × 100. Measurements using the same tissue sample in several consecutive runs made without sulfide showed that the ratio of initial rate to final rate was a constant. Consumption of oxygen due to chemical oxidation of sulfide was subtracted from the rates measured in the presence of sulfide. Sodium sulfide solutions were prepared from a concentrated stock solution sealed from air immediately before respiration measurements or enzyme assays. The stock solutions were prepared from large crystals of Na_2S that were thoroughly rinsed with distilled water to remove oxidized sulfur compounds from the surface of the crystals. The oxygen electrode was calibrated between each run with air-saturated seawater, and the stability of the electrode (which could be affected by sulfide) was tested daily. No sulfide poisoning of the electrode occurred during the short periods of the respiration measurements.

Tissue	Percent inhibition by 0.6 m*M* sulfide
Riftia pachyptila	
Plume	11 ± 5 (3)
Plume homogenate	13 ± 4 (2)
Yellowfin tuna (*Thunnus albacares*) gill	52 ± 11 (2)
Shark (*Carcharhinus longimanus*) gill	59 ± 5 (3)
Rockfish (*Sebastes carnatus*) gill	77 ± 7 (3)

moles per liter (*10*). Arp and Childress (*11*) have shown, however, that the vascular blood of *R. pachyptila* contains sulfide at concentrations ranging up to 1.1 m*M*. During the Oasis Expedition to the 21°N hydrothermal vent site on the East Pacific Rise, we investigated the mechanisms used by *R. pachyptila* to avoid poisoning of sulfide-sensitive enzymes such as the cytochrome c oxidase system, which is the primary locus of sulfide toxicity in aerobic organisms (*10*).

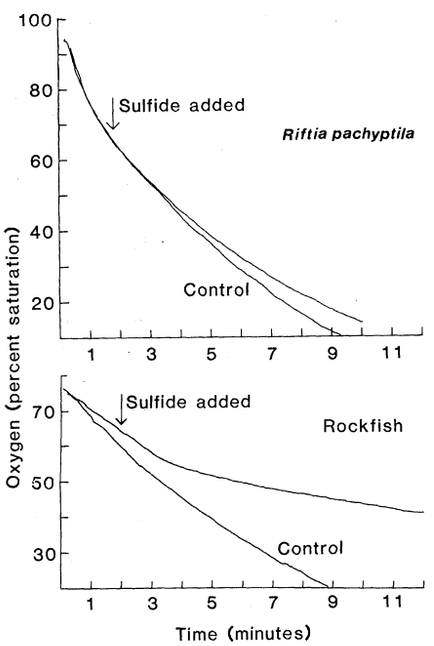

Fig. 1. Representative tracings showing the effects of sulfide on respiration of *R. pachyptila* obturacular plume and rockfish (*Sebastes carnatus*) gill. The control traces were obtained with no sulfide added. The arrow indicates the time of sulfide addition (final sulfide concentration was 0.6 m*M*). For experimental details, see legend to Table 1.

Three potential mechanisms for avoiding sulfide poisoning of aerobic metabolism were studied: (i) permeability barriers that could prevent sulfide from reaching the intracellular compartments where sulfide-sensitive enzymes are present—for example, the cytochrome c oxidase system of mitochondria; (ii) sulfide-insensitive forms of cytochrome c oxidase that could function in the presence of sulfide concentrations normally toxic to organisms that do not inhabit vents; and (iii) protective factors that could prevent sulfide poisoning of respiration by tightly binding sulfide and preventing accumulation of toxic levels of free sulfide within the cells.

We initially examined the sulfide sensitivity of respiration of tissues from *R. pachyptila* and non-vent organisms (Fig. 1 and Table 1). We used the highly vascularized obturacular plume from *R. pachyptila*, a tissue that serves as the major exchange site for materials transferred between the worm and the ambient seawater. The plume is the only portion of the worm to contact seawater directly, and it consists of numerous small, fused tentacles attached to a central supporting obturaculum (*5*). The sulfide sensitivities of gill tissues from surface-living fishes were used for comparison. The rate of oxygen consumption by plume tissue was only slightly inhibited by high (0.6 m*M*) concentrations of sulfide, whereas respiration of fish gills was rapidly and strongly inhibited (Fig. 1 and Table 1). To determine whether insensitivity of plume respiration to sulfide was due to an inability of sulfide to penetrate the plume, we examined the effects of 0.6 m*M* sulfide on the respiration of plume homogenates having protein contents roughly equal to those in the intact tissue used for respiration measurements (Table 1). Homogenization of the plume did not lead to an increase in sulfide sensitivity, suggesting that sulfide permeability barriers are not involved in the resistance to sulfide poisoning of respiration.

To determine whether the insensitivity of plume respiration to sulfide was due to the existence of a sulfide-insensitive form of cytochrome c oxidase, we partially purified this enzyme from plume tissue. The cytochrome c oxidase of plume, assayed spectrophotometrically (*12*) at very low protein concentration, exhibited a sulfide sensitivity comparable to homologs of the enzyme from nonvent organisms; sulfide concentrations of less than 10 μ*M* led to 90 percent inhibition of the most purified enzyme preparation (Fig. 2). We noted, however, that the effect of sulfide on tube worm

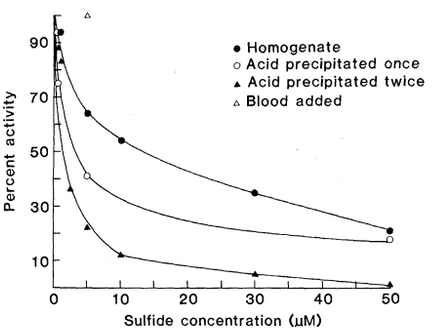

Fig. 2. The effects of varying concentrations of sulfide on cytochrome c oxidase activity of obturacular plume of *R. pachyptila*. Cytochrome c oxidase was prepared (*13*) and assayed spectrophotometrically (*12*). Reduced cytochrome c (final concentration in the cuvette was 1.0 m*M*) was prepared by reduction with sodium dithionite, followed by chromatography on Sephadex G-25 to remove low molecular weight sulfur compounds. The "blood added" point corresponds to the addition of fresh vascular blood from *R. pachytila* to a final concentration of 0.45 mg of protein per milliliter in the assay cuvette. Protein content of the undiluted blood sample was 45 mg/ml, as determined by the method of Peterson (*15*).

cytochrome c oxidase was dependent on the extent of enzyme purification (Fig. 2). Cytochrome c oxidase that had been acid-precipitated twice (*13*) was significantly more sensitive to sulfide than, for example, the crude supernatant preparation. This finding suggested that the mechanisms for protecting respiration from sulfide poisoning might involve factors present in the blood or cytosol that render sulfide unable to inhibit cytochrome c oxidase.

To examine whether the relative insensitivity to sulfide of cytochrome c oxidase in crude supernatant preparations was due to a general protein binding of sulfide, we added bovine serum albumin (BSA) to the assay cuvette to a final BSA concentration of 100 mg/ml. No protection against sulfide poisoning occurred. When we tested the vascular blood from *R. pachyptila* for its protective effects, however, we found that blood concentrations as low as 0.45 mg of protein per milliliter in the cuvette could completely reverse the inhibitory effects of sulfide (Fig. 2). In contrast, whole blood from a freshly collected whitetip shark (*Carcharhinus longimanus*) had no disinhibitory effects. This indicates that the factors present in *R. pachyptila* blood are not ubiquitous blood constituents. The protective factors do not appear to be present in the cytosol of *R. pachyptila* tissues. Portions of homogenates of *R. pachyptila* vestimental muscle had no disinhibitory effects. Because vestimental muscle, unlike the plume, is very poorly vascularized, extremely little blood was present in the muscle homogenates in comparison with the plume homogenates, which were bright red in color. We conclude that blood-borne factors are responsible for protecting the respiration of *R. pachyptila* from sulfide poisoning.

Arp and Childress (*11*) provided strong evidence that these blood-borne factors are sulfide-binding proteins. These factors appear not to be previously characterized forms of modified hemoglobin, such as methemoglobin, which bind sulfide in other organisms (*14*). Nor are they enzymes capable of oxidizing sulfide to less toxic sulfur compounds. Although sulfide-oxidizing enzymes are present in the bacteria-containing trophosome of *R. pachyptila*, no capacities for sulfide oxidation were observed in plume or blood (*6, 7*). Thus, the sulfide-binding proteins in the blood of *R. pachyptila* may prevent sulfide poisoning of aerobic metabolism because these proteins have higher binding affinities for sulfide than do sulfide-sensitive enzymes like cytochrome c oxidase. These sulfide-binding proteins may have the dual role of sulfide transport (*11*) and protection against sulfide poisoning.

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Insulin Receptor: Evidence That It Is a Protein Kinase

Abstract. *Highly purified preparations of insulin receptor catalyzed the phosphorylation of the 95,000-dalton subunit of the insulin receptor. This subunit of the insulin receptor was also labeled with [α-^{32}P]8-azidoadenosine 5′-triphosphate, a photoaffinity label for adenosine triphosphate binding sites. The identity of the 95,000-dalton band was confirmed in both cases by precipitation with a monoclonal antibody to the insulin receptor. These results suggest that the insulin receptor is itself a protein kinase.*

Insulin binds to specific membrane receptors on the surface of target cells and then regulates a variety of metabolic processes (*1*). However, it is not known how the interaction of insulin with its receptor elicits changes in cellular functions (*1*). Since insulin can regulate the phosphorylation of several proteins (*2*), one possibility is that insulin exerts its effects via the stimulation of a protein kinase or inhibition of a protein phosphatase. Kasuga *et al.* (*3*) reported that the insulin receptor is itself phosphorylated in intact cells and that the extent of this phosphorylation is regulated by insulin. Recently, the phosphorylation of the insulin receptor was observed in solubilized rat liver plasma membranes and the phosphorylation of the receptor was shown to be on a tyrosine residue (*4*). These studies raise the possibility that the insulin receptor is itself a protein kinase. The present studies were designed to test this hypothesis by using a highly purified insulin receptor preparation and a photoaffinity label for proteins binding adenosine triphosphate (ATP).

Insulin receptors were purified from cultured lymphocytes (IM-9 cells) by using an affinity column containing a monoclonal antibody to the insulin receptor (*5*). Since the binding of this antibody to the receptor is competitive with that of insulin (*5*), the receptor could be specifically eluted from the column with 1 μ*M* insulin. The insulin receptor was then further purified on a wheat germ agglutinin column (*6*). To determine the purity of the insulin receptor preparations, the IM-9 cells were first cultured in the presence of [^{35}S]methionine for 16 hours to label all the proteins in the cell. The labeled receptor preparation could then be visualized by autoradiography of sodium dodecyl sulfate (SDS)–polyacrylamide gels. Under reducing conditions, the purified insulin receptor preparation exhibited two major bands (Fig. 1A) with apparent molecular weights of 135,000 (135K) and 95,000 (95K), values identical to those reported for the α and β subunits, respectively, of the insulin receptor (*1*). Densitometric scans of the autoradiographs showed that more than 90